Vivek Kumar · Manoj Kumar
Ram Prasad *Editors*

Phytobiont and Ecosystem Restitution

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Editors
Vivek Kumar
Himalayan School of Biosciences
Swami Rama Himalayan University
Dehradun, Uttarakhand, India

Ram Prasad
Amity Institute of Microbial Technology
Amity University
Noida, Uttar Pradesh, India

School of Environmental Sciences
and Engineering
Sun Yat-Sen University
Guangzhou, China

Manoj Kumar
Department of Life Science, School of Natural Sciences
Central University of Jharkhand
Ranchi, Jharkhand, India

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About the Editors

Vivek Kumar is an associate professor working at the Himalayan School of Biosciences, Swami Rama Himalayan University, Jolly Grant, Dehradun, India. He currently serves on the editorial boards of various respected international journals, including *EnvironmentAsia*, *International Journal of Biological and Chemical Sciences*, *Environmental Sustainability*, *Journal of Advanced Botany and Zoology*, and *Journal of Ecobiotechnology*. He is also a reviewer for prestigious international journals such as the *Journal of Hazardous Materials*, *Environmental Sustainability, Science International*, *Acta Physiologiae Plantarum*, *Environment Science and Pollution Research*, and *Rhizosphere*. He has authored more than 100 publications, including research papers, review articles, and book chapters, and also edited several Springer books. Dr. Kumar has served as a microbiologist in the Department of Soil and Water Research, Public Authority of Agricultural Affairs and Fish Resources, Kuwait, for 8 years. He has been credited with first reporting and identification of Pink Rot inflorescence disease of date palms in Kuwait caused by *Serratia marcescens*. He was awarded the 2002 “Young Scientist Award” in agricultural microbiology by the Association of Microbiologists of India (AMI). Dr. Kumar’s research areas include plant-microbe interactions, environmental microbiology, and bioremediation. He has also organized various outreach activities.

Manoj Kumar is an associate professor at Centre for Life Sciences, Central University of Jharkhand, Ranchi, India. He is committed to high-quality science that contributes to both increasing intellectual knowledge of plant development and increasing the ecological niche. He has pursued his PhD in plant biotechnology at the prestigious Jawaharlal Nehru University and was then awarded two postdoctoral fellowships: DBT-PDF from IISc Bangalore in 2005 and then NRF-PDF from the Universities of Pretoria and Melbourne. He is leading a diverse research group in Life Sciences supported by DBT-BUILDER program, Ministry of Sciences and Technology, Government of India. He referees for several journals, including *Phytoremediation* and the *Journal of Soil Sediments*. Dr. Kumar’s research focuses on the integration of microbial genetics with a range of plant physiological approaches to enable novel gene discovery and conferring of metabolites.

Ram Prasad is associated with Amity Institute of Microbial Technology, Amity University, Uttar Pradesh, India. His research interests include plant-microbe interactions, sustainable agriculture, and microbial nanobiotechnology. Dr. Prasad has written more than hundred publications, including research papers, review articles, and book chapters. He has five patents issued or pending and has edited or authored several books. Dr. Prasad has 12 years of teaching experience and was awarded the Young Scientist Award (2007) and Prof. J.S. Datta Munshi Gold Medal (2009) by the International Society for Ecological Communications; FSAB fellowship (2010) by the Society for Applied Biotechnology; the American Cancer Society UICC International Fellowship for Beginning Investigators, USA (2014); Outstanding Scientist Award (2015) in the field of microbiology by Venus International Foundation; BRICPL Science Investigator Award (ICAABT-2017); and Research Excellence Award (2018). Dr. Prasad served as visiting assistant professor, Whiting School of Engineering, Department of Mechanical Engineering at Johns Hopkins University, USA, and is currently a research associate professor at the School of Environmental Sciences and Engineering, Sun Yat-Sen University, Guangzhou, China.

Nanobiotechnology Approaches for Crop Protection

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Gerardo Rodríguez-Cutiño, Juan J. Gaytán-Andrade,
Ariel García-Cruz, Rodolfo Ramos-González,
Mónica L. Chávez-González, Elda P. Segura-Ceniceros,
José L. Martínez-Hernández, Mayela Govea-Salas, and Anna Ilyina

Abstract

Modern agriculture uses nanobiotechnology development as one of the most valuable tools. Using biomolecules in nanotechnology provides new agrochemical nanostructured formulations with different action mechanisms to increase crop productivity and improve their protection decreasing chemical pesticide use. Sustainability and safety of agriculturally cultivated crops are achieved by application of nanoformulations used for control of plant disease related with microorganisms, insects and environmental factors. Nanostructures are also applied for controlled release of nutrients and growth regulators. Safety of nanomaterials use and their environmental impacts are the important factors to acceptance of these new technologies by consumer and agricultural companies.

1.1 Introduction

Nanobiotechnology applied for crop production and protection is a relatively recent subject in comparison with pharmaceutical nanotechnology (Corradetti and Ferrari 2016). As nanobiotechnology tools are considered nanosystems that contain biomolecules (nanoparticles obtained from biochemical compounds, or coated with biopolymers, nanoformulations from plant extracts and metal or metal oxide nanoparticles) (Elemike et al. 2017; Kumar et al. 2015; MubarakAli et al. 2011; Narendhran and Sivaraj 2016). Nanobiotechnology alludes to the comprehension

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G. Rodríguez-Cutiño · J. J. Gaytán-Andrade · A. García-Cruz · M. L. Chávez-González
E. P. Segura-Ceniceros · J. L. Martínez-Hernández · M. Govea-Salas · A. Ilyina (✉)
Nanobioscience Group, Chemistry School of the Autonomous University of Coahuila,
Coahuila, Mexico

R. Ramos-González
CONACYT – Autonomous University of Coahuila, Coahuila, Mexico

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and regulation of this content at the nanoscale. These tools have the potential to protect plants against microbial diseases and insects, stimulate plant growth, enhance food quality, reduce wastes, and control environmental contamination. Nanosystems are characterized by size dimensions up to 100 nm. It is well known that the properties of nanomaterials differ on scale. Also a material having particles or constituents of nanoscale dimensions is known as a nanomaterial. Moreover, as nanosystems are considered manufactured or natural materials which contain unbound or agglomerated particles with size distribution on at least one dimension between 1 and 100 nm for 50% or more particles (Corradetti and Ferrari 2016). Nanoscale materials that have one dimension are surface coatings or thin films (extended in two dimensions). Nanoscale that are in two dimensions are recognized as nanowire and nanotubes. At the same time, materials with nanosized in three dimensions are fullerenes, colloids, particles, and dendrimers, among others. The improved properties of nanomaterials originate principally from their amplified specific surface area and quantum effects. Different biological molecules have nanodimensions: proteins, cell membranes, sugars, and polysaccharides.

Nanobiosystems applied in agriculture may be useful for improving use water by plants, as well as effect of pesticides, fertilizers, and growth regulators. Nanobiotechnology use may bring potential benefits for crop production, protection, and environmental preservation. Nanobiotechnology approaches include nanomaterials for controlled-release and effective application of water, nutrients, and fertilizers, nanocapsules for products distribution, and antimicrobial nanoemulsions for control of pathogen contamination (Prasad et al. 2014, 2017; Bhattacharyya et al. 2016). Nanobiotechnology attempts are focused on to decrease the unfavorable effect of agrochemical products in crops and surroundings and human well-being. In addition, nanobiotechnology aims to solve problems related to the use of water, soil, water, impacts on the environment and the health of field workers. The present overview is intended to demonstrate potential uses and benefits of nanobiotechnology.

1.2 Nanoparticles Against Pathogens

In the last years, many types of research have been carried out in the field of nanotechnology, since their properties are linked to their size and morphology. Nanoparticles are characterized by being highly reactive and possessing a large surface area. The nanotechnology has undergone fast growth in divergent fields, incorporating microbiology and infectious diseases. Especially, nanostructures carry enormous prospect as effective drug distribution method to tend microbial infections as they display more advantages as compared to standard antibiotic formulations (Aziz et al. 2015, 2016). Nanotechnology points to new solutions of different problems which are faced by humanity, and it promises benefits of all kinds, to mention a few: innovations in medical applications, solutions to environmental problems such as pollution and water treatment, food processing, transgenic foods obtaining, molecular thermal changes, and computer science.

Foodborne illness is the product of consumption of food contaminated with a broad range of pathogenic microorganisms, their toxins, or chemicals. Therefore, there is a need to find methods that contribute to the control of these pathogens in food during processing, packaging, and distribution. One of the most striking applications of nanotechnology has focused on the production of modified packages with silver nanoparticles (AgNPs) (Jo et al. 2009). Thus, the nanoparticles are a classification of materials mixed using different metals or using the nonmetal elements with clear features and large uses in various branches of scientific research. Nanoparticles have possible biological roles and applications, like biosensing the small amount, catalytic reactions, drug delivery, visual representation, and nanodevice fabrication, and may be utilized as antimicrobial representatives (Faraz et al. 2018). The synthesis of NPs utilizing chemical and physical technique has been exhaustively all over society, although these techniques are frequently environmentally poisonous, strictly arduos, and financially costly. Appropiately, biological techniques for synthesis using plants, plant extracts, microbes, and their enzymes have been recomended as great environmental friendly substitutes (Prasad 2014; Prasad et al. 2016, 2018). The use of vegetal extracts as reducing agents during NP synthesis or covering agents of NP is useful over other biological procedures. In this case, the procedures of cultivation and conservation of biological cells are not required, as in the case of tissues or cell cultures, and the nanoparticle synthesis procedure can be scaled. Altogether, plant-mediated synthesis of nanoparticles is a less expensive process, ecologically sustainable, and a one-phase technique for biological synthesis procedures that is secured for different individual curative and nutritional-based applications. Recently, the application of industrial and agricultural waste in the biosynthesis of dissimilar kinds of metallic nanoparticles has been broadly considered (Patra and Baek 2017; Sangeetha et al. 2017). These pathogens which are drug resistant are more pathogenic having enhanced rate of transience compared to that of wild or non-mutated strain. The scientific society is constantly investigating for novel types of decontamination methods and approaches that could act effectively against the potential pathogens.

Numerous researchers on the effectiveness of nanoparticles systems with plant extracts have been published (Table 1.1). The main nanoparticle systems are silver, copper, and zinc; these nanoparticles are capable of decrease microbial growth and also are excellent inhibitors of pathogens. Dhand et al. (2016) reported the synthesis of silver nanoparticles (AgNP), using *Coffea arabica* (seed) extract. These NPs have been tested against pathogen like *E. coli* and *S. aureus* and an excellent antibacterial activity was observed. Extracts of *Vernonia cinerea* (L.) Less. (Asteraceae) were tested and highly efficient against *Xanthomonas campestris* pv. Malvacearum (pathogen of the cotton plant) (Sahayaraj et al. 2015). Rao et al. (2016) reported the action of crude root extract of *Diospyros paniculata* against *B. subtilis*, *E. coli*, and *P. aeruginosa*. Use of peel aqueous extract of citrus (Citrus × clementina) has also been tested and has demonstrated its effectiveness to inhibit *E. coli*, *B. cereus*, and *S. aureus* (Saratale et al. 2017) and extract of *Dodonaea viscosa* against *E. coli*, *K. pneumonia*, *P. fluorescens, S. aureus*, and *B. subtilis* (Kiruba et al. 2013). In the same way, extract of *Tamarindus indica* fruit was used to synthesize AgNP through

Table 1.1 Plant extract used in synthesis of nanoparticles

Plant material	Nanoparticle	Action against pathogen	References
Mentha piperita	AgNPs	*Escherichia coli*	MubarakAli et al. (2011)
Caesalpinia coriaria (leaf)	AgNPs	*E. coli*, *P. aeruginosa*, *K. pneumoniae*, *S. aureus*	Jeeva et al. (2014)
Lippia citriodora	AgNPs	*S. aureus*	Elemike et al. (2017)
		B. subtilis	
		S. typhi	
		E. coli	
		C. albicans	
Tamarind (fruit extract)	AgNPs	*Bacillus cereus*, *Staphylococcus aureus*	Jayaprakash et al. (2017)
		Micrococcus luteus, Bacillus subtilis	
		Enterococcus sp., *Pseudomonas aeruginosa*, *Salmonella typhi*	
		Escherichia coli	
		Klebsiella pneumonia	
Mangifera indica, *Eucalyptus tereticornis*	AgNPs	*Aeromonas hydrophila (aquatic pathogen)*	Mahanty et al. (2013)
Carica papaya			
Musa paradisiaca			
Zingiber officinale (root extract)	AgNPs	*Staphylococcus* spp., *Listeria* spp.	Velmurugan et al. (2014)
Lantana aculeata Linn leaf	ZnO NPs	*Aspergillus flavus*, *Fusarium oxysporum*	Narendhran and Sivaraj (2016)
Brassica rapa L	AgNPs	*Gloeophyllum abietinum*, *G. trabeum*, *Chaetomium globosum*, and *Phanerochaete sordida*	Narayanan and Park (2014)
Mangifera indica, *Eucalyptus tereticornis*, *Carica papaya*	AgNPs	*Aeromonas hydrophila* (fish pathogen)	Mahanty et al. (2013)
Musa paradisiaca			
Salvadora oleoides	ZnO NPs	*Bacillus cereus* (BC) ATCC11778, *S. aureus* (SA) ATCC29737	Padalia et al. (2017)
		C. rubrum (CR) ATCC14898	
		E. coli (EC) NCIM2931	
		P. aeruginosa (PA) ATCC9027	
		K. pneumoniae (KP) NCIM2719	
		S. typhimurium (ST) ATCC23564	
Aloe vera (leaf extract)	CuO NPs	*Aeromonas hydrophila*, *Pseudomonas fluorescens*, and *Flavobacterium branchiophilum*	Kumar et al. (2015)

(continued)

Table 1.1 (continued)

Plant material	Nanoparticle	Action against pathogen	References
Citrus × clementines (peel extract)	AgNPs	*Escherichia coli*	Saratale et al. (2017)
		Bacillus cereus	
		Staphylococcus aureus	
Psidium guajava	AgNPs	*P. aeruginosa* MTCC 741	Bose and Chatterjee (2016)
Dodonaea viscosa	Cu and Ag nanoparticles	*Escherichia coli*	Kiruba et al. (2013)
		Klebsiella pneumoniae, *Pseudomonas fluorescens*, *Staphylococcus aureus*, and *Bacillus subtilis*	
Sargassum wightii (brown marine seaweed)	AgNPs	*S. aureus*	Shanmugam et al. (2014)
		K. pneumoniae	
		S. typhi	
Diospyros paniculata (root extract)	AgNPs	*B. subtilis*	Janmohammadi et al. (2016)
		E. coli	
		P. aeruginosa	
		K. pneumonia	
		S. pyogenes	
		S. aureus	
		B. pumilus	
		P. vulgaris	
Parthenium hysterophorus L.	ZnO NPs	*Aspergillus flavus*, *A. niger*	Rajiv et al. (2013)
Coffea arabica (seed extract)	AgNPs	*E. coli* and *S. aureus*	Dhand et al. (2016)

microwave irradiation (Jayaprakash et al. 2017). Narayanan and Park (2014) managed to synthesize AgNPs using turnip extract and tested its action against fungal pathogens of genera *Gloeophyllum*, *Chaetomium*, and *Phanerochaete* obtaining excellent results. Papaya leaf extract synthesized AgNPs has been reported to achieve inhibition of *Aeromonas hydrophila* an important aquatic pathogen (Mahanty et al. 2013).

Other investigations using an extract substance of brown marine seaweed (*Sargassum wightii)* showed a significant potential antibacterial action against *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Salmonella typhi*, surpassing twice its action compared to the control of antibiotic (Shanmugam et al. 2014). Even extract of *Cordyceps militaris*, a fungus medicinal, was employed in the synthesis of AgNPs and its antibacterial action against clinical infectious agents like *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Bacillus subtilis* (Wang et al. 2016). Until now, for nanoparticles production, most of the methods reported synthesis with only aqueous extract (Rajan et al. 2015).

Copper oxide nanoparticles were synthesized utilizing a watery solution extract of *Acalypha indica* leaf and tested against *E. coli*, *P. fluorescens*, and *C. albicans* showing inhibition (Sivaraj et al. 2014). Thatoi et al. (2016) evaluated the impact of ZnONPs with two extracts of plant species of mangrove on different pathogenic bacteria; nanoparticles only showed action against *Shigella flexneri*. Also, extract of *Aloe vera* (leaf) was used to synthesized copper oxide nanoparticles; its potential to inhibit *P. fluorescens*, *A. hydrophila*, and *F. branchiophilum* was tested (Kumar et al. 2015).

Furthermore, fabrication of zinc oxide nanoparticles (ZnONPs) has been reported, and different natural extracts are used, for example, extract of *Parthenium hysterophorus* L. showed an excellent inhibition of *A. flavus* and *A. niger* (Rajiv et al. 2013); ZnONPs with extract of *Salvadora oleoides* (leaf) show action against bacteria such as *E. coli B. cereus*, *S. typhimurium*, *S. aureus*, and *C. rubrum*, among others (Padalia et al. 2017). *Anchusa italica* (flower extract) showed that Gram-positive bacteria have an increase of susceptibility than Gram-negative bacteria to the system of ZnO nanoparticles (Azizi et al. 2016). Other reports have described effect of ZnO nanoparticles against fungus in the presence of an extract of *Suaeda aegyptiaca* plant with achieving *Candida albicans* and *Aspergillus oryzae* inhibition.

1.3 Nanoparticles for Plant Disease Suppression

Nanotechnology has the possibility to transform the food and agricultural industries by developing new instruments for the management of plant diseases. It is possible that in the coming days nanostructured catalysts will advance the effectiveness of pesticides and insecticides and will also lower the doses level needed for crop plants (Khan and Rizvi 2014; Servin et al. 2015). The use of nanostructured materials in plant disease management is a novel approach that has the potential to act against pathogens like bacteria, fungi, and nematodes (Gupta et al. 2018; Abd-Elsalam and Prasad 2018). Table 1.2 shows various nanostructured materials utilized against diverse crop pathogens.

The agriculture production is influenced worldwide yearly due to plant pathogens; therefore, several investigations have been realized in an effort to control these plant diseases. Among methods of control or protection of crops against pathogens, the use of nanotechnology is a suitable alternative to protect crops from several pathogens. In this field, silver nanoparticles have been studied and applied as antibacterial material. Recently, Jo et al. (2015) used Ag nanoparticles of 7.5 nm against *Gibberella fujikuroi* in order to protect rice seedlings. Also, Cromwell et al. (2014) studied the application of silver nanoparticles to soil and control the propagation of the nematode *Meloidogyne* spp. and protect Bermuda grass plants. Small Ag nanoparticles (4–8 nm), were sprayed to pepper leaves plants to control the fungus pathogen *Colletotrichum* sp. (Lamsal et al. 2011).

