Fungal Biology

Ram Prasad
S. Chandra Nayak
Ravindra Nath Kharwar
Nawal Kishor Dubey *Editors*

Mycoremediation and Environmental Sustainability

Volume 3

Springer

Fungal Biology

Series Editors

Vijai Kumar Gupta
Biorefining and Advanced Materials Research Center
Scotland's Rural College (SRUC)
SRUC Barony Campus, Parkgate
Dumfries DG1 3NE
Scotland, United Kingdom

Maria G. Tuohy
School of Natural Sciences
National University of Ireland Galway
Galway, Ireland

Fungal biology has an integral role to play in the development of the biotechnology and biomedical sectors. It has become a subject of increasing importance as new fungi and their associated biomolecules are identified. The interaction between fungi and their environment is central to many natural processes that occur in the biosphere. The hosts and habitats of these eukaryotic microorganisms are very diverse; fungi are present in every ecosystem on Earth. The fungal kingdom is equally diverse, consisting of seven different known phyla. Yet detailed knowledge is limited to relatively few species. The relationship between fungi and humans has been characterized by the juxtaposed viewpoints of fungi as infectious agents of much dread and their exploitation as highly versatile systems for a range of economically important biotechnological applications. Understanding the biology of different fungi in diverse ecosystems as well as their interactions with living and non-living is essential to underpin effective and innovative technological developments. This series will provide a detailed compendium of methods and information used to investigate different aspects of mycology, including fungal biology and biochemistry, genetics, phylogenetics, genomics, proteomics, molecular enzymology, and biotechnological applications in a manner that reflects the many recent developments of relevance to researchers and scientists investigating the Kingdom Fungi. Rapid screening techniques based on screening specific regions in the DNA of fungi have been used in species comparison and identification, and are now being extended across fungal phyla. The majorities of fungi are multicellular eukaryotic systems and therefore may be excellent model systems by which to answer fundamental biological questions. A greater understanding of the cell biology of these versatile eukaryotes will underpin efforts to engineer certain fungal species to provide novel cell factories for production of proteins for pharmaceutical applications. Renewed interest in all aspects of the biology and biotechnology of fungi may also enable the development of "one pot" microbial cell factories to meet consumer energy needs in the 21st century. To realize this potential and to truly understand the diversity and biology of these eukaryotes, continued development of scientific tools and techniques is essential. As a professional reference, this series will be very helpful to all people who work with fungi and should be useful both to academic institutions and research teams, as well as to teachers, and graduate and postgraduate students with its information on the continuous developments in fungal biology with the publication of each volume.

More information about this series at http://www.springer.com/series/11224

Ram Prasad • S. Chandra Nayak
Ravindra Nath Kharwar • Nawal Kishor Dubey
Editors

Mycoremediation and Environmental Sustainability

Volume 3

Springer

Editors
Ram Prasad
Department of Botany
Mahatma Gandhi Central University
Motihari, Bihar, India

S. Chandra Nayak
DoS in Biotechnology
University of Mysore
Manasagangotri, Mysore, India

Ravindra Nath Kharwar
Department of Botany
Banaras Hindu University
Varanasi, Uttar Pradesh, India

Nawal Kishor Dubey
Department of Botany
Banaras Hindu University
Varanasi, Uttar Pradesh, India

ISSN 2198-7777 ISSN 2198-7785 (electronic)
Fungal Biology
ISBN 978-3-030-54421-8 ISBN 978-3-030-54422-5 (eBook)
https://doi.org/10.1007/978-3-030-54422-5

This Springer imprint is published by the registered company Springer Nature Switzerland AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Preface

Mycoremediation is a process of bioremediation in which fungal-based technology is used to decontaminate the environment. Fungi have been confirmed to be a very cost-effective and environmentally sound way for helping to remove an extensive array of contaminants from damaged environments or wastewater. The contaminants include heavy metals, persistent organic pollutants [polycyclic aromatic hydrocarbons (PAHs), pesticides, and herbicide], textile dyes, leather tanning industry chemicals and wastewater, petroleum fuels, pharmaceuticals, and personal care products. The by-products of the remediation can be appreciated constituents themselves, such as enzymes (like laccase) and edible or medicinal mushrooms, making the remediation process even lucrative. Mycoremediation practices involve placing of mycelium into contaminated soil and placing mycelial mats over toxic sites or a combination of these techniques in one or more treatments. Toxins in our food chain (including heavy metals, PCBs, and dioxins) become more concentrated at each step, with those at the top being contaminated by ingesting toxins consumed by those lower on the food chain. Fungal mycelia can destroy these toxins in the soil before they enter our food supply.

Fungi are among the primary saprotrophic organisms in an ecosystem, as they are efficient in the decomposition of material. Wood-decay fungi, especially white rot, secrete extracellular enzymes and acids that break down lignin and cellulose. Fungi feature among nature's most vital agents for the decomposition of waste matter and are crucial components of the soil food web, providing nourishment for the supplementary biota that live in the soil environment. The degree of sustainability of the physical environment is an index of the survival and well-being of the all-inclusive components in it. Additionally, it is not sufficient to try disposing toxic/deleterious substances with any known method. The best method of sustaining the environment is to return all the components (wastes) in a recyclable way so that the waste becomes useful and helps the biotic and abiotic relationship to maintain an aesthetic and healthy equilibrium that characterizes an ideal environment.

This book should be immensely valuable for researchers, technocrats, policy makers, and scientists of fungal biology and those who are interested in environmental sustainability. We are honored that leading scientists who have extensive,

in-depth understanding and expertise in fungal biology and environmental concern took the time and effort to develop these outstanding chapters. Each chapter is written by globally recognized academicians, so the reader is given an up-to-date and detailed account of our knowledge of the fungal system and numerous applications of fungi.

We are indebted to the many people who helped bring this book to light. The Editors wish to thank Series Editors Dr. Vijai Kumar Gupta and Dr. Maria G. Tuohy as well as Dr. Eric Stannard, Senior Editor, Botany, Springer, for their generous assistance, constant support, and patience in initializing the volume. Editors in particular are very thankful to Springer's Nicholas DiBenedetto, Anthony Dunlap, and Rahul Sharma (Project Coordinator) for the kind care and constant encouragement received. Ram Prasad thanks honorable Vice Chancellor Dr. Sanjeev Kumar for continuous support and inspiration in putting everything together. Special thanks are due to our well-wishers and friends.

Motihari, Bihar, India — Ram Prasad
Mysore, India — S. Chandra Nayak
Varanasi, India — Ravindra Nath Kharwar
Varanasi, India — Nawal Kishor Dubey

Contents

About the Editor

Ram Prasad is associated with the Department of Botany, Mahatma Gandhi Central University, Motihari, Bihar, India. His research interest includes applied and environmental microbiology, plant–microbe interactions, sustainable agriculture, and nanobiotechnology. Dr. Prasad has more than one hundred seventy five publications to his credit, including research papers, review articles and book chapters and five patents issued or pending, and edited or authored several books. Dr. Prasad has 12 years of teaching experience and has been awarded the Young Scientist Award and Prof. J.S. Datta Munshi Gold Medal by the International Society for Ecological Communications, FSAB Fellowship by the Society for Applied Biotechnology, the American Cancer Society UICC International Fellowship for Beginning Investigators; Outstanding Scientist Award in the field of Microbiology by Venus International Foundation; and BRICPL Science Investigator Award and Research Excellence Award. He has been serving as Editorial Board Member of *Frontiers in Microbiology, Frontiers in Nutrition*, *Archives of Phytopathology and Plant Protection*, *Phyton-International Journal of Experimental Botany*, *Biocell, IET Nanobiotechnology*, and *Journal of Renewable Materials* as well as Series Editor of *Nanotechnology in the Life Sciences*, Springer Nature, USA. Previously, Dr. Prasad served as Assistant Professor at Amity University Uttar Pradesh, India; Visiting Assistant Professor, Whiting School of Engineering, Department of Mechanical Engineering, Johns Hopkins University, Baltimore, United States; and Research Associate Professor at the School of Environmental Science and Engineering, Sun Yat-sen University, Guangzhou, China.

S. Chandra Nayak is an Agricultural Biotechnology Researcher specialized in the application of biotechnology/nanotechnology tools in plant protection, pathogen detection, and plant–pathogen interaction. He has 20 years of experience in research, including Visiting Scientist at Danish Government Institute of Seed Pathology, Denmark; China Agricultural University, Beijing, China; and Principal Investigator for thirteen ICAR-AICRP-Pearl Millet Pathology Research Centre. Dr. Nayak is one of the members of the team that established the Asian Seed Health Centre at the University of Mysore, with the assistance from DANIDA, Denmark. His laboratory is one of the leading research laboratories on seed health and plant protection research in India. Dr. Nayak is recipient of the prestigious Japan International Award for Young Agricultural Researchers and The Millennium Plaques Honor (Prime Minister Award for Contribution in the field of Science & Technology) by the Indian Science Congress Association, DST, Govt. of India. Dr. Nayak has more than 2000 research citations with 27 h Index and 4 national patents; published 90 research articles in reputed national and international journals, 8 books, 15 book chapters (CABI/Springer/Elsevier/Nova Science, etc.), 3 application notes (Agilent Technologies, USA), 6 technical bulletins, and 16 short publications/popular articles; and submitted more than 1000 nucleotide sequences of seed-borne pathogens and bio-control agents to NCBI-USA, EMBL-Europe, and DDBJ-Japan database.

Ravindra Nath Kharwar is currently serving as a Professor at the Centre of Advanced Study in Botany, Institute of Science, Banaras Hindu University, Varanasi. He has over 30 years of experience in teaching and research. Prof. Kharwar has more than 90 research papers, 11 reviews in journals of repute, and over 22 book chapters are to his credit. The highest impact factors of journals in which he has published are 14.34 and 12.00, that is, *Trends in Biotechnology* and *Natural Product Reports*. He is Fellow of the Mycological Society of India, the Indian Phytopathological Society, and BOYSCAST and is the recipient of Dr. S.K. Shome Memorial Lecture Award in 2012, Dr. V. Agnihothrudu Memorial Lecture Award in 2016, Dr. AK Sarbhoy Memorial Award in 2019, and Professor P.C.Jain Memorial Award in 2021.

Prof. Kharwar is associated with various reputed journals in capacities of either Editor-in-Chief/Editor or member of editorial board. He has worked with Prof. Gary Strobel, Department of Plant Sciences, Montana State University, Bozeman, USA, as a BOYSCAST Fellow. Prof. Kharwar has been working on fungal and actinobacterial endophytes. His research focus is on isolation, purification, and characterization of bioactive molecules from fungal and actinobacterial endophytes along with documenting their diversity and ecology. Abiotic stress alleviation and

metal removal activities are also performed. He has been conducting epigenetic study for cryptic metabolites from endophytic microbes. In addition, endophytes derived biosynthesis (Green Synthesis) of metal nanoparticles and their usages and understanding the mechanism of induced resistance in plants against diseases by using endophytes/or PGPR are also his focus points. He has guided 15 Ph. D. candidates and over 50 dissertation students, and 8 Ph.D. students are working currently under him. Prof. Kharwar has visited countries like Iran, UAE, the Netherland, the USA, Sweden, China, and Thailand in different capacities.

Nawal Kishor Dubey has significantly contributed to the important area of botanical pesticides. He has formulated several novel plant-based preservatives that exhibit significant potency in control of biodeterioration of food from fungi, mycotoxins, and insects as well as from lipid peroxidation. Prof. Dubey has been granted 3 patents; published 360 research papers, review articles, and 13 books; and is a recipient of several awards including Prof. M. J. Narshimhan Award and Young Scientist Award. He acted as Chairperson, Session Coordinator, and Key Speaker at the 9th ICPP 2008, held between August 24 and 29, 2008, Torino, Italy. Dr. Dubey has been awarded several fellowships: Visiting Fellow, University of Iri, South Korea, under INSA Exchange (1991); Tokyo University of Pharmacy and Life sciences, Tokyo, Japan, under INSA-JSPS Exchange Programme (1996); INSA visiting Fellow CFTRI, Mysore (1995). He has been invited Professor at the University De French, Besancon, France (1997), and Kings College, London, under INSA-Royal Society Exchange Programme for 3 months (2001) and Coordinator, for Applied Microbiology at BHU (2012–2015). Prof. Dubey has been Fellow and Member of National Academy of Sciences (FNASc) and National Academy of Agricultural Sciences (NAAS), India.

Contributors

Mohd. Aamir Khan Centre for Rural Development and Technology, IIT-Delhi, New Delhi, India

O. P. Abioye Department of Microbiology, Federal University of Technology, Minna, Nigeria

Ricardo Aguilar-López Department of Biotechnology and Bioengineering. CINVESTAV, Instituto Politécnico Nacional, México City, México

Sandra Pérez Álvarez Facultad de Ciencias Agrícolas y Forestales, Universidad Autónoma de Chihuahua, Chihuahua, Mexico

S. A. Aransiola Bioresources Development Centre, National Biotechnology Development Agency, Ogbomoso, Nigeria

Eduardo Fidel Héctor Ardisana Instituto de Posgrado, Universidad Técnica de Manabí, Portoviejo, Ecuador

S. H. Auta Department of Microbiology, Federal University of Technology, Minna, Nigeria

Geeta Bhandari Department of Biochemistry and Biotechnology, SBS University, Dehradun, Uttarakhand, India

Edenis Cesar de Oliveira Federal University of São Carlos, São Paulo, Brazil

Neha Dhingra Department of Zoology, University of Delhi, New Delhi, India

Eva Duborská Institute of Laboratory Research on Geomaterials, Faculty of Natural Sciences, Comenius University in Bratislava, Bratislava, Slovakia

Neeraj Dwivedi TERI-Deakin Nano Biotechnology Centre, The Energy and Resources Institute (TERI), TERI GRAM, Gurgaon, India

S. A. Dwivedi Department of Entomology, School of Agriculture, Lovely Professional University, Phagwara, Punjab, India

Yit Kheng Goh School of Science, Monash University Malaysia, Bandar Sunway, Selangor Darul Ehsan, Malaysia

Advanced Agriecological Research Sdn. Bhd., Petaling Jaya, Selangor Darul Ehsan, Malaysia

Mayurika Goel TERI-Deakin Nano Biotechnology Centre, The Energy and Resources Institute (TERI), TERI GRAM, Gurgaon, India

Saurabh Gupta Department of Microbiology, Mata Gujri College, Fatehgarh Sahib, India

Ingrid Hagarová Institute of Laboratory Research on Geomaterials, Faculty of Natural Sciences, Comenius University in Bratislava, Bratislava, Slovakia

U. J. J. Ijah Department of Microbiology, Federal University of Technology, Minna, Nigeria

Rishu Kalra TERI-Deakin Nano Biotechnology Centre, The Energy and Resources Institute (TERI), TERI GRAM, Gurgaon, India

Gaganpreet Kaur Department of Biotechnology & Medical Sciences, Baba Farid Group of Institutions, Baba Farid College, Bathinda, India

Malavika Ram Amanthra Keloth Department of Plant Pathology, School of Agriculture, Lovely Professional University, Phagwara, Punjab, India

Hyunjung Kim Department of Mineral Resources and Energy Engineering, Jeonbuk National University, Jeonju, Republic of Korea

Deepak Kumar Department of Botany, Institute of Science, Banaras Hindu University, Varanasi, Uttar Pradesh, India

Vankayalapati Vijaya Kumar Natems Sugar Private Limited, Gachibowli, Hyderabad, Telangana, India

Juana Lira-Pérez Department of Biotechnology and Bioengineering. CINVESTAV, Instituto Politécnico Nacional, México City, México

Pavol Littera Institute of Laboratory Research on Geomaterials, Faculty of Natural Sciences, Comenius University in Bratislava, Bratislava, Slovakia

Peter Matúš Institute of Laboratory Research on Geomaterials, Faculty of Natural Sciences, Comenius University in Bratislava, Bratislava, Slovakia

Rahul Chandra Mishra TERI-Deakin Nano Biotechnology Centre, The Energy and Resources Institute (TERI), TERI GRAM, Gurgaon, India

Jashan Nirwan Department of Microbiology, Mata Gujri College, Fatehgarh Sahib, India

M. I. Ojeba Department of Microbiology, Federal University of Technology, Minna, Nigeria

Riya Rachel Department of Plant Pathology, School of Agriculture, Lovely Professional University, Phagwara, Punjab, India

Meenakshi Raina Department of Botany, Central University of Jammu, Rahya Suchani (Bagla), Samba, Jammu and Kashmir, India

Department of Biotechnology, Government Degree College (Boys), Udhampur, Jammu and Kashmir, India

Adriana de J. Ramírez-Castillo National Center for Disaster Prevention, Ministry of Interior, México City, México

Chilkuri Udaykiran Reddy Department of Plant Pathology, School of Agriculture, Lovely Professional University, Phagwara, Punjab, India

Refugio Rodríguez-Vázquez Department of Biotechnology and Bioengineering. CINVESTAV, Instituto Politécnico Nacional, México City, México

Akanksha Sharma Department of Botany, Central University of Jammu, Rahya Suchani (Bagla), Jammu and Kashmir, India

Mukund Sharma Department of Biochemistry and Biotechnology, SBS University, Dehradun, Uttarakhand, India

Ranju Sharma Department of Zoology, University of Delhi, New Delhi, India

Aditya Singh Department of Botany, Central University of Jammu, Rahya Suchani (Bagla), Jammu and Kashmir, India

Ngangbam Sarat Singh Department of Zoology, Dr. SRK Government Arts College, Yanam, Puducherry, India

Ramji Singh Department of Plant Pathology, Sardar Vallabhbhi Patel University of Agriculture & Technology, Meerut, Uttar Pradesh, India

Saurabh Kumar Singh Department of Horticulture, Janta Vedic College, Baraut, Uttar Pradesh, India

Jyoti Srivastava Department of Botany, GSRMM P.G. College, Bhadoi, Lucknow, Uttar Pradesh, India

Edgar N. Tec-Caamal Department of Biotechnology and Bioengineering. CINVESTAV, Instituto Politécnico Nacional, México City, México

Adeline Su Yien Ting School of Science, Monash University Malaysia, Bandar Sunway, Selangor Darul Ehsan, Malaysia

Ajay Tomer Department of Plant Pathology, School of Agriculture, Lovely Professional University, Phagwara, Punjab, India

Martin Urík Institute of Laboratory Research on Geomaterials, Faculty of Natural Sciences, Comenius University in Bratislava, Bratislava, Slovakia

Shulbhi Verma Department of Biotechnology, College of Basic Science & Humanities, SDAU, S.K Nagar, Gujarat, India

Laíze Aparecida Ferreira Vilela Federal University of São Carlos, São Paulo, Brazil

Sonal Yadav Centre for Rural Development and Technology, IIT-Delhi, New Delhi, India

Chapter 1
Bioremediation of Toxic Pesticides in Soil Using Microbial Products

O. P. Abioye, U. J. J. Ijah, S. A. Aransiola, S. H. Auta, and M. I. Ojeba

Contents

1.1 Introduction

Soil pollution is a serious global threat and, hence, an effective remediation technology is of great importance (Abioye et al. 2019). Rapid industrialization along with increasing population has resulted in a wide accumulation of chemicals (Aransiola et al. 2013). The recurrence and enormous utilization of 'xenobiotic' chemicals have prompted an amazing push toward new innovations in order to reduce or eliminate these contaminants from the environment. The techniques traditionally used for the

O. P. Abioye (✉) · U. J. J. Ijah · S. H. Auta · M. I. Ojeba
Department of Microbiology, Federal University of Technology, Minna, Nigeria
e-mail: bisyem2603@yhoo.com

S. A. Aransiola
Bioresources Development Centre, National Biotechnology Development Agency, Ogbomoso, Nigeria

R. Prasad et al. (eds.), *Mycoremediation and Environmental Sustainability*, Fungal Biology, https://doi.org/10.1007/978-3-030-54422-5_1

remediation of polluted sites (e.g., recycling, landfilling, pyrolysis, and incineration) affect the environment as well, which can cause the release of toxic intermediates (Debarati et al. 2005; Prasad 2021). Moreover, these techniques are expensive and sometimes difficult to execute, particularly in broad agricultural areas (Jain et al. 2005). One promising technique is bioremediation which exploits the capacity of microorganisms to expel toxins from polluted environment, an option that is viable, negligibly hazardous, economical, flexible, and environmentally friendly (Finley et al. 2010). Pesticides have turned into an unavoidable part of present-day agriculture because of their need in economical pest management and in the enhancement of product quality (Gouma 2009). Be that as it may, increased use of pesticide significantly affects climate, around 90% of pesticides applied by farmers failed to completely achieved the set objectives as it affect farmers health directly, escaped into the soil, air and washed into water bodies. Out of the aggregate unpredictable outflow to nature, 63% are pesticides (Yates et al. 2011). Overall, their capacity to collect into the tissues of living beings prompting bioaccumulation is the real concern. Each of these factors contributes to environmental contamination and significant strides are taken to handle this issue. The conventional methods utilized for the treatment of these contaminants are compelling and additionally have certain disadvantages; for example they are expensive and the quality of these procedures is low. Likewise, most of the time, these systems are not adequate (Dixon 1996). Pesticide management should essentially maintain soil quality which is of high concern. Pesticides constitute the key control system for crop pest and disease management. Nonstop application of these pesticides to the soil and aquatic system poses risks to well-being and results in environmental contamination, which has activated much open concern. Consistence application of these pesticides throughout the years has brought about issues created by their cooperation with the biological framework in nature. Despite the risks, pesticides will continue to be a crucial component in agriculture in years to come as there is no reasonable other option to absolutely supplant them. Considering the lethal impact of the pesticides, it is fundamental to expel them from the environment with appropriate remediation measures. Bioremediation is one of the current methods utilized for environmental cleanup. In this process, heterotrophic microorganisms are used to separate carbon and other vital compounds from perilous mixtures. Organophosphorous compounds alone compensate for 70% of the pesticides utilized around the world.

It has been found that microorganisms can alter and degrade xenobiotics; researchers have been investigating different microbial qualities, especially around polluted environments looking for microorganisms that can help in the remediation of an extensive variety of contaminations. Subsequently, biotransformation of environmental contaminants in the regular habitat has been widely considered to comprehend microbial ecology, physiology, and development because of their bioremediation potential (Mishra et al. 2001; Kumar M et al. 2017; Kumar V et al. 2017). The biochemical and genetic basis of microbial degradation has gotten impressive consideration. A few genes/enzymes, which furnish microorganisms with the capacity to remediate organopesticides, have been recognized and portrayed. In this manner, microorganisms has proved to be a better and safer option in the biodegradation of pesticides. The capacity of these microorganisms to degrade xenobiotics is specifically connected to their adaptation to conditions where these

compound exist. Also, genetic engineering might be utilized to upgrade the properties of such microorganisms that have the desirable characteristics required for biodegradation (Schroll et al. 2004). Around 30% of agricultural produce is lost because of pests. Consequently, increased utilization of pesticides has turned out to be irreplaceable in agribusiness and has become a part of agribusiness. Nonetheless, the unpredictable utilization of pesticides also poses serious threats and issues to people and the biodiversity (Gavrilescu 2005; Hussain et al. 2009). Environmental pollution caused by pesticides is also noted in regions where pesticides are not used. The agricultural pesticides applications gets to the soil and can diffuse quickly until it reaches the water table at noticeable concentration which affects different categories of living organisms. Therefore, the fate of pesticides is unpredictable and they can degrade different regions apart from where they were initially utilized. Hence, cleaning pesticide-contaminated zones becomes an extremely complex errand (Gavrilescu 2005).

Organochlorine pesticides were generally in use during the 1970s, especially in the United States. Although their utilization has been ousted in numerous nations, they are still used in many developing countries. Organochlorine pesticides get aggregated in living beings and pose interminable risks to well-being, for example, cancer, neurological, and teratogenic impacts (Vaccari et al. 2006). Numerous xenobiotic compounds are unmanageable and resistant to biodegradation, especially organochlorine pesticides (Chaudhry and Chapalamadugu 1991; Dua et al. 2002). As a result, these exceedingly dangerous and cancer-causing compounds hold on in the environment for a relatively long time. But in reality organophosphorus pesticides are generally utilized in the United States. These pesticides affect the nervous system of insects and humans, in addition to influencing the reproductive system (Colosio et al. 2009; Jokanovic and Prostran 2009). Increased utilization of organophosphorus in agribusiness has begun to result in different environmental issues (Singh and Walker 2006). In spite of the fact that these pesticides degrade rapidly in water, there is a possibility that the buildups and by-products of these pesticides remain in unsafe levels in living beings (Silva et al. 1999; Ragnarsdottir 2000). Carbamate pesticides are imperative in the farming because of their wide movement range. Notwithstanding an extensive variety of compound, they are moderately pollute the environment and for the most part are less harmful to people (Wolfe et al. 1978). Nonetheless, they interfere with the activity of enzyme acetylcholinesterase, thereby inhibiting the hydrolysis of acetylcholine (AcH) which results in the accumulation of AcH. This leads to different manifestations, for example, sweating, lacrimation, hypersalivation, and convulsion of extremities (Suzuki and Watanabe 2005). Hence, this class of pesticides are considered lethal. Cleaning the pesticide-infested environment is a troublesome matter and can be exorbitant. Indeed, the negative effects from pesticides in the environment are for all intents and out-weighed its usefulness. Any measure used to diminish the impacts of pesticides on the environment will only be a palliative measure and not a solution. Unfortunately, there is a constant threat to the organisms and environment, for instance, the annihilation of the avian species and microorganisms on the planet. Organic strategies are more reliable to disinfect regions that have been contaminated by pesticides. These techniques use a large

number of microorganisms in the environment, whose specific end goal is to eliminate pesticides from the contaminated zone. Numerous native microorganisms develop complex and viable metabolic pathways that allow the biodegradation of pollutants that are discharged into nature. In spite of the fact that the metabolic procedure is long, it is considered a more suitable option for evacuating the wellsprings of xenobiotic compound and the contamination they cause (Diaz 2004; Schoefs et al. 2004; Finley et al. 2010). By virtue of the deadly dangers synthetic pesticides stance to the living beings, there is an unending quest for environmentally friendly pesticides that can support agricultural enterprise. Organic pesticides depend on common exacerbates that viably control the invasion of bugs in agribusiness. As opposed to synthetic pesticides, organic pesticides are advantageous in that they are efficient and do not cause inadvertent blowback (Gerhardson 2002; Raaijmakers et al. 2002; Fravel 2005). This chapter discusses the degradation of pesticides using microorganisms and their metabolites. This topic is infinite, and we are going to underscore the most recent points, including studies on the biodegradation of organochlorine, organophosphorus, and carbamate pesticides by microbiological processes.

1.2 Pesticides

A pesticide can be defined as any substance or mixture of substances that counteract, devastate, repulse, or destruct any pest (e.g., nematodes, insects, parasites, rats, weeds). Pesticides like herbicides, fungicides, and insecticides and different materials are utilized to control pests (EPA 2015).

Every year, millions of tons of pesticides are used throughout the world. The expenditures on pesticides were 35.8 billion in 2006, which increased to 39.4 billion US dollars in 2007. One of the essential concerns is to limit hurtful impacts brought by organisms including viruses, bacteria, fungi and insects (Liu et al. 2001). The broad utilization of pesticides causes environmental worries, as just 5% or less from the applied pesticides achieve the objective living beings which brought about contamination of soil and water bodies (major environmental problem of current age). Occasional utilization of pesticides results in the process of pesting. This redundancy in the long time application without remediation, essentially prompts pesticides and their deposits in the environments, endangering the whole populace by their multifaceted toxicity (Bouziani 2007).

1.2.1 Types of Pesticides

Synthetic pesticides (Table 1.1) offer many benefits to agriculture; however, as discussed before, they are lethal to other non-target life forms and cause environmental contamination. Therefore, research works are focusing on new pests control choices due to the impacts of these compounds on human well-being and on the environment. The persistence of pesticides in soil differs from 7 days to quite a while relying on the

Table 1.1 Summary of types of pesticides and their effects

Pesticides	Class	Examples	Health effects
Insecticides	Organophosphates	Parathion, malathion, methyl parathion, chlorpyrifos, diazinon, dichlorvos, phosmet, fenitrothion tetrachlorvinphos and azinphos methyl	Neuropathy, myopathy, tremors, irritability, convulsions, inhibiting the enzyme acetylcholinesterase, paralysis
	Carbamates	Aldicarb, carbofuran (Furadan), fenoxycarb, carbaryl (Sevin), ethienocarb and fenobucarb	Inhibition of acetylcholinesterase enzyme, paralysis
	Organochlorines (dichlorodiphenyle thanes and cyclodienes)	DDT, dicofol, heptachlor, endosulfan, chlordane, aldrin, dieldrin, endrin, mirex and pentachlorophenol	Stimulation of the nervous system by disrupting the sodium/potassium balance of the nerve fiber, tremors, irritability, convulsions, hyperexcitable state of the brain, cardiac arrhythmiatic and reproductive problems
Herbicides	Phenoxy and benzoic acids, triazines, ureas, and Chloroacetanilides	Chlorophenoxy acids, hexachlorobenzene (HCB), Picloram, atrazine, simazine, propazine, diquat, paraquat, oxyfluorfen, alachlor, fluroxypyr	Dermal toxicity, carcinogenic effect, damage to the liver, thyroid, nervous system, bones, kidneys, blood and immune system.
Fungicides	Substituted benzenes, thiocarbamates, thiophthalimides, organomercury compounds, etc.	Chloroneb, chlorothalanil, hexachlorobenzene, ferbam, metam sodium, thiram, ziram, ethyl mercury	Damage to the liver, thyroid, nervous system, bones, kidneys, blood and immune system, carcinogenic property also
Rodenticides	Coumarins,1,3-indandione	Warfarin, coumatetralyl, difenacoum, brodifacoum, flocoumafen, bromadiolone diphacinone, chlorophacinone, pindone	
Nematicides		Aldicarb, dibromochloropropane	
Bactericides		Metiram, difolatan	
Botanicals		Perethrin, permethrin	

structure of the pesticide and penetration through the soil. For instance, the exceedingly toxic phosphates do not hold on for more than 3 months, while chlorinated hydrocarbon insecticides like chlordane are known to continue in any event for 4–5 years and a few times over 15 years. Constancy of pesticides represents a danger to domesticated animals and human well-being. Longer aplications of pesticides prompts the amassing of its deposits in soil which may come about into the expanded bioaccumulation by plants to the level at which the utilization of plant items may demonstrate harmful to

human being and also animals. Pesticides buildups in different environmental frameworks (soil and additionally water) have been reported around the world.

1.2.2 Biological Pesticides

As per the Environmental Protection Agency (EPA 2015), biopesticides are characterized as naturally occurring pest control substances. They are categorized into three groups (Joshi 2006):

Microbial pesticides: a microbial living thing (microorganisms, protozoans, parasites) is the dynamic control agent

Plant pesticides: pesticidal substances produced by plants from presented genetic material (plant consolidated protectants)

Biochemical pesticides: naturally occurring substances that control pests by nontoxic components. These incorporate substances that meddle with development or mating, for example, pheromones.

The good thing about biopesticides is their safety to non-target life form, biodegradability and their specificity, which allows the utilization of little measurements and power presentation, thus maintaining a strategic distance from contamination created by conventional pesticides (Rosell et al. 2008). Notwithstanding being less harmful than chemical pesticides, biopesticides are significantly utilized in integrated pest management (IPM) procedures, where they incredibly diminish the utilization of chemicals, thereby increasing harvest yields. The specificity of biopesticides varies widely depending on their chemical counterparts.

1.2.2.1 Organochlorine, Organophosphate, and Carbamate Pesticides

Organochlorine pesticides (Fig. 1.1) are being used widely throughout the world for public health and farming purposes. As of now, their utilization is being eliminated in light of their toxic quality, environmental industriousness and collection in the environmental way of life. Hexachlorocyclohexane (HCH) is a standout among the most widely used organochlorine pesticides for both agriculture and medical purposes. Although the use of a specialized mixture containing eight stereoisomers of organochlorine compounds was restricted in a few developing countries in the 1970s, many developed nations continue to use lindane (γ-HCH) for monetary reasons. Hence, new destinations are consistently being polluted by γ-HCH and its stereoisomers (Blais et al. 1998; Iwata et al. 1993).

As of now, among the different groups of pesticides used around the world, organophosphorus pesticides are the major and most widely used, accounting for more than 36% of the total world market. The most utilized among these is methyl parathion. Its accumulation causes numerous health risks; therefore, its degradation becomes vital (Ghosh et al. 2010). Organophosphorus pesticides (OP) are esters of phosphoric acid, also called organophosphates, which includes aliphatic, phenyl, and heterocyclic derivatives (Fig. 1.2). Organophosphates are used to control the

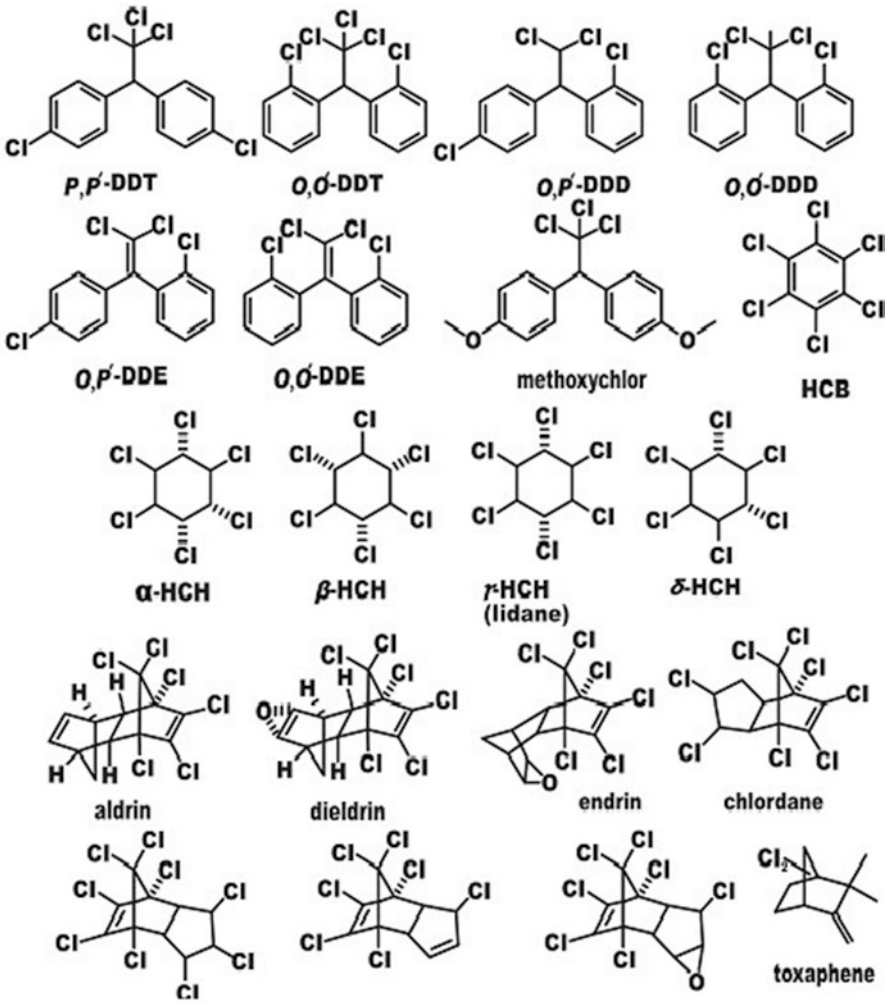


Fig. 1.1 Structure of organochlorine pesticide

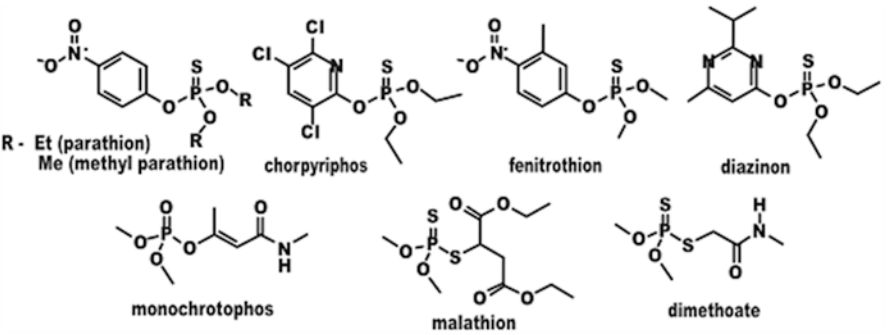


Fig. 1.2 Structure of organophosphate pesticide

sucking, biting, and boring insects, arachnid, aphids, and pests that assault crops like cotton, sugarcane, peanuts, tobacco, vegetables and other products of the soil. Organophosphorus pesticides are advertised by a considerable lot of the world's major agrochemical organizations. Few principal agricultural products are parathion, methyl parathion, chlorpyriphos, malathion, monocrotophos, diazinon, fenitrothion, and dimethoate (Fig. 1.2).

Carbamates were introduced as pesticides in the early 1950s and are still used extensively in pest control due to their effectiveness and broad spectrum of biological activity (insecticides, fungicides, herbicides). High polarity and solubility in water and thermal instability are typical characteristics of carbamate pesticides, as well as high acute toxicity. The carbamates are transformed into various products in consequence of several processes such as hydrolysis, biodegradation, oxidation, photolysis, biotransformation, and metabolic reactions in living organisms (Soriano et al. 2001). Chemically, the carbamate pesticides are esters of carbamates and organic compounds derived from carbamic acid (Fig. 1.3). This group of pesticides can be divided into benzimidazole-, *N*-methyl-, *N*-phenyl-, and thiocarbamates. The compounds derived from carbamic acid are probably the insecticides with the widest range of biocidic activities (Sogorb and Vilanova 2002).

1.2.3 Importance of Pesticides

The important goal of using pesticides in agricultural fields is to control vermins and disease vectors. This has been ponder upon as human efforts through research could be used in expanding agricultural yields and enhancing general wellbeing when pesticides are applied (Helweg 2003). Pesticides discharged into the environment may have a few unfriendly environmental impacts extending from long time impacts to numerous changes in biological community. In spite of the great consequences of utilizing pesticides in agriculture and public health, their utilization is typically with pernicious environmental and general well-being impacts. Pesticides are considered remarkable environmental contaminants because of their high organic toxicity (acute and chronic). Pesticides by definition are lethal compound operators. A pesticide is normally equipped with harmful substances to all types of life other than the focused pests. Because of this property, they can be best defined as biocides (fit to destroy all forms of life). Albeit a few pesticides are produced to be specific in their method of action, that their scope of selectivity is just restricted to the targeted pest.

Fig. 1.3 General structures of carbamate pesticides

1.2.4 Environmental Impact of Pesticides

The broad usage and transfer of pesticides by agriculturists, establishments and the overall population give numerous conceivable wellsprings of pesticides in the environment. Pesticides once discharged into the environment may have a wide range of destinies. Pesticides that are released can travel through the air and may in the long run get accumulated in different parts of the environment, for example, in soil or water. Pesticides that are connected specifically to the soil may be washed off the soil into nearby surface water bodies or may permeate through the soil to lower soil layers and groundwater (Harrison 1990). However, these exchanges not only happen between ranges that are near one another (for example, a neighborhood lake getting a portion of the herbicides connected to an adjoining land) but also additionally may include transportation of pesticides over long distance in the environment. The applications of DDT and the nearness of pesticides in waterways, for example, causes threat to the living organisms in such an environment (Fig. 1.4). Besides being toxic to people, pesticides are highly dangerous to the biological community (Veiga et al. 2006). Volatilization of sprayed pesticides typically hit (straightforwardly) non-target vegetation, which results in the contamination of air, soil, and non-target plants (Johnson and Ware 1992). There are constant dangers to human life, brought about by long time applications of pesticides. It can bring about hormonal disturbance and can bring about brain degeneration (Gupta 2004). The steady release of pesticides through draining, sorption, and volatilization brings about pollution of various levels in the environment (Nawab et al. 2003; Andreu and Picó 2004).

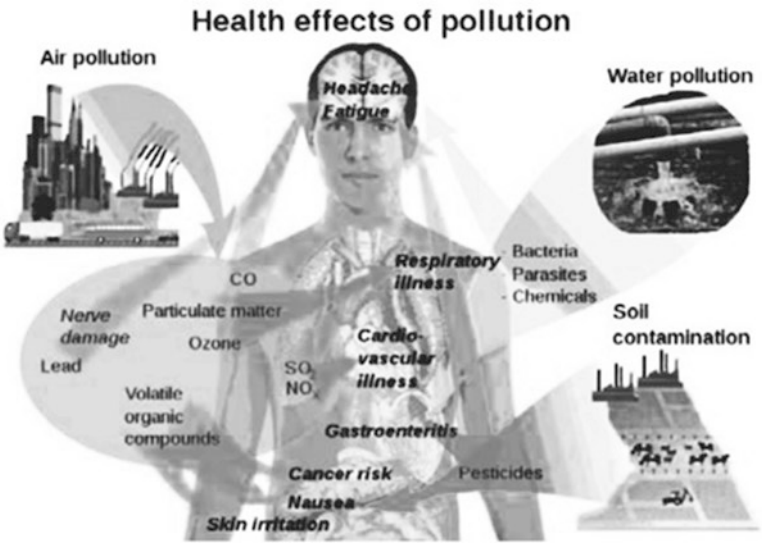


Fig. 1.4 Impact of pesticides on human health

1.2.4.1 Effects of Pesticides on the Environment

Pesticides applied to the environment have appeared to have long-term leftover impacts, while others have appeared to have intense lethal impacts when not appropriately taken care of. Organochlorine pesticides, for instance, remain stable in the environment, due to which they contaminate groundwater, surface water, sustenance items, air, and soil, and may affect people through direct contact. Accumulation of pesticides in human being has been very much archived to be the reasons for the occurrences of few illnesses such as cancer, respiratory ailments, skin diseases, endocrine disturbance, and reproduction disorders. This has raised concern among environmental researchers to focus their research on environment and to obtain a solution to protect human population from the unfavorable impacts of pesticides. Even 50 years (half a century) after Rachel Carson's cautioned the world about the staggering impact that pesticides have on birds and useful insects, pesticides is still being an inescapable and tricky danger to the world's biological systems. An enormous substance assault on our environment is propelled every year. This harmful flow of pesticides affect biological systems, for example, in growing sub-urban development and dammed streams, debilitating the survival of many fowls, fish, insects, and little oceanic living beings that frame the premise of the sustenance web. All the more for the most part, pesticides decrease species of all animals and add to populace decrease microorganisms and plants by destroying environmental surroundings, diminishing nourishment supplies and impeding proliferation (Kegley 1999).

1.2.4.2 Effects Involving Pollinators

Some common pollinators, for example, honeybees and butterflies, are exceptionally delicate to pesticides. Pesticides can kill honey bees and are clearly involved in eliminating pollinators, through the component of Colony Collapse Disorder (Hackenberg 2007), in which worker bees from a beehive or Western honey bee colony unexpectedly vanish. Since honey bees are vital pollinators of both harvests and local plants, a decreased number of common pollinators can result in decreased seed production and other environmental products. This has a strong impact on the environment. Honey bees are very essential for the pollination of crops and wild plants. In spite of the fact that pesticides are screened for their toxic effects on honey bees and the utilization of these pesticides is allowed under stringent conditions, numerous honey bees are being killed by pesticides, which results in extremely less yield of harvests, which rely on honey bee pollination (Miller 2004).

1.2.4.3 Effects on Soil Erosion, Structure, and Fertility

A significant number of the chemicals used in the production of pesticides are always soil contaminants, whose effect may persevere for a considerable length of time and antagonistically influence soil fertility. A smaller substance of environmental matter in the soil builds the measure of pesticide and leave other part of the soil, since organic matter ties to and separates pesticides (Lotter et al. 2003). Herbicides for instance can lessen vegetative cover of the ground thereby advancing soil disintegration by means of overflow and wind. Soil disintegration distorts the soil structure and results in lopsidedness in soil fertility. An exposed land with poor soil structure and poor soil fertility can't bolster the development of plants on it. Biologically, such lands can't bolster different types of life on it; consequently, they may prompt the fall of specific ecosystem.

1.2.4.4 Effects on Water Quality

Water bodies are the destination for pesticides applied in the environment either from the air, or by overflow or by permeation to groundwater. There are four ways through which pesticides can reach water bodies: it might float outside of the proposed zone when it is sprayed, it might permeate or filter through the soil, it might be conveyed to the water as spillover, or it might be spilled, for instance, coincidentally or through carelessness. They may also be conveyed to water by disintegrating soil. When pesticides enter water bodies, they can possibly bring about destructive impacts on the well-being of humans and amphibians and can interfere with oceanic biological systems. This may affect fish production in streams and vast water bodies, particularly where angling is one among the major financial exercises of a specific group. In the United States, for instance, pesticides were found to contaminate each stream and more than 90% of wells that were tested in a review by the US Geological Survey (Gillion 2007). Pesticide deposits have likewise been found in rain and groundwater. The UK government demonstrated that pesticide fixations surpassed those permissible for savoring water a few specimens of stream water and groundwater (Bingham 2007).

1.2.4.5 Effects on Birds

Pesticides have had some of their most striking impacts on birds, especially those at the higher trophic levels, for example, bald eagles, hawks, and owls. These feathered creatures are regularly uncommon, jeopardized, and helpless to pesticide buildups; for example, these species get affected due to the bioconcentration of organochlorine insecticides through terrestrial food chain. Pesticides may kill grain- and plant-nourishing birds and also deaths of numerous uncommon types of ducks and geese have been reported. Insect-eating birds, for example, partridges, grouse,

and pheasants, have diminished in farming fields by applications insecticides. Application of pesticides incorporated with diazinon and carbofuran has resulted in killing of many birds across the world (Kegley 1999). Organochlorine bug sprays, for example, DDT, are reported to have destroyed avian species even after their use is restricted. Weight reduction, helplessness to predation, a decrease in illness resistance, absence of enthusiasm for mating and safeguarding region, and deserting of nestlings are the impacts of pesticides introduction.

1.2.4.6 Effects on Human Beings

Pesticides can enter human body through inhalation of aerosols, dust, and vapor that contain pesticides; through oral exposure by consuming contaminated food and water; and through dermal exposure by direct contact of pesticides with skin (Fig. 1.4; Sacramento 2008). Pesticides are sprayed onto food, especially fruits and vegetables, and they secrete into soils and groundwater which can end up in drinking water; pesticide spray can drift and pollute the air. Pesticides have more harmful effects on human health, which is based on the toxicity of the chemicals and the duration and magnitude of exposure (Lorenz 2009). Farmworkers and their families are mostly exposed to agricultural pesticides as the directly deal with chemical pesticides. But every human contain a percentage of pesticides in their fat portions of body. Children are most susceptible and sensitive to pesticides due developing organs. The chemicals can bioaccumulate in the body over time. Exposure to pesticides can result in mild skin irritation, birth defects, tumors, genetic changes, blood and nerve disorders, endocrine disruption, and even coma or death (Miller 2004).

1.3 Microbial Degradation of Pesticides

Within the environment, the fate of pesticides is determined by abiotic and biotic factors. The rate at which completely different pesticides are biodegraded varies widely. Some pesticides like dieldrin and DDT are recalcitrant. Consequently, they remain in the environment for a long time and accumulate into food chains for decades after their application to the soil (Kannan et al. 1994). Biodegradation of DDT residues largely involves co-metabolism, that is, it requires the presence of an alternative carbon source, in which microorganisms growing at the expense of a substrate are able to transform DDT residues without deriving any nutrient or energy for growth from the process (Bollag and Liu 1990). Under reducing conditions, reductive dechlorination is the major mechanism for the microbial conversion of both the o,p'-DDT and p,p'-DDT isomers of DDT to DDD (Fries et al. 1969). The reaction involves the substitution of an aliphatic chlorine for a hydrogen atom. Using metabolic inhibitors together with changes in pH and temperature. Wedemeyer (1967) found that discrete enzymes were involved in the metabolism of DDT by *Aerobacter aerogenes*. The pathway for the anaerobic transformation of DDT by

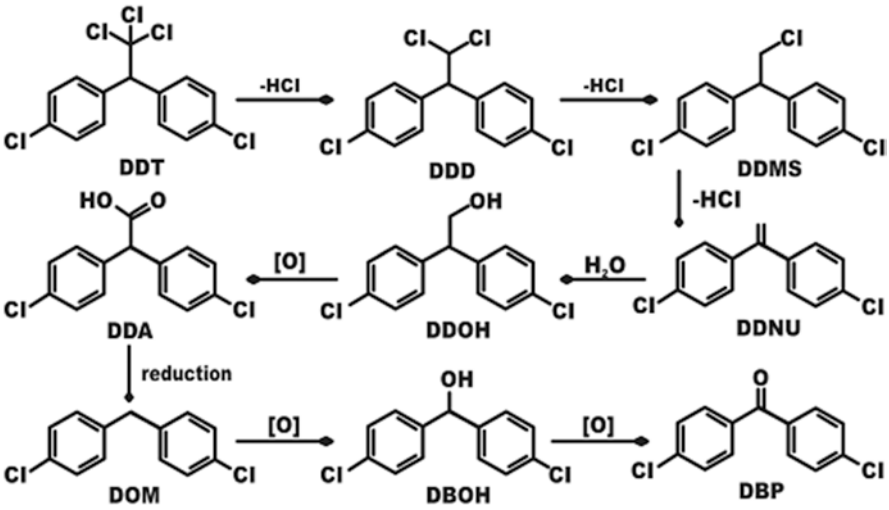


Fig. 1.5 Proposed pathway for bacterial metabolism of DDT. (Source: Aislabie et al. 1997)

microorganism is shown in Fig. 1.5. Degradation proceeds by successive reductive dechlorination reactions of DDT to yield 2,2-bis(p-chlorophenyl)ethylene (DDNU), which is then oxidised to 2,2- bis(pchlorophenyl) ethanol (DDOH). More oxidization of DDOH yields bis(p-chlorophenyl) acetic acid (DDA) that is decarboxylated to bis(p-chlorophenyl)methane (DDM). DDM is metabolized to4′dichlorobenzophenone (DBP) or, instead, might bear cleavage of one of the aromatic rings to form p-chlorophenylacetic acid (PCPA). Below anaerobic conditions, DBP was not more metabolized. Through an investigation of the co-metabolism of DDT metabolites by a number of fungi (Subba-Rao & Alexander, 1985) were able to substantiate the pathway proposed by Wedemeyer (1967). There has been one report describing the conversion of DDE to 1-chloro-2,2-bis(p-chlorophenyl)ethylene – DDMU by microorganisms. Some studies have given notable results on the biodegradation of organochlorine pesticides. Table 1.2 presents a variety of microorganisms that were able to degrade organochlorine pesticides. Among the various microorganisms, bacteria comprise the most cluster with regard to organochlorine degradation, particularly soil habitants belonging to genera *Bacillus*, *Pseudomonas*, *Arthrobacter,* and *Micrococcus* (Langlois et al. 1970). Thus, to predict the different factors that influence the ability of *Sphingobacterium* sp. in the biodegradation of insecticides, Fang et al. (2010) studied biodegradation at completely different temperatures, pH levels, concentrations of insecticides, and with an extra supply of carbon. Results showed that the degradation rates were proportional to the concentrations of p,p′-DDT, o,p′-DDT, p,p′-DDD and p,p′-DDE ranging from 1 to 50 mg.L^{-1}. The ability of *Sphingobacterium* sp. to degrade DDTs was somewhat repressed by DDTs at a concentration as high as 50 mg.L^{-1}. In step with the authors, this would possibly ensue to the actual fact that DDTs at high concentration

Table 1.2 Microorganisms having potential for remediation of pesticides

Microorganism involved in the degradation	Pesticides
Pseudomonas	Cypermethrin, oxyfluorfen, chlorphyrifos, iprodione (fungicide), atrazine (Mercadier et al. 1997; Kaneva and Chen 1999; Martínez et al. 2001; Fulekar and Geetha 2008; Jung et al. 2008)
Bacillus	Lindane, endosulfan, oxyflurfen (Benimeli et al. 2008; Mervat 2009; Mohamed et al. 2011)
Rhodococcus	Metamitron (Pesce and Wunderlin 2004; Kumar and Philip 2006)
Arthrobacter	Metamitron, atrazine (Kumar and Philip 2006)
Staphylococcus	Endosulfan (Mohamed et al. 2011)
Stenotrophomonas	Tetrachlorvinphos, chlorpyriphos (Parekh et al. 1994; Aislabie et al. 2005)
Bjerkandera	Terbufos, azinphosmethyl, phosmet and tribufos (Kumar and Philip 2006)
Pleurotus	Terbufos, azinphosmethyl, phosmet and tribufos (Kumar and Philip 2006)
Proteus	Tetrachlorvinphos (Parekh et al. 1994)
Proteus	Tetrachlorvinphos (Parekh et al. 1994)
Vibrio	Tetrachlorvinphos (Parekh et al. 1994)
Yersinia	Tetrachlorvinphos (Parekh et al. 1994)
Serratia	Tetrachlorvinphos (Parekh et al. 1994)
Synechocystis (cyanobacterium)	Chlorpyrifos (Ortiz-Hernández and Sánchez-Salinas 2010)
Brucella	Chlorpyrifos (Yang et al. 2006)
Trichoderma	Malathion (Jauregui et al. 2003)
Micococcus	Cypermetherin (Singh et al. 2011)
Sphingomonas	Oxyfluorfen (Vidya Lakshmi et al. 2008)
Enterobacter	Chlorpyrifos (Chawla et al. 2013)

Sources: Chawla et al. (2013)

are cytotoxic to *Sphingobacterium* sp. and inhibit degradation. The experiment was conjointly tested at different pH levels; 5, 7, and 9. The results indicated that neutral pH condition is favorable for the degradation of insecticide by *Sphingobacterium* sp., whereas higher or lower pH scale inhibits degradation. The influence of temperature on the biodegradation was investigated by playacting the experiments at temperatures of 20, 30 and 40 °C. The results indicated that the optimum temperature for the biodegradation of DDTs by a *Sphingobacterium* sp. in pure culture was 30 °C.

Studies with fungi have conjointly proven the biodegradation of organochlorine pesticides. Ortega et al. (2011) evaluated marine fungi collected off the coast of São Sebastião, north of urban center state, Brazil. The fungi strains were obtained from marine sponges. The fungi *Penicillium miczynskii*, *Aspergillus sydowii*, *Trichoderma sp., Penicillium raistrickii, Aspergillus sydowii,* and *Bionectria sp*. were antecedently tested in solid medium containing 5, 10, and 15 mg of DDD. The tests were conjointly administered with liquid medium throughout a rotary shaker, with

identical quantity of DDD per a 100 cm^3 liquid medium. The results showed that the fungi *P. miczynskii*, *A. sydowii,* and *Trichoderma sp*. presented good growth in the presence of the pesticide.

1.4 Bioremediation of Toxic Pesticides with Microbial Products

Different microorganisms (Table 1.2) can be utilized to degrade specific pesticides, though this largely depends on the chemical constituents of the pesticide. The selection of these microorganisms should be done carefully for effective remediation as they can continue to exist within a narrow range of contaminants (Prescott et al. 2002; Dubey 2004). Bacteria, mainly of the genus *Alcaligenes*, *Pseudomonas*, *Flavobacterium, and Rhodococcus*, are the potent degraders of pesticides (Larkin et al. 2005). Actinomycetes also show an impressive ability to biodegrade pesticides. Research revealed that these microorganisms produce various extracellular enzymes that enable them to degrade different types of complex organic compounds. These actinomycetes work under aerobic conditions, and an extensive feature is the presence of monooxygenases and dioxygenases (Bastos and Magan 2009). The major genera involved are *Rhodococcus*, *Streptomyces*, *Clavibacter*, *Arthrobacter,* and *Nocardia.* Recent studies have shown the capacity of actinomycetes in deterioration of pesticides. White rot fungi such as *Phanerochaete chrysosporium* and *Trametes versicolor* have played a significant role in biodegradation of pesticides like lindane, atrazine, metalaxyl, DDT, dieldrin, aldrin, mirex and chlordane, diuron, etc. (Pointing 2001; Bending et al. 2002; Shanahan 2004; Tortella 2005; Rubilar et al. 2007; Fragoeiro and Magan 2008). Pesticides have different chemical structures including s-triazines, carbamates, triazinonones, organophosphates, acetanilides, etc. Because of this variation, their mineralization is difficult by single isolates; therefore, consortia of bacteria must be used for complete and effective degradation. The degradation of pesticides results in the production of carbon dioxide (CO_2) and water (H_2O) via oxidation of parent compounds. The bacterium involved in the degradation process derives its energy from the degradation of the products. The optimum atmospheric conditions, that is, temperature, pH of soil, moisture contents, etc., are what determines the efficiency of the degradation process.

Genetic modification and mutations of different bacterial isolates also enhance the effectiveness of applied microbes. The breakdown of pesticides has positive effects on the fertility of agricultural soil. Chlorpyrifos has a massive effect on contaminating soil and water bodies. Microbial breakdown is very useful for the detoxification of such (chloroorganic) pesticides (Chishti et al. 2013).

The importance of microbial degradation of pesticides cannot be overemphasised. This is due to the diversity, wide distribution, and adaptation of variable metabolic pathways (Cui et al. 2012). Microbial strain screening and isolation are very

effective for the degradation of carbendazim in mineral culture medium. *Sphingobium japonicum* is a strain that degrades chlorinated pesticides, that is, hexachlorocyclohexane. This strain (*Sphingobium japonicum* LZ-2) can completely decompose lindane at a concentration of 20 mg/L in 10 h (Liu et al. 2007). An aerobic bacterium (*Burkholderia cepacia* strain CH-9) can be used for the degradation of imidacloprid and metribuzin. Sixty-nine percent degradation of imidacloprid and 86% degradation of metribuzin can be obtained in 20 days with an initial dose of 50 mg/L in a mineral salt medium. Bifenthrin (BF) is a synthetic pesticide that can be degraded by pyrethroid bacteria (*Acinetobacter calcoaceticus*). A 56.4% degradation rate could be achieved with an initial concentration of 100 mg/L at a pH range of 6.0–8.0 and 5% inoculation (Tingting et al. 2012). *Streptomycetes* strains have enormous applications in the degradation of chlorpyrifos (CP) pesticide. The ability of these strains to carry out biodegradation can be studied in an agar medium. Alterations of the pH can affect the efficiency of the degradation process (Briceño et al. 2012). Liquid chromatography (HPLC) analysis of bacterial strains shows their ability to degrade methomyl and carbofuran. Acetonitrile and water were used as mobile phases. Carbofuran-degrading strains are very close to the genera *Flavobacterium* and *Alcaligenes* and that of methomyl-degrading strains. Photosynthetic bacterium (GJ-22) is capable of degrading cypermethrin (CMP). CMP degradation by GJ-22 is very productive at 25–35 °C and pH of 7.0. By performing gas chromatography/mass spectrometry (GC-MS), metabolic products are detected. Lactic acid bacteria can degrade organophosphorus insecticides through fermentation. Lactic acid bacteria utilize organophosphate as a source of carbon and phosphorus (Kye et al. 2009). Highly efficient bacterial strain of *Enterobacter aerogenes* can degrade many other pesticides, such as bifenthrin, cypermethrin, and so on (Lio and Xie 2009).

Sphingomonas a Gram-negative bacterial strain possesses high potential for degrading DDT (Shunpeng and Mingxing 2006). Pyrethroid insecticide which is an allethrin can be degraded by *Acidomonas* sp. Eight bacterial strains potentially degrade PCNP pesticide. Better results were obtained when all these strains were collectively used (Ning et al. 2005). Two bacteria cad1 and cad2 that degraded cadusafos in mineral salt medium with nitrogen (MSMN) were also able to degrade ethoprophos nematicide completely (Karpouzas et al. 2005). Psychrotrophic bacterium can degrade Me-parathion. This biodegradation is sensitive to pH and temperature variations (Krishna and Philip 2009). Six genera are able to degrade organochlorine pesticides, that is, endosulfan. Different genera have different potential to degrade them, from which *Micrococcus* and *Pseudomonas* were highly active compared to others (Li et al. 2004). Immobilized *Escherichia coli* (a well-known bacterium) was able to degrade organochlorine insecticide which had an ester bond (Singh et al. 2003). Fungi from the environment can be properly screened as an effective tool for biodegradation of toxic organic chemicals. *Fusarium verticillioides* is a fungal strain capable of using lindane as a carbon and energy source under aerobic conditions. This strain can be isolated from *Agave tequilana* leaves using enrichment techniques. *Fusarium oxysporum*, *Lentinula edodes*, *Penicillium brevicompactum* and *Lecanicillium saksenae* possess great potential for the

biodegradation of pesticides like pendimethalin, difenoconazole and terbuthylazine in batch liquid cultures. These fungal strains are investigated to be valuable as active microorganisms for pesticides degradation (Hai et al. 2012). Endosulfan-degrading, aerobic fungal strains are effective for soil contaminated with organochlorine pesticides. These strains (*Mortierella* sp. strains W8 and Cm1–45) resulted in 50–70% degradation in 28 days at 25 °C. During degradation, diol formation of endosulfan takes place first and then endosulfan lactone conversion.

1.4.1 Remediation of Toxic Pesticides by Microbial Enzymes

Microorganisms in soil (bacteria and fungi) are responsible for the degradation of glyphosate via two chemical pathways. The first pathway produces a compound known as AMPA (aminomethylphosphonic acid) which is found in soils treated with glyphosate. This is thought to be mildly toxic to plant growth. The second pathway produces the compound sarcosine. The microorganisms responsible for the degradation use enzymes to break down glyphosate, so as to get phosphorus, nitrogen, and carbon sources for themselves. Studies examining the rate of glyphosate degradation revealed some variability in results, and the process can depend on a range of factors. There is some evidence for the rate of degradation being correlated with the population size of bacteria in the soils (Gimsing et al. 2004). Overall, sorption of glyphosate onto soil particles is thought to decrease degradation, but glyphosate that has been sorbed can still be degraded by microorganisms. Rates vary with topographical features that affect water availability (Stenrod et al. 2006) and soil type and increase with temperature.

Enzymes produced during different metabolic pathways in plants as well as in microbes present in soil are key for bioremediation of pesticides. Optimum environmental conditions such as pH and temperature support fast rate of removal of toxic intermediates. The engineered bacteria were used to produce esterase genes which specifically act on a substrate and degrade more than 65% methyl parathion within 3 h (Li-Qing et al. 2008). Carbofuran, an insecticide present in contaminated soil, can be treated with *Paracoccus* sp. YM3, by MSM method, which enzymatically degrades carbofuran into its metabolites which were analyzed by HPLC. This bacterium uses carbofuran as a sole source of carbon (Peng et al. 2008). Genetically modified *E. coli* enzymatically degrades methyl parathion and many other OPs, that is, PNP, which is detected by HPLC (Zhang et al. 2008). *Micrococcus* sp. has been found to have a versatile ability to degrade OPs pesticide like cypermethrin by enzymatic action (Tallur et al. 2008). Lindane is degraded by fungus *Conidiobolus* through enzyme action. GC-ECD and GC/MS confirm that there is no metabolite; this proved that lindane is completely degraded by this fungus (Nagpal et al. 2008). In a study of atrazine (AT) and alachlor (AL), their degradation by treating them with extracellular enzyme extracted from fungi was determined (Chirnside et al. 2007). FDS-1 strain of *Burkholderia* sp. can degrade nitrophenyl enzymatically at 30 °C and pH of 7.0 taken as optimized conditions (Lan et al. 2006). Strains of

genetically modified bacteria contain enzymes, which potentially can degrade a number of pesticides including OPs, carbamates, and pyrethroids (Liu et al. 2006). A study revealed that different enzymes specifically degrade different pesticides (OPs) in wheat kernels (Yoshii et al. 2006). Thirty fungal strains were used to investigate the degradation rate of Diuron and pyrithiobac-sodium. Results suggested that the highest degrading rate was by ligninolytic enzymes (Gondim-Tomaz et al. 2005). *Enterobacter* enzymatically degrades chlorpyrifos and many other OPs. It degrades them and uses them as carbon and phosphorus source (sole source) (Singh et al. 2004). Some Gram-negative bacteria have the ability to degrade dimethoate. They use it as a sole source of carbon. Bacteria hydrolyze insecticides by using different enzymes, namely, phosphatases and esterases (Kadam et al. 2003). More than 15 fungal strains were capable of degrading different OPs up to 96% by enzyme-catalysed pathways (Jauregui et al. 2003). Enzymes for the degradation of organochlorinated pesticide are mainly dehydrochlorination enzymes, hydrolytic enzymes, and dehydrogenases. The genes related are Lin family genes with typical functional codes. Further studies are needed to find an effective tool for the complete removal of these pesticides (Zhang et al. 2012). The amino acid sequence of phosphotriesterase mutant is very effective in organophosphorus pesticide degradation (Xiang-Ming and Ping-Ping 2012).

The first signs of the aerobic lindane degradation were determined by Nagata et al. (1999), who demonstrated that *Sphingobium japonicum* UT26 possesses a dechlorinase enzyme, LinA (γ-hexachlorocyclohexane dehydrochlorinase, EC 4.5.1), encoded by the linA gene that catalyzes two dehydrochlorination steps: γ-HCH to 1,3,4,6-tetrachloro-1,4-cyclohexadiene (1,4-TCDN) via γ-pentachlorocyclohexene (γ-PCCH). In addition to γ-HCH and γ-PCCH, α- and δ-isomers of HCH were also dehydrochlorinated by LinA, whereas γ-HCH was not (Nagata et al. 1999). Furthermore, it was experimentally confirmed that dehydrochlorination of γ-HCH proceeds by a 1,2-ante dehydrochlorination reaction (Nagata et al. 2007). Regarding the environmental problems caused by lindane and the current lack of information about the presence of dechlorinase activity in *Streptomyces*, the aim of this point was to demonstrate, for the first time, a specific dechlorinase activity in *Streptomyces* using lindane as a substrate. In order to determine lindane and metabolites in cell-free extract of *Streptomyces* sp. M7, the strain was grown in flasks with 250 mL of MM containing γ-HCH 100 μg mL^{-1} and incubated at 30 °C at 100 rpm for 48 and 96 h. At the beginning of the experiment, the inoculum contained 150 μL of concentrated spore suspension (109 CFU ml^{-1}). Lindane and its metabolites were extracted by solid-phase extraction (SPE) using C18 columns, evaporated to dryness under reduced pressure, and the residue was resuspended in hexane. Routine quantitative determinations of lindane (γ-HCH), γ-pentachlorocyclohexene (γ-PCCH), and 1,3,4,6-tetrachloro-1,4-cyclohexadiene (1,4-TCDN) were carried out with gas chromatography-micro-electron capture detector (GC-μECD) [37–38]. The gas chromatography results of the cell-free extracts obtained at 48 and 96 h of growth of *Streptomyces* sp. M7 revealed the appearance of γ-PCCH (Rt 6.26 min) and 1,4-TCDN (Rt 5.29 min), the first and second products of the lindane catabolism by the specific dechlorinase in the

catabolic way proposed by Nagata et al. (1999). The relative abundance of γ-PCCH and the 1,4-TCDN increased one and a half times, at 96 h compared to 48 h of growth. However, these results indirectly demonstrated the presence of one specific enzyme in the lindane degradation pathway from *Streptomyces* sp. M7. This is one of the recent studies on dehalogenase activity in actinomycetes with lindane as the specific substrate. It has only been reported in *Sphingomonas* (Nagata et al. 1999) and a putative 2,5-dichloro-2,5cyclohexadiene-1,4-diol dehydrogenase (2,5-DDOL dehydrogenase) was reported in *Frankia* (Normand et al. 2007). Genetic studies of this strain are necessary for a proper understanding of the principle of its ability to degrade different chlorinated hydrocarbon compounds.

1.4.2 Remediation of Toxic Pesticides by Biosurfactant

A wide variety of microorganisms such as bacteria, fungi, and yeast can naturally be used to produce biosurfactants extracellularly or as part of the cell membrane. Some examples are *Pseudomonas aeruginosa* (produces rhamnolipids), *Bacillus subtilis* (produces a lipopeptide called surfactin) (Ron and Rosenberg 2001; Mata-Sandoval et al. 2002; Mulligan 2005), *Nocardia amarae* (Moussa et al. 2006), and *Saccharomyces lipolytica* CCT- 0913 (Lima and Alegre 2009). Barkay et al. (1999) examined the influence of the bioemulsifier alasan on the biodegradation fates of PAHs. The presence of alasan (500 μg mL^{-1}) more than doubled the rate of fluoranthene mineralization and significantly increased the rate of phenanthrene mineralization by *Sphingomonas paucimobilis* EPA505. Shin et al. (2006) used a rhamnolipid from *Pseudomonas* to remediate soil contaminated with phenanthrene by the combined solubilization biodegradation process. They reported a high percentage of removal in the solubilization step and a significant decrease of phenanthrene in the soil sample during biodegradation. They suggested that the degradation of contaminants by specific species might not be affected by the residual biosurfactants following the application of the solubilization process, that they would not present negative effects to the environment, and that they could be combined with the biodegradation process to improve the removal efficiency. In general, most pesticides used in agriculture are moderately hydrophobic compounds, with complex molecular structures that differ from hydrocarbons in their lower hydrophobicity and in the presence of a polar functional group. These compounds are also strongly adsorbed by soil organic matter and desorption is limited (Rodríguez-Cruz et al. 2004). Their desorption rate decreased with an increase in aging time. Wattanaphon et al. (2008) evaluated the ability of a BS biosurfactant produced by *Burkholderia cenocepacia* BSP3 to enhance pesticide solubilization for further application in environmental remediation. Moreover, it lowered the surface tension of deionized water to 25 ± 0.2 mN m^{-1} and exhibited good emulsion stability. Many microbes have been discovered with abilities to degrade different pesticides and toxic compounds. Research works are carried out to isolate and characterize those

microorganisms that are responsible for the degradation of carbfuran, carbaryl, and Baygon (Sutherland et al. 2002). Genes responsible for the degradation of pesticides and hazardous chemicals were present on the plasmids. It was observed that sequences of Esd gene have the same homology to monooxygenase family that requires reduced flavin, presented by a separate flavin reductase enzyme, found in *Mycobacterium smegmatis* as co-substrates (Weir et al. 2006). Esd gene has the ability to catalyze the oxygenation of β-endosulfan to endosulfan monoaldehyde to endosulfan hydroxyether, but it lacks the ability to degrade either α-endosulfan or the metabolites of endosulfan and endosulfan sulpfate. Coding enzyme of the gene Ese, from the monooxygenase family, has also been reported (Lal et al. 2006), which has the ability to break both endosulfan α and β using *Arthrobacter* species.

1.4.3 Remediation of Toxic Pesticides by Microbial Pigments

Filamentous fungi produce several non-carotenoid pigments (quinones). Anthraquinone pigments are produced by *Eurotium* spp., *Fusarium* spp., *Curvularia lunata,* and *Drechslera* spp. The yellow pigments epurpurins A to C were isolated from *Emericella falconensis* and *Emericella fruticulosa.* Moreover, *Monascus* spp. produce azaphilone pigments. A red colorant of the anthraquinone class, it may be produced by a variety of *Penicillium oxalicum.* The pigments produced by microorganisms that are commercially used are riboflavin (vitamin B2), a yellow pigment accepted in many countries and produced by *Eremothecium ashbyii* and *Ashbya gossypi*, and the pigments from *Monascus purpureus* and *M. ruber.* Carotenoids (yellow pigments) are being produced by several microorganisms, but to this moment commercial production is only from microalgae, such as β-carotene using *Dunaliella salina* and *D. bardawil* and astaxanthin by *Haematococcus pluvialis.*

Microbial pigments are advantageous, when compared to similar pigments extracted from plants or animals. However, the isolation and development of new strains may provide new, different pigments which could be effective in biodegradation of pesticides (Babitha 2009).

Fungi often act as important natural control agents against insects, pathogenic, nematodes and as herbicide. Some fungi that are utilized as biopesticides are pathogenic to insect hosts and are referred to as entomopathogenic fungi; examples are members of *Entomophtorales* (*Zygomycota*) and *Hyphomycetes* currently under research (Srivastava et al. 2009). Fungal strains are considered suitable for biopesticide development because, unlike other microorganisms, the infectious propagules (conidia) need to be ingested and contact with cuticle allows the fungi to enter the insect body (Thomas and Read 2007).

Fungi can act as an insecticide in two ways:

(a) *Infection*: many of the fungi species cause death to the insect through asexual spores called conidia. The infective unit (conidium) of entomopathogenic fungi binds to the host cuticle by nonspecific interaction mediated by cuticle-

degrading enzymes present on the conidia or by fungal lectins. These conidia enter through the body wall of the host pest by dissolving the body wall by the combined action of enzymes, i.e., chitinase and protease, secreted by the fungi. Fungal penetration is further enhanced by mechanical force.

(b) *Mycotoxins*: another fungi mode can cause the death of the host by the production of mycotoxins, which can interfere in the nervous system of insects. Mycotoxins such as aflatoxin B, trichothecenes, patulin, and ochratoxin are reported to be toxic to insects (Srivastava et al. 2009).

Virus-based biopesticides have been used as insect control agents. The larvae of many insect species are vulnerable to viral diseases. Baculoviruses are a large virus group belonging to the family *Baculoviridae* and can infect different insect orders, particularly *Lepidoptera* and *Diptera* (Theilmann et al. 2005). Baculoviruses are classified into two genera: nuclear polyhedrovirus (NPV) and granulovirus (GV) (Cory and Hails 1997; McCutchen and Flexner 1999). Two morphologically distinct forms of infectious particles are generated in the baculovirus cycle, the occlusion derived virus (ODVs), comprising enveloped virions embedded within a crystalline matrix of protein (polyhedrin for NPVs and granulin for GVs), and budded virus (BVs), consisting of a single virion enveloped by a plasma membrane. Due to their specificity and high virulence to a number of insect pest species, they have been used worldwide to control lepidopteran pests in many crops. BVs are responsible for the systemic or cell-to-cell spread of the virus within an infected insect. OVs, in turn, are responsible for the larva-to-larva transmission of the virus (Inceoglu et al. 2006). Like bacteria, viruses must be ingested to infect the insect hosts. Forest pests are good targets for viral pesticides because the permanence in the forest environment contributes to the pathogen cycle and the forest canopy also helps to protect viral particles from radiation. Different approaches targeted at enhancing the role of baculovirus as effective biopesticides. For example, the effect of baculovirus may be enhanced by the synergistic action of specific chemical insecticides, such as the pyrethroids deltamethrin and permethrin (McCutchen and Flexner 1999).

Few protozoan pathogens can kill insect hosts; however, many of them cause serious infections with debilitating effects (Lacey and Goettel 1995). The consequence of protozoan infection is the reduction in the number of offspring by the infected insects. Species of the genera *Nosema* sp. and *Vairimorpha necatrix* offer the greatest biopesticide potential. *Nosema locustae* is a species of *Microsporidium* commercially available to control grasshoppers and crickets. It is most effective when ingested by immature grasshoppers (early nymphal stages). Spore formed by the protozoan is the infection stage of insusceptible insects; it germinates in the midgut and causes a slow progressive infection where the pathogen causes death 3–6 weeks after the initial infection (Rosell et al. 2008). *Ostrinia nubilalis* that causes important damages to corn was controlled by *Nosema pyrausta* infection, which reduced the egg production per female by 53% and 11% at 16 and 27 °C temperature, respectively (Bruck et al. 2001). *Nosema locustae* has been used to reduce grasshopper population in rangeland areas; although not all insects are killed, the infected grasshoppers

consume less forage, and the females produce fewer eggs. However, the utility of *N. locustae* as biopesticide remains questionable because of the difficulty to determine the treatment efficacy in this highly mobile insect.

1.4.4 Remediation of Toxic Pesticides by Microbial Crystal Protein and Toxins

Most biopesticides available in the market are bacterial products. The well-known and widely used bacterial biopesticide comprises Gram-positive, spore-forming bacteria belonging to the genus *Bacillus* that are commonly found in soil. The majority of commercial microbial insecticides are preparations based on strains of *Bacillus thuringiensis* (Bt) that produces a crystalline inclusion body during sporulation (Frankenhuyzen 2009).

The crystal proteins (Cry proteins) are toxic to many insects and are defined as endotoxins (Bt toxin) that are generally encoded by bacterial plasmids. Both spores and inclusion bodies are released upon lysis of the parent bacterium at the end of the sporulation cycle, and if ingested, the spores and crystals act as poisons in certain insects. The protein is activated by alkaline conditions and enzyme activity of the insect's gut; hence, Bt is referred to as a stomach poison (Chattopadhyay et al. 2004). The toxicity of the activated protein is dependent on the presence of receptor sites on the insect's gut wall. This match between toxin and receptor sites determines the range of insect species killed by each Bt subspecies and isolates (Frankenhuyzen 2009). Cry proteins are produced as protoxins that are proteolytically converted into a combination of up to four smaller toxins upon ingestion. These proteins bind to specific receptors in the larval midgut epithelium causing the formation of large cation-selective pores that increase the water permeability of the cell membrane. A large uptake of water then causes cell swelling and rupture of the midgut. Poisoned insects can die quickly from the toxin activity or may die within 2–3 days from septicemia due to the entering of gut contents into the bloodstream. Bt strains containing mixtures of up to 6–8 Cry proteins have been used as microbial pesticides since Bt var. kurstaki have been commercially available since 1961 (Montesinos 2003). Formulations are active against insect order *Lepidoptera* (moths and butterflies), *Diptera* (flies and mosquitoes), *Coleoptera* (beetles and weevils), and *Hymenoptera* (bee and wasps) larvae (Frankenhuyzen 2009). Of the recognized subspecies of Bt, var. kurstaki is toxic to gypsy moth, cabbage looper, and caterpillars (order Lepidoptera); var. israelensis is toxic to fungus gnat larvae, mosquitoes (species of *Aedes* and *Psorophora*), black fly, and some midges (order Diptera); var. san diego is effective against potato beetle, elm leaf beetle, and boll weevils (Whalon and McGaughey 1998); var. aizawai is effective against wax moth larvae and diamondback moth caterpillar; and var. morrisoni is toxic against moth and butterfly

caterpillars (order Lepidoptera) (Chattopadhyay et al. 2004). Cytolysins interact with phospholipid receptors on the cell membrane in a detergent-like manner (Gill et al. 1987). The hydrophobic portion of the cytolysins binds the amphipathic phospholipids; transmembrane pores are formed and cells are lysed by osmotic lysis (Knowles and Ellar 1987). Spore inclusions contain many proteins, have distinct activities, and may act in a synergistic manner (Yokoyama et al. 1998). Cry proteins are non-toxic to vertebrate species even at doses higher than 1×10^6 μg/kg body weight, while dosages acutely toxic to susceptible insects are about μg/kg body weight (Rosell et al. 2008); however, Bt formulations can lead to skin and eye irritation (Siegel and Shadduck 1990). The mammalian stomach which has an acidic environment does not enhance solubilization and activation of the Cry proteins. These proteins are broken down very fast (often in few seconds) – from 60–130 kDa to polypeptides less than 2 kDa that corresponds to peptides with 10 amino acids in length. Research into vertebrates has failed to find high-affinity Cry protein binding sites on gut epithelial cell membranes (Rosell et al. 2008). Bt has thus become a bioinsecticide of great agronomical importance and is classified as toxicity class III pesticide (slightly toxic). Commercial Bt products are powders that contain a mixture of dried spores and toxin crystal proteins, and these are applied to the leaves and roots where insects feed. Other species of *Bacillus*, including *B. firmus*, *B. pumilus*, *B. subtilis*, *B. lentimorbus*, *B. popilliae*, and *B. sphaericus*, have been applied as biopesticides (Schisler et al. 2004). Bacteria belonging to other genera such as *Pseudomonas fluorescens*, *P. syringae*, *P. putida*, *P. chlororaphis*, *Burkholderia cepacia,* and *Streptomyces griseoviridis* have also been used as biopesticides (Montesinos 2003). Bacteria generally lose viability when stored for a few weeks, a disadvantage when compared with *Bacillus* (spore-forming) that shows better shelf life and facilitates the development of commercial products. Insect resistance to Bt toxins has led to pursue suitable alternatives. Two more bacteria that are also known to produce insecticidal toxins are *Xenorhabdus* and *Photorhabdus* (both of these belong to the family *Enterobacteriaceae*). Both bacteria are entomophathogens; *Xenorhabdus luminescens* is found to occur in a specialized intestinal vesicle of the nematode *Steinernema carpocapsae* with which it maintains a symbiotic relationship. *Photorhabdus luminescens* maintains a symbiotic relationship with nematodes of the family *Heterorhabditidae* (Poinar 1990) and is present throughout the intestinal tract of these nematodes. In both mutualistic associations, the nematodes and the bacteria complement each other: the nematode acts as a vector and transports the bacteria into the target insect larva where it bores holes in the intestinal walls of the insect and releases the bacteria in the hemolymph. In the absence of the nematode, the bacteria cannot penetrate into the hemocoel. Both the nematode and the bacteria release insecticidal toxins, which eventually kill the insect (Poinar et al. 1977). Septicemia in insects is caused by bacteria, the insect is killed, and its tissues are used as nutrients (Kaya and Gaugler 1993). Bacteria are needed by the nematodes in their developmental stage into the infective juvenile stage and thus are needed for efficient completion of the nematode life cycle.

1.5 Advantages of Using Microbial Products

The main advantage of using microbial products in bioremediation strategies is that the toxic compound to be treated is neutralized or removed totally, which produces a waste material that is easily disposed. At times, there is no requirement for disposal by any means (Gold et al. 2005).

The issue that needs to be solved regarding the use of microbial pesticides is their specificity because they are not effective against a wide range of pests. Specificity is sometimes considered advantageous because the commercial potential gets limited and costs get increased compared to synthetic pesticides. Moreover, biopesticide preparations are sensitive to heat, desiccation, and ultraviolet radiation, which reduce their effectiveness. Storage conditions and special formulations are important; this in turn can negatively affect the distribution and use of products. Genetic engineering technology and molecular genetics of microorganisms will help in finding out new ways for biopesticide improvement and its use. Further studies should be done to enhance shelf-life, the speed of killing, the biological spectrum, and the field efficacy of biopesticides (Bhattacharyya et al. 2016).

Bioremediation is a natural process seen by the public as an acceptable waste treatment process to treat contaminated material such as soil. Microorganisms that can degrade contaminants increase in numbers when the contaminant is present; when the contaminant is degraded or broken down, the microbial population declines. The remains from the treatment are mostly harmless products that include carbon dioxide, water, and cell biomass. This puts to rest the chance of future liability associated with treatment and disposal of contaminated material. Transferring contaminants from one environmental medium to another, for example, from land to water or air is not necessary anymore because complete destruction of target pollutants is possible. Bioremediation can often be carried out onsite, often without causing a major disruption of normal activities. This also eliminates the need to transport quantities of waste offsite and the potential threats to human health and the environment that can arise during transportation. Bioremediation is less expensive than other technologies that are used for cleanup of hazardous waste. Bioremediation is limited to those compounds that are biodegradable. Not all compounds are susceptible to rapid and complete degradation. There are some concerns that the products of biodegradation may be more persistence or toxic than the parent compound. Biological processes are often highly specific. Factors necessary for success include the presence of metabolically competent microbial populations, optimal environmental growth conditions, and optimal levels of nutrients and contaminants.

Many toxic chemical compounds have been degraded by utilizing different microorganisms, and their enzymatic activity can be increased by using various genetic engineering techniques (Prasad 2017, 2018). Genetically engineered microorganisms have certain advantages such as rapid growth affinity, fast growth rate, and resistance to toxicity (Gold et al. 2005). The potential results of releasing such genetically engineered microorganisms into the environment cannot be predicted practically because the conditions of the field are not always optimal; also, there are indigenous communities.

1.6 Future Prospects of Microbial Products in Bioremediation of Toxic Pesticides

There are diverse bioremediation techniques that have proven effective in restoring sites polluted with different pollutants including pesticides. The role of microorganisms is very important in bioremediation; molecular techniques with suffix "omics" such as genomics, metabolomics, proteomics, and transcriptomics have contributed toward the understanding of microbial identification, functions, and metabolic and catabolic pathways, thereby overcoming the limitations associated with microbial culture-dependent methods. Nutrient limitation lowers the population or results in the absence of microbes with degradative capabilities and pollutant bioavailability, which are among the major pitfalls. Bioremediation depends on microbial process; two major approaches to increase microbial activities in polluted sites are biostimulation and bioaugmentation. Biostimulation involves the addition of nutrients or substrates to a polluted sample in order to stimulate the activities of autochthonous microbes. As microorganisms are ubiquitous, it is apparent that pollutant degraders are naturally present in the polluted site and their numbers and metabolic activities may increase or decrease in response to pollutant concentration; hence, the use of agroindustrial wastes with appropriate nutrient composition, especially nitrogen, phosphorus, and potassium, will help solve the challenge of nutrient limitation in most polluted sites. Excessive addition of stimulant resulted in suppressed microbial metabolic activity and diversity (Wang et al. 2012). While bioaugmentation is an approach aimed at introducing or increasing microbial population with degradative capabilities, microbial consortium has been reported to degrade pollutants more efficiently than pure isolates (Silva-Castro et al. 2012). This is due to metabolic diversities of individual isolates, which might originate from their isolation source or adaptation process or as a result of pollutant composition and will bring about synergistic effects, which may lead to complete and rapid degradation of pollutants when such isolates are mixed together. Although bioaugmentation has proven effective, competition between endogenous and exogenous microbial populations, the risk of introducing pathogenic organisms into an environment, and the possibility that the inoculated microorganisms may not survive in the new environment make bioaugmentation a very skeptical approach. The use of agar, agarose, alginate, gelatin, gellan gum, and polyurethane as carrier materials will help solve some of the challenges associated with bioaugmentation (Tyagi et al. 2011).

Simultaneous multiple bioremediation techniques during remediation will help increase remediation efficacy (by reducing the weakness of individual techniques) and at the same time reduce cost (Cassidy et al. 2015; Garcia-Delgado et al. 2015; Martinez-Pascual et al. 2015). Application of combined metric of spatial configuration of bacterial dispersal networks will be a good indicator of biodegradation performance (Banitz et al. 2016). Enhancing bioremediation efficacy with controlled use of genetically engineered microorganisms (GEM) is a promising approach. Nevertheless, horizontal gene transfer and uncontrolled multiplication of GEM in an environment limit the application of such a promising approach. Notwithstanding,

bacterial biofilm will be killed by induction of controlled suicide systems which will help gain public acceptance of using GEM to restore polluted environment. The use of nanomaterials could help reduce toxicity of pollutant to microorganisms. Nanomaterials increase surface area and lower activation energy, thereby increasing the efficiency of microorganisms in the degradation of waste and toxic materials, resulting in overall reduction in remediation time and cost (Rizwan et al. 2014; Prasad et al. 2016; Prasad and Aranda 2018).

1.7 Conclusion

Results brought about by pesticides utilization of contaminated environment is need in this current time. The application of ordinary methods, that is, physicochemical techniques, for the degradation of harmful chemicals are not extremely proficient. This strategy is costly and furthermore not agreeable in the biological community. For the degradation of pesticides and extreme disinfecting of contaminated territories, biodegradation by microscopic organisms is exceptionally proficient as they are financially savvy and additionally ecofriendly. These methods can possibly break down pesticides into their less dangerous results. There is a need of further review for the examination of instruments of microorganisms and their proteins amid degradation potentials. The comprehension of enzymatic activities, particularly ideas identified with pesticides mode of action, resistance, selectivity, resilience, and environmental purpose, vitally affects the learning of pesticide science and applications.

References

Abioye OP, Aina PF, Ijah UJJ, Aransiola SA (2019) Effects of cadmium and lead on the biodegradation of diesel-contaminated soil. J Taibah Univ Sci 13(1):628–638

Aislabie J, Richards NK, Boul HL (1997) Microbial degradation of DDT and its residues – a review. N Z J Agric Res 40:269–282

Aislabie J, Bej AK, Ryburn J, Lloyd N, Wilkins A (2005) Characterization of Arthrobacter nicotinovorans HIM, an atrazine degrading bacterium, from agricultural soil New Zealand, FEMS Microbiol Ecol 52:279–286

Andreu V, Picó Y (2004) Determination of pesticides and their degradation products in soil. Trends Anal Chem 23(10–11):772–789

Aransiola SA, Ijah UJJ, Abioye OP (2013) Phytoremediation of lead polluted soil by Glycine max L. Appl Environ Soil Sci 2013. Article ID: 631619, https://doi.org/10.1155/2013/631619

Babitha S (2009) Microbial pigments. In: Singh-Nee Nigam P, Pandey A (eds) Biotechnology for agroindustrial residues utilisation. Springer & Business Media B.V., Dordrecht, pp 147–162, ISBN 978-1-4020-9942-7

Banitz T, Frank K, Wick LY, Harms H, Johst K (2016) Spatial metrics as indicators of biodegradation benefits from bacterial dispersal networks. Ecol Indic 60:54–63

Barkay T, Navon-Venezia S, Ron EZ, Rosenberg E (1999) Enhancement of solubilization and biodegradation of polyaromatic hydrocarbons by the bioemulsifier alasan. Appl Environ Microbiol 65:2697–2702

Bastos AC, Magan N (2009) Trametesversicolor: potential for atrazine bioremediation in calcareous clay soil, under low water availability conditions. Int Biodeterior Biodegrad 63:389–394

Bending GD, Friloux M, Walker A (2002) Degradation of contrasting pesticides by white rot fungi and its relationship with ligninolytic potential. FEMS Microbiol Lett 212:59–63

Benimeli CS, Fuentes MS, Abate CM, Amoroso MJ (2008) Bioremediation of lindane-contaminated soil by Streptomyces sp. M7 and its effects on Zea mays growth, Int. Biodeterior. Biodegradation 61:233–239

Bhattacharyya A, Duraisamy P, Govindarajan M, Buhroo AA and Prasad R (2016) Nano-biofungicides: Emerging trend in insect pest control. In: R. Prasad (ed.), *Advances and Applications through Fungal Nanobiotechnology*, Springer International Publishing Switzerland 307–319

Bingham S (2007) Pesticides in rivers and groundwater. Environment Agency, UK. Heather B, et al (1997) Movement of pesticides and best management practices, Ground Water Pollution Primer, Virginia

Blais JM, Schindler DW, Mair DC, Kimpe LE, Donald DB, Rosenberg B (1998) Acclimation of persistent organochlorine compounds in mountains of Western Canada. Nature 395:585–588

Bollag JM, Liu SY (1990) Biological transformation processes of pesticides. In: Cheng HH (ed) Pesticides in the soil environment processes, impacts and modeling. Soil Science Society of America, Madison, pp 169–211, ISBN 0–89118-791-X

Bouziani M (2007) L'usage immod´er´e des pesticides. De graves cons´equences sanitaires, Le Guide de laM´edecine et de la Sant´e, Sant´emaghreb

Briceño G, Fuentes MS, Palma G, Jorquera MA, Amoroso MJ, Diez MC (2012) Chlorpyrifos biodegradation and 3,5,6-trichloro-2-pyridinol production by actinobacteria isolated from soil. Int Biodeterior Biodegrad 73:1–7

Bruck DJ, Lewis LC, Gunnarson RD (2001) Interaction of *Nosema pyrausta* and temperature on *Ostrinia nubilalis* egg production and hatch. J Invertebr Pathol 78(4):210–214

Cassidy DP, Srivastava VJ, Dombrowski FJ, Lingle JW (2015) Combining *in situ* chemical oxidation, stabilization, and anaerobic bioremediation in a single application to reduce contaminant mass and leachability in soil. J Hazard Mater 297:347–355

Chattopadhyay A, Bhatnagar NB, Bhatnagar R (2004) Bacterial insecticidal toxins. Crit Rev Microbiol 30(1):33–54

Chishti Z, Hussain S, Arshad KR, Khalid A, Arshad M (2013) Microbial degradation of chlorpyrifos in liquid media and soil. J Environ Manag 114:372–380

Chirnside AEM, Ritter WF, Radosevich M (2007) Bioremediation strategies for pesticide-contaminated Soil: IV. Biodegradation using a selected microbial consortium following pretreatment with fungal Enzymes. In: Proceedings of the 9th International In situ and On-Site Bioremediation Symposium, pp 1233–1240, Baltimore, MD, May 2007

Chawla N, Suneja S, Kukreja K, Kumar R (2013) Bioremediation: an emerging technology for remediation of pesticides. Res J Chem Environ 17(4):88–105

Chaudhry GR, Chapalamadugu S (1991) Biodegradation of halogenated organic compounds microbiological reviews. Microbiol Mol Biol Rev 55(1):59–79

Colosio C, Tiramani M, Brambilla G, Colombi A, Moretto A (2009) Neurobehavioural effects of pesticides with special focus on organophosphorus compounds: which is the real size of the problem? Neurotoxicology 30(6):1155–1161

Cory JS, Hails RS (1997) The ecology and biosafety of baculoviruses. Curr Opin Biotechnol 8(3):323–327

Cui ZI, Cui LX, Huang Y, Yan X, He J, Li SP (2012) Advances and application of microbial degradation in pesticides pollution remediation. Nanjing Nongye Daxue Xuebao 35(5):93–102

Debarati P, Gunjan P, Janmejay P, Rakesh VJK (2005) Accessing microbial diversity for bioremediation and environmental restoration. Trends Biotechnol 23(3):135–142

Diaz E (2004) Bacterial degradation of aromatic pollutants: a paradigm of metabolic versatility. Int Microbiol 7:173–180
Dixon B (1996) Bioremediation is here to stay. ASM News 62:527528
Dua M, Singh A, Sethunathan N, Johri AK (2002) Biotechnology and bioremediation: successes and limitations. Appl Microbiol Biotechnol 59(2–3):143–152
Dubey RC (2004) A text book of biotechnology, 3rd edn. S. Chand and Company Ltd., New Delhi, pp 365–375
EPA (2015) What is a Pesticide, April 2015, http://www.epa.gov
Fang H, Dong B, Yan H, Tang F, Yunlong Y (2010) Characterization of a bacterial strain capable of degrading DDT congeners and its use in bioremediation of contaminated soil. J Hazard Mater 184(1–3):281–289
Finley SD, Broadbelt LJ, Hatzimanikatis V (2010) In Silico feasibility of novel biodegradation pathways for 1,2,4-Trichlorobenzene. BMC Syst Biol 4(7):4–14
Fragoeiro S, Magan N (2008) Impact of *Trametes versicolor* and *Phanerochaete crysosporium* on differential breakdown of pesticide mixtures in soil microcosms at two water potentials and associated respiration and enzyme activity, Int. Biodeterior Biodegrad 62:376–383
Frankenhuyzen KV (2009) Insecticidal activity of *Bacillus thuringiensis* crystal proteins. J Invertebr Pathol 101(1):1–16
Fravel DR (2005) Commercialization and implementation of biocontrol. Annu Rev Phytopathol 43:337–359
Fries GR, Marrow GS, Gordon H (1969) Metabolism of *o,p'*-DDT by rumen microorganisms. J Agric Food Chem 17(4):860–862
Fulekar MH, Geetha M (2008) Bioremediation of chlorpyrifos in surface soil treatment unit using microbial consortium. Can J Pure Appl Sci 2(2):267–273
Garcia-Delgado C, Alfaro-Barta I, Eymar E (2015) Combination of biochar amendment and mycoremediation for polycyclic aromatic hydrocarbons immobilization and biodegradation in creosote-contaminated soil. J Hazard Mater 285:259–266
Gavrilescu M (2005) Fate of pesticides in the environment and its bioremediation. Eng Life Sci 5(6):497–526
Gerhardson B (2002) Biological Substitutes for Pesticides. Trends Biotechnol 20(8):338–343
Ghosh PG, Sawant NA, Patil SN, Aglave BA (2010) Microbial biodegradation of organophosphate pesticides. Int J Biotechnol Biochem 6(6):871–876
Gill SS, Singh GJP, Hornung JM (1987) Cell membrane interaction of *Bacillus thuringiensis* Subsp. israelensis cytolytic toxins. Infect Immun 55(5):1300–1308
Gillion RJ (2007) The Quality of our nation's waters: pesticides in the nation's streams and ground water, 1992–2001. US Geological Survey
Gimsing AL, Borggaard OK, Jacobsen OS, Aamand J, Sorensen J (2004) Chemical and microbiological soil characteristics controlling glyphosate mineralization in Danish surface soils. Appl Soil Ecol 2:233–242
Gold RS, Maxim J, Johnson DA (2005) Electron beam irradiation as protection against the environmental release of recombinant molecules for biomaterials applications. J Biomater Sci Polym Ed 16:79–89
Gondim-Tomaz MA, Franco TT, Durrant LR (2005) Biodegradation of diuron and pyruthiobac-sodium by white rot and soil fungi. Contam Soils Sediments Water 9:21–32
Gouma S (2009) Biodegradation of mixtures of pesticides by bacteria and white rot fungi, Ph.D. Thesis, School of Health Cranfield University, 416
Gupta PK (2004) Pesticide exposure—Indian scene. Toxicology 198(1–3):83–90
Hackenberg D (2007) Letter from David Hackenberg to American growers. Plattform Imkerinnen Austria
Hai FI, Modin O, Yamamoto K, Fukushi K, Nakajima F, Nghiem LD (2012) Pesticide removal by a mixed culture of bacteria Scientifica 9 and white-rot fungi. J Taiwan Inst Chem Eng 43(3):459–462

Harrison SA (1990) The fate of pesticides in the environment, Agrochemical Fact Sheet # 8, Penn, USA

Helweg (2003) Fate of pesticides in surface waters, Laboratory and Field Experiments; Ministry of Environment, Danish Environmental Protection Agency, Pesticides Research No. 68

Hussain S, Siddique T, Arshad M, Saleem M (2009) Bioremediation and phytoremediation of pesticides: recent advances. Crit Rev Environ Sci Technol 39(10):843–907

Inceoglu AB, Kamita SG, Hammock BD (2006) Genetically modified baculoviruses: a historical overview and future outlook. Adv Virus Res 68:323–360

Iwata H, Tanabe S, Sakai N, Tatsukawa R (1993) Distribution of persistent organochlorine in the oceanic air and surface seawater and the role of ocean on their global transport and fate. Environ Sci Technol 27:1080–1098

Jain RK, Kapur M, Labana S, Lal B, Sarma PM, Bhattacharya D, Thakur IS (2005) Microbial diversity: application of microorganisms for the biodegradation of xenobiotics. Curr Sci 89(1):101–112

Jauregui J, Valderrama B, Albores A, Vazquez-Duhalt R (2003) Microsomal transformation of organophosphorus pesticides by white rot fungi. Biodegradation 14:397–406

Jokanovic M, Prostran M (2009) Pyridinium oximes as cholinesterase reactivators. Structure-activity relationship and efficacy in the treatment of poisoning with organophosphorus compounds. Curr Med Chem 16(17):2177–2188

Johnson J, Ware WG (1992) Pesticide litigation manual 1992 edition, Clark Boardman Callaghan Environmental Law Series, New York, NY

Joshi SR (2006) Biopesticides: a biotechnological approach, New Age International Publishers, New Delhi, ISBN 81–224–1781-7

Jung H, Sohn KD, Neppolian B, Choi H (2008) Effect of soil organic matter (SOM) and soil texture on the fatality of indigenous microorganisms in intergrated ozonation and biodegradation. J Hazard Mater 150:809–817

Kadam TA, Jadhav VD, Gyananath G (2003) Microbial degradation of dimethoate by Gram negative soil isolates from cotton field. Pollut Res 22(3):443–445

Kannan K, Tanabe S, Willians RJ, Tatsukawa R (1994) Persistent organochlorine residues in foodstuffs from Australia, Papua New Guinea and the Solomon Islands: contamination levels and dietary exposure. Sci Total Environ 153:29–49

Karpouzas DG, Fotopoulou A, Menkissoglu-Spiroudi U, Singh BK (2005) Non-specific biodegradation of the organophosphorus pesticides, cadusafos and ethoprophos, by two bacterial isolates. FEMS Microbiol Ecol 53(3):369–378

Kaya HK, Gaugler R (1993) Entomopathogenic nematodes. Annu Rev Entomol 38:181–206

Kaneva I, Chen W (1999) Detoxification of organophosphate pesticides by immobilized Escherichia coli expressing organophosphorus hydrolase on cell surface. Biotechnol Bioeng 63:216–223

Kegley S (1999) Disrupting the balance, ecological impacts of pesticides in California, California, USA

Knowles BH, Ellar D (1987) Colloid-osmotic Lysis is a general feature of the mechanism of action of *Bacillus thuringiensis* δ-endotoxins with different insect specificity. Biochim Biophys Acta 924(3):509–518

Krishna KR, Philip L (2009) Biodegradation of mixed pesticides by mixed pesticide enriched cultures. J Environ Sci Health B 44(1):18–30

Kumar M, Philip L (2006) Bioremediation of endosulfan contaminated soil and water-optimization of operating conditions in laboratory scale reactors. J Hazard Mater 136:354–364

Kumar M, Prasad R, Goyal P, Teotia P, Tuteja N, Varma A, Kumar V (2017) Environmental biodegradation of xenobiotics: role of potential microflora. In: Xenobiotics in the Soil Environment (ed. Hashmi MZ, Kumar V and Varma A) Springer International Publishing Switzerland 319–334

Kumar V, Prasad R, Goyal P, Tuteja N, Singh J, Varma A, Kumar M (2017) Prominences on xenobiotic degradation underneath of ecological sanitary. In: Xenobiotics in the Soil Environment (ed. Hashmi MZ, Kumar V and Varma A) Springer International Publishing Switzerland 373–383

Kye CM, Reukaradhya M, Islam K (2009) Biodegradation of chlorpyrifos by lactic acid bacteria during kimchi fermentation. J Agric Food Chem 57(5):1882–1889
Lacey LA, Goettel MS (1995) Current developments in microbial control of insect pests and prospects for the early 21st century. Entomophaga 40:3–27
Langlois BE, Collins JA, Sides KG (1970) Some factors affecting degradation of organochlorine pesticide by bacteria. J Dairy Sci 53(12):1671–1675
Lan WS, Gu JD, Zhang JL (2006) Co-expression of two detoxifying pesticide-degrading enzymes in a genetically engineered bacterium. Int Biodeterior Biodegrad 58(2):70–76
Lal R, Dogra C, Malhotra S, Sharma P, Pal R (2006) Diversity, distribution and divergence of lin genes in hexachlorocyclohexane degrading sphingomonads. Trends Biotechnol 24:121–129
Larkin M, Kulakov L, Allen C (2005) Biodegradation and Rhodococcus-masters of catabolic versatility. Curr Opin Biotech 16:282–290
Li H, Liang W, Wu X, Liu Y (2004) Research on biodegradation of organophosphorus insecticide by a novel psychrotrophic bacterium SA-8. Zhongshan Daxue Xuebao, Ziran Kexueban 43(3):131–132
Li-Qing BL, Hong-Xia J, Yan-Chun Y et al (2008) Degradation of organophosphorus pesticide by engineered bacteria and immobilized cell. Huanjing Kexue Yu Jishu 31(5):45–48
Lorenz ES (2009) Potential health effects of pesticides. Ag Communications and Marketing: 1–8
Lio M, Xie X (2009) Application of enterobacteraerogenes in degrading pyrethroid pesticide residue, and preparation with enterobacteraerogenes, Faming Zhuanli Shenqing, CN102021135 A
Lima AS, Alegre RM (2009) Evaluation of emulsifier stability of biosurfactant produced by *Saccharomyces lipolytica* CCT-0913. Braz Arch Biol Technol 52:285–290
Lotter DW, Seidel R, Liebhardt W (2003) The performance of organic and conventional cropping systems in an extreme climate year. Am J Altern Agric 18:146–154
Liu YH, Chung YC, Xiong Y (2001) Purification and characterization of a dimethoate-degrading enzyme of *Aspergillus niger* ZHY256, isolated from sewage. Appl Environ Microbiol 67(8):3746–3749
Liu Z, Hong Q, Xu JH, Jun W, Li SP (2006) Construction of a genetically engineered microorganism for degrading organophosphate and carbamate pesticides. Int Biodeterior Biodegrad 58(2):65–69
Liu Z, Hong Q, Xu JH, Jun W, Li SP (2007) Construction of a genetically engineered microorganism for degrading organophosphate and carbamate pesticides, International Biodeterioration and Biodegradation 58(2):65–69
Martınez-Pascual E, Grotenhuis T, Solanas AM, Vinas M (2001) Coupling chemical oxidation and biostimulation: effects on the natural attenuation capacity and resilience of the native microbial community in alkylbenzene-polluted soil. J. Hazard Mater 300:135–143
Martinez-Pascual E, Grotenhuis T, Solanas AM, Vinas M (2015) Coupling chemical oxidation and biostimulation: effects on the natural attenuation capacity and resilience of the native microbial community in alkylbenzene-polluted soil. J Hazard Mater 300:135–143
Mata-Sandoval JC, Karns J, Torrents A (2002) Influence of rhamnolipids and Triton X-100 on the desorption of pesticides from soils. Environ Sci Technol 36:4669–4675
McCutchen WF, Flexner L (1999) Join action of Baculovirus and other control agents. In: Hall DR, Menn JJ (eds) Biopesticides use and delivery. Humana Press, New Jersey, pp 341–355, ISBN 0–89603–515-8
Mercadier C, Vega D, Bastide J (1997) Iprodione degradation by isolated soil microorganisms. FEMS Microbiol Ecol 23:207–215
Mervat SM (2009) Degradation of methomyl by the novel bacterial strain *Stenotrophomonas maltophilia* M1. Electron J Biotechnol 12(4):1–6
Miller GT (2004) Sustaining the earth, 6th edn. Thompson Learning, Inc., Pacific Grove
Mishra V, Lal R, Srinivasan S (2001) Enzymes and operons mediating xenobiotic degradation in bacteria. Crit Rev Microbiol 27(2):133–166
Mohamed AT, El Hussein AA, El Siddig MA, Osman AG (2011) Degradation of oxyfluorfen herbicide by soil microorganisms: biodegradation of herbicides. Biotechnology 10:274–279

Montesinos E (2003) Development, registration and commercialization of microbial pesticides for plant protection, Int. Microbiology 6(4):245–252
Moussa TAA, Ahmed GM, Abdel-hamid SMS (2006) Optimization of cultural conditions for biosurfactants production from *Nocardia amarae*. J Appl Sci Res 2:844–850
Mulligan CN (2005) Environmental applications for biosurfactants. Environ Pollut 133:183–198
Nagata Y, Futamura AA, Miyauchi K, Takagi M (1999) Two different types of dehalogenases, Lin A and Lin B, involved in γ- hexachlorocyclohexane degradation in *Sphingomonas paucimobilis* UT26 are localized in the periplasmic space without molecular processing. J Bacteriol 181:5409–5413
Nagata Y, Endo R, Ito M, Ohtsubo Y, Tsuda M (2007) Aerobic degradation of lindane (hexachlorocyclohexane) in bacteria and its biochemical and molecular basis. Appl Microbiol Biotechnol 76:741–752
Nagpal V, Srinivasan MC, Paknikar KM (2008) Biodegradation of γ-hexachlorocyclohexane (Lindane) by a non-white rot fungus conidiobolus 03-1-56 isolated from litter. Indian J Microbiol 48(1):134–141
Nawab A, Aleem A, Malik A (2003) Determination of organochlorine pesticides in agricultural soil with special reference to γ-HCH degradation by *Pseudomonas* strains. Bioresour Technol 88(1):41–46
Ning L, Jing-ying C, Li L (2005) Isolation and selection of strains used to degrade organic PCNB pesticides and their application effects. Jilin Nongye Daxue Xuebao 27(2):205–208
Normand P, Queiroux C, Tisa LS, Benson DR, Rouy Z, Cruvellier S, Medigue C (2007) Exploring the genomes of Frankia. Physiol Plant 130:331–343
Ortega NO, Nitschke M, Mouad AM, Landgraf MD, Rezende MOO, Seleghim MHR, Sette LD, Porto ALM (2011) Isolation of Brazilian marine Fungi capable of growing on DDD pesticide. Biodegradation 22:43–50
Ortiz-Hernández ML, Sánchez-Salinas E (2010) Biodegradation of the organophosphate pesticide tetrachlorvinphos by bacteria isolated from agricultural soils in México. Rev Int Contam Ambient 26(1):27–38
Parekh NR, Walker A, Roberts SJ, Welch SJ (1994) Rapid degradation of the triazinone herbicide metamitron by a Rhodococcus sp. isolated from treated soil. J Appl Bacteriol 77(5):467–475
Pesce S, Wunderlin D (2004) Biodegradation of lindane by a native bacterial consortium isolated from contaminated river sediment. Int Biodeterior Biodegradation 54:255–260
Peng X, Zhang JS, Li YY, Li W, Xu GM, Yan YC (2008) Biodegradation of insecticide carbofuran by *Paracoccus* sp. YM3. J Environ Sci Health B 43(7):588–594
Pointing SB (2001) Feasibility of bioremediation by white-rot fungi. Appl Microbiol Biotechnol 57:20–33
Poinar GO, Thomas GM, Hess R (1977) Characteristics of the specific bacterium associated with Heterorhabditis Bacteriophora (Heterorhabditidae: Rhabditida). Nematologica 23(1):97–102
Poinar G (1990) Biology and taxonomy of Steinernematidae and Heterorhabditidae. In: Gaugler RR, Kaya RK (eds) Entomopathogenic nematodes in biological control. CRC Press, Boca Raton, pp 23–62
Prasad R (2017) Mycoremediation and Environmental Sustainability. Volume 1. Springer International Publishing (ISBN 978-3-319-68957-9) https://link.springer.com/book/10.1007/978-3-319-68957-9
Prasad R (2018) Mycoremediation and Environmental Sustainability, Volume 2. Springer International Publishing (ISBN 978-3-319-77386-5) https://www.springer.com/us/book/9783319773858
Prasad R, Pandey R, Barman I (2016) Engineering tailored nanoparticles with microbes: quo vadis. WIREs Nanomed Nanobiotechnol 8:316–330. https://doi.org/10.1002/wnan.1363
Prasad R, Aranda E (2018) Approaches in Bioremediation. Springer International Publishing https://www.springer.com/de/book/9783030023683
Prasad R (2021) Environmental Pollution and Remediation. Springer (ISBN 978-981-15-5499-5) (in press) https://www.springer.com/gp/book/9789811554988

Prescott LM, Harley JP, Klein DA (2002) Microbiology. Fundamental of applied microbiology. Microbiology 2:1012–1014

Raaijmakers JM, Vlami M, De Souza TJ (2002) Antibiotic production by bacterial biocontrol agents. Antonie Van Leeuwenhoek 81(1–4):537–547

Ragnarsdottir K (2000) Environmental fate and toxicology of organophosphate pesticides. J Geol Soc London 157(4):859–876

Rizwan M, Singh M, Mitra CK, Morve RK (2014) Ecofriendly application of nanomaterials: nanobioremediation. J Nanoparticles 2014:1–7

Rodríguez-Cruz MS, Sánchez-Martín MJ, Sánchez-Camazano M (2004) Enhanced desorption of herbicides sorbed on soils by addition of Triton X-100. J Environ Qual 33:920–929

Ron EZ, Rosenberg E (2001) Minireview: natural roles of biosurfactants. Environ Microbiol 3:229–236

Rosell G, Quero C, Coll J, Guerrero A (2008) Biorational insecticides in pest management. J Pestic Sci 33(2):103–121

Rubilar O, Feijoo G, Diez MC, Lu-Chau TA, Moreira MT, Lema JM (2007) Biodegradation of pentachlorophenol in soil slurry cultures by Bjerkanderaadusta and Anthracophyllumdiscolour. Ind Eng Chem Res 4:6744–6751

Sacramento CA (2008) Department of pesticide regulation "What are the potential health effects of pesticides?" Community guide to recognizing and reporting pesticide problems, pp 27–29

Schisler DA, Slininger PJ, Behle RW, Jackson MA (2004) Formulation of *Bacillus* spp. for biological control of plant diseases. Phytopathology 94(11):1267–1271

Schoefs O, Perrier M, Samson R (2004) Estimation of contaminant depletion in unsaturated soils using a reduced-order biodegradation model and carbon dioxide measurement. Appl Microbiol Biotechnol 64(1):53–61

Schroll R, Brahushi R, Dorfler U, Kuhn S, Fekete J, Munch JC (2004) Biomineralization of 1,2,4-Trichlorobenzene in soils by an adapted microbial population. Environ Pollut 127(3):395–401

Shanahan P (2004, Spring) Bioremediation, waste containment and remediation technology, Massachusetts Institute of Technology, MIT Open Course Ware

Shin KH, Kim KW, Ahn Y (2006) Use of biosurfactant to remediate phenanthrene-contaminated soil by the combined solubilization-biodegradation process. J Hazard Mater B 137:1831–1837

Shunpeng L, Mingxing Z (2006) Sphingomonas strain for degrading chlorophenothane pesticide residue and bacteria agent containing this strain, Faming Zhuanli Shenqing Gongkai Shuomingshu

Siegel JP, Shadduck JA (1990) Clearence of *B. sphaericus* and *B. thuringiensis* spp. israelensis from mammals. J Eco Entomol 83(2):347–355

Singh A, Ranjit JA, Dhasarathan P (2003) Isolation and characterization of pesticide metabolizing bacteria from groundnut soil system. Asian J Microbiol Biotechnol Environ Sci 5(3):323–332

Singh BK, Walker A, Morgan JAW, Wright DJ (2004) Biodegradation of chlorpyrifos by *Enterobacter* strain B-14 and its use in bioremediation of contaminated soils. Appl Environ Microbiol 70(8):4855–4863

Silva-Castro GA, Uad I, Gonzalez-Lopez J, Fandino CG, Toledo FL, Calvo C (2012) Application of selected microbial consortia combined with inorganic and oleophilic fertilizers to recuperate oil-polluted soil using land farming technology. Clean Techn Environ Policy 14:719–726

Silva FC, Cardeal ZL, De Carvalho CR (1999) Determination of organophosphorus pesticides in water using SPME-GC-MS. Química Nova 22(2):197–200

Singh BK, Walker A (2006) Microbial degradation of organophosphorus compounds. FEMS Microbiol Rev 30(3):428–471

Singh DP, Khattar JI, Nadda J, Singh Y, Garg A, Kaur N, Gulati A (2011) Chlorpyriphos degradation by the cyanobacterium Synechocystis sp. strain PUPCCC 64. Environ Sci Poll Res Int 18(8):1351–1359

Sogorb MA, Vilanova E (2002) Enzymes involved in the detoxification of organophosphorus, carbamate and pyrethroid insecticides through hydrolysis. Toxicol Lett 128(1–3):215–228

Soriano JM, Jiménez B, Font G, Moltó JC (2001) Analysis of carbamate pesticides and their metabolites in water by solid phase extraction and liquid chromatography: a review. Crit Rev Anal Chem 31(1):19–52

Srivastava CN, Maurya P, Sharma P, Mohan L (2009) Prospective role of insecticides of fungal origin: review. Entomol Res 39(6):341–355

Stenrod M, Charnay MP, Benoit P, Eklo OM (2006) Spatial variability of glyphosate mineralization and soil microbial characteristics in two Norwegian sandy loam soils as affected by surface topographical features. Soil Biol Biochem 38:962–971

Subba-Rao RV, Alexander M (1985) Bacterial and fungal cometabolism of 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (DDT) and its breakdown products. Appl Environ Microbiol 49:509–516

Sutherland TD, Horne I, Harcourt RL, Russel RJ, Oakeshott JG (2002) Isolation and characterization of a Mycobacterium strain that metabolizes the insecticide endosulfan. J Appl Microbiol 93:380–389

Suzuki O, Watanabe K (2005) Drugs and poisons in humans – a handbook of practical analysis. In: Carbamate pesticides. Springer, pp 559–570. https://doi.org/10.1007/3-540-27579-7-62

Tallur PN, Megadi VB, Ninnekar HZ (2008) Biodegradation of Cypermethrin by *Micrococcus* sp. strain CPN 1. Biodegradation 19(1):77–82

Theilmann DA, Blissard GW, Bonning B, Jehle JA, O'Reilly DR, Rohrmann GF (2005) Baculoviridae. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, Ball LA (eds) Virus taxonomy classification and nomenclature of viruses: eighth report of the international committee on the taxonomy of viruses. Elsevier, New York, pp 177–185

Thomas MB, Read AF (2007) Can fungal biopesticides control malaria? Nat Rev Microbiol 5(5):377–383

Tingting L, Kunming D, Miao L (2012) Isolation, identification and biodegradation characteristics of a bacterial strain able to degrade bifenthrin. Nongye Huanjing KexueXuebao 31(6):1147–1152

Tortella G (2005) Fungal diversity and use in decomposition of environmental pollutants. Crit Rev Microbiol 31:197–212

Tyagi M, da Fonseca MMR, de Carvalho CCCR (2011) Bioaugmentation and biostimulation strategies to improve the effectiveness of bioremediation processes. Biodegradation 22:231–241. https://doi.org/10.1007/s10532-010-9394-4

Vaccari DA, Strom PF, Alleman JE (2006) Environmental biology for engineers and scientists. John Wiley & Sons, New York. https://doi.org/10.1002/0471741795

Vidya Lakshmi C, Kumar M, Khanna S (2008) Biotransformation of chlorpyrifos and bioremediation of contaminated soil. Int Biodeter Biodegrad 62(2):204–209

Veiga MM, Silva DM, Veiga LBE, de Castro Faria MV (2006) Pesticide pollution in water systems in a small rural community in Southeast Brazil. Cad Saude Publica 22:2391–2399

Wang X, Wang Q, Wang S, Li F, Guo G (2012) Effect of biostimulation on community level physiological profiles of microorganisms in field-scale biopiles composed of aged oil sludge. Bioresour Technol 111:308–315

Wattanaphon HT, Kerdsin A, Thammacharoen C, Sangvanich P, Vangnai AS (2008) A biosurfactant from *Burkholderia cenocepacia* BSP3 and its enhancement of pesticide solubilization. J Appl Microbiol 105:416–423

Wedemeyer G (1967) Dechlorination of 1,1,1-Trichloro-2,2-bis(*p*-chlorophenyl)ethane by Aerobacter aerogene. J Appl Microbiol 15(3):569–574

Weir KM, Sutherland TD, Horne I, Russell RJ, Oakeshott JG (2006) A single Monooxygenase, Ese, is involved in the metabolism of the organochlorides endosulfan and endosulfate in an Arthrobacter sp. Appl Environ Microbiol 72:3524–3530

Whalon ME, McGaughey WH (1998) BT: use and resistance management. In: Ishaaya I, Degheele D (eds) Insecticides with novel modes of action, mechanism and application. Springer, Berlin, pp 106–137, ISBN 3–5406–3058-9

Wolfe NL, Zepp RG, Paris DF (1978) Use of structure-reactivity relationships to estimate hydrolytic persistence of Carbamate pesticides. Water Res 12(8):561–563, ISSN 0043–1354

Xiang-Ming L, Ping-Ping C (2012) Progress in degradation of organo-chlorinated pesticides by microorganism. Huanjing Kexue Yu Jishu 35(6):89–93

Yang C, Liu N, Guo X, Qiao C (2006) Cloning of mpd gene from a chlorpyriphos-degrading bacterium and use of this strain in bioremediation of contaminated soil. FEMS Microbiol Lett 265:118–125

Yates SR, McConnell LL, Hapeman CJ, Papiernik SK, Gao S, Trabue SL (2011) Managing agricultural emissions to the atmosphere: state of the science, fate and mitigation and identifying research gaps. J Environ Qual 40:1347–1358

Yokoyama Y, Kohda K, Okamoto M (1998) CytA protein, a Dendotoxin of *Bacillus thuringiensis* subsp. Israelensis is associated with DNA. Biol Pharm Bull 21(12):1263–1266, ISSN 0918–6158

Yoshii K, Tonogai Y, Ueno H, Nakamuro K (2006) Malathion residue in wheat kernels is degraded by thion organophosphorus pesticide-specific carboxylesterase. J Health Sci 52(3):221–227

Zhang FY, Jiao YA, Guangyu Y (2012) Sequence of Geobacillus phosphotriesterase mutant and its uses in decomposing organophosphorus pesticide, Faming Zhuanli Shenqing

Zhang H, Yang C, Li C, Li L, Zhao Q, Qiao C (2008) Functional assembly of a microbial consortium with autofluorescent and mineralizing activity for the biodegradation of organophosphates. J Agric Food Chem 56:7897–7902

Chapter 2
Arbuscular Mycorrhizal Fungi and Remediation Potential of Soils Contaminated by Potentially Toxic Elements

Laíze Aparecida Ferreira Vilela and Edenis Cesar de Oliveira

Contents

L. A. F. Vilela (✉) · E. C. de Oliveira
Federal University of São Carlos, São Paulo, Brazil

R. Prasad et al. (eds.), *Mycoremediation and Environmental Sustainability*, Fungal Biology, https://doi.org/10.1007/978-3-030-54422-5_2

2.1 Introduction

Many countries have faced increasing challenges in protecting their soils from contamination caused by dizzying population growth (Tume et al. 2018; Yang et al. 2018), aggravated by rapid industrialization (Sarwar et al. 2017), and unprecedented urbanization rate, especially in the last three decades. Studies draw attention to the fact that a considerable amount of financial resources must be invested by governments and industries from various economic segments, among other anthropogenic sources, to remediate contaminated sites (Swartjes et al. 2012; Cappuyns 2016).

A significant and alarming number of contaminated areas are estimated to exist worldwide, which represent a missed economic opportunity and a direct threat to human health and well-being and the environment (CRCCARE 2013; Gasparatos 2013). Although soil contamination was recognized in the 1960s due to the legacy of industrialization, less than one-tenth of potentially contaminated land was only remedied due to the challenging nature of the contamination itself, as well as cost, technical impracticality, legislation, and soil use impossibility (Naidu et al. 2008).

Many countries do not have comprehensive and systematic studies about the identification and assessment of contaminated sites, which substantially increases the difficulty of accurately quantifying currently affected areas and the actual cost of remediation. However, the extent of contamination is growing at a rate of approximately 3% per year. The global remediation market estimated at about $ 59.5 billion in 2013 is expected to expand to nearly $ 80.5 billion in 2019, with a compound annual growth rate of 5.5% during 2013 and 2019 (Kuppusamy et al. 2017).

Contaminated land management has become a global challenge (Phoungthong et al. 2016; Järup 2003). Even so, the remediation process needs to deliver tangible benefits. However, if remediation practices are not properly selected and implemented, more environmental impact may arise in addition to the impacts directly associated with the contamination itself. Stated another way, corrective action itself in addressing the contamination problem can have negative effects on soil ecological functions (Groot et al. 2002; Volchko et al. 2013) – soil compaction, loss of organic matter, decline in biodiversity, and nutrient deficiency, among others. In turn, these negative effects contribute to a drastic reduction in the provision of soil ecosystem services (Volchko et al. 2014). Main soil functions or soil ecosystem services are (i) biomass production, including agriculture and forestry; (ii) storage, filtration, and transformation of nutrients, substances, and water; (iii) biodiversity shelter; (iv) physical and cultural environment for humans and the development of their activities; (v) raw materials source; (vi) carbon storage; and (vii) refuge of geological and archaeological heritage (CEC 2006). Considering the criticality of soil functions for the survival of the ecosystem, it is essential to evaluate its performance from a sustainability perspective in remediation projects (Volchko et al. 2013).

Over the years, scientists in partnership with governments and other interested institutions have made efforts to create decontamination processes and technologies to meet sustainability goals in environmental remediation (Gavrilescu and Macoveanu 2000; Gavrilescu and Macoveanu 1999; Khan et al. 2004). Integrating

sustainability practices into contaminated soil remediation provides an opportunity for the process's social, environmental, and economic benefits to be considered and optimized (Schädler et al. 2011; Rosén et al. 2015; Gill et al. 2016; Behera and Prasad 2020).

Remediation process, which also includes sustainable remediation or green remediation, consists in reducing or removing unwanted contaminants from the soil (Prasad 2021). According to the technique, soil remediation activities themselves can become an additional source of contamination. Since the mid-1990s, due to significant increase in remediation sites, scientific community has been paying increasing attention to these additional threats introduced by remediation efforts, increasing the need for better evaluation and management of these projects. The Sustainable Remediation Forum (SuRF) played an important role in connecting diverse stakeholders, disseminating knowledge, and building and developing a framework based on a criteria set to evaluating remediation activities (Rosén et al. 2015; Bardos et al. 2016; Yasutaka et al. 2016).

Although SuRF originally started in the United States in 2006, it achieved greater projection in Europe, driving an increase in the number of evaluation activities across the continent (Bardos et al. 2016). These SuRFs have produced publications covering guidance recommendations (NICOLE-2011 2011), assessment frameworks (Holland et al. 2011), assessment standards (ASTM 2013; ISO 2016), and assessment tools (Volchko et al. 2014; Rosén et al. 2015; Perini and Rosasco 2013; Lemming et al. 2010; Beames et al. 2014). Importantly, for the effective monitoring of the pace of these developments, it is necessary to establish processes of continuous assessment of recommended correction efforts, as well as the accuracy of newly introduced assessment tools through case studies. Such studies may facilitate the refinement of the methods, as well as expand their application, enabling greater understanding and wider adoption of these methods, especially focusing on the development of sustainable remediation methods (Bardos et al. 2016).

Sustainable remediation can be defined as a treatment or a combination of treatments whose benefit to human health and the environment is maximized by the judicious use of limited resources Bardos et al. (2009). Sometimes, organizations refer to green remediation, which can be defined as the practice of considering all environmental effects of treatment implementation and incorporating options to maximize the benefit of environmental cleansing actions (EPA 2008). ISO 18504 defines sustainable remediation as the safe and appropriate elimination and/or control of unacceptable risks, with a focus on optimizing the environmental, social, and economic value of the activity (ISO 2016). The UK Sustainable Remediation Forum (SuRF-UK) established clear principles for effective sustainable remediation with a fundamental emphasis on decision-making on risk assessment and management of contaminated land (Bardos et al. 2016). Table 2.1 presents the six key principles.

These key principles should be considered by professionals in preparing, implementing, and reporting sustainable remediation systems. Balancing environmental, social, and economic costs and benefits in identifying the best remediation solution needs to be achieved while meeting key principles (Bardos et al. 2011). Admittedly, selection of any practice or alternative to environmental remediation has historically been made according to the contaminant type, environmental component affected

Table 2.1 Key principles of sustainable remediation established by UK Sustainable Remediation Forum (UK-SuRF)

Principles	Description	Definition – scope
1	Protection of human health and the wider environment	Remediation (site-specific risk management) should remove unacceptable risks to human health and protect the wider environment now and in the future for the agreed land use, and give due consideration to the costs, benefits, effectiveness, durability, and technical feasibility of available options
2	Safe working practices	Remediation works should be safe for all workers and for local communities and should minimize impacts on the environment
3	Consistent, clear, and reproducible evidence-based decision-making	Sustainable risk-based remediation decisions are made having regard to environmental, social, and economic factors and consider both current and likely future implications. Such sustainable and risk-based remediation solutions maximize the potential benefits achieved. Where benefits and impacts are aggregated or traded in some way, this process should be explained and a clear rationale provided
4	Record keeping and transparent reporting	Remediation decisions, including the assumptions and supporting data used to reach them, should be documented in a clear and easily understood format in order to demonstrate to interested parties that a sustainable (or otherwise) solution has been adopted
5	Good governance and stakeholder involvement	Remediation decisions should be made having regard to the views of stakeholders and following a clear process within which they can participate
6	Sound science	Decisions should be made on the basis of sound science, relevant and accurate data, and clearly explained assumptions, uncertainties, and professional judgment. This will ensure that decisions are based upon the best available information and are justifiable and reproducible

Source: Bardos et al. (2011)

by the contamination, location, and the potentially exposed and affected receptors (Fortuna et al. 2011).

Considering the sustainable remediation principles proposed by SuRF, it becomes evident the viability of using arbuscular mycorrhizal fungi in remediation processes of contaminated areas, since they are beneficial microorganisms to plant growth and widespread occurrence in ecosystems, do not present risks to human health. Thus, the use of these microorganisms for soil decontamination mainly meets the following principles: (1) protection of human health and the wider environment, (2) safe working practices, (3) consistent, clear, and reproducible evidence-based decision-making, and (4) sound science. In this chapter, we approach research on the potential of arbuscular mycorrhizal fungi in the remediation of contaminated area by potentially toxic elements and their importance in environmental sustainability. We also discuss the physiological and biochemical mechanisms of these microorganisms and plants related to tolerance to these elements and explore relevant aspects of the phytoremediation process involving arbuscular mycorrhizal fungi.

2.2 What Are the Potentially Toxic Elements (PTE)?

Usually, under natural conditions, chemical elements do not pose an environmental risk, as they occur naturally in low-concentration soils and rocks (Kabata-Pendias 2011; Cao et al. 2015). Some of these elements perform indispensable physiological functions for plant metabolism, and therefore, elements such as cobalt (Co), copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn) are considered essential mineral elements for plants (Table 2.2) but, in high concentrations, are toxic to living organisms.

On the other hand, elements such as cadmium (Cd), lead (Pb), and mercury (Hg) and metalloids such as arsenic (As) are highly toxic to the environment and human health (Cabral et al. 2015), since they do not have biological functions. Given the wide variety of chemical elements capable of promoting toxicity to plants, this chapter will address potentially toxic elements (PTE). In this sense, all essential and nonessential chemical elements, formerly called heavy metals, trace elements,

Table 2.2 Functions and benefits of some potentially toxic elements (PTE) in plant metabolism

Group	Element	Ionic species uptake by plant	Essential/beneficial biological function	Reference
Essential elements				
Metalloid	Boron (B)	H_3BO_3, $H_2BO_3^-$ and $B(OH)_4^-$	Enzyme activator, cell wall component, carbohydrate transport	Prado (2008)
Transition metals	Zinc (Zn)	Zn^{2+}	Indoleacetic acid (AIA) synthesis, protein synthesis, nitrate reduction, enzyme component and activator	Prado (2008)
Transition metals	Manganese (Mn)	Mn^{2+}	Enzyme component and activator	Prado (2008)
Transition metals	Iron (Fe)	Fe^{2+}, Fe^{3+}, and chelated-Fe	Chlorophyll and protein synthesis, protein and enzyme component, and enzyme activator	Prado (2008)
Transition metals	Cupper (Cu)	Cu^{2+} and chelated-Cu	Electron transport, activator and enzyme component, nodulation	Prado (2008)
Transition metals	Molybdenum (Mo)	MoO_4^{2-} and $HMoO_4^-$	Biological nitrogen fixation, enzymatic component, sulfur metabolism	Prado (2008)
Beneficial elements				
Transition metals	Cobalt (Co)	Co^{2+}	Nitrogenase component (biological nitrogen fixation)	Marschner (2012)
Transition metals	Nickel (Ni)	Ni^{2+}	Urease component	Marschner (2012)
Metalloid	Silicon (Si)	H_4SiO_4 (pH < 9)	Cell wall strengthening, plant structural defense system that increases plant resistance against pests and pathogens, improved plant tolerance to drought and heavy metal excess	Guntzer, et al. (2012)

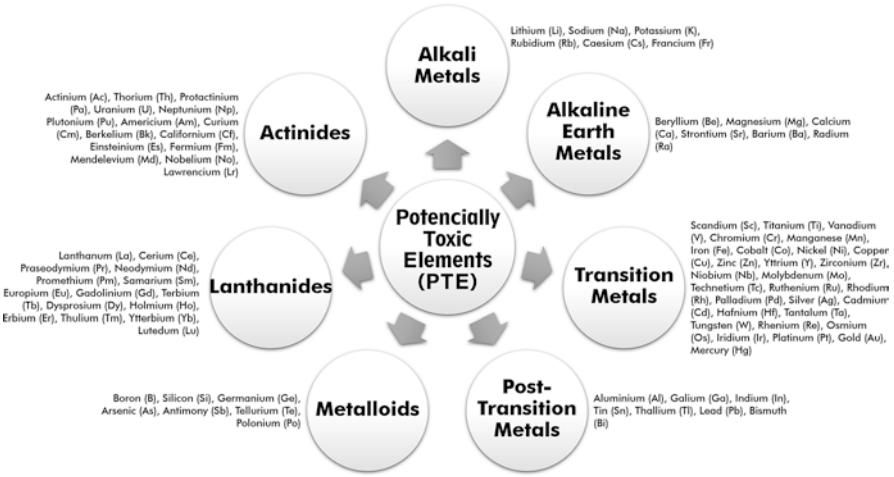


Fig. 2.1 Chemical elements of the periodic table considered in this chapter as potentially toxic elements (PTE)

alkali metals, alkaline earth metals, transition metals, metalloids, post-transition metals, lanthanides, and actinides (Fig. 2.1) are considered as potentially toxic elements (PTE) (Kasemodel et al. 2019; Halka and Nordstrom 2011; Göhre and Paszkowski 2006).

2.3 Contamination Inputs in Biosystems and Negative Consequences of Soil Contamination by Potentially Toxic Elements (PTE)

PTE sources are generally classified into two categories: (i) lithogenic (natural) and (ii) anthropogenic (man-made) (Karimi et al. 2017). Most PTEs that occur in the environment originate from lithogenic (natural) sources. The most important natural sources of pollution are volcanic activity, erosion, and weathering of minerals (Coninx et al. 2017). However, anthropogenic sources stand out as the main cause of soil contamination by PTEs (Kabata-Pendias 2011). The intensification of industrial and agricultural activities on the planet as well as population growth, responsible for the rapid and unorganized urbanization of many areas, provides a large contribution of PTEs to ecosystems (Dankoub et al. 2012; Esmaeili et al. 2014; Li et al. 2015). This situation aggravates the inadequate disposal of urban and industrial waste, the intensification of mining activities, and the use of high doses of agricultural inputs (pesticides, herbicides, mineral fertilizers, sewage sludge, etc.) (Yesilonis et al. 2008; Xu et al. 2014; Barbieri 2016). Mining activities usually result in the most extreme cases of PTE soil contamination (Cabral et al. 2015; Göhre and Paszkowski 2006; Perveen et al. 2015). Table 2.3 presents examples of

Table 2.3 Anthropogenic sources of some potentially toxic elements (PTE)

PTE	Sources	References
Pb	Chemical and organic fertilizer usage, agricultural practices, traffic emission, industrial activities	Lv (2019), Jiang et al. (2017), Liang et al. (2017), Huang et al. (2015), Mihailović et al. (2015), Maas et al. (2010), Sun et al. (2010), Lee et al. (2006), Zhang (2006), Li et al. (2004), Facchinelli et al. (2001)
Zn	Electroplating industries and livestock/poultry breeding, chemical and organic fertilizer usage, traffic emission	Lv (2019), Jiang et al. (2017), Liang et al. (2017), Huang et al. (2015), Mihailović et al. (2015), Kelepertzis (2014), Maas et al. (2010), Sun et al. (2010), Zhang (2006), Li et al. (2004)
Cu	Atmospheric deposition from coal combustion, chemical and organic fertilizer usage, traffic emission	Liang et al. (2017), Krishna and Mohan (2016), Luo et al. (2015), Mihailović et al. (2015), Kelepertzis (2014), Sun et al. (2010), Zhang (2006), Li et al. (2004)
Ni	Atmospheric deposition from coal combustion, traffic emission, surgical instruments, kitchen appliances	Liang et al. (2017), Sarwar et al. (2017), Tume et al. (2018), Luo et al. (2015), Sun et al. (2010), Tariq et al. (2006), Zhang (2006), Li et al. (2004)
Cr	Atmospheric deposition from coal combustion, electroplating industries and livestock/poultry breeding, metallurgical and steel industry, chrome plating and pigment production, leather industry	Tume et al. (2018), Jiang et al. (2017), Liang et al. (2017), Sarwar et al. (2017), Krishna and Mohan (2016), Huang et al. (2015), Luo et al. (2015), Mihailović et al. (2015), Zhang (2006), Li et al. (2004)
Cd	Chemical and organic fertilizer usage, agricultural practices, cement industry, power stations, metal industries	Lv (2019), Sarwar et al. (2017), Huang et al. (2015), Kelepertzis (2014)
Hg	Gold mining industry, cement industry, chemical and organic fertilizer usage, traffic emission, coal combustion, surgical instruments, hospital waste	Liang et al. (2017), Huang et al. (2015), Kelepertzis (2014), Mason et al. (2012), Pacyna et al. (2010)
As	Chemical and organic fertilizer usage, coal and peat combustion for home heating, wood preservatives	Tóth et al. (2016), Huang et al. (2015), Kelepertzis (2014), Khan et al. (2007), Zhang (2006), Ursitti et al. (2004)
Mn	Waste incineration and the textile/dyeing industries Mining activities	Tume et al. (2018), Jiang et al. (2017), Rivera-Becerril et al. (2013)
V	Electroplating industries and livestock/poultry breeding	Jiang et al. (2017), Wang et al. (2013)
Fe	Chemical and organic fertilizer usage (insecticides, fungicides, and herbicides)	Myers and Thorbjornsen (2004)

anthropogenic sources of some PTE. The first six (Pb, Zn, Cu, Ni, Cr, and Cd) are the most intensely studied, and these are listed in descending order of frequency (Hou et al. 2017).

Essential or not, excess of PTE in the soil impairs plant development, impeding several important metabolic processes. Considering the physicochemical properties, some PTE can be separated into redox and non-redox active metals. The first

group includes elements such as Cr, Cu, Mn, and Fe and generally promotes oxidative damage to plant metabolism, which leads to ROS production and, consequently, disruption of cellular homeostasis due to DNA molecule disruption, lipid peroxidation, defragmentation of proteins or cell membrane, and damage to photosynthetic pigments, which can trigger cell death. On the other hand, those non-redox active metals (e.g., Cd, Ni, Hg, Zn, and Al) trigger oxidative stress through other mechanisms such as by binding with sulfhydryl protein groups, inhibiting antioxidant enzymes, downregulating glutathione, or upregulating ROS-producing enzymes such as NADPH oxidases (Emamverdian et al. 2015). In general, PTE toxicity can cause the following (Göhre and Paszkowski 2006; Emamverdian et al. 2015):

1. Changes in or displacement of protein structure blocks arising from the formation of bonds between PTE and sulfhydryl groups
2. Downregulation of important molecular functional groups, compromising plant physiological balance
3. Replacement of essential metals in root uptake and, consequently, interruption of the functionality of these elements in biomolecules such as pigments or enzymes
4. Induction of nutritional deficiency caused by nutrient replace in root uptake
5. Reduction of plasma membrane integrity by promoting alterations of important membrane proteins such as H^+-ATPases
6. Suppression of vital processes such as photosynthesis, respiration, and enzymatic activities
7. Stimulation of the production of reactive oxygen species (ROS), such as superoxide free radicals (O2•⁻), and hydroxyl free radicals OH•⁻), or free radical species such as singlet oxygen (O_2) and hydrogen peroxide (H_2O_2)

2.4 Plant Defense Strategies Against Toxicity of Potentially Toxic Elements (PTE)

Plants usually have a wide range of defense strategies against toxicity caused by excess PTE. These various strategies can be grouped into (1) the creation of physical barriers against absorption of TEP, (2) changes in plant metabolism, and (3) activation of the antioxidant defense mechanism, as shown in Fig. 2.2 (Emamverdian et al. 2015; Bhandari and Garg 2017; Garg and Bhandari 2014).

Under PTE excess in the soil, the first strategy of plants is to avoid the uptake of these elements by creating physical barriers (Fig. 2.2). Under these conditions, morphological changes such as root cuticle thickening and increased production of biologically active tissues such as trichomes are found to impair root uptake and secrete secondary metabolites capable of chelating PTE, respectively (Emamverdian et al. 2015). Another strategy, already well described in the literature, is the development of mycorrhizal symbiosis, which will be further explored in Sect. 2.7 of this chapter.