Moreover, other nanostructured materials have been studied as crop protection alternatives. Kanhed et al. (2014) evaluated the antifungal efficacy of copper nanoparticles of 5–10 nm against potential plant pathogenic fungi such as *Alternaria alternata*, *Phoma destructiva*, *Fusarium oxysporum*, and *Curvularia lunata*. In the study, they found that Cu nanoparticles are better than the

Table 1.2 Examples of nanoparticles utilized against crop pathogens

Nanoparticle	Particle size	Mode of exposure (conc.)	Plant	Pathogen	References
DNA-directed AgNPs grown on graphene oxide	5, 18 nm	Sprayed (16, 50 and 100 ppm)	Tomato	*Xanthomonas perforans*	Ocsoy et al. (2013)
Ag ions and NPs	20–30 nm	Sprayed (25–100 ppm)	*Lolium perenne*	*Bipolaris sorokiniana* and *Magnaporthe grisea*	Jo et al. (2009)
SiO_2–Ag	20, 100 nm	Sprayed (0.3–100 ppm)	Cucumber, pansy, and green squash	Fungus: *Botrytis cinerea*, *Rhizoctonia solani*, *Colletotrichum gloeosporioides*, *Magnaporthe grisea*, and *Pythium ultimum* Bacteria: *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas syringae*, *Xanthomonas campestris*, *Azotobacter chroococcum*, and *Rhizobium tropici*	Hae-Jun et al. (2006)
AgNPs	4–8 nm	Sprayed on the leaves (10, 30, 50, and 100 ppm)	Pepper	*Colletotrichum* sp.	Lamsal et al. (2011)
AgNPs	n/a	Applied to soil (1.5, 3, 13, 30 and 150 μg/ml)	Bermuda grass	*Meloidogyne* spp.	Cromwell et al. (2014)
AgNPs	~7.5 nm	Seeds submerged in AgNPs suspension (150 μg/ml)	Rice seedlings	*Gibberella fujikuroi*	Jo et al. (2015)
SeNPs	49–300 nm	–	*Pennisetum glaucum*	*Sclerospora graminicola*	Nandini et al. (2017)

commercial fungicide. Selenium nanoparticles result to be an accurate alternative to protect pearl millet plants against the *Sclerospora graminicola* (Nandini et al. 2017). Rajiv et al. (2013) synthesized zinc oxide nanoparticles and report its antifungal action against the plant pathogens *Aspergillus flavus* and *Aspergillus niger*.

1.4 Nano-encapsulated Phytoextracts for the Control of Pest

This information offers an existing viewpoint and trend of the futuristic prospective of agricultural nanotechnology to improve the effectiveness of botanical insecticides by encapsulating phytoextracts to control insects or plagues, the so-called green technology. Agricultural nanotechnology promises not only new products but also a vast potential as "upgrading technology" in combination with current technical solutions and nano-encapsulation trends of secondary compounds called active phytochemicals of plant extracts. Phytoextract is a plant extract of complex substances of bioactive chemical compounds obtained by physicochemical or microbiological processes (Medina 2001). The use of phytoextracts is known from remote times. They were applied as treatment of common diseases such as indigestion or pneumonia as well as as food preservatives. Numerous phytochemical compounds are considered as plant secondary derivatives or metabolites which were created during evolution to support different functions such as a defensive and protective barrier for the plants, or prevention against attack of microorganisms or insects, between others.

These substances are alkaloids, phenolics, terpenoids, and many others, the secondary metabolites are present in the whole plant in different proportions, and for that reason they are analyzed and obtained by the process of extraction with different organic or aqueous solvents. The application of nanotechnology provides a way to develop novel preparations and systems capable of improving the effectiveness of this kind of insecticides, to be incorporated into the ecosystem, as discussed below with the help to encapsulate this phytoextracts.

The advantage of the nanotechnology strategy is attractive since it can contribute to the benefits of the phytochemical compound in the ecosystem and to anthropoid health as it is easy to assimilate and nontoxic; also its advantages are biodegradability and high structural diversity, which can delay the occurrence of resistance (Raskin et al. 2002).

As a consequence or outcome of several research findings in this field from the study of phytoextracts for insect control, the foremost areas of opportunity to be studied are the scalability of the production of nano-carrier systems before commercializing this technology completely. However, in the manufacture of isolated extracts and active ingredients, a good amount is necessary to regulate some agrarian production in the presence of a pest. The prospective of these systems is to resolve difficulties that will associate the advantages of phytoextracts with nanotechnology, in a potential preparation that will be more efficient for the environment in general.

There are active compounds extracted and widely employed in agricultural sector to control the proliferation of devastating insects, harmful nematodes, and pathogenic fungi and bacteria. Conversely, on the other hand, some may lose their activity in a short period. On the other hand, some of them may lose their activity in a short period. Due to the current trend is focused to the remediation of land, aquifers, and natural systems associated with compounds that affect them, it is intended to increase the use of alternatives based on natural products and pest control agents that are more compatible with the new ecological policy (McSpadden and Fravel 2002). However, these products so far represent a small percentage of the total of the inputs used. Faced with this and the lack of a wider range of active ingredients, these have been gaining and begin to have an important participation not only in small-scale production systems in development with nano-encapsulation of this type of active compounds for their greater stability and effectiveness in the application. The encapsulation of phytoextracts in the encapsulated particles offers an effective potential for future agricultural applications. If the efficiency encapsulation is greater than 90%, the profile will be excellent and will be taken into consideration for the application compared to others that are already on the market that is harmful.

In general, reported results for pesticide-based formulations are encouraging, though more research work will be required to manufacture actual products that are benign, safe, and affordable.

There are comparatively very few reports in the scientific literature regarding the alliance of phytoextracts with encapsulated nanoparticles, and it would be essential to develop accessible systems appropriate for use in agricultural sector in general or green agriculture for pest control.

The next step in the encapsulated phytoextracts will be the activity in the soil and in the plant material of application, together with an efficient carrier system, also against an effect in conjunction with active agents extrinsic to the system that is applied.

The formulations are characterized by particle size and analyzed by scanning electron microscopy (SEM), differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), atomic force microscopy (AFM), and transmission electron microscopy (TEM). All these analyze and verify the potential of the nanoparticles and the availability of the vegetative material.

There are active compounds such as phenylpropanoids, monoterpenoids, and sesquiterpenoids (Nigam and Purohit 1962; Gupta et al. 1985; Ramos et al. 1986) that could be investigated for the development of nano-encapsulates for pest control. Valdés et al. (2006) evaluated the phytoextracts effect on *Scyphophorus acupunctatus* pest. Treatment with Dysphania ambrosioides extract led to 40% mortality in larvae and pupae of *S. acupunctatus*, with *Castela tortuosa* extract on 70% and *Tagetes erecta* at 48%. Moreover, larvae weight decrease was also reported that was considered as growth inhibition.

Table 1.3 shows studies reporting a comparison of phytoextracted compounds reported for botanical pesticides extracted from plants using the encapsulated micro- and nanosystems.

Table 1.3 Studies reporting a comparison between phytoextracts biological active complexes, their resources, and carrier systems employing nano- or microtechnology

Bioactive compound	Source	Carrier system	References
Azadirachtin	Chiefly extracted from the leaves and seeds	Microcapsules of the poly(vinyl acetate)	Riyajan and Sakdapipanich (2009)
	Azadirachta indica (Meliaceae) insecticidal and acaricidal activity	Capsules of sodium alginate/glutaraldehyde	Riyajan and Sakdapipanich (2009)
		Carboxymethyl chitosan/ricinoleic acid-coated nanoparticles	Feng and Peng (2012)
		Sodium alginate spheres	Jerobin et al. (2012)
		Polymeric nano- or microparticles	Forim et al. (2013)
		Polymeric nanocapsules	Da Costa et al. (2014)
Rotenone	Found in *Derris* genera species, *Lonchocarpus*, and *Tephrosia* (Fabaceae) insecticidal and pesticidal activity	Chitosan nanoparticles	Martin et al. (2013)
		Polymeric microparticles	Keawchaoon and Yoksan (2011)
Carvacrol	Present in oregano and thyme essential oils	Nanoparticles of chitosan	Higueras et al. (2013)
	Insecticidal and bactericidal activity	Chitosan/β-cyclodextrin	Higueras et al. (2013)
Thymol	Present in thyme and pepper-rosmarin essential oils. Display bactericidal and insecticidal activity	Film of nano clay	Lim et al. (2010)
		Polymeric microparticles	Guarda et al. (2011)
		Zein nanoparticles	Zhang et al. (2014)
Eugenol	Found in clove, cinnamon, myrrh, and sassafras essential oils. Display bactericidal, nematicidal and insecticidal activity	Cyclodextrins	Choi et al. (2009)
		Solid lipid nanoparticles	Garg and Singh (2011)
		Chitosan/β-cyclodextrin	Sajomsang et al. (2012)
	The active component of turmeric (*Curcuma longa* Zingiberaceae)	Zein nanoparticles	Gomez-Estaca et al. (2012)
Curcumin	Insecticidal action	Nanoparticles of hydroxypropyl cellulose	Bielska et al. (2013)
Limonoids	Anti-food effects on insect pests belonging to Lepidoptera	Phytoextract particles	Suresh et al. (2002)

Research has evaluated the use of synergism based on phytoextracts, used in formulations of natural pesticides observed an appreciable increase in the mortality of the pests.

1.5 Nanoparticles in Bioremediation

Heavy metals affect human, animals, and plants because of they are not biodegradable. Metabolic activities in plans are directly affected by heavy metals leading to people's health affectation and the decreasing of food production (Singh and Prasad 2015).

Dixit et al. (2015) cited that the presence in the environment of heavy metals comes from anthropogenic sources, such as pesticides, paints, fertilizers, tanneries effluents, and steel industries among others, and from natural sources, such as erosion and volcanic activities, particles released by vegetation, and forest fires. On the other hand, heavy metals have toxic effects on the human health producing skin damages, cardiac arrhythmias, and respiratory, fertility, and gastrointestinal problems.

Agrochemicals are the principal source of heavy metal contamination, phosphate fertilizers being the principal source. Getting heavy metal from soil, plants produces exudate which chelates metal ions and facilitates their solubility and extraction (Mustafa and Komatsu 2016).

Vegetables contaminated with heavy metals are a risk to the human health. It is important to know the concentration of these metals in vegetables to avoid human contamination. For instance, it was found a high concentration of heavy metals in vegetables sold in markets at Riyadh, Saudi Arabia, and Varanasi, India, which is a problem to people that consumed these vegetables. This pollution could be originated from industries and vehicles, and they could be deposited on the vegetable surfaces (Ali and Al-Qahtani 2012).

Nanotechnology gives to plants tools to treat diseases and take nutrients from soil leading to increasing crop production. Nanoparticles can be absorbed onto the clay lattice improving soil health and efficient use of nutrients by plants. Polymeric carriers in nanostructures can enhance soil stability and hydraulic characteristics to remove sodium from the soil. Nanoscale gypsum particles encapsulated in polymers would be better to improve the ground quality than gypsum used alone (Patra et al. 2016).

Bioremediation is a microbe-mediated and promising technology to remove heavy metals in polluted water and lands. Some microorganisms use different detoxification methods such as biosorption, bioaccumulation, biotransformation, and biomineralization (Ali and Al-Qahtani 2012).

Nanoparticles used for metal remediation is an attracting idea because of their properties such as unique electrical property, high chemical stability, and large specific surface area (Singh and Prasad 2015).

Nanostructures help to improve crop productivity using nano-nutrients, nano-pesticides, and nano-fertilizers. Nano-pesticides keep a long effect in the plant producing some advantages such as the reduction of the utilization of dangerous traditional chemical pesticides. On the other hand, nano-fertilizers improve the crop growth and productivity (Subramanian et al. 2016; Singhal et al. 2017).

On the contrary, nanosystems are introduced in agriculture and in other applications such as nutrients delivery systems and antifungal and bactericidal uses. Currently, nutrients applied in soils can be incorporated into nanotubes and nanopores, using a coat polymer film. Also, nutrient could be made at nanoscales (Robles-García et al. 2016), and they could be incorporated into the soil.

Bradfield et al. (2017) cited that metals such as zinc and copper had an adverse effect on tuber biomass of potato at the highest concentration analyzed. On the other hand, low and high concentration of cerium did not affect the yield; besides its high levels have a positive effect on tuber diameter. The bioavailable of these metals to the plant was increased from zinc to cerium.

Aluminum is not essential for plants; its toxicity is caused by the trivalent ion which has an adverse effect at high concentrations in acidic soils. It avoids cellular processes, interacting with PO_4^{3-}, SO_4^{2-} and carbonyl groups. Cadmium accesses plant roots as a divalent ion, and it affects plants inducing the oxidative stress and replaces zinc, iron, and calcium in the prosthetic groups of many proteins. Animals and plants need zinc as an essential micronutrient, and it is presented in the 10% of protein binding sites, but higher concentrations have adverse effects in plants provoking growth inhibition. At this high concentration, zinc reduces root growth and increase root thickening amount affectations. Copper is also a micronutrient for the plant, but it is toxic at a high concentration because it is capable of catalyzing Haber-Weiss and Fenton reactions producing cellular damage. Arsenic appears in soils due to pesticides applications changing the properties of soils and reducing crop growth and productivity. Other metals such as chromium, iron, and mercury have appreciable affectations over crops (Mustafa and Komatsu 2016). The Table 1.4 describes the nanosystems effect over the crop-soil surrounding system and their characteristics.

1.6 Nanosystems in Biofertilizers

The use of indiscriminate of pesticides and fertilizers has been causing irreversible damages to the environment. Our communities are being exposed to serious problems such as the pollution of the environment, appearance of agricultural pests and pathogens, and loss of diversity in biology. These problems have led scientists to explore the agriculture area, especially for the protection of the plants and productions (Ghormade et al. 2011).

Introductory studies show the capacity of nanomaterials (in different sizes and shapes) in enhancing of the seed sprouting and development, plant conservation, pathogen recognition, and pesticide/herbicide residue recognition. The use of nanomaterials in these fields has been successful and safe. These improvements are the foundation for future engineering allowing to generate unique properties aiming in a direction of specific applications (Khot et al. 2012).

Table 1.4 Nanosystems effect over the crop-soil surrounding system

Nanosystem	Characteristics/effect	References
TiO_2 and CuO magnetic nanoparticles	Nanoparticles change the nutrient bioavailability reducing the microbial biomass in flooded paddy soil, the total phospholipid fatty acid, and enzymes activities	Xu et al. (2015)
$CoFe_2O_4$	$CoFe_2O_4$ did not have any affectation over germination and growth of plants. 1000 mgL^{-1} of $CoFe_2O_4$-NPs significantly increases the tomato roots compared to the control (30%). However, the micronutrient uptake was affected	López-Moreno et al. (2016)
CeO_2	Although exposition to 2000–4000 mgL^{-1} of CeO_2-engineered nanoparticles increased peroxidase activity in roots and the highest CeO_2 treatments increased the antioxidant enzyme activity, the treatment with nanoparticles provoked a reduction in micronutrients	Hernandez-Viezcas et al. (2016)
Attapulgite micro-nano-network	A high-energy electron beam irradiation of thiosulfate sodium supported by attapulgite network was used as a remediation agent on soil contaminated with chromium VI. In this work, the inhibition effect, on the corn growth, of this ion was reduced	He et al. (2015)
Silicon	The effect of silicon nanoparticles to decrease the chromium (VI) phytotoxicity on the decline the pea growth was studied. Authors, also suggested the use of this information against heavy metal toxicity to understand the interaction mechanism amount silicon nanoparticles and chromium VI in plants	Kumar et al. (2015)
Magnesium hydroxide nanoparticles [Mg $(OH)_2$NPs]	Magnesium hydroxide nanoparticles affect levels of cadmium toxicity on *Brassica juncea*. These nanoparticles, synthesized by sol–gel methods, had a protected effect over cadmium toxicity improving the chlorophyll and carotenoids content, the root–shoot length, seedling size germination percentage, and biomass accumulation. Also, the authors concluded that zinc oxide nanoparticles increase the tolerance mechanisms of *Brassica juncea* contaminated with cadmium	Bohra et al. (2016)
Nanosilica Schiff-base copper (II) bactericide	This environment-friendly bactericide has an excellent antimicrobial activity against four bacterial. At the same time, this nanosystem intensified disease resistance and reduced the phytotoxicity and genotoxicity of copper in plants as a cucumber	Zhang et al. (2014)
Nanoparticles of zinc oxide	They were synthesized to control on rice blast fungus (*P. guise*) and rice brown spot fungus (*H. oryzae*) in vitro under greenhouse conditions. The average ratio of these nanoparticles was around 6.5–71.4 nm, and they have better antifungal effect than zinc oxide	Kalboush et al. (2016)

Nanotechnology, by nanomaterials related property, has agrarian biotechnology applications capacity to mitigate such matters. Reports regarding the function of nanotechnology in soil and plant systems reveal that nanomaterials may help in the following: (1) the regulated release of agrochemicals for nutrition and preservation toward pests and pathogens, (2) distribution of genetic component, (3) fragile identification of pollutants and plant disease, and (4) conservation and development of soil composition (Ghormade et al. 2011).

For example, biodegradable polymeric chitosan (78 nm) and porous silica (15 nm) nanoparticles show steady discharge of enclosed fertilizer and pesticide, appropriately. Furthermore, nano-sized gold particles (5–25 nm) distributed DNA to the plant cells, while the iron oxide (30 nm)-based nano-sensors discovered pesticides at molecular degrees (Ghormade et al. 2011).

The evaluation of the consequences of nitroxin and nanosilver on produce and yield elements of the mini potato tubers raised in the field concludes that by application of nitroxin, the quantity of mineral nitrogen fertilizer can be diminished to half building positive environmental influence. Fascinatingly, the use of nanosilver in merging with nitroxin produce notably higher tuber yield probably attributable to its antimicrobial cause. This antimicrobial effect of nanosilver may have lend seed tubers to remain in healthier condition for an extended period of time in the soil and eventually generated healthier plants. On the other hand, the fabrication of diverse antibiotics by the bacteria existent in nitroxin in rhizospheres of roots may avert the invasion of the root and seed tuber by infectious soilborne organisms and nematodes and expand the defense of plants to these harmful agents (Davod et al. 2011).

Potassium nano-fertilizer and nitrogenous bioinoculants has a positive effect on yield and components of the red bean (*Phaseolus vulgaris L.*), on enlarging the leaf area ratio, grain weight, and number of grain per pod of the treatment. Subject on the testing results, combined application of N biofertilizer and Khazraa K chelate Nano-fertilizer (KKCNF) has a better result on red bean productivity than the lone application of the above inoculants. As a result, chemical fertilizers can be substitute by these fertilizers for more effectiveness. They are more inexpensive and environmental friendly and have more useful crop execution (Farnia and Ghorbani 2014).

The effects of seed sowing date and nano-biofertilizers (Biozar®) containing *Azotobacter* and *Pseudomonas* bacteria and nano-fertilizers such as Fe, Zn, and Mn on yield and yield components of wheat indicate that late seed sowing decreases wheat vegetative growth and growing period length, which are the main reasons for yield loss. The addition of nano-biofertilizer application (4-l ha-1) increased spike length, spike number in m^2, seed number in m^2, seed number in spike, and seed weight some days until physiological maturity. However, there was no further increase with increasing nano-biofertilizer application, except for seed weight, some days until physiological maturity. In sum, early seed sowing and application of 4-l ha-1 nano-biofertilizer are recommended to gain the desirable yield (Farnia and Omidi 2015).

Results of evaluation of effect of nanoparticles and biofertilizers on the chlorophyll and carbohydrate content in forage sorghum (Speedfeed hybrid) show that these treatments allow to synergistic effect of nourishments, can provide the necessary plant nourishment and preserve soil microbes, providing the better condition for plant development without environmental contamination (Mir et al. 2015).

The result of zinc doses and seed treatment with biofertilizer on quality and amount of produce and soybean nodulation shows that the utilization of biofertilizers and zinc performs a major part in yield produce and amount of soybean produced.

Therefore, the raised nodules weight per plant, quantity of plant pods and weight of grains per plant, and the oil content were acquired via application of high quantity of zinc and *Bradyrhizobium japonicum* inoculation along with the plant growth-enhancing bacteria. Also, the stearic and palmitic acids (saturated fatty acids) decrease in the seeds implanted with bioinoculants besides the uninoculated control, whereas in linoleic, linolenic, and oleic acids, the common unsaturated fatty acids were found to be increasing. Therefore, it provides a notion that appropriate quantities of zinc (i.e., 0.9 g L^{-1}) used and inoculation of *Bradyrhizobium japonicum* and plant growth-promoting rhizobacteria (PGPR) can be suggested for succesful lucrative production of soybean (Sharifi 2016).

The evaluation of the impact of biofertilizers and nano-fertilizers under full and deficit irrigation on growth, plant yield, and yield components of maize was performed in northwest of Iran. With the efficiency of biological and nano-fertilizers under various moisture levels, deficit irrigation (25 and 50% of field capacity (FC)) was applied during the reproductive growth stage. Findings showed that although the utilization of biological fertilizers increased growth and some of the yield components under the well-irrigated condition, they failed to maintain their superiority under deficit irrigation. Our results revealed that a maize hybrid (704 single-cross) is well suited for rational deficit irrigation applied during the reproductive growth stages in northwestern Iran. The fundamental aim of the irrigation deficit technique is to increase water use efficiency by reducing the volume of water at each irrigation level. Also, our results indicate that application of bulk NPK, nano-chelated Zn, and nano-fertilizers of complete micronutrients under mild deficit irrigation (50% FC) could significantly improve plant performance. With increasing pressure on limited water resources and growing water shortage, the need for application of deficit irrigation will be more sensible, especially in dry and semidry areas (Janmohammadi et al. 2016). Table 1.5 describes the different applications of nano-biofertilizer system and their application mode.

1.7 Conclusions

The application of nanotechnology in modern agriculture is an invaluable tool for the crop protection. The use of biomolecules to develop nanostructured systems permits elaborate new agrochemical formulations with enhanced action mechanisms that provide crop protection against several pathogenic organisms, increase the crop production, and also reduce the utilization of agrochemicals. Nanotechnology is an appropriate alternative to protect plants from pathogens like bacteria, fungi, and

Table 1.5 Different applications of nano-biofertilizer system

Nanosystem	Application mode	References
Nanosilver	The irrigation method was sprinkler raining systems	Davod et al. (2011)
Khazraa K chelate Nano-fertilizer (KKCNF)	Foliar application	Farnia and Ghorbani (2014)
Fe, Zn, and Mn	Foliar application	Mardalipour et al. (2014)
Nano-zinc chelate	Foliar application	Farnia and Omidi (2015)
Iron chelate nano-fertilizer, potassium chelates nano-fertilizer	Mixed by soil before the sowing	Mir et al. (2015)
Nano zinc oxide and five biofertilizer	Mixed by soil before the sowing	Sharifi (2016)
Nano-chelated boron, nano-chelated zinc, and biofertilizer	Foliar application	Janmohammadi et al. (2016)

nematodes. Diverse nanosystems have been studied in plants and over pathogens to evaluate its activity. In several cases, the use of nanostructured materials exhibits better activity against pathogens than industrial chemicals. The use of nanomaterials improves the growth of crops and seed germination, which makes these nanostructured systems an advantage alternative to protect and promote plant growth.

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Roles of Plants and Bacteria in Bioremediation of Petroleum in Contaminated Soils

2

Abbas Alemzadeh

Abstract

Large amounts of petroleum compounds are released into the environment every year as a result of industrial activities causing serious damages to the environment and human health. Various methods may be applied to remove the petroleum pollutants, but bioremediation is a cost-effective and sustainable process to remove these hazardous organic pollutants. The utilization of organisms such as plants and bacteria for biodegradation of pollutants is an inexpensive, environmentally friendly, and efficient approach to clean up polluted soils. Bacteria are ubiquitous in polluted environments and may develop different strategies to utilize pollutants. Plants may also be used to degrade the pollutants; that is called phytoremediation which is a promising method for reclaiming contaminated sites. Most plants associate with different bacteria that live around their roots, and this association can increase the biodegradation rate of organic compounds. In fact, plants and bacteria play pivotal roles in cleaning up the environment and can accelerate the remediation process of petroleum waste.

2.1 Introduction

Bioremediation, which is also referred to as biotreatment, bioreclamation, and biorestoration (Ahluwalia and Sekhon 2012), has been defined as the use of living organisms, plants, bacteria, or fungi, to detoxify, remove, or reduce the concentration of pollutants from the environment (Boopathy 2000; Vidali 2001), or may be defined as a biological response to environmental abuse (Hamer 1993). We must be

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A. Alemzadeh (✉)
Department of Crop Production and Plant Breeding, School of Agriculture, Shiraz University, Shiraz, Iran
e-mail: alemzadeh@shirazu.ac.ir

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careful in the use of our environment inasmuch as there are limited lands and resources, but studies worldwide show our carelessness in using them. Contaminated soils result from human activities when hazardous substances are produced. In the past, awareness of the potential adverse health and the environmental effects associated with these substances were less well recognized, but today, it is well known that contaminated soil is a threat to human health, leading to many efforts to remedy these sites, although the number of them is significant (Hou and Al-Tabbaa 2014; Sam et al. 2017).

The traditional techniques used for remediation of contaminated soils have usually been to excavate and remove it to landfill or cap and contain the contaminated areas of a site (Vidali 2001). Each method has some disadvantages. In the first method, it is expensive and difficult to find new sites for disposal of hazardous substances, and it may also create significant risks in the excavation, handling, and transport of these materials. The second method is an interim solution because the contamination remains on-site. A better approach is to destroy the contaminants or to transform them to innocuous substances (Vidali 2001). Hence, bioremediation, as a biological method, in which microorganisms and/or plants (phytoremediation) are used to destroy, transform, or in other ways detoxify wastes, may be considered as a substitution approach. In this method, microorganisms and plants have a critical role, and the increase in information about the parameters controlling the growth and metabolism of them in contaminated areas can predict the activity of plants or microorganisms that are involved in bioremediation and transform this technique from an empirical practice into a science (Lovley 2003).

2.2 Fate of Petroleum in Soil

The name petroleum covers both petroleum products and naturally occurring unprocessed crude oil (Jukic 2013; Matejicek 2017) and is constituted of hydrocarbons mixed with nitrogen, oxygen, and sulfur compounds and trace amounts of metals (Jukic 2013). Crude oil contains four major fractions including alkanes (paraffins), aromatics, cycloalkanes (naphthenes) and nitrogen-sulfur-oxygen (NSO) compounds (resins and asphaltenes) (El Nemr 2005; Franco et al. 2010; Jukic 2013). Alkane fraction mostly contains saturated hydrocarbons which occur as straight- or branched-chain (Jukic 2013). Aromatic fraction is composed of benzene rings, and their amounts are relatively small compared to naphthenes and paraffins in crude oil (El Nemr 2005). Cycloalkanes are saturated cyclic hydrocarbons which have one or more carbon rings, and their ratio depends on the crude oil type (Jukic 2013). NSO compounds consist of one or more aromatic rings and at least one heteroatom which can be oxygen, sulfur, or nitrogen atom (Oberoi et al. 2015).

The composition and inherent biodegradability of the hydrocarbon compounds are the most important factors in biodeterioration process. Polycyclic aromatic hydrocarbons (PAHs) and benzene, toluene, ethylbenzene, and xylene compounds (BTEX compounds) have been considered as main soil and water pollutant groups (Kuhad and Gupta 2009) inasmuch as they have low water solubility, high chemical stability, and resistance to biodeterioration. There are other factors that affect biodegradation of the compound in the soil as well as chemical characterizations. Among physical factors, temperature has an important role in biodegradation, affecting chemical characteristics of the compound and diversity of the microbial flora (Das and Chandran 2011). It has been shown that maximum degradation is obtained in the range of 30–40, 20–30, and 15–20 °C, in soil, freshwater, and marine environments, respectively (Das and Chandran 2011). In lower temperature, the volatility of low molecular weight hydrocarbons is decreased, while the viscosity of the oil increases, delaying in hydrocarbon biodeterioration (Atlas 1975). In addition, hydrocarbon biodegradation is mainly affected by the composition of the environmental pollutants (Huesemann 1995). There is a report to show that when the population of biodegrading microorganisms is more than 105 CFU/g of soil, efficient biodeterioration occurs (Forsyth et al. 1995). In addition, the rate of biodegradation is affected by the kind of microorganisms in the soil. It has been shown that the different population of microorganisms results in different rates of biodeterioration inasmuch as each microorganism has its own unique characteristics (Xu et al. 2009).

2.3 Use Bacteria in Bioremediation

Bacteria play a critical role in the sustainability of any ecosystem forasmuch as they are present everywhere and able to adapt to environmental changes. Bacteria are the most important groups in biodegradation of hydrocarbons although this process has been done by a wide range of microorganisms (Song et al. 1986). There is a wide group of bacteria that can decompose hydrocarbons derived from crude oil. They belong to different genera such as *Aeromonas*, *Alcaligenes*, *Achromobacter*, *Bacillus*, *Beijerinckia*, *Brevibacillus*, *Burkholderia*, *Corynebacterium*, *Delftia*, *Enterobacter*, *Flavobacterium*, *Gordonia*, *Lysinibacillus*, *Microbacterium*, *Mycobacterium*, *Nocardia*, *Paenibacillus*, *Paracoccus*, *Pseudomonas*, *Sphingomonas*, and *Stenotrophomonas* (Bartha and Bossert 1984; Paixão et al. 2010; Roy et al. 2014; Mojarad et al. 2016). Of these, some bacteria such as *Pseudomonas* and *Enterobacter* genera are the most predominant bacteria in environments contaminated by petroleum and have been applied in different studies as the main bacteria (Paixão et al. 2010; Mojarad et al. 2016; Ghoreishi et al. 2017). It has been found that some bacteria from the genus *Pseudomonas* are able to degrade many polycyclic aromatic hydrocarbons (Mrozik et al. 2003). The biodeterioration potential of bacteria isolated from contaminated environment is generally more than others isolated from non-contaminated environments, as these bacteria have adapted to contaminated area (Van der Meer 2006). It has been reported that the biodegradation rate of petroleum for soil bacteria is close to 70% (Mojarad et al. 2016; Ghoreishi et al. 2017), but it is more for marine bacteria, up to 100% (Mulkins-Phillips and Stewart 1974).

Bioremediation of petroleum using bacterial consortium is more effective compared to individual strain since there are various hydrocarbons in petroleum and they have various susceptibility to microbial attack, ranked as follows: linear alkanes>branched alkanes>aromatic compounds>cyclic alkanes>NSO compounds (Huesemann 1995). It should be considered that each environment harbors only a few strains and it can be an effective way to use various strains in bacterial consortium to increase the bioremediation efficiency (Lafortune et al. 2009). It has been shown that the diversity of bacterial species between different consortia can result in different rates of biodegradation. In addition to diversity, the bacterial community of different consortia is also important and can affect the biodegradation potential of a consortium (Ledezma-Villanueva et al. 2015).

2.3.1 Thermophilic Bacteria

Bacteria having an optimum growth temperature of above 40 °C are considered as thermophilic, in contrast to mesophilic bacteria that grow best in moderate temperature, between 20 and 40 °C (Margesin and Schinner 2001). These bacteria are divided into moderate thermophilic bacteria which refer to bacteria that grow optimally from about 50 to about 70 °C, extreme thermophilic bacteria which refer to bacteria that grow optimally from 70 to 80 °C, and finally hyperthermophiles which refer to bacteria having an optimum growth temperature of above 70 °C (Stetter 1998).

It has been shown that the biodeterioration of a wide range of hydrocarbons occurs in high-temperature habitats and many thermophilic bacteria have been isolated for use in petroleum-contaminated bioremediation (Margesin and Schinner 2001). Some thermophilic bacteria with potential for bioremediation are shown in Table 2.1. Of these, most bacteria belong to *Bacillus* and *Geobacillus* genera (Zhou et al. 2016; Elumalai et al. 2017; Singh et al. 2017) which are capable of degrading hydrocarbons, including all major classes, when the temperature is raised above 40 °C. This property of these bacteria has been of special interest in the bioremediation of contaminated sites mostly in naturally hot environments (Perfumo et al. 2007). Compared to mesophilic bacteria, thermophilic bacteria have great advantages in cleaning up oil-contaminated soils in terms of their properties, such as faster reaction rates and higher mass transfer rates (Feitkenhauer et al. 2003; Adhikari and Satyanarayana 2007).

It should be noted that hydrocarbons are naturally present in uncontaminated soils as a result of biotransformation of organic materials; therefore the presence of bacteria with biodegrading capacity is natural. Then biodeterioration of petroleum is a natural process. Although thermophilic bacteria naturally exist in hot environment like desert soils and hot springs (Aanniz et al. 2015), it has been reported some thermophiles exist in cool soil environments (Zeigler 2014). Both of these bacteria, isolated from hot environments or mesophilic environments, have the potential to be used in bioremediation of petroleum in contaminated soils.

Table 2.1 Some thermophilic bacteria with potential for bioremediation

Bacteria	Temperature	Carbohydrate	Reference
Bacillus licheniformis	50 °C	Alkanes	Garcia-Alcántara et al. (2016)
Bacillus licheniformis	50 °C	Alkanes	Elumalai et al. (2017)
Bacillus licheniformis	55 °C	Crude oil	Liu et al. (2016)
Bacillus stearothermophilus	60 °C	Hexadecane	Sorkhoh et al. (1993)
Bacillus thermoleovorans	70 °C	Alkanes	Kato et al. (2001)
Bacillus thermoleovorans	60 °C	Naphthalene	Annweiler et al. (2000)
Nocardia otitidiscaviarum	50 °C	Naphthalene	Zeinali et al. (2008)
Geobacillus kaustophilus	55 °C	Paraffin	Sood and Lal (2008)
Geobacillus pallidus	60 °C	Polycyclic aromatic	Zheng et al. (2011)
Geobacillus stearothermophilus	50 °C	Alkanes	Elumalai et al. (2017)
Geobacillus stearothermophilus	60 °C	Alkanes and aromatic	Zhou et al. (2016)
Geobacillus thermoleovorans	60 °C	Alkanes	Perfumo et al. (2007)
Geobacillus thermoparaffinivorans	50 °C	Alkanes	Elumalai et al. (2017)
Pseudomonas aeruginosa	45 °C	Crude oil and diesel oil	Perfumo et al. (2006)
Pseudomonas oleovorans	55 °C	Crude oil	Meintanis et al. (2006)
Pseudomonas putida	55 °C	Crude oil	Meintanis et al. (2006)
Thermus brockii	70 °C	Polycyclic aromatic	Feitkenhauer et al. (2003)

2.3.2 Bacterial Populations

In addition to abiotic factors that can affect the bioremediation rate of contaminated soils, bacterial population is also an important factor for hydrocarbons biodegradation. It has been shown that the soil bacterial population has a critical role in the biodeterioration of petroleum. Addition of organic materials instead of inorganic nutrients could not increase the degradation of hydrocarbons, indicating the lack of suitable strains in the environment (Vasudevan and Rajaram 2001). Forsyth et al. (1995) has been shown that biodegradation rate will not significantly occur when the population of biodegraders is less than 10^5 CFU (colony-forming units) g^{-1} of soil. In addition, it has also been previously reported that efficient biodegradation of crude oil was achieved when biodegraders were 1.7×10^6 CFU g^{-1} (Bello 2007). Although biodegraders are ubiquitous, the environmental conditions in petroleum-contaminated soils negatively affected the growth of these bacteria (Liu et al. 2010). It has been reported that manure increases the population of bacteria capable of degrading hydrocarbons in contaminated soils (Fallgren and Jin 2008; Liu et al. 2009). In all of these studies, the treatments led to increase in heterotrophic bacterial counts in contaminated soils. It was cleared that the bacterial counts increased in the first days after treatments and then gradually decreased, but always, the bacterial counts in treated soils were higher than control (Liu et al. 2009).

With gaining information about the bacterial communities in the contaminated soils, the bioremediation of contaminated sites can be performed in a more efficient way. It was shown that bacterial community dynamics during bioremediation of contaminated sites and the diversity of the community are decreased with increased biodegradation of petroleum (Ruberto et al. 2003; Katsivela et al. 2005; Leal et al. 2017). Different studies have showed that various biotic and abiotic factors affect the size of a bacterial community and principally depend on previous exposure to the contamination present in bacterial habitats and their adaptive capacity (Ruberto et al. 2003). It has been shown that a small fraction of indigenous flora is able to biodegrade hydrocarbons and usually needs to add exogenous bacteria (Ruberto et al. 2003).

2.3.3 Bioaugmentation

Bioaugmentation or biological augmentation is the addition of bacterial cultures to contaminated sites to speed up the rate of biodeterioration of undesired compounds (Van Limbergen et al. 1998). This method could serve as a powerful tool to improve biodegradation of recalcitrant compounds. There are several successful cases of biodeterioration of petroleum compounds that improved bioremediation efficiency in treated soils compared to control (bioremediation only by indigenous microorganisms) (Table 2.2). Bioaugmentation is often compared with biostimulation, which involves the modification of the environment to stimulate indigenous bacteria capable of bioremediation. Literature reviews indicated that both of these technologies can become environmentally friendly and economic approaches especially when applied together (Tyagi et al. 2011). These methods are the most common approaches for in situ bioremediation of contaminated environment, and in both strategies, the improvement of biodegrader population is the goal. Many factors such as applied bacteria, indigenous microbial community, competition with autochthonous microorganisms, type and concentration of pollutants, their availability to bacteria, the presence of roots that release organic compounds, and physicochemical characteristics of environment may affect the bioaugmentation process (El Fantroussi and Agathos 2005; Tyagi et al. 2011). Different strategies may be used for bioaugmentation, and the most common ones are addition of a bacterial strain, addition of a bacterial consortium, addition of genetically engineered bacteria, and biodeterioration of relevant genes into a vector to be transferred by conjugation to indigenous bacteria (El Fantroussi and Agathos 2005).

Although the introduction of exogenous bacteria into different environments is not new, bioaugmentation remains an experimental technique for in situ bioremediation of polluted soils (El Fantroussi and Agathos 2005). The success of any bioaugmentation depends on the relationship of exogenous bacteria with its new environmental conditions, and it is especially true in a dynamic and complex

Table 2.2 Successful cases of bioaugmentation of petroleum compounds

Bacteria	Strain	Percentage	Contamination	Reference
Acinetobacter sp.	B-2-2	75% $_{35\%}$	TPH	Ruberto et al. (2003)
Acinetobacter SZ-1	KF453955	34% $_{16\%}$	TPH	Wu et al. (2016)
Bacterial consortium	–	61% $_{26\%}$	TPH	Xu and Lu (2010)
Bacterial consortium	–	84% $_{47\%}$	TPH	Bento et al. (2005)
Bacterial consortium	–	85% $_{30\%}$	Alkanes	Tahhan et al. (2011)
Bacterial consortium	–	61% $_{6\%}$	Aromatics	Tahhan et al. (2011)
Bacterial consortium	–	43% $_{0\%}$	Asphaltenes	Tahhan et al. (2011)
Bacterial consortium	–	97.5% $_{62\%}$	TPH	Taccari et al. (2012)
Bacterial consortium	–	62.1% $_{16.6\%}$	PAH	Wu et al. (2013)
Bacterial consortium	–	75% $_{-}$	Total diesel hydrocarbons	Alisi et al. (2009)
Bacterial consortium	–	78% $_{-}$	PAH	Jacques et al. (2008)
Indigenous bacteria	–	$\underline{95.5\%}$ 64% $_{35\%}$	C_{10}–C_{22}	Lebkowska et al. (2011)
Indigenous bacteria	–	$\underline{98.2\%}$ 52% $_{31\%}$	TPH	Lebkowska et al. (2011)
Paracoccus sp.	HPD-2	23.2% $_{3.4\%}$	PAH	Teng et al. (2010)
Pseudomonas aeruginosa	WatG	57% $_{35\%}$	TPH	Ueno et al. (2007)
Rhodococcus sp.	–	97% $_{65\%}$	Diesel (C_{13}–C_{28})	Suja et al. (2014)

Subscript numbers correspond to the biodegradation percentage of control (without adding bacteria) and "–" indicates that data is not available. Underlined numbers indicate the biodegradation percentage of area where the inoculation was repeated every 3 days

PAH Polycyclic aromatic hydrocarbons, *TPH* Total petroleum hydrocarbons

environment such as soil. Therefore, it can be inferred that sterile soil is usually more hospitable to exogenous bacteria than natural soil (El Fantroussi et al. 1999). Hence, to increase the initial establishment of exogenous bacteria in natural soil, one must provide protection from harmful circumstances and predictable ecological selectively.