The strategies outlined in Fig. 2.3 are commonly effective under conditions of low toxicity (low PTE concentrations). On the other hand, under high soil

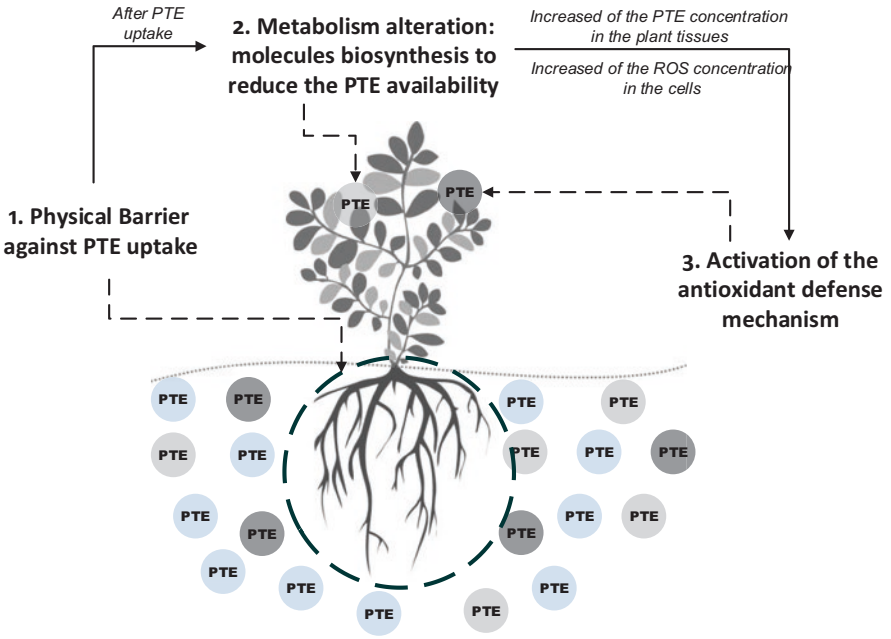


Fig. 2.2 Plant defense strategies against the toxicity of potentially toxic elements. PTE, potentially toxic elements; ROS, reactive oxygen species

concentrations, plant uptake of PTE is unavoidable. Thus, the second group of defense mechanisms comes into operation: alteration in metabolism, upregulating the molecules biosynthesis capable of altering the PTE availability within cells and, consequently, attenuating the harmful effects of these elements on physiological processes (Fig. 2.4; Cabral et al. 2015; Dutta and Neog 2016; Garg and Singh 2018; Kapoor et al. 2013; Dietterich et al. 2017; Lenoir et al. 2016; Cicatelli et al. 2014).

Upregulation of low-molecular-weight chelating proteins such as metallochaperones has been reported in plants under PTE toxicity. Among metallochaperones, nicotinamide, spermine, muginic acids, organic acids, phytochelatins, and metallothioneins stand out. Cellular exudates such as flavonoids and other phenolic compounds, as well as heat shock proteins, protons, amino acids (proline and histidine), and hormones (ethylene, jasmonic acid, and salicylic acid), also minimize internal damage caused by PTE toxicity (Göhre and Paszkowski 2006; Perveen et al. 2015; Emamverdian et al. 2015; Garg and Bhandari 2014; Gratão et al. 2005; Shi et al. 2019). Phytochelatins are low-molecular-weight molecules synthesized from glutathione and catalyzed by the enzyme phytochelatin synthase (PC synthase). These molecules act on cellular homeostasis and PTE detoxification, as they have a high capacity to bind to various metals, including Cd, Cu, Zn, and As through sulfhydryl and carboxyl residues (Inouhe 2005). Phytochelatins are synthesized in the cytosol and, after binding to PTE, are actively transported as high-molecular-weight metal-phytochelatin complexes to the plant cell vacuole, their final destination.

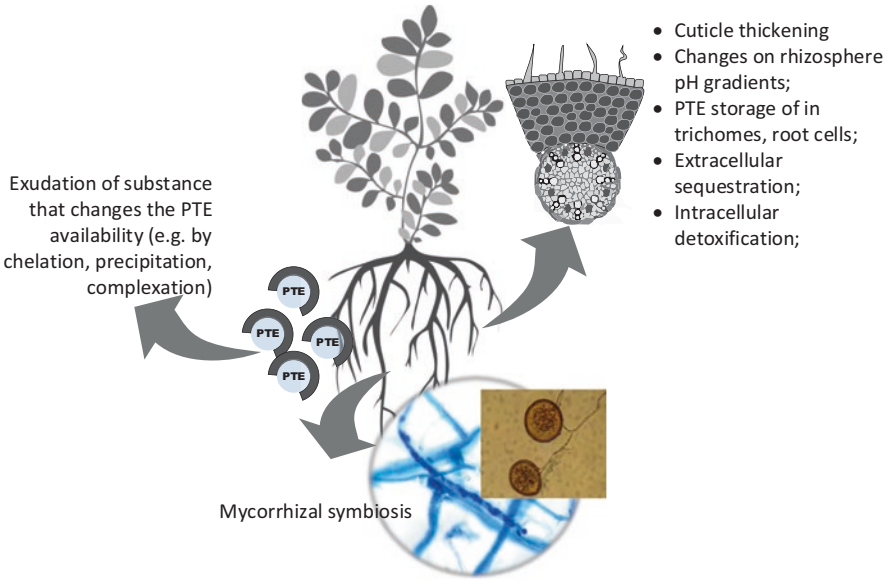


Fig. 2.3 Plant defense strategies that performance as physical barriers to prevent or reduce the uptake of potentially toxic metals (PTE)

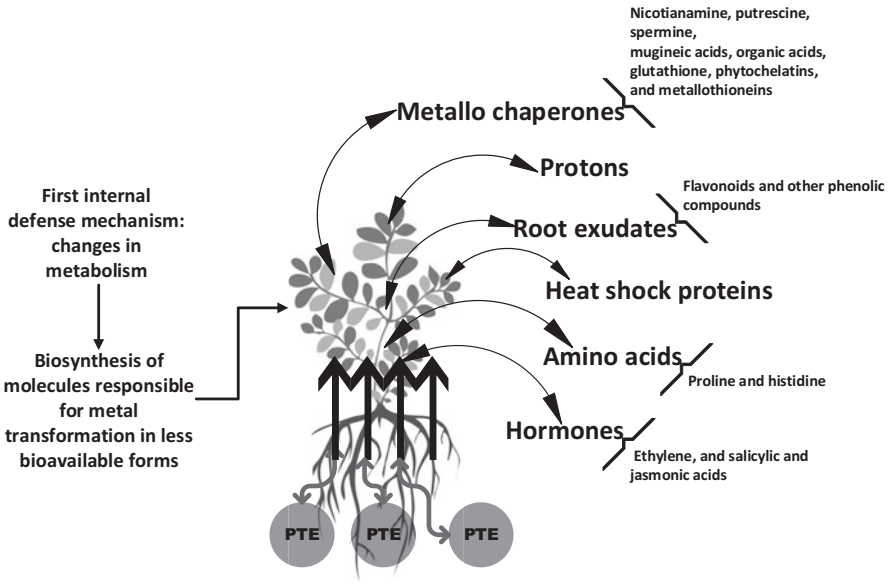


Fig. 2.4 Plant defense strategies under stress after the uptake of potentially toxic metals (PTE)

Phytochelatin synthesis occurs in both roots and shoots but tends to accumulate in roots. Thus, upregulation of phytochelatins is a relevant strategy to reduce PTE translocation to plant shoots (Emamverdian et al. 2015; Garg and Bhandari 2014). In turn, metallothioneins make up a family of cysteine-rich low-molecular-weight cytoplasmic metal-binding proteins produced by a wide variety of eukaryotic organisms, including fungi and plants, as well as some prokaryotes. Unlike phytochelatins (enzymatically synthesized peptide product), metallothioneins are synthesized from mRNA translation. In addition, they have affinity for a wider range of PTE. In plants, these molecules act to mitigate PTE toxicity through cell sequestration, cellular homeostasis, metal transport adjustment, ROS scavenging, cell proliferation, and restoration of the plasma membrane and damaged DNA (Emamverdian et al. 2015; Garg and Bhandari 2014). Proline, another plant metabolite, is an amino acid that acts as a compatible metabolic osmolyte, free-radical scavenger, antioxidant, and macromolecule stabilizer. Increased proline cell levels are a characteristic response of higher plants under biotic and abiotic stress conditions (e.g., PTE). This amino acid can act as an osmoregulator or osmoprotectant as well as stimulating antioxidant enzyme activities, preserving cellular redox homeostasis, restructuring the chlorophyll molecule, and stabilizing intracellular pH (Emamverdian et al. 2015).

These mechanisms are not always able to mitigate the negative effects of toxic elements on plant metabolism. Thus, there is an excessive increase in the production and accumulation of reactive oxygen species (ROS) in the cell, generating a series of physiological and molecular damages, briefly reported in Sect. 2.7.5. Thus, the ROS increase in the cell triggers the antioxidant defense mechanism of plants that promote the upregulation of enzymatic and nonenzymatic components (Fig. 2.5).

Enzymatic components mainly include the enzymes superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GPX), and glutathione reductase (GR). Briefly, SOD catalyzes the conversion of the highly reactive superoxide radical to H_2O_2, which is removed by the CAT, APX, and GPX

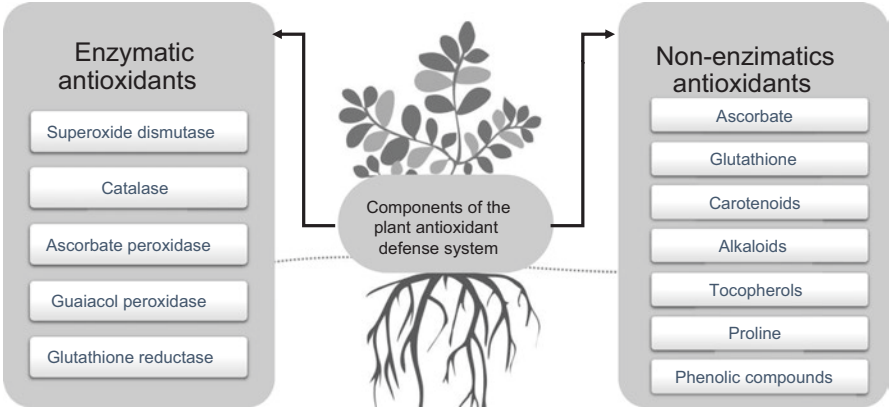


Fig. 2.5 Enzymatic and nonenzymatic components of the plant antioxidant defense system responsible for scavenging reactive oxygen species from cells

activity (Gomes et al. 2014a; Nath et al. 2016, 2017; Kapoor et al. 2019). Activity of these oxidative enzymes associated with the action of phytochelatins may present an important synergy in the defense of plants subjected to PTE high concentrations (Emamverdian et al. 2015). In nonenzymatic components, there are molecules such as ascorbate, glutathione, carotenoids, alkaloids, tocopherols, proline, and phenolic compounds (e.g., flavonoids) which constitute acting as free-radical scavengers, chelators, and/or antioxidants. Upregulation of these compounds varies by PTE type, contamination level, plant species, plant species tolerance to PTE, and plant developmental stage (Perveen et al. 2015; Emamverdian et al. 2015; Garg and Bhandari 2014; Gratão et al. 2005).

2.5 Remediation Alternatives for Contaminated Soils by Potentially Toxic Elements (PTE)

Given the extent of soil contamination sources and the risk of these PTE to human and animal health, it is necessary to remediate contaminated sites (Coninx et al. 2017). Restoration of PTE-contaminated areas can be made possible through the implementation of remediation techniques. These techniques encompass a set of practices designed to mitigate or suppress the impacts caused by contaminants (Cabral et al. 2015).

Bioremediation and phytoremediation are efficient techniques for environmental restoration, as they optimize existing natural resources (microorganisms and plants) and biological processes (Cabral et al. 2015). Bioremediation processes consist of degrading soil contaminants using efficient species of microorganisms. Bioremediation (from Greek "bios," life, and Latin "remedium," clean or restore) includes in situ (performed at the contaminated site) and ex situ (with physical removal of contaminated material off-site) techniques. Microorganisms used in these processes release enzymes that degrade toxic compounds, such as industrial waste, oils, and pesticides, reducing their toxicity. Importantly, although PTE cannot be decomposed, many microorganisms are able to alter their availability through changes in oxidation state, chelation processes, complexation, etc. (Arya et al. 2018; Chibuike 2013). Phytoremediation (from the Greek "phyto" meaning plant, and the Latin "remedium" meaning clean or restore) refers to a diverse set of technologies based on the use of natural or genetically modified plants to mitigate or clear soil and water contamination (Prasad and Freitas 2003; Koch et al. 2021). This set of techniques includes (Cabral et al. 2015; Göhre and Paszkowski 2006; Coninx et al. 2017; Chibuike 2013; Hassan et al. 2017; Meier et al. 2012):

1. *Phytoextraction* includes the uptake, translocation, and accumulation processes of contaminants by plants in their aboveground biomass. This technique is often used in areas contaminated by PTE.
2. *Phytodegradation* refers to the degradation/decomposition of organic contaminants (e.g., herbicides, insecticides, chlorinated solvents) through internal and

external metabolic processes to plant tissue, however, always driven by the plant. However, this process does not apply to PTE as they are not biodegradable.

3. *Rhizodegradation* promotes the organic contaminants decomposition by microorganisms present in the rhizosphere. Plant roots release exudates that stimulate microbial activity and, consequently, accelerate the pollutant degradation. In addition, plants also release enzymes that have the ability to degrade soil organic pollutants.
4. *Phytofiltration* is the removal of contaminants from soil or wastewater by plants. In this technique, PTE uptake or adsorption and its movement in groundwater are minimized.
5. *Phytostabilization* includes the reduction of PTE bioavailable forms and subsequent immobilization by microorganisms and plants at the contaminated site, either by sorption, precipitation, or chemical complexation processes.
6. *Phytovolatilization* is the transformation of contaminants into volatiles and consequent release to the atmosphere. This technique is widely used in soils contaminated with organic pollutants and has restricted application to PTE.

Unlike organic pollutants, PTE are nonbiodegradable and persistent elements, and therefore, low-cost phytoremediation techniques used for the organic pollutant remediation (e.g., phytodegradation and rhizodegradation) are not applicable to PTE-contaminated soils. Phytovolatilization techniques, for example, are applicable for a small amount of PTE (e.g., mercury and selenium) (Coninx et al. 2017; Emamverdian et al. 2015; Prasad and Freitas 2003). Among these techniques, phytostabilization and phytoextraction are the most studied PTE phytoremediation processes (Cabral et al. 2015; Chibuike 2013). In phytoextraction, plants, usually hyperaccumulating species, uptake large amounts of soil contaminants, storing them in their shoots. On the other hand, in phytostabilization, the contaminants are not extracted from the soil; however, they are immobilized, especially in the roots. As a result, the translocation of contaminants to the shoots is reduced, which reduces the pollutant toxicity (Cabral et al. 2015). Since both of these processes require plant cultivation, their survival in PTE-contaminated soils is critical to successful phytoremediation (Coninx et al. 2017). In this perspective, considering the ubiquitous occurrence of arbuscular mycorrhizal fungi and, consequently, mycorrhizal symbiosis formation and its benefits in various ecosystems types, it is remarkable that these microorganisms can be used in phytoremediation processes. In addition, along with phytostabilization, exploitation of mycorrhizal symbiosis increases the success chance, since arbuscular mycorrhizal fungi also stabilize contaminants in their biomass, reducing their translocation to the aerial part of plants (Cabral et al. 2015). The potential of arbuscular mycorrhizal fungi for PTE remediation will be explored in the following topics.

2.6 What Are Arbuscular Mycorrhizal Fungi (AMF) and Mycorrhizal Symbiosis?

Arbuscular mycorrhizal fungi (AMF) are obligate ubiquitous biotrophic microorganisms that establish the symbiosis called mycorrhizae with most superior plants (Delavaux et al. 2017; Dubchak 2017; Stürmer et al. 2018). These fungi thus constitute important soil microbiome communities. AMF omnipresence is verified even in degraded ecosystems, and much research data confirms that these microorganisms can reduce the PTE toxicity in host plants (Cabral et al. 2015; Coninx et al. 2017). AMF are probably determining factors in the structure and function of plant communities. These fungi are often predominant in soil microbial biomass and therefore occupy a prominent position at the soil-plant interface (Van der Heijden et al. 1998a, b, 2008; Wagg et al. 2014).

The main ecosystem service provided by these fungi is their role as extension of plant roots. This is because after intracellular colonization, AMF develop an abundant extraradicular mycelium, which uptakes nutrients and water into the host plant in exchange for photoassimilates. Such a benefit is widely known as the biofertilizer function (Giri et al. 2019). Opportunely, AMF promote non-nutritional effects on plants that may be as important as nutritional benefits. Thus, mycorrhizal symbiosis can increase water uptake, alter plant metabolism, and improve soil aggregation, plant defense mechanisms, and tolerance to biotic and abiotic stresses. The non-nutritional benefits of mycorrhizal symbiosis may not be independent of the others. Generally, these benefits derive from the AMF nutritional action on plants. Thus, better-nourished plants tend to produce more defense compounds or have greater tolerance to biotic and abiotic stresses (Delavaux et al. 2017).

In the current classification, AMF belong to the phylum *Glomeromycota* (Schüβler et al. 2001) and have about 222 species described so far (Stürmer et al. 2018). AMF diversity species in contaminated areas closely matches the abundance of plant species present. In addition, the AMF isolate tends to determine which plant species can be selected for application and, therefore, the success of phytostabilization strongly depends on the selection of AMF isolated. On the other hand, it is important to highlight that the AMF species richness in the environment varies in terms of soil attributes, contamination level, and type of available PTE, which makes it difficult to directly relate the proportion of plants and the AMF species abundance in PTE-contaminated areas (Coninx et al. 2017; Van der Heijden et al. 1998a, b, 2008; Wagg et al. 2014).

Many studies point out that plants that colonize PTE-contaminated soils possibly have great survival advantage if mycorrhized. This behavior is evident from the observation that non-mycotrophic species can develop symbolic mycorrhizal when growing in contaminated soil (Coninx et al. 2017). In this context, we present in the next topic the numerous benefits of mycorrhizal symbiosis under PTE toxicity.

2.7 Benefits of Mycorrhizal Association in Mitigating the Toxicity of Potentially Toxic Elements (PTE)

AMF can mitigate the PTE toxicity in plants through several direct and indirect mechanisms (Fig. 2.6). In general, such mechanisms favor mainly the processes of phytostabilization and/or phytoextraction of PTE (Cabral et al. 2015; Coninx et al. 2017; Perveen et al. 2015; Kapoor et al. 2013; Meier et al. 2012; Kaur and Garg 2018; Krishnamoorthy et al. 2019).

2.7.1 Physical Barrier Promoted by Arbuscular Mycorrhizal Fungi on Plants in Soil Contaminated by Potentially Toxic Elements

The first mechanism of AMF defense to plants is to act as a physical barrier, by means of exclusion mechanisms, to reduce the PTE uptake in high concentrations (Fig. 2.6). In this sense, AMF may release chelating substances (metallothionein, siderophores, glomalin, oxalic acid, formic acid, malic acid, and succinic acid) to

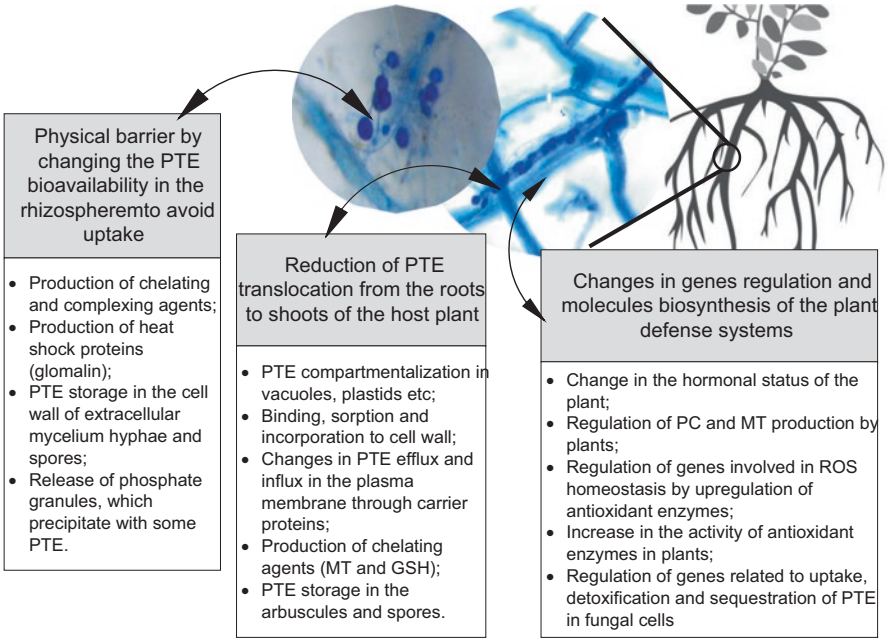


Fig. 2.6 Possible mechanisms of arbuscular mycorrhizal fungi to mitigate the toxicity of potentially toxic metals to plants and to promote phytoremediation

reduce the bioavailability of these elements in soil. Chelation is a primary defense mechanism of AMF under PTE toxicity. The efficiency of this mechanism varies depending on the PTE type and AMF isolate. Under these conditions, various organic molecules excreted by the fungi are capable of chelating metals, reducing their uptake by the host plant. There is also the release of polyphosphate granules that promote the PTE exclusion by precipitation (Coninx et al. 2017; Bhandari and Garg 2017; Garg and Bhandari 2014; Kaur and Garg 2017).

Glomalin role in the PTE retention is an important strategy to reduce the uptake of these elements by plants or to mitigate toxic effects (Garg and Bhandari 2014). Glomalin is possibly the highly conserved heat shock proteins. Its production may be induced by several factors, including abiotic stress such as excess PTM (Kaur and Garg 2018; Gadkar and Rillig 2006). Purin and Rillig (Purin and Rillig 2007) suggest that glomalin has (or had) primary cellular function, related to the properties of heat shock proteins, such as chaperonins (stress signalers). Subsequently, glomalin acquired function over the extraradical mycelium, reducing its palatability against microarthropods and saprophytic fungi to reduce predation. Secondary to the primary physiological functions, glomalin would act in the soil aggregation process due to its high persistence in the environment. It is a fact that glomalin promotes the sequestration of PTE in contaminated soils. Jia et al. (2016) reported this behavior in the rhizosphere of *Robinia pseudoacacia*, a tree legume, grown in Cd- and Pb-contaminated soil. This research showed that as contamination increased, total glomalin-related soil protein (T-GRSP) contents and easily extractable GRSP (EE-GRSP) increased proportionally. Moreover, over time, there was an increase in the amount of GRSP-bound Pb and Cd, reducing the concentration of DTPA-Pb and DTPA-Cd in the soil. An interesting observed fact was the higher GRSP-bound Pb/total ratio compared to GRSP-bound Cd/total, which suggests a higher affinity of Pb with glomalin molecules than Cd. Another study in a sand culture system with a clover plant, increasing Pb concentrations increased the immunoreactive glomalin (IR) and Bradford-reactive glomalin content. At the highest Pb dose (450 μM), IR amount increased more than twofold compared to the control. Apparently, increasing Pb doses induce protein upregulation, reinforcing the AMF protection mechanism to plants under these conditions. In addition, there was significant Pb sequestration in the glomalin fractions. In hyphal compartment, glomalin Pb sequestration reached 665.72 mg Pb mg^{-1} glomalin at the highest dose (450 μM) (Malekzadeh et al. 2016a). Fungal hyphae are the major site of glomalin-related gene expression (Gadkar and Rillig 2006). Many other studies have evaluated the involvement of glomalin in the PTE sequestration and retention in contaminated soils (Jia et al. 2016; Malekzadeh et al. 2016a, b; Wu et al. 2014; Gil-Cardeza et al. 2014; Jia et al. 2018; Singh 2015; Siani et al. 2017).

2.7.2 Mechanisms to Reduce the Potentially Toxic Elements Translocation in Mycorrhizal Plants

Reducing PTE translocation from roots to the shoots of mycorrhizal plants is another efficient mechanism for mitigating the harmful effects of toxicity and includes some PTE exclusion mechanisms (precipitation, chelation, compartmentalization in organelles). For this, AMF have capacity to store PTE in their biomass, which reduces the transfer of these elements to the shoots. Storage of these elements may occur by binding to amino acids, carboxyl and hydroxyl groups, chitin, and negatively charged phosphates on the hyphae cell wall. In addition, these fungi can compartmentalize PTE in their vacuoles, plastids, and other membrane-rich organelles in both hyphae and arbuscules and spores (Cornejo et al. 2013).

Another outstanding AMF mechanism to reduce soil PTE availability is the release of heat shock protein and glutathione to promote precipitation or chelation of PTE and formation of metal-phosphate complexes within hyphae (Emamverdian et al. 2015). In addition, AMF activity can alter soil pH, causing metal immobilization in the mycorrhizospheric region (Kapoor et al. 2013; Kaur and Garg 2018; Bano and Ashfaq 2013). Change in soil pH and consequent change in the Cd and Pb availability were observed by Zhan et al. (2019) by inoculating separately *Funneliformis mosseae* and *Diversispora spurcum* in bermudagrass. Soil pH ranged from 5.4 (uninoculated treatment) to 5.8 with *D. spurcum* inoculation; however, both AMF species were able to significantly reduce soil acidity. As a result, there was a highlighted reduction in the Pb and Cd content available in the soil.

Finally, especially in cases of high toxicity and considerable PTE uptake and translocation, AMF promote mechanisms that help plants mitigate the deleterious effects of high PTE concentrations on plant biomass (Fig. 2.6). Under these conditions, one of the simplest mechanisms refers to the favoring of plant growth through symbiosis, which results in a dilution effect of PTE on plant biomass. Thus, AMF exerts an antagonistic effect on PTE, as they promote rapid plant growth, which is not accompanied by an increase in PTE accumulation in plant tissues, resulting in a reduction in the concentration of these elements, characterizing the dilution effect (Marschner 2012). This occurs mainly through the AMF biofertilizer function, as it ensures nutrient uptake and thus stimulates plant growth (Shi et al. 2019; Kaur and Garg 2018) as noted by Gomes et al. (2014b). These authors verified that plants of *Anadenanthera peregrina* inoculated with *Acaulospora scrobiculata* had higher leaf P content and, consequently, higher growth in As-contaminated soil (539.33 mg As kg^{-1}). The dilution effect also occurred in maize submitted to Cd levels (1 and 5 mg kg^{-1}), where the individual inoculation of *Rhizophagus intraradices* and *Glomus versiforme* promoted increase in shoot and root dry weight; however, there was a drastic reduction in the Cd concentration in plant tissues (Zhang et al. 2019). In *Robinia pseudoacacia*, inoculation with *Funneliformis mosseae* and *Rhizophagus intraradices* increased growth to the detriment of Pb concentration, especially in leaves (Yang et al. 2015). PTE dilution effect on plant biomass caused in

mycorrhizal plants is frequently reported (Hu et al. 2013; Zhang et al. 2015; Wang et al. 2017; Zhipeng et al. 2016).

Many studies show that not only the nutritional but also water status of mycorrhizal plants is higher to those not colonized, even in soils with high PTE concentrations. Biofertilizing action along with the increase in water uptake occurs through extraradical mycelium from the AMF that reach places beyond the root zone. Another benefit is that better-nourished plants can alter the biosynthesis of various metabolites (amino acids, hormones, antioxidant defense components) to withstand high levels of PTE contamination (Shi et al. 2019).

The challenge of using these benefits is in the fact that the effect of mycorrhizal symbiosis on plant growth in contaminated locations varies with a large number of factors, which makes the interpretation of research results quite complicated and often with inconclusive results. Thus, the interpretation of the benefits of mycorrhizal symbiosis under these conditions must consider the chemical and physical attributes of contaminated soil; quality, PTE quantity and availability, level of mycorrhizal dependence on plant species, and plant and fungal ecotypes tolerance to PTE (Takács 2012).

2.7.3 Arbuscular Mycorrhizal Fungi Mechanisms to Exclude or Alter the Availability of Potentially Toxic Elements and Metabolic Alteration of Colonized Plants

Analyzing the relationship between PTE uptake and mycorrhizal plant growth parameters in contaminated soils, research points to two more recurrent response patterns: first, increased PTE uptake via mycorrhizosphere under conditions of low PTE concentrations and, second, reduced PTE uptake at high concentrations due to decreased PTE bioavailability through specific fungal protection mechanisms or toxicity mitigation (Takács 2012). In reducing uptake, PTE immobilization in the fungal mycelium is the main protection mechanism to plants in contaminated soils. Molecules such as free amino acids, hydroxyl and carboxyl groups of the AMF, and chaperone cell walls (heat shock proteins, e.g., glomalin) act as PTE binding points. In corn plants under Cd (1 and 5 mg kg^{-1}) addition, AMF inoculation not only reduced Cd concentration in the shoot and roots but also changed the storage pattern of this PTE in the plant cell. In this case, the proportion of accumulated Cd in the cell wall and soluble fractions (vacuoles) of the cell increased along with increasing Cd doses. This behavior was even more significant in the roots. On the other hand, there was a reduction in the accumulated Cd fraction in cell organelles (Zhang et al. 2019). Cd compartmentalization in the vacuoles and cell wall and simultaneous reduction in accumulated organelle fractions show that AMF inoculation assists the plant in its toxicity defense strategies and preserves cellular processes, especially those occurring in organelles.

AMF's role in altering the PTE available forms in soil may also contribute to the reduction of uptake of these elements under high toxicity. Moshiri et al. (2019) verified this behavior in Pb- and Zn-contaminated soil (DTPA extractable Zn 8.66 mg kg^{-1}; total Zn 126 mg kg^{-1}; DTPA extractable Pb 3.12 mg kg^{-1}; total Pb 34 mg kg^{-1}). Under these conditions, AMF inoculation in alfalfa increased the less labile fractions of these elements in the soil (organic and oxide-linked fractions) to the disadvantage of the more labile fractions (exchangeable and readily available fractions), elucidating one of the factors that may contribute to the reduction. PTE uptake by plants in contaminated soil (Moshiri et al. 2019). Similar behavior showed the *Rhizophagus intraradices* inoculation in rice cultivated in Cd-spiked soil (10 mg k^{-1}). Compared to uninoculated treatment, this AMF species reduced the extractable Cd content of the soil while reducing the Cd concentration in the shoots and roots (Chen et al. 2019), demonstrating that the concentration reduction of this PTE in the soil did not occur by the increase in the uptake by the plants, but, probably, by the AMF participation in the alteration of the soil Cd fractions.

Changes in PTE fractions within the plant cell are also influenced by the AMF action. Zhang et al. (2019) verified that the *Rhizophagus intraradices* and *Glomus versiforme* inoculation in maize with Cd addition decreased the proportions of inorganic Cd form (extracted with 80% ethanol, FE) and organic water-soluble form (more toxic fractions). On the other hand, the pectates and proteins-integrated Cd fraction increased prominently, which may be responsible for the adaptation of the plant to the stress caused by Cd. The latter fraction refers to Cd bound to pectates and proteins such as phytochelatins, which complex Cd to phytochelatin-Cd complexes which are subsequently compartmentalized in vacuoles. This was corroborated by the increase in Cd content in the soluble fraction (mainly vacuoles) of plant cells in inoculated plants, demonstrating the importance of AMF in reducing the toxicity of this PTE. Similarly, Li et al. (Li et al. 2016) found that rice inoculated with *Rhizophagus intraradices* and *Funnelliformis mosseae* (inoculated separately) reduced the active and more toxic Cd proportions, contributing to higher tolerance of plants to the addition of Cd (0.05 and 0.1 mM).

Importantly, there are also reports of no response (Cui et al. 2019) or negative response of mycorrhizal symbiosis on plant development in PTE-contaminated soils (Aguirre et al. 2018). Interestingly, plant colonization by AMF can increase PTE uptake; however, in many cases, even with increased PTE uptake, mycorrhized plants may show higher growth, suggesting the presence of detoxifying mechanisms from molecular processes and/or biochemicals as yet unknown (Cicatelli et al. 2014). Mycorrhizal colonization affects the PTE biogeochemical fractions and their accumulation in plant tissues, and this varies as to the level of soil contamination and the nature of PTE. Moshiri et al. (2019) found that AMF inoculation in alfalfa altered the chemical behavior of Zn and Pb, notably the mobility of these PTE in the soil. In highly contaminated soil (DTPA extractable Zn 89.6 mg kg^{-1}; total Zn 258.1 mg kg^{-1}; DTPA extractable Pb 9.9 mg kg^{-1}; total Pb 91.6 mg kg^{-1}), mycorrhiza increased the Zn- and Pb-labile forms (exchangeable and readily available fractions – Ca $(NO_3)_2$) to the detriment of less labile forms (oxide-bound and

organic fractions). These changes in soil PTE lability may also explain the increased uptake of these elements by mycorrhizal plants.

In addition to all primary and external mechanisms of defense of AMF to plants under stress, these fungi also act as mechanisms of PTE exclusion in internal processes. In fungal cell cytosol, for example, there may be the production of molecules that promote PTE chelation to reduce their deleterious action on plant metabolism. Among the intracellular chelators, we highlight metallothionein, glutathione, polyamines, amino acids, and chaperones (some of them are heat shock proteins). Once chelated, these metal complexes can also be transported. PTE compartmentalization mechanism in fungal structures (Fig. 2.6) includes the action of carrier proteins present in the plasma membrane of AMF. These metal carrier proteins can alleviate toxicity by transporting metals out of the cell or into intracellular compartments. In addition, metal uptake through specific transport systems located on the AMF plasma membrane can be downregulated under conditions of high PTE contamination (Coninx et al. 2017).

Exposed to excess PTE, plants synthesize various low-molecular-weight metabolites in the cytoplasm, together referred to as compatible solutes. This set of molecules includes specific amino acids (proline, total soluble sugars, glycine betaine, trehalose, sorbitol, etc.) that have the osmoprotective function, inhibit lipid peroxidation, stabilize proteins, and sequester free radicals (Garg and Bhandari 2014; Kaur and Garg 2018). Proteomic analysis performed on leaves of *Populus alba* showed that *Glomus intraradices* inoculation largely altered proteins belonging to the functional groups "photosynthesis and carbon fixation" and "sugar metabolism." In this research, after 4 months of clone transplantation into contaminated soil, a significant number of proteins were involved in the protein folding functional group. Sixteen months after transplantation, there was a greater representation of protein groups linked to "oxidative damage" and "glutathione metabolism" to the detriment of previous groups (Lingua et al. 2012). Mycorrhizal symbiosis can stimulate plant production of these molecules by favoring plant defense systems (Cabral et al. 2015). In corn plants under Cu addition (500 μg g^{-1}), *Rhizophagus irregularis* inoculation increased the phytochelatin concentration in leaves in the Cu-sensitive cultivar (Merlos et al. 2016). Increased phytochelatin content by AMF inoculation was reported in *Lonicera japonica* plants inoculated with *Glomus versiforme and Rhizophagus intraradices*, even at the highest dose of Cd – 20 μg g^{-1} (Jiang et al. 2016a).

Metallothioneins, low-molecular-weight proteins, are synthesized by numerous organisms, including plants and AMF (Cabral et al. 2015). These proteins are encoded by a multigene family, which appear to be regulated according to the organ and development stage and in response to various stimuli, including PTE contamination (Cicatelli et al. 2014). Metallothioneins play a complex role in both PTE homeostasis and protection against oxidative stress of organisms under toxic conditions (Cabral et al. 2015; Cicatelli et al. 2014). It is important to mention that AMF inoculation can regulate the metallothionein gene expression in plants and thus favor the survival of these plants under toxicity conditions. This behavior was studied in *Festuca arundinacea* plants under Ni (30, 90, and 180 mg kg^{-1}) and

Funneliformis mosseae inoculation. In these growing conditions, compared to uninoculated plants, inoculated plants upregulated the MET gene in the roots at all Ni levels and in the shoot from 90 mg kg^{-1} (Shabani et al. 2016). Modulation of metallothionein gene transcription levels in *Populus alba* inoculated with *Glomus mosseae* or *Glomus intraradices* showed that after 16 months of growth in PTE-contaminated soil, there was upregulation of the PAMT1, PAMT2, and PAMT3 genes (in both isoforms, a and b) related to metallothionein (Cicatelli et al. 2014).

Polyamines, another important intracellular chelator, are organic polycations considered to be plant growth regulators. Putrescine (Put), spermidine (Spd), and spermine (Spm) are the most abundant polyamines in plants and occur in both free and conjugated forms. Since these molecules act on cell growth and proliferation and protein and nucleic acid synthesis, their biosynthesis is essential for normal growth and development of eukaryotic organisms (Cicatelli et al. 2014). Fortunately, mycorrhizal symbiosis can stimulate plant production of these chelators in PTE-contaminated environments. Polyamines can protect against PTE contamination by both their antioxidant activity and metal chelation. Inoculation of *Populus alba* with *Glomus mosseae* or *Glomus intraradices* upregulated PaSPDS1 and PaSPD2 gene expression in Zn and Cu contaminated soil. As a result, free spermidine levels were higher in the presence than in the absence of AMF and correlated with better plant growth. In addition, conjugated spermidine and spermine levels also expanded significantly in plants inoculated with *G. intraradices* relative to control (Cicatelli et al. 2010).

2.7.4 Changes in Gene Expression in Arbuscular Mycorrhizal Fungi and Plants (Stimulated by Mycorrhization)

Advances in molecular techniques have allowed for more in-depth studies of the PTE effects on AMF gene expression and their effect on plant gene expression. AMF can mediate the up- and downregulation of specific genes as well as different chemical components (Cabral et al. 2015), in both plant and fungal cells. AMF can favor plant defense through non-nutritive mechanisms, for example, by regulating the expression of specific plant metabolism genes, resulting in induced tolerance that plants can develop in response to AMF colonization (Delavaux et al. 2017). Large-scale molecular analyses reinforce that mycorrhizal symbiosis determines important changes at the transcriptional/translational levels. These changes may help clarify the reasons for the increased growth of mycorrhizal plants under PTE toxicity (Cicatelli et al. 2014).

Some studies indicate that AMF inoculation strongly modifies transcriptome in plants subjected to PTE toxicity, partially restoring it to the control profile (Cicatelli et al. 2014). In a transcriptome analysis of *Populus alba* leaves subjected to PTE contamination, Cicatelli et al. (2014) found that AMF inoculation strongly modified the transcriptome of plants grown in contaminated soil. PTE contamination did not

affect the regulation of most genes evaluated. However, inoculation with *Glomus mosseae* and *Glomus intraradices* upregulated defense gene groups (thaumatin, glutathione synthase, and metallothioneins) and genes related to primary metabolism and transcription. Moreover, observation of the gene expression pattern showed that the AMF species differed from each other, since *Glomus mosseae* showed greater activation capacity of the plant defense system. Additionally, colonized plants obtained higher PTE concentration in the biomass; however, they also achieved higher growth and negative regulation of most antioxidant genes, suggesting a higher degree of protection in these plants and a lower need for the activation of the antioxidant pathways involved in ROS scavenging.

In addition to changes in plant gene expression at toxic PTE levels, AMF show changes in their own gene expression to overcome stress. Benabdellah et al. (2009a, b) identified the first glomeromycotan dithiol glutaredoxin gene (GintGRX1) from the *Glomus intraradices*. Cu induced the ROS accumulation in the extraradicular mycelium of *Glomus intraradices* and further upregulated GintGRX1 transcription in the fungus. This gene encodes a multifunctional protein with oxidoreductase, peroxidase, and glutathione S-transferase activity, suggesting the role of the GintGRX1 gene in protecting the fungus against oxidative damage induced directly by superoxide anions or indirectly by copper (Benabdellah et al. 2009a). Additionally, Benabdellah et al. (2009b) have shown that GintGRX1 gene encodes the PDX protein involved in vitamin B6 biosynthesis. PDX 1 and PDX2 are proteins involved in the alternative pathway of vitamin B6 biosynthesis. Importantly, this vitamin has recently been implicated in the defense against cellular oxidative stress.

2.7.5 Changes in Antioxidant Defense System of Mycorrhizal Plants

PTE have the ability to interact with various cellular biomolecules, such as nuclear proteins and DNA, leading to excessive increase in reactive oxygen species (ROS). Due to the induction of oxidative stress caused by PTE excess, AMF can stimulate antioxidant enzyme activity and also trigger the upregulation of enzymatic and non-enzymatic antioxidants to protect the plant cell from ROS (Emamverdian et al. 2015; Shi et al. 2019).

Higher concentrations of enzymatic antioxidants (Fig. 2.5) are often found in mycorrhizal plants compared to control plants (Coninx et al. 2017; Garg and Bhandari 2014; Shi et al. 2019; Abdelhameed and Metwally 2019). This was observed in fenugreek (*Trigonella foenumgraecum* L.) plants submitted to Cd application (2.25 and 6.25 mM), where the inoculation with *Glomus monosporum, G. clarum, Gigaspora nigra,* and *Acaulospora laevis* mixture significantly increased the activity of the enzymes superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) (Abdelhameed and Metwally 2019). The stimulus for the enzyme activity by AMF is important because of their functions in the antioxidant defense

system of plants, as they act to suppress or prevent the formation of free radicals or reactive species in cells. SOD, POD, and CAT are the first-line defense antioxidants since they neutralize free-radical precursor molecules or the free radicals themselves capable of inducing the production of other harmful molecules (Ighodaro and Akinloye, 2018). SOD, for example, catalyzes the conversion of superoxide anion (O_2 • -) to oxygen and hydrogen peroxide (H_2O_2). Superoxide anion is a byproduct of many metabolic processes, including mitochondrial respiration; however, its excessive accumulation in cells is deleterious. In addition to superoxide anion, SOD activity controls the levels of a variety of ROS and reactive nitrogen species (e.g., peroxynitrite – $ONOO^-$), restricting the toxic potential of these molecules (Wang et al. 2018). Already CAT, abundant in peroxisomes, transforms H_2O_2 into water and molecular oxygen. However, there is no catalase in mitochondria, so in this, organelle glutathione peroxidase (GPx) is responsible for decomposing to H_2O_2. The role and effectiveness of first-line defense antioxidants (SOD, CAT, and GPx) is therefore indispensable in any antioxidant defense strategy (Ighodaro and Akinloye 2018) and may be positively influenced by mycorrhizal symbiosis in several plant species (Sharma et al. 2017; Schneider et al. 2017; Jiang et al. 2016b; Spagnoletti et al. 2016; Sarathambal et al. 2017).

In addition to ROS accumulation in cells, high concentrations of PTE uptaked by plants cause malondialdehyde (MDA) accumulation, impairing plasma membrane stability (Jiang et al. 2016b; Hashem et al. 2016; Shahabivand et al. 2016). MDA generation is clearly related to the symptoms of plants stressed by the environment (Morales and Munné-Bosch 2019). MDA is a small reactive organic molecule that occurs omnipresent among eukaryotes. Formation of this molecule may be induced by lipoxygenase activity or ROS accumulation. As most MDA is derived from lipid peroxidation of polyunsaturated fatty acid in response to oxidative stress (via ROS and/or lipoxygenase), MDA content is widely used as an indicator of membrane damage in plants (Morales and Munné-Bosch 2019). Fenugreek (*Trigonella foenumgraecum* L.) plants inoculated with mix of *Glomus monosporum*, *G. clarum*, *Gigaspora nigra*, and *Acaulospora laevis* showed lower MDA concentration than uninoculated plants, both at 2.25 and 6.25 mM Cd (Abdelhameed and Metwally 2019). Similar results are reported in tomato under Cd (Jiang et al. 2016b; Hashem et al. 2016), *Lonicera japonica* – Cd (Jiang et al. 2016a), maize – Cu (Merlos et al. 2016), wheat – Cd (Shahabivand et al. 2016), *Phragmites australis* – Cd (Wang et al. 2017), and sunflower – Cd (Abd-Allah et al. 2015).

In the nonenzymatic antioxidant defense system (Fig. 2.5), AMF acts by increasing the concentration of these components in plant biomass (Spagnoletti et al. 2016; Spagnoletti and Lavado 2015). The addition of 1 mg Cd kg^{-1} in maize inoculated with *Rhizophagus intraradices* and *Glomus versiforme* did not change glutathione concentration in leaves and roots. However, the increase in Cd concentration (5 mg kg^{-1}) significantly enhanced the concentration of this antioxidant, especially in plants inoculated with *G. versiform* (Zhang et al. 2019). Glutathione is a low-molecular-weight water-soluble thiol compound, widely distributed in most plant tissues. Besides storing and transporting reduced sulfur, this metabolite can protect plant cells of excess PTE in three possible ways: (1) direct ROS scavenging (second

component with greater H_2O_2 neutralizing capacity), (2) PTE chelation/complexation, and (3) phytochelatin precursor (Noctor et al. 2012; Hasanuzzaman et al. 2017), which in turn are capable of chelating PTE and reducing its deleterious action on plant cells. The increase in phytochelatin concentration in maize under Cd levels (1 and 5 mg kg^{-1}) was directly proportional to the increase of glutathione content, especially in plants inoculated with *G. versiforme* (Zhang et al. 2019). Thus, we can see the wide range of processes beneficially influenced by AMF, protecting the plant against excess PTE.

Ascorbate concentration in plants under PTE toxicity is also favored by AMF inoculation (Jiang et al. 2016a; Hristozkova et al. 2016). This antioxidant is very abundant and occurs in all plant tissues, especially those rich in photosynthetic cells and meristems. Its main role in plant defense refers to the elimination of free radicals $O_2^{\bullet -}$ and OH. The rise in ascobartum levels has already been reported for *Glomus versiforme* and *Rhizophagus intraradices* species in Cd-spiked soil (Jiang et al. 2016a) and *Glomus versiforme* and *Glomus mosseae* under Cs addition (Huang et al. 2016). Increase in leaf content by mycorrhizal inoculation is not verified only for the ascorbate. Inoculation with various AMF species was able to increase concentrations of polyphenols (Hristozkova et al. 2016; Ibiang et al. 2017), carotenoids (Sharma et al. 2017; Abd-Allah et al. 2015; Hristozkova et al. 2016), proline (Kaur and Garg 2017; Sharma et al. 2017; Hashem et al. 2016), tocopherols (Sharma et al. 2017), and flavonoids (Hristozkova et al. 2016).

Although numerous studies demonstrate the beneficial action of AMF to the antioxidant defense system of plants, there is a lack of studies that elucidate the pathways of AMF action in increasing nonenzymatic antioxidant concentrations as well as in the activity of antioxidant enzymes.

2.8 Phytoremediation Involving Arbuscular Mycorrhizal Fungi (AMF)

Filtration, electrochemical application, reverse osmosis, and chemical precipitation, among others, are some of the remediation techniques that make the process of recovering contaminated soil very expensive or harmful to the environment. In this sense, AMF benefits to promote plant growth and even enhance phytoremediation in contaminated areas make these microorganisms a lower-cost alternative and especially with no environmental impact, given that these microorganisms are native to many ecosystems, disturbed and undisturbed (Hassan et al. 2017).

However, for the phytoremediation process associated with AMF mycorrhizal symbiosis to be successful, several factors and some steps need to be considered and carefully planned (Fig. 2.7). Obtaining a detailed diagnosis of the contaminated area should be the first step in the process. This diagnosis should start with soil characterization (step 1), especially with the chemical, physical, and mineralogical attributes analysis, since these will directly influence the PTE bioavailability.

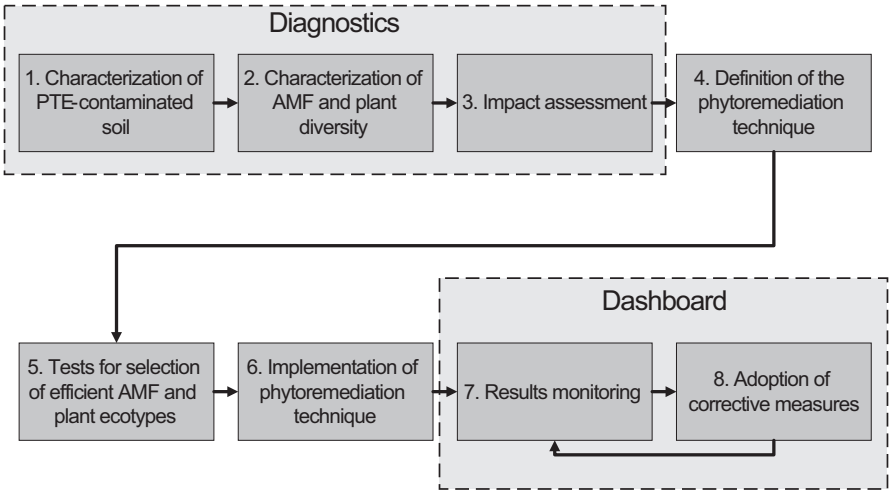


Fig. 2.7 Summary of the implementation steps of phytoremediation techniques using arbuscular mycorrhizal fungi species associated with plants

Together, identifying the contaminants present, it is possible to predict the intrinsic affinity of ions with soil adsorption surfaces (e.g., humus, silicate, and oxides clays) as well as the influence of more variable soil attributes (e.g., pH, redox potential, water content, temperature, biological activity, ionic strength) on PTE availability (Kabata-Pendias 2011). Identification of the contamination degree and extent as well as the origin and conditions of contamination formation is essential (Takács 2012).

In the second stage, the detailed survey of the contaminated area will point out the diversity of plant species as well as the AMF species that colonize the roots of these plants. These results will mainly lead to step 5 (Fig. 2.7). Generally, AMF ecotypes that develop in contaminated areas have greater PTE contamination tolerance. These ecotypes are assumed to evolve as contamination increases over time and gradually develop mechanisms to resist or tolerate PTE stress (Wei et al. 2015).

Risk assessment, step 3, is a useful tool to enable a rational and objective basis to assist in priority making and decision-making (Ferguson et al. 1998). There are many ecological risk assessment methodologies. This assessment may include collecting, organizing, and analyzing environmental data to estimate the contamination risks to ecosystems (Weeks and Comber 2005) exposed to PTE excess or a description or estimate of changes in populations or ecosystems. In the latter case, it may also be presented as an impact assessment rather than a risk assessment (Jensen et al. 2006). Following the US National Research Council (NRC) risk assessment process report, we can list four distinct phases for this step in contaminated soil: (1) identification of the present PTE that may cause harmful effects; (2) estimated dose-response relationship of PTE, i.e., quantitative relationship between plant exposure and adverse effects or epidemiological studies; (3) analysis of plant exposure to PTE by estimating the intensity, frequency, and duration of exposure these

elements; and (4) risk characterization through the interpretation of data collected in previous phases. In general, many countries have a similar framework for risk assessment procedures for contaminated areas. Thus, some important parameters are considered (Fig. 2.8) (Takács 2012; Ferguson et al. 1998).

Information and data obtained from the diagnostic steps enable the precise choice of phytoremediation technology as well as the definition of priorities (Fig. 2.7). The main phytoremediation techniques were discussed in Sect. 2.5, and in this chapter, we will emphasize those with the possibility of applying mycorrhizal symbiosis, especially phytostabilization and phytoextraction. Once the phytoremediation technique is defined, step 5 begins (Fig. 2.7), which consists of performing tests to select efficient host plants for the chosen technique and effective and infectious AMF strains for these plant species. This choice can be driven mainly by the data obtained in step 2. The botanical families Asteraceae, Brassicaceae, Caryophyllaceae, Cyperaceae, Cunoniaceae, Fabaceae, Flacourtiaceae, Lamiaceae, Poaceae, Violaceae, and Euphorbiaceae together contain over 500 hyperaccumulating plants species (Prasad and Freitas 2003; Krishnamoorthy et al. 2019). However, many hyperaccumulators species such as Brassicaceae, Juncaceae, Caryophyllaceae, Amaranthaceae, and Cyperaceae and some members of the Fabaceae family do not develop arbuscular mycorrhizae (Liu et al. 2015). This factor is extremely relevant and should be considered when choosing which species to associate with AMF in contaminated areas. Therefore, the choice of plant species for efficient methods of

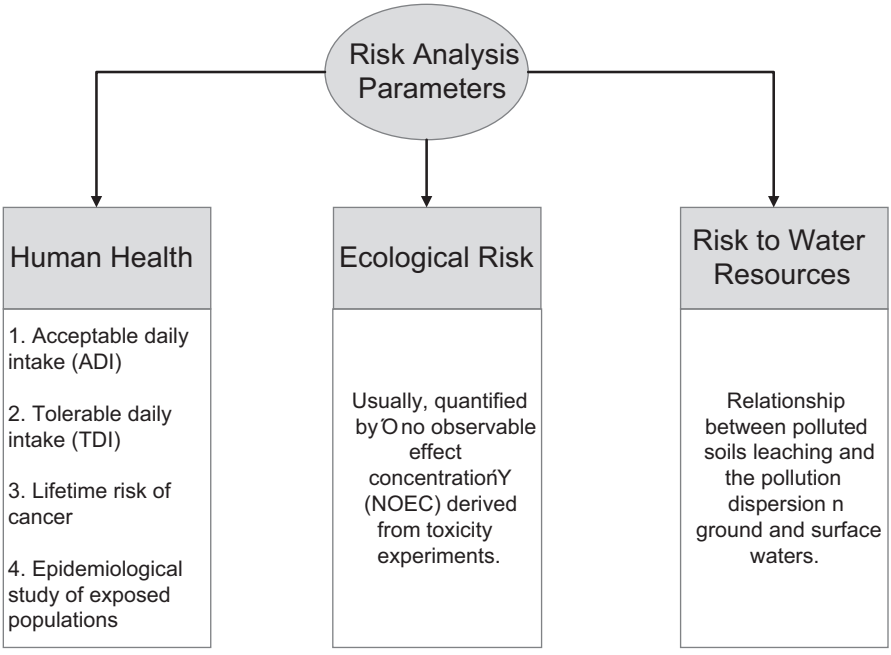


Fig. 2.8 Main parameters observed in risk analysis for the employment of remediation techniques of soils contaminated with potentially toxic elements

phytoremediation using AMF should prioritize species that have considerable mycorrhizal dependence. Takács (2012) highlights three plants categories that grow in PTE-contaminated soils:

1. Excluding plants (excluders): plants that uptake PTE by storing them in the roots to the detriment of the shoots
2. Indicator plants: plants that present PTE concentration in their organs directly proportional to the concentration found in the soil
3. Hyperaccumulator plants: plants in which PTE concentration in the roots exceeds 1000 mg kg^{-1} dry weight, a higher dose than the soil

From the choice of plant species, it is possible to select AMF species to be tested. The choice of the most appropriate species and ecotypes can start from the data collected in step 2. Indigenous AMF ecotypes from contaminated areas may be more efficient than others, as they have already developed considerable tolerance and adaptation to contamination (Wei et al. 2015). Furthermore, PTE contamination tends to suppress the most sensitive species. In Sb mining area in China, S contamination was the factor that predominantly influenced the AMF community (Wei et al. 2015). However, combining indigenous with exogenous AMF species may also be a successful option (Takács 2012). Nevertheless, many studies of diversity, infectivity, adaptation, and tolerance of AMF under PTE contamination show that these elements may retard fungal development but never completely suppress it (Lenoir et al. 2016; Wang 2017). Consequently, much research data attests to the ubiquitous presence of MFA in contaminated locations (Wei et al. 2014, 2015; Ban et al. 2017; Mikryukov et al. 2015; Buck et al. 2019; Sun et al. 2016). In a gold mining area in Brazil, Schneider et al. (2013) found that the *Glomus minutum*, *G. fasciculatum*, *Acaulospora spinosa*, *A. scrobiculata*, *A. tuberculata*, *Scutellospora biornata*, *Racocetra fulgida*, and *R. persica* were identified only in native areas without contamination, indicating that these species were more susceptible to contamination than *A. morrowiae*, *Paraglomus occultum*, and *Glomus clarum*, for example, which had the highest frequencies of occurrence in both native and contaminated areas. Paraglomeraceae and Glomeraceae species also had higher frequency of occurrence in Pb contaminated área (Faggioli et al. 2019). In Zn Foundry Unit, Lopes Leal et al. (2016) found a higher occurrence of *Glomus* sp. and *Paraglomus occultum*. Moreover, these authors showed that there was a reduction in the frequency of occurrence of *P. occultum* in the areas under rehabilitation. These data may indicate high adaptability of these species under these conditions, placing them as good options for remediation processes. In China, phylogenetic analysis of soil in the Sb mining area showed that most AMF species belonged to the genus *Glomus*. Although the frequent identification of *Glomus* in contaminated areas may represent the largest AMG group, it is still suggested that this genus has greater adaptability to stressful conditions than the others (Wei et al. 2015). In South Africa, Buck et al. (2019) identified substantial AMF species diversity in an inactive Au and U mining. These authors have identified species of the genera *Claroideoglomus* (2), *Diversispora* (4), *Scutellospora* (Swartjes et al. 2012), *Rhizophagus* (3), *Sclerocystis* (1), *Glomus* (1), *Cetraspora* (1), and *Redecker* (1). However, the highest occurrence was of the

species *Claroideoglomus lamellosum*, *C. etunicatum*, and *Diversispora celata*. In multicontaminated area with PTE (Cd, Zn, Cu, Pb, Cr, and Ni), Sidhoum and Fortas (2019) reported significant occurrence of the genera *Glomus* (1 spp.), *Funneliformis* (4 spp.), *Rhizoglomus* (3 spp.), and *Sclerocystis* (2 spp.). Besides, species of *Septoglomus* (1 sp.) *Acaulosporaceae*/*Acaulospora* (11 spp.) were present. These authors also highlighted the occurrence of Archaeosporaceae (*Archaeospora* 2 spp.), Claroideoglomeraceae (*Claroideoglomus* 2 sp.), Diversisporaceae (*Diversispora* 1 spp. and *Tricispora* 1 sp.), Paraglomeraceae (*Paraglomus* 2 sp.), and Ambisporaceae (*Ambispora* 1 sp.), which had a rare occurrence. Despite the extremely varied results, it is clear that there are few reports of the occurrence of the genera *Diversispora*, *Redeckera*, *Pacispora*, *Racocetra*, *Cetraspora*, *Dentiscutata*, *Septoglomus*, *Sclerocystis*, *Archaeospora*, *Ambisporaceae*, *Ambispora*, and *Entrophospora*, which strongly suggests their very low competitive capacity under PTE contamination conditions (Wei et al. 2015; Ban et al. 2017; Buck et al. 2019; Lopes Leal et al. 2016; Vilela et al. 2018; Vilela and Barbosa 2019).

With the best results obtained in the selection tests of efficient plant and fungal species, it is possible to perform step 6 of the phytoremediation process, implementation of the in situ phytoremediation technique (Fig. 2.7). From this, it is necessary to continuously monitor the entire process through the dashboard, which consists of uninterrupted monitoring of the effectiveness of the chosen technology through the evaluation of ecological, environmental, and economic indicators (step 7) that will enable the adoption of corrective measures (step 8) throughout the phytoremediation process.

Conventionally, AMF inoculum production occurs in cultivated pots containing sterile soil and inoculated with fungal spores. This cultivation takes place in greenhouses using plants with high mycorrhizal dependence. After the plant growth cycle, soil is collected containing spores, extraradicular mycelium and mycorrhized roots that are used as a source of inoculum (Kumar et al. 2017). Despite its relative simplicity, this technique requires for at least 4 months to produce viable spores (https://invam.wvu.edu/) and can become unviable for large-scale inoculum production. There are other more expensive and more technological techniques for the production of AMF inoculum, hydroponics, and aeroponics. Hydroponics is a system in which host plants are inoculated with AMF and grown in aqueous solution that provides nutrients to plants. In aeroponics, plants are also inoculated with AMF spores; however, cultivation is suspended in the air, with plants supported by the root of the roots, which are sprayed with a mist of nutrient solution. In addition to their high cost, these cropping systems require constant monitoring, are susceptible to widespread disease occurrence, and have large-scale production limitations (Kumar et al. 2017). Due to their characteristic biotrophic, AMF are not produced in monoxenic culture (in vitro culture system) without the presence of metabolically active roots. For these conditions, there is a need for the use of transformed plant roots (induced by *Agrobacterium rhizogenes* Ri-TDA) or autotrophic plants with the shoot outside the Petri dish or in a sterile tube connected vertically to the petri dish. Despite the high cost, in vitro crops continually produce pure, contamination-free, and traceable concentrate products (Kumar et al. 2017). In addition to care in

inoculum production, the inoculum must be properly supplied to the plant. For herbaceous or arboreal species, the seedlings are usually grown in seedling nursery, where they receive the AMF inoculum. In this way, the plant begins the establishment of mycorrhizal symbiosis before transplantation in contaminated soil, i.e., being placed in contact with contamination is already benefiting from symbiosis.

2.9 Closing Remarks

The main sources of PTE contamination of soil have anthropogenic cause, demonstrating that the number of contaminated areas in the world tends to increase exponentially in the coming years. Worryingly, some remediation techniques in these areas tend to be as environmentally impactful as the contamination itself. In this sense, techniques that prioritize the use of natural resources for soil detoxification gain more space in remediation projects because they are environmentally friendly. Of these, we mention phytoremediation (phytoextraction and phytostabilization) that use plants to extract or immobilize potentially toxic elements in the soil, making them less harmful. Although many plants have a considerable range of external and internal defense strategies, they are not always effective. Therefore, arbuscular mycorrhizal fungi may favor plant growth in contaminated sites given their tolerance to these environments as well as the plant defense processes, as discussed in this chapter. Effects of arbuscular mycorrhizal fungi on the PTE uptake by host plants can be influenced by the metal specifications and their total concentrations, substrate physicochemical properties, combinations between mycorrhizal fungi isolates and host plants, and cultivation conditions. Currently, there are numerous research results demonstrating that AMF inoculation in plants grown in contaminated soil can stimulate photosynthetic activity, increase nutrient uptake, increase production of secondary metabolites, and modify the expression of plant defense-related genes, which promotes greater tolerance to plants. However, research has not yet directed how these positive results can turn into applicable remediation, bioremediation, or phytoremediation projects for contaminated areas. Major difficulty is in the absence of specific information on the remediation capacity of each AMF species for certain types of PTE, i.e., there are no specific remediation programs yet to mention which AMF species and plants should be used for each kind of contamination. It is noteworthy that the latest research has focused on metagenomic and metatranscriptomic techniques to broaden knowledge about new genes and proteins responsible for AMF tolerance and mycorrhizal plants in PTE-contaminated soils. Possibly, these results will expand understanding of the diversity, functionality, and adaptability of different AMF species in contaminated soils. In addition, there is a great need for advances in research into large-scale AMF inoculum production or the production of mycorrhizal stimulants for native AMF populations with applicability to contaminated soil remediation programs.

References

Abd-Allah EF, Hashem A, Alqarawi AA, Alwathnani HA (2015) Alleviation of adverse impact of cadmium stress in sunflower (*Helianthus annuus* L.) by arbuscular mycorrhizal fungi. Pak J Bot 47:785–795

Abdelhameed RE, Metwally RA (2019) Alleviation of cadmium stress by arbuscular mycorrhizal symbiosis. Int J Phytoremediation 21:663–671. https://doi.org/10.1080/15226514.2018.1556584

Aguirre CFV, Páez FAR, Vargas SE (2018) Effect of arbuscular mycorrhizae and mercury on *Lactuca sativa* (Asteraceae) seedling morpho-histology. Environ Exp Bot 156:197–202. https://doi.org/10.1016/j.envexpbot.2018.09.005

Arya A, Ojha S, Singh S (2018) In: Fungi and their role in sustainable development: current perspectives (eds: Gehlot P, Singh J). Springer, Singapore, pp 255–265

ASTM (2013). Standard guide for greener cleanup, E2893–13. ASTM International

Ban Y et al (2017) Homogenous stands of a wetland grass living in heavy metal polluted wetlands harbor diverse consortia of arbuscular mycorrhizal fungi. Chemosphere 181:699–709. https://doi.org/10.1016/j.chemosphere.2017.04.135

Bano SA, Ashfaq D (2013) Role of mycorrhiza to reduce heavy metal stress. Nat Sci 5:16–20. https://doi.org/10.4236/ns.2013.512A003

Barbieri M (2016) The importance of enrichment factor (EF) and geoaccumulation index (Igeo) to evaluate the soil contamination. Geol Geophys 5:2. https://doi.org/10.4172/2381-8719.1000237

Bardos P, Lazar A, Willenbrock N (2009) A review of published sustainability indicator sets: How applicable are they to contaminated land remediation indicator-set development. In: Contaminated Land: Applications in Real Environments (CL:AIRE), London

Bardos R, et al (2011) The SuRF-UK indicator set for sustainable remediation assessment, http://www.claire.co.uk/surfuk

Bardos RP et al (2016) The rationale for simple approaches for sustainability assessment and management in contaminated land practice. Sci Total Environ 563:755–768. https://doi.org/10.1016/j.scitotenv.2015.12.001

Beames A, Broekx S, Lookman R, Touchant K, Seuntjens P (2014) Sustainability appraisal tools for soil and groundwater remediation: how is the choice of remediation alternative influenced by different sets of sustainability indicators and tool structures? Sci Total Environ 470:954–966. https://doi.org/10.1016/j.scitotenv.2013.10.044

Behera BK, Prasad R (2020) Environmental Technology and Sustainability. Elsevier (ISBN: 9780128191033) https://www.elsevier.com/books/environmental-technology-and-sustainability/behera/978-0-12-819103-3

Benabdellah K, Merlos M-Á, Azcón-Aguilar C, Ferrol N (2009a) GintGRX1, the first characterized glomeromycotan glutaredoxin, is a multifunctional enzyme that responds to oxidative stress. Fungal Genet Biol 46:94–103. https://doi.org/10.1016/j.fgb.2008.09.013

Benabdellah K et al (2009b) GintPDX1 encodes a protein involved in vitamin B6 biosynthesis that is up-regulated by oxidative stress in the arbuscular mycorrhizal fungus *Glomus intraradices*. New Phytol 184:682–693. https://doi.org/10.3389/fpls.2016.01251

Bhandari P, Garg N (2017) In: Plant-microbe interactions in agro-ecological perspectives (eds: Singh D, Singh H, Prabha R). Springer, Singapore, pp 377–402

Buck M, Straker C, Mavri-Damelin D, Weiersbye I (2019) Diversity of arbuscular mycorrhizal (AM) fungi colonising roots of indigenous *Vachellia* and *Senegalia* trees on gold and uranium mine tailings in South Africa. S Afr J Bot 121:34–44. https://doi.org/10.1016/j.sajb.2018.10.014

Cabral L, Soares CRFS, Giachini AJ, Siqueira JO (2015) Arbuscular mycorrhizal fungi in phytoremediation of contaminated areas by trace elements: mechanisms and major benefits of their applications. World J Microbiol Biotechnol 31:1655–1664. https://doi.org/10.1007/s11274-015-1918-y

Cao S et al (2015) Health risk assessment of various metal(loid)s via multiple exposure pathways on children living near a typical lead-acid battery plant, China. Environ Pollut 200:16–23. https://doi.org/10.1016/j.envpol.2015.02.010

Cappuyns V (2016) Inclusion of social indicators in decision support tools for the selection of sustainable site remediation options. J Environ Manag 184:45–56. https://doi.org/10.1016/j.jenvman.2016.07.035

CEC (2006) Proposal for a Directive of the European Parliament and of the Council establishing a framework for the protection of soil and amending Directive 2004/35/EC, https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:52006PC0232&from=EN

Chen XW et al (2019) Arbuscular mycorrhizal fungi and the associated bacterial community influence the uptake of cadmium in rice. Geoderma 337:749–757. https://doi.org/10.1016/j.geoderma.2018.10.029

Chibuike G (2013) Use of mycorrhiza in soil remediation: a review. Sci Res Essays 8:679–1687. https://doi.org/10.5897/SRE2013.5605

Cicatelli A et al (2010) Arbuscular mycorrhizal fungi restore normal growth in a white poplar clone grown on heavy metal-contaminated soil, and this is associated with upregulation of foliar metallothionein and polyamine biosynthetic gene expression. Ann Bot 106:791–802. https://doi.org/10.1093/aob%2Fmcq170

Cicatelli A et al (2014) Arbuscular mycorrhizal fungi as a tool to ameliorate the phytoremediation potential of poplar: biochemical and molecular aspects. iForest-Biogeosci For 7:333. https://doi.org/10.3832/ifor1045-007

Coninx L, Martinova V, Rineau F (2017) Mycorrhiza-assisted phytoremediation. Adv Bot Res 83:127–188. https://doi.org/10.1016/bs.abr.2016.12.005

Cornejo P et al (2013) Copper compartmentalization in spores as a survival strategy of arbuscular mycorrhizal fungi in Cu-polluted environments. Soil Biol Biochem 57:925–928. https://doi.org/10.1016/j.soilbio.2012.10.031

CRCCARE (2013) Global contamination initiative e a proposal for a new global initiative addressing one of the Most serious threats to our planet and our future, Cooperative Research Centre for Contamination Assessment and Remediation of Environment, pp 1–5

Cui G, Ai S, Chen K, Wang X (2019) Arbuscular mycorrhiza augments cadmium tolerance in soybean by altering accumulation and partitioning of nutrient elements, and related gene expression. Ecotoxicol Environ Saf 171:231–239. https://doi.org/10.1016/j.ecoenv.2018.12.093

Dankoub Z, Ayoubi S, Khademi H, Sheng-Gao L (2012) Spatial distribution of magnetic properties and selected heavy metals in calcareous soils as affected by land use in the Isfahan region, Central Iran. Pedosphere 22:33–47. https://doi.org/10.1016/S1002-0160(11)60189-6

Delavaux CS, Smith-Ramesh LM, Kuebbing SE (2017) Beyond nutrients: a meta-analysis of the diverse effects of arbuscular mycorrhizal fungi on plants and soils. Ecology 98:2111–2119. https://doi.org/10.1002/ecy.1892

Dietterich LH, Gonneau C, Casper BB (2017) Arbuscular mycorrhizal colonization has little consequence for plant heavy metal uptake in contaminated field soils. Ecol Appl 27:1862–1875. https://doi.org/10.1002/eap.1573

Dubchak S (2017) In: Impact of cesium on plants and the environment (eds: Gupta D, Walther C). Springer, Singapore, pp 35–50

Dutta SC, Neog B (2016) Accumulation of secondary metabolites in response to antioxidant activity of turmeric rhizomes co-inoculated with native arbuscular mycorrhizal fungi and plant growth promoting rhizobacteria. Sci Hortic 204:179–184. https://doi.org/10.1016/j.scienta.2016.03.028

Emamverdian A, Ding Y, Mokhberdoran F, Xie Y (2015) Heavy metal stress and some mechanisms of plant defense response. Sci World J 2015. https://doi.org/10.1155/2015/756120

EPA (2008) EPA – Environmental Protection Agency, Illinois

Esmaeili A, Moore F, Keshavarzi B, Jaafarzadeh N, Kermani M (2014) A geochemical survey of heavy metals in agricultural and background soils of the Isfahan industrial zone, Iran. Catena 121:88–98. https://doi.org/10.1016/j.catena.2014.05.003

Facchinelli A, Sacchi E, Mallen L (2001) Multivariate statistical and GIS-based approach to identify heavy metal sources in soils. Environ Pollut 114:313–324. https://doi.org/10.1016/S0269-7491(00)00243-8

Faggioli V et al (2019) Soil lead pollution modifies the structure of arbuscular mycorrhizal fungal communities. Mycorrhiza:1–11. https://doi.org/10.1007/s00572-019-00895-1

Ferguson C et al (1998) Risk assessment for contaminated sites in Europe: scientific basis, vol 1. LQM Press, Nottingham, p 170

Fortuna ME, Simion IM, Gavrilescu M (2011) Sustainability in environmental remediation. Environ Eng Manag J 10, pp. 1987–1996

Gadkar V, Rillig MC (2006) The arbuscular mycorrhizal fungal protein glomalin is a putative homolog of heat shock protein 60. FEMS Microbiol Lett 263:93–101. https://doi.org/10.1111/j.1574-6968.2006.00412.x

Garg N, Bhandari P (2014) Cadmium toxicity in crop plants and its alleviation by arbuscular mycorrhizal (AM) fungi: an overview. Plant Biosyst 148:609–621. https://doi.org/10.1080/11263504.2013.788096

Garg N, Singh S (2018) Arbuscular mycorrhiza *Rhizophagus irregularis* and silicon modulate growth, proline biosynthesis and yield in *Cajanus cajan* L. Millsp.(pigeonpea) genotypes under cadmium and zinc stress. J Plant Growth Regul 37:46–63. https://doi.org/10.1007/s00344-017-9708-4

Gasparatos D (2013) Sequestration of heavy metals from soil with Fe - Mn concretions and nodules. Environ Chem Lett 11:1–9. https://doi.org/10.1007/s10311-012-0386-y

Gavrilescu M, Macoveanu M (1999) Process engineering in biological aerobic waste-water treatment. Acta Biotechnol 19:111–145. https://doi.org/10.1002/abio.370190205

Gavrilescu M, Macoveanu M (2000) Attached-growth process engineering in wastewater treatment. Bioprocess Eng 23:95–106. https://doi.org/10.1007/s004490050030

Gil-Cardeza ML, Ferri A, Cornejo P, Gomez E (2014) Distribution of chromium species in a Cr-polluted soil: presence of Cr (III) in glomalin related protein fraction. Sci Total Environ 493:828–833. https://doi.org/10.1016/j.scitotenv.2014.06.080

Gill R, Thornton S, Harbottle MJ, Smith J (2016) Sustainability assessment of electrokinetic bioremediation compared with alternative remediation options for a petroleum release site. J Environ Manag 184:120–131. https://doi.org/10.1016/j.jenvman.2016.07.036

Giri B, Prasad R, Wu Q-S, Varma A (2019) Biofertilizers for Sustainable Agriculture and Environment. Springer International Publishing (ISBN 978-3-030-18932-7) https://www.springer.com/gp/book/9783030189327

Göhre V, Paszkowski U (2006) Contribution of the arbuscular mycorrhizal symbiosis to heavy metal phytoremediation. Planta 223:1115–1122. https://doi.org/10.1007/s00425-006-0225-0

Gomes MP, Carneiro MMLC, Garcia QS (2014a) In: Oxidative damage to plants: antioxidant networks and signaling (ed: Ahmad P) Ch. 17. Academic Press, San Diego, CA, pp 523–540

Gomes MP, Andrade ML, Nascentes CC, Scotti MR (2014b) Arsenic root sequestration by a tropical woody legume as affected by arbuscular mycorrhizal fungi and organic matter: implications for land reclamation. Water Air Soil Pollut 225:1919. San Diego, CA. https://doi.org/10.1007/s11270-014-1919-8

Gratão PL, Polle A, Lea PJ, Azevedo RA (2005) Making the life of heavy metal-stressed plants a little easier. Funct Plant Biol 32:481–494. https://doi.org/10.1071/FP05016

Groot RS, Wilson MA, Boumans R (2002) A typology for the classification, description and valuation of ecosystem functions, goods and services. Ecol Econ 41:393–408. https://doi.org/10.1016/S0921-8009(02)00089-7

Guntzer F, Keller C, Meunier J-D (2012) Benefits of plant silicon for crops: a review. Agron Sustain Dev 32:201–213. https://doi.org/10.1007/s13593-011-0039-8

Halka M, Nordstrom B (2011) Metals and metalloids. Facts On File, New York, p 193

Hasanuzzaman M, Nahar K, Anee TI, Fujita M (2017) Glutathione in plants: biosynthesis and physiological role in environmental stress tolerance. Physiol Mol Biol Plants 23:249–268. https://doi.org/10.1007/s12298-017-0422-2

Hashem A et al (2016) Alleviation of cadmium stress in *Solanum lycopersicum* L. by arbuscular mycorrhizal fungi via induction of acquired systemic tolerance. Saudi J Biol Sci 23:272–281. https://doi.org/10.1016/j.sjbs.2015.11.002

Hassan Z et al (2017) In: Probiotics in agroecosystem (eds: Kumar V, Kumar M, Sharma S, Prasad R). Springer, Singapore, pp 517–537

Holland KS et al (2011) Framework for integrating sustainability into remediation projects. Remediat J 21:7–38. https://doi.org/10.1002/rem.20288

Hou D, O'Connor D, Nathanail P, Tian L, Ma Y (2017) Integrated GIS and multivariate statistical analysis for regional scale assessment of heavy metal soil contamination: a critical review. Environ Pollut 231:1188–1200. https://doi.org/10.1016/j.envpol.2017.07.021

Hristozkova M, Geneva M, Stancheva I, Boychinova M, Djonova E (2016) Contribution of arbuscular mycorrhizal fungi in attenuation of heavy metal impact on *Calendula officinalis* development. Appl Soil Ecol 101:57–63. https://doi.org/10.1016/j.apsoil.2016.01.008

Hu J et al (2013) Arbuscular mycorrhizal fungi induce differential Cd and P acquisition by Alfred stonecrop (*Sedum alfredii* Hance) and upland kangkong (*Ipomoea aquatica* Forsk.) in an intercropping system. Appl Soil Ecol 63:29–35. https://doi.org/10.1016/j.apsoil.2012.09.002

Huang Y et al (2015) An integrated approach to assess heavy metal source apportionment in peri-urban agricultural soils. J Hazard Mater 299:540–549. https://doi.org/10.1016/j.jhazmat.2015.07.041

Huang R-H, Lu Y-M, Yang H-L, Huang W, Chen K (2016) Effects of arbuscular mycorrhizal fungi on caesium accumulation and the ascorbate-glutathione cycle of *Sorghum halepense*. Sci Asia 42:323–331. https://doi.org/10.2306/scienceasia1513-1874.2016.42.323

Ibiang YB, Mitsumoto H, Sakamoto K (2017) Bradyrhizobia and arbuscular mycorrhizal fungi modulate manganese, iron, phosphorus, and polyphenols in soybean (*Glycine max* (L.) Merr.) under excess zinc. Environ Exp Bot 137:1–13. https://doi.org/10.1016/j.envexpbot.2017.01.011

Ighodaro O, Akinloye O (2018) First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): their fundamental role in the entire antioxidant defence grid. Alexandria J Med 54:287–293. https://doi.org/10.1016/j.ajme.2017.09.001

Inouhe M (2005) Phytochelatins. Braz J Plant Physiol 17:65–78. https://doi.org/10.1590/S1677-04202005000100006

ISO (2016) International Standards Organization, ISO 2016 ISO/DIS 18504 Soil quality, Guidance on sustainable remediation, http://www.iso.org/iso/home/store/catalogue_tc/catalogue_detail.htm?csnumber=62688

Järup L (2003) Hazards of heavy metal contamination. Br Med Bull 68:167–182. https://doi.org/10.1093/bmb/ldg032

Jensen J, Mesman M, Bierkens J, Rutgers M (2006) In: Ecological risk assessment of contaminated land: decision support for site specific investigations (eds: Jensen J, Mesman M) Ch. 1. National Institute for Puclic Health and the Environment, Bilthoven, pp 11–18

Jia X, Zhao Y, Liu T, Huang S, Chang Y (2016) Elevated CO_2 increases glomalin-related soil protein (GRSP) in the rhizosphere of *Robinia pseudoacacia* L. seedlings in Pb-and Cd-contaminated soils. Environ Pollut 218:349–357. https://doi.org/10.1016/j.envpol.2016.07.010

Jia X, Zhao Y, He Y, Chang Y (2018) Glomalin-related soil protein in the rhizosphere of *Robinia pseudoacacia* L. seedlings under higher air temperature combined with Cd-contaminated soil. Eur J Soil Sci 69:634–645. https://doi.org/10.1111/ejss.12671

Jiang Q-Y et al (2016a) Can arbuscular mycorrhizal fungi reduce Cd uptake and alleviate Cd toxicity of Lonicera japonica grown in Cd-added soils? Sci Rep 6:21805. https://doi.org/10.1038/srep21805

Jiang Q-Y et al (2016b) Effect of *Funneliformis mosseae* on the growth, cadmium accumulation and antioxidant activities of *Solanum nigrum*. Appl Soil Ecol 98:112–120. https://doi.org/10.1016/j.apsoil.2015.10.003

Jiang Y et al (2017) Source apportionment and health risk assessment of heavy metals in soil for a township in Jiangsu Province, China. Chemosphere 168:1658–1668. https://doi.org/10.1016/j.chemosphere.2016.11.088

Kabata-Pendias A (2011) Trace elements in soils and plants, 4th edn. CRC Press, Boca Raton, p 548

Kapoor R, Evelin H, Mathur P, Giri B (2013) In: Plant acclimation to environmental stress (eds: Tuteja N, Singh Gill S). Springer, Singapore, pp 359–401

Karimi A, Khodaverdiloo H, Sadaghiani MR (2017) Characterisation of growth and biochemical response of *Onopordum acanthium* L. under lead stress as affected by microbial inoculation. Chem Ecol 33:963–976. https://doi.org/10.1080/02757540.2017.1391798

Kasemodel MC, Papa TBR, Sígolo JB, Rodrigues VGS (2019) Assessment of the mobility, bioaccessibility, and ecological risk of Pb and Zn on a dirt road located in a former mining area Ribeira Valley – Brazil. Environ Monit Assess 191:101. https://doi.org/10.1007/s10661-019-7238-1

Kaur H, Garg N (2017) Zinc-arbuscular mycorrhizal interactions: effect on nutrient pool, enzymatic antioxidants, and osmolyte synthesis in pigeonpea nodules subjected to Cd stress. Commun Soil Sci Plant Anal 48:1684–1700. https://doi.org/10.1080/00103624.2017.1374400

Kaur H, Garg N (2018) Recent perspectives on cross talk between cadmium, zinc, and arbuscular mycorrhizal fungi in plants. J Plant Growth Regul 37:680–693. https://doi.org/10.1007/s00344-017-9750-2

Kelepertzis E (2014) Accumulation of heavy metals in agricultural soils of Mediterranean: insights from Argolida basin, Peloponnese, Greece. Geoderma 221:82–90. https://doi.org/10.1016/j.geoderma.2014.01.007

Khan FI, Husain T, Hejazi R (2004) An overview and analysis of site remediation technologies. J Environ Manag 71:95–122. https://doi.org/10.1016/j.jenvman.2004.02.003

Khan MA, Ahmad I, Rahman IU (2007) Effect of environmental pollution on heavy metals content of *Withania somnifera*. J Chin Chem Soc 54:339–343. https://doi.org/10.1002/jccs.200700049

Kapoor D, Singh S, Kumar V, Romero R, Prasad R, Singh J (2019) Antioxidant enzymes regulation in plants in reference to reactive oxygen species (ROS) and reactive nitrogen species (RNS). Plant Gene https://doi.org/10.1016/j.plgene.2019.100182

Krishna AK, Mohan KR (2016) Distribution, correlation, ecological and health risk assessment of heavy metal contamination in surface soils around an industrial area, Hyderabad, India. Environ Earth Sci 75:411. https://doi.org/10.1007/s12665-015-5151-7

Krishnamoorthy R et al (2019) In: Sustainable green technologies for environmental management (eds: Shah S, Venkatramanan V, Prasad R). Springer, Singapore, pp 67–85

Kumar A, Singh R, Adholeya A (2017) In: Developments in fungal biology and applied mycology (eds: Satyanarayana T, Deshmukh S, Johri B). Springer, Singapore, pp 413–431

Kuppusamy S et al (2017) Remediation approaches for polycyclic aromatic hydrocarbons (PAHs) contaminated soils: technological constraints, emerging trends and future directions. Chemosphere 168:944–968. https://doi.org/10.1016/j.chemosphere.2016.10.115

Lee CSL, Li X, Shi W, Cheung SC-N, Thornton I (2006) Metal contamination in urban, suburban, and country park soils of Hong Kong: a study based on GIS and multivariate statistics. Sci Total Environ 356:45–61. https://doi.org/10.1016/j.scitotenv.2005.03.024

Lemming G et al (2010) Environmental impacts of remediation of a trichloroethene-contaminated site: life cycle assessment of remediation alternatives. Environ Sci Technol 44:9163–9169. https://doi.org/10.1021/es102007s

Lenoir I, Fontaine J, Sahraoui AL-H (2016) Arbuscular mycorrhizal fungal responses to abiotic stresses: a review. Phytochemistry 123:4–15. https://doi.org/10.1016/j.phytochem.2016.01.002

Li X, Lee S-L, Wong S-C, Shi W, Thornton I (2004) The study of metal contamination in urban soils of Hong Kong using a GIS-based approach. Environ Pollut 129:113–124. https://doi.org/10.1016/j.envpol.2003.09.030

Li N et al (2015) Concentration and transportation of heavy metals in vegetables and risk assessment of human exposure to bioaccessible heavy metals in soil near a waste-incinerator site, South China. Sci Total Environ 521:144–151. https://doi.org/10.1016/j.scitotenv.2015.03.081

Li H et al (2016) Do arbuscular mycorrhizal fungi affect cadmium uptake kinetics, subcellular distribution and chemical forms in rice? Sci Total Environ 571:1183–1190. https://doi.org/10.1016/j.scitotenv.2016.07.124

Liang J et al (2017) Spatial distribution and source identification of heavy metals in surface soils in a typical coal mine city, Lianyuan, China. Environ Pollut 225:681–690. https://doi.org/10.1016/j.envpol.2017.03.057

Lingua G et al (2012) Effects of heavy metals and arbuscular mycorrhiza on the leaf proteome of a selected poplar clone: a time course analysis. PLoS One 7:e38662. https://doi.org/10.1371/journal.pone.0038662

Liu H et al (2015) Enhancement of arbuscular mycorrhizal fungus (*Glomus versiforme*) on the growth and Cd uptake by Cd-hyperaccumulator *Solanum nigrum*. Appl Soil Ecol 89:44–49. https://doi.org/10.1016/j.apsoil.2015.01.006

Lopes Leal P et al (2016) Enrichment of arbuscular mycorrhizal fungi in a contaminated soil after rehabilitation. Braz J Microbiol 47:853–862. https://doi.org/10.1016/j.bjm.2016.06.001

Luo X-S et al (2015) Source identification and apportionment of heavy metals in urban soil profiles. Chemosphere 127:152–157. https://doi.org/10.1016/j.chemosphere.2015.01.048

Lv JJ (2019) Multivariate receptor models and robust geostatistics to estimate source apportionment of heavy metals in soils. Environ Pollut 244:72–83. https://doi.org/10.1016/j.envpol.2018.09.147

Maas S et al (2010) Spatial distribution of heavy metal concentrations in urban, suburban and agricultural soils in a Mediterranean city of Algeria. Environ Pollut 158:2294–2301. https://doi.org/10.1016/j.envpol.2010.02.001

Malekzadeh E, Aliasgharzad N, Majidi J, Abdolalizadeh J, Aghebati-Maleki L (2016a) Contribution of glomalin to Pb sequestration by arbuscular mycorrhizal fungus in a sand culture system with clover plant. Eur J Soil Biol 74:45–51. https://doi.org/10.1016/j.ejsobi.2016.03.003

Malekzadeh E, Aliasgharzad N, Majidi J, Aghebati-Maleki L, Abdolalizadeh J (2016b) Cd-induced production of glomalin by arbuscular mycorrhizal fungus (*Rhizophagus irregularis*) as estimated by monoclonal antibody assay. Environ Sci Pollut Res 23:20711–20718. https://doi.org/10.1007/s11356-016-7283-z

Marschner H (2012) Mineral nutrition of higher plants, 3rd edn. Academic Press, San Diego, p 650

Mason RP et al (2012) Mercury biogeochemical cycling in the ocean and policy implications. Environ Res 119:101–117. https://doi.org/10.1016/j.envres.2012.03.013

Meier S, Borie F, Bolan N, Cornejo P (2012) Phytoremediation of metal-polluted soils by arbuscular mycorrhizal fungi. Crit Rev Environ Sci Technol 42:741–775. https://doi.org/10.1080/10643389.2010.528518

Merlos MA, Zitka O, Vojtech A, Azcón-Aguilar C, Ferrol N (2016) The arbuscular mycorrhizal fungus *Rhizophagus irregularis* differentially regulates the copper response of two maize cultivars differing in copper tolerance. Plant Sci 253:68–76. https://doi.org/10.1016/j.plantsci.2016.09.010

Mihailović A et al (2015) Spatial distribution of metals in urban soil of Novi Sad, Serbia: GIS based approach. J Geochem Explor 150:104–114. https://doi.org/10.1016/j.gexplo.2014.12.017

Mikryukov V, Dulya O, Vorobeichik E (2015) Diversity and spatial structure of soil fungi and arbuscular mycorrhizal fungi in forest litter contaminated with copper smelter emissions. Water Air Soil Pollut 226:114. https://doi.org/10.1007/s11270-014-2244-y

Morales M, Munné-Bosch S (2019) Malondialdehyde: facts and artifacts. Plant Physiol 180:1246–1250. https://doi.org/10.1104/pp.19.00405

Moshiri F, Ebrahimi H, Ardakani MR, Rejali F, Mousavi SM (2019) Biogeochemical distribution of Pb and Zn forms in two calcareous soils affected by mycorrhizal symbiosis and alfalfa rhizosphere. Ecotoxicol Environ Saf 179:241–248. https://doi.org/10.1016/j.ecoenv.2019.04.055

Myers J, Thorbjornsen K (2004) Identifying metals contamination in soil: a geochemical approach. Soil Sediment Contam 13:1–16. https://doi.org/10.1080/10588330490269732

Naidu R, Pollard S, Bolan N, Owens G, Pruszinski A (2008) Bioavailability: the underlying basis for risk-based land management. Dev Soil Sci 32:53–72. https://doi.org/10.1016/S0166-2481(07)32004-7

Nath M, Bhatt D, Prasad R, Gill SS, Anjum NA, Tuteja N (2016) Reactive oxygen species generation-scavenging and signaling during plant-arbuscular mycorrhizal and *Piriformospora indica* interaction under stress condition. Frontiers in Plant Science 7:1574. https://doi.org/10.3389/fpls.2016.01574

Nath M, Bhatt D, Prasad R, Tuteja N (2017) Reactive oxygen species (ROS) metabolism and signaling in plant-mycorrhizal association under biotic and abiotic stress conditions. In: Varma A, Prasad R and Tuteja N (eds.), *Mycorrhiza*, Springer International Publishing AG 223-232

NICOLE-2011 (2011) Road map for sustainable remediation (co-author) NICOLE secretariat, Dutch Sino Business Promotions, Postbus 28249, 3003, http://www.nicole.org/pagina/22/Thematic_Documents.html

Noctor G et al (2012) Glutathione in plants: an integrated overview. Plant Cell Environ 35:454–484. https://doi.org/10.1111/j.1365-3040.2011.02400.x

Pacyna EG et al (2010) Global emission of mercury to the atmosphere from anthropogenic sources in 2005 and projections to 2020. Atmos Environ 44:2487–2499. https://doi.org/10.1016/j.atmosenv.2009.06.009

Perini K, Rosasco P (2013) Cost–benefit analysis for green façades and living wall systems. Build Environ 70:110–121. https://doi.org/10.1016/j.buildenv.2013.08.012

Perveen R, Faizan S, Ansari AA (2015) In: Phytoremediation (eds: Ansari A et al). Springer, Singapore, pp 131–142

Phoungthong K, Xia Y, Zhang H, Shao L, He P (2016) Leaching toxicity characteristics of municipal solid waste incineration bottom ash. Front Environ Sci Eng 10:399–411. https://doi.org/10.1007/s11783-015-0819-5

Prado RM (2008) Nutrição de Plantas, 1st edn. Editora UNESP, p 407

Prasad MNV, Freitas HMDO (2003) Metal hyperaccumulation in plants: biodiversity prospecting for phytoremediation technology. Electron J Biotechnol 6:285–321. https://doi.org/10.4067/S0717-34582003000300012

Prasad R (2021) Environmental Pollution and Remediation. Springer (ISBN 978-981-15-5499-5) https://www.springer.com/gp/book/9789811554988

Purin S, Rillig MC (2007) The arbuscular mycorrhizal fungal protein glomalin: limitations, progress, and a new hypothesis for its function. Pedobiologia 51:123–130. https://doi.org/10.1016/j.pedobi.2007.03.002

Rivera-Becerril F et al (2013) Impacts of manganese mining activity on the environment: interactions among soil, plants, and arbuscular mycorrhiza. Arch Environ Contam Toxicol 64:219–227. https://doi.org/10.1007/s00244-012-9827-7

Rosén L et al (2015) SCORE: a novel multi-criteria decision analysis approach to assessing the sustainability of contaminated land remediation. Sci Total Environ 511:621–638. https://doi.org/10.1016/j.scitotenv.2014.12.058

Sarathambal C et al (2017) The effect of plant growth-promoting rhizobacteria on the growth, physiology, and Cd uptake of *Arundo donax* L. Int J Phytoremediation 19:360–370. https://doi.org/10.1080/15226514.2016.1225289

Koch N, Islam NF, Sonowal S, Prasad R, Sarma H (2021) Environmental antibiotics and resistance genes as emerging contaminants: methods of detection and bioremediation. Current Research in Microbial Sciences https://doi.org/10.1016/j.crmicr.2021.100027

Sarwar N et al (2017) Phytoremediation strategies for soils contaminated with heavy metals: modifications and future perspectives. Chemosphere 171:710–721. https://doi.org/10.1016/j.chemosphere.2016.12.116

Schädler S, Morio M, Bartke S, Rohr-Zaenker R, Finkel M (2011) Designing sustainable and economically attractive brownfield revitalization options using an integrated assessment model. J Environ Manag 92:827–837. https://doi.org/10.1016/j.jenvman.2010.10.026

Schneider J, Stürmer SL, Guilherme LRG, Moreira FMDS, Soares CRFDS (2013) Arbuscular mycorrhizal fungi in arsenic-contaminated areas in Brazil. J Hazard Mater 262:1105–1115. https://doi.org/10.1016/j.jhazmat.2012.09.063

Schneider J, Bundschuh J, Rangel WDM, Guilherme LRG (2017) Potential of different AM fungi (native from As-contaminated and uncontaminated soils) for supporting *Leucaena leucocephala* growth in As-contaminated soil. Environ Pollut 224:125–135. https://doi.org/10.1016/j.envpol.2017.01.071

Schüßler A, Schwarzott D, Walker C (2001) A new fungal phylum, the Glomeromycota: phylogeny and evolution. Mycol Res 105:1413–1421. https://doi.org/10.1017/S0953756201005196

Shabani L, Sabzalian MR, Pour SM (2016) Arbuscular mycorrhiza affects nickel translocation and expression of ABC transporter and metallothionein genes in *Festuca arundinacea*. Mycorrhiza 26:67–76. https://doi.org/10.1007/s00572-015-0647-2

Shahabivand S, Aliloo AA, Maivan HZ (2016) Wheat biochemical response to cadmium toxicity under *Funneliformis mosseae* and *Piriformospora indica* symbiosis. Bot Lith 22:169–177. https://doi.org/10.1515/botlit-2016-0018

Sharma S, Anand G, Singh N, Kapoor R (2017) Arbuscular mycorrhiza augments arsenic tolerance in wheat (*Triticum aestivum* L.) by strengthening antioxidant defense system and thiol metabolism. Front Plant Sci 8:906. https://doi.org/10.3389/fpls.2017.00906

Shi W et al (2019) Physiological and molecular mechanisms of heavy metal accumulation in nonmycorrhizal versus mycorrhizal plants. *Plant*. Cell Environ 42:1087–1103. https://doi.org/10.1111/pce13471

Siani NG, Fallah S, Pokhrel LR, Rostamnejadi A (2017) Natural amelioration of zinc oxide nanoparticle toxicity in fenugreek (*Trigonella foenum-gracum*) by arbuscular mycorrhizal (*Glomus intraradices*) secretion of glomalin. Plant Physiol Biochem 112:227–238. https://doi.org/10.1016/j.plaphy.2017.01.001

Sidhoum W, Fortas Z (2019) The beneficial role of indigenous arbuscular mycorrhizal fungi in phytoremediation of wetland plants and tolerance to metal stress. Arch Environ Prot 45:103–114. https://doi.org/10.24425/aep.2019.125916

Singh PK (2015) In Vitro Cu-Sequestration by Glomalin from *Acaulospora spinosa* Walker and Trappe. Natl Acad Sci Lett 38:183–185. https://doi.org/10.1007/s40009-014-0309-5

Spagnoletti F, Lavado R (2015) The arbuscular mycorrhiza *Rhizophagus intraradices* reduces the negative effects of arsenic on soybean plants. Agronomy 5:188–199. https://doi.org/10.3390/agronomy5020188

Spagnoletti FN, Balestrasse K, Lavado RS, Giacometti R (2016) Arbuscular mycorrhiza detoxifying response against arsenic and pathogenic fungus in soybean. Ecotoxicol Environ Saf 133:47–56. https://doi.org/10.1016/j.ecoenv.2016.06.012

Stürmer SL, Bever JD, Morton JB (2018) Biogeography of arbuscular mycorrhizal fungi (Glomeromycota): a phylogenetic perspective on species distribution patterns. Mycorrhiza 28:587–603. https://doi.org/10.1007/s00572-018-0864-6

Sun Y, Zhou Q, Xie X, Liu R (2010) Spatial, sources and risk assessment of heavy metal contamination of urban soils in typical regions of Shenyang, China. J Hazard Mater 174:455–462. https://doi.org/10.1016/j.jhazmat.2009.09.074

Sun Y et al (2016) The molecular diversity of arbuscular mycorrhizal fungi in the arsenic mining impacted sites in Hunan Province of China. J Environ Sci 39:110–118. https://doi.org/10.1016/j.jes.2015.10.005

Swartjes F et al (2012) State of the art of contaminated site management in the Netherlands: policy framework and risk assessment tools. Sci Total Environ 427:1–10. https://doi.org/10.1016/j.scitotenv.2012.02.078

Takács T (2012) In: Toxicity of heavy metals to legumes and bioremediation (eds: Zaidi A, Wani P, Khan M). Springer, Singapore, pp 179–202

Tariq M, Ali M, Shah Z (2006) Characteristics of industrial effluents and their possible impacts on quality of underground water. Soil and Environment 25:64–69

Tóth G, Hermann T, Da Silva M, Montanarella L (2016) Heavy metals in agricultural soils of the European Union with implications for food safety. Environ Int 88:299–309. https://doi.org/10.1016/j.envint.2015.12.017

Tume P et al (2018) Distinguishing between natural and anthropogenic sources for potentially toxic elements in urban soils of Talcahuano, Chile. J Soils Sediments 18:2335–2349. https://doi.org/10.1007/s11368-017-1750-0

Ursitti F, Vanderlinden L, Watson R, Campbell M (2004) Assessing and managing exposure from arsenic in CCA-treated wood play structures. Can J Public Health 95:429–433. https://doi.org/10.1007/BF03403987

Van der Heijden MG, Boller T, Wiemken A, Sanders IR (1998a) Different arbuscular mycorrhizal fungal species are potential determinants of plant community structure. Ecology 79:2082–2091. https://doi.org/10.1890/0012-9658(1998)079[2082:DAMFSA]2.0.CO;2

Van der Heijden MG et al (1998b) Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature 396:69

Van Der Heijden MG, Bardgett RD, Van Straalen NM (2008) The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecol Lett 11:296–310. https://doi.org/10.1111/j.1461-0248.2007.01139.x

Vilela LAF, Barbosa MV (2019) In: Cadmium tolerance in plants: agronomic, molecular, signaling, and omic approaches (eds: Hasanuzzaman M, Narasimha M, Prasad V, Nahar K). Academic Press, San Diego, pp 553–586

Vilela LAF, Teixeira AFS, Lourenço FMO, Souza MD (2018) In: Plants under metal and metalloid stress (eds: Hasanuzzaman M, Nahar K, Fujita M). Springer, Singapore, pp 337–366

Volchko Y, Norrman J, Bergknut M, Rosén L, Söderqvist T (2013) Incorporating the soil function concept into sustainability appraisal of remediation alternatives. J Environ Manag 129:367–376. https://doi.org/10.1016/j.jenvman.2013.07.025

Volchko Y et al (2014) Using soil function evaluation in multi-criteria decision analysis for sustainability appraisal of remediation alternatives. Sci Total Environ 485:785–791. https://doi.org/10.1016/j.scitotenv.2014.01.087

Wagg C, Bender SF, Widmer F, van der Heijden MG (2014) Soil biodiversity and soil community composition determine ecosystem multifunctionality. Proc Natl Acad Sci 111:5266–5270. https://doi.org/10.1073/pnas.1320054111

Wang F (2017) Occurrence of arbuscular mycorrhizal fungi in mining-impacted sites and their contribution to ecological restoration: mechanisms and applications. Crit Rev Environ Sci Technol 47:1901–1957. https://doi.org/10.1080/10643389.2017.1400853

Wang C, Ji J, Yang Z, Chen L (2013) The contamination and transfer of potentially toxic elements and their relations with iron, vanadium and titanium in the soil-rice system from Suzhou region, China. Environ Earth Sci 68:13–21. https://doi.org/10.1007/s12665-012-1699-7

Wang L et al (2017) Role of *Rhizophagus irregularis* in alleviating cadmium toxicity via improving the growth, micro-and macroelements uptake in *Phragmites australis*. Environ Sci Pollut Res 24:3593–3607. https://doi.org/10.1007/s11356-016-7984-3

Wang Y, Branicky R, Noë A, Hekimi S (2018) Superoxide dismutases: dual roles in controlling ROS damage and regulating ROS signaling. J Cell Biol 217:1915–1928. https://doi.org/10.1083/jcb.201708007

Weeks J, Comber S (2005) Ecological risk assessment of contaminated soil. Mineral Mag 69:601–613. https://doi.org/10.1180/0026461056950274

Wei Y et al (2014) Molecular diversity of arbuscular mycorrhizal fungi associated with an Mn hyperaccumulator – *Phytolacca americana*, in Mn mining area. Appl Soil Ecol 82:11–17. https://doi.org/10.1016/j.apsoil.2014.05.005

Wei Y et al (2015) Diversity of arbuscular mycorrhizal fungi associated with a sb accumulator plant, ramie (*Boehmeria nivea*), in an active Sb mining. J Microbiol Biotechnol 25:1205–1215. https://doi.org/10.4014/jmb.1411.11033

Wu Z et al (2014) Decomposition and the contribution of glomalin-related soil protein (GRSP) in heavy metal sequestration: field experiment. Soil Biol Biochem 68:283–290. https://doi.org/10.1016/j.soilbio.2013.10.010

Xu X, Zhao Y, Zhao X, Wang Y, Deng W (2014) Sources of heavy metal pollution in agricultural soils of a rapidly industrializing area in the Yangtze Delta of China. Ecotoxicol Environ Saf 108:161–167. https://doi.org/10.1016/j.ecoenv.2014.07.001

Yang Y et al (2015) The combined effects of arbuscular mycorrhizal fungi (AMF) and lead (Pb) stress on Pb accumulation, plant growth parameters, photosynthesis, and antioxidant enzymes in *Robinia pseudoacacia* L. PLoS One 10:e0145726. https://doi.org/10.1371/journal.pone.0145726

Yang H, Ma M, Thompson JR, Flower RJ (2018) Waste management, informal recycling, environmental pollution and public health. Epidemiol Community Health 72:237–243. https://doi.org/10.1136/jech-2016-208597

Yasutaka T et al (2016) Development of a green remediation tool in Japan. Sci Total Environ 563:813–821. https://doi.org/10.1016/j.scitotenv.2016.01.018

Yesilonis I, Pouyat R, Neerchal N (2008) Spatial distribution of metals in soils in Baltimore, Maryland: role of native parent material, proximity to major roads, housing age and screening guidelines. Environ Pollut 156:723–731. https://doi.org/10.1016/j.envpol.2008.06.010

Zhan F et al (2019) Effects of arbuscular mycorrhizal fungi on the growth and heavy metal accumulation of bermudagrass [*Cynodon dactylon* (L.) Pers.] grown in a lead-zinc mine wasteland. Int J Phytoremediation:1–8. https://doi.org/10.1080/15226514.2019.1577353

Zhang C (2006) Using multivariate analyses and GIS to identify pollutants and their spatial patterns in urban soils in Galway, Ireland. Environ Pollut 142:501–511. https://doi.org/10.1016/j.envpol.2005.10.028

Zhang X, Chen B, Ohtomo R (2015) Mycorrhizal effects on growth, P uptake and Cd tolerance of the host plant vary among different AM fungal species. Soil Sci Plant Nutr 61:359–368. https://doi.org/10.1080/00380768.2014.985578

Zhang X-F et al (2019) Arbuscular mycorrhizal fungi alleviate Cd phytotoxicity by altering Cd subcellular distribution and chemical forms in *Zea mays*. Ecotoxicol Environ Saf 171:352–360. https://doi.org/10.1016/j.ecoenv.2018.12.097

Zhipeng WU, Weidong WU, Shenglu ZH, Shaohua WU (2016) Mycorrhizal inoculation affects Pb and Cd accumulation and translocation in Pakchoi (*Brassica chinensis* L.). Pedosphere 26:13–26. https://doi.org/10.1016/S1002-0160(15)60018-2

Chapter 3
Microbial Enzymes in the Bioremediation of Pollutants: Emerging Potential and Challenges

Geeta Bhandari and Mukund Sharma

Contents

3.1 Introduction

A large number of pollutants such as polychlorinated biphenyl compounds (PCBs), hydrocarbons, dyes, pesticides, esters, heavy metals, petroleum products, and nitrogen-containing chemicals persist in the environment, which are released from various industrial and agricultural resources (Dua et al. 2002; Prasad 2021). These pollutants are highly toxic and carcinogenic in nature, and accumulations of these chemicals become hazardous to the environment and also flora and fauna living in the environment (Wasilkowski et al. 2012). As the increase of contaminated sites poses a major environmental and human health problem, it appears mandatory to decontaminate the environment. Wastes released from various industries and agricultural resources are treated by dumping off in a hole, high-temperature-based incineration, and using UV rays. But these methods don't prove very effective due to their less effectiveness, complex nature, high cost, and formation of other

G. Bhandari (✉) · M. Sharma
Department of Biochemistry and Biotechnology, SBS University, Dehradun, Uttarakhand, India

R. Prasad et al. (eds.), *Mycoremediation and Environmental Sustainability*, Fungal Biology, https://doi.org/10.1007/978-3-030-54422-5_3

recalcitrant derivatives (Vidali 2001). Bioremediation provides a way for the degradation of these chemicals (Dzionek et al. 2016). Bioremediation involves the use of microorganisms and their enzymes for the degradation and transformation of pollutants into another form which is less toxic to the environment. Various species of archaea, bacteria, algae, and fungi demonstrating bioremediation ability have been discovered (Dua et al. 2002). Use of microbes and their enzymes for the removal of pollutants is an effective, safe, and less expensive method (Karigar and Rao 2011; Behera and Prasad 2020).

3.2 Microbial Enzymes in Bioremediation

Enzymes are complex biological macromolecules which act as a catalyst for a number of biochemical reactions involved in the pollutant degradation pathways (Kalogerakis et al. 2017). Enzymes can enhance the rate of a reaction by lowering the activation energy of molecules. Enzymes are central to the biology of many pesticides, influencing their modes of action, environmental fates, and mechanisms of target species resistance. In the last few years, microbial enzymes separated from their cells have been used for bioremediation as compared to using whole microorganisms (Thatoi et al. 2014). They are the main effectors of all the transformations occurring in the biota. They are catalysts with either narrow (chemo-, region-, and stereo-selectivity) or broad specificity and, therefore, they can be applied to a large range of different compounds in a mixture, as well. They may produce extensive transformations of structural and toxicological properties of contaminants and even their complete conversion into innocuous inorganic end products. They may perform processes for which no efficient chemical transformations have been devised. Bioremediation based on purified and partially purified enzymes does not depend on the growth of a particular microorganism in a polluted environment, but it depends upon the catalytic activity of the enzyme secreted by microbes (Ruggaber and Talley 2006). Moreover, enzymes may present advantages over traditional technologies and also over microbial remediation. In nutrient-poor soil, bioremediation can be possible by using a purified enzyme. Toxic side products produced by microbial biotransformation are not produced by using enzymatic biotransformation which is safe to the environment. Indeed, enzymes are not inhibited by inhibitors of microbial metabolism. They can be used under extreme conditions limiting microbial activity. They are effective at low pollutant concentrations and are active in the presence of microbial predators or antagonists and are more mobile than microorganisms because of their smaller size (Gianfreda and Bollag 2002). In comparison to microbes, enzymes are more specific to their substrate and mobile in nature because of their smaller size (Gianfreda and Bollag 2002). There are several types of enzymes such as oxidoreductases, laccases, hydrolases, and peroxidases actively involved in the bioremediation process (Table 3.1; Kadri et al. 2017; Prasad 2017, 2018; Prasad and Aranda 2018).

Table 3.1 Enzymes used in the bioremediation of different pollutants

Enzymes	Microorganisms	Pollutant	Reference
Carbamate hydrolase	*Achromobacter* sp., *Pseudomonas* sp.	Carbofuran, carbaryl	Derbyshire et al. (1987), Mulbry and Eaton (1991)
Laccase	*P. sanguineis*	Bleach plant effluents	Archibald et al. (1990), Limura et al. (1996)
LiP, MnP	*Coriolopsis polyzona*, *Pleurotus ostreatus*, *T. versicolor*	PCBs	Zeddel et al. (1993), Novotny et al. (1997)
LiP, MnP	White-rot fungi	Biopolymers (kraft, lignin)	Reddy (1995), Cameron et al. (2000), Pointing (2001)
Lignin degrading enzymes, Laccase	*Phanerochaete chrysosporium*, *Trametes versicolor*	PAHs	Bogan and Lamar (1996), Bumpus (1989)
MnP, LiP, laccase, chloroperoxidases, peroxidase	White-rot fungi	Phenols, PAH	Gianfreda et al. (1998), Bollag et al. (1988), Nicell (2001)
LiP, MnP, Cellobiose dehydrogenase	*Phanerochaete chrysosporium*	CCl_4, $CHCl_3$	Cameron and Aust (1999)
Peroxidases	*Phanerochaete chrysosporium*	TNT (2,4,6-trinitrotoluene), Nitroaromatic compounds	Cameron et al. (2000)
Laccase	*Pycnoporus sanguineis*	Azo dyes	Pointing and Vrijmoed (2000)
Laccase	*Pycnoporous sanguineus*	Dyes such as bromophenol blue, malachite green	Mayer and Staples (2002)
Carboxylesterases	*Pseudomonas aeruginosa* PA1	Malathion and Parathion	Qiao et al. (2003), Singh et al. (2012)
Laccase	*Trametes versicolor*, *Pleurotus ostreatus*	PCBs	Dodor et al. (2004)
Alkane hydroxylases (monooxygenase and dioxygenase)	*Arthrobacter*, *Burkholderia*, *Mycobacterium*, *Pseudomonas*, *Sphingomonas*, *Rhodococcus*	Hydrocarbon (aromatic and aliphatic)	Das and Chandran (2010)
Atrazine dechlorinase, triazine hydrolase	*Nocardioides* sp. C190, *Pseudomonas*, *Rhodococcus*, *Erythropolis*	Triazine herbicides	Scott et al. (2010)

(continued)

Table 3.1 (continued)

Enzymes	Microorganisms	Pollutant	Reference
Chromium reductase	*Pseudomonas*, *Bacillus*, *Enterobacter*, *Deinococcus*, *Shewanella*, *Agrobacterium*, *Escherichia*, *Thermus*	Chromium	Thatoi et al. (2014)
Phytase	*Aspergillus niger* NCIM 563	Organophosphate	Shah et al. (2017)

3.2.1 Oxidoreductases

The detoxification of toxic organic compounds by various bacteria and fungi and higher plants through oxidative coupling is mediated with oxidoreductases. Microbes extract energy via energy-yielding biochemical reactions mediated by these enzymes to cleave chemical bonds and to assist the transfer of electrons from reductants to oxidants resulting in releasing of chloride ions, CO_2, and methanol. Heat or energy is generated as a result of the degradation of pollutants oxidoreductases, which is utilized by microorganisms for their metabolic activities. During such oxidation-reduction reactions, the contaminants are finally oxidized to harmless compounds (Interstate Technology and Regulatory Council (ITRC) 2002). Oxidoreductases have been used in the degradation of many natural and manmade pollutants. A Gram-positive bacteria *Bacillus safensis* CFA-06 produces oxidoreductase to degrade the petroleum compounds. Lignin degradation in nature produces various kinds of phenolic substances which have been converted into another form by oxidoreductases through polymerization and copolymerization by binding with other compounds (Husain 2006). Color compounds produced from textile industries are released into the environment degraded by various oxidoreductases enzymes such as peroxidases and laccase (Novotny et al. 2004). It was reported that phenols, color, and organic load from olive-mill wastewater were removed by white-rot fungi, *Panus tigrinus*, and its extracellular oxidoreductases such as laccase, Mn-dependent peroxidase, and lignin peroxidase (Annibale et al. 2004). Many bacterial species release oxidoreductase enzymes for the reduction of radioactive metals as a result of redox reactions.

3.2.1.1 Oxygenases

Oxygenases belong to the oxidoreductase group of enzymes. Oxygenases are the main enzymes in the aerobic degradation of aromatic compounds that catalyze the cleavage of the ring in aromatic compounds by transferring oxygen from molecular oxygen (O_2) utilizing FAD/NADH/NADPH as a cosubstrate. On the basis of the number of oxygen molecules involved, oxygenases are classified into two subclasses: monooxygenase (catalyzing the addition of one molecule of an oxygen

atom) and dioxygenase (catalyzing the addition of two molecules of oxygen atom). They play a key role in the metabolism of organic compounds by increasing their reactivity or water solubility or bringing about cleavage of the aromatic ring. Oxygenases have a broad substrate range and are active against a wide range of compounds, including chlorinated aliphatics. Generally, the introduction of O_2 atoms into the organic molecule by oxygenase results in the cleavage of the aromatic rings. Historically, the most studied enzymes in bioremediation are bacterial mono- or dioxygenases (Arora et al. 2009; Fetzner and Lingens 1994; Fetzner 2003).

Monooxygenases

Monooxygenases catalyze the degradation of aromatic compounds by adding one molecule of oxygen into the substrate and enhance their reactivity and solubility. It has been reported that monooxygenases involved in dehalogenation, desulfurization, denitrification, ammonification, hydroxylation, biotransformation, and biodegradation of various aromatic and aliphatic compounds (Arora et al. 2010). Monooxygenases are classified into two groups based on their cofactor used: flavin-dependent monooxygenases and P450 monooxygenases. The monooxygenases comprise a versatile superfamily of enzymes that catalyze oxidative reactions of substrates ranging from alkanes to complex endogenous molecules such as steroids and fatty acids. Monooxygenases act as biocatalysts in the bioremediation process and synthetic chemistry due to their high region selectivity and stereoselectivity on a wide range of substrates. A tightly bound flavin cofactor is present in the flavin-dependent monooxygenase family, reduced by NAD(P)H. Esd (endosulfan diol) and Ese (endosulfan ether) are the members of the TC-FDM (two-component flavin diffusible monooxygenase) family used for the degradation of chlorine-containing pesticides such as endosulfan (Bajaj et al. 2010). Genes encoding the Ese and Esd enzymes were identified in bacteria isolated from endosulfan-exposed soil upon enrichment in sulfur-deficient media with endosulfan or endosulfate supplied as the sole source of sulfur, a technique that targeted the relatively reactive sulfur moiety (Sutherland et al. 2000, 2002c). Ese performs oxidation of one of the methylene groups of endosulfan or endosulfate, producing an unstable intermediate that spontaneously dehydrates the methylene group, allowing bond cleavage and leading to the generation of a sulfur-containing intermediate. The sulfur-containing intermediate of endosulfate metabolism has been identified as endosulfan hemisulfate (Weir et al. 2006). The equivalent metabolite for endosulfan metabolism, endosulfan hemisulfite, was not detected and likely undergoes rapid desulfurization to form endosulfan monoalcohol. Esd works by differential metabolism of the two isomers of endosulfan, with no detectable activity on the alpha isomer. Esd catalyzes the oxidation of one or both of the methylene groups present in β-endosulfan, resulting in the formation of the endosulfan monoalcohol metabolite or endosulfan hydroxyether, respectively (Sutherland et al. 2002a, b).

P450 monooxygenases found in both prokaryotes and eukaryotes are heme-containing enzymes (Galan et al. 2000). P450 enzymes require a non-covalently

bound cofactor to recycle their redox center. A variant of $P450_{cam}$ (F87W\ Y96F\ L244A\V247L) from *Pseudomonas putida* that has been demonstrated to have significant activity against the key chlorinated pollutants pentachlorobenzene (a k_{cat} of 82.5 min^{-1}) and hexachlorobenzene (a k_{cat} of 2.5 min^{-1}) (Chen et al. 2002). This variant of the $P450_{cam}$ enzyme has now been used to endow the capacity to completely degrade hexachlorobenzane upon a *Sphingobium chlorophenolicum* species (Yan et al. 2006). P450 monooxygenase isolated from bacterium *Bacillus megaterium* BM3 has the capacity to degrade a variety of substrates such as fatty acid and aromatic compounds (Roccatano 2015). Methane monooxygenase enzyme is the best characterized one, among monooxygenases. This enzyme is involved in the degradation of hydrocarbon such as substituted methanes, alkanes, cycloalkanes, alkenes, haloalkenes, ethers, and aromatic and heterocyclic hydrocarbons (Fox et al. 1990; Grosse et al. 1999). Methane monooxygenase occurs in two forms: first is found in the cytoplasmic membrane and the second is located in the cytoplasm. Soluble MMOs isolated from bacterium *Methylocella palustris* are capable of degrading a wide range of pollutants such as hydrocarbon, aliphatic, and aromatic compounds (Singh and Singh 2017). Some monooxygenases have also been isolated and characterized that do not require any cofactor for their activity like tetracenomycin F1 monooxygenase (TcmH) isolated from bacterium *Streptomyces glaucescens* and quinol monooxygenase (YgiN) from *E. coli* (Shen and Hutchinson 1993; Arora et al. 2010). Cytochrome P450 is also an important class of monooxygenase family used in many industries to oxidize the contaminant released. More than 200 subfamilies of P450 oxidoreductase are present in both prokaryotes and eukaryotes. All members of P450 oxidoreductase have an iron-containing porphyrin group, and to recycle their redox center, they use a non-covalently bound cofactor.

Dioxygenases

Dioxygenases are multicomponent enzyme systems that introduce molecular oxygen into their substrate. Aromatic hydrocarbon dioxygenases belong to a large family of Rieske nonheme iron oxygenases. These dioxygenases catalyze enantiospecifically the oxygenation of a wide range of substrates. Dioxygenases primarily oxidize aromatic compounds and, therefore, have applications in environmental remediation. All members of this family have one or two electron transport proteins preceding their oxygenase components. On the basis of their mode of action, aromatic dioxygenases can be classified into (1) aromatic ring hydroxylation dioxygenases (ARHDs) and (2) aromatic ring cleavage dioxygenases (ARCDs). ARHDs degrade the chemical compounds by the addition of two molecules of oxygen into the ring, while ARCDs cleave the aromatic rings of compounds (Parales and Ju 2011).

Toluene dioxygenase (TOD) produced by *Pseudomonas putida* F1 catalyzes the first reaction in the degradation of toluene by *P. putida* F1 (Yeh et al. 1977; Gibson et al. 1982). This multicomponent enzyme not only has extremely broad substrate specificity but also acts as a dioxygenase or monooxygenase. TOD acts as a

dioxygenase against a range of compounds including monocyclic aromatics, fused aromatics, linked aromatics, and aliphatic olefins. TOD also acts as a monooxygenase on monocyclic aromatics, aliphatic olefins, and other miscellaneous substrates (Mukherjee and Roy 2013). By these means, it converts different isomers of dimethylbenzene into dimethyl phenols and isomers of nitrotoluene into nitrobenzyl alcohols and nitrophenols (Whited and Gibson 1991; Lange and Wackett 1997). Allylic methyl group monooxygenation can be seen with different halo-propene and halobutene isomers which are converted into butene-1-ol and propene-1-ol, respectively (Resnick et al. 1996).

TOD also has the capacity to catalyze sulfoxidation reactions, converting compounds such as ethyl phenyl sulfide, methyl phenyl sulfide, methyl p-nitophenyl sulfide, and p-methoxymethyl sulfide into their respective sulfoxides (Resnick et al. 1996). TOD has been shown to work efficiently for detoxifying cation of polychlorinated hydrocarbons, chlorotoluenes, and BTEX residues (benzene, toluene, ethylbenzene, and p-xylene) (Resnick et al. 1996). The broad substrate specificity of TOD makes it an ideal enzyme for bioremediation of several key pollutants, including certain pesticide residues. The TOD enzyme complex has been resolved into three components: an iron-sulfur protein (ISP TOL), a flavoprotein (reductase TOL), and an iron sulfur-dependent ferredoxin (ferredoxin TOL) (Yeh et al. 1977; Gibson et al. 1982; Subramanian et al. 1979, 1981, 1985). The reductase TOL initially accepts electrons from NADH and then transfers these electrons to ferredoxin TOL. The latter reduces the terminal ISP TOL that functions as the oxygenase component. Reduced ISP TOL catalyzes the addition of both atoms of molecular oxygen into the aromatic nucleus of toluene to form *cis*-toluene dihydrodiol, which is eventually mineralized by other enzymes encoded by the toluene dioxygenase gene cluster (*tod* operon) (Zylstra and Gibson 1989).

The catechol dioxygenases are found in the soil bacteria causes the biotransformation of aromatic precursors into aliphatic products (Muthukamalam et al. 2017). Ring-opening 2,4 dioxygenases help in the bioremediation of quinaldine, and 1H-4-oxoquinoline catalyzes the breakdown of two carbon-carbon bonds with formation of carbon monoxide (Ali et al. 2017). A large number of aromatic compounds are released into the environment from various pharmaceutical, chemical, and dye industries. To incorporate two molecules of oxygen into the substrate, dioxygenase breaks down the aromatic ring at 1, 2-position (Guzik et al. 2013). Naphthalene dioxygenases isolated from *Pseudomonas putida* involve in the naphthalene degradation (Gennaro et al. 1997).

3.2.1.2 Laccases

Laccases are copper-containing oxidases, which catalyzes the oxidation of a wide range of phenolic and aromatic compounds. It is found in multiple isoforms produced by varying species of bacteria, fungi, insects, and plants. Laccases are always produced in the cell but can be secreted extracellular, able to degrade the ortho- and paradiphenols, amino group containing phenols, and lignin and aryl groups

containing diamines (Mai et al. 2000). Laccase also decolorized azo dyes by oxidizing their bonds and transformed into less harmful substances present in the environment (Legerska et al. 2016).

Many microorganisms produce intra- and extracellular laccases capable of catalyzing the oxidation of ortho and paradiphenols, aminophenols, polyphenols, polyamines, lignins, and aryl diamines as well as some inorganic ions (Mai et al. 2000; Ullah et al. 2000; Couto and Herrera 2006). Laccases not only oxidize phenolic and methoxyphenolic acids but also decarboxylate them and attack their methoxy groups (demethylation). These enzymes are involved in the depolymerization of lignin, which results in a variety of phenols. In addition, these compounds are utilized as nutrients for microorganisms or repolymerized to humic materials by laccase (Kim et al. 2002).

Laccases produced by *R. practicola* have the ability to degrade and biotransform phenolic compounds (Strong and Claus 2011). Laccase immobilization on solid support increases their stability, half-life, and resistance to protease enzymes (Dodor et al. 2004). It proves a powerful enzyme for the bioremediation of a wide range of pollutants such as phenolic compounds, aromatic heterocyclic compounds, and amine-containing aromatic compounds. Laccase can reduce the dioxygen molecules of pollutants into the water by the removal of electrons from the organic substrate (Chakroun et al. 2010). The X-ray crystal structures of laccases deposited in Protein Data Bank (PDB) were used for docking studies with two-dimensional structures of pollutants, downloaded from the NCBI database. An online tool, CORINA, was used for the conversion of 2-D structures of pollutants into three-dimensional structures. Further, GOLD was used for docking of protein-ligand. Nearly 30% and 17% of the selected datasets showed the best average GOLD fitness score for fungal and bacterial laccase enzymes, respectively, thereby suggesting that laccase might be able to oxidize these pollutants (Suresh et al. 2008).

3.2.2 Peroxidases

Peroxidases are ubiquitous in nature, produced by animals, plants, fungi, and bacteria. Peroxidases help in the degradation of lignin, phenolic, and other aromatic compounds by using hydrogen peroxide in the presence of a mediator. Phenolic radicals produced by oxidation of phenolic compounds and aggregates become less soluble and precipitated quickly. These peroxidases can be heme and nonheme proteins. (Bansal and Kanwar 2013). The heme-containing peroxidases can be divided into two groups: one group found only in animals and the other group found in fungi, bacteria, and plants. Peroxidases found in bacteria, fungi, and plants are further divided into three classes: intracellular enzyme found in class 1 including cytochrome c peroxidase produced by yeast, ascorbate peroxidase (APX) produced by some species of plants, and bacterial catalase peroxidases. Class 2 containing secreted fungal enzyme include lignin peroxidase (LiP) and manganese peroxidase (MnP). The main role of class II peroxidases appears to be the degradation of lignin

in wood. Class 3 contains plant-secreted peroxides such as horseradish peroxidases (HRP) from horseradish plants. Nonheme peroxidases are from five different families, thiol peroxidase, alkylhydroperoxidase, haloperoxidase, manganese catalase, and NADH peroxidase (Koua et al. 2009). Among all, lignin peroxidase and manganese peroxidase have greater potential for the degradation of toxic substances and most studied enzymes. Horseradish peroxidase-immobilized cross-linked enzyme aggregates (HRP-CLEAs) were produced using a cross-linking agent, i.e., ethylene glycol-bis[succinic acid Nhydroxysuccinimide, (EG-NHS)]. The efficiency of biodegradation of HRP-CLEAs was tested by using a packed bed reactor system (Bilal et al. 2017). HRP also causes the oxidative para-dechlorination of toxic contaminant and carcinogen 2,4,6-trichlorophenol (Sumithran et al. 2012). Soybean peroxidase and chloroperoxidase were studied for the degradation of thiazole compounds (Alneyadi and Ashraf 2016).

3.2.2.1 Lignin Peroxidases

Lignin peroxidases (LiPs) are monomeric heme-containing proteins and secondary metabolites secreted by fungi such as *Phanerochaete chrysosporium* and *Trametes versicolor* and bacteria (Xu et al. 2014). In LiPs, Fe (III) is pentacoordinated with histidine residue and four heme tetrapyrrole nitrogens. It catalyzes the oxidation of toxic pollutants in the presence of cosubstrate hydrogen peroxide and mediator veratryl alcohol. The reaction mechanism of LiPs involved the two-electron oxidation of the native ferric enzyme (Fe (III)) by H_2O_2 to form a compound I in the initial phase that is reduced by a reducing substrate with gaining of one electron to form compound II. In the final phase, compound II obtains a second electron from the reduced substrate with the returning of the enzyme in their native ferric oxidation state (Abdel-Hamid et al. 2013). LiPs show great application for the treatment of wastewater and in the field of bioremediation (Tuomela and Hatakka 2011).

3.2.2.2 Manganese Peroxidases

Manganese peroxidases (MnPs) are heme-containing extracellular enzymes produced by lignin-degrading fungi that can oxidize Mn^{2+} into Mn^{3+} by the multistep reaction. Several acidic amino acid residues and one heme group containing a manganese binding site are present in enzyme MnP. Additionally, Mn^{2+} contributes a single electron to compound I of MnP and acts as best reducing substrates. This chelator is considered to act indirectly to degrade lignin and xenobiotic compounds. These catalyze the degradation of several phenols, amine-containing aromatic compounds, and dyes (Have and Teunissen 2001). MnPTra- 48424 was identified and purified from white-rot fungi *Trametes* sp. 48424. This enzyme has strong capability to decolorize different kinds of dyes such as indigo, anthraquinone, azo, and triphenylmethane, while other dyes such as indigo carmine and methyl green combined with heavy metal ions and organic solvent can also be degraded by

MnP-Tra-48424 enzyme. Different polycyclic aromatic hydrocarbons (PAHs) are also degraded by MnP-Tra- 48424 purified (Zhanga et al. 2016). During the degradation of anthracene, gene (pimp1) encoding manganese-dependent peroxidase was found in *P. incarnata* KUC8836. This gene was further expressed in fungi *Saccharomyces cerevisiae* to enhance the bioremediation process (Lee 2016).

3.2.3 Hydrolases

Hydrolytic enzymes are most commonly used for the bioremediation of pesticides and insecticides and reduction in their toxicity. Major chemical bonds such as esters, peptide bonds, and carbon-halide bonds are disrupted by different hydrolytic enzymes and generally operate in the absence of redox cofactors. Using bioremediation for the degradation of toxic organic compounds is safe and economical as compared to physicochemical treatment (Karigar and Rao 2011). An extracellular hydrolase secreted by microbes catalyzes the bioremediation of organic polymers, toxic compounds with less than 600 Da molecular weights that can pass through cell pores (Vasileva-Tonkova and Galabova 2003). Bioremediation of oil spill, organophosphate, and carbamate insecticides by using hydrolytic enzyme is very effective. Extracellular hydrolytic enzymes used in the food industry and chemical industry include proteases, lipases, xylanases, DNAses, and amylases. The hemicellulase, cellulase, and glycosidase are used for biomass degradation (Porro et al. 2003). Recently, carbendazim, widely used fungicide, hydrolyzing enzyme encoding gene has been isolated from *Microbacterium* sp. djl-6F and cloned into *Escherichia coli* BL21 (DE3) by Lei et al. (2017) to increase the levels of the enzyme. This enzyme was able to hydrolyze carbendazim into 2-aminobenzimidazole.

3.2.3.1 Lipases

Lipases are ubiquitous in nature and catalyze the breakdown of triacylglycerols into glycerol and free-fatty acids which are major constituents of hydrocarbons. Lipases are produced by many species of bacteria, plants, actinomycetes, and animal cells (Shukla and Gupta 2007). Hydrolysis, interesterification, esterification, alcoholics, and aminolysis reactions are carried out by lipases (Prasad and Manjunath 2011). The level of hydrocarbon in the contaminated soil was decreased due to lipase activity. These enzymes hydrolyze the fatty acids into triglycerol, diacylglycerol, monoacylglycerol, and glycerol (Ghafil et al. 2016). Different statistical tools were used to optimize media to enhance the production of microbial lipases. Using statistical tools, the medium was optimized for the production of a novel crude oil-degrading lipase from fungus *Pseudomonas aeruginosa* SL-72 for the bioremediation of crude oils (Verma et al. 2012).

3.2.3.2 Cellulases

Cellulases are the key enzymes for the degradation of cellulose, the most abundant biopolymer found on the earth. Cellulase produced by microorganisms can be cell-bound, associated with the cell envelope, and extracellular (Yang et al. 2016). Cellulases are used in the detergent manufacturing industries, where cellulose microfibrils produced during processes are removed by these enzymes. Some alkaline cellulases are produced by *Bacillus* strains and neutral and acidic cellulases by *Trichoderma* and *Humicola* fungi (Hmad and Gargouri 2017). These cellulases have been employed for the bioremediation of ink in paper and pulp industry during the recycling of paper (Karigar and Rao 2011).

3.2.3.3 Carboxylesterases

Degradation of synthetic compounds and natural products such as organophosphates, ester bond of carbamates, and chlorine-containing organic compounds has been catalyzed by enzyme carboxylesterases (Cummins et al. 2007). Carboxylesterases has been used for the degradation of pesticides, insecticides, and fungicide spray in the fields. For the absorption of mercury in the polluted site, carboxylesterases E2 from strain *P. aeruginosa* PA1 was displayed on the outer membrane of *E. coli* (Yin et al. 2016). Ester bond of synthetic pyrethroids has been hydrolyzed by carboxylesterases using a common pathway for the degradation of all types of pyrethroids insecticides.

3.2.3.4 Phosphotriesterases

Phosphotriesterases have the potential for the degradation of chemical waste released from industries and pesticides such as parathion used in crop fields (Romeh and Hendawi 2014). The bacterial phosphotriesterases are a subgroup of the amidohydrolase metalloenzyme family. The phosphotriesterases primarily catalyze the hydrolysis of OP trimesters. Two closely related bacterial phosphotriesterases have been extensively characterized: OpdA from *Agrobacterium radiobacter* (Harcourt et al. 2002) and OPH from *Pseudomonas diminuta* (Serdar et al. 1985) and *Flavobacterium* (Mulbry et al. 1986). Field trials of OpdA as a bioremediation agent have been conducted (Sutherland et al. 2004), and it is already in use as a commercial product to detoxify OP residues in various contaminated wastes, sold under the brand name LandGuard™ from Orica Watercare (Australia) at a cost to user considerably lower than the pesticides themselves. Both OPH and OpdA display extraordinary catalytic efficiency for OPs, vastly superior to that of the E3 mutants described above; for instance, the k cat /K m of OpdA for the pesticide methyl parathion is in the order of 3×106 sec^{-1} M^{-1} (Yang et al. 2003). The catalytic mechanism is thought to proceed via direct in-line nucleophilic attack from a water

molecule, activated through its interaction with the Fe2+ ion, at the electrophilic phosphorus of the substrate, which coordinates to the Zn2+ ion (Jackson et al. 2005).

Parathion is an organophosphate containing compound, used as a component in herbicides and insecticides (Gao et al. 2014). Organophosphate is an ester of phosphoric acid degraded by phosphotriesterases also known as aryldialkylphosphatase and organophosphorus hydrolase. Three recombinants thermostable phosphotriesterase, SsoPox W263F, and SsoPox C258L/I261F/ W263A, whose gene isolated from wild-type *Sulfolobus solfataricus*, and SacPox isolated from *Sulfolobus acidocaldarius* were produced and purified (Restaino et al. 2016). Some strains of marine bacteria such as *Phaeobacter* sp., *Ruegeria mobilis*, and *Thalassospira tepidiphila* have ability to degrade the phosphate triester present in coastal oceanic conditions (Yamaguchi et al. 2016). From bacteria *Geobacillus stearothermophilus* (GsP), a new enzyme homologous to phosphotriesterases was identified which has the capacity to hydrolyze both lactone- and organophosphate-containing compounds. Phosphotriesterase-like lactonase (PLL) isolated from bacteria *Geobacillus stearothermophilus* (GsP) are extremely thermostable and can be active at temperature 100 °C (Hawwa et al. 2009).

3.2.3.5 Haloalkane Dehalogenases

Halogenated compounds produced as a result of both natural activities and man-made efforts are present everywhere in soil and can be hazardous, toxic, mutagenic, or carcinogenic (Koudelakova et al. 2013). Haloalkane dehalogenases used for the hydrolysis of carbon halogen bond present in the various halogens containing contaminants produce alcohol and halides (Kotik and Famerova 2012). The active site of haloalkane dehalogenase is present between two domains. The main domain of enzyme composed of an eight stranded b-sheet surrounded by a-helices (Pavlova et al. 2007). First haloalkane dehalogenase discovered in bacterium *Xanthobacter autotrophicus* GJ10 has the ability to degrade 1, 2- dichloroethane (Nagata et al. 2015). After that, several dehalogenases have been cloned and characterized from Gram-positive and Gram-negative haloalkane-degrading bacteria.

Genes encoding the enzymes responsible for bacterial degradation of γ-isomer of hexachlorocyclohexane (γ-HCH, commonly known as lindane) have been cloned and studied extensively. The two key enzymes are encoded by the *linA* and *linB* genes. LinB is a haloalkane dehalogenase of the α/β−hydrolase fold family of enzymes that shows significant similarity to three other α/β-hydrolase fold enzymes: haloalkane dehalogenase (DhlA) from *Xanthobacter autotrophicus* GJ10, haloacetate dehalogenase (DehH1) from *Moraxella* sp. B, and 2-hydroxymuconic semialdehyde hydrolase (DmpD) from *Pseudomonas* sp. CF600 (Nagata et al. 1993a, b). LinB mediates the two sequential chlorohydrolase reactions converting 2,3,5,6-tetr achloro-1,4-cyclohexadiene to 3,6-dichloro-2,5-dihydoxy-1,4-cyclohexadiene (Negri et al. 2007). In addition, LinB has been found to be involved in the degradation of β-HCH in *Sphingomonas paucimobilis* (Nagata et al. 2005) and of β- and δ-HCH in *Sphingobium indicum* B90A, *Sphingobium francense* SpC, and

Sphingobium japonicum UT26, although the ability to degrade β-HCH and δ-HCH differs between these strains (Sharma et al. 2006). The reaction mechanism of LinB involves nucleophilic attack from the aspartic acid residue 108 at an electrophilic carbon of the substrate, followed by the formation of a covalent alkyl-enzyme intermediate. The catalytic aspartic acid is then regenerated through a nucleophilic attack at Asp108 upon the activation of a water molecule by histidine 272 (Prokop et al. 2003).

3.2.4 Lyases

Lyases catalyze the cleavage of bonds in the absence of redox cofactors or water, including the energetically demanding cleavage of carbon-carbon bonds (pyruvate-formate lyase, for example) (Sawers 1998) and carbon bonds with phosphorus, oxygen, nitrogen, halides, and sulfur.

3.2.4.1 Haloalkane Dehydrochlorinases: LinA

The *linA*-encoded HCH dehydrochlorinase (LinA) mediates the first two steps of dehydrochlorination of the insecticide γ-HCH (Nagata et al. 1993a, b), which is further catabolized by the remaining enzymes encoded by the *lin* operon. The structure of LinA has not yet been resolved, but it is predicted to belong to a novel superfamily which includes scytalone dehydratase and naphthalene dioxygenase (Nagata et al. 2001). The reaction mechanism proposed for LinA is dependent upon a catalytic dyad (Asp25 and His73) (Trantirek et al. 2001), where a proton is abstracted from HCH by His73 followed by the release of a chloride ion and formation of a carbon-carbon double bond. This process is then repeated with the product (pentchlorocyclohexene) to ultimately yield 2,3,5,6-tetrachloro-1,4-cyclohexadiene (Trantirek et al. 2001). HCH-contaminated soil was treated by the controlled release of a bacterium (*Sphingobium indicum*) containing the naturally occurring *lin*-operon which has led to significant remediation of the pesticide residue (up to 95%) (Raina et al. 2008).

3.3 Conclusion

Due to increased population, urbanization, and industrialization, accumulation of harmful pollutants in the environment has reached an alarming level. Bioremediation is a promising approach for the removal and decontamination of these pollutants. Bioremediation using specific enzymes is a more efficient and cost-effective alternative. A diverse range of microbes from different natural sources have been

explored in the isolation of enzymes containing biodegradative ability. A diverse family of enzymes such as oxidoreductase, laccases, and peroxidases demonstrating bioremediation ability has been isolated and characterized. Enzymatic bioremediation can provide real benefits to the environment, avoiding the conditions that are required for whole-cell applications, especially in extreme environments. Furthermore, enzymatic effectiveness can be improved in vitro also using molecular tools, such as DNA engineering, to generate super bioremediators, which can present advantages in field. Additionally, the catalytic activity, self-life, and stability in stress conditions of enzymes can be enhanced up to remarkable levels by enzyme engineering and immobilization techniques. Recently, cost-effective strategies in the production of nanoparticles and nanoparticle-based materials are attracting great interest for their unique properties and immense application potential in diverse areas. Single-enzyme nanoparticles (SENs) related to nanoparticles have been developed in which each enzyme molecule is surrounded by a hybrid organic/inorganic polymer network. These nanoparticles have the potential to bind with the xenobiotic compounds and degrade them completely or transform in less harmful derivatives which further help in cleaning the environment. Nevertheless, the technologies described above are complete for effectual bioremediation, and the need for the optimal technological intervention is the key for evolved, efficient, and eco-friendly strategies.