One way in which to overcome the problems in bioaugmentation is to use bacteria from the same niche as the contaminated soil as illustrated by the literature. The indigenous bacteria can be easily acclimatized and various studies showed this capacity is tremendous than previously thought inasmuch as environmental stresses may increase mutation rate. As these rapidly acclimating bacteria are to be used in bioaugmentation process, it should be for their applicability to remove pollutants from contaminated soils. Indigenous bacteria are often faster distributed than exogenous ones and can be better established in contaminated area. Another important concept is the distance between bacteria and target compound(s); it is thought that indigenous bacteria are closer to old contamination, while exogenous bacteria are closer to recent contamination (Vogel 1996). It has been recently shown that the addition of activated soils, soils containing indigenous biodegraders that are exposed to the pollutant, may be effective in bioaugmentation. However, the capacity of applied bacteria in bioaugmentation to survive and perform the biodegradation activity under conditions prevailing in the contaminated soil undergoing the bioremediation process is consequential.

2.3.4 Effects of Abiotic Factors

In biological process, abiotic factors usually play a pivotal role and determine the success or failure of the process. These factors are divided into two categories: those that limit the transfer of pollutants to bacteria such as soil texture and those that affect the activity of bacteria such as humidity and temperature (Zekri and Chaalal 2005; Leal et al. 2017). Here, we focus on those factors that affect the growth and activity of bacteria.

2.3.4.1 Temperature

There are some reports that showed the biodegradation increases in high temperature since increase in temperature accelerates the growth and activity of bacteria that results in increase of pollutants' deterioration (Zekri and Chaalal 2005). The results of some studies have shown that high temperature can increase removal of contaminants near to two times (Perfumo et al. 2007), although the best temperature for bacterial activity is the optimum temperature for bacterial growth (Ghoreishi et al. 2017). For example, the bacteria isolated from an area with yearly average high temperature of 32 °C could biodegrade petroleum pollutants at 28 °C very well, close to optimum temperature for their growth (Mojarad et al. 2016). Hence, when thermophilic bacterial strains are applied for biodegradation of unwanted compounds, the best result is obtained at high temperature (Elumalai et al. 2017); but the highest biodeterioration by mesophilic bacteria was obtained at 25 °C, and with increase in temperature, the biodegradation rate often decreases (Hesnawi and Mogadami 2013). In general, the optimum biodegradation has been carried out in the range of 25–40 °C for mesophilic bacteria (Kuhad and Gupta 2009) and well done at 50 °C or higher for thermophilic bacteria (Hesnawi and Mogadami 2013).

Cold temperature delays the biodegradation of petroleum-contaminated soils since microbial growth and activity are very negligible under low temperature. It means that pollutions remain for many years and natural remediation is believed not to be enough to rapidly clean up the contaminated environment. In situ bioremediation methods can be considered as a relatively low-cost way to remove pollutants in cold area with reasonable environmental safety. One of the most important factors to enhance bioremediation process in cold areas is the selection of suitable strains. It has been previously shown gram-negative bacteria, such as genus *Pseudomonas*, are the predominant bacteria in these areas, and therefore, they can be suitable candidates for petroleum bioremediation in cold environments (Labbé et al. 2007). Psychrophiles or cryophiles are the extremophilic bacteria that are capable of growth at temperature about 0 °C with an optimum growth temperature of <15 °C and are not able to grow above 20 °C and are usually found at temperature below 5 °C. Psychrotrophs or psychrotrophic bacteria are those able to grow at temperature about 0 °C with an optimum growth temperature >15 °C and are able to grow above 20 °C (Margesin and Schinner 2001).

The biodeterioration of different petroleum hydrocarbons by cold-adapted psychrophilic and psychrotrophic bacteria has been reported from various cold environments. These areas possess enough indigenous cold-adapted bacteria that can adapt rapidly to pollution inasmuch as after a contamination event, the number of biodegraders increase. As mentioned above, the temperature threshold for cold-adapted bacteria is around 0 °C, so lower temperature is not favorable for biodegradation. Bioaugmentation may be used as a useful bioremediation strategy in cold area, but the studies showed that indigenous cold-adapted bacteria can biodegrade pollutants more efficiently than exogenous strains (Margesin and Schinner 2001). One important factor that may limit pollutant biodeterioration by biodegraders in cold environments is the availability of nutrients like P and N. It was revealed that ammonium affects the biodegradation of petroleum components through progressive acidification to cause the inhibition of aromatic biodegradation. Biologically induced mineralization and biologically controlled mineralization are two models for the synthesis of minerals by bacteria that are affected by different factors. The mineralization of petroleum compounds decreases by some nutrients; for example, dodecane mineralization, any of the oily paraffin hydrocarbons having the formula $C_{12}H_{26}$, is limited by P and N at low temperature (Margesin and Schinner 2001).

Bacterial pathways responsible for deterioration of hydrocarbons, in petroleum contaminations, are spread in cold environments, and some of them can naturally coexist in the same bacterium. Some cold-adapted bacteria, such as *Rhodococcus* species, isolated from contaminated areas in cold environments are able to retain their metabolic activity even at sub-zero temperatures. The decreased availability of hydrocarbons at low temperature may be a reason for decreased biodeterioration; at 0 °C, bacteria mineralize the long-chain hydrocarbons to a greater extent than the long-chain ones because of their low bioavailability that may be responsible for their recalcitrance (Margesin and Schinner 2001).

2.3.4.2 pH

Although the optimum pH for petroleum compounds is at a range of 6–8, near-neutral pH values, biodeterioration process may occur over a wide pH range. If the pH of soil is less than 6, acidic soils, lime is generally added to increase the pH, and if the pH of soil is more than 8, alkaline soils, ammonium sulfate is generally added to decrease the pH (Kuhad and Gupta 2009). Different bacteria may have different pH optima; the soil pH plays a critical role in determining which bacterial strains become dominant during bioremediation process. Biodeterioration of aromatic petroleum hydrocarbons by bacteria seems to be sensitive to pH, which may be responsible for the persistence of aromatic hydrocarbons in environments. A pH of around 7 is often favorable for most biodegraders and can degrade the highest proportion of petroleum (Palanisamy et al. 2014).

2.3.4.3 Other Factors

In addition to temperature and pH, other factors have been shown to have a significant impact on bacterial growth and activity and consequently affect biodegradation activity. The growth of biodegraders in contaminated soils and their activity are affected by various factors; nutrient availability is one of them. Among nutrients, phosphorous and nitrogen are considered as the most important ones inasmuch as they are required for incorporation of carbon into biomass (Kuhad and Gupta 2009). The condition is unfavorable for bacterial growth when the ratio of C to N or P is increased. The optimal ratio of C/N and C/P for effective biodegradation of petroleum is usually 60/1 and 800/1, respectively (Kuhad and Gupta 2009).

Oxygen is another important factor in bioremediation process forasmuch as the main degradative pathways in most hydrocarbons in bacteria involve oxygenases for which oxygen is required (Kuhad and Gupta 2009). In addition, oxygen plays a role as an electron acceptor in the biodeterioration of petroleum compounds. Therefore, the depth of oxygen penetration in contaminated soils plays an important role in acceleration of biodegradation process, and it depends on the magnitude of the diffusion coefficient for oxygen in soils (Huesemann and Truex 1996). The oxygen availability is very important for metabolic activities of bacterial cells; hence it is necessary that oxygen supply rate should be more than oxygen consumption rate.

It has been shown that some other substances such as NaCl and chemical surfactants like Tween 80 and sodium dodecyl sulfate (SDS) affect the biodegradation rate through influence on bacterial growth (Palanisamy et al. 2014). Although it has been reported that NaCl increases biodegradation of petroleum products, but, in general, the effect of NaCl on the biodegradation process by bacterial cell somewhat depends on the strain involved (Palanisamy et al. 2014). It also showed that some surfactants, especially ionic ones, may enhance the growth of bacteria cells which leads to an increase in biodegradation rate (Palanisamy et al. 2014).

2.3.5 Genetic Engineering

Many bioremediating bacteria have been isolated and used to remove environmental pollutants; however, those that are present in high concentrations or some recalcitrant compounds do not occur in nature (xenobiotics), which are new compounds, that bacteria cannot catabolize them in an efficient way or have not usually evolved suitable catabolic pathways for them. Hence, application of genetically modified bacteria (GMB) with novel catabolic capabilities in the bioremediation of petroleum-contaminated sites can be considered as a new approach. GMB have shown high potential for petroleum biodegradation in contaminated soils although most of studies have been carried out in laboratory-based experiments and there are a few examples of GMB applications in the environment. Over the past years, application of GMB in bioremediation has been little developed, and its future is obscure for a number of underlying reasons. One of these is the high public sensitivity to genetically modified organisms (GMOs).

Recent advances in molecular biotechnology have been used to create new strains regarding bioremediation of petroleum-contaminated sites. These include development of new pathways and/or extension of existing pathways to catabolize a wider range of substrates, modification of catabolic enzyme specificity, cellular localization, substrate affinity, expression, increasing bioavailability contaminants, and creation and development of processes for monitoring and tracking of GMB (Urgun-Demirtas et al. 2006). In the case of pathways, various genes are obtained from different bacteria and combined together in one host to create an efficient pathway to catabolize xenobiotics and recalcitrant compounds. Site-directed mutagenesis has been used to increase properties of existing enzymes or alternative promoters used to enhance the expression level of enzyme genes. Oxygenases are required for the biodeterioration of many organic contaminants especially aromatic ones, and oxygen is necessary for optimal function of these enzymes. In the past decade, bacterial hemoglobin (VHB), which is synthesized in response to low-oxygen conditions, has been attracting more attention for use in bioremediation process because of their ability to provide sufficient oxygen to support enzyme activity. Expression or overexpression of VHB in bacterial cells can help them to overcome the problem in bioremediation of organic compounds under low oxygen availability (Urgun-Demirtas et al. 2006; Stark et al. 2015).

The first GMB, derived from genus *Pseudomonas*, was generated by an Indian scientist, Ananda Chakrabarty, in 1971 (Ezezika and Singer 2010). This strain harbored genes from four different *Pseudomonas* strains encoding different oil-degrading enzymes and enabled to break down crude oil very well, but unfortunately due to public concerns about genetically modified organisms (GMOs), it still sits on a shelf (Ezezika and Singer 2010). Following this effort, *Pseudomonas fluorescens* strain HK44, designed by the University of Tennessee in collaboration with Oak Ridge National Laboratory, was the first GMB released into the environment for bioremediation of a contaminated soil (Ripp et al. 2000). Strain HK44 derived from the parental strain was isolated from a polyaromatic hydrocarbon-contaminated site and harbors pUTK21 plasmid, naphthalene catabolic plasmid. In addition, this strain contains a *lux* gene fused to naphthalene catabolic promoter that shows a bioluminescent response when exposed to naphthalene (Ripp et al. 2000). Therefore, the use of *lux* gene in this GMB promises an efficient approach to monitor GMB in the environment that can facilitate the use of these bacteria in bioremediation process.

Potential environmental risks and biosafety issues for the release of GMOs should be considered as one of the most important aspects of using GMB in bioremediation process in the field. It is recommended that environmental risk assessment should be identified through the use of a model before release of GMB (Urgun-Demirtas et al. 2006). Another important reason for the limitation of application of GMB in the field may be a result of the instability of transferred genetic elements that includes two aspects: first, the functioning GMB depends on their ability to carry the genetic elements stably. Second, transfer of genetic elements to autochthonous bacteria may negatively affect other indigenous microorganisms (Ezezika and Singer 2010).

In spite of the advantages of GMB and recent advances in monitoring and tracking strategies for GMB, there are still concerns that their application in bioremediation of contaminated sites may have environmental risks such gene flow. Horizontal DNA transfer among bacteria is a widespread and natural process that plays a pivotal role in bacterial evolution. It has been suggested that this phenomenon contributes to the development of new strains with higher biodegradation potential for xenobiotics when bacterial cells are exposed to contaminants (Shintani and Nojiri 2013). It seems that horizontal transfer rate depends on both substrate concentration and growth rate of plasmid-bearing cells (Urgun-Demirtas et al. 2006). Hence, GMB introduced into contaminated sites may have unknown and undesired effects on indigenous bacterial communities because of horizontal DNA transfer, and this effect is not uniform, and therefore, it is necessary to evaluate case by case. Thus, it is required to find a way to overcome concerns about the use of GMB in bioremediation of contaminated sites. The use of antibiotic resistance genes as selectable markers is an important concern with the use of these bacteria, and replacing them with other kinds of markers, such as genes for herbicide or heavy metal resistance, can be a solution. Another solution may be use of transposons as a way for stable integration of transgenes into bacterial chromosome instead of recombinant plasmid. Suicide systems can be used as another solution for this problem. In these systems, GMB will be dead after finishing its job. Although the use of GMB in bioremediation project has been limited due to public concern and little application in the field over the past years, recent advances in molecular biology allow us to create “suicidal genetically modified bacteria” (S-GMB) to minimize the hazards and clean up the contaminated sites in a safer way (Singh et al. 2011).

2.3.6 Tracking GMB

It is very important that we are able to use some methods to track released GMB to determine the effects of them on the environment. The used methods should be simple, accurate, inexpensive, and applicable in the environment. It is also necessary and useful to monitor the recombinant plasmid and transgene by which the GMB has been genetically modified as well as GMB itself to determine the potential loss of recombinant DNA from the GMB and transferring to nontarget organisms. Growth of colonies on plates has traditionally been used as a simple method to monitor GMB, but this method has some limitations that methods based on molecular biology techniques overcome these limitations. Various molecular techniques including Southern blotting, polymerase chain reaction (PCR), reverse transcription-PCR (RT-PCR), real-time PCR, and denaturing gradient gel electrophoresis (DGGE) have been used in tracking GMB or recombinant DNA (Urgun-Demirtas et al. 2006; Han et al. 2012). Molecular methods are suitable ones because they are applied directly to contaminated sites and it is not necessary to culture bacterial cell outside the environment. Hence, in these methods bacterial communities do not change, and all microorganisms, culturable and non-culturable ones, will be used in the experiment. Anyway, using molecular methods in combination with traditional ones may give a strength method for monitoring GMB and/or recombinant DNA.

2.4 Phytoremediation: Role of Plants

The use of plants to clean up contaminated soils is called phytoremediation that has been considered as a promising method for the bioremediation of petroleum-contaminated soils. In phytoremediation technology, the plants usually absorb the pollutants from the soils, but in the case of petroleum or its products, the plants are not able to take up most of them inasmuch as they are water insoluble. Petroleum contains PAHs that may be taken up by plants through several transferring mechanisms of PAHs from soil to plant roots and leaves, including volatilization, transpiration stream, and direct absorption from soils (Watts et al. 2006). The solubility of pollutants is a critical factor to uptake, translocate, and catabolize by plants that is usually reflected by log K_{ow}, octanol-water partition coefficient of the pollutant. Plants are able to uptake pollutants with log K_{ow} values less than 3 and translocate and catabolize those pollutants with log K_{ow} values less than 1 (Alkorta and Garbisu 2001).

2.4.1 The Rhizosphere

Indeed, in phytoremediation of petroleum-contaminated sites, plants are usually used to stimulate rhizosphere bacteria, namely, plants that have an indirect effect in cleaning up petroleum-contaminated sites (Merkl et al. 2005), although they may play a pivotal role in direct removal of pollutants. The rhizosphere is the thin layer of soil that contains plant roots and is influenced by root system and associated soil bacteria. Hence, rhizosphere is a suitable environment for microorganism's life inasmuch as the moisture, oxygen, and organic matter content of rhizosphere is usually higher than other parts of the soil, and the bacterial communities in rhizosphere are usually different from non-rhizosphere area (Shrivastava et al. 2014; Prasad et al. 2015). On the other hand, some species such as bacteria belonging to the genera *Pseudomonas* and *Azospirillum* affect the plants and may increase the release of organic compounds from roots (Curl and Truelove 1986).

Bacterial numbers increase around the plant roots and decrease with increasing distance from the roots; that is called "rhizosphere effect." Rhizosphere effect is calculated using the ratio of bacterial numbers in the rhizosphere to non-rhizosphere soil which is between 1 and more than 100 (Jones et al. 2004). Rhizosphere effect is affected by many factors that one of them is the quality of the soil, and in contaminated soils, the ratio is dramatically decreased (Jones et al. 2004). It is expected that there is a high potential for deterioration of petroleum compounds in rhizosphere for more bacterial activities, although the bacterial numbers decrease in contaminated soils.

2.4.2 Potential Use of Plants

Petroleum-contaminated soils may affect plant growth and production in different ways. Pollutants influence both abiotic and biotic factors, microorganisms, and parts of soil that leads to damage of cell membranes of plant roots and shoots. But, different plants have different potentials to use in phytoremediation of petroleum-contaminated sites, and some plants can grow well in contaminated soils, whereas the growth of some species is inhibited after germination or in other growth phases (Kaimi et al. 2007). The toxicity of petroleum compounds on the plant growth may be caused by parameters: hydrophobicity in contaminated soils and/or volatile hydrocarbons (Kaimi et al. 2007). Hydrophobicity causes the decrease of aeration rate that results in growth inhibition of plant roots, and volatile compounds cause toxic effects moving through the plant cells. In most studies, legumes and grasses have been used for phytoremediation of petroleum contaminants because grasses have well-developed root system with maximum root surface area and can deeply penetrate in the soil and legumes have the potential for nitrogen fixation (Merkl et al. 2005; Kaimi et al. 2007). However, it is expected that legumes are able to grow in contaminated soils due to their capacity for symbiotic relationship, but some of them showed poor growth in these soils (Merkl et al. 2005; Kaimi et al. 2007). In addition to these plants, some other species are also used for phytoremediation of petroleum pollutants that the result was positive. *Mirabilis jalapa* is one of these species that show a high potential for phytoremediation and can be effectively used for phytoremediation of highly contaminated soils (Peng et al. 2009). Hence, it is necessary to test the efficiency of other plants from other families for phytoremediation of petroleum-contaminated soils.

A long exposure to pollutants may prolong the toxic effects of petroleum compounds on the plant, since the pollutants can accumulate and persist for a long period of time (Merkl et al. 2005). Hence, some species may germinate and grow in contaminated soils in a short time, but they died when exposed to contaminants for a long time. The sensitivity of plant to contaminants can limit the phytoremediation of petroleum-contaminated soils due to reduced plant growth, soil fertility, and bacterial numbers. Plant root exudates including amino acids, simple carbohydrates, vitamins, alkaloids, tannins, phosphatides, and other organic materials induce some microbial metabolites and increase bacterial communities in soils which results in higher efficiency of contaminant biodeterioration (Mehmannavaz et al. 2002). Not only fertilizers can increase the growth of plants resulting in higher biomass production but also enhance the bacterial growth and their activities since sufficient fertilizers in contaminated soils reduce the competition for nutrients. However, it should be considered that the high levels of fertilizers may damage plants due to the hydrophobic nature of petroleum-contaminated soils and accumulation of the fertilizers on the soil surface (Merkl et al. 2005).

In addition to root exudates that stimulate the growth and activities of rhizosphere bacteria, some degradative enzymes including dehalogenases, laccases, nitrilases, nitroreductases, tyrosinase (monophenol monooxygenase), and peroxidase are also released from roots into the rhizosphere (Alkorta and Garbisu 2001; Gianfreda and Rao 2004). Different plants have been studied for the exudation of enzymes from their roots, and it has been revealed that several members of Solanaceae, Gramineae, and Fabaceae are able to efficiently secrete various enzymes into the rhizosphere (Gianfreda and Rao 2004). There is no doubt that these enzymes play a pivotal role in phytoremediation of organic compounds, but there is a paucity of information on the amount of secreted enzymes which lead to the deterioration of organic substrates, although the calculation of half-life of the enzymes suggests that they are able to remain active and degrade the compounds for days (Alkorta and Garbisu 2001). Frick et al. (1999) states *n*-alkanes can be assimilated in both roots and leaves of plants and the pathway of conversion for *n*-alkanes is generalized as:

$$\begin{aligned} n\text{-alkanes} &\rightarrow \text{Primary alcohols} \rightarrow \text{fatty acids} \rightarrow \text{acetyl-CoA} \\ &\rightarrow \text{various compounds} \end{aligned}$$

2.4.3 Plant-Bacteria Interactions

Although both bacteria and plants can directly and indirectly degrade hydrocarbons into products in petroleum-contaminated soils independently from each other, it is thought that the interaction between bacteria and plants is the primary mechanism in phytoremediation (Frick et al. 1999). Plant-bacteria interactions are important processes defining the efficiency of phytoremediation of petroleum-contaminated soils; however our knowledge about the mechanisms of these interactions remains very incomplete. The interactions between bacteria and plants can be divided into two types, specific and non-specific interactions depending on root exudates. Specific interactions occur when the roots exude the specific compound(s) in response to the presence of specific pollutant and the plant provides a specific carbon source for the bacteria, but in non-specific interactions, normal plant processes stimulate the bacterial cells. Both of these interactions enhance the biodegradation rate of organic compounds by bacteria associated with plant roots, and in return, the capacity of plants increases for petroleum biodegradation or reduces the phytotoxicity of the contaminated soil (Siciliano and Germida 1998). As mentioned above, the plant also releases some enzymes from roots that play a pivotal role in deterioration of organic compounds and altering the environment to promote bioremediation by bacteria (Frick et al. 1999). In fact, the external degradation of contaminants by rhizosphere bacteria is a way to reduce the harmful effects of petroleum compounds, and it is believed that plants and bacteria have coevolved to develop a mutually beneficial strategy to reduce phytotoxicity (Frick et al. 1999). Plant roots enhance the degradation of contaminants by affecting the chemical and physical properties of the soil.

The presence of roots in the contaminated soil brings plants, bacteria, pollutants, and nutrients into contact with each other.

It has been shown that the interaction between plants and biodegraders is relatively stronger during vegetative phase than reproductive phase. The roots have very important roles in interaction between plants and bacteria, and its traits vary faster than other organs in response to the presence of contaminants in the soil (Nie et al. 2011).

2.4.4 Limitations of Petroleum Phytoremediation

The depth of contamination is a limitation factor for phytoremediation and it can be effective only in the surface area (Anyasi and Atagana 2011). The roots of most plants, except trees, cannot penetrate very deeply into the soil, and root density decreases with increase in depth. In addition, there is an increase in immobile pollutants that cannot be taken up by the roots when the depth increases more than 2 m that can negatively affect the efficiency of phytoremediation.

In spite of positive aspects of phytoremediation, it is a slow process and needs a long period, usually several years, to clean up contaminated soils (Van Epps 2006). The time required will increase in the presence of hydrophobic contaminants which are tightly bound to soil particles. There are some approaches such as chemically enhanced phytoextraction, application of genetically modified plants, and developing plant-bacteria system that may be used to enhance the phytoremediation rate (Oh et al. 2013).

The high concentrations of contaminants may not allow plants to grow and extend the roots due to oxidative stress and toxic effects (Van Epps 2006). Hence, this technology can be effective when used to clean up low contaminant concentrations (Yavari et al. 2015). In highly contaminated soils, the contamination should be reduced before applying phytoremediation to remediate contaminated sites, and then it can be used as a final treatment (Yavari et al. 2015).

Environmental factors such as temperature, soil texture, oxygen availability, pH, salinity, and nutrient availability can affect the efficiency of phytoremediation (Frick et al. 1999; Brandt et al. 2006). The effectiveness of phytoremediation will be enhanced when the environmental conditions are optimum for plant growth. For example, phytoremediation is not an effective technology in low-temperature environment forasmuch as the plant growth is slow or stopped (Frick et al. 1999; Brandt et al. 2006; Yavari et al. 2015). It has been shown that the sufficient nutrient can enhance plant growth parameters in contaminated soils that results in increase of phytoremediation rate (Brandt et al. 2006).

The chemical properties of contaminants also play important roles in phytoremediation efficiency. Water solubility of contaminants is an important factor in biodegradation of them; i.e., the more water-soluble the contaminant, the more rapidly it is biodegraded (Frick et al. 1999). The compounds with smaller molecular weight are more easily dissolved in water and biodegraded by living organisms (Yavari et al. 2015). Although, water solubility of the compound is a favorable property, but it may cause leaching of compounds and resulting in contamination of underground water (Frick et al. 1999).

2.5 Conclusion

In conclusion, the removal of contaminants from the environment by bacteria (bioremediation) and plants (phytoremediation) has been used as a reasonable approach to clean up the soil in recent decades. However, this technology has developed into an acceptable alternative to physical methods, but numerous biotic and abiotic factors involved in the successful implementation of the technology. Hence, even under optimal conditions, it is unlikely that petroleum contaminants have been completely removed from contaminated sites. It is important to keep in mind that when bioremediation is applied to a contaminated site, increased biodeterioration may not immediately occur due to various factors. Some new strategies, such as the use of GMB, may increase the efficiency of bioremediation, but the stability of GMB and the horizontal transfer of the recombinant DNA should be considered. Finally, recent advances in our knowledge of associations between plants and bacteria and the mechanisms by which bioremediation occur can help us develop practical soil bioremediation strategies.

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Recent Advances in Microbial Remediation of Textile Azo Dyes

3

Amrit Saini, Alisha Doda, and Baljinder Singh

Abstract

Azodyes, as used in textile, paper, food, leather, cosmetics and pharmaceutical industries, are major environment pollutants. Existing chemical and physical methods are not able to remove these recalcitrant dyes completely. But microbial methods, on the other hand, have the potential to decolorize as well as completely mineralize these azodyes. Moreover these methods are inexpensive, ecofriendly and are applicable to a large range of azodyes. This chapter focuses mainly on bacterial methods for decolourization and degradation of azo dyes. Different bacterial strains give optimum result at different physiological conditions. Also, different strains use different enzymes for decolourization as well as degradation. Individual strains as well as consortia can be used for the decolourization and degradation. We summarize the bacterial strains isolated, enzymes identified, general mechanism of action and methods involved in decolourization and degradation of these dyes.

3.1 Introduction

Azo dyes represent an important class of synthetic dyes characterized by presence of one or more chromophoric azo group (-N=N-). They are extensively utilized as coloring agents in textile, food, paper, printing, leather and cosmetic industries (Chang and Lin 2001). Based on the number of azo groups present, these dyes can be classified into monoazo dyes (e.g. Reactive yellow 201, acid orange 52 etc.), diazo dyes (e.g. acid black 1, reactive brown 1 etc.), trisazo dyes (e.g. direct black 19) and polyazodyes (direct black 80) (Jeong 2008).

Authors Amrit Saini and Alisha Doda have been equally contributed to this chapter.

A. Saini · A. Doda · B. Singh (✉)
Department of Biotechnology, Panjab University, Chandigarh, India
e-mail: sbaljinder@pu.ac.in

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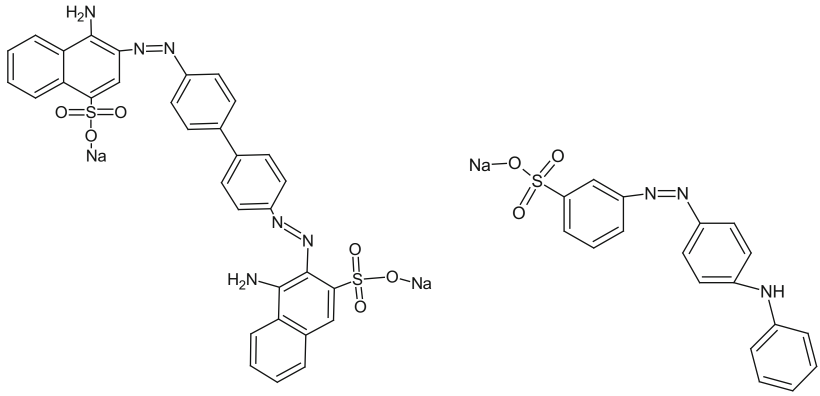
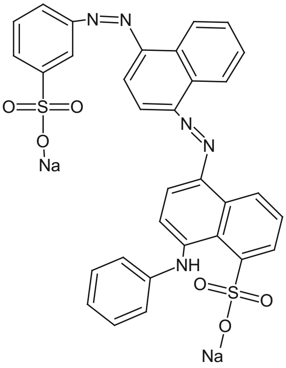


Congo red (C.I. Name Direct Red 28)

Metanil Yellow

Methyl Red

Disperse Red 78



Ranocid Fast Blue (C.I. name
Acid Blue 113)

Fig. 3.1 Structures of some common azo dyes. Congo red (C.I. Name Direct Red 28)

Among 100,000 dyes present, over 2000 are azo dyes, making them the largest class of synthetic dyes currently in use. Worldwide production of dyes is 70,000 tones annually, of which more than 50% are azo dyes (Vijaykumar et al. 2007). The structures of some common azodyes are shown in Fig. 3.1. It has been estimated that approximately 10–15% of the total dyes following dyeing process in textile, plastics and biomedical industries is discharged into the environment as effluents (Asad et al. 2007).

Azo dyes absorb light in the visible region of electromagnetic spectrum because of the presence of azo group (Chang et al. 2000). Release of these colored dyes into water bodies is not only aesthetically unpleasant but it also leads to a reduction in sunlight penetration, which in turn results in decreased photosynthetic activity, dissolved oxygen concentration and pH levels, damaging the aquatic ecosystem (Molina-Guijarro et al. 2009). In addition, many azo dyes and their metabolites (colorless amines), produced by reductive cleavage of azo linkages, are toxic, carcinogenic and mutagenic to living organisms (Singh et al. 2015). Thus presence of these dyes in the water ecosystem is the cause of serious environmental and health problems. Therefore, treatment of industrial effluents containing azo dyes and their breakdown products is necessary prior to their final discharge in the environment.

Different factors which reduce degradation of these dyes by natural environmental activities include high water solubility, high molecular weight, fused aromatic ring structures and xenobiotic nature (Singh et al. 2014; Mohamed 2011). The recalcitrance of these dyes is because of the azo groups (–N=N–) which is not found naturally in nature (Bafana et al. 2008). Thus conventional wastewater treatment remains ineffective. Although several physicochemical methods are available for treatment of textile effluents such as adsorption, coagulation or flocculation techniques, ion extractions etc., these approaches have serious drawbacks like high cost, low efficiency, limited versatility, production of large amounts of sludge and handling of the effluents generated (Saroj et al. 2014).

Biological treatment methods consist of enzymatic methods (using enzyme isolates) and microbial methods (using microorganisms) (Saratale et al. 2011). Synthetic dyes can be treated using enzyme isolates, but production cost of enzymes at large scale is quite high and also the activity levels of these isolates decrease very rapidly under non sterile conditions (Saroj et al. 2014). Therefore, in such a scenario, microbial treatment method is a real hope. Microbial decolourization and degradation is an eco-friendly, cost-competitive and less sludge producing alternative to physiochemical treatment methods. (Rai et al. 2005; Verma and Madamwar 2003; Chen 2006). Different taxonomic groups of microorganisms such as bacteria, fungi, yeast, and algae have developed enzyme systems for the decolourization and mineralization of azo dyes under certain environmental conditions (Mohamed 2011). In this chapter we shall focus on biological methods for the treatment of textile effluent containing azo dyes, and thus describe their importance.

3.2 Toxicity of Azo Dyes

To reduce the adverse effects of azo dyes, the study of their toxicity is very important. Azo Dyes constitute the largest group of colorants that has been certified by Food and Drug Administration (FDA) (Puvaneswari et al. 2006). The entry of these colorants into environment is generally through their own manufacture process but there are also some other methods through which they can enter into the environment (Ratna 2012). The various toxic effects of azo dyes are summarized in Table 3.1.

Toxicity of azo dyes is derived from the reductive cleavage of one or more azo groups by azoreductase leading to release of one or more aromatic amines (Smith and John). This toxicity of azo dye due to reductive cleavage is usually only feasible with exposure to certain ecological conditions and anaerobic bacteria, the most common of which is present in the microbiota of our intestine and digestive tract of various mammals (Smith and John; Pinheiro et al. 2004). Bacterial azoreductases reduce azo dyes more actively than hepatic azoreductases (Puvaneswari et al. 2006).

In aquatic environment, these dyes undergo biological and photochemical degradation which may cause serious environmental problems (Ventura-camargo and Marin-morales 2013; Ratna 2012). The reduced product may be more or less harmful than the original dye depending on its chemical structure (Ventura-camargo and Marin-morales 2013; Ratna 2012). The reduction of azo dye generally gives rise to potential toxic mutagenic and carcinogenic amines (Puvaneswari et al. 2006; Ratna 2012). The reduction product includes DNA adducts which can be toxic even for microorganisms which are responsible for discoloration of azo dyes (Ventura-camargo and Marin-morales 2013).

Various azo dyes are known to cause human bladder cancer, hepatocarcinomas, splenic sarcomas and nuclear anomalies in experimental animals and are also known to cause chromosomal aberration in mammalian cells (Ratna 2012). Liver nodules are also caused by some azodyes in experimental animals (Puvaneswari et al. 2006).

Various assays can be performed to evaluate the toxicity of azo dyes. Some of most commonly used bioassays are bioluminescence and respirometric methods (Ratna 2012).

3.3 Biodegradation Azo Dyes

Generally, the bacterial biodegradation takes place in two stages (Fig. 3.2):

3.3.1 Reduction of Azo Dye

It involves reductive cleavage of dye’s azo linkages, resulting in the formation of aromatic amines which are generally colourless and potentially hazardous. It usually requires anaerobic conditions. It is non-specific and presumably extracellular process in which reducing equivalents from either chemical or biological source are transferred to the dye. Various bacteria can be involved in this stage like ɣ-proteobactera (Pandey et al. 2007), sulphate reducing bacteria (Pandey et al. 2007), *Pseudomonas putida* (Chengalroyen and Dabbs 2013), *Clostridium* sp. (Chengalroyen and Dabbs 2013), etc.

Table 3.1 Some examples of azo dyes and their toxic effects

S. No.	Dye	Toxic effects	References
1.	Malachite green	Potential genotoxic and carcinogenic agent and affects reproductive and immune system	Ratna (2012)
2.	CI disperse blue	Frame-shift mutation and base pair substitution in *Salmonella*	Ratna (2012)
		In humans, genotoxic and cytotoxic effects are seen, foration of micronuclei is there due to chromosomal breakage (clatogencity) and aneuploidy. Also, DNA fragmentation due to double and single strand breaks is also observed	
3.	p-dimethylaminobenzene (p-DAB)	Cytotoxic and genotoxic effects in the chromosome aberrations test, micronucleus test, and mitotic index in the bone marrow cells and spermatozoids of rats	Ventura-Camargo and Marin-morales (2013)
4.	Pigment red 3	Weakly mutagenic	Ventura-Camargo and Marin-morales (2013)
5.	Benzidine	Carcinogenic	Ratna (2012) and Puvaneswari et al. (2006)
6.	Tartrazine and Carmoisine	Can induce oxidative stress by forming free radicals	Ventura-camargo and Marin-morales (2013)
7.	1-amino-naphthalene (1-AN)	Carcinogen	Puvaneswari et al. (2006)
8.	Disperse red 1 and disperse orange 1	Increase the frequency of micronuclei in human lymphocytes and HepG2 cells (genotoxic)	Chequer et al. (2009)
9.	Disperse red 13	Shows mutagenic potential by inducing chromosomal damage in human lymphocytes. Also, its reduction and oxidation products showed mutagenic activity as detected by *Salmonella*/microsome assay	Chequer et al. (2015)
10.	Acid violet 7	Shows significant ability to induce chromosomal aberrations, lipid peroxidation and acetylcholinesterase inhibitory effect	Ben et al. (2010)
11.	Scarlet RR	Produces various chromosomal aberrations and depression in mitotic index in time and dose dependent manner in root tips cells of *Allium cepa* (cytotoxic and mutagenic)	Dutta (2015)

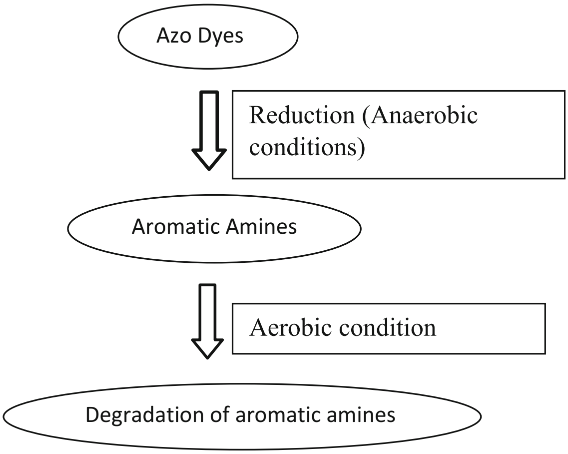


Fig. 3.2 General overview of biodegradation of azo dye

3.3.2 Degradation of Aromatic Amines

Bacterial biodegradation of aromatic amines usually occurs almost exclusively under aerobic conditions. The organisms generally involved in this stage are *Sphignomonas* sp., *Pseudomonas aeruginosa*, *Xenophilus azovorans* KF 46, *Pigmentiphaga kullae* K24 (previously, *Psuedomonas* sp. K24), etc. (Pandey et al. 2007).

3.3.3 Decolourisation

Removal of color is mainly associated with the anaerobic reduction of the azo dyes whereas further decolourization during the aerobic degradation is usually limited to a few extra percents (Van Der Zee and Villaverde 2005). The aerobic decolourization of azo dyes depend upon the oxidation potential of microorganisms. The rate of decolourization is dependent on the dye structure as well as added organic carbon source (Pandey et al. 2007). Dyes with lower molecular weight and simple structure generally exhibit higher rate of decolourization whereas high molecular weight dyes experience difficulty in decolourization (Patel et al. 2012; Franciscon et al. 2009). Several bacteria are also there which can decolorize the dye under aerobic conditions but many of these dyes cannot utilize dye as growth substrate and require organic carbon sources (Pandey et al. 2007).