References

Abdel-Hamid AM, Solbiati JO, Cann IK (2013) Insights into lignin degradation and its potential industrial applications. Adv Appl Microbiol 82:28

Ali MI, Khatoon N, Jamal A (2017) Polymeric pollutant biodegradation through microbial oxidoreductase; a better strategy to safe environment. Int J Biol Macromol 105:9–16. https://doi.org/10.1016/j.ijbiomac.2017.06.047

Alneyadi AH, Ashraf SS (2016) Differential enzymatic degradation of thiazole pollutants by two different peroxidases: a comparative study. Chem Eng J 303:529–538

Annibale AD, Ricci M, Quaratino D, Federici F, Fenice M (2004) *Panus tigrinus* efficiently removes phenols, color and organic load from olive-mill wastewater. Res Microbiol 155:596–603

Archibald FS, Paice MG, Jurasek L (1990) Decolorization of kraft bleachery effluent chromophores by *Coriolus* (*Trametes*) *versicolor*. Enzym Microb Technol 12:846–853

Arora PK, Kumar M, Chauhan A, Raghava GP, Jain RK (2009) OxDBase: a database of oxygenases involved in biodegradation. BMC Res Notes 2:67

Arora PK, Srivastava A, Singh VP (2010) Application of monooxygenases in dehalogenation, desulphurization, denitrification and hydroxylation of aromatic compounds. J Bioremed Biodegr 1:112

Bajaj A, Pathak A, Mudiam MR, Mayilraj S, Manickam N (2010) Isolation and characterization of a Pseudomonas sp. strain IITR01 capable of degrading endosulfan and endosulfan sulfate. J Appl Microbiol 109:2135–2143

Bansal N, Kanwar SS (2013) Peroxidase(s) in environment protection. Sci World J. https://doi.org/10.1155/2013/714639

Behera BK, Prasad R (2020) Environmental Technology and Sustainability. Elsevier (ISBN: 9780128191033) https://www.elsevier.com/books/environmental-technology-and-sustainability/behera/978-0-12-819103-3

Bilal M, Asgherb M, Parra-Saldivarc R, Hua H, Wanga W, Zhanga X, Iqbal HMN (2017) Immobilized ligninolytic enzymes: an innovative and environmental responsive technology to tackle dye-based industrial pollutants e a review. Sci Total Environ 576:646–659

Bogan BW, Lamar RT (1996) Polycyclic aromatic hydrocarbon-degrading capabilities of *Phanerochaete laevis* HHB-1625 and its extra cellular ligninolytic enzymes. Appl Environ Microbiol 62:1597–1603

Bollag JM, Shuttleworth KL, Anderson DH (1988) Laccase-mediated detoxification of phenolic compounds. Appl Environ Microbiol 54:3086–3091

Bumpus JA (1989) Biodegradation of polycyclic aromatic hydrocarbons by *Phanerochaete chrysosporium*. Appl Environ Microbiol 55:154–158

Cameron MD, Aust SD (1999) Degradation of chemicals by reactive radicals produced by cellobiose dehydrogenase from *Phanerochaete chrysosporium*. Arch Biochem Biophys 367:115–121

Cameron MD, Timofeevski S, Aust SD (2000) Enzymology of *Phanerochaete chrysosporium* with respect to the degradation of recalcitrant compounds and xenobiotics. Appl Microbiol Biotechnol 54:751–758

Chakroun H, Mechichi T, Martinez MJ, Dhouib A, Sayadi S (2010) Purification and characterization of a novel laccase from the ascomycete *Trichoderma atroviride*: application on bioremediation of phenolic compounds. Process Biochem 45:507–513

Chen X, Christopher A, Jones JP, Bell SG, Guo Q, Xu F, Roa Z, Wong LL (2002) Crystal structure of the F87W/ Y96F/V247L mutant of cytochrome P-450_{cam} with 1,3,5-trichlorobenzene bound and further protein engineering for the oxidation of pentachlorobenzene and hexachlorobenezene. J Biol Chem 277:37519–37526

Couto SR, Herrera JLT (2006) Industrial and biotechnological applications of laccases: a review. Biotechnol Adv 24:500–513

Cummins I, Landrum M, Steel PG, Edwards R (2007) Structure activity studies with xenobiotic substrates using carboxylesterases isolated from *Arabidopsis thaliana*. Phytochemistry 68:811–818

Das N, Chandran P (2010) Microbial degradation of petroleum hydrocarbon contaminants: an overview. Biotechnol Res Int 2011:1–13

Derbyshire MK, Karns JS, Kearney PC, Nelson JO (1987) Purification and characterization of an *N*-methylcarbamate pesticide hydrolyzing enzyme. J Agric Food Chem 35:871–877

Dodor DE, Hwang HM, Ekunwe SI (2004) Oxidation of anthracene and benzo[a] pyrene by immobilized laccase from *Trametes versicolors*. Enzyme Microbial Technol 35:3210–3217

Dua M, Singh A, Sethunathan N, Johri AK (2002) Biotechnology and bioremediation: successes and limitations. Appl Microbiol Biotechnol 59:143–152

Dzionek A, Wojcieszynska D, Guzik U (2016) Natural carriers in bioremediation: a review. Electron J Biotechnol 23:28–36

Fetzner S (2003) Oxygenases without requirement for cofactors or metal ions. Appl Microbiol Biotechnol 3:243–257

Fetzner S, Lingens F (1994) Bacterial dehalogenases: biochemistry, genetics, and biotechnological applications. Microbiol Rev 58:641–685

Fox BG, Borneman JG, Wackett LP, Lipscomb JD (1990) Haloalkene oxidation by the soluble methane monooxygenase from *Methylosinus trichosporium* OB3b: mechanistic and environmental implications. Biochemist 29:6419–6427

Galan B, Diaz E, Prieto MA, Garcia JL (2000) Functional analysis of the small component of the 4-hydroxyphenylacetate 3-monooxygenase of *Escherichia coli* W: a prototype of a new flavin: NAD(P)H reductase subfamily. J Bacteriol 182:627–636

Gao Y, Truong YB, Cacioli P, Butler P, Kyratzis IL (2014) Bioremediation of pesticide contaminated water using an organophosphate degrading enzyme immobilized on nonwoven polyester textiles. Enzym Microb Technol 54:38–44

Gennaro PD, Galli E, Albini G, Pelizzoni F, Sello G, Bestetti G (1997) Production of substituted naphthalene dihydrodiols by engineered *Escherichia coli* containing the cloned naphthalene 1,2-dioxygenase gene from *Pseudomonas fluorescens*N3. Res Microbiol 148:355–436

Ghafil JA, Hassan SS, Zgair AK (2016) Use of immobilized lipase in cleaning up soil contaminated with oil. World J Exp Biosci 4:53–57
Gianfreda L, Bollag JM (2002) Isolated enzymes for the transformation and detoxification of organic pollutants. Marcel Dekker, New York, pp 495–538
Gianfreda L, Sannino F, Filazzola MT, Leonowicz A (1998) Catalytic behavior and detoxifying ability of a laccase from the fungal strain *Cerrena unicolor*. J Mol Catal B 14:13–23
Gibson DT, Yeh WK, Liu TN, Subramanian V (1982) Toluene dioxygenase: a multicomponent enzyme system from *Pseudomonas putida*. In: Nozaki M, Yamamoto S, Ishimura Y, Coon MJ, Ernster L, Estabrook RW (eds) Oxygenases and oxygen metabolism. Academic Press, Inc., New York, pp 51–62
Grosse S, Laramee L, Wendlandt KD, McDonald IR, Miguez CB, Kleber HP (1999) Purification and characterization of the soluble methane monooxygenase of the type II methanotrophic bacterium *Methylocystis* sp. strain WI 14. Appl Environ Microbiol 65:3929–3935
Guzik U, Kocurek KH, Wojcieszynska D (2013) Intradiol dioxygenases: the key enzymes in xenobiotics degradation. In: Biodegradation of hazardous and special products. https://doi.org/10.5772/56205
Harcourt RL, Horne I, Sutherland TD, Hammock BD, Russell RJ, Oakeshott JG (2002) Development of a simple and sensitive fl uorimetric method for isolation of coumaphos-hydrolysing bacteria. Lett Appl Microbiol 34:263–268
Have RT, Teunissen PJ (2001) Oxidative mechanisms involved in lignin degradation by white-rot fungi. Chem Rev 101:3397–3413
Hawwa R, Aikens J, Turner RJ, Santarsiero BD, Mesecar AD (2009) Structural basis for thermostability revealed through the identification and characterization of a highly thermostable phosphotriesterase-like lactonase from *Geobacillus stearothermophilus*. Arch Biochem Biophys 488:109–120
Hmad IB, Gargouri A (2017) Neutral and alkaline cellulases: production, engineering, and applications. J Basic Microbiol 57:653–658
Husain Q (2006) Potential applications of the oxidoreductive enzymes in the decolorization and detoxification of textile. Crit Rev Biotechnol 26:201–221
Interstate Technology and Regulatory Council (ITRC) (2002) A systematic approach to in situ bioremediation in ground eater including decisions tees on in situ bioremediation for nitrates, carbon tetrachloride and perchlorate: technical regulatory guidelines
Jackson C, Kim HK, Carr PD, Liu JW, Ollis DL (2005) The structure of an enzyme-product complex reveals the critical role of a terminal hydroxide nucleophile in the bacterial phosphotriesterase mechanism. Biochim Biophys Acta 1752:56–64
Kadri T, Rouissi T, Brar SK, Cledon M, Sharma S, Verma M (2017) Biodegradation of polycyclic aromatic hydrocarbons (PAHs) by fungal enzymes: a review. J Environ Sci 51:52–74
Kalogerakis N, Fava F, Corvine PF (2017) Bioremediation advances. N Biotech 38:41–42
Karigar CS, Rao SS (2011) Role of microbial enzymes in the bioremediation of pollutants: a review. Enzym Res 7. https://doi.org/10.4061/2011/805187
Kim JS, Park W, Lee SE, Kim JE (2002) Formation of bound residues of 8-hydroxybentazon by oxidoreductive catalysts in soil. J Agric Food Chem 50:3507–3511
Kotik M, Famerova V (2012) Sequence diversity in haloalkane dehalogenases, as revealed by PCR using family-specific primers. J Microbiol Methods 88:212–217
Koua D, Cerutti L, Falquet L (2009) PeroxiBase: a database with new tools for peroxidase family classification. Nucleic Acids Res 37:D261–D266
Koudelakova T, Bidmanova S, Dvorak P, Pavelka A, Chaloupkova R, Prokop Z, Damborsky J (2013) Haloalkane dehalogenases: biotechnological applications. Biotechnol J 8:32–45
Lange CC, Wackett LP (1997) Oxidation of aliphatic olefi ns by toluene dioxygenase: enzyme rates and product identifi cation. J Bacteriol 179:3858–3865
Lee AH (2016) Heterologous expression of a new manganese-dependent peroxidase gene from Peniophora incarnata KUC8836 and its ability to remove anthracene in *Saccharomyces cerevisiae*. J Biosci Bioeng 122:716–721

Legerska B, Chmelova D, Ondrejovic M (2016) Degradation of synthetic dyes by laccase- a mini review. Nova Biotechnologica et Chimica 15e1, https://doi.org/10.1515/nbec-2016-0010

Lei J, Wei S, Ren L, Hu S, Chen P (2017) Hydrolysis mechanism of carbendazim hydrolase from the strain *Microbacterium* sp. djl-6F. J Environ Sci 54:171–177

Limura Y, Hartikainen P, Tatsumi K (1996) Dechlorination of tetrachloroguaiacol by laccase of white-rot basidiomycete *Coriolus versicolor*. Appl Microbiol Biotechnol 45:434–439

Mai C, Schormann W, Milstein O, Huttermann A (2000) Enhanced stability of laccase in the presence of phenolic compounds. Appl Microbiol Biotechnol 54:510–514

Mayer AM, Staples RC (2002) Laccase: new functions for an old enzyme. Phytochemistry 60:551–565

Mukherjee P, Roy P (2013) Cloning, sequencing and expression of novel trichloroethylene degradation genes from *Stenotrophomonas maltophilia* PM102: a case of gene duplication. J Bioremed Biodegr 4:1000177

Mulbry WW, Eaton RW (1991) Purification and characterization of the *N*-methylcarbamate hydrolase from *Pseudomonas* strain CRL-OK. Appl Environ Microbiol 57:3679–3682

Mulbry WW, Karns JS, Kearney PC, Nelson JO, McDaniel CS, Wild JR (1986) Identification of a plasmid-borne parathion hydrolase gene from Flavobacterium sp. by southern hybridization with opd from *Pseudomonas diminuta*. Appl Environ Microbiol 51:926–930

Muthukamalam S, Sivagangavathi S, Dhrishya D, Ran S (2017) Characterization of dioxygenases and biosurfactants produced by crude oil degrading soil bacteria. Braz J Microbiol. https://doi.org/10.1016/j.bjm.2017.02.007

Nagata Y, Nariya T, Ohtomo R, Fukuda M, Yano K, Takagi M (1993a) Cloning and sequencing of a dehalogenase gene encoding an enzyme with hydrolase activity involved in the degradation of γ-hexachlorohexane in *Pseudomonas paucimobilis*. J Bacteriol 175:6403–6410

Nagata Y, Imai R, Sakai A, Fukuda M, Yano K, Takagi M (1993b) Isolation and characterisation of Tn5-induced mutants of *Pseudomonas paucimobilis* UT26 defective in γ-hexachlorocyclohexane dehydrochlorinase (LinA). Biosci Biotechnol Biochem 57:703–709

Nagata Y, Mori K, Takagi M, Murzin AG, Damborsky J (2001) Identification of protein fold and catalytic residues of γ-hexachlorocyclohexane dehydrochlorinase *Lin*A. Proteins 45:471–477

Nagata Y, Prokop Z, Sato Y, Jerabek P, Kumar A, Ohtsubo Y, Tsuda M, Damborsky J (2005) Degradation of β-hexachlorcyclohexane by haloalkane dehalogenase LinB from *Sphingomonas paucimobilis* UT26. Appl Environ Microbiol 71:2183–2185

Nagata Y, Ohtsubo Y, Tsuda M (2015) Properties and biotechnological applications of natural and engineered haloalkane dehalogenases. Appl Microbiol Biotechnol 99:9865–9881

Negri A, Marco E, Damborsky J, Gago F (2007) Stepwise dissection and visualization of the catalytic mechanism of haloalkane dehalogenase LinB using molecular dynamics simulations and computer graphics. J Mol Graph Model 26:643–651

Nicell JA (2001) Environmental applications of enzymes. Interdisci Environ Rev 3:14–41

Novotny C, Vyas BRM, Erbanova P, Kubatova A, Sasek V (1997) Removal of various PCBs by various white-rot fungi in liquid cultures. Folia Microbiol 42:136–140

Novotny C, Svobodova K, Erbanova P, Cajthamal T, Kasinath A, Lang E, Sasek V (2004) Ligninolytic fungi in bioremediation: extracellular enzyme production and degradation rate. Soil Biol Biochem 36:1545–1551

Parales RE, Ju KS (2011) Rieske-type dioxygenases: key enzymes in degradation of aromatic hydrocarbons. In: Comprehensive Biotechnology, vol 6: Environmental biotechnology and safety, second ed., pp. 115–134. Elsevier, UK

Pavlova M, Klvaka M, Jesenska A, Prokop Z, Konebna H, Sato T, Tsuda M, Nagata Y, Damborsky J (2007) The identification of catalytic pentad in the haloalkane dehalogenase Dhm A from *Mycobacterium avium* N85: reaction mechanism and molecular evolution. J Struct Biol 157:384–392

Pointing SB (2001) Feasibility of bioremediation by white-rot fungi. Appl Microbiol Biotechnol 57:20–32

Pointing SB, Vrijmoed LLP (2000) Decolorization of azo and triphenylmethane dyes by *Pycnoporus sanguineus* producing laccase as the sole phenoloxidase. World J Microbiol Biotechnol 16:317–318

Porro CS, Martın S, Mellado E, Ventosa A (2003) Diversity of moderately halophilic bacteria producing extracellular hydrolytic enzymes. J Appl Microbiol 94:295–300

Prasad MP, Manjunath K (2011) Comparative study on biodegradation of lipid-rich wastewater using lipase producing bacterial species. Indian J Biotechnol 10:121–124

Prasad R (2017) Mycoremediation and Environmental Sustainability. Volume 1. Springer International Publishing (ISBN 978-3-319-68957-9) https://link.springer.com/book/10.1007/978-3-319-68957-9

Prasad R (2018) Mycoremediation and Environmental Sustainability, Volume 2. Springer International Publishing (ISBN 978-3-319-77386-5) https://www.springer.com/us/book/9783319773858

Prasad R (2021) Environmental Pollution and Remediation. Springer (ISBN 978-981-15-5499-5) https://www.springer.com/gp/book/9789811554988

Prasad R and Aranda E (2018) Approaches in Bioremediation: The New Era of Environmental Microbiology and Nanobiotechnology. Springer International Publishing (978-3-030-02369-0) https://www.springer.com/gp/book/9783030023683

Prokop Z, Moninvoca M, Chaloupkova R, Klvana M, Nagata Y, Janssen DB, Damborsky J (2003) Catalytic mechanism of the haloalkane dehalogenase LinB from *Sphingomonas paucimobilis* UT26. J Biol Chem 278:45094–45100

Qiao CL, Huang J, Li X, Shen BC, Zhang JL (2003) Bioremediation of organophosphate pollutants by a genetically-engineered enzyme. Bull Environ Contam Toxicol 70:455–461

Raina V, Suar M, Singh A, Prakash O, Dadhwal M, Gupta SK, Dogra C, Lawlor K, Lal S, van der Meer JR, Holliger C, Lal R (2008) Enhanced biodegradation of hexachlorocyclohexane (HCH) in contaminated soils via inoculation with *Sphingobium indicum* B90A. Biodegradation 19:27–40

Reddy CA (1995) The potential for white-rot fungi in the treatment of pollutants. Curr Opin Biotechnol 6:320–328

Resnick SM, Lee K, Gibson DT (1996) Diverse reactions catalyzed by naphthalene dioxygenase from *Pseudomonas* sp strain NCIB 9816. J Ind Microbiol Biotechnol 17:438–457

Restaino OF, Borzacchiello MG, Ilaria S, Fedele L, Schiraldi C (2016) Biotechnological process design for the production and purification of three recombinant thermophilic phosphotriesterases. N Biotech 33:15

Roccatano D (2015) Structure, dynamics, and function of the monooxygenase P450 BM-3: insights from computer simulations studies. J Phys Condens Matter 27:273102

Romeh AA, Hendawi MY (2014) Bioremediation of certain organophosphorus pesticides by two biofertilizers, Paenibacillus (Bacillus) polymyxa (Prazmowski) and *Azospirillum lipoferum* (Beijerinck). J Agric Sci Technol 16:265–276

Ruggaber TP, Talley JW (2006) Enhancing bioremediation with enzymatic processes: a review. Pract Period Hazard Toxic Radioact Waste Manag 10:73–85

Sawers G (1998) Biochemistry, physiology and molecular biology of glycyl radical enzymes. FEMS Microbiol Rev 22:543–551

Scott C, Lewis SE, Milla R, Taylor MC, Rodgers AJW, Dumsday G, Brodie JE, Oakeshott JG, Russell RJ (2010) A free-enzyme catalyst for the bioremediation of environmental atrazine contamination. J Environ Manag 91:2075–2078

Serdar CM, Gibson DT, Munnecke DM, Lancaster JH (1985) Enzymatic hydrolysis of organophosphates: cloning and expression of a parathion hydrolase gene from Pseudomonas diminuta. Biotechnol 3:367–371

Shah PC, Kumar VR, Dastager SG, Khire JM (2017) Phytase production by *Aspergillus Niger* NCIM 563 for a novel application to degrade organophosphorus pesticides. AMB Express 7:66

Sharma P, Raina V, Kumari R, Shweta M, Dogra C, Kumari H, Kohler HPE, Holliger C, Lal R (2006) Haloalkane dehalogenase LinB is responsible for β- and δ-hexachlorocyclohexane transformation in *Sphingobium indicum* B90A. Appl Environ Microbiol 72:5720–5727

Shen B, Hutchinson CR (1993) Tetracenomycin F1 monooxygenase: oxidation of a naphthacenone to a naphthacenequinone in the biosynthesis of tetracenomycin C in *Streptomyces glaucescens*. Biochemist 32:6656–6663

Shukla P, Gupta K (2007) Ecological screening for lipolytic molds and process optimization for lipase production from *Rhizopus oryzae* KG-5. J Appl Sci Environ Sanit Sby 2:35–42

Singh JS, Singh DP (2017) Methanotrophs: an emerging bioremediation tool with unique broad spectrum methanotrophs. In: Agro-environmental sustainability. Springer, Cham. https://doi.org/10.1007/978-3-319-49727-3_1

Singh B, Kaur J, Singh K (2012) Biodegradation of malathion by Brevibacillus sp. strain KB2 and *Bacillus cereus* strain PU. World J Microbiol Biotechnol 28:1133–1141

Strong PJ, Claus H (2011) Laccase: a review of its past and its future in bioremediation. Crit Rev Environ Sci Technol 41:373–434

Subramanian V, Liu TN, Yeh WK, Gibson DT (1979) Toluene dioxygenase – purification of an iron-sulfur protein by affinity-chromatography. Biochem Biophys Res Comm 91:1131–1139

Subramanian V, Liu TN, Yeh WK, Narro M, Gibson DT (1981) Purification and properties of NADH-ferredoxintol reductase – a component of toluene dioxygenase from *Pseudomonas putida*. J Biol Chem 256:2723–2730

Subramanian V, Liu TN, Yeh WK, Serdar CM, Wackett LP, Gibson DT (1985) Purification and properties of ferredoxintol – a component of toluene dioxygenase from *Pseudomonas putida* F1. J Biol Chem 260:2355–2363

Sumithran S, Sono M, Raner GM, Dawson JH (2012) Single turnover studies of oxidative halophenol dehalogenation by horseradish peroxidase reveal a mechanism involving two consecutive one electron steps: toward a functional halophenol bioremediation catalyst. J Inorg Biochem 117:316–321

Suresh PS, Kumar A, Kumar R, Singh VP (2008) An insilco approach to bioremediation: laccase as a case study. J Mol Graph Model 26:845–849

Sutherland TD, Horne I, Lacey MJ, Harcourt RL, Russell RJ, Oakeshott JG (2000) Enrichment of an endosulfan degrading mixed bacterial culture. Appl Environ Microbiol 66:2822–2828

Sutherland TD, Horne I, Russell RJ, Oakeshott JG (2002a) Gene cloning and molecular characterization of a two-enzyme system catalyzing the oxidative detoxifi cation of beta-endosulfan. Appl Environ Microbiol 68:6237–6245

Sutherland TD, Horne I, Harcourt RL, Russell RJ, Oakeshott JG (2002b) Isolation and characterization of a *Mycobacterium* strain that metabolizes the insecticide endosulfan. J Appl Microbiol 93:380–389

Sutherland TD, Weir KM, Lacey MJ, Horne I, Russell RJ, Oakeshott JG (2002c) Enrichment of a microbial culture capable of degrading endosulphate, the toxic metabolite of endosulfan. J Appl Microbiol 92:541–548

Sutherland TD, Horne I, Weir KM, Coppin CW, Williams MR, Selleck M, Russell RJ, Oakeshott JG (2004) Enzymatic bioremediation: from enzyme discovery to applications. Clinic Exper Pharmacol Physiol 31:817–821

Thatoi H, Das S, Mishra J, Rath BP, Das N (2014) Bacterial chromate reductase, a potential enzyme for bioremediation of hexavalent chromium: a review. J Environ Manag 146:383–399

Trantirek L, Hybkova K, Nagata Y, Murzin A, Ansorgova A, Sklenar V, Damborsky J (2001) Reaction mechanism and sterochemistry of γ-hexachlorocyclohexane dehydrochlorinase *Lin*A. J Biol Chem 276:7734–7740

Tuomela M, Hatakka A (2011) Oxidative fungal enzymes for bioremediation. In: Comprehensive biotechnology: environmental biotechnology and safety, 2nd ed., vol. 6. https://doi.org/10.1016/b978-0-08-088504-9.00370-6

Ullah MA, Bedford CT, Evans CS (2000) Reactions of pentachlorophenol with laccase from *Coriolus versicolor*. Appl Microbiol Biotechnol 53:230–234

Vasileva-Tonkova E, Galabova D (2003) Hydrolytic enzymes and surfactants of bacterial isolates from lubricant-contaminated wastewater. Z Naturforsch 58:87–92

Verma S, Saxena J, Prasanna R, Sharma V, Nain L (2012) Medium optimization for a novel crude-oil degrading lipase from *Pseudomonas aeruginosa* SL-72 using statistical approaches for bio-remediation of crude-oil. Biocatal Agric Biotechnol 1:321–329

Vidali M (2001) Bioremediation: an overview. Pure Appl Chem 73:1163–1172

Wasilkowski D, Swedziol Z, Mrozik A (2012) The applicability of genetically modified microorganisms in bioremediation of contaminated environments. Chemik 66:817–826

Weir KM, Sutherland TD, Horne I, Russell RJ, Oakeshott JG (2006) A single monooxygenase, *ese*, is involved in the metabolism of the organochlorides endosulfan and endosulfate in an *Arthrobacter* sp. Appl Environ Microbiol 72:3524–3530

Whited GM, Gibson DT (1991) Toluene-4-monooxygenase, a 3-component enzyme-system that catalyzes the oxidation of toluene to para-cresol in *Pseudomonas mendocina* KR1. J Bacteriol 173:3010–3016

Xu P, Liu L, Zeng G, Huang D, Lai C, Zhao M, Huang C, Li N, Wei Z, Wu H, Zhang C (2014) Heavy metal-induced glutathione accumulation and its role in heavy metal detoxification in *Phanerochaete chrysosporium*. Appl Microbiol Biotechnol 98:6409–6418

Yamaguchi H, Arisaka H, Seki M, Adachi M, Kimura K, Tomaru Y (2016) Phosphotriesterase activity in marine bacteria of the genera Phaeobacter, Ruegeria, and Thalassospira. Int Biodeterior Biodegrad 115:186–191

Yan DZ, Lui H, Zhou NY (2006) Conversion of *Sphingobium chlorophenolicum* ATCC 39723 to a hexachlorobenzene degrader by metabolic engineering. Appl Environ Microbiol 72:2283–2286

Yang H, Carr PD, McLoughlin SY, Liu JW, Horne I, Qiu X, Jeffries CM, Russell RJ, Oakeshott JG, Ollis DL (2003) Evolution of an organophosphate-degrading enzyme: a comparison of natural and directed evolution. Protein Eng 16:135–145

Yang C, Xia Y, Qu H, Li AD, Liu R, Wang Y, Zhang T (2016) Discovery of new cellulases from the metagenome by a metagenomics-guided strategy. Biotechnol Biofuels 9:138

Yeh WK, Gibson DT, Liu TN (1977) Toluene dioxygenase: a multicomponent enzyme system. Biochem Biophys Res Commun 78:401–410

Yin K, Lv M, Wang Q, Wu Y, Liao C, Zhan GW, Chen L (2016) Simultaneous bioremediation and biodetection of mercury ion through surface display of carboxylesterase E2 from *Pseudomonas aeruginosa* PA1. Water Res 103:383–390

Zeddel A, Majcherczyk A, Hutterman A (1993) Degradation of polychlorinated biphenyls by white-rot fungi *Pleurotus ostreatus* and *Trametes versicolor* in a solid state system. Toxicol Environ Chem. 40:225–266

Zhanga H, Zhanga S, Hea F, Qina X, Zhanga X, Yang Y (2016) Characterization of a manganese peroxidase from white-rot fungus Trametes sp. 48424 with strong ability of degrading different types of dyes and polycyclic aromatic hydrocarbons. J Hazard Mater 320:265–277

Zylstra GJ, Gibson DT (1989) Toluene degradation by *Pseudomonas putida* F1 – nucleotide-sequence of the *todc1c2*BADE genes and their expression in *Escherichia coli*. J Biol Chem 264:14940–14946

Chapter 4
Mycoremediation Through Redox Mechanisms of Organic Pollutants

Refugio Rodríguez-Vázquez, Edgar N. Tec-Caamal, Juana Lira-Pérez, Ricardo Aguilar-López, and Adriana de J. Ramírez-Castillo

Contents

4.1 Introduction

Nowadays, environmental pollution has become a global concern; however, due to population growth, there is an increased demand for products and services, which translates into greater production of basic products by industries. This results in an increase in pollutants generated by anthropogenic causes, which are released into the environment and cause damage to ecosystems. Because toxins in the environment endanger human health, physicochemical and biological procedures have been developed as an attempt to mitigate the pollution. Biological methods seem to

R. Rodríguez-Vázquez (✉) · E. N. Tec-Caamal · J. Lira-Pérez · R. Aguilar-López
Department of Biotechnology and Bioengineering. CINVESTAV, Instituto Politécnico Nacional, México City, México
e-mail: rrodrig@cinvestav.mx

A. d. J. Ramírez-Castillo
National Center for Disaster Prevention, Ministry of Interior, México City, México

R. Prasad et al. (eds.), *Mycoremediation and Environmental Sustainability*, Fungal Biology, https://doi.org/10.1007/978-3-030-54422-5_4

be promising solutions, due to low operating costs, low energy consumption, and the production of less harmful compounds from microbial metabolism. Among biological methods, mycoremediation has been explored for the removal of a wide range of toxins (Prasad 2017, 2018). Fungi can chemically transform contaminants into less toxic compounds, or they can be used as a biological sorbent material to remove target compounds. This chapter shows the use of fungi for bioremediation purposes, i.e., the removal of benzene, methyl tert-butyl ether (MTBE), and vat blue dye (VBD). Possible fungal removal mechanisms are discussed, including redox reactions and biosorption processes. It was observed that the oxidation of contaminants is related to hydrogen peroxide (H_2O_2) production that in the presence of iron may lead to the formation of reactive species, which due to its high oxidizing strength can degrade contaminants.

4.2 Fungi for Remediation Purposes

Mycoremediation is the biological procedure based on the use of fungi for toxic removal from the environment. This technique commonly involves biosorption, adsorption, bioaccumulation/biovolatilization, or biodegradation processes (Fig. 4.1). Biosorption consists in the passive uptake of toxins by inactive biological materials or its derivatives. The potential of any biomaterial as a biosorbent is determined by the nature of biomass, the concentration, and the pH and ionic strength. Adsorption could be defined as the physical adherence or bonding on to a given

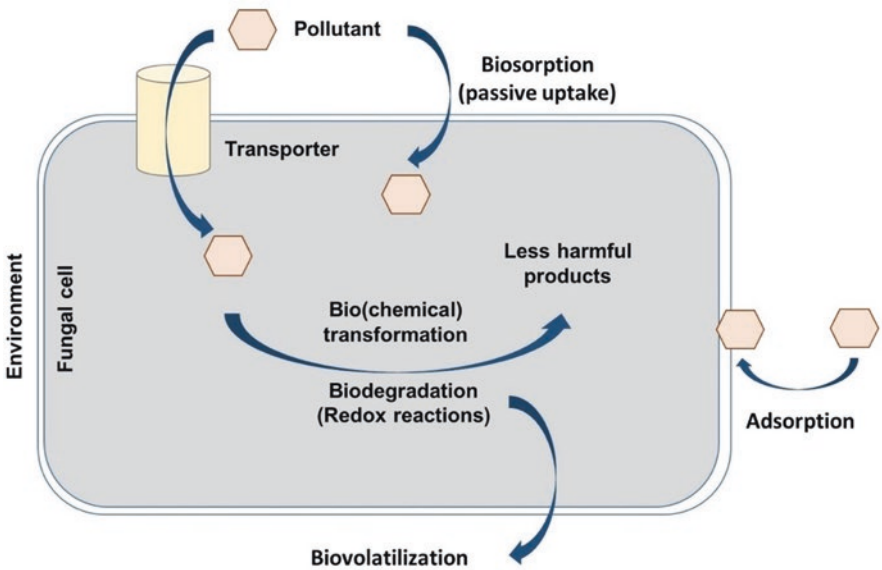


Fig. 4.1 Schematic diagram of fungal mechanisms for the removal of contaminants

surface (fungal biomass) (Dhankhar and Hooda 2011; Puchana-Rosero et al. 2017). When intracellular uptake of toxins into living cells takes place, the process is known as bioaccumulation, where chemical transformations of toxins result in methylated or alkylated products that are often volatile. The release of gaseous compounds to the environment from cells is known as biovolatilization (Boriová et al. 2014). Moreover, biodegradation is the use of living organisms to carry out transformations of a substance into new compounds by enzymatic reactions. Fungi can be used for remediation since these microorganisms can chemically modify hazardous contaminants to less harmful compounds (Pérez-Armendáriz et al. 2010; Pinedo Pinedo-Rivilla et al. 2009).

Basidiomycetes and ascomycetes are fungi that can be exploited for biodegradation purposes (Table 4.1). Basidiomycetes are commonly classified into white-rot and brown-rot fungi, which are responsible for the wood decay by the decomposition of cellulose, hemicellulose, and lignin (Mäkelä et al. 2015). These mechanisms of degradation can be harnessed in the bioremediation of pollutants with similar chemical structures, such as hydrocarbons, pesticides, and PCBs, among others (Acosta-Rubí et al. 2017; Gayosso-Canales et al. 2011). It is worth noting that these fungi can also be used to remove or accumulate metals. Their degradative capacity of organic compounds is associated with an unspecific enzymatic system where extracellular ligninolytic enzymes, such as lignin peroxidase (LiP) and manganese peroxidase (MnP), play an important role. Both enzymes are capable to oxidize lignin and its derivatives, as well as a wide array of compounds. Due to its relative specificity, LiP can oxidize aromatic compounds such as veratrilic alcohol, methoxybenzenes, and aromatic hydrocarbons, among others, while MnP produces Mn^{3+} which can oxidize phenolic compounds. Among ligninolytic fungi, lacasse phenol oxidase is an enzyme that catalyzes the oxidation of a wide range of phenolic compounds and aromatic amines using molecular oxygen as electron acceptor to form water (Janusz et al. 2013).

Ascomycota division of fungi is a group that presents the ability to degrade organic compounds and remove metals. Like basidiomycetes, some ascomycetes may secrete lignin-modifying enzymes; however, these enzymes may not play an important role in contaminated environments, since its expression is mediated by the use of lignocellulosic substrates (Aranda 2016). Nevertheless, ascomycetes are capable to produce reactive oxygen species (ROS) to oxidize the target contaminants. The production of ROS by these fungi is related to a wide range of enzymes, such as glucose oxidase, that catalyze the oxidation of glucose in the presence of molecular oxygen to form gluconolactone and hydrogen peroxide (H_2O_2). Then, H_2O_2 is decomposed to form water and oxygen, while gluconolactone is converted to gluconic acid spontaneously (Eqs. 4.1–4.3).

$$\text{Glucose} + O_2 \xrightarrow{\text{Glucose oxidase}} \text{Gluconolactone} + H_2O_2 \tag{4.1}$$

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2 \tag{4.2}$$

Table 4.1 Basidiomycetes and ascomycetes for remediation purposes

Fungus species	Pollutant	Matrix	Reference
Basidiomycetes			
Pycnoporus sanguineus	Tetrabromobisphenol A (TBBPA)	Liquid culture medium	Feng et al. (2019)
Pycnoporus sanguineus	2,2′,4,4′-tetrabromodiphenyl ether	Liquid media	Wang et al. (2019)
Pleurotus ostreatus	Endocrine disruptors	Urban wastewater	Kresinová et al. (2018)
Bjerkandera adusta Pleurotus ostreatus	2-Naphthalensulfonic acid polymers	Petrochemical wastewater	Palli et al. (2016)
Pleurotus ostreatus	Polychlorinated biphenyls	Mineral media	Cvančarová et al. (2012)
Irpex lacteus Pleorutus ostreatus Bjerkandera adusta	Chlorobenzoic acids	Soil	Muzikář et al. (2011)
Boletus edulis, Gomphidius viscidus, Laccaria bicolor Leccinum scabrum	DDT	Liquid media	Huang et al. (2007)
Ascomycetes			
Fusarium solani	Methane	Vermiculite as solid support and mineral media	Vergara-Fernández et al. (2019)
Aspergillus niger	Hydrocarbons	Liquid media	Hassaine and Bordjiba (2019)
Penicillium oxalicum	Diclofenac	Liquid medium	Olicón-Hernández et al. (2019)
Trichoderma koningii	Alachlor	Liquid culture	Nykiel-Szymanska et al. 2018
Trichoderma tomentosum	Petroleum	Liquid media	Marchand et al. (2017)
Aspergillus niger	Atrazine	Model wastewaters	Marinho et al. (2017)
Penicillium simplicissimum	Triphenylmethane dyes	Liquid culture	Hui-Chen and Yien-Ting (2015)

$$\text{Gluconolactone} + H_2O \xrightarrow{\text{Lactonase}} \text{Glucose acid} \tag{4.3}$$

The enzymatic activity of glucose oxidase when H_2O_2 is accumulated, which inactivates the enzyme. Moreover, the accumulation of gluconic acid promotes a decrease in the pH of the solution (Liaud et al. 2014). Under these acidic conditions, the Fenton reaction can take place (Jung et al. 2009). The understanding of the

biodegradation mechanisms in ascomycetes seems to be a key aspect that can serve to propose improvement strategies of the process.

4.3 Fenton Reaction for Toxins Oxidation with Fungi

Oxidation reactions are widely used for the degradation of toxic compounds. Due to its high reduction potential, strong oxidants such as hydrogen peroxide, ozone, chlorine, or manganese oxide are commonly used for environmental applications. When hydrogen peroxide is coupled with ferrous iron (Fe^{2+}), the Fenton reaction can take place (Eq. 4.4; Jung et al. 2009). Generally, this reaction is combined with another set of reactions related to the decomposition of H_2O_2, which are known as the Haber-Weiss reaction (Eqs. 4.4, 4.5, 4.6, and 4.7). From Fenton reaction, hydroxyl radicals ($HO^{\bullet}$) are produced, which are considered as nonselective strong oxidants:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^{\bullet} + OH^{-} \tag{4.4}$$

$$HO^{\bullet} + H_2O_2 \rightarrow H_2O + O_2^{-} + H \tag{4.5}$$

$$Fe^{3+} + O_2^{-} \rightarrow Fe^{2+} + O_2 \tag{4.6}$$

$$O_2^{-} + H + H_2O_2 \rightarrow O_2 + HO^{\bullet} + H_2O \tag{4.7}$$

Besides, the coupling of Fe^{2+} with H_2O_2 and molecular oxygen may result in other reactive species that can oxidize simultaneously organic or inorganic compounds (Eqs. 4.8 and 4.9; Hug and Leupin 2003). If H_2O_2 is available in the system, the reduction/oxidation cycle of iron proceeds, unless insoluble oxides and hydroxides of iron are formed:

$$Fe^{2+} + O_2 \rightarrow O_2^{-} + Fe^{3+} \tag{4.8}$$

$$Fe^{2+} + O_2^{-} + 2H^{+} \rightarrow Fe^{3+} + H_2O_2 \tag{4.9}$$

$$HO_2^{\bullet} + HO_2^{\bullet} \rightarrow H_2O_2 + O_2 \tag{4.10}$$

In biological systems, the $HO^{\bullet}$ radical is generally produced by a Fenton reaction. Fungi can produce hydrogen peroxide, to degrade complex carbon sources which can lead to the formation of reactive species (Ko et al. 2005). To use $HO^{\bullet}$ as an oxidant agent, microorganisms need to reduce Fe^{3+} and produce H_2O_2. Ascomycetes can produce H_2O_2 by the enzymatic activity of different enzymes, such as glucose oxidase, glyoxal oxidase, alcohol oxidase, glyoxylate oxidase, and pyranose oxidase, among others. Once H_2O_2 is released to the extracellular environment, it can react with Fe^{2+} ions, and the Fenton reaction occurs (Izcapa-Treviño

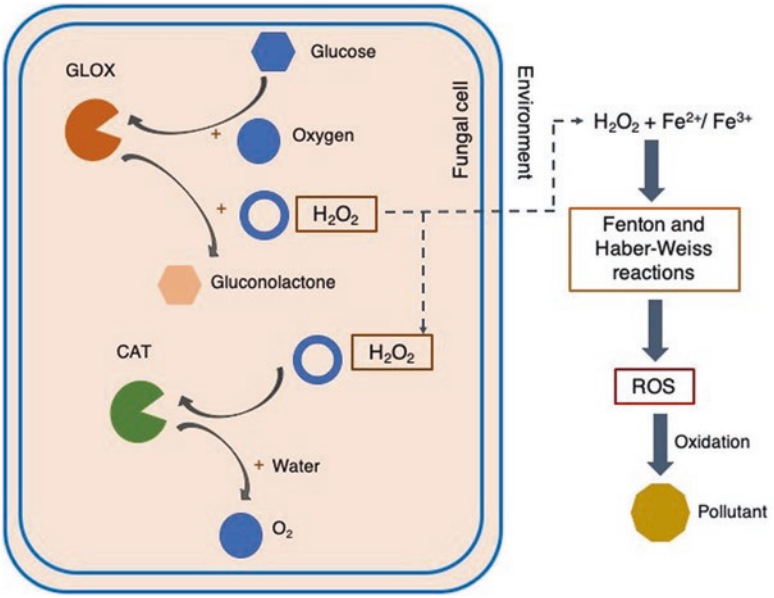


Fig. 4.2 Fungi mechanism for the oxidation of pollutants involving the formation of ROS. As an example, we consider GLOX (or another enzyme as mentioned in the text) as a peroxide producing enzyme, which is released to the environment. Moreover, CAT can decompose H_2O_2 to form water an oxygen as a mechanism to avoid oxidative stress. However, if H_2O_2 is released, and considering the presence of Fe^{2+} ions, the Fenton and Haber-Weiss reactions take place. The produced ROS can oxidize the target pollutants. GLOX glyoxal oxidase, CAT catalase, ROS reactive oxygen species

et al. 2009). Figure 4.2 shows a schematic diagram of a proposed mechanism for the oxidation of target pollutants in fungi, involving Fenton and Haber-Weiss reactions.

4.4 Remediation of Contaminated Water with Benzene and MTBE Using Fungi Associated with Green Coffee Beans: The Role of Reactive Species

Environmental pollution by hydrocarbon represents a global concern, since offshore extraction, fuel leakage during storage, refinement, or transportation of oil sometimes result in oil spills in soil and water, which cause severe damages to marine ecosystems and, thus, to activities dependent on these resources (Liua and Wirtzb 2009). Among monoaromatic hydrocarbons of petroleum (BTEX), benzene is of the highest environmental concern since it is considered as a stable volatile hydrocarbon with carcinogenic effects (Mosmeri et al. 2019). On the other hand, methyl tert-butyl ether (MTBE) is an oxygenate widely used to improve both the octane number and the combustion efficiency. However, the intensive use of these compounds often results in soil, surface water, and groundwater pollution due to their high solubility and mobility (Table 4.2; Alfonso-Gordillo et al. 2016). This has led

Table 4.2 Physical properties of benzene and MTBE at 25 °C

Property	Benzene	MTBE
Melting point (°C)	5.5	−108.6
Boiling point (°C)	80.1	55.2
Density (g/mL)	0.87	0.74
Vapor pressure (KPa)	12.7	33.5
Solubility in water (g/L)	1.8	48

to the application of a wide range of physicochemical techniques to remediate contaminated sites. However, many of these technologies do not destroy pollutants but are only moved from one place to another and may need further treatment for final disposal.

In the last decades, bioremediation has emerged as a technology compatible with the environment, since these techniques use living organisms that can modify the chemical structure of contaminants to compounds with less harmful effects. Moreover, the use of biological procedures can present the capability of high removal efficiency with low energy consumption. Thus, these techniques can be considered as cost-effective methods (AI-Hawash et al. 2018). Mycoremediation is the use of fungi to detoxify the environment or wastewater of toxic compounds. Fungi can be used for remediation purposes due to its ability to generate suitable environmental conditions to achieve the oxidation of organic and inorganic contaminants such as hydrocarbons, pharmaceuticals, pesticides, herbicides, textile dyes, or heavy metals (Deshmukh et al. 2016; Thakare et al. 2021).

Agroindustrial products/wastes can be a source of potential microorganisms for bioremediation. Green coffee beans (GCB) are known to host a microbiota consisting of bacteria, yeast, and fungi. Ascomycetes such as *Penicillium* and *Aspergillus* have been isolated from coffee and coffee fermentation (Huch and Franz 2015). Besides, ascomycetes have been successfully used to remove aromatic hydrocarbons from soil and water (Winquist et al. 2014). This allows us to take advantage of coffee beans as an agricultural resource for remediation aims (Rodríguez-Vázquez

Box 4.1 Materials and Procedures

One-liter surface water samples were obtained from an oil-contaminated site (the ex-petroleum refinery 18 de Marzo, Ciudad de México, México, 19°28′00.1″N 99°12′13.0″W) and stored in glass bottles at 4 °C for further analysis. Green coffee beans (GCBs) have been studied previously as a source of potential microorganisms for remediation. The microbiota of GCBs was identified by molecular (18s-ITS1-5.8S-ITS2-28s rRNA), microscopic, and macroscopic techniques in two previous works by Acosta-Rubí et al. 2017 and Roldán-Martín et al. 2007, finding fungal strains of *Aspergillus niger*, *Fusarium* sp., *Mucor* sp., and *Penicillium* sp. among other microorganisms. Fungi and bacterial count (UFC) were performed by diluting 500 μL contaminated water samples in 4.5 mL NaCl solution (0.9%). Once the dilutions in the range of $1 \times 10^{-1} - 1 \times 10^{-6}$ were prepared, 30 μL was poured into petri dishes with nutritive agar for bacteria (at 37 °C)

(continued)

and rose Bengal agar for fungi (at 28 °C). In the case of GCBs, 0.5 g ground grain was used for dilutions. Furthermore, solid-state cultures were performed using raw GCBs at different particle sizes, and water was added to achieve the desired moisture content in the medium. For liquid cultures, the Wunder medium (modified) was used (Wunder et al. 1994), containing (g/L) 10 glucose, 1 polypeptide, 1 $(NH_4)_2SO_4$, 0.5 $MgSO_4$-$7H_2O$, 0.875 KH_2PO_4, 0.125 K_2HPO_4, 0.1 $CaCl_2$-$2H_2O$, 0.1 NaCl, 0.02 $MnSO_4$-H_2O, and 0.001 $FeSO_4$-$7H_2O$. 40 mL of Wunder medium was inoculated with 0.5% GCBs (from SSC) in 125 mL shake flasks.

For chemical oxygen demand (COD) determinations, three solutions were prepared by duplicate. For the control blank, the standard solution, and the sample preparation, 5 mL of distilled water, 5 mL potassium hydrogen phthalate (0.0024 M), and 5 mL contaminated water sample were poured into separated round bottom flasks with three glass beads, respectively. Then, 2.5 mL potassium dichromate (0.041 M) and 7.5 mL concentrated sulfuric acid were added to each flask. Digestion was performed in a reflux apparatus with cooled water for 2 h. Then, two drops of ferroin indicator to each flask were instilled, and titration was carried out with ammonium ferric sulfate until to observe a color change from blue green to reddish brown. Ferroin indicator was prepared by dissolving 1.485 g 1,10-phenanthroline monohydrate and 0.695 g ferrous sulfate heptahydrate in 100 mL distilled water. Equations 4.11 and 4.12 were used for sample and standard COD calculations:

$$\mathrm{COD}_{\mathrm{Sample}} = \frac{\left(\mathrm{mL}_{\mathrm{Blank}} - \mathrm{mL}_{\mathrm{Sample}}\right) \cdot 25 \cdot 8000}{5 \cdot \mathrm{Dilution}} \tag{4.11}$$

$$\mathrm{COD}_{\mathrm{Standard}} = \frac{\left(\mathrm{mL}_{\mathrm{Blank}} - \mathrm{mL}_{\mathrm{Standard}}\right) \cdot 25 \cdot 8000}{5} \tag{4.12}$$

The pH of water samples was determined with a pH meter (Jenway Mod. 3020, UK). For pH determinations of GCBs, a suspension was prepared using a 1:10 GCBs-distilled water ratio. The GCB moisture was determined with a thermobalance (Kern MLB 50–3, Germany). Total nitrogen was determined according to the Kjeldahl method suggested by the Association of Official Analytical Chemists (AOAC). The HgO-K_2SO_4 catalyzer was prepared as follows: 50 g potassium sulfate and 2 g mercury oxide were mixed in a ball mill until a homogeneous powder is obtained. NaOH-$Na_2S_2O_3$ solution was prepared by dissolving 600 g NaOH and 50 g $Na_2S_2O_3$ in 1-L distilled water. The H_3BO_3 solution was prepared by dissolving 50 g H_3BO_3 in 1-L water. Methyl red and methylene blue were prepared by dissolving 200 mg methyl red and 200 mg methylene blue in 100 mL absolute ethanol, respectively. To prepare the indicator solution, 100 mL of methyl red and 50 mL of methylene solutions were mixed in a vortex. Then, 15–40 mg samples were collocated in a micro Kjeldahl flask; then, 2 g HgO-K_2SO_4 catalyzer and 2.5 mL sulfuric acid (0.01 N) were added. The samples were digested in a digestion unit for 2 h until samples were clarified and were allowed to cool to room temperature. Then, approximately 100 mL distilled water was added. The outlet of the

(continued)

condenser was submerged into a solution composed of 50 mL H_3BO_3 solution (5%) and two drops of indicator solution into an Erlenmeyer flask. 10 mL NaOH-$Na_2S_2O_3$ solution was added to the sample. Steam distillation is performed, and 75–100 mL distillated solution is recovered. The solution is titrated with hydrochloric acid (0.01 N) until a color change from green to red violet. The blank was determined with the same procedure. The following formula is used for nitrogen determinations:

$$\%N = \frac{\left(V_2 - V_1\right)\cdot\left(N_{HCl}\right)\cdot 14.007}{M}\cdot 100 \tag{4.13}$$

where %N is the percentage of total nitrogen, V_1 is the spent volume of HCl for blank titration (mL), V_2 is the spent HCl for sample titration (mL), N_{HCl} is the normality of HCl (N), and M is the sample weight (mg).

Metals were determined by atomic absorption spectroscopy. Sample digestion was performed as follows: 10 mL samples were poured into microwave tubes (Mars 230/60, USA) and 5 mL concentrated nitric acid was added. Digestion was done for 40 min. Then, by evaporation on a hot plate, 2 mL of samples was obtained. Samples were filtrated in vacuo using filter paper (Whatman grade 42) and finally analyzed. The identification of organic compounds in water was carried out by gas chromatography/mass spectroscopy. A previous extraction of samples with dichloromethane was done. For this, a capillary column (ZB-5, 5% phenyl, 95% dimethylpolysiloxane) was used. The injector temperature was set at 200 °C. The oven starts with a temperature of 35 °C (for 1 min), and a single ramp temperature program was used (35–300 °C, 25 °C/min). The concentrations of benzene and MTBE were determined with a gas chromatograph (FOCUS, GC) equipped with an FID detector and a TR-5 MS column (30 m × 0.32 mm), using nitrogen as carrier gas at 1.5 mL/min. The temperature configuration was as follows: 35 °C for oven, 200 °C for injector, and 180 °C for detector. For MTBE determinations, 100 μL of samples was taken from vials' headspace, which was submerged previously in water bath at 65 °C. For benzene, vials were maintained at 30 °C for 20 min before headspace sampling. Organic acid determinations were carried out with HPLC with a UV detector (210 nm), using sulfuric acid as a mobile phase at 0.4 mL/min and an HPX-87H column (300 mm length, 7.8 mm diameter). The H_2O_2 production and free-radical (FR) formation were evaluated by luminescence in a luminometer (TD2020, Turner Designs). In 4 mL vials, 200 μL culture medium, 10 μL luminol, and 790 μL Tris buffer (pH = 8) were mixed and measured. For the detection of FRs, 100 μL culture medium, 800 μL buffer, and 100 μL Lucigenin (bis-N-methylacridinium nitrate) (1 mM) were mixed and measured.

Microbial respirometry (CO_2 production, O_2 consumption, and respiratory quotient) was determined using a Go-Mac 550 gas chromatograph equipped with a thermal conductivity detector and a CTR-1 column. Helium was used as carrier gas with a flow rate of 55 mL/min. The temperature configuration was as follows: 25 °C for column, 30 °C for injector, and 100 °C for detector.

et al. 2011; Roldán-Martín et al. 2007). GCB can also be used as a carrier/support material to promote the formation of biofilms that can increase the resistance to adverse environmental conditions and increase the tolerance to high pollutant concentrations in addition to remove pesticides (Barragán-Huerta et al. 2007).

4.4.1 *Contaminated Water with Benzene and MTBE: Fungi for Remediation*

Due to the intense refining activities (7500 barrels of oil per day) of the ex-petroleum refinery "18 de Marzo" (México), a serious subsoil and surface water pollution was generated (SEMARNAT 2019). The characterization of the contaminated water showed the presence of petroleum-derived products, such as benzene, cyclohexene, and MTBE (as gasoline additive) (Table 4.3). Moreover, low nitrogen content and low COD were found. The COD level was near to the common low limit of raw municipal wastewater (500 mg/L) that suggests a small amount of oxidable pollutants. It is worth noting that fungi were not detected in water samples, probably due to the lack of nutrients (nitrogen and phosphorus). Besides, GCBs showed acid pH and the presence of both bacteria and fungi (Table 4.4). Fungal strains of the genera *Penicillium*, *Mucor*, and *Aspergillus* were identified previously in GCBs (Barragán-Huerta et al. 2007; Acosta-Rubí et al. 2017), which was corroborated by microscopic and macroscopic techniques. A factorial design (2^3, eight treatments, Table 4.5) was used as a first assessment of microbial activity and for inoculum selection. For this, solid-state cultures (SSC) were carried out using GCBs as carbon and energy source and as a source of microorganisms. Moisture, grain amount, and particle size were used as independent variables. CO_2 production and respiratory quotient (RQ) were used as response variables.

RQ is defined as the ratio of microbial CO_2 production and O_2 consumption when a given substrate is used for microbial metabolic activities. This indicator has been used as a unique parameter to estimate the biodegradation process of organic compounds and is related to microbial growth (Lamy et al. 2013). RQ values near

Table 4.3 Characterization of the contaminated water samples from the ex-petroleum refinery "18 de Marzo," México

Parameter/compound	Value	Parameter/compound (μg/L)	Value
pH	7.44	Cd	ND
COD (mg/L)	252.83	Ni	ND
Nitrogen (%)	0.000029	Pb	0.09
Phosphorus (mg/L)	0.51	Mn	0.067
Total bacterial count (UFC/L)	2×10^2	Ca	62.28
Total fungal count (UFC/L)	ND	MTBE	20.47
Cu (μg/mL)	ND	Benzene	15.02

ND not detected

Table 4.4 Characterization of the GCBs

Parameter	Value
pH	5.84
Nitrogen (%)	0.062
Humidity (%)	0.11
Total bacterial count (UFC/L)	7×10^2
Total fungal count (UFC/L)	4×10^3

Table 4.5 Factorial design (2^3) matrix with natural values

Inoculum	Particle size (mm)	Moisture (% CC)	GCB amount (g)
I1	2	20	1
I2	10	20	1
I3	2	40	1
I4	10	40	1
I5	2	20	4
I6	10	20	4
I7	2	40	4
I8	10	40	4

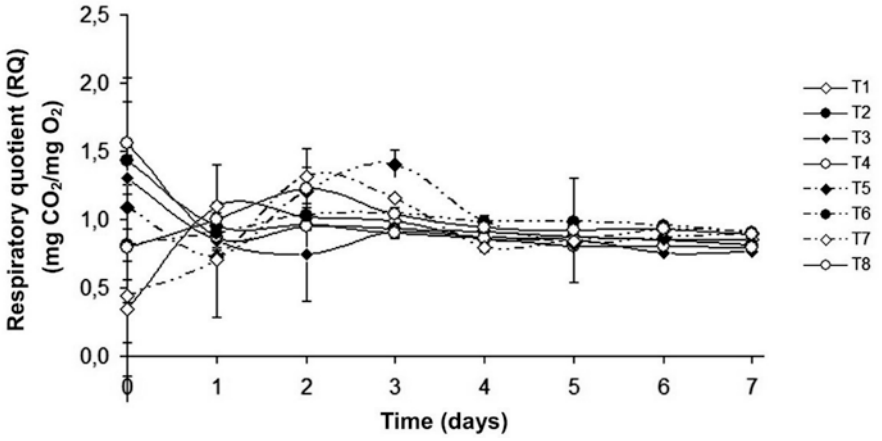


Fig. 4.3 Respiratory quotient of the microorganisms associated with GCBs for 4 days' culture in solid state

or below to 1 are suggested for the utilization of monomers of complex carbon sources by ascomycetes, under aerobic conditions (Lameiras et al. 2018). Figure 4.3 shows the RQ performance for 4 days' culture in solid state, finding RQ values near to 1 from day 2, for all treatments tested (Table 4.5). Moreover, inoculums I1–I4 were selected for reactive species formation and biodegradation tests in liquid

medium, because these conditions presented the highest microbial activity under aerobic conditions and low GCB amounts were needed.

4.4.1.1 Reactive Species and Organic Acid Production by Fungi in Liquid Medium

Ascomycetes are able to degrade toxic compounds by redox mechanisms. On the one hand, this biodegradative capacity can be correlated with the production of enzymes such as lignin peroxidase and manganese peroxidase, which are able to degrade a wide range of pollutants. On the other hand, ascomycetes can produce and release hydrogen peroxide (Ko et al. 2005), which can be used to oxidize target contaminants.

For submerged liquid cultures, inoculums from solid-state culture were taken from day two, since at this time, a higher fungal growth was observed by microscopic and macroscopic techniques. Moreover, the identified bacterial strains (*Pseudomonas putida* and *Klebsiella variicola*) are not known as H_2O_2 producers, and therefore, the H_2O_2 production can be attributed to fungal strains. First, to

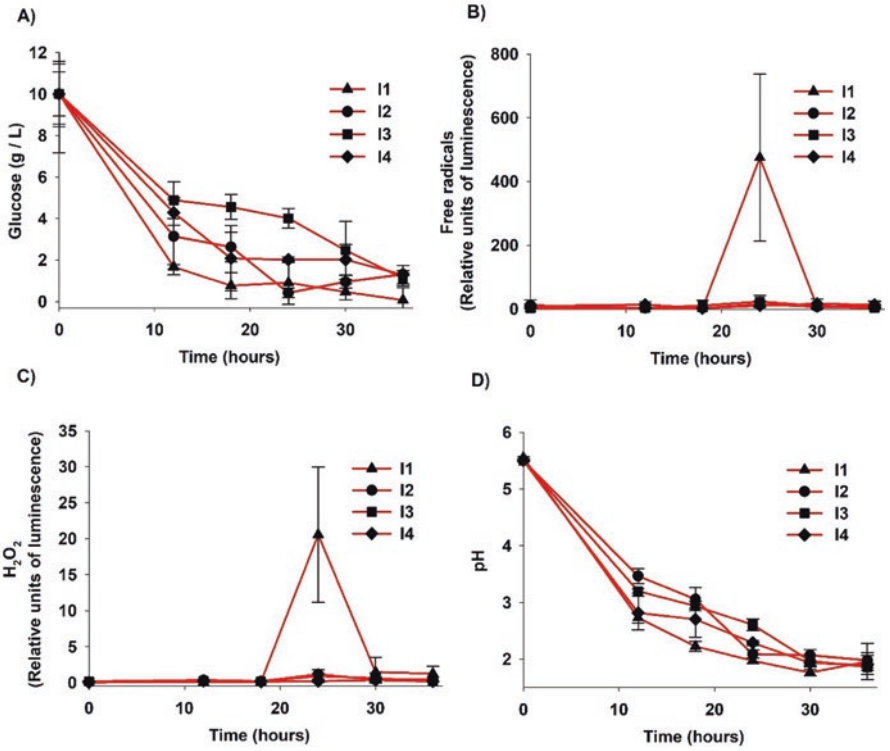


Fig. 4.4 Liquid culture performance for 36 h, using the inoculums obtained from SSC (Table 4.5, I1-I4). (**a**) Glucose consumption, (**b**) FR formation, (**c**) H_2O_2 production, and (**d**) pH of the liquid medium

determine suitable conditions (i.e., redox potential, pH, and H_2O_2 concentration) to perform the removal assays of benzene and MTBE, liquid cultures were performed with glucose as simple carbon and energy source. The H_2O_2 production is related to the enzymatic activity of fungi. According to Magnuson and Lasure (2004), *A. Niger* lowers the pH of its environment by releasing glucose oxidase to the outside of the cell wall. This enzyme can produce gluconic acid and hydrogen peroxide from glucose. This trend is shown in Fig. 4.4a, where consumption of glucose from 78% to 97% for all treatments is observed. Besides from Fig. 4.4d, a decrease of pH from 5.5 to 1.9 is observed for all treatments.

The acidification of the culture medium can promote the inhibition of rapidly growing bacterial species and fungi that are not able to grow below pH 3 (Magnuson and Lasure 2004). Figure 4.4c shows the production of H_2O_2 by the associated microorganisms to GCBs, cultivated in liquid medium for 36 h. It can be observed that the highest peak of relative units of luminescence (RUL) was obtained at 24 h, and the same tendency was obtained for all treatments.

The highest RUL was obtained for I1 and I2, 20.5 and 1.14 RUL, respectively. The difference in H_2O_2 production between I1-I2 and I3-I4 can be related to conditions of SSC for the obtaining of inoculums. For instance, I1 and I2 were grown with a moisture content of 20%, while I3 and I4 were obtained with 40% moisture. Nuñez-Gaona et al. (2010) indicated when ascomycetes are cultured in solid state, an increase of the moisture content leads to the decrease of the conidial yield. This fact can be explained by the resistance of oxygen mass transfer when water fills the spores. Furthermore, since the culture medium contains iron, it is possible that the Fenton reaction can occur. The formation of free radicals (FRs) was determined by luminescence (Fig. 4.4b). As expected, the I1 showed the highest FR formation at 24 h, which was at least 23-fold higher than the other inoculums. This was due to the high H_2O_2 concentration in the liquid medium that can react with Fe^{2+} to form FRs, according to Eq. (4.4). From this equation, one can observe that hydroxyl radical is formed. This product is known as the most reactive oxygen species that can react with organic and inorganic compounds. Once FRs are produced, it is expected that oxidation of the pollutant takes place (Lira-Pérez et al. 2019). Redox potential (E_h,) is a parameter that measure (mV) the electron availability of an environment and show its tendency to oxidize or reduce substrates. For instance, in well-oxidized water with oxygen concentrations above 1 ppm, the E_h will be highly positive, above 300–500 mV. Besides, in reduced environments, the E_h will be low, below 100 mV or negative.

Figure 4.5a shows the profiles of E_h of the culture medium for 36 h, where similar tendency was observed for all experiments, reaching maximum values of 273 mV that indicate oxidant conditions. E_h is modified by two main effects: oxygen concentration and pH. On the one hand, if oxygen concentration decreases, it is expected that E_h also decreases. However, oxygen concentration may not diminish drastically because the experiments were performed with constant agitation, which imply continuous mass transfer of oxygen to the aqueous phase consumption. On the other

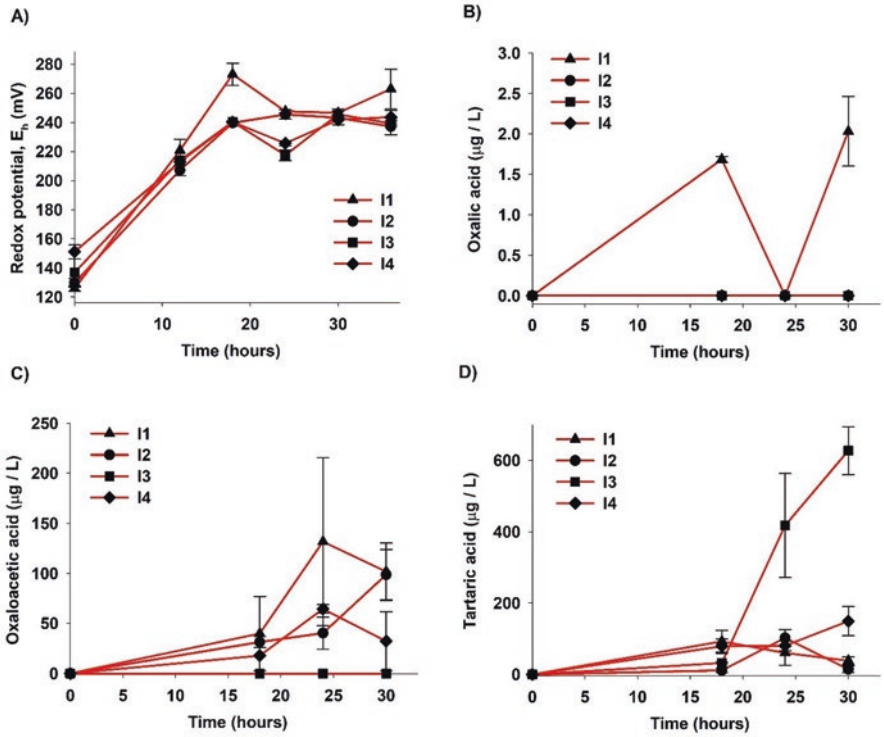


Fig. 4.5 Redox potential and organic acids produced by the microorganisms associated with GCBs (I1-I4)

hand, E_h is modified significantly with changes in the concentration of hydrogen protons. Because of this, it is expected that E_h changes, since pH varies with time. Nevertheless, the obtained E_h values were positive for all experiments, which are recommended to promote biodegradation processes (Maier and Gentry 2015). As depicted in Fig. 4.4, the decrease of pH could be related to the production of organic acids, when fungi use glucose or lignocellulosic biomass as carbon and energy source (Dörsam et al. 2017; Magnuson and Lasure 2004). Fungi are capable to produce organic acids such as oxaloacetic, oxalic, tartaric, citric, lactic, or succinic acids that lead to acidify the surroundings of the cells to very low pH values. In *A. niger*, the production of oxalic acid from oxaloacetate is mediated by oxaloacetate hydrolase (EC3.7.1.1), which is in the cytosol. The expression of this enzyme is induced at pH greater than 4 (Izcapa-Treviño et al. 2009). Our results show oxaloacetic acid concentrations up to 131.6 µg/L, which was obtained with the inoculum I1 (Fig. 4.5c). Besides, Fig. 4.5b shows that the maximum oxalic acid concentration (2.03 µg/L) was obtained for the same inoculum. The low formation of oxalic acid may be explained by the fact that the decrease of pH with time may decrease the

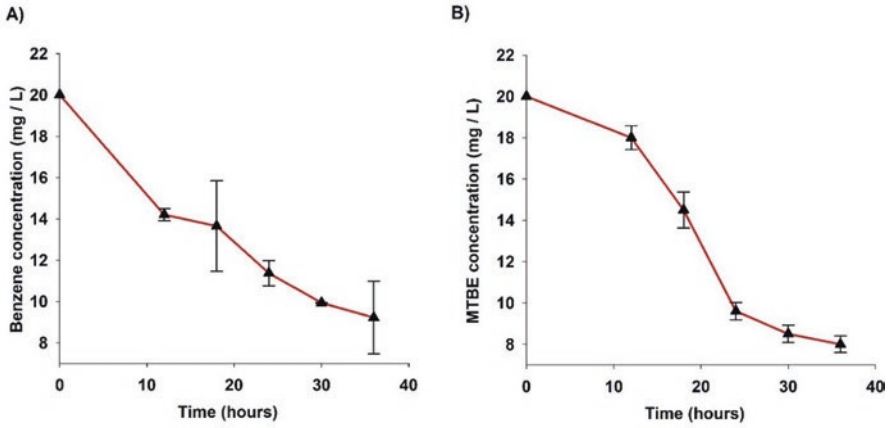


Fig. 4.6 Benzene and MTBE removal using the inoculum I1

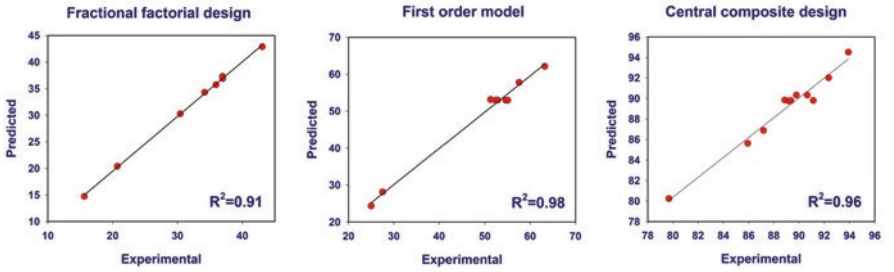


Fig. 4.7 Determination coefficients (R^2) for each experimental statistical design (obtained from ANOVA) in the optimization process of VBD biosorption in *A. niger*. (Lira-Pérez et al. 2019)

activity of oxaloacetate hydrolase, since the optimal pH for oxalic acid formation is in the range of 4–6.

Finally, since inoculum I1 showed adequate conditions for biodegradation to take place, that is, high hydrogen peroxide production, high FR formation, and low pH, it was used to perform benzene and MTBE removal assays. For this, 20 µg/L of both benzene and MTBE was added to liquid cultures. The same initial culture conditions were used for both removal assays. Figure 4.6 shows the biodegradation of benzene and MTBE by the microorganisms associated with GCBs for 36 h, where removal percentages of 60% and 52% were observed for benzene and MTBE, respectively. Fungal species of GCBs are reported to be able to mineralize alkanes or aromatic hydrocarbons in liquid medium (Govarthanan et al. 2017). The diminish of benzene and MTBE in the first 18 h where H_2O_2 and FRs are not yet formed can be related to biodegrading processes. However, between 18 and 30 h culture, H_2O_2 and FRs are produced, which can improve the biodegradation of both pollutants.

4.5 Dyeing Pollution and Dye Biosorption to Active Fungal Biomass

Dyeing printing, tanning, leather, cosmetics, paper, food, painting, and textile industries are the main sources of dyes released to the environment. In the textile industry, during the dyeing processes, approximately 1–20% of color is discarded, which generates large quantities of wastewater containing a wide variety of pollutants with different toxicities (Venkatesha et al. 2012). Since textile pollution represents a problem of global concern, physical, chemical, and biological technologies are used to treat those effluents. Azo-type (–N=N–) and anthraquinone dyes are widely used in the world for cotton and cellulose fibers dyeing (Lira-Pérez et al. 2019). The release of these hazardous pollutants to the environment may generate by-products that are considered toxic or carcinogenic agents for humans and for aquatic living organisms. In humans, these toxins can cause dermatitis, skin and gastrointestinal irritation, or allergies. Moreover, the textile industry can damage the environment in two ways: first, by huge water consumption and secondly by the use of complex chemicals (Nawaz and Ahsan 2014). All these have led to the use of physical and chemical techniques for dye removal, such as precipitation, coagulation, ozonation, and filtration, among others. However, these techniques can be expensive, generate toxic by-products, and not be adaptable to other dyes (El-Hosiny et al. 2018). Nowadays, biological procedures are used as an attempt to mitigate pollution in an environment-friendly way (Kumar et al. 2018). Mycoremediation is an effective and affordable method for the decolorization of dye-bearing effluents (Lu et al. 2017). Biosorption is considered as the most advantageous process for colored waters and living or inactive fungal cells have been studied as biosorbents

Box 4.2 Improvement of Vat Blue Dye Removal from Water with Active Biomass of *Aspergillus niger*

Aspergillus niger CDBB-H-175 was cultured in potato dextrose agar (PDA) at 28 °C for 5 days. After that, 6 mm-diameter agar disks were placed into 500 mL Erlenmeyer flask with 170 mL of modified Wunder medium (Wunder et al. 1994). Incubation was done at 28 °C, 125 rpm for 72 h. 1.5 g fungal biomass was then used as inoculum. To evaluate the capacity of *A. niger* for vat blue dye (VBD) removal, VBD (Químicos y Colorantes S.A. de C.V.) solutions were prepared at different concentrations. The VBD concentration in the culture medium was determined with a UV-spectrophotometer (Shimadzu model UV-1800) at a wavelength of 630 nm. Decolorization (%) was calculated as the difference in concentration before and after adsorption. Furthermore, the glucose oxidase (GOX) activity was determined by measuring the hydrogen peroxide produced from glucose (Eq. 4.1), using peroxidase and ABTS as its substrate. The absorbances were measured at 420 nm. One unit of GOX activity was defined as 1 μmol of hydrogen peroxide generated per minute (Tahir and Ali 2016).

(continued)

The improvement of dye removal was performed as follows: first, to identify the variables that affect the sorption of VBD into active biomass of *A. niger*, a 2^{4-1} fractional factorial experimental design (FFD) was performed, considering dye concentration (50 and 100 mg/L), pH (3 and 5 units), agitation (60 and 180 rpm), and exposure time (30 and 90 min) as independent variables and the percentage of decolorization as a response variable. From here, since agitation and pH were not statistically significant, these variables were maintained constant at its optimal values, i.e., pH = 5 and 180 rpm. Then, to improve the decolorization, a 2^2 factorial experimental design with central points was applied to obtain a first-order model (FOM), which is used in the steepest ascent step (SAS) methodology. Finally, the determination of the maximum decolorization percentage was carried out using a central composite design (CCD) of a response surface methodology. Each experiment was done in triplicate, and an analysis of variance (ANOVA) was performed for means comparison using the least significant difference (LSD) method. More experimental details can be found in Lira-Pérez et al. (2019).

(Kaushik and Malik 2009). The biosorption process involves the passive uptake of pollutants by biological materials. The biosorption capacity of a given biomaterial is determined by the composition of the biomass and the concentration or pH, among other factors (Dhankhar and Hooda 2011). Fungal strains of *Trametes*, *Phanerochaete*, *Penicillium*, *Pleurotus*, *Rhizopus*, and *Aspergillus* have been used for sorption studies (Salvi and Chattopadhyay 2017; Rodríguez-Couto 2009). For *A. niger*, several compounds have been identified from its cell wall, which can play an important role in the biosorption process.

4.5.1 *Biosorption Optimization of VBD in Fungal Biomass and the Role of Hydrogen Peroxide in Decolorization of Water*

Optimization techniques are useful tools that allow to find conditions that improve a desired response variable (Sandoval-Espinola et al. 2015). On one hand, the FFD showed that the VBD concentration and exposure time are the most significant

Table 4.6 Results obtained for each experimental statistical design (Lira-Pérez et al. 2019)

ESD	VBD (mg/L)	Exposure time (min)	pH	Agitation (rpm)	Decolorization (%)
FFD	100	30	3	180	43.07
FOM	150	150	5	180	62.91
SAS	450	142.5	5	180	90.85
CCD	520	143	5	180	94.06

variables in the VBD decolorization. On the other hand, optimization by the ascent step and the response surface methodologies were accomplished, finding the following optimal conditions: 450–500 mg/L VBD and exposure times from 133 to 150 min. Under these conditions, a maximum of 94% decolorization was obtained (Lira-Pérez et al. 2019). Table 4.6 displays the best results obtained for each experimental statistical design, where we can observe that the decolorization increases from 43% (FFD) to 94% (CCD). Figure 4.2 shows the determination coefficients obtained from the analysis of variance. Electrostatic attractions between VBD (negative charge) and positive charges of functional groups found in the cell wall of *Aspergillus niger* can be related to the biosorption process. Binding sites such as amino and carboxyl groups are the main responsible for sorption (Fu and Viraraghavan 2002). For *A. niger* cells at an early age, the main carbohydrates of the cell wall are mannose and galactose, but for aged cells, the percentage of glucose increase up to 90% of the carbohydrate portion. In this study, *A. niger* cells with a physiological age of ~80 h were used for assays. Due to the high content of glucose in cell wall composition and its neutral charge, carbohydrates could not play a relevant role in biosorption. However, the negative charges of the VBD structure can interact with the positive charges of chitin components. Besides, the negative charge of glucans might interact with the positive charges of VBD. Both interactions can allow the sorption of VBD to the cell wall of *A. niger* (Lira-Pérez et al. 2019).

Notwithstanding, the removal of VBD can be due to an oxidative mechanism mediated by the production of reactive species that can oxidize contaminants. Results from FOM showed a decolorization percentage of 62.91%, where 28% was attributed to the hydrogen peroxide production by GOX activity (Eq. 4.1). The GOX activity and hydrogen peroxide production were 1.98 ± 0.16 U/mL and 1.43 ± 0.25 mg/L, respectively. Since the culture medium contains iron (Wunder et al. 1994), it is possible that the production of hydrogen peroxide could promote the formation of reactive oxygen species (ROS), which can oxidize VBD due to its high oxidizing strength (0.695 V) (Heiser et al. 1998; Tec-Caamal et al. 2019; Ko et al. 2005). These results suggest that the decolorization of VBD using active fungal biomass can be carried out by simultaneous sorption and redox reactions. It is important to note that although the agitation and pH were set as constant for most of the experiments, the study of these variables in other bioreactor configurations such as mechanically or pneumatic bioreactors could be of importance, since changes in pH and agitation are related to changes in the metabolism of microorganisms. Moreover, the temperature and inoculum amount could be also evaluated.

4.6 Conclusions

The use of fungi for bioremediation purposes is widely used; however, there is a lack of information that should be covered in relation to the mechanisms used by ascomycetes to remove contaminants, as well as the culture conditions that could enhance the biodegradation process. Although the production of hydrogen peroxide by ascomycetes is reported in the literature, there is little information about the

redox reactions that can take place outside the cell for the elimination of toxins, such as dyes, hydrocarbons, and metals. This work emphasizes the capacity of ascomycetes to produce strong oxidants by means of Fenton and Haber-Weiss reactions. Once reactive species are formed enzymatically by a wide variety of enzymes, such as glucose oxidase, glyoxal oxidase, alcohol oxidase, and glyoxylate oxidase, among others, the pH of the environment is low by the production of organic acids, and there are oxidant conditions (positive redox potential) the oxidation of the pollutant can occur. Moreover, it is possible that the simultaneous removal process can occur, such as the case of *Aspergillus niger*, where biosorption and hydrogen peroxide production were documented.

References

Acosta-Rubí S, Tomasini Campocosio A, Montes-Horcasitas MC et al (2017) Production of a halotolerant biofilm from green coffee beans immobilized on loofah fiber (*Luffa cylindrica*) and its effect on phenanthrene degradation in seawater. J Environ Sci Health A. https://doi.org/10.1080/10934529.2017.1294965

Al-Hawash AB, Alkooranee JT, Abbood HA et al (2018) Isolation and characterization of two crude oil-degrading fungi strains from Rumaila oil field, Iraq. Biotechnol Rep 17:104–109. https://doi.org/10.1016/j.btre.2017.12.006

Alfonso-Gordillo G, Flores-Ortiz CM, Morales-Barrera L et al (2016) Biodegradation of Methyl Tertiary Butyl Ether (MTBE) by a microbial consortium in a continuous up-flow packed-bed biofilm reactor: kinetic study, metabolite identification and toxicity bioassays. PLos One 11:e0167494. https://doi.org/10.1371/journal.pone.0167494

Aranda E (2016) Promising approaches towards biotransformation of polycyclic aromatic hydrocarbons with Ascomycota fungi. Curr Opin Biotech 38:1–8. https://doi.org/10.1016/j.copbio.2015.12.002

Barragán-Huerta BE, Costa-Pérez C, Peralta-Cruz J et al (2007) Biodegradation of organochlorine pesticides by bacteria grown in microniches of the porous structure of green bean coffee. Int Biodeterior Biodegrad 59:239–244. https://doi.org/10.1016/j.ibiod.2006.11.001

Boriová K, Cerňanský S, Matúš P et al (2014) Bioaccumulation and biovolatilization of various elements using filamentous fungus *Scopulariopsis brevicaulis*. Lett Appl Microbiol 59:217–223. https://doi.org/10.1111/lam.12266

Cvančarová M, Křesinová Z, Filipová A (2012) Biodegradation of PCBs by ligninolytic fungi and characterization of the degradation products. Chemosphere 88:1317–1323. https://doi.org/10.1016/j.chemosphere.2012.03.107

Deshmukh R, Khardenavis AA, Purohit HJ (2016) Diverse metabolic capacities of Fungi for bioremediation. Indian J Microbiol 56:247–264. https://doi.org/10.1007/s12088-016-0584-6

Dhankhar R, Hooda A (2011) Fungal biosorption--an alternative to meet the challenges of heavy metal pollution in aqueous solutions. Environ Technol 32:467–491. https://doi.org/10.1080/09593330.2011.572922

Dörsam S, Fesseler J, Gorte O et al (2017) Sustainable carbon sources for microbial organic acid production with filamentous fungi. Biotechnol Biofuels 10:242. https://doi.org/10.1186/s13068-017-0930-x

El-Hosiny FI, Abdel-Khalek MA, Selim KA et al (2018). Physicochemical study of dye removal using electro-coagulation-flotation process. Physicochem Probl Miner Process 54:321–333. https://doi.org/10.5277/ppmp1825

Feng M, Li H, You S et al (2019) Effect of hexavalent chromium on the biodegradation of tetrabromobisfhenol A (TBBA) by *Pycnoporus sanguineus*. Chemosphere 235:995–1006. https://www.x-mol.com/paperRedirect/5757338

Fu Y, Viraraghavan T (2002) Dye biosorption sites in *Aspergillus niger*. Bioresour Technol 82:139–145. https://doi.org/10.1016/S0960-8524(01)00172-9

Gayosso-Canales M, Esparza-García FJ, Bermúdez-Cruz RM et al (2011) Application of 2 $_{III}^{7-3}$ fractional factorial experimental design to enhance enzymatic activities of *Pleurotus ostreatus* with high concentrations of polychlorinated biphenyls. J Environ Sci Health A 46(3):298–305. Online: http://www.tandf.co.uk/journals/titles/10934529.asp

Govarthanan M, Fuzisawa S, Hosogai T et al (2017) Biodegradation of aliphatic and aromatic hydrocarbons using the filamentous fungus *Penicillium* sp. CHY-2 and characterization of its manganese peroxidase activity. RSC Adv 7:20716–20723. https://doi.org/10.1039/C6RA28687A

Hassaine A, Bordjiba O (2019) Removal of hydrocarbons from liquid media by *Aspergillus niger*. Acta Ecol Sin 39:300–305. https://doi.org/10.1016/j.chnaes.2018.11.009

Heiser I, Oßwald W, Elstner EF (1998) The formation of reactive oxygen species by fungal and bacterial phytotoxins. Plant Physiol Biochem 36:703–713. https://doi.org/10.1016/S0981-9428(98)80020-X

Huang Y, Xi Z, Luan S (2007) Uptake and bidegradation of DDT by 4 ectomycorrhizal fungi. Sci Total Environ 385:235–241. https://doi.org/10.1016/j.scitotenv.2007.04.023

Huch M, Franz CMAP (2015) Coffee: fermentation and microbiota. In: Advances in Fermented Foods and Beverages: Improving Quality, Technology and Health Benefits. Elsevier, Oxford

Hug ST, Leupin O (2003) Iron-catalyzed oxidation of arsenic(III) by oxygen and by hydrogen peroxide: pH-dependent formation of oxidants in the fenton reaction. Environ Sci Technol 37:2734–2742. https://doi.org/10.1021/es026208x

Hui-Chen S, Yien-Ting AY (2015) Biosorption and biodegradation potential of triphenylmethane dyes by newly discovered *Penicillium simplicissimum* isolated from indoor wastewater sample. Int Biodeterior Biodegrad 103:1–7. https://doi.org/10.1016/j.ibiod.2015.04.004

Izcapa-Treviño C, Loera O, Tomasini-Campocosio A et al (2009) Fenton (H_2O_2/Fe) reaction involved in *Penicillium* sp. culture for DDT [1,1,1-trichloro-2,2-bis(p-chlorophenyl) ethane] degradation. J Environ Sci Heal B 44:798–804. https://doi.org/10.1080/03601230903238368

Janusz G, Kucharzyk KH, Pawlik A et al (2013) Fungal laccase, manganese peroxidase and lignin peroxidase: gene expression and regulation. Enzym Microb Technol 52:1–12. https://doi.org/10.1016/j.enzmictec.2012.10.003

Jung YS, Lim WT, Park JY et al (2009) Effect of pH on Fenton and Fenton-like oxidation. Environ Technol 30:183–190. https://doi.org/10.1080/09593330802468848

Kaushik P, Malik A (2009) Fungal dye decolourization: recent advances and future potential. Environ Internat 35:127–141. https://doi.org/10.1016/j.envint.2008.05.010

Ko HS, Fujiwara H, Yokoyama Y et al (2005) Inducible production of alcohol oxidase and catalase in a pectin medium by *Thermoascus aurantiacus* IFO 31693. J Biosci Bioeng 99:290–292. https://doi.org/10.1263/jbb.99.290

Kresinová Z, Linhartová L, Filipová A et al (2018) Biodegradation of endocrine disruptors in urban wastewater using *Pleurotus ostreatus* bioreactor. New Biotechnol 162:1–9. https://doi.org/10.1016/j.nbt.2017.05.004

Kumar R, Dhiman N, Negi S et al (2018) Role of fungi in dye removal. In: Phytobiont and ecosystem restitution. Springer, Singapore

Lameiras F, Ras C, ten Pierick A et al (2018) Stoichiometry and kinetics of single and mixed substrate uptake in *Aspergillus niger*. Bioprocess Biosyst Eng 41:157. https://doi.org/10.1007/s00449-017-1854-3

Lamy E, Chi Tran T, Mottelet S et al (2013) Relationships of respiratory quotient to microbial biomass and hydrocarbon contaminant degradation during soil bioremediation. Int Biodeterior Biodegrad 83:85–91. https://doi.org/10.1016/j.ibiod.2013.04.015

Liaud N, Giniés C, Navarro D et al (2014) Exploring fungal biodiversity: organic acid production by 66 strains of filamentous fungi. Fungal Biol Biotechnol 1, 1. https://doi.org/10.1186/s40694-014-0001-z

Lira-Pérez J, Hidalgo-Lara ME, Melendez-Estrada J et al (2019) The contribution of H_2O_2 produced by *Aspergillus niger* in vat blue dye discoloration: enhancement by statistical optimization methodology. RMIQ 18:701–714. https://doi.org/10.24275/uam/izt/dcbi/revmexingquim/2019v18n2/Lira

Liua X, Wirtzb W (2009) The economy of oil spills: direct and indirect costs as a function of spill size. J Hazard Mater 171:471–477. https://doi.org/10.1016/j.jhazmat.2009.06.028

Lu T, Zhang Q, Yao S (2017) Removal of dyes from wastewater by growing fungal pellets in a semi-continuous mode. Front Chem Sci Eng 11:338. https://doi.org/10.1007/s11705-017-1644-0

Magnuson JK, Lasure LL (2004) Organic acid production by filamentous Fungi. In: Advances in fungal biotechnology for industry, agriculture, and medicine. Springer, Boston

Maier RM, Gentry TJ (2015) Microorganisms and organic pollutants. In: Environmental microbiology. Academic Press, San Diego, pp 377–413

Mäkelä MR, Marinović M, Nousiainen P (2015) Aromatic metabolism of filamentous fungi in relation to the presence of aromatic compounds in plant biomass. In: Advances in applied microbiology. Academic Press, San Diego, pp 63–137. https://doi.org/10.1016/bs.aambs.2014.12.001

Marchand C, St-Arnaud M, Hogland W et al (2017) Petroleum biodegradation capacity of bacteria and fungi isolated from petroleum-contaminated soil. Int Biodeterior Biodegrad 116:48–57. https://doi.org/10.1016/j.ibiod.2016.09.030

Marinho G, Barbosa BCA, Rodriguez K et al (2017) Potential of the filamentous fungus *Aspergillus niger* AN 400 to degrade Atrazine in wastewaters. Biocatal Agric Biotechnol 9:162–167. https://doi.org/10.1016/j.bcab.2016.12.013

Mosmeri H, Gholami F, Shavandi M et al (2019) Bioremediation of benzene-contaminated groundwater by calcium peroxide (CaO_2) nanoparticles: continuous-flow and biodiversity studies. J Hazard Mater 371:183–190. https://doi.org/10.1016/j.jhazmat.2019.02.071

Muzikář M, Křesinová Z, Svobodová K et al (2011) Biodegradation of chlorobenzoic acids by ligninolytic fungi. J Hazard Mater 196:386–394. https://doi.org/10.1016/j.jhazmat.2011.09.041

Nawaz MS, Ahsan M (2014) Comparison of physico-chemical, advanced oxidation and biological techniques for the textile wastewater treatment. Alexandria Eng J 53:717–722. https://doi.org/10.1016/j.aej.2014.06.007

Nuñez-Gaona O, Saucedo-Castañeda G, Raquel Alatorre-Rosas et al (2010). Effect of moisture content and inoculum on the growth and conidia production by Beauveria bassiana on wheat bran. Braz Arch Biol Technol 53:771–777. https://doi.org/10.1590/S1516-89132010000400004

Nykiel-Szymanska J, Bernat P, Slaba M (2018) Potential of *Trichoderma kiningii* to eliminate alachlor in the presence of copper ions. Ecotox Environ Safe 162:1–9. https://doi.org/10.1016/j.ecoenv.2018.06.060

Olicón-Hernández DR, Camacho-Morales RL, Pozo C et al (2019) Evaluation of diclofenac biodegradation by the ascomycete fungus *Penicillium oxalicum* at flaks and bench bioreactors scales. Sci Total Environ 662:607–6014. https://doi.org/10.1016/j.scitotenv.2019.01.248

Palli L, Gullotto A, Tilli S et al (2016) Bidegradation of 2-naphthalensulfonic acid polymers by white-rot fungi: scale-up into non-sterile packed bed bioreactors. Chemosphere 164:120–127. https://doi.org/10.1016/j.chemosphere.2016.08.071

Pérez-Armendáriz B, Martínez-Carrera D, Calixto-Mosqueda M et al. (2010) Filamentous fungi remove weathered hydrocarbons from polluted soil of tropical México. Rev Int Contam Ambie. ISSN. 0188-4999. rvp@atmosfera.unam.mx. Universidad Nacional Autónoma de México, México 26(3):193–199

Pinedo-Rivilla C, Aleu J, Collado IG (2009) Pollutants biodegradation by fungi. Curr Org Chem 13:12. https://doi.org/10.2174/138527209788921774

Prasad R (2017) Mycoremediation and Environmental Sustainability. Volume 1. Springer International Publishing (ISBN 978-3-319-68957-9) https://link.springer.com/book/10.1007/978-3-319-68957-9

Prasad R (2018) Mycoremediation and Environmental Sustainability, Volume 2. Springer International Publishing (ISBN 978-3-319-77386-5) https://www.springer.com/us/book/9783319773858

Puchana-Rosero MJ, Lima EC, Ortiz-Monsalve S et al (2017) Fungal biomass as biosorbent for the removal of Acid Blue 161 dye in aqueous solution. Environ Sci Pollut Res 24:4200. https://doi.org/10.1007/s11356-016-8153-4

Rodríguez-Couto S (2009) Dye removal by immobilised fungi. Biotechnol Adv 27:227–235. https://doi.org/10.1016/j.biotechadv.2008.12.001

Rodríguez-Vázquez R, Roldán-Martín GA, del Río-Galván CP (2011, November) Proceso de biorremediación en cultivo sólido con la adición de granos de café No. Mexican Patent No 291975, INPI

Roldán-Martín A, Calva-Calva G, Rojas-Avelizapa N et al (2007) Solid culture amended with small amounts of raw coffee beans for the removal of petroleum hydrocarbon from weathered contaminated soil. Int Biodeterior Biodegrad 60:35–39. https://doi.org/10.1016/j.ibiod.2006.10.008

Salvi NA, Chattopadhyay S (2017) Biosorption of Azo dyes by spent Rhizopus arrhizus biomass. Appl Water Sci 7:3041. https://doi.org/10.1007/s13201-016-0417-0

Sandoval-Espinola WJ, Chinn M, Bruno-Barcena JM (2015) Inoculum optimization of Clostridium beijerinckii for reproducible growth. FEMS Microbiol Lett 362. https://doi.org/10.1093/femsle/fnv164

SEMARNAT (2019). Retrieved from http://www.parquebicentenario.gob.mx/acerca/refineria.html

Tahir A, Ali S (2016) Isolation and purification of glucose oxidase from different fungal sources. Adv Pharm J 1:71–79

Tec-Caamal EN, Rodríguez-Vázquez R, Aguilar-López R (2019) Kinetic analysis of arsenic and iron oxidation by *Acidianus brierleyi* for biogenic scorodite formation. Chem Pap 73:811. https://doi.org/10.1007/s11696-018-0653-x

Thakare M, Sarma H, Datar S, Roy A, Pawar P, Gupta K, Pandit S, Prasad R (2021) Understanding the holistic approach to plant-microbe remediation technologies for removing heavy metals and radionuclides from soil. Current Research in Biotechnology https://doi.org/10.1016/j.crbiot.2021.02.004

Venkatesha TG, Viswanatha R, Nayaka YA et al (2012) Kinetics and thermodynamics of reactive and dyes adsorption on MgO nanoparticles. Chem Eng J 198:1–10. https://doi.org/10.1016/j.cej.2012.05.071

Vergara-Fernández A, Morales P, Scott F et al (2019) Methane biodegradation and enhanced methane solubilization by the filamentous fungi *Fusarium solani*. Chemosphere 226:24–35. https://doi.org/10.1016/j.chemosphere.2019.03.116

Wang M, Yin H, Peng H et al (2019) Degradation of 2,2´,4,4´-tetrabromodiphenyl ether by *Pycnoporus sanguineus* in the presence of cooper ions. J Environ Sci 83:133–143. https://doi.org/10.1016/j.jes.2019.03.020

Winquist E, Björklöf K, Schultz E et al (2014) Bioremediation of PAH-contaminated soil with fungi – from laboratory to field scale. Int Biodeterior Biodegrad 86:238–247. https://doi.org/10.1016/j.ibiod.2013.09.012

Wunder T, Kremer S, Sterner O et al (1994) Metabolism of the polycyclic aromatic hydrocarbon pyrene by *Aspergillus niger* SK 9317. Appl Microbiol Biotechnol 42:636–641. https://doi.org/10.1007/BF00173932

Chapter 5
Mycoremediation: A Novel Approach to Rescue Soil from Heavy Metal Contamination

Shulbhi Verma and Jyoti Srivastava

Contents

The authors Shulbhi Verma and Jyoti Srivastava are equally contributed to this chapter.

S. Verma (✉)
Department of Biotechnology, College of Basic Science & Humanities, SDAU, S.K Nagar, Gujarat, India
e-mail: shulbhiverma@sdau.edu.in

J. Srivastava
Department of Botany, GSRMM P.G. College, Bhadoi, Lucknow, Uttar Pradesh, India

R. Prasad et al. (eds.), *Mycoremediation and Environmental Sustainability*, Fungal Biology, https://doi.org/10.1007/978-3-030-54422-5_5

5.1 Introduction

The incessant and indiscriminate use of chemicals, agriculture fertilizers, sewage disposal, tar, accidental spillages, and explosives has been cardinally contaminating soil, water bodies, and air, which has created an alarming situation globally. The exuberant industrial growth and various developments and establishments have added to the exponential increase in the production of various municipal, industrial, and domestic wastes. All these waste materials are discarded either in landfill/soil or in the sea without undergoing initial treatment, thus annexing to the contamination of the environment as a whole.

5.1.1 The Problems

Amongst the various kinds of pollution, soil pollution has recently gained tremendous momentum across the global communities due to the proliferating number of problems associated with its contamination. Soil pollution can be reflected as a steady accumulation of toxic compounds, salts, chemicals, radioactive materials, or disease-causing agents having harmful effects on the growth and health of plants and animals.

The major soil pollutants can be grouped under two broad genres of compounds: (i) heavy metals and (ii) mephitic organic chemicals. Most of the organic chemicals like polycyclic aromatic hydrocarbons (PAHs), pentachlorophenols (PCP), polychlorinated biphenyls (PCBs), 1,1,1-trichloro-2,2-bis(4- chlorophenyl)ethane (DDT), and trinitrotoluene (TNT) are recognized as mutagenic and carcinogenic agents apart from their high degree of persistence in nature. The term heavy metal has been used in a different context by various authors as there is no clear definition by IUPAC. In some research, it has been cited in relation to density or specific gravity, while in others, it has been described in terms of atomic mass atomic number or toxicity, while most of them are not pure metals like arsenic, selenium, and geranium which is a metalloid. Here, we use the term heavy metals in context of all the metals occurring in the periodic table except for the ones present in group I and group II. Metals are an inherent part of the soil texture. A few of them are essential micronutrients. Metals in the soil are ascribed toxic when they exceed their bioavailability threshold in living organisms. The immutable nature of the metals accounts for their bioaccumulation in the environment through the food chain. The percolation of these heavy metals in soil and water supplies eventually piles up to a toxic level and thereby contaminates the surrounding of the living beings. Some commonly detected heavy metal contaminants are mercury, lead, cadmium, and chromium (VI) which are regarded as toxic. Radionuclides, such as uranium, possess high toxicity and radioactivity and exhibit a serious threat, even at small concentrations (Thakare et al. 2021), whereas others such as copper, nickel, cobalt, and zinc are an integral part of various enzymes, and some like magnesium, potassium,

calcium, and sodium are required for the proper sustenance of living system and are not toxic, but their extensive usage and increasing levels in the environment are of serious concern. The metals have a propensity to form a toxic complex with the cellular proteins or inactivate the enzyme, thereby disrupting the metabolic machinery which results in either malfunctioning or the death of the cells (Thakare et al. 2021). Bioaugmentation following bioaccumulation of the metals negatively affect the food chain and thus pose risk. Thus, remedial measure to detoxify agricultural land as well as other land masses becomes top priority once contaminated with the metals. Human beings are the worst sufferers as they are both primary and secondary consumers in the food chain. The effects of the quantity of heavy metal in the environment and its subsequent repercussions on health and their resistance are an exhaustive issue demanding significant attention. Nevertheless, the source effects of metal toxicity associated with human health hazards is the same which are summarized in Table 5.1 (Dhankhar and Hooda 2011).

5.1.2 The Remedies

The existing decontamination methodologies through the chemical process like precipitation and ion exchange solvent extraction often involve low or incomplete metal contaminant removal including the high-cost expenditure, extensive reagents, and energy requirements incurred through the existing methodologies. These methods are susceptible at producing mephitic intermediate waste or toxic sludge which demands proper disposal.

5.1.2.1 Bioremediation

There has been a tremendous upsurge in recent years to harness the efficiency of microorganisms to degrade contaminants on a more global scale commercially while offering a safer and holistic treatment. Bioremediation entertains the use of microorganisms as well as plants which employ their efficient enzyme system for the metabolism of various kinds of xenobiotics and other man-made chemicals, thereby detoxifying and degrading the environmental pollutant (Kumar et al. 2017). Bioremediation encompasses within itself a large body of biotic mechanism and can be described under three principal methods: (i) the process of natural degradation of pollutant and noxious chemicals or natural attenuation, (ii) biostimulation which involves the alteration in the physical environment to insinuate biodegradation of contaminant, and (iii) bioaugmentation, where exogenous or foreign organisms are ingressed at the contaminated site to initiate decomposition of the contaminant (Anza et al. 2019). A significant number of plants and plant products have been associated with alleviating metal stress caused by heavy metals, also known as phytoremediation. The presence of well-bodied defence mechanism ensures the survival of plants at metal contaminated sites, but beyond a certain capacity, the plant living system fails to tolerate the heavy metal (HM) load, and its growth and

Table 5.1 Toxicity of heavy metals on human health (Dhankhar and Hooda 2011)

S. no	Metal	Primary sources	Biological effects
1	Mercury	Industries: pesticides, batteries, pulp, and paper	Damage to the nervous system, protoplasm poisoning
2	Cadmium	Welding, electroplating, pesticide fertilizer, Cd and Ni batteries, nuclear fission plant	Kidney damage, bronchitis, gastrointestinal disorder, bone marrow, cancer
3	Lead	Paints, pesticides, smoking, automobile emission, mining, burning of coal	Liver, kidney, and gastrointestinal damage, mental retardation in children
4	Chromium	Chrome plating, ceramics, metallurgical processes, paints, dyes, magnetic tapes	Persisting diarrhoea, skin ulceration, "chrome holes", bronchial asthma
5	Copper	Agricultural fungicides, algicides, fertilizers, plumbing corrosion	Gastrointestinal disorder, liver and kidney malfunctioning, nausea, vomiting, diarrhoea, and intestinal cramps, anaemia
6	Arsenic	Pesticides, fungicides, metal smelters	Bronchitis, dermatitis
7	Manganese	Welding, fuel addition, ferromanganese production	Inhalation or contact causes damage to central nervous system
8	Nickel	Nickel- or chromium-plated taps, bore-hole equipment	Skin sensitizer, dermatitis, prenatal mortality
9	Cobalt	Aircraft engines, magnets, grinding and cutting tools, artificial hip and knee joints, glass, ceramics, and paints	Congestive heart failure, dermatitis, liver and kidney effects, nausea, vomiting, diarrhoea, bleeding, coma
10	Zinc	Refineries, brass manufacture, metal plating, plumbing	Zinc fumes have corrosive effect on skin, cause damage to the nervous membrane
11	Iron	Blister packaging, iron pipes, and cookware	Liver, cardiovascular system, and kidney malfunctioning
12	Palladium	Automobile catalytic converters, electronic equipment, jewellery, glass production industry	Allergic reactions, including contact eczema chronic fatigue syndrome, multiple sclerosis, fibromyalgia, multiple, autism
13	Platinum	Automobile exhaust, roadside soil	Allergic effects, liver and kidney damage
14	Thorium	Electric lamps, metallurgical industries, laboratory crucibles, glass industry, nuclear fuel industry	Lung diseases, pancreas cancer, genetic alterations, bone cancer
15	Uranium	Phosphate fertilizers, ceramics, mining	Kidney damage
16.	Arsenic	Paints, drugs, dyes, soaps, metals and semiconductors, agricultural applications, mining, and smelting	Hyper pigmentation and keratoses, gastrointestinal, cardiovascular, haematological, pulmonary, neurological, immunological, and reproductive malfunctioning

sustenance are severely hampered and may even be fatal. It is chiefly the microorganisms which have garnered special interest as a cost-effective and efficient alternative to overcome the menace of metal toxicity in the soil.

Bacterial bioremediation encompasses the use of bacterial consortia to degrade the contaminants. Presently, it is the most extensively explored area as the growth, sustenance, and maintenance of the bacterial species require minimal investment time and space. Pires et al. (2017) have reported the presence of *Firmicutes*, *Proteobacteria*, and *Actinobacteria* at sites with heavy metal toxicant load comprising mostly of *Bacillus*, *Pseudomonas*, and *Arthobacter* genera. There is substantial evidence indicating the efficiency of legume-rhizobia symbiosis in curtailing the HM stress, but it has also been found to greatly enhance the quality of the contaminated soil in spite of the sensitivity of the nitrogenase and nodulation activities to the heavy metal (Checcucci et al. 2017).

5.1.2.2 Mycoremediation and Its Current Significance

Mycoremediation is rapidly emerging as a robust methodology to deal with abiotic metal/organic contaminant stress. Fungi can act as pivotal role because their efficient adaptation in varied surroundings and emerge as key players in reducing the heavy metal contamination, high tolerance to lethal metal environments, and an inherent elaborate detoxification mechanism make them an ideal tool against heavy metal toxicants. The ease of genetic and morphological manipulation besides short multiplication cycle makes their growth easier and economical on a large scale. They show pronounced intracellular ingestion of heavy metal subject to decreased fluctuations in pH, temperature, aeration, and nutrition. The metal-fungal associations in the rhizosphere are extremely stringent and depend upon various parameters such as physicochemical texture of soil, concentration and the kind of metal species, metabolic activity, and diversity of microbes (Mishra et al. 2017).

The first fungus which was reported to successfully degrade a diverse group of environmental pollutants was *Phanerochaete chrysosporium* (Bumpus et al. 1985; Eaton 1985). The fungi are able to procure the contaminant from the environment and store it in their tissue such as mycelia or fruiting mushroom bodies. Fungi belonging to phyla *Ascomycota* and *Basidiomycota* commonly occur at HM contamination sites. Diligent observations have led to the conclusion that nutrient-deprived soil profiles with huge loads of HM toxicants are often colonized by arbuscular mycorrhizae. Fungi have an elaborate cell wall composition which enhances its potencies of binding different kinds of metal through various possible functional group ligands and thus helps in metal sequestration. The presence of various transporter proteins, intracellular as well as extracellular enzymes such as lignin-modifying enzymes which have low substrate specificity, allows them to target many organic as well as inorganic/metal pollutants in this way fungi emerge as key player in bioremediation (Hyde et al. 2019).

5.2 Fungal Group Participation

The highly oxidative extracellular enzyme system enlarges the fungus degradative influence beyond the hyphae, thus rendering them profoundly efficient in oxidizing extremely hydrophobic substrate. It is of importance that the fungus does not utilize the contaminant for growth, and hence, the quantity of the contaminant degraded is not a function of the concentration of the fungus within the soil. The presence of enzymes like lignin peroxidases (LiP), manganese peroxidases (MnP), and laccases (LAC) exuded by fungi are tremendous at degrading organo and heavy metal pollutants. Successful treatment of herbicides and pesticide containing heavy metal contaminated site has been reported with fungi such as *Lentinus subnudus, Phlebia acanthocystis*, and *Pleurotus ostreatus* (Kamei et al. 2011; Nyakundi et al. 2011; Xiao et al. 2011).

The fungi belonging to White rot fungi (WRF) group or the basidiomycetes are commonly saprophytes having dikaryotic hyphae and clamp connections along the septation, e.g. *Pleurotus ostreatus* (oyster mushroom), *Lentinulus edodes* (Shitake), and *Agaricus bisporus* (white button mushroom). The growth of the white rot fungi through hyphal extension makes them adept in reaching out to the contaminated sites unlike the other organism like bacteria with low colonizing capacity (Reddy and Mathew 2001).

Various edible mushroom species from the Bucegi mountain forest area were reported to uptake heavy metal. The mean values of the metal concentration in the fruiting body of the mushrooms were reported: 17.49 mg/kg for Mn, 1163.86 mg/kg for Bi, 11.94 mg/kg for Ti, and 1.07 mg/kg for Sr. Amongst the eight mushroom species evaluated, *Hypholoma capnoides* species was the most efficient and absorbed the highest concentrations of Ti, Sr, and Mn followed by *Marasmius oreades* which reported crucial bioconversion values of Bi and Ti (Carmen and Gabriela 2013; Asiriuwa et al. 2013) employed mushrooms at Cd, Zn, Cu, and Pd metal-contaminated sites. Efficient bioaccumulation of the Cu metals was reported with a minimum value of 10.60 and a maximum value of 41.80 mg/kg. The maximum concentration of Cu accumulation was recorded without any amendment in the soil apart from the fungal inoculum. Out of the four mentioned metals, Cd was the least biosorbed by the mushrooms. *Galerinavitti formis* species from *Strophariacea* family was found to be efficient in the uptake of Cu, Cd, Cr, Pb, and Zn, from the contaminated soils of Dakshina Kannada, Karnataka, India, in about 30 days. At 1, 5, and 10 mmol/kg, both the biological and chemical chelators (citric acid and gallic acid) increased the metal uptake capacity of the mushrooms. Mycorrhizal fungi also have tremendous potencies in alleviating metal toxicity for their host plants. Several researchers have successfully demonstrated that *Aspergillus* sp., *Penicillium* sp., and *Fusarium* sp. can be used to remove heavy metals, i.e. Cr, Zn, Ni, Pd, and Cd (Sen 2018; Khodja et al. 2018; Das and Osborne 2018). Similarly, in another finding, *Aspergillus niger* and other *Aspergillus* sp. showed more tolerance to heavy metals (Zn > Ni > Pd > Cd) as compared to *Penicillium* sp. and *Fusarium* sp. (El Hameed et al. 2015). AM fungi have been documented as

promoting the survival and growth of plant at metal-contaminated sites. Ruscitti et al. (2017) subjected pepper plant to an increasing load of Cu concentration in the soil after the inoculation with AM fungi. Phenomenal increase in the total dry weight and leaf area in the mycorrhizal plants was observed.

Lamar and White (2001) suggested a four-step strategy for the practical implementation of fungi for the mycoremediation of contaminated sites. The steps include (i) laboratory-scale experiments to establish preparation methods, (ii) comprehensive on-site pilot testing to understand the technical and engineering methodology details, (iii) and the production of inoculum enhanced nutrients to warrant the growth and finally full-scale application.

The propensity of the fungi to survive in extreme conditions of pH, temperature, and nutrient availability has conferred excellent metal bonding properties by the cell wall. Fungal species such as *Aspergillus* sp. and *Sterigmatomyces halophilus* have been employed to remove metal contaminant which is then physically removed by harvesting the fungus (Bano et al. 2018; Baldrian 2003). The biosurfactants of fungal species have been reported to eliminate heavy metals such as Fe, Zn, and Pb (Igiri et al. 2018). For example, an anionic biosurfactant from *Candida sphaerica* was tested on cleaning soil collected from an automotive battery industry, and the heavy metal removal success rate was 95%, 90%, and 79% for Fe, Zn, and Pb, respectively (Luna et al. 2016).

5.3 Metal Detoxification Mechanisms

The defence mechanism in fungi and most microorganisms, under the influence of the contaminant stress, operates through two major mechanisms: (i) the *metabolically active processes* viz. biomineralization, biotransformation, bioprecipitation, and bioaccumulation and (ii) *metabolically passive process* like biosorptive processes. The metal detoxification in fungi employs primarily the following two steps:

(a) Extracellular mechanism: Incorporates the binding of the metal to the cell wall and extracellular material (performed by both live and dead fungal cells); the extracellular mechanism is concerned with inhibiting the entry of metal and involves biosorptive procedures which is a common feature of both the living and the dead fungal biomass.
(b) Intracellular mechanism: Incorporates the intracellular uptake, bioaccumulation, and final compartmentation or sequestration of the metal (can be performed only by live cells). The intracellular mechanisms are concerned with reducing the load of the metal in the cytosol. Bioaccumulation procedures are energy exhaustive and are only performed in a live system.

Fungi have illustrated some tremendous results in the remediation of heavy metal contaminants through biosorptive and bioaccumulation procedures (Singh 2015).

The two processes are discussed in detail in the following sections.

5.3.1 Biosorption Versus Bioaccumulation

Bioaccumulation can be referred to as the process by which living cell biomass uptake the contaminants from the surroundings, whereas biosorption principally involves the use of dead or living biomass for toxicant removal from the environment. The biosorptive procedures are more feasible and reliable than bioaccumulation procedures. During bioaccumulation, the living cell can transport the toxicant into the cell and pile it up intracellular across the cell membrane via the cell metabolic cycle. After reaching the threshold, the metabolic machinery could be disrupted due to an overload of the toxicant, thereby causing untimely death of the same, whereas biosorptive procedures seem to escape this as it is a surface phenomenon involving the cell wall components, and there is no direct need for active live metabolic machinery. Some of the characteristic features of biosorption and bioaccumulation have been discussed in Table 5.2.

5.3.2 Bioaccumulation: Mechanism and Effects

Bioaccumulation involves both extracellular and intracellular processes that comprise diverse physical, chemical, and biological mechanisms. A diverse combination of extracellular chelation, intracellular complexation, and transport mechanism operates during bioaccumulation.

5.3.2.1 Extracellular Chelation and Cell Wall Binding

Fungal cell exudes various organic molecules, in particular di- and tricarboxylic acids, to chelate metal ions. Citrate ion has been identified as the most significant Al311 complex-forming agent in soil sample obtained from podzolized forest soil. Brown rot fungi have often been found to exude oxalic acid in response to Cu tolerance. The overproduction of oxalic acid has been observed in *Beauveria caledonica* towards Cd, Cu, Pb, and Zn metal stress. The oxalate crystals produced by the mycorrhizae tend to immobilize and detoxify heavy metals. X-ray microanalysis (SEM-EDXA) and X-ray powder diffraction studies (XPRD) in wood rooting fungal species such as *Fomitopsis* cf. *meliae* and *Ganoderma* aff. *steyaertanum* have revealed that the oxalic acids extruded by them react with the metals and convert them into the lesser toxic metal oxalates, e.g. zinc sulphate into zinc oxalate dihydrate, copper sulphate into copper oxalate dihydrate, cadmium sulphate into cadmium oxalate trihydrate, and lead nitrate into lead oxalate (Kaewdoung et al. (2016). Soil acidification usually results because of the exudation of organic acids. Since the growth and metabolism of the metal sensitive fungal isolate are affected more rapidly than the metal tolerant isolates, they dissolve lesser amounts of metal contaminant. Glomalin proteins secreted by the arbuscular mycorrhizal fungi have been

Table 5.2 Features of biosorption and bioaccumulation

S. no	Characteristic	Biosorption	Bioaccumulation
1	Economical	Highly cost-effective, the biosorbents mainly comprise of waste biomass obtained from industrial, agricultural, and other sources. Transportation and other simple processing charges are required	Expensive Maintenance of living system is cost-prone
2	pH	Process can occur at broad pH range, metal ingression is hugely dependent on pH. Metal uptake is strongly influenced by pH; however, process can be operated under wide range of pH conditions	Metal uptake and the living cells both are sensitive towards extreme pH fluctuations
3	Temperature	Rarely affects biosorption	Severely affects bioaccumulation
4	Maintenance	Depends on the kind of biomass: living biomass or dead is relatively easy	Is complex. External metabolic energy is needed in maintenance of the living cell/biomass
5	Selectivity	Poor, enhanced through modification/ processing of biomass	Highly selective
6	Specificity	Versatile, the binding sites can accommodate a variety of ions	Stringent, the process has low substrate to metal specificity, is prone to high metal/salt conditions
7	Uptake capacity	Large, reports illustrate the quantity of metal uptake can be as high as the dry weight of the dead biomass	Small, high toxicant concentration hampers the metabolic machinery and may even be fatal
8	Uptake rate	Fast	It is a time-consuming process as compared to biosorption
9	Reusability	High, the biomass can be repeatedly reused for repeated with possible reuse over a number of cycles	Low, toxicants are intracellularly accumulated
10	Toxicant recovery	Proper selection of eluents can recover the metal from the biomass	Not feasible

documented to sequester metal ion such as Cu, Pb, and Cd, in the polluted soils. Presence of melanin amongst the cell wall components further increases extracellular metal chelation (Bellion et al. 2006).

5.3.2.2 Intracellular Complexation by Peptides

The presence of metallothionein [MT] like peptides in metal stressed fungal species was first reported in *Pisolithus tinctorius*. Metallothioneins are ubiquitous, low-molecular-weight cysteine- and metal-rich proteins with a sulphur-based metal

cluster. When exposed to metal stress, fungi synthesize two types of MTs, class II type and phytochelatin PC or class III type. PC derivatives are primarily glutathione-related peptides and are not the gene products (Kameo et al. 2000)

The cellular resistance to heavy metal cytotoxicity inside the fungus is conferred by the binding of the MT, OR, PC to the metal ligands. Fungal MT have been reported to chiefly consist of copper ions that differ from their vertebrate counterparts which bind different metal ions. The major role of these proteins is in imparting homeostasis of trace metals like Zn, Cu or the sequestration of noxious metals like Cd and Hg.

Another compound of importance during metal toxicity in fungi is the non-protein thiol glutathione which is reported to be increased under Cd exposure in *Paxillus involutus*, as well as g-glutamylcysteine a compound mostly related to a metallothionein. The significance of glutathione as a metal chelator is now well-established intracellular glutathione inhibits the progression of heavy metal-initiated cell injury by chelating and sequestering the metal ions themselves (Bellion et al. 2006)

5.3.2.3 Transport Mechanisms Involved in Metal Tolerance

It has been illustrated that metal tolerance in fungi could be attributed to the involvement of certain transport proteins which either extrude lethal metal ions from the cytosol out of the cell or by allowing its sequestration in the intracellular compartments such as vacuole (Fig. 5.1). Blaudez et al. (2000) used radiotracer flux analysis and reported that the excessive quantity of Cd in the vacuole compartment in ectomycorrhizal fungus *Paxillus involutus* is because of the cd – conjugated glutathione or cd conjugated phytochelatins in the vacuolar compartments. The above process is mediated by ATP-binding cassette transporter Hmt1 located on the vacuolar membrane or the tonoplast. Mycorrhizal fungi chiefly depict vacuolar compartmentation of heavy metals. Guerrero et al. (2008) reported that in the extra- radical mycelium of *Glomus intraradices* now *Rhizophagus irregularis* the metal like Zn, Cu, and Cd were compartmentalized into the vacuolar compartments after their uptake inside the cell. Yao et al. (2014) also examined the extra-radical mycelium of *R. irrregularis* in symbiotic association with clover and found excessive Cd accumulation in the vacuole. It has been documented that the vacuole deficient fungal strains showed increased susceptibility to metal stress resulting in a concomitant decrease in the biosorption of Zn, Mn, Co, and Ni metal ions (Ramsay and Gadd 1997). Increased susceptibility to chromate and tellurite following a gradual decrease of the metals in the cytosol was observed in defective mutants and vacuole lacking strains of *S. cerevisiae*, Gharieb et al. (1998), whereas increased tolerance to selenite was observed followed by its gradual increase of Se in the cytosol. A large number of genes associated with the metal detoxification mechanism have been identified which includes Arr4p which induces tolerance to various metals ion species like As^{3+}, As^{5+}, Co^{2+}, $Cr^{3+,}$ Cu^{2+}, VO^{3-} (Rosen 2002; Shen et al. 2003). Similarly the presence of specific permeases such as MgATP- energized glutathione S-conjugate transporter responsible for the vacuolar sequestration of

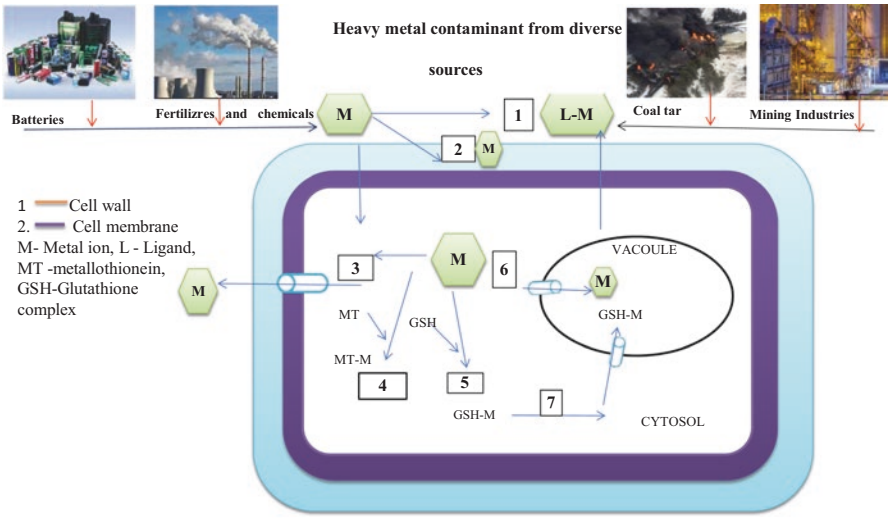


Fig. 5.1 Detoxification mechanism against heavy metal by a typical fungal cell

bis(glutathionato- cadmium) as well as bis(glutathionato-mercury) encoded by the yeast cadmium factor (Ycf1) gene is responsible the metal detoxification mechanism in the vacuole tonoplast of *Paxillus involutus* (Blaudez et al. 2000)

5.3.3 Biosorption Mechanism and Effects

Biosorption processes basically indulge in physiochemical interactions between the metal ions and the functional groups projecting from the cell surface. There is sufficient data envisaging that biosorption by fungi generally follows the Langmuir or the Freundlich model, which is in accordance with the primary role of the fungal cell wall. Metal tethering involves a two-step procedure. The primary step involves stoichiometric interaction between the metal and the reactive functional groups in the cell wall, while the secondary step involves a continuous inorganic deposition of the metal. The metal binding to the cell wall components is illustrative of complex ion exchanger closely resembling to that of resin.

Electrostatic, ion exchange and metal chelation are some of the key interactions which occur during biosorption of the heavy metals. The kind of interactions is largely determined by the composition of the cell wall and the type of metallic ions. The most common functional groups elicited in the biosorption process include carboxylate (COOH), sulphydryl (−SH), hydroxyl (−OH), amines ($-NH_2$), and phosphoryl groups ($-H_2PO_4$) present within the cell wall components such as polysaccharide lipids proteins. The binding is usually swift and requires marginal activation energy (approx. 21 kJ mol^{-1}), thus allowing for repeated metal sorption and

desorption cycles by the biosorbent materials. Since biosorption is a surface phenomenon, the ionic state and surface area chiefly determine the biosorption capacity of a biosorbent. Fungi emerge as promising candidates as their vast and hugely complex cell wall composition offers a wide array of functional groups for metal binding thus enhancing their metal sequestration capabilities.

5.3.3.1 Merits of Fungal Biosorbent

Biosorptive techniques have emerged as frontier techniques in removing heavy metal and other possible contaminants as compared to various other techniques because of the following features: (a) low operation cost, (b) high efficiency, (c) enhanced sensitivity, (d) fewer technological requirements, (e) minimal nutrient and growth requirement, and effective regeneration and reuse of the biosorbent material with the possibility of metal recovery. Fungi enjoy a majority of the features required in a good biosorbent during biosorptive processes. Fungi possess profound metal tethering capacities owing to the presence of a diverse range of functional groups present in the cell wall matrix. They are comparatively easy to cultivate on a large scale when compared to the other biosorbent living systems such as algal biomass bacteria and plant products. They require inexpensive cultural media waste, e.g. *Aspergillus niger* (waste from citric acid production) and *Saccharomyces cerevisiae* (brewery industry). Majority of the fungi used as biosorbents are nonpathogenic and are safe; therefore, they are easily accepted by the public at large when applied practically. *Saccharomyces cerevisiae* has been acknowledged as a model organism to understand the intricacies of biosorptive procedure; the complete availability of the genomic sequence and the ease of genetic manipulation offer to understand and explore the mechanism of biosorption of metal ion removal in greater depths.

There are several parameters which affect the biosorption capacity of the heavy metal, which include biotic factors (such as the type of biomass, biomass concentration) as well as abiotic factors (such as pH, temperature, and ionic strength).

Biotic Factors

Type of biomass Biosorption can be achieved by utilizing either dead/living, free/immobilized, raw/pretreated, wild/mutant, and genetically engineered/non-engineered biomass. The metal sorption achieved through various biomasses can provide us with useful data in understanding the strategies involved in metal detoxification. A large initial concentration of the metal-solute results in higher ingression of the metal ion.

Biomass concentration A poor ratio of the initial concentration of the metal to a high surface area provided by the fungal cells is responsible for the narrow metal sorption. The crucial limiting factor is the maximum saturation potential of the fungal biomass. A large amount of the fungal biomass provides greater binding sites for

the metal ion, therefore ensuring large uptakes of the metal contaminant. A significant increase in cu metal uptake up to 29.83 mg from an artificial solution of 30 mg/mL was reported when *R. arrhizus* biomass concentration was scaled from 0.15 g/L to 0.50 g (Subudhi and Kar 2008).

Abiotic Factors

pH: It largely affects the chemical association of the metal ligand to the cell wall involving hydrolysis, complexation, redox, and biosorption reactions. A high pH decreases the metal complex solubility and allows precipitation which lowers the metal sorption. The sorption of metal like Cu, Cd, Ni, and Zn is often reduced at an acidic pH, while for some metals like Au, Ag, and Hg, the sorption is pH-independent.

Temperature: Most sorption processes are not affected in the range 20–35 °C. The increase in temperature is associated with an increase in the kinetic energy of the molecules, thereby enhancing sorption process, but extreme temperature may harm the integrity of the fungal biosorbent and may even cause irreparable damage suggesting that biosorption process should be carefully evaluated and initiated at room temperatures. Brady and Ducan (1994) have reported that accumulation of Cu^{2+}, Co^{2+} or Cd^{2+} by *S. cerevisiae* in suspension is scarcely affected between 3 and 40 °C.

Ionic strength: Adsorption is decreased with an increase in the ionic strength as reported by (Dönmez and Aksu 2002). The competition between the ions and the changes in the metal activity are greatly influenced by the ionic strength (Dhankhar and Hooda 2011).

5.4 Biotechnology Perspective in Mycoremediation

Soil heavy metals are very resistant and impact deleterious effect in the environment which causes life-threatening diseases. Microbes remediation can be proved as an effective method to eliminate the toxicity of heavy metals from soil (Thakare et al. 2021). Generally, microbes have few basic attributes such as biosorption, bioaccumulation, siderophore formation, bioleaching and biotransformation. Naturally, microbes have some limitation in the process, but their attributes can be increased through technology. Amongst the various technologies available, high-throughput technology impacts valuable contribution in enhancing the knowledge for remediation. These attributes compel to gain intimate knowledge internally into the genome level to explore the mechanism to intensify the potential. Through those candidate gene related to heavy metal response used in the engineering for the detoxification of heavy metal from soil. Few heavy metals are recalcitrant in their nature due to that their degradation is almost impossible there; also, mycoremediation is the most possible way to eradicate the heavy metal.

5.4.1 Genetic Engineering in Mycoremediation

Natural fungal attributes are limited in uptaking heavy metal, but their efficiency can be increased through genetic engineering technology. In terms of biosorption, genetic engineering can be done on binding proteins of metal, peptide/anionic moieties present on the cell wall, and chelator in this perspective biomass of fungus also plays essential participation in the process, whereas in bioaccumulation, engineering can be done at the level of gene/protein for more storage of heavy metal. Engineering of fungal enzymes, siderophores, transporter gene/channels and implementation regarding the enhancement for improvement in bioremediation which provides the broader range in the clean-up of heavy metals from the soil can be a milestone in mycoremediation. These fungal enzymes and other attributes enhanced the uphold capacity of heavy metal in the organism through the change in their gene level. Fungus possesses several enzymatic systems such as catalase, oxidative, and hydrolytic related enzymes. This genetic engineering technology can be used in those fungi which do not support in producing candidate enzymes essential for heavy metal consumption. Engineering technology is not limited to one aspect this can be done on metal-binding proteins their sites and ligands which enhance the accumulation of toxic metal.

Many studies reveal that fungus *Rhizopus arrhizus, Penidiella* sp. T9 for yttrium, *Aspergillus niger, Aspergillus flavus*, screened for cadmium and lead while *Penicillium notat*um for nickel their adsorption capacity measured by atomic absorption spectrophotometer. *Penicillium spinulum, Phanerochaete chrysosporium*, and *Penicillium canescens* (2195) tested the adsorption capacity of cadmium, lead, mercuric, and arsenic; *Trametes versicolor, Bjerkandera adjusta,* and *Pleurotus* sp.*,* have high capacity of biosorption due to ligninolytic enzymes, viz. laccases, catalases, and peroxidases. *Suillus bovines, rhizopogon roseolus, and* ectomycorrhizal fungi in association with *Pinus* assist in cadmium removal; these symbiotic relationships enhanced the process of heavy metal immobilization, *Curvularia, Aspergillus, Cryptococcus,* and *Penicillium* used for uranium removal from the soil through bioadsorption. *Acremonium* and *Pythium* fungi species assist in heavy metal eradication. Mushrooms also participate in alleviating the heavy metal from contaminated site naturally through several enzymes such as oxalic acid, citric acid and several ions such as carboxylic, hydroxyl, phosphate, sulfhydryl and MTs present in the cell wall to bind the heavy metal. Their efficiency can be more extent through high-throughput techniques.

Metallothioneins (MTs) are the largest storage proteins they are ubiquitous, polyphyletic super family metal-binding proteins. These metallothioneins have small cysteine-rich peptides assist in homeostasis, metal tolerance and detoxification. Different fungi have different MTs, fungal MTs genes are either respond to single or multiple metal signals, but the range of inducing metals corresponds to the metal specificity to specific MTs. These MTs participate in heavy metal binding; their binding gets enhanced in the presence of fusion proteins for heavy metal sequestration so their engineering assists in the more bioaccumulation. Such as in

Saccharomyces cerevisiae, two MTs were identified, CUP1 gene which induced only by Cu, Cd, and Ag, while CRS5 second MT gene binds Zn, Cu, and stress related to oxidation. These CUP 1 and CRS 5 MTs of *S. cerevisiae* engineered in the inner surface of yeast plasma membrane and also engineering in their fusion protein partners such as GST, glutathione-S-transferase, for first CUP1 MT and GSS, glutathione synthetase, for second CRS 5 MT for enhance the capacity of MTs, *Candida glabrata* MTs induced by Ag and Cu but not by Cd. *Hebeloma mesophaeum* have three MTs H*m*Mt1(induced by Zn, Cu, and Cd), H*m*Mt2, and H*m*Mt3 (both induced by Ag); popular fusion soluble proteins include maltose-binding protein and glutathione-S-transferase (Diep et al. 2018)). Engineering the overexpression of *S. cerevisiae* protein improves Cd tolerance; same can be done in other fungi to broaden mycoremediation. *Pleurotus* species of mushrooms show higher resistance to Cu, Cd, Zn, Ni, Co, and Hg, in fungus *Gigaspora margarita* (BEG 34) *Gmar MT 1* metallothionein identified for Cd and Cu tolerance so engineering in this species within metallothionenin will enhance the toxic metal uptake. Artificial designer proteins and fusion protein can be used as a metalloprotein and its enhancer is used to accelerate the binding capacity of heavy metals.

5.4.2 Participation of Genomics and Transcriptome

Though many fungi are known for their enormous potential in heavy metal remediation, the genomic data of most of them are unavailable. Fungal genomics widens the range of its bioremediation process. Few fungus libraries have already been created to study genetic basis through sequences. Theses sequences assist in several types of investigation regarding the candidate gene responsible for remediation. Transcriptome analysis and genome sequencing is the tool for understanding gene expression patterns in fungus for more heavy metal accumulation. Novel fungi sequences can be known through whole-genome sequencing. Transcriptome analysis is done for the specific part of the organism. The sequences obtained from the next generation reveal several metabolic pathways, genes related to the biological function, molecular function and cellular function, and relation of genes in the metabolism; ultimately, this advance tool provides vast information related to the mycoremediation process. Apart from small and agriculture crop, tree symbiosis with fungus also plays an essential role in eradicating the heavy metal from soil (Deshmukh et al. 2016). Genome sequencing of mercury and chromium heavy metal resistant fungus *Rhodotorula taiwanensis* MD1149, basidiomycetes group member is already done through the genome information closely related fungus can be traced, biological, molecular and cellular genes can be analyzed, several pathways came into the existence, genome used in gene editing for broadening the heavy metal cleanup (Tkavc et al. 2018). Transcriptome sequencing of *Ganoderma* reveals the presence of degrading enzymes such as oxidoreductase, laccases, xylanases, cellulases, chitinase which assist in lignocelluloses degradation their effect increases in the presence of Cu^{2+} heavy metal (Jain et al. 2020). Genome analysis of

legumes–fungus interaction in heavy metals soil confirms the different fungal transporter involved in heavy metal tolerance. Therefore, RNA seq approach utilized in Cd tolerant fungus strain *Exophiala pisciphila* and Cd stress fungus Dark septate endophytic fungal strain for the purpose to know about the gene related to the heavy metal resistance. Genome analysis revealed about novel transporter natural resistance-associated macrophage protein (Nramp) from *Exophiala pisciphila* which assist in more accumulation of Cd^{2+} heavy metal (Mosa et al. 2016). In that naturally growing tree plant near mining sites, soil is *Clethra barbinervis* Sieb. Their roots have symbiosis with three types of fungi: *Phialocephala fortinii*, *Rhizodermea veluwensis*, and *Rhizoscyphus* sp., which reduce pressures due to heavy metal such as Cu, Zn, Ni, Cd, and Pb from soil. Genome sequencing of plants and tress which possess the symbiosis relationship in their roots can be widely used in heavy metal eradication (Yamaji et al. 2016) and other fungi such as *Penicillium canescens*, *P. simplicissimum,* and *Talaromyces macrosporus*, as well as *Talaromyces* sp. are utilized for Pb(II) uptake from soil (Maini et al. 2019).

De nova transcriptome analysis of Salix sp. root rhizosphere of fungal microbes reveals very interesting facts regarding mycoremediation. Transcripts identified in rhizospheric interaction indicate the fungal diversity of several families under the ground that includes 40,352 distinct contigs of *Pyronemataceae* (23.8%), *Hydnangiaceae* (11.7%), *Tuberaceae* (8.0%), *Polyporaceae* (6.3), *Gloeophyllaceae* (3.3%), *Hymenogastraceae* (3.3%), *Marasmiaceae* (2.9%), *Serpulaceae* (2.7%), *Psathyrellaceae* (2.7%), and *Pleosporaceae* (2.6%). Another fact related to the experiment narrates that contamination of heavy metal downregulated the constitutive fungal expression. In a broader way, along with distinct families, different phylum also exist in the root zone; amongst those Ascomycetes and Basidiomycetes are predominantly found. Major species such as *Pyronema omphalodes* and *Tuber melanosporum* belong to *Ascomycota* and reflect downregulation of fungal constitutive gene expression and abundance of RAS protein in contaminated sites; unlike *Ascomycota*, the closely related species of *Basidiomycota* are *Hymenogastraceae* and *Strophariaceae (Agaricoid* family), *Heboloma cylindrosporum* (an ECM fungi) *Galerina marginata* (predominantly white rot) and *Hypholoma sublateritium* (white rot) which shows downregulation of fungal constitutive gene expression and upregulation of same gene in other *Basidiomycetes* such as *Scleroderma citrinum, Paxillus involutus*, *Pleurotus ostreatus,* and *Trametes versicolor* in the contamination site. Few genes function were recognized in *Basidiomycetes* contaminated sites they are cryptic plasma membrane proteolipid 3, small hydrophic pmp3 are highly conserved in stress condition, cytotoxic cation tolerance and sphingolipid synthesis related to cell membrane integrity in the adverse condition, during adverse situation carbohydrate import, nitrogen transport and metabolism related gene also gets upregulated. Apart from these genes, three dioxygenase genes identified in low abundance, glutathione peroxidase like protein, and thioredoxin-dependent peroxidase genes are upregulated in the differential gene expression. Carbohydrate transport plays a very essential role in heavy metal stress condition because it is responsible for hexose transportation that maintains the energy level in fungus. The monosaccharide transporter MST1 is upregulated in *Amanita muscaria, Laccaria*

bicolor, other monosaccharides transporter reported in *Serendipita vermifera, Piriformospora indica*, and analogous of the transporter found in *Saccharomyces cerevisiae* in the form of extracellular glucose sensor rgt2;these hexose transporters generally occur in ECM-type *Basidiomycetes*. These *Basidiomycetes* possess carbohydrate-related enzymes in the upregulated form during heavy metal condition, amongst that are different contigs of a gene related to glycosyl hydrolase (GH) families, a gene to glycosyl transferase (GT), and a gene without any contig related to pectin/pectate lyase. The CAZy GH45 belongs to expansin family proteins which are the most abundant in ECM-type basidiomycetes; another CAZy GH131 includes cellulose-binding module with beta glucanase activity in the *Plicaturopsis*, *Laccaria*, *Jaapia*, *Tulasnella*, and *Gelatoporia*, *Hebeloma,* and *Jaapia argillacea*. Three contigs of GH5 (exo beta 1,3 glucanase), carbohydrate-binding module family 13 (CMB 13), assist in binding with cell wall of plant for energy extraction Fig 5.2.

Transcriptome analysis identifies the nitrogen related compound transporter in ECM fungi such as in *Paxillus involutus* and *Laccaria bicolor*. Other classes of gene related to protein degradation of macromolecules, amino acid, and heavy metal degradation occurred both in EMC and saprophytic basidiomycetes. Several Ras-like proteins Rab-5B, Ras protin, Rab-type small GTPase, GTPase forz1, and sar1-like proteins are involved in endocytotic vascular trafficking to reduce the toxicity of heavy metals (Gonzalez et al. 2018)

Transcriptome analysis of wheat root possess the symbiosis relation with arbuscular mycorrhizal fungi (AMF) *Rhizoglomus irregular* for the heavy metal remediation of soil. For this an experiment is conducted with/without AMF and contaminated/noncontaminated sites. Mycorrhizal fungi have the capability to immobilize metals in their biomass, cell wall, plasma membrane, vesicles, vacuoles and the glomalin. Plants-AMF interaction increases the upregulation of those genes which either

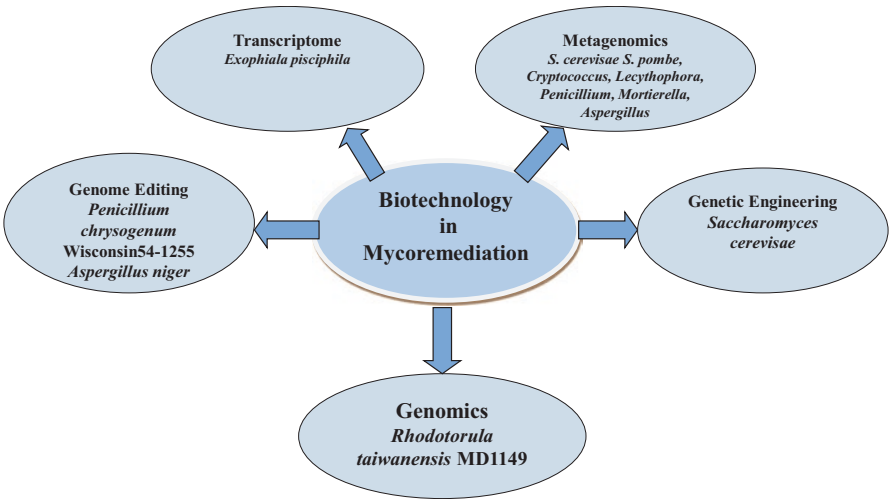


Fig. 5.2 Biotechnology approaches

assist in the detoxification or produce secondary metabolite of soil for heavy metal remediation. In fact, their symbiosis in contaminated site upregulates the gene related to the metal ion binding for sequestration in the microbe organelles (Compos et al. 2019) Fig. 5.2.

5.4.3 Proteomics in Mycoremediation

Proteomics technology also predicts the molecular level in the gene through protein analysis. The mechanism to predict the relation between expression of mRNA and their corresponding proteins is quite ambiguous, so for widening the proteome level it is quite necessary to detail the study of translation and post-translational for more understanding. Proteins participate actively in the enzyme formation which play an essential part in metabolite level. *Populus alba* roots have symbiosis with *Glomus intraradices* fungus which enhanced the capacity of Cu and Zn heavy metal absorbance; this phenomenon was confirmed through proteomic approaches (Lingua et al. 2012). White rot fungus *Phanerochaete chrysosporium* was investigated for protein in the presence of Pb heavy metal. A total of 14 proteins are upregulated and 21 protein downregulated. These upregulated proteins participate in the production of lipid peroxidase, redox metabolism, defence against oxidative damage related to heavy metal, transcription, recombination, and DNA repair. Amongst the upregulated protein isoforms of glyceraldehydes 3 phosphate dehydrogenase, alcohol dehydrogenase class V in a metal stress situation, mRNA splicing factor, ATP-dependent RNA helicase, thioredoxin reductase, actin-related protein which induce hyperactivation of Ras signalling pathway which assist in metal response in stress condition, protein related to amino acid, Ras GTPase protein required in the response of heavy metal stress, RNA binding proteins activated in stress carbohydrate, lipid transport and metabolism, protein related to G protein assist in signal transduction and in heavy metal stress, protein related to the thioredoxin reductase (Yildirim et al. 2011) (Fig. 5.2).