The decolorizing activity is expressed in terms of percentage decolourization.

$$\%\text{Decolorization} = \left\{\frac{\text{Initial Absorbance-Final Absorbance}}{\text{Initial Absorbance}}\right\} \times 100$$

(Prasad and Rao 2011)

$$\text{Average Decolourization Rate} = \frac{C \times \%D}{100 \times \tau}$$

(Saratale et al. 2013)
Where,

C=Initial concentration of dye (mg/l)
$\%D$= Dye decolourization (%) after time t (h)

Absorbance is taken at different wavelengths for different dyes.

As the concentration of dye increases the decolourization efficiency decreases. This may be due to the toxic effects of dye on bacteria and/or less bacterial biomass for the uptake of higher dye concentration (Saratale et al. 2011). Both individual strains and microbial consortium can be used for the decolourization of azo dyes. In microbial consortium, the individual strains may attack the dye molecules at various positions or they may utilize the degradation metabolites generated by co-existing strains for further degradation which results in enhanced decolourization of dyes (Saratale et al. 2011).

Various microorganisms capable of degrading azo dyes are shown in Table 3.2. Enzymes produced by bacterial and fungal cultures are used for degradation of azo dyes. These enzymes can be non-specific or specific, catalyzing only azo dye reduction (Pandey et al. 2007). Some of these enzymes are azoreductase, laccases (multicopper phenol oxidases), peroxidises, polyphenol oxidases, etc. (Singh et al. 2015).

Following are some of the common examples related to degradation of azo dyes by microorganisms.

3.4 Congo Red, Bordeaux, Ranocid Fast Blue and Blue

The development of bacterial consortia SKB II from the samples obtained from the waste disposal sites from textile industry indicates the natural adaptation of these organisms to survive in the presence of toxic dyes. As the concentration of these dyes is increased, the decolourization capability of SKB II decreases. This bacterial consortium can also decolorize Remazol golden yellow RML and Lavender red but only to a very minimal level but despite the minimal decolourization, the culture showed minimal decolourization in the presence of these two dyes in the basal medium indicating they were not toxic to the culture. Starch is the favored supplementary carbon source for efficient degradation of azo dyes in basal media for this consortium (Tony et al. 2009).

Table 3.2 Bacterial Strains involved in azo dye decolorization and degradation

Sr. No.	Azo dyes	Microorganisms	Isolation source	Conditions [temperature, pH, agitation]	Degradation Pathway	Percentage degradation	Detection techniques	Metabolites	References
1.	Congo red, Bordeaux, Ranocid fast blue and blue BCC	SKB-II (bacterial mixed culture) consisting of 5 strains namely *Bacillus vallismortis, Bacillus pumilus, Bacillus cereus, Bacillus subtilis* and *Bacillus*	Textile wastewater treatment plant	37;	Reductive	50–60% decolorizatoin of dye mixture at 10 mg L^{-1} concentration	UV-Vis	–	Tony et al. (2009).
		Megaterium		7.0; Rotator shaking (130 rpm)		86–90% individual dye decolorization at 10 mg L^{-1} dye concentration by the end of 120 h			
2.	Reactive black 5, reactive Orange 16, disperse red 78 and direct red 81	Bacterial consortium consisting of *Providenciarettgeri*strain HSL1 and *Pseudomonas* sp. SUK1	Textile effluent contaminated soil	30 ± 0.2;	Reductive;	98–99% decolorization of dye mixture at 100 mg/ml concentration within 12–30 hunder microaerophilic, sequential aerobic/ microaerophilic and microaerophilic/ aerobic processes	UV-Vis	–	Saratale et al. (2011)
				7.0; For aerobic conditions shaking at 120 rpm	Oxidative	62–72% degradation in all the dyes decolorized broths under sequential microaerophilic/ aerobic processes			

3.	Reactive black-B	*Morganella* sp. HK-1	Dye contaminated industrial landfill	30; – Static	Reductive	Complete degradation at 20 g/L dye concentration within 24 h	UV-vis, FTIR and GC-MS	Disodium 3,4,6-triamino-5-hydroxynaphthalene-2,7-disulfonate, 4-aminophenylsulfonylethyl hydrogen sulfate, naphthalene-1-ol, aniline and benzene	Pathak et al. (2014)
4.	Reactive blue 160	BDN (bacterial mixed culture) Consisting of 8 strains namely *Alcaligenes* sp. BDN1, *Bacillus* sp. BDN2, *Escherichia* sp. BDN3, *Pseudomonas* sp. BDN4, *Provedencia* sp. BDN5, *Acine-tobacter*sp. BDN6, *Bacillus* sp. BDN7 and *Bacillus* sp. BDN8	Soil samples con-taminated with anthropogenic activities	37; 7.0; Microareophillic conditions along with yeast extract (0.5%)	Reductive Oxidative	Complete decolourization and degradation at 100 mg/L dye concentration within 4 h	UV-Vis, FTIR, HNMR, HPTLC and GC–MS	1-phenylmethanediamine, benzene sulfonate, benzene-1,4-disulfonate, benzene and aniline	Balapure et al. (2014)
5.	C.I. Remazol red	*Lysinibacillus* sp. RGS	Soil of Ichalkaranji textile industrial area	30; 7.0; Static	Reductive Oxidative	Complete decolorization and degradation within 6 h	UV-Vis HPLC FTIR GC-MS	4-[(6-amino-4-chloro-1,2,3,4-tetrahydro-1,3,5-triazin-2-yl) amino] decahydronaphthalene-2,7-disulfonate], 4-[(4-chloro-1,3,5-triazin-2-yl)amino] naphthalene-2,7-disulfonate], 4-chloro-1,3,5-triazin-2-amine and 1,3,5-triazine as a final product	Saratale et al. (2013)
6.	Acid maroon V	EDPA bacterial consortium consisting of *Enterobacter dissolvens*AGYP1 and *Pseudomonas aeruginosa*AGYP2	Dye contaminated site	30; Range of pH values (6.0–9.0) with peak activity at pH 7.0; Static	Reductive	93% decolorization of dye (100 mg/L) in 20 h COD reduced by 31%	UV-Vis, HPTLC and FTIR	–	Patel et al. (2012)

(continued)

Table 3.2 (continued)

Sr. No.	Azo dyes	Microorganisms	Isolation source	Conditions [temperature, pH, agitation]	Degradation Pathway	Percentage degradation	Detection techniques	Metabolites	References
7.	Red 3BN	Two bacterial species *Bacillus cereus* and *B. megaterium*	Dying industry located at Peenya, Bangalore (Karnataka)	Optimal condition for *B. cereus* 37; 7.0; shaking (120 rpm) Optimal condition for *B. megaterium* 37; 6.0; shaking (120 rpm)	Reductive	Decolorization 93.64% and 96.88% respectively for *B. cereus and B. megaterium*	UV-Vis	–	Praveen and Bhat (2012)
8.	Reactive violet 5R	SB4 (bacterial mixed culture) consisting of 6 strains namely *Bacillus* sp. V1DMK, *Lysinibacillus* sp. V3DMK, *Bacillus* sp. V5DMK, *Bacillus* sp. V7DMK, *Ochrobacterium* sp. V10DMK, *Bacillus* sp. V12DMK	Anthropogenic dye contaminated soil	37; 7.0; Static; Microareophilic conditions	Reductive	Decolorized 200 mg/L of RV5 within 18 h	FTIR, NMR and GC-MS	1-diazo-2-naphthol, 4-hydroxybenzenesulphonic acid, 2-naphthol and benzenesulphonic acid	Jain et al. (2012)
9.	Methyl red	*Sphingomonaspaucimobilis*	Effluent treatment plant of a textile and dyeing industry	30; 9.0; Shaking		99.63% decolorization at 750 ppm dye concentration within 10 h	UV-Vis, FTIR	–	Ayed et al. (2011)
10.	Acid orange 10	*Pseudomonas putida*	Purchased from NCL, Pune, India	37; 7.0; Static	Reductive	90% decolorization at 250 mg/ml dye concentration within 24 h	UV-Vis	–	Tripathi and Srivastava (2011)
11.	Hexavalent chromium and reactive black-5	Bacterial strainsKI (*Pseudomonas putida*) and SL14 (*Serratiaproteamaculans*)	Tannery wastewater and sludge	35; 7.2; Static	Reductive	Simultaneously reduced 93% of hexavalent chromium (2 mg/L) and 100% of reactive black 5 (100 mg/L) within 12 h	UV-Vis	–	Mahmood et al. (2013)

12.	Metanil yellow	*Bacillus* sp. strainAK1 and *Lysinibacillus* sp. strainAK2	Dye contaminated soil sample collected from dyeing industry	40; pH range 5.5–9.0; optimum pH 7.2; Static	Reductive	Individual bacterial strains *Bacillus* sp. AK1 and *Lysinibacillus* sp. AK2 decolorized Metanil yellow (200 mg/L) completely within 27 and 12 h respectively	UV–Vis, TLC, HPLC and GC/MS	Metanillic acid, p-aminodiphenylamine,	Anjaneya et al. (2011)
13.	Remazol black-B	Bacterial isolate SS1	Dye-contaminated textile wastewater	35; 7.0; –	–	Complete decolorization of dye (100 mg/L) in 18 h	UV-Vis	–	Mohamed (2011)
14.	Scarlet R	Microbial consortium-GR, consisting of *Proteus vulgaris* NCIM-2027 (PV) and *Micrococcus glutamicus* NCIM-2168 (MG)	National Chemical Laboratory (NCL, Pune, India)	37;	Reductive	Complete decolorization with an average decolorization rate of 16,666 μg/h	UV-Vis, FTIR, HPLC, and GC-MS	1,4-benzenediamine	Saratale et al. (2009)
				7.0;static anoxic condition		Over 90% TOC and COD reduction within 3 h			
15.	Reactive yellow 107, reactive red 198, reactive black 5 and direct blue 71	*Klebsiella* sp. strain VN-31	Activated sludge process of the textile industry	For decolorization:	Reductive	>94% decolorization within 168 h; 100 mg/L dye concentration	UV-Vis and FTIR	–	Franciscon et al. (2009)
				30; 7.0; microaerophilic conditions for degradation: Aerobic conditions (stirring)	Oxidative	TOC reduction ~50% in microaerophilic stage and ~80% in aerobic stage			

3.4.1 Reactive Red Black 5, Reactive Orange 16, Disperse Red 78 and Direct Red 81

Individual strains of *Providencia rettgeri* HSL1 and *Pseudomonas* sp. SUK1 as well as their consortium was unable to completely decolorize these dyes (100 mg/ml) under aerobic conditions and only 12–22% performance was achieved within 48 h. On the other hand, individual cultures were able to completely decolorize Reactive Orange 16 and Disperse Red 78 and partially decolorize Direct Red 81 and Reactive Red Black 5 in microaerophilic conditions. Dyes with monoazo bonds most likely decolorize faster than dyes with diazo or triazo group. Because of this reason, Reactive Orange 16 and Disperse Red 78 (both monoazo dyes) show shorter decolourization time than highly substituted diazo dyes Reactive Red Black 5 and Direct Red 81 (Saratale et al. 2011).

3.4.2 Reactive Black-B

This dye has a multi sulfonated complex structure comprising of naphthalene and benzene rings and one or more azo linkages. The dye is decolorized by the isolate *Morganella* sp. HK1 by enzymatic reduction with azoreductase enzyme. The metabolites obtained after the degradation of Reactive Black-B are non-phytotoxic and non-cytotoxic in nature (Pathak et al. 2014).

3.4.3 Reactive Blue 160

Mixed culture BDN is used for decolorizing Reactive Blue 160. It consists of eight bacterial strains and was developed through culture enrichment method from soil samples contaminated with anthropogenic activities. Maximum dye decolourization often requires optimum growth conditions and BDN is very well adapted for a wide range of environmental conditions. Azoreductase activity in the strains BDN4, BDN7, BDN5 and BDN6 is higher as compared to the other five strains so they carry out the activity of azo dye reductionBDN6, BDN8, BDN5 and BDN1 exhibit higher lignin peroxidase activity whereas BDN6, BDN5 and BDN1 exhibit higher laccase activity. Therefore, the reduced dye products can further be detoxified by the collective action of oxidative enzymes where each bacterium of BDN plays their respective significant role in dye mineralisation. RB160 is symmetrically cleaved into lower molecular weight compounds. The toxicity of this dye is generally to original compound instead of its biotransformed products (Balapure et al. 2014).

3.4.4 C.I. Remazol Red

Remazol Red can be decolorized using *Lysinibacillus* sp. RGS. *Lysinibacillus sp. RGS* can also decolorize Golden Yellow HER. It can decolorize Remazol Red more efficiently under static conditions. There is no change in pH under static conditions which indicate that decolourization is due to microbial action and not because of pH. The *Lysinibacillus* sp. RGS can decolorize up to 90% in the broad pH range (7.0–9.0) and temperature (30–35 °C) but maximum decolourization was achieved at pH 7.0 and temperature 30 °C. *Lysinibacillus* sp. RGS is more efficient in decolorizing Remazol Red in the presence of additional carbon and nitrogen source and extract of agricultural by-products. The decolourization is more efficient in the presence of nitrogen sources like peptone and beef extract as compared to decolourization in the presence of carbon sources. Oxidoreductive enzymes are primarily involved in the decolourization process. Non toxic residual metabolites are formed during decolourization process (Saratale et al. 2013).

3.4.5 Acid Maroon V

It is a sulfonated di-azo dye which can be decolourized by consortium EDPA. The decolourization of Acid Maroon V using consortium EDPA is correlated with dissolved oxygen content and is independent of biomass concentration. Static condition is preferred for effective decolourization of the dye. The consortium EDPA can work in broad range pH (4.0–10.0) and the optimum pH is 7.0 and the optimum temperature is 30 °C. The decolourization performance of consortium EDPA is better in the presence of sucrose [0.4% (w/v)] than in the presence of commonly metabolizable sugar glucose. Also, the decolourization capability improves in the presence of ammonium dihydrogen phosphate. An addition of co-substrates like carbon and/or nitrogen enhance the decolourization efficiency of the consortium by allowing faster growth of actively respiring bacteria resulting in the rapid depletion of oxygen from medium favouring anaerobic reduction. The parent compound, that is, Acid maroon V is considerably more toxic as compared to its metabolites (Patel et al. 2012).

3.4.6 Red 3BN

Sucrose or glucose is the ideal carbon source and peptone or yeast extract is the ideal nitrogen source for the activity of *B. cereus* under in vitro condition.

Optimal conditions for *B. cereus*- 1% sucrose, 0.25% peptone, pH 7, 37 °C and 8% inoculum.

Optimal conditions for *B. megaterium*- 1% glucose, 0.25% yeast extract, pH 6, 37 °C and 10% inoculums. Under ideal conditions *B. megaterium* is better than *B. cereus* (Praveen and Bhat 2012).

3.4.7 Reactive Violet 5R

This dye can be decolorized using bacterial mixed cultures SB4.The decolourization is efficient under static conditions. Glucose (1 g/L) is the preferred source of carbon and yeast (1 g/L) extract the preferred source of nitrogen. SB4 can work within a broad range of pH (5–8.5) but optimum pH is 7 and optimum temperature is 37 °C. SB4 can decolorize various dyes at a time. Individual organisms of the bacterial mixed culture failed to decolorize the dye even when the individual strains were taken at very high concentration. So, the higher decolourization efficiency can be attributed to the synthrophic and catabolic interactions of indigenous species leading to complete degradation of azo dye (Jain et al. 2012).

3.4.8 Methyl Red

Methyl Red can be decolorized by using *Sphingomonas paucimobilis*. *S. Paucimobilis* can degrade Methyl red in a wide range of temperature (5–40 °C) and pH (3–11) but the optimum temperature is 30 °C and optimum pH is 9. The decolourization occurs more efficiently in shaking condition. The metabolites formed after the degradation of azo dyes are non-toxic in nature (Ayed et al. 2011).

3.4.9 Acid Orange 10

Pseudomonas putida is the most potent decolourizer for Acid orange 10. The optimum temperature for decolourization is 37 °C and optimum pH is 7.0 in static conditions. The decolourization efficiency of *P. putida* is also good in alkaline conditions (Tripathi and Srivastava 2011).

3.4.10 Hexavalent Chromium and Reactive Black-5

Bacteria which are capable of removing toxic Hexavalent chromium and Reactive Black-5 simultaneously can be isolated in liquid mineral salt medium (MSM). The optimum temperature and pH for bacterial strains KI (*Pseudomonas putida*) and SL14 (*Serratia proteamaculans*) for reduction of both the dyes simultaneously are 35 °C and 7.2 respectively. The reduction is carried out efficiently under static (batch) condition. These organisms can reduce the dyes completely in liquid medium but the efficiency decreases in broth medium (Mahmood et al. 2013).

3.4.11 Metanil Yellow

It is a sulfonated azo dye. Two strains can be used for decolourization of Metanil Yellow – *Bacillus* sp. Strain AK1 and *Lysinibacillus* sp. Strain AK2. For best results, the decolourization should be carried under static conditions. The decolourization of this dye involves the activity of enzyme azoreductase. The decolourization can take place in a broad range of pH (5.5–9.0) but the optimum pH is 7.2 and the optimum temperature is 37 °C but decolourization efficiency increases as temperature increases up to 40 °C. If temperature increases above 40 °C, the efficiency of decolourization decreases. The metabolite is very less toxic as compared to the parent dye (Anjaneya et al. 2011).

3.4.12 Remazol Black-B

The bacteria, SS1 which can be used to decolorize Remazol Black-B, is enriched by using MSM medium with an azo dye Remazol Black-B as sole carbon source. For optimal results, Yeast extract is used as carbon source for the decolourization of the dye. The decolourization efficiency is considerable between 25 and 30 °C and pH 7–9 but the optimum temperature and pH for the decolourization process are 35 °C and 7 respectively. If the pH rises above 7 or if the temperature increases above 35 °C then the decolourisation efficiency decreases (Mohamed 2011).

3.4.13 Scarlet R

The decolourization of Scarlet R s carried out by microbial consortium-GR under static condition. Faster decolourization occurs at 37 °C for both pure cultures of the consortium as well as for the consortium. For both the pure cultures and the consortium, highest decolourization activity was achieved over the range of pH (5–8) but the optimum pH is 7 while there is no decolourization activity at alkaline pH (9–12). Riboflavin reductase and NADH-DCIP reductase, both these enzymes are involved in the decolourization activity by microbial consortium-GR (Saratale et al. 2009).

3.4.14 Reactive Yellow 107, Reactive Red 198, Reactive Black-5 and Direct Blue 71

All these dyes can be decolorized and degraded in a sequential microaerophilic-aerobic process by a facultative *Klebsiella*sp strain VN-31. The decolourization of the strain is achieved in microaerophilic stage and no decolourization occurs in aerobic stage. This strain has an obligate requirement for pyruvate and glucose as supplementary carbon source as *Klebsiella*sp. Strain VN-31 cannot grow in the absence of glucose and pyruvate. Aromatic amines formed in microaerophilic stage are toxic but in aerobic stage the aromatic amines are degraded (non-toxic). A little percentage of toxic compounds was left of Reactive Red 198 even after the aerobic stage which may be due to the presence of triazine reactive group which persisted in the aerobic treated effluent due to its slower reaction rates (Franciscon et al. 2009).

3.5 Conclusion and Future Prospective

The degradation of azo dyes using microorganisms is more efficient, cost-effective, eco-friendly, method as compared to chemical and other conventional methods. Complete mineralization is possible using microorganisms for degradation which is not quite possible while using other methods. Incentivised research in this area may lead to development of better microbial strains or consortia for complete mineralization of azo dyes. The present chapter provide an important insight in determining the bioremediation potential of isolated bacterial strains and further improving the setup to clean the azo dyes contaminated sites by bioremediation. Further study is required at genetic level that provides insight into the molecular basis of the versatility bacterial strains and also identifying niches in which degrading genes prevail and the conditions under which the population of microbes bearing these genes increases.

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4

Application of Plant-Microbe Interactions in Contaminated Agroecosystem Management

Fredrick Fidelis Umaru and Chikezie I. Owuama

Abstract

Agroecosystem is often confronted with a variety of pollutants. The application of plant-microbe interactions in remedying the ecosystem is called phytoremediation. Within the rhizosphere, plant roots interact with microorganisms and the soil, and plants usually secrete substances which affect microbial growth. Some plant-microbe relationships are beneficial to the plant while others are not. However, these interactions largely ensure a healthy plant growth while eliminating plant pathogens from the soil either by separate or combined activities of the plant exudates and beneficial microbes. The nature of microbes associated with each plant is apparently related to the exudates and signal molecules emanating from the plant and the interactive signals of the microbes. Sometimes, the soil is contaminated either deliberately or inadvertently by a variety of chemicals and heavy metals. To control or eliminate these contaminants, chemical and physical means have largely been applied. Unfortunately, some of these control measures introduce their own contaminants thereby causing secondary contamination. This necessitates the need and application of eco-friendly and sustainable solar-driven technology, viz., phytoremediation, to restitute the soils. Microbe-plant interactions sometimes improve the absorptive capacity of the plant for contaminants. Some microbes modify soil contaminants by using organic acids, redox reactions, producing siderophores, metal chelators, biosurfactants, causing bioleaching, biosorption, and bioexclusion. These microbes-contaminants interactions boost the reduction of toxicity and elimination of contaminants via various phytoremediation processes, viz., phytostimulation, phytodegradation, phytoextraction/phytoaccumulation,

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F. F. Umaru (✉)
Department of Biological Sciences, Faculty of Science, Taraba State University, Jalingo, Nigeria

C. I. Owuama
Department of Microbiology, School of Life Sciences, Modibbo Adama University of Technology, Yola, Nigeria

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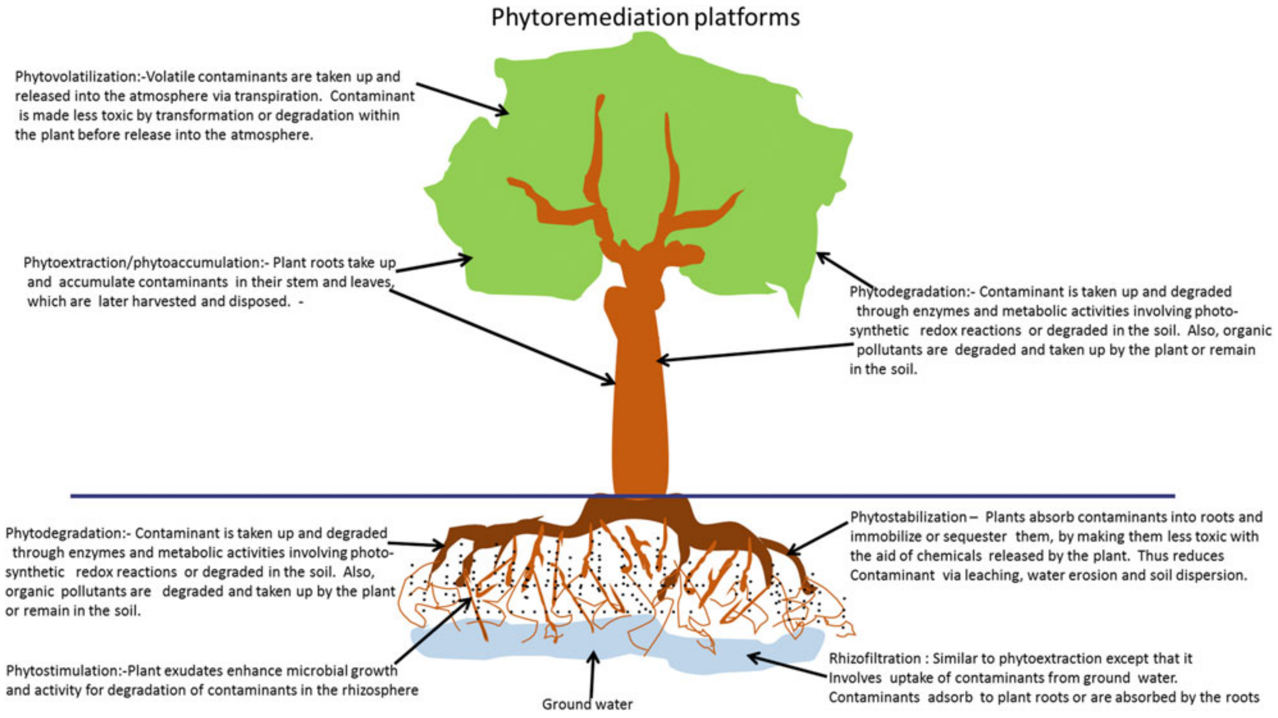
phytostabilization, phytovolatilization, and rhizofiltration. Nevertheless, phytoremediation faces certain major challenges as regards to its commercial-scale application in the field. To overcome these limitations, it is essential to have a better understanding of the relationships among plant microbes, soil types, chemicals, and heavy metal contaminants within an agroecosystem. Besides, it is important to develop phyto-hyper-accumulators and super microbial solubilizers, for various soil types.

4.1 Introduction

The agroecosystem is construed as a coherent functional unit of interactions that exist between living and nonliving components of the environment as well as their activities (Jalloh et al. 2012). The release of prevalent chemical contaminants into the environment due to increased anthropogenic activities has been on elevated levels. Chemical contaminants, such as polycyclic aromatic hydrocarbons (PAHs), petroleum hydrocarbons (PHCs), pesticides, halogenated hydrocarbons, metals, salts, and solvents, have been prompting stress on the ecosystem and human health (Gerhardt et al. 2009; Meagher 2000). The continuous discharge of pollutants into the environment poses a great threat to the microbiota, to flora and fauna, and also to human and animal health, as these chemicals are transferred down the food chain in the ecosystem (Esedafe et al. 2015). The pressure on production and application of agrochemicals either for yield improvement or insect and weed control has also caused perpetual stress and distortions in the ecosystems (Singh 2015; Vimal et al. 2017). The turnover of chemical contaminants in the ecosystem is a function of the viability and coherence of the functional interactions of the participating organisms in that environment. In-depth understanding of the processes involved in plant-microbe interactions will make the management of contaminated agroecosystems quite efficient (Fester et al. 2014). Some of these contaminants are recalcitrant and can persist for longer periods in the environment. Traditional methods of remediation employ the chemical or physical approaches which involve extraction of the contaminants onsite or removal of the contaminated soil. However, beneficial microbes and plants with tendencies for hyper-accumulation of metals have been identified as a promising approach for the cleanup of contaminants in the environment through phytovolatilization, phytostabilization, or phytoextraction processes (Glick 2010; Lebeau et al. 2008) (see Fig. 4.1).

Naturally, plants depend on soil, air, and water as essential ingredients for growth and development. They also interact with myriads of unicellular and multicellular organisms in mutualistic, pathogenic, or parasitic relationships (Ahmad et al. 2008; Lau and Lennon 2011; Siebers et al. 2016), which are triggered by exchange of molecular information when a microbial invader comes into contact with a host plant (Siebers et al. 2016). Microbes interacting with plant hosts can be found in the rhizosphere, root tissues, rhizoplane, and root nodules of leguminous plants (Antoun



Phytovolatilization:-Volatile contaminants are taken up and released into the atmosphere via transpiration. Contaminant is made less toxic by transformation or degradation within the plant before release into the atmosphere.

Phytoextraction/phytoaccumulation:- Plant roots take up and accumulate contaminants in their stem and leaves, which are later harvested and disposed. -

Phytodegradation:- Contaminant is taken up and degraded through enzymes and metabolic activities involving photo-synthetic redox reactions or degraded in the soil. Also, organic pollutants are degraded and taken up by the plant or remain in the soil.

Phytodegradation:- Contaminant is taken up and degraded through enzymes and metabolic activities involving photo-synthetic redox reactions or degraded in the soil. Also, organic pollutants are degraded and taken up by the plant or remain in the soil.

Phytostabilization – Plants absorb contaminants into roots and immobilize or sequester them, by making them less toxic with the aid of chemicals released by the plant. Thus reduces Contaminant via leaching, water erosion and soil dispersion.

Phytostimulation:-Plant exudates enhance microbial growth and activity for degradation of contaminants in the rhizosphere

Rhizofiltration : Similar to phytoextraction except that it Involves uptake of contaminants from ground water. Contaminants adsorb to plant roots or are absorbed by the roots

Fig. 4.1 Illustration of various phytoremediation processes involved in the removal of contaminants in an ecosystem and Prévost 2005). Microbes such as fungi, bacteria, actinomycetes, algae, and protozoa have been identified in close interactions with plants roots and demonstrated proven characteristics of enhancing growth and development of plant (Bhattacharyya and Jha 2012; Hayat et al. 2010; Saharan and Nehra 2011). The plant growth-promoting rhizobacteria (PGPR), including members of the genera *Bacillus*, *Enterobacter*, *Pseudomonas*, *Klebsiella*, *Azospirillum*, *Variovorax*, *Burkholderia*, *Azotobacter*, *Alcaligenes*, *Rhizobium*, *Xanthomonas*, *Proteus*, *Flavobacterium*, *Erwinia*, *Arthrobacter*, and *Serratia*, constitute the rhizosphere microbiota producing noticeable impacts on host plants (Kaymak 2010; Nadeem et al. 2014; Prasad et al. 2015). Other than bacterial species abundance, fungi also constitute a large proportion of the rhizosphere microflora valuable for enhanced plant growth. Fungal symbiotic mycorrhizae are fundamental at increasing the surface area of the root, which invariably results in more effective water and nutrient uptake from the soil (Prasad et al. 2017). Ecto- and endo-mycorrhizae are known to associate with a host of plant species. Other than facilitating water and mineral uptake, mycorrhizae also provide the host plant with protection from certain abiotic stress factors (Miransari 2010).

In their coevolutionary history, plants and microbes interact in either beneficial or detrimental manner for survival, a relationship that is inevitable for their individual existence. Exudates produced from these interactions greatly enhance movements of nutrients and metals by (1) enzymatic transfer of electrons in the rhizosphere, (2) acidification and formation of complexes due to released proton (H^+) molecule, and (3) indirect enhancement of microbial activity in the rhizosphere, resulting in effective phytoremediation (Pérez-Esteban et al. 2013; Sessitsch et al. 2013). Phytoremediation of heavy metal-contaminated soil is a promising relatively new technology that is eco-friendly, solar-driven, and potentially cost-effective. The technology aims at managing the agroecosystems by exploiting the healthy interaction between soil, plant roots, and microbes in the rhizosphere to rid the soil of heavy metals. The effectiveness of phytoremediation depends on soil temperature, moisture, nutrients, microorganisms, and herbivory as well as contaminant distribution, soil type, soil pH, soil texture, variety of plant roots, and metal uptake capacity (Vangronsveld et al. 2009).

4.2 Beneficial Plant-Microbe Interactions

In nature, the interactions between plants and microbes are diverse and dynamic due to their coevolutionary pressures (Chaparro et al. 2013). Beneficial plant-microbe interactions in the environment have been severally reported as one of the major drivers of a functional ecosystem (Nadeem et al. 2014; Rashid et al. 2016; Singh et al. 2016). These interactions significantly alter the microbiological, biochemical, and physicochemical parameters of the rhizosphere as it is evident that the microbial population in the root environment is usually more enriched than the surrounding environment. The beneficial plant-microbe relationship is a critical factor that helps to determine and improve plant health and fertility of the soil (Hayat et al. 2010) and root growth and development (Gamalero et al. 2004) and promote resistance to environmental stress factors (Glick 2004). Plants are naturally capable of determining their root microbiota from the soil environment, and the microbiota species abundance is typical of each plant species (Hartmann et al. 2009), a feature that can be attributed to the composition of the root exudates and the characteristics of the rhizosphere soil (Chaparro et al. 2013; Ma et al. 2016). Some of these organic chemicals released from the plant roots function as effective signaling molecules that foster effective communications between the plant and its microbial associates (Bulgarelli et al. 2013; Doornbos et al. 2012; Drogue et al. 2012). It may also dictate the order of interactions among the participating microbial partners in the rhizosphere. Signaling molecules in plant-microbe interactions show reciprocal effects on the partners involved. The plant signals are perceived by the microbial partners, while the microbial signal molecules affect the plant physiology (Nadeem et al. 2014).

The root exudates of plants are composed of diverse organic compounds which have been classified into three major classes: (1) high molecular weight compounds, (2) low molecular weight compounds, and (3) volatile organic compounds (VOCs). The bulk of the root exudates are the low molecular weight compounds which contain organic acids, sugars, phenolics, vitamins, amino acids, and certain secondary metabolite compounds. Compounds such as aldehydes, alcohols, carbon dioxide, mucilage and proteins, and various secondary metabolites constitute the high molecular weight compounds (Badri and Vivanco 2009; Schulz and Dickschat 2007). Cultivated plants on phosphate-deficient soil or polluted soil with high aluminum concentration often demonstrate increased exudation of certain organic acids such as malic acid, citric acid, and oxalic acids (Lopez-Bucio et al. 2000; Neumann and Römheld 1999). These exudate organic molecules may function as chemical attractants to microbes and also as carbon source for enhanced microbial proliferations in the rhizosphere (Ortíz-Castro et al. 2009). The rhizosphere is a dynamic component of plant-microbe interactions that promotes plants growth and productivity. The ecological, physical, and biochemical features of the rhizosphere remain a function of the type and nature of exudates released and the timing for the release. About 20–40% of fixed carbon has been estimated to be released back into the rhizosphere. These events make the rhizosphere a significant integral component for enhanced processes such as water and nutrient uptake and promotion of beneficial microbial interactions (Badri and Vivanco 2009; Bais et al. 2004). Malic acid exuded from the root attracts the beneficial soil bacteria, *B. subtilis*, in an interaction that confers protection against *Pseudomonas syringae*, the foliar pathogen (Rudrappa et al. 2008). As well, root colonization by rhizobacteria and mycorrhizal fungi was increased in alfalfa and tobacco plants genetically manipulated to overproduce malic acid. Invariably, malic acid plays a significant role in plant-microbe interaction, and root exudates of plant contribute in determining the rhizosphere structure and composition of the soil. This presents a great potential to be exploited for biotechnological advancement of the rhizosphere and its application in agricultural productions.

Interactions between plant and arbuscular mycorrhizal fungi (AMF) help to confer resistance to the plant against biotic and abiotic factors, resulting in improved plant health (Nadeem et al. 2014). The water relations of plants can be affected by AMF through mechanisms such as regulation of host stomatal organelle, increase in absorption of water by extending hyphae, enhanced phosphorus uptake, and antioxidant enzyme activity (Birhane et al. 2012; Habibzadeh et al. 2013; Younesi et al. 2013). The activity of antioxidant enzymes (peroxidase and catalase) was found to increase compared to uninoculated plants in a study on wheat-AMF interactions under stressed environment (Khalafallah and Abo-Ghalia 2008; Nadeem et al. 2014). However, other than enhancing phosphorus uptake, AMF facilitate the availability of micro- and macronutrients such as Zn, Cu, N, Mg, and K in absorbable forms from the soil (Meding and Zasoski 2008). In addition, the AMF functions to maintain the stability of the soil structure and also the performance of the plant under environmental stress conditions (Smith et al. 2010).

Plant-microbe interactions can be beneficial to all participating partners of the association. The mutualism can occur between plant-microbe and microbe-microbe with effects on both partners involved. These synergies generate positive impacts and beneficial coexistence toward promoting each other's proliferation (Nadeem et al. 2014; Richardson et al. 2009). For example, *Pseudomonas* spp. produced certain antifungal secondary metabolite which was nontoxic to its fungal counterpart, *Glomus mosseae*; however when applied in combination with the fungus, the bacteria enhanced the fungal hyphae to colonize the roots (Barea et al. 1998). The exopolysaccharides produced by the PGPR are important at facilitating effective attachment of bacterial cells to mycorrhizal roots of plants (Bianciotto et al. 2009). Furthermore, while mycorrhizae facilitate nitrogen fixation and improved phosphorus solubilization (Linderman 1992), the bacteria promote fungal hyphal proliferation by enabling plant root permeability for ease of fungal hyphal penetration (Jeffries et al. 2003). For PGPMs, fungi offer better comparative advantage over bacteria by being capable of extending their mycelia to spread long distances in the soil and rhizosphere environments. Usually, plant growth promotion by fungi is achieved through mechanisms such as antibiotic production, competition with invading fungal pathogens, and invocation of host defense reactions. Additionally, certain beneficial fungi possess the ability to successfully parasitize the conidia, hyphae, or sclerotia of phytopathogens thereby enabling biocontrol of pathogens. Mycoparasitism is preceded by the fungal ability to sense a suitable host, toward which the hyphae grow. This is followed by the ability of the fungi to recognize, penetrate, and degrade the encountered host. Degradative enzymes such as the proteinases, chitinases, and glucanases are important integral components of the biocontrol activity (Harman et al. 2004).

Trichoderma species are beneficial fungi which are found as free-living soil inhabitants or in association with plant roots in the rhizosphere. Although they are known mycoparasites in nature, many strains are capable of colonizing plant roots for improved growth and development. Plant-*Trichoderma* interactions are usually beneficial with no harmful effects (Harman et al. 2004). Colonization by the fungus results in induced localized and systemic resistance due to the secretion of a protein elicitor called *small protein 1* (*Sm1*). *Sm1* is nontoxic to plants and microbes. The native purified form is able to stimulate the production of reactive oxygen species

(ROS) in cotton and rice seedlings and trigger both localized and systemic defense-like gene expression (Djonović et al. 2006; Ortíz-Castro et al. 2009). However, for plant growth enhancement, *Trichoderma* species including *T. atroviride* and *T. virens* employ mechanisms such as the production of indole-3-acetic acid (IAA) and some forms of auxin-like compounds (Contreras-Cornejo et al. 2009).

4.3 Harmful Plant-Microbe Interactions

Despite the myriad growth-promoting advantages conferred by the PGPR, there also exist harmful effects of these interactions in the ecosystem (Saharan and Nehra 2011). These harmful effects may be attributed to certain unique conditions and some specifically endowed traits (Nadeem et al. 2014). For example, cyanide produced by some *Pseudomonas* species plays dual role of plant growth promotion as well as a growth inhibition. Though the cyanide produced plays the role of biological control of certain phytopathogens for enhanced plant growth (Martínez-Viveros et al. 2010), it has been reported to demonstrate some harmful effects on plant growth (Bakker and Schippers 1987). The production of auxin, a plant hormone, by PGPR can impact negatively on plant growth (Vacheron et al. 2013), depending on its concentration. Auxins at low concentrations promote plant growth (Patten and Glick 2002), but at a much higher concentrations, and affect root growth (Xie et al. 1996). Similarly, *Bradyrhizobium elkanii* produces a secondary metabolite called rhizobitoxine, which plays dual role: inhibits ethylene production in order to reduce the effect on nodulation in legumes (Vijayan et al. 2013) or functions as a plant toxin that stimulates chlorosis in soybeans (Xiong and Fuhrmann 1996). In another perspective, it was observed that, though the PGPR are nonpathogenic, their combined application with fungi can trigger pathogenicity among partners (Dewey et al. 1999). This may be attributed to horizontal gene transfers within the gene pool of the rhizosphere. This could be possible due to the continuous microbial activity around the plant root environment under optimum conditions.