5.4.4 Genome Editing in Fungi

Genome editing is another advance technology which has the capability of DNA manipulation (deletion or insertion) and extent the opportunity in heavy metal mycoremediation. In the process, guide sequences designed complementary to the target sequence which assist in recognition of breaking point and repair through homologous recombination. Generally, CRISPER-CAS, TALEN and ZFN are three main gene editing methods used. CRISPER CAS is the most prominent technology in the modification. There are three types and many subtypes of systems that exist; though all types are of CRISPER, still there are specific Cas (DNA endonuclease) guided by RNA complementary sequence (approx. 20–30 bp) of targeted sequence

to cut at a specific site and afterwards the lesion gets repaired. Gene of interest can be manipulated with the help of CRISPER-Cas9 system (Sarma et al. 2021). The CRISPER technology is the natural phenomenon of bacteria they scarp the part of invading virus nucleic acid as CRISPER array to remember the virus which assist in destroying immediately in the next invasion without delay (Jaiswal et al. 2019) CRISPER-CAS 9 system used in a filamentous fungal host cells of *Aspergillus niger* CBS513.88, *Penicillium chrysogenum* Wisconsin54–1255 for genome editing (Meijrink et al. 2016). TALENs (transcription activator-like effector nucleases) are in the category of gene modification and editing. In disparity to CRISPER, TALENs use TAL protein which is artificial molecular scissor cleaves at specific target site. TAL protein is very effective; it binds even very short nucleotide sequence, that is, evenone to two nucleotides. Interestingly, nucleases are involved in the binding phenomenon due to the presence of 34 amino acid tandem repeats. TALENs possess two protein domains, one for sequencing and the other for recognizing and binding. TAL proteins are extracted from *Xanthomonas*, a pathogenic bacterium where they form naturally. In this series, another eukaryotic and prokaryotic genome editing manipulator is zinc finger nucleases (ZFNs); here, ZFP proteins are used as scissors. ZIP protein used artificial in the system originally obtained from *Flavonbacterium okeanokoites*; in contrast, they are eukaryotic transcription factor that acts as a DNA binding domain Fig. 5.2.

5.4.5 Metagenomics Technique in Fungi

Metagenomics approaches deal with microbial capacity in the heavy metal degradation from the soil. Metagenomics can be used in both culturable and non-culturable microbes because it deals with the DNA extraction, sequencing and analysis of related microbes. In Metagenome annotation of genome allowed the identification of functional genes involved in the bioremediation. Further metagenome assists in the recognition of metabolic pathways involved in the heavy metal rescue from the soil. Researchers utilized the metagenome tool in investigating the microbes for cadmium in soil. As a result, they found that higher species have a diverse number of microbes in the site that decrease the cadmium in the soil and, through KEGG pathways, analysed functional annotation of genes from blast; as a result, it concluded that enzymes present in the pathways active during the process, ABC transporter, play an important role in many biological functions involved in coping up of cadmium stress apart. In this way, metagenomic is a remarkable tool in the investigation of microbial structure and function related to the Cd exist in the soil. This research reveals that *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae* have potential in detoxification of heavy metals from soil (Feng et al. 2018). Metagenomic evaluation of fungal assemblies enriched within diffusion chambers and microbial traps containing uraniferous soils plays an important role in every event of the living organism process, the same miracle they did in the removal of heavy metal from soil through microbes. Here, metagenome narrated the presence

of relative fungal community in uranium contaminated soil, the *Ascomycota* phylum presence is highest, followed by *Basidiomycota* phylum and least abundance of *Zygomycota*. At the genus level, *Cryptococcus* has highest abundant followed by *Trichoderma* in uranium-contaminated soil. Apart from these two, *Lecythophora, Penicillium, Mortierella*, and *Aspergillus* also assist in rescue in uranium (Jaswal et al. 2019) Fig. 5.2.

5.5 Transporter Gene

Transporters are considered as the first line of defence in metal homeostasis. Major transporters are classified under three sections. Channels are the first in the transporter classification; they are simple in nature and comprise only single alpha helical protein component which facilitates passive diffusion of heavy metal. Due to being passive in nature, they do not need any proton motive force or any energy molecule for the process of substrate translocation; this channel improved in *Saccharomyces cerevisiae* from the transporter *Fps1* for As^{3+} uptake. These importers belong to the major intrinsic protein superfamily. Second are the secondary carriers use the energy in the form of ATP and acts as uniporters, symporters and antiporters single component protein. Here, for the accumulation of heavy metals, symporters have been used to import As^{4+} from *S. cerevisiae* through transporter *Hxt7* and *Pho84*. The former is a uniporter belongs to the sugar porter family and the latter is a symporter belongs to the PO^{4-}: H^{+} Family, both belongs to the major facilitator superfamily. Uniporters activity depends on the proton motive force they use energy from the charge occurs at the across the inner membrane they assist in positive charge heavy metal translocation whereas symporter too depends on proton motive force as they use the protons to generate the charge difference for energy production. Third is primary active transporter that comprises multicomponent protein complexes containing a transmembrane component for the translocation pathways, a cytoplasmic energy coupling ATPase component (approx. 30 kDa) that uses phosphoanhydride bond hydrolysis to drive the translocation of substrates and sometimes a periplasmic solute-binding component (30–70 kDa) depending on the superfamily. These importers also need proton motive force to carry their substrate against a concentration gradient using ATP and GTP (Diep et al. 2018). Arbuscular mycorrhizal fungi *Rhizophagus irregularis* establish mutual symbiosis with higher plants, and different heavy metal transporter assist in the uptake of heavy metal through the fungi. Generally, this fungus shows Cu, Fe, and Zn transporter in the fungus. Copper transporter belongs to CTR family utilized for Cu transpotation, identifies in *R. irregularis* genome; P18 ATPase family proteins exist for Cu transport, SIT family proteins for Sid-Fe transportation, OFet family protein used for sequestration of reduced Fe through transporter complex comprise from ferroxidase and Fe permease, generally fungal vacuoles utilized for storage and detoxification site for heavy metal CCC1, a member of VIT family transport Fe/Mn, ZIP family mainly for Zn only ATX2 protein for Mn, CDF family protein is for Zn (ZnT1,

ZnT2, MSC2, ZRG 17), same CDF family member MMT1 for Fe, MnT1 for Mn, NRAMP family for Mn/Fe (SMF1), SMF2 for Mn,SMF3.1, SMF3.2 for Fe. *Ustilago maydis, S. pombe, A. fumigates*, and *Fusarium graminesrum* able to sequester the siderophores-Fe, *S. cerevisiae, A. niger* and *B. cinerea* possess (OFeT) low-affinity Fe transporter system. For balancing Zn, two transporters are responsible: ZIP (zinc iron permease) and CDF (cation diffusion facilitator). Three Zn transporters of CDF family were identified in *R. irregularis, Hebeloma cylindrosporum, Oidiodendron maius;* ZIP in *S. cerevisiae* has Zn transporters such as ZRT1, ZRT2, ZRT3, ATX 2, and YKE4 and *R. irregularis* has Zn transporter such as ZRT1 and ZRT2 in plasma membrane, ATX2 protein is involved in Mn trafficking in yeast, YKE4 acts as bidirectional Zn transporter, and VIT transporter is identified in *Aspergillus, Rhizopus, R. irregularis, S. cerevisiae.* The NRAMP family transporter occurs in *S. cerevisiae, R. irregularis* (Tamayo et al. 2014). Different classes of transporters are involved in the uptake of different metal ions. Uptake of heavy metals occurs mainly by cation channels and symporters such as ZRT/IRT1 protein family which is responsible for iron zinc uptake and copper transporter COPT1 for copper uptake. To export these metal ions inside P-type ATPase required exporting zinc into the xylem and to shoot, whereas antiporters also release iron into xylem through iron exporter ferroportin. *Schizosaccharomyces Pombe* has vacuolar phytochelatin transporter named as SpHMT1; this transporter requires glutathione for function. The first ABC transporter, YCF1P, was identified in *S. cerevisiae* which also requires glutathione chelators for the detoxification of heavy metals such as As, Sb, and Cd. For more insightful knowledge, X-ray crystallography and genetic analysis tools are employed. These ABC proteins play an important role in cellular and biological processes; members of the ABC superfamily are conserved in nature. These ABC transporters consist of two domains; the first one is core domain that consists of two homologous halves, each containing membrane-spanning domain with multiple transmembrane spans and cytosolic loop, and the second part is nucleotide-binding domain which couples nucleotide hydrolysis to substrate transport. The yeast genome contains 30 ABC proteins; amongst those, 22 proteins are related to multiple membrane spans that are part of ABC transporter, while 8 proteins do not possess any membrane spans; they function apart from transportation in the cell. Yeast ABC transporters are divided into four families: ABCB, ABCC, ABCD, and ABCG, and several transporters classified under family and their location vary in the cell organelle according to their function. In family ABCB, MDL1 is located on the inner membrane of mitochondria and plays an important role in oxidative stress. In family ABCC, YCF1 transporter is present on vacuole and assists in the accumulation and detoxification of heavy metal, and BPT1 assists only in detoxification. In family ABCG, PDR15 presents on the plasma membrane and assists in cellular detoxification (Paumi et al. 2009). Evolution and diversity of ABC proteins in basidiomycetes reveal more than 1000 genes coding for ABC proteins (Kovalchuk and Driessen 2010) and the same type of research done on 27 fungal species within fungal kingdom concluded that ABC proteins are highly conserved in fungi but reduced in the number of ABC protein in *S. cerevisiae* and *S. pombe*, while in others, these proteins undergo group-specific diversification (Kovalchuk

et al. 2013). *Amanita strobiliformis*, known mushrooms, have the capacity to uptake Cu and Ag heavy metal through the same transporter, same metallothionenins, and sequestered in vacuoles. Transcriptome study of $P_{1B\text{-}1}$-ATPase transporter which encode *AsCRD1* and *AsCCC2* gene. Gene *AsCRD1* is involved in the increased manifold accumulation of copper and silver heavy metals, whereas *AsCCC2* assists in the trafficking of Cu from the cytoplasm to Golgi bodies and charges the endomembrane system and helps in detoxification (Benes et al. 2018). In Ectomycorrhizal fungi *Suillus luteus* ZIP transporter *SIZRT2* was characterized for Zn importer located on plasma membrane. Trace amount of Zn is essential, so Zn can enter the cytoplasm through ZIP transporter; *SIZRT2* contains the histidine-rich domain which is essential for metal binding (Coninx et al. 2019).

5.6 Conclusion

Increasing urbanization and global industrialization have adversely impacted the environment in substantial ways. Soil pollution has witnessed an alarming threshold in recent years. It is thus of paramount importance to not only deal with the soil pollution but also amalgamate such procedures which help in retaining and improving the soil fertility and texture. Metal toxicity in the soil is a significant challenge due to the persistent and recalcitrant nature of the metals and their toxic effects on the living system, more so in the case of human beings.

Mycoremediation technologies are currently at the forefront due to their immense diverse potentialities. They are cost-effective and allow for easy maintenance reproducibility and growth requirements. The nonpathogenicity of many species towards many living systems particularly humans and the utilization of both dead and living fungal biomass is a tremendous advantage and provides an edge over other bioremediation techniques. It has become an essential part of a wider approach called GRO (gentle remediation options) which focuses on conserving the net functionality of soil along with the risk management. The essence of GRO concept is to provide biologically more productive soil with the aid of diverse technologies like phytoremediation microremediation along with mycoremediation with or without biological (farmyard vermicompost, cow dung, etc.) and chemical additives (fertilizers, pesticides, herbicides), although minimalist use of chemical additives is often the primary concern of GRO methodologies. GRO is currently popular in certain European countries, but it may be more helpful when it has global acceptance and is practised worldwide.

The biotechnological tools have come up with a finer understanding of their fungal detoxification mechanism and the corresponding involvement of the gene in some species, but a more comprehensive knowledge of genetic mechanism in different fungal groups is yet to be explored. Biotechnological tools such as genetic engineering, gene editing, metagenomics, transcriptomics, and system biology can greatly assist us in our understanding and refining our approach towards the use of fungi in improving soil health besides contaminant removal from soil. Thus, fungal

species play quintessential role in the removal of heavy metal toxicants and in rejuvenating soil health. Their exquisite feature as a novel bioremediating agents demands a more in-depth research at both the molecular and gene level while offering a wider commercial aspect.

References

Anza M, Salaza O, Epelde L, María J, Alkorta BI, Garbisu C (2019) Remediation of organically contaminated soil through the combination of assisted phytoremediation and bioaugmentation. Appl Sci 9:4757. https://doi.org/10.3390/app9224757

Asiriuwa OD, Ikhuoria JU, Ilori EG (2013) Myco-remediation potential of heavy metals from contaminated soil. Bull Environ Pharmacol Life Sci 2(5):16–22

Baldrian P (2003) Interactions of heavy metals with white-rot fungi. Enzyme Microbial Technol 32(1):78–91

Bano A, Hussain J, Akbar A, Mehmood K et al (2018) Biosorption of heavy metals by obligate halophilic fungi. Chemosphere 199:218–222. https://doi.org/10.1016/j.chemosphere.2018.02.043

Bellion M, Courbot M, Jacob C, Blaudez D, Chalot M (2006) Extracellular and cellular mechanisms sustaining metal tolerance in ectomycorrhizal fungi. FEMS Microbiol Lett 254:173–181

Benes V, Leonhardt T, Sacky J, Kotrba P (2018) Two P_{1B-1}-ATPases of *Amanita strobiliformis* with distinct properties in Cu/Ag transport. Front Microbial 9:747. https://doi.org/10.3389/fmicb.2018.00747

Blaudez D, Botton B, Chalot M (2000) Cadmium uptake and subcellular compartmentation in the ectomycorrhizal fungus *Paxillus involutus*. Microbiology 146:1109–1111

Brady D, Ducan JR (1994) Bioaccumulation of metal-cations by *Saccharomyces cerevisae*. Appl Microbiol Biotechnol 41:149–154

Bumpus JA, Tien M, Wright D, Aust SD (1985) Oxidation of persistent environmental pollutants by a white rot fungus. Science 228:1434–1436

Carmen CE, Gabriela B (2013) The mycoremediation of metals polluted soils using wild growing species of mushrooms. Latest Trends Eng Educ 38:147–151. ISSN: 1792-426X 36-39

Checcucci A, Bazzicalupo M, Mengoni A (2017) Exploiting nitrogen fixing rhizobial symbionts genetic resources for improving phytoremediation of contaminated soils. In: Naser A, Anjum A, Gill SS, Tuteja N (eds) Enhancing cleanup of environmental pollutants: biological approaches. Springer International Publishing, Cham, pp 275–288

Compos C, Nobre T, Goss MJ, Faria J, Barrulas P, Carvalho M (2019) Transcriptome analysis of wheat roots reveals a differential regulation of stress responses related to arbuscular mycorrhizal fungi and soil disturbance. Biology 8(4):93. https://doi.org/10.3390/biology8040093

Coninx L, Smisdom N, Kohler A, Natascha A et al (2019) SIZRT2 encoded a ZIP family Zn transporter with dual localization in the ectomycorrhizal fungus. Front Microbiol 10:2251. https://doi.org/10.3389/fmicb.2019.02251

Das A, Osborne JW (2018) Bioremediation of heavy metals. In: Nanotechnology, food security and water treatment. Springer, Cham, pp 277–311

Deshmukh R, Khardenavis AA, Purohit HJ (2016) Diverse metabolic capacities of fungi for bioremediation. Indian J Microbiol 56(3):247–264

Dhankhar R, Hooda A (2011) Fungal biosorption – an alternative to meet the challenges of heavy metal pollution in aqueous solutions. Environ Technol, Taylor and Francis group 32(5):467–491

Diep P, Mahadevan R, Yakunin AF (2018) Heavy metal removal by bioaccumulation using genetically engineered microorganisms. Front Bioeng Biotechnol 6:157

Dönmez G, Aksu Z (2002) Removal of chromium(VI) from saline wastewaters by Dunaliella species. Process Biochem 38:751–762

Eaton DC (1985) Mineralization of polychlorinated biphenyls by *Phanerochaete chrysosporium*: a ligninolytic fungus. Enzyme Microb Technol 7:194–196

El Hameed AHA, Eweda WE, Abou-Taleb KA, Mira H (2015) Biosorption of uranium and heavy metals using some local fungi isolated from phosphatic fertilizers. Ann Agric Sci 60(2):345–351

Feng G, Xie T, Wang X, Bai J, Tang L, Zhao H, Wei W, Wang M, Zhao Y (2018) Metagenomic analysis of microbial community and function involved in cd-contaminated soil. BMC Microbiol 18:11. https://doi.org/10.1186/s12866-018-1152-5

Gharieb MM, Sayed JA, Gadd GM (1998) Solubilization of natural ypsum($CaSO_4.2H_2O$) and the formation of Calcium Oxalate by *Aspergillus Niger* and *Serpula humatioides*. Mycol Res 102:825–830

Gonzalez E, Pitre FE, Pagé AP et al (2018) Trees, fungi and bacteria: tripartite metatranscriptomics of a root microbiome responding to soil contamination. Microbiome 6(53):1–30

Guerrero GM, Melville LH, Ferrol N, Lott JN, Azcon-Aguilar C, Peterson RL (2008) Ultrastructural localization of heavy metals in the extraradical mycelium and spores of the arbuscular mycorrhizal fungus *Glomus intraradices*. Can J Microbiol 54:103–110. https://doi.org/10.1139/w07-119

Hyde KD, Xu J, Rapior S, Jeewon R, Lumyong S, Grace A et al (2019) The amazing potential of fungi: 50 ways we can exploit fungi industrially. Fungal Divers 97:1–136. https://doi.org/10.1007/s13225-019-00430-9

Igiri BE, Okoduwa SIR, Idoko GO, Akabuogu EP et al (2018) Toxicity and bioremediation of heavy metals contaminated ecosystem from Tannery wastewater: a review. J Toxicol 2018:1–16

Jain KK, Kumar A et al (2020) De nova transcriptome assembly and protein profiling of copper induced lignocellulolytic fungus *Ganoderma lucidum* MDU-7 reveals genes involved in lignocelluloses degradation and terpenoid biosynthetic pathways. Genomics 112:184–198

Jaiswal S, Singh DK, Shukla P (2019) Gene editing and systems biology tools for pesticide bioremediation: a review. Front Microbiol 10:87

Jaswal R, Pathak A, Chauhan A (2019) Metagenomic evaluation of bacterial and fungal assemblages enriched within diffusion chambers and microbial traps containing uraniferous soils. Microorganisms 7:324. https://doi.org/10.3390/microorganisms7090324

Kaewdoung B, Sutjaritvorakul T, Gadd GM, Whalley AJS, Sihanonth P (2016) Heavy metal tolerance and biotransformation of toxic metal compounds by new isolates of wood-rotting fungi from Thailand. Geo Microbiol J 33:283–288. https://doi.org/10.1080/01490451.2015.1048394

Kamei I, Takagi K, Kondo R (2011) Degradation of endosulfan and endosulfan sulfate by white-rot fungus *Trametes hirsuta*. J Wood Sci 57:317–322

Kameo S, Iwahashi H, Kojima Y, Satoh H (2000) Induction of metallothioneins in the heavy metal resistant fungus *Beauveria bassiana* exposed to copper or cadmium. Analusis 28:382–385

Khodja H, Iddou A, Aguedal H, Aziz A, Shishkin A (2018) Bioremoval of lead (II) and cadmium (II) in single and multicomponent systems using Penicillium sp. Key Eng Mater Trans Tech Publ 762:93–98

Kovalchuk A, Driessen AJM (2010) Phylogenetic analysis of fungal ABC transporters. BMC Genomics 11:177. https://doi.org/10.1186/1471-2164-11-177

Kovalchuk A, Lee YH, Asiegbu FO (2013) Diversity and evolution of ABC proteins in basidiomycetes. Mycologia 105(6):1456–1470. https://doi.org/10.3852/13-001

Kumar M, Prasad R, Goyal P, Teotia P, Tuteja N, Varma A, Kumar V (2017) Environmental biodegradation of xenobiotics: role of potential microflora. In: Hashmi MZ, Kumar V and Varma A (eds.), *Xenobiotics in the Soil Environment*, Springer International Publishing Switzerland 319–334

Lamar RT, White RB (2001) Mycoremediation: commercial status and recent developments. In: Magar VS et al (eds) Proceedings of the sixth international symposium on in situ and on-site bioremediation, vol 6, San Diego, pp 263–278

Lingua G, Bona E, Todeschini V, Cattaneo C, Marsano F, Berta G, Cavalet M (2012) Effects of heavy metals and arbuscular mycorrhiza on the leaf proteome of a selected poplar clone: a time course analysis. PLoS One 7(6):e38662. https://doi.org/10.1371/journal.pone.0038662

Luna JM, Rufino RD, Sarubbo LA (2016) Biosurfactant from Candida sphaerica UCP0995 exhibiting heavy metal remediation properties. Process Safe Environ 102:558–566

Maini ZAN, Aribal KMJ, Narag RMA et al (2019) Lead (II) tolerance and uptake capacities of fungi isolated from a polluted tributary in the Philippines. Appl Environ Biotechnol 4(1):18–29. https://doi.org/10.26789/AEB.2019.01.004

Meijrink M, Verwaal R, Gielesen BEM, Roubos JA, Nygård YI, Bovenberg RAL, Driessen AJM, Pohl C (2016) A CRISPR-CAS system for a filamentous fungal host cell. WO2016110453A8 WIPO (PCT) patent

Mishra J, Singh R, Arora NK (2017) Alleviation of heavy metal stress in plants and remediation of soil by rhizosphere microorganisms. Front Microbiol 8:1706

Mosa KA, Saadoun I, Kumar K, Helmy M, Dhankher OP (2016) Potential biotechnological strategies for the cleanup of heavy metal and metalloids. Front Plant Sci 7:303

Nyakundi WO, Magoma G, Ochora J, Nyende AB (2011) Biodegradation of diazinon and methomyl pesticides by white rot fungi from selected horticultural farms in rift valley and Central Kenya. J Appl Technol Environ Sanit 1:107–1249

Paumi CM, Chuk M, Snider J, Stagljar I, Michaelis S (2009) ABC transporters in *Saccharomyces cerevisiae* and their interactors: new technology advances the biology of the ABCC (MRP) subfamily. Microbiol Mol Biol Rev 73(4):577–593

Pires C, Franco AR, Pereira SIA, Henriques I, Correia A, Magan N et al (2017) Metal(loid)-contaminated soils as a source of culturable heterotrophic aerobic bacteria for remediation applications. Geomicrobiol J 1:9

Ramsay LM, Gadd GM (1997) Mutants of *Saccharomyces cerevisiae* defective in vacuolar function confirms a role for the vacuole in toxic metal ion detoxification. FEMS Microbiol Lett 152:293–298

Reddy CA, Mathew Z (2001) Bioremediation potential of white rot fungi (2018). In: Gadd G (ed) Fungi in bioremediation. Cambridge University Press, Cambridge

Rosen BP (2002) Transport and detoxification systems for transition metals, heavy metals and metalloids in eukaryotic and prokaryotic microbes. Comp Biochem Physiol A Mol Integr Physiol 133:689–693

Ruscitti M, Arango M, Belgrano J (2017) Improvement of copper stress tolerance in pepper plants (*Capsicum annuum* L.) by inoculation with arbuscular mycorrhizal fungi. Theor Exp Plant Physiol 29:37–49. https://doi.org/10.1007/s40626-016-0081-7

Sarma H, Forid N, Prasad R, Prasad MNV, Ma LQ, Rinklebe J (2021) Enhancing phytoremediation of hazardous metal(loid)s using genome engineering CRISPR–Cas9 technology. Journal of Hazardous Materials https://doi.org/10.1016/j.jhazmat.2021.125493

Sen M (2018) Enhanced biological removal of Cr (VI) in continuous stirred tank reactor (CSTR) using Aspergillus sp. Braz J Biol Sci 5(9):33–50

Shen J, Hsu CM, Kang BK, Rosen BP, Bhattacharjee H (2003) The Saccharomyces cerevisiae Arr4p is involved in metal and heat tolerance. Biometals 16:369–378

Singh JS (2015) Microbes: the chief ecological engineers in reinstating equilibrium in degraded ecosystems. Agric Ecosyst Environ 203:80–82

Subudhi E, Kar RN (2008) *Rhizopus arrhizus*- an efficient fungus for copper effluent treatment. Int J Integr Biol 2:166–171

Tamayo E, Gallego TG, Aguilar CA, Ferrol N (2014) Genome-wide analysis of copper, iron and zinc transporters in the arbuscular mycorrhizal fungus *Rhizophagus irregularis*. Front Plant Sci 5:547

Thakare M, Sarma H, Datar S, Roy A, Pawar P, Gupta K, Pandit S, Prasad R (2021) Understanding the holistic approach to plant-microbe remediation technologies for removing heavy metals and radionuclides from soil. Current Research in Biotechnology https://doi.org/10.1016/j.crbiot.2021.02.004

Tkavc R, Matrosova VY, Grichenko OE et al (2018) Prospects for fungal bioremediation of acidic radioactive waste sites: characterization and genome sequence of *Rhodotorula taiwanensis* MD1149. Front Microbiol 08. https://doi.org/10.3389/fmicb.2017.02528

Xiao P, Mori T, Kamei I, Kiyota H et al (2011) Novel metabolic pathways of organochlorine pesticides dieldrin and aldrin by the white rot fungi of the genus Phlebia. Chemosphere 85:218–224

Yamaji K, Watanabe Y, Masuya H, Shigeto A, Yui H, Haruma T (2016) Root fungal endophytes enhance heavy metal stress tolerance of *Clethra barbinerv* is growing naturally at mining sites via growth enhancement, promotion of nutrient uptake and decrease of heavy metal concentration. PLoS One 11(12):e0169089. https://doi.org/10.1371/journal.pone.0169089

Yao Q, Yang R, Long L, Zhu H (2014) Phosphate application enhances the resistance of arbuscular mycorrhizae in clover plants to cadmium via polyphosphate accumulation in fungal hyphae. Environ Exp Bot 108:63–70. https://doi.org/10.1016/j.envexpbot.2013.11.007

Yildirim V, Ozcan S, Becher D, Buttner K, Hecker M, Ozcengiz G (2011) Characterization of Proteome alterations on *Phanerochaete chrysosporium* in response to lead exposure. Proteome Sci 9:12

Chapter 6
Role of Endophytes in Plant-Associated Remediation and Plant Growth Promotion: A Deep Insight

Saurabh Gupta, Gaganpreet Kaur, and Jashan Nirwan

Contents

6.1 Introduction

Due to consistent increase in urbanisation and industrialisation, our environment has been contaminated by a diverse array of perilous chemicals released by various industries including pesticides, heavy metals, fuel waste, harmful solvents, alkanes, polycyclic aromatic hydrocarbons (PAHs), explosives, dyes, etc. (Gianfreda and Rao 2004; Ma et al. 2011). These anthropogenic sources of pollution are affecting

S. Gupta (✉) · J. Nirwan
Department of Microbiology, Mata Gujri College, Fatehgarh Sahib, India

G. Kaur
Department of Biotechnology & Medical Sciences, Baba Farid Group of Institutions, Baba Farid College, Bathinda, India

R. Prasad et al. (eds.), *Mycoremediation and Environmental Sustainability*, Fungal Biology, https://doi.org/10.1007/978-3-030-54422-5_6

naturally existing abiotic components including air, water and soil under the impact of modernisation (Gianfreda and Rao 2004). These contaminants are of eminent concern for mankind as they exhibit a mutagenic, carcinogenic and cytotoxic effect on humans along with having adverse implications on our surrounding environment (Gianfreda and Rao 2004; Ma et al. 2011). The majority of risk exerted on humans as well as their surroundings is through contamination by heavy metals and radioactive compounds such as lead, zinc, cadmium, selenium, chromium, manganese, cobalt, copper, nickel, mercury arsenic, sodium, nitrate, ammonia and phosphate (Glick 2003). Large-scale technological advancements lead to the dissemination of heavy metals and other pollutants to a much greater extent having detrimental effects on not only humans but also our ecosystem worldwide (Luo et al. 2012). The elimination of these toxic contaminants from the environment is necessary but is still potentially complicated due to the extensive scattering of these pollutants. For decades, several groups of researchers are developing contemporary and cost-effective technological methods using plants to remove these contaminants from the soil along with the positive influence on plant growth (Glick 2003). Different methods employed for remediation of heavy metal are shown in Fig. 6.1.

The most prevalent traditional method used for treating contaminants is excavating, pumping then treating, soil washing followed by the addition of chemical reactants, solidification and stabilising, vitrification or transportation to the off-site for degradation process involving an excessive amount of energy consumption. For a future perspective, more economical, energy-efficient and environmentally friendly techniques have been developed (Doty et al. 2007). Bioremediation is based on the natural diminution of various contaminants' influence, and currently, it is more acceptable than other technologies and is a safer approach which utilises microbial metabolites for the elimination of these undesirable contaminants. Phytoremediation (that uses green plants in situ) is particularly enhanced by endophytes (Stępniewska and Kuźniar 2013). It is being an inexpensive and safer choice for protecting humans

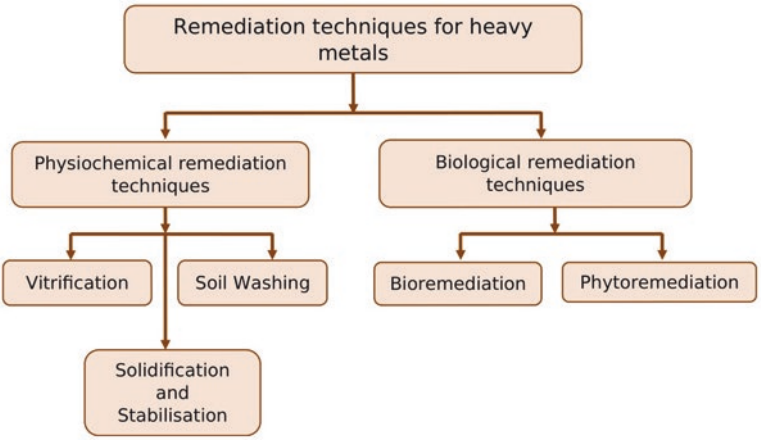


Fig. 6.1 Variable remediation methods for heavy metal pollution

and the environment as compared to conventional approaches for removing contaminants from the soil (Luo et al. 2011). Phytoremediation is also an aesthetically pleasing mode for eradicating pollutants that uses the natural efficiency of kingdom Plantae to decrease the effect of pollutants in the environment (Doty et al. 2007). This book chapter focuses on the contribution of plants through phytoremediation and importantly plant-microbe interactions that help in promoting growth in plants, i.e. studied in depth to inculcate the knowledge about the role of endophytic microbes in biodegradation of pollutants as well as maintaining plants as a valuable resource.

6.2 Phytoremediation: A Promising Approach for the Future

Phytoremediation is a comparatively new approach that utilises green plants for the treatment of hazardous chemical contaminants from the environmental resources, i.e. air, soil and water (Glick 2003; Ho et al. 2013; Macek et al. 2000; Ho et al. 2012; Chaudhry et al. 2005; Khan et al. 2015; Rajkumar et al. 2009; Germaine et al. 2006; Gerhardt et al. 2009). Phytoremediation is a versatile, solar-driven pump (Yadav et al. 2010) that can extract and accumulate specific compounds from the environment making it a promising bioresource technology for the future (Yadav et al. 2010). Phytoremediation utilises numerous types of plant processes as well as the external characteristics of plants for remediating polluted sites (Dixit et al. 2015). Phytoremediation is an economical and environmentally friendly technology for removing toxic contaminants from polluted sites (Ho et al. 2012, 2013; Ma et al. 2011; Chen et al. 2014; Khan et al. 2014; Feng et al. 2017; Dixit et al. 2015; Azubuike et al. 2016; Germaine et al. 2009 and Arshad et al. 2007; Sarma et al. 2021). It usually includes the removal of various classes of pollution caused by industrial contaminants (Anyasi and Atagana 2015) such as radioactive elements, toxic organic chemicals (Macek et al. 2000; Dixit et al. 2015), volatile organic compounds such as trichloroethylene (TCE) and BTEX (benzene, toluene, ethylbenzene, xylene) (Wu et al. 2009), metal-contaminated soils (Ma et al. 2016; Meharg and Cairney 2000), toxic heavy metals (Chaudhry et al. 2005; Dixit et al. 2015), xenobiotic compounds (Eapen et al. 2007; Ijaz et al. 2016), recalcitrant chemicals, polyaromatic hydrocarbons (PAHs) (Germaine et al. 2009) and agricultural run-off water containing toxic chemical fertiliser and nutrients including metals, arsenic, selenium, boron, organic pesticides as well as herbicides (Pilon-Smits 2005). Although this technique takes an extended time for the treatment of low level of contaminants, nonetheless it is efficient in remediating polluted sites in comparison with conventional methods of remediation (Ho et al. 2012, 2013) including excavation and incineration from the site of contamination, storage at off-site areas, washing of soil and stabilising by in situ cappings (Gerhardt et al. 2009). This process is advantageous over conventional technologies as it is ten times less expensive (Doty 2008). Plants help in stabilising the soil, minimising the amount of polluted dust that may leave the site and enter the areas surrounding (Azubuike et al. 2016). The prerequisite for an effective process

of phytoremediation is an extensive root growth system of plants (Arshad et al. 2007). The plant generally involves a passive process of eliminating contaminants through uptake by roots and then translocation from parts below from roots towards parts above the soil, i.e. shoots, which is then carried out through vascular tissue via xylem flow later on accumulating inside the shoot. The plant utilises its metabolic enzymes which metabolise and biodegrade contaminants by acting as filters or traps (Kabra et al. 2013). Plants adapt in stress induced by organic pollutants through the establishment of a detoxification system inside the cells where the reduction in these contaminants occur, which are then catabolised or eradicated, giving plants capability in minimising the hazardous effects of the contaminants. This complete process is known as the "Green Liver" model. It was first demonstrated in carrot plants, proving the ability to degrade phthalate esters. Many enzymes produced by plants during their metabolic processes such as glutathione-S-transferase, cytochrome 450 monooxygenase, glycosyltransferase, etc. help by directly participating for tolerance, stabilisation, deposition and detoxification processing of the organic pollutants (Feng et al. 2017). A plant may continue towards absorbing pollutants until it is harvested. The important points to be considered while choosing phytoremediation plant include its root system (depending upon the depth of pollutant accumulation), biomass present above the ground available for consumption of animals, toxicity concentration of pollutant to plant, survival rate, growth rate and adaptability of the plant towards environmental conditions, monitoring of the site as well as resistance to pests and diseases (Azubuike et al. 2016; Sarma et al. 2021).

Phytoremediation is an environmentally friendly mechanism which is utilised to remove, hold or modify toxic products into nontoxic contaminants in terrestrial land or water employing fast-growing plants. Phytoremediation uses different processes for the degradation of toxic contaminants including phytoextraction, phytofiltration, phytodegradation, phytostabilisation, phytovolatilisation and rhizoremediation which are depicted in Fig. 6.2.

6.2.1 Phytoextraction

Phytoextraction includes uptake and movement of metal contaminants from the soil by roots of plants into the plant parts that are present above the ground based on the mechanism known as hyperaccumulation (Ojuederie and Babalola 2017). Plants that are good in concentrating the contaminants are known as hyperaccumulators (Ma et al. 2011; Doty 2008). A hyperaccumulator especially metals is classified as a plant that can accumulate the metals to a level of 1% (10,000 ppm) of zinc; 0.1% (1000 ppm) of metals such as copper, cobalt, lead and nickel; and 0.01% (100 ppm) of cadmium (Doty 2008; Brown et al. 1994). For instance, a Chinese fern *Pteris vittata* can accumulate arsenic in its fronds and removed efficiently from the soil, highly carcinogenic and toxic metal cadmium is concentrated by *Thlaspi caerulescens* (Doty 2008). Hyperaccumulators have received great attention because they exhibit good efficacy towards tolerance of heavy metals and their accumulation

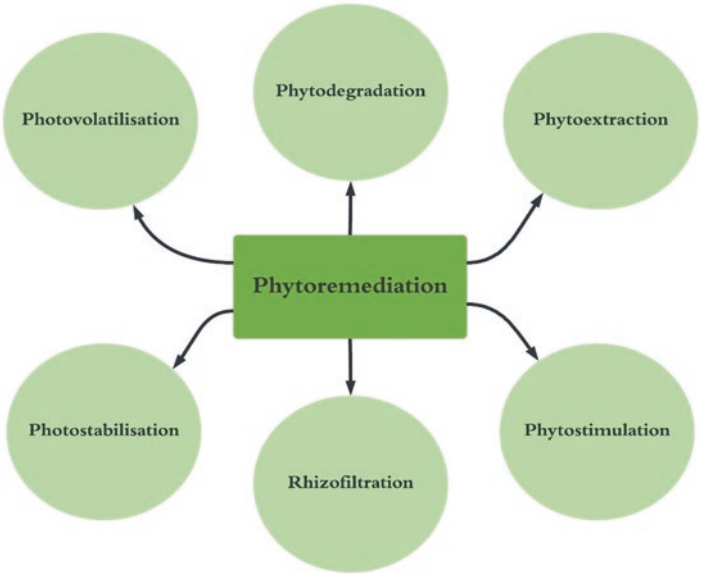


Fig. 6.2 Different processes of phytoremediation

efficiency is higher as compared to other plants (Dixit et al. 2015). Other examples of plants as hyperaccumulators for various contaminants are mentioned in Table 6.1 given below. On the other hand, non-hyperaccumulator plants did not exceed 10 ppm, accumulation of metals for metabolic processes, whereas hyperaccumulators can accumulate metals 100-fold greater than non-accumulators (Lasat 1999). This process of phytoextraction occurs continuously by the use of hyperaccumulators with enhanced bioavailability by the addition of chelates. The advantage of this process is that some valuable metals get bioaccumulated inside plants which can be later recovered after phytoremediation of contaminated sites known as phytomining (Azubuike et al. 2016).

6.2.2 Phytofiltration

This process includes rhizofiltration that utilises plant roots (Glick 2003; Dixit et al. 2015) or blastofiltration by the use of seedlings (Dixit et al. 2015) through either adsorption or precipitation of pollutants when they are present in a soluble form (Kabra et al. 2013). This process usually involves the remediation of polluted underground water. Plants used for this method are not sown directly to the site which is to be remediated; instead, they are first grown in clean water using hydroponics until a large root system is formed; after its acclimatisation, it is further planted in contaminated areas where roots uptake pollutants; and upon obtaining a saturation limit, they are harvested and disposed under benign conditions. Further additional

Table 6.1 Plant species with phytoremediation capacity of various pollutants

Host plant	Substrate degraded	References
Pteris vittata	Arsenic	Doty et al. (2007), Pilon-Smits (2005)
Thlaspi caerulescens	Cadmium and zinc	Doty et al. (2007), Ebbs et al. (1997), Mastretta et al. (2006), Lasat (1999), Li et al. (2012), Pilon-Smits (2005)
Salix matsudana, *Salix alba*	Cadmium	Doty et al. 2007)
Salix viminalis	Cadmium and zinc	Doty et al. 2007)
Populus trichocarpa	Trichloroethylene (TCE)	Doty et al. 2007)
Populus deltoids	4-Amino-2,6-dinitrotoluene (TNT)	Doty et al. 2007)
Leucaena leucocephala, *Ipomoea batatas*	4-Amino-2,6-dinitrotoluene (TNT) and trichloroethylene (TCE)	Doty et al. 2007), Eapen et al. (2007)
Populus nigra	Polyaromatic hydrocarbons (PAHs) and 4-amino-2,6-dinitrotoluene (TNT)	Doty et al. 2007)
Salix spp. (EW-20)	4-Amino-2,6-dinitrotoluene (TNT)	Doty et al. 2007)
Myriophyllum aquaticum, *Catharanthus roseus*	Royal demolition explosives (RDX) & 4-amino-2,6-dinitrotoluene (TNT)	Doty et al. 2007), Eapen et al. (2007), Macek et al. (2000)
Thlaspi goesingense	Nickel	Macek et al. (2000)
Brassica napus	2,4-Dichlorophenol (2,4-D) and 4-amino-2,6-dinitrotoluene (TNT)	Eapen et al. (2007)
Arabidopsis	4-amino-2,6-dinitrotoluene (TNT)	Eapen et al. (2007)
Methylobacterium oryzae	Royal demolition explosives (RDX) and 4-amino-2,6-dinitrotoluene (TNT)	Eapen et al. (2007)
Panicum virgatum, *Medicago sativa*, *Lolium perenne*, *Vicia faba*, *Schizachyrium scoparium*	Polyaromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs)	Eapen et al. (2007), Macek et al. (2000), Pilon-Smits (2005)
Brassica juncea, *B. napus*, *B. rapa*	Cadmium and zinc	Lasat (1999)
Agrostis capillaris, *Festuca rubra*	Cadmium and zinc	Lasat (1999)
Alyssum bertolonii, *Alnus firma*, *Brassica napus*, *Nicotiana tabacum*, *Solanum nigrum*	Metal hyperaccumulator (nickel, copper, zinc and lead)	Li et al. (2012), Pilon-Smits (2005)

(continued)

Table 6.1 (continued)

Host plant	Substrate degraded	References
Arabis hirsuta, *Acacia decurrens*, *Symplocos paniculata*	Metal non-hyperaccumulator (nickel, copper, zinc, lead and cadmium)	Li et al. (2012), Pilon-Smits (2005)
Lolium multiflorum, *Lotus corniculatus*, *Methylobacterium oryzae*, *Burkholderia* spp.	Nickel and cadmium	Pilon-Smits (2005)
Gluconacetobacter diazotrophicus, *Salix caprea*	Zinc	Pilon-Smits (2005)
Brassica napus	Lead, copper	Li et al. (2012), Ma et al. (2016)
Lupinus luteus	Heavy metals and organic pollutants	Ijaz et al. (2016)
Pisum sativum	2,4-Dichlorophenol (2,4-D)	Ijaz et al. (2016)
Populus tremula	Volatile toxic compounds: trichloroethylene (TCE), vinyl chloride, carbon tetrachloride, chloroform, benzene	Kang (2014)
Populus alba	Volatile toxic compounds: trichloroethylene (TCE), vinyl chloride, carbon tetrachloride, chloroform, benzene	Kang (2014), Yadav et al. (2010)
Solanum nigrum	Cadmium hyperaccumulator	Ma et al. (2016), Luo et al. (2011)
Sorghum bicolor	Heavy metals (cadmium, lead and copper)	Luo et al. (2012), Raskin et al. (1997)
Catharanthus roseus	4-Amino-2,6-dinitrotoluene (TNT)	Macek et al. (2000)
Poplar trees	Trichloroethylene (TCE)	Macek et al. (2000)
Betula celtiberica	Arsenic	Mesa et al. (2017)
Buchloe dactyloides, *Azolla*, rabbitfoot grass	Selenium	Pilon-Smits (2005)

studies are being carried out to successfully study the plant parts that are efficient in accumulating contaminants (Ojuederie and Babalola 2017).

6.2.3 Phytodegradation

This process includes the biodegradation of organic contaminants metabolically through the use of biocatalyst, namely, enzymes (Doty 2008; Pilon-Smits 2005), which further reduces mobility (Glick 2003; Dixit et al. 2015) and bioavailability of metals in the surrounding environment to prevent their transfer into the food chain or underground water (Dixit et al. 2015). Specific enzymes must be utilised under

optimum conditions such as temperature and pH for the degradation of pollutants. Examples of enzymes used for the degradation of contaminants are nitroreductases and dehalogenases. The phytodegradation can be enhanced through the microorganisms present in the rhizospheric region, and the process is termed as rhizodegradation (Ojuederie and Babalola 2017). Rhizodegradation is accountable for the augmented elimination of total petroleum hydrocarbons from the soil by deeply rooted trees, whereas the fate of polyaromatic hydrocarbons (PAHs) and other related contaminants in the surrounding environment is dependent on biotic and abiotic processes (Singh and Jain 2003).

6.2.4 Phytostabilisation

This process refers to stabilising the polluted soil and sediments at a particular place through plantation and immobilisation of pollutants in soils, rendering them into harmless state and preventing their further spread into the environment (Ojuederie and Babalola 2017). The establishment of plantation and immobilisation prevents exposure of toxic site waste to mankind. The hydraulic control method can be implied in some of the cases because a larger volume of water transpired via plants prevents migration of leachate towards underground water or through stabilising pollutants in the sub-surface layer by preventing contact between water and waste (Pilon-Smits 2005). The plants that are used in this process should have a broad network of roots and a lower rate of mobilisation of metals from roots towards apical shoots. This process can be improved adjusting pH and organic content (adding biochar or compost) which in turn improves plant yield and immobilisation of metals (Ojuederie and Babalola 2017).

6.2.5 Phytovolatilisation

When the plants convert toxic contaminants into volatile forms (Glick 2003; Dixit et al. 2015), through evapotranspiration (Kabra et al. 2013) or volatilisation from the plant leaf, stomata or stem, before releasing into the atmosphere is known as phytovolatilisation (Glick 2003; Dixit et al. 2015). A very few pollutants are adequately soluble in water and volatilise enough to reach up to atmospheric concentration levels through evapotranspiration (Singh and Jain 2003). For instance, tobacco plants can transform toxic methyl mercury into a less toxic elemental form of mercury which escapes into the atmosphere through leaves by the process of volatilisation in a volatile form (Ojuederie and Babalola 2017). This method can also be used for volatile organic compounds such as trichloroethylene (TCE) and inorganic compounds such as selenium (Pilon-Smits 2005).

6.2.6 Rhizoremediation

Limitations in the process of phytoremediation leads to using an alternative method of remediation i.e. utilising naturally occurring microorganisms in the rhizospheric region called rhizoremediation. These microorgainsms may be further isolated by enrichment method for biodegradation of selected pollutants by Insitu ioculation known as bioaugmentation. The rhizospheric region has inordinate potential to remediate contaminants because the microbes that are present in root exudates help in stimulation as well as for attaining better soil moisture, aeration and nutrient conditions near root region which enhances the capability of the process.

However, the success of any of the approaches of phytoremediation mentioned above depends on the optimisation of the remediation potential of native plants growing in the polluted sites. It is a time-consuming process that depends on multiple characteristics such as bioavailability, toxicity and concentration of pollutants along with certain properties of plants (Azubuike et al. 2016). It can be converted into an efficient biotechnological approach. Moreover, for effective biodegradation of organic chemicals, plants are dependent on the microbes associated with them (Weyens et al. 2009a). Synergistic association of endophytic microbes with their host plant forms symbiotic relationships in which the plant helps the microbe by supplying nutrients and providing protection, and on the contrary, microbes provide some essential nutrients to the plants (Newman and Reynolds 2005). Several studies have demonstrated that endophytes speed up the phytoremediation techniques effectively through close interaction with plants that act as a host for it (Stępniewska and Kuźniar 2013). Recently, bacterial endophyte-assisted phytoremediation is recommended highly for eradicating pollutants at polluted sites (Ma et al. 2016).

6.3 An Introduction to Endophytes

Interaction among plants and microorganisms is a fundamental part of the terrestrial ecosystem (Wu et al. 2009). Therefore, plant and endophyte synergism provides a magnificent way to restore the polluted ecosystem (Ijaz et al. 2016). Endophytes are the microorganisms colonising the plant (Patle et al. 2018; Deng and Cao 2017; Li et al. 2012; Stępniewska and Kuźniar 2013) and live inside the microenvironment of the host plant (Patle et al. 2018), beneath the epidermal cell layers (Ma et al. 2016) where it receives protection from the stress of the environment, experiences less competition from other microorganisms, remediates the contaminated soil (Patle et al. 2018) through synthesis of natural products, promotes plant growth (Anyasi and Atagana 2015; Dowling and Doty 2009), helps in nitrogen fixation of plants (Doty et al. 2009; Taghavi et al. 2009; Feng et al. 2017) and reduces phytotoxicity (Taghavi et al. 2009; Soleimani et al. 2010). Endophytes may act as biocontrol agents (Ryan et al. 2007; Sgroy et al. 2009; Patle et al. 2018; Feng et al. 2017; Dobbelaere et al. 2003; Verma et al. 2011; Gaiero et al. 2013; Rashid et al. 2012), as

these microbes get carbohydrates from plants and improve abiotic and biotic stress of the plants (Li et al. 2012; Khan et al. 2014; Feng et al. 2017). Endophytes generate a close linkage inside plant tissues that facilitates the exchange of nutrients and enzyme activity. A variety of endophytes produces phytohormones, which help in growth-promoting mechanisms, to maintain a dynamic balance of hormones in host plants and modulates stress response of hosts. These phytohormones such as indole-3-acetic acid (auxin) also help in the colonisation of endophytes in plants, probably by interference with the defence mechanism of the host plant (Feng et al. 2017). Endophytes can also be helpful to the host for the production of natural products that could be used for medicinal, agricultural or industrial purposes. An endophytic organism not only improves the process of phytoremediation but also enhances soil fertility through solubilisation of phosphate present in soil and nitrogen fixation (Patle et al. 2018; Mishra et al. 2015).

6.4 History of Endophytic Microorganisms

The term "endophyte" was first coined by de Bary in 1886 for microorganisms including fungi, yeast and bacteria that reside in tissues of a plant. Further in 1887, Victor Gallipe postulated that microorganisms that are present in soil can penetrate healthy tissues of plants. After 120 years, Carol during 1986 postulated that fungi which are responsible for asymptomatic infections completely in the host plant tissues are endophytes. In 1991, Petrini studied all microorganisms that may colonise plant tissues without any viable symptoms. Later in 1992, Hirsch and Braun named a group of microorganisms that can colonise plant tissues without resulting in any sort of infection as endobionts. During the period between 1933 and 1989, intensive studies were carried out on the development of research of endophytes that are typically focused on different species of grass. Recently in 2005, Posada and Vega gave a new definition to endophytes and used the term to describe all the microorganisms that inhabit inside different plant parts. In the year 2008, Sánchez and Márquez used this technique as an effective sterilisation tool for isolating endophytic organisms from spp. of grass *Dactylis glomerata*. Nowadays, researchers are focused on the isolation procedures of endophytes, studying the biodiversity of endophytes, secondary metabolites produced and endophyte-host interaction mechanisms (Stępniewska and Kuźniar 2013; Mishra et al. 2015). In 2010, Sikora defined endophyte as an organism that colonises into the internal tissues of a plant during its lifetime, where it can prove to be either helpful, neutral or detrimental. There is an extensive amount of research carried out on endophytes during the past which inculcates millions of plant species in number and their existence under specified circumstances in plant cell microtubule and intercellular spaces (Anyasi and Atagana 2015). The community structure of endophytic microbes within the plant is very much dynamic, depending upon both biotic and abiotic surrounding factors inclusively condition of the soil, biogeography of the region and inter- and intraspecies (plant and microbe) interactions (Deng and Cao 2017; Chadha et al. 2015).

6.5 Endophytes: A Synergistic Approach Towards Phytoremediation

Endophytic microbes play a valuable role in phytoremediation in comparison to that of rhizospheric microorganisms (Doty 2008; Santoyo et al. 2016; Deng and Cao 2017). The microbes present in the rhizosphere colonise themselves within the close vicinity of plant roots, while endophytic bacteria inhabit within the plant (Khan et al. 2015; Feng et al. 2017). This colonisation by endophytes can be carried out inside the tissues or throughout the plant with colonies of bacteria and biofilms inhabiting in intercellular spaces or either inside the vascular tissues (Germaine et al. 2004). The contaminants that are highly soluble in water can readily enter into the stream of xylem before any action of rhizospheric organisms (Weyens et al. 2009a; Ijaz et al. 2016) through the apoplastic pathway (van der Lelie et al. 2009; Ijaz et al. 2016). The population of microbes present in the rhizosphere is not easy to control and also there is a competition among these organisms, the reduction in the desired strains occurs until or unless selective metabolic processes are carried out for remediating a contaminant (Doty 2008; Santoyo et al. 2016). On the other hand, endophytes that reside naturally in the plant reduce the competition with the other microorganisms (Doty 2008). Endophytes have been studied over a wide range of geographic areas as well as in variable climatic zones (Li et al. 2012; Ijaz et al. 2016), and they are found ubiquitously among the plants that are examined (Taghavi et al. 2009; Li et al. 2012; Ijaz et al. 2016; Santoyo et al. 2016; Feng et al. 2017). The interface occurring between the roots of plants and microbes has a greater significance on the growth and survival capability of plants (Deng and Cao 2017). Endophytes have the inbuilt capability to degrade pollutants, and for the promotion of plant growth, therefore endophyte-assisted phytoremediation of organic contaminants and heavy metals could be successfully utilised, keeping in view beneficial traits of endophytes (Feng et al. 2017; Khan et al. 2014; Sarma et al. 2021). Naturally occurring endophytes have the efficiency for phytoremediation of contaminants from polluted soil and groundwater; contaminant-degrading endophytic microbes were isolated and studied from the plants that were growing in polluted areas (Kang et al. 2012). The essential association among plants and endophytic organisms complement each other naturally by stimulation of biological activities as well as in the enhancement of efficiency of the process of phytoremediation alongside enhancing the production of the biomass (Kang et al. 2012).

6.6 Organisms Involved in Endophyte-Assisted Phytoremediation

According to prior research conducted, more than 129 different bacterial endophytes have been isolated from various crop plants. Endophytes include bacteria, fungus as well as actinomycetes that are observed in various plant species and are

extensively studied. In recent times, it has been proposed that phytoremediation of organic compounds can be enhanced by actively degrading compounds translocated by the plants (Moore et al. 2006). Bacterial endophytes may act more efficiently than bacteria added for the bioaugmentation process into the soil; many bacterial species which have been isolated and examined from grapevine were resistant to many heavy metals including lead, nickel, mercury, zinc and manganese (Stępniewska and Kuźniar 2013). Moreover, endophytes especially endophytic bacteria improvise the adaptation and growth of plants through plant growth activities and simultaneously increasing the process of phytoremediation (Khan et al. 2014) involving both Gram-negative and Gram-positive bacteria (Anyasi and Atagana 2015). Endophytes especially bacteria have been studied after isolation from variable species of plants, which helps in the stimulation of growth of the host plant through various mechanisms involving biological control (Ma et al. 2011), induction of systemic resistance of plants towards pathogens (Ma et al. 2011; Ryan et al. 2007), production of plant growth regulators or promoters (Moore et al. 2006 Ma et al. 2011), improvement in uptake of water (Ma et al. 2011; Feng et al. 2017) and mineral nutrients (Ma et al. 2011). Endophytic actinomycetes are also important microorganisms that were isolated from the medicinal plants (Anyasi and Atagana 2015). The endophytic fungus also plays a significant role in organic and inorganic modifications, cycling of elements, mineral formation and fungal interactions with metal or clay, and apart from this, endophytic fungus showed a greater efficiency for the enhancement of phytoremediation. Furthermore, endophytic fungus possesses metal chelation systems to accelerate their tolerance capacity towards heavy metals maintaining a higher rate of biomass production which is suitable for the process of biodegradation (Deng and Cao 2017). Endophytic microorganisms are believed to interact very closely with the host in which they inhabit, and under adverse modifications in the surrounding environment, they are even more protected (Deng and Cao 2017). Endophytes have an intensive obligation to depend strictly on the host plants for survival in nature or either through a facultative method in which microbes complete one stage of their life cycle outside the plant entering plant system in later stage (Li et al. 2012; Rajkumar et al. 2009; Hardoim et al. 2008). Various examples of endophytic organisms involved in the process of phytoremediation are mentioned below in Table 6.2.

6.7 Metabolic Substances or Products of Endophytes That Promotes Phytoremediation

Bacterial endophytes have a greater efficiency for the enhancement in solubilisation of mineral and metals from the soil, by secretion of metal-specific chelating ligands called siderophores (Ma et al. 2016; Rajkumar et al. 2012). In recent investigations, it has been studied that bacterial endophytes release organic acids that are involved in the uptake of heavy metals from the soil which increases nutrient uptake

Table 6.2 Endophytic organisms inhabiting plant and types of substrate degraded

Host plant	Endophytic organism associated	Substrate degraded	References
Populus trichocarpa, *Populus deltoides*	*Rhizobium tropici*	Royal demolition explosives (RDX) and 4-amino-2,6-dinitrotoluene (TNT)	Doty et al. (2009), Doty (2008), Van der Lelie (2009)
Salix sitchensis	*Burkholderia*, *Rahnella*, *Acinetobacter*, *Pseudomonas*, *Herbaspirillum*, *Sphingomonas*	4-Amino-2,6-dinitrotoluene (TNT)	Doty (2008)
Populus vittata	*Arbuscular mycorrhizae fungi*	Arsenic	Doty (2008)
Populus trichocarpa, *Populus deltoides*	*Pseudomonas putida*	2,4-Dichlorophenol (2,4-D)	Doty (2008)
Alyssum serpyllifolium	*Pseudomonas* sp.	Nickel	Khan and Doty (2011), Li et al. (2012), Ma et al. (2016)
Nicotiana tabacum	*Sanguibacter* spp.	Cadmium	Li et al. (2012)
Lolium perenne	*Herbaspirillum seropedicae*	Nickel	Li et al. (2012)
Solanum lycoperisicum	*Methylobacterium oryzae*, *Burkholderia* spp.	Nickel, cadmium	Li et al. (2012)
Megathyrsus maximus	*Pantoea* spp.	Copper	Li et al. (2012)
Brachiaria mutica, *Leptochloa fusca*	*Acinetobacter* spp. *Pseudomonas aeruginosa*	Oil degradation	Li et al.(2012), Fatima et al. (2016)
Festuca arundinacea, *Festuca pratensis*	*Neotyphodium coenophialum*, *Neotyphodium uncinatum*	Polyaromatic hydrocarbons (PAHs)	Feng et al. (2017)
Chrysopogon zizanioides	*Achromobacter xylosoxidans*	Heavy metals (cadmium, zinc, nickel, arsenic and lead), monoaromatic hydrocarbons	Ho et al. (2013)
Astragalus bisulcatus Stanleya pinnata	*Bacillus*, *Pseudomonas*, *Staphylococcus Pantoea*, *Paenibacillus*, *Arthrobacter*, *Advenella*, *Variovorax*	Selenium	Ijaz et al. (2016)

(continued)

Table 6.2 (continued)

Host plant	Endophytic organism associated	Substrate degraded	References
Typha domingensis	*Microbacterium arborescens*, *Bacillus pumilus*	Textile effluent	Ijaz et al. (2016)
Festuca arundinacea, *Lolium perenne*	*Neotyphodium*	Zinc	Ijaz et al. (2016)
Prosopis juliflora	*Microbacterium arborescens*, *Pantoea stewartii*, *Enterobacter*	Heavy metals	Ijaz et al. (2016)
Arabidopsis	*Enterobacter cloacae*	4-Amino-2,6-dinitrotoluene (TNT)	Ijaz et al. (2016)
Poplar trees	*Methylobacterium populi*	Trichloroethylene (TCE) and royal demolition explosives (RDX)	Khan et al. (2014)
Hybrid poplar	*Enterobacter* spp.	Trichloroethylene (TCE)	Khan et al. (2014)
Pisum sativum	*Pseudomonas putida*	2,4-Dichlorophenol (2,4-D)	Khan et al. (2014)
Lupinus arboreus	*Burkholderia cepacia*	Toluene	Khan and Doty (2011)
Arabidopsis thaliana	*Achromobacter xylosoxidans*	Phenolic pollutants	Khan and Doty (2011)
Phytolacca acinosa, *Solanum nigrum*	*Bacillus* spp.	Heavy metals	Luo et al. (2011)
Populus trichocarpa, *Salix sitchensis*	*Burkholderia*, *Rahnella*, *Sphingomonas*, *Acinetobacter*	Nitrogen	Ma et al. (2016)
Brassica napus	*Pseudomonas fluorescens*, *Microbacterium*	Lead	Ma et al. (2016)
Alnus firma	*Bacillus* spp.	Cadmium	Ma et al. (2016)
Triticum aestivum	*Methylobacterium oryzae*, *Burkholderia* spp.	Nickel and cadmium	Ma et al. (2016)
Alyssum bertolonii, *Alyssum murale*	*Pseudomonas* spp.	Nickel	Ma et al. (2016), Mastretta et al. (2006)
Solanum nigrum	*Agrobacterium rhizogenes*	Polychlorinated biphenyls (PCBs)	Macek et al. (2000)
Poplar spp.	*Pseudomonas*, *Bacillus*, *Enterobacter*, *Stenotrophomonas*, *Arthrobacter*	BTEX Compounds	Mastretta et al. (2006), Phillips et al. (2008)

(continued)

Table 6.2 (continued)

Host plant	Endophytic organism associated	Substrate degraded	References
Populus trichocarpa, *Populus deltoides*	*Gammaproteobacteria*, *Pseudomonas* spp., *Xanthomonas* spp., *Acinetobacter* spp., *Enterobacter* spp. *Betaproteobacteria Arthrobacter* spp., *Bacillus* spp., *Paenibacillus* spp., *Agreia* spp.	BTEX compounds	Mastretta et al. (2006)
Oryza sativa	*Bradyrhizobium*, *Rhizobium*		Mastretta et al. (2006)
Medicago sativa	*Rhizobium meliloti*		Mastretta et al. (2006)
Meloidogyne incognita	*Pseudomonas chlororaphis*		Mastretta et al. (2006)
Vitis vinifera	*Xylella fastidiosa*		Mastretta et al. (2006)
Lupinus luteus	*Burkholderia cepacia*, *Pseudomonas putida*	Nickel, BTEX (benzene, toluene, ethylbenzene, xylene) compounds, trichloroethylene (TCE), 2,4-dichlorophenol (2,4-D); 4-amino-2,6-dinitrotoluene (TNT)	Weyens et al. (2011), Moore et al. (2006), Wu et al. (2009), Phillips et al. (2008), Weyens et al. (2009b)
Arabidopsis	*Pseudomonas putida*	4-Amino-2,6-dinitrotoluene (TNT), polychlorinated biphenyls (PCBs)	Dowling and Doty (2009), Wu et al. (2009)
Thlaspi goesingense	*Gammaproteobacteria Pseudomonas* spp., *Xanthomonas* spp., *Acinetobacter* spp., *Enterobacter* spp.	Nickel	Rajkumar et al. (2009)
Thlaspi caerulescens	*Methylobacterium*, *Sphingomonas*	Nickel	Rajkumar et al. (2009)
Alyssum bertolonii	*Pseudomonas* spp., *Micrococcus* spp., *Microbacterium* spp., *Curtobacterium* spp.	Nickel, chromium, zinc, copper	Rajkumar et al. (2009)
Nicotiana tabacum	*Pseudomonas fluorescens*	Cadmium	Rajkumar et al. (2009)
Brassica napus	*Microbacterium* spp.	Nickel	Rajkumar et al. (2009)
Lycopersicon esculentum	*Methylobacterium oryzae*, *Burkholderia* spp.	Nickel and cadmium	Rajkumar et al. (2009)

(continued)

Table 6.2 (continued)

Host plant	Endophytic organism associated	Substrate degraded	References
Pisum sativum	*Pseudomonas* spp.	2,4-Dichlorophenol (2,4-D)	Ryan et al. (2008), Stępniewska and Kuźniar (2013)
Lycopersicon esculentum	*Gammaproteobacteria Pseudomonas* spp., *Xanthomonas* spp., *Acinetobacter* spp., *Enterobacter* spp.	2,4-Dichlorophenol (2,4-D)	Santoyo et al. (2016)
Vitis vinifera	*Bacillus* spp.	2,4-Dichlorophenol (2,4-D), heavy metals	Stępniewska and Kuźniar (2013)
Solanum nigrum	*Serratia nematodiphila*	Cadmium	Stępniewska and Kuźniar (2013)
Festuca arundinacea, *Festuca pratensis*	*Neotyphodium coenophialum*, *Neotyphodium uncinatum*	Polyaromatic hydrocarbons (PAHs)	Stępniewska and Kuźniar (2013)
Phragmites australis, *Ipomoea aquatic*	*Achromobacter xylosoxidans*	Aromatic compounds	Stępniewska and Kuźniar (2013)
Sphagnum spp.	*Methylocella palustris*	Methane	Stępniewska and Kuźniar (2013)
Pinus nigra, *Salix caprea*	*Rhodococcus* spp.	Polychlorinated biphenyls (PCBs)	Wu et al. (2009)
Sinorhizobium meliloti	*Pseudomonas fluorescens*	Polychlorinated biphenyls (PCBs)	Wu et al. (2009)
Salix caprea	*Streptomyces*, *Agromyces terreus*	Cadmium and zinc	Wu et al. (2009)
Helianthus annuus	*Pseudomonas putida*	Cadmium	Wu et al. (2009)
M. sativa	*Pseudomonas fluorescens*, *Hebeloma crustuliniforme*	Polychlorinated biphenyls (PCBs)	Wu et al. (2009)
P. canadensis	*Paxillus involutus*	Cadmium	Yadav et al. (2010)
Eucalyptus camaldulensis	*Ochrobactrum intermedium*	Lead	Kabra et al. (2013)
Populus nigra	*Pseudomonas putida*	Diesel oil	Kabra et al. (2013)
Brassica juncea	*Bacillus subtilis*	Nickel	Kabra et al. (2013)
Zinnia angustifolia	*Exiguobacterium aestuarii*	Remazol Black B	Kabra et al. (2013)

additionally (Ma et al. 2016). Siderophores are the compounds that have low molecular mass with the higher rate of association constants to complex with iron (Fe), but these chelates also form complexes with other metals, namely, aluminium (Al), cadmium (Cd), copper (Cu), lead (Pb) and zinc (Zn) (Das et al. 2007). The formation of siderophores by microorganisms is generally regulated by various factors such as availability of iron, pH of the soil, nutrient availability and the type of heavy metals present along with its concentration in the soil. On the other hand, it is stated after various investigations by the researchers that siderophore formed does not always result in enhanced metal uptake by the host plants. Due to variation in the inherited ability of a host to take up metals from the surrounding, it directly relies on the availability of metal in soil, type of host plant and their potential of transporting metal from root to the upper parts of the plant, i.e. shoot (Rajkumar et al. 2012).

Poorly soluble metals are made bio-available by production and release of the low molecular weight compounds i.e. biosurfactants by the endophytic bacteria into the niches of the host as root exudates which enhance the rate of phytoremediation (Chaudhry et al. 2005; Ma et al. 2016). Evidence from the previous studies suggests that the organic chemicals secreted by the root exudates such as organic acids, amino acids and phenolic compounds play an active part in the communication carried out between root and microbes (Abhilash et al. 2012). These are an amphiphilic molecule that comprises two moieties, i.e. hydrophilic and hydrophobic, forming a diverse range of chemical structures including glycolipids, mycolic acid, lipopeptides, polysaccharide-protein complexes, fatty acids, phospholipids, etc. These biosurfactant molecules interact and form a complex with insoluble metals on the interface of soil particles present in the rhizosphere and is followed by desorption of metals from the matrix of soil and change in the mobility as well as bioavailability of metal in the soil (Ma et al. 2016). The specific endophytic microbes that colonise host plants may stimulate a certain level of transcription genes that are responsible for the degradation of pollutants, thus exerting a vital impact on the metabolism of degrading enzymes. These endophytic organisms represent a vast range of enzymes that are required for the identification of pollutants and enhancing the process of phytoremediation of polluted sites. On exposure to organic pollutants, some of the endophytic microbes can stimulate and regulate the enzymes produced in plants or endophytes themselves, thus increasing the metabolism of organic contaminants (Feng et al. 2017). These contaminant-biodegrading enzymes include peroxidases, nitrilases, laccases, dehalogenases, monooxygenases and nitroreductases present inside both the host plant and endophytic microbes (Feng et al. 2017; Macek et al. 2000).

6.8 Endophytes: A Boon in Plant Growth Promotion

Plants persistently undergo interactions with a large variety of bacterial populations colonising as rhizobacteria, epiphytes and endophytes (Bai et al. 2002). Among these endophytes are the microbes which are protected from stresses of the

environment (Bai et al. 2002; Compant et al. 2005) and competition of other microbes by the plant enacting as host. Moreover, they are ubiquitous inside the tissues of the plant (Bai et al. 2002; Sheng et al. 2008). The distribution of endophytic organisms in plants relies on both their ability to inhabit and the location of resources of plants (Gaiero et al. 2013). Endophytic microbes are reported to undergo active or passive processes of translocation inside their host plant, from outer rhizoplane to the cortical region of the root system. On reaching the cortex of root, the endodermis portion permits only a few bacteria to pass through it. Endophytic organisms secrete a specific cell wall-degrading enzyme that helps them to disrupt the endodermal cell layers, or it passively enters the endodermis during the process of secondary growth of plant roots. After entering the pericycle through the endodermis barrier, it reaches the xylem vessels of their host (root cortex-endodermis-pericycle-xylem). There are some systemic species of endophytic bacteria which utilise intercellular spaces of plants (Compant et al. 2010). The plant growth-promoting bacterial endophytes colonise plant tissues forming a close linkage that helps in facilitating the exchange of nutrients and activity of enzymes (Hassan 2017). The bacteria that act as plant growth-promoting endophytes enter and inhabit the healthy tissues of a plant without causing any symptoms of disease in the host (Barka et al. 2002; Waqas et al. 2012; Hassan 2017; Ji et al. 2014). Many isolates of the bacteria play a vital role to protect the plant from soil-borne pathogens (Barka et al. 2002; de Melo Pereira et al. 2012; Jha and Kumar 2009) including a broad range of parasites such as fungi, bacteria, viruses, nematodes and insects (Ji et al. 2014) through producing hydrogen cyanide, siderophores and antibiotics (de Melo Pereira et al. 2012) and resulting in the higher yield and productivity of crops (Barka et al. 2002).

Firstly Honma and Shimomura initiated work and characterised the ACC deaminase enzyme and showed that it is involved in the promotion of plant growth by the bacteria. The compound aminocyclopropane-1-carboxylic acid is involved in the biosynthesis of ethylene, by acting as an intermediate converting methionine into ethylene (Ma et al. 2011). The enzyme (ACC) aminocyclopropane-1-carboxylic acid deaminase is produced by endophytic microbes (Ali et al. 2014; Hardoim et al. 2008; Ma et al. 2011; Weilharter et al. 2011) which cleaves ACC into ammonia and alpha-ketobutyrate (Hardoim et al. 2008; Rashid et al. 2012; Souza et al. 2015; Santoyo et al. 2016) decreasing the production of plant growth-regulating gaseous hormone ethylene produced endogenously (Ali et al. 2014; Souza et al. 2015) and is secreted in a very low amount under normal conditions and helps to carry out functions involving root initiation in plants, ripening process of fruits, germination of seeds, wilting of flowers, abscission of leaves, biosynthesis of plant hormones and stress signalling. When a plant undergoes abnormal conditions, it starts producing a significantly high level of ethylene biosynthesis; this condition is called “stress ethylene” (Ali et al. 2014). This stressful condition may cause wounding, flooding, drought, salinity, temperature extremes and insect predation (Ali et al. 2014; Santoyo et al. 2016). Under these conditions of stress, the endophytic organisms exhibit ACC deaminase activity which can be beneficial in lowering ethylene stress (Ali et al. 2014; de Melo Pereira et al. 2012; Jha and Kumar 2009; Sgroy et al. 2009; Sheng et al. 2008). The endophytic bacterium uses the ammonia produced from

ACC which is a sole source of nitrogen and decreases the level of ACC in the plant along with the reduction in ethylene level. On the other hand, when ACC using bacteria is absent, ACC is oxidised by enzyme ACC oxidase catalysing the formation of ethylene hormone, carbon dioxide and cyanide (Ma et al. 2011). The enzyme ACC deaminase produced by the endophytic bacteria is usually taken through stems of plucked flowers that lead to delaying senescence for many days (Santoyo et al. 2016). The most phylum, i.e. *Proteobacteria* involving all the three classes *Alpha-*, *Beta-* and *Gammaproteobacteria*, dominates in the diversity of endophytic microbes, while the occurrences of other classes of bacteria including *Bacteroidetes* and *Planctomycetes* are less commonly found as endophytic bacteria. The bacterial genera *Pseudomonas*, *Bacillus*, *Burkholderia*, *Stenotrophomonas*, *Micrococcus*, *Pantoea* and *Microbacterium* are the most common genera that are found to be inhabited as endophytes in plants (Santoyo et al. 2016). Endophytes are capable of many processes which are responsible for direct plant growth promotion which includes:

1. Solubilising the nutrients that are in the immobilised state, for example, phosphorus (P) and zinc (Zn) (Ali et al. 2014; de Melo Pereira et al. 2012; Gaiero et al. 2013; Hardoim et al. 2012; Jha and Kumar 2009; Li et al. 2008; Rashid et al. 2012; Walitang et al. 2017; Zhu and She 2018).

   Phosphorus is a necessary macronutrient which participates as a structural part of nucleic acid, phosphates and energy-producing element, i.e. ATP, in the plants. Plants absorb the phosphorus in soluble form from the soil (Souza et al. 2015). The endophytic bacteria provide solubilised form of phosphates to the plants and in turn gain root-borne organic compounds such as sugars and organic acids for their growth (Otieno et al. 2015). The plant growth-promoting endophytic bacteria that have been studied for solubilisation of phosphorus are *Achromobacter xylosoxidans* and *Bacillus pumilus* (Gaiero et al. 2013).
2. Association in nitrogen fixation (Shishido et al. 1999; Ali et al. 2014; de Melo Pereira et al. 2012; Gaiero et al. 2013; Hardoim et al. 2012; Li et al. 2008; Santoyo et al. 2016; Walitang et al. 2017; Zhu and She 2018).

   There are many examples in which the plant growth-promoting endophytic bacteria have been studied that are responsible for nitrogen fixation such as *Azospirillum* spp. (Souza et al. 2015; Gaiero et al. 2013), *Pantoea agglomerans* and *Azoarcus* spp. (Gaiero et al. 2013). Studies have shown that endophytic bacterium *Gluconacetobacter diazotrophicus* gene gum D is involved in the biosynthesis of exopolysaccharide which is required for biofilm formation and subsequently to colonise plant. It was observed later that this bacterium is involved in the fixation of nitrogen in plants (Santoyo et al. 2016).
3. Production of the hormones in plants (Shishido et al. 1999; Ali et al. 2014; de Melo Pereira et al. 2012; Jha and Kumar 2009; Ji et al. 2014 Kuklinsky-Sobral et al. 2004) called as plant growth regulators (Dobbelaere et al. 2003; Santoyo et al. 2016).

   The hormones that are produced by endophytic bacteria associated with plant include auxins (indole-3-acetic acid, gibberellic acid) (Ma et al. 2011; Sgroy

et al. 2009), cytokinins (zeatin) (Sgroy et al. 2009) and gibberellins that stimulate germination, growth, reproduction and protection under both stressed and non-stressed situations (Ma et al. 2011; Santoyo et al. 2016).
4. Production of iron chelating-agents called siderophores (Ali et al. 2014; Jha and Kumar 2009; Ji et al. 2014; Santoyo et al. 2016; Sgroy et al. 2009).

   Endophytic bacteria such as *Burkholderia*, *Fusarium verticillioides*, *Colletotrichum graminicola*, *Bipolaris maydis* and *Cercospora zea-maydis* have been studied and are found responsible for producing siderophores ultimately helping in plant growth promotion (Souza et al. 2015).
5. Sulphur oxidation.
6. Production of metabolically active enzyme (ACC) aminocyclopropane-1-carboxylic acid deaminase (Ali et al. 2014).
7. Producing growth stimulants that are volatile in nature, for example, acetoin and 2,3-butanediol (Santoyo et al. 2016).

On the other hand, indirect ways of plant growth promotion include:

1. Antibiosis
2. Induced systemic resistance (Shishido et al. 1999; Kuklinsky-Sobral et al. 2004; Ramamoorthy et al. 2001; Rashid et al. 2012)
3. Competition for limited sources
4. Production of hydrogen cyanide (HCN)
5. Producing a diverse array of enzymes that plays a role in cell wall degradation

Several bacterial endophytes that are efficient in plant growth promotion are sequenced including *Azoarcus* spp., *Azospirillum lipoferum*, *Azospirillum* spp. B510, *B. phytofirmans*, *Burkholderia* spp., *Gluconacetobacter diazotrophicus*, *Pseudomonas putida*, *Pseudomonas stutzeri*, *Serratia proteamaculans* and *Stenotrophomonas maltophilia.* All these species have various gene encoding for plant growth-promoting traits which are mentioned above (Santoyo et al. 2016).

6.9 Modification of Endophytes by the Use of Various Techniques

In comparison with conventional physiochemical-based approaches, the utilisation of genetically engineered plant-based bioremediation of heavy metals is a leading approach because it is environment-friendly as well as causes fewer health hazards (Dixit et al. 2015; Thakare et al. 2021). The genetic engineering of the plant by insertion or overexpression of particular genes into the genome of host plants delivers an effective method to improve phytoremediation capability of plants (Kang et al. 2012; Sarma et al. 2021). These modifications of host plant species can be achieved by transgenic methods which include enhanced uptake of metals, transportation and biodegradation of contaminants along with plant growth and development of the root system. There are multiple examples of successfully incorporated genes of microbes into the host tissue of plants for enhanced phytoremediation

(Abhilash et al. 2012). For instance, genetically modified plants including willows, poplar and *Jatropha* could be used for two purposes: biodegradation, i.e. phytoremediation, as well as the production of bioenergy (Dixit et al. 2015; Abhilash et al. 2012). Likewise, bacterial reductase-containing transgenic plants can increase the process of volatilisation of selenium (Se) and mercury (Hg) along with the accumulation of arsenic in shoots of plants (Dixit et al. 2015). The studies demonstrated that the genetically engineered endophytic bacteria could be utilised to decelerate the phytotoxicity of organic pollutants (van der Lelie et al. 2005). The expression of the genes responsible for contaminant degradation in efficient bioenergised plants should decrease the organic contaminant load in the tissue of plants as well as facilitate the usage of the product of plants generated from the plantation for phytoremediation. Taking an example of poplar plants that have been modified genetically using the catabolic gene of microbes for enhanced bioremediation and the genes particularly, microbial mercuric reductase genes expressed by poplar plants have proved increased resistance to mercury (Hg) (Abhilash et al. 2012). The genes that encode toluene monooxygenase (TOM) of *Burkholderia cepacia* strain G4 that oxidises trichloroethylene (TCE), vinyl chloride and dichloroethylenes were inserted into bacteria particularly from the rhizospheric zone of poplar trees for the enhanced efficiency of trichloroethylene (TCE) metabolism. The endophytes were engineered for the enhancement of metal remediation including nickel tolerance genes (*ncc-nre* nickel-cadmium-cobalt resistance) from *Ralstonia metallidurans* 31A into two endophytes named *Burkholderia cepacia* and *Herbaspirillum seropedicae* with TOM system; the genes are inserted into the chromosomes of the endophytes. These *ncc-nre*-containing engineered endophytes were inoculated into the host plant *Lupinus luteus* and *Lolium perenne* seeds, and further modified endophytic microbes have enhanced resistance towards toxic effects of nickel in the inoculated plants (Doty et al. 2009). The endophytes that are being engineered with genetic information required for catabolism may increase the biodegradation of the contaminants in the vascular system of plants. These genetically engineered plants having recombinant endophytic microbes, particularly bacteria with engineered catabolic pathways, are responsible for effective colonisation and protection against the phytotoxic effect of contaminant naphthalene when compared with the normal plants which are not inoculated with modified endophytes. For example, *Burkholderia cepacia* VM1468 is a genetically engineered endophyte when inoculated into poplar and yellow lupine plants increases biomass and decreases phytotoxicity as well as evapotranspiration of toluene and trichloroethylene (TCE) (Feng et al. 2017). Some of the complex organic contaminants such as polyaromatic hydrocarbons (PAHs) require a multienzyme system for their degradation. Recently a fruitful attempt was carried out to from the multigene system to produce an active enzyme system complex for the process of phytoremediation including four genes that encode for naphthalene dioxygenases responsible for the metabolism of polyaromatic hydrocarbons (PAHs) in *Pseudomonas putida* G7 were genetically engineered in host plant *Arabidopsis thaliana* via expression cassette followed by the enhanced capacity of assimilation of target contaminants through the transgenic plant (Ijaz et al. 2016). The genes can be modified in both plants and bacteria; the genetic engineering of

these organisms is a comparatively easier process than the manipulation of genes in higher organisms (Ijaz et al. 2016).

A designer plant utilises the combination of host plants, bacteria as well as fungus present in soil and endophytic bacteria that helps to eliminate the contaminants present in the soil. This approach avails a strong mode for the production of a customised system of plants, in which microorganisms present on roots help in the degradation of complex organic pollutants; on the other hand, the microorganisms that are efficient in biodegradation of pollutants are introduced into the host plants, where they reduce these contaminants to a greater extent. If this technology is implemented in fields successfully, it could greatly enhance the process of removal of pollutants from the contaminated soil and plant tissues alongside boosting plant biomass growth for the production of bioenergy via pyrolysis. Metals accumulated in the tissues of a plant could be extracted in the form of biochar which is produced, while the process of pyrolysis of this biochar can be further used in fields to improvise the nutrient content in soil (Abhilash et al. 2012). A diverse array of microbial populations is efficient for gene exchange as well as gene rearrangements. The wide range of catabolic pathways that are being encoded by genes are generally situated on plasmids or transposons which are self-transferable entities, and this property makes possible horizontal gene transfer among an endogenous community of bacteria. Horizontal gene transfer is a non-vertical transfer of genes between two or more organisms to bring novel evolution and adaptation of bacteria into new environments. The natural transfer of genes through horizontal gene transfer carries a vast efficiency in developing genetically engineered endophytic bacteria that possess accurate genes for catabolism as well as heterologous expression, particularly when both the recipient and the donor belong to the similar species. The heterologous expression in the community of endophytic organisms followed by horizontal gene transfer is successful when the genetic information is generally carried on vectors over a broad range of hosts. This not only eradicates the need for selection as well as isolation of suitable endophytic strain from the plant that acts as host but also there is no need to optimise and establish the endophytic inoculum which is aimed to persist in endogenous populations (Ijaz et al. 2016).

The recent advancement in the genomics provides opportunities to explore the maximum benefits of bioremediation approaches and allows manipulation towards tolerance, accumulation and biodegradation efficiency of host plant-microbe against various contaminants (Dixit et al. 2015; Abhilash et al. 2012). The metagenomics analysis of hyperaccumulator plants is carried out to identify the full bacteria population present in the rhizospheric and endosphere region. The DNA is extracted without culturing bacteria present in these regions and is used for the construction of 16S RNA libraries of a clone. Metagenomics coupled with next-generation sequencing methods such as pyrosequencing has made it feasible to examine the complex structure of microbial systems in the environment and assign their gene function. Moreover, functional metagenomics and transcriptomics when combined with microarray technology and RNA sequence analysis may assist to identify the genes, gene products and biodegradation pathways that are potentially required in the host plants for hyperaccumulator bacterial interactions and for analysing the plant-rhizome interactions that occur at the level of transcriptomics. These studies

will increase the knowledge about host plant-endophytic microbe interactions as well as will bring out progress in novel technologies of phytoremediation (Ijaz et al. 2016; Sarma et al. 2021). To sum up, the above-mentioned details of genetic engineering and genetic modification of endophytic microbes, especially bacteria, are easy in comparison to plants. Gene expression within the endophytic microbes is also helpful as a tool for monitoring the sites of contamination. Nevertheless, releasing the concept of recombinant bacteria into the environment must be tackled to assure the public that science is not harming their surroundings with these modifications in the organisms.

6.10 Conclusion

To conclude, removing toxic chemical pollutants that are harming the natural resources of our environment is necessary for better survival of all forms of life and to maintain an ecological balance on earth. Bioremediation has proved beneficial for resolving this critical matter of concern. Phytoremediation is a better field of interest for researchers working in this area, as it is an alternative plant-assisted technology of bioremediation. Endophytic organisms are now being targeted for a more precised field of bioremediation, i.e. endophyte-assisted phytoremediation. This technique has been a boon for the degradation of hazardous contaminants present in the environment. Many plants help in the remediation of heavy metals present in the soil, water or air. Endophytic organisms and plants in association exhibit a combined action towards organic as well as inorganic contaminants, the results of this synergistic approach have not only influenced the improvement in biodegradation process but have also been advantageous for both plants and endophytic microbes. Scientists are focusing on improvising this mutualistic relationship to strengthen up the degradation practices through genetic engineering, designer plant and omics tools. The plant-microbe interaction will be encouraging technology to combat the rising pollution around the globe because this technology not only remediates the contaminants but also improves the condition of plants via its plant growth promotion activity. Thus, endophytic organisms will be a future alternative for fertilisers because of their beneficial powers as biofertilisers and a cleaner way for bioremediation.

References

Abhilash PC, Powell JR et al (2012) Plant–microbe interactions: novel applications for exploitation in multipurpose remediation technologies. Trends Biotechnol 30(8):416–420

Ali S, Charles TC, Glick BR (2014) Amelioration of high salinity stress damage by plant growth-promoting bacterial endophytes that contain ACC deaminase. Plant Physiol Biochem 80:160–167

Anyasi RO, Atagana HI (2015) Endophytes: an Indicator for improved phytoremediation of environmental pollutants. Journal of Environmental Indicators, 9:27

Arshad M, Saleem M, Hussain S (2007) Perspectives of bacterial ACC deaminase in phytoremediation. Trends Biotechnol 25(8):356–362

Azubuike CC, Chikere CB, Okpokwasili GC (2016) Bioremediation techniques–classification based on site of application: principles, advantages, limitations and prospects. World J Microbiol Biotechnol 32(11):180

Bai Y, D'Aoust F, Smith DL, Driscoll BT (2002) Isolation of plant-growth-promoting Bacillus strains from soybean root nodules. Can J Microbiol 48(3):230–238

Barka EA, Gognies S, Nowak J et al (2002) Inhibitory effect of endophyte bacteria on Botrytis cinerea and its influence to promote the grapevine growth. Biol Control 24(2):135–142

Brown SL, Chaney RL, Angle JS et al (1994) Phytoremediation potential of Thlaspi caerulescens and bladder campion for zinc-and cadmium-contaminated soil. J Environ Qual 23(6):1151–1157

Chadha N, Prasad R, Varma A (2015) Plant promoting activities of fungal endophytes associated with tomato roots from central Himalaya, India and their interaction with *Piriformospora indica*. International Journal of Pharma and BioSciences 6(1): 333–343

Chaudhry Q, Blom-Zandstra M et al (2005) Utilising the synergy between plants and rhizosphere microorganisms to enhance breakdown of organic pollutants in the environment. Environ Sci Pollut Res Int 12(1):34–48

Chen B, Shen J, Zhang X et al (2014) The endophytic bacterium, Sphingomonas SaMR12, improves the potential for zinc phytoremediation by its host, Sedum alfredii. PLoS One 9(9):e106826

Compant S, Reiter B, Sessitsch A et al (2005) Endophytic colonization of *Vitis vinifera* L. by plant growth-promoting bacterium *Burkholderia* sp. strain PsJN. Appl Environ Microbiol 71(4):1685–1693

Compant S, Clément C, Sessitsch A (2010) Plant growth-promoting bacteria in the rhizo-and endosphere of plants: their role, colonization, mechanisms involved and prospects for utilization. Soil Biol Biochem 42(5):669–678

Das A, Prasad R, Srivastava A, Giang PH, Bhatnagar K and Varma A (2007) Fungal siderophores: structure, functions and regulations. In: Varma A and Chincholkar SB (eds.), *Microbial Siderophores*, Springer-Verlag Berlin Heidelberg 12: 1–42

de Melo Pereira GV, Magalhães KT, Lorenzetii ER (2012) A multiphasic approach for the identification of endophytic bacterial in strawberry fruit and their potential for plant growth promotion. Microb Ecol 63(2):405–417

Deng Z, Cao L (2017) Fungal endophytes and their interactions with plants in phytoremediation: a review. Chemosphere 168:1100–1106

Dixit R, Wasiullah, Malaviya D et al (2015) Bioremediation of Heavy Metals from Soil and Aquatic Environment: An Overview of Principles and Criteria of Fundamental Processes. Sustainability, 7:2189–2212.

Dobbelaere S, Vanderleyden J, Okon Y (2003) Plant growth-promoting effects of diazotrophs in the rhizosphere. Crit Rev Plant Sci 22(2):107–149

Doty SL, James CA, Moore AL, et al (2007) Enhanced phytoremediation of volatile environmental pollutants with transgenic trees. Proceedings of the National Academy of Sciences, USA 104: 16816–16821.

Doty SL (2008) Enhancing phytoremediation through the use of transgenics and endophytes. New Phytol 179(2):318–333

Doty SL, Oakley B, Xin G et al (2009) Diazotrophic endophytes of native black cottonwood and willow. Curr Microbiol 47:23–33. https://doi.org/10.1007/BF03179967

Dowling DN, Doty SL (2009) Improving phytoremediation through biotechnology. Curr Opin Biotechnol 20:1–3

Eapen S, Singh S, D'souza SF (2007) Advances in development of transgenic plants for remediation of xenobiotic pollutants. Biotechnol Adv 25(5):442–451

Ebbs SD, Lasat MM, Brady DJ et al (1997) Phytoextraction of cadmium and zinc from a contaminated soil. J Environ Qual 26(5):1424–1430

Fatima K, Imran A, Amin I et al (2016) Plant species affect colonization patterns and metabolic activity of associated endophytes during phytoremediation of crude oil-contaminated soil. Environ Sci Pollut Res Int 23(7):6188–6196

Feng NX, Yu J, Zhao HM et al (2017) Efficient phytoremediation of organic contaminants in soils using plant–endophyte partnerships. Sci Total Environ 583:352–368
Gaiero JR, McCall CA, Thompson KA (2013) Inside the root microbiome: bacterial root endophytes and plant growth promotion. Am J Bot 100(9):1738–1750
Gerhardt KE, Huang XD, Glick BR, Greenberg BM (2009) Phytoremediation and rhizoremediation of organic soil contaminants: potential and challenges. Plant Sci 176(1):20–30
Germaine K, Keogh E, Garcia-Cabellos G (2004) Colonisation of poplar trees by gfp expressing bacterial endophytes. FEMS Microbiol Ecol 48(1):109–118
Germaine KJ, Liu X, Cabellos GG et al (2006) Bacterial endophyte-enhanced phytoremediation of the organochlorine herbicide 2, 4-dichlorophenoxyacetic acid. FEMS Microbiol Ecol 57(2):302–310
Germaine KJ, Keogh E, Ryan D, Dowling DN (2009) Bacterial endophyte-mediated naphthalene phytoprotection and phytoremediation. FEMS Microbiol Lett 296(2):226–234
Gianfreda, L., & Rao, M. A. (2004). Potential of extra cellular enzymes in remediation of polluted soils: a review. Enzyme and Microbial Technology, 35(4), 339–354. doi:10.1016/j.enzmictec.2004.05.006
Glick BR (2003) Phytoremediation: synergistic use of plants and bacteria to clean up the environment. Biotechnol Adv 21(5):383–393
Hardoim PR, van Overbeek LS, van Elsas JD (2008) Properties of bacterial endophytes and their proposed role in plant growth. Trends Microbiol 16(10):463–471
Hardoim PR, Hardoim CC, Van Overbeek LS, Van Elsas JD (2012) Dynamics of seed-borne rice endophytes on early plant growth stages. PLoS One 7(2):e30438
Hassan SED (2017) Plant growth-promoting activities for bacterial and fungal endophytes isolated from medicinal plant of Teucrium polium L. J Adv Res 8(6):687–695
Ho YN, Mathew DC, Hsiao SC et al (2012) Selection and application of endophytic bacterium Achromobacter xylosoxidans strain F3B for improving phytoremediation of phenolic pollutants. J Hazard Mater 219:43–49
Ho YN, Hsieh JL, Huang CC (2013) Construction of a plant–microbe phytoremediation system: combination of vetiver grass with a functional endophytic bacterium, Achromobacter xylosoxidans F3B, for aromatic pollutants removal. Bioresour Technol 145:43–47
Ijaz A, Imran A, ul Haq MA et al (2016) Phytoremediation: recent advances in plant-endophytic synergistic interactions. Plant Soil 405(1–2):179–195
Jha P, Kumar A (2009) Characterization of novel plant growth promoting endophytic bacterium Achromobacter xylosoxidans from wheat plant. Microb Ecol 58(1):179–188
Ji SH, Gururani MA, Chun SC (2014) Isolation and characterization of plant growth promoting endophytic diazotrophic bacteria from Korean rice cultivars. Microbiol Res 169(1):83–98
Kabra AN, Khandare RV, Govindwar SP (2013) Development of a bioreactor for remediation of textile effluent and dye mixture: a plant–bacterial synergistic strategy. Water Res 47(3):1035–1048
Kang JW (2014) Removing environmental organic pollutants with bioremediation and phytoremediation. Biotechnol Lett 36(6):1129–1139
Kang JW, Khan Z, Doty SL (2012) Biodegradation of trichloroethylene by an endophyte of hybrid poplar. Appl Environ Microbiol 78(9):3504–3507
Khan Z, Doty S (2011) Endophyte-assisted phytoremediation. Plant Biol 12:97–105
Khan Z, Roman D, Kintz T et al (2014) Degradation, phytoprotection and phytoremediation of phenanthrene by endophyte *Pseudomonas putida*, PD1. Environ Sci Technol 48(20):12221–12228
Khan MU, Sessitsch A, Harris M et al (2015) Cr-resistant rhizo-and endophytic bacteria associated with Prosopis juliflora and their potential as phytoremediation enhancing agents in metal-degraded soils. Front Plant Sci 5:755
Kuklinsky-Sobral J, Araújo WL, Mendes R et al (2004) Isolation and characterization of soybean-associated bacteria and their potential for plant growth promotion. Environ Microbiol 6(12):1244–1251

Lasat MM (1999) Phytoextraction of Metals from Contaminated Soil: A Review of Plant/Soil/Metal Interaction and Assessment of Pertinent Agronomic Issues, Journal of Hazardous Substance Research: Vol. 2. https://doi.org/10.4148/1090-7025.1015

Li JH, Wang ET, Chen WF, Chen WX (2008) Genetic diversity and potential for promotion of plant growth detected in nodule endophytic bacteria of soybean grown in Heilongjiang province of China. Soil Biol Biochem 40(1):238–246

Li HY, Wei DQ, Shen M, Zhou ZP (2012) Endophytes and their role in phytoremediation. Fungal Divers 54(1):11–18

Luo S, Wan Y, Xiao X et al (2011) Isolation and characterization of endophytic bacterium LRE07 from cadmium hyperaccumulator Solanum nigrum L. and its potential for remediation. Appl Microbiol Biotechnol 89(5):1637–1644

Luo S, Xu T, Chen L et al (2012) Endophyte-assisted promotion of biomass production and metal-uptake of energy crop sweet sorghum by plant-growth-promoting endophyte Bacillus sp. SLS18. Appl Microbiol Biotechnol 93(4):1745–1753

Ma Y, Prasad MNV, Rajkumar M, Freitas H (2011) Plant growth promoting rhizobacteria and endophytes accelerate phytoremediation of metalliferous soils. Biotechnol Adv 29(2):248–258

Ma Y, Rajkumar M, Zhang C, Freitas H (2016) Beneficial role of bacterial endophytes in heavy metal phytoremediation. J Environ Manag 174:14–25

Macek T, Mackova M, Káš J (2000) Exploitation of plants for the removal of organics in environmental remediation. Biotechnol Adv 18(1):23–34

Mastretta C, Barac T, Vangronsveld J et al (2006) Endophytic bacteria and their potential application to improve the phytoremediation of contaminated environments. Biotechnol Genet Eng Rev 23(1):175–188

Meharg AA, Cairney JW (2000) Ectomycorrhizas—extending the capabilities of rhizosphere remediation? Soil Biol Biochem 32(11–12):1475–1484

Mesa V, Navazas A, González-Gil R et al (2017) Use of endophytic and rhizosphere bacteria to improve phytoremediation of arsenic-contaminated industrial soils by autochthonous Betula celtiberica. Appl Environ Microbiol 83(8):e03411–e03416

Mishra M, Prasad R, Varma A (2015) Endophytic fungi: Biodiversity and functions. International Journal of Pharma and BioSciences 6(1): 18–46

Moore FP, Barac T, Borremans B (2006) Endophytic bacterial diversity in poplar trees growing on a BTEX-contaminated site: the characterisation of isolates with potential to enhance phytoremediation. Syst Appl Microbiol 29(7):539–556

Newman LA, Reynolds CM (2005) Bacteria and phytoremediation: new uses for endophytic bacteria in plants. Trends Biotechnol 23(1):6–8

Ojuederie O, Babalola O (2017) Microbial and plant-assisted bioremediation of heavy metal polluted environments: a review. Int J Environ Res Public Health 14(12):1504

Otieno N, Lally RD, Kiwanuka S (2015) Plant growth promotion induced by phosphate solubilizing endophytic Pseudomonas isolates. Front Microbiol 6:745

Patle PN, Navnage NP, Ramteke PR (2018) Endophytes in plant system: roles in growth promotion, mechanism and their potentiality in achieving agriculture sustainability. Int J Chem Stud 6:270–274

Phillips LA, Germida JJ, Farrell RE, Greer CW (2008) Hydrocarbon degradation potential and activity of endophytic bacteria associated with prairie plants. Soil Biol Biochem 40(12):3054–3064

Pilon-Smits E. A. H. (2005). Phytoremediation. Annu. Rev. Plant Biol. 56, 15–39. 10.1146/annurev.arplant.56.032604.144214

Rajkumar M, Ae N, Freitas H (2009) Endophytic bacteria and their potential to enhance heavy metal phytoextraction. Chemosphere 77(2):153–160

Rajkumar M, Sandhya S, Prasad MNV, Freitas H (2012) Perspectives of plant-associated microbes in heavy metal phytoremediation. Biotechnol Adv 30(6):1562–1574

Ramamoorthy V, Viswanathan R, Raguchander T (2001) Induction of systemic resistance by plant growth promoting rhizobacteria in crop plants against pests and diseases. Crop Prot 20(1):1–11

Rashid S, Charles TC, Glick BR (2012) Isolation and characterization of new plant growth-promoting bacterial endophytes. Appl Soil Ecol 61:217–224
Raskin I, Smith RD, Salt DE (1997) Phytoremediation of metals: using plants to remove pollutants from the environment. Curr Opin Biotechnol 8(2):221–226
Ryan RP, Germaine K, Franks A et al (2008) Bacterial endophytes: recent developments and applications. FEMS Microbiol Lett 278(1):1–9
Santoyo G, Moreno-Hagelsieb G, del Carmen Orozco-Mosqueda M, Glick BR (2016) Plant growth-promoting bacterial endophytes. Microbiol Res 183:92–99
Sarma H, Forid N, Prasad R, Prasad MNV, Ma LQ, Rinklebe J (2021) Enhancing phytoremediation of hazardous metal(loid)s using genome engineering CRISPR–Cas9 technology. Journal of Hazardous Materials https://doi.org/10.1016/j.jhazmat.2021.125493
Sgroy V, Cassán F, Masciarelli O et al (2009) Isolation and characterization of endophytic plant growth-promoting (PGPB) or stress homeostasis-regulating (PSHB) bacteria associated to the halophyte Prosopis strombulifera. Appl Microbiol Biotechnol 85(2):371–381
Sheng XF, Xia JJ, Jiang CY et al (2008) Characterization of heavy metal-resistant endophytic bacteria from rape (Brassica napus) roots and their potential in promoting the growth and lead accumulation of rape. Environ Pollut 156(3):1164–1170
Shishido M, Breuil C, Chanway CP (1999) Endophytic colonization of spruce by plant growth-promoting rhizobacteria. FEMS Microbiol Ecol 29(2):191–196
Singh OV, Jain RK (2003) Phytoremediation of toxic aromatic pollutants from soil. App Microbiol Biotechnol 63(2):128–135
Soleimani M, Afyuni M, Hajabbasi MA (2010) Phytoremediation of an aged petroleum contaminated soil using endophyte infected and non-infected grasses. Chemosphere 81(9):1084–1090
Souza RD, Ambrosini A, Passaglia LM (2015) Plant growth-promoting bacteria as inoculants in agricultural soils. Genet Mol Biol 38(4):401–419
Stępniewska Z, Kuźniar A (2013) Endophytic microorganisms-promising applications in bioremediation of greenhouse gases. App Microbiol Biotechnol 97(22):9589–9596
Taghavi S, Garafola C, Monchy S et al (2009) Genome survey and characterization of endophytic bacteria exhibiting a beneficial effect on growth and development of poplar trees. Appl Environ Microbiol 75(3):748–757
Thakare M, Sarma H, Datar S, Roy A, Pawar P, Gupta K, Pandit S, Prasad R (2021) Understanding the holistic approach to plant-microbe remediation technologies for removing heavy metals and radionuclides from soil. Current Research in Biotechnology https://doi.org/10.1016/j.crbiot.2021.02.004
van der Lelie D, Barac T, Taghavi S, Vangronsveld J (2005) Response to Newman: new uses of endophytic bacteria to improve phytoremediation. Trends Biotechnol 23(1):8–9
van der Lelie D, Taghavi S, Monchy S et al (2009) Poplar and its bacterial endophytes: coexistence and harmony. Crit Rev Plant Sci 28(5):346–358
Verma VC, Singh SK, Prakash S (2011) Bio-control and plant growth promotion potential of siderophore producing endophytic Streptomyces from Azadirachta indica A Juss. J Basic Microbiol 51(5):550–556
Walitang DI, Kim K, Madhaiyan M (2017) Characterizing endophytic competence and plant growth promotion of bacterial endophytes inhabiting the seed endosphere of Rice. BMC Microbiol 17(1):209
Waqas M, Khan AL, Kamran M (2012) Endophytic fungi produce gibberellins and indoleacetic acid and promotes host-plant growth during stress. Molecules 17(9):10754–10773
Weilharter A, Mitter B, Shin MV (2011) Complete genome sequence of the plant growth-promoting endophyte Burkholderia phytofirmans strain Ps JN. J Bacteriol 193(13):1–2
Weyens N, van der Lelie D, Taghavi S et al (2009a) Phytoremediation: plant–endophyte partnerships take the challenge. Curr Opin Biotechnol 20(2):248–254
Weyens N, van der Lelie D, Taghavi S et al (2009b) Exploiting plant–microbe partnerships to improve biomass production and remediation. Trends Biotechnol 27(10):591–598

Weyens N, Truyens S, Saenen E et al (2011) Endophytes and their potential to deal with co-contamination of organic contaminants (toluene) and toxic metals (nickel) during phytoremediation. Int J Phytoremediation 13(3):244–255

Wu CH, Bernard SM, Andersen GL, Chen W (2009) Developing microbe–plant interactions for applications in plant-growth promotion and disease control, production of useful compounds, remediation and carbon sequestration. Microb Biotechnol 2(4):428–440

Yadav R, Arora P, Kumar S, Chaudhury A (2010) Perspectives for genetic engineering of poplars for enhanced phytoremediation abilities. Ecotoxicology 19(8):1574–1588

Zhu Y, She X (2018) Evaluation of the plant-growth-promoting abilities of endophytic bacteria from the psammophyte *Ammodendron bifolium*. Can J Microbiol 64(4):253–264

Chapter 7
Microbial Remediation: A Natural Approach for Environmental Pollution Management

Vankayalapati Vijaya Kumar

Contents

V. V. Kumar (✉)
Natems Sugar Private Limited, Gachibowli, Hyderabad, Telangana, India

R. Prasad et al. (eds.), *Mycoremediation and Environmental Sustainability*, Fungal Biology, https://doi.org/10.1007/978-3-030-54422-5_7

7.1 Introduction

The dependence of humans on industries and agriculture for their needs such as clothing, shelter, and food led to the release of many unwanted contaminants into the environment which caused the environmental pollution. The industrialization has caused the burning of fossil fuels, which releases carbon dioxide, carbon monoxide, oxides of nitrogen and sulfur, particulate matter, and heavy metals. By the excessive use of chemicals in agriculture, such as fertilizers, pesticides, synthetic organic chemicals, heavy metals have been accumulated in the environment resulting in environmental pollution (Steph 1988; Prasad 2021). The accumulation of these chemicals from agriculture in ponds/rivers through runoff streams caused eutrophication, leading to oxygen depletion conditions, harming the aquatic life.

Bioremediation is defined as the process of stimulation of microorganisms for faster degradation of hazardous organic pollutants to environmentally safe levels in soil, sediments, substances, materials, and groundwater. Inorganic pollutants such as heavy metals are either precipitated or immobilized using the bioremediation techniques (Pandey and Fulekar 2012). Bioremediation is also defined as the process whereby organic wastes are biologically degraded under controlled conditions to an innocuous state or to levels below concentration limits established by regulatory authorities (Mueller et al. 1996). Bioremediation mainly employs the use of living organisms, mainly microorganism for degrading the toxic pollutants to less toxic forms.

Bioremediation is of two types such as in situ, when bioremediation carried out in the same site where the pollutants/contaminants are accumulated, and ex situ when the bioremediation is performed at a site away from the site of occurrence of pollutants/contaminants, and this involves the additional cost of transportation. In situ bioremediation strategies include biosparging, bioventing, and bioaugmentation, and ex situ bioremediation strategies include land farming, composting, bioreactors, and biopiling (Kumar et al. 2011; Vijaya Kumar 2017).

Bioremediation by employing the microorganisms is called microbial bioremediation. When plants are used in bioremediation, it is called phytoremediation, and when mycorrhizal fungi are used in bioremediation, it is known as mycorrhizoremediation. Mycorrhizoremediation is the advanced form of phytoremediation, in which the mycorrhizal fungi living in the plant roots participate in bioremediation process. This chapter highlights the role of microorganisms (bacteria, fungi, and mycorrhizal fungi) in bioremediation process.

The advantages and disadvantage of bioremediation are given below.

7.2 Advantages of Bioremediation

1. Bioremediation is a natural process and is recognized by the public as the acceptable way of waste treatment process.
2. In the presence of contaminant/pollutant, the microorganisms increase in their number and degrade the contaminant. The number of microorganisms will

decrease upon the degradation of contaminant. The harmless degradation products include water, carbon dioxide, and microbial biomass.
3. Many contaminants that are considered as hazardous legally can be completely degraded to non-hazardous form without leaving any residues.
4. Bioremediation can be performed at the same site where the contaminants are present, avoiding the transportation costs off site and alleviating the potential health risks to humans and the environment arising during the transportation of contaminants.
5. Bioremediation is inexpensive compared to the conventional remediation methods such as incineration, etc.

7.3 Disadvantages of Bioremediation

1. Bioremediation is limited to the compounds that are biodegradable. All the compounds are not susceptible to complete and faster degradation.
2. Sometimes the degradation products are more toxic than the parent compounds.
3. Bioremediation is extremely specific and requires favorable conditions such as sufficient microbial populations, suitable environmental conditions for growth, and accessibility to the essential nutrients and contaminants.
4. Scale-up from bench-/pilot-scale studies to field-level studies is difficult.
5. Bioremediation takes longer time compared to other treatment options, for example, excavation, removal of soil, or incineration.
6. Pollutants may be present at any state such as solid, liquid, or gaseous state.
7. More studies/research is required to adopt strategies for developing bioremediation technologies suitable for sites where the contaminants are not uniformly distributed and complex mixtures of contaminants are present at the same site (Kumar et al. 2011; Santra 2010; Sharma 2012; Mary Kensa 2011).

7.4 Microbial Remediation

Many microorganisms, belonging to bacteria and fungi, are involved in the remediation of environmental pollution. The important inorganic contaminants comprise of heavy metals generated from various industries such as mining, metallurgy, chemical processing, and power plants. The enormous usage of organic compounds such as biocides, polymers, crude oil, explosives, flame retardants, polymers, solvents, and chlorinated organic compounds has polluted the environment (Srivastava et al. 2014). The following groups of microorganisms are involved in bioremediation.

7.4.1 Bacteria

Both aerobic bacteria and anaerobic bacteria are identified for their capacity to degrade pollutants.

(a) Aerobic bacteria: *Pseudomonas*, *Sphingomonas*, *Rhodococcus*, *Alcaligenes*, and *Mycobacterium* are able to degrade alkanes and polyaromatic compounds which are the ingredients in pesticides and hydrocarbons. These bacteria utilize contaminants as the only source of carbon and energy. The other bacteria that degrade the contaminants include *Bacillus*, *Acinetobacter*, *Arthrobacter*, *Citrobacter*, *Corynebacterium*, etc.
(b) Anaerobic bacteria: There is a growing interest recently in using anaerobic bacteria for degradation of polychlorinated biphenyls (PCBs) and dechlorination of trichloroethane and chloroform. The anaerobic bacteria belonging to *Desulfitobacterium*, *Desulfuromonas*, *Dehalospirillum*, *Dehalobacter*, and *Dehalococcoides* are able to degrade the organohalide contaminants.
(c) Methylotrophs: These bacteria utilize methanol as a sole carbon source. Due to carbon recycling across the globe, methane and methanol formed as intermediates in the atmosphere. Methanol is also generated by the decay of lignin and pectin from plant parts. *Methylosinus*, *Methylobacterium*, *Methylocapsa*, *Methylovirgula*, *Ochrobactrum*, etc. are the few methylotrophic bacteria involved in the bioremediation of methane or methanol.

7.4.2 Fungi

Fungi degrade a variety of contaminants such as lignocellulosic wastes originating from forestry, pulp and paper industries, food and agriculture industries, municipal wastes, etc. (Prasad 2017, 2018). The fungi belonging to the genera *Phanerochaete*, *Armillaria*, *Pleurotus*, *Trametes*, *Ganoderma*, *Laetiporus*, *Serpula*, *Fibroporia*, *Phaeolus*, *Fomitopsis*, *Chaetomium*, *Ceratocystis*, etc. degrade the lignocellulosic waste. The other soil fungi that reduce the heavy metal contamination by biosorption include *Mucor*, *Aspergillus*, *Rhizopus*, *Saccharomyces*, *Botrytis*, *Neurospora*, *Phanerochaete*, etc.

Mycorrhizal fungi living in association with the plant roots reduce the heavy metal contamination in the soil by accumulating the heavy metals either in the roots or in their hyphae (Kamal et al. 2010; Kumar et al. 2011; Vijaya Kumar 2017; Abatenh et al. 2017; Matthew Lee et al. 2015).

7.5 Factors Affecting Microbial Bioremediation

For effective degradation of pollutants, various biological and environmental factors play a key role. Microorganisms through their enzymatic pathways act as biocatalysts and expedite the biochemical processes in degrading the desired pollutants.

Nutrients (nitrogen and phosphorus), oxygen, and electron acceptors promote the microbial growth.

7.5.1 Biological Factors

The presence of sufficient quantities of microorganisms and the secretion of specific enzyme are essential for the degradation of pollutants. The availability of contaminants to the microorganisms is also important for effective degradation of pollutants.

7.5.2 Environmental Factors

The environmental factors such as type of soil, temperature, pH, the presence of oxygen or other electron acceptors, soil moisture, and nutrients are important in the efficient degradation of pollutants.

7.5.3 Soil

Soils containing high concentration of contaminants (above 5%) are agitated in water solution with interface-active agents and then separated from oils, and then the bioremediation process can be started to remove the pollutants. The bioremediation process can be initiated immediately in soils containing about 2% heavy oils, and it takes 6 months to 1 year for the cleanup of soil, whereas it will take 1 or 2 months to clean up the soils containing less than 0.8% of the oils.

7.5.4 Temperature

Temperature is an important factor for growth and multiplication of microorganisms. The enzymes secreted by microbes need an optimum temperature for their activity. The high temperature leads to the drying of microbes due to dehydration, whereas lower temperature reduces the microbial growth, multiplication, and enzyme activity. The optimum temperature for microbial activity for bioremediation is 20–30 °C.

7.5.5 pH

pH is an important factor for microbial growth and optimum enzyme activity for pollutant degradation process. The pH of the acidic soils can be raised by adding lime. The optimum pH for bioremediation by microorganisms is 6.5–7.5.

7.5.6 Oxygen Concentration

Some microbes (aerobic) require oxygen for their growth and accelerate the biodegradation process. The optimum oxygen concentration is above 0.2 mg/L dissolved oxygen, more than 10% air-filled pore space for aerobic degradation.

7.5.7 Moisture Content

Adequate moisture is required for the growth of microbes and for degradation of pollutants. Water holding capacity of 25–28% is required for the microbial activity in bioremediation process**.**

7.5.8 Nutrients

Various nutrients in the form of carbon, hydrogen, oxygen, nitrogen, phosphorus, sulfur, and other minerals are required for the growth and multiplication of the microorganisms in the contaminated soils. The nutrients are supplied in the form of fertilizers. Carbon is the most essential element required in greater quantities than the other elements. It constitutes more than 95% of the weight of the cells. Phosphorus and sulfur represent 70% of the rest. The carbon-to-nitrogen nutritional requirement ratio is 10:1, and the ratio of carbon to phosphorus is 30:1. The optimum (nutritional requirement) C:N:P ratio for oil degradation is 100:10:1 (Vidali 2001; Sasikumar and Papinazat 2003; Sharma 2012).

7.6 Bioremediation by Bacteria

Many aerobic and anaerobic bacteria degrade a variety of contaminants such as alkanes and polyaromatic compounds in pesticides and hydrocarbons, polychlorinated biphenyls, organohalides, etc.

7.6.1 Bioremediation by Aerobic Bacteria

7.6.1.1 Heavy Metal Bioremediation

Microorganisms require metal ions in various biochemical processes. The presence of metal ions in higher concentration inhibits the microbial growth by forming complexes in the microbial cells. Bacteria develop resistance to heavy metal toxicity by

siderophore production and compartmentalization inside the cells, formation of complexes, and synthesis of binding proteins such as metallothioneins (MTs) and phytochelatins (PCs) (Rajendran et al. 2003). Endophytic bacteria reduce heavy metal toxicity by precipitation, buildup of heavy metals, and sequestration, adsorption, and biotransformation to less toxic forms.

The removal of various heavy metals such as arsenic, copper, chromium, lead, and strontium was demonstrated by Achal et al. (2011, 2012, 2013). *Sporosarcina ginsengisoli* bacterium isolated from the arsenic-contaminated soil removed 96.3% of exchangeable arsenic from the liquid solution. Similarly, the bacterium *Kocuria flava* isolated from mining soil showed higher urease and tolerance to copper. The maximum removal of copper was due to high calcite precipitation through more degradation of urea by urease production. These bacteria also removed lead from the contaminated soil through microbial induced calcite precipitation through urease production. Cadmium bioremediation was demonstrated by Kang et al. (2014) by using the bacterium *Lysinibacillus sphaericus* through urease production and calcite precipitation. 99.95% of cadmium was removed from sand and is converted into stable biomineral in 48 hours. Similar results of the removal of chromium from contaminated soil by the bacteria *Bacillus cereus* were demonstrated by Kumari et al. (2014). The *Pisum sativum* seeds were planted in the soil containing chromium and treated with *Bacillus cereus*, and the uptake of chromium was measured. The *Pisum* plants from the treated soil showed negligible uptake of chromium compared to the controls.

Osman et al. (2015) reported the reduction of heavy metals by the bacteria (*Myroides* spp. and *Micrococcus* spp.) identified from the water and soil samples of the downstream of Galing River, Kuantan, Malaysia. These bacteria reduced the concentrations of heavy metals such as zinc, lead, arsenic, cadmium, manganese, selenium, and indium when these bacteria were cultured in the basal salt medium mixture with these heavy metals (1 ppm each) for 7 days. In *Myroides* spp.-inoculated medium, the highest reduction was observed in zinc from 1 ppm (control) to 0.513 ppm (in bacteria inoculated) followed by cadmium 1–0.523 ppm and selenium 1–0.533 ppm. In *Micrococcus* spp.-treated medium mixture, the highest reduction was observed in selenium 1–0.205 ppm, followed by lead 1–0.405 ppm and cadmium 1–0.493 ppm.

Sarkar and Ghosh (2012) reported the isolation of solvent-tolerant strain of *Bacillus thermophilus* PS11 from the soil by cyclohexane enrichment. The strain was adapted to the solvent by accumulating it in the cytoplasm during the initial 48 hours of incubation, and this accumulation was confirmed by transmission electron microscopy. Upon further incubation up to 96 hours, the decline in solvent accumulation and the reorganization of the cell membrane were observed. During the initial growth of 12 hours, it accumulated 50 nm/ml of uranium exhibiting metal resistance capability. This bacterial strain was also tolerated the other solvents such as isooctane, toluene, benzene, chloroform, etc.

7.6.1.2 Polycyclic Aromatic Hydrocarbon Bioremediation

The bioremediation of polycyclic aromatic hydrocarbon was confirmed by Al-Haditi et al. (2017) in Iraq. They have screened 105 samples from municipal drinking water, soil, and surface water sample from Shatt Al-Arab waterway area, Basrah, Iraq, polluted with diesel oil. They got 10 isolates of *Acinetobacter* from 3 sources and identified as *A. lwoffii* (8%), whereas *A. calcoaceticus* (2%) was isolated from soil only. *A. lwoffii* and *A. calcoaceticus* bacteria (1×10^8 cells/ml) were inoculated in mineral salt medium (250 ml.) supplemented with 0.1 gm of PAH. The bacterial cell count and PAH reduction were measured in 2, 4, and 8 weeks, respectively. In *A. lwoffii* inoculated medium, the cell count was reduced from the initial 5×10^8 cells/ml to 0.6×10^2 cells/ml, and the PAH concentration was reduced from 0.1 to 0.003 g, while in *A. calcoaceticus* inoculated medium, the cell count was reduced from the initial 5×10^8 cells/ml to 0.2×10^2 cells/ml, and the PAH concentration was reduced from 0.1 to 0.001 gm in 2 months of incubation.

Similar results of 4T engine oil degradation (84–86%) by the bacterium *Acinetobacter calcoaceticus* BD4 isolated from a coastal area in Mumbai were obtained by Sihag et al.(2013). In this experiment 1% 4T engine oil was used as a carbon source. During the degradation of hydrocarbons, maximum CO_2 (792 μ mol) evolved on the 30th day, and thereafter, the CO_2 evolution was reduced.

7.6.1.3 Herbicide and Pesticide Degradation

The biodegradation ability of *Arthrobacter* in the degradation of herbicides (pendimethalin, thiobencarb, and bromoxynil) was studied by Ashour et al. (2005). They have identified *Arthrobacter* from the 25 bacterial isolates that were isolated from the heavily polluted soil from 7 different soil samples collected from the warehouses of herbicides in Egypt. One ml of bacterial sample containing 10^8 cells were inoculated in 100 ml of nutrient broth containing different concentrations of herbicides (up to 250 ppm) and incubated for 7 days on shaker incubator. The growth of *Arthrobacter* was severely affected by bromoxynil, followed by thiobencarb and pendimethalin. In the presence of pendimethalin, the specific growth rate ($\mu\ hr^{-1}$) of *Arthrobacter* was reduced from 0.294 in control to 0.077 in 250 ppm, and the doubling time was increased from 2.36 hours in control to 8.97 hours in 250 ppm. Similarly, the specific growth rate and doubling time in thiobencarb and bromoxynil in control and treated medium were 0.295 and 0.055 $\mu\ h^{-1}$ and 2.36 and 12.55 h and 0.294 and 0.047 $\mu\ h^{-1}$ and 2.36 and 14.89 h, respectively. Mandal et al. (2012) reported the degradation of more than 2,00,000 tons of oil sludge by consortium of bacteria isolated from various hydrocarbon-polluted sites in India by using ex situ and in situ remediation techniques. The initial total petroleum hydrocarbon (TPH) from 5% to 52% present in the polluted soil was reduced to less than 1% in 2–12-month period.

7.6.2 Bioremediation by Anaerobic Bacteria

Anaerobic bacteria are useful in the degradation of organohalides. Organohalides are the organic compounds containing C1 and C2 aliphatics and dioxins which are complex polyaromatic compounds. They are the recalcitrant contaminants polluting soil and water. The organohalides include hexachlorobenzene (HCB), trichloromethane (TCM), polychlorinated biphenyls (PCBs), perchloroethene (PCE), trichloroethene (TCE), dichloroethanes (DCA), polybrominated diphenyl ethers (PBDEs), chlorinated/brominated phenols, and dioxins. They are used in a variety of applications such as in the manufacture of polyvinyl chloride (PVC), perchloromethane as dry cleaning solvent, and trichloromethane (chloroform) as precursor in chlorofluorocarbon refrigerant gases (Matthew Lee et al. 2015; Jugder et al. 2016).

The dehalogenation of various halogenated substrates by using the bacteria *Dehalococcoides* isolated from the contaminated groundwater from the area of Bitterfeld-Wolfen, Germany, was reported by Kaufhold et al. (2012). The isolated bacteria converted chlorinated ethenes to ethane. These bacteria also converted vinyl bromide (VB) and 1,2-dichloroethane to ethane. 1,2,3,4- and 1,2,3,5-tetrachlorobenzene (TeCB) and penta- and hexachlorobenzene (PeCB and HCB) are converted to trichlorobenzenes (TCB), lindane is converted to monochlorobenzene (MCB), and pentachlorophenol (PCP) was converted to 2,3,4,6-tetrachlorophenol (TeCP).

Similarly, the *Dehalobacter* sp. strain TeCB1 isolated from the groundwater near Sydney dehalogenated the 1,2,4,5-tetrachlorobenzene to 1,3- and 1,4-dichlorobenzene. During this reductive chlorination process, 1,2,4-trichlorobenzene was formed as an intermediate product (Alfan-Guzman et al. 2017). Drzyzga and Gottschal (2002) demonstrated the dependence of *Desulfitobacterium frappieri* TCE1 growth on the activity of *Desulfovibrio fructosivorans.* When both the bacteria *Desulfitobacterium frappieri* TCE1 and *Desulfovibrio fructosivorans* are cocultivated at different concentrations of sulfate and without sulfate, the growth of TCE1 strain outnumbered the sulfate-reducing bacteria at 2.5 mM sulfate concentration, and trace amounts of PCE were degraded, whereas at 1 mM sulfate and without sulfate, the TCE1 strain degraded the PCE to cis-dichloroethane (cis-DCE).

7.6.3 Bioremediation by Methylotrophs

Methylobacteria utilize C1 compounds as a source of carbon and energy and play a key role in global carbon recycling and reduce the greenhouse gas emissions. Tambekar et al. (2014) reported that the bacterium *Ochrobactrum oryzae* isolated from the alkaline Lonar lake in the Buldhana district of Maharashtra, India, utilized maximum amount of methanol (78%) in 96 hours. At pH 7 and pH 8, the highest methanol degradation (0.042 mg/h) was noted in 72 hours.

Tambeker and Rajgire (2015) identified *Pseudomonas aeruginosa* (DHT 2) and *Enterobacter cloacae* (DHT 8) from sediment samples collected from the alkaline Lonar lake by using 2% methanol as carbon source in minimal salt medium. *P. aeruginosa* utilized 78% (rate of utilization 0.0406 mg/ml), and *E. cloacae* utilized 75% of methanol (rate of utilization 0.0390 mg/ml) in 96 hours.

7.7 Bioremediation by Fungi

Mycoremediation is the term used when fungi are used in the bioremediation process. In the forests the fallen leaf and wood litter contain many nutrients locked in them, which cannot be utilized by the plants. The fungi are the fastest decomposers of the forest litter which degrade the dead wood and leaves and release the nutrients locked in the litter and supply them to the plants and other living biota. The vegetative part of the fungus – the mycelium which is a thread-like structure – releases a diverse extracellular enzymes and acids, which degrade cellulose and lignin, the important constituent of plant fiber. During the breakdown of lignin and cellulose, humus is formed which helps in the growth of the plants (Rhodes 2014).

7.7.1 Mechanisms of Remediation by Fungi

Fungi degrade various lignocellulosic materials by the secretion of enzymes such as ligninolytic enzymes (oxidases and peroxidases) and extracellular cellulolytic enzymes. These enzymes act as biocatalysts and help in the degradation of lignin and cellulose.

Biosorption of metals from aqueous solution by fungi is achieved by surface binding which includes ion exchange reactions and formation of complexes with the functional groups existing on the cell surfaces.

Based on the participation of various fungi in pollutant degradation, the remediation process is categorized into:

- Ligninolytic fungal degradation
- Soil fungal biosorption
- Mycorrhizoremediation (mycorrhizal fungal degradation)

7.7.2 Ligninolytic Fungal Degradation

Lignocellulosic wastes are primarily generated from various industries such as pulp and paper industry, food, and agriculture. It is the major component of plants. These fungi also degrade various other pollutants such as polycyclic aromatic hydrocarbons (PAHs), pesticides, etc.

The fungi degrading the lignocellulosic materials are classified into three groups such as white rot fungi, brown rot fungi, and soft rot fungi (Hickman et al. 2011).

White rot fungi belonging to the genera *Phanerochaete chrysosporium*, *Armillaria* spp. (honey mushroom), *Pleurotus*, and other oyster mushrooms, *Trametes versicolor* (turkey tail), *Trametes hirsute* (hairy turkey tail), *Ganoderma applanatum* (artist's conk), and *Fomes fomentarius* (tinder fungus) degrade the cellulose and lignin in the wood.

Brown rot fungi belonging to the genera *Laetiporus sulphureus* (sulfur fungus), *Serpula lacrymans* (true dry rot), *Fibroporia vaillantii* (mine fungus), *Phaeolus schweinitzii*, and *Fomitopsis pinicola* are the degraders of cellulose and hemicellulose in wood.

Soft rot fungi belonging to the genera *Chaetomium*, *Ceratocystis*, and *Kretzschmaria deusta* degrade cellulose, hemicellulose, and lignin.

Zhu et al. (2011) reported the degradation of lignocellulose, production of enzymes, and protein enrichment in the solid-state fermentation of corn stover by *Trametes versicolor*. Under optimal conditions, high laccase activity (20-fold increase, 45.1 U/g of corn stover), moderate xylanase activity, and little carboxymethyl cellulase (CMCase) activity were recorded. The highest degradation of lignin up to 34.8%, followed by hemicellulose 21.9%, and the low cellulose degradation of less than 10.5 were observed. Bishnoi et al. (2008) demonstrated the degradation of five PAHs in unsterile and sterile soil using the *Phanerochaete chrysosporium* isolated from the soil sample of petroleum refinery. At optimum conditions of pH 7.0, temperature 30 °C, and 5 μg/gm of PAH concentration, the maximum degradation occurred in 42 days. In sterile soil, the maximum degradation occurred in phenanthracene (98.96%), followed by anthracene (92.60%), pyrene (92.2%), acenaphthene (83.8%), and fluoranthene (79.8), whereas in unsterile soil, the low degradation was noticed (38.94–62.89%).

Mtui and Masalu (2008) reported the extracellular enzymes (manganese peroxidase 2.5 U/ml and lignin peroxidase 1 U/ml) produced by *Laetiporus sulphureus* isolated from mangrove forests, and it has the ability to oxidize the rhemazol brilliant blue-R (RBB-R) dye and phenol and removed 90% of the color from raw textile effluents in immobilized culture.

7.7.3 Soil Fungal Biosorption

Many soil fungi, for example, *Mucor* sp., *Aspergillus carbonarius*, *Aspergillus niger*, *Rhizopus* sp., *Saccharomyces cerevisiae*, *Botrytis cinerea*, *Neurospora crassa*, etc., are having the capability of biosorption of heavy metals.

Ahmad et al. (2005) isolated *Aspergillus* and *Rhizopus* sp. from the agriculture field treated with sewage/industrial effluent in Aligarh city. They have used dead biomass of the above fungus for biosorption experiment for the metals cadmium (Cd) and chromium (Cr). *Aspergillus* and *Rhizopus* biosorbed 6.20–9.5 mg/g of Cr and 2.3–8.21 mg/g of Cd. The biosorption capacity of *Rhizopus* sp. is higher

compared to *Aspergillus* sp. Similarly, *Mucor racemosus* isolated from the polluted water in Northern delta of Egypt was used to study the biosorption of copper (Cu), zinc (Zn) and lead (Pb) by El-Morsy et al. (2013). The highest uptake was noticed for Cu (60.13 mg/g), followed by Zn (57.67 mg/g) and Pb (21.97 mg/g) at 200 mg/l biomass.

7.7.4 Mycorrhizal Fungal Degradation

Mycorrhizal fungi are having symbiotic association with plants roots and are having the capacity to degrade the organic pollutants and accumulate heavy metals by avoiding them reaching the food web. The different types of mycorrhiza include ectomycorrhiza, endomycorrhiza (arbuscular mycorrhiza, AM), ectendomycorrhiza, ericoid mycorrhiza, monotropoid mycorrhiza, arbutoid mycorrhiza, and orchidoid mycorrhiza.

Arbuscular mycorrhizal fungi play a key role in imparting heavy metal tolerance by the plants. The heavy metals bind to the chitin, cellulose, cellulose derivatives, and melanin. The high concentration of S&N in polyphosphate granules signifies the existence of heavy metal-thiolate binding by metallothionein-like peptides. The cell wall proteins of AM fungi have the capability to sequester the heavy metals by absorption. The glomalin present on the hyphae of AM fungi can increase the sequestering of the heavy metals. Glomalin plays a key role in the sorption and sequestering of heavy metals, reducing their bioavailability (Galli et al. 1994).

Al-Garni (2006) reported the increased tolerance of cowpea plants in a pot culture experiment supplemented with zinc (Zn) and cadmium (Cd) at various concentrations, inoculated with or without AM and *Rhizobium*. The metals accumulated in the roots of cowpea plants. The dry weight, leaf number and area, plant length, leaf pigments, total carbohydrates, and total P&N increased in cowpea plants, indicating the stoppage of heavy metals reaching the aerial portion of the cowpea plants.

Huang et al. (2007) studied the impact of *Glomus caledonium* on atrazine accumulation and metabolism in maize in the pot culture experiment. More atrazine was accumulated in the roots of mycorrhiza applied maize plants compared to the non-mycorrhizal plant roots. In shoots the less deposition of atrazine was noticed in mycorrhiza applied plants compared to the control plants.

7.8 Conclusion and Future Prospects

Pollution control is the major task in the present scenario. Even though measures are being taken by governments and industries for treating the effluents in a scientific manner, they are yielding short-term results. The drawback in implementing the pollution-preventing devises is due to their higher initial establishment costs, as well as operating costs. Bioremediation is a natural and cost-effective treatment

process, which uses microorganisms and plants. Bioremediation is not widely used for reclamation of polluted soils due various reasons such as lack of awareness, non-availability of suitable inocula for the contaminated sites, difficulties in replication of the lab results at field level, etc. The required microbes can be easily produced and supplied for the bioremediation processes in large scale in a shorter period of time. Analyzing various soil parameters and creating the field conditions at the laboratory level will give a better understanding of bioremediation process.

Acknowledgment The author is thankful to the Management of Natems Sugar Private Limited for the encouragement and support during the preparation of this manuscript.

References

Abatenh E, Gizaw B, Tsegaye Z, Wassie M (2017) Application of microorganisms in bioremediation-review. J Environ Microbiol 1:2–9

Achal V, Pan X, Zhang D (2011) Remediation of copper-contaminated soil by *Kocuria flava* CR1, based on microbially induced calcite precipitation. Ecol Eng 37:1601–1605

Achal V, Pan X, Fu Q, Zhang D (2012) Biomineralization based remediation of As(III) contaminated soil by *Sporosarcina ginsengisoli*. J Hazard Mater 201–202:178–184

Achal V, Pan X, Lee DJ, Kumari D, Zhang D (2013) Remediation of Cr(VI) from chromium slag by biocementation. Chemosphere 93:1352–1358

Ahmad I, Zafar S, Ahmad F (2005) Heavy metal biosorption potential of *Aspergillus* and *Rhizopus* sp. isolated from wastewater treated soil. J Appl Sci Environ Mgt 9:123–126

Alfán-Guzmán R, Ertan H, Manefield M, Lee M (2017) Isolation and characterization of *Dehalobacter* sp. Strain TeCB1 including identification of TcbA: a novel tetra- and trichlorobenzene reductive dehalogenase. Front Microbiol 8:1–13. (Article 558)

Al-Garni SMS (2006) Increased heavy metal tolerance of cowpea plants by dual inoculation of an arbuscular mycorrhizal fungi and nitrogen-fixer *Rhizobium* bacterium. Afr J Biotechnol 5:133–142

Al-Hadithi HT, Al-Razzaq EA, Fadhil GF (2017) Bioremediation of polycyclic aromatic hydrocarbons by *Acinetobacter* species isolated from ecological sources. J Environ Biol 38:785–789

Ashour EH, Bayoumy SMM, El-Sawah MA, Shady SA (2005) Biodegradation of certain herbicides by *Arthrobacter* and the use of this isolate in bioremediation of contaminated soil. N Egypt J Microbiol 1:184–198

Bishnoi K, Kumar R, Bishnoi NR (2008) Biodegradation of polycyclic aromatic hydrocarbons by white rot fungi *Phanerochaete chrysosporium* in sterile and unsterile soil. J Sci Ind Res 67:538–542

Drzyzga O, Gottschal JC (2002) Tetrachloroethene dehalorespiration and growth of *Desulfitobacterium frappieri* TCE1 in strict dependence on the activity of *Desulfovibrio fructosivorans*. Appl Environ Microbiol 68:642–649

El-Morsy EM, Nour El-Dein MM, El-Didamoney SMM (2013) *Mucor racemosus* as a biosorbent of metal ions from polluted water in Northern Delta of Egypt. Mycosphere 4:1118–1131

Galli U, Schuepp H, Brunold C (1994) Heavy metal binding by Mycorrhizal fungi. Physiol Plant 92:364–368

Hickman GW, Perry EJ, Davis RM (2011) Wood decay fungi in landscape trees. University of California, p4.UC ANR Publication 74109. http://www.ipm.ucdavis.edu/PMG/PESTNOTES/

Huang H, Zhang S, Shan X, Chen BD, Zhu YG, Bell JNB (2007) Effect of Arbuscular mycorrhizal fungus (*Glomus caledonium*) on the accumulation and metabolism of atrazine in maize (*Zea mays* L.) and atrazine dissipation in soil. Environ Pollut 146:452–457

Jugder BE, Ertan H, Bohl S, Lee M, Marquis CP, Manefield M (2016) Organohalide respiring bacteria and reductive dehalogenases: key tools in organohalide bioremediation. Front Microbiol 7:1–12. (Article 249)

Kamal S, Prasad R, Varma A (2010) Soil microbial diversity in relation to heavy metals. In: Sherameti I, Varma A (eds) Soil heavy metals, vol 19. Springer-Verlag, Berlin, Heidelberg, pp 31–63

Kang CH, Han SH, Shin Y, Oh SJ, So JS (2014) Bioremediation of Cd by microbially induced calcite precipitation. Appl Biochem Biotechnol 172:1929–1937

Kaufhold T, Schmidt M, Cichocka D, Nikolausz M, Nijenhuis I (2012) Dehalogenation of diverse halogenated substrates by a highly enriched *Dehalococcoides*-containing culture derived from the contaminated mega-site in Bitterfeld. FEMS Microbiol Ecol 83:176–188

Kumar A, Bisht BS, Joshi VD, Dhewa T (2011) Review on bioremediation of polluted environment: a management tool. Int J Environ Sci 1:1079–1093

Kumari D, Li M, Pan X, Xin-Yi Q (2014) Effect of bacterial treatment on Cr(VI) remediation from soil and subsequent plantation of *Pisum sativum*. Ecol Eng 73:404–408

Mandal AK, Sarma P, Singh B, Jeyaseelan C, Channashettar V, Lal B, Datta J (2012) Bioremediation: an environment friendly sustainable biotechnological solution for remediation of petroleum hydrocarbon contaminated waste. ARPN J Sci Technol 2:1–11

Mary Kensa V (2011) Bioremediation – an overview. J Ind Pollut Control 27:161–168

Matthew Lee, Chris Marquis, Bat-Erdene Judger, Mike Manefield (2015) Anaerobic microorganisms and bioremediation of organohalide pollution. Microbiology Australia. pp A–D. https://doi.org/10.1071/MA15044

Mtui G, Masalu R (2008) Extracellular enzymes from brown-rot fungus *Laetiporus sulphureus* isolated from mangrove forests of coastal Tanzania. Sci Res Essay 3:154–161

Mueller JG, Cerniglia CE, Pritchard PH (1996) Bioremediation of environments contaminated by polycyclic aromatic hydrocarbons. In: R. Crawford & D. Crawford (Eds.), Bioremediation: principles and applications. Cambridge University Press, Cambridge, pp 125–194

Osman MR, Azid A, Yunus K, Mustafa AD, Amran MA, Azaman F, Suhaili Z, Bakar YA, Zainuddin SFM (2015) Assessment on bacteria in the heavy metal bioremediation. Malaysian J Anal Sci 19:1405–1414

Pandey B, Fulekar MH (2012) Bioremediation – a natural way for cleaner environment. Biol Med 4:51–59

Prasad R (2017) Mycoremediation and environmental sustainability. vol 1. Springer International Publishing (ISBN 978-3-319-68957-9). https://link.springer.com/book/10.1007/978-3-319-68957-9

Prasad R (2018) Mycoremediation and environmental sustainability, vol 2. Springer International Publishing (ISBN 978-3-319-77386-5). https://www.springer.com/us/book/9783319773858

Prasad R (2021) Environmental Pollution and Remediation. Springer (ISBN 978-981-15-5499-5) https://www.springer.com/gp/book/9789811554988

Rajendran P, Muthukrishnan J, Gunasekhran P (2003) Microbes in heavy metal bioremediation. Indian J Exp Biol 41:935–944

Rhodes CJ (2014) Mycoremediation (bioremediation with fungi) – growing mushrooms to clean the earth. Chem Speciat Bioavailab 26:196–198

Santra SC (2010) Bioremediation: a prudent approach in environmental pollution management. Envis Centre Environ Biotechnol 17:3–14

Sarkar P, Ghosh S (2012) Bioremediation potential of a newly isolate solvent tolerant strain *Bacillus thermophilus* PS11. J BioSci Biotechnol 1:141–147

Sasikumar CS, Papinazat T (2003) Environmental management: bioremediation of polluted environment. In: Bunch MJ, Madha Suresh V, Vasantha Kumaran T (eds) Proceedings of the Third International Conference on Environment and Health, Chennai, India, 15–17 December, 2003. Chennai, Department of Geography, University of Madras and Faculty of Environmental Studies, York University, pp 465–469

Sharma S (2012) Bioremediation: features, strategies and applications. Asian J Pharm Life Sci 2:202–213

Sihag S, Sharma S, Pathak H, Dave S, Jaroli DP (2013) Biodegradation of engine oil by *Acinetobacter calcoaceticus* BD4, isolated from coastal area Mumbai. Int J Biotechnol Bioeng Res 4:235–242

Srivastava J, Naraian R, Kalra SJS, Chandra H (2014) Advances in microbial bioremediation and the factors influencing the process. Int J Environ Sci Technol 11:1787–1800

Steph JG (1988) Environmental pollution. A long-term perspective. In: Earth'88 changing geographic perspectives. Published by the National Geographic Society, pp 262–282

Tambekar DH, Rajgire AV (2015) Bioremediation of methanol by halo-alkaliphilic methylotrophs from Lonar crater. Indian J Pharm Sci Res 5:290–294

Tambekar DH, Rajgire AV, Gaikwad JN (2014) Bioremediation of C1 compounds from Methylotrophic bacteria isolated from Lonar lake. Int J Adv Pharm Biol Chem 3:612–616

Vidali M (2001) Bioremediation. An overview. Pure Appl Chem 73:1163–1172

Vijaya Kumar V (2017) Mycoremediation: a step towards cleaner environment. In: Prasad R (ed) Mycoremediation and environmental sustainability. Springer International Publishing AG, Switzerland. pp 171–187

Zhu YS, Zhang HB, Zhang YL, Huang F (2011) Lignocellulose degradation, enzyme production and protein enrichment *by Trametes versicolor* during solid-state fermentation of corn stover. Afr J Biotechnol 10:9182–9192

Chapter 8
Role of Fungi in Bioremediation and Environmental Sustainability

Ajay Tomer, Ramji Singh, Saurabh Kumar Singh, S. A. Dwivedi, Chilkuri Udaykiran Reddy, Malavika Ram Amanthra Keloth, and Riya Rachel

Contents

A. Tomer (✉) · C. U. Reddy · M. R. A. Keloth · R. Rachel
Department of Plant Pathology, School of Agriculture, Lovely Professional University, Phagwara, Punjab, India
e-mail: ajay.20547@lpu.co.n

R. Singh
Department of Plant Pathology, Sardar Vallabhbhi Patel University of Agriculture & Technology, Meerut, Uttar Pradesh, India

S. K. Singh
Department of Horticulture, Janta Vedic College, Baraut, Uttar Pradesh, India

S. A. Dwivedi
Department of Entomology, School of Agriculture, Lovely Professional University, Phagwara, Punjab, India

R. Prasad et al. (eds.), *Mycoremediation and Environmental Sustainability*, Fungal Biology, https://doi.org/10.1007/978-3-030-54422-5_8

8.1 Introduction

Bioremediation is an environmentally friendly process using many different microbes to weaken and detoxify harmful pollutants in a parallel or sequential manner. Microorganisms (e.g., fungi and bacteria), green plants, or combinations of them used together can convert toxic pollutants into carbon dioxide (CO_2) and water (H_2O), inorganic salts, microbial biomass, and other products that are less toxic—in effect, accelerating natural metabolic processes that result in these outcomes (Egamberdieva et al. 2008; Gupta and Sinha 2007; Pawar 2012; Mohammadi-Sichani et al. 2019).

In recent years, interest in exploring microbial biodegradation of toxins has been amplified by human attempts to achieve a sustainable approach to purification and restoration of polluted habitats. Cleaning up polluted soil and water by use of organisms—including fungi, bacteria, and their enzymes—is a cost-efficient, sustainable, and natural approach (in comparison with other typical techniques) (Kumar and Dwivedi 2019). In bioremedial technologies, microbes are introduced to improve decomposition or elimination of organic and inorganic pollutants and harmful contaminants. Pollutant bioremediation can be achieved by various methods such as natural attenuation, biostimulation, bioaugmentation, or combinations of these methods (Bisht et al. 2019). Because of their consistent morphology and versatile metabolic ability, fungi play crucial roles as degraders and symbionts in the environment as a whole, including soil and aquatic habitats; thus, they are particularly suitable for bioremediation. Mycoremediation is a method of bioremediation using fungi to decontaminate contaminated areas. Arbuscular mycorrhizal fungi (AMF) primarily perform their functions in soil, achieving and altering the soil microbial balance. AMF primarily enhance soil microbe growth and restrict plant pathogen proliferation. Because of their symbiotic nature, AMF depend on plant roots to provide them with the carbon and sugar they need in order to grow and proliferate. Eventually, as the plants and fungi grow together, they both benefit from their association. Mycorrhizal combinations make plants soil tolerant, enhance their growth, and keep them healthier than nonmycorrhizal plants. The main division of the book provides an overview of bioremediation and main emphasis of this book is on microbial process because the cycling of organic compounds in the environment is an important part of bioremediation (Zhang et al. 2019).

8.2 Mechanisms of Bioremediation

Bioremediation is a biological degradation mechanism using microbial capacities to minimize the concentrations and toxicity of a large variety of contaminants, whereby areas contaminated with harmful pollutants are treated with the help of microbial processes. Microorganisms interact physically and chemically with pollutants, leading to structural changes or total disintegration of those pollutants. An amalgam of electrons and electron acceptors can be used to accelerate their metabolism by

microbes. Microbes utilize organic matter from pollutants for their proper growth and development. Moreover, proliferation of other important nutrients (including nitrogen and phosphorus), as well as minor nutrients (including sulfur and trace elements) occurs (US National Research Council 2000; Qin et al. 2013; Lacerda et al. 2019; Magnin et al. 2019). Microbes also acquire energy by catalyzing energy-efficient chemical reactions that dissociate chemicals from contaminants and transmit electrons (Friesen 2013). These types of reaction are known as oxidation and reduction reactions. In natural conditions, it has often been observed that transformation of molecules and other xenobiotics accompanies their degradation (Fig. 8.1). This process includes phenomena such as co-oxidation, gratuitous metabolism, co-metabolism, and free or accidental metabolism (Tegli et al. 2014; Zengguang et al. 2015). Co-metabolism is a type of metabolism in the presence of an organically active substrate as the primary carbon and energy source, without any nutritional gains. This type of metabolism is a regular microbial activity (Pickering 2000). The metabolic enzymes secreted by bacteria break down the complex organic materials around them to make digestion easier (Segura and Ramos 2013; Kameshwar and Qin 2019). Such enzymes are usually nonspecific and can function on various types of substrate, including substrate materials that are not beneficial to the bacteria themselves (Ganley et al. 2004; Neifar et al. 2015).

Microbes utilize contaminants as sources of carbon for their growth and reproduction. In this way, they break down the contaminants and transform them into simpler compounds. From this breakdown of contaminants, they obtain energy to reproduce and give rise to new microbial cells. The microorganisms degrade chemical bonds and release electrons, which are then used in production of new microbial cells. When a chemical compound loses electrons, it becomes oxidized, and when it gains electrons, it becomes reduced. This phenomenon is known as a redox reaction,

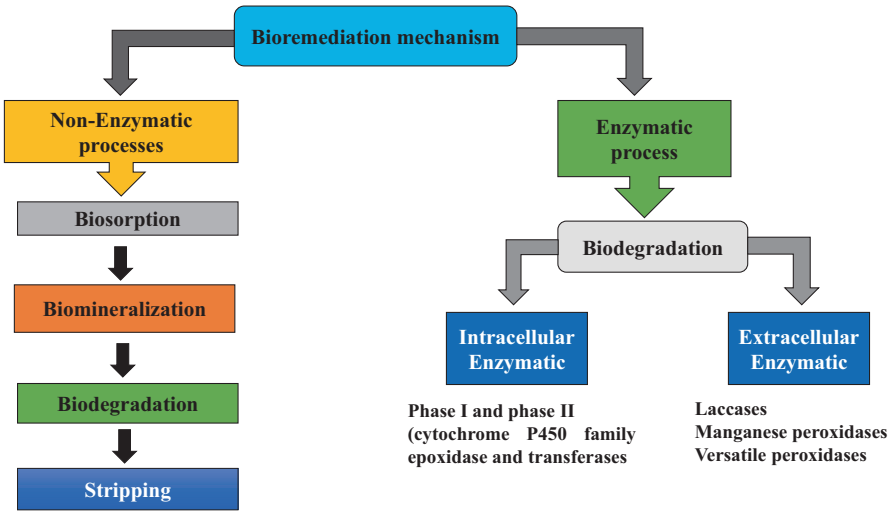


Fig. 8.1 Bioremediation mechanisms

where reduction and oxidation occur simultaneously. Most living organisms use oxygen (O_2) as an electron acceptor. Thus, we can conclude that organisms degrade organic compounds into simpler molecules such as H_2O and CO_2 in the presence of O_2; this process is known as aerobic respiration. As a result of evolution, some microorganisms do not require O_2 to break down chemical compounds (Villela et al. 2019). In their processes, contaminants are degraded by nitrate (NO_3^-) and sulfate (SO_4^{2-}), and the end products are nitrogen gas (N_2), hydrogen sulfide (H_2S), and methane (CH_4); this process is known as anerobic respiration. The energy released in this process is utilized in cell synthesis. Fermentation is a process in which reactions occur in the absence of oxygen, where microbes convert contaminants into simpler by-products such as ethanol, hydrogen, and carbon. In this reaction, the contaminants behave as electron acceptors and electron donors (Cecchi et al. 2019).

Some microorganisms convert contaminants into simpler forms that have no beneficial requirements, and this phenomenon is known as secondary utilization. One other phenomenon that occurs simultaneously is co-metabolism, in which the by-products help to detoxify the effects of the reaction. When bacteria are used in degradation of CH_4, certain enzymes are produced that degrade the chlorinated solvent, which plays no vital role in the growth of the bacteria. Here, the chlorinated solvent serves as a secondary substrate, as it has no role in the maintenance of bacterial growth. Another variation due to evolution is reductive dehalogenation, in which halogen atoms in the compound are replaced by hydrogen atoms. Here, detoxification of the halogen atom occurs, with addition of two electrons to the organic chemical compound in the presence of lactate, glucose, and acetate, acting as electron donors. In this reaction, there is no release of energy, but the reaction has a detoxifying effect, with removal of a toxic compound, and this is beneficial for production and proliferation of new cells.

Nowadays, various bioremediation methods are used to convert toxic organic materials in pesticides, industrial waste, oil spills, etc. into harmless compounds by degrading them. Their transformation into CO_2, H_2O, N_2, hydrochloric acid (HCl), etc. is known as mineralization, and this is the ultimate goal of bioremediation. Heavy metals and radioactive cations are physically removed by phytoremediation or mycoremediation through harvesting of the entire plant or fungus, although they cannot be decomposed (Ceci et al. 2019). Degrading microorganisms obtain carbon, nitrogen, or energy from pesticide molecules. Thus, the most important pesticide degradation process in soil is microbial metabolism. Fungi are known to play a vital role in leaf litter degradation; moreover, they are the only organisms on earth that decompose wood. Lignin and cellulose are essential components of plant fiber, which is decomposed into humus by extracellular enzymes and acids exuded from fungal mycelia. It is possible to increase the rate of degradation by supplying nitrogen, phosphorus, potassium, and other inorganic elements. Decomposition of starches, celluloses, hemicelluloses, other sugar polymers, and pectins is carried out by molds such as *Aspergillus* and *Botrytis*. They are also capable of degrading fats,

oils, chitin, and keratin. These molds can be used for biodegradation, in which they degrade paper and textile raw materials such as cotton, linen, and jute. Fungi such as *Mucor thermohyalospora*, *Cladosporium oxysporum*, *Phanerochaete chrysosporium*, *Trichoderma harzianum*, and *Aspergillus* spp. (e.g., *Aspergillus niger* and *Aspergillus terreus*) have the ability to degrade endosulfan, which causes problems in the environment and in living organisms. Fungi can transform pesticides into innocuous substances via certain processes such as esterification, hydroxylation, deoxygenation, and dehydrogenation. A few examples are mentioned below. 3-Phenoxybenzoic acid is hydroxylated into 3-hydroxy-5-phenoxybenzoic acid, which is further deoxygenated into gallic acid and phenol. A fungal strain of *Rhizopus oryzae* (CDBB-H-1877) can be used for biosorption of pentachlorophenol through dechlorination and methylation. *Aspergillus* and *Zygomycetes* fungi can decolorize and detoxify textile wastewater. Polychlorinated biphenyls (PCBs) are degraded by nonligninolytic enzymes produced by fungi such as *Penicillium digitatum*, *Penicillium chrysogenum*, *Fusarium solani*, and *Scedosporium apiospermum*. White rot fungi are preferred as robust and protective tools in soil bioremediation, as they can tolerate high concentrations of pollutant chemicals. Reports have shown that brown rot fungi degrade cellulose, leaving lignin undissolved as brown deposits, while white rot fungi digest lignin, leaving cellulose intact and giving a bleached appearance to wood. Some white rot fungi are also capable of degrading persistent xenobiotic compounds. They include *Pleurotus ostreatus*, *Pleurotus tuber regium*, *Pleurotus pulmonarius*, *Agaricus bisporus*, *Lentinula edodes*, *Bjerkandera adusta*, *Irpex lacteus*, and *Trametes versicolor*. In addition, these white rot fungi can degrade pesticides, phenols, chlorophenols, polychlorinated biphenyls and dioxins, heavy metals, dyestuffs, and effluent from pulp and paper mills (Singh 2006). They are also capable of degrading environmental pollutants such as CO_2, dichlorodiphenyltrichloroethane (DDT), lindane, and chlordane. This wide range of activity of white rot fungi is due to (1) production of lignin peroxidase (LiP), manganese peroxidase (MnP), lactase, and various hydrogen peroxide (H_2O_2)–producing enzymes and (2) their mycelial growth habit, which allows rapid colonization of substrates and hyphal extension, enabling penetration of soil to reach pollutants (Park et al. 2020). LiP and MnP are also produced by *Phanerochaete chrysosporium*. Studies have shown that *Lentinus subnudus* has the ability to degrade both metolachlor and heptachlor by up to 94% and atrazine by up to 78%. *Phanerochaete ostreatus* has the potential to degrade heptachlor and heptachlor epoxide by up to 89% and 32%, respectively. Aldrin and Dieldrin pesticides can be degraded by *Phlebia acanthocystis*, *Phlebia brevispora*, and *Phlebia aurea*. Degradation of effluent from textile industries has been carried out using several hyphomycetes, ascomycetes, and basidiomycetes fungi isolated from marine environments.

8.3 Bioremediation of Contaminated Land

Use of microbes for disintegration of contaminants in soil, as well as in water, can be defined as bioremediation. For efficient mycoremediation, it is important to perform screening to select suitable fungal species that can degrade the relevant contaminants. Bioremediation can be done using in situ or ex situ approaches (Akcil et al. 2015). The main difference between these two methods is that bioremediation performed on-site as classified as in situ, while bioremediation performed after physical removal of the contaminant substance from the site is classified as ex situ (Margesin et al. 2003). Ex situ treatment for cost-efficient remediation of soil pollutants requires chemicals and incineration (Rodriguez et al. 2008; Gillespie and Philp 2013; Mishra and Malik 2014). The main objective of bioremediation is to mineralize pollutants through their transformation into CO_2, H_2O, N_2, HCl, etc. It is difficult to decompose heavy metals and radioactive ions, as they are converted into less soluble forms. One example is oxidation of uranium(IV) into uranium dioxide (UO_2), a less dangerous form that can be removed physically with the help of phytoremediation or mycoremediation, which may include use of co-cultivation of fungi and plants (Richardson et al. 1992; Graham and Eissenstat 1998; McGrath and Zhao 2003; Megharaj et al. 2011; Haq et al. 2020; Thakare et al. 2021).

8.4 Bioremediation Potential of Fungi

Fungi have been shown to play significant roles in bioremediation of contaminants such as persistent organic pollutants (POPs), textile dyes, coal, chemicals used in paper production and leather tanning, pharmaceuticals and personal care products (PPCPs), polycyclic aromatic hydrocarbons (PAHs), and pesticides (Prasad 2017, 2018). Various reports have described use of fungi from different groups—including *Aspergillus*, *Penicillium*, and alkalophilic white-rod fungi—for bioremediation and decolorization of textile dyes, sugar industry effluent, chemicals used in kraft pulp mills, and leather tanning effluent, indicating the diverse substrate choices of these fungi (Redman et al. 2001; Redman et al. 2002; Rockne and Reddy 2003). Substantial removal of petrol and diesel contaminants from soil by short-term incubation of *Aspergillus niger* and *Phanerochaete chrysosporium* with petroleum hydrocarbons was shown in conjunction with total organic carbon (TOC) elimination, which helps in bioremediation (Fig. 8.2) (Timmis 2010; Redman et al. 2011; Echeveria et al. 2020).

8.5 Use of Fungal Enzymes in Bioremediation

Cellulases, xylanases, amylases, proteases, lipases, laccases, peroxidases, catalases, chitinases, etc. are fungal enzymes with industrial value and can be used in organic waste management—for example, in organic fractionation (Betancor et al. 2013;

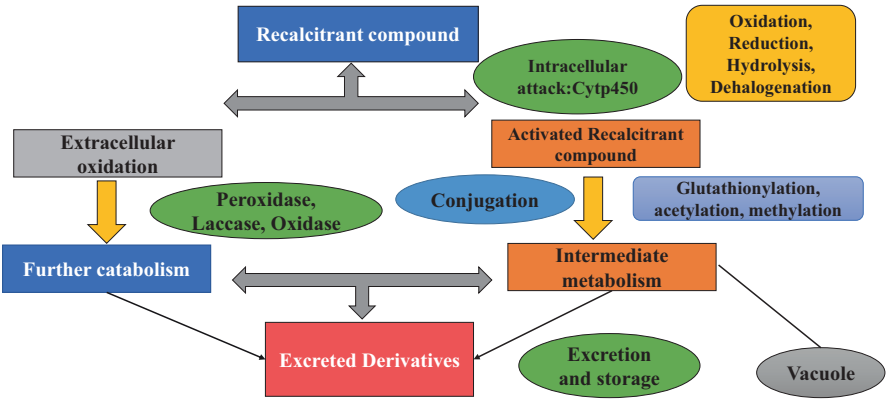


Fig. 8.2 Mechanisms adopted by fungi for bioremediation of toxic, recalcitrant compounds

Narayanan et al. 2013; Claus 2014). White rot fungi give rise to one or more types of enzyme, depending on the species and environmental conditions. Their role is not limited to degradation of natural lignocellulose substrates; they can also be used in bioremediation requiring degradation of numerous xenobiotic compounds, including dyes (Nigam 2013; Kumar et al. 2017). The ligninolytic enzymes secreted by white rot fungi are classified into two categories—MnPs and LiPs—which can be used for lignin oxidation in fungal cells. Laccases and certain fungal class II peroxidases produced by white rot basidiomycetes are well known to degrade organic pollutants (Naranjo-Briceño et al. 2013; Quintella et al. 2019).

8.6 Mycoremediation Using Fungi

The name *white rot fungi* refers to the secretion of enzymes that break down cellulose and lignin, giving the cellulose a white color. About 30% of the bioremediation linked to literature, by means of fungi (Cruz-Hernández et al. 2013). Bacteria must be adapted for synthesis of specific enzymes that can achieve degradation of the relevant pollutant(s). Various organic molecules, including untraceable and persistent components such as PAHs, are susceptible, to differing degrees, to various strains of the white rot fungi that can degrade them (Egamberdieva and Lugtenberg 2014; Sayyed et al. 2020). Soil polluted with crude oil can be mixed with a lignocellulose substrate—for example, sawdust or maize cob—allowing the fungal species to proliferate in the soil and decompose the crude oil. Moreover, white rot fungi have been shown to effectively disintegrate harmful elements such as dioxins, pesticides, phenols, chlorophenols, polychlorinated biphenyls, effluent, dyestuffs, and heavy metals.

8.7 Advanced Technologies Used in Fungal Bioremediation

In the field of fungal bioremediation, many technical advances have been made in order to overcome the associated shortcomings. These developments include use of enzymes to reduce the bioremediation time and simplify the process, with greater control over fungal biomass. Bioremediation using immobilized fungi in various bioreactors such as rotating biological contactors and fluidized bed reactors has recently been introduced (Tordoff et al. 2000; Lien et al. 2015; Roccuzzo et al. 2020). Bioremediation of benzo[a]pyrene under nutrient-enhanced conditions (involving ligninolysis) results in PAH oxidant monooxygenesis, which was also removed during a subsequent nonligninolytic process (Joutey et al. 2013; Tian et al. 2019). Bioremediation of wastewater sludge from sewage treatment plants, mixed with a filament inoculum in a broad-scale bioreactor, has been shown to be sustainable and environmentally friendly when performed using a continuous process (Connell and Staudigel 2013; Yadav et al. 2019; Singh et al. 2020). In a further innovative approach to removal of PAHs by establishing permeable new reactive biobarriers of *Trichoderma longibrachiatum* on nylon sponges, 90% removal was achieved over a period of 14 days (Tyagi et al. 2011; Li et al. 2013).

8.8 Bioremediation Using Fungal Cytochromes

Fungi have complex enzyme detoxification mechanisms in their bodies for oxidative and hydrolyte detoxification. In addition to these structures, some fungi have intracellular Genome networks consisting of cytochrome P450 monooxygenases and glutathione transferases, which enable them to cope with various different pollutants. The fungal cytochrome P450 system is a flexible catalyst for region-specific and stereospecific oxidation of nonactivated hydrocarbons. Eradication of pollutants can be achieved by use of molecular instruments to generate cytochrome P450 monooxygenases quickly and abundantly, including by use of a wide range of yeast expression systems with a viral vector (*Arxula adeninivorans*) (Tangahu 2011; Singh et al. 2013).

8.8.1 Factors Affecting Bioremediation

The availability of nutrients affects the process of microbial detoxification of contaminants. Insufficiency of nutrients can directly inhibit the proliferation and enzyme activity of pollutant-degrading organisms. For cell metabolism and efficient proliferation in contaminated environments, microbes require nutrients such as nitrogen, phosphorus, potassium, and minerals (Sagarkar 2013).

Environmental conditions such as the pH, temperature, salinity, oxygen level, and availability of water vary from site to site and can inhibit development of the contaminant-degrading microbes that are needed to drive the bioremediation process. Pathogen break down complex organic pollutant matter and grow on them and microbes can metabolize more contaminants under optimal environmental conditions (Egamberdieva and Lugtenberg 2014).

Some specific organism they interact organic pollutant and they utilize for proper growth and devlopment and decontaminats the pollutant.

The ability of the microbial community to remove pollutants from a contaminated site depends on the numbers of microbes at the site and their catabolic effectiveness. The presence of soil pathogens can be regulated by both environmental and nutritional factors.

8.9 Phytoremediation Using Arbuscular Mycorrhizal Fungi

Bioremediation is a method using microbes to treat contaminated soil. In the general phytoremediation cycle, the combination of AMFs and plants—also known as root–colonizer symbiosis—is involved in soil remediation. AMFs have been found to reduce metal toxicity to plants through a decrease in the rate of root-to-shoot translocation (Fan and Liu 2011). Phytoextraction requires plants that are capable of storing significant amounts of heavy metals and discard oraganic pollutant and remove complex substance into simple substances. Organic pollutants (such as PAHs) are transformed by the microbial activity that is commonly seen around plant roots (Gianinazzi et al. 2010). PAHs are degraded by exudates from plant roots and make detoxify pollutant substances. In a case study in which different methods of soil quality improvement were assessed, noninoculated soil and soil inoculated with a single AMF mix (indigenous AMF) were studied (Kumar et al. 2008, 2017; Sim et al. 2019). The AMF in the soil inoculated with the indigenous mix were found to be effective in soil quality improvement. The presence of AMF nodules in the soil increased plant growth, water infiltration, and soil aeration through soil agitation. Phosphorus inoculation in rhizosphere of crops by AMF (Francis and Read 1995; Tang 2019).

8.9.1 Fungi as Symbionts

In various parts of the world, systematic use of large quantities of fertilizers containing phosphorus has contributed to accumulation of phosphorus in various soil types. Plants roots can be utilize the phosphorus from soil that converted into soluble form by Arbuscular Mycorrhizal Fungi (AMF). AMF hyphae perform two main functions: (1) they serve as a system that absorbs nutrients, and (2) newly formed roots act as plows, breaking the soil hyphal network and hindering its functions (Rodriguez

et al. 2009; Mishra et al. 2020). Monoculture of a single crop dominates production of certain fungi that are capable of growing in symbiosis and leads to declines in various other AMF. Continuous monoculture of a single crop with the same AMF species results in decreased yields. There are a few crops that inhibit root colonization by AMF (Ruiz-Lozano 2003; Franken 2012), such as *Brassica* perviridis Asiatic plant cultivated for its swollen root crown and edible foliage. If *Brassica* crops are grown in the same rotation, AMF growth in the soil is suppressed. Therefore, an interspersed mixture of supplemental plants is needed to facilitate AMF growth to support AMF inoculants in cultivation of these crops. After 8 years of transition from conventional to organic farming in the farming systems trial at the Rodale Institute in the USA, it became clear that larger quantities of fungal spores were produced in organic farming than in traditional farming (Ruiz Sanchez et al. 2010; Kumar et al. 2018).

8.10 Conclusion

Bioremediation is a versatile and environmentally friendly treatment solution and a rapidly growing practice. The capacity of microbes to deal with environmental pollutants can be used to disintegrate and/or detoxify them into less harmful forms (US Environmental Protection Agency 1999). Recent research to improve our understanding of bioremediation mechanisms and genomic developments has shown that whole-genome studies can help to explain and explore bioremediation pathways. Land that is polluted or otherwise unfit for agriculture can be remediated via use of arbuscular mycorrhizal fungi (AMF) to make it suitable for agriculture. The yield and nutritional value of crops are also increased by use of AMF. Strong degraders of polycyclic aromatic hydrocarbons are found in AMF. Because of their sensitivity to a great variety of pollutants, AMF can also be used in bioassays to test soil and its toxicity levels.

References

Akcil A, Erust C, Ozdemiroglu S, Fonti V, Beolchini F (2015) A review of approaches and techniques used in aquatic contaminated sediments: metal removal and stabilization by chemical and biotechnological processes. J Clean Prod 86:24–36

Betancor L, Johnson GR, Luckarift HR (2013) Stabilized laccases as heterogeneous bioelectrocatalysts. Chem Cat Chem 5(1):46–60

Bisht J, Harsh NS, Palni LM, Agnihotri V, Kumar A (2019) Biodegradation of chlorinated organic pesticides endosulfan and chlorpyrifos in soil extract broth using fungi. Remediat J 29(3):63–77

Cecchi G, Vagge G, Cutroneo L, Greco G, Di Piazza S, Faga M, Zotti M, Capello M (2019) Fungi as potential tool for polluted port sediment remediation. Environ Sci Pollut Res 26(35):35602–35609

Ceci A, Pinzari F, Russo F, Persiani AM, Gadd GM (2019) Roles of saprotrophic fungi in biodegradation or transformation of organic and inorganic pollutants in co-contaminated sites. Appl Microbiol Biotechnol 103(1):53–68

Claus H (2014) Microbial degradation of 2,4,6-trinitrotoluene in vitro and in natural environments. In: Singh SN (ed) Biological remediation of explosive residues. Springer, Cham, pp 15–38

Connell L, Staudigel H (2013) Fungal diversity in a dark oligotrophic volcanic ecosystem (DOVE) on Mount Erebus, Antarctica. Biology 2(2):798–809

Cruz-Hernández A, Tomasini-Campocosio A, Pérez-Flores LJ, Fernández-Perrino FJ, Gutiérrez-Rojas M (2013) Inoculation of seed-borne fungus in the rhizosphere of *Festuca arundinacea* promotes hydrocarbon removal and pyrene accumulation in roots. Plant Soil 362(1–2):261–270

Echeveria L, Gilmore S, Harrison S, Heinz K, Chang A, Nunz-Conti G, Cosi F, Singh P, Bond T (2020) Versatile bio-organism detection using microspheres for future biodegradation and bioremediation studies. In: Kudryashov AV, Paxton AH, Ilchenko VS, Armani AM (eds) Laser resonators, microresonators, and beam control XXII, SPIE proceedings volume 11266. SPIE, Bellingham

Egamberdieva D, Lugtenberg B (2014) Use of plant growth–promoting rhizobacteria to alleviate salinity stress in plants. In: Miransari M (ed) Use of microbes for the alleviation of soil stresses, vol 1. Springer, New York, pp 73–96

Egamberdieva D, Kamilova F, Validov S, Gafurova L, Kucharova Z, Lugtenberg B (2008) High incidence of plant growth–stimulating bacteria associated with the rhizosphere of wheat grown on salinated soil in Uzbekistan. Environ Microbiol 10(1):1–9

Fan QJ, Liu JH (2011) Colonization with arbuscular mycorrhizal fungus affects growth, drought tolerance and expression of stress-responsive genes in *Poncirus trifoliata*. Acta Physiol Plant 33(4):1533

Francis R, Read DJ (1995) Mutualism and antagonism in the mycorrhizal symbiosis, with special reference to impacts on plant community structure. Can J Bot 73(S1):1301–1309

Franken P (2012) The plant strengthening root endophyte *Piriformospora indica*: potential application and the biology behind. Appl Microbiol Biotechnol 96(6):1455–1464

Friesen ML (2013) Microbially mediated plant functional traits. Mol Microbial Ecol Rhizosphere 1:87–102

Ganley RJ, Brunsfeld SJ, Newcombe G (2004) A community of unknown, endophytic fungi in western white pine. Proc Natl Acad Sci U S A 101(27):10107–10112

Gianinazzi S, Gollotte A, Binet MN, van Tuinen D, Redecker D, Wipf D (2010) Agroecology: the key role of arbuscular mycorrhizas in ecosystem services. Mycorrhiza 20(8):519–530

Gillespie IM, Philp JC (2013) Bioremediation, an environmental remediation technology for the bioeconomy. Trends Biotechnol 31(6):329–332

Graham JH, Eissenstat DM (1998) Field evidence for the carbon cost of citrus mycorrhizas. New Phytol 140(1):103–110

Gupta AK, Sinha S (2007) Phytoextraction capacity of the *Chenopodium album* L. grown on soil amended with tannery sludge. Bioresour Technol 98(2):442–446

Haq BU, AlFaify S, Alshahrani T, Ahmed R, Butt FK, Rehman SU, Tariq Z (2020) Devising square-and hexagonal-shaped monolayer's of ZnO for nanoscale electronic and optoelectronic applications. Solar Energy 211:920-927

Joutey NT, Bahafid W, Sayel H, El Ghachtouli N (2013) Biodegradation: involved microorganisms and genetically engineered microorganisms. In: Chamy R (ed) Biodegradation: life of science. InTechOpen, London, pp 289–320

Kameshwar AK, Qin W (2019) Systematic review of publicly available non-Dikarya fungal proteomes for understanding their plant biomass–degrading and bioremediation potentials. Bioresour Bioprocess 6(1):30

Kumar V, Dwivedi SK (2019) Hexavalent chromium reduction ability and bioremediation potential of *Aspergillus flavus* CR500 isolated from electroplating wastewater. Chemosphere 237:124567

Kumar KC, Chandrashekar KR, Lakshmipathy R (2008) Variation in arbuscular mycorrhizal fungi and phosphatase activity associated with *Sida cardifoliain* Karnataka. World J Agric Sci 4(6):770–774

Kumar M, Prasad R, Goyal P, Teotia P, Tuteja N, Varma A, Kumar V (2017) Environmental biodegradation of xenobiotics: role of potential microflora. In: Hashmi MZ, Kumar V, Varma A (eds) Xenobiotics in the soil environment: monitoring, toxicity and management. Springer, Cham, pp 319–334

Kumar V, Shahi SK, Singh S (2018) Bioremediation: an eco-sustainable approach for restoration of contaminated sites. In: Singh J, Sharma D, Kumar G, Sharma NR (eds) Microbial bioprospecting for sustainable development. Springer, Singapore, pp 115–136

Lacerda EC, Baltazar MD, dos Reis TA, do Nascimento CA, Côrrea B, Gimenes LJ (2019) Copper biosorption from an aqueous solution by the dead biomass of *Penicillium ochrochloron*. Environ Monit Assess 191(4):247. https://doi.org/10.1007/s10661-019-7399-y

Li Y, Fu K, Gao S, Wu Q, Fan L, Li Y, Chen J (2013) Increased virulence of transgenic *Trichoderma koningi* strains to the Asian corn borer larvae by over expressing heterologous chit42 gene with chitin-binding domains. J Environ Sci Health 48(5):376–383

Lien PJ, Ho HJ, Lee TH, Lai WL, Kao CM (2015) Effects of aquifer heterogeneity and geochemical variation on petroleum-hydrocarbon biodegradation at a gasoline spill site, Advanced materials research 1079. Trans Tech Publications Ltd, pp 584–588

Magnin A, Hoornaert L, Pollet E, Laurichesse S, Phalip V, Avérous L (2019) Isolation and characterization of different promising fungi for biological waste management of polyurethanes. Microb Biotechnol 12(3):544–555

Margesin R, Labbe D, Schinner F, Greer CW, Whyte LG (2003) Characterization of hydrocarbon-degrading microbial populations in contaminated and pristine alpine soils. Appl Environ Microbiol 69(6):3085–3092

McGrath SP, Zhao FJ (2003) Phytoextraction of metals and metalloids from contaminated soils. Curr Opin Biotechnol 14(3):277–282

Megharaj M, Ramakrishnan B, Venkateswarlu K, Sethunathan N, Naidu R (2011) Bioremediation approaches for organic pollutants: a critical perspective. Environ Int 37(8):1362–1375

Mishra A, Malik A (2014) Novel fungal consortium for bioremediation of metals and dyes from mixed waste stream. Bioresour Technol 171:217–226

Mishra A, Mishra SP, Arshi A, Agarwal A, Dwivedi SK (2020) Plant–microbe interactions for bioremediation and phytoremediation of environmental pollutants and agro-ecosystem development. In: Saxena G, Bharagava RN (eds) Bioremediation of industrial waste for environmental safety. Springer, Singapore, pp 415–436

Mohammadi-Sichani M, Assadi MM, Farazmand A, Kianirad M, Ahadi AM, Hadian-Ghahderijani H (2019) Ability of *Agaricus bisporus*, *Pleurotus ostreatus* and *Ganoderma lucidum* compost in biodegradation of petroleum hydrocarbon–contaminated soil. Int J Environ Sci Technol 16(5):2313–2320

Naranjo-Briceño L, Pernía B, Guerra M, Demey JR, De Sisto Á, Inojosa Y, González M, Fusella E, Freites M, Yegres F (2013) Potential role of oxidative exoenzymes of the extremophilic fungus *P. estalotiopsis* palmarum BM-04 in biotransformation of extra-heavy crude oil. Microb Biotechnol 6:720–730

Narayanan K, Chopade N, Raj PV, Subrahmanyam VM, Rao JV (2013) Fungal chitinase production and its application in biowaste management. J Sci Ind Res 72:393–399

Neifar M, Maktouf S, Ghorbel RE, Jaouani A, Cherif A (2015) Extremophiles as source of novel bioactive compounds with industrial potential. In: Gupta VK, Tuohy MG, O'Donovan A, Lohani M (eds) Biotechnology of bioactive compounds: sources and applications. Wiley, Hoboken, pp 245–268

Nigam PS (2013) Microbial enzymes with special characteristics for biotechnological applications. Biomol Ther 3(3):597–611

Park Y, Liu S, Gardner T, Johnson D, Keeler A, Ortiz N, Rabah G, Ford E (2020) Biohybrid nanofibres containing manganese oxide–forming fungi for heavy metal removal from water. J Eng Fibers Fabr. https://doi.org/10.1177/1558925019898954

Pawar RM (2012) The effect of soil pH on degradation of polycyclic aromatic hydrocarbons. Dissertation, University of Hertfordshire. https://uhra.herts.ac.uk/handle/2299/8965
Pickering IJ (2000) Reduction and coordination of arsenic in Indian mustard. Plant Physiol 122(4):1171–1178
Prasad R (ed) (2017) Mycoremediation and environmental sustainability, vol 1. Springer, Cham. https://link.springer.com/book/10.1007/978-3-319-68957-9
Prasad R (ed) (2018) Mycoremediation and environmental sustainability, vol 2. Springer, Cham. https://www.springer.com/us/book/9783319773858
Qin G, Gong D, Fan MY (2013) Bioremediation of petroleum-contaminated soil by biostimulation amended with biochar. Int Biodeterior Biodegradation 85:150–155
Quintella CM, Mata AM, Lima LC (2019) Overview of bioremediation with technology assessment and emphasis on fungal bioremediation of oil contaminated soils. J Environ Manag 241:156–166
Redman RS, Sheehan KB, Stout RG, Rodriguez RJ, Henson JM (2002) Thermotolerance generated by plant/fungal symbiosis. Science 298(5598):1581
Redman RS, Kim YO, Woodward CJ, Greer C, Espino L, Doty SL, Rodriguez RJ (2011) Increased fitness of rice plants to abiotic stress via habitat adapted symbiosis: a strategy for mitigating impacts of climate change. PLoS One 6(7):e14823
Redman RS, Dunigan DD, Rodriguez RJ (2001) Fungal symbiosis from mutualism to parasitism: who controls the outcome, host or invader? New Phytol 151(3):705–716
Richardson MD, Chapman GW, Hovel CS, Bacon CW (1992) Sugar alcohols in endophyte-infected tall fescue under drought. Crop Sci 32(4):1060–1061
Roccuzzo S, Beckerman AP, Trögl J (2020) New perspectives on the bioremediation of endocrine disrupting compounds from wastewater using algae-, bacteria- and fungi-based technologies. Int J Environ Sci Technol. https://doi.org/10.1007/s13762-020-02691-3
Rockne KJ, Reddy KR (2003) Bioremediation of contaminated sites. Presented at the International e-Conference on Modern Trends in Foundation Engineering: Geotechnical Challenges and Solutions. Indian Institute of Technology, Madras, 21–26 Jan 2003
Rodriguez RJ, Henson J, Van Volkenburgh E, Hoy M, Wright L, Beckwith F, Kim YO, Redman RS (2008) Stress tolerance in plants via habitat-adapted symbiosis. ISME J 4:404–411
Rodriguez RJ, White JF Jr, Arnold AE, Redman AR (2009) Fungal endophytes: diversity and functional roles. New Phytol 182(2):314–330
Ruiz-Lozano JM (2003) Arbuscular mycorrhizal symbiosis and alleviation of osmotic stress: new perspectives for molecular studies. Mycorrhiza 13(6):309–317
Ruiz-Sánchez M, Aroca R, Muñoz Y, Polón R, Ruiz-Lozano JM (2010) The arbuscular mycorrhizal symbiosis enhances the photosynthetic efficiency and the antioxidative response of rice plants subjected to drought stress. J Plant Physiol 167(11):862–869
Sagarkar S (2013) Monitoring bioremediation of atrazine in soil microcosms using molecular tools. Environ Pollut 172:108–115
Sayyed RZ, Bhamare HM, Sapna MN, Elgorban AM, Syed A, El-Enshasy HA, Dailin DJ (2020) Tree bark scrape fungus: a potential source of laccase for application in bioremediation of non-textile dyes. PLoS One 15(6):e0229968. https://doi.org/10.1371/journal.pone.0229968
Segura A, Ramos JL (2013) Plant–bacteria interactions in the removal of pollutants. Curr Opin Biotechnol 24(3):467–473
Sim CS, Chen SH, Ting AS (2019) Endophytes: emerging tools for the bioremediation of pollutants. In: Bharagava RN, Chowdhary P (eds) Emerging and eco-friendly approaches for waste management. Springer, Singapore, pp 189–217
Singh P, Raghukumar C, Parvatkar RR, Mascarenhas-Pereira MB (2013) Heavy metal tolerance in the psychro tolerant *Cryptococcus* sp. isolated from deep-sea sediments of the Central Indian Basin. Yeast 30(3):93–101
Singh S, Kumar V, Dhanjal DS, Datta S, Bhatia D, Dhiman J, Samuela J, Prasad R, Singh J (2020) A sustainable paradigm of sewage sludge biochar: Valorization, opportunities, challenges and future prospects. Journal of Cleaner Production https://doi.org/10.1016/j.jclepro.2020.122259

Tang KHD (2019) Phytoremediation of soil contaminated with petroleum hydrocarbons: a review of recent literature. Glob J Civil Environ Eng 1(1):33–42. https://doi.org/10.36811/gjcee.2019.110006

Tangahu BV (2011) A review on heavy metals (As, Pb, and Hg) uptake by plants through phytoremediation. Int J Chem Eng 2011:939161. https://doi.org/10.1155/2011/939161

Tegli S, Cerboneschi M, Corsi M, Bonnanni M, Bianchini R (2014) Water recycle as a must: decolorization of textile wastewaters by plant-associated fungi. J Basic Microbiol 54(2):120–132

Thakare M, Sarma H, Datar S, Roy A, Pawar P, Gupta K, Pandit S, Prasad R (2021) Understanding the holistic approach to plant-microbe remediation technologies for removing heavy metals and radionuclides from soil. Current Research in Biotechnology https://doi.org/10.1016/j.crbiot.2021.02.004

Tian D, Jiang Z, Jiang L, Su M, Feng Z, Zhang L, Wang S, Li Z, Hu S (2019) A new insight into lead(II) tolerance of environmental fungi based on a study of *Aspergillus niger* and *Penicillium oxalicum*. Environ Microbiol 21(1):471–479

Timmis KN (ed) (2010) Handbook of hydrocarbon and lipid microbiology. Springer, Berlin

Tordoff GM, Baker AJ, Willis AJ (2000) Current approaches to the revegetation and reclamation of metalliferous mine wastes. Chemosphere 41(1–2):219–228

Tyagi M, da Fonseca MM, de Carvalho CC (2011) Bioaugmentation and biostimulation strategies to improve the effectiveness of bioremediation processes. Biodegradation 22(2):231–241

US Environmental Protection Agency (1999) Use of monitored natural attenuation at superfund, RCRA corrective action, and underground storage tank sites [directive number 9200.4-17P]. Washington, DC: US Environmental Protection Agency

US National Research Council (2000) Natural attenuation for groundwater remediation. National Academies Press, Washington, DC

Villela HD, Peixoto RS, Soriano AU, Carmo FL (2019) Microbial bioremediation of oil contaminated seawater: a survey of patent deposits and the characterization of the top genera applied. Sci Total Environ 666:743–758

Yadav AN, Mishra S, Singh S, Gupta A (eds) (2019) Recent advancement in white biotechnology through fungi. Springer, Cham

Zengguang X, Yanlong L, Junrui C, Ronggao Q, Rong Y (2015) Bioremediation modelling of an aquifer contaminated by benzene using the slow-release oxygen source technique. Arab J Sci Eng 40(9):2457–2463

Zhang B, Wang S, Diao M, Fu J, Xie M, Shi J, Liu Z, Jiang Y, Cao X, Borthwick AG (2019) Microbial community responses to vanadium distributions in mining geological environments and bioremediation assessment. J Geophys Res Biogeo 124(3):601–615

Chapter 9
Sorptive and Redox Interactions of Humic Substances and Metal(loid)s in the Presence of Microorganisms

Martin Urík, Pavol Littera, Hyunjung Kim, Ingrid Hagarová, Eva Duborská, and Peter Matúš

Contents

9.1 Introduction

Humic substances are considered a chemically heterogeneous group and a major component of natural organic matter. As a part of practically all terrestrial and aquatic environments and their distinct physicochemical properties, the role of humic substances in the ecosystem is highly diversified (Evangelou and Marsi 2001; Trevisan et al. 2010). They can promote extracellular electron transfer where they can serve as both an electron donor and acceptor (Keller et al. 2009; Tian et al. 2018). They also facilitate transformation of various elements, including potentially toxic metals and metalloids, due to their heterogeneous chemical composition, surface properties and differences in physical appearances (dissolved, colloidal or solid-phase state) (Burlakovs et al. 2013); and finally, they intimately interact with

M. Urík (✉) · P. Littera · I. Hagarová · E. Duborská · P. Matúš
Institute of Laboratory Research on Geomaterials, Faculty of Natural Sciences, Comenius University in Bratislava, Bratislava, Slovakia
e-mail: martin.urik@uniba.sk

H. Kim
Department of Mineral Resources and Energy Engineering, Jeonbuk National University, Jeonju, Republic of Korea

R. Prasad et al. (eds.), *Mycoremediation and Environmental Sustainability*, Fungal Biology, https://doi.org/10.1007/978-3-030-54422-5_9

various biological and mineral surfaces, which ultimately leads to changes in activity of indigenous microbial communities, as well as bioavailability of elements (Rieuwerts et al. 1998; Pospíšilová et al. 2011). Implication of these interactions is in particular interest of geochemists and ecotoxicologists who explore mobility, availability and transformation of hazardous elements in the environment in order to predict behaviour and effects of these substances at the contaminated sites. Thus, this short review provides an insight into mutual interactions of metals and metalloids with humic substances, which are linked to the presence of microorganisms.

9.2 Redox Transformation of Metals and Metalloids by Humic Substances

Humic substances' involvement in redox transformation of metals and metalloids in near-surface environments is critical for mobility and bioavailability of various nutrients, as well as potentially toxic metals and metalloids (Singh et al. 2020). The most studied redox transformation enabled by the presence of humic substances is that of chromium(VI).

The chromium(VI) reduction by humic substances (as well as by their precursors) is favourable in acidic environments (Nakayasu et al. 1999). Although this process is not restricted to acidic conditions and reduction occurs at neutral and slightly alkaline environments as well, pH significantly affects the chromium(VI) transformation rate. Wittbrodt and Palmer (1997) reported that the apparent reduction rate coefficient decreases by three orders with increasing solution pH from value of 2 to 7. Due to decrease in number of repulsive interactions between chromium(VI) oxyanion and humic substances' surfaces at low pH, the sorption, a prerequisite for successful reduction, is enhanced. Thus, the subsequent electron transport is facilitated, and chromium(VI) reduction rate increases (Hsu et al. 2009).

Aldmour et al. (2019) suggested that the first step in chromium(VI) reduction mechanism by humic substances is controlled by chromate-ester formation, most likely between chromium(VI) and phenolic and hydroxyl moieties of humic substances. More statistically complex two-dimensional correlation spectroscopy analysis of the various spectral data reported by Zhang et al. (2017) indicated that after electrostatic attraction of chromium(VI) toward humic acids' surfaces, the complexation of chromium(VI) by carboxyl and ester groups of undissolved humic acids takes place under acidic conditions; this is followed by reduction to chromium(III) by polysaccharides and phenols. Also, thiols have been reported to contribute to the chromium(VI) reduction (Scaglia et al. 2013). Hence, the humic substances with higher densities of polar functional sites, including carboxylic and phenolic groups, have greater capacities for chromium(VI) retention and reduction (Chen et al. 2011).

Aforementioned mechanism expects the presence of adjacent electron donor group (hence, the suggested phenols or polysaccharides as reductive agents) and, thus, can be described as indirect mechanism. Another distinguishable mechanism

of chromium(VI) reduction by humic substances, recognized by Janoš et al. (2009) as "direct" reduction, is its redox transformation in the aqueous phase after interacting with electron donor functional group. This is followed by ion-exchange binding of chromium(III) with humic substances' surface groups, or the reduced chromium product remains in the aqueous phase. In case of humic acids, after reduction, the generated chromium(III) forms inner and outer sphere complexes with hydroxyl and carboxyl groups, respectively (Wu et al. 2017). Similarly, Krajnc et al. (1995) concluded that the formed chromium(III) is coordinated in fulvic acids by carboxylic groups and also hypothesized that some other functional groups are involved, most likely phenolic and alcoholic groups. However, fulvic acids form soluble high-molecular hydroxofulvate complex compounds of chromium(III) and its hydrolytic species. Thus, the presence of fulvic acids significantly increases the chromium(III) solubility, which may facilitate its migration in natural environments (Koshcheeva et al. 2007), while its sorption onto high-molecular-weight organic matter efficiently decreases chromium's environmental mobility (Kyziol et al. 2006).

Similarly to chromium(VI), copper(II) has been shown to be reducible by humic substances to copper(I) even under oxic conditions; and there, it is stabilized by the three- to fourfold coordination (Fulda et al. 2013). Under anoxic conditions, it can be reduced even further into elemental copper (Maurer et al. 2013). Exceptional reducing and stabilizing properties of humic acids have been successfully applied also for synthesis of copper nanoparticles, which have been found extremely stable and resisting oxidation for several months even after exposure to air (Wang et al. 2015). Unfortunately, while the most recent publication regarding copper immobilization provides interesting information on effects of solid mineral phases on humic acids' sorption properties (Ding et al. 2019; Menad et al. 2019), they usually omit the speciation analysis or thermodynamic calculations of copper species distribution in solid and aqueous phases. Thus, the quantification of the copper(II) reduction process is rare (Maurer et al. 2013).

Mercury(II) also undergoes both immobilization via strong complexation with humic substances, forming thermodynamically stable complexes via ionic binding (Vudamala and Chakraborty 2016), and abiotic reduction by humic substances to elemental mercury via photochemically and nonphotochemically induced reactions (Amyot et al. 1997; Jiskra et al. 2015). However, the abiotic reduction rate is possibly less significant in soils and sediments at the interfaces of solid phases compared to biotic transformation of mercury (Allard and Arsenie 1991). In the aquatic environments, the high reductive capacities of dissolved aquatic humic substances (Scott et al. 1998) play significant role in abiotic formation of elemental mercury, especially at low humic substances' concentrations (Rocha et al. 2000).

As the interaction of mercury and humic substances via complexation and reduction has competitive character, the predominance of each process significantly affects the mobility and bioavailability of mercury in both oxic and anoxic environments (Gu et al. 2011; Jiang et al. 2014). Mercury(II) abiotic reduction occurs largely by the action of reduced quinones or semiquinones, while, surprisingly, the oxidation of mercury by humic substances takes place simultaneously due to oxidative capacity of thiol moieties under anoxic conditions (Zheng et al. 2012, 2013;

Chakraborty et al. 2015). Thus, besides metal(loid)s' reduction, humic substances can also serve as an electron acceptor in redox transformations of metals and metalloids.

This dualistic character of humic substances has some severe environmental consequences. While it has been shown that the humic substances behave like an electron shuttle for arsenic(V) reduction under anoxic conditions (Qiao et al. 2019), Fakour and Lin (2014) noted that the arsenic(III) oxidation by humic acids is favourable process in oxygenated systems. The latter is advantageous, as the arsenic(V) forms more stable bounds with humic substances and mineral surfaces (Buschmann et al. 2006) and is considered less environmentally mobile, as well as less toxic compared to arsenic(III) (Hughes 2002). However, Zhai et al. (2019) indicated that humic acids are also capable of reducing arsenic(V) to arsenic(III), which is most likely enabled by quinone moieties (Palmer and von Wandruszka 2010). Furthermore, besides being redox-active agents themselves, humic acids are associated with mineral phases and microorganisms that may directly contribute to arsenic redox transformation and channel the electron transfer (Redman et al. 2002; Jiang and Kappler 2008).

9.3 Microbially Induced Oxidation and Reduction of Humic Substances

As indicated, humic acids play significant, and in some cases essential, role in redox transformation of various elements directly as an electron donor/acceptor or as an electron shuttle (Lee et al. 2019). Ratasuk and Nanny (2007) suggested three main redox-active groups in humic substances – two distinct quinone redox sites and one non-quinone redox site (e.g. thiols and disulphides). Similarly, using sophisticated electrochemical in situ FITR spectroscopic (EC-FTIRS) technique coupled with 2D-COS, Yuan et al. (2018) concluded that the quinones and phenols are the major active redox sites in terrestrial solid-phase humic substances. Aeschbacher et al. (2012) noted that the phenolic moieties serve as a major electron-donating group in humic substances. He also concluded that the electron-donating capacities of terrestrial humic substances tend to be smaller, while their electron-accepting capacitates are higher compared to aquatic natural organic matter and humic substances.

The electron-transferring capacity of humic acids can be determined chemically by using a reductive system. This experimental approach comprises the measurement of redox sites in humic acids by repeating sequential reduction and oxidation of the same sample, usually applying Pd(powder)/H_2 as a reductive system and the air for oxidation. Electrochemical approach to accurately determine redox properties of humic substances was also proposed using direct or mediated electrochemical reduction (Aeschbacher et al. 2010). Among other methods, there is also unique biological approach to determine electron-accepting capacity of humic substances. This relates to reported capability of diverse microbial groups to reduce humic

substances in various environments (Coates et al. 1998). Thus, Scott et al. (1998) successfully applied metal-reducing strain *Geobacter metallireducens* and acetone, which served as an electron donor for strain metabolism, to determine reducing capacity of various humic substances. This method was originally proposed by Lovley et al. (1996) applying poorly crystalline iron(III) as the electron acceptor. Coates et al. (1998) also highlighted that humic acids can be utilized as an exclusive terminal acceptor by bacterial species of *Geobacteriaceae* family.

The process of humic substances' reduction is not restricted to iron(III)-reducing bacteria but has been reported for various physiologically distinctive microbial groups (Martinez et al. 2013). Fermenting bacteria, including *Propionibacterium freudenreichii*, *Lactococcus lactis* and *Enterococcus cecorum*, are capable to funnel electrons from anaerobic oxidation of organic substrates to humic acids (Benz et al. 1998). Similar potential has been shown for hyperthermophilic microorganisms and methanogenic archaea (Lovley et al. 2000), as well as halo-respiring and sulphate-reducing bacteria (Cervantes et al. 2002).

The biologically induced redox transformation of humic substances, utilized by microorganism to transfer electrons to mineral surfaces (Roden et al. 2010), has severe consequences in geochemistry of various elements in subsurface environments. As indicated, the iron-reducing bacteria are capable to utilize humic substances as an electron shuttle in the process of respiration and subsequent transfer of electrons from humic substances to solubilize crystalline and low-crystalline iron(III) phases (Lovley and Phillips 1986; Nevin and Lovley 2000). Chen et al. (2003) noted that the content of polycondensed and conjugated aromatic moieties of natural organic matter is critical factor affecting iron(III) reduction in circumneutral and slightly acidic pH conditions. The presence of humic acids also significantly enhanced reduction of manganese(IV) from MnO_2 ores by dissimilatory manganese-reducing microbial consortia (Aishvarya et al. 2019). Furthermore, the coupling of redox cycles of iron and dissolved organic matter enhances chromium(VI) reduction in the presence of quinone-reducing bacteria when compared to the individual cycles (Huang et al. 2016). Arsenic(V) reduction was also promoted by the microbially reduced humic substances in arsenic-contaminated paddy soil (Qiao et al. 2019).

The reduced quinone moieties in humic substances do not serve only as an electron-donating substrate for mineral surfaces but also as a substrate for bacterial respiration. Therefore, oxidation of humic substances has been hypothesized to be coupled with significant percentage of nitrate reduction process in agricultural soils (Van Trump et al. 2011). Involvement of humic acids as an electron shuttle or donor during denitrification process was also confirmed by Dong et al. (2017). Stern et al. (2018) proposed that humic acids could donate electron for dissimilatory iron reduction. Interestingly, electron shuttling via humic substances has been reported coupling acetate oxidation by *Geobacter sulfurreducens* and nitrate reduction by *Thiobacillus denitrificans* (Zheng et al. 2019).

This highlights environmental role of redox-active humic substances as an electron transfer network connecting various microbial physiological groups and mineral and other spatial electron acceptors. Thus, the humic substances can affect extracellular electron transfer which positively correlates with physiochemical

properties of humic acids, including their electron-accepting capacity and wettability (Yuan et al. 2017).

Fungal enzymatic oxidative transformation is a redox mechanism that affects the stability of humic substances as well, usually increasing their low molecular weight fraction and hydrophilic constituents (Fedoseeva et al. 2019; Řezáčová et al. 2006). In some cases, polymerization of humic substances occurs, which mostly involves ascomycetes (Grinhut et al. 2007). Fungal oxidative biodegradation is considered a cometabolic process where decomposition of humic substances is associated with the presence of easily metabolizable carbon source (Zavarzina et al. 2004). Biodegradation and transformation involve activity of some nonspecific oxidizing enzymes, such as laccase and manganese peroxidase (Zahmatkesh et al. 2017) and small extracellular oxidants as well (Rojas-Jimenez et al. 2017). As a result, the chemical modification of humic substances' active functional groups may appear (Xiao et al. 2018). This affects their natural metal-binding capacity and, thus, the bioavailability of metals and metalloids in the contaminated substrates (Burlakovs et al. 2013).

9.4 Sorptive Interactions of Humic Substances with Metal(loid)s and Microbial Surfaces

Regardless of the particular mechanism of the redox transformation of metals and metalloids by direct or indirect action of humic substances, these processes generally result in two possibilities – release or immobilization of newly formed chemical entities of metals and metalloids. Especially in the case of solid surfaces of humic substances, the immobilization efficiency is often characterized by the sorption performance of particular humic substance (Kerndorff and Schnitzer 1980). Furthermore, humic substances also play a role as a sorbate and accumulate onto naturally occurring inorganic surfaces of amorphous and mineral phases (Weng et al. 2007; Gardošová et al. 2011), as well as materials of biological origin, including microorganisms (Campbell et al. 1997; Urík et al. 2014).

Because of their highly irregular and heterogeneous structural and elemental composition, humic substances provide various distinguishable organic fractions. For example, hydrophobic free and bound lipid classes, as well as hydrophilic constituents of proteinaceous materials and carbohydrates, can be identified in humic acids (Allard 2006). Thus, besides carboxylic, phenolic and aliphatic hydroxyl groups (Dell'Agnola and Ferrari 1971), humic substances can also provide various S- and N-functional groups, including sulfhydryl and amino groups (Vasilevich and Beznosikov 2015).

Due to prevalence of carboxylic and phenolic functional groups, it is expected that the affinity of metallic cations, such as copper(II), cadmium(II), nickel(II), mercury and lead(II), towards humic substances is high (Kerndorff and Schnitzer 1980; El-Eswed and Khalili 2006; Vetrova et al. 2014). Thus, various models suggest that

the humic substances form relative stable complexes and that the soil organic matter provides main sorption sites for metallic cations in soil surface horizons and subsoils (Weng et al. 2001; Tiberg et al. 2018). However, the precise modelling is highly affected by the soil composition and mostly influenced, besides organic matter quality (Weng et al. 2002), by the presence and abundance of metallic oxides and oxyhydroxides (Cancès et al. 2003). Therefore, usually simplified methods to evaluate the sorption behaviour of humic substances are used which omit the complex soil environment completely and focus on the sorption of desired metals and metalloids onto (immobilized) humic substances from aqueous solutions (Urík et al. 2014; Vetrova et al. 2014).

The humic substances' complex interactions with solid surfaces of silicates, mineral oxides and oxyhydroxides include sorption and aggregation via cation bridging, as well as specific ligand exchange mechanism (Parfitt et al. 1977; Chorover and Amistadi 2001; Gardošová et al. 2012). This interaction consequently affects immobilization of metals and metalloids in soils and sediments, since it changes the reactivity and availability of sorption sites and decreases the minerals' surface charge (Chorover et al. 1999).

Wang and Mulligan (2006) indicated that humic substances enhanced leachability of arsenic in soils. This was most likely due to their competitive interaction for sorption sites on the solid mineral surfaces. Besides the site competition, the electrostatic repulsive interactions between sorbed humic substances and negatively charged ions of metals and metalloids take place at the near-mineral surfaces (Weng et al. 2009). Verbeeck et al. (2019) concluded that the soil organic matter has negative effect on soil-binding capacity for antimony(V) as the preferential sorption sites located on aluminium and iron oxides and oxyhydroxides are competitively blocked by organic matter. On the contrary, Dousova et al. (2015) demonstrated that antimony(V) is effectively immobilized in organic O horizons indicating formation of complexes with the surface of co-occurred organic matter, or it is complexed in iron-organic matter aggregates. This most likely relates to different acidity of soils used in previously mentioned works as the pH controls surface charge. Thus, it also plays significant role in sorption of metals and metalloids in the soils. It has been suggested that metals bind directly to iron and manganese oxyhydroxides' surfaces in circumneutral environments, while in acidic region the binding to the functional groups of humic substances is preferential (Tessier et al. 1996).

Furthermore, the sorptive interactions may also affect redox transformation and subsequent complexation of metals and metalloids by humic substances. As no significant shifts in peaks were found in excitation/emission matrices of humic acids and fulvic acids in the presence of chromium(VI) and iron(0) fillings, Mak and Lo (2011) concluded that zero-valent iron was exclusive reducent of chromium(VI). Therefore, most likely as a result of sorptive inhibition of iron fillings' surface or its passivation via complexation, the humic acids decreased the observable chromium(VI) reduction rate constant by up to 9% (Liu et al. 2008)

Sorption of humic substances onto microbial biomasses' surfaces is most favourable in acidic solutions, where neither the humic acids nor microbial surfaces are charged (Fein et al. 1999). Tikhonov et al. (2013) suggested that size exclusion also

plays significant role in humic acids' absorption by bacteria, since outer cell wall layer of Gram-negative bacteria hinders the uptake of humic acids with molecular weight over 20 kDa, while in Gram-positive bacteria, humic acids diffuse into the peptidoglycan layer easier.

The intimate interactions between humic substances and microorganism affect the microorganism both directly and indirectly. The physiological effect of humic substances is complex. They can serve as a carbon and energy source for some microbial groups (Tikhonov et al. 2010), while it can also suppress growth of other microbial species (Loffredo and Senesi 2009). Despite their direct physiological action (Visser 1985), capability of humic substances to form stable complexes with potentially toxic metals and metalloids may mitigate the mobility and bioavailability of hazardous compounds for soil organisms (He et al. 2017) and allow them to grow in unfavourable environments (Urík et al. 2018). Therefore, the presence of humic substances seems essential with regard to efficient decrease of the reactive content of pollutants (Fonesca et al. 2013) as the microbial surfaces are generally negatively charged at pH values above 3 (Harden and Harris 1953) and, thus, easily interact with metal cations under normal environmental conditions. This was clearly demonstrated by Perelomov et al. (2018) who showed that the humic substances decreased toxicity of zinc(II) and lead(II) for bacterial *Pseudomonas chlororaphis*, *P. fluorescens* and *Rhodococcus* sp. strains. Similarly, Malcová et al. (2002) highlighted the role of fulvic acids in mitigation of manganese(II) and lead(II) toxic effects on arbuscular mycorrhizal fungus *Rhizophagus irregularis* (formerly *Glomus intraradices*) and three strains of filamentous fungi (*Fusarium solani*, *Cladosporium sphaerospermum* and *Gliocladium roseum*). Also Abdel Aziz et al. (2016) suggested that sorption of caesium(II) and lead(II) onto both *F. oxysporum* strain's and humic acids' surfaces decreased the bioavailability of these toxic metals for lettuce.

The aforementioned effect is of particular importance in the acidic environments where the biological surfaces may provide sorption sites with high affinity for toxic element, such is the case of binary mineral-microbial composites of goethite-*Burkholderia cepacia* (Templeton et al. 2003) and amorphous hydrous ferric oxide-*Shewanella alga* (Small et al. 1999). Du et al. (2016) indicated that the bacterial surfaces still provided binding sites with higher affinity for cadmium(II) even in the presence of humic acids and montmorillonite at mid-low pH. However, even the majority of copper(II) was bound to bacterial surface in goethite-humic acid-*P. putida* system, the humic acid component in this ternary aggregate was capable to block sorption sites on bacterial surfaces to some extent (Du et al. 2017).

9.5 Concluding Remarks

The complicated nature of mutual interactions of humic substances, microorganism and metal(loid)s highlights the scientific struggle to grasp the complexity and an overall picture of metals' and metalloids' behaviour in the environment. Although we have intentionally simplified these interactions to redox and/or sorptive

behaviour of these components, there is still great uncertainty in an impact of the biochemically active microorganisms on this system (e.g. via selective bioaccumulation). Nevertheless, in this review, we have highlighted that the humic substances provide active redox and sorption sites for various environmentally significant contaminants and that the microbial interaction with humic substances and mineral surfaces plays vital role in mobility and transformation of metals and metalloids. Therefore, we hope that this short review will inspire the reader to explore new ideas regarding microbial and humic acid involvement in the natural geochemical cycles, as well as their implication for the remediation of contaminated sites.

Acknowledgements This work was supported by the Scientific Grant Agency of the Slovak Republic Ministry of Education and the Slovak Academy of Sciences under VEGA contract Nos. 1/0164/17, 1/0153/17 and 1/0146/18.

References

Abdel Aziz OA, Lotfy SM, Zhran MA (2016) Effect of humic acids and fungal inoculations on cesium and lead concentrations in lettuce plants grown in contaminated soils. Remed J 26:183–194. https://doi.org/10.1002/rem.21476

Aeschbacher M, Sander M, Schwarzenbach RP (2010) Novel electrochemical approach to assess the redox properties of humic substances. Environ Sci Technol 44:87–93. https://doi.org/10.1021/es902627p

Aeschbacher M, Graf C, Schwarzenbach RP, Sander M (2012) Antioxidant properties of humic substances. Environ Sci Technol 46:4916–4925. https://doi.org/10.1021/es300039h

Aishvarya V, Barman S, Pradhan N, Ghosh MK (2019) Selective enhancement of Mn bioleaching from ferromanganese ores in presence of electron shuttles using dissimilatory Mn reducing consortia. Hydrometallurgy 186:269–274. https://doi.org/10.1016/j.hydromet.2019.04.029

Aldmour ST, Burke IT, Bray AW et al (2019) Abiotic reduction of Cr(VI) by humic acids derived from peat and lignite: kinetics and removal mechanism. Environ Sci Pollut Res 26:4717–4729. https://doi.org/10.1007/s11356-018-3902-1

Allard B (2006) A comparative study on the chemical composition of humic acids from forest soil, agricultural soil and lignite deposit: bound lipid, carbohydrate and amino acid distributions. Geoderma 130:77–96. https://doi.org/10.1016/j.geoderma.2005.01.010

Allard B, Arsenie I (1991) Abiotic reduction of mercury by humic substances in aquatic system — an important process for the mercury cycle. Water Air Soil Pollut 56:457–464. https://doi.org/10.1007/BF00342291

Amyot M, Mierle G, Lean D, Mc Queen DJ (1997) Effect of solar radiation on the formation of dissolved gaseous mercury in temperate lakes. Geochim Cosmochim Acta 61:975–987. https://doi.org/10.1016/S0016-7037(96)00390-0

Benz M, Schink B, Brune A (1998) Humic acid reduction by Propionibacterium freudenreichii and other fermenting bacteria. Appl Environ Microbiol 64:4507–4512

Burlakovs J, Kļaviņš M, Osinska L, Purmalis O (2013) The impact of humic substances as remediation agents to the speciation forms of metals in soil. APCBEE Proc 5:192–196. https://doi.org/10.1016/j.apcbee.2013.05.034

Buschmann J, Kappeler A, Lindauer U et al (2006) Arsenite and arsenate binding to dissolved humic acids: influence of pH, type of humic acid, and aluminum. Environ Sci Technol 40:6015–6020. https://doi.org/10.1021/es061057+

Campbell PGC, Twiss MR, Wilkinson KJ (1997) Accumulation of natural organic matter on the surfaces of living cells: implications for the interaction of toxic solutes with aquatic biota. Can J Fish Aquat Sci 54:2543–2554. https://doi.ogr/10.1139/f97-161

Cancès B, Ponthieu M, Castrec-Rouelle M et al (2003) Metal ions speciation in a soil and its solution: experimental data and model results. Geoderma 113:341–355. https://doi.org/10.1016/S0016-7061(02)00369-5

Cervantes FJ, de Bok FAM, Duong-Dac T et al (2002) Reduction of humic substances by halorespiring, sulphate-reducing and methanogenic microorganisms. Environ Microbiol 4:51–57. https://doi.org/10.1046/j.1462-2920.2002.00258.x

Chakraborty P, Vudamala K, Coulibaly M et al (2015) Reduction of mercury (II) by humic substances—influence of pH, salinity of aquatic system. Environ Sci Pollut Res 22:10529–10538. https://doi.or/10.1007/s11356-015-4258-4

Chen J, Gu B, Royer RA, Burgos WD (2003) The roles of natural organic matter in chemical and microbial reduction of ferric iron. Sci Total Environ 307:167–178. https://doi.org/10.1016/S0048-9697(02)00538-7

Chen SY, Huang SW, Chiang PN et al (2011) Influence of chemical compositions and molecular weights of humic acids on Cr(VI) photo-reduction. J Hazard Mater 197:337–344. https://doi.org/10.1016/j.jhazmat.2011.09.091

Chorover J, Amistadi MK (2001) Reaction of forest floor organic matter at goethite, birnessite and smectite surfaces. Geochim Cosmochim Acta 65:95–109. https://doi.org/10.1016/S0016-7037(00)00511-1

Chorover J, Amistadi MK, Burgos WD, Hatcher PG (1999) Quinoline sorption on kaolinite–humic acid complexes contribution from the Dep. of Agronomy, Pennsylvania State Univ. Soil Sci Soc Am J 63:850–857. https://doi.org/10.2136/sssaj1999.634850x

Coates JD, Ellis DJ, Blunt-Harris EL et al (1998) Recovery of humic-reducing bacteria from a diversity of environments. Appl Environ Microbiol 64:1504–1509

Dell'Agnola G, Ferrari G (1971) Molecular sizes and functional groups of humic substances extracted by 0.1M pyrophosphate from soil aggregates of different stability. J Soil Sci 22:342–349. https://doi.org/10.1111/j.1365-2389.1971.tb01622.x

Ding Y, Liu M, Peng S et al (2019) Binding characteristics of heavy metals to humic acid before and after fractionation by ferrihydrite. Chemosphere 226:140–148. https://doi.org/10.1016/j.chemosphere.2019.03.124

Dong S, Li M, Chen Y (2017) Inherent humic substance promotes microbial denitrification of landfill leachate via shifting bacterial community, improving enzyme activity and up-regulating gene. Sci Rep 7:12215. https://doi.org/10.1038/s41598-017-12565-3

Dousova B, Buzek F, Herzogova L et al (2015) Effect of organic matter on arsenic(V) and antimony(V) adsorption in soils. Eur J Soil Sci 66:74–82. https://doi.org/10.1111/ejss.12206

Du H, Chen W, Cai P et al (2016) Cd(II) Sorption on montmorillonite-humic acid-bacteria composites. Sci Rep 6:19499. https://doi.org/10.1038/srep19499

Du HH, Lin YP, Chen WL et al (2017) Copper adsorption on composites of goethite, cells of *Pseudomonas putida* and humic acid. Eur J Soil Sci 68:514–523. https://doi.org/10.1111/ejss.12430

El-Eswed B, Khalili F (2006) Adsorption of Cu(II) and Ni(II) on solid humic acid from the Azraq area, Jordan. J Colloid Interface Sci 299:497–503. https://doi.org/10.1016/j.jcis.2006.02.048

Evangelou VP, Marsi M (2001) Composition and metal ion complexation behaviour of humic fractions derived from corn tissue. Plant Soil 229:13–24. https://doi.org/10.1023/A:1004862100925

Fakour H, Lin TF (2014) Effect of humic acid on as redox transformation and kinetic adsorption onto iron oxide based adsorbent (IBA). Int J Environ Res Public Health 11:10710–10736. https://doi.org/10.3390/ijerph111010710

Fedoseeva E, Stepanov A, Yakimenko O et al (2019) Biodegradation of humic substances by microscopic filamentous fungi: chromatographic and spectroscopic proxies. J Soils Sediments 19:2676–2687. https://doi.org/10.1007/s11368-018-2209-7

Fein JB, Boily J-F, Güçlü K, Kaulbach E (1999) Experimental study of humic acid adsorption onto bacteria and Al-oxide mineral surfaces. Chem Geol 162:33–45. https://doi.org/10.1016/S0009-2541(99)00075-3

Fonesca EM, Neto JA, McAlister J et al (2013) The role of the humic substances in the fractioning of heavy metals in Rodrigo de Freitas Lagoon, Rio de Janeiro – Brazil. An Acad Bras Cienc 85:1289–1301. https://doi.org/10.1590/0001-3765201371011

Fulda B, Voegelin A, Maurer F et al (2013) Copper redox transformation and complexation by reduced and oxidized soil humic acid. 1. X-ray absorption spectroscopy study. Environ Sci Technol 47:10903–10911. https://doi.org/10.1021/es4024089

Gardošová K, Urík M, Littera P et al (2011) Sorption of humic substances onto natural zeolite and their effect on arsenic immobilization. Fresenius Environ Bull 20:2939–2942

Gardošová K, Urík M, Littera P et al (2012) The effect of pH on the sorption of humic acids on bentonite. Fresenius Environ Bull 21:2977–2980

Grinhut T, Hadar Y, Chen Y (2007) Degradation and transformation of humic substances by saprotrophic fungi: processes and mechanisms. Fungal Biol Rev 21:179–189. https://doi.org/10.1016/j.fbr.2007.09.003

Gu B, Bian Y, Miller CL et al (2011) Mercury reduction and complexation by natural organic matter in anoxic environments. Proc Natl Acad Sci 108:1479–1483. https://doi.org/10.1073/pnas.1008747108

Harden VP, Harris JO (1953) The isoelectric point of bacterial cells. J Bacteriol 65:198–202

He E, Qiu H, Qiu R et al (2017) Time-dependent uptake and toxicity of nickel to *Enchytraeus crypticus* in the presence of humic acid and fulvic acid. Environ Toxicol Chem 36:3019–3027. https://dor.irg/10.1002/etc.3870

Hsu N-H, Wang S-L, Lin Y-C et al (2009) Reduction of Cr(VI) by crop-residue-derived black carbon. Environ Sci Technol 43:8801–8806. https://doi.org/10.1021/es901872x

Huang B, Gu L, He H et al (2016) Enhanced biotic and abiotic transformation of Cr(VI) by quinone-reducing bacteria/dissolved organic matter/Fe(III) in anaerobic environment. Environ Sci: Processes Impacts 18:1185–1192. https://doi.org/10.1039/C6EM00229C

Hughes MF (2002) Arsenic toxicity and potential mechanisms of action. Toxicol Lett 133:1–16. https://doi.org/10.1016/S0378-4274(02)00084-X

Janoš P, Hůla V, Bradnová P et al (2009) Reduction and immobilization of hexavalent chromium with coal- and humate-based sorbents. Chemosphere 75:732–738. https://doi.org/10.1016/j.chemosphere.2009.01.037

Jiang J, Kappler A (2008) Kinetics of microbial and chemical reduction of humic substances: implications for electron shuttling. Environ Sci Technol 42:3563–3569. https://doi.org/10.1021/es7023803

Jiang T, Wei S-Q, Flanagan DC et al (2014) Effect of abiotic factors on the mercury reduction process by humic acids in aqueous systems. Pedosphere 24:125–136. https://doi.org/10.1016/S1002-0160(13)60087-9

Jiskra M, Wiederhold JG, Skyllberg U et al (2015) Mercury deposition and re-emission pathways in boreal forest soils investigated with Hg isotope signatures. Environ Sci Technol 49:7188–7196. https://doi.org/10.1021/acs.est.5b00742

Keller JK, Weisenhorn PB, Megonigal JP (2009) Humic acids as electron acceptors in wetland decomposition. Soil Biol Biochem 41:1518–1522. https://doi.org/10.1016/j.soilbio.2009.04.008

Kerndorff H, Schnitzer M (1980) Sorption of metals on humic acid. Geochim Cosmochim Acta 44:1701–1708. https://doi.org/10.1016/0016-7037(80)90221-5

Koshcheeva IY, Khushvakhtova SD, Levinskii VV et al (2007) Interaction of Cr(III) with the humus acids of soil, water, and bottom sediments. Geochem Int 45:178–184. https://doi.org/10.1134/S0016702907020061

Krajnc M, Štupar J, Milićev S (1995) Characterization of chromium and copper complexes with fulvic acids isolated from soils in Slovenia. Sci Total Environ 159:23–31. https://doi.org/10.1016/0048-9697(94)04316-F

Kyziol J, Twardowska I, Schmitt-Kopplin P (2006) The role of humic substances in chromium sorption onto natural organic matter (peat). Chemosphere 63:1974–1982. https://doi.org/10.1016/j.chemosphere.2005.09.042

Lee S, Roh Y, Koh D-C (2019) Oxidation and reduction of redox-sensitive elements in the presence of humic substances in subsurface environments: a review. Chemosphere 220:86–97. https://doi.org/10.1016/j.chemosphere.2018.11.143

Liu T, Tsang DCW, Lo IMC (2008) Chromium(VI) reduction kinetics by zero-valent iron in moderately hard water with humic acid: iron dissolution and humic acid adsorption. Environ Sci Technol 42:2092–2098. https://doi.org/10.1021/es072059c

Loffredo E, Senesi N (2009) In vitro and in vivo assessment of the potential of compost and its humic acid fraction to protect ornamental plants from soil-borne pathogenic fungi. Sci Hortic 122:432–439. https://doi.org/10.1016/j.scienta.2009.05.030

Lovley DR, Phillips EJP (1986) Organic matter mineralization with reduction of ferric iron on anaerobic sediments. Appl Environ Microbiol 51:683–689

Lovley DR, Coates JD, Blunt-Harris EL et al (1996) Humic substances as electron acceptors for microbial respiration. Nature 382:445–448

Lovley DR, Kashefi K, Vargas M et al (2000) Reduction of humic substances and Fe(III) by hyperthermophilic microorganisms. Chem Geol 169:289–298. https://doi.org/10.1016/S0009-2541(00)00209-6

Mak MSH, Lo IMC (2011) Influences of redox transformation, metal complexation and aggregation of fulvic acid and humic acid on Cr(VI) and As(V) removal by zero-valent iron. Chemosphere 84:234–240. https://doi.org/10.1016/j.chemosphere.2011.04.024

Malcová R, Gryndler M, Hršelová H, Vosátka M (2002) The effect of fulvic acids on the toxicity of lead and manganese to arbuscular mycorrhizal fungus *Glomus intraradices*. Folia Microbiol 47:521–526. https://doi.org/10.1007/BF02818792

Martinez CM, Alvarez LH, Celis LB, Cervantes FJ (2013) Humus-reducing microorganisms and their valuable contribution in environmental processes. Appl Microbiol Biotechnol 97:10293–10308. https://doi.org/10.1007/s00253-013-5350-7

Maurer F, Christl I, Fulda B et al (2013) Copper redox transformation and complexation by reduced and oxidized soil humic acid. 2. Potentiometric titrations and dialysis cell experiments. Environ Sci Technol 47:10912–10921. https://doi.org/10.1021/es4024095

Menad K, Feddag A, Juhna T (2019) Copper(II)–humic acid adsorption process using microporous-zeolite Na-X. J Inorg Organomet Polym Mater 29. https://doi.org/10.1007/s10904-018-0958-9

Nakayasu K, Fukushima M, Sasaki K et al (1999) Comparative studies of the reduction behavior of chromium(VI) by humic substances and their precursors. Environ Toxicol Chem 18:1085–1090. https://doi.org/10.1002/etc.5620180603

Nevin KP, Lovley DR (2000) Potential for nonenzymatic reduction of Fe(III) via electron shuttling in subsurface sediments. Environ Sci Technol 34:2472–2478. https://doi.org/10.1021/es991181b

Palmer NE, von Wandruszka R (2010) Humic acids as reducing agents: the involvement of quinoid moieties in arsenate reduction. Environ Sci Pollut Res 17:1362–1370. https://doi.org/10.1007/s11356-010-0322-2

Parfitt RL, Fraser AR, Farmer VC (1977) Adsorption on hydrous oxides. III. Fulvic acids and humic acid on goethite, gibbsite and imogolite. J Soil Sci 28:289–296. https://doi.org/10.1111/j.1365-2389.1977.tb02237.x

Perelomov LV, Sarkar B, Sizova OI et al (2018) Zinc and lead detoxifying abilities of humic substances relevant to environmental bacterial species. Ecotoxicol Environ Saf 151:178–183. https://doi.org/10.1016/j.ecoenv.2018.01.018

Pospíšilová L, Formanek P, Kucerik J et al (2011) Land use effects on carbon quality and soil biological properties in Eutric Cambisol. Acta Agric Scand Sect B 61:661–669. https://doi.org/10.1080/09064710.2010.539576

Qiao J, Li X, Li F et al (2019) Humic substances facilitate arsenic reduction and release in flooded paddy soil. Environ Sci Technol 53:5034–5042. https://doi.org/10.1021/acs.est.8b06333

Ratasuk N, Nanny MA (2007) Characterization and quantification of reversible redox sites in humic substances. Environ Sci Technol 41:7844–7850. https://doi.org/10.1021/es071389u

Redman AD, Macalady DL, Ahmann D (2002) Natural organic matter affects arsenic speciation and sorption onto hematite. Environ Sci Technol 36:2889–2896. https://doi.org/10.1021/es0112801

Řezáčová V, Hršelová H, Gryndlerová H et al (2006) Modifications of degradation-resistant soil organic matter by soil saprobic microfungi. Soil Biol Biochem 38:2292–2299. https://doi.org/10.1016/j.soilbio.2006.02.011

Rieuwerts JS, Thornton I, Farago ME, Ashmore MR (1998) Factors influencing metal bioavailability in soils: preliminary investigations for the development of a critical loads approach for metals. Chem Speciat Bioavailab 10:61–75. https://doi.org/10.3184/095422998782775835

Rocha JC, Junior ÉS, Zara LF et al (2000) Reduction of mercury(II) by tropical river humic substances (Rio Negro) — a possible process of the mercury cycle in Brazil. Talanta 53:551–559. https://doi.org/10.1016/S0039-9140(00)00532-4

Roden EE, Kappler A, Bauer I et al (2010) Extracellular electron transfer through microbial reduction of solid-phase humic substances. Nat Geosci 3:417. https://doi.org/10.1038/ngeo870

Rojas-Jimenez K, Fonvielle JA, Ma H, Grossart H-P (2017) Transformation of humic substances by the freshwater ascomycete *Cladosporium* sp. Limnol Oceanogr 62:1955–1962. https://doi.org/10.1002/lno.10545

Scaglia B, Tambone F, Adani F (2013) Cr(VI) reduction capability of humic acid extracted from the organic component of municipal solid waste. J Environ Sci 25:487–494. https://doi.org/10.1016/S1001-0742(12)60078-3

Scott DT, McKnight DM, Blunt-Harris EL et al (1998) Quinone moieties act as electron acceptors in the reduction of humic substances by humics-reducing microorganisms. Environ Sci Technol 32:2984–2989. https://doi.org/10.1021/es980272q

Singh S, Kumar V, Datta S, Dhanjal DS, Singh S, Kumar S, Kapoor D, Prasad R, Singh J (2020) Physiological responses, tolerance and remediation strategies in plants exposed to metalloids. Environmental Science and Pollution Research https://link.springer.com/article/10.1007/s11356-020-10293-2

Small TD, Warren LA, Roden EE, Ferris FG (1999) Sorption of strontium by bacteria, Fe(III) oxide, and bacteria−Fe(III) oxide composites. Environ Sci Technol 33:4465–4470. https://doi.org/10.1021/es9905694

Stern N, Mejia J, He S et al (2018) Dual role of humic substances as electron donor and shuttle for dissimilatory iron reduction. Environ Sci Technol 52:5691–5699. https://doi.org/10.1021/acs.est.7b06574

Templeton AS, Spormann AM, Brown GE (2003) Speciation of Pb(II) sorbed by Burkholderia cepacia/Goethite composites. Environ Sci Technol 37:2166–2172. https://doi.org/10.1021/es026081b

Tessier A, Fortin D, Belzile N et al (1996) Metal sorption to diagenetic iron and manganese oxyhydroxides and associated organic matter: narrowing the gap between field and laboratory measurements. Geochim Cosmochim Acta 60:387–404. https://doi.org/10.1016/0016-7037(95)00413-0

Tian W, Yang Z, Zhang X et al (2018) Redox properties of humic substances under different environmental conditions. Environ Sci Pollut Res 25:25734–25743. https://doi.org/10.1007/s11356-017-9506-3

Tiberg C, Sjöstedt C, Gustafsson JP (2018) Metal sorption to Spodosol Bs horizons: organic matter complexes predominate. Chemosphere 196:556–565. https://doi.org/10.1016/j.chemosphere.2018.01.004

Tikhonov VV, Yakushev AV, Zavgorodnyaya YA et al (2010) Effects of humic acids on the growth of bacteria. Eurasian Soil Sci 43:305–313. https://doi.org/10.1134/S1064229310030087

Tikhonov VV, Orlov DS, Lisovitskaya OV et al (2013) Sorption of humic acids by bacteria. Microbiology 82:707–712. https://doi.org/10.1134/S0026261713060106

Trevisan S, Francioso O, Quaggiotti S, Nardi S (2010) Humic substances biological activity at the plant-soil interface: from environmental aspects to molecular factors. Plant Signal Behav 5:635–643. https://doi.org/10.4161/psb.5.6.11211

Urík M, Gardošová K, Bujdoš M, Matúš P (2014) Sorption of humic acids onto fungal surfaces and its effect on heavy metal mobility. Water Air Soil Pollut 225. https://doi.org/10.1007/s11270-013-1839-z

Urík M, Bujdoš M, Gardošová K et al (2018) Selenite distribution in multicomponent system consisting of filamentous fungus, humic acids, bentonite, and ferric oxyhydroxides. Water Air Soil Pollut 229. https://doi.org/10.1007/s11270-018-3719-z

Van Trump JI, Wrighton KC, Thrash JC et al (2011) Humic acid-oxidizing, nitrate-reducing bacteria in agricultural soils. MBio 2:e00044–e00011. https://doi.org/10.1128/mBio.00044-11

Vasilevich RS, Beznosikov VA (2015) Amino acid composition of humic substances in tundra soils. Eurasian Soil Sci 48:593–599. https://doi.org/10.1134/S1064229315060125

Verbeeck M, Warrinnier R, Gustafsson JP et al (2019) Soil organic matter increases antimonate mobility in soil: an Sb(OH)6 sorption and modelling study. Appl Geochem 104:33–41. https://doi.org/10.1016/j.apgeochem.2019.03.012

Vetrova OV, Konovalov KB, Gavrilenko MA (2014) Application of humic sorbents for Pb2+, Cu2+ and Hg2+ ions preconcentration from aqueous solutions. Procedia Chem 10:120–126. https://doi.org/10.1016/j.proche.2014.10.022

Visser SA (1985) Physiological action of humic substances on microbial cells. Soil Biol Biochem 17:457–462. https://doi.org/10.1016/0038-0717(85)90009-4

Vudamala K, Chakraborty P (2016) Kinetic speciation of mercury–humate complexes in aqueous solutions by using competing ligand exchange method. Microchem J 126:551–557. https://doi.org/10.1016/j.microc.2016.01.012

Wang S, Mulligan CN (2006) Effect of natural organic matter on arsenic release from soils and sediments into groundwater. Environ Geochem Health 28:197–214. https://doi.org/10.1007/s10653-005-9032-y

Wang N, Li B, Qiao F et al (2015) Humic acid-assisted synthesis of stable copper nanoparticles as a peroxidase mimetic and their application in glucose detection. J Mater Chem B 3:7718–7723. https://doi.org/10.1039/C5TB00684H

Weng L, Temminghoff EJM, Van Riemsdijk WH (2001) Contribution of individual sorbents to the control of heavy metal activity in sandy soil. Environ Sci Technol 35:4436–4443. https://doi.org/10.1021/es010085j

Weng L, Fest EPMJ, Fillius J et al (2002) Transport of humic and fulvic acids in relation to metal mobility in a copper-contaminated acid sandy soil. Environ Sci Technol 36:1699–1704. https://doi.org/10.1021/es010283a

Weng L, Van Riemsdijk WH, Hiemstra T (2007) Adsorption of humic acids onto goethite: effects of molar mass, pH and ionic strength. J Colloid Interface Sci 314:107–118. https://doi.org/10.1016/j.jcis.2007.05.039

Weng L, Van Riemsdijk WH, Hiemstra T (2009) Effects of fulvic and humic acids on arsenate adsorption to goethite: experiments and modeling. Environ Sci Technol 43:7198–7204. https://doi.org/10.1021/es9000196

Wittbrodt PR, Palmer CD (1997) Reduction of Cr(VI) by soil humic acids. Eur J Soil Sci 48:151–162. https://doi.org/10.1111/j.1365-2389.1997.tb00194.x

Wu M, Li G, Jiang X et al (2017) Non-biological reduction of Cr(VI) by reacting with humic acids composted from cattle manure. RSC Adv 7:26903–26911. https://doi.org/10.1039/c6ra28253a

Xiao L, Li Y, Liao Y et al (2018) Bioconversion of lignite humic acid by white-rot fungi and characterization of products. 3 Biotech 8:258–258. https://doi.org/10.1007/s13205-018-1281-4

Yuan Y, Cai X, Wang Y, Zhou S (2017) Electron transfer at microbe-humic substances interfaces: electrochemical, microscopic and bacterial community characterizations. Chem Geol 456:1–9. https://doi.org/10.1016/j.chemgeo.2017.02.020

Yuan Y, Cai X, Tan B et al (2018) Molecular insights into reversible redox sites in solid-phase humic substances as examined by electrochemical in situ FTIR and two-dimensional correlation spectroscopy. Chem Geol 494:136–143. https://doi.org/10.1016/j.chemgeo.2018.07.029

Zahmatkesh M, Spanjers H, van Lier JB (2017) Fungal treatment of humic-rich industrial wastewater: application of white rot fungi in remediation of food-processing wastewater. Environ Technol 38:2752–2762. https://doi.org/10.1080/09593330.2016.1276969

Zavarzina AG, Leontievsky AA, Golovleva LA, Trofimov SY (2004) Biotransformation of soil humic acids by blue laccase of Panus tigrinus 8/18: an in vitro study. Soil Biol Biochem 36:359–369. https://doi.org/10.1016/j.soilbio.2003.10.010

Zhai H, Wang L, Hövelmann J et al (2019) Humic acids limit the precipitation of cadmium and arsenate at the brushite–fluid interface. Environ Sci Technol 53:194–202. https://doi.org/10.1021/acs.est.8b05584

Zhang J, Chen L, Yin H et al (2017) Mechanism study of humic acid functional groups for Cr(VI) retention: two-dimensional FTIR and 13C CP/MAS NMR correlation spectroscopic analysis. Environ Pollut 225:86–92. https://doi.org/10.1016/j.envpol.2017.03.047

Zheng W, Liang L, Gu B (2012) Mercury reduction and oxidation by reduced natural organic matter in anoxic environments. Environ Sci Technol 46:292–299. https://doi.org/10.1021/es203402p

Zheng W, Lin H, Mann BF et al (2013) Oxidation of dissolved elemental mercury by thiol compounds under anoxic conditions. Environ Sci Technol 47:12827–12834. https://doi.org/10.1021/es402697u

Zheng Y, Kappler A, Xiao Y et al (2019) Redox-active humics support interspecies syntrophy and shift microbial community. Sci China Technol Sci. https://doi.org/10.1007/s11431-018-9360-5

Chapter 10
Microbial Biocontrol Agents for Agricultural Soil Remediation: Prospects and Application

Yit Kheng Goh and Adeline Su Yien Ting

Contents

Y. K. Goh
School of Science, Monash University Malaysia, Bandar Sunway, Selangor Darul Ehsan, Malaysia

Advanced Agriecological Research Sdn. Bhd, Petaling Jaya, Selangor Darul Ehsan, Malaysia

A. S. Y. Ting (✉)
School of Science, Monash University Malaysia, Bandar Sunway, Selangor Darul Ehsan, Malaysia
e-mail: adeline.ting@monash.edu

R. Prasad et al. (eds.), *Mycoremediation and Environmental Sustainability*, Fungal Biology, https://doi.org/10.1007/978-3-030-54422-5_10

10.1 Introduction

Soil is a highly dynamic medium, essential in ensuring the survival and continuation of all life on Earth (Liu et al. 2010; Montanarella et al. 2016). Soils are, unfortunately, constantly exposed to soil deterioration factors such as erosion, compaction, deprivation of soil organic carbon, loss in soil biodiversity, acidification, salinization, and contamination/pollution. As a result, soils are degraded and have poor quality and functions (Lal 2015). Soil degradation is more serious in the tropical and subtropical regions, especially in developing countries (Lal 2015), where approximately 60% decrease in ecosystem functions were reported from 1950 to 2010 (Leon and Osorio 2014).

Soil pollution occurs when xenobiotic compounds or chemicals exist at levels above the ordinary threshold levels, causing detrimental effects to living organisms and reducing and degrading soil quality (Mirsal 2008; Wong 2012; Masindi and Muedi 2016; Rodríguez-Eugenio et al. 2018). Soil pollutants are normally derived from two major sources: (a) natural or geogenic, due to soil formation and nature of the soil parent materials, and (b) anthropogenic, due to human intervention or activities (Fig. 10.1) (Petruzzelli et al. 2010; Barbieri et al. 2018; Rodríguez-Eugenio et al. 2018). Of the two, anthropogenic soil contamination is of greater concern as it is accelerated by (a) industrialization, manufacturing, and energy-related processes; (b) urbanization, where over the past few decades, the urban population has surpassed rural population in year 2008 (Fig. 10.2); (c) expansion of human population with the world population projected to be over nine billion by year 2050 (Fig. 10.2); and (d) agricultural activities or intensification (Pain et al. 1991; Chaney and Oliver 1996).

Pain et al. (1991) and Chaney and Oliver (1996) have summarized a few common sources of agricultural pollutants. They include fertilizers, limestone, organic

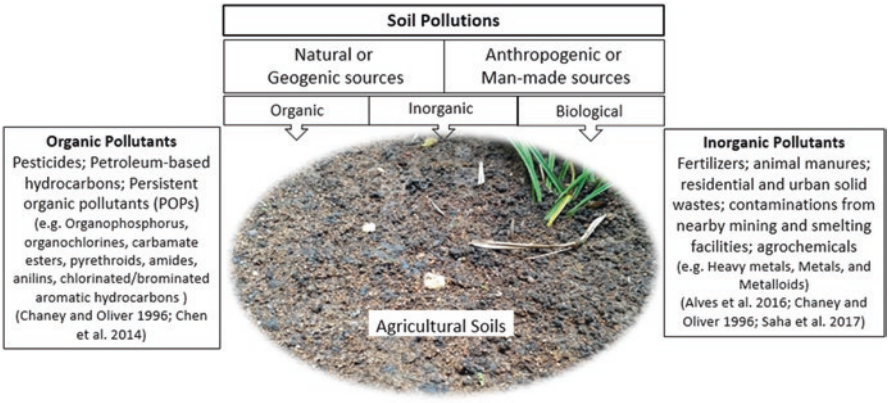


Fig. 10.1 Soil pollutions, sources, and types of common pollutants. (Sources from: Alves et al. (2016), Saha et al. (2017), Chaney and Oliver (1996), Lal (2015), Masindi and Muedi (2018), Pain et al. (1991), Rodríguez-Eugenio et al. (2018), Su et al. (2014) and Ye et al. (2017))

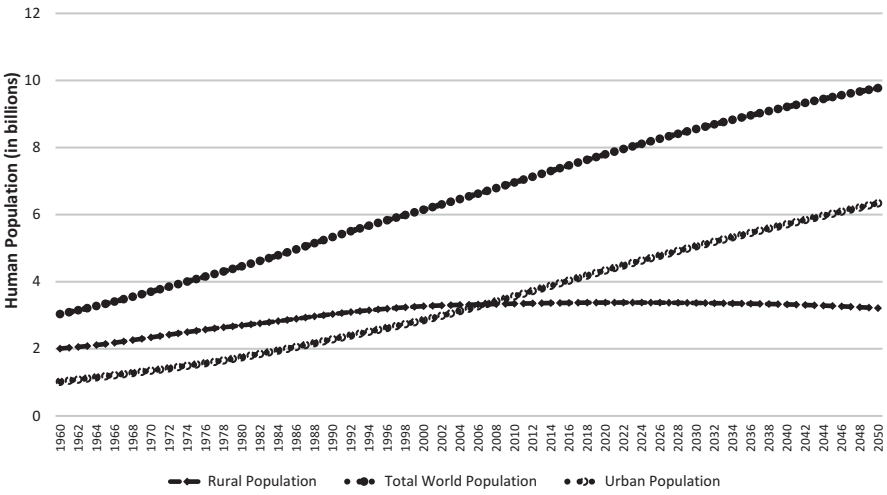


Fig. 10.2 Total estimated and projected world, rural, and urban populations. (Data adopted from FAOSTAT (2018))

contaminants, animal manures, residential or urban solid wastes, aerosol pollution from the smelting facilities, and contaminations from mining and smelting facilities through water and wind routes. These pollutants degrade agricultural soils making them less suitable for crops (Pain et al. 1991; Chaney and Oliver 1996; Abbasi et al. 2014). Soil pollution, if not manage efficiently and strategically, will be a major limiting factor in achieving high and sustainable agricultural production in the future (Lal 2015; Aragón and Rud 2016).

Polluted soils can be managed by adopting the physicochemical or the biological approach. The physicochemical soil remediation can be conducted in situ or ex situ, which includes a variety of techniques. Some common physicochemical approaches include soil removal/replacement, isolation and containment of polluted soils via capping with synthetic membranes (barrier technology) (Mulligan et al. 2001), solidification/stabilization of soil pollutants via immobilization or encapsulation with monomer (Tajudin et al. 2016), alteration of the pollutant properties through chemical reaction (Tajudin et al. 2016), vitrification through immobilization of pollutants through thermally enhanced solidification/stabilization process (Mallampati et al. 2015), and chemical oxidation or reduction activities (Su et al. 2014; Ye et al. 2017). Although most of the conventional remediation techniques are effective, they are costly, laborious, and not applicable for large-scale field conditions. Furthermore, physicochemical remediation approaches also result in the generation of hazardous waste and the loss of soil fertility (low sustainability), and the techniques are often highly complex and therefore have low acceptance from the public (Khalid et al. 2017).

As an alternative, biological remediation is explored as this approach is less destructive, environmental-friendly, safe, and economical and has high public acceptance (Khalid et al. 2017; Mishra 2017). Biological remediation is typically

via microbial remediation or phytoremediation. Various species of fungi and bacteria, as well as the phytoremediator plants, have been used for bioremediation (Madigan 2000; Selbmann et al. 2013). Over the past three decades, studies with a more holistic and versatile approach aimed at soil bioremediation have been initiated. The use of xenobiotic-tolerant microbial agents armed with growth-promoting and biocontrol traits is explored (Jacobsen 1997; Harman et al. 2004; Zafra and Cortés-Espinosa 2015; Gkorezis et al. 2016; Ting and Jioe 2016; Sim et al. 2019a).

This chapter proposes the prospect of exploring microbial biocontrol agents for the removal of pollutants (toxic metals, metalloids) from soils. Microbial biocontrol agents, especially fungi and bacteria, are often introduced into soils (specifically agricultural soils) to control the development of plant diseases or to improve plant growth (Varma et al. 2017). They are introduced into soils which may be subjected to various agrochemical pollutants. The discovery of metal-tolerant microbial species from phytoremediator plants and soil and the use of these potential biocontrol agents will be discussed. This approach promotes the search and adoption of biocontrol candidates as bioremediators to control disease and manage soil pollution at the same time. This chapter will therefore describe the microbial biocontrol agents and their potential, mechanisms, and applications for the remediation of polluted soils, particularly agricultural soils.

10.2 Soil Pollution

Soil pollution has become one of the most alarming global environmental issues, with more contaminated soils revealed in the past three decades due to industrialization, urbanization, globalization, expansion in human population, and intensification of agricultural activities (Norse and Ju 2015; Rodríguez-Eugenio et al. 2018; Yu and Wu 2018; Li et al. 2019; Behera and Prasad 2020). In 2014, China has surveyed and reported approximately 19.4% of its agricultural soils have been polluted by heavy metals, polycyclic aromatic hydrocarbons (PAHs), and dichlorodiphenyltrichloroethane (DDT) (Chen et al. 2014; Li et al. 2019). Soil pollutants are categorized into organic, inorganic, and biological types (Masindi and Muedi 2016) (Table 10.1). Organic and inorganic pollutants are the most common contaminants reported in agricultural soils.

10.2.1 Organic Pollutants in Agricultural Soils

Organic pollutants are environmental pollutants that can be oxidized, degraded, and utilized by living organisms (Masindi and Muedi 2016). They originate from industrial, manufacturing, and petrochemical industries and agricultural activities. The main constituents of the organic pollutants in agricultural soils are persistent organic pollutants (POPs), PAHs and petroleum-based pollutants, as well as various types of

Table 10.1 Types of soil pollutants, their sources/contributors, and their detrimental effects

Types	Pollutants	Sources/contributors	Negative and hazardous effects	References
Organic	Persistent organic pollutants (POP) (chlorinated and brominated aromatic hydrocarbons, organochlorine-based pesticides, and dioxins)	Industrial chemicals, industrial products, chemical manufacturing, agrochemicals, agricultural practices	Persistence in the ecosystem and long half-life, biomagnification in food chains, carcinogenic, mutagenic, teratogenic, affect health and reproduction of humans and animals	El-Shahawi et al. (2010), Jones and de Voogt (1999), Colborn et al. (1993)
	Polycyclic aromatic hydrocarbons (PAHs) and petroleum-based hydrocarbons			Cao et al. (2017), Cocârţă et al. (2017), and Marinescu et al. (2010)
	Pesticides (herbicides, insecticides, fungicides, nematicide, rodenticide, and others)		Adverse effects to non-targeted organisms and vegetation, affecting the health of human and wildlife	Wasim Aktar et al. (2009), and Colborn et al. (1993)
Inorganic	Heavy metals and metalloids	Wastes from industrial, agricultural, and domestic sectors, human activities or intervention (mining, smelting, chemical processing and manufacturing), agrochemicals, natural processes	Impairment of human and animal health, affect human and wildlife reproduction, affect the soil health and sustainability of the ecosystem, biomagnification in food chains	Colborn et al. (1993), Masindi and Muedi (2016), Rhind (2009), Salomons et al. (1995), and Wong (2012)
Biological	Microbial agents (fungi, bacteria, and viruses); macro-organisms (mites, nematodes, and cockroaches); others (pollens and animal urine and saliva)	Human activities or actions, animal faeces, domestic wastewater, livestock manure	Biohazards, pathogenic to human and wildlife, impairment of environment and ecosystem quality and services; food contamination and poisoning	Elliott (2003), Masindi and Muedi (2016), Rodríguez-Eugenio et al. (2018)

pesticides (Khanif and Salmijah 1996; Manz et al. 2001; Padilla-Sánchez et al. 2014; Sun et al. 2018). Organic pollutants are persistent, have long half-life and low degradability, and are lipophilic and hydrophobic in nature. These characteristics have enabled organic pollutants to accumulate in human and animal fatty tissues

(Rhind 2009) and biomagnify in the food chains or food webs (Kelly et al. 2007). Exposure to POPs, PAHs, and pesticides increases the risk of cancers (Dich et al. 1997), disrupts the development of respiratory and immune systems (Gascon et al. 2013), and affects reproductive systems and fertility in humans and animals (Rhind 2009; Ramesh and Archibong 2011). Organic pollutants also implicate soil microbial diversity, stability, and ecosystem service and functions (Hafez and Elbesawy 2009; Tejada et al. 2015).

10.2.2 Inorganic Pollutants in Agricultural Soils

Inorganic pollutants are typically contaminants originating from natural minerals. They are generated from agricultural activities, human interferences, and natural or geogenic activities (Masindi and Muedi 2018). The main constituents of the inorganic pollutants are impurities in phosphorus fertilizers and limestones, manures from livestock, domestic or human solid wastes, contaminations from mining or smelting facilities, and pesticides (Pain et al. 1991; Chaney and Oliver 1996; Khanif and Salmijah 1996; Kelepertzis 2014; Su et al. 2014; Alves et al. 2016; Yang et al. 2018). Metals, metalloids, and nuclides are some of the common inorganic pollutants which are non-degradable through biological or chemical means (Knox et al. 2000). Approximately ten million surveyed sites around the world revealed that they are contaminated by various pollutants, with the primary source attributed to metals and/or metalloids (He et al. 2015).

Metals, in minute amounts, are essential for various stages of plant and human development and reproduction (Emamverdian et al. 2015). These include cobalt (Co), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni), and zinc (Zn). In high concentrations though, these metals can cause health risks and toxic effects to living organisms (Alves et al. 2016). On the other hand, heavy metals, namely, arsenic (As), lead (Pb), cadmium (Cd), chromium (Cr), and mercury (Hg), are the non-essential metals with high atomic number and are highly hazardous. These metals can cause toxicity and detrimental effects even at low concentrations (Peralta-Videa et al. 2009). Due to the non-biodegradable, hazardous, and persistent nature of the pollutants, metals (either singly or in combinations) are highly toxic to humans and animals when ingested, consumed, or inhaled (Colborn et al. 1993; Rhind 2009; Ali and Khan 2018; Masindi and Muedi 2018) (Table 10.1). Metals, particularly heavy metals, cause denaturation of protein synthesis, inhibition of cell division and enzymatic activity, and disruption to DNA transcription and cell membrane formation, which are all severe implications to biological organisms (Khan et al. 2009; Rhind 2009).

Coexistence of two or more inorganic pollutants (any combination of organic and inorganic contaminants) will aggravate the negative effects, even when concentrations are much lower compared to when the pollutant is present on its own (Rhind

Table 10.2 The standard regulatory or intervention thresholds of a few essential metals and non-essential hazardous heavy metals in agricultural soils

	Metal/heavy metal concentration (mg/kg)									
	Non-essential hazardous heavy metals					Essential metals				
Country	As	Cr	Hg	Pb	Cd	Ni	Cu	Zn	Co	References
Europe (EU) (2002)	–	150	–	300	3	75	140	300	–	European Union (2002)
Netherlands (1995)	55	380	10	530	12	210	190	720	–	Van den Berg (1995)
China (2007)	25–30	200–300	0.5	80	0.3	50	100–200	250	–	EPMC (2015)
Taiwan (2000)	60	250	5	500	5	200	200	600	–	TEPA (2000)
India (1998)				250–500	3–6	75–150	135–270	300–600		Awasthi (1998)
FAO/WHO (2001)	20	100	–	100	3	50	100	300	50	FAO/WHO (2001) Chiroma et al. (2014)
Nigeria (2002)	200	20	85	35	100	140	0.3	–	–	DPR-EGASPIN (2003)

2009). Standard regulatory thresholds for a few major heavy metal and metal pollutants have been established and used in environmental assessments, monitoring, and management of metal pollutants in agricultural sectors (Table 10.2) (Chen et al. 2007; He et al. 2015). Apart from the regulated metal limits for agricultural soils, other information related to various characteristics and properties of metals, and polluted sites, type of other pollutants (single metal or multiple or combination of both inorganic and organic sources), distribution of the pollution, soil physicochemical and biological features, and hydrogeological details, are also important for soil remediation (Das et al. 2012; Su et al. 2014).

10.3 Soil Remediation

Soil remediation approaches can be categorized into two broad groups; the physicochemical and biological remediation techniques (Table 10.3). Traditional and conventional physicochemical remediation techniques have been demonstrated to be highly effective for small- to medium-scaled areas. However, physicochemical approaches are less economical and cause more disturbance to the targeted sites. Furthermore, these technologies also interfere with existing soil biological and chemical quality and properties and are less practical for vast areas of affected

Table 10.3 Physiochemical and biological remediation techniques or technologies for remediating soil pollution and their respective advantages and disadvantages

Physiochemical		Biological	
In situ	Ex situ	In situ	Ex situ
Containment and barrier	Landfilling	Phytoremediation	Landfarming
Encapsulation	Soil washing	Microbial remediation	Biopile
Solidification/stabilization	Physical separation	Phytobial remediation	Windrow
Electrokinetic/vitrification	Pyrometallurgical	Bioventing	Bioreactor
Soil flushing	Oxidation and reduction	Biosparging	
Nanomaterials		Composting	
(a) Required shorter treatment time	*Advantages*	(a) Generally high to very high public acceptability	
(b) Highly effective for multiple metals pollution		(b) Medium- to large-scale	
(c) Highly effective for high to very high level of pollutants		(c) Economic and environmental-friendly	
		(d) Less laborious and user-friendly	
		(e) Low energy input (energy saving)	
		(f) Less disruptive to the soil properties and fertility	
(a) Generally low to very low public acceptability	*Disadvantages*	(a) Required longer treatment time	
(b) Small- to medium-scale (c) Expensive equipment and facilities required		(b) Low to moderately effective for multiple metals pollution (c) Constant monitoring of the sites required	
(d) Costly and laborious		(d) Dependent on the growth condition of bioremediators	
(e) Destructive to the soil functions and fertility		(e) Dependent on the metal-tolerant capacity of bioremediators	
(f) May release some other chemical compounds		(f) Dependent on the metal solubility in soil	
(g) Some required high input of energy		(g) Effectiveness restricted to low to medium level of pollutants	
		(h) Plants can be vulnerable to diseases	
		(i) Suitability and efficacy will be affected by soils and climate	

Sources: Azubuike et al. (2016), Jin et al. (2018), Khalid et al. (2017), Sabir et al. (2015), Su et al. (2014), Masindi and Muedi (2018), Mulligan et al. (2001) and Ye et al. (2017)

agricultural soils (Table 10.3) (Lynch and Moffat 2005; Lim et al. 2016; Khalid et al. 2017; Sun et al. 2018). Therefore, there is a need to explore, assess, and adopt soil remediation methods that are more environmental- and user-friendly, sustainable, less destructive to the soil functions and properties, and applicable for large-scale application (Lynch and Moffat 2005; Sun et al. 2018; Behera and Prasad 2020). This approach is known as bioremediation.

10.4 Bioremediation

Biological remediation refers to the exploitation of living organisms for metabolizing, removing, degrading, transforming, and attenuating the pollutants through catabolic, metabolic, and biological activities (BBSRC 1999; Lynch and Moffat 2005; Griffin 2014). Transformation, immobilization, attenuation, and detoxification of toxic metal(loid)s by bioremediators (plants and microorganisms) are achieved through a few common biological mechanisms. These mechanisms include reduction-oxidation processes, methylation/chelation, biosorption, and bioaccumulation (Dixit et al. 2015; Ye et al. 2017; Prasad and Aranda 2018; Thakare et al. 2021). Bioremediation can encompass phytoremediation, microbial remediation, and macro-organism or animal remediation, depending on the type of organisms used (Su et al. 2014; Song et al. 2017; Prasad 2017, 2018). Of these, phytoremediation and microbial remediation are the two bioremediation techniques that are more commonly explored and adopted.

10.4.1 Phytoremediation

Phytoremediation was first adopted in 1983 for the decontamination of metal(loid)s using plants. These methods comprise phytoextraction or phytoaccumulation, phytovolatilization or phytoevaporation, phytostabilization or phytodeposition, and phytofiltration (Sabir et al. 2015; Khalid et al. 2017; Mishra 2017; Ye et al. 2017). Phytoextraction or phytoaccumulation refers to the removal of metals from the soils by plants through roots, in which the metal(loid)s will then be translocated to and accumulated in the aboveground plant parts. Phytovolatilization or phytoevaporation on the other hand is specific to only a few heavy metals, namely, Hg, Se, and As. These heavy metals can be biotransformed into volatile compounds via microbial regulated activity, and the transformed compounds are gradually removed via plant transpiration. Phytostabilization or phytodeposition refers to removal of metals via immobilization in which movement of metals to other sites is minimized. The last approach, phytofiltration, is used to extract metal pollutants from the aqueous environments and mostly involves aquatic plants. To date, there are over 500 vascular plant species (terrestrial or wetland plant species) from approximately 50 families that are established with hyperaccumulation capability and studied for phytoremediation of heavy metals in soils (Li et al. 2011; van der Ent et al. 2012; Gall and Rajakaruna 2013; Su et al. 2014; Neilson and Rajakaruna 2015; Sarma et al. 2021).

10.4.2 Microbial Bioremediation

Microbial bioremediation is the use of microbial agents in removing pollutants from the polluted environment (Garbisu and Alkorta 1997). Microorganisms, through a series of metabolic and enzymatic processes, are able to transform metal(loid)s from the existing oxidative state to other chemical forms with reduced toxicity/reactivity. The transformed forms are then either highly soluble in water and have low toxicity or have low bioavailability as they are less water-soluble (Garbisu and Alkorta 1997; Gadd 2010; Coelho et al. 2015). Common microbial remediation approaches adopted for remediating soil metal(loid)s pollution are bioaugmentation, biostimulation, and bioattenuation (Mishra 2017; Ye et al. 2017; Emenika et al. 2018). Bioaugmentation refers to the introduction of indigenous or foreign metal-tolerant microbes, either from a specific group or a consortium of microbial agents, to transform or remove the metal(loid)s. Biostimulation, on the other hand, involves alteration of the existing environment through supplementation of essential nutrients, growth hormones or promoters, and/or optimum growth conditions to promote proliferation of the indigenous metal-tolerant microbes for remediating the contaminated soils. The last approach, bioattenuation, encompasses utilization of naturally occurring microbes to reduce or transform metals into non-toxic or less toxic forms, and the process is carried out without human intervention. Various metal-tolerant bacterial, fungal, and algal agents have been studied for soil microbial bioremediation. A brief summary on the microbes explored for metal(loid)s remediation has been outlined in Table 10.4 and elaborated in Sect. 10.5.

10.4.3 Integrated Bioremediation Approach

Microbial-mediated or microbial-assisted phytoremediation (also known as phytobial remediation) (Harman et al. 2004) has gained attention in the recent decades. Phytobial remediation is established through introduction of beneficial symbiotic microbes or endophytic microbes into the root system or other plant tissues of the phytoremediators to improve plant growth, metal tolerance, and remediation efficacy (Lynch and Moffat 2005; Abhilash et al. 2012; Phieler et al. 2013; Waigi et al. 2017). Plant growth-promoting bacteria (PGPB), endophytic microorganisms (bacterial or fungal endophytes), and mycorrhizal strains are among the common microbial strains studied for integrated bioremediation approaches (Phieler et al. 2013). This approach has yielded successful outcomes. For example, the introduction of the metal-tolerant rhizospheric *Trichoderma virens* PDR-28 improved accumulation of metals in maize (*Zea mays*) roots and shoots and reduced metal residues in the soils (Babu et al. 2014). This *Trichoderma* strain was incorporated into the phytostabilization technique as well, to aid in removing and immobilizing metals in soils. The use of *T. virens* PDR-28 also improved the production of dry root and shoot biomass in maize. In another study, Mao et al. (2016) integrated the

Table 10.4 Bacterial and fungal bioremediators for microbial remediation of xenobiotic contaminants

Type of microbe	Genus/species	Origin	Target pollutant	Application	Tolerance concentration	Reference
Bacteria	*Bacillus subtilis* *Bacillus cereus*	Industrial dumpsite and agricultural soils (Mauritius)	Hg^{2+}, Pb^{2+}, Ag^{+}, Zn^{2+}, Cu^{2+}	Remediation of heavy metal-polluted agricultural soils (bioaugmentation)	Hg^{2+} (0.5–1 mM) Cu^{2+} (0.5–3 mM) Pb^{2+} (3–5 mM) Ag^{+} (1–5 mM) Zn^{2+} (1–5 mM)	Hookoom and Puchooa (2013)
	Pseudomonas spp.	Agricultural soils (India)	Zn^{2+}, Co^{2+}, Hg^{2+}, Cd^{2+}, Ni^{2+}, Pb^{2+}, Cr^{3+}	Remediation of heavy metal-polluted agricultural soils (bioaccumulation of Cu and Zn)	Zn^{2+} (800–1600 ug/mL) Co^{2+} (1600 ug/mL) Hg^{2+} (50 ug/mL) Cd^{2+} (100–200 ug/mL) Ni^{2+} (1600 ug/mL) Pb^{2+} (2400 ug/mL) Cr^{3+} (2400 ug/mL) Cr^{6+} (400–800 ug/mL)	Ahemad and Malik (2012)
	Pseudomonas sp. *Acinetobacter* sp. *Klebsiella* sp. *Comamonas* sp.	Agricultural soils (Taiwan)	As^{3+}, As^{5+}	Soil bioremediation, plant growth promotion, and nutrient solubilization	As^{3+} (10–30 mM) As^{5+} (150–320 mM)	Das et al. (2014)
	Azotobacter chroococcum	Agricultural soils (Cairo)	Hg^{2+}, Cd^{2+}, Cu^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Pb^{2+}, Cr^{3+}, 2,4-D	Agricultural soil heavy metal and 2,4-D (herbicide) remediation	Hg^{2+} (0.4 mM) Cd^{2+} (2.2 mM) Cu^{2+} (4.7 mM) Co^{2+} (8.1 mM) Ni^{2+} (6.2 mM) Zn^{2+} (5.9 mM) Pb^{2+} (3.7 mM) Cr^{3+} (5.1 mM)	Abo-Amer et al. (2014)
	Cupriavidus taiwanensis *Pseudomonas aeruginosa*	Paddy soils (Thailand)	Cd^{2+}	Reduced uptake of Cd by rice, potential Cd bioremediation	Cd^{2+} (2500 uM)	Siripornadulsil and Siripornadulsil (2013)
	Kluyvera ascorbata	Polluted wetland soils (Canada)	Ni^{2+}, Pb^{2+}, Zn^{2+}	Reduction of metal toxicity in plants, plant growth promotion, metal tolerance in plant	–	Burd et al. (2000)
	Bipolaris sp., *Diaporthe miriciae, Phomopsis asparagi, Saccharicola bicolor*	*Phragmites* sp. (phytoremediator) (Malaysia)	Cd^{2+}, Cu^{2+}, Zn^{2+}, Pb^{2+}, Al^{3+}	Microbial phytoremediation, biocontrol	–	Sim et al. (2018, 2019b)

(continued)

Table 10.4 (continued)

Type of microbe	Genus/species	Origin	Target pollutant	Application	Tolerance concentration	Reference
Fungi	*Trichoderma* sp., *Neocosmospora* sp., *Rhizopus* sp.	Agricultural soils (India)	As	Biovolatilization	Arsenate (5000 mg/L)	Srivastava et al. (2011)
	Trichoderma virens	Rhizospheric soil (Korea)	Cu^{2+}, Cd^{2+}, Pb^{2+}, Zn^{2+}	Microbial phytoremediation, plant growth promotion	–	Babu et al. (2014)
	Aspergillus sp. *Rhizopus* sp.	Agricultural soils (India)	Cd^{2+}, Cr^{3+}, Cu^{2+}, Co^{2+}, Ni^{2+}	Remediation of heavy metal-polluted agricultural soils (biosorption of Cr and Cd)	Cd^{2+} (1000–2000 ug/mL) Cr^{2+} (200–400 ug/mL) Cu^{2+} (100–600 ug/mL) Co^{2+} (100–400 ug/mL) Ni^{2+} (400 ug/mL)	Ahmad et al. (2005)
	Fusarium sp. *Mucor* sp. *Aspergillus niger*	Agricultural soils (Pakistan)	Zn^{2+}, Ni^{2+}	Remediation of heavy metal-polluted agricultural soils (Zn and Ni)	20 mM $ZnCl_2$ and $NiCl_2$	Iram et al. (2009)
	Aspergillus oryzae, Fusarium sp., *Aspergillus nidulans, Rhizomucor variabilis, Emericella* sp.	Agricultural soils (India)	As	Bioaccumulation and biovolatilization and plant growth promotion	Arsenate (10,000 mg/L)	Singh et al. (2015a, b)
	Oudemansiella radicata	Mushroom farming (China)	Cd^{2+}, pyrene	Bioaccumulation of Cd was enhanced with pyrene, remediation of organic and inorganic pollutants	–	Chen et al. (2015)
	Aspergillus flavus, Aspergillus niger, Fusarium sp.	Agricultural soils (Pakistan)	Cr^{3+}, Pb^{2+}	Remediation of Cr- and Pb-polluted soils	Cr^{3+} (1000 mg/L) Pb^{2+} (800–1000 mg/L)	Iram et al. (2012)
	Scutellospora heterogama, Gigaspora gigantea	Polluted soils (India)	Cu^{2+}, Zn^{2+}, Pb^{2+}, Ni^{2+}, Cd^{2+}	Remediation of heavy metal-contaminated soils, enzymatic degradation	–	Sambandan et al. (1992)
Fungi and bacteria	*Glomus* sp. *Acaulospora* sp. *Scutellospora* sp. *Streptomyces* sp. *Azotobacter* sp. *Pseudomonas* sp. *Paenibacillus* sp.	Soil (India)	Fe^{3+}	Remediation of iron-contaminated soils with phytoremediation assisted by arbuscular mycorrhizal and PGPR	–	Mishra et al. (2016)

Abbreviations for the metals or heavy metals or pesticides: mercury (Hg), lead (Pb), silver (Ag), zinc (Zn), copper (Cu), nickel (Ni), cobalt (Co), arsenic (As), iron (Fe), 2,4-dichlorophenoxyacetic acid (2,4-D)

electrokinetic field remediation approach with phytoremediation and observed an increase in the translocation of metals from roots to shoots of mustard (*Brassica juncea*) and spinach (*Spinacia oleracea*). The combination of electrokinetic field remediation and phytoremediation improved the bioaccumulation of Pb, As, and cesium (Cs). This study has illustrated the enhanced metal remediation by incorporation of physical and biological remediation techniques.

10.5 Microbes for Bioremediation

Various microorganisms have been studied for applications in bioremediation. Bacterial and fungal agents are the most commonly studied for metal bioremediation with more than 200 publications annually in the past one decade. On the other hand, algae and yeast are less common.

10.5.1 Bacteria

Bacteria, being one of the most dominant inhabitants in the soils, are ubiquitous in the environment and very versatile, capable of thriving in diverse and extreme environments (Rampelotto 2013). To survive in environments polluted by metal(loid)s, bacteria evolve and adapt to polluted environments. Their mechanisms of adaptation may include various passive intra- and extracellular metal-sequestrating mechanisms and energy-driven metal efflux transporters or pumps to modulate the toxic metal ions (Nies 1999; Ma et al. 2009; Choudhary et al. 2017). Three commonly known metal(loid)s transportation systems are CBA (capsule biogenesis/assembly family); P-type ATPases; and cation diffusion facilitator (CDF) (or chemiosmotic ion-proton exchangers). CBA is a transmembrane pump with three major components: resistance-nodulation-division (RND) protein, membrane fusion protein (MFP), and outer membrane factor (OMF), used to sequestrate metals off the outer cell membrane from the cytoplasm or periplasm (Franke et al. 2003). P-type ATPases utilize ATP energy to transport metal ions out from cytoplasm into periplasm (Rensing et al. 1999). CDF, on the other hand, is employed to pump metal ions from cytoplasm into the periplasm (Nies 1999). Bacteria also produce siderophores, iron-chelating compounds, and specific metal-binding proteins (e.g. metallothioneins) to immobilize metal(loid)s, facilitate ion complexation, and maintain homeostasis of metal ions (Cobbett and Goldsbrough 2002; Blindauer 2011; Schalk et al. 2011; Saha et al. 2013).

Metal-tolerant bacterial strains, from genera *Bacillus*, *Azotobacter*, *Pseudomonas*, *Rhizobium*, *Streptomyces*, *Amycolatopsis*, *Acinetobacter*, *Klebsiella*, *Comamonas*, *Cupriavidus*, and *Kluyvera*, have been isolated from various polluted ecosystems (Table 10.4). These microbes showed promising potential for bioremediation through bioaugmentation, bioaccumulation, phytoremediation, and

phytovolatilization (Burd et al. 2000; Tunali et al. 2006; Nanda and Abraham 2011; Ahemad and Malik 2012; Hookoom and Puchooa 2013; Siripornadulsil and Siripornadulsil 2013; Abo-Amer et al. 2014; Das et al. 2014; Choudhary et al. 2017; Emenike et al. 2018).

10.5.2 Fungi

Biosorption (passive) and bioaccumulation (active) are the common mechanisms adopted by fungi to remove, detoxify, or transform metal(loid)s in the polluted environments (Singh et al. 2018). Fungal cell walls have multiple functional groups, namely, sulfhydryl, carboxyl, hydroxyl, amine, and phosphate, to facilitate the adsorption and complexation of metal(loids) (Gupta et al. 2000). Fungal cell walls also consist of carbohydrates (e.g. polysaccharide and glycoproteins) (Ahluwalia and Goyal 2007) and other components (e.g. chitin and chitosan) (Das et al. 2008), which are important in chelating and sequestrating metal(loids) (Gadd 1990) and in metal binding (Abbas et al. 2014). The biosorption process is generally more rapid compared to the energy-driven bioaccumulation process. Furthermore, biosorption is not affected by metal toxicity and is not metabolic-dependent (Gadd 1990; Abbas et al. 2014). On the other hand, bioaccumulation mechanisms, which require living cells, utilize metal transportation services to modulate ion metals (Abbas et al. 2014). Ascomycetous and basidiomycetous fungi utilize intra- and extracellular enzymes, namely, laccases, tyrosinases, and peroxidases, to metabolize metals (Baldrian 2006; Halaouli et al. 2006; Hofrichter et al. 2010). In addition, fungi also produce organic acids (e.g. oxalic and citric acids) to assist in the immobilization of metal ions (via complexation and formation of insoluble metal oxalates) for detoxification (Gadd 1999; Franceschi and Nakata 2005).

A wide array of fungal species (i.e. *Bipolaris*, *Diaporthe*, *Phomopsis*, *Saccharicola*, *Trichoderma*, *Rhizopus*, *Aspergillus*, *Mucor*, *Rhizomucor*) and mycorrhizae have been isolated from metal-polluted agricultural soils and the phytoremediator plant (*Phragmites* sp.). These isolates have demonstrated potential as bioremediators for remediating metal-contaminated agricultural soils (Sambandan et al. 1992; Ahmad et al. 2005; Iram et al. 2009; Srivastava et al. 2011; Iram et al. 2012; Babu et al. 2014; Chen et al. 2015; Singh et al. 2015a; Sim et al. 2018, 2019b) (Table 10.4). Some of these metal-tolerant fungal strains can be integrated into phytoremediation system for agricultural soils polluted with metal pollutants.

10.5.3 Consortium of Microbial Agents

Both bacteria and fungi can be applied together as a mixed consortium to enhance removal of metal(loid)s. The consortium or combination of microbial agents, either bacterial-bacterial, fungal-fungal, or bacterial-fungal mixtures, is capable of

minimizing toxic effects towards host plant while improving plant growth. Mishra et al. (2016) reported that the combination of arbuscular mycorrhizal fungi (AMF) and plant growth-promoting rhizobacteria (PGPR), such as the use of *Glomus*, *Acaulospora*, and *Scutellospora*, and a different combination of *Streptomyces*, *Azotobacter*, *Pseudomonas*, and *Paenibacillus* were able to improve iron absorption and phytoremediation, respectively. This is when compared with the use of single AMF or PGPR. Sim and Ting (2017) elucidated the combination of bacterium *Stenotrophomonas maltophilia* and fungus *Saccharicola bicolor* from the phytoremediator plant *Phragmites* sp. and observed improved metal biosorption for metals from single- and multi-metal ions (Pb, Cu, Zn, and Cd), compared to when *S. bicolor* was applied singly.

10.6 Metal-Tolerant Biological Control Agents (BCAs) for Bioremediation

Microbial agents with tolerance to pollutants and also with disease suppression trait make ideal candidates for agricultural and bioremediation uses. The discovery of metal-tolerant endophytic fungi from phytoremediator plant and other potential metal-tolerant bacterial and fungal species from metal-laden environments or media with disease suppression trait has showed the possibility of using biocontrol agents for bioremediation (Table 10.5). Biological control (or biocontrol) is defined as the use or introduction of desirable living organisms to suppress the growth and activity of the undesirable organism (e.g. weeds, pests, plant pathogens) (Gnanamanickam et al. 2002; Pal and McSpadden Gardener 2006). Common biological control mechanisms employed are direct interactions via mycoparasitism and predation, antimicrobial mechanisms (antibiotic compounds, degrading enzymes), and indirect interactions via competition and induction of host defensive mechanisms (Whipps 2001; Pal and McSpadden Gardener 2006; Cortes-Penagos et al. 2007).

Rhizospheric soils and various metal-polluted samples or substrates have been explored extensively to bio-prospect for potential BCAs to be used in bioremediation (Table 10.5). Sayyed and Patel (2011) isolated two Ni- and Mn-resistant bacterial strains with biocontrol trait, namely, *Alcaligenes* sp. and *Pseudomonas aeruginosa*, from Indian soils. *Alcaligenes* sp. and *P. aeruginosa* strains with antimicrobial and siderophoregenic activities inhibited the growth of pathogens such as *Aspergillus niger*, *A. flavus*, *Fusarium oxysporum*, *Cercospora arachichola*, *Metarhizium anisopliae*, *Pseudomonas solanacerum*, and *Alternaria alternata* in in vitro bioassays. In a separate study, Pb-tolerant *P. aeruginosa* from surface water of Mandovi estuary in India was equipped with the metallothionein gene (*bmtA* – for metal sequestration) and hydrogen cyanide and siderophore production capabilities (Naik et al. 2012). Pb accumulation in the root of *P. sativum* L. was reduced by 51.69% in the treatment with Pb-tolerant *P. aeruginosa*. This bacterium also improved seed germination and plant growth with 48.83% and 43.83% root and

Table 10.5 Bacterial and fungal biocontrol agents and bioremediators for heavy metal(loid)s

Type of microbe	Genus/species	Origin	Metal(loids) stressor	Biocontrol target	Biocontrol mechanism	Plant growth promotion	Application	Reference
Bacteria	*Alcaligenes* sp. *Pseudomonas aeruginosa*	Soils (India)	Ni^{2+}, Mn^{2+}	*Aspergillus flavus, A. niger, Fusarium oxysporum, Alternaria alternate, Metarhizium anisopliae* (groundnut and other crops)	Siderophore production (antifungal)	–	Metal-tolerant BCA of *Aspergillus, Fusarium, Alternaria,* and *Metarhizium* pathogen under metal-stressed soils	Sayyed and Patel (2011)
	Pseudomonas aeruginosa	Surface water (India)	Pb^{2+}, Cd^{2+}, Hg^{2+}	*Fusarium oxysporum* (*Pisum sativum* L)	Siderophore production	Seed germination and growth promotion	Metallothionein and bioaccumulation, reduce Pb accumulation in plant	Naik et al. (2012)
	Pseudomonas protegens	Agricultural well water (Algeria)	Cr^{2+}, Co^{2+}, Hg^{2+}, Cd^{2+}, Pb^{2+}	*Botrytis cinerea, Verticillium dahlia, Fusarium graminearum, Aspergillus niger, A. flavus*	Siderophores, chitinases, and polymer-degrading enzymes, insect toxin gene	IAA, siderophores, phosphate solubilization, seed germination, and plant growth	Metal-tolerant BCA for agricultural uses as biofertilizer, biopesticide, and bioremediator	Leila et al. (2016)
	Serratia plymuthica	Polluted soils (Italy)	Cd^{2+}	*Phytophthora megasperma*	Antagonistic	Auxin-like hormone	Metal-tolerant BCA with metal accumulation ability for bioremediation	Carlot et al. (2002)
	P. aeruginosa, Alcaligenes faecalis, B. subtilis	Mine tailings (South Africa)	Ni^{2+}, Cr^{2+}, Cd^{2+}	*Fusarium solani*	Antagonistic or antifungal activity	IAA, HCN, and NH_3 production, phosphorus solubilization, catalase activity, seed germination, plant growth promotion, metal protection for plant	Metal-tolerant BCA with metal accumulation ability for bioremediation	Aka and Babalola (2016)

Fungal	*Diaporthe miriciae*, *Trichoderma asperellum*	*Phragmites* sp. (Malaysia)	Cu^{2+}, Pb^{2+}, Zn^{2+}, Cd^{2+}	*Ganoderma boninense* (pathogen of oil palm)	Parasitism, competition, antibiosis (alkaloids, terpenoids, sterols)	–	Metal-tolerant BCA of *G. boninense* under metal-laden agricultural soils	Sim et al. (2018, 2019b, c)
	Trichoderma spp.	–	Cyanide and metallocyanide	Root diseases	Parasitism, antagonistic	Plant growth, seed germination, alleviate cyanide effects	Metal-tolerant BCA of various plant diseases and can be adopted in phytoremediators and other crops	Ezzi and Lynch (2002), Harman et al. (2004), Lynch and Moffat (2005)
	Trichoderma sp.	–	Cd^{2+}	*Sclerotium rolfsii, Rhizoctonia solani* (*Cicer arietinum* L.)	Parasitism, antagonism	Siderophore, IAA, HCN, NH_3		Rawat and Tewari (2011)

Abbreviations: *BCA* biocontrol agent, *GA* gibberellic acid, *HCN* hydrogen cyanide, *IAA* indole acetic acid

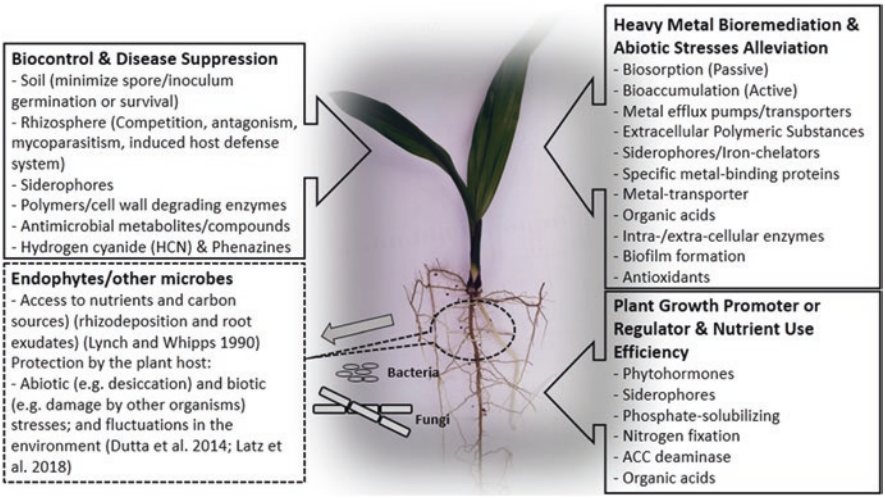


Fig. 10.3 Various types of mechanisms and activities provided by the potential metal-tolerant or metal-resistant microbial biocontrol agents to the plant hosts in exchange for nutrient and energy sources and protection from the plant hosts. Mechanisms that contribute to metal-tolerant or metal-resistant microbial agents also included. (References: Deketelaere et al. (2017), Purohit et al. (2018), Taj and Rajkumar (2016) and Wu et al. (2009))

shoot growth improvements, respectively. This *P. aeruginosa* strain also demonstrated tolerance towards Cd and Hg. In another study, two strains of *P. protegens* were isolated from waters containing agricultural effluents in Algeria (Leila et al. 2016). These two isolates showed metal resistance, plant growth-promoting (PGP) characteristics (i.e. phosphate-solubilizing ability, production of phytohormones), and the ability to suppress pathogens via production of chitinase and cell wall-degrading enzymes. These mechanisms were effective in inhibiting mycelial growth of *Botrytis cinerea*, *Verticillium dahliae*, *F. graminearum*, *A. niger*, and *A. flavus* with 48–88% inhibition. In addition, these bacterial strains also improved plant growth and seed germination in *Hordeum vulgare* L. (barley) (Leila et al. 2016). In separate study by Carlot et al. (2002), Cd-tolerant PGP *Serratia plymuthica* was isolated from soils polluted with metals. This isolate suppressed the growth of pathogenic *Phytophthora megasperma* while able to adsorb and accumulate Cd. Aka and Babalola (2016) further discovered three metal-tolerant bacterial strains (*P. aeruginosa*, *Alcaligenes faecalis*, and *B. subtilis*) with the ability to tolerate high Cd, Cr, and Ni, from mine tailings in South Africa. These bacterial strains were also able to increase the solubility of heavy metals in soil (Cr, 50%; Cd, 50%; and Ni, 44%), enhancing metal accumulation by the metal accumulator *Brassica juncea* L. (canola). *Pseudomonas aeruginosa* improved Cr accumulation in plant root and shoot tissues by 56% and 73%, respectively. Furthermore, through inoculation of *B. subtilis*, Ni accumulation in root and shoot tissues increased by 55.9% and 32%, respectively. *Alcaligenes faecalis* improved Cd accumulation in root and shoot

tissues by 73% and 14%, respectively. These microbes could be the potential biocontrol candidates to be incorporated into phytoextraction remediation approach.

In other studies, metal-tolerant endophytes from phytoremediator plants have been discovered to have both metal tolerance and biocontrol activities. Sim et al. (2018, 2019b, 2019c) established this when the metal-tolerant endophytic *Diaporthe miriciae* and *Trichoderma asperellum* isolated from *Phragmites* sp. (phytoremediator) were found to inhibit the growth of *Ganoderma boninense* in bioassays under metal stress. *Trichoderma asperellum* inhibited the growth of *G. boninense* through mycoparasitism, whereas *D. miriciae* adopted competitive exclusion to inhibit the growth of *G. boninense.* Both fungal isolates produced secondary metabolites (e.g. alkaloids, sterols, and terpenoids) with antifungal activities to suppress *G. boninense* as well. These fungal endophytes reduced basal stem rot disease incidences and severity in oil palm (*Elaeis guineensis*) seedlings when challenged with *G. boninense*. *Diaporthe miriciae* demonstrated better reduction in *G. boninense* disease scores compared to *T. asperellum* under in vivo experiment.

Other *Trichoderma* species, namely, *T. harzianum*, *T. viride*, *T. atroviride*, and *T. pseudokoningi*, with metal-, cyanide-, or metallocyanide-tolerant traits, have also been studied for their potential in remediating metals and other xenobiotic pollutants (Harman et al. 2004; Lynch and Moffat 2005; Tripathi et al. 2013). *Trichoderma harzianum* with biocontrol traits improved plant growth and seed germination in soils amended with cyanide as this isolate is known to detoxify cyanide and metallocyanide (Ezzi and Lynch 2002; Lynch and Moffat 2005). Rawat and Tewari (2011) examined two isolates of *T. virens*, one *T. viride*, and one *Aspergillus flavus*, for their potential in antagonizing and parasitizing *Sclerotium rolfsii* and *Rhizoctonia solani* fungal pathogens of *Cicer arietinum* L. (chickpea). They found these BCAs were able to undergo phosphate solubilization under different abiotic stresses, namely, Cd, pH, and temperature stress. All tested fungal isolates produced siderophore and NH_3, while only *T. virens* and *A. flavus* produced hydrogen cyanide (HCN). *Trichoderma viride* retained its phosphate-solubilizing capability despite Cd amendments (0–1000 μg/mL).

Studies on highly versatile microbes with multiple traits (Fig. 10.3), namely, disease-suppressing and metal(loid)-tolerant, are crucial to bio-prospect for potential BCAs for bioremediation. These microbes can also be incorporated into the agricultural systems polluted with metal(loids) to sustain plant growth, survival, and crop production and minimize economic losses due to pest and diseases.

Microbes also improve survival, growth, nutrient solubility, and nutrient uptake of the host plant through production of the following valuable compounds – phytohormones, siderophores, indole acetic acid (IAA), 1-aminocyclopropane-1-carboxylate (ACC) deaminases, and organic acids (Ojuederie and Babalola 2017) (Fig. 10.3). Microbial ACC deaminases are commonly reported in bacteria and fungi to assist plants to survive under abiotic stresses via ACC (the precursor of ethylene) metabolism (Singh et al. 2015b). ACC will be converted into ammonia and α-ketobutyrate (readily accessible molecules for the microbes to grow) to reduce the accumulation of stress ethylene and improve plant growth. Various metal-tolerant bacterial and fungal species with biocontrol traits have been studied

for their potential in improving seed germination, plant growth, survival of the plant, and efficiency of nutrient use under metal-stressed conditions. Metal-tolerant bacterial *Pseudomonas protegens* produced 3.1–4.0 μg/mL of IAA (in 500 μg/mL of L-tryptophan) and solubilized phosphate (Leila et al. 2016). *Pseudomonas protegens* also significantly improved the growth whereby shoot (fresh weight from 0.06 to 0.1 g and dry weight from 0.025 to 0.04 g) and root (fresh weight from 0.047 to 0.075 g and dry weight from 0.016 to 0.03 g) were enhanced in *Hordeum vulgare* L. Vivas et al. (2003) reported that the inoculation of both Pb-tolerant *Brevibacillus* sp. and arbuscular mycorrhizae in the *Trifolium pratense* L. improved shoot and root dry weight when cultivated in Pb-amended soils.