4.4 The Role of Plant Signal Molecules in Plant-Microbe Interactions

Cuticular waxes formed due to plant-microbe interactions create a physical bridge on epidermal cell layers, regulate host-microbe communications, function as signaling molecules and affect pathogen proliferation, or modulate the recognition of pathogen invasion by elicitor molecules. Elicitors serve as signal-inducing molecules and also as recognized components of the innate immune defense system of the host. They can be produced by both beneficial and pathogenic microorganisms. Usually, elicitor molecules such as microbe-associated molecular patterns (MAMPs), derived from beneficial microbes, or pathogen-associated molecular patterns (PAMPs), derived from pathogenic microbes in interactions with host plant, can elicit the immune reaction of the host. The recognition of these elicitor molecules is mediated by the transmembrane pattern-recognition receptors (PRRs), and pathogens can only proliferate inside the host when these responses are suppressed (Jones and Dangl 2006; Siebers et al. 2016). Basically, plant-stimulated roots exude different varieties of organic compounds than non-stimulated ones. For example, when defense signaling molecules (DSM) such as methyl, jasmonate, salicylic acid, and nitric oxide were exogenously applied on plants, the formation of diverse secondary metabolites such as phytoalexins, alkamides, and indole glucosinolates was induced which is critical in enhancing effective microbial communications (Zhao et al. 2005). Thus, the role of plant metabolites in determining the microbial structure of the rhizosphere is based on the ability of the host to selectively secrete organic compounds that signal the presence of either bacteria or fungi (Ortíz-Castro et al. 2009).

Flavonoids exuded from plant roots are vital signaling molecules in different plant-microbe interactions such as the legume-rhizobia symbiotic interactions and mycorrhiza formation (Steinkellner et al. 2007). Flavonoids stimulate the proliferation of host-specific rhizobacteria and also act as chemoattractants which can regulate the *nod* gene to stimulate nodule formation during synthesis of the Nod factor (lipochitin oligosaccharide) signaling molecule (Ma et al. 2016; Mandal et al. 2010). Exuded flavonoids from plant roots can be recognized by the transcriptional regulator molecules, the *nodD* proteins which are fundamental at determining the synthesis and transport of the *nod* gene. Other than inducing the expression of *nod* genes, flavonoids greatly impact on bacterial chemotaxis and multiplication (Bais et al. 2006), a characteristic that pairs rhizobia to root hairs of their ideal plant hosts. Isoflavonoids and plant flavone are effective inducers of *nod* gene expression in rhizobia (Zhang et al. 2007). In plant-AMF interactions, flavonoids play key role as effective activators of conidial germination, growth of hyphae, plant root colonization, and sporulation (Mandal et al. 2010). However, the role of flavonoids on AMF growth is significantly relative as it can be of negative or neutral effect depending on the fungal species involved in the symbiotic mycorrhizal interactions (Scervino et al. 2005).

4.5 Role of Microbial Signal Molecules in Plant-Microbe Interactions

During plant-microbe interactions, communications between interspecies and intraspecies can occur within the rhizosphere either through direct cell-cell interactions or via chemical signaling molecules (Badri et al. 2009). In nature, microbes involved in interactions with plants are capable of transforming the chemical composition of plant root exudates to alter its physiology by way of secretion of certain signal molecules such as the Nod factors, Myc factors, volatile organic compounds (VOCs), exopolysaccharides, and microbe-assisted molecular patterns (MAMPs) (Goh et al. 2014; Ma et al. 2016). VOCs are defined as organic compounds of high vapor pressure and can vaporize into the atmosphere under certain conditions. They are low molecular weight (usually ⍰300 $\mathrm{g/mol}^{-1}$) compounds such as aldehydes, alcohols, ketones, and hydrocarbons (Ortíz-Castro et al. 2009). VOCs of bacterial origin such as 2,3-butanediol and acetoin can stimulate host defense and enhance growth of host plant through a mechanism that enables the plant to thrive on soil depleted of essential nutrients like sulfur and iron (Bailly and Weisskopf 2012). VOCs produced and secreted by PGPMs can function as (1) phytostimulators, stimulate the various hormonal networks required for signals to any given stimulus; (2) bioprotectants, provoke induced systemic resistance in plants (ISR); and (3) biopesticides, kill plant vectors (Ma et al. 2016). The positive effects of VOCs can enhance plant growth promotion for effective phytoremediation. For example, the VOCs produced by *Bacillus* B55 improved sulfur uptake by *Nicotiana attenuata* (Hofmann 2013). VOCs produced and secreted by *Bacillus amyloliquefaciens* and *B. subtilis* activated the ISR of *Arabidopsis* seedlings compromised by the phytopathogen, *Erwinia carotovora* (Ryu et al. 2004). As well, other bacterial VOCs such as hydrogen cyanide, ammonia, phenazine-1-carboxylic acid, butyrolactones, and certain alcohols affect fungal conidial sporulation and mycelial mat formations (Kai et al. 2009). This implies that VOCs can function as an effective signaling molecule between the prokaryotes and the eukaryotes colonizing the plant roots (Ma et al. 2016). Furthermore, the AM fungus *Glomus intraradices* is able to produce a variety of lipochito-oligosaccharides (LCOs) containing both sulfated and non-sulfated derivatives (Myc factors), signaling molecules similar to the Nod factors of rhizobia. The Myc factor and the Nod factor signaling molecules are important in determining plant root organization such as development of lateral roots and stimulation of organogenesis (Maillet et al. 2011; Oláh et al. 2005). Plants have developed mechanisms to initiate non-specific immunity against phytopathogens via the activity of elicitor molecules such as the MAMPs (Newman et al. 2013). Novel MAMPs, rhamnolipids produced by *Pseudomonas aeruginosa*, have been shown to effectively confer resistance to grapevine plant against the phytopathogen, *Botrytis cinerea* (Varnier et al. 2009). In addition, MAMPs isolated from three PGPBs including *P. fluorescens*, *Chryseobacterium balustinum*, and *Stenotrophomonas maltophilia* were found to stimulate germination in *Papaver somniferum* (Bonilla et al. 2014).

4.6 Bacterial Quorum-Sensing Signals

Quorum sensing (QS) is a genetic mechanism that regulates the functioning and structure of a bacterial community (Bhattacharyya and Jha 2012). It is a communication process that occurs during bacterial cell to cell interactions, thereby monitoring population growth and density, while signaling molecules produced by individual cells control the expression and alteration of genes in the community (Daniels et al. 2004; Ma et al. 2016). The discovery and understanding of the role of bacterial signaling molecules have enabled the identification of two principal mechanisms of interference in microbial QS signaling, the enzymatic and the nonenzymatic microbial signal interferences which possess the ability of regulating QS signaling (Zhang and Dong 2004) and preventing microbial biofilm formation (Ren et al. 2001). Individual cell to cell QS communication signals are usually activated by certain autoinducer molecules that regulate bacterial actions. The N-acyl homoserine lactones (AHLs) are the most reported signaling autoinducers (von Bodman et al. 2003). AHLs possess the ability to promote or inhibit various phenotypes of either pathogenic or beneficial bacteria (Ortíz-Castro et al. 2009). The production of AHL signaling molecule is sacrosanct for the establishment of quorum sensing among Gram-negative bacteria such as *Pseudomonas aeruginosa*, *Erwinia carotovora*, and *Rhizobium radiobacter*. Production of AHLs signaling has been observed among PGPBs including *Gluconacetobacter diazotrophicus* and *Burkholderia graminis* (Cha et al. 1998) and also among strains of *Agrobacterium*, *Pectobacterium*, and *Chromobacterium* (Chernin et al. 2011). It has been observed recently that AHLs of bacterial origin can be well recognized by plants, to regulate gene expression in tissues, host defenses, and homeostatic balance (Daniels et al. 2004). It was recently shown that related types of AHLs (including *N*-octanoyl homoserine lactone, the 3-oxo and 3-hydroxy derivatives) secreted by members of the *Rhizobia*, *R. sullae*, *R. rhizobium*, and *Sinorhizobium fredii* mediated effective interactions with their legume host plant (Pérez-Montaño et al. 2011). Interaction between *Arabidopsis thaliana* root and *N*-hexanoyl-DL-homoserine-lactone (C6-HSL) resulted in obvious transcriptional alterations in roots and shoots systems (von Rad et al. 2008). However, higher plants possess the ability to produce certain AHLs mimic compounds which play critical role in the structure composition of the microbe community population density. For example, mimic compounds such as furanones secreted by plants such as soybean, barrel clover, and rice are able to interfere with or manipulate bacterial QS behaviors (Pérez-Montaño et al. 2013). The AHL mimic molecules can interfere structure-wise with the bacterial AHLs by binding to bacterial AHL receptors to antagonize its signaling (Bauer and Mathesius 2004). In addition, the flavonoids and genistein components of plant root exudates play critical role in QS communication among bacteria, considering their ability to act as chemoattractants of rhizobia to colonize and regulate the expression of genes responsible for nodulation in legumes (Loh et al. 2002). However, in spite of the biological significance of QS, bacterial VOCs are known to exhibit quorum-quenching effect on bacterial cell to cell communications during QS network (Chernin et al. 2011; Dong et al. 2001).

4.7 Management of Contaminants in Agroecosystem

Under the stress of an agroecosystem contamination, growth-enhancing nutrients become a limiting factor to plant. Essential nutrients like phosphorus may be lacking either due to total absence from the soil or due to the antagonistic effect of other nutrients (Nadeem et al. 2014). However, the inoculation of microbial consortia into contaminated environments is capable of restoring deficient nutrients for enhanced plant growth. For example, a saline-stressed environment was restored via the application of PGPR and AMF. The application of a combination of PGPR and AMF significantly elevated uptake of essential nutrients by sunflower (*Helianthus annuus* L.) (Shirmardi et al. 2010). Also, the interactions between the PGPR and

AMF significantly improved nutrient and water absorption by the roots of barley plant (Najafi et al. 2012). These interactions aided better colonization of barley plant roots and increased grain yields. Both traditional and conventional bioremediation techniques are essential requirements in the management of contaminated agroecosystem. The emerging technology of phytoremediation by using heavy metals hyper-accumulating or genetically modified plants is also an immense palliative toward agroecosystem restitution.

4.8 Advances in Microbial Bioremediation

Bioremediation is the technology that harnesses the natural ability of living organisms to breakdown toxic chemical materials in the environment. Traditionally, the remediation of pollutant-contaminated soils was done by excavating and transporting the contaminated soil off-site for treatments such as thermal alkaline dechlorination, incineration, solvent extraction, or landfilling (Campanella et al. 2002). But because of the possible damages, cost implications, and the extent of contaminations in the environment, the method is considered cumbersome, rather cost-effective approaches based on plants and microbes are being developed (McCutcheon et al. 1995). Living organisms are constantly faced with the challenges of toxic chemical contamination from allelochemicals (natural toxic chemicals) or xenobiotics (man-made toxic chemicals) (see Tables 4.1 and 4.2), leading to supposedly avoidable environmental deterioration. These contaminations could sometimes result from intentional disposal or unintentional discharge due to the pressures of expanding societal development (Srivastava et al. 2014; Van Aken et al. 2010). The bioaccumulation of heavy metals and its toxicity on animals, humans, plants, and microbes constitute a global concern for the health and safety of the

Table 4.1 Ranking of substances that pose significant threat to human health due to their toxicity and threat of exposure according to the US Department of Health and Human Services (2015)

Rank	Substance
1	Arsenic
2	Lead
3	Mercury
4	Vinyl chloride
5	Polychlorinated
6	Benzene
7	Cadmium
8	Benzo(a)pyrene
9	Polycyclic Aromatic Hydrocarbons (PAHs)
10	Benzo(b)fluoranthene
11	Chloroform
12	Aroclor 1260
13	DDT, P,P
14	Aroclor 1254
15	Dibenzo(a,h)anthracene

Table 4.2 List of common environmental contaminants

Type of contaminant	Example
Metals and metalloids	Cr, Ni, Cd, Hg, Pb, Mn, etc.
Petroleum hydrocarbons	Benzene, toluene, hexane, naphthalene, xylenes, etc.
Organic pollutants	Dioxins, aldrin, chlordane, dieldrin, heptachlor, endosulfans, toxaphene, chlordecone, mirex, PCB, HCB, DDT, PCDF, etc.
Organophosphate insecticides	Dimethoate, parathion, chlorpyrifos, dichlorvos, phenthoate, parathion-methyl, phorate, etc.
Herbicides	Atrazine, 2,4-D, glyphosate, simazine, etc.
Carbamate insecticides	Carbofuran, aldicarb, carbaryl, aminocarb, methomyl, fenoxycarb, methiocarb, etc.
Radionuclides	Uranium, plutonium, thorium, cesium, strontium, etc.
Nanoparticles	Carbon nanotubes, metal phosphates, TiO_2, SiO_2, aluminosilicates, fullerenes, ZnO nanoparticles, silver nanoparticles, etc.
New and emerging pollutants	Antibiotics, anti-inflammatories, antiepileptics, analgesics, lipid regulators, psychostimulants, diuretics, beta-blockers, cosmetics, disinfectants, plasticizers and phthalates, antidepressants, paint additives, wood preservatives, etc.

environment (Mani and Kumar 2014). Bioremediation is the widely practiced approach for the natural attenuation of chemical contaminants of human and environmental health concerns (Abhilash et al. 2012; Fester et al. 2014); and the methods often focus on the use of either single microbial species, single microbial gene, microbial consortia, or interactions such as in phytoremediation (Fester et al. 2014).

The biodegradation of these pollutant materials in the soil is well enhanced in the rhizosphere. Root exudates are released to encourage the rapid proliferation of microbial biomass which in turn affects the growth of the plant (Kloepper et al. 1989). The consequence of the nutrients released as exudates from the plant root is the elevation in microbial concentration in the root environment compared to the nearby vicinity. For bacteria, the population in the rhizosphere is often 10- to 1000-fold higher compared to the population in the soil vicinity. The degradative potential of some rhizosphere microbes enables them to degrade (rhizodegradation or phytodegradation) organic or inorganic pollutants in the vicinity (Kuiper et al. 2004). In dealing with contaminants in the environment, microorganisms utilize them as sources of carbon and energy via co-metabolism with any suitable substrates. For example, the carbon material exuded from the plant root plays a significant role in the co-metabolism of certain pollutant materials. Under this condition, certain electron-donating contaminants get oxidized under both aerobic and anoxic conditions. Additionally, halogen-containing (halogenated) organic compounds can function as terminal electron acceptors to support de-halorespiration in microbes, or they are de-halogenated for lack of enzyme specificity during the co-metabolism processes. However, these organic contaminants (growth-supporting and co-metabolized) can be broken down to yield carbon dioxide and water (Fester et al. 2014). Table 4.2 shows the common environmental contaminants, which includes inorganic as well as organic sources.

Fungi are endowed with the capacity to break down environmental organic pollutants in order to alleviate the environment from the risks commonly associated with these chemical contaminants either through structural modifications or facilitating their bioavailability for degradation (Harms et al. 2011). During plant-fungal symbiotic interactions, ectomycorrhizae (ECM) are known to demonstrate high levels of efficiency in the degradation of chemical contaminants. For example, chemicals such as the explosive, 2, 4, 6-tinitrotoluene (TNT), polycyclic aromatic hydrocarbon (PAH), and certain chloro-aromatics have been reported to be successfully degraded by axenic cultures of ECM. However, AMF, though are less studied for bioremediation purposes, possess the ability to scale up the dissipation of atrazine and PAH in soils (Harms et al. 2011). A few investigations have shown the ability of AMF to colonize plant roots and elevate PAH uptake by a plant via its roots (Gao et al. 2010; Sun et al. 2012). Nevertheless, not all mycorrhizal interactions are capable of enhancing pollutants degradation during phytoremediation (Joner et al. 2006). However, endophytic fungi have also been implicated as beneficial agents of pollutants degradation, through efficient removal of chemical contaminants in soils (Cruz-Hernández et al. 2013). During phytoremediation, endophytic fungi also metabolize and detoxify plant defense materials secreted around the root environment, as well as express enzymes with efficient specificity for contaminants degradation (Zikmundova et al. 2002).

Microbial biodegradation has mostly been practiced as an effective biotechnological approach for environmental restitution (Biswas et al. 2015; Srivastava et al. 2014). The application of microbes for the degradation of environmental contaminants was due to the ability of microbes to acclimatize and proliferate at environmental extremes. However, these adaptations should not be seen only at the level of the microbial cells but also at the level of enzymes secreted and the metabolites released in these extreme environments (Srivastava et al. 2014).

Remarkable advances have been made in biotechnological techniques for bioremediation of contaminated ecosystems. Approaches like the use of renewable plant, live and dead microbial biomass, immobilization in the roots of plants (phytostabilization), synthesis of certain minerals by biological systems (bio-mineralization), uptake, translocation and concentration of metals or organic pollutants on plant tissues (hyper-accumulation), stimulation for increase in microbial population (bio-stimulation), stimulation of algal bloom (cyano-remediation), cultivation of crops in contaminated ecosystems (dendro-remediation), stimulation of fungal proliferation (myco-remediation), and stimulation of gene expression for remediation of contaminants (geno-remediation) (Mani and Kumar 2014). Concerted integration of these advances with the existing traditional approaches will greatly enhance effective ecosystem restitution.

4.9 Advances in Phytoremediation

The technology that employs plants and their microbial symbionts for the treatment and restoration of groundwater and contaminated soils is referred to as phytoremediation. This technology relies heavily on the performance and contributions of plant-associated microbes to achieving desired results (Van Aken et al. 2010). The idea of applying phytoremediation technology for environmental restitution was conceived some decades ago when plants were found to possess the ability to metabolize certain toxic pollutants such as benzopyrene and 1,1,1-trichloro-2,2-*bis*-(4′-chlorophenyl) ethane (DDT) (Castelfranco et al. 1961). The ability of plants to metabolize toxic chemical contaminants has been likened to the capability of the mammalian liver to metabolize and detoxify injurious chemicals, a phenomenon referred to as “green liver” for plants (Coleman et al. 1997; Sandermann 1994; Van Aken 2008). Although phytoremediation is considered as an efficient technology for the degradation of chemical contaminants, the difficulty surrounding its extensive large-scale applications for the restitution of contaminated fields negates its known significance (Eapen et al. 2007).

Plants determine to a large extent the diversity of a microbial community of highly contaminated soil. They possess elaborate enzyme systems that enable them to degrade contaminating organic pollutants. However, the driving force behind phytoremediation of organic contaminants may largely be the symbiotic microbes with which they coexist (Fester et al. 2014; van Loon 2016). On the contrary, plants lack the required catabolic machineries for total metabolism of organic pollutants of high recalcitrance (Eapen et al. 2007); nevertheless, the degradation of chemical pollutants around the root environment may be by enzymes secreted as natural defense mechanisms against allelochemicals (Gerhardt et al. 2009). Plant roots produce certain substance generally referred to as root exudates; and the phytoremediation of organic compounds occurs around the root environment because of the high turnover rate (Gerhardt et al. 2009). These are organic metabolite substances released by plant roots as critical metabolic components during the developmental stages of the plant. These organic chemicals play some critical roles in phytoremediation by enhancing the adjustment and survival of plants under stressed conditions by way of allelopathy or detoxification. The organic acid components of root exudates are good sorption vehicles of metals for enhanced solubility, bioavailability, and mobility in the soil (Luo