10.7 Benefits and Challenges of Using BCAs for Bioremediation

Adoption of BCAs for bioremediation does have its merits. Incorporation of BCAs into bioremediation systems can be environmental-friendly and capable of maintaining or improving soil fertility and functions (Ye et al. 2017). Furthermore, indigenous metal-tolerant or metal-resistant BCAs isolated from metal-polluted soils can be reintroduced back into original environment to facilitate the bioremediation process and protect the crops from pest and diseases. These BCAs can also ameliorate the adverse effects caused by heavy metals, improve the growth of the crops, and sustain crop production. In addition, BCAs with multiple biocidal properties, namely, fungicidal, bactericidal, and insecticidal, can be the potential candidates for managing both pest and disease (Leila et al. 2016) at the same time. For phytoremediation, metal-tolerant or metal-resistant BCAs with the ability to promote plant growth, immobilize soluble complexes, solubilize accessible metal complex, and accumulate metal(loid)s were isolated and studied for bioremediation. These microbes can be utilized for phytostabilization, phytoextraction, and phytovolatilization processes and protect the host plant from pest and diseases at the same time. Meanwhile, for the agricultural soils with heavy metal pollution, metal-tolerant or metal-resistant BCAs can be used for minimizing the metal uptake by plant and improving the seed germination, survival, and growth of the crops.

Unfortunately, the adoption of BCAs for bioremediation processes does have its own limitations. Suitability of the microbial-mediated bioremediation approaches was reported to be generally low in highly contaminated sites (Prasad and de Oliveira Freistas 2003). Furthermore, most of the studies conducted are mainly under artificial or controlled conditions, namely, laboratory, glasshouse, or small experimental plots (Ye et al. 2017). Therefore, it is essential to conduct experiments in the original contaminated sites to assess the potential of BCAs for bioremediation. Microbial population and activity can be affected by various soil types, conditions, and properties (Prasad and de Oliveira Freistas 2003). Survival, growth, proliferation, and also the competitiveness of the introduced microbial agents in the

actual contaminated fields differ and warrant more detailed studies (Waigi et al. 2017). Coexistence of organic and inorganic pollutants or multiple heavy metals may pose adverse effects on BCAs and the host plant. These phenomena may also reduce the efficacy of BCAs in disease control and decrease the efficiency of microbial-mediated phytoremediation (Rhind 2009; Ye et al. 2017).

10.8 Conclusions and Future Prospects

Bioremediation is an environmental-friendly, sustainable, and cost-effective technology that has great potential to be adopted in remediating the contaminated soils. Microbial-mediated phytoremediation or phytobial remediation has attracted attention in recent decades due to the potential use of microorganisms with various important roles and traits (Mishra et al. 2017; Ojuederie and Babalola 2017; Waigi et al. 2017; Hrynkiewicz et al. 2018; Singh et al. 2018). The use of metal-tolerant BCAs will serve as metal ameliorator to reduce metal toxicity, as well as to improve plant growth, reduce metal accumulation, and sustain the yield for agricultural crops.

Sim et al. (2018, 2019b, c) isolated metal-tolerant *D. miriciae* and *T. asperellum* endophytic fungi from the phytoremediator *Phragmites* sp. (leachate treatment site). These endophytes were able to slow down the development of disease symptoms in *E. guineensis* seedlings challenged with *G. boninense* in metal-spiked soils in the nursery set-up. Both *D. miriciae* and *T. asperellum* could be potential BCAs for agricultural applications, such as for protecting oil palm from *G. boninense* and for phytobial remediation in the metal-stressed soils. This is the first experiment conducted in the nursery settings to study the potential of metal-tolerant BCAs in suppressing *G. boninense.* Most of other previous studies on metal-tolerant BCAs evaluation were mainly conducted in the laboratory set-up or in vitro conditions to determine the capability of potential microbial agents in controlling plant pathogens. Therefore, more detailed nursery and field experiments with potential BCAs for bioremediation should be conducted to assess the efficacy of BCAs in remediating the polluted soils (Ye et al. 2017).

Rhind (2009) and Ye et al. (2017) emphasized the importance of potential detrimental effects from coexistence of organic and inorganic pollutants or multiple heavy metal(loids) on the efficacy of bioremediation and survival and growth of bioremediators. Therefore, more in-depth studies are required to understand the effects of metal(loids) and organic pollutant coexistence on the efficacy of potential BCAs with bioremediation capability. Combination of either conventional and biological or multiple remediation technologies for cleaning up the soils with co-contaminants has been proposed for improving the remediation process (Ye et al. 2017). Megharaj and Naidu (2017) and Jin et al. (2018) suggested the utilization of conventional physicochemical remediation followed by biological remediation methods to improve the soil quality. It is worthwhile to explore further on the adoption of multiple remediation technologies, by integrating biological and non-biological techniques together with the BCAs, for remediating soil pollutants.

Furthermore, an array of '-omics' researches, namely, metagenomics, transcriptomics, proteomics, and metabolomics (Malla et al. 2018), have been initiated for understanding multi-trophic relationships and cell function in the ecosystem. The '-omics' technology can be adopted to generate information on the metabolic networks and corporations among the microorganisms and microbial groups or communities in the polluted soils. Information produced through various '-omics' studies will be beneficial for the selection of potential BCA/bioremediators and understanding of pathways for BCA-mediated bioremediation. All the additional future researches and information on metal-tolerant microbial bioremediators with biological control trait will be beneficial for improving efficacy of biocontrol and bioremediation in the soils with metal pollutants. These studies and information will enhance formulation of metal-tolerant BCAs, minimize the limitations of BCAs, and select for the highly metal-tolerating microbial candidates.

Acknowledgement GYK would like to thank Monash University Malaysia for the scholarship provided to support his study.

References

Abbas SH, Ismail IM, Mostafa TM et al (2014) Biosorption of heavy metals: a review. J Chem Sci Technol 3:74–102

Abbasi A, Sajid A, Haq N et al (2014) Agricultural pollution: an emerging issue. In: Ahmad P, Wani M, Azooz M et al (eds) Improvement of crops in the era of climatic changes. Springer, New York, pp 347–387

Abhilash PC, Powell JR, Sing HB et al (2012) Plant-microbe interactions: novel applications for exploitation in multipurpose remediation technologies. Trends Biotechnol 30:416–420

Abo-amer AE, Abu-Gharbia MA, Soltan EM et al (2014) Isolation and molecular characterization of heavy metal-resistant *Azotobacter chroococcum* from agricultural soil and their potential application in bioremediation. Geomicrobiol J 31:551–561

Ahemad M, Malik A (2012) Bioaccumulation of heavy metals by zinc resistant bacteria isolated from agricultural soils irrigated with wastewater. Bacteriol J 2:12–21

Ahluwalia SS, Goyal D (2007) Microbial and plant derived biomass for removal of heavy metals from wastewater. Bioresour Technol 98:2243–2257

Ahmad I, Zafar S, Ahmad F (2005) Heavy metal biosorption potential of *Aspergillus* and *Rhizopus* sp. isolated from wastewater treated soil. J Appl Sci Environ Mgt 9:123–126

Aka RJN, Babalola OO (2016) Effect of bacterial inoculation of strains of *Pseudomonas aeruginosa, Alcaligenes faecalis* and *Bacillus subtilis* on germination, growth and heavy metal (Cd, Cr, and Ni) uptake of *Brassica juncea*. Int J Phytoremediation 18:200–209

Ali H, Khan E (2018) Trophic transfer, bioaccumulation, and biomagnification of non-essential hazardous heavy metals and metalloids in food chains/webs – concepts and implications for wildlife and human health. Hum Ecol Risk Assess. https://doi.org/10.1080/10807039.2018.1469398

Alves LR, Reis ARD, Gratão PL (2016) Heavy metals in agricultural soils: from plants to our daily life. Cientifica 44:346–361

Aragón F, Rud JP (2016) Polluting industries and agricultural productivity: evidence from mining in Ghana. Econ J 126:1980–2011

Awasthi SK (1998) Prevention of food adulteration act No 37 of 1954. Central and state rules as amended for 1999. Ashoka Law House

Azubuike CC, Chikere CB, Okpokwasili GC (2016) Bioremediation techniques – classification based on site of application: principles, advantages, limitations and prospects. World J Microbiol Biotechnol 32:180

Babu AG, Shim J, Bang KS et al (2014) *Trichoderma virens* PDR-28: a heavy metal-tolerant and plant growth-promoting fungus for remediation and bioenergy crop production on mine tailing soil. J Environ Manag 132:129–134

Baldrian P (2006) Fungal laccases – occurrence and properties. FEMS Microbiol Rev 30:215–242

Barbieri M, Sappa G, Nigro A (2018) Soil pollution: anthropogenic versus geogenic contributions over large areas of the Lazio region. J Geochem Explor 195:78–86

BBSRC (1999) A joint research council review of bioremediation research in the United Kingdom. BBRRC, EPSRC, and NERC, Swindon, UK, p 55

Behera BK, Prasad R (2020) Strategies for soil management. In: Behera BK, Prasad R (eds) Environmental Technology and Sustainability. Elsevier 143–167

Blindauer CA (2011) Bacterial metallothioneins: past, present, and questions for the future. J Biol Inorg Chem 16:1011–1024

Burd GI, Dixon DG, Glick BR (2000) Plant growth-promoting bacteria that decrease heavy metal toxicity in plants. Can J Microbiol 46:237–245

Cao W, Yin L, Zhang D, Wang Y, Yuan J, Zhu Y, Dou J (2017) Contamination, sources, and health risks associated with soil PAHs in rebuilt land from a coking plant, Beijing, China. Int J Environ Res Public Health 16:670

Carlot M, Giacomini A, Casella S (2002) Aspects of plant-microbe interactions in heavy metal polluted soil. Acta Biotechnol 22:13–20

Chaney RL, Oliver DP (1996) Sources, potential adverse effects and remediation of agricultural soil contaminants. In: Naidu R, Kookana RS, Oliver DP et al (eds) Contaminants and the soil environment in the Australasia-Pacific region. Springer, Dordrecht, pp 323–359

Chen Z, Su S, Guo H et al (2007) New aspects of collaborative research on soil pollution, food safety, and soil remediation techniques in Asia. Technical Bull No. 175. Food and Fertilizer Technology Center (FFTC), Taipei, Taiwan, pp 1–35

Chen R, de Sherbinin A, Ye C et al (2014) China's soil pollution: farms on the frontline. Science 344:691

Chen R, Zhou Z, Liu Y et al (2015) Mycoremediation potential and tolerance responses of *Oudemansiella radicata* in cadmium-pyrene co-contaminated soil. J Soils Sediments 15:1083–1093

Chiroma TM, Ebewele RO, Hymore K (2014) Comparative assessment of heavy metal levels in soil, vegetables, and urban grey waste water used for irrigation in Yola and Kano. Int Ref J Eng Sci 3:1–9

Choudhary M, Kumar R, Datta A et al (2017) Bioremediation of heavy metals by microbes. In: Arora S, Singh A, Singh Y (eds) Bioremediation of salt affected soils: an Indian perspective. Springer, Cham, pp 233–255

Cobbett C, Goldsbrough P (2002) Phytochelatins and metallothioneins: roles in heavy metal detoxification and homeostasis. Annu Rev Plant Biol 53:159–182

Cocârță DM, Stoian MA, Karademir A (2017) Crude oil contaminated sites: evaluation by using risk assessment approach. Sustainability 9:1365

Coelho LM, Rezende HC, Coelho LM et al (2015) Bioremediation of polluted waters using microorganisms. In: Shiomi N (ed) Advances in bioremediation of wastewater and polluted soil. IntechOpen, London, UK, pp 1–22

Colborn T, vom Saal FS, Soto AM (1993) Developmental effects of endocrine-disrupting chemicals in wildlife and humans. Environ Health Perspect 101:378–384

Cortes-Penagos C, Olmedo-Monfil V, Herrera-Estrella A (2007) The nature of fungal mycoparasitic biocontrol agents. In: Chincholkar SB, Mukerji KG (eds) Biological control of plant diseases. Haworth Press, New York, pp 327–353

Das N, Vimala R, Karthika P (2008) Biosorption of heavy metals – an overview. Indian J Biotechnol 7:159–169

Das A, Sherameti I, Varma A (2012) Contaminated soil: physical, chemical, and biological components. In: Kothe E, Varma A (eds) Bio-geo interactions in metal-contaminated soils. Springer-Verlag, Heidelberg, Berlin, pp 1–15
Das S, Jean J, Kar S et al (2014) Screening of plant growth-promoting traits in arsenic-resistant bacteria isolated from agricultural soil and their potential implication for arsenic bioremediation. J Hazard Mater 272:112–120
Deketelaere S, Tyvaert L, Franca SC et al (2017) Desirable traits of a good biocontrol agent against Verticillium wilt. Front Microbiol 8:1186
Dich J, Zahm SH, Hanberg A, Adami H-O (1997) Pesticides and cancer. Cancer Causes Control 8:420–443
Dixit R, Wasiullah MD et al (2015) Bioremediation of heavy metals from soil and aquatic environment: an overview of principles and criteria of fundamental processes. Sustainability 7:2189–2212
DPR-EGASPIN (2003) Environmental guidelines and standards for the petroleum industry in Nigeria (EGASPIN). Department of Petroleum Resources, Lagos, Nigeria
Elliott M (2003) Biological pollutants and biological pollution – an increasing cause for concern. Mar Pollut Bull 46:275–280
El-Shahawi MS, Hamza A, Bashammakhb AS, Al-Saggaf WT (2010) An overview on the accumulation, distribution, transformation, toxicity, and analytical methods for the monitoring of persistent organic pollutants. Talanta 80:1587–1597
Emamverdian A, Ding Y, Mokhberdoran F et al (2015) Heavy metal stress and some mechanisms of plant defense response. Sci World J 2015:1–18
Emenike CU, Jayanthi B, Agamuthu P et al (2018) Biotransformation and removal of heavy metals: a review of phytoremediation and microbial remediation assessment on contaminated soil. Environ Rev 26:156–168
Environmental Protection Ministry of China (EPMC) (2015) Standards of soil environmental quality of agricultural land. Environmental Protection of Ministry of China, Beijing, China
European Union (2002) Heavy metals in wastes, European Commission on Environment. http://ec.europa.eu/environment/waste/studies/pdf/heavy_metalsreport.pdf. Accessed on 15 May 2019
Ezzi M, Lynch JM (2002) Cyanide catabolizing enzymes in *Trichoderma* spp. Enzyme Microb Technol 31:1042–1047
FAO/WHO (2001) Codex alimentarius commission, food additives and contamination, Joint FAO/WHO Food standard program, p 289
FAOSTAT (2018) FAOSTAT Inputs/Annual population. [online]. Cited 9 May 2019. http://www.fao.org/faostat/en/#data/OA
Franceschi VR, Nakata PA (2005) Calcium oxalate in plants: formation and function. Annu Rev Plant Biol 56:41–71
Franke S, Grass G, Rensing C et al (2003) Molecular analysis of the copper-transporting efflux system CusCFBA of *Escherichia coli*. J Bacteriol 185:3804–3812
Gadd GM (1990) Fungi and yeasts for metal binding. In: Ehrlich H, Brierley CL (eds) Microbial mineral recovery. McGraw-Hill, New York, pp 249–275
Gadd GM (1999) Fungal production of citric and oxalic acid: importance in metal speciation, physiology and biogeochemical process. Adv Microb Physiol 11:47–91
Gadd GM (2010) Metals, minerals and microbes: geomicrobiology and bioremediation. Microbiology 156:609–643
Gall JE, Rajakaruna N (2013) The physiology, functional genomics, and applied ecology of heavy metal-tolerant Brassicaceae. In: Lang M (ed) Brassica: characterization, functional genomics and health benefits. Nova, New York, pp 121–148
Garbisu C, Alkorta I (1997) Bioremediation: principles and future. J Clean Technol Environ Occup Med 6:1–16
Gascon M, Morales D, Sunyer J et al (2013) Effects of persistent organic pollutants on the developing respiratory and immune systems: a systematic review. Environ Int 52:51–65
Gkorezis P, Daghio M, Franzetti A et al (2016) The interaction between plants and bacteria in the remediation of petroleum hydrocarbons: an environmental perspective. Front Microbiol 7:1836

Gnanamanickam SS, Vasudevan P, Reddy MS et al (2002) Principles of biological control. In: Gnanamanickam SS (ed) Biological control of crop diseases. Marcel Dekker, New York, pp 1–9
Griffin MR (2014) Biocontrol and bioremediation: two areas of endophytic research which hold great promise. In: Verma VC, Gange AC (eds) Advances in endophytic research. Springer, India, pp 257–282
Gupta R, Ahuja P, Khan S et al (2000) Microbial biosorbents: meeting challenges of heavy metal pollution in aqueous solutions. Curr Sci 78:967–973
Hafez EE, Elbesawy E (2009) Molecular characterization of soil microorganisms: effect of industrial pollution on distribution and biodiversity. World J Microbiol Biotechnol 25:215–224
Halaouli S, Asther M, Sigoillot JC et al (2006) Fungal tyrosinases: new prospects in molecular characteristics, bioengineering and biotechnological applications. J Appl Microbiol 100:219–232
Harman GE, Lorito M, Lynch JM (2004) Uses of *Trichoderma* spp. to alleviate soil or water pollution. Adv Appl Microbiol 56:313–330
He Z, Shentu J, Yang X et al (2015) Heavy metal contamination of soils: sources, indicators, and assessment. J Environ Indic 9:17–18
Hofrichter M, Ullrich R, Pecyna M et al (2010) New and classic families of secreted fungal heme peroxidases. Appl Microbiol Biotechnol 87:871–897
Hookoom M, Puchooa D (2013) Isolation and identification of heavy metals tolerant bacteria from industrial and agricultural areas in Mauritius. Curr Res Microbiol Biotechnol 1:119–123
Hrynkiewicz K, Złoch M, Kowalkowski T et al (2018) Efficiency of microbially assisted phytoremediation of heavy-metal contaminated soils. Environ Rev 26:316–332
Iram S, Ahmad I, Stuben D (2009) Analysis of mines and contaminated agricultural soil samples for fungal diversity and tolerance to heavy metals. Pak J Bot 41:885–895
Iram S, Parveen K, Usman J et al (2012) Heavy metal tolerance of filamentous fungal strains isolated from soil irrigated with industrial wastewater. Biologija 58:107–116
Jacobsen CS (1997) Plant protection and rhizosphere colonization of barley by seed inoculated herbicide degrading *Burkholderia* (*Pseudomonas*) *cepacia* DBO1 (pRO101) in 2,4-D contaminated soil. Plant Soil 189:139–144
Jin Y, Luan Y, Ning Y et al (2018) Effects and mechanisms of microbial remediation of heavy metals in soil: a critical review. Appl Sci 8:1336
Jones KC, de Voogt P (1999) Persistent organic pollutants (POPs): state of the science. Environ Pollut 100:209–221
Kelepertzis E (2014) Accumulation of heavy metals in agricultural soils of Mediterranean: insights from Argolida basin, Peloponnese, Greece. Geoderma 221–222:82–90
Kelly BC, Ikonomou MG, Blair JD et al (2007) Food web – specific biomagnification of persistent organic pollutants. Science 317:236
Khalid S, Shahid M, Niazi NK et al (2017) A comparison of technologies for remediation of heavy metal contaminated soils. J Geochem Explor 182:247–268
Khan MS, Zaidi A, Wani PA et al (2009) Role of plant growth promoting rhizobacteria in the remediation of metal contaminated soils. Environ Chem Lett 7:1–19
Khanif YM, Salmijah S (1996) Contaminants and the soil environment in Malaysia. In: Naidu R, Kookana RS, Oliver DP et al (eds) Contaminants and the soil environment in the Australasia-Pacific region. Springer, Netherlands, pp 563–578
Knox AS, Seamans JC, Mench MJ et al (2000) Remediation of metals and radionuclides. Contaminated soil using in situ stabilization techniques. Macmillan, New York, pp 21–26
Lal R (2015) Restoring soil quality to mitigate soil degradation. Sustainability 7:5875–5895
Leila B, Nabti E, Tabli N et al (2016) Heavy metal tolerant *Pseudomonas protegens* isolates from agricultural well water in northeastern Algeria with plant growth promoting, insecticidal and antifungal activities. Eur J Soil Biol 75:38–46
Leon J, Osorio N (2014) Role of litter turnover in soil quality in tropical degraded lands of Colombia. Sci World J 13:1–11
Li GY, Hu N, Ding DX et al (2011) Screening of plant species for phytoremediation of uranium, thorium, barium, nickel, strontium, and lead contaminated soil from uranium mill tailings repository in South China. Bull Environ Contam Toxicol 86:646–652

Li T, Liu Y, Lin S, Liu Y, Xie Y (2019) Soil pollution management in China: a brief introduction. Sustainability 11:1–15
Lim MW, Von Lau E, Poh PE (2016) A comprehensive guide of remediation technologies for oil contaminated soil – present works and future directions. Mar Pollut Bull 109:14–45
Liu XB, Zhang XY, Wang XY et al (2010) Soil degradation: a problem threatening the sustainable development of agriculture in Northeast China. Plant Soil Environ 56:87–97
Lynch JM, Moffat AJ (2005) Bioremediation – prospects for the future application of innovative applied biological research. Ann Appl Biol 146:217–221
Ma Z, Jacobsen FE, Giedroc DP (2009) Metal transporters and metal sensors: how coordination chemistry controls bacterial metal homeostasis. Chem Rev 109:4644–4681
Madigan MT (2000) Bacterial habitats in extreme environments. In: Seckbach J (ed) Journey to diverse microbial worlds. Cellular origin and life in extreme habitats, vol 2. Springer, Dordrecht, pp 61–72
Malla MA, Dubey A, Yadav S et al (2018) Understanding and designing the strategies for the microbe-mediated remediation of environmental contaminants using omics approaches. Front Microbiol 9:1132
Mallampati SR, Mitoma Y, Okuda T, Simion C, Lee BK (2015) Dynamic immobilization of simulated radionuclide 133 Cs in soil by thermal treatment/vitrification with nanometallic Ca/CaO composites. J Environ Radioact 139:118–124
Manz M, Wenzel KD, Dietze G et al (2001) Persistent organic pollutants in agricultural soils of Central Germany. Sci Total Environ 277:187–198
Mao X, Han FX, Shao X et al (2016) Electro-kinetic remediation coupled with phytoremediation to remove lead, arsenic and cesium from contaminated paddy soil. Ecotoxicol Environ Saf 125:16–24
Marinescu M, Toti M, Tanase V et al (2010) An assessment of the effects of crude oil pollution on soil properties. Annu Food Sci Technol 11:94–99
Masindi V, Muedi KL (2016) Environmental contamination by heavy metals. In: Larramendy M, Soloneski S (eds) Environmental health risk: hazardous factors to living species. IntechOpen, London, UK, pp 115–133
Masindi V, Muedi KL (2018) Environmental Contamination by Heavy Metals. In Heavy Metals; IntechOpen: Aglan, France, pp. 115–133
Megharaj M, Naidu R (2017) Soil and brownfield bioremediation. Microb Biotechnol 10:1244–1249
Mirsal IA (2008) Soil pollution: origin, monitoring and remediation. Springer, Berlin, Heidelberg, 312 p
Mishra GK (2017) Microbes in heavy metal remediation: a review on current trends and patents. Recent Pat Biotechnol 11:188–196
Mishra J, Singh R, Arora NK (2017) Alleviation of heavy metal stress in plants and remediation of soil by rhizosphere microorganisms. Front Microbial 8:1706
Mishra V, Gupta A, Kaur P et al (2016) Synergistic effects of arbuscular mycorrhizal fungi and plant growth promoting rhizobacteria in bioremediation of iron contaminated soils. Int J Phytoremediation 18:697–703
Montanarella L, Pennock DJ, McKenzie N et al (2016) World's soils are under threat. Soil 2:79–82
Mulligan CN, Yong RN, Gibbs BF (2001) Remediation technologies for metal-contaminated soils and groundwater: an evaluation. Eng Geol 60:193–207
Naik MM, Pandey A, Dubey SK (2012) *Pseudomonas aeruginosa* strain WI-1 from Mandovi estuary possess metallothionein to alleviate lead toxicity and promotes plant growth. Ecotoxicol Environ Saf 79:129–133
Nanda S, Abraham J (2011) Impact of heavy metals on the rhizosphere microflora of *Jatropha multifida* and their effective remediation. Afr J Biotechnol 10:11948–11955
Neilson S, Rajakaruna N (2015) Phytoremediation of agricultural soils: using plants to clean metal-contaminated arable land. In: Ansari AA, Gill SS, Gill R et al (eds) Phytoremediation: management of environmental contaminants. Springer International, Switzerland, pp 159–168
Nies DH (1999) Microbial heavy-metal resistance. Appl Microbiol Biotechnol 51:730–750
Norse D, Ju X (2015) Environmental costs of China's food security. Agric Ecosyst Environ 209:5–14

Ojuederie OB, Babalola OO (2017) Microbial and plant-assisted bioremediation of heavy metal polluted environments: a review. Int J Environ Res Public Health 14:1504

Padilla-Sánchez JA, Romero-González R, Plaza-Bolaños P et al (2014) Residues and organic contaminants in agricultural soils in intensive agricultural areas of Spain: a three years survey. CLEAN-Soil Air Water 43:746–753

Pain B, Jarvis S, Clements B (1991) Impact of agricultural practices on soil pollution. Outlook Agric 20:153–160

Pal KK, McSpadden Gardener B (2006) Biological control of plant pathogens. The Plant Health Instructor. https://doi.org/10.1094/PHI-A-2006-1117-02

Peralta-Videa JR, Lopez ML, Narayana M et al (2009) The biochemistry of environmental heavy metal uptake by plants: implications for the food chain. Int J Biochem Cell Biol 41:1665–1677

Petruzzelli G, Gorini F, Pezzarossa B, Pedron F (2010) The fate of pollutants in soil. In: Fabrizio B, Liliana C, Pier FM (eds) Environmental health interdepartment project. Consglio Nazionale delle Ricerche, Rome, pp 1–31

Phieler R, Voit A, Kothe E (2013) Microbially supported phytoremediation of heavy metal contaminated soils: strategies and application. Adv Biochem Eng Biotechnol 141:211–235

Prasad R (2017) Mycoremediation and environmental sustainability, vol 1. Springer International Publishing (ISBN 978-3-319-68957-9). https://link.springer.com/book/10.1007/978-3-319-68957-9

Prasad R (2018) Mycoremediation and environmental sustainability, vol 2. Springer International Publishing (ISBN 978-3-319-77386-5). https://www.springer.com/us/book/9783319773858

Prasad R, Aranda E (2018) Approaches in bioremediation: the new era of environmental microbiology and nanobiotechnology. Springer International Publishing (978-3-030-02369-0) https://www.springer.com/gp/book/9783030023683

Prasad M, de Oliveira Freistas H (2003) Metal hyperaccumulation in plants – biodiversity prospecting for phytoremediation technology. Electron J Biotechnol 6:617

Purohit J, Chattopadhyay A, Biswas MK et al (2018) Mycoremediation of agricultural soil: bioprospection for sustainable development. In: Prasad R (ed) Mycoremediation and environmental sustainability: fungal biology. Springer, Cham, pp 91–120

Ramesh A, Archibong AE (2011) Reproductive toxicity of polycyclic aromatic hydrocarbons: occupational relevance. In: Gupta RC (ed) Reproductive and developmental toxicology. Academic Press, San Diego, CA, pp 577–591

Rampelotto PH (2013) Extremophiles and extreme environments. Life (Basel) 3:482–485

Rawat R, Tewari L (2011) Effect of abiotic stress on phosphate solubilization by biocontrol fungus *Trichoderma* sp. Curr Microbiol 62:1521–1526

Rensing C, Ghosh M, Rosen BR (1999) Families of soft-metal-ion-transporting ATPases. J Bacteriol 181:5891–5897

Rhind SM (2009) Anthropogenic pollutants: a threat to ecosystem sustainability? Philos Trans R Soc 364:3391–3401

Rodríguez-Eugenio N, McLaughlin M, Pennock D (2018) Soil pollution: a hidden reality. FAO, Rome, 142 p

Sabir M, Waraich EA, Hakeem KR et al (2015) Phytoremediation. In: Hakeem K, Sabir M, Ozturk M et al (eds) Soil remediation and plants: prospects and challenges, contaminated soil is indispensable. Elsevier, Boston, pp 85–105

Saha R, Saha N, Donofrio RS et al (2013) Microbial siderophores: a mini review. J Basic Microbiol 53:303–317

Saha N, Rahman MS, Ahmed MB et al (2017) Industrial metal pollution in water and probabilistic assessment of human health risk. J Environ Manag 185:70–78

Salomons W, Forstner U, Mader P (1995) Heavy metals: problems and solutions. Springer-Verlag, Berlin, Germany, 414 pp

Sambandan K, Kannan K, Raman N (1992) Distribution of vesicular-arbuscular mycorrhizal fungi in heavy metal polluted soils of Tamil Nadu, India. J Environ Biol 13:159–167

Sayyed RZ, Patel PR (2011) Biocontrol potential of siderophore producing heavy metal resistant *Alcaligenes* sp. and *Pseudomonas aeruginosa* RZS3 vis-à-vis organophosphorus fungicide. Indian J Microbiol 51:266–272

Schalk IJ, Hannauer M, Braud A (2011) New roles for bacterial siderophores in metal transport and tolerance. Environ Microbiol 13:2844–2854
Selbmann L, Egidi E, Isola D et al (2013) Biodiversity, evolution and adaptation of fungi in extreme environments. Plant Biosys 147:237–246
Sarma H, Forid N, Prasad R, Prasad MNV, Ma LQ, Rinklebe J (2021) Enhancing phytoremediation of hazardous metal(loid)s using genome engineering CRISPR–Cas9 technology. Journal of Hazardous Materials https://doi.org/10.1016/j.jhazmat.2021.125493
Sim CSF, Ting ASY (2017) Metal biosorption in single- and multi-metal solutions by biosorbents: indicators of efficacy in natural wastewater. Clean Soil Air Water 45:1600049
Sim CSF, Cheow YL, Ng SL, Ting ASY (2018) Discovering metal-tolerant endophytic fungi from the phytoremediator plant *Phragmites*. Water Air Soil Pollut 229:68
Sim CSF, Chen SH, Ting ASY (2019a) Endophytes: emerging tools for the bioremediation of pollutants. In: Bharagava RN, Chowdhary P (eds) Emerging and eco-friendly approaches for waste management. Springer, Singapore, pp 189–217
Sim CSF, Cheow YL, Ng SL, Ting ASY (2019b) Antifungal activities of metal-tolerant endophytes against *Ganoderma boninense* under the influence of metal stress. Biol Control 130:9–17
Sim CSF, Cheow YL, Ng SL, Ting ASY (2019c) Biocontrol activities of metal-tolerant endophytes against *Ganoderma boninense* in oil palm seedlings cultivated under metal stress. Biol Control 132:66–71
Singh M, Srivastava PK, Verma PC et al (2015a) Soil fungi for mycoremediation of arsenic pollution in agriculture soils. J Appl Microbiol 119:1278–1290
Singh RP, Shelke GM, Jha PN (2015b) Biochemistry and genetics of ACC deaminase: a weapon to "stress ethylene" produced in plants. Front Microbiol 6:937
Singh PC, Srivastava S, Shukla D et al (2018) Mycoremediation mechanisms for heavy metal resistance/tolerance in plants. In: Prasad R (ed) Mycoremediation and environmental sustainability: fungal biology. Springer, Cham, pp 351–380
Siripornadulsil S, Siripornadulsil W (2013) Cadmium-tolerant bacteria reduce the uptake of cadmium in rice: potential for microbial bioremediation. Ecotoxicol Environ Saf 94:94–103
Song B, Zeng G, Gong J et al (2017) Evaluation methods for assessing effectiveness of in situ remediation of soil and sediment contaminated with organic pollutants and heavy metals. Environ Int 105:43–55
Srivastava PK, Vaish A, Dwivedi S et al (2011) Biological removal of arsenic pollution by soil fungi. Sci Total Environ 409:2430–2442
Su C, Jiang L, Zhang W (2014) A review on heavy metal contamination in the soil worldwide: situation, impact and remediation techniques. Environ Skep Crit 3:24–38
Sun J, Pan L, Tsang DCW et al (2018) Organic contamination and remediation in the agricultural soils of China: a critical review. Sci Total Environ 615:724–740
Taiwan Environment Protection Agency (TEPA) (2000) Soil and groundwater pollution and remediation. Available: http://sgw.epa.gov.tw/public/En/index.htm. Accessed on: 15 May 2019
Taj ZZ, Rajkumar M (2016) Perspectives of Plant Growth-Promoting Actinomycetes in Heavy Metal Phytoremediation. In: Subramaniam G., Arumugam S., Rajendran V. (eds) Plant Growth Promoting Actinobacteria. Springer, Singapore
Tajudin SAA, Azmi MAM, Nabila ATA (2016) Stabilization/solidification remediation method for contaminated soil: a review. IOP Conf Ser Mater Sci Eng 136:012043
Tejada M, García C, Hernández T et al (2015) Response of soil microbial activity and biodiversity in soils polluted with different concentrations of cypermethrin insecticide. Arch Environ Contam Toxicol 69:8–19
Thakare M, Sarma H, Datar S, Roy A, Pawar P, Gupta K, Pandit S, Prasad R (2021) Understanding the holistic approach to plant-microbe remediation technologies for removing heavy metals and radionuclides from soil. Current Research in Biotechnology https://doi.org/10.1016/j.crbiot.2021.02.004
Ting ASY, Jioe E (2016) *In vitro* assessment of antifungal activities of antagonistic fungi towards pathogenic *Ganoderma boninense* under metal stress. Biol Control 96:57–63

Tripathi P, Singh P, Mishra A et al (2013) *Trichoderma*: a potential bioremediatory for environmental clean up. Clean Techn Environ Policy 15:541–550

Tunali S, Cabuk A, Akar T (2006) Removal of lead and copper ion from aqueous solutions by bacterial strain isolated from soil. Chem Eng J 115:203–211

Van den Berg R (1995) Human exposure to soil contamination: a qualitative and quantitative analysis towards proposals for human toxicological intervention values. RIVM Report No. 725201011. National Institute of Public Health and Environmental Protection, Bilthoven, The Netherlands

Van der Ent A, Baker AJM, Reeves RD et al (2012) Hyperaccumulators of metal and metalloid trace elements: facts and fiction. Plant Soil 362:1–16

Varma A, Prasad R, Tuteja N (2017) Mycorrhiza: nutrient uptake, biocontrol, ecorestoration. Springer International Publishing (ISBN: 978-3-319-68867-1). http://www.springer.com/us/book/9783319688664

Vivas A, Azcón R, Biró B et al (2003) Influence of bacterial strains isolated from lead-polluted soil and their interactions with arbuscular mycorrhizae on the growth of *Trifolium pratense* L. under lead toxicity. Can J Microbiol 49:577–588

Waigi MG, Sun K, Gao Y (2017) Sphingomonads in microbe-assisted phytoremediation: tackling soil pollution. Trends Biotechnol 35:883–899

Wasim Aktar M, Sengupta D, Chowdhury A (2009) Impact of pesticides use in agriculture: their benefits and hazards. Interdiscip Toxicol 2:1–12

Whipps JM (2001) Microbial interactions and biocontrol in the rhizosphere. J Exp Bot 52:487–511

Wong MH (2012) Environmental contamination: health risks and ecological restoration. Taylor and Francis Group, USA, 518 p

Wu CH, Bernard SM, Andersen GL et al (2009) Developing microbe-plant interactions for applications in plant-growth promotion and disease control, production of useful compounds, remediation and carbon sequestration. Microb Biotechnol 2:428–440

Yang Q, Li Z, Lu X et al (2018) A review of soil heavy metal pollution from industrial and agricultural regions in China: pollution and risk assessment. Sci Total Environ 642:690–700

Ye S, Zeng G, Wu H et al (2017) Biological technologies for the remediation of co-contaminated soil. Crit Rev Biotechnol 37:1062–1076

Yu J, Wu J (2018) The sustainability of agricultural development in China: the agriculture-environment nexus. Sustainability 10:1776

Zafra G, Cortés-Espinosa DV (2015) Biodegradation of polycyclic aromatic hydrocarbons by *Trichoderma* species: a mini review. Environ Sci Pollut Res Int 22:19426–19433

Chapter 11
Application of Mycobiocontrol Agent in Biodergradation and Pest Management

S. A. Dwivedi and Ajay Tomer

Contents

11.1 Introduction

Extension dealing and proceed share directly or indirectly escalate the introduction of insect pests into new areas, where they become serious pests. Continued use of inorganic insecticides by farmers (mainly for management of pests) over a period of years causes environmental and human health problems despite initially achieving success and being economically viable. Integrated pest management (IPM) involves surveying, recognition, and effective action for pest management. Treatment is generally started after proper inspection and identification of pests, with consideration

S. A. Dwivedi (✉)
Department of Entomology, School of Agriculture, Lovely Professional University, Phagwara, Punjab, India
e-mail: sunil.21186@lpu.co.in

A. Tomer
Department of Plant Pathology, School of Agriculture, Lovely Professional University, Phagwara, Punjab, India

R. Prasad et al. (eds.), *Mycoremediation and Environmental Sustainability*, Fungal Biology, https://doi.org/10.1007/978-3-030-54422-5_11

of economic viability and environmental safety, including use of safe, pest-specific chemicals with limited persistence in the environment. Biological control is a valuable tool in integrated pest management. It came out proposition to modify as the welfare matter by covering all pest management alternatives into deliberation and encourages an equitable action plan that is eco-friendly, workable, inexpensive, and culturally appropriate, utilizing different frameworks for management of insect pests that cause problems in crop production (Dara 2019).

Pests are accountable for approximately 25–42% of losses in crop production. Substantial utilization of synthetic chemical pesticides causes pests to develop resistance against the chemicals and creates environmental pollution and hazards to the health of humans and other organisms. As a result, there is now a call to decrease chemical utilization in agricultural crop production, giving momentum for expansion of different ways of reduction pest populations. An acceptable alternative to chemical control is biocontrol, using microbes to destroy pests without causing harmful effects on the health of humans and the environment. Because of the complex modes of activity of microbes, it is difficult for pests to develop resistance against effective microbes even when they are used frequently. Use of bacteria, fungi, viruses, nematodes, and other microbes is now widespread and is achieving excellent results. Among all of these different types of microbe, fungal microbial agents have the greatest potential because of their easy transportability in appropriate formulations, the extensive number of pathogenic strains available, simple engineering skill and overexploitation of endogenous proteinous or exogenous contamination (St Leger and Wang 2009; Wang and St Leger 2007a; Butt et al. 2001; St Leger et al. 1996). Reason behind wide host assortment, direction of pathogenomic and capacity to control sap feeders and soft-bodied pests such as aphids (which feed on plants), mosquitos (which feed on human blood and act as a vector for disease), whitefly, jassids, thrips (which are phytophagous in crop ecosystems) (Thomas and Read 2007; Fan et al. 2007; Qazi and Khachatourians 2005; Butt 2002), and solid feeders such as various type of caterpillar (de Faria and Wraight 2007; Hajek and St Leger 1994). Several major pests belong to the orders Coleoptera, Lepidoptera, Orthoptera, Thysanoptera, Hemiptera, and Hymenoptera. Crop ecosystems that have been a targeted by different manage tactics covering microbial techniques with native or exotic biopesticides. Molds descend from a group of ancestral organisms that depended on complex organic substances for their nutrition, and they are eukaryotic pathogens of pests, utilizing the bodies of target hosts to complete part of their life cycle (Wraight et al. 2007; Samson et al. 1988). More than 700 entomopathogenic species have now been identified in the Chromista kingdom (Goettel et al. 2000). Other major valuable species include members of the Ascomycota phylum, the Hypocreales order, the Neozygitales order, and *Entomophthora* (which belongs to the Entomophthorales order). These fungi are outstanding candidates for microbial pest management (Hajek 1997; Roy et al. 2010). Even so, only a few members of these taxa—such as *Beauveria bassiana* Petch, *Metarhizium anisopliae* (Metschn.), *Lecanicillium lecanii* (Zimm.), and *Isaria fumosorosea* (Wize)—are vigorously marketed for application as biopesticides to obtain high yields of good-quality crops (Humber 2010). The significance

of entomopathogenic fungi in relation to agriculture and forest pests has recently been highlighted (Augustyniuk-Kram and Kram 2012).

11.2 Entomopathogenic Fungi

Entomopathogenic fungi can be extensively sprayed in both limited and broad host ranges for biocontrol of arthropods and insects on plants. These fungi were the first organisms to be applied as biopesticides for management of pests. Ninety genera, including more than 700 species, have been recognized as infective agents that can be used against insect pests (Khachatourians and Sohail 2008). The Ascomycota and Zygomycota are two important phyla in this regard. In the past, ascomycete fungi were classified into two subgroups: Ascomycota and Deuteromycota. The Deuteromycota (also known as "imperfect fungi") have no sexual stage in their life cycle. Molecular and cultural research has shown that some of these (which were previously formally classed among the Hyphomycetes in the Deuteromycota) are asexual Ascomycota that belong to the order Hypocreales in the family Clavicipitaceae (Hodge 2003; Fukatzu et al. 1997; Krasnoff et al. 1995). In the division Zygomycota, the majority of entomological fungi are part of the order Entomophthorales (Roy et al. 2006). Because they are saprotrophic in nature, these fungi fulfill their nutritional requirements by being rhizophagous and phyllophagous. Endophagous saprotrophic, hemibiotrophic, necrotrophic on greenery, fungal parasitic and few among them take on various econutritional food material.

11.3 Life Processes of Entomologically Useful Molds

During their life cycles, molds develop from spores and grow into mature fungi that produce spores. The life cycle of these fungi comprises two stages: normal growth of mycelia occurs in the stage of externally body on insect pests as well as yeast manners promising stage mainly fluid material of target host body such modification occurred manner of dimorphism way of growing in *Beauveria bassiana* (Alves et al. 2002), and elliptical blastospore-like propagules are produced by *Metarhizium flavoviride* (Fargues et al. 2002). The life cycle processes of green muscardine fungus have been studied in fluid media (Uribe and Khachatourians 2008). *Beauveria bassiana* absence in particular insect body grows via vegetative life-form of asexual condition connected emergence of thread form development and formation of fruiting bodies called sympoduloconidia. *Beauveria* conidiospores grow on the integument of the host insect and penetrate the cuticle as the fungal structure expands. It undergoes a change in its external shape, like a yeast stage, and creates mold fruiting bodies during its expansion, which are transported throughout the insect's body via its blood and kill it. When it is fully grown, the mold grows fully turn blackish to well develop mold structure convert in to saprotrophic decomposing

stage. The capability to change into a yeast form may be a precondition for pathogenesis of entomopathogenic fungi. *Verticillium lecanii*, *Beauveria bassiana*, and *Metarhizium anisopliae* are assiduously deliberate as normal bioagents and valuable for control of plant lice and other soft-bodied pests that feed on crops (Li and Sheng 2007; Thomas and Read 2007; Roberts and St Leger 2004; Milner 1997). White muscardine disease is caused by important entomopathogenic fungi, which contaminate nearly 95% of winged plant lice, particularly green peach aphids (*Myzus persicae*) (Chen et al. 2008). Both types of entomopathogenic fungus have dual biocontrol attributes, as they act as bioagents on target pests and as pathogens on plants (Goettel et al. 2008; Bonnie et al. 2009). Formulations of *Verticillium lecanii* can be applied for management of sucking pests, and it is also effective as an antagonistic mold for curing plant diseases such as powdery mold (Miller et al. 2004; Askary et al. 1997; Dik et al. 1998), decaying green fungus, *Fusarium*, *Verticillium dahliae*, and *Pythium ultimum* (Spencer and Atkey 1981; Benhamou and Brodeur 2000, 2001; Kusunoki et al. 2006). *Tritirachium shiotae* is announce as limiting of the expansion of this fungus outside of test tube, mass multiplication endophytically many crops and persuade integral resistance when microbes contaminate crops and decreases infections caused by soilborne microbes such as *Fusarium*, *Pythium* and *Rhizoctonia* (Ownley et al. 2010). Microspores of these molds are commonly eco-friendly, with insignificant or only minor toxicity to mammals and no residual effects (see Fig. 11.1) (Copping 2004), and they are effective fungal biopesticides against plant lice (Milner 1997; Shah and Pell 2003). To date, a few mycobiopesticides have been formulated and utilized in different countries, including the USA (Goettel et al. 2005; Kiss 2003). Mycobiopesticides have a broad range, without affecting other bioagents used against their target host or affecting economically important microbes with beneficial effects. They can also be used to manage different pests and plant diseases at the same time (Wraight and Carruthers 1999). Molds that are effective for management of agricultural pests are listed in Table 11.1.

11.4 Modes of Action of Fungi

The molds used to control insect pests are pathogenically dissimilar to bacteria and viruses. Normally, they enter the target host by damaging its cuticle. The integument is made up of polysaccharide glucosamine polyose fibrils inset with pigments, proteins, lipids, and N-acyl catecholamines (Richard et al. 2010). Extracellular fluid containing enzymes, lipases, proteases, and chitinases is released and degrades the main components of the cuticle (i.e., lipids, proteins, and chitin), allowing penetration of the fungus (Wang et al. 2005; Cho et al. 2006a). Lipase enzymes have complicated pathogen virulence and play various roles in the process of microbial infection (Stehr et al. 2003). Successful contamination is directly related to release of the extracellular enzymes (Khachatourians 1996). Such fungi are trusted for unemotional force and the action of enzymes are evolved hemocoel of the insect and

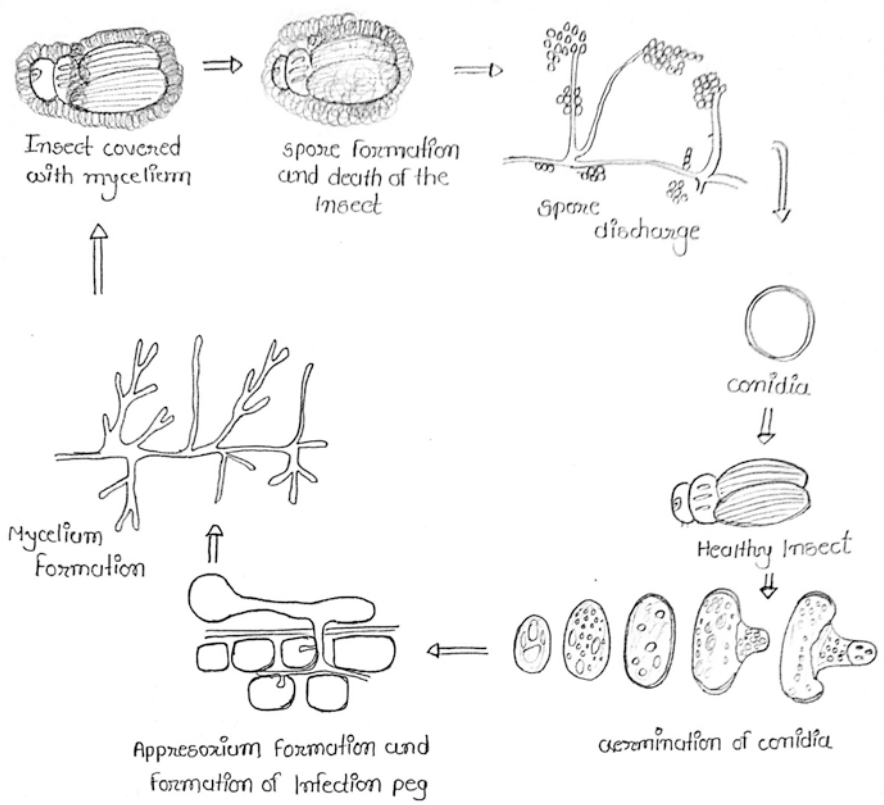


Fig. 11.1 Life cycle of an entomopathogenic fungi

enter via opening of the cuticle. An overview of the actions of chitinase, protease, and lipase enzymes in this pathological process is shown in Fig. 11.2. Exoenzymes produced by entomological molds have been shown to release toxic proteins and metabolites in vitro and in field conditions. At that place have few harmful content in extracted fungi with tiny collateral metabolites, cyclic peptides, and macroparticle proteins. White muscardine fungi have been shown to produce low molecular weight cyclic peptides and A-type and C-type cyclosporines with toxic attributes, such as bassianolide, enniatins, beauvericin (Vey et al. 2001; Roberts 1981), and oosporein, as well as cyclic peptides with immunosuppressant actions. A few biopesticides containing cyclic peptides such as beauvericin and bassianolide have been extracted from the same molds. One fungal strain has been used to create the high molecular weight toxic compound hirsutellin A for natural management of pests (Enrique and Alain. 2004). However, the main problem limiting the marketability of mycobiopesticides is that they take more time to kill their target hosts than chemical pesticides do (St Leger and Wang 2009). Great worked carried in point of view enhance as well as ameliorate acerbity of such mold to a bigger expanse than

Table 11.1 Commercially available entomopathogenic fungi used to control target pests

Fungal species	Trade names	Producers	Origins	Target pests	Hosts
Culicinomyces clavisporus			Austria, Belgium	Mosquito larvae	Humans
Hirsutella thompsonii	Mycar		Austria, Belgium	Citrus rust mites	Citrus
Metarhizium anisopliae	Meta-Sin, Green Muscle, Bioblast, Biomagic			Spittle bugs, sugarcane froghoppers, locusts, brown planthoppers, termites	Sugarcane, rice
Nomuraea rileyi				Lepidopteran larvae	Cotton
Verticillium lecanii	Vertalec			Aphids, coffee green bugs, greenhouse whiteflies, thrips	Greenhouse crops
Beauveria bassiana	Bio-Power	Stanes	India	Mites, coffee green bugs	Tea, coffee
	BotaniGard ES, BotaniGard 22WP	Laverlam International, Betel	USA	Scarab beetle larvae	Sugarcane
	Boverol	Fytovita	Czech Republic		
	Conidia	Live Systems Technology	Columbia		
	Naturalis	Intrachem	Italy	Aphids, spittle bugs, sugarcane	
Beauveria brongniartii (*Beauveria tenella*)	Beauveria Schweizer	Lbu (formerly Eric Schweizer Seeds)	Switzerland	Greenhouse whiteflies, thrips, mosquito larvae	Polyhouse pests
	Betel	Arysta	France	Scarab beetle larvae	Pasture
	Biolisa-Kamikir	Nitto Denko	Japan		
	Engerlingspilz	Andermatt Biocontrol	Switzerland		
	Melocont-Pilzgerste	Agrifutur-Kwizda	Italy, Austria		
Hirsutella thompsonii	Mycohit			Mites	Citrus

Data sources: Butt et al. (2001), Wraight et al. (2001), Copping (2004), Zimmermann (2007), and Khachatourians (1986)

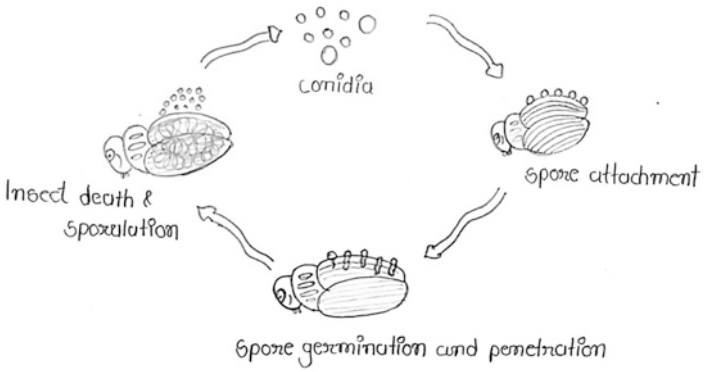


Fig. 11.2 Mode of action of an entomopathogenic fungi

its personal action that accelerates application of formulated products present in market. Excellent quantity of transcribe and gene modification work of entomopathogenic molds contamination procedure let out availability few different genes participate in the pathogenic action in the same way as chitinase enzymes (Cho et al. 2006b, 2007; Wang et al. 2005; Bagga et al. 2004). Guanine nucleotide–binding protein and its regulator (Fang et al. 2007, 2008), adhesin, aid the attachment of spores. A perilipin-like protein regulates appressorium turgor pressure and differentiation, and a cell protective coat protein helps the pathogen to avoid being recognized by the host's immune system (Wang and St Leger 2006). Likewise, increased virulence of entomopathogenic mold is seen with overexpression of virulence genes such as protease PR1A and subtilisin protease PII (Ahman et al. 2002; St Leger et al. 1996). A hybrid chitinase containing a chitin-binding domain (Wang and St Leger 2007b; Fan et al. 2007) alter tarantula nerve toxic peptide, applying the genetic code of *Metarhizium* particular under management of gene MC11 advocate for mold change. The 50% lethal concentration (LC_{50}) of the transgenic strain AaIT-Ma549 was decreased 22-fold when this strain was tested on Carolina sphinx moths (*Manduca sexta*) and 9-fold when it was tested on mosquitos (Wang and St Leger 2007b). When the AaIT-Ma549 strain was tested on coffee borer beetles (*Hypothenemus hampei*), the LC_{50} was decreased 15.7-fold and the mean survival time was reduced by 20% (Pava Ripoll et al. 2008).

11.5 White Muscardine Fungi (*Beauveria bassiana* (Clavicipitaceae))

This is a valuable microbe for use against insect pests. It has been used to develop biopesticides for management of many key pests that cause problems for agricultural crops and animals, and also for management of aquatic pests (Faria and Wraight 2007; Charnley and Collins 2007). These biopesticides are utilized mainly

against key lepidopteran, orthopteran, and hemipteran pests. Of the mycobiopesticides that are used commercially, 33.9% are based on *Beauveria bassiana*, 33.9% on *Metarhizium anisopliae*, 5.8% on *Isaria fumosorosea*, and 4.1% on *Beauveria brongniartii*. The increasing contribution of *Beauveria bassiana* to the biopesticide market means that the main obstacles that restrict its utilization as a mycobiopesticide are being overcome. Acting biotic strains of such mold frequently absence of enough antagonism to misfortune (St Leger and Wang 2009; Rangel et al. 2005; Ying and Feng 2004) inherited manipulate requisite upgrade their effectiveness as well as natural strength (Roberts and St Leger 2004). The significance of white muscardine, caused by *Beauveria brongniartii*, is easy to understand. Experimental work has been performed to develop more advanced formulations and to utilize mold inoculates to improve the potency of fungal biopesticides by gene moderation. Research on the potency of entomological molds has improved our understanding of the most relevant integument-degrading enzymes, which can then be further developed through overexpression in engineered strains for greater lethality to pests. It is likely that overexpression of the chitinase gene Bbchit1 increases the effectiveness of *Beauveria bassiana* against populations of *Myzus persicae* plant lice (St Leger and Wang 2009; Wraight et al. 2001; St Leger et al. 1996).

11.6 Fungi Utilized for Bioremediation

Molds can live in various environmental conditions. Complex soil matrices are the main setting for mold colonization, together with fresh as well as marine water that represent steady colonize of mold. They mostly flourish in soils in various weather conditions covering extremely virulent strains of antagonist via scattering of fruiting bodies in the air, which assists in maintaining the equilibrium of the environment (Anastasi et al. 2013). It has been reported that some fungi live in effluent treatment plants (ETPs) and treated sewage water (Badia-Fabregat et al. 2015; Zhang et al. 2013). The variety of their habitats and their capacity to produce a host of enzymes makes fungi capable of being useful for bioremediation in different settings. Molds degrade chemical molecules by causing minor structural modifications. This means they can detoxify insecticide residues in soil, in some instances enabling additionally degradation of such residues by bacteria. Some molds—such as *Auricularia auricula*, *Flammulina velupites*, *Agrocybe semiorbicularis*, *Coriolus versicolor*, *Pleurotus ostreatus*, *Stereum hirsutum*, *Dichomitus squalens*, Avatha, and *Hypholoma fasciculare* colorless—have the capability to degrade different groups of pesticides such as phenylureas, dicarboximides, phenylamides, triazines, and chlorinated and organophosphorus compounds. Some group of pesticides—such as dichlorodiphenyltrichloroethane (DDT), gamma-hexachlorocyclohexane, dieldrin, aldrin, heptachlor, chlordane, lindane, mirex, atrazine, diuron, terbuthylazine, and metalaxyl—have been shown to be degraded to various extents by white rot mold. This is sort out from soil habitat can decrease oil contamination (Das and Chandran 2011).

11.7 White Rot Mold (*Ceriporiopsis subvermispora*)

This is the main biodegrader of ligneous substances in the environment and plays a vital role in the carbon cycle. Human use of endocrine-disrupting chemicals, pharmaceuticals, and personal care products results in environmental effects such as bioaccumulation, severe toxicity to aquatic organisms, and possibly also unfavorable effects on human health. These problems have caused widespread concern and drawn considerable attention to the potential for degradation of these environmental contaminants by this type of fungus. Most of this research work has explored possibilities for bioremediation using the fungi *Trametes versicolor*, *Bjerkandera adjusta*, *Pleurotus* spp., and *Phanerochaete chrysosporium*, which produce various ligninolytic enzymes such as laccases and peroxidases (dos Santos Bazanella et al. 2013). The ligninolytic enzymes produced by white rot mold have been utilized for modification of a variety of forms of organic toxic waste (such as pesticides in polluted wastewater) by enhancing the microorganisms' activity in a biopurification system (Rodríguez-Rodríguez et al. 2013) unsettled to constricted entry enzymes ligninolytic for lignin particles are accumulated to exterior of lignocellulosic threads, compulsion purify is tested for detachment of thread in lignocellulosic substance. This approach intensifies the ligninolytic ability of enzymes produced by *Ceriporiopsis subvermispora*. In one study, this fungus was shown to manifest greater removal of lignin woody tissue when grown on pressure-refined *Miscanthus* than when grown on milled *Miscanthus* (Baker et al. 2015). Extracellular ligninolytic enzymes from fungi have the ability to adsorb color. Reports have described decolorization of Direct Blue 14 by species of *Pleurotus* and decolorization of Remazol Brilliant Blue-R by Agaricomycetes, a class of white rot fungus from the Amazon forest (Singh et al. 2013; dos Santos et al. 2015). Use of fungi such as *Hirschioporus larincinus*, *Phanerochaete chrysosporium*, *Phlebia tremellosa*, *Coriolus versicolor*, and *Inonotus hispidus* has been described for decolorization of colorant sewage spell 38 species of white rot fungi is express reason of decrease in total phenolics (60%) and color (B70%) from brown mill wastewater. Likewise, such types of fungus have been utilized for treatment of cresolate-polluted soil with bioaugmentation of two strains: *Lentinus tigrinus* and *Trametes versicolor* (Ntougias et al. 2015; Llado et al. 2013). In cresolate-polluted soil containing residual intractable crude oil, hydrocarbons, and high molecular weight fragment remains after biopiling treatment, effective decreases in the remaining contaminants can be achieved through biostimulation with the help of a lignocellulosic substratum bioaugmented with a suitable mold. There is always the possibility that such treatment will encourage growth of a community of more potent microorganisms, and so appropriate studies should be performed on a small scale prior to field utilization. In adding use enzyme ligninolytic for bioremediation of different compounds, another attributes asses has engage by fungi for discrediting of exchange of living composite at increase expulsion of planning (Fan et al. 2013; Purnomo et al. 2013; Cutright and Erdem 2012). Bearing in mind the attributes that are effective in bioremediation, enhancement of laccase production by the fungi *T. versicolor* and *Pleurotus*

ostreatus was studied via solid-state fermentation using orange peel waste come after by more distant treated of its capability for bioremediation of polycyclic aromatic hydrocarbons (PAHs) such as pyrene and phenanthrene (Rosales et al. 2013). Although a higher concentration of laccase was produced with use of *Trametes versicolor* media (3000 U/L) than with *Pleurotus ostreatus* media (2700 U/L), *Pleurotus ostreatus* manifested superior removal of phenanthrene and pyrene. Best comprehension as well as misuse of bioremediation strength mold to the complete, require for more experiment on that type fungi at genomic parameter.

11.8 Roles of Microbes in Biodegradation of Pesticide Molecules in the Environment

Various types of microbes have the capability to biodegrade pesticide residues. This is because different pesticides are generally utilized on agricultural crops, and soil is the substratum that is mainly contaminated by pesticide molecules, apart from pesticide production effluent, sewage sludge, activated sludge, wastewater, natural freshwater, sediment, the surroundings of pesticide manufacturing plants, and a few living organisms. Generally, microbes that are recognized as biodegrading chemicals come from a extensive variety of environments polluted with different pesticides. At present, in various laboratories around the world, there are collections of microbes selected for their ability to be cultured and to biodegrade pesticide compounds in the environment. Screening and identification of microbes that are capable of degrading pesticide compounds have been enhanced by newly developed techniques for polluted weather, count wastes prior to the final deposition. The activities of microorganisms reduce pollution in valuable ecosystems. Progress in pollutant degradation biotechnology depends on the basic sciences of microbiology and analytical geochemistry, which is used to assess the status of habitats. Latest key detection promotes information aromatic hydrocarbon biodegradation have depends on the attributes of microbes, pure-culture separate, laboratory enhancement media as well as polluted area of field. Recently developed systematics and molecular implements have intensified our awareness of the mechanisms (how), the events (what), and the specifications (who) of the microbes that are active in reducing pollution in organic systems. Pesticides that can be biodegraded by microbes are listed in Table 11.2.

11.9 Pesticide Biodegradation Mechanisms Used by Fungi

Microbes have the capacity to interrelate, both chemically and physically, with media in order to structurally modify or fully biodegrade selected particles. Among the different types of microbe, fungi and bacteria are the key participants in

biodegradation of chemical compounds (Briceño et al. 2007). Fungi usually biotransform pesticides and other xenobiotics by making minor structural modifications in their molecules that make them nontoxic. The biotransformed chemicals are released into the soil, where they are vulnerable to additional degradation by bacteria (Diez 2010). Fungi and bacteria are appraised as the extracellular enzyme releasing microorganisms for superiority. It has been suggested that white rot mold encourages the activity of biodegrading microbes, mostly against composites that

Table 11.2 Examples of pesticides that can be degraded by microorganisms

Types of microorganism	Microorganism species	Pesticides
Bacteria	*Pseudomonas*	Aldrin, chlorpyrifos, dichlorodiphenyltrichloroethane, endosulfan, endrin, BHC, monocrotophos (Verma et al. 2014); coumaphos (Upadhyay and Dutt 2017); diazinon, methyl parathion, parathion (Verma et al. 2014; Upadhyay and Dutt 2017)
	Bacillus	Parathion glyphosate, methyl parathion, chlorpyrifos (Verma et al. 2014; Upadhyay and Dutt 2017); coumaphos (Upadhyay and Dutt 2017); diazinon, dichlorodiphenyltrichloroethane, dieldrin, endosulfan, endrin, monocrotophos, polycyclic aromatic hydrocarbons (Verma et al. 2014)
	Alcaligenes	Chlorpyrifos (Verma et al. 2014); endosulfan (Jayabarath et al. 2010)
	Flavobacterium	Diazinon, glyphosate, methyl parathion, parathion (Upadhyay and Dutt 2017)
Fungi	White rot fungi, *Rhizopus*, *Cladosporium*, *Aspergillus fumigatus*, *Penicillium*, *Aspergillus* spp., *Fusarium*, *Mucor*, *Trichoderma*, *Mortierella*	Alachlor, aldicarb (Hai et al. 2012); atrazine (Hai et al. 2012; Elgueta et al. 2016); pentachlorophenol, malathion, carbofuran, chlordane, chlorpyrifos, dichlorodiphenyltrichloroethane, 2,4-dichlorophenoxyacetic acid (Maloney 2001); diuron (Bending et al. 2002); endosulfan (Bhandari 2017; Romero-Aguilar et al. 2014; Kataoka et al. 2010); fenvalerate (Birolli et al. 2016); fenitrothion, fenitrooxon (Baarschers and Heitland 1986); fipronil (Wolfand et al. 2016); heptachlor epoxide (Xiao et al. 2012); lindane (Maloney 2001; Sagar and Singh 2011); metalaxyl (Martins et al. 2017); terbuthylazine (Bending et al. 2002)
Actinomycetes	*Micromonospora*, *Actinomyces*, *Nocardia*, *Streptomyces*	Aldrin (Verma et al. 2014); carbofuran (Jayabarath et al. 2010); chlorpyrifos (Verma et al. 2014; Briceño et al. 2018); diazinon (Briceño et al. 2018); diuron (Esposito et al. 1998)
Algae	Small green algae	Phorate, parathion (Tang 2018)
	Chlamydomonas	Atrazine (Kabra et al. 2014); fenvalerate (Day and Kaushik 1987)
	Diatoms	Dichlorodiphenyltrichloroethane, patoran (Shehata et al. 1997)

are not easily broken down by bacteria. This capacity arises from mass formation of extracellular enzymes that act on a wide variety of organic composites. A few of these are involved in degradation of lignin, such as manganese peroxidases, lignin peroxidases, laccases, and oxidases. Some bacterial degrading pesticide molecules have sort out and increase in size rapidly recorded. Three major enzyme families involved in pesticide resistance are glutathione S-transferases (GSTs), cytochrome P450 monooxygenases, and esterases (Bass and Field 2011).

11.10 Genetic Modifications for Pesticide Degradation

In order to inspect genetic assist in pesticide molecule biodegradation, some research having special prominence part of declining genes as well as utilization of fingerprint DNA technology, has describe. A classification of pesticides on the basis of their composition is given in Table 11.3. Its residues discrediting genes found in of only a few microbes having this attributed. The majority of genes that control biodegradation are found on the chromosomal body; only in a few cases do such genes occur in transposons or plasmids. The latest progress in metagenomics and full genome succession has pioneered new directions for discovery of novel toxic waste degradation genes and their governing components from culturable and non-culturable microbes in the environment. Plasmids and transposons adoptable genetic component have reflected to make secret enzymatic that control degradation of a few types of pesticide residue. The discovery of pesticide molecule–degrading microorganisms and description of genes encoding enzymes that degrade pesticide compounds, combinations of new methods for screening, and examination of nucleic acids from soil microbes provides new insights into the molecular mechanisms that enable them to evolve more potent ability to degrade pesticide residues.

11.11 Plan of Action for Intensifying Effectiveness for Pesticide Degradation: Cell Immobilization

It engages for biological control decline of pesticide compounds since, grants to chance provide for catalytic action for prolong duration. Fully cell immobilize has reflect into astonish lead on regular biocontrol applying free cells, probability utilize a density of high cell, avoid washout cell, smoothly good infusion rates, uncomplicated sorting of cells through reaction system, frequent application of cells, get better defense of cells from rough climatic conditions. Previous reports have proposed that these increased productiveness outcomes of cellular or genetic quality are due to immobilization. There is evidence that immobilized cells are much more tolerant of disruptions in climatic conditions and less vulnerable to toxic materials, making immobilized cell systems particularly attractive for treatment of compounds

Table 11.3 Classification of pesticides on the basis of their chemical composition

Groups	Main composition
Organochlorines	Carbon atoms, chlorine, hydrogen, and occasionally oxygen; they are nonpolar and lipophilic
Organophosphates	Molecules contain a central phosphorus atom; in comparison with organochlorines, these compounds are more stable and less toxic in the environment; organophosphate pesticides can be aliphatic, cyclic, and heterocyclic
Carbamates	Chemical structure based on a plant (*Physostigma venenosum*) alkaloid
Pyrethroids	Compounds similar to synthetic pyrethrins (alkaloids obtained from *Chysanthemun cinerariefolium* petals)
Botanical origin	Products derived directly from plants, not chemically synthesized
Biological agents	Viruses, microorganisms, or their metabolic products
Copper compounds	Inorganic compounds of copper
Thiocarbamates	Different from carbamates in their molecular structure, containing an S-group in their composition
Organotin compounds	Molecules contain a central tin atom
Organosulfur compounds	Molecules contain a central sulfur atom; very toxic to mites and insects
Dinitrophenols	Recognized by the presence of two nitro groups (NO_2) bonded to a phenol ring
Urea derivatives	Include urea bound to aromatic compounds
Diverse composition	Triazines, talimides, carboxyamides, trichloroacetic and trichloropicolinic acid derivatives, guanidines, naphthoquinones

Data source: Ortiz-Hernández (2002)

such as pesticide residues (Ha et al. 2008). The reason why immobilized cells are more efficient at degradation is that they are protected from inhibitory substances in the substrate solution. In repeated operations, the rates of degradation of successive batches increase; this suggests that over time, the cells become better adapted to the reaction conditions (Ha et al. 2009).

11.12 Conclusion

Entomopathogenic fungi have excellent potential for specificity in pest management without harming the predators of those pests and useful parasites. Unlike widespread application of pesticides, use of appropriately selected fungi for pest control poses no risk to the health of ecosystems or mammals. Because they have different modes of entry into insect bodies, the insects are unable to develop resistance to them, and they are effective for pest management over a long duration by having genes release toxic formation in host responsible good capability for additional evolution of biotechnical experimental works. Several endophytic fungi play a crucial role in activating the defence system in host. Selection of appropriate fungi

for use in a particular habitat achieves more durable results in defeating pests. Microbial degradation work on pesticide molecules had been considerable expand with pesticide substance decline microbial strains recognized but its utilization for microbial bioremediation restricted, for less degradable effect in habitat. Mineralization and metabolism are key mechanisms in degradation of pesticide compounds, as well as their intermediate outcomes; the overall and component structures of pesticides determine the ways in which they are degraded in the microbial habitat. Their chemical components determine their solubility, and attributes such as particle attraction, the dimensional structure, chemical functional groups, and attraction/repulsion between molecules determine whether pesticide compounds can be ingested by microbes. Leading research work in this area is focused on microbial degradation of pesticides by highly effective cultures combining pesticide-degrading bacteria and fungi, inability of degrading microorganisms, novel experiments with pesticide compound–degrading molds, and insecticide molecule biodegradation strategies.

References

Ahman J, Johansson T, Olsson M, Punt PJ, van den Hondel CA, Tunlid A (2002) Improving the pathogenicity of a nematode trapping fungus by genetic engineering of a subtilisin with nematotoxic activity. Appl Environ Microbiol 73:295–302

Alves SB, Rossi LS, Lopes RB, Tamai MA, Pereira RM (2002) *Beauveria bassiana* yeast phase on agar medium and its pathogenicity against *Diatraea saccharalis* (Lepidoptera: Crambidae) and *Tetranychus urticae* (Acari: Tetranychidae). J Invertebr Pathol 81:70–77. https://doi.org/10.1016/S0022-2011(02)00147-7

Anastasi A, Tigini V, Varese GC (2013) The bioremediation potential of different ecophysiological groups of fungi. In: Goltapeh EM, Danesh YR, Varma A (eds) Fungi as bioremediators. Springer, Berlin, pp 29–49

Askary H, Benhamou N, Brodeur J (1997) Ultrastructural and cytochemical investigations of the antagonistic effect of *Verticillium lecanii* on cucumber powdery mildew. Phytopathology 87:359–368. https://doi.org/10.1094/PHYTO.1997.87.3.359

Augustyniuk-Kram A, Kram KJ (2012) Entomopathogenic fungi as an important natural regulator of insect outbreaks in forests (review). In: Blanco JA, Lo YH (eds) Forest ecosystems—more than just trees. InTechOpen, London, pp 265–294

Baarschers WH, Heitland HS (1986) Biodegradation of fenitrothion and fenitrooxon by the fungus *Trichoderma viride*. J Agric Food Chem 34:707–709

Badia-Fabregat M, Lucas D, Gros M, Rodríguez-Mozaz S, Barceló D, Caminal G, Vicent T (2015) Identification of some factors affecting pharmaceutical active compounds (PhACs) removal in real wastewater: case study of fungal treatment of reverse osmosis concentrate. J Hazard Mater 283:663–671

Bagga S, Hu G, Screen SE, St Leger RJ (2004) Reconstructing the diversification of subtilisins in the pathogenic fungus *Metarhizium anisopliae*. Gene 324:159–169. https://doi.org/10.1016/j.gene.2003.09.031

Baker PW, Charlton A, Hale MD (2015) Increased delignification by white-rot fungi after pressure refining *Miscanthus*. Bioresour Technol 189:81–86. https://doi.org/10.1016/j.biortech.2015.03.056

Bass C, Field LM (2011) Gene amplification and insecticide resistance. Pest Manag Sci 67(8):886–890. https://doi.org/10.1002/ps.2189

Bending GD, Friloux M, Walker A (2002) Degradation of contrasting pesticides by white rot fungi and its relationship with ligninolytic potential. FEMS Microbiol Lett 212:59–63

Benhamou N, Brodeur J (2000) Evidence of antibiosis and induced host defense reaction in the interaction between *Verticillium lecanii* and *Penicillium digitatum*, the causal agent of green mold. Phytopathology 90:932–943. https://doi.org/10.1094/PHYTO.2000.90.9.932

Benhamou N, Brodeur J (2001) Pre-inoculation of RiT-DNA transformed cucumber roots with the mycoparasite, Verticillium lecanii, induces host defense reactions against Pythium ultimum infection. Physiol Mol Plant Pathol 58:133–146. https://doi.org/10.1006/pmpp.2001.0322

Bhandari G (2017) Mycoremediation: an eco-friendly approach for degradation of pesticides. In: Prasad R (ed) Mycoremediation and environmental sustainability. Springer, Cham, pp 119–131

Birolli WG, Alvarenga N, Seleghim MH, Porto AL (2016) Biodegradation of the pyrethroid pesticide esfenvalerate by marine-derived fungi. Mar Biotechnol 18(4):511–520

Bonnie H, Kimberly D, Fernando E (2009) Endophytic fungal entomopathogens with activity against plant pathogens: ecology and evolution. Biol Control 55:113–128

Briceño G, Palma G, Duran N (2007) Influence of organic amendment on the biodegradation and movement of pesticides. Crit Rev Environ Sci Technol 37:233–271. https://doi.org/10.1080/10643380600987406

Briceño G, Vergara K, Schalchli H, Palma G, Tortell G, Fuentes MS, Diez MC (2018) Organophosphorus pesticide mixture removal from environmental matrices by a soil streptomyces mixed culture. Environ Sci Pollut Res Int 25(22):21296–21307. https://doi.org/10.1007/s11356-017-9790-y

Butt TM (2002) Use of entomogenous fungi for the control of insect pests. In: Esser K, Bennett JW (eds) Agricultural applications. The Mycota, vol 11. Springer, Berlin, pp 111–134

Butt TM, Jackson C, Magan N (2001) Introduction—fungal biological control agents: progress, problems and potential. In: Butt T, Jackson C, Magan N (eds) Fungi as biocontrol agents: progress, problems and potential. CAB International, Wallingford, pp 1–8

Charnley A, Collins SA (2007) Entomopathogenic fungi and their role in pest control. In: Howard DH, Miller JD (eds) The mycota IV: environmental and microbial relationships. Springer, Berlin, pp 159–187

Chen C, Li ZY, Feng MG (2008) Occurrence of entomopathogenic fungi in migratory alate aphids in Yunnan Province of China. Biol Control 53:317–326. https://doi.org/10.1007/s10526-006-9063

Cho EM, Boucias D, Keyhani NO (2006a) EST analysis of cDNA libraries from the entomopathogenic fungus *Beauveria* (Cordyceps) *bassiana*. II. Fungal cells sporulating on chitin and producing oosporein. Microbiology 152:2855–2864. https://doi.org/10.1099/mic.0.28845-0

Cho EM, Liu L, Farmerie W, Keyhani NO (2006b) EST analysis of cDNA libraries from the entomopathogenic fungus *Beauveria* (Cordyceps) *bassiana*. I. Evidence for stage-specific gene expression in aerial conidia, in vitro blastospores and submerged conidia. Microbiology 152:2843–2854. https://doi.org/10.1099/mic.0.28844-0

Cho EM, Kirkland BH, Holder DJ, Keyhani NO (2007) Phage display cDNA cloning and expression analysis of hydrophobins from the entomopathogenic fungus *Beauveria* (Cordyceps) *bassiana*. Microbiology 153:3438–3447. https://doi.org/10.1099/mic.0.2007/008532-0

Copping LG (2004) The manual of biocontrol agents, British Crop Protection Council. Crop Prot 23:275–285

Cutright TJ, Erdem Z (2012) Overview of the bioremediation and the degradation pathways of DDT. J Adnan Menderes Univ Agric Fac 9:39–45

Dara SK (2019) The new integrated pest management paradigm for the modern age. J Integr Pest Manag 10(1):12

Das N, Chandran P (2011) Microbial degradation of petroleum hydrocarbon contaminants: an overview. Biotechnol Res Int 2011:941810. https://doi.org/10.4061/2011/941810

Day K, Kaushik NK (1987) The adsorption of fenvalerate to laboratory glassware and the alga *Chlamydomonas reinhardii*, and its effect on uptake of the pesticide by *Daphnia galeata mendotae*. Aquat Toxicol (10):131–142

de Faria MR, Wraight SP (2007) Mycoinsecticides and mycoacaricides: a comprehensive list with worldwide coverage and international classification of formulation types. Biol Control 43:237–256. https://doi.org/10.1016/j.biocontrol.2007.08.001

Diez MC (2010) Biological aspects involved in the degradation of organic pollutants. J Soil Sci Plant Nutr 10(3):244–267

Dik AJ, Verhaar MA, Be Langer RR (1998) Comparison of three biological control agents against cucumber powdery mildew (*Sphaerotheca fuliginea*) in semi commercial-scale glasshouse trials. Eur J Plant Pathol 104:413–423. https://doi.org/10.1023/A:1008025416672

dos Santos Bazanella GC, Araujo AV, Castoldi R, Maciel GM, Inacio FD, de Souza CGM, Bracht A, Peralta RM (2013) Ligninolytic enzymes from white-rot fungi and application in the removal of synthetic dyes. In: Polizeli TM, Rai M, De Lourdes M (eds) Fungal enzymes. CRC, Boca Raton, pp 258–279

dos Santos YVS, Freire DA, Pinheiro SB, de Lima LF, de Souza JVB, Cavallazzi JRP (2015) Production of laccase from a white rot fungi isolated from the Amazon forest for oxidation of Remazol Brilliant Blue-R. Sci Res Essays 10:132–136. https://doi.org/10.5897/SRE2013.5695

Elgueta S, Santos C, Lima N, Diez MC (2016) Immobilization of the white-rot fungus *Anthracophyllum discolor* to degrade the herbicide atrazine. AMB Express 6:104

Enrique Q, Alain VEY (2004) Bassiacridin, a protein toxic for locusts secreted by the entomopathogenic fungus *Beauveria bassiana*. Mycol Res 108(4):441–452. https://doi.org/10.1017/S0953756204009724

Esposito E, Paulillo SM, Manfio GP (1998) Biodegradation of the herbicide diuron in soil by indigenous actinomycetes. Chemosphere 37:541–548

Fan Y, Fang W, Guo S, Pei X, Zhang Y, Xiao Y, Li D, Jin K, Bidochka MJ, Pei Y (2007) Increased insect virulence in *Beauveria bassiana* strains overexpressing an engineered chitinase. Appl Environ Microbiol 73:295–302. https://doi.org/10.1128/AEM.01974-06

Fan B, Zhao Y, Mo G, Ma W, Wu J (2013) Co-remediation of DDT-contaminated soil using white-rot fungi and laccase extract from white-rot fungi. J Soils Sediments 13:1232–1245

Fang W, Pei Y, Bidochka MJ (2007) A regulator of a G protein signalling (RGS) gene, cag8, from the insect-pathogenic fungus *Metarhizium anisopliae* is involved in conidiation, virulence and hydrophobin synthesis. Microbiology 153:1017–1025. https://doi.org/10.1099/mic.0.2006/002105-0

Fang W, Scully LR, Zhang L, Pei Y, Bidochka MJ (2008) Implication of a regulator of G protein signalling (BbRGS1) in conidiation and conidial thermo tolerance of the insect pathogenic fungus *Beauveria bassiana*. FEMS Microbiol Lett 279:146–156. https://doi.org/10.1111/j.1574-6968.2007.00978.x

Fargues J, Smiths N, Viial C, Vey A, Vega F, Mercadier G, Quimby P (2002) Effect of liquid culture media on morphology, growth, propagule production, and pathogenic activity of the hyphomycete, *Metarhizium flavoviride*. Mycopathologia 154:127–138

Fukatzu T, Sato H, Kuriyama H (1997) Isolation, inoculation to insect host, and molecular phylogeny of an entomogenous fungus *Paecilomyces tenuipes*. J Invertebr Pathol 70:203–208

Goettel M, Inglis G, Wraight S (2000) Fungi. In: Lacey L, Kaya H (eds) Field manual of techniques in invertebrate pathology, 1st edn. Kluwer, London, pp 255–282

Goettel MS, Eilenberg J, Glare TR (2005) Entomopathogenic fungi and their role in regulation of insect populations. In: Gilbert LI, Iatrou K, Gill S (eds) Comprehensive molecular insect science. Elsevier, Amsterdam, pp 361–406

Goettel MS, Koike M, Kim JJ, Aiuchi D, Shinya R, Brodeur J (2008) Potential of *Lecanicillium* spp. for management of insects, nematodes and plant diseases. J Invertebr Pathol 98:256–261

Ha J, Engler CR, Lee SJ (2008) Determination of diffusion coefficients and diffusion characteristics for chlorferon and diethylthiophosphate in Ca-alginate gel beads. Biotechnol Bioeng 100(4):698–706

Ha J, Engler CR, Wild J (2009) Biodegradation of coumaphos, chlorferon, and diethylthiophosphate using bacteria immobilized in Ca-alginate gel beads. Bioresour Technol 100:1138–1142

Hai FI, Modin O, Yamamoto K, Fukushi K, Nakajima F, Nghiem LD (2012) Pesticide removal by a mixed culture of bacteria and white-rot fungi. J Taiwan Inst Chem Eng 43:459–462

Hajek A (1997) Ecology of terrestrial fungal entomopathogens. In: Jones GJ (ed) Advances in microbial ecology, 1st edn. Springer, New York, pp 193–249

Hajek AE, St Leger RJ (1994) Interactions between fungal athogenesis and insect hosts. Annu Rev Entomol 39:293–322. https://doi.org/10.1146/annurev.en.39.010194.001453

Hodge KT (2003) Clavicipitaceous anamorphs. In: White JF, Bacon CW, Hywel-Jones NL, Spatafora JW (eds) Clavicipitalean fungi: evolutionary biology, chemistry, biocontrol and cultural impacts. Marcel Dekker, New York, pp 75–123. https://doi.org/10.1201/9780203912706.ch3

Humber R (2010) Seeking stability for research and applied uses of entomopathogenic fungi as biological control agents. J Asia Pac Entomol 19:1019–1025

Jayabarath J, Musfira SA, Giridhar R, Arulmurugan R (2010) Biodegradation of carbofuran pesticide by saline soil actinomycetes. Int J Biotechnol Biochem 6:187–193

Kabra AN, Ji MK, Choi J, Kim JR, Govindwar SP, Jeon BH (2014) Toxicity of atrazine and its bioaccumulation and biodegradation in a green microalga, *Chlamydomonas mexicana*. Environ Sci Pollut (21):12270–12278

Kataoka R, Takagi K, Sakakibara FA (2010) New endosulfan-degrading fungus, *Mortierella* species, isolated from a soil contaminated with organochlorine pesticides. J Pestic Sci 35:326–332

Khachatourians GG (1986) Production and use of biological pest control agents. Tibtech 12:120–124. https://doi.org/10.1016/0167-7799(86)90144-7

Khachatourians GG (1996) Biochemistry and molecular biology of entomopathogenic fungi. In: Howard DH, Miller JD (eds), Human and animal relationships, Mycota VI, Springer, Heidelberg, pp 331–363

Khachatourians GG, Sohail SQ (2008) Entomopathogenic fungi: biochemistry and molecular biology. In: Brakhage AA, Zipfel PF (eds) Human and animal relationships. The Mycota VI, 2nd edn, Berlin, Springer, pp 33–61

Kiss L (2003) A review of fungal antagonists of powdery mildews and their potential as biocontrol agents. Pest Manag Sci 59:475–483. https://doi.org/10.1002/ps.689

Krasnoff SB, Watson DW, Gibson DM, Kwan EC (1995) Behavioral effects of the entomopathogenic fungus, *Entomophthora muscae* on its host *Musca domestica*: postural changes in dying hosts and gated pattern of mortality. J Insect Physiol 41:895–903. https://doi.org/10.1016/0022-1910(95)00026-Q

Kusunoki K, Kawai A, Aiuchi D, Koike M, Tani M, Kuramochi K (2006) Biological control of *Verticillium* black-spot of Japanese radish by entomopathogenic *Verticillium lecanii* (*Lecanicillium* spp.). Res Bull Obihiro Univ 27:99–107

Li W, Sheng C (2007) Occurrence and distribution of entomophthoralean fungi infecting aphids in mainland China. Biocon Sci Technol 17:433–439. https://doi.org/10.1080/09583150701213802

Lladó S, Covino S, Solanas AM, Vinas M, Petruccioli M, Dannibale A (2013) Comparative assessment of bioremediation approaches to highly recalcitrant PAH degradation in a real industrial polluted soil. J Hazard Mater 248–249:407–414. https://doi.org/10.1016/j.jhazmat.2013.01.020

Maloney SE (2001) Pesticide degradation. In: Gadd GM (ed) Fungi in bioremediation. The British Mycological Society, Cambridge, pp 188–223

Martins MR, Santos C, Pereira P, Cruz-Morais J, Lima N (2017) Metalaxyl degradation by *Mucorales* strains *Gongronella* sp. and *Rhizopus oryzae*. Molecules (22):2225

Miller TC, Gubler WD, Laemmlen FF, Geng S, Rizzo DM (2004) Potential for using *Lecanicillium lecanii* for suppression of strawberry powdery mildew. Biocon Sci Technol 14:215–220. https://doi.org/10.1080/09583150310001639204

Milner RJ (1997) Prospects for biopesticides for aphid control. Entomophaga 42:227–239. https://doi.org/10.1007/BF02769900

Ntougias S, Baldrian P, Ehaliotis C, Nerud F, Merhautová V, Zervakis GI (2015) Olive mill wastewater biodegradation potential of white-rot fungi—mode of action of fungal culture extracts and effects of ligninolytic enzymes. Bioresour Technol 189:121–130

Ortiz-Hernández ML (2002) Biodegradación de plaguicidas organofosforados por nuevas bacterias aisladas del suelo. Thesis, Universidad Autónoma del Estado de Morelos

Ownley BH, Pereira RM, Klineman WE, Quigley NB, Leckie BM (2004) *Beauveria bassiana*, a dual purpose biocontrol organism, with activity against insect pests and plant pathogens. In:

Lartey RT, Caeser A (eds) Emerging concepts in plant health management. Research Signpost, Kerala, pp 255–269
Ownley BH, Kimberly DG, Fernando EV (2010) Endophytic fungal entomopathogens with activity against plant pathogens: ecology and evolution. BioControl 55:113–128. https://doi.org/10.1007/s10526-009-9241-x
Pava-Ripoll M, Posada F, Momen B, Wang C, St Leger RJ (2008) Increased pathogenicity against coffee berry borer, *Hypothenemus hampei* (Coleoptera: Curculionidae) by *Metarhizium anisopliae* expressing the scorpion toxin (AaIT) gene. J Invertebr Pathol 99(2):220–226. https://doi.org/10.1016/j.jip.2008.05.004
Purnomo AS, Mori T, Putra SR, Kondo R (2013) Biotransformation of heptachlor and heptachlor epoxide by white-rot fungus *Pleurotus ostreatus*. Int Biodeterior Biodegradation 82:40–44
Qazi SS, Khachatourians GG (2005) Insect pests of Pakistan and their management practices: prospects for the use of entomopathogenic fungi. Biopest Int 1:13–24
Rangel DE, Braga GU, Anderson AJ, Roberts DW (2005) Influence of growth environment on tolerance to UVB radiation, germination speed, and morphology of *Metarhizium anisopliae* var. *acridum* conidia. J Invertebr Pathol 90:55–58. https://doi.org/10.1016/j.jip.2005.05.005
Richard JS, Neal TD, Karl JK, Michael RK (2010) Model reactions for insect cuticle sclerotization: participation of amino groups in the cross-linking of *Manduca sexta* cuticle protein MsCP36. Insect Biochem Mol Biol 40:252–258
Roberts DW (1981) Toxins of entomopathogenic fungi. In: Burges HD (ed) Microbial control of pests and plant diseases 1970–1980. Academic, New York, pp 441–464
Roberts DW, St Leger RJ (2004) *Metarhizium* spp., cosmopolitan insect-pathogenic fungi: mycological aspects. Adv Appl Microbiol 54:1–70. https://doi.org/10.1016/S0065-2164(04)54001-7
Rodríguez-Rodríguez CE, Castro-Gutiérrez V, Chin-Pampillo JS, Ruiz-Hidalgo K (2013) On-farm biopurification systems: role of white-rot fungi in depuration of pesticide-containing wastewaters. FEMS Microbiol Lett 345:1–12. https://doi.org/10.1111/1574-6968.12161
Romero-Aguilar M, Tovar-Sánchez E, Sánchez-Salinas E, Mussali-Galante P, Sánchez-Meza JC, Castrejón-Godínez ML, Dantán-González E, Trujillo-Vera MA, Ortiz-Hernández ML (2014) *Penicillium* sp. as an organism that degrades endosulfan and reduces its genotoxic effects. Springer Plus 3:536
Rosales E, Pazos M, Ángeles Sanromán M (2013) Feasibility of solid-state fermentation using spent fungi-substrate in the biodegradation of PAHs. Clean Soil Air Water 41:610–615. https://doi.org/10.1002/clen.201100305
Roy HE, Steinkraus DC, Eilenberg J, Hajek AE, Pell JK (2006) Bizarre interactions and endgames: entomopathogenic fungi and their arthropod hosts. Annu Rev Entomol 51:331–357. https://doi.org/10.1146/annurev.ento.51.110104.150941
Roy H, Brodie E, Chandler D, Goettel M, Pell J, Wajnberg E, Vega F (2010) Deep space and hidden depths: understanding the evolution and ecology of fungal entomopathogens. Biol Control 55:1–6
Sagar V, Singh DP (2011) Biodegradation of lindane pesticide by non-white-rot soil fungus *Fusarium* sp. World J Microbiol Biotechnol (27):1747–1754
Samson R, Evans HC, Latgé JP (1988) Atlas of entomopathogenic fungi, 1st edn. Springer, Berlin/Heidelberg, pp 187
Shah PA, Pell JK (2003) Entomopathogenic fungi as biological control agents. Appl Microbiol Biotechnol 61:413–423
Shehata SA, El-Dib MA, Waly HA (1997) Effect of certain herbicides on the growth of freshwater algae. Water Air Soil Pollut (100):1–12
Singh MP, Vishwakarma SK, Srivastava AK (2013) Bioremediation of Direct Blue 14 and extracellular ligninolytic enzyme production by white-rot fungi: *Pleurotus* sp. Biomed Res Int 2013:180156. https://doi.org/10.1155/2013/180156
Spencer DM, Atkey PT (1981) Parasitic effects of *Verticillium lecanii* on two rust fungi. Trans Br Mycol Soc 77:535–542. https://doi.org/10.1016/S0007-1536(81)80101-5
St Leger RJ, Wang C (2009) Entomopathogenic fungi and the genomic era. In: Stock SP, Vandenberg J, Glazer I, Boemare N (eds) Insect pathogens: molecular approaches and techniques. CAB International, Wallingford, pp 366–400

St Leger RJ, Joshi L, Bidochka MJ, Roberts DW (1996) Construction of an improved mycoinsecticide overexpressing a toxic protease. Proc Natl Acad Sci U S A 93:6349–6354

Stehr F, Kretschmar M, Kroger C, Hube B, Schafer W (2003) Microbial lipases as virulence factors. J Mol Catal B Enzym 22:347–355. https://doi.org/10.1016/S1381-1177(03)00049-3

Tang W (2018) Research progress of microbial degradation of organophosphorus pesticides. Prog Appl Microbiol (1):29–35

Thomas MB, Read AF (2007) Can fungal biopesticides control malaria? Nat Rev Microbiol 5:377–383

Upadhyay LS, Dutt A (2017) Microbial detoxification of residual organophosphate pesticides in agricultural practices. In: Patra J, Vishnuprasad C, Das G (eds) Microbial biotechnology. Springer, Singapore, pp 225–242

Uribe D, Khachatourians GG (2008) Identification and characterization of an alternative oxidase in the entomopathogenic fungus *Metarhizium anisopliae*. Can J Microbiol 54:1–9. https://doi.org/10.1139/W07127

Verma JP, Jaiswal DK, Sagar R (2014) Pesticide relevance and their microbial degradation: a-state-of-art. Rev Environ Sci Technol 13:429–466

Vey A, Hoagland R, Butt TM (2001) Toxic metabolites of fungal biocontrol agents. In: Butt TM, Jackson CW, Magan N (eds) Fungi as biocontrol agents. CAB International, Wallingford, pp 311–345

Wang MY, Shao Z (2006) Pseudomonas, the dominant polycyclic aromatic hydrocarbon-degrading bacteria isolated from antarctic soils and the role of large plasmids in horizontal gene tansfer. Environ Microbiol (8):455–465

Wang C, St Leger RJ (2007a) The *Metarhizium anisopliae* perilipin homolog MPL1 regulates lipid metabolism, appressorial turgor pressure, and virulence. J Biol Chem 282:21110–21115. https://doi.org/10.1074/jbc.M609592200

Wang C, St Leger RJ (2007b) A scorpion neurotoxin increases the potency of a fungal insecticide. Nat Biotechnol 25(12):1455–1456. https://doi.org/10.1038/nbt1357

Wang C, Hu G, St Leger RJ (2005) Differential gene expression by *Metarhizium anisopliae* growing in root exudates and host (*Manduca sexta*) cuticle or hemolymph reveals mechanisms of physiological adaptation. Fungal Genet Biol 42(8):704–718. https://doi.org/10.1016/j.fgb.2005.04.006

Wolfand JM, LeFevre GH, Luthy RG (2016) Metabolization and degradation kinetics of the urban-use pesticide fipronil by white rot fungus *Trametes versicolor*. Environ Sci Process Impacts (18):1256–1265

Wraight SP, Carruthers RI (1999) Production, delivery and use of mycoinsecticides for control of insect pests of field crops. In: Hall FR, Menn JJ (eds) Biopesticides: use and delivery. Humana, Totowa, pp 233–269

Wraight SP, Jackson MA, de Kock SL (2001) Production, stabilization and formulation of fungal biological agents. In: Butt TM, Jackson C, Magan N (eds) Fungi as biocontrol agents. CAB International, Wallingford, pp 253–287. https://doi.org/10.1079/9780851993560.0253

Wraight S, Inglis G, Goettel MS (2007) Fungi. In: Lacey L, Kaya H (eds) Field manual of techniques in invertebrate pathology 2nd edn. Dordrecht: Springer; pp 223–248

Xiao PF, Mori T, Kondo R (2012) Bioconversion of heptachlor epoxide by wood-decay fungi and detection of metabolites. Adv Mater Res (518):29–33

Ying SH, Feng MG (2004) Relationship between thermo tolerance and hydrophobin-like proteins in aerial conidia of *Beauveria bassiana* and *Paecilomyces fumosoroseus* as fungal biocontrol agents. J Appl Microbiol 97:323–331. https://doi.org/10.1111/j.1365-2672.2004.02311.x

Zhang Y, Xie J, Liu M, Tian Z, He Z, van Nostrand JD, Ren L, Zhou J, Yang M (2013) Microbial community functional structure in response to antibiotics in pharmaceutical wastewater treatment systems. Water Res 47:6298–6308. https://doi.org/10.1016/j.watres.2013.08.003

Zimmermann G (2007) Review on safety of the entomopathogenic fungus *Beauveria bassiana* and *Beauveria brongniartii*. Biocontrol Sci Tech 17:553–596. https://doi.org/10.1080/09583150701

Chapter 12
Biotechnology of Beneficial Bacteria and Fungi Useful in Agriculture

Sandra Pérez Álvarez and Eduardo Fidel Héctor Ardisana

Contents

12.1 Introduction

The majority of plant species in terrestrial ecosystems establish more or less close relationships with rhizospheric microorganisms that somehow make it easier for them to live in normal or stressful environments. The numerous microorganisms that inhabit the rhizosphere include symbiotic nitrogen-fixing bacteria, mycorrhizae and plant-growth-promoting rhizobacteria (Prasad et al. 2015). However, the microorganisms natural role have been marginalized due to modifications induced by tillage and the excessive use of inorganic fertilizers, herbicides and pesticides. Current methods of crop production have created a series of environmental and human health problems. Nowadays, the increase in the appearance of emerging, pre-emergent and endemic pathogens and weeds challenges our ability to protect the growth and health of crops (Miller et al. 2009). That is why, among other

S. P. Álvarez (✉)
Facultad de Ciencias Agrícolas y Forestales, Universidad Autónoma de Chihuahua, Chihuahua, Mexico

E. F. H. Ardisana
Instituto de Posgrado, Universidad Técnica de Manabí, Portoviejo, Ecuador

R. Prasad et al. (eds.), *Mycoremediation and Environmental Sustainability*, Fungal Biology, https://doi.org/10.1007/978-3-030-54422-5_12

reasons, there is a growing demand for more ecological strategies in agriculture. Plant biotechnology has contributed to the development of new crop varieties tolerant or resistant to diseases, drought and salinity, and that are of greater nutritional value (Garg and Chandel 2010).

For about 150 years, it has been shown that bacteria and fungi have an intimate relationship with plants; some are pathogenic, others are neutral, while many of them are beneficial. The rhizosphere of plants is highly colonized by microorganisms; of all of them, between 1 and 35% of the arable crops, show antagonism against pathogens, while two-thirds promote plant growth (Singh et al. 2011). The latter can provide both macro- and micronutrients, release phosphorus from organic compounds, modify the pH of the soil, especially that surrounding the root, thereby increasing the availability of phosphorus and other elements (Berg 2009).

These days sustainable agriculture has gained more attention, because it guarantees productivity of plants and animals using their natural adaptive potentials, with a minimal disturbance to the environment (Noble and Ruaysoongnern 2010). To accomplish this goal, it is necessary to reduce the use of harmful agrochemicals (mineral fertilizers, pesticides) and to use more environment-friendly preparations of symbiotic microorganisms, which could improve the nutrition of crops and cattle, as well as their protection from biotic (pathogens, pests) and abiotic (salinity, drought) stresses (Yang et al. 2009). Consequently, agricultural microbiology is a great research field to transfer and apply knowledge to the agricultural biotechnologies (Mohammed et al. 2008).

12.2 Beneficial Fungi in Agriculture

Biotechnology is in use for more than thousand years in the production of beer, bread, wine, through the fermentation of sugar and starch. In the twentieth and twenty-first centuries, biotechnology has evolved and is being used in the synthesis of many useful molecules and has become a very productive industry (Show et al. 2015); for example, the estimated market volume for plant-degrading enzymes from filamentous fungi in 2016 was €4.7 billion, and it is expected to reach up to €10 billion within the next decade (Meyer et al. 2016).

Several microorganisms are found in agricultural soils, and they can have different applications which tend to improve plant development, such as biofertilizers and biopesticides (Prasad et al. 2020). These microorganisms that live in the soil can help plants in nutrients uptake and a symbiotic relationship is established where plants provide their waste by-products for the microbes as food and microbes help the plant to "take up" essential energy sources (Mosttafiz et al. 2012).

Fungi are eukaryotic organism that in agriculture behave as pathogens of many crops *(Magnaporthe oryzae*, *Botrytis cinerea*, *Puccinia* spp, *Fusarium graminearum*, *Fusarium oxysporum*, *Blumeria graminis*, *Colletotrichum* spp, *Ustilago maydis*, and some others) (Dean et al. 2012) and entomopathogens [*Verticillium lecanii*, *Beauveria bassiana, Metarhizium anisopliae* (Li and Sheng 2007),

Paecilomyces fumosoroseus (Chan-Cupul et al. 2010), *Trichoderma* spp (Zeilinger and Omann 2007)]. Insect populations are regulated mostly by entomopathogenic fungi and the first study was about the silkworm industry (Steinhaus 1975). According to Steinhaus (1975), Bassi in 1835, demonstrated the germ theory using silkworms and muscardine fungus, which was later named *Beauveria bassiana* in his honour.

12.2.1 Mycoinsecticides

The increasing soil and environmental contamination, and the use of chemical pesticides, has increased pest resistance; the need of controlling pests efficiently with non-harming techniques has led to the improvement of friendly insect control methods, such as the use of entomopathogenic fungi that are biological control with a wide host range. These fungi are distributed in a group of over 90 reported genera with approximately 750 species from different insects, and they do not inflict any damage to the environment (Rai et al. 2014).

Fermentation is the process commonly used to produce fungi massively, spores are stored and packed for further field application. The fungi spores contain enzymes that break down the outer surface of the insects' bodies inducing death after they grow; this allow that fugi become into a useful strategy for long-term insect control. These bioinsecticides act in several ways at the same time, which makes the insects almost impossible develop resistance (Zarafi and Dauda 2019).

Bioinsecticides do not persist long in the environment and have shorter shelf-lives; they are effective in small quantities, safer to humans and animals compared to synthetic insecticides; they are very specific, often affecting only a single species of insect and have complicated modes of action; they are slow in action and the timing of their application is relatively critical. Use of fungi as insecticides has been utilized effectively to control devastating insect pests (Zarafi and Dauda 2019). Some examples of fungi controlling insects are as follows:

- The pathogenicity and virulence of fungi ranging from *Metarhizium anisopliae* to *Blissus antillus* (Hemiptera: Lygaeidae) eggs under field conditions were determined and verified that those formulated in mineral oil and in Tween 80 generated 63.5% and 27.1% of mortality, respectively (Samuels et al. 2002).
- The effectiveness of three entomopathogenic fungi (*Beauveria bassiana*, *M. anisopliae* and *Paecilomyces fumosoroseus*) for the control of pests in vegetable crops was evaluated. The fungi were emulsified in diatomaceous earth in proportion 1:10 and was applied in a concentration of 1.2×10^{12} spores ha^{-1} generating mortality higher than 80% after 72 hours of application (García-Gutiérrez and González-Maldonado 2010).
- The effectiveness of *B. bassiana* production in liquid medium for the control of the coffee berry borer (*Hypothenemus hampei*) was evaluated, finding that the culture medium consisting of sugar, yeast extract and peptone is where the best

growth of the fungus occurs on the fourth day, without being affected by the initial pH, nor the temperature of 28 °C; also, it generates mortality of 86.7% (Mata and Barquero 2010).

- Fifteen strains of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* were evaluated on adult one-day-old fruit fly *Anastrepha obliqua* and no significant differences were found in mortality. Mortality of 34–48% during the first 120 hours of evaluation was obtained by applying *B. bassiana* and *M. anisopliae* in a targeted manner to young adults under the canopy of trees (Osorio-Fajardo and Canal 2011).
- Autochthonous isolates of *Beauveria* spp. controlled the white worm (*Premnotrypes vorax* Hustache) in a 77%; this insect causes considerable losses in the cultivation of potatoes, which can reach up to 100% depending on the level of infestation and crop management (Villamil et al. 2016).
- *Beauveria bassiana* and *Metarhizium anisopliae* were used to control the Red Palm Weevils (*Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae), a major/main palm pest in the Mediterranean Basin (Yasin et al. 2017).
- Another entomopathogenic fungi that is a dimorphic hyphomycete that can cause epizootic death in various insects is *Nomuraea rileyi*. Several insect species belonging to Lepidoptera including *Spodoptera litura* and some belonging to Coleoptera are susceptible to *N. rileyi* (Ignoffo 1981). Also, several insects are hosts of *N. rileyi* such as *Trichoplusiani*, *Heliothis zea*, *Plathypena scabra*, *Bombyx mori*, *Pseudoplusia includes* and *Anticarsia gemmatalis*.

These days it is necessary to understand entomopathogenic fungi ecology outside of the insect host, specifically fungi strategies and their role in the ecosystem. Some discoveries suggesting that the way to control insect with entomopathogenic fungi must be reviewed. As an example, *M. anisopliae* strain compete for the rhizosphere and this depends on the plant community and not on the insect host presence (Hu and St. Leger 2002), whereas *B. bassiana* strains exist as endophytes in several crops and have the potential for insect and plant disease suppression (Vega 2008).

12.2.2 Mycoherbicides

Weeds are unwanted plants when they grow around crops. The intensive uses of herbicides to their elimination and the resistance that weeds develop against these products has created the necessity to look for new and friendly strategies. The application of fungi to control weeds opens a new field to get this goal because the use of microorganisms is friendly with the environment, they are more specific to the target and less expensive that traditional herbicides. The fungi genera that have been used effectively are *Colletotrichum*, *Phoma* and *Sclerotinia* (Harding and Raizada 2015).

Bioherbicides as definition are products made of phytopathogenic microorganisms or microbial phytotoxins useful for weed control, and they are used in similar

way to conventional herbicides (Boyetchko et al. 2002; Boyetchko and Peng 2004). The active ingredient in a bioherbicide is, however, a living microorganism that usually is a fungus, so the term mycoherbicide is frequently used in these cases (Auld and McRae 1997).

The majority of the weeds control in North America has been based on fungi formulations, but just a few of these products were successful in the long term. Here is a list of several examples: a formulation called BioMal that was made with *Colletotrichum gloeosporioides* f.sp. *malvae*, introduced for the control of round leaf mallow (*Malva pusilla* L.) (Mortensen 1988; PMRA 2006); another formulation, Sarritor, includes *Sclerotinia minor* for the control of dandelion (*Taraxacum officinale* (L.) Weber ex F.H. Wigg., Prim. FL. Holsat), white clover (*Trifolium repens* L.) and broadleaf plantain (*Plantago major* L.) in turf (PMRA 2010).

In Brazil, fungi were selected for production of secondary metabolites with herbicidal activity using biological resources of the Brazilian Pampa biome; for this purpose, phytopathogenic fungi were isolated from infected tissues of weeds and the phytotoxicity of fungal metabolites was evaluated using a biological test with *Cucumis sativus* L. Thirty-nine fungi were isolated, and 28 presented some phytotoxic symptoms against the target plant. The best strain was identified through molecular studies. Fungus VP51 belonging to the genus *Diaporthe* showed the most effective herbicidal activity (Castro de Souza et al. 2017).

Hoagland et al. (2007) studied a strain of *Myrothecium verrucaria*, isolated from sicklepod (*Senna obtusifolia* L.), a plant that has bioherbicidal activity against kudzu (*Pueraria lobata* (Willd.) Ohwil) and some other weeds. Those authors found that *M. verrucaria* caused great reductions of kudzu plant biomass production at 30 °C, compared to 20 °C or 40 °C, under experimental conditions.

In a study carried out in West Africa, *Fusarium oxysporum* (PSM 197) controlled 91.3% of *Striga asiatica* (L.) Kuntze (a hemiparasitic plant in the broomrape family), 81.8% of *S. gesneroides* and 94.3% of *S. hermonthica* (Marley et al. 2005). An isolate from Italy of *M. verrucaria* produced trichothecenes (a very large family of chemically related mycotoxins produced by various species of fungi) that could inhibit seed germination of the parasitic plant *Orobanche ramosa* Delile ex Decne. 1824 (Andolfi et al. 2005). Another study with *M. verrucaria* in the south-eastern United States showed that this fungus is very virulent against *Portulaca oleracea* L., *Sesuvium portulacastrum* L., *Euphorbia maculata* L. and *Euphorbia prostrata* Aiton in cultivated tomato (*Lycopersicon esculentum* L.) (Boyette et al. 2007).

Microsphaeropsis amaranthi and a mixture of *Microsphaeropsis amaranthi* and *Phomopsis amaranthicola* were used to control eight *Amaranthus* species, and as a result, severe disease ratings were showed 15 days after treatment (DAT), and mortality ranged from 74% to 100% (Ortiz-Ribbing and Williams 2006).

Microsphaeropsis amaranthi and *P. amaranthicola* have been used as bioherbicide for the control of water hemp [*Amaranthus rudis* (Moq.) J. D. Sauer] and pigweeds (*Amaranthus* spp.); these are weeds that affect many crops and have become resistant to several herbicides. Results showed significant reductions in weed biomass when one or both of the fungal organisms were used; nevertheless, it is

necessary to control leaf surface moisture and air temperatures following application because inconsistencies in field results may occur (Ortiz-Ribbing et al. 2011).

According to Hetherington et al. (2002), bioherbicides can improve seedlings growth through the infection and delay of the growth of weed.

12.2.3 Fungal Symbiosis

Since plants first colonized terrestrial ecosystems developed several strategies to survive biotic and/or abiotic stresses; among these strategies are symbiosis that they can establish through root systems with microorganisms (Gianinazzi-Pearson 1984; Varma et al. 2020). In this relationship, both plant and microorganisms get something necessary for their growth and development (Fig. 12.1).

The majority of crops are capable of forming symbiosis associations with soil fungi; to facilitate or improve such association, the crops or the fungi can be genetically modified, so it is necessary to identify the genes involved in this relationship (Behie and Bidochka 2013). One study made in *Medicago truncatula* Gaertn. showed that 29 genes were upregulated during mycorrhizal association, 11 of which were not upregulated in plants during bacterial colonization, suggesting that only certain genes play a role in plant-fungal interactions (Weidmann et al. 2004). In this sense, some genes have been identified such as MtScp1, a gene that encodes a carboxypeptidase-related transmission of fungal specific signals; mad229 and myc control the regulation of molecules secreted from the fungus prior to association that stimulate root development and expression of plant genes required for intercellular fungal interaction (Bucher et al. 2009).

Mycorrhizae are fungi that establish a symbiotic relationship with the roots of terrestrial plants and seven associations can be identified: (1) Ectoendomycorrhizae:

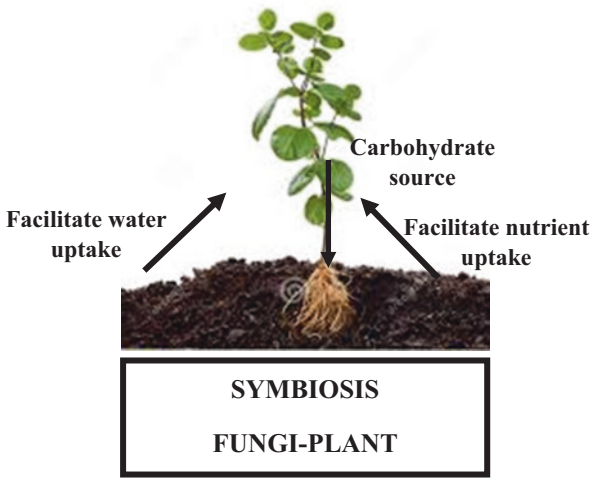


Fig. 12.1 Symbiotic relationship between plant and soil fungi

association of Ascomycetes and the genera *Pinus* and *Larix* of Coniferae (Yu et al. 2001); (2) Ericoid: they are unique mycorrhizae to the families of order Ericales (Cairney and Ashford 2002); (3) Arbutoid: typical arbutoid mycorrhizae are formed with two genera of Ericaceae family (*Arbutus* and *Arctostaphylos*) and several genera of the family Pyrolaceae (Molina and Trappe 1982); (4) Monotropoid: plants that have this kind of mycorrhizae are non-photosynthetic, but this fungi can associate with neighbouring trees that are photosynthetically active to get their photosynthates (Björkman 1960); (5) Orchid: they only exist in the Orchidaceae family (Smith and Read 1997); (6): Ectomycorrhiza has three characteristics that are typical of these mycorrhizae: (a) the formation of a hyphae mantle on portions of the laterals roots, (b) the formation of the Hartig net between the roots cells and, (c) hyphae that emanates from the mantle and grow in the soil (Peterson et al. 2004); (7) Arbuscular: association between most of vascular plants roots and fungi from a new phylum named *Glomeromycota* (Schübler et al. 2001). The last two mycorrhizae described are the most abundant in earth.

Mycorrhizae can protect plants against root pathogens and toxic stresses, and another important role that these fungi could play is the restoration and the improvement of revegetation in soils mined, even when this practice is not well implemented in many parts of the world (Prasad et al. 2017; Varma et al. 2017). One of the main results of soil damage is the destruction of mycorrhizal fungal network, so the restoration of these fungi is essential for the soil habitat (Quoreshi 2008). In vitro culture is an important tool to achieve this result because with this technique, it is possible to obtain a great volume of inoculum and to transport it cheaply (Ceballos et al. 2013).

Ceballos et al. (2013) evaluated the in vitro production of *Rhizophagus irregularis* (mycorrhizal fungus) and its effect on cassava yield; though good production was obtained, no greater return on investment than conventional cultivation was achieved.

The inoculation effect of nine consortiums of arbuscular mycorrhizal fungi (AMF) in coffee seedlings of *Coffea Arabica* (Caturra variety) was compared with a control without inoculation during seven months under greenhouse conditions; three of the nine consortia studied were more efficient during the growth and development of coffee plants seedlings (Del Aguila et al. 2018).

12.2.4 Fungi and Biodegradation

Biodegradation can be defined as the decomposition of dead plant and animals by microorganisms (Kakde and Jamdhade 2009). Plant biomass contributes with sources of carbon on earth and fungi are efficient degraders of this biomass (Mäkelä et al. 2014). Fungi also can degrade polysaccharides in the environment, and 218 have been sequenced, allowing the identification of genes and proteins implicated in this degradation (Berlemont 2017).

The degradation of polysaccharides such as xylan and cellulose from plants and chitin produced by fungi is very important for several ecosystem processes that include nutrient cycles (like carbon cycle) (Nielsen et al. 2011) and the nutrition of animals (herbivores) (El Kaoutari et al. 2013). Cellulose, xylan and chitin are hydrolyzed mainly by microorganisms such as bacteria and fungi through different ways like enzymes that sometimes can be associated with non-catalytic domain (multi-domain glycoside hydrolases [GHs]) (Hervé et al. 2010; Várnai et al. 2013), multi-activity GHs and synthesis of some multi-protein complexes named cellulosomes (Gefen et al. 2012). Multi-domain GHs and cellulosomes can degrade biopolymers (VanFossen et al. 2011; Talamantes et al. 2016), so it is possible to use them for successful processes like biofuel industries.

There are several enzymes that can degrade plant polymers; such enzymes are produced by fungi and they belong to six groups: the glycoside hydrolases (GHs), glycosyltransferases (GTs), polysaccharide lyases (PLs), carbohydrate esterases (CEs), auxiliary activities (AAs) and carbohydrate-binding modules (CBMs) (Aspeborg et al. 2012).

Pesticides, that can persist in soils for many years, could be degraded by microorganisms. This is possible because physical, chemical and biological processes such as accumulation in plants, volatilization and others are associated with several soil characteristics like pH, salt content and presence of organic matter (Boivin et al. 2004).

White rot fungi are widely used for bioremediation processes that use microorganisms to degrade contaminants such as heavy metals and pesticides in soil and water. These fungi (white rot) degrade lignin and others polymers using enzymes (Pointing 2001) that are extracellular oxidases and peroxidases: lactases, manganese peroxidases, lignin peroxidases, among others (Novotný et al. 2004).

Brown rot fungi are also used with the same purpose that white rod. These fungi can degrade cellulose and hemicellulose (Schlosser et al. 2000; Newcombe et al. 2002). One example of bioremediation by these fungi is the degradation of DDT by *Fomitopsis pinicola* and *Daedalea dickinsii*, which can transform DDT to DDE 1,1-dichloro-2,2-bis (4-chlorophenyl) ethylene and DDD (1,1-dichloro-2,2-bis (4-chlorophenyl) ethane) via the Fenton reaction (Purnomo et al. 2010, 2011).

To biodegradate pentachlorophenol, several fungi have been used like *Phanerochaete chrysosporium*, *Berjkandera adusta* and *Pleurotus ostreatus*, getting the highest percentage (96%) with *P. chrysosporium* (Lamar et al. 1990; Ruttimann and Lamar 1997). *Trametes hirsuta*, *Pleurotus eryngii* and *P. chrysosporium* have been used for the degradation of lindane (insecticide) and the best results were obtained with *T. hirsute* (10.6% to 96%) (Singh and Kuhad 1999; Quintero et al. 2007).

Tejomyee and Pravin (2007) studied the biodegradation of the insecticide endosulfan, and they demonstrated that *Aspergillus niger* can eliminate a concentration of 400 ppm endosulfan after 12 days of incubation. According to Kamei et al. (2011) *T. hirsute* is able to remove up to 90% of endosulfan and endosulfan sulfate after 14 days of incubation.

12.3 Beneficial Bacteria in Agriculture

Many microorganisms coexist in soils, and the survival capacities of some of them are being studied with increasing interest, mainly as alternatives for the control of pathogenic fungi. Plant-growth-promoting bacteria (PGPB) are recognized for their bio-stimulating, biofertilizing and stress-regulating capacity in vegetables (Lugtenberg and Kamilova 2009; Prasad et al. 2015, 2020; Basu et al. 2021). These bacteria are able to colonize the rhizosphere of the plant and survive in it (Raaijmakers et al. 2009) through mechanisms that allow them to compete successfully with other microbes. For these reasons, they have been studied as potential antagonists/biological controllers of plant pathogens.

Undoubtedly, the bacterial genus that has generated the most research and applications in terms of biological control is *Bhurkolderia* sp. However, in recent years, interest has developed in other genera of bacteria that also show potential in this regard.

12.3.1 The Genus Burkholderia

Although the bacteria that grow in the rhizosphere are a useful source for the protection of plants against pathogenic fungi, it would be preferable for the resistance to be present inside all plant tissues. Certain bacteria – among which are several of the genus *Bhurkolderia* – are able to grow and develop inside the plant, which is why they are called endophytic bacteria. This characteristic means that they can interact with the plant more effectively than those that live in the rhizosphere. Thus, the biocontrol metabolic products expressed by the endophytic bacteria could act more efficiently in the protection of plants against pathogenic microbes or predators.

The genus *Burkholderia* groups bacteria that usually grow in the rhizosphere of numerous plants; consequently, several of their species have been observed with interest to know how they can compete with other bacteria and mainly with phytopathogenic fungi. The identification of the antagonist mechanisms and the metabolites participating in this competition could help the formulation of biopesticides. But also, several of the species of the genus are able to grow in an endophytic manner. *Burkholderia phytofirmans* PsJN, for example, can migrate to the aerial parts of the grape plants and form a biofilm on the leaf surface that restricts the growth of the *Botrytis cinerea* mycelium (Miotto-Vilanova et al. 2016).

Simonetti et al. (2018) isolated the T16 line of *Burkholderia ambifaria* that grows in the rhizosphere of barley plants (*Hordeum vulgare*). This line uses fusaric acid (the main toxic metabolite produced by *Fusarium* spp.) as the sole source of carbon, nitrogen and energy for its own growth in vitro, and is also able to detoxify fusaric acid in barley seeds. Before, Utsumi et al. (1991) had obtained similar results in vitro for a line of *Burkholderia cepacia*.

Through comparison with the genome of other bacteria, Ali et al. (2014) identified genes putatively responsible for the endophytic behaviour of several *Burkholderia* species. On the other hand, it is known that the different *Burkholderia* lines can live in different environments, because they have a large, complex (4.6–9 Mb) and variable genome, with three chromosomes and large plasmids (Esmaeel et al. 2016). As for the metabolites involved in the antagonistic activity of *Burkholderia*, Esmaeel et al. (2017) cite a group of authors who have detected several substances with different properties; among these, the lipopeptides synthesized by several lines of *B. cepacia*, *B.ambifaria* and *B. contaminans* have specifically antifungal activity.

Several authors (cited by Haidar et al. 2016) have reported the antagonistic activity of endophytic bacterial genera such as *Bacillus*, *Pseudomonas*, *Streptomyces* and *Bhurkolderia*, among others, against *Botrytis cinerea*, a necrophyte fungus that inflicts large losses among the plantations of grapes and strawberries. Among the ways in which this antagonism occurs are the synthesis of various antifungal compounds such as antibiotics and lytic enzymes that destroy the cell walls of fungi, the induction of resistance in the host and competition for nutrients (Koch et al. 2021).

Some of these genera – but not *Bhurkolderia* – have been used in the formulation of biopesticides for the control of *B. cinerea*. However, it has been shown that *Bhurkolderia* produces metabolites capable of controlling various fungal species. Mahenthiralingham et al. (Mahenthiralingam et al. 2011) and Masschelein et al. (2017) report that the various species and lines of the genus synthesize several substances (pyrrolnitrin, occidiofungin, cepafungin and burkholdines) and other compounds such as the cepacines that have a broad spectrum of action. Although the focus of these two studies was mainly on the medical applications of such products, the production of these antifungal metabolites demonstrates the potential of the *Burkholderia* genus as biological control in agriculture.

Rika Fithri et al. (2014) tested the application of several isolates of *Burkholderia* sp. in the attempt to control the root rot in oil palm, caused by the fungus *Ganoderma boninense*. As part of this investigation, they detected the synthesis of pyrrolnitrin in the *Burkholderia* 312 isolate, which led to the best results in the plants. Pyrrolnitrin is a secondary metabolite derived from tryptophan (Kirner et al. 1998) that has strong antibiotic activity on various fungi. Ramli et al. (2016) found that the isolates of three endophytic bacteria, including *Burkholderia cepacia*, were able to control the in vitro development of *G. boninense*, and to delay the onset of disease symptoms in the oil palm when the seeds had been pre-treated with these microorganisms.

Bach et al. (2016) analysed the bacterial properties of *Bacillus mycoides* B38 V, *Burkholderia cepacia* 89 and *Paenibacillus riograndensis* SBR5, microbes of the PGPB type isolated from Brazilian soils. It was observed that the three bacteria produce amylases, catalases, esterases and proteases. Aktuganov et al. (2008) have reported that these extracellular enzymes can affect the cell walls of pathogenic fungi. In addition, in the assays by Bach et al. (2016), *Burkholderia cepacia* 89 showed antagonistic activity against several filamentous fungi; under greenhouse conditions, the combined inoculation of wheat plants with this bacterium and the

pathogens *Bipolaris cynodontis*, *Drechslera tritici-repentis* and *Fusarium graminearum* led to dry weight values of roots and stems superior to plants inoculated only with pathogens. These values were also higher than those of the plants treated only with fungicides, possibly due to the growth-promoting effect that the PGPBs also provide. Additionally, *Burkholderia cepacia* 89 produced a metabolite with antifungal activity, which can become an important biological fungicide.

The effectiveness of the line JP2-270 of *Burkholderia cepacia*, isolated from the rhizosphere of rice, in the control of the fungus *Rhizoctonia solani* was demonstrated by Song et al. (2018). The analysis of the genome showed that the gene bysR (DM992_17470) is essential for the antifungal activity of *B. cepacia* JP2-270 against *R. solani*. The nature of this gene, which belongs to the lysR family of transcriptional regulators (Lu et al. 2009), allows to suppose that the antagonist activity is exerted through an affectation to the synthesis processes of secondary metabolites. This gene could then become a potential target for its use in genetic engineering in order to take advantage of the controlling potentials of *B. cepacia* JP2-270 (Song et al. 2018).

Kim et al. (2019) studied the activity of *Burkholderia stabilis*, endophytic bacteria isolated from ginseng (*Panax ginseng*), on several pathogens. Both the bacteria and their extracts were able to control the development of *B. cinerea*, *R. solani*, *A. panax*, *Phytium* sp. and in particular of *Cylindrocarpon destructans*, the main pathogen of ginseng, which causes severe root rot. The separation of *B. stabilis* extracts by column chromatography allowed to collect a fraction that inhibited the growth of the five pathogens; another of the fractions was also able to control *C. destructans*.

Mullins et al. (2019) demonstrated that cepacin A synthesized by *Bhurkolderia ambifaria* is an efficient metabolite in the biological control of *Pythium ultimum*, a pathogenic fungus that causes decay in hundreds of useful plant species such as potatoes, wheat and soybeans. Sandani et al. (2019) identified five isolates of four bacteria (*Pseudomonas aeruginosa*, *Burkholderia arboris*, *Burkholderia gladioli* and *Burkholderia rinojensis*) capable of 100% effective inhibition of germination of the spores of *Colletotrichum truncatum*, a pathogenic fungus responsible for anthracnose in chili pepper. In addition, the metabolites secreted by the isolates controlled the development of the disease to a large extent. These compounds, of diffusible nature, could be of various types, such as antibiotics, hydrolytic enzymes of cell walls or other secondary metabolites (Beneduzi et al. 2012).

What has been reviewed up to here suggests that *Burkholderia* is useful and can be applied as a biological fungal control agent, given the effects demonstrated as an antagonist of various fungi. In fact, in the 1990s, several *Burkholderia* lines began to be used as fungi biocontrol in American agriculture. However, risk studies (derived from their pathogenic potential to animals and people) advised their withdrawal from the market (Eberl and Vandamme 2016). What happens is that the genus *Bhurkolderia* can cause opportunistic infections to the plants, becoming a pathogenic agent. This would limit its generalized application as biological control; however, Bolívar et al. (2016) indicate that the genus is divided into two large groups, the so-called *Bhurkolderia cepacea* complex (BCC) constituted by

opportunistic pathogenic species, and another phylogenetically distant group composed of beneficial species, promoters of growth and with biotic activity against known pathogens. Also, Eberl and Vandamme (2016) point out that the genus can be divided into two clades genetically separated from each other: one which contains pathogenic species to plants, animals and humans, and other grouping species that promote plant growth and protection of plants against numerous pathogens. It has even been proposed and accepted to rename this second group as a new genus (*Paraburkholderia*) (Sawana et al. 2014; Oren and Garrity 2015).

Regardless of the potential dangers of using *Bhurkolderia* in agriculture, the possibility of modifying the genome of the genus with useful characteristics opens up new possibilities of employment in plant production. Li et al. (2017) introduced the cry218 gene of *Bacillus thuringiensis* by electroporation into the genome of *Burkholderia pyrrocinia* JKSH007, which lives as endophyte in the poplar. The transgenic bacterium thus obtained was effective in the control of the larvae (second instar) of *Bombyx mori* (silkworm) which is a lepidopteran used as a model in these investigations. Consequently, it could potentially be used for the control of harmful lepidoptera.

12.3.2 Other Bacterial Genera

In addition to *Bhurkolderia* sp., other bacterial genera have been studied with the aim of using them directly as biological controls or of using the metabolites that they synthesize and that have an antagonistic effect with pathogenic microbes. The main approaches have been directed towards the genera *Pseudomonas* sp. and *Bacillus* sp.

Several species of the genus *Pseudomonas* exhibit antifungal activity, and have been used for the control of various pathogens in beet, tobacco, cucumber, cotton, wheat, rice, eucalyptus and other species (several authors, cited by Sindhu et al. 2016). *Pseudomonas aeruginosa* and *Pseudomonas viridiflava* were useful in the control of *Lasiodiplodia theobromae*, the main causal agent of crown rot in banana (Thangavelu et al. 2007). Other species are able to act as antagonists only under certain conditions; for example *Pseudomonas fluorescens* controls *Rhizoctonia solani* and *Pythium aphanidermatum* when the culture medium is rich in nitrogen, but not when it is rich in carbon (Michelsen and Stougaard 2012).

From wheat leaves, Müller et al. (2015) isolated 20 lines of *Pseudomonas fluorescens* and *Pseudomonas gessardii*, carriers of the gene phlD, which codes for the synthesis of the antibiotic 2,4-diacetylphloroglucinol, and are able to suppress in vitro *Fusarium* and *Alternaria*, important pathogens of this and other crops. The role of antibiotics such as pyrrolnitrin is decisive in the control of other microbes by *P. fluorescens*, as in the case of the prevention of damage caused by *R. solani* in cotton (Hill et al. 1994) or phenazine in the control of *F. oxysporum* and *G. graminis* (Chin-A-Woeng et al. 2003). The production of phenazine by species of the genus *Pseudomonas* is the control route of several fungi (Suryadi et al. 2014; Parvin et al.

2016; Irma et al. 2018). The MP12 line of *Pseudomonas protegens*, isolated from the soil and identified by Andreolli et al. (2019), carries phlD, pltB and prnC genes, which encode the synthesis of 2,4-diacetylphloroglucinol, pyoluteorin and pyrrolnitrin, respectively. This bacterium inhibits the in vitro growth of several phytopathogenic fungi of the vine: *Phaeomoniella chlamydospora* and *Phaeoacremonium aleophilum*, and these are responsible for the esca disease, not controllable by the methods available in agriculture.

The ability of *Pseudomonas* to colonize different organs of the plant, its versatility in terms of the use of organic substrates exuded by seeds and roots, the diversity of metabolites that they synthesize and their compatibility with other biological control agents and chemical pesticides make this genus a powerful candidate for its use in the control of damage caused by pathogens (Sindhu et al. 2016).

Within the genus *Bacillus*, both those who live in the rhizosphere and in an endophytic form have been studied for purposes of biological control. In wheat, three endophytic isolates of *Bacillus subtillis* and one of *Bacillus megaterium* inhibited the in vitro growth of *Fusarium graminearum*; the *B. megaterium* isolate is the most effective in field conditions (Pan et al. 2015). In corn, Figueroa-López et al. (2016) found three rhizospheric isolates of species of the genus (*B. megaterium*, *B. cereus* sensu lato and *Bacillus* sp.) that reduce the damage caused by *Fusarium verticillioides*, apparently thanks to the synthesis of glucanases, proteases, chitinases and substances that stimulate growth, such as siderophores and auxins.

Two endophytic isolates, one from *Bacillus cereus* and the other from *Bacillus mojavensis*, inhibit the development of *F. proliferum*, *F. verticillioides* and *F. fujikuroi*, rice pathogens (Etesami and Alikhani 2017). Melnick et al. (2008) were successful in controlling *Phytophthora capsici* in cocoa by applying *B. cereus* isolated from tomatoes and potatoes, and also *Bacillus* sp. from the cocoa plants themselves; equivalent results in the control of *Moniliophthora roreri* with *Bacillus* sp. in cocoa were obtained by Villamil et al. (2015). The genus *Bacillus* is able to synthesize lytic enzymes that, by destroying the cell walls of pathogens, impedes their growth (Tirado-Gallego et al. 2016).

Finally, the known toxicity of certain proteins of *Bacillus thuringiensis* on insects is another promising route (Malathi et al. 2006; Sujatha et al. 2009), taking advantage in this case of the facilities of genetic engineering. However, genetic engineering processes to control insects with *Bacillus thuringiensis* must be carried out with great foresight, since Bt toxins can be dangerous for useful insects such as the silkworm (Kumar et al. 2016). Although it is feared that insects may develop resistance to *B. thuringiensis* toxins, Badran et al. (2016) have discovered mechanisms to obtain new Bt toxins that do not adhere to their traditional receptors but to new adhesion sites in *Trichoplusia ni*. In this way, the resistance to the Bt toxins that already begins to appear in the field could be overcome.

The potentialities of the genus *Bacillus* as a biological control agent are given not only by its antagonistic capacity, but because it produces stable endospores that are able to withstand high temperatures and desiccation (Sindhu et al. 2016).

The production of antibiotics and hydrolytic enzymes are not the only mechanism important in the biological control of diseases that some bacteria exert. In

addition to these, other mechanisms are known, such as the production of phytoalexins, the induction of systemic resistance, the synthesis of secondary metabolites of various types and the production of siderophores (Sindhu et al. 2016). The genera *Arthrobacter*, *Curtobacterium*, *Enterobacter*, *Microbacterium*, *Stenotrophomonas* and even *Pseudomonas*, which are able to control the damage caused by *Xanthomonas axonopodis* pv. *passiflorae*, do it through competition for iron and nitrogen compounds (Halfeld-Vieira et al. 2014).

Indirectly, in addition, the protection of the plants can be carried out in ways that improve their constitution and nutritional status, which makes them more resistant to pathogenic infections. The genus *Rhizobium* form nodules in the roots of Fabaceae (Fig. 12.2), reducing atmospheric N_2, which is very stable and relatively inert, to ammonium ions (NH_4^+) easily assimilated by most plant species (Marquina et al. 2011). This association between bacteria and plants from Fabaceae family is an efficient process in the biological fixation of atmospheric nitrogen (BFAN). According to Ángeles-Núñez and Cruz-Acosta (2015), nitrogen fixation could vary from 24 to 584 kg ha^{-1} and may supply up to 90% of the needs of the plant. Also, BFAN can reduce drastically the application of nitrogen fertilizers, which brings less contamination of soil and water, also reducing production costs (Yadegari and Rahmani 2010; Granda et al. 2014). The final result is a vigorous and healthy plant, more able to defend itself from pathogenic infections.

Rhizobium characterization studies have been carried out in order to know their growth and nodulation properties with a view to their use in agriculture. Morphological and biochemical traits from several *Rhizobium* strains (9 of them from wild common bean roots and 11 from domesticated bean roots from Western Mexico) were characterized by López-Alcocer et al. (2017). Results from the morphological characterization showed that all strains had a rapid growth (2–3 days),

Fig. 12.2 Nodules of *Rhizobium* (www.farmersjournal.ie)

white colour and smooth border; 14 had a convex shape, and 12 were translucent. With respect to biochemical characterization, all strains grew at a pH of 6.0 or higher, and when a pH from 4.0 to 5.5 was fixed, four strains did not grow. A great variability between strains was found in this study showing generally rapid growth, tolerance to acid pH values, tolerance to moderate concentrations of sodium chloride, susceptibility to heavy metals and resistance to antibiotics, which is consistent with bacteria of the genus *Rhizobium* (López-Alcocer et al. 2017).

Gómez-Padilla et al. (2017) characterized six bacteria isolated from roots of *Vigna unguiculata*; they were subjected to different salt concentrations (0.17–6.6 dSm^{-1} of NaCl), pH levels (4.5–9.0) and temperatures (28–45 °C). The variation of 16S rRNA gene was examined by amplified 16S rDNA restriction analysis (ARDRA) and direct sequencing to show genetic diversity. Three isolates (VIBA-1, VIBA-2 and VIBA-6) achieved similar results as the control with 2.6 and 3.4 dSm^{-1} of NaCl. All of the isolates could grow at pH 7 and 9 and could grow until 40 °C, meanwhile only two of them (VIBA-4 and VIBA-5) grew at 45 °C. VIBA-1 was closely related to *Bradyrhizobium liaoningense*, VIBA-4 to *Rhizobium radiobacter* and the remaining to *Bradyrhizobium yuanmingense.* All of them, with the exception of VIBA-4, were able to nodulate in the plants when they were inoculated.

Bacteria producing organic acids such as lactic acid and acetic acid are used in the biopreservation of plant products (Trias et al. 2008a) mainly because the low pH prevents the growth of fungi that rot the edible fruits and leaves. *Enterococcus*, *Lactobacillus*, *Leuconostoc*, *Lactococcus* and *Pediococcus* produce various antifungal compounds, among which are protein molecules, peptides, fatty acids, organic acids and reuterin, a metabolite resulting from the degradation of glycerol. Although the use of these bacterial genera as biological controls of fungi has not been widely studied, their antagonist activity has been reported in some cases (Sathe et al. 2007; Rouse et al. 2008; Trias et al. 2008b; Lan et al. 2012, and others) which allows considering them as potential candidates for this purpose. In addition, unlike other microorganisms such as *Bhurkolderia*, there are no reports of toxicity to plants, animals or humans related to these bacterial genera, and they are easy to isolate from different environments, including the aerial parts of plants (Gajbhiye and Kapadnis 2016).

An important and recent application of the properties of bacteria is the control of weeds. Four main reports were pioneers in this topic: a limited effect of *P. fluorescens* strain D7 on *Bromus tectorum* (Kennedy et al. 1991), the control of *Poa annua* and *Poa attenuata* by *Xanthomonas campestris* pv. *poae* JT-P482 (Imaizumi et al. 1997), the antagonist activity of *P. fluorescens* strain BRG100 on *Setaria viridis* (Quail et al. 2002) and the inhibition of 29 species between monocotyledonous and dicotyledonous plants by *P. fluorescens* strain WH6 (Banowetz et al. 2008). In recent years, several reports have appeared on the herbicidal activity of other genera (Patil 2014; Sayed et al. 2014; Juan et al. 2015; Boyette and Hoagland 2013, 2015). Recently, *P. fluorescens* strain BRG100 has been used successfully in the formulation of a bioherbicide (Agriculture and Agri-Food Canada 2019).

The use of bacteria for the control of insects and other invertebrates has also been limited to some genera (Lacey et al. 2015). First, there are the subspecies of *Bacillus*

thuringiensis, which in addition to their well-known success in the suppression of lepidoptera have achieved success in nematodes (Carneiro et al. 1998; Wei et al. 2003; Khan et al. 2010), coleoptera (Suzuki et al. 1992) and hymenoptera (Porcar et al. 2008). In 2014, only four biopesticides (three based on B. *thuringiensis* and one based on *B. firmus*) were registered in Europe for use in the greenhouse (Gwynn 2014); the subspecies *israelensis*, *japonensis* and *galleriae* (all of *B. thuringiensis*) began to be used experimentally for the control of insects in peanuts, vegetables, grass and turf (Kergunteuil et al. 2016). However, future employment prospects are broad, since 150 proteins of *B. thuringiensis* toxic to insects have been isolated (Crickmore et al. 2018). The toxins of *B. thuringiensis* have been the main base for the creation of transgenic crops resistant to lepidoptera, although their biosecurity for other insects and humans has been questioned; they also have the fact that they generate resistance in the target insects (Lacey et al. 2015). However, the above-mentioned results of Badran et al. (2016) promise substantial improvements in this last direction.

A promising prospect – at least for greenhouse plants – seems to be the combined use of bacterial biopesticides with the natural enemies of insects, in particular using the former as correction tools in cases where the latter do not work at all to the extent to which it is needed (Gonzalez et al. 2016).

12.4 Conclusions and Future Outlook

Fungi and bacteria can play an important role in agriculture on the basis of their properties that help commercial crops to acquire nutrients and water through symbiotic associations, stimulating their growth and development and/or protecting them against infections of other microbes, competition with undesirable vegetation and attacks from other predatory organisms.

Biotechnology has been useful in the identification and characterization of useful fungi and bacteria and their metabolites, as well as in the formulation of bioinsecticides, biofungicides and bioherbicides that begin to be used in a larger or smaller scale. The possibilities opened by the use of genetic engineering in the transformation of beneficial microorganisms make it a useful tool for the more exact and targeted application of these microbes and the products obtained from them.

References

Agriculture and Agri-Food Canada (2019) Agriculture and Agri Food Canada Biopesticide: *Pseudomonas fluorescens* BRG100. In: https://www5.agr.gc.ca/eng/science-and-innovation/agricultural-practices/agricultural-pest-management/agricultural-pest-management-resources/agriculture-and-agri-food-canada-biopesticide-pseudomonas-fluorescens-brg100/?id=1553177949347

Aktuganov G, Melentjev A, Galimzianova N, Khalikova E, Korpela T, Susi P (2008) Wide-range antifungal antagonism of *Paenibacillus ehimensis* IB-X-b and its dependence on chitinase and b-1,3-glucanase production. Can J Microbiol 54:577–587

Ali S, Duan J, Charles TC, Glick BR (2014) A bioinformatics approach to the determination of genes involved in endophytic behavior in *Burkholderia* spp. J Theor Biol 343:193–198

Andolfi A, Boari A, Evidente A, Vurro M (2005) Metabolites inhibiting germination of *Orobanche ramosa* seeds produced by *Myrothecium verrucaria* and *Fusarium compactum*. J Agric Food Chem 53(5):1598–1603

Andreolli M, Zapparoli G, Angelini E, Lucchetta G, Lampis S, Vallini G (2019) *Pseudomonas protegens* MP12: a plant growth-promoting endophytic bacterium with broad-spectrum antifungal activity against grapevine phytopathogens. Microbiol Res 219:123–131

Ángeles-Núñez JG, Cruz-Acosta T (2015) Aislamiento, caracterización molecular y evaluación de cepas fijadoras de nitrógeno en la promoción del crecimiento de frijol. Rev Mex Cienc Agríc 6:929–942

Aspeborg H, Coutinho PM, Wang Y, Brumer H, Henrissat B (2012) Evolution, substrate specificity and subfamily classification of glycoside hydrolase family 5 (GH5). BMC Evol Biol 12:186

Auld BA, McRae C (1997) Emerging technologies in plant protection – bioherbicides. Proc. 50th N.Z. Plant Protection Conference, pp 191–194

Bach E, Dubal dos Santos G, de Carvalho G, Brito B, Pereira LM (2016) Evaluation of biological control and rhizosphere competence of plant growth promoting bacteria. Appl Soil Ecol 99:141–149

Badran AH, Guzov VM, Huai Q, Kemp MM, Vishwanath P, Kain W, Nance AM, Evdokimov A, Moshiri F, Turner KH, Wang P, Malvar T, Liu DR (2016) Continuous evolution of *Bacillus thuringiensis* toxins overcomes insect resistance. Nature 533(7601):58–63

Banowetz GM, Azevedo MD, Armstrong DJ, Halgren AB, Mills DI (2008) Germination-Arrest Factor (GAF): biological properties of a novel, naturally-occurring herbicide produced by selected isolates of rhizosphere bacteria. Biol Control 46(3):380–390

Basu S, Kumar G, Chhabra S, Prasad R (2021) Role of soil microbes in biogeochemical cycle for enhancing soil fertility. In: Verma JP, Macdonald C, Gupta VK, Podile AR (eds) New and Future Developments in Microbial Biotechnology and Bioengineering: Phytomicrobiome for Sustainable Agriculture. Elsevier 149–157

Behie SW, Bidochka MJ (2013) Potential agricultural benefits through biotechnological manipulation of plant fungal associations. Bioassays 35:328–331

Beneduzi A, Ambrosini A, Passaglia LMP (2012) Plant growth-promoting rhizobacteria (PGPR): their potential as antagonists and biocontrol agents. Genet Mol Biol 35(4):1044–1051

Berg G (2009) Plant-microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. Appl Microbiol Biotechnol 84:11–18

Berlemont R (2017) Distribution and diversity of enzymes for polysaccharide degradation in fungi. Sci Rep 7(222):1–11

Björkman E (1960) *Monotropa hypopithys* L. An epiparasite on tree roots. Physiol Plant 13:308–327

Boivin A, Cherrier R, Perrin-Ganier C, Schiavon M (2004) Time effect on bentazone sorption and degradation in soil. Pest Manag Sci 60(8):809–814

Bolívar HJ, Contreras ML, Teherán LG (2016) *Burkholderia tropica,* una bacteria con gran potencial para su uso en la agricultura. TIP 19(2):102–108

Boyetchko SM, Peng G (2004) Challenges and strategies for development of mycoherbicides. In: Arora DK (ed) Fungal biotechnology in agricultural, food, and environmental applications. Marcel Dekker, New York, pp 11–121

Boyetchko SM, Rosskopf EN, Caesar AJ, Charudattan R (2002) Biological weed control with pathogens: search for candidates to applications. In: Khachatourians GG, Arora DK (eds) Applied mycology and biotechnology, vol 2. Elsevier, Amsterdam, pp 239–274

Boyette CD, Hoagland RE (2013) Bioherbicidal potential of a strain of *Xanthomonas* spp. for control of common Cocklebur (*Xanthium strumarium*). Biocontrol Sci Tech 23(2):183–196

Boyette CD, Hoagland RE (2015) Bioherbicidal potential of *Xanthomonas campestris* for controlling *Conyza canadensis*. Biocontrol Sci Tech 25(2):229–237

Boyette CD, Hoagland RE, Abbas HK (2007) Evaluation of the bioherbicide *Myrothecium verrucaria* for weed control in tomato (*Lycopersicon esculentum*). Biocontrol Sci Tech 17(2):171–178

Bucher M, Wegmuller S, Drissner D (2009) Chasing the structures of small molecules in arbuscular mycorrhizal signaling. Curr Opin Plant Biol 12:500–507

Cairney JWG, Ashford AE (2002) Biology of mycorrhizal associations of epacrids (Ericaceae). New Phytol 154:305–326

Carneiro RMDG, Souza IS, Belarmino LC (1998) Nematicidal activity of *Bacillus* spp. 2378 strains on juveniles of *Meloidogyne javanica*. Nematol Bras 22(1):12–21

Castro de Souza AR, Baldoni DB, Lima J, Porto V, Marcuz C, Machado C, Camargo RF, Kuhn RC, Jacques RJS, Guedes JVC, Mazuttia MA (2017) Selection, isolation, and identification of fungi for bioherbicide production. Braz J Microbiol 48(1):101–108

Ceballos I, Ruiz M, Fernández C, Peña R, Rodríguez A, Sanders IR (2013) The in vitro mass-produced model mycorrhizal fungus, *Rhizophagus irregularis*, significantly increases yields of the globally important food security crop cassava. PLoS One 8(8):e70633

Chan-Cupul W, Ruiz-Sánchez E, Cristóbal-Alejo J, Pérez-Gutiérrez A, Munguía-Rosales R, Lara-Reyna J (2010) Desarrollo in vitro de cuatro cepas nativas de *Paecilomyces fumosoroseus* y su patogenicidad en estados inmaduros de mosquita blanca. Agrociencia 44(5):587–597

Chin-A-Woeng TFC, Bloemberg GV, Lugtenberg BJJ (2003) Phenazines and their role in biocontrol by *Pseudomonas* bacteria. New Phytol 157:503–523

Crickmore N, Baum J, Bravo A, Lereclus D, Narva K, Sampson K, Schnepf E, Sun M, Zeigler DR (2018) *Bacillus thuringiensis* toxin nomenclature. In: http://www.btnomenclature.info/

Dean R, Van Kan JAL, Pretorius ZA, Hammond-Kosack KE, Di Pietro A, Spanu PD, Rudd JJ, Dickman M, Kahmann R, Ellis J, Foster GD (2012) The top 10 fungal pathogens in molecular plant pathology. Mol Plant Pathol 13(4):414–430

Del Aguila KM, Vallejos-Torres G, Arévalo LA, Becerra AG (2018) Inoculación de consorcios micorrícicos arbusculares en *Coffea arabica*, variedad Caturra en la región San Martín. Información Tecnológica 29(1):137–146

Eberl L, Vandamme P (2016) Members of the genus *Burkholderia*: good and bad guys. F1000Res 5:1–10

El Kaoutari A, Armougom F, Gordon JI, Raoult D, Henrissat B (2013) The abundance and variety of carbohydrate-active enzymes in the human gut microbiota. Nat Rev Microbiol 11:497–504

Esmaeel Q, Pupin M, Kieu NP, Chataigné G, Béchet M, Deravel J, Krier F, Höfte M, Jacques P, Leclère V (2016) *Burkholderia* genome mining for non-ribosomal peptide synthetases reveals a great potential for novel siderophores and lipopeptides synthesis. Microbiol Open 5(3):512–526

Esmaeel Q, Pupin M, Jacques P, Leclère V (2017) Nonribosomal peptides and polyketides of *Burkholderia*: new compounds potentially implicated in biocontrol and pharmaceuticals. Environ Sci Pollut Res 25(30):29794–29807

Etesami H, Alikhani HA (2017) Evaluation of Gram-positive rhizosphere and endophytic bacteria for biological control of fungal rice (*Oryza sativa* L.) pathogens. Eur J Plant Pathol 147(1):7–14

Figueroa-López AM, Cordero-Ramírez JD, Martínez-Álvarez JC, López-Meyer M, Lizárraga-Sánchez GJ, Gastélum RF, Castro-Martínez M-MIE (2016) Rhizospheric bacteria of maize with potential for biocontrol of *Fusarium verticillioides*. Springerplus 5:330

Gajbhiye MH, Kapadnis BP (2016) Antifungal-activity-producing lactic acid bacteria as biocontrol agents in plants. Biocontrol Sci Technol 26(11):1451–1470

García-Gutiérrez C, González-Maldonado MB (2010) Uso de bioinsecticidas para el control de plagas de hortalizas en comunidades rurales. Ra Ximhai 6(1):17–22

Garg N, Chandel S (2010) Arbuscular mycorrhizal networks: process and functions. A review. Agron Sustain Dev 30:581–599

Gefen G, Anbar M, Morag E, Lamed R, Bayer EA (2012) Enhanced cellulose degradation by targeted integration of a cohesin-fused β-glucosidase into the *Clostridium thermocellum* cellulosome. Proc Natl Acad Sci U S A 109:10298–10303

Gianinazzi-Pearson V (1984) Host-fungus specificity, recognition and compatibility in mycorrhizae. In: Verma DPS, Hohn T (eds) Genes involved in microbe-plant interactions. Springer-Verlag, Vienna, New York, pp 225–253

Gómez-Padilla E, Ruiz-Díez B, Fajardo S, Eichler-Loebermann B, Samson R, Van-Damme P, López-Sánchez R, Fernández-Pascual M (2017) Caracterización de rizobios aislados de nódulos de frijol caupí, en suelos salinos de cuba. Cultivos Tropicales 38(4):39–49

Gonzalez F, Tkaczuk C, Dinu MM, Fiedler Z, Vidal S, Zchori-Fein E, Messelink GJ (2016) New opportunities for the integration of microorganisms into biological pest control systems in greenhouse crops. J Pest Sci 89:295–311

Granda MK, Ochoa MT, Ruilova VV, Guamán FD, Torres RG (2014) Evaluación de cepas nativas de *Rhizobium* sobre parámetros fenotípicos en frijol común (*Phaseolus vulgaris* L.). Centro de Biotecnología 3:25–37

Gwynn RL (2014) The manual of biocontrol agents: a world compendium. BCPC, Alton, British Crop Protection Council, 852 p

Haidar R, Fermaud M, Calvo-Garrido C, Roudet J, Deschamps A (2016) Modes of action for biological control of *Botrytis cinerea* by antagonistic bacteria. Phytopathol Mediterr 55(3):301–322

Halfeld-Vieira BA, da Silva WLM, Schurt DA, Ishida AKN, de Souza GR, Nechet KL (2014) Understanding the mechanism of biological control of passionfruit bacterial blight promoted by autochthonous phylloplane bacteria. Biol Control 80:40–49

Harding DP, Raizada MN (2015) Controlling weeds with fungi, bacteria and viruses: a review. Front Plant Sci 6:1–14

Hervé C, Rogowski A, Blake AW, Marcus SE, Gilbert HJ, Knox JP (2010) Carbohydrate-binding modules promote the enzymatic deconstruction of intact plant cell walls by targeting and proximity effects. Proc Natl Acad Sci U S A 107:15293–15298

Hetherington SD, Smith HE, Scanes MG (2002) Effects of some environmental conditions on the effectiveness of *Drechslera avenacea* (Curtis ex Cooke) Shoem: a potential bioherbicidal organism for *Avena fatua* L. Biol Control 24:103–109

Hill DS, Stein JI, Torkewitz NR, Morse AM, Howell CR, Pachlatko JP, Becker JO, Ligon JM (1994) Cloning of genes involved in the synthesis of pyrrolnitrin from *Pseudomonas fluorescens* and role of pyrrolnitrin synthesis in biological control of plant disease. Appl Environ Microbiol 60:78–85

Hoagland RE, Weaver MA, Boyett CD (2007) *Myrothecium verrucariu* fungus; A bioherbicide and strategies to reduce its non-target risks. Allelopathy J 19(1):179–119

Hu G, St Leger RJ (2002) Field studies using a recombinant mycoinsecticide (*Metarhizium anisopliae*) reveals that it is rhizosphere competent. Appl Environ Microbiol 68:6383–6387

Ignoffo CM (1981) The fungus *Nomuraea rileyi* as a microbial insecticide. In: Burges HD (ed) Microbial control of pests and plant diseases. Academic Press, London, UK, pp 513–538

Imaizumi S, Nishino T, Miyabe K, Fujimori T, Yamada M (1997) Biological control of annual bluegrass (*Poa annua* L.) with a Japanese isolate of *Xanthomonas campestris* pv. *poae* (JT-P482). Biol Control 8(1):7–14

Irma A, Meryandini A, Rupaedah B (2018) Biofungicide producing bacteria: an *in vitro* inhibitor of *Ganoderma boninense*. Hayati J Biosci 25(4):151–159

Juan Y, Wei W, Peng Y, Bu T, Zheng Y, Li-hui Z, Jin-gao D (2015) Isolation and identification of *Serratia marcescens* Ha1 and herbicidal activity of Ha1 'pesta' granular formulation. J Integr Agric 14(7):1348–1355

Kakde UB, Jamdhade VM (2009) Studies on fungi responsible for biodegradation and humification of organic matter. Bionano Frontier 2(2):77–81

Kamei I, Takagi K, Kondo R (2011) Degradation of endosulfan and endosulfan sulfate by white-rot fungus *Trametes hirsuta*. J Wood Sci 57(4):317–322

Kennedy AC, Elliott LF, Young FL, Douglas CL (1991) Rhizobacteria suppressive to the weed downy brome. Am J Soil Sci Soc 55(3):722–727

Kergunteuil A, Bakhtiari M, Formenti L, Xiao Z, Defossez E, Rasmann S (2016) Biological control beneath the feet: a review of crop protection against insect root herbivores. Insects 7(4):1–22

Khan MQ, Abbasi MW, Zaki MJ, Khan SA (2010) Evaluation of *Bacillus thuringiensis* isolates against root-knot nematodes following seed application in okra and mungbean. Pak J Bot 42(4):2903–2910

Kim H, Rim SO, Bae H (2019) Antimicrobial potential of metabolites extracted from ginseng bacterial endophyte *Burkholderia stabilis* against ginseng pathogens. Biol Control 128:24–30

Kirner S, Hammer PE, Hill S, Altmann A, Fischer I, Weislo LJ, Lanahan M, vanPee KH, Ligon JM (1998) Functions encoded by pyrrolnitrin biosynthetic genes from *Pseudomonas fluorescens*. J Bacteriol 180(7):1939–1943

Koch N, Islam NF, Sonowal S, Prasad R, Sarma H (2021) Environmental antibiotics and resistance genes as emerging contaminants: methods of detection and bioremediation. Current Research in Microbial Sciences https://doi.org/10.1016/j.crmicr.2021.100027

Kumar DS, Tarakeswari M, Lakshminarayana M, Sujatha M (2016) Toxicity of *Bacillus thuringiensis* crystal proteins against eri silkworm, *Samia cynthia ricini* (Lepidoptera: Saturniidae). J Invertebr Pathol 138:116–119

Lacey LA, Grzywacz D, Shapiro-Ilan DI, Frutos R, Brownbridge M, Goettel MS (2015) Insect pathogens as biological control agents: back to the future. J Invertebr Pathol 132:1–41

Lamar RT, Larsen MJ, Kirk TK (1990) Sensitivity to and degradation of pentachlorophenol PCP by *Phanerochaete chrysosporium*. Appl Environ Microbiol 56(11):3519–3526

Lan W, Chen Y, Wu H, Yanagida F (2012) Bio-protective potential of lactic acid bacteria isolated from fermented wax gourd. Folia Microbiol 57(2):90–105

Li W, Sheng C (2007) Occurrence and distribution of entomo-phthoralean fungi infecting aphids in mainland China. Biocon Sci Technol 17:433–439

Li Y, Wu C, Xing Z, Gao B, Zhang L (2017) Engineering the bacterial endophyte *Burkholderia pyrrocinia* JK-SH007 for the control of Lepidoptera larvae by introducing the cry218 genes of *Bacillus thuringiensis*. Biotechnol Biotechnol Equip 31(6):1167–1172

López-Alcocer JJ, Lépiz-Ildefonso R, González-Eguiarte DR, Rodríguez-Macías R, López-Alcocer E, Olalde-Portugal V (2017) Caracterización morfológica y bioquímica de cepas de Rhizobium colectadas en frijol común silvestre y domesticado. Rev Fitotec Mex 40(1):73–81

Lu J, Huang X, Li K, Li S, Zhang M, Wang Y, Jiang H, Xu Y (2009) LysR family transcriptional regulator PqsR as repressor of pyoluteorin biosynthesis and activator of phenazine-1-carboxylic acid biosynthesis in *Pseudomonas* sp. M18. J Biotechnol 143:1–9

Lugtenberg B, Kamilova F (2009) Plant-growth-promoting rhizobacteria. Annu Rev Microbiol 63:541–556

Mahenthiralingam E, Boaisha O, Song L, Paine J, Sass A, Knight D, White J, Challis GL (2011) Enacyloxins are products of an unusual hybrid modular polyketide synthase encoded by a cryptic *Burkholderia ambifaria* genomic island. Chem Biol 18:665–677

Mäkelä MR, Donofrio N, de Vries RD (2014) Plant biomass degradation by fungi. Fungal Genet Biol 72:2–9

Malathi B, Ramesh S, Venkateswara Rao K, Dashavantha Reddy V (2006) *Agrobacterium*-mediated genetic transformation and production of semilooper resistant transgenic castor (*Ricinus communis* L.). Euphytica 147(3):441–449

Marley PS, Kroschel J, Elzien A (2005) Host specificity of *Fusarium oxysporum* Schlect (Isolate PSM 197), a potential mycoherbicide for controlling *Striga* spp. in West Africa. Weed Res 45(6):407–412

Marquina ME, González NE, Castro Y (2011) Caracterización fenotípica y genotípica de doce rizobios aislados de diversas regiones geográficas de Venezuela. Rev Biol Trop 59:1017–1036

Masschelein J, Jenner M, Challis GL (2017) Antibiotics from Gram-negative bacteria: a comprehensive overview and selected biosynthetic highlights. Nat Prod Rep 34(7):679–934

Mata M, Barquero M (2010) Evaluación de la fermentación sumergida del hongo entomopatógeno *Beauveria bassiana* como parte de un proceso de escalamiento y producción de bioplaguicidas. PROMECAFE 122:8–19

Melnick RL, Zidack NK, Bailey BA, Maximova SN, Guiltinan M, Backman PA (2008) Bacterial endophytes: *Bacillus* spp. from annual crops as potential biological control agents of black pod rot of cacao. Biol Control 46(1):46–56

Meyer V, Andersen MR, Brakhage AA, Braus GH, Caddick MX, Cairns TC, de Vries RP, Haarmann T, Hansen K, Hertz-Fowler C, Krappmann S, Mortensen UH, Peñalva MA, Ram AF, Head RM (2016) Current challenges of research on filamentous fungi in relation to human welfare and a sustainable bio-economy: a white paper. Fungal Biol Biotechnol 3(6):1–17

Michelsen CF, Stougaard P (2012) Hydrogen cyanide synthesis and antifungal activity of the biocontrol strain *Pseudomonas fluorescens* In5 from Greenland is highly dependent on growth medium. Can J Microbiol 58:381–390

Miller SA, Beed FB, Harmon CL (2009) Plant disease diagnostic capabilities and networks. Annu Rev Phytopathol 47:15–38

Miotto-Vilanova L, Jacquard C, Courteaux B, Wortham L, Michel J, Clément C, Barka EA, Sanchez L (2016) *Burkholderia phytofirmans* PsJN confers grapevine resistance against *Botrytis cinerea* via a direct antimicrobial effect combined with a better resource mobilization. Front Plant Sci 7:1–15

Mohammed SH, Seady MA, Enan MR, Ibrahim NE, Ghareeb A, Moustafa SA (2008) Biocontrol efficiency of *Bacillus thuringiensis* toxins against root-knot nematode, *Meloidogyne incognita*. J Cell Mol Biol 7:57–66

Molina R, Trappe JM (1982) Lack of mycorrhizal specificity by the ericaceous host *Arbutus menziesii* and *Arctostaphylos uva ursi*. New Phytol 90:495–509

Mortensen K (1988) The potential of an endemic fungus, *Colletotrichum gloeosporioides*, for biological control of round-leaved mallow (*Malva pusilla*) and velvet leaf (*Abutilon theophrasti*). Weed Sci 36:473–478

Mosttafiz SB, Rahman M, Rahman M (2012) Biotechnology: role of microbes in sustainable agriculture and environmental health. Int J Microbiol 10(1):1–7

Müller T, Behrendt U, Ruppel S, von der Waydbrink G, Müller MEH (2015) Fluorescent Pseudomonads in the phyllosphere of wheat: potential antagonists against fungal phytopathogens. Curr Microbiol 72(4):383–389

Mullins AJ, Murray JAH, Bull MJ, Jenner M, Jones C, Webster G, Green AE, Neill DR, Connor TR, Parkhill J, Challis GL, Mahenthiralingam E (2019) Genome mining identifies cepacin as a plant protective metabolite of the biopesticidal bacterium *Burkholderia ambifaria*. Nat Microbiol 4:996–1005

Newcombe D, Paszcynsky A, Gajewska W, Kroger M, Feis G, Crawford R (2002) Production of small molecular weight catalyst and the mechanism of trinitrotoluene degradation by several *Gloeophyllum* species. Enzyme Microb Technol 30:506–517

Nielsen UN, Ayres E, Wall DH, Bardgett RD (2011) Soil biodiversity and carbon cycling: a review and synthesis of studies examining diversity-function relationships. Eur J Soil Sci 62:105–116

Noble AD, Ruaysoongnern S (2010) The nature of sustainable agriculture. In: Dixon R, Tilston E (eds) Soil microbiology and sustainable crop production. Springer Science and Business Media B.V, Berlin, Heidelberg, Germany, pp 1–25

Novotný C, Svobodová K, Erbanová P, Cajthaml T, Kasinath A, Lang E, Šašek V (2004) Ligninolytic fungi in bioremediation: extracellular enzyme production and degradation rate. Soil Biol Biochem 36:1545–1551

Oren A, Garrity GM (2015) List of new names and new combinations previously effectively, but not validly, published. Int J Syst Evol Microbiol 65(11):3763–3767

Ortiz-Ribbing L, Williams MM (2006) Potential of *Phomopsis amaranthicola* and *Microsphaeropsis amaranthi*, as bioherbicides for several weedy *Amaranthus* species. Crop Prot 25(1):39–46

Ortiz-Ribbing LM, Glassman KR, Roskamp GK, Hallett SG (2011) Performance of two bioherbicide fungi for waterhemp and pig-weed control in pumpkin and soybean. Plant Dis 95:469–477

Osorio-Fajardo A, Canal NA (2011) Selección de cepas de hongos entomopatógenos para el manejo de *Anastrepha obliqua* (Macquart, 1835) (Diptera: Tephritidae) en Colombia. Rev Fac Nal Agr Medellín 64(2):6129–6139

Pan D, Mionetto A, Tiscornia S, Bettucci L (2015) Endophytic bacteria from wheat grain as biocontrol agents of *Fusarium graminearum* and deoxynivalenol production in wheat. Mycotoxin Res 31(3):137–143

Parvin W, Othman R, Jaafar H, Wong MY (2016) Detection of phenazines from UPMP3 strain of *Pseudomonas aeruginosa* and its antagonistic effects against *Ganoderma boninense*. Int J Agric Biol 8:483–488

Patil VS (2014) Isolation, characterization and identification of rhizospheric bacteria with the potential for biological control of *Sida acuta*. J Environ Res Dev 8(3):411–417

Peterson L, Massicotte HB, Melville LH (2004) Mycorrhizas: anatomy and cell biology. NRC Research Press, USA, p 196

PMRA (2006) Re-evaluation of *Colletotrichum gloeosporioides* f.sp. *malvae* [CGM]. REV2006-10. Health Canada, Ottawa, ON, 7p

PMRA (2010) *Sclerotinia minor* Strain IMI 34414. RD2010-08. Health Canada, Ottawa, ON, 30p

Pointing SB (2001) Feasibility of bioremediation by white-rot fungi. Appl Microbiol Biotechnol 57:20–33

Porcar M, Gómez F, Gruppe A, Gómez-Pajuelo A, Segura I, Schroder R (2008) Hymenopteran specificity of *Bacillus thuringiensis* strain PS86Q3. Biol Control 45(3):427–432

Prasad R, Kumar M, Varma A (2015) Role of PGPR in soil fertility and plant health. In: Egamberdieva D, Shrivastava S, Varma A (eds) Plant growth-promoting rhizobacteria (PGPR) and medicinal plants. Springer International Publishing, Switzerland, pp 247–260

Prasad R, Bhola D, Akdi K, Cruz C, Sairam KVSS, Tuteja N, Varma A (2017) Introduction to mycorrhiza: historical development. In: Varma A, Prasad R, Tuteja N (eds) Mycorrhiza. Springer International Publishing AG, Cham

Prasad R, Chhabra S, Gill SS, Singh PK, Tuteja N (2020) The microbial symbionts: potential for the crop improvement in changing environments. In: Tuteja N, Tuteja R, Passricha N, Saifi SK (eds) Advancement in crop improvement techniques. Elsevier, Amsterdam, Netherlands, pp 233–240

Purnomo AS, Mori T, Ryuichiro K (2010) Involvement of Fenton reaction in DDT degradation by brown-rot fungi. Int Biodeterior Biodegrad 64:560–565

Purnomo AS, Morib TK, Ichiro KR (2011) Basic studies and applications on bioremediation of DDT: a review. Int Biodeterior Biodegrad 65:921–930

Quail JW, Ismail N, Pedras SC, Boyetchko SM (2002) Pseudophomins A and B, a class of cyclic lipodepsipeptides isolated from a Pseudomonas species. Acta Crystallogr C 58(5):268–271

Quintero JC, Lú-Chau TL, Moreira MT, Feijoo G, Lema JM (2007) Bioremediation of HCH present in soil by the white-rot fungus *Bjerkandera adusta* in a slurry batch bioreactor. Int Biodeterior Biodegrad 60(4):319–326

Quoreshi AM (2008) The use of mycorrhizal biotechnology in restoration of disturbed ecosystem. In: Siddiqui ZA, Akhtar MS, Futai K (eds) Mycorrhizae: sustainable agriculture and forestry. Springer, Dordrecht, pp 303–320

Raaijmakers J, Paulitz T, Steinberg C, Alabouvette C, Moënne-Loccoz Y (2009) The rhizosphere: a playground and battlefield for soilborne pathogens and beneficial microorganisms. Plant Soil 321(1–2):341–361

Rai D, Updhyay V, Mehra P, Rana M, Pandey AK (2014) Potential of entomopathogenic fungi as biopesticides. Ind J Sci Res Technol 2(5):7–13

Ramli NR, Mohamed MS, Seman IA, Zairun MA, Mohamad N (2016) The potential of endophytic bacteria as a biological control agent for ganoderma disease in oil palm. Sains Malaysia 45:401–409

Rika Fithri NB, Aris Tri W, Nurita TM (2014) Control activity of potential antifungal-producing *Burkholderia* sp. in suppressing *Ganoderma boninense* growth in oil palm. Asian J Agric Res 8:259–268

Rouse S, Harnett D, Vaughan A, van Sinderen D (2008) Lactic acid bacteria with potential to eliminate fungal spoilage in foods. J Appl Microbiol 104(3):915–923

Ruttimann C, Lamar R (1997) Binding of substances in pentachlorophenol to humic soil by the action of white rot fungi. Soil Biol Biochem 9(7):1143–1148

Samuels RI, Coracini DLA, Martins Dos Santos CA, Gava CAT (2002) Infection of *Blissus antillus* (Hemiptera: Lygaeidae) eggs by the entomopathogenic fungi *Metarhizium anisopliae* and *Beaveria bassiana*. Biol Control 23(3):269–273

Sandani HBP, Ranathunge NP, Lakshman PLN, Weerakoon WMW (2019) Biocontrol potential of five *Burkholderia* and *Pseudomonas* strains against *Colletotrichum truncatum* infecting chilli pepper. Biochem Sci Technol. https://doi.org/10.1080/09583157.2019.1597331

Sathe SJ, Nawani NN, Dhakephalkar PK, Kapadnis BP (2007) Antifungal lactic acid bacteria with potential to prolong shelf-life of fresh vegetables. J Appl Microbiol 103(6):2622–2628

Sawana A, Adeolu M, Gupta RS (2014) Molecular signatures and phylogenomic analysis of the genus *Burkholderia*: proposal for division of this genus into the emended genus *Burkholderia* containing pathogenic organisms and a new genus *Paraburkholderia* gen. nov. harboring environmental species. Front Genet 5:1–22

Sayed MHE, Aziz ZKA, Abouzaid AM (2014) Efficacy of extracellular metabolite produced by *Streptomyces levis* strain LX-65 as a potential herbicidal agent. J Am Sci 10(11):169–180

Schlosser D, Fahr K, Karl W, Wetzstein HG (2000) Hydroxylated metabolites of 2,4-dichlorophenol imply a Fenton-type reaction in *Gloeophyllum striatum*. Appl Environ Microbiol 66:2479–2483

Schübler A, Schwarzott D, Walker C (2001) A new fungal phylum, the Glomeromycota: phylogeny and evolution. Mycol Res 105:1413–1421

Show PL, Oladele KO, Siew QY, Aziz Zakry FA, Lan JC-W, Ling TC (2015) Overview of citric acid production from *Aspergillus niger*. Front Life Sci 8:271–283

Simonetti E, Roberts IN, Montecchia MS, Gutierrez-Boem FH, Gomez FM, Ruiz JA (2018) A novel *Burkholderia ambifaria* strain able to degrade the mycotoxin fusaric acid and to inhibit *Fusarium* spp. growth. Microbiol Res 206:50–59

Sindhu SS, Sehrawat A, Sharma R, Dahiya A (2016) Biopesticides: use of rhizosphere bacteria for biological control of plant pathogens. Def Life Sci J 1(2):135–148

Singh BK, Kuhad RC (1999) Biodegradation of lindane gamma-hexachlorocyclohexane by the white-rot fungus *Trametes hirsutus*. Lett Appl Microbiol 28(3):238–241

Singh JS, Pandey VC, Singh DP (2011) Efficient soil microorganisms: a new dimension for sustainable agriculture and environmental development. Agric Ecosyst Environ 140:339–353

Smith SE, Read DJ (1997) Mycorrhizal symbiosis. Academic Press, London, p 605

Song D, Chen G, Liu S, Khaskheli MA, Wu L (2018) Complete genome sequence of *Burkholderia* sp. JP2-270, a rhizosphere isolate of rice with antifungal activity against *Rhizoctonia solani*. Microb Pathogen 127:1–6

Steinhaus EA (1975) Disease in a minor chord. Ohio State University Press, Columbus, pp 1–508

Sujatha M, Lakshminarayana M, Tarakeswari M, Singh PK, Tuli R (2009) Expression of the *cry1EC* gene in castor (*Ricinus communis* L.) confers field resistance to tobacco caterpillar (*Spodoptera litura* Fabr) and castor semilooper (*Achoea janata* L.). Plant Cell Rep 28:935–946

Suryadi Y, Susilowati DN, Lestari P, Priyatno TP, Samudra IM, Hikmawati N, Mubarik NR (2014) Characterization of bacterial isolates producing chitinase and glucanase for biocontrol of plant fungal pathogens. J Agric Technol 10(4):983–999

Suzuki N, Hori H, Ogiwara K, Asano S, Sato R, Ohba M, Iwahana H (1992) Insecticidal spectrum of a novel isolate of *Bacillus thuringiensis* serovar *japonensis*. Biol Control 2(2):138–142

Talamantes D, Biabini N, Dang H, Abdoun K, Berlemont R (2016) Natural diversity of cellulases, xylanases, and chitinases in bacteria. Biotechnol Biofuels 9:133

Tejomyee SB, Pravin RP (2007) Biodegradation of organochlorine pesticide, endosulfan, by a fungal soil isolate, *Aspergillus niger*. Int Biodeterior Biodegrad 59(4):315–321

Thangavelu R, Sangeetha G, Mustaffa MM (2007) Cross-infection potential of crown rot pathogen (*Lasiodiplodia theobromae*) isolates and their management using potential native bioagents in banana. Aust Plant Pathol 36(6):595–605

Tirado-Gallego PA, Lopera-Álvarez A, Ríos-Osorio LA (2016) Estrategias de control de *Moniliophthora roreri* y *Moniliophthora perniciosa* en *Theobroma cacao* L.: revisión sistemática. Corpoica Cienc Tecnol Agrop 17(3):417–430

Trias R, Bañeras L, Badosa E, Montesinos E (2008a) Bioprotection of golden delicious apples and Iceberg lettuce against foodborne bacterial pathogens by lactic acid bacteria. Int J Food Microbiol 123(1–2):50–60

Trias R, Bañeras L, Montesinos E, Badosa E (2008b) Lactic acid bacteria from fresh fruit and vegetables as biocontrol agents of phytopathogenic bacteria and fungi. Int Microbiol 11(4):231–236

Utsumi R, Yagi T, Katayama S, Katsuragi K, Tachibana K, Toyoda H, Ouchi S, Obata K, Shibano Y, Noda M (1991) Molecular cloning and characterization of the fusaric acid-resistance gene from *Pseudomonas cepacia*. Agric Biol Chem 55(7):1913–1918

VanFossen AL, Ozdemir I, Zelin SL, Kelly RM (2011) Glycoside hydrolase inventory drives plant polysaccharide deconstruction by the extremely thermophilic bacterium *Caldicellulosiruptor saccharolyticus*. Biotechnol Bioeng 108:1559–1569

Varma A, Prasad R, Tuteja N (2017) Mycorrhiza: nutrient uptake, biocontrol, ecorestoration. Springer International Publishing. http://www.springer.com/us/book/9783319688664

Varma A, Swati T, Prasad R (2020) Plant Microbe Symbiosis. Springer International Publishing (ISBN 978-3-030-36247-8) https://www.springer.com/gp/book/9783030362478

Várnai A, Siika-Aho M, Viikari L (2013) Carbohydrate-binding modules (CBMs) revisited: reduced amount of water counterbalances the need for CBMs. Biotechnol Biofuels 6(1):1–11

Vega FE (2008) Insect pathology and fungal endophytes. In: Vincent C, Goettel MS, Lazarovits G (eds) Biological control: a global perspective. CAB International/AAFC, Wallingford, United Kingdom, 432 p

Villamil JE, Viteri SE, Villegas WL (2015) Aplicación de antagonistas microbianos para el control biológico de *Moniliophthora roreri* Cif & Par en *Theobroma cacao* L. bajo condiciones de campo. Rev Fac Nal Agr Medellín 68(1):7441–7450

Villamil J, Martínez J, Pinzón E (2016) Actividad biológica de hongos entomopatógenos sobre *Premnotrypes vorax* Hustache (Coleoptera: Curculionidae). Rev Cienc Agric 33(1):34–42

Wei JZ, Hale K, Carta L, Platzer E, Wong C, Fang SC, Aroian RV (2003) *Bacillus thuringiensis* crystal proteins that target nematodes. Proc Natl Acad Sci U S A 100(5):2760–2765

Weidmann S, Sanchez L, Descombin J, Chataqnier O, Gianinazzi S, Gianinazzi-Pearson V (2004) Fungal elicitation of signal transduction related plant genes precedes mycorrhiza establishment and requires the dmi3 gene in *Medicago truncatula*. Mol Plant-Microbe Interact 17:1385–1393

Yadegari M, Rahmani HA (2010) Evaluation of bean (*Phaseolus vulgaris*) seeds' inoculation with *Rhizobium phaseoli* and plant growth promoting Rhizobacteria (PGPR) on yield and yield components. Afr J Agric Res 5:792–799

Yang J, Kloepper JW, Ryu CM (2009) Rhizosphere bacteria help plants tolerate abiotic stress. Trends Plant Sci 14:1–4

Yasin M, Wakil W, Elshafie H, Miller TA (2017) Potential role of microbial pathogens in control of red palm weevil (*Rhynchophorus ferrugineus*). A review. Entomol Res 47:219–234

Yu TE, Egger KN, Peterson RL (2001) Ectendomycorrhizal associations-characteristics and functions. Mycorrhiza 11:167–177

Zarafi AB, Dauda WP (2019) Exploring the importance of fungi in agricultural biotechnology. Int J Agric Sci Vet Med 7(1):1–12

Zeilinger S, Omann M (2007) Trichoderma biocontrol: signal transduction pathways involved in host sensing and mycoparasitism. Gene Regul Syst Biol 1:227–234

Chapter 13
Plant-Fungal Association: An Ideal Contrivance for Combating Plant Stress Tolerance

Akanksha Sharma, Aditya Singh, Meenakshi Raina, and Deepak Kumar

Contents

Akanksha Sharma and Aditya Singh contributed equally with all other contributors.

A. Sharma · A. Singh
Department of Botany, Central University of Jammu, Rahya Suchani (Bagla), Jammu and Kashmir, India

M. Raina
Department of Botany, Central University of Jammu, Rahya Suchani (Bagla), Samba, Jammu and Kashmir, India

Department of Biotechnology, Government Degree College (Boys), Udhampur, Jammu and Kashmir, India

D. Kumar (✉)
Department of Botany, Institute of Science, Banaras Hindu University, Varanasi, Uttar Pradesh, India
e-mail: deepakkumar@bhu.ac.in

R. Prasad et al. (eds.), *Mycoremediation and Environmental Sustainability*, Fungal Biology, https://doi.org/10.1007/978-3-030-54422-5_13

13.1 Introduction

Plant stress is a condition wherein the normal growth and development of a plant is severely hampered due to certain external conditions (Verma et al. 2013). Stress in plants can result in a cascade of changes ranging from altered gene expression, cellular metabolism, plant productivity, etc. A plant stress more often than not is an indication of an abrupt environmental change. Notwithstanding the previous statement, prolonged stress exposure may result in plants adapting various strategies to counter the altered conditions for the sake of their survival during a period of time (Verma et al. 2013). Plant stress can either be biotic stress or abiotic stress. Biotic stress exposed to plants is a biological unit like disease, insect, and phytopathogens, while abiotic stress imposed on plants by environment may be either physical or chemical. Abiotic and biotic stresses contribute 50% and 30%, respectively, to losses in agricultural productivity worldwide (Chodak et al. 2015).

It is pertinent to mention here that plants are not an isolated entity growing in total seclusion, rather plants grow in a dynamic environment with various interactions among them and other biotic and abiotic factors. In this context, it is important to highlight that most plants in natural ecosystems have symbiotic associations with fungi. Symbiosis (from the Greek *sumbiōsis*, living together) was first described by Anton de Barry and later interpreted by Hertig et al. (1937). Modern day studies indicate that almost all plant life on earth is inextricably linked with fungi. These fungi are necessary for maintaining the structure, function and health of the plant. In this scenario, studying them becomes even more essential to understand the effect of fungi on plant stress. In fact, symbiotic fungi have shown to impart stress tolerance and adaptations in plants for thriving in difficult conditions (Rodriguez et al. 2003).

Plant fungal symbionts can either be endophytes or mycorrhiza. Endophytes reside inside plant tissues and are found in root, stem or leaf, whereas mycorrhizal fungi are found in close association with the roots extending into the rhizosphere. Carroll (1988) have divided endophytic fungi into two main classes. Class I comprises of the constitutive mutualists, which infect grasses only and show vertical transmission via seeds. These are systemic in nature (e.g. *Epichloë/neotyphodium*). Class II contains the inducible mutualists, which infect a wide range of plants and show horizontal transmission (e.g. *Aspergillus niger, Aspergillus terreus, Aspergillus ochraceous and Trichoderma viride*). Fungal symbionts exhibit different modes of lifestyles like mutualism, commensalism and parasitism (Rodriguez et al. 2003; Varma et al. 2020). Among all the above-mentioned lifestyles, mutualism/symbiosis is considered to be the most effective in conferring host fitness benefits that will ultimately result in stress tolerance and higher plant productivity. Plant association with fungus helps to reduce the deleterious effects of stress on plants by improving absorption and translocation of nutrients, aiding in nutrient cycling etc. (Kumar and Verma 2018; Prasad et al. 2020).

Plants have evolved and adapted to persist and thrive in stressed conditions by forming symbiotic relations with organisms like fungi that have imparted these plants a tool necessary to tide over the unfavourable conditions (Chadha et al. 2015). Plant-fungal associations are not only economically sound but also environment friendly in terms of combating various plant stresses (Kumar and Verma 2018). In

this chapter, we will discuss the various strategies and mechanisms that involve plant-fungal symbiosis for countering various biotic and abiotic stresses.

13.1.1 Arbuscular Mycorrhizal Fungi: Mechanism of Action

Arbuscular mycorrhizal fungi (AMF) not only supply essential inorganic nutrients to plants but also upregulate plant defence mechanisms against various environmental stresses. AMF provides plant with unique strategies to cope with stressful conditions by playing a crucial link between the plant and the fungi, resulting in an increased photosynthetic rate as well as higher gas-exchange-related traits (Birhane et al. 2012). Most AMF members belong to the sub-phylum Glomeromycotina of the phylum *Mucoromycota* (Spatafora et al. 2016). *Paraglomerales, Glomerales, Diversisporales* and *Archaeosporales* which collectively include 25 genera are the four orders that have been identified in the sub-phylum Glomeromycotina (Redecker et al. 2013). Most bryophytes, pteridophytes and flowering plants, that is ranging up to 90%, can form AMF associations (Zhu et al. 2010a, b; Ahanger et al. 2014). AMF can form arbuscules, vesicles and hyphae in roots along with spores and hyphae in the rhizosphere (Bowles et al. 2016). Fungal hyphae extensively increase the absorptive surface area of the plant roots, thereby enhancing both water and nutrient absorption. Not only this, fungal hyphae also enhance the soil quality and texture along with aiding in the process of decomposition of organic matter (Zou et al. 2016; Paterson et al. 2016; Thirkell et al. 2017). Moreover, AMF also increase the translocation of photo-assimilates to the various plant parts and may also increase the "sink effect" by positively influencing atmospheric CO_2 fixation in plants.

AMF association apart from providing nutrients and minerals also defends plants against several fungal pathogens (Jung et al. 2012; Smith and Read 2008). Under abiotic stress, many nutrients relocate from fungus to the plant itself, thus helping the host to tide over unfavourable conditions (Plassard and Dell 2010). Many physiological functions of plants including growth, CO_2 assimilation, relative water content (RWC), PSII efficiency, stomatal conductance, leaf water potential, etc., are regulated by AMF association (Chandrasekaran et al. 2019; He et al. 2017). Thus, under abiotic stress, these functions are adjusted by the fungal partners to increase the plant efficiency during fluctuating environmental conditions. Barzana et al. (2012) observed AMF association to strengthen water stress tolerance of plants by altering the physiology of the shoot system. In case of drought or salinity stress, AMF can increase plant dry matter along with higher water moisture uptake, resulting in better plant tolerance. It is evident that a plant with better growth will possess greater vigour to tolerate abiotic stresses. In this context, plants with AMF association had improved uptake of almost all nutrients with decreased uptake of Na^+ and Cl^-, resulting in growth stimulation (Evelin et al. 2012). Similarly, uptake of macronutrient N is greatly increased due to extensive underground network of fungal mycelia. In addition to greater absorption of N, almost 20–75% of the N absorbed by the AMF is directly translocated to the plant (Hashem et al. 2018; Ahanger et al. 2014; Hameed et al. 2014; Tanaka and Yano 2005; Govindarajulu et al. 2005).

Wang et al. (2018) observed that AMF during salt stress affects the N:P ratio in plant to maximize plant's performance under stress. Under high metal stress, the translocation of metal ions with the help of AMF association further strengthens the role played by AMF in abiotic stress mitigation. AMF like *Glomus mosseae* and *Rhizophagus irregularis* showed a significantly higher metal translocation in the plant shoot (Ali et al. 2015; Zaefarian et al. 2013). Also, it has been shown by Asrar et al. (2012) that the concentration of macronutrients such as N, P, K, Ca and Mg can be increased by specific fungal association under conditions of drought in *Antirrhinum majus*. Moreover, Bati et al. (2015) showed that plant-AMF association also aids in restricting the high accumulation of Na, Mn, Mg and Fe in roots. Rouphael et al. (2015) reported that pH regulation could aid in the diminution of abiotic stress by AMF, thereby conserving its horticultural value.

AMF association in case of biotic stress follows a similar pattern to that of abiotic stresses, with alteration of plant physiology resulting in heightened state of plant defence mechanism. In plants, phytohormones like salicylic acid (SA), jasmonate (JA), ethylene (ET) and abscisic acid (ABA) are in the frontline of plant defence response (Pieterse et al. 2009). In case of plants with AMF association, the level of these hormones appears to be altered (López-Ráez et al. 2010). AMF association activates phenylpropanoid and oxylipin metabolism along with accumulation of reactive oxygen species and plant-defence-related enzymes (López-Ráez et al. 2010). Similarly, Pozo et al. (2009) had shown that mycorrhizal association in tomato accentuated the expression of defence-related genes which were known to get activated by JA (jasmonate) under biotic stresses.

13.2 Biotic Stress in a Nutshell

Biotic stress in plants is caused by various living organisms like insects, arachnids, nematodes, viruses, bacteria, fungi and weeds, Fig. 13.1. These organisms may, in extreme cases, starve the host plant of its nutrients, ultimately resulting in the plant's death. Economically, biotic stress is a major contributor for agricultural yield loss, resulting in food shortage and poor nutrient quality. In the continuous race for survival, plants have devised various strategies to counter such biotic stresses. These defence mechanisms help plants to perpetuate their species even in harsh conditions.

13.2.1 Role of Fungal Endophytes in Alleviating Plant Biotic Stress

Endophytic fungi (EF) are organisms that reside inside a healthy plant tissue without inflicting any morbid change in the structure or causing any diseases for most part of its life cycle (Rajamanikyam et al. 2017). An endophytic fungus generally lives in its mycelial form in biological association with the living plant and is found

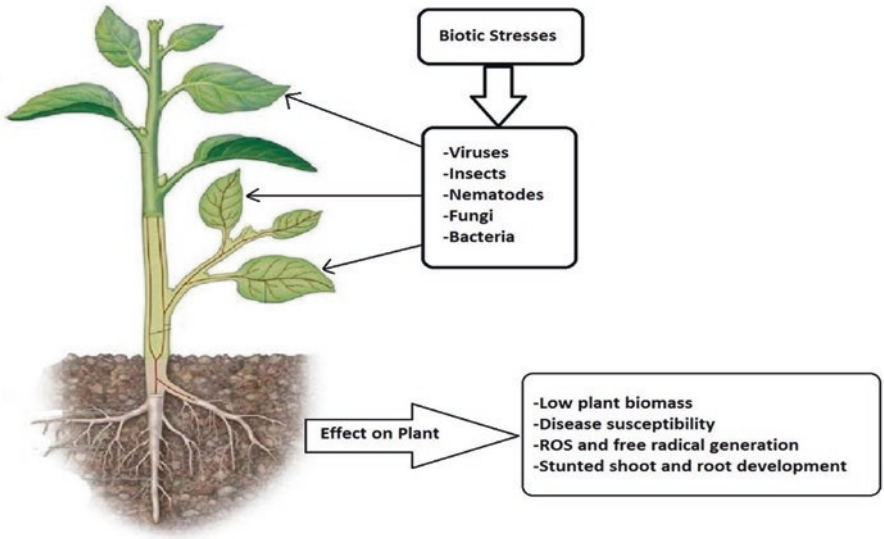


Fig. 13.1 Various agents responsible for biotic stress and their effect on plants

in all kinds of plants: trees, grasses, algae and herbaceous plants. EF is an important source of a plethora of plant metabolites. These bioactive compounds not only play a key role in suppressing biotic stress, but they may also trigger plant immune responses against invading pathogens (Rajamanikyam et al. 2017).

Endophytic fungi mainly consist of members of the Ascomycota or their mitosporic fungi, as well as some taxa of the Basidiomycota, Zygomycota and Oomycota, as depicted in Fig. 13.2.

13.2.2 Classification of Plant Pathogens Causing Biotic Stress

Biotic stresses caused by agents like pests, microbes, and insects decrease not only the productivity of the plant but also severely impact the health of the plant in general. Chadha et al. (2015) have broadly classified the different types of plant pathogens interacting with fungal endophyte into three categories: endophyte and nematode, endophyte and plant pathogenic fungi and endophyte with other plant pathogens, as depicted in Fig. 13.3.

13.2.2.1 Impact of Fungal Endophytes on Biotic Stress Amelioration Caused by Nematodes

According to Zabalgogeazcoa (2008), The occurrence of inhibitory effect is due to the translocation of fungal alkaloids from the aerial parts (where the plant is infected with the endophyte) to the roots, as shown by Timper et al. (2005). Plants infected

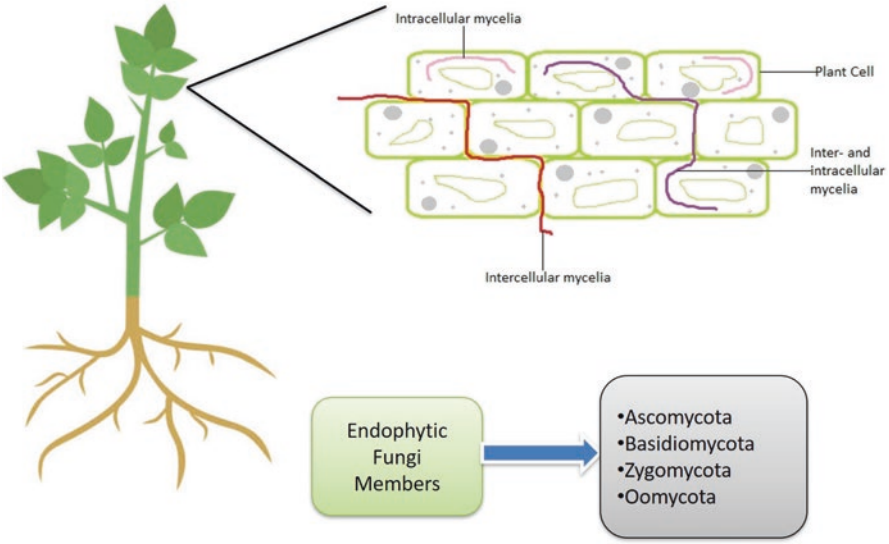


Fig. 13.2 Classification of endophytic fungi and existence in plant cells

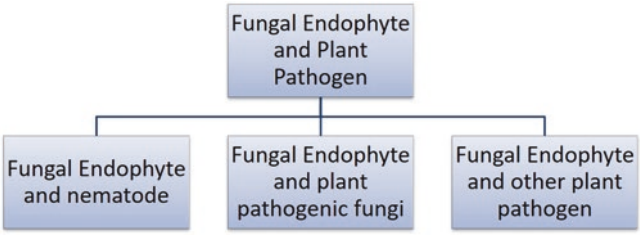


Fig. 13.3 A classification of various types of plant pathogens interacting with fungal endophyte

with Neotyphodium strains that did not produce ergot alkaloids were unable to defend themselves against Pratylenchus sp. (nematode) in contrast to the plants infected with ergot alkaloid producing endophyte (Timper et al. 2005). According to Timper et al. (2005), translocation of fungal alkaloids forms aerial parts to roots imparted plants toward off these nematodes as *Neotyphodium* strains deficient in the production of ergot alkaloids were not able to protect themselves against nematodes like *Pratylenchus* sp.

Though there are doubts as to whether ergot alkaloids are essential for plant defence against nematodes, but nevertheless, *Neotyphodium* sp. are known to produce various types of alkaloids exhibiting anti-herbivore activity along with the production of phenolic compounds (Malinowski and Belesky 2000). Although, the exact mechanism is still not known, but it is safe to say that *Neotyphodium* endophytes protect plant from various nematodes, exhibiting alleviation of biotic stress.

13.2.3 Impact of Fungal Endophytes on Biotic Stress Amelioration Caused by Fungal Pathogens

A diverse range of fungal endophytic species produce antibiotic substances which have the capacity to stop the growth of many plant pathogenic fungi (Zabalgogeazcoa 2008; Koch et al. 2021). It was observed that if six species of fungal endophytes commonly isolated from cocoa (*Theobroma cacao* L.) trees were used to inoculate leaves of endophyte free seedlings of the same plant species, the effect of leaf disease caused by *Phytophthora* sp. was drastically reduced. One plausible mechanism for plant defence in the above said case could be due to a direct competition between the endophytes and fungal pathogen (Arnold et al. 2003). The already present fungal endophyte occupies most of the leaf tissue required for fungal pathogen along with producing zones of inhibition restricting the growth of fungal pathogen, thereby decreasing the impact of biotic stress. Similarly, in another study, it has been seen that fungal endophyte infection may result in changed plant biochemistry such that it could be used against plant pathogens. Root endophyte *Piriformospora indica* has a diverse range of hosts which includes cereals, pulses, vegetables and medicinal plants (Vadassery et al. 2009; Bagde et al. 2010a,b, 2011; Prasad et al. 2008, 2013). Cereals like barley when inoculated with this endophyte showed resistance to a vascular pathogen *Fusarium culmorum* and *Blumeriagramini* (Waller et al. 2005; Gill et al. 2016).

Fungal endophytes may also be mycoparasites by nature as in the case of *Acremonium strictum* isolated from *Dactylis glomerata* L. The fungus *Acremonium strictum* is a mycoparasite of *Helminthosporium solani,* which invades potato plants (Rivera-Varas et al. 2007). Interestingly, among all the fungal species that infect plants only a relatively small proportion causes disease, that is acts as pathogens, thus indicating that part of plant disease cycle is shared by pathogens of endophytes. Once a plant is infected by a fungus, the fungus can either act as a pathogen or an endophyte, though majority of them behave as endophytes. In some cases, mutation in a single locus converts pathogens such as *Colletotrichum magna* to a mutualistic endophyte (Freeman and Rodriguez 1993). However, in some cases, the above-mentioned fungal species behave as pathogen in cucurbits or as an endophyte in other plant species (Redman et al. 2001)

13.2.3.1 Impact of Fungal Endophytes on Biotic Stress Caused by Other Plant Pathogens

Effects of endophytes on plant pathogens like bacteria and virus are not numerous compared to other plant pathogens. Viruses like barley yellow dwarf virus (BYDV) showed reduced pathogenicity in *Lolium pratense* infected by endophyte

Neotyphodium compared to plants not infected by this fungal endophyte. The release of toxic fungal alkaloids from *Neotyphodium* sp. inhibits the aphid vectors carrying the BYDV pathogen, as aphid reproduction was significantly lower in plants infected by these endophytes (Lehtonen et al. 2006).

13.2.4 Mechanism of Biotic Stress Alleviation in Plants via Fungal Endophytes

The myriad of ways via. which the fungal endophytes can alleviate biotic stress in plants can be broadly classified into three main groups: (a) direct inhibition of plant pathogens, (b) indirect inhibition of plant pathogens, (c) ecological effects (Gao et al. 2010).

13.2.4.1 Direct Inhibition of Plant Pathogens Causing Biotic Stress

In direct inhibition, endophytes suppress pathogens by producing antibiotics, lytic enzyme secretion, etc. Direct interactions between pathogen and fungi are quite complex and vary from species to species (Gao et al. 2010).

Antibiotics Produced by Endophytes and Their Role in Biotic Stress Tolerance

Fungal endophytes have an innate capacity of producing diverse range of secondary metabolites exhibiting strong antifungal and antibacterial properties which stop the growth of many plant pathogens (Gao et al. 2010). Such endophytes have the capacity to produce multiple kinds of antibiotics including terpenoids, alkaloids, aromatic compounds and polypeptides. For example *Phomopis cassia*, an endophytic fungus isolated from *Cassia spectabili*, produces five cadinene sesquiterpenes, and among them 3,11,12-trihydroxy cadinene acts as the strongest antifungal compound against *Cladosporium sphaerospermum* and *Cladosporium cladsporioides* (Gao et al. 2010).

Lytic Enzymes Secreted from Endophytes and Their Role in Biotic Stress Tolerance

In order to effectively penetrate their hosts, endophytes release various enzymes to hydrolyze the rigid cell wall and cell surface. These enzymes also show the ability to degrade the cell walls of fungi and oomycetes pathogens, thus barring their entry.

Among the many enzymes produced, few prominent ones are chitinases, cellulases and 1,3-glucanase (Gao et al. 2010).

13.2.4.2 Indirect Inhibition of Plant Pathogens Causing Biotic Stress

Plants during evolution have developed two types of resistance against biotic stresses: non-specific (general) resistance and specific resistance (Gao et al. 2010). Since, it is known that fungal endophytes may evolve from plant pathogenic fungi, plant defence can be activated with the help of fungal endophytes similar to plant pathogens. This defence results from plant resistance enhancement and secondary metabolite production.

Role of Endophytes in Induction of Plant Resistance to Biotic Stress Alleviation

Fungal endophytes like *Fusarium solani* found in the roots of tomato are responsible for inducing systematic resistance against tomato foliar pathogen *Septoria lycopersici* and expressed PR gene, PR5 and PR7 in its roots (Kavroulakis et al. 2007). Similarly, endophyte *Neotyphodiumlolii* decreased lesions caused on leaves due to pathogens by activating enzymes superoxide dismutase (SOD) and peroxidase (POD) (Tian et al. 2008).

Role of Endophytes in Stimulation of Plant Secondary Metabolites for Biotic Stress Alleviation

Secondary metabolites more commonly called plant natural products are not essential for plants survival but play major role in plant defence and allelopathic interactions. Secondary metabolites known as phytoalexins are low molecular weight antimicrobial molecules (Smith 1996), containing many flavonoids, terpenoids etc., and are central to plant defence. These phytoalexins were first discovered in *Orchis morio* and *Loroglossumhircinum* in response to a fungal attack.

Role of Endophytes in Promoting Plant Growth and Physiology for Biotic Stress Alleviation

Endophytes may also control plant physiology and contribute in plant defence against biotic stresses (Giménez et al. 2007). Increased plant growth is an indication of strong plant vigour, thus helping plants to tide over biotic stresses. Fungal endophytes like *Colletotrichum* sp. found in *Artemisia annua* produce

phytohormones like indole acetic acid (IAA) to regulate plant biotic response (Lu et al. 2000).

13.2.4.3 Ecological Effects and Their Role in Biotic Stress Alleviation Through Fungal Endophytes

The interaction of plant pathogen and fungal endophyte for the use of limited resources and space for growth results in formation of a niche for each of the competing member. Sometimes, as seen in the case of endophytes, this niche may not permit the fungal pathogen altogether to infect the plant host or in some cases the endophyte may use this as an opportunity to kill the pathogen and obtain its nutrition. Thus, ecological niche both at occupational and nutritional level works simultaneously (Table 13.1).

Impact of Occupational Endophytic Ecological Niche on Biotic Stress Alleviation

Endophytes occupy a specific niche inside their host such that an equilibrium between the host and fungal endophyte gets established. Endophyte obtains nutrition from its host, whereas the host benefits from the various exudates and leachates that protect the host against other pathogens. Fungal endophytes rapidly colonize and exhaust the substrates so that pathogens found it difficult to establish themselves for longer period of time (Pal and Gardener 2006). As a defence response, plants produce lignin and other exudates to stop further infection by endophytes (Harman et al. 2004). Thus, plant adaptation to prevent endophyte infection becomes a barrier for fungal pathogen too.

Impact of Endophytic-Hyper-Parasitism and Predation on Biotic Stress Alleviation

Fungal endophytes protect their host plant from biotic stress by directly attacking the plant pathogen (Tripathi et al. 2008). *Trichoderma* sp. is a good example as it directly parasitizes hyphae of fungal pathogens like *Rhizoctonia solani*. Such responses of endophytes also constitute bio-control methods (Grosch et al. 2006).

Predation though like parasitism is a more general method to eliminate plant pathogens. The same endophyte *Trichoderma* under nutrient-deficient conditions produces enzymes to assimilate fragments of fungal pathogen (Benhamou and Chet 1997).

Table 13.1 Plant-endophyte associations in combating various biotic stresses

Biotic stress	Endophyte genus	Endophyte family	Plant species	Mechanism	Reference
Pratylenchus sp.	*Neotyphodium* sp.	Clavicipitaceae	Grasses	Production of anti-herbivore alkaloids and phenolic production in infected roots	Timper et al. (2005)
Fusarium culmorum	*Piriformospora indica*	Sebacinaceae	*Arabidopsis* and cereals	Altered plant biochemistry to induce plant defence	Waller et al. (2005)
Helminthosporium solani	*Acremonium strictum*	*Incertae sedis*	*Dactylis glomerata* L	Mycoparasitism	Rivera-Varas et al. (2007)
BYDV virus	*Neotyphodium* sp.	Clavicipitaceae	*Lolium pratense*	Inhibition of aphid vectors carrying BYDV due to toxic alkaloids released from endophyte	Lehtonen et al. (2006)
Cladosporium sphaerospermum	*Phomopis cassia*	Valsaceae	*Cassia spectabili*	Production of antifungal cadinene sesquiterpene by endophytes	Gao et al. (2010)
Septoria lycopersici	*Fusarium solani*	Nectriaceae	*Solanum lycopersicum*	Expression of PR genes: PR5 and PR7 in roots	Kavroulakis et al. (2007)
Fusarium oxysporum	*Penicillium citrinum*	Trichocomaceae	*Musa* sp.	Higher level of enzyme production in banana	Ting et al. (2012)
Pyrenophora tritici	*Chaetomium globosum*	Chaetomiaceae	*Triticum aestivium*	Activation of host defence	Istifadah and McGee (2006)
Puccinia recondita	*Phoma*sp.	Didymellaceae	*Triticum aestivum*	Release of inhibitory substances along with activation of host defence	Dingle and McGee (2003)
Plasmodiophora brassicae	Heteroconium chaetospira	Antennulariellaceae	*Brassica campestris*	Not known	Usuki et al. (2002)
Plutellaxlostlla	*Acremonium alternatum*	*Incertae sedis*	*Brassica oleracea* var. *gemmifera*	Inhibition of larval growth	Raps and Vidal (1998)
Helicoverpa armigera	*Acremonium strictum*	*Incertae sedis*	*Lycopersicum esculentum*	Decreased development of pupae and larva	Jallow et al. (2004)
Meliodogyne incognita	*Fusarium oxysporum*	Nectriaceae	*Lycopersicum esculentum*	Inhibition through release of antimicrobial compounds	Hallman and Sikora (1994, 1996)

13.2.5 Role of Mycorrhizal Fungi in Plant Biotic Stress Alleviation

The most widely studied plant fungal interaction is mycorrhiza, which is the quintessential symbiotic relation. This relation between plant roots and fungus is widespread in nature. Many fungi form such associations but arbuscular mycorrhizal fungi (AMF) of phylum Glomeromycota are the most important (Gosling et al. 2006). More than 80% of land plant families form AMF type association. AMF consist of an external network of fungal mycelia and an internal phase which penetrate the cortical cells of the root (Prasad et al. 2017). Fitter and Moyersoen (1996) defined AMF association as "a sustainable non-pathogenic bio-trophic interaction between a fungus and a root".

AMF associations represent an evolutionary strategy that led to sharing of functions and responsibilities of both plant and fungal symbionts (Varma et al. 2017a, b, c). The changes in the physiology have a significant impact on plant response to biotic stress. In the following sections, some major impacts of AMF on biotic stress alleviation are discussed.

13.2.5.1 Impact of AMF on Biotic Stress Alleviation Caused by Soil-Borne Pathogens

AM symbioses are known to protect plants from soil-borne pathogens. Diseases like wilting or root rot caused by fungi such as *Macrophomina, Fusarium, Verticillum, Rhizoctoni*a and oomycetes like *Pythium, Phytophtohra* and *Apahnomyces* can be suppressed to some extent by AM symbioses. Mycorrhizal plants defend against these biotic stresses by using many different mechanisms. Competition between the AMF and pathogen for both the photosynthates and space for growth has been demonstrated. Cordier et al. (1998) depicted that tomato roots with arbusculated cells were able to exclude *Phytophthora.*

13.2.5.2 Impact of AMF on Biotic Stress Alleviation Caused by Root Parasitic Plants

Plants like *Striga* and *Orobanche* are plant parasites severely impacting agricultural crops. These plants act as obligate parasites and attach themselves to the roots of their host and exhaust them of their nutrients (Bouwmeester et al. 2003). Lopez-Raez et al. (2009) showed that growth of hemiparasite *Striga hermonthica* found in African fields was inhibited when its host maize and sorghum were inoculated with AMF. Thus, AMF are advised for integrated management of parasitic weeds.

13.2.5.3 Impact of AMF on Biotic Stress Alleviation Caused by Phytophagous Plants

Insect herbivory is a common biotic stress encountered by many plants. Mycorrhizal association is known to influence such stresses, but the lifestyle and feeding mechanism of the insects also greatly determine the insect herbivory performance (Hartley and Gange 2009; Koricheva et al. 2009). Hartley and Gange (2009) deduced that mycorrhiza have strong negative effects on rhizophagous insects, but such effects are variable in shoot-feeding insects. One reason for such variability is due to the fact that generalist insects are sensitive to improved defence capacity of the plant due to AM association, whereas specialist insects circumvent such defences and in turn possibly benefit from improved nutritional status of the plant.

13.2.6 Molecular Mechanism of Fungal Endophytes to Biotic Stress

Environmental changes along with the increment of various biotic stresses have led to the accumulation of various adaptations in plants to maximize their chances of survival. One such adaptation is the use of endophytic fungi for evading biotic stresses such as pathogenic fungi, bacteria, nematodes and herbivory. Among the many endophytic fungi which can alleviate these stresses, none is more studied than *Piriformospora indica* (Johnson et al. 2014). *P. indica* is root-colonizing endophyte which lacks host specificity and is similar to AMF but unlike AMF, it can be cultured on an artificial medium (Johnson et al. 2014; Prasad et al. 2005). Various studies on the interaction between host plant and *P. indica* have been shown to upregulate many defence-related genes including those belonging to the pathogenesis related PR genes like jasmonate (*VSP, PDF1.2, LOX2)* and ethylene (*ERF1*) which play the role of signalling genes during a pathogenic attack (Camehl et al. 2010; Molitor et al. 2011). In addition to these genes, colonization of the plant by *P. indica* could lead to the induction of reactive oxygen species (ROS) scavenging genes and enzymes in leaves (Johnson et al. 2014; Nath et al. 2016). In one study, after infection of plant by *P. indica,* an influx in Ca^{2+}, short ROS burst, sudden apoplastic alkalization, CDPK and MAPK activation along with induction of defence genes was observed in the early stages both in *Arabidopsis* and barley independently (Johnson et al. 2014; Nath et al. 2018).

13.3 Abiotic Stress: A Havoc for Plant Growth

Plants are prone to a wide range of abiotic stresses which show detrimental effects on their growth and productivity. These stresses can reduce the plant survival, yield and biomass production to up to 70% and, thus, form a major threat to the global food security (Kumar and Verma 2018). The major abiotic stresses affecting plants are drought, salinity, heat, cold and heavy metals (Fig. 13.4). The various abiotic stresses and their possible mechanism to cope up under stress condition are discussed in detail below.

13.3.1 Drought and Its Impacts on Plant Growth

Drought is a serious abiotic environmental stress which results due to water scarcity owing to a period of below average rainfall. It often lasts longer and is more severe than dry spells. It adversely affects plant growth and productivity by hindering nutrient assimilation, ion uptake, activity of enzymes, etc. (Ahanger et al. 2017; Ahanger and Agarwal 2017). Water-deficit plants have reduced cell size, decreased membrane integrity, reduced rate of transpiration, photosynthesis, etc. They also produce reactive oxygen species which may lead to leaf senescence, protein degradation, lipid peroxidation, membrane injury and cell death (Tiwari et al. 2015).

However, plants associated with mycorrhizal fungi can tolerate drought stress because of the increased absorption of water and minerals. The fungal hyphae significantly increase the absorptive surface area of these plants. Also, trehalose

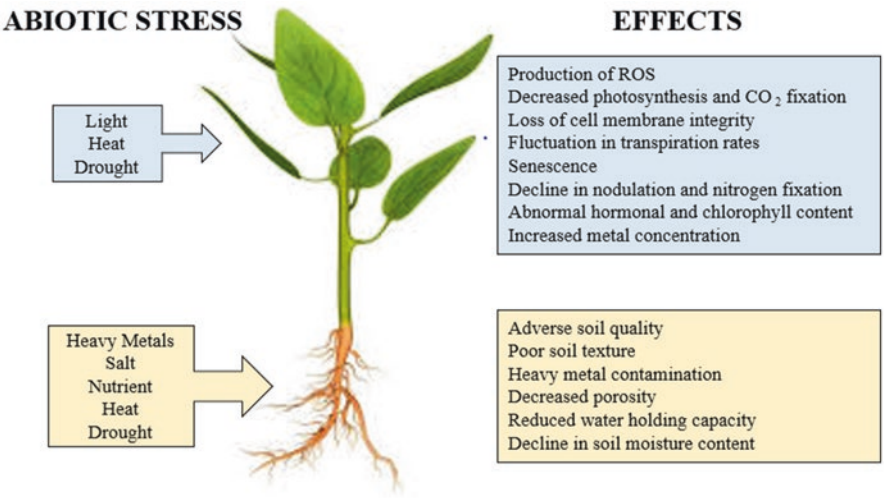


Fig. 13.4 Various abiotic stress and their effect on plants

production by fungi (Jiménez Zacarías et al. 2004; Farías-Rodríguez et al. 1998), along with increased synthesis of antioxidants like peroxidase (POD), catalase (CAT), superoxide dismutase (SOD), etc., also aids in protecting the plant from oxidative stress caused due to drought conditions (Ruiz-Lozano 2003). Fungi are also reported to positively affect the processes of growth, cell expansion, stomatal opening, etc. (Dar et al. 2018).

Maize, wheat, barley, onion, strawberry, soybean, etc., are some of the plants where the role of AMF in combating drought stress has been extensively studied (Mena-Violante et al. 2006; Moradtalab et al. 2019; Yooyongwech et al. 2016; Ruiz-Lozano et al. 2015). Most AMF members belong to family *Glomeraceae* which is also regarded as the "Global Family" in terms of forming association with plants (Bahadur et al. 2019). Basidiomycete member, *Piriformospora indica*, belonging to family Sebacinaceae is also reported to alleviate drought stress in plants like *Arabidopsis* sp., *Brassica campestris* sp. Chinensis etc. (Sherameti et al. 2008; Sun et al. 2010). Amiri et al. (2017) reported that mycorrhizal association increased the concentrations of P, N and Fe in *Pelargonium graveolens* L. under drought stress. The concentrations of K, P, Zn and Mn were also found to be higher in AMF-associated *Pistachio* plants grown under water scarce conditions (Bagheri et al. 2012). In AMF-inoculated *Poncirus trifoliata* and *Rosmarinuso cinalis* plants, stomatal conductance was reported to increase in conditions of water scarcity (Bahadur et al. 2019). Wu et al. (2019) reported that association of trifoliate orange (*Poncirus trifoliata*) with mycorrhizal fungi *Funneliformis mosseae* changed the composition and unsaturation of fatty acids to combat stress. Also, strigolactone level was found to increase in tomato and lettuce to cope up with drought stress (Ruiz-Lozano et al. 2016).

13.3.1.1 Mitigation of Drought Stress at the Genic Level

Genes involved in combating drought tolerance are mainly divided into two main categories (Seki et al. 2002). The first category includes proteins which directly regulate abiotic stress, while the second category comprises of proteins which play an indirect role in stress tolerance by controlling the functioning of stress-responsive genes along with aiding in signal transduction (Shinozaki et al. 2003). Under conditions of drought stress, the amounts of plant hormones like abscisic acid, jasmonates, and strigolactones vary considerably (Fernández-Lizarazo and Moreno-Fonseca 2016). Xu et al. (2018) reported the increased expression of 14-3-3 genes (TFT1-TFT12) involved in abscisic acid signalling pathway in drought-affected, AMF-associated *Solanum lycopersicum* plants. Similarly, Oelmüller et al. (2009) while working with Chinese cabbage observed several changes in the plant in response to treatment with polyethylene glycol (stimulates drought-like conditions). Activity of drought-associated genes like CBL1, RD29A, DREB2A and ANAC072 and antioxidant enzymes like SOD, POD and CAT was identified to increase in several folds. Also, an enhancement was seen in the concentration of CAS protein and CAS mRNA level for Ca^{2+}-sensing regulator linked with the thylakoid membrane.

13.3.2 Salinity Stress and Its Implication in Agriculture

Almost 7% of the earth's surface is occupied by saline soils (Ruiz-Lozano et al. 2001). Fertilizers used in agriculture and soluble salts in water (Al-Karaki 2000; Copeman et al. 1996; Abrol 1986) accompanied by high temperatures and water stress lead to high salt content – approximately 0.1% of the total soil (Richards 1954; Juniper and Abbott 1993) in most of the areas (Cantrell and Linderman 2001; Al-Karaki 2006; Mouk and Ishii 2006). Salinity is a serious problem that causes poor microbial activity because of osmotic stress and toxicity, leading to low water potential. Anions such as NO_3^- (nitrate), Cl^- (chloride) and cations like K^+ (potassium), Na^+ (sodium), Ca^{2+} (calcium) are primarily responsible for saline soils. Salt stress has both direct and indirect effects on crop productivity. Directly, it decreases the amount of available water to plants due to the reduction of osmotic potential of soil solution, leading to conditions of a physiological drought (Feng et al. 2002; Jahromi et al. 2008). Plant productivity, germination rates, water and mineral uptake, ecological and physiological balance are all adversely affected. Also, the activity of nitrogenase enzyme is severely affected by salt stress, thereby showing detrimental effects on crop yield, nitrogen fixation and nodule formation. Indirect effects include imbalance in nutrient uptake and transport (Adiku et al. 2001; Marschner 1995), membrane disruption, damaged structure of macromolecules, enzymes and organelles, etc., due to Na^+ and Cl^- toxicity (Feng et al. 2002; Juniper and Abbott 1993).

However, the positive impact of AMF on alleviating salt stress has been extensively studied. El-Nashar (2017) and Ait-El-Mokhtar et al. (2019) have recently reported an increase in growth rate, stomatal conductance, rate of photosynthesis and an improvement in leaf-water relations in AMF-associated *Antirrhinum majus* plants under conditions of saline stress. Shukla et al. (2012) demonstrated the role of *Trichoderma harzianum* in alleviating salt stress in rice plants. Borde et al. (2010) and Elhindi et al. (2017) have independently reported the positive effects of AMF in *Ocimum basilicum* L. and *Allium sativum* plants under salt stress, respectively. Navarro et al. (2014) reported a decline in the absorption rates of Na^+ and Cl^- in Citrus plants under saline conditions. Gomez-Bellot et al. (2015) demonstrated an increase in the levels of Ca^{2+}, K^+ and P in AMF-associated *Euonymus japonica* under salt stress. Increased nitrogen concentration in AMF-associated plant root and shoot along with enhancement in fresh and dry weights under moderate salt stress has also been reported (Wang et al. 2018). Various researchers like Cekic et al. (2012); Aroca et al. (2013); Hameed et al. (2014); Talaat and Shawky (2014); Hashem et al. (2018); Santander et al. (2019), etc., have independently shown an increase in the concentrations of chlorophyll, strigolactone, cytokinin, jasmonic acid, salicylic, Ca^{2+}, P, N, Mg^{2+}, and K^+ and osmolyte proline in plant species like *Capsicum annuum, Lactuca sativa* and *Cucumis sativus*.

13.3.2.1 Molecular Mechanism of Salt Tolerance in AMF-Associated Plants

Baltruschat et al. (2008) reported that plants may alleviate salinity stress by mechanisms involving lipid desaturation. This research was further supported by Zhao and Qin (2005) who showed that application of unsaturated fatty acids can alleviate NaCl stress in *Hordeum* sp. Also, Zhang et al. (2002) and Liang et al. (2005) independently demonstrated the decline in concentration of oleic acid in stressed barley roots. Similar results are observed in barley leaves when inoculated with *P. indica* (Baltruschat et al. 2008). The activities of GR, DHAR, CAT, MDHAR and APX are also observed to increase in stressed barley roots. Miller et al. (2007) made an astounding observation in *Arabidopsis* double mutants showing enhanced stress tolerance despite lacking thylakoid and cytosolic APX, thus concluding that reactive oxygen species likes peroxidases might be responsible for initiation of abiotic stress signal in conditions of salt stress. The activity of DHAR and ratio of reduced to oxidized ascorbate was reported to be improved in *Hordeum* sp. (Waller et al. 2005). However, opposite results were reported in case of salt-stressed barley where there was a decline in both the above-said parameters (Baltruschat et al. 2008). Similarly, Mittova et al. (2004) had demonstrated an increase and decrease in the ratio of ascorbate to DHAR in salt-tolerant *L. pennellii* and salt-sensitive *L. esculentum*, respectively.

13.4 Temperature Fluctuations: A Bane for Plants

Thermal stresses including both heat and cold stress are wreaking havoc on the plant growth and development. Varied temperatures lead to reduced germination and photosynthesis rates, loss of plant vigour, retarded growth, yield and biomass production, cell death, abnormal enzyme activity, increase in oxidative stress, abscission and senescence of leaves, wilting and thermal damage of plant parts and discolouration of fruits (Wahid et al. 2007; Hasanuzzaman et al. 2013). For example cells become more rigid during cold stress, while heat stress increases the fluidity of cells. Plants adopt different strategies to counter heat stress like production and accumulation of enzymes and osmolytes. The concentration of jasmonic acid (JA) is also found to elevate significantly during stress condition. Heat shock proteins (HSP100, HSP 90, HSP70, HSP 60, HSP20) and ROS-scavenging enzymes (ascorbate peroxidase and catalase) are major functional proteins synthesized enormously during temperature stress in plants (Qu et al. 2013; Kotak et al. 2007).

Maya and Matsubara (2013) showed the positive effects of AMF *Glomus fasciculatum* on plant growth and development under heat stress. Also, numerous reports suggest better growth rates in some AMF-associated plants when grown at low temperature (Chen et al. 2013; Liu et al. 2013; Abdel Latef and Chaoxing

2011a, b; Zhu et al. 2010a, b). AMF association aids the plants in surviving under cold stress along with enhancing their growth and development (Gamalero et al. 2009; Birhane et al. 2012). AMF helps the plant by strengthening its immunity owing to increased production of secondary metabolites and various proteins (Abdel Latef and Chaoxing 2011a, b). It also aids the plant in efficiently retaining moisture (Zhu et al. 2010a, b). Also, AMF-associated plants have improved rates of chlorophyll production (Abdel Latef and Chaoxing 2011a, b; Zhu et al. 2010a, b), better osmotic adjustment capacity and gas exchange potential along with enhanced plant-water relationship (Zhu et al. 2012).

13.4.1 Heavy Metals and Their Deleterious Effects on Plants

Non-degradable, metallic elements with density higher than 4 g/cm^3 are called heavy metals. These are also hazardous at even low concentration (Ma et al. 2016a, b; Duruibe et al. 2007). Heavy metals (HMs) like Cu, Fe, Zn, Co and Mn constitute the mineral micronutrients and are essential for the proper growth and functioning of plants. However, increased concentration of these heavy metals results in the production of reactive oxygen species which have various negative effects on the plant (Palmer and Guerinot 2009; Puig and Penarrubia 2009). Approximately 30–35% reduction in length, mass and shoot and root ratio is observed in plants grown under conditions of heavy metal stress.

HM toxicity in plants results from increased absorption of both essential and non-essential metals from the soil. Nowadays, microbe-mediated phytoremediation is gaining wide attention because of its sustainability, cost efficiency and environment-friendly nature (Broos et al. 2004; Thakare et al. 2021). AMF-associated plants are shown to easily thrive in excess metal stress conditions. Hashem et al. (2016) showed the positive effects of AMF by decreasing the amount of hydrogen peroxide and malonaldehyde under conditions of cadmium stress. Similarly, clone of *Schizosaccharomyces pombe* has been reported to improve sequestering of heavy metals from polluted environments (Yong et al. 2014). However, the impact of AMF varies considerably with change in plant species, fungal species and the heavy metal involved. AMF can, thus, both decrease and increase the heavy metal concentration in plants, depending on the prevalent conditions (de Souza et al. 2012; de Andrade et al. 2008; Carvalho et al. 2006; Joner and Leyval 2001). Interestingly, it has also been observed that AMF can simultaneously increase and decrease the heavy metal concentration in root and shoot respectively (Wu et al. 2016a, b; Sheikh-Assadi et al. 2015; Chen et al. 2005; Joner and Leyval 1997). Joner and Leyval (1997) demonstrated that increased accumulation of Cd in roots of *Trifolium subterraneum.* Contrary to this, researchers like Tullio et al. (2003); Li and Christie (2001); Heggo et al. (1990) have shown reduced uptake of heavy metal in AMF-associated plants. Reduced Zn uptake in root and shoot concentrations has been reported in mycorrhizal tomato (Watts-Williams and Cavagnaro 2014) and red clover (Li and Christie 2001) when grown under high Zn conditions. Several

Table 13.2 Plant-AMF associations in combating various abiotic stresses

Stress	Plant species	Fungus species	Family	References
Drought	*Poncirus trifoliata, Pelargonium graveolens, Robinia pseudoacacia* L., *Cicer arietinum, Poncirus trifoliata* L.	*Funneliformis mosseae*	Glomeraceae	Zhang et al. (2018), Amiri et al. (2015), Yang et al. (2014), Hashem et al. (2018), and Huang et al. (2017)
Drought	*Fragaria ananassa*	*F. mosseae BEG25, F. geosporus* strain BEG11	Glomeraceae	Boyer et al. (2014)
Drought	*Phoenix dactylifera* L.	*Funneliformis monosporum*	Glomeraceae	Meddich et al. (2015)
Drought	*Triticum aestivum* L.	*Glomus fasciculatum*	Glomeraceae	Pal and Pandey (2016)
Drought	*Triticum aestivum* L.	*Glomus mosseae*	Glomeraceae	Pal and Pandey (2016), Rani (2016)
Drought	*Lactuca sativa, Solanum lycopersicum, Vigna subterranean, Hordeum vulgare, Calotropis procera*Ait	*Glomus intraradices*	Glomeraceae	Ruiz-Lozano et al. (2015), Tsoata et al. (2015), Bayani et al. (2015), and Bahmani et al. (2018)
Drought	*Glycine max*	*Glomus aggregatum*	Glomeraceae	Grümberg et al. (2015)
Drought	*Antirrhinum majus* L., *Phoenix dactylifera* L.	*Glomus deserticola*	Glomeraceae	Asrar et al. (2012), and Meddich et al. (2015)
Drought	*Ipomoea batatas*	*Glomus spp.*	Glomeraceae	Yooyongwech et al. (2016)
Drought	*Saccharum arundinaceum* Retz.	*Glomus spp.*	Glomeraceae	Mirshad and Puthur (2016)
Drought	*Cicer arietinum*	*Claroideoglomus etunicatum*	Glomeraceae	Hashem et al. (2018)
Drought	*Digitariaeriantha, Lactuca sativa, Solanum lycopersicum, Cicerarietinum, Glycyrrhiza uralensis* Fisch. ex DC., *Pelargonium graveolens* L. Herit., *Zea mays* L.	*Rhizophagus irregularis*	Glomeraceae	Pedranzani et al. (2016), Ruiz-Lozano et al. (2015), Hashem et al. (2018), Xie et al. (2018), Amiri et al. (2015), and Zhao et al. (2015)
Drought	*Triticum durum, Pelargonium graveolens, Robiniapseudoacacia* L., *Solanum Lycopersicum* L.	*Rhizophagus intraradices*	Glomeraceae	Goicoechea et al. (2016), Goicoechea and Antol (2017), Amiri et al. (2015), Yang et al. (2014), and Chitarra et al. (2016)

(continued)

Table 13.2 (continued)

Stress	Plant species	Fungus species	Family	References
Drought	*Zea mays*	*Rhizophagus intraradices*, strain BGCBJ09	Glomeraceae	Zhao et al. (2015)
Drought	*Phoenix dactylifera* L.	*Rhizophagus clarus*	Glomeraceae	Meddich et al. (2015)
Drought	*Triticum aestivum, L. esculentum* cv. Big Beef, Seattle's Best, *C. annuum* cv. Calif. Wonder, Watermelon	*Colletotrichum magna*	Glomeraceae	Rodriguez and Redman (2008)
Drought	*L. esculentum, Capsicum annuum*	*Colletotrichum magna* (path 1)	Glomerellaceae	Redman et al. (2001)
Drought	*L. esculentum, Capsicum annuum*	*C. musae* (927)	Glomerellaceae	Redman et al. (2001)
Drought	*L. esculentum*	*C. orbiculare* (683)	Glomerellaceae	Redman et al. (2001)
Drought	*Capsicum annuum, L. esculentum* cv. Big Beef, *C. annuum* cv. Calif. Wonder	*C. gloeosporioides*	Glomerellaceae	Redman et al. (2001), and Rodriguez and Redman (2008)
Drought	*L. esculentum*	*C. gloeosporioides* (95-41A)	Glomerellaceae	Redman et al. (2001)
Drought	*L. esculentum* cv. Big Beef	*C. orbiculare*	Glomerellaceae	Rodriguez and Redman (2008)
Drought	*Poncirus trifoliata*	*Paraglomusoccultum*	Paraglomaceae	Zhang et al. (2018)
Drought	*Poncirus trifoliata* L.	*Diversispora versiformis*	Diversisporaceae	Zou et al. (2017)
Drought	*Triticum aestivum L.*	*Gigasporadecipiens*	Gigasporaceae	Pal and Pandey (2016)
Drought	*Vigna subterranea*	*Gigasporagregaria*	Gigasporaceae	Tsoata et al. (2015)
Drought	*Vigna subterranea*	*Scutellospora gregaria*	Gigasporaceae	Tsoata et al. (2015)
Drought	*Leymusmollis, Oryza sativa, L. esculentum*	*Fusarium culmorum* (Fc18)	Nectriaceae	Rodriguez et al. (2008)
Drought	*L. mollis, O. sativa, L. esculentum, D. lanuginosum*	*F. culmorum* (FcRed1)	Nectriaceae	Rodriguez et al. (2008)
Drought	*Festuca pratensis, F. arizonica, Loliumperenne*	*Neotyphodium* sp.	Clavicipitaceae	Malinowski et al. (1997), Morse et al. (2002), and Barker et al. (1997)
Drought	*Loliumperenne*	*Neotyphodiumlolii*	Clavicipitaceae	Latch et al. (1985), and Ravel et al. (1997)

Stress	Plant species	Fungus species	Family	References
Drought	*Festuca arundinacea*	*N. coenophialum*	Clavicipitaceae	Belesky et al. (1989), and de Battista et al. (1990)
Drought	*Festuca arundinacea*	*Acremonium* sp.	Hypocreaceae	White et al. (1992)
Drought	*Theobroma cacao*	*Trichoderma hamatum* (DIS 219b)	Hypocreaceae	Bae et al. (2009)
Drought	*Festuca pratensis*	*Phialophora* sp.	Herpotrichiellaceae	Malinowski et al. (1997)
Drought	*L. esculentum*	*Alternaria* sp.	Pleosporaceae	Rodriguez and Redman (2008)
Drought	*D. lanuginosum, Leymusmollis, Oryza sativa, Lycopersicon esculentum*	*Curvularia protuberate* (Cp4666D)	Pleosporaceae	Rodriguez et al. (2008)
Drought	*D. lanuginosum, L. esculentum*	*C. protuberate* (CpMH206)	Pleosporaceae	Rodriguez et al. (2008)
Drought	*Triticum aestivum, Citrullus lanatus*	*C. protuberate* (Cp4666D)	Pleosporaceae	Rodriguez et al. (2008)
Drought	*Arabidopsis thaliana*, *Brassica campestris* sp. Chinensis	*Piriformospora indica*	*Sebacinaceae*	Sherameti et al. (2008), and Sun et al. (2010)
Salinity	*Solanum lycopersicum* L., *Panicum turgidum*	*Glomus intraradices*	Glomeraceae	Hajiboland et al. (2010), and Hashem et al. (2015)
Salinity	*Acacia nilotica, Solanum lycopersicum* L.	*Glomus fasciculatum*	Glomeraceae	Giri et al. (2007), and Ebrahim and Saleem (2017)
Salinity: alkali	*Leymus chinensis*	*Glomus mosseae*	Glomeraceae	Jixiang et al. (2017)
Salinity	*Cucumis sativus* L.	*Glomus etunicatum, G. intraradices, G. mosseae*	Glomeraceae	Hashem et al. (2018)
Salinity	*Solanum lycopersicum* L. *Panicum turgidum*	*Rhizophagus irregularis*	Glomeraceae	Khalloufi et al. (2017)
Salinity	*Oryza sativa* L., *Aeluropus littoralis, Panicum turgidum*	*Claroideoglomus etunicatum*	Glomeraceae	Porcel et al. (2015), Hajiboland et al. (2015), and Hashem et al. (2015)

(continued)

Table 13.2 (continued)

Stress	Plant species	Fungus species	Family	References
Salinity	*Chrysanthemum morifolium*	*Funneliformis mosseae*	Glomeraceae	Wang et al. (2018), and Hashem et al. (2015)
Salinity	*Chrysanthemum morifolium*	*Diversispora versiformis*	Diversisporaceae	Wang et al. (2018)
Salinity	*L. mollis, O. sativa, L. esculentum, D. lanuginosum*	*Fusarium culmorum* (FcRed1)	Nectriaceae	Rodriguez et al. (2008)
Salinity	*Hordeum vulgare, Medicago truncatula*	*Piriformospora indica*	Sebacinaceae	Waller et al. (2005); Li et al. (2017)
Salinity	*Hordeum vulgare* cv. Ingrid	*Piriformospora indica*	Sebacinaceae	Baltruschat et al. (2008)
Salinity	*Porostereumspadiceum*	*Glycine max*	Phanerochaetaceae	Hamayun et al. (2017)
Heat	*Triticum aestivum*	*Rhizophagus intraradices*	Glomeraceae	Cabral et al. (2016)
High temperature/ heat	*Solanum lycopersicum, Triticum aestivum*	*Rhizophagus irregularis*	Glomeraceae	Calvo-Polanco et al. (2016), and Cabral et al. (2016)
Heat	*Triticum aestivum*	*Claroideoglomus claroideum*	Glomeraceae	Cabral et al. (2016)
High temperature/ heat	*Zea mays, Triticum aestivum*	*Funneliformis mosseae*	Glomeraceae	Mathur et al. (2016), and Cabral et al. (2016)
High temperature/ heat	*Zea mays, Triticum aestivum*	*F. geosporium*	Glomeraceae	Mathur et al. (2016), and Cabral et al. (2016)
Heat	*Dichanthelium lanuginosum,* L. *esculentum*	*Curvularia protuberate*	Pleosporaceae	Redman et al. (2002), and Rodriguez et al. (2008)
Heat	*L. esculentum*	*Curvularia spp.*	Pleosporaceae	Rodriguez and Redman (2008)
Heavy metal	*Sesbania rostrate, Trifolium* sp., *Schedonorus arundinaceus, Helianthus annuus* L.	*Glomus mosseae*	Glomeraceae	Lin et al. (2007); Vivas et al. (2003); Miransari (2017)
Heavy metal	*Trigonellafoenum-graecum*	*G. clarum*	Glomeraceae	Abdel Hameed and Rabab (2019)
Heavy metal	*Trigonellafoenum-graecum*	*Glomus monosporum*	Glomeraceae	Abdel Hameed and Rabab (2019)

Stress	Plant species	Fungus species	Family	References
Heavy metal	*Helianthus annuus* L	*Glomus fasciculatum*	Glomeraceae	Mani et al. (2016)
Heavy metal	*Ricinus communis, Conium maculatum*	*Glomus intraradices*	Glomeraceae	Miransari (2017)
Heavy metal	*Lycopersicon esculentum*	*Glomus intraradices*	Glomeraceae	Ferrol et al. (2016)
Heavy metal	*Calopogonium mucunoide*	*Glomus etunicatu*	Glomeraceae	De Souza et al. (2012)
Heavy metal	*Cajanuscajan* L	*Rhizophagus irregularis*	Glomeraceae	Garg and Singh (2017)
Heavy metal	*Calendula officinalis* L.	*Claroideoglomus claroideum*	Glomeraceae	Hristozkova et al. (2016)
Heavy metal	*Calendula officinalis* L.	*Funneliformis mosseae*	Glomeraceae	Hristozkova et al. (2016)
Heavy metal	*Clethra barbinervis* Sieb. et Zucc.	*Rhizoscyphus* sp.	Ericaceae	Yamaji et al. (2016)
Heavy metal	*Clethra barbinervis* Sieb. et Zucc.	*Phialocephala fortinii*	Ericaceae	Yamaji et al. (2016)
Heavy metal	*Clethra barbinervis* Sieb. et Zucc.	*Rhizodermeaveluwensis*	Ericaceae	Yamaji et al. (2016)
Heavy metal	*Trigonellafoenum-graecum*	*Gigasporanigra*	Gigasporaceae	Abdel Hameed and Rabab (2019)
Heavy metal	*Trigonellafoenum-graecum*	*Acaulospora laevis*	Acaulosporaceae	Abdel Hameed and Rabab (2019)
Heavy metal	*Triticum aestivum*	*Penicillium ruqueforti*	Trichocomaceae	Ikram et al. (2018)
Heavy metal	*Solanum surattense*	*Penicillium sp.*	Trichocomaceae	Ikram et al. (2018)
Heavy metal	*Pinus*	*Paxillus involutus*	Paxillaceae	Andres and Andrea (2001)

researchers like Chen et al. (2013); Christophersen et al. (2012); Ultra et al. (2007); Gonzalez-Chavez et al. (2002) have obtained similar results for metalloid Arsenic as well. Table 13.2 shows the various plant-AMF associations in combating the above-mentioned abiotic stresses.

13.5 Conclusion and Future Perspective

Plant stress is one of the major culprits for damaging plants, often rendering them unfit for consumption. Use of pesticides and fertilizers has, to a certain extent, helped in containing biotic and abiotic stress, respectively, but its overuse has now led to new problems, not to mention its harmful impact on environment. In this scenario, it becomes essential to identify a more viable solution to the problem. Many bio-control agents are known to inhibit plant stress. Among them, fungi can play a pivotal role. Their close association with plants either in the form of endophytes or as mycorrhiza makes them ideal for stress alleviation. Also, due to their various other allied processes, plants become more vigorous and can thrive in harsh conditions. Many fungi like *Trichoderma* sp. are already used powerful bio-control agents to ward off pathogens responsible for biotic stress injuries. More studies are important in this field as a cursory glance is enough to realize that fungi are one of the largest groups of plant pathogens inflicting a major chunk of all the stresses. Thus, it becomes critical to understand these pathogens, since they may act as a stress-alleviating agent in some plants or in the same members but in different conditions. Similarly, fungal members mainly belonging to sub-phylum Glomeromycotina are ideal in combating many abiotic plant stresses. Thus, a comprehensive study into the role of these fungi in plant stress alleviation can be a panacea for our current challenges.

Acknowledgments Dr. Deepak Kumar is thankful to the Science and Engineering Research Board (Grant No- EEQ/2016/000487) and UGC startup research grant (No. F.30-352/2017 (BSR), New Delhi, Government of India for providing the financial support to the laboratory.

References

Abdel Latef AA, Chaoxing H (2011a) Arbuscular mycorrhizal influence on growth, photosynthetic pigments, osmotic adjustment and oxidative stress in tomato plants subjected to low temperature stress. Acta Physiol Plant 33:1217–1225

Abdel Latef AA, Chaoxing H (2011b) Effect of arbuscular mycorrhizal fungi on growth, mineral nutrition, antioxidant enzymes activity and fruit yield of tomato grown under salinity stress. Sci Hortic 127:228–233

Abdel Hameed RE and Rabab AM (2019). Alleviation of cadmium stress by arbuscular mycorrhizal symbiosis. International Journal of Phytoremediation 21(7):1–9

Abrol JP (1986) Fuel and forage production from salt affected wasteland in India. Reclamat Reveg Res 5:65–74

Adiku G, Renger M, Wessolek G, Facklam M, Hech-Bischoltz C (2001) Simulation of dry matter production and seed yield of common beans under varying soil water and salinity conditions. Agric Water Manag 47:55–68

Ahanger MA, Agarwal RM (2017) Potassium up-regulates antioxidant metabolism and alleviates growth inhibition under water and osmotic stress in wheat (*Triticum aestivum* L.). Protoplasma 254(4):1471–1486

Ahanger MA, Tyagi SR, Wani MR, Ahmad P (2014) Drought tolerance: role of organic osmolytes, growth regulators, and mineral nutrients. In: Ahmad P, Wani MR (eds) Physiological mechanisms and adaptation strategies in plants under changing environment, vol 1. Springer, New York, pp 25–55

Ahanger MA, Tittal M, Mir RA, Agarwal RM (2017) Alleviation of water and osmotic stress-induced changes in nitrogen metabolizing enzymes in *Triticum aestivum* L. cultivars by potassium. Protoplasma 254(5):1953–1963

Ait-El-Mokhtar M, Laouane RB, Anli M, Boutasknit A, Wahbi S, Meddich A (2019) Use of mycorrhizal fungi in improving tolerance of the date palm (*Phoenix dactylifera* L.) seedlings to salt stress. Sci Hortic 253:429–438

Ali N, Masood S, Mukhtar T, Kamran MA, Rafique M, Munis MFH et al (2015) Differential effects of cadmium and chromium on growth, photosynthetic activity, and metal uptake of *Linum usitatissimum* in association with *Glomus intraradices*. Environ Monit Assess 187(6):311

Al-Karaki GN (2000) Growth of mycorrhizal tomato and mineral acquisition under salt stress. Mycorrhiza 10:51–54

Al-Karaki GN (2006) Nursery inoculation of tomato with arbuscular mycorrhizal fungi and subsequent performance under irrigation with saline water. Sci Hortic 109:1–7

Amiri R, Nikbakht A, Etemadi N (2015) Alleviation of drought stress on rose geranium Pelargonium graveolen L Herit. in terms of antioxidant activity and secondary metabolites by mycorrhizal inoculation. Sci Hortic 197:373–380

Amiri R, Ali N, Nematollah E, Mohammad RS (2017) Nutritional status, essential oil changes and water-use efficiency of rose geranium in response to arbuscular mycorrhizal fungi and water deficiency stress. Symbiosis 73:15–25

Andres S, Andrea P (2001) Plant responses to abiotic stresses: heavy metal-induced oxidative stress and protection by mycorrhization. J Exp Bot 53:372

Arnold AE, Mejia LC, Kyllo D, Rojas EI, Maynard Z, Robbins N, Herre EA (2003) Fungal endophytes limit pathogen damage in a tropical tree. Proc Natl Acad Sci U S A 100:15649–15654

Aroca R, Ruiz-Lozano JM, Zamarreño AM, Paz JA, García-Mina JM, Pozo JA et al (2013) Arbuscular mycorrhizal symbiosis influences strigolactone production under salinity and alleviates salt stress in lettuce plants. J Plant Physiol 170:47–55

Asrar AA, Abdel-Fattah GM, Elhindi KM (2012) Improving growth, flower yield, and water relations of snapdragon *Antirhinum majus* L. plants grown under well-watered and water-stress conditions using arbuscular mycorrhizal fungi. Photosynthetica 50:305–316

Bae H, Sicher RC, Kim MS, Kim SH, Strem MD, Melnick RL et al (2009) The beneficial endophyte *Trichoderma hamatum* isolate DIS 219b promotes growth and delays the onset of the drought response in *Theobroma cacao*. J Exp Bot 60:3279–3295

Bagde US, Prasad R, Varma A (2010a) Interaction of *Piriformospora indica* with medicinal plants and of economic importance. Afr J Biotechnol 9(54):9214–9226

Bagde US, Prasad R, Varma A (2010b) Mass cultivation of *Piriformopsora indica* in new Brunswick fermenter and its formulation as biofertilizer. Asian J Microbiol Biotechnol Environ Sci 12:911–916

Bagde US, Prasad R, Varma A (2011) Influence of culture filtrate of *Piriformospora indica* on growth and yield of seed oil in *Helianthus annus*. Symbiosis 53:83–88

Bagheri V, Shamshiri MH, Shirani H, Roosta H (2012) Nutrient uptake and distribution in mycorrhizal pistachio seedlings under drought stress. J Agric Sci Technol 14:1591–1604

Bahadur A, Batool A, Nasir F et al (2019) Mechanistic insights into arbuscular mycorrhizal fungi-mediated drought stress tolerance in plants. Int J Mol Sci 20:4199

Bahmani M, Naghdi R, Kartoolinejad D (2018) Milkweed seedlings tolerance against water stress: comparison of inoculations with *Rhizophagus irregularis* and *Pseudomonas putida*. Environ Technol 10:111–121

Baltruschat H, Fodor J, Harrach BD, Niemczyk E, Barna B, Gullner G et al (2008) Salt tolerance of barley induced by the root endophyte *Piriformospora indica* is associated with a strong increase in antioxidants. New Phytol 180:501–510

Barker DJ, Hume DE, Quigley PE (1997) Negligible physiological responses to water deficit in endophyte-infected and uninfected perennial ryegrass. In: Bacon CW, Hill NS (eds) Neotyphodium/grass interactions. Plenum Press, New York, pp 137–139

Bárzana G, Aroca R, Paz JA, Chaumont F, Martinez-Ballesta MC, Carvajal M et al (2012) Arbuscular mycorrhizal symbiosis increases relative apoplastic water flow in roots of the host plant under both well-watered and drought stress conditions. Ann Bot 109:1009–1017

Bati CB, Santilli E, Lombardo L (2015) Effect of arbuscular mycorrhizal fungi on growth and on micronutrient and macronutrient uptake and allocation in olive plantlets growing under high total Mn levels. Mycorrhiza 25(2):97–108

Bayani R, Saateyi A, Faghani E (2015) Influence of arbuscular mycorrhiza in phosphorus acquisition efficiency and drought-tolerance mechanisms in barley *Hordeum vulgare* L. Int J Agric Biosci 7:86–94

Belesky DP, Stringer WC, Hill NS (1989) Influence of endophyte and water regime upon tall fescue accessions. I. Growth characteristics. Ann Bot 63:495–503

Benhamou N, Chet I (1997) Cellular and molecular mechanisms involved in the interaction between *Trichoderma harzianum* and *Pythium ultimum*. Appl Environ Microbiol 63:2095–2099

Birhane E, Sterck F, Fetene M, Bongers F, Kuyper T (2012) Arbuscular mycorrhizal fungi enhance photosynthesis, water use efficiency, and growth of frankincense seedlings under pulsed water availability conditions. Oecologia 169:895–904

Borde M, Dudhane M, Jite PK (2010) AM fungi influences the photosynthetic activity, growth and antioxidant enzymes in *Allium sativum* L. under salinity condition. Not Sci Biol 2:64–71

Bouwmeester HJ, Matusova R, Zhongkui S, Beale MH (2003) Secondary metabolite signalling in host-parasitic plant interactions. Curr Opin Plant Biol 6:358–364

Bowles TM, Barrios-Masias FH, Carlisle EA, Cavagnaro TR, Jackson LE (2016) Effects of arbuscular mycorrhizae on tomato yield, nutrient uptake, water relations, and soil carbon dynamics under deficit irrigation in field conditions. Sci Total Environ 566:1223–1234

Boyer LR, Brain P, Xu XM, Jeffries P (2014) Inoculation of drought-stressed strawberry with a mixed inoculum of two arbuscular mycorrhizal fungi: effects on population dynamics of fungal species in roots and consequential plant tolerance to water. Mycorrhiza 25(3):215–227

Broos K et al (2004) A survey of symbiotic nitrogen fixation by white clover grown on metal contaminated soils. Soil Biol Biochem 36:633–640

Cabral C, Sabine R, Ivanka T, Bernd W (2016) Arbuscular mycorrhizal fungi modify nutrient allocation and composition in wheat (*Triticum aestivum* L.) subjected to heat-stress. Plant Soil 408(1–2):385–399

Calvo-Polanco M, Sanchez-Romera B, Aroca R, Asins MJ, Declerck S, Dodd IC et al (2016) Exploring the use of recombinant inbred lines in combination with beneficial microbial inoculants (AM fungus and PGPR) to improve drought stress tolerance in tomato. Environ Exp Bot 131:47–57

Camehl I, Sherameti I, Venus Y, Bethke G, Varma A, Lee J et al (2010) Ethylene signaling and ethylene-targeted transcription factors are required to balance beneficial and nonbeneficial traits in the symbiosis between the endophytic fungus *Piriformospora indica* and *Arabidopsis thaliana*. New Phytol 185:1062–1073

Cantrell IC, Linderman RG (2001) Preinoculation of lettuce and onion with VA mycorrhizal fungi reduces deleterious effects of soil salinity. Plant Soil 233:269–281

Carroll G (1988) Fungal endophytes in stems and leaves- from latent pathogen to mutualistic symbiont. Ecology 69:2–9

Carvalho LM, Caçador I, Martins-Louçao MA (2006) Arbuscular mycorrhizal fungi enhance root cadmium and copper accumulation in the roots of the salt marsh plant *Aster tripolium* L. Plant Soil 285:161–169

Cekic FO, Unyayar S, Ortas I (2012) Effects of arbuscular mycorrhizal inoculation on biochemical parameters in *Capsicum annuum* grown under long term salt stress. Turk J Bot 36:63–72

Chadha N, Mishra M, Rajpal K, Bajaj R, Choudhary DK, Verma A (2015) An ecological role of fungal endophytes to ameliorate plants under biotic stress. Arch Microbiol 197:869–881

Chandrasekaran M, Chanratana M, Kim K, Seshadri S, Sa T (2019) Impact of arbuscular mycorrhizal fungi on photosynthesis, water status, and gas exchange of plants under salt stress-a meta-analysis. Front Plant Sci 10:457

Chen X, Wu C, Tang J, Hu S (2005) Arbuscular mycorrhizae enhance metal lead uptake and growth of host plants under a sand culture experiment. Chemosphere 60:665–671

Chen XW, Wu FY, Li H, Chan WF, Wu C, Wu SC, Wong MH (2013) Phosphate transporters expression in rice (*Oryza sativa* L.) associated with arbuscular mycorrhizal fungi (AMF) colonization under different levels of arsenate stress. Environ Exp Bot 87:92–99

Chitarra W, Pagliarani C, Maserti B, Lumini E, Siciliano I, Cascone P, Schubert A, Gambino G, Balestrini R, Guerrieri E (2016). Insights on the impact of arbuscular mycorrhizal symbiosis on tomato tolerance to water stress. Plant Physiology 171: 1009–1023

Chodak M, Gołębiewski M, Morawska-Płoskonka J, Kuduk K, Niklińska M (2015) Soil chemical properties affect the reaction of forest soil bacteria to drought and rewetting stress. Ann Microbiol 65:1627–1637

Christophersen HM, Smith AF, Smith SE (2012) Unraveling the influence of arbuscular mycorrhizal colonization on arsenic tolerance in *Medicago*: *Glomus mosseae* is more effective than *G. intraradices*, associated with lower expression of root epidermal Pi transporter genes. Front Physiol 3:91

Copeman RH, Martin CA, Stutz JC (1996) Tomato growth in response to salinity and mycorrhizal fungi from saline or non-saline soils. Hortic Sci 31:341–344

Cordier C, Pozo MJ, Barea JM, Gianinazzi S, Gianinazzi-Pearson V (1998) Cell defense responses associated with localized and systemic resistance to *Phytophthora* induced in tomato by an arbuscular mycorrhizal fungus. Mol Plant-Microbe Interact 11:1017–1028

Dar ZM, Masood A, Asif M, Malik MA (2018) Review on arbuscular mycorrhizal fungi: an approach to overcome drought adversities in plants. Int J Curr Microbiol App Sci 7:1040–1049

de Andrade SAL, da Silveira APD, Jorge RA, de Abreu MF (2008) Cadmium accumulation in sunflower plants influenced by arbuscular mycorrhiza. Int J Phytoremediation 10:1–13

de Battista JP, Bouton JH, Bacon CW, Siegel MR (1990) Rhizome and herbage production of endophyte-removed tall fescue clones and populations. Agron J 82:651–654

de Souza LA, de Andrade SAL, de Souza SCR, Schiavinato MA (2012) Arbuscular mycorrhiza confers Pb tolerance in *Calopogonium mucunoides*. Acta Physiol Plant 34:523–531

Dingle J, McGee PA (2003) Some endophytic fungi reduce the density of pustules of *Puccinia recondite* f. sp. *tritici* in wheat. Mycol Res 107:310–316

Duruibe JO, Ogwuegbu MOC, Egwurugwu JN (2007) Heavy metal pollution and human bio toxic effects. Int J Phys Sci 2:112–118

Ebrahim MEH, Saleem AR (2017) Alleviating salt stress in tomato inoculated with mycorrhizae: photosynthetic performance and enzymatic antioxidants. J Taibah Univ Sci 11(6):850–860

Elhindi KM, El-Din SA, Elgorban AM (2017) The impact of arbuscular mycorrhizal fungi in mitigating salt-induced adverse effects in sweet basil (*Ocimum basilicum* L.). Saudi J Biol Sci 24:170–179

El-Nashar YI (2017) Response of snapdragon *Antirrhinum majus* L. to blended water irrigation and arbuscular mycorrhizal fungi inoculation: uptake of minerals and leaf water relations. Photosynthetica 55(2):201–209

Evelin H, Giri B, Kapoor R (2012) Contribution of *Glomus intraradices* inoculation to nutrient acquisition and mitigation of ionic imbalance in NaCl-stressed *Trigonella foenum-graecum*. Mycorrhiza 22:203–217

Farías-Rodríguez R, Mellor RB, Arias C, Peña Cabriales JJ (1998) The accumulation of trehalose in nodules of several cultivars of common bean (*Phaseolus vulgaris*) and its correlation with resistance to drought stress. Physiol Plant 102:353–359

Feng G, Zhang FS, Xl L, Tian CY, Tang C, Rengel Z (2002) Improved tolerance of maize plants to salt stress by arbuscular mycorrhiza is related to higher accumulation of soluble sugars in roots. Mycorrhiza 12:185–190

Fernández-Lizarazo JC, Moreno-Fonseca LP (2016) Mechanisms for tolerance to water-deficit stress in plants inoculated with arbuscular mycorrhizal fungi. A review. Agron Colomb 34:179–189

Ferrol N, Tamayo E, Vargas P (2016) The heavy metal paradox in arbuscular mycorrhizas: from mechanisms to biotechnological applications. J Exp Bot 67(22):6253–6265

Fitter AH, Moyersoen B (1996) Evolutionary trends in root-microbe symbioses. Philos Trans R Soc B Biol Sci 35:1367–1375

Freeman S, Rodriguez RJ (1993) Genetic conversion of a fungal plant pathogen to a non-pathogenic, endophytic mutualist. Science 260:75–78

Gamalero E, Lingua G, Berta G, Glick BR (2009) Beneficial role of plant growth promoting bacteria and arbuscular mycorrhizal fungi on plant responses to heavy metal stress. Can J Microbiol 55:501–514

Gao FK, Cc D, Liu X (2010) Mechanisms of fungal endophytes in plant protection against pathogens. Afr J Microbiol Res 4(13):1346–1351

Garg N, Singh S (2017) Arbuscular mycorrhiza *Rhizophagus irregularis*, and silicon modulate growth, proline biosynthesis and yield in *Cajanus cajan*, L. Mill sp. (pigeon pea) genotypes under cadmium and zinc stress. J Plant Growth Regul 37:1–18

Gill SS, Gill R, Trivedi DK, Anjum NA, Sharma KK, Ansari MW, Ansari AA, Johri AK, Prasad R, Pereira E, Varma A, Tuteja N (2016) *Piriformospora indica*: potential and significance in plant stress tolerance. Front Microbiol 7:332. https://doi.org/10.3389/fmicb.2016.00332

Giménez C, Cabrera R, Reina M, González-Coloma A (2007) Fungal endophytes and their role in plant protection. Curr Org Chem 11:707–720

Giri B, Kapoor R, Mukerji KG (2007) Improved tolerance of *Acacia nilotica*, to salt stress by arbuscular mycorrhiza, *Glomus fasciculatum*, may be partly related to elevated K/Na ratios in root and shoot tissues. Microb Ecol 54:753–760

Goicoechea N, Antol MC (2017) Increased nutritional value in food crops. J Microbiol Biotechnol 10:1004–1007

Goicoechea N, Bettoni M, Fuertes-Mendiza´bal T, Gonzalez-Murua C, Aranjuelo I (2016) Durum wheat quality traits affected by mycorrhizal inoculation, water availability and atmospheric CO_2 concentration. Crop Pasture Sci 67:147–155

Gomez-Bellot MJ, Ortuño MF, Nortes PA, Vicente-Sánchez J, Bañón S, Sánchez Blanco MJ (2015) Mycorrhizal euonymus plants and reclaimed water: biomass, water status and nutritional responses. Sci Hortic 186:61–69

Gonzalez-Chavez C, D'Haen J, Vangronsveld J, Dodd JC (2002) Copper sorption and accumulation by the extraradical mycelium of different *Glomus* spp. (arbuscular mycorrhizal fungi) isolated from the same polluted soil. Plant Soil 240:287–297

Gosling P, Hodge A, Goodlass G, Bending GD (2006) Arbuscular mycorrhizal fungi and organic farming. Agric Ecosyst Environ 113:17–35

Govindarajulu M, Pfeffer PE, Jin HR, Abubaker J, Douds DD, Allen JW et al (2005) Nitrogen transfer in the arbuscular mycorrhizal symbiosis. Nature 435:819–823

Grosch R, Scherwinski K, Lottmann J, Berg G (2006) Fungal antagonists of the plant pathogen *Rhizoctonia solani*: selection, control efficacy and influence on the indigenous microbial community. Mycol Res 110:1464–1474

Grümberg BC, María UC, Shroeder A, Vargas-Gil S, Luna CM (2015) The role of inoculum identity in drought stress mitigation by arbuscular mycorrhizal fungi in soybean. Biol Fertil Soils 51:1–10

Hajiboland R, Aliasgharzadeh N, Laiegh SF, Poschenrieder C (2010) Colonization with arbuscular mycorrhizal fungi improves salinity tolerance of tomato *Solanum lycopersicum* L. plants. Plant Soil 331:313–327

Hajiboland R, Dashtebani F, Aliasgharzad N (2015) Physiological responses of halophytic C4 grass, *Aeluropus littoralis* to salinity and arbuscular mycorrhizal fungi colonization. Photosynthetica 53(4):572–584

Hallmann J, Sikora RA (1994) Influence of *Fusarium oxysporum*, a mutualistic fungal endophyte, on *Meloidogyne incognita* infection of tomato. J Plant Dis Plant Protect 101:475–481

Hallmann J, Sikora RA (1996) Toxicity of fungal endophyte secondary metabolites to plant-parasitic nematodes and soil-borne plant-pathogenic fungi. Eur J Plant Pathol 102:155–162

Hamayun M, Hussain A, Khan SA, Kim HY, Khan A et al (2017) Gibberellins producing endophytic fungus *Porostereum spadiceum* AGH786 rescues growth of salt affected soybean. Front Microbiol 8:686

Hameed A, Dilfuza E, Abd-Allah EF, Hashem A, Kumar A, Ahmad P (2014) Salinity stress and arbuscular mycorrhizal symbiosis in plants. In: Miransari M (ed) Use of microbes for the alleviation of soil stresses, vol 1. Springer Science+Business Media, New York, pp 139–159

Harman GE, Howell CR, Viterbo A, Chet I, Lorito M (2004) *Trichoderma* species-opportunistic, avirulent plant symbionts. Nat Rev 2:43–55

Hartley SE, Gange AC (2009) Impacts of plant symbiotic fungi on insect herbivores: mutualism in a multitrophic context. Annu Rev Entomol 54:323–342

Hasanuzzaman M, Gill SS, Fujita M (2013) Physiological role of nitric oxide in plants grown under adverse environmental conditions. In: Tuteja N, Gill SS (eds) Plant acclimation to environmental stress. Springer Science+Business Media, New York, pp 269–322

Hashem A, Allah EF, Alqarawi AA, Aldubise ED (2015) Arbuscular mycorrhizal fungi enhance salinity tolerance of Panicum turgidum Forssk by altering photosynthetic and antioxidant pathways. J Plant Microorgan Interact 10(1):230–242

Hashem A et al (2016) Alleviation of cadmium stress in *Solanum lycopersicum* L. by arbuscular mycorrhizal fungi via induction of acquired systemic tolerance. Saudi J Biol Sci 23:272–281

Hashem A, Alqarawi AA, Radhakrishnan R, Al-Arjani AF, Aldehaish HA, Egamberdieva D et al (2018) Arbuscular mycorrhizal fungi regulate the oxidative system, hormones and ionic equilibrium to trigger salt stress tolerance in *Cucumis sativus* L. Saudi J Biol Sci 25(6):1102–1114

He F, Sheng M, Tang M (2017) Effects of *Rhizophagus irregularis* on photosynthesis and antioxidative enzymatic system in *Robinia pseudoacacia* L. under drought stress. Front Plant Sci 8:183

Heggo A, Angle JS, Chaney RL (1990) Effects of vesicular-arbuscular mycorrhizal fungi on heavy metal uptake by soybeans. Soil Biol Biochem 22:865–869

Hertig M, Taliaferro WH, Schwartz B (1937) The terms symbiosis, symbiont and symbiote. J Parasitol 23:326–329

Hristozkova M et al (2016) The contribution of arbuscular mycorrhizal fungi in attenuation of heavy metal impact on Calendula officinalis development. Appl Soil Ecol 101:57–63

Huang YM, Zou YN, Wu QS (2017) Alleviation of drought stress by mycorrhizas is related to increased root H_2O_2 efflux in trifoliate orange. Sci Rep 7:42335

Ikram M, Ali N, Jan G, Jan FG, Rahman IU, Iqbal A et al (2018) IAA producing fungal endophyte *Penicillium roqueforti* Thom., enhances stress tolerance and nutrients uptake in wheat plants grown on heavy metal contaminated soils. PLoS One 13(11):e0208150

Istifadah N, McGee PA (2006) Endophytic *Chaetomium globosum* reduces development of tan spot in wheat caused by *Pyrenophora tritici-repentis*. Aust J Plant Pathol 35:411–418

Jahromi F, Aroca R, Porcel R, Ruiz-Lozano JM (2008) Influence of salinity on the in vitro development of Glomus intraradices and on the in vivo physiological and molecular responses of mycorrhizal lettuce plants. Microb Ecol 55:45–53

Jallow MFA, Dugassa-Gobena D, Vidal S (2004) Indirect interaction between an unspecialized endophytic fungus and a polyphagous moth. Basic Appl Ecol 5:183–191

Jiménez Zacarías JJ, Altamirano-Hernández J, Peña Cabriales JJ (2004) Nitrogenase activity and trehalose content of nodules of drought-stressed common beans infected with effective (Fix+) and ineffective (Fix^-) rhizobia. Soil Biol Biochem 36:1975–1981

Jixiang L, Yingnan W, Shengnan S, Chunsheng M, Xiufeng Y (2017) Effects of arbuscular mycorrhizal fungi on the growth, photosynthesis and photosynthetic pigments of *Leymus chinensis* seedlings under salt-alkali stress and nitrogen deposition. Sci Total Environ 576:234–241

Johnson JM, Alex T, Oelmuller R (2014) *Piriformospora indica*: the versatile and multifunctional root endophytic fungus for enhanced yield and tolerance to biotic and abiotic stress in crop plants. J Trop Agric 52(2):103–122

Joner EJ, Leyval C (1997) Uptake of 109Cd by roots and hyphae of a *Glomus mosseae/Trifolium subterraneum* mycorrhiza from soil amended with high and low concentrations of cadmium. New Phytol 135:353–360

Joner EJ, Leyval C (2001) Time-course of heavy metal uptake in maize and clover as affected by root density and different mycorrhizal inoculation regimes. Biol Fertil Soils 33:351–357

Jung SC, Martinez-Medina A, Lopez-Raez JA, Pozo MJ (2012) Mycorrhiza-induced resistance and priming of plant defenses. J Chem Ecol 38:651–664

Juniper S, Abbott LK (1993) Vesicular-arbuscular mycorrhizas and soil salinity. Mycorrhiza 4:45–57

Kavroulakis NS, Zervakis GI, Ehaliotis C, Haralampidis K, Papadopoulou KK (2007) Role of ethylene in the protection of tomato plants against soil-borne fungal pathogens conferred by an endophytic *Fusarium solani* strain. J Exp Bot 58:3853–3864

Khalloufi M, Martínez-Andújar C, Lachaâl M, Karray-Bouraoui N, Pérez- Alfocea F, Albacete A (2017) The interaction between foliar GA3 application and arbuscular mycorrhizal fungi inoculation improves growth in salinized tomato *Solanum lycopersicum* L. plants by modifying the hormonal balance. J Plant Physiol 214:134–144

Koch N, Islam NF, Sonowal S, Prasad R, Sarma H (2021) Environmental antibiotics and resistance genes as emerging contaminants: methods of detection and bioremediation. Current Research in Microbial Sciences https://doi.org/10.1016/j.crmicr.2021.100027

Koricheva J, Gange AC, Jones T (2009) Effects of mycorrhizal fungi on insect herbivores: a meta-analysis. Ecology 90:2088–2097

Kotak S et al (2007) The complexity of the heat stress response in plants. Curr Opin Plant Biol 10:310–316

Kumar A, Verma JP (2018) Does plant-microbe interaction confer stress tolerance in plants: a review? Microbiol Res 207:41–52

Latch GCM, Hunt WF, Musgrave DR (1985) Endophytic fungi affect growth of perennial ryegrass. N Z J Agric Res 28:165–168

Lehtonen PT, Helander M, Siddiqui SA, Lehto K, Saikkonen K (2006) Endophytic fungus decreases plant virus infections in meadow ryegrass (*Lolium pratense*). Biol Lett 2:620–623

Li X, Christie P (2001) Changes in soil solution Zn and pH and uptake of Zn by arbuscular mycorrhizal red clover in Zn-contaminated soil. Chemosphere 42:201–207

Li L, Li L, Wang X, Zhu P, Wu H, Qi S (2017) Plant growth-promoting endophyte Piriformospora indica alleviates salinity stress in Medicago truncatula. Plant Physiol Biochem 119:211–223

Liang YC, Zhang WH, Chen Q, Liu YL, Ding RX (2005) Effects of silicon on H^+-ATPase and H^+-PPase activity, fatty acid composition and fluidity of tonoplast vesicles from roots of salt-stressed barley (*Hordeum vulgare* L.). Environ Exp Bot 53:29–37

Lin AJ, Zhang XH, Wong MH, Ye ZH, Lou LQ, Wang YS (2007) Increase of multi-metal tolerance of three leguminous plants by arbuscular mycorrhizal fungi colonization. Environ Geochem Health 29:473–481

Liu X, Song Q, Tang Y, Li W, Xu J, Wu J et al (2013) Human health risk assessment of heavy metals in soil–vegetable system: a multi-medium analysis. Sci Total Environ 463–464:530–540

López-Ráez JA, Matusova R, Cardoso C, Jamil M, Charnikhova T, Kohlen W, Verstappen F, Ruyter-Spira C, Bouwmeester HJ (2009) Strigolactones: ecological significance and use as a target for parasitic plant control. Pest Manag Sci 64:471–477

López-Ráez JA, Verhage A, Fernández I, García JM, Azcón-Aguilar C, Flors V, Pozo MJ (2010) Hormonal and transcriptional profiles highlight common and differential host responses to arbuscular mycorrhizal fungi and the regulation of the oxylipin pathway. J Exp Bot 61:2589–26001

Lu H, Zou WX, Meng JC, Hu J, Tan RX (2000) New bioactive metabolites produced by *Colletotrichum* sp., an endophytic fungus in *Artemisia annua*. Plant Sci 151:67–73

Ma Y et al (2016a) Inoculation of Brassica oxyrrhina with plant growth promoting bacteria for the improvement of heavy metal phytoremediation under drought conditions. J Hazard Mater 320:36–44

Ma Y et al (2016b) The beneficial role of bacterial endophytes in heavy metal phytoremediation. J Environ Biol 174:14–25

Malinowski DP, Belesky DP (2000) Adaptations of endophyte-infected cool-season grasses to environmental stresses: mechanisms of drought and mineral stress tolerance. Crop Sci 40:923–940

Malinowski D, Leuchtmann A, Schmidt D, Nsberger J (1997) Growth and water status in meadow fescue (*Festuca pratensis*) is differently affected by its two natural endophytes. Agron J 89:673–678

Mani D, Kumar C, Patel NK (2016) Integrated micro-biochemical approach for phytoremediation of cadmium and lead contaminated soils using *Gladiolus grandiflorus* L. cut flower. Ecotoxicol Environ Saf 124:435–446

Marschner H (1995) Mineral nutrition of higher plants, 2nd edn. Academic Press, New York, NY

Mathur S, Sharma MP, Jajoo A (2016) Improved photosynthetic efficacy of maize *Zea mays* plants with arbuscular mycorrhizal fungi (AMF) under high temperature stress. J Photochem Photobiol 180:149–154

Maya MA, Matsubara Y (2013) Influence of arbuscular mycorrhiza on the growth and antioxidative activity in *Cyclamen* under heat stress. Mycorrhiza 23(5):381–390

Meddich A, Jaiti F, Bourzik W, Asli AE, Hafidi M (2015) Use of mycorrhizal fungi as a strategy for improving the drought tolerance in date palm (Phoenix dactylifera). Sci Hortic 192:468–474

Mena-Violante HG, Ocampo-Jimenez O, Dendooven L, Martinez-Soto G, Gonzalez-Castafeda J, Davies FT et al (2006) Arbuscular mycorrhizal fungi enhance fruit growth and quality of chile ancho *Capsicum annuum* L. cv San Luis plants exposed to drought. Mycorrhiza 16:261–267

Miller G, Suzuki N, Rizhsky L, Hegie A, Koussevitzky S, Mittler R (2007) Double mutants deficient in cytosolic and thylakoid ascorbate peroxidase reveal a complex modeof interaction between reactive oxygen species, plant development and response to abiotic stresses. Plant Physiol 144:1777–1785

Miransari M (2017) Arbuscular mycorrhizal fungi and heavy metal tolerance in plants. In: Wu QS (ed) Arbuscular mycorrhizas and stress tolerance of plants. Springer Nature, Singapore, pp 174–161

Mirshad PP, Puthur JT (2016) Arbuscular mycorrhizal association enhances drought tolerance potential of promising bioenergy grass *Saccharum arundinaceum*. Retz Environ Monit Assess 188:425

Mittova V, Guy M, Tal M, Volokita M (2004) Salinity upregulates the antioxidative system in root mitochondria and peroxisomes of the wildsalt-tolerant tomato species *Lycopersicon pennellii*. J Exp Bot 55:1105–1113

Molitor A, Zajic D, Voll L, Pons-Kuehnemann J, Samans B, Kogel KH et al (2011) Barley leaf transcriptome and metabolite analysis reveals new aspects of compatibility and *Piriformospora indica* mediated systemic induced resistance to powdery mildew. Mol Plant-Microbe Interact 24:1427–1439

Moradtalab N, Roghieh H, Nasser A, Tobias EH, Günter N (2019) Silicon and the association with an arbuscular-mycorrhizal fungus (*Rhizophagus clarus*) mitigate the adverse effects of drought stress on strawberry. Agronomy 9:41

Morse LJ, Day TA, Faeth SH (2002) Effect of *Neotyphodium* endophyte infection on growth and leaf gas exchange of Arizona fescue under contrasting water availability regimes. Environ Exp Bot 48:257–268

Mouk BO, Ishii T (2006) Effect of arbuscular mycorrhizal fungi on tree growth and nutrient uptake of *Sclerocarya birrea* under water stress, salt stress and flooding. J Jpn Soc Hortic Sci 75:26–31

Nath M, Bhatt D, Prasad R, Gill SS, Anjum NA and Tuteja N (2016) Reactive oxygen species generation-scavenging and signaling during plant-arbuscular mycorrhizal and *Piriformospora*

indica interaction under stress condition. Frontiers in Plant Science 7:1574. https://doi.org/10.3389/fpls.2016.01574

Nath M, Bhatt D, Jain A, Saxena SC, Saifi SK, Yadav S, Negi M, Prasad R, Tuteja N (2018) Salt stress triggers augmented levels of Na+, Ca2+ and ROS and alter stress-responsive gene expression in roots of CBL9 and CIPK23 knockout mutants of *Arabidopsis thaliana*. Environmental and Experimental Botany 161: 265-276. https://doi.org/10.1016/j.envexpbot.2018.10.005

Navarro JM, Perez-Tornero O, Morte A (2014) Alleviation of salt stress in citrus seedlings inoculated with arbuscular mycorrhizal fungi depends on the root stock salt tolerance. J Plant Physiol 171(1):76–85

Oelmüller R, Sherameti I, Tripathi S, Varma A (2009) *Piriformospora indica*, a cultivable root endophyte with multiple biotechnological applications. Symbiosis 49:1–17

Pal KK, Gardener BM (2006) Biological control of plant pathogens. The plant health Instructor

Pal A, Pandey S (2016) Role of arbuscular mycorrhizal fungi on plant growth and reclamation of barren soil with wheat (*Triticum aestivum* L.) crop. Int J Soil Sci 12:25–31

Palmer C, Guerinot ML (2009) A question of balance: facing the challenges of Cu, Fe and Zn homeostasis. Nat Chem Biol 5:333–340

Paterson E, Sim A, Davidson J, Daniell TJ (2016) Arbuscular mycorrhizal hyphae promote priming of native soil organic matter mineralization. Plant Soil 408:243–254

Pedranzani H, RodrãGuez-Rivera M, GutiaRrez M, Porcel R, Hause B, Ruiz-Lozano JM (2016) Arbuscular mycorrhizal symbiosis regulates physiology and performance of *Digitaria eriantha* plants subjected to abiotic stresses by modulating antioxidant and jasmonate levels. Mycorrhiza 26:141–152

Pieterse CMJ, León-Reyes A, Van der Ent S, Van Wees SCM (2009) Networking by small-molecule hormones in plant immunity. Nat Chem Biol 5:308–316

Plassard C, Dell B (2010) Phosphorus nutrition of mycorrhizal trees. Tree Physiol 30:1129–1139

Porcel R, Redondogómez S, Mateosnaranjo E, Aroca R, Garcia R, Ruiz-lozano JM (2015) Arbuscular mycorrhizal symbiosis ameliorates the optimum quantum yield of photosystem II and reduces non-photochemical quenching in rice plants subjected to salt stress. J Plant Physiol 185:75–83

Pozo MJ, Verhage A, García-Andrade J, García JM, Azcón-Aguilar C (2009) Priming plant defences against pathogens by arbuscular mycorrhizal fungi. In: Azcón-Aguilar C, Barea JM, Gianinazzi S, Gianinazzi-Pearson V (eds) Mycorrhizas: functional processes and ecological impact. Springer, Heidelberg, pp 123–136

Prasad R, Pham GH, Kumari R, Singh A, Yadav V, Sachdev M, PeskanT, Hehl S, Oelmuller R, Garg AP and Varma A (2005) Sebacinaceae: culturable mycorrhiza–like endosymbiotic fungi and their interaction with non-transformed and transformed roots. In: In vitro Culture of Mycorrhizas (eds. Declerck S, Strullu DG, Fortin JA), Springer-Verlag Berlin Heidelberg, 4: 291–312

Prasad R, Bagde US, Pushpangdan P, Varma A (2008) *Bacopa monniera* L.: pharmacological aspects and case study involving *Piriformospora indica*. Int J Integr Biol 3:100–110

Prasad R, Kamal S, Sharma PK, Oelmueller R, Varma A (2013) Root endophyte *Piriformospora indica* DSM 11827 alters plant morphology, enhances biomass and antioxidant activity of medicinal plant Bacopa monniera. J Basic Microbiol 53(12):1016–1024

Prasad R, Bhola D, Akdi K, Cruz C, Sairam KVSS, Tuteja N, Varma A (2017) Introduction to mycorrhiza: Historical development. In: Varma A, Prasad R, Tuteja N (eds), *Mycorrhiza*, Springer International Publishing AG 1–7

Prasad R, Chhabra S, Gill SS, Singh PK, Tuteja N (2020) The microbial symbionts: potential for the crop improvement in changing environments. In: Tuteja N, Tuteja R, Passricha N, Saifi SK (eds) Advancement in crop improvement techniques. Elsevier, Amsterdam, Netherlands, pp 233–240

Puig S, Penarrubia L (2009). Placing metal micronutrients in context: transport and distribution in plants. Current Opinion in Plant Biology 12: 299–306

Qu AL et al (2013) Molecular mechanisms of the plant heat stress response. Biochem Biophys Res Commun 432:203–207

Rajamanikyam M, Vadlapudi V, Amanchy R, Upadhyayula SM (2017) Endophytic fungi as novel resources of natural therapeutics. Braz Arch Biol Technol 60:e17160542

Rani B (2016) Effect of arbuscular mycorrhiza fungi on biochemical parameters in wheat Triticum aestivum L. under drought conditions. Doctoral dissertation, CCSHAU, Hisar

Raps A, Vidal S (1998) Indirect effects of an unspecialized endophytic fungus on specialized plant-herbivorous insect interactions. Oecologia 114:541–547

Ravel C, Courty C, Coudret A, Charmet G (1997) Beneficial effects of *Neotyphodium lolii* on the growth and water status in perennial ryegrass cultivated under nitrogen deficiency or drought stress. Agronomie 17:173–181

Redecker D, Schüssler A, Stockinger H, Stürmer SL, Morton JB, Walker C (2013) An evidence-based consensus for the classification of arbuscular mycorrhizal fungi (Glomeromycota). Mycorrhiza 23(7):515–531

Redman RS, Dunigan DD, Rodriguez RJ (2001) Fungal symbiosis: from mutualism to parasitism, who controls the outcome, host or invader? New Phytol 151:705–716

Redman RS, Sheehan KB, Stout RG, Rodriguez RJ, Henson JM (2002) Thermotolerance conferred to plant host and fungal endophyte during mutualistic symbiosis. Science 298:1581

Richards LA (ed) (1954) Diagnosis and improvement of saline and alkali soils. United States Department of Agriculture, Handbook no. 60, Washington, DC, pp 4–18

Rivera Varas VV, Freeman TA, Gusmestad NC, Secor GA (2007) Mycoparasitism of *Helminthosporium solani* by *Acremonium strictum*. Phytopathology 97:1331–1337

Rodriguez RJ, Redman RS and Henson JM (2003). The role of fungal symbioses in the adaptation of plants to high stress environments. Mitigations and adaptation strategies for global climate change 9: 261–272

Rodriguez RJ, Redman RS (2008) More than 400 million years of evolution and some plants still can't make it on their own: plant stress tolerance via fungal symbiosis. J Exp Bot 59:1109–1114

Rodriguez RJ, Henson J, Van Volkenburgh E, Hoy M, Wright L, Beckwith F et al (2008) Stress tolerance in plants via habitat-adapted symbiosis. ISME J 2:404–416

Rouphael Y, Franken P, Schneider C, Schwarz D, Giovannetti M, Agnolucci M (2015) Arbuscular mycorrhizal fungi act as bio-stimulants in horticultural crops. Sci Hortic 196:91–108

Ruiz-Lozano JM, Collados C, Barea JM, Azcon R (2001). Arbuscular mycorrhizal symbiosis can alleviate drought—induce nodule senescence in soybean plants. New Phytologist 151:493–502

Ruiz-Lozano JM (2003) Arbuscular mycorrhizal symbiosis and alleviation of osmotic stress. New perspectives for molecular studies. Mycorrhiza 13:309–317

Ruiz-Lozano JM, Aroca R, Zamarreño ÁM, Molina S, Andreo-Jiménez B, Porcel R et al (2015) Arbuscular mycorrhizal symbiosis induces strigolactone biosynthesis under drought and improves drought tolerance in lettuce and tomato. Plant Cell Environ 39(2):441–452

Ruiz-Lozano, et al. (2016). Arbuscular mycorrhizal symbiosis induces strigolactone biosynthesis under drought and improves drought tolerance in lettuce and tomato. Plant Cell Environment 39: 441–452

Santander C, Sanhueza M, Olave J, Borie F, Valentine C and Cornejo P (2019). Arbuscular mycorrhizal colonization promotes the tolerance to salt stress in lettuce plants through an efficient modification of ionic balance. Journal of Soil Science and Plant Nutrition 19 (2): 321–331

Seki M, Narusaka M, Ishida J, Nanjo T, Fujita M et al (2002) Monitoring the expression profiles of 7,000 *Arabidopsis* genes under drought, cold and high-salinity stresses using a full-length cDNA microarray. Plant J 31:279–292

Sheikh-Assadi M, Khandan-Mirkohi A, Alemardan A, Moreno- Jiménez E (2015) Mycorrhizal *Limonium sinuatum* (L.) mill. enhances accumulation of lead and cadmium. Int J Phytoremediation 17:556–562

Sherameti I, Tripathi S, Varma A, Oelmüller R (2008) The root-colonizing endophyte *Piriformospora indica* confers drought tolerance in *Arabidopsis* by stimulating the expression of drought stress-related genes in leaves. Mol Plant-Microbe Interact 21:799–807

Shinozaki K, Yamaguchi-Shinozaki K, Seki M (2003) Regulatory network of gene expression in the drought and cold stress responses. Curr Opin Plant Biol 6:410–417

Shukla N et al (2012) Biochemical and physiological responses of rice (*Oryza sativa* L.) as influenced by *Trichoderma harzianum* under drought stress. Plant Physiol Biochem 54:78–88

Smith CJ (1996) Accumulation of phytoalexins: defense mechanism and stimulus response system. New Phytol 32:1–45

Smith S, Read D (2008) Mycorrhiza symbiosis, 3rd edn. Academic Press, San Diego, CA

Spatafora JW, Chang Y, Benny GL, Lazarus K, Smith ME, Berbee ML et al (2016) A phylum-level phylogenetic classification of zygomycete fungi based on genome-scale data. Mycologia 108:1028–1046

Sun C, Johnson JM, Cai D, Sherameti I, Oelmüller R, Lou B (2010) Piriformospora indica confers drought tolerance in Chinese cabbage leaves by stimulating antioxidant enzymes, the expression of drought-related genes and the plastid-localized CAS protein. J Plant Physiol 167:1009–1017

Talaat NB, Shawky BT (2014) Protective effects of arbuscular mycorrhizal fungi on wheat (*Triticum aestivum* L.) plants exposed to salinity. Environ Exp Bot 98:20–31

Tanaka Y, Yano Y (2005) Nitrogen delivery to maize *via* mycorrhizal hyphae depends on the form of N supplied. Plant Cell Environ 28:1247–1254

Thakare M, Sarma H, Datar S, Roy A, Pawar P, Gupta K, Pandit S, Prasad R (2021) Understanding the holistic approach to plant-microbe remediation technologies for removing heavy metals and radionuclides from soil. Current Research in Biotechnology https://doi.org/10.1016/j.crbiot.2021.02.004

Thirkell TJ, Charters MD, Elliott AJ, Sait SM, Field KJ (2017) Are mycorrhizal fungi our sustainable saviours considerations for achieving food security. J Ecol 105:921–929

Tian P, Nan Z, Li C (2008) Effect of the endophyte *Neotyphodium lolii* on susceptibility and host physiological response of perennial ryegrass to fungal pathogens. Eur J Plant Pathol 122:593–602

Timper P, Gates RN, Bouton JH (2005) Response of *Pratylenchus* spp. in tall fescue infected with different strains of the fungal endophyte *Neotyphodium coenophialum*. Nematology 7:105–110

Ting ASY, Mah SW, Tee CS (2012) Evaluating the feasibility of induced host resistance by endophytic isolate *Penicillium citrinum* BTF08 as a control mechanism for *Fusarium* wilt in banana plantlets. Biol Control 61:155–159

Tiwari S et al (2015) *Pseudomonas putida* attunes morphophysiological, biochemical and molecular responses in *Cicer arietinum* L. during drought stress and recovery. Plant Physiol Biochem 99:108–117

Tripathi S, Kamal S, Sheramati I, Oelmuller R, Varma A (2008) Mycorrhizal fungi and other root endophytes as biocontrol agents against root pathogens. Mycorrhiza, 3rd edn, pp 281–306

Tsoata E, Njock SR, Youmbi E, Nwaga D (2015) Early effects of water stress on some biochemical and mineral parameters of mycorrhizal *Vigna subterranea* (L.) Verdc. (Fabaceae) cultivated in Cameroon. Int J Agron Agric Res 7:21–35

Tullio M, Pierandrei F, Salerno A, Rea E (2003) Tolerance to cadmium of vesicular arbuscular mycorrhizae spores isolated from a cadmium polluted and unpolluted soil. Biol Fertil Soils 37:211–214

Ultra VU, Tanaka S, Sakurai K, Iwasaki K (2007) Effects of arbuscular mycorrhiza and phosphorus application on arsenic toxicity in sunflower (*Helianthus annuus* L.) and on the transformation of arsenic in the rhizosphere. Plant Soil 290:29–41

Usuki F, Narisawa K, Yonezawa M, Kakishima M, Hashiba T (2002) An efficient inoculation method for colonization of Chinese cabbage seedlings by the root endophytic fungus *Heteroconium chaetospira*. J Gen Plant Pathol 68:326–332

Vadassery J, Tripathi S, Prasad R, Varma A, Oelmüller R (2009) Monodehydroascorbate reductase 2 and dehydroascorbate reductase 5 are crucial for a mutualistic interaction between *Piriformospora indica* and Arabidopsis. J Plant Physiol 166:1263–1274. https://doi.org/10.1016/j.jplph.2008.12.016

Varma A, Prasad R, Tuteja N (2017a) Mycorrhiza: Function, Diversity and State-of-Art. Springer International Publishing (ISBN: 978-3-319-53064-2) http://www.springer.com/us/book/9783319530635

Varma A, Prasad R, Tuteja N (2017b) Mycorrhiza: Eco-Physiology, Secondary Metabolites, Nanomaterials. Springer International Publishing(ISBN: 978-3-319-57849-1) http://www.springer.com/us/book/9783319578484

Varma A, Prasad R, Tuteja N (2017c) Mycorrhiza: Nutrient Uptake, Biocontrol, Ecorestoration. Springer International Publishing (ISBN: 978-3-319-68867-1) http://www.springer.com/us/book/9783319688664

Varma A, Swati T, Prasad R (2020) Plant Microbe Symbiosis. Springer International Publishing (ISBN 978-3-030-36247-8) https://www.springer.com/gp/book/9783030362478

Verma S, Nizam S, Verma PK (2013) Biotic and abiotic stress signalling in plants. Stress signaling in plants: genomics and proteomics perspective, vol 1, pp 25–4

Vivas A, Vörös A, Biró B, Barea JM, Ruiz-Lozano JM, Azcóna R (2003) Beneficial effects of indigenous Cd-tolerant and Cd-sensitive *Glomus mosseae* associated with a Cd-adapted strain of *Brevibacillus* sp. in improving plant tolerance to Cd contamination. Appl Soil Ecol 24(2):177–186

Wahid A, Gelani S, Ashraf M, Foolad MR (2007) Heat tolerance in plants: an overview. Environ Exp Bot 61:199–223

Waller F, Achatz B, Baltruschath H, Fodor J, Becker K, Fischer M, Heier T, Huckelhoven R, Neuman C, Von WD, Franken P, Kogel KH (2005) The endophytic fungus *Piriformospora indica* reprograms barley to salt-stress tolerance, disease resistance, and higher yield. Proc Natl Acad Sci U S A 102(6):13386–13391

Wang Y, Wang M, Li Y, Wu A, Huang J (2018) Effects of arbuscular mycorrhizal fungi on growth and nitrogen uptake of *Chrysanthemum morifolium* under salt stress. PLoS One 13(4):e0196408

Watts-Williams SJ, Cavagnaro TR (2014) Nutrient interactions and arbuscular mycorrhizas: a meta-analysis of a mycorrhiza-defective mutant and wild-type tomato genotype pair. Plant Soil 384:79–92

White RH, Engelke MC, Morton SJ, Johnson-Cicalese JM, Ruemmele BA (1992) *Acremonium* endophyte effects on tall fescue drought tolerance. Crop Sci 32:1392–1396

Wu S, Zhang X, Chen B, Wu Z, Li T, Hu Y, Sun Y, Wang Y (2016a) Chromium immobilization by extraradical mycelium of arbuscular mycorrhiza contributes to plant chromium tolerance. Environ Exp Bot 122:10–18

Wu S, Zhang X, Sun Y et al (2016b) Chromium immobilization by extra-and intraradical fungal structures of arbuscular mycorrhizal symbioses. J Hazard Mater 316:34–42

Wu QS, He JD, Srivastava AK, Zou YN, Kuča K (2019) Mycorrhizas enhance drought tolerance of citrus by altering root fatty acid compositions and their saturation levels. Tree Physiol 39(7):1149–1158

Xie W, Hao Z, Zhou X, Jiang X, Xu L, Wu S, Zhao A, Zhang X, Chen B (2018) Arbuscular mycorrhiza facilitates the accumulation of glycyrrhizin and liquiritin in *Glycyrrhiza uralensis* under drought stress. Mycorrhiza 28:285–300

Xu L, Li T, Wu Z, Feng H, Yu M, Zhang X, Chen B (2018) Arbuscular mycorrhiza enhances drought tolerance of tomato plants by regulating the 14-3-3 genes in the ABA signaling pathway. Appl Soil Ecol 125:213–221

Yamaji K, Watanabe Y, Masuya H, Shigeto A, Yui H, Haruma T (2016) Root fungal endophytes enhance heavy-metal stress tolerance of *Clethra barbinervis* growing naturally at mining sites via growth enhancement, promotion of nutrient uptake and decrease of heavy-metal concentration. PLoS One 11(12):e0169089

Yang Y, Tang M, Sulpice R, Chen H, Tian S, Ban Y (2014) Arbuscular mycorrhizal fungi alter fractal dimension characteristics of *Robinia pseudoacacia*, L. seedlings through regulating plant growth, leaf water status, photosynthesis, and nutrient concentration under drought stress. J Plant Growth Regul 33:612–625

Yong X et al (2014) Enhanced cadmium resistance and accumulation in *Pseudomonas putida* KT2440 expressing the phytochelatin synthase gene of *Schizosaccharomyces pombe*. Lett Appl Microbiol 58:255–226

Yooyongwech S, Samphumphuang T, Tisarum R, Theerawitaya C, Chaum S (2016) Arbuscular mycorrhizal fungi (AMF) improved water deficit tolerance in two different sweet potato genotypes involves osmotic adjustments via soluble sugar and free proline. Sci Hortic 198:107–117

Zabalgogeazcoa I (2008) Fungal endophytes and their interaction with plant pathogens. Span J Agric Res 6:138–146

Zaefarian F, Rezvani M, Ardakani MR, Rejali F, Miransari M (2013) Impact of mycorrhizae formation on the phosphorus and heavy-metal uptake of *Alfalfa*. Commun Soil Sci Plant Anal 44:1340–1352

Zhang WH, Chen Q, Liu YL (2002) Relationship between H^+-ATPase activity and fluidity of tonoplast in barley roots under NaCl stress. J Integr Plant Biol 44:292–296

Zhang F, Jia-Dong HE, Qiu-Dan NI, Qiang-Sheng WU, Zou YN (2018) Enhancement of drought tolerance in trifoliate orange by mycorrhiza: changes in root sucrose and proline metabolisms. Notulae Botanicae *Horti* Agrobotanici Cluj-Napoca 46:270

Zhao FG, Qin P (2005) Protective effects of exogenous fatty acids on root tonoplast function against salt stress in barley seedlings. Environ Exp Bot 53:215–223

Zhao R, Guo W, Bi N, Guo J, Wang L, Zhao J et al (2015) Arbuscular mycorrhizal fungi affect the growth, nutrient uptake and water status of maize (*Zea mays*, L.) grown in two types of coal mine spoils under drought stress. Appl Soil Ecol 88:41–49

Zhu XC, Song FB, Xu HW (2010a) Arbuscular mycorrhizae improve low temperature stress in maize *via* alterations in host water status and photosynthesis. Plant Soil 331:129–137

Zhu XC, Song FB, Xu HW (2010b) Effects of arbuscular mycorrhizal fungi on photosynthetic characteristics of maize under low temperature stress. Acta Ecol Sin 21:470–475

Zhu XC, Song FB, Liu S, Liu T, Zhou X (2012) Arbuscular mycorrhizae improve photosynthesis and water status of *Zea may*s L. under drought stress. Plant Soil Environ 58:186–191

Zou YN, Srivastava AK, Wu QS (2016) Glomalin: a potential soil conditioner for perennial fruits. Int J Agric Biol 18:293–297

Zou YN, Wang P, Liu CY, Ni QD, Zhang DJ, Wu QS (2017) Mycorrhizal trifoliate orange has greater root adaptation of morphology and phytohormones in response to drought stress. Sci Rep 7:41134

Chapter 14
Recent Trends in Nanobioremediation

Ranju Sharma, Ngangbam Sarat Singh, Neha Dhingra, Sonal Yadav, and Mohd. Aamir Khan

Contents

R. Sharma (✉) · N. Dhingra
Department of Zoology, University of Delhi, New Delhi, India

N. S. Singh
Department of Zoology, Dr. SRK Government Arts College, Yanam, Puducherry, India

S. Yadav · M. Aamir Khan
Centre for Rural Development and Technology, IIT-Delhi, New Delhi, India

R. Prasad et al. (eds.), *Mycoremediation and Environmental Sustainability*, Fungal Biology, https://doi.org/10.1007/978-3-030-54422-5_14

14.1 Introduction

Environmental pollution is one of the most serious global problems facing today. Industrialization and uncontrolled use of chemical pesticides leads to release of toxic compounds directly into the environment. This causes various adverse effects on our environment and our earth's ecosystem. Earlier, conventional methods were used to dispose waste materials, and through these methods, toxic wastes were disposed in pits dug in soil. The disadvantages of these conventional methods include the need of a new place each and every time and the serious problem of soil and water pollution caused by the disposed wastes. These led to the development of newer technologies that used high-temperature incineration and chemical decomposition. Although these methods are very effective, they are uneconomical and lead to air pollution. All these pollutions pose a serious threat to human health (Fig. 14.1).

14.2 Bioremediation

Bioremediation is defined as "*the process whereby organic wastes are biologically degraded under controlled conditions to an innocuous state, or to levels below concentration limits established by regulatory authorities*" (Mueller et al. 1996). It is an

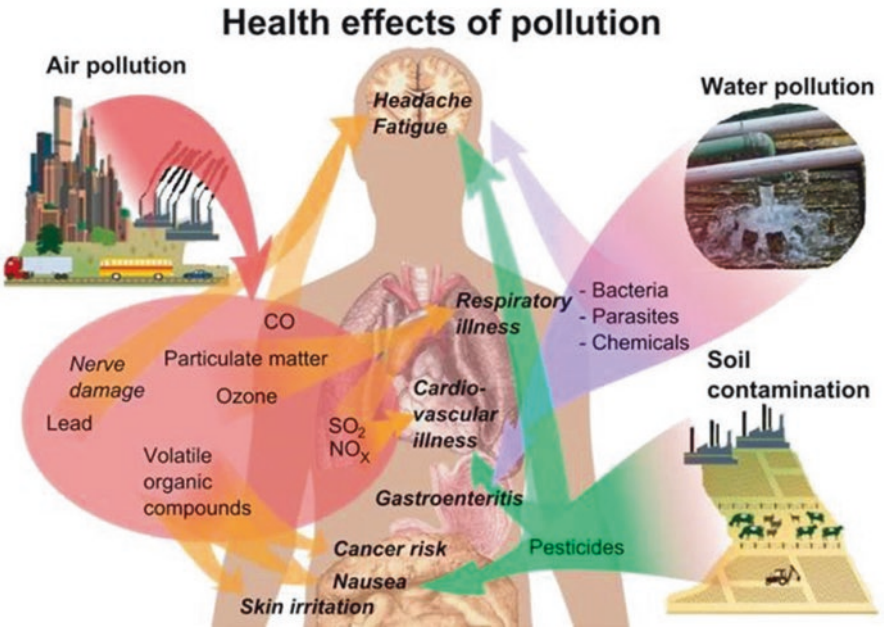


Fig. 14.1 Various harmful effects of soil, water, and air pollution on human health. (http://mpenvis.nic.in/index1.aspx?lid=1470&mid=1&langid=1&linkid=1044)

approach in which efficient microbes are used to degrade toxic material in the environment. Bioremediation is a molecular biology tool by which we modify microorganism in such a way that it enables to use these compounds as an energy source and degrade them or to convert them into nontoxic compounds. This transformation of organic contaminants by microbes usually occurs because they use these contaminants for their growth and reproduction. Organic contaminants serve as source of carbon, i.e., they serve as the key constituent of a new cell and also provide electrons, which help microbes to obtain energy (https://www.nap.edu/read/2131/chapter/4).

In the environment, biodegradation of a compound is the result of action of multiple organisms. Microorganisms used in bioremediation are either indigenous to contaminated site or may be isolated from somewhere under stress conditions and brought to the contaminated site. The process under which we imported microbes to contaminated site is known as bioaugmentation. There are various techniques similar to bioremediation like phytoremediation, mycoremediation, bioventing, bioleaching, land farming, bioreactor, composting, bioaugmentation, rhizofiltration, and biostimulation (Vidali 2001; Behera and Prasad 2020). Among all these techniques, bioremediation is effective, consumes less time, cost-efficient, and is a popularly accepted technique. One of the major advantages of this technique that it is carried out on the contaminated site. Due to rapidly increasing research in the field of bioremediation, it has been used at a number of sites worldwide (Elekwachi et al. 2014). For an effective bioremediation, (1) microbial enzyme must convert the toxic compound into nontoxic metabolites and end products and (2) environmental conditions should promote the microbial growth and activity. We may manipulate the environmental conditions, which favors the microbial growth so that bioremediation occurs at faster rate.

14.2.1 Types of Bioremediation Techniques

On the basis of site of application, degree of saturation, and aeration of an area, bioremediation techniques are mainly divided into two groups: in situ and ex situ (Vidali 2001).

14.2.1.1 In Situ Technique

Bioremediation take place at the contaminated site with minimal disturbance. Due to less disturbance, it is most desirable and less expensive technique. In this technique, we generally add nutrients at the contaminated site, which increase the degradation ability of already present microorganisms (USEPA/625/K-96/001; US EPA/540/2-90/002). Based on the mode of remediation, in situ techniques are divided into different types.

Bioventing In this technique, air and nutrients are supplied at the contaminated site through well to increase the degradation ability of indigenous microbes. It requires

low air flow rate to minimize the volatilization and increase the release of contaminants to the atmosphere. This technique is generally applied where the contamination is deep under the soil/water surface. Sometimes nitrogen and phosphorus also added to maximize the rate of degradation. In some cases where contamination reaches the ground water, hydrogen peroxide is added through injection.

Biosparsing In this technique, we increase the oxygen concentration of ground water table by injecting air with pressure. This will enhance the mixing at saturated zone, thus increasing the contact between soil and groundwater, which further increases the biological degradation. This requires low cost for installing air injection point of small diameter, which allows flexibility in the construction of the system.

Bioaugmentation In this technique, Culture microorganisms has been added to the contaminated site. In this technique, degradation rate depends upon two main factors: (1) either the imported microbes compete with indigenous microbes and attain useful population level or not, (2) in most soil environment, which has long-term exposure to biodegradable waste, indigenous microorganisms are effective degraders.

14.2.1.2 Ex Situ Techniques

These techniques generally involve excavation (soil) and pumping (water) of contaminated site. The following techniques are generally used under this category.

Land Farming This technique involves the stimulation of indigenous microorganisms. During this technique, we excavate the contaminated soil and spread over a prepared bed and till periodically until the contaminants are completely degraded. This will facilitate aerobic degradation. This technique has received much attention due to low monitoring and maintenance cost and clean-up liabilities. This technique is effective to the superficial treatment of soil up to 10–35 cm.

Composting During this technique, amendment of manure or agriculture waste (straw, hay or corn cobs) is done in contaminated soil. This will support the microbial population of detritus-eating organisms by maximizing the water and air levels and increase the degradation rate. It is a multistep, closely monitored process having measured input of water and air (carbon- and nitrogen-rich material). There are three main stages of composting cycle. In the first stage, mesophilic microorganisms thrive at 25–45 °C and due to high temperature, physical breakdown of biodegradable compounds begins. After this, second stage begins, in which temperature increases up to 65 °C, mesophilic microbial community turns into thermophilic. They break down protein, fats, and complex carbohydrates. At this stage, we also provide additional oxygen and new sources for breakdown. Finally, at the third stage, which typically of several months, thermophillic microorganism break down all most all the compounds and temperature begin to drop this will takes several months and then again mesophilic microbes resume and complete the process by

breaking the remaining organic compound into humus (https://www.livescience.com/63559-composting.html).

Biopiles In this technology, excavated soil was mixed with soil amendments and enclosed in above-ground treatment enclosure. Biopile system consists of treatment bed, an aeration system, irrigation system, and a leachate collection system. Physicochemical parameters like oxygen, moisture, nutrients, heat, and pH are controlled in order to enhance the rate of biodegradation. In biopile system, air and nutrient are provided through irrigation and nutrient system present below the ground level. This system is generally 20 feet in height covered with plastic sheet to control evaporation, volatilization, and run-off, as well as it acts as solar heater. If there are volatile compounds present in the soil, they volatilize and present in air stream; thus, regular air treatment is required. It is a short-term technology and runs from few weeks to fewer months (http://www.cpeo.org/techtree/ttdescript/biopil.htm).

Bioreactor It is a highly controlled technology to treat contamination in soil and ground water. This may be an open or closed system. In batch- or continuously fed reactors, pH, nutrient level, and agitation can be controlled, which optimized the microbial activity and thus degradation of contaminants. There are two types of bioreactors, compost-based bioreactor and Slurry bioreactor. *Compost based bioreactor* is a closed in vessel approach in which biodegradation takes place due to high temperature (Cookson 1995). This can be applied to soil, lagoon, and municipal sludge having biodegradable organic contamination. Composting also is useful for explosives, pentachlorophenol (PCP), polycyclic aromatic hydrocarbons (PAHs), ethylene glycol, and insecticides. Composting is well performed in enclosed reactor, and the curing may be accompanied in a reactor or an exterior pile (Norris 1994). *Slurry-based bioreactors* are used to remediate a mixture of water and excavated soil. It provides three-phase (solid, liquid, and gas) mixing condition, which increases the rate of bioremediation of soil-bound as well as water-soluble pollutants. Generally, the rate and extent of biodegradation is greater in bioreactor because it is more manageable and predictable than other techniques. In spite of this, contaminated soil requires pretreatment like excavation or contaminant that can be stripped via soil washing or physical extraction (https://www.hawaii.edu/abrp/Technologies/slurry.html).

14.3 Advantages and Disadvantages

14.3.1 Advantages of Bioremediation

- It is a natural process carried out by naturally occurring microorganisms, which produce harmless products: carbon dioxide, water, and microbial biomass.
- It results in complete mineralization of harmful compounds at the contaminated site, which decreases the further treatment or disposal of contaminated material.

- It can be carried out at the site of contamination, which reduces the cost of transportation and potential threats to human health and the environment that may arise during transportation.
- It is environment friendly and economically cheaper technique for the removal of hazardous waste.

14.3.2 Disadvantages of Bioremediation

- Bioremediation is limited to biodegradable compounds only. All compounds are not susceptible to complete degradation.
- Sometimes, metabolites are more toxic than their parent compounds.
- It is highly specific biological process and requires suitable environment for metabolically capable microorganisms with appropriate level of nutrients.
- Sometimes, results from pilot-scale studies to field-scale studies may vary due to naturally occurring environmental conditions.

14.4 Need of Nanotechnology in Bioremediation

Further research is needed to develop such bioremediation technologies, which are suitable for remediation of complex mixture of contaminants. In recent years, nanotechnology is a topic of extensive research, involving all forms of life science (Baker and Satish 2012). It is an umbrella term covering wide variety of technologies, which comprise processes and structures at nanoscale (Abbasi et al. 2009). Richard Feynman, in 1960, introduced the concept of nanotechnology, which grew faster worldwide in the area of science and technology research and is known as "*Next Industrial Revolution*" (Feynman 1960; Roco 2005).

The potential use of nanotechnology has been divided into three categories: treatment and remediation, sensing and detection, and pollution prevention. Here we discuss the potential role of nanotechnology in site remediation. While selecting a remediating technology, we should focus on many factors like efficiency and coast, its ease to use, time required, availability of resources, etc. Nowadays, various technologies are available for the bioremediation of toxic compounds at the contaminated site, but use of single technology may be expensive and not effective as much. It does not sustain in the environment for longer period (Kim et al. 2011; Le et al. 2015; Nemecek et al. 2016). Thus, it is necessary to combine two or more remediation technologies and develop a new technology, which overcomes the gap of each other. This reduces the cost of the effective technology, time, and resources requirement (He et al. 2006; Dinesh et al. 2012; Koenig et al. 2016). Thus, based on all these facts about remediation technology, the concept of *nanobioremediation* is generated, which involves the use of fat and expensive physicochemical technology followed by cheap and slow biological technology. This is a viable and sustainable

alternative to in situ and ex situ bioremediation technologies and an emerging field, which is commercially applied in various clean-up sites around the world (Pandey et al. 2015; Prasad and Aranda 2018).

14.5 Different Types of Nanoparticles and Their Role in Bioremediation

Nanotechnology is the branch of science, which deals with various approaches of nanoparticle. In nanotechnology, the two mostly used terms are nanomaterial and nanoparticle. Although these both terms are synonyms, the American Society for Testing and Materials (ASTM) has stated that a "nanoparticle (NP) is a subclassification of ultrafine particle with lengths in two or three dimensions greater than 0.001 lm (1 nm) and smaller than about 0.1 lm (100 nm) and which may or may not exhibit a size-related intensive property" (ASTM 2012). On the other hand, the European Union stated that "nanomaterial means a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where*, for 50% or more of the particles in the number size distribution, one or more external dimensions are in the size range 1–100 nm"* (http://ec.europa.eu/environment/index_en.htm).

Nanoparticle is ultrafine aggregates of atomic and molecular particle with size 10^{-9} m (<100 nm) (Prathna et al. 2010). These are also known as nanoscale particle (NSPs). Their activity depends upon their chemical composition and shape and size of particle. Due to their small size, their physicochemical properties are significantly different from their parent compounds. They are more reactive then their parent compounds. Nanoparticle are generally classified into two groups: organic and inorganic nanoparticles. Organic nanoparticle are carbon nanoparticle (dendrimers, liposomes, and micelles), while inorganic are metallic (gold NPs, Qdots), magnetic, and semi-conductor nanoparticles (silicones and germanium). Ruffin Castiglione and Cremonini discovered three types of nanoparticles on the basis of their manufacturing process: natural, incidental, and engineered (Ruffini-Castiglione and Cremonini 2009). Bionanoparticles develop from naturally occurring parent materials such as mineral composite and volcanic or lunar dust, while incidental NPSs develop as a result of anthropogenic activities, thatis, diesel exhaust, coal combustion, and welding fumes. Engineered nanoparticles are metal-based engineered nanoparticles, which include nanogold, nanoiron, and nanocadmium. (Lin and Xing 2007).

Today, researchers have developed an efficient, cost-effective, and eco-friendly alternative to existing parent material and demonstrated their use in both resource conservation and environmental remediation (Friedrich et al. 1998; Dimitrov 2006; Dastjerdi and Montazer 2010). This is known as green approach or biological synthesis of nanoparticle in which plant- and microbes-based nanoparticles are produced, which show significant importance and numerous application in the area of

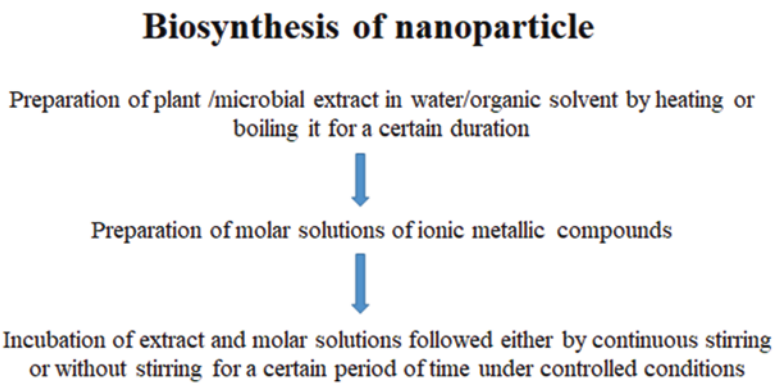


Fig. 14.2 Flowchart representation of key steps involved in biosynthesis of nanoparticles

medicine, agriculture, and electronics (Mishra et al. 2014; Kasthuri et al. 2009). Green technology is the widely accepted technology for the synthesis of nanoparticles due to its ecofriendly nature, cost effectiveness, and stability in nature (Ingale and Chaudhari 2013). Due to the insignificant role of chemically synthesized nanoparticles in bioremediation, in this chapter, we are only concerned with the biological methods of nanoparticle production instead of chemical synthesis. Biological synthesis of nanoparticle includes plants, bacteria, algae, yeast, and fungi (Prasad et al. 2016, 2018; Srivastava et al. 2021). Figure 14.2 shows the flowchart representation of biosynthesis of nanoparticle (Thakkar et al. 2010; Rauwel et al. 2015).

14.5.1 Plant-Based Nanoparticles

These nanoparticles are processed from single-step synthesis process from various plant parts such as leaf extract, seed extract, plant resins and oils, secondary metabolites, and gums (Prasad 2014; Haleemkhan and Naseem 2015). It includes the use of natural capping agent and lack of toxicants (Gurunathan et al. 2009). Plant-based nanoparticles are advantageous over microbial based due to their easy availability and safe handling. They also process variables of secondary metabolites and require less time for reduction, therefore better option for NPSs synthesis than microbial-based ones. By the application of plant tissue culture technique and downstream processing approach, it is possible to synthesize metallic as well as oxides nanoparticle for industrial purpose. Nanoparticles differ in their effect on the basis of plant type from which they are processed, as well as their mode of action, size, and concentration (Manzer et al. 2015). Research on plant-based nanoparticle is at its initial stage. This will require more understanding of biochemical, physiological, and molecular mechanism of plants. Further research is needed to evaluate and uncover the mode of action of NPSs and their interaction with biomolecule as well as with

gene expression in plants. *Example:* Leaves extract of *Mentha, Ocimum,* and *Eucalyptus* were reported for the synthesis of gold nanoparticles (Haleemkhan and Naseem 2015).

14.5.2 Microbe-Based Nanoparticle

Microbes have high tolerance and reproduction power; due to this reason, they are commercially used for decontamination process. Microbial synthesis of nanoparticle with different shapes and sizes is an important aspect of nanobiotechnology. It is bottom-up approach where the synthesis of nanoparticle occurs due to oxidation/reduction reaction. Microbial enzyme reduces the metal compound and biologically generated nanoparticle with higher catalytic reactivity and specific surface area (Prathna et al. 2010; Riddin et al. 2010). It is a dose-dependent process and also related to the type of microbes used. Biomolecules secreted by specific microbe responsible for the reduction of metal such as peptides and polysaccharides. Bionanoparticles are stable and do not aggregate due to presence of capping agent secreted by respective microbes such as proteins and sulfated polysaccharides (Singaravelu et al. 2007). Microbial synthesis of NPSs is at much slower rate as compared to plant based. Microbial-based NPSs may be synthesized either by bacteria, algae, fungi, and yeast (Prasad et al. 2016).

14.5.2.1 Bacteria-Based Nanoparticle

Bacterial strains, which have the ability to precipitate metal at nanometer scale, are considered as a potential source of nanoparticle production. Metals, like gold, silver, platinum, palladium, titanium, iron, zinc, aluminum, magnetite, cadmium sulfide, etc., are used for this purpose. Enzymes, vitamins, polysaccharides, and polymers produced by bacterial species catalyze specific reaction, leading to the production of inorganic nanoparticles (Iravani 2014). Nanoparticle may be synthesized via extracellular or intracellular process. Extracellular biosynthesis of NPSs is widely in use due to its low cost and no downstream processing requirement (Mishra et al. 2014). During the extracellular biosynthesis, secondary metabolites present in cell-free extract are responsible for redox reaction after the addition of precursor molecule. Due to variation in biological and physical parameters, particle characteristics can also vary. These properties may be controlled by optimization of growth condition of the required bacterial strain, which further controls the cellular and enzyme activity (Iravani 2014). These particles are further characterized by SEM, TEM, FTIR, etc. Example*: Bacillus subtilis* is well reported for the synthesis of silver nanoparticles (Saifuddin et al. 2009) (Fig. 14.3).

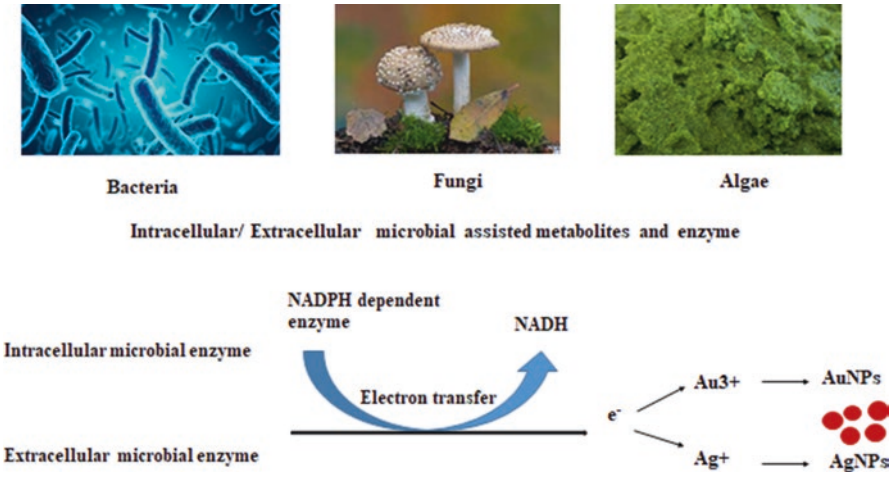


Fig. 14.3 Microbial biosynthesis of metal nanoparticles

14.5.2.2 Yeast- and Fungi-Based Nanoparticle

Fungi play promising role in the production of NPSs as they are excellent sources of extracellular enzymes compared to bacteria. They secrete higher amount of proteins and result in the higher production of well-defined dimensional nanoparticles with monodispersity (Mohanpuria et al. 2008). Instead of culture, isolated proteins are also used for the synthesis of nanoparticles. Fungi have an edge over other biological systems for NPS production due to its higher growth rate, and they are easy to culture, easy to handle, require less time, and cost-effective (Vahabi et al. 2011; Prasad 2016, 2017; Abdel-Aziz et al. 2018; Aziz et al. 2016, 2019). All these properties are responsible for its better industrial application over the bacteria. Besides all these properties, there is one drawback with the fungal synthesized nanoparticles i.e the fungal enzyme reduces salt into its metallic nanoparticles due to its catalytic effect (Oksanen et al., 2000). Example*: Fusarium oxysporum* is well reported for the synthesis of gold and silver nanoparticles (Popescu et al. 2010).

14.5.2.3 Algae-Based Nanoparticles

Algae are known as *bionanofactories* because both live and dead biomass of algae are utilized for the synthesis of metallic nanoparticle (Davis et al. 1998). The study that deals with the synthesis of nanoparticles from algae is known as phyconanotechnology, a new branch of science and technology. Algae-based synthesis of nanoparticle requires less time as compared to other biosynthesizing methods (Thakkar et al. 2010; Rauwel et al. 2015; Aziz et al. 2014, 2015). Seaweeds like *Sargassum wightii* and *Fucus vesiculosus* have also been reported for synthesizing AgNPs of different sizes and shapes (Singaravelu et al. 2007; Mata et al. 2009). Microalgae such as diatoms (*N. atomus* and *D. gallica*) have also shown the ability

to synthesize gold, and silica–gold bionanocomposites (Mubarak Ali et al. 2013). Marine algae are also miserably explored for the synthesis of nanoparticles. *For example*: *Chlorella vulgaris* has strong binding capability toward tetrachloroaurate ions to form algal-bound gold. Approximately 88% of algal-bound gold attained metallic state, accumulated in the form of tetrahedral, decahedral, and icosahedral crystalline structures on the inner and outer parts of cell surface (Jianping et al. 2007). *Spirulina platensis* has been reported for the extracellular synthesis of gold, silver, and Au/Ag bimetallic NPs, while *T. kochinensis* for extracellular synthesis of gold nanoparticles (Chakraborty et al. 2009; Mata et al. 2009; Senapati et al. 2012).

14.6 Role of Nanotechnology in Bioremediation

Nanotechnology increases its utility day by day in the field of bioremediation. It remediates the contaminant in a very cost-effective manner. There are various mechanisms, which have been applied to decontaminate the heavy metals and polycyclic aromatic hydrocarbons (Prasad and Aranda 2018). Many of them are commercialized in the present-day market.

14.6.1 Mechanism Used to Remediate the Pollutants

Nanoiron and its derivatives Nanoscale zerovalent iron is widely used for the removal of highly mobile and toxic heavy metal in the soil as well as in the ground water. It rapidly immobilizes Cr (VI) and Pd (II) and reduces them to Cr (III) and Pd (0), while in case of iron, Fe oxidizes into goethite (α-FeOOH) (Ponder et al. 2000). Iron nanoparticle supported with polyacrylic acid (Fe/PAA) and hydrophilic anionic carbon (Fe/C) have been reported as a reactive material for the degradation of halogenated chlorinated hydrocarbon (Schrick et al. 2004). Nickel-iron nanoparticles having high surface area have also been studied for the degradation of trichloroethylene (TCE). Organochlorine compounds like PCP (penta chlorophenol), DDT, DDD, and DDE were also reported for their anaerobic degradation by zerovalent iron at 20 °C in the presence of nonionic surfactant Triton X-114 (Sayles et al. 1997).

Dendrimers Dendrimers are the highly branched monodispersive macromolecule that consists of central core, interior branch cell, and terminal branch cell (Tomalia et al. 1985; Newkome et al. 1985; Undre et al. 2013a, b). First dendrimer was reported by Buhleier et al. (1978). Dendrimer is a Greek word: it means branch of a tree having many void spaces due to which it easily interacts with other substances and is also used to enhance catalytic activity (Undre et al. 2013a, b). It is more reactive, less toxic with larger surface area due to which it has its application in water treatment and textile industries. Today, TiO_2 porous ceramic filters impregnated with alkylated poly(propylene imine) dendrimer, poly(ethyleneimine) hyperbranched polymer, or β-cyclodextrin are also developed for removal of organic pol-

lutants. They have hybrid organic/inorganic filters modules with high mechanical strength and larger surface area (Guo et al. 2012).

Nanocrystals and Carbon Nanotubes Carbon-based nanoparticles such as carbon nanotubes, *for example*, single-walled carbon nanotubes (SWCNTs), multiwalled carbon nanotubes (MWCNTs), and hybrid carbon nanotubes are effective pollution preventive strategies. These are renewable energy technologies acting as sorbents, antimicrobial agents, depth filters, high flux membrane, and environmental sensors (Mauter and Elimelech 2008). BET isotherm expression indicates that SWNCTs are rapid and efficient adsorbent for ethylbenzene and have good potential for the maintenance for high-quality water (Bina et al. 2012). Recently, CDcohexamethylene-/toluene-di-isocyanate polyurethanes modified CNTs have been developed with high potential for removing organic (p-nitrophenol) as well as inorganic contaminants (Cd^{2+}, Pb^{2+}). Carbon nanotubes immobilized with calcium alginate (CNTs/CA) were also used for copper adsorption, and it was observed that it has 69.9% Cu removal efficiency even at pH 2.1 (Li et al. 2010). MWCNTs were also used in waste water treatment to remove nickel ions (Gong et al. 2009).

Single-Enzyme NPs Enzymes are highly specific and effective proteins used as biocatalyst in the field of bioremediation. Due to oxidation reaction, they lose their catalytic activity after a short period of time and remain unstable, which limits its application as a cost-effective alternative to synthetic catalyst. Recent research has proved that magnetic FeNPs attach with enzyme and increases the catalytic activity, stability, and reusability of enzymes. *For example:* The activity of MNP attach trypsine and peroxidase increases from hours to weeks and are more stable, efficient and economical. Magnetic nanoparticles inhibit the enzyme oxidation and increase its life, which makes them more productive (Qiang et al. 2007).

14.6.2 Engineered Polymeric NPs

Engineered polymeric nanoparticles are currently used in bioremediation of hydrophobic contaminants, *for example*: polycyclic aromatic hydrocarbons (PAHs). Polymer NPs limit the solubility and mobility rate and also reduce the bioavailability of PAHs by its sequestration to soil or by partitioning in nonaqueous phase liquids (NAPLs). In case of phenanthrene (PHEN), hydrophobic organic contaminants poly(ethylene)glycol-modified urethane acrylate (PMUA) precursor chain has been developed which not only enhance the bioavailability of PHEN but also increase its mineralization rate. The properties of PMUA NPs to remain stable in the presence of active heterogeneous bacterial population enable them to be reused after the particles bounded PHEN are degraded by bacteria (Tungittiplakorn et al. 2005).

14.6.3 Biogenic Uraninite NPs

Today, scientists have great interest in the production of biogenic uraninite due to its small particle size, biological origin, molecular scale structure, energetics, and surface area. Due to all these properties, it appears similar to coarse particles, abiotic, stoichiometric UO_2 and has potential role in bioremediation of subsurface U(VI) contamination (Bargar et al. 2008).

14.6.4 Nanoparticle as a Biosensor

Before applying any bioremediation technique, we should first sense the pollutant in the environment, which makes our work easier. In this direction, various techniques have been developed, but to determine exact position and composition of contaminant is impossible. Nowadays, nanotechnology plays an important role in improving the sensitivity of the sensor by targeting the binding between the contaminant and the recognition element or optimizing the transduction and electronic interface to the sensing layer. Contaminant may be organic, inorganic, or biological. Immobilized enzymes in carbon nanotubes, single enzyme nanoparticles, or polymeric nanoparticles are used as environmental biosensors (Kim et al. 2006). *For example:* Tyrosinase (TYR, EC 1.14.18.1) is a copper-containing oxidorectase enzyme, which catalyzes the o-hydroxylation of monophenol (cresolase activity) as well as oxidoreduction of o-diphenols to o-quinones (catecholase activity) (Seo et al. 2003). It displays great potential as a sensor against mono- and diphenolics compounds (Alkasir et al. 2010). Laccase enzyme is also used as a biosensor against catechol, a hazardous phenolic compound (Tang et al. 2008).

14.7 Remediation of Water Pollutants

Water is basic need of day-to-day life. It is linked with economic development. According to Leonardo Da Vinci "*water is the vehicle of nature*". Due to rapidly increasing industrialization and urbanization, various hazardous chemicals are directly mixed with water. The major contaminants are pesticides, organochlorine compounds, and heavy metals (Behera and Prasad 2020a). Instead of conventional methods of water treatment, nanotechnology presents better way with less time consuming, cost effective, and in efficient manner. Due to small particle size, adsorption efficiency increases significantly with increase in surface area energy (Prasad and Thirugnanasanbandham 2019).

Zerovalent iron nanoparticles are widely used for dechlorination in waste water treatment plants as the iron acts as reducing agent (Chuang et al. 1995). Chlorinated hydrocarbons have the same oxidation potential as oxygen and thus acts as electron acceptor. During dechlorination, ground water oxidizes the iron nanoparticle from Fe^0 to Fe^{2+} (ferrous iron) with the release of two electrons. As the surface area of

iron nanoparticle increases, the rate of carbon to act as electron acceptor also increases, which results in the release of chloride ion (Junyapoon 2005). Coating of iron nanoparticle with catalytic metal, such as Pd, Ni, Ag, and Pt, will not only increase the dichlorination process but also inhibit the formation of toxic by-products (Xu and Zhang 2000; Schrick et al. 2002). These are widely used in environmental decontamination process as they detoxify organic and inorganic contaminants due to their redox activity. Iron nanoparticles are also employed for the removal of heavy metal such as (III), Cu (II) Pb (II), Hg (II), Cd (II) from water. Nanoparticles of iron oxides, that is, magnetite (Fe_3O_4), maghemite (γ-Fe_2O_3), and hematite (Fe_2O_3)-based nanoabsorbents, also have their application in removal of heavy metals from wastewater (Dave and Chopda 2014).

CNTs (carbon nanotubes) and MWCNTs (Multi-walled carbon nanotubes) are also used as good absorbers for metals, like Cu, Ni, Pb, Ag etc., as well as volatile organic compounds and dyes like ethidium bromide, eosin bluish, etc. (Li et al. 2003; Fugetsu et al. 2004; Liang et al. 2005; Chen and Wang 2006; Ding et al. 2006). CNTs also showed positive relationship with pH as their absorbance capacity increases with increase in pH. Titanium dioxide NPs also have great catalytic and redox activity due to their optical properties (Obare and Meyer 2004). Due to this property, they are also applied in purification of water involving both oxidation/reduction reaction in presence of UV light. In presence of UV light, they photocatalyze the organic contaminants as well as reduce the toxic metal ions: Cr (VI), Ag (I), and Pt (II) (Savage and Diallo 2005). Recently, N-doped TiO_2 NPs are also used to degrade methylene blue under visible light conditions (Asahi et al. 2001).

Van der Bruggen and Vandecasteele (2003) have reviewed the concept of desalination of water by using nanofiltration to remove cations, natural organic matter, biological contaminants, organic pollutants, nitrates, arsenic, and microbes from groundwater and surface water; U (VI) can be removed from sea water by nanofiltration and reverse osmosis (Van der Bruggen and Vandecasteele 2003). *For example:* carbon nanotubes filter are successfully used for removing pathogenic microbes from contaminated water, such as *Escherichia coli, Staphylococcus aureus* and *Poliovirus sabin 1* (Srivastava et al. 2004). Fe- and Mn-doped alumina ultrafine membranes consisting of alumina (A-alumoxanes) nanoparticles (7–25 nm) also are used against chlorinated hydrocarbons (DeFriend et al. 2003). Silver nanoparticles embedded with cellulose acetate fibers also are found to be effective against *Staphylococcus aureus*, *E. coli*, *Klebsiella pneumonia,* and *Pseudomonas aeruginosa* (Son et al. 2004). MgO nanoparticles are an effective biocide applied against Gram-positive bacteria (*E. coli)*, Gram-negative bacteria (*Bacillus megaterium*), and bacterial spores (*Bacillus subtilis*).

Dendrimers are also used to remove organic and inorganic solutes, radionuclides, microbes, and toxic metal ions during the purification of drinking water. *For example:* Ag (I) and quaternary ammonium chlorides have been successfully applied as antimicrobial agents. They are found in different forms dendrimers, dendrigraft polymers, random hyperbranched polymers, and dendrons (Balogh et al. 2001; Chen and Cooper 2002).

14.8 Remediation of Soil Contaminants

Soil contributes 70% to Indian economy as it is a key constituent of agriculture. Soil contamination occurs due to various anthropogenic activities, industrialization, and urbanization (Behera and Prasad 2020b). Contaminants may be either organic or inorganic, and based on this, remediation strategy may be applied.

Inorganic Contaminants Persistence of heavy metals is the major source of inorganic contaminants. It is generally caused by industries, coal combustion, electroplating, municipal incineration, fuel production, etc. Adsorbance of heavy metal through iron nanoparticle is the best way to decontaminate them from soil. *For example*: Mercury (Hg^{2+}) and its derivative come from seed treatment and dental filling is the major contamination of soil. Chromium used in various industries, that is, textile, electronic, electroplating, etc., gets discharged in a very large quantity in the environment in a very insignificant manner. Cr (VI) is a very toxic and mobile state of chromium (Kimbrough et al. 1999). Zerovalent Fe^0 nanoparticles adsorb these heavy metals and oxidize them into nontoxic state. There are several indigenous microbes present in the contaminated soil, which adsorb heavy metals and convert them into nanoparticles, which generally are used for industrial purpose (Salvadori et al. 2014; Thakare et al. 2021). *For example:* The dead biomass of *H. lixii* is an efficient adsorbent for copper ions and converted them into nanoparticle (Salvadori et al. 2013).

Organic Contaminants Incomplete combustion of fossil fuel leads to the production of highly toxic organic contaminants, that is, polycyclic aromatic hydrocarbon, into the environment (Gibson and Subramanian 1984; Johnsen et al. 2005). *Anthracophyllum discolour,* a white rot fungus, secretes enzyme, that is, manganese peroxidase, lignin peroxidase, and laccase. These enzymes are reported for their role in PAHs degradation (Collins et al. 1996; Steffen et al. 2002). This fungus is also responsible for the secretion of nanoclay having their potential role in degrading PAHs in aqueous system also. Cultured microbial cells immobilized on nanoparticles are more effective than immobilization occurs on any other media. Iron nanoparticles (Fe_3O_4) functionalized with ammonium oleate when coated on the surface of *Pseudomonas delafieldii*, by applying an external magnetic field, has been clubbed at one place, and separated from the solution. They were recycled for the treatment of the same substrate. The microbial cell of *P. delafieldii* desulfurizes dibenzothiophene (organic sulfur) from the fossil fuel (Shan et al. 2005). Dyes used in textile industries are heterocyclic organic compounds responsible for soil, air, and water pollution. They hinder the incidence of sunlight, which reduces the photosynthesis rate and decreases the release of oxygen. This has become a serious issue for plant and water animals. *For example:* Methylene blue degrades titanium dioxide (photocatalyst) in the presence of sunlight and also decomposes pathogenic bacteria, that is, *P. aeruginosa*, *E. coli*, and ammonia (Jang et al. 2001).

14.9 Remediation of Air Contaminants

Greenhouse gases include CO_2, CH_4, N_2O, and CFCs, which cause serious threat to our environment and cause global warming (IPCC 2014; Behera and Prasad 2020c). Nanotechnology plays an important role in the adsorption of these contaminants from the environment via noncovalent forces such as hydrogen bonding, electrostatic forces, π–π and hydrophobic interactions, and van der Waals forces (Ren et al. 2011; Gupta and Saleh 2013; Wang et al. 2013; Bergmann and Machado 2015). *For example:* CNTs, due to their high electric and thermal conductivity, high strength, and specific adsorption capacity, increase the combination of one or more functional groups on the surface and thus increase the adsorption capacity (Gupta and Saleh 2013; Wang et al. 2014). Interaction between solid surface and molecule depends on the pore size and its geometry. Likewise, CNTs are more graphitic in nature than activated carbon; thus, they have more adsorption capacity (Ren et al. 2011). Today, concept of self-cleaning is very much exploited. In this technique, nanoparticles of titanium oxides are coated on the surface, which becomes self-capable to decontaminate the air contaminants (nitrogen oxides and VOCs) (Shen et al. 2015). To increase the efficiency of these TiO_2, CNTs, and graphene, nanosheets have been used which facilitate the movements of electron and inhibit electron and hole recombination (Low et al. 2017).

14.10 Conclusion

Global environmental pollution is increasing day by day and is becoming a major concern. To overcome this problem, bioremediation is a cost-effective and eco-friendly way. Various potential in situ and ex situ technologies have been used to overcome these problems, but they also have their own drawbacks. To overcome these, nanotechnology has emerged as a powerful tool. Being on its developmental stage, it is considered as potential tool for bioremediation. Nanobioremediation is an efficient tool to compete with the global pollution problem. Zerovalent iron, gold, silver, titanium dioxide, quantum dots, and carbon nanotubes can not only help to remediate these contaminations but also play a role of sensor for various types of toxic contaminants also. Plants and microbially synthesize nanoparticles also have an advantage for environmental remediation as a green technology. Nanobioremediation is a nanorenovogen to remediate the environmental pollution.

14.11 Future Prospects

Though the field of nanobioremediation is rapidly developing, the mechanism of remediation still needs to be understood. Despite being a great tool of bioremediation, microbes also synthesize nanoparticles, thus exploring the field of

nanobioremediation. This nascent field of green technology is in cradle and needs to bloom up to overcome this global problem of pollution. These technologies are better option to replace conventional technologies but also have their own potential risk. Toxicity due to nanomaterial is still unclear. We have to uncover these research gaps to make nanobioremediation a promising tool and, thus, have environmental sustainability.

References

Abbasi T, Jayaraman A, Abbasi SA (2009) Nanotechnology and its potential in revolutionizing the pollution control scenario. J Inst Public Health Eng 10:1–12

Abdel-Aziz SM, Prasad R, Hamed AA, Abdelraof M (2018) Fungal nanoparticles: a novel tool for a green biotechnology? In: Prasad R, Kumar V, Kumar M, Wang S (eds) Fungal nanobionics: principles and applications. Springer Singapore Pte Ltd, Singapore, pp 61–87

Alkasir RSJ, Ganesana M, Won YH, Stanciu L, Andreeceu S (2010) Enzyme functionalized nanoparticles for electrochemical biosensors: A comparative study with applications for the detection of bisphenols. Biosens Bioelectron 26:43–49

American Society for Testing and Materials (2012) Standard terminology relating to nanotechnology. E 2456-06. ASTM, West Conshohocken

Asahi R, Morikawa T, Ohwaki T, Aoki K, Taga Y (2001) Visible-light photocatalysis in nitrogen-doped titanium oxides. Science 293:269–271

Aziz N, Fatma T, Varma A, Prasad R (2014) Biogenic synthesis of silver nanoparticles using *Scenedesmus abundans* and evaluation of their antibacterial activity. J Nanopart 2014:689419. https://doi.org/10.1155/2014/689419

Aziz N, Faraz M, Pandey R, Sakir M, Fatma T, Varma A, Barman I, Prasad R (2015) Facile algae-derived route to biogenic silver nanoparticles: synthesis, antibacterial and photocatalytic properties. Langmuir 31:11605–11612. https://doi.org/10.1021/acs.langmuir.5b03081

Aziz N, Pandey R, Barman I, Prasad R (2016) Leveraging the attributes of *Mucor hiemalis*-derived silver nanoparticles for a synergistic broad-spectrum antimicrobial platform. Front Microbiol 7:1984. https://doi.org/10.3389/fmicb.2016.01984

Aziz N, Faraz M, Sherwani MA, Fatma T, Prasad R (2019) Illuminating the anticancerous efficacy of a new fungal chassis for silver nanoparticle synthesis. Front Chem 7:65. https://doi.org/10.3389/fchem.2019.00065

Baker S, Satish S (2012) Endophytes: toward a vision in synthesis of nanoparticles for future therapeutic agents. Int J Bio 1:1–11

Balogh L, Swanson DR, Tomalia DA, Hagnauer GL, McManus AT (2001) Dendrimersilver complexes and nanocomposites as antimicrobial agents. Nano Lett 1:18–21

Bargar JR, Bernier-Latmani R, Giammar DE, Tebo BM (2008) Biogenic uraninite nanoparticles and their importance for uranium remediation. Elements 4:407–412

Behera BK, Prasad R (2020) Environmental Technology and Sustainability. Elsevier (ISBN: 9780128191033) https://www.elsevier.com/books/environmental-technology-and-sustainability/behera/978-0-12-819103-3

Behera BK, Prasad R (2020a) Aqueous-phase conservation and management. In: Behera BK, Prasad R (eds) Environmental Technology and Sustainability. Elsevier 73–141

Behera BK, Prasad R (2020b) Strategies for soil management. In: Behera BK, Prasad R (eds) Environmental Technology and Sustainability. Elsevier 143–167

Behera BK, Prasad R (2020c) Greenhouse gas capture and conversion. In: Behera BK, Prasad R (eds) Environmental Technology and Sustainability. Elsevier 41–71

Bergmann CP, Machado F (2015) Carbon nanomaterials as adsorbents for environmental and biological applications. In: Araujo P, Tuscaloosa AL (eds) Carbon nanostructures library of

congress. Springer International Publishing, New York/Dordrecht/London/Cham/Heidelberg, pp 1–126
Bina B, Pourzamani H, Rashidi A, Amin MM (2012) Ethylbenzene removal by carbon nanotubes from aqueous solution. J Environ Public Health 817187:8
Biopiles (n.d.) Center for public environmental oversight. http://www.cpeo.org/techtree/ttdescript/biopil.htm. Assessed on 12 April 2019
Buhleier E, Wehner W, ogtle FV (1978) 'Cascade'- and "nonskidchain-like" syntheses of molecular cavity topologies. Synthesis 2:55–158
Chakraborty N, Banerjee A, Lahiri S, Panda A, Ghosh AN, Pal R (2009) Biorecovery of gold using cyanobacteria and an eukaryotic alga with special reference to nanogold formation-a novel phenomenon. J Appl Phycol 21:145–152
Chen CZS, Cooper S (2002) Interactions between dendrimer biocides and bacterial membranes. Biomaterials 23:3359–3368
Chen CL, Wang XK (2006) Adsorption of Ni (II) from aqueous solution using oxidized multiwall carbon nanotubes. Ind Eng Chem Res 45:9144–9149
Chuang FW, Larson RA, Wessman MS (1995) Zerovalent iron-promoted dechlorination of polychlorinated biphenyls. Environ Sci Technol 29:2460–2463
Collins PJ, Kotterman M, Field JA, Dobson A (1996) Oxidation of anthracene and benzo [a] pyrene by Laccases from trametes versicolor. Appl Environ Microbiol 62:4563–4567
Commission Joint Research Centre Reference Report (2011) Off J Eur Union. http://ec.europa.eu/environment/index_en.htm
Cookson JT Jr (1995) Bioremediation engineering design and application. McGraw-Hill, Inc., New York
Dastjerdi R, Montazer M (2010) A review on the application of inorganic nano-structured materials in the modification of textiles: focus on anti-microbial properties. Colloids Surf B Biointerfaces 79:5–18
Dave PN, Chopda LV (2014) Application of iron oxide nanomaterials for the removal of heavy metals. J Nanotechnol 2014:398569
Davis SA, Patel HM, Mayes EL, Mendelson NH, Franco G, Mann S (1998) Brittle bacteria: a biomimetic approach to the formation of fibrous composite materials. Chem Mater 10:2516–2524
DeFriend KA, Wiesner MR, Barron AR (2003) Alumina and aluminate ultrafiltration membranes derived from alumina nanoparticles. J Membr Sci 224:11–28
Dimitrov D (2006) Interactions of antibody conjugated nanoparticles with biological surfaces. Colloids Surf A Physicochem Eng Asp 8:282–283
Dinesh R, Anandaraj M, Srinivasan V, Hamza S (2012) Engineered nanoparticles in the soil and their potential implications to microbial activity. Geoderma 173–174:19–27
Ding Q, Liang P, Song F, Xiang A (2006) Separation and preconcentration of silver ion using multiwalled carbon nanotubes as solid phase extraction sorbent. Sep Sci Technol 41:2723–2732
Elekwachi CO, Andresen J, Hodgman TC (2014) Global use of bioremediation technologies for decontamination of ecosystems. J Bioremed Biodegr 5:225
Feynman R (1960) There's plenty of room at the bottom. Eng Sci 23:22–36
Friedrich KA, Henglein F, Stimming U, Unkauf W (1998) Investigation of Pt particles on gold substrates by IR spectroscopy particle structure and catalytic activity. Colloids Surf A Physicochem Eng Asp 134:193–206
Fugetsu B, Satoh S, Shiba T, Mizutani T, Lin YB, Terui N et al (2004) Caged multiwalled carbon nanotubes as the adsorbents for affinity-based elimination of ionic dyes. Environ Sci Technol 38:6890–6896
Gibson DT, Subramanian V (1984) Microbial degradation of aromatic compounds. In: Gibson DT (ed) Microbial degradation of organic compounds. Marcel Dekker Inc, New York, pp 181–252
Gong JL, Wang B, Zeng GM, Yang CP, Niu CG, Ya Q, Jin NW, Liang ZY (2009) Removal of cationic dyes from aqueous solution using magnetic multi-wall carbon nanotube nanocomposite as adsorbent. J Hazard Mater 164:1517–1522

Guo R, Guo X, Yu D, Hu J (2012) Application research in water treatment of PAMAM dendrimer. J Ind Eng Chem 31:671–675
Gupta VK, Saleh TA (2013) Sorption of pollutants by porous carbon, carbon nanotubes and fullerene – an overview. Environ Sci Pollut Res 20:2828–2843
Gurunathan S, Kalishwaralal K, Vaidyanathan R, Venkataraman D, Pandian SR, Muniyandi J, Hariharan N, Eom SH (2009) Biosynthesis, purification and characterization of silver nanoparticles using Escherichia coli. Colloids Surf B Biointerfaces 74:328–335
Haleemkhan AA, Naseem VBV (2015) Synthesis of nanoparticles from plant extracts. Int J Mod Chem Appl Sci 2:195–203
He N, Li P, Zhou Y, Fan S, Ren W (2006) Degradation of pentachlorobiphenyl by a sequential treatment using Pd coating iron and aerobic bacterium (H1). Chemosphere 76:1491–1497
Ingale AG, Chaudhari AN (2013) Biogenic synthesis of nanoparticles and potential applications: an EcoFriendly approach. J Nanomed Nanotechol 4:165
IPCC (2014) Climate change 2014: Working group III contributing the fifth Assessment Report of the inter-governmental panel on climate change – mitigation of climate change. Cambridge University Press, New York, p 56
Iravani S (2014) Bacteria in nanoparticle synthesis: current status and future prospects. Int Sch Res Notices 2014:359316
Jang HD, Kim SK, Kim SJ (2001) Effect of particle size and phase composition of titanium dioxide nanoparticles on the photocatalytic properties. J Nanopart Res 3:141–147
Jianping X, Jim YL, Daniel ICW, Yen PT (2007) Identification of active biomolecules in the high-yield synthesis of single-crystalline gold nanoplates in algal solutions. Small 3:668–672
Johnsen AR, Wick LY, Harms H (2005) Principles of microbial PAH-degradation in soil. Environ Pollut 133:71–84
Junyapoon S (2005) Use of zero-valent iron for waste water treatment. KMITL Sci Technol J 5:587–595
Kasthuri J, Veerapandian S, Rajendiran N (2009) Biological synthesis of silver and gold nanoparticles using apiin as reducing agent. Colloids Surf B Biointerfaces 68:55–60
Kim J, Grate JW, Wang P (2006) Nanostructures for enzyme stabilization. Chem Eng Sci 61:1017–1026
Kim YM, Murugesan K, Chang YY, Kim EJ, Chang YS (2011) Degradation of polybrominated diphenyl ethers by a sequential treatment with nanoscale zero valent iron and aerobic biodegradation. J Chem Technol Biotechnol 87:216–224
Kimbrough DE, Cohen Y, Winer AM, Creelman L, Mabuni C (1999) Critical assessment of chromium in the environment. Crit Rev Environ Sci Technol 29:1–46
Koenig JC, Boparai HK, Lee MJ, O'Carroll DM, Barnes RJ, Manefield MJ (2016) Particles and enzymes: combining nanoscale zero valent iron and organochlorine respiring bacteria for the detoxification of chloroethane mixtures. J Hazard Mater 308:106–112
Le TT, Nguyen KH, Jeon JR, Francis AJ, Chang YS (2015) Nano/bio treatment of polychlorinated biphenyls with evaluation of comparative toxicity. J Hazard Mater 287:335–341
Li YH, Wang SG, Luan ZK, Ding J, Xu CL, Wu DH (2003) Adsorption of cadmium (II) from aqueous solution by surface oxidized carbon nanotubes. Carbon 41:1057–1062
Li Y, Liu F, Xia B, Du Q, Zhang P, Wang D, Wanga Z, Xia Y (2010) Removal of copper from aqueous solution by carbon nanotube/calcium alginate composites. J Hazard Mater 177:876–880
Liang P, Ding Q, Song F (2005) Application of multiwalled carbon nanotubes as solid phase extraction sorbent for preconcentration of trace copper in water samples. J Sep Sci 28:2339–2343
Lin DH, Xing BS (2007) Phytotoxicity of nanoparticles: inhibition of seed germination and root growth. Environ Pollut 150:243–250
Live science (n.d.). https://www.livescience.com/63559-composting.html. Accessed on 21 May 2019
Low J, Cheng B, Yu J (2017) Surface modification and enhanced photocatalytic CO2 reduction performance of TiO2: a review. Appl Surf Sci 392:658–686

Manzer H, Mohamed HS, Whaibi A, Firoz M, Mutahhar Y, Khaishany A (2015) Nanotechnology and plant science. Springer International Publishing, Cham

Mata YN, Torres E, Blázquez ML, Ballester A, González F, Munoz JA (2009) Gold (III) biosorption and bioreduction with the brown alga *Fucus vesiculosus*. J Hazard Mater 166:612–618

Mauter MS, Elimelech M (2008) Environmental applications of carbon-based nanomaterials. Environ Sci Technol 42:5843–5859

Mishra A, Kumari M, Pandey S, Chaudhary V, Gupta KC, Nautiyal CS (2014) Biocatalytic and antimicrobial activities of gold nanoparticles synthesized by *Trichoderma sp*. Bioresour Technol 166:235–242

Mohanpuria P, Rana KN, Yadav SK (2008) Biosynthesis of nanoparticles: technological concepts and future applications. J Nanopart Res 10:507–517

Mubarak Ali D, Sasikala M, Gunasekaran M, Thajuddin N (2013) Biosynthesis and characterization of silver nanoparticles using marine *cyanobacterium*, *Oscillatoria willei* NTDM01. Dig J Nanomater Biostruct 6:385–390

Mueller JG, Cerniglia CE, Pritchard PH (1996) Bioremediation of environments contaminated by polycyclic aromatic hydrocarbons. In: Crawford RL, Crawford DL (eds) Bioremediation: principles and applications. Cambridge University Press, Cambridge, pp 125–194

Nemecek J, Pokorný P, Lhotský O, Knytl V, Najmanova P, Steinova J, Cerník M, Filipova A, Filip J, Cajthaml T (2016) Combined nano-biotechnology for in-situ remediation of mixed contamination of groundwater by hexavalent chromium and chlorinated solvents. Sci Total Environ 563–564:822–834

Newkome GR, Yao ZQ, Baker GR, Gupta VK (1985) Cascade molecules: a new approach to micelles. A [27]-arborol. J Org Chem 50:2003–2004

Norris RD (1994) Handbook of bioremediation. CRC Press, Boca Raton. Slurry based bioreactors. University of Hawai. https://www.hawaii.edu/abrp/Technologies/slurry.html. Accessed 12 April, 2019

Obare SO, Meyer GJ (2004) Nanostructured materials for environmental remediation of organic contaminants in water. J Environ Sci Health A 39:2549–2582

Oksanen T, Pere J, Paavilainen L, Buchert J, Viikari L (2000) Treatment of recycled Kraft pulps with *Trichoderma reesei* hemicellulases and cellulases. J Biotechnol 78:39–44

Pandey S, Kumari M, Singh SP, Bhattacharya A, Mishra S, Chauhan PS, Mishra A (2015) Bioremediation via nanoparticles: an innovative microbial approach. In: Sharma AK, Khanna DR, Saxena DK, Hooda PS, Singh S, Vimala Y (eds) Handbook of research on uncovering new methods for ecosystem management through bioremediation. IGI Global, Hershey, pp 491–515

Pollution ENVIS Centre of Madhya Pradesh's State of Environment (n.d.) Disaster Management Institute (DMI), Bhopal, Ministry of Environment, Forests & Climate Change, Govt of India. http://mpenvis.nic.in/index1.aspx?lid=1470&mid=1&langid=1&linkid=1044. Accessed on 22 May 2019

Ponder SM, Darab JG, Mallouk TE (2000) Remediation of Cr(VI) and Pb(II) aqueous solutions using supported, nanoscale zero-valent iron. Environ Sci Technol 34:2564–2569

Popescu M, Alin V, Lőrinczi A (2010) Biogenic production of nanoparticles. Dig J Nanomater Biostruct 5:1035–1040

Prasad R (2014) Synthesis of silver nanoparticles in photosynthetic plants. J Nanopart 2014:1–8. https://doi.org/10.1155/2014/963961

Prasad R (2016) Advances and applications through fungal nanobiotechnology. Springer International Publishing, Cham, (ISBN: 978-3-319-42989-2)

Prasad R (2017) Fungal nanotechnology: applications in agriculture, industry, and medicine. Springer Nature Singapore Pte Ltd, Singapore, (ISBN 978-3-319-68423-9)

Prasad R, Aranda E (2018) Approaches in Bioremediation: The New Era of Environmental Microbiology and Nanobiotechnology. Springer International Publishing (978-3-030-02369-0) https://www.springer.com/gp/book/9783030023683

Prasad R, Pandey R, Barman I (2016) Engineering tailored nanoparticles with microbes: quo vadis. Nanomed Nanobiotechnol 8:316–330. https://doi.org/10.1002/wnan.1363

Prasad R, Jha A, Prasad K (2018) Exploring the realms of nature for nanosynthesis. Springer International Publishing, (ISBN 978-3-319-99570-0). https://www.springer.com/978-3-319-99570-0

Prasad R, Thirugnanasanbandham K (2019) Advances Research on Nanotechnology for Water Technology. Springer International Publishing https://www.springer.com/us/book/9783030023805

Prathna TC, Mathew L, Chandrasekaran N, Raichur AM, Mukherjee A (2010) Biomimetic synthesis of nanoparticles: science, technology and applicability. In: Mukherjee A (ed) Biomimetics, learning from nature. Intech, Croatia, pp 1–21

Principals of Bioremediation: In situ bioremediation (1993) the national academies of science engineering medicine, 500 Fifth St., NW|Washington, DC 20001. https://www.nap.edu/read/2131/chapter/4. Accessed 15 June 2019

Qiang Y, Sharma A, Paszczynski A, Meyer D (2007) Conjugates of magnetic nanoparticle-enzyme for bioremediation. In: Proceedings of the 2007 NSTI Nanotechnology Conference and Trade Show 4, pp 656–659

Rauwel P, Küünal S, Ferdov S, Rauwel E (2015) A review on the green synthesis of silver nanoparticles and their morphologies studied via TEM. Adv Mater Sci Eng 682749:9

Ren X, Chen C, Nagatsu M, Wang X (2011) Carbon nanotubes as adsorbents in environmental pollution management: a review. Chem Eng J 170:395–410

Riddin T, Gerickeb M, Whiteleya CG (2010) Biological synthesis of platinum nanoparticles: effect of initial metal concentration. Enzym Microb Technol 46:501–505

Roco MC (2005) The emergence and policy implications of converging new technologies integrated from the nanoscale. J Nanopart Res 7:129–143

Ruffini-Castiglione M, Cremonini R (2009) Nanoparticles and higher plants. Caryologia 62:161–165

Saifuddin N, Wong CW, Yasimura AAN (2009) Rapid biosynthesis of silver nanoparticles using culture supernatant of bacteria with microwave irradiation. E-J Chem 6:61–70

Salvadori MR, Lepre LF, Ando RA, Oller doNascimento CA, Correa B (2013) Biosynthesis and uptake of copper nanoparticles by dead biomass of *Hypocrea lixii* isolated from the metal mines in the Brazilian Amazon region. PLoS One 8:e80519

Salvadori MR, Ando RA, Oller doNascimento CA, Corréa B (2014) Intracellular biosynthesis and removal of copper nanoparticles by dead biomass of yeast isolated from the wastewater of a mine in the Brazilian Amazonia. PLoS ONE 9:e-87968

Savage N, Diallo MS (2005) Nanomaterials and water purification: opportunities and challenges. J Nanopart Res 7:331–342

Sayles GD, You G, Wang M, Kupferle MJ (1997) DDT, DDD, and DDE dechlorination by zerovalent iron. Environ Sci Technol 31:3448–3454

Schrick B, Blough JL, Jones AD, Mallouk TE (2002) Hydrodechlorination of trichloroethylene to hydrocarbons using bimetallic nickel-iron nanoparticles. Chem Mater 14:5140–5147

Schrick B, Hydutsky BW, Blough JL, Mallouk TE (2004) Delivery vehicles for zerovalentmetal nanoparticles in soil and groundwater. Chem Mater 16:2187–2193

Senapati S, Syed A, Moeez S, Kumar A, Ahmad A (2012) Intracellular synthesis of gold nanoparticles using alga *Tetraselmis kochinensis*. Mater Lett 79:116–118

Seo SY, Sharma VK, Sharma N (2003) Mushroom tyrosinase: recent prospects. J Agric Food Chem 51:2837–2853

Shan GB, Xing JM, Zhang HY, Liu HZ (2005) Biodesulfurization of dibenzothiophene by microbial cells coated with magnetite nanoparticles. Appl Environ Microbiol 71:4497–4502

Shen W, Zhang C, Li Q, Zhang W, Cao L, Ye J (2015) Preparation of titanium dioxide nanoparticle modified photocatalytic self-cleaning concrete. J Clean Prod 87:762–765

Singaravelu G, Arockiyamari J, Ganesh Kumar V, Govindaraju K (2007) A novel extracellular synthesis of monodisperse gold nanoparticles using marine alga, *Sargassum wightii Greville*. Colloids Surf B Biointerfaces 57:97–101

Son WK, Youk JH, Lee T, Park WH (2004) Preparation of antimicrobial ultrafine cellulose acetate fibres with silver nanoparticles. Macromol Rapid Commun 25:1632–1637

Srivastava A, Srivastava ON, Talapatra S, Vajtai R, Ajayan PM (2004) Carbon nanotube filters. Nat Mater 3:610–614

Srivastava S, Usmani Z, Atanasov AG, Singh VK, Singh NP, Abdel-Azeem AM, Prasad R, Gupta G, Sharma M, Bhargava A (2021) Biological nanofactories: Using living forms for metal nanoparticle synthesis. Mini-Reviews in Medicinal Chemistry 21(2): 245–265

Steffen K, Hatakka A, Hofrichter M (2002) Removal and mineralization of polycyclic aromatic hydrocarbons by litter-decomposing basidiomycetous fungi. Appl Microbiol Biotechnol 60:212

Tang L, Zeng G, Liu J, Xu X, Zhang Y, Shen G et al (2008) Catechol determination in compost bioremediation using a laccase sensor and artificial neural networks. Anal Bioanal Chem 391:679–685

Thakkar KN, Mhatre SS, Parikh RY (2010) Biological synthesis of metallic nanoparticles. Nanomed Nanotechnol Biomed 6:257–262

Thakare M, Sarma H, Datar S, Roy A, Pawar P, Gupta K, Pandit S, Prasad R (2021) Understanding the holistic approach to plant-microbe remediation technologies for removing heavy metals and radionuclides from soil. Current Research in Biotechnology https://doi.org/10.1016/j.crbiot.2021.02.004

Tomalia DA, Baker H, Dewald J, Hall M, Kallos G, Martin S, Roeck J, Ryder J, Smith P (1985) A new class of polymers: starburst-dendritic macromolecules. Polym J 17:117–132

Tungittiplakorn W, Cohen C, Lion LW (2005) Engineered polymeric nanoparticles for bioremediation of hydrophobic contaminants. Environ Sci Technol 39:1354–1358

U.S. EPA. Handbook on in situ treatment of hazardous waste contaminated soils, EPA/540/2-90/002

U.S. EPA Seminars. Bioremediation of hazardous waste sites: practical approach to implementation, EPA/625/K-96/001

Undre SB, Singh M, Kale RK (2013a) Interaction behaviour of trimesoyl chloride derived 1st tier dendrimers determined with structural and physicochemical properties required for drug designing. J Mol Liq 182:106–120

Undre SB, Singh M, Kale RK, Rizwan M (2013b) Silibinin binding and release activitiesmoderated by interstices of trimesoyl, tridimethyl, and tridiethyl malonate first-tier dendrimers. J Appl Polm Sci 130:3537–3554

Vahabi K, Mansoori GL, Karimi S (2011) Biosynthesis of silver nanoparticles by fungus *Trichoderma Reesei* (A route for large-scale production of agapes). Int Sci J 1:65–79

Van der Bruggen B, Vandecasteele C (2003) Removal of pollutants from surface water and groundwater by nanofiltration overview of possible applications in the drinking water industry. Environ Pollut 122:435–445

Vidali M (2001) Bioremediation. An overview. Pure Appl Chem 73:1163–1172

Wang S, Sun H, Ang HM, Tadé MO (2013) Adsorptive remediation of environmental pollutants using novel graphene-based nanomaterials. Rev Chem Eng 226:336–347

Wang W, Liao S, Liu M, Zhao Q, Zhu Y (2014) Polymer compositesre in forced by nanotubes as scaffolds for tissue engineering. Int J Polym Sci 2014:1–14

Xu Y, Zhang WX (2000) Sub colloidal Fe/Ag particles for reductive dehalogenation of chlorinated benzenes. J Ind Eng Chem 39:2238–2244

Chapter 15
Exploring Endophytes Using "Omics": An Approach for Sustainable Production of Bioactive Metabolites

Rahul Chandra Mishra, Rishu Kalra, Neeraj Dwivedi, and Mayurika Goel

Contents

15.1 Introduction

An endophytic relationship is the occurrence of an interdependent conjunction of a plant and a microbe wherein the microorganism resides in the plant tissue asymptomatically. However, it may cause biological changes in the plant by producing diverse chemical entities, such as plant growth hormones, or by harmonizing the gene expression of defense and other secondary metabolic pathways of the host. Novel biologically active secondary metabolites—viz., alkaloids, benzopyranones,

R. C. Mishra · R. Kalra · N. Dwivedi · M. Goel (✉)
TERI-Deakin Nano Biotechnology Centre, The Energy and Resources Institute (TERI), TERI GRAM, Gurgaon, India
e-mail: mayurika.goel@teri.res.in

R. Prasad et al. (eds.), *Mycoremediation and Environmental Sustainability*, Fungal Biology, https://doi.org/10.1007/978-3-030-54422-5_15

chinones, flavonoids, phenolic acids, quinones, steroids, terpenoids, tetralones, xanthones, and similar compounds—are produced by endophytes (Tan and Zou 2001). These secondary metabolites are known for their pharmaceutical properties, along with their varied utility as antimicrobial agents, antioxidants, antiparasitic agents, anticancer agents, immune modulators, and pigments (Wang et al. 2011; Zhao et al. 2011; Deshmukh et al. 2015; Kalra et al. 2020; Vasundhara et al. 2016; Mishra et al. 2020). Fungal endophytes play a pivotal role in secondary metabolite production, which has also been ascertained from the fact that inducing factors produced by both host plants and endophytic fungi increase the aggregation of bioactive compounds when used in consolidation (Bagde et al. 2010; Prasad et al. 2008, 2013; Prasad 2017).

Thus, the mutual relation and corresponding effects of the plant and the endophyte on each other need to be studied by utilizing genetic and metabolic engineering approaches. Advanced endophytic research using an omics-based approach not only serves as a source of novel scaffolds for future production of natural pharmacological products but also could act as a reservoir for sustainable production of these metabolites without harming natural resources (Fig. 15.1) (Komaraiah et al. 2003).

Endophytic fungi are diversified polyphyletic ascomycetes that dwell inside host plant tissue, at most for a part of their life cycle, without inducing any immediate obvious effects on the plants (Hyde and Soytong 2008; Debbab et al. 2013). They are believed to interact mutualistically with their host plants, mainly by increasing host resistance to herbivores (Faeth and Fagan 2002). They play pivotal roles in ecological transformations such as nutrient cycling and decomposition, and have advantageous symbiotic relationships with the roots of many plants (Sun et al. 2011).

Endophytes have both antagonistic and mutualistic relationships with their host plants. However, for coexistence and evolution, both the host and the fungus need to

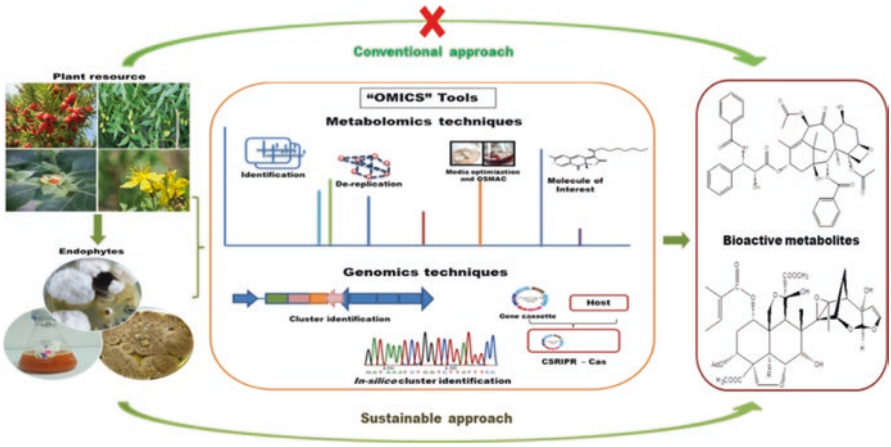


Fig. 15.1 "Omics"-based approaches for sustainable discovery of novel secondary metabolites through harnessing of endophytes

have strongly balanced morphology, physiology, and life cycle characteristics (Saikkonen et al. 1998; Chadha et al. 2014; Mishra et al. 2015). This mutual relationship depends on a fragile equilibrium between the two parties; if one party weakens, the relationship is broken. This relationship can be maintained using three key elements. Firstly, some endophytic fungi produce plant growth regulators, which assist the growth of the host plants (Waqas et al. 2012; Prasad et al. 2020). Secondly, endophytes produce a plethora of bioactive secondary metabolites, which increase the hosts' resistance against various stresses (Firáková et al. 2007; Rodriguez et al. 2009; Gill et al. 2016). Lastly, these endophytes boost the accumulation of secondary metabolites, including pharmacologically important compounds, originally produced by the host plant (Bajaj et al. 2018). This greatly helps in protecting part of the host plant or the entire plant from being used up for pharmaceutical use (Fig. 15.2) (Shwab and Keller 2008).

Endophytic fungi are prolific producers of metabolites and have the capability to produce compounds that are isolated exclusively from higher plants. Recent studies have shown that 51% of secondary metabolites obtained from endophytic fungi remain uncharacterized, in comparison with 38% of those isolated from soil fungi. Therefore, endophytic fungi should be exhaustively explored, as the majority of them still remain cryptic (Strobel 2003).

Natural resources, especially medicinal ones belonging to the vegetable kingdom, have been extensively explored and exploited to furnish raw materials for the pharmaceutical and cosmeceutical industries. This consequently has led to the diminution and extinction of plant resources to a great extent. One of the major drawbacks is that in many cases, the planting and harvesting cycle takes more than 12 months and, even with support from expensive techniques such as plant tissue

Fig. 15.2 The well-established advantageous relationship between endophytic fungi and their host plants

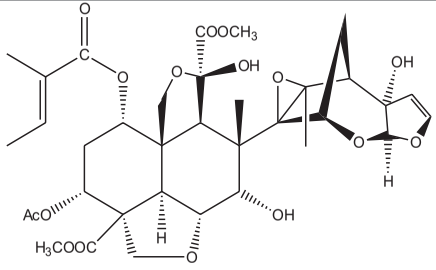
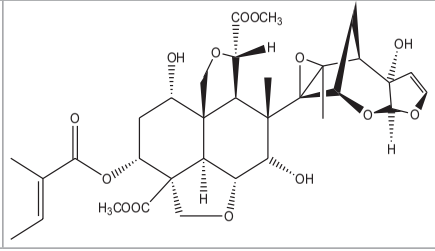
culture, metabolic engineering produces very low-yield results, which are quite inadequate to meet the demand. In contrast, endophytes are untapped sources of novel natural bioactive compounds and their analogues, which could sustainably reduce the burden on these environmental resources. These endophytes can competently produce bioactive metabolites, which are also biosynthesized by their respective host plants. These include the insecticides azadirachtin A and B (Kusari et al. 2012), the anticancer drug baccatin (Zaiyou et al. 2013) and its analogue 10-deacetylbaccatin (Zaiyou et al. 2013; Sreekanth et al. 2009), camptothecin and its structural analogues 10-hydroxycamptothecin and 9-methoxycamptothecin (Shweta et al. 2010), the antidepressant compounds emodin and hypericin (Kusari et al. 2009b), the anticancer agent paclitaxel (Stierle et al. 1993), the anticancer drug precursor podophyllotoxin (Eyberger et al. 2006) and its analogue with additional antiviral properties deoxypodophyllotoxin (Kusari et al. 2009a), the anticancer drugs vincristine and vinblastine (Kumar et al. 2013), and the withanolides—a large group of steroid compounds with utility in numerous pharmaceutical applications such as cardiovascular and anti-Alzheimer drugs (Sathiyabama and Parthasarathy 2018) (Table 15.1).

The quest for new bioactive metabolites for use in pharmaceuticals and nutraceuticals is an ongoing process that requires constant optimization (Dreyfuss and Chapela 1994). With contemporary progress in drug discovery, bioinvestigation of fungal endophytes for identification of pharmaceutically important novel metabolites has been the prime objective globally. Fungal endophytes have unquestionably been recognized as potential candidates for biosynthesis of plant metabolites. Nevertheless, no major breakthrough has been achieved for biotechnological production of these bioactive metabolites through use of endophytes. For better understanding and utilization of endophytes for commercial production of well-known metabolites, there is a great need to study the metabolomes and genetic makeup of endophytes with respect to the associated plant metabolomes. Previously, screening of approximately 10,000 natural metabolites would result in one commercial product. However, with the advent of combinatorial chemistry (i.e., structural chemistry along with natural product screening), we are now able to screen a million bioactive structures a day. Metabolomics-based studies are providing new findings regarding the evolution and chemistry of plant–fungus interactions (Kaul et al. 2016).

Although the biosynthetic pathways responsible for secondary metabolite production have a genetic basis, the gene clusters that account for metabolite production remain cryptic under laboratory conditions. Large-scale production of these metabolites is greatly afflicted by exhaustion in growth cultures. A spectrum of silent pathway–targeted and pleiotropic approaches—such as alteration of culture conditions, co-cultivation with different microorganisms, use of chemical elicitors, and genetic alterations—have been employed to activate reticent gene clusters responsible for bioactive metabolite production.

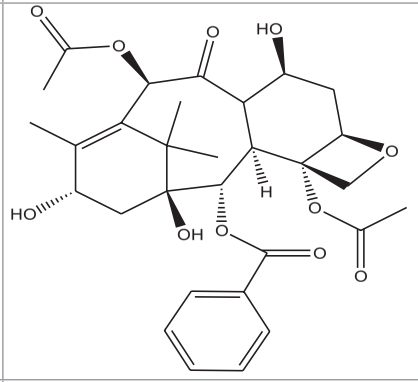
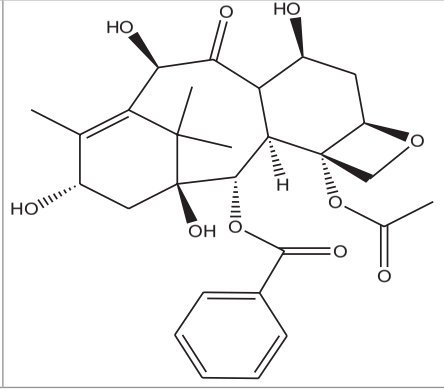
Genomics has evolved as a potent tool for exploring novel natural products, with a plethora of advantages over trivial approaches. For instance, by predicting the structural aspects of silent biosynthetic gene clusters, it promotes dereplication and inhibits rediscovery of previously known compounds, significantly accelerating the

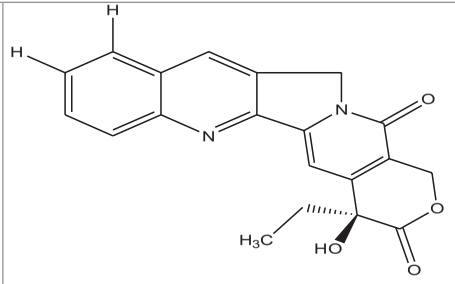
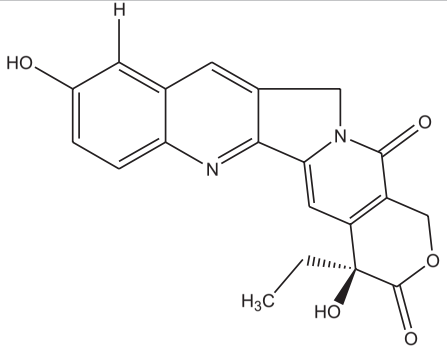
Table 15.1 Some important bioactive compounds produced by endophytic fungi and also biosynthesized by their host plants

Bioactive compound	Structure	Endophytic fungus	Host plant	Bioactivity of the compound	Reference
Azadirachtin A		*Eupenicillium parvum*	*Azadirachta indica* A. Juss.	Insecticidal compound	Kusari et al. (2012)
Azadirachtin B		*Eupenicillium parvum*	*Azadirachta indica* A. Juss.	Insecticidal compound	Kusari et al. (2012)

(continued)

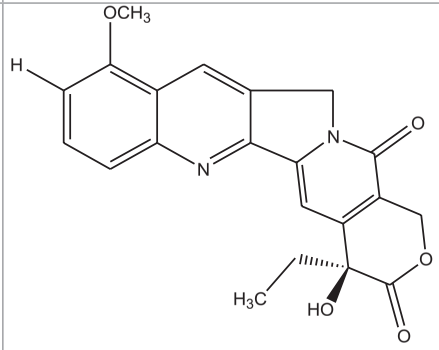
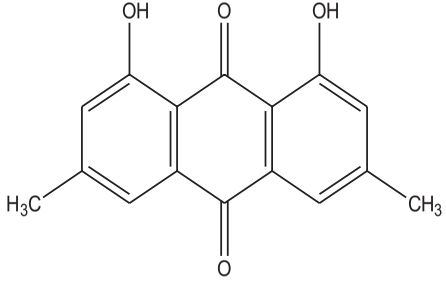
Table 15.1 (continued)

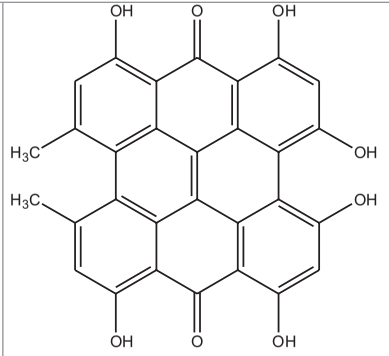
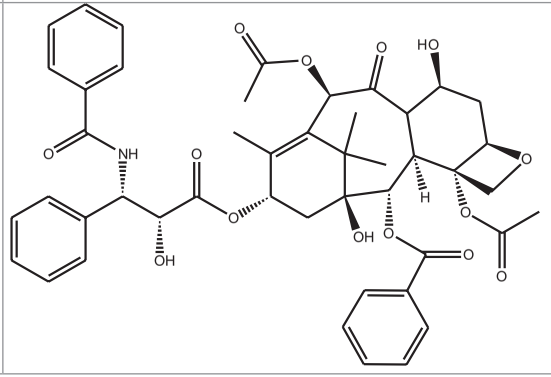
Bioactive compound	Structure	Endophytic fungus	Host plant	Bioactivity of the compound	Reference
Baccatin		*Diaporthe phaseolorum*	*Taxus wallichiana* var. *mairei*	Anticancer compound	Zaiyou et al. (2013)
10-Deacetylbaccatin		*Liocladium* sp.	*Taxus baccata*	Anticancer compound	Sreekanth et al. (2009)

Camptothecin		*Fusarium solani*	*Apodytes dimidiata*	Anticancer compound	Shweta et al. (2010)
10-Hydroxycamptothecin		*Fusarium solani*	*Apodytes dimidiata*	Anticancer compound	Shweta et al. (2010)

(continued)

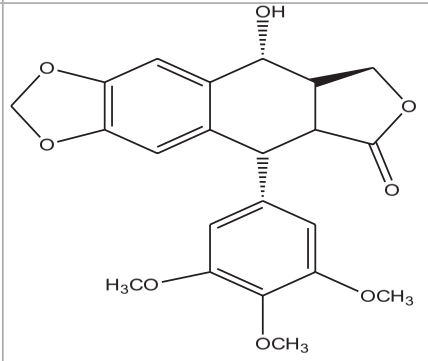
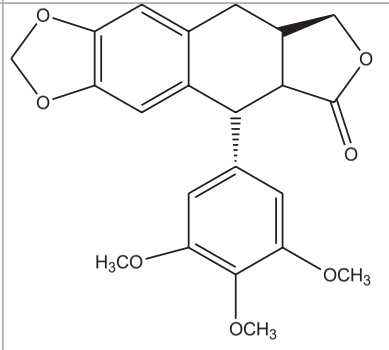
Table 15.1 (continued)

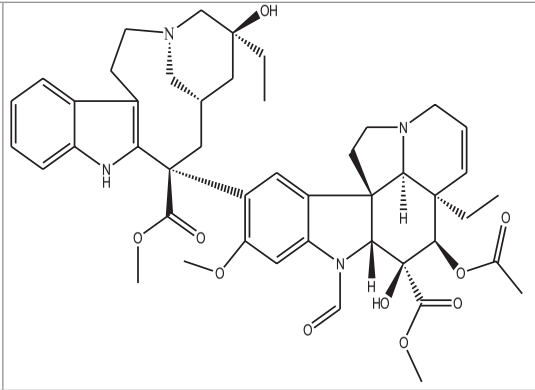
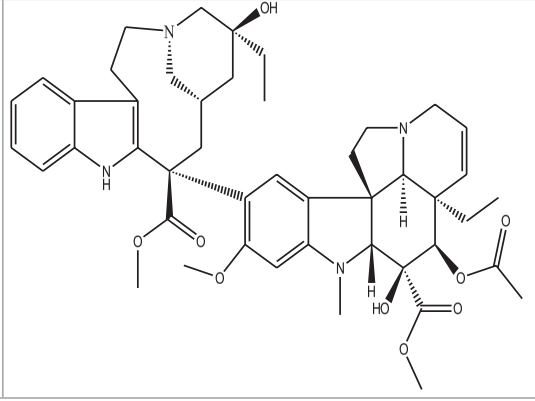
Bioactive compound	Structure	Endophytic fungus	Host plant	Bioactivity of the compound	Reference
9-Methoxycamptothecin		*Fusarium solani*	*Apodytes dimidiata*	Anticancer compound	Shweta et al. (2010)
Emodin		*Thielavia subthermophila*	*Hypericum perforatum*	Antidepressant compound	Kusari et al. (2009a, b)

Hypericin		*Thielavia subthermophila*	*Hypericum perforatum*	Antidepressant compound	Kusari et al. (2009a, b)
Paclitaxel		*Taxomyces andreanae*	*Taxas brevifolia*	Anticancer compound	Stierle et al. (1993)

(continued)

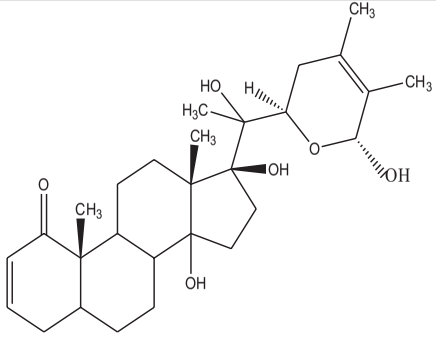
Table 15.1 (continued)

Bioactive compound	Structure	Endophytic fungus	Host plant	Bioactivity of the compound	Reference
Podophyllotoxin		*Phialocephala fortinii*	*Podophyllum peltatum*	Anticancer and antiviral compound	Eyberger et al. (2006)
Deoxypodophyllotoxin		*Aspergillus fumigatus*	*Juniperus communis*	Anticancer and antiviral compound	Kusari et al. (2009a, b)

Vincristine		*Fusarium oxysporum*	*Catharanthus roseus*	Anticancer compound	Kumar et al. (2013)
Vinblastine		*Fusarium oxysporum*	*Catharanthus roseus*	Anticancer compound	Kumar et al. (2013)

(continued)

Table 15.1 (continued)

Bioactive compound	Structure	Endophytic fungus	Host plant	Bioactivity of the compound	Reference
Withanolide		*Taleromyces pinophilus*	*Withania somnifera*	Cardiovascular and anti-Alzheimer compound	Sathiyabama and Parthasarathy (2018)

process of spectroscopic structural elucidation of new compounds. In parallel, combinatorial use of genome sequencing with transcriptomics alongside genetic manipulation promotes rational approaches for maximal and diversified utilization of a strain for bioactive metabolite production.

With the help of multidisciplinary biotechnological approaches—bioinformatics, molecular genetics, genome mining, metabolomics, etc.—we can sustainably explore the hidden treasures of diversified fungal endophytes without diminishing their host flora. Integration of all of these approaches into endophytic research, together with traditional approaches, not only leads to discovery of novel metabolites but also helps to expedite identification of novel sources for recovery of known useful metabolites (Kaul et al. 2016).

This chapter presents a comprehensive picture of use of both metabolomic and genomic approaches to harness the entire biosynthetic potential of endophytic fungi. It highlights the importance of metabolomics in identifying novel metabolites from endophytic fungi. Moreover, metabolic screening of known metabolites from new endophytic sources may increase the chances of obtaining specific metabolites in bulk quantities without harming the environmental sources of those metabolites. Insights into novel methods for activating these cryptic gene clusters, their regulation, and their expression can lead to exploration of novel bioactive metabolites.

15.2 Use of Metabolomics in the Study of Fungal Metabolites

Metabolomics is considered an extension of analysis and identification of the whole repertoire of biomolecules synthesized in an organism, and is an important and expeditiously evolving part of new systems biology. Metabolite profiling of microbial, plant, and natural resource metabolites has been part of fundamental research in biological studies since the 1960s (Want et al. 2005). Recent studies have highlighted the importance of metabolomics as a chief contributor in systems biology–based studies and have provided a holistic overview of the biochemical status of biological systems (Sévin et al. 2015). However, in comparison with other omics-based techniques, metabolomics is considered a comparatively young discipline. Metabolomic techniques, together with other drug discovery methods, can be implemented to obtain a wide array of novel and sustainable sources of pharmacologically active drugs (Rochfort 2005). These processes not only help in the search for novel molecules but also are helpful in expediting the process of drug discovery. The capacity of metabolomics to provide significantly larger numbers of molecules than other standardized techniques has made it a vital tool for the future of drug development, molecular medicine, and drug target discovery.

High-throughput analysis of metabolic profiling and simultaneous comparison of various fungal samples provide critical insight into the fundamental role of these metabolites in cellular processes (Aldridge and Rhee 2014). Utilization of this information under laboratory conditions may assist in triggering the capacity of fungi to enhance the production of specific metabolites. Metabolomics has made a

substantial impact on the drug discovery and development process by providing manifold advantages. The foremost benefits are its precision and accuracy in qualitative and quantitative analyses of biochemical modifications (Bedair and Sumner 2008). In recent decades, remarkable progress has been achieved in improvement of the sensitivity and resolution of various analytical methods used in natural product discovery. Robust hyphenation systems make possible coherent work between chromatographic and spectroscopic instruments and thus enable identification of even trace-level metabolites in complex mixtures of compounds. Metabolomic mapping provides another advantage in that identified metabolites can then be linked with genetic and biochemical pathway information for exploration of genetic information for the purpose of production of specific metabolites (Oppong-Danquah et al. 2018).

On the other hand, untargeted metabolomics provides the fingerprint of the entire metabolome of an organism under study using mass spectrometry (MS) or nuclear magnetic resonance (NMR) data (Kluger et al. 2015). This technique aids understanding of the complete biochemical potential of the fungus under study. Taking into account the vast structural diversity found in fungal metabolites, which belong to various chemical classes of metabolites, it is obvious that no single analytical technique is able to study the entirety of these molecules with equal sensitivity and precision. Thus, untargeted metabolomics–based experiments should be designed in a way to capture the maximum number of metabolites as compared with experimental targeting of specific metabolites. Also, the quantities of raw data created using a metabolomic strategy are huge and cannot be evaluated manually (Gertsman and Barshop 2018; Cambiaghi et al. 2017). This often represents the major bottleneck in metabolomic work flows. However, various recently developed novel computational tools can facilitate processing and statistical analysis of the big data that are generated.

The key focus areas of metabolomics-based studies in endophytic research are (i) to achieve a more comprehensive view of secondary metabolite profiling (Son et al. 2018), (ii) to discover novel sources of host-based metabolites (Zhang et al. 2015), (iii) to link natural products to biosynthetic pathways (Trautman and Crawford 2016), (iv) to disclose minor or hidden metabolites in secondary metabolomes, and (v) to uncover host–fungus interactions and regulatory mechanisms of specific metabolites (Pusztahelyi et al. 2015). The next subsections of this chapter discuss various metabolomic techniques, classified into the groups of targeted and untargeted metabolomic studies, with the objectives of sustainable supply and identification of novel molecules, respectively.

15.2.1 Targeted Metabolomics

Although untargeted/global metabolomics has been strongly emphasized in recent years because of its capacity to discover novel bioactive scaffolds, application of targeted metabolomics aimed at specific classes of metabolites also offers some benefits in addressing the necessity of specific groups of metabolites for certain applications. Targeted analyses are carefully designed to seek information regarding a specific set of metabolites from a complex mixture of metabolites from various novel sources,

with the purpose of obtaining good and sustainable yields of them. Targeted metabolic investigations are usually hypothesis-driven studies with a particular rationale for the selection of the targeted group of metabolites (Roberts et al. 2012). The approaches used in such studies mainly target selective extraction of specific compounds from the biological sample and/or selection of detector systems in a manner that targets the analysis of specific metabolites. For example, MS-based studies such as high-performance liquid chromatography (HPLC) separation with electrospray ionization (ESI) and tandem MS (HPLC-ESI-MS/MS) and gas chromatography (GS) with MS (GC-MS) target identification of compounds on the basis of fragmentation patterns and specific ions formed during ionization of the compounds (Xiao et al. 2012).

Some studies targeting host-specific metabolites from endophytic fungi obtained from particular host plants employ experiments based on targeted metabolomics. In a recent experiment, endophytic fungi isolated from the medicinal herb *Hypericum perforatum* (St. John's wort) were analyzed for production of the metabolite hypericin. With the help of HPLC with ultraviolet absorption (HPLC-UV) and ultra-HPLC with high-resolution MS (UHPLC-HRMS) techniques in full scan and MS/MS mode, researchers were able to identify its suspected precursor, emodin, in three strains and hypericin in one strain. The isolate that yields both emodin and hypericin was identified as *Epicoccum nigrum*, whereas strains yielding only emodin belong to the species *Alternaria alternata* (Kusari et al. 2008).

15.2.2 Untargeted Metabolomics

Because of their high sensitivity, chromatographic techniques coupled with MS and NMR have been recognized as the paramount techniques for untargeted/global metabolomic study of fungal metabolites. These techniques are used for metabolic profiling of biological systems with remarkable precision and sensitivity. High-resolution data sets provided by both techniques have revolutionized research on fungal metabolites (Forseth and Schroeder 2011; Wolfender et al. 2015). Implementation of novel computational algorithms involving appropriate data mining and spectral interpretation for the purpose of decoding such complex data is the need of the hour. Various other analytical strategies such as dereplication, advancements in analytical technologies, and availability of suitable databases are critical aspects for successful recovery of fungal metabolites.

15.2.3 Dereplication

Dereplication is used at the early stage of screening and aims to detect and eliminate bioactive metabolites that have previously been identified from a specific fungus. MS-based and NMR-based techniques are extensively used for identifying known metabolites prior to the bioactivity-guided isolation study process. Rapid identification of already known metabolites at the early stage of a screening campaign is

particularly helpful to detect pan-assay interference compounds (PAINS; sometimes referred to as "frequent hitters") (Baell 2016). This kind of work is ultimately helpful to prioritize isolation procedures targeting novel scaffolds and eliminate redundant isolation work on already well-studied natural products. MS is a rapid, sensitive, and accurate technique, and is the one most commonly used in dereplication-based studies. The combination of liquid chromatography with high-resolution mass spectrometers (such as Fourier transform (FT), time-of-flight (TOF), or Orbitrap devices) presently constitutes the most rugged "high-throughput screening" forum for online identification of metabolites in natural resources (Hubert et al. 2017). In a recent investigation of antifungal metabolites from an endophytic extract, dereplication-based study was conducted on an accurate mass obtained from UPLC-MS, employing the METLIN database. Several bioactive components in the fractions were exhaustively characterized, and systematically demarcated chromatographic and hyphenated spectroscopic techniques led to identification of the well-described antifungal metabolite sulfamethazine (a sulfonamide derivative) (Chowdhary and Kaushik 2019).

On the other hand, NMR-based studies provide much richer structural information on a compound than MS or UV detection. One of the major setbacks associated with mass-based studies is the limitation of identifying regioisomers or stereoisomers of known compounds (Pérez-Victoria et al. 2016). The advent of various cryogenic and capillary probes and high-field magnets counterbalance the previously recognized lower sensitivity of NMR. Thus, identification of minor compounds within mixtures becomes possible.

15.2.4 Computational Data Mining

As mentioned earlier, the major challenge in metabolomics-based study is metabolite annotation. Characteristically, an indeterminately large number of metabolites or mass data can be obtained in an untargeted metabolomic experiment. Simplification of these data and annotation of all of those metabolites is very challenging (Schrimpe-Rutledge et al. 2016). Recent progress in this direction has highlighted the emergence of various computational tools and generation of large numbers of online public tandem mass spectral databases. Apart from the competence of computational tools in simplification of data, the success of any metabolomic study or dereplication approach is based on the availability and quality of the database and/or libraries used for identification of metabolites (Hubert et al. 2017).

Preprocessing of raw data—which includes noise filtering, peak alignment, baseline correction, peak extraction, feature detection, normalization, and deconvolution—is a prerequisite step to reduce interference factors (Gorrochategui et al. 2016). A number of software packages such as MZmine, MetAlign, METIDEA, XCMS, MSFACTS, and AMDIS can be used for the aforementioned preprocessing (Schlotterbeck et al. 2006). Numerous instruments are equipped with their own registered software, which helps to facilitate processing and analysis of samples.

Examples of such software are Progenesis QI, MarkerLynx, and MassLynx, owned by Waters (Milford, MA, USA) (Yao et al. 2020); MassProfiler and MetAlign, associated with Agilent Technologies (Santa Clara, CA, USA) (Lommen 2009; Robbat et al. 2017); and SIEVE and MarkerView, associated with Thermo Fisher Scientific (Waltham, MA, USA) (Völker-Albert et al. 2016).

Preprocessed data are subsequently analyzed using various bioinformatic and multivariate statistical analyses. These include various unsupervised methods and supervised methods. Unsupervised analyses mainly include hierarchical cluster analysis (HCA) and principal component analysis (PCA) (Granato et al. 2018). Supervised analyses include multiple univariate data analysis (MUDA), orthogonal partial least-squares discriminant analysis (OPLS-DA), partial least-squares discriminant analysis (PLS-DA), neural networks (NNs), and linear discriminant analysis (LDA) (Chen et al. 2019). As yet, no established metabolic database comparable to those available for genomics and proteomics is available. There is a great need for more databases specifically for fungal metabolites. Some recently established chemical databases that facilitate the structural elucidation process and serve as a foundation for known and well-researched metabolites are KEGG, mzCloud, PubChem, and DNP (Mohimani et al. 2018).

15.2.5 Media Optimization and Co-culturing

Systematic alteration of cultivation parameters and the cultivation environment—the media composition and type, temperature, pH value, level of hydration, etc.—has been found to be the most effective and modest approach for enhancement of production of specific metabolites from endophytic fungi and is known as "one strain, many compounds" (OSMAC) (Bode et al. 2002). This approach is also useful to elucidate the whole profile of an endophyte, as some gene clusters that normally behave as silent genes in standard laboratory settings become activated under stress conditions and produce specific or novel metabolites. Besides this, the yield of some specific metabolites can be increased greatly by optimization of the medium. For example, one study (Chaichanan et al. 2014) found that the yield of exopolysaccharide (EPS) (Mahapatra and Banerjee 2013) from an endophytic fungus named *Xylaria* sp. Acra L38 was increased using this method. Another study (Li et al. 2012) reported that the EPS yield from *Berkleasmium* sp. Dzf12 was increased 6.29-fold (from 2.22 g/L to 13.97 g/L) by medium optimization in comparison with use of the original basal medium. In another study (Peng et al. 2011), a novel cyclopentanol pyridine alkaloid was identified from the mangrove-derived endophyte *Wallemia sebi* PXP-89 when the normal growth medium was replaced with 10% NaCl broth.

Co-cultivation, or co-culturing, is an another approach in which two or more strains are cultivated together, with the purpose of amplifying production of specific metabolites or detecting novel cryptic metabolites that are usually not produced in an axenic culture (Bertrand et al. 2014; Marmann et al. 2014). These effects can be

achieved through epigenetic modifications of the producer strain by the co-cultivated strain or activation of specific enzymes or silent biosynthetic pathways that may trigger metabolite precursors and yield new metabolites. Thus, the cross-talk between the co-cultivated species not only increases the chemical diversity but also provides a conceptual framework for revealing novel bioactive compounds. In one study, co-cultivation of the endophytic strain *Acremonium* sp. Tbp-5 (isolated from *Taxus baccata* L.) with *Mycogone rosea* DSM 12973 led to identification of new lipoaminopeptides (Degenkolb et al. 2002).

15.3 Use of Genomics to Reveal Silent Secondary Metabolite–Producing Gene Clusters

15.3.1 Genome Mining

DNA libraries are well capable of revealing key information leading to the study of genetic sequences encoding the biosynthetic pathways responsible for formation of unexplored metabolites. Use of DNA libraries can open new pathways to novel bioactive compounds and gene clusters for known metabolites as well (Van Lanen and Shen 2006).

Data from genomic studies on endophytic fungi have revealed that they possess many more genetic clusters for biosynthesis of bioactive metabolites than those that have already been identified. Signature genes are the foundation for genetic clusters that encode diverse bioactive metabolites—viz., nonribosomal peptide synthetases, terpene synthases, and polyketide synthases. These also consist of modifying enzymes that tailor the scaffold of bioactive metabolites—e.g., acyltransferases, oxidoreductases, glycosyltransferases, and methyltransferases (Osbourn 2010). Through employment of a genome-mining approach in *Aspergillus* spp., almost 40 silent gene clusters for bioactive metabolites were revealed per genome. It was also found that *Aspergillus nidulans* is capable of producing 32 different polyketide synthetases (PKSs), 14 nonribosomal peptides, and two indole alkaloids (Brakhage et al. 2008).

Novel derivatives and first-rate natural products can be produced by utilization of these genome manipulation approaches in filamentous fungi. These approaches also assist in comparison and elucidation of fully or partially discovered biosynthetic pathways responsible for a particular metabolite that is common, known in different plant taxa, and produced by endophytes (Van Lanen and Shen 2006; Kusari and Spiteller 2011). Additionally, genome mining conjugates the genes used in biosynthesis and exploration of new secondary metabolites; these further aid in acquisition of the entire data set needed for producing analogues of natural compounds, using structure–activity relationships.

15.3.2 Study of Fungal Genome Sequences

Complete genomic sequences and their data are the major prerequisites for applying genome mining as a tool for novel natural product discovery. Previously, acquisition of these data was tedious and expensive, but, with rapid advancement in DNA sequencing and technology, genome mining as an approach has become part of the arsenal of every natural product laboratory. It has made thousands of cryptic bioactive metabolite gene clusters available for study. These strategies for analyzing the products of these cryptic metabolic gene clusters come with both pros and cons, depending on the accuracy of the bioinformatic studies, the physiochemical nature of the natural product being used, the size of the cluster, and its expression in vivo. All of these factors should be carefully considered prior to commitment to any particular approaches. Presumably, novel strategies for metabolic product discovery will continue to be added to the already available versatile pool; nevertheless, the potentiality of this approach has already been established. With further research and observations, this will definitely become an intrinsic part of natural product laboratories globally. With the increasing number of fungal genomes being sequenced (Grigoriev et al. 2014) and mining strategies being employed for biosynthetic gene clusters, and with their identification becoming widely accessible, the number of characterized biosynthetic pathways and newly discovered products is likely to increase rapidly in the future.

15.3.3 In Silico Predictors

Bioinformatic tools are principally designed to anticipate the assembly of nonribosomal protein synthetases (NRPSs) and/or polyketide synthetases (PKSs) and their corresponding substrates, alongside the physiochemical properties of potential natural products. This approach is solely based on the fundamentals of synthesis of these multimodular enzyme systems. It subsequently facilitates isolation of metabolites from a particular endophytic fungal strain (Scherlach and Hertweck 2006). The ClustScan Database (CSDB) and Recombinant ClustScan Database (rCSDB) are the major databases that focus on NRPS and PKS gene clusters (Schmitt et al. 2004). A frequently discussed database that supervises the tailoring of enzymes in the design of PKS and NRPS biosynthetic gene clusters is the Database of Biosynthesis Clusters Curated and Integrated (DoBISCUIT). Apart from these, SEARCHPKS, MAPSI, and Natural Product Domain Seeker (NaPDos) are software packages that address particular enzyme classes (Ichikawa et al. 2012). Chemical and structural data on these metabolites are accessible via databases such as the Human Metabolome Database (HMDB) (Wishart et al. 2009), METLIN (Smith et al. 2005), and the Madison Metabolomics Consortium Database (MMDB) (Cui et al. 2008).

15.4 Use of Methods to Unravel Cryptic Gene Clusters for Improved and Sustainable Production of Bioactive Secondary Metabolites

Secondary metabolites produced by endophytic fungi hold great promise for varied usage in the pharmaceutical industry; however, the amounts of metabolites that are produced are usually very small. Therefore, various strategies such as the OSMAC approach (using modification of the composition of the culture medium, temperature, or agitation conditions) and co-culturing methods (using interspecies crosstalk for increased and diversified production) are required in order to increase the yield of bioactive metabolites from microorganisms.

Many studies have shown that biosynthetic gene clusters in microorganisms are poorly expressed under normal laboratory conditions (Scherlach and Hertweck 2009). To activate such silent gene clusters, chemicals that act as DNA methyltransferase (DNMT) inhibitors or histone deacetylase (HDAC) inhibitors, which in turn stimulate genes at the transcriptional level, are being extensively used to increase the chemical diversity and enhance the yield of the spectrum of natural compounds produced by these microorganisms. Some details of these methods are highlighted in the following subsections.

15.4.1 Epigenetic Modifications

Recent studies have confirmed that epigenetic modulators such as DNMT inhibitors and HDAC inhibitors lead to upregulation and expression of silent/cryptic genes in endophytic fungi that are normally poorly expressed under laboratory conditions. Histone modification is a method used to activate silent gene clusters by treating fungi with inhibitors of histone acetyltransferase (HAT) or DMAT. HAT removes acetyl groups from the amino tails of histones and maintains chromatin in a state that is inaccessible to the transcriptional machinery (Bulger 2005). Compounds such as 5-azacytidine (a DMAT inhibitor) and suberoylanilide hydroxamic acid (SAHA; an HDAC inhibitor) have been used in several laboratories to activate silent biosynthetic pathways. This technique does not require strain-dependent genetic manipulation and can thus be applied to any fungal strain (Williams et al. 2008).

Genetic studies have confirmed the presence of secondary metabolites' biosynthetic genes as cryptic gene clusters on fungal chromosomes. However, because these genes are silent under normal laboratory conditions or are expressed only marginally, it is a challenging task to understand the biology of these genes. Activation of these genes is controlled by complex regulatory networks involving multiple transcriptional factors, which respond to environmental stimuli such as nutritional, physical, and interactive signals (Brakhage 2013). For induction of secondary metabolite production and enhanced accumulation in fungi, manipulation of global gene regulators, such as deletion or overexpression, has also been used (Bok

and Keller 2004). Molecular biology approaches such as generation of gene knockouts, promoter exchange, overexpression of transcription factors, or use of other pleiotropic regulators are among the successful strategies used for induction of silent biosynthetic pathways (Brakhage and Schroeckh 2011).

15.4.2 CRISPR-Cas9-Based Approaches: The Genome-Editing Era

Since its introduction, use of CRISPR-Cas9 (clustered regularly interspaced short palindromic repeats–CRISPR associated protein 9) has revolutionized the technique of genome editing (Sarma et al. 2021). It has empowered the world with more precision for modifying genomic sequences (Knott and Doudna 2018). Two key elements of the CRISPR-Cas9 type II method for gene targeting and cleavage are the RNA guide (subgenomic messenger RNA (sgRNA)) and the Cas9 endonuclease. sgRNA (chimeric RNA strands) guides Cas9 to the genetic target in the genome whose expression needs to be intercepted. Cas9 binds to the target and produces a double-stranded nick, and this in turn activates the cell repair enzyme system—a nonhomologous end-joining system— which ensures flawless sealing of the nicks, avoiding any extra insertions or deletions of nucleotide(s). Such insertions and deletions alter the reading frame, leading to nonsense sequences and or untimely introduction of stop codons, thereby arresting the transcription of the target genetic sequence (Bono et al. 2015). In fungi, application of this has provided the necessary proof of concept; after its employment in the host, the target gene can be tuned through changes in the sequences of sgRNA (Konermann et al. 2015). Apart from this, one of the fascinating aspects is that it can execute deletions without prerequisite markers by employing transitory expression plasmids, which replicate under antibiotic stress.

Genetic modulation of biosynthetic metabolic pathways can induce otherwise cryptic secondary metabolites, thus providing novel fungal strains with reinforced bioactivity. Studies have reported that changes in environmental conditions induce synthesis of bioactive secondary metabolites by clustered genes. However, in most instances, these genetic clusters remain cryptic (Osbourn 2010). Studies done by Bok et al. (2009) confirmed the expression and yield of novel secondary metabolites by suppressing the transcription factor responsible for methylation of lysine 4 of histone H3 in *Aspergillus nidulans*. Similarly, a significant improvement in bioactivity against *Fusarium oxysporum* and *Rhizoctonia solani* was reported when the *ace1* gene was silenced, resulting in induction and upregulation of four polyketide biosynthetic gene clusters in *Trichoderma atroviride* that regulate production of antibiotics and other bioactive secondary metabolites (Fang and Chen 2018). Subsequently, this approach was used to induce activation of unexplored clusters in the studied fungal strains by using CRISPR-Cas9, aiding the exploration of novel bioactive secondary metabolites that can cross-talk with plants or phytopathogens.

Thus, the CRISPR-Cas system not only efficiently provides an easy and economical pathway to execution of genomic analysis but also facilitates production of unique fungal genotypes and could be a key player in galvanizing plant defenses against plant pathogens (Katayama et al. 2016; Nødvig et al. 2015; Schuster et al. 2016; Zhang et al. 2016; Liu et al. 2017; Wenderoth et al. 2017; Weyda et al. 2017; Wang et al. 2018). Thereby, it could strongly bolster sustainable biocontrol of endophytic fungal strains, which could be effectively utilized to avoid introduction of transgenes into the environment.

15.5 Conclusion

Filamentous fungi are abundant sources of bioactive secondary metabolites with prominent potential to be administered as medicinal drugs for treating various different human diseases.

These secondary metabolites have the potential for use as antimicrobial agents, antioxidants, pigments, and toxins in key applications in the pharmaceutical, nutraceutical, and biomedical industries.

This chapter has provided a broad insight into current genetic and metabolomic approaches for development of novel bioactive secondary metabolites. The metabolomic approach aids in identification and characterization of unique metabolites. The explored fungal strains are usually unable to produce adequate quantities of these metabolites, but gene-editing tools for gene cloning, tailoring, and deletion enable native strains to supply novel metabolites in larger quantities, which could potentially be scaled up to meet the growing global demand for these natural products. Additionally, these robust approaches anticipate the structural aspects of silent biosynthetic gene clusters, greatly facilitating dereplication and avoiding re-exploration of already known compounds or compound classes. This automatically speeds up the process of spectroscopic elucidation of the structures of novel metabolites.

The genome-based advent of bioprospecting and production of new bioactive compounds is promising, but the major hurdle lies in comprehension of the regulatory pathways that drive the expression of these silent/cryptic genes. Acquisition of knowledge on different strategies for the identification of gene clusters and induction of these silent gene clusters is the need of the hour. Nevertheless, although these omics-based approaches hold great promise as potential tools, key challenges include the cost effectiveness of application of combinatorial methods for screening and characterization of novel metabolites. Moreover, these approaches require high-end tools for throughput screening of the whole cellular genome and proteome in less time. With these challenges in mind, tenable exploitation of integrated omics-based approaches can be made more efficient, and sustainable production of hitherto unavailable plant compounds can be achieved by employing endophytes as a resource.

References

Aldridge BB, Rhee KY (2014) Microbial metabolomics: innovation, application, insight. Curr Opin Microbiol 19:90–96

Baell JB (2016) Feeling nature's PAINS: natural products, natural product drugs, and pan assay interference compounds (PAINS). J Nat Prod 79(3):616–628. https://doi.org/10.1021/acs.jnatprod.5b00947

Bagde US, Prasad R, Varma A (2010) Interaction of *Piriformospora indica* with medicinal plants and of economic importance. Afr J Biotechnol 9(54):9214–9226

Bajaj R, Huang Y, Gebrechristos S, Mikolajczyk B, Brown H, Prasad R, Varma A, Bushley K (2018) Transcriptional responses of soybean roots to colonization with the root endophytic fungus *Piriformospora indica* reveals altered phenylpropanoid and secondary metabolism. Sci Reports 8:10227. https://doi.org/10.1038/s41598-018-26809-3

Bedair M, Sumner LW (2008) Current and emerging mass-spectrometry technologies for metabolomics. TrAC Trends Anal Chem 27(3):238–250

Bertrand S, Bohni N, Schnee S, Schumpp O, Gindro K, Wolfender J-L (2014) Metabolite induction via microorganism co-culture: a potential way to enhance chemical diversity for drug discovery. Biotechnol Adv 32(6):1180–1204

Bode HB, Bethe B, Höfs R, Zeeck A (2002) Big effects from small changes: possible ways to explore nature's chemical diversity. Chembiochem 3(7):619–627

Bok JW, Chiang Y-M, Szewczyk E, Reyes-Dominguez Y, Davidson AD, Sanchez JF, Lo H-C, Watanabe K, Strauss J, Oakley BR (2009) Chromatin-level regulation of biosynthetic gene clusters. Nat Chem Biol 5(7):462–464

Bok JW, Keller NP (2004) LaeA, a regulator of secondary metabolism in *Aspergillus* spp. Eukaryotic Cell 3(2):527–535. https://doi.org/10.1128/ec.3.2.527-535.2004

Bono JM, Olesnicky EC, Matzkin LM (2015) Connecting genotypes, phenotypes and fitness: harnessing the power of CRISPR/Cas9 genome editing. Mol Ecol 24(15):3810–3822

Brakhage AA (2013) Regulation of fungal secondary metabolism. Nat Rev Microbiol 11(1):21–32. https://doi.org/10.1038/nrmicro2916

Brakhage AA, Schroeckh V (2011) Fungal secondary metabolites—strategies to activate silent gene clusters. Fungal Genet Biol 48(1):15–22. https://doi.org/10.1016/j.fgb.2010.04.004

Brakhage AA, Schuemann J, Bergmann S, Scherlach K, Schroeckh V, Hertweck C (2008) Activation of fungal silent gene clusters: a new avenue to drug discovery. In: Petersen F, Amstutz R (eds) Natural compounds as drugs, vol II. Springer, Basel, pp 1–12

Bulger M (2005) Hyperacetylated chromatin domains: lessons from heterochromatin. J Biol Chem 280(23):21689–21692. https://doi.org/10.1074/jbc.R500004200

Cambiaghi A, Ferrario M, Masseroli M (2017) Analysis of metabolomic data: tools, current strategies and future challenges for omics data integration. Brief Bioinform 18(3):498–510

Chadha N, Mishra M, Prasad R, Varma A (2014) Root endophytic fungi: research update. J Biol Life Sci USA 5(2):135–158

Chaichanan J, Wiyakrutta S, Pongtharangkul T, Isarangkul D, Meevootisom V (2014) Optimization of zofimarin production by an endophytic fungus, *Xylaria* sp. Acra L38. Braz J Microbiol 45(1):287–293

Chen F, Ma R, Chen X-L (2019) Advances of metabolomics in fungal pathogen–plant interactions. Metabolites 9(8):169

Chowdhary K, Kaushik N (2019) UPLC-MS and dereplication-based identification of metabolites in antifungal extracts of fungal endophytes. Proc Natl Acad Sci India Sect A 89(4):1379–1387

Cui Q, Lewis IA, Hegeman AD, Anderson ME, Li J, Schulte CF, Westler WM, Eghbalnia HR, Sussman MR, Markley JL (2008) Metabolite identification via the Madison Metabolomics Consortium Database. Nature Biotechnol 26(2):162–164

Debbab A, Aly AH, Proksch P (2013) Mangrove derived fungal endophytes—a chemical and biological perception. Fungal Diver 61(1):1–27

Degenkolb T, Heinze S, Schlegel B, Strobel G, Gräfe U (2002) Formation of new lipoaminopeptides, acremostatins A, B, and C, by co-cultivation of *Acremonium* sp. Tbp-5 and *Mycogone rosea* DSM 12973. Biosci Biotechnol Biochem 66(4):883–886. https://doi.org/10.1271/bbb.66.883

Deshmukh SK, Verekar SA, Bhave SV (2015) Endophytic fungi: a reservoir of antibacterials. Front Microbiol 5:715

Dreyfuss M, Chapela IH (1994) Potential of fungi in the discovery of novel, low-molecular weight pharmaceuticals. In: Gullo VP (ed) Discovery of novel natural products with therapeutic potential. Elsevier, St. Louis, pp 49–80. https://doi.org/10.1016/B978-0-7506-9003-4.50009-5

Eyberger AL, Dondapati R, Porter JR (2006) Endophyte fungal isolates from *Podophyllum peltatum* produce podophyllotoxin. J Nat Prod 69(8):1121–1124

Faeth SH, Fagan WF (2002) Fungal endophytes: common host plant symbionts but uncommon mutualists. Integr Comp Biol 42(2):360–368

Fang C, Chen X (2018) Potential biocontrol efficacy of *Trichoderma atroviride* with cellulase expression regulator ace1 gene knock-out. 3 Biotech 8(7):302. https://doi.org/10.1007/s13205-018-1314-z

Fráková S, Šturdíková M, Múčková M (2007) Bioactive secondary metabolites produced by microorganisms associated with plants. Biologia 62(3):251–257

Forseth RR, Schroeder FC (2011) NMR-spectroscopic analysis of mixtures: from structure to function. Curr Opin Chem Biol 15(1):38–47

Gertsman I, Barshop BA (2018) Promises and pitfalls of untargeted metabolomics. J Inherit Metab Dis 41(3):355–366

Gill SS, Gill R, Trivedi DK, Anjum NA, Sharma KK, Ansari MW, Ansari AA, Johri AK, Prasad R, Pereira E, Varma A, Tuteja N (2016) *Piriformospora indica*: potential and significance in plant stress tolerance. Front Microbiol 7:332. https://doi.org/10.3389/fmicb.2016.00332

Gorrochategui E, Jaumot J, Lacorte S, Tauler R (2016) Data analysis strategies for targeted and untargeted LC-MS metabolomic studies: overview and workflow. Trends Anal Chem 82:425–442

Granato D, Santos JS, Escher GB, Ferreira BL, Maggio RM (2018) Use of principal component analysis (PCA) and hierarchical cluster analysis (HCA) for multivariate association between bioactive compounds and functional properties in foods: a critical perspective. Trends Food Sci Technol 72:83–90

Grigoriev IV, Nikitin R, Haridas S, Kuo A, Ohm R, Otillar R, Riley R, Salamov A, Zhao X, Korzeniewski F (2014) MycoCosm portal: gearing up for 1000 fungal genomes. Nucleic Acids Res 42(D1):D699–D704

Hubert J, Nuzillard J-M, Renault J-H (2017) Dereplication strategies in natural product research: how many tools and methodologies behind the same concept? Phytochem Rev 16(1):55–95. https://doi.org/10.1007/s11101-015-9448-7

Hyde K, Soytong K (2008) The fungal endophyte dilemma. Fungal Divers 33(163):e173

Ichikawa N, Sasagawa M, Yamamoto M, Komaki H, Yoshida Y, Yamazaki S, Fujita N (2012) DoBISCUIT: a database of secondary metabolite biosynthetic gene clusters. Nucleic Acids Res 41(D1):D408–D414

Kalra R, Conlan XA, Goel M (2020) Fungi as a potential source of pigments: harnessing filamentous fungi. Front Chem 8:369. https://doi.org/10.3389/fchem.2020.00369

Katayama T, Tanaka Y, Okabe T, Nakamura H, Fujii W, Kitamoto K, Maruyama J-I (2016) Development of a genome editing technique using the CRISPR/Cas9 system in the industrial filamentous fungus *Aspergillus oryzae*. Biotechnol Lett 38(4):637–642

Kaul S, Sharma T, Dhar K, Fips MJ (2016) "Omics" tools for better understanding the plant–endophyte interactions. Front Plant Sci 7:955. https://doi.org/10.3389/fpls.2016.00955

Kluger B, Lehner S, Schuhmacher R (2015) Metabolomics and secondary metabolite profiling of filamentous fungi. In: Zeilinger S, Martín J-F, García-Estrada C (eds) Biosynthesis and molecular genetics of fungal secondary metabolites, vol 2. Springer, New York, pp 81–101

Knott GJ, Doudna JA (2018) CRISPR-Cas guides the future of genetic engineering. Science 361(6405):866–869

Komaraiah P, Ramakrishna S, Reddanna P, Kishor PK (2003) Enhanced production of plumbagin in immobilized cells of *Plumbago rosea* by elicitation and in situ adsorption. J Biotechnol 101(2):181–187

Konermann S, Brigham MD, Trevino AE, Joung J, Abudayyeh OO, Barcena C, Hsu PD, Habib N, Gootenberg JS, Nishimasu H (2015) Genome-scale transcriptional activation by an engineered CRISPR-Cas9 complex. Nature 517(7536):583. https://doi.org/10.1038/nature14136

Kumar A, Patil D, Rajamohanan PR, Ahmad A (2013) Isolation, purification and characterization of vinblastine and vincristine from endophytic fungus *Fusarium oxysporum* isolated from *Catharanthus roseus*. PloS One 8(9):e71805

Kusari S, Spiteller M (2011) Are we ready for industrial production of bioactive plant secondary metabolites utilizing endophytes? Nat Prod Rep 28(7):1203–1207

Kusari S, Lamshöft M, Zühlke S, Spiteller M (2008) An endophytic fungus from *Hypericum perforatum* that produces hypericin. J Nat Prod 71(2):159–162

Kusari S, Lamshöft M, Spiteller M (2009a) *Aspergillus fumigatus* Fresenius, an endophytic fungus from *Juniperus communis* L. Horstmann as a novel source of the anticancer pro-drug deoxypodophyllotoxin. J Appl Microbiol 107(3):1019–1030. https://doi.org/10.1111/j.1365-2672.2009.04285.x

Kusari S, Zühlke S, Kosuth J, Cellarova E, Spiteller M (2009b) Light-independent metabolomics of endophytic *Thielavia subthermophila* provides insight into microbial hypericin biosynthesis. J Nat Prod 72(10):1825–1835

Kusari S, Verma VC, Lamshoeft M, Spiteller M (2012) An endophytic fungus from *Azadirachta indica* A. Juss. that produces azadirachtin. World J Microbiol Biotechnol 28(3):1287–1294

Li P, Xu L, Mou Y, Shan T, Mao Z, Lu S, Peng Y, Zhou L (2012) Medium optimization for exopolysaccharide production in liquid culture of endophytic fungus *Berkleasmium* sp. Dzf12. Mol Divers Preserv Int 13(9):11411–11426. https://doi.org/10.3390/ijms130911411

Liu Q, Gao R, Li J, Lin L, Zhao J, Sun W, Tian C (2017) Development of a genome-editing CRISPR/Cas9 system in thermophilic fungal *Myceliophthora* species and its application to hyper-cellulase production strain engineering. Biotechnol Biofuels 10(1):1. https://doi.org/10.1186/s13068-016-0693-9

Lommen A (2009) MetAlign: interface-driven, versatile metabolomics tool for hyphenated full-scan mass spectrometry data preprocessing. Anal Chem 81(8):3079–3086

Mahapatra S, Banerjee D (2013) Fungal exopolysaccharide: production, composition and applications. Microbiol Insights 6:1–16:S10957. https://doi.org/10.4137/MBI.S10957

Marmann A, Aly AH, Lin W, Wang B, Proksch P (2014) Co-cultivation—a powerful emerging tool for enhancing the chemical diversity of microorganisms. Mar Drugs 12(2):1043–1065

Mishra M, Prasad R, Varma A (2015) Endophytic fungi: biodiversity and functions. Int J Pharma BioSci 6(1):18–46

Mishra RC, Goel M, Barow JC, Deshmukh SK (2020) Endophytic fungi—an untapped source of potential antioxidants. Curr Bioact Compd 15(1):1. https://doi.org/10.2174/1573407215666191007113837

Mohimani H, Gurevich A, Shlemov A, Mikheenko A, Korobeynikov A, Cao L, Shcherbin E, Nothias L-F, Dorrestein PC, Pevzner PA (2018) Dereplication of microbial metabolites through database search of mass spectra. Nat Commun 9(1):4035. https://doi.org/10.1038/s41467-018-06082-8

Nødvig CS, Nielsen JB, Kogle ME, Mortensen UH (2015) A CRISPR-Cas9 system for genetic engineering of filamentous fungi. PloS One 10(7):e0133085

Oppong-Danquah E, Parrot D, Blümel M, Labes A, Tasdemir D (2018) Molecular networking–based metabolome and bioactivity analyses of marine-adapted fungi co-cultivated with phytopathogens. Front Microbiol 9:2072

Osbourn A (2010) Gene clusters for secondary metabolic pathways: an emerging theme in plant biology. Plant Physiology 154(2):531–535

Peng X-P, Wang Y, Liu P-P, Hong K, Chen H, Yin X, Zhu W-M (2011) Aromatic compounds from the halotolerant fungal strain of *Wallemia sebi* PXP-89 in a hypersaline medium. Arch Pharm Res 34(6):907–912

Pérez-Victoria I, Martín J, Reyes F (2016) Combined LC/UV/MS and NMR strategies for the dereplication of marine natural products. Planta Med 82(09/10):857–871

Prasad R (2017) Fungal nanotechnology: applications in agriculture, industry, and medicine. Springer International Publishing, Singapore, (ISBN 978-3-319-68423-9) https://www.springer.com/gb/book/9783319684239

Prasad R, Sharma M, Kamal S, Rai MK, Rawat AKS, Pushpangdan P, Varma A (2008) Interaction of *Piriformospora indica* with medicinal plants. In: Varma A (ed) Mycorrhiza. Springer-Verlag, Berlin Heidelberg, pp 655–678

Prasad R, Kamal S, Sharma PK, Oelmueller R, Varma A (2013) Root endophyte *Piriformospora indica* DSM 11827 alters plant morphology, enhances biomass and antioxidant activity of medicinal plant *Bacopa monniera*. J Basic Microbiol 53(12):1016–1024

Prasad R, Chhabra S, Gill SS, Singh PK, Tuteja N (2020) The microbial symbionts: potential for the crop improvement in changing environments. In: Tuteja N, Tuteja R, Passricha N, Saifi SK (eds) Advancement in crop improvement techniques. Elsevier, San Diego, pp 233–239

Pusztahelyi T, Holb IJ, Pócsi I (2015) Secondary metabolites in fungus–plant interactions. Front Plant Sci 6:573

Robbat A Jr, Kfoury N, Baydakov E, Gankin Y (2017) Optimizing targeted/untargeted metabolomics by automating gas chromatography/mass spectrometry workflows. J Chromatogr A 1505:96–105

Roberts LD, Souza AL, Gerszten RE, Clish CB (2012) Targeted metabolomics. Curr Protoc Mol Biol 98(1):30.2.1–30.2.24. https://doi.org/10.1002/0471142727.mb3002s98

Rochfort S (2005) Metabolomics reviewed: a new "omics" platform technology for systems biology and implications for natural products research. J Nat Prod 68(12):1813–1820

Rodriguez R, White J Jr, Arnold AE, Redman RS (2009) Fungal endophytes: diversity and functional roles. New Phytol 182(2):314–330

Saikkonen K, Faeth SH, Helander M, Sullivan T (1998) Fungal endophytes: a continuum of interactions with host plants. Ann Rev Ecol Syst 29(1):319–343

Sarma H, Forid N, Prasad R, Prasad MNV, Ma LQ, Rinklebe J (2021) Enhancing phytoremediation of hazardous metal(loid)s using genome engineering CRISPR–Cas9 technology. Journal of Hazardous Materials https://doi.org/10.1016/j.jhazmat.2021.125493

Sathiyabama M, Parthasarathy R (2018) Withanolide production by fungal endophyte isolated from *Withania somnifera*. Nat Prod Res 32(13):1573–1577

Scherlach K, Hertweck C (2006) Discovery of aspoquinolones A–D, prenylated quinoline-2-one alkaloids from *Aspergillus nidulans*, motivated by genome mining. Org Biomol Chem 4(18):3517–3520

Scherlach K, Hertweck C (2009) Triggering cryptic natural product biosynthesis in microorganisms. Org Biomol Chem 7(9):1753–1760. https://doi.org/10.1039/b821578b

Schlotterbeck G, Ross A, Dieterle F, Senn H (2006) Metabolic profiling technologies for biomarker discovery in biomedicine and drug development. Pharmacogenomics 7(7):1055–1075. https://doi.org/10.2217/14622416.7.7.1055

Schmitt EK, Hoff B, Kück U (2004) AcFKH1, a novel member of the forkhead family, associates with the RFX transcription factor CPCR1 in the cephalosporin C–producing fungus *Acremonium chrysogenum*. Gene 342(2):269–281

Schrimpe-Rutledge AC, Codreanu SG, Sherrod SD, McLean JA (2016) Untargeted metabolomics strategies—challenges and emerging directions. J Am Soc Mass Spectrom 27(12):1897–1905

Schuster M, Schweizer G, Reissmann S, Kahmann R (2016) Genome editing in *Ustilago maydis* using the CRISPR-Cas system. Fungal Genet Biol 89:3–9

Sévin DC, Kuehne A, Zamboni N, Sauer U (2015) Biological insights through nontargeted metabolomics. Curr Opin Biotechnol 34:1–8. https://doi.org/10.1016/j.copbio.2014.10.001

Shwab EK, Keller NP (2008) Regulation of secondary metabolite production in filamentous ascomycetes. Mycol Res 112(2):225–230

Shweta S, Zuehlke S, Ramesha B, Priti V, Kumar PM, Ravikanth G, Spiteller M, Vasudeva R, Shaanker RU (2010) Endophytic fungal strains of *Fusarium solani*, from *Apodytes dimidiata* E. Mey. ex Arn (Icacinaceae) produce camptothecin, 10-hydroxycamptothecin and 9-methoxycamptothecin. Phytochemistry 71(1):117–122

Smith CA, O'Maille G, Want EJ, Qin C, Trauger SA, Brandon TR, Custodio DE, Abagyan R, Siuzdak G (2005) METLIN: a metabolite mass spectral database. Ther Drug Monit 27(6):747–751

Son SY, Lee S, Singh D, Lee N-R, Lee D-Y, Lee CH (2018) Comprehensive secondary metabolite profiling toward delineating the solid and submerged-state fermentation of *Aspergillus oryzae* KCCM 12698. Front Microbiol 9:1076

Sreekanth D, Syed A, Sarkar S, Sarkar D, Santhakumari B, Ahmad A, Khan M (2009) Production, purification and characterization of taxol and 10DAB III from a new endophytic fungus *Gliocladium* sp. isolated from the Indian yew tree, *Taxus baccata*. J Microbiol Biotechnol 19(11):1342–1347

Stierle A, Strobel G, Stierle D (1993) Taxol and taxane production by *Taxomyces andreanae*, an endophytic fungus of Pacific yew. Science 260(5105):214–216

Strobel GA (2003) Endophytes as sources of bioactive products. Microbes Infect 5(6):535–544. https://doi.org/10.1016/S1286-4579(03)00073-X

Sun X, Guo L-D, Hyde K (2011) Community composition of endophytic fungi in *Acer truncatum* and their role in decomposition. Fungal Divers 47(1):85–95

Tan RX, Zou WX (2001) Endophytes: a rich source of functional metabolites. Natural Prod Rep 18(4):448–459

Trautman EP, Crawford JM (2016) Linking biosynthetic gene clusters to their metabolites via pathway-targeted molecular networking. Curr Top Med Chem 16(15):1705–1716

Van Lanen SG, Shen B (2006) Microbial genomics for the improvement of natural product discovery. Current Opin Microbiol 9(3):252–260

Vasundhara M, Baranwal M, Kumar A (2016) *Fusarium tricinctum*, an endophytic fungus exhibits cell growth inhibition and antioxidant activity. Indian J Microbiol 56(4):433–438

Völker-Albert MC, Pusch MC, Schmidt A, Imhof A (2016) Data on the kinetics of in vitro assembled chromatin. Data Brief 8:353–359

Wang L-W, Zhang Y-L, Lin F-C, Hu Y-Z, Zhang C-L (2011) Natural products with antitumor activity from endophytic fungi. Mini Rev Med Chem 11(12):1056–1074

Wang Q, Cobine PA, Coleman JJ (2018) Efficient genome editing in *Fusarium oxysporum* based on CRISPR/Cas9 ribonucleoprotein complexes. Fungal Genet Biol 117:21–29

Want EJ, Cravatt BF, Siuzdak GJC (2005) The expanding role of mass spectrometry in metabolite profiling and characterization. Chembiochem 6(11):1941–1951

Waqas M, Khan AL, Kamran M, Hamayun M, Kang S-M, Kim Y-H, Lee I-J (2012) Endophytic fungi produce gibberellins and indoleacetic acid and promotes host-plant growth during stress. Molecules 17(9):10754–10773

Wenderoth M, Pinecker C, Voß B, Fischer R (2017) Establishment of CRISPR/Cas9 in *Alternaria alternata*. Fungal Genet Biol 101:55–60

Weyda I, Yang L, Vang J, Ahring BK, Lübeck M, Lübeck PS (2017) A comparison of *Agrobacterium*-mediated transformation and protoplast-mediated transformation with CRISPR-Cas9 and bipartite gene targeting substrates, as effective gene targeting tools for *Aspergillus carbonarius*. J Microbiol Methods 135:26–34

Williams RB, Henrikson JC, Hoover AR, Lee AE, Cichewicz RH (2008) Epigenetic remodeling of the fungal secondary metabolome. Org Biomol Chem 6(11):1895–1897. https://doi.org/10.1039/b804701d

Wishart DS, Knox C, Guo AC, Eisner R, Young N, Gautam B, Hau DD, Psychogios N, Dong E, Bouatra S (2009) HMDB: a knowledgebase for the human metabolome. Nucleic Acids Res 37(Suppl 1):D603–D610

Wolfender J-L, Marti G, Thomas A, Bertrand S (2015) Current approaches and challenges for the metabolite profiling of complex natural extracts. J Chromatogr 1382:136–164

Xiao JF, Zhou B, Ressom HW (2012) Metabolite identification and quantitation in LC-MS/MS-based metabolomics. Trends Analyt Chem 32:1–14. https://doi.org/10.1016/j.trac.2011.08.009

Yao H, Yu P-C, Jiang C-M (2020) Metabolomics-driven identification of perturbations in amino acid and sphingolipid metabolism as therapeutic targets in a rat model of anorexia nervosa disease using chemometric analysis and a multivariate analysis platform. RSC Adv 10(9):4928–4941

Zaiyou J, Li M, Guifang X, Xiuren Z (2013) Isolation of an endophytic fungus producing baccatin III from *Taxus wallichiana* var. *mairei*. J Ind Microbiol Biotechnol 40(11):1297–1302

Zhang A, Sun H, Yan G, Wang P, Wang X (2015) Metabolomics for biomarker discovery: moving to the clinic. Biomed Res Int 2015:354671

Zhang C, Meng X, Wei X, Lu L (2016) Highly efficient CRISPR mutagenesis by microhomology-mediated end joining in *Aspergillus fumigatus*. Fungal Genet Biol 86:47–57

Zhao J, Shan T, Mou Y, Zhou L (2011) Plant-derived bioactive compounds produced by endophytic fungi. Mini Rev Med Chem 11(2):159–168

Index

R. Prasad et al. (eds.), *Mycoremediation and Environmental Sustainability*, Fungal Biology, https://doi.org/10.1007/978-3-030-54422-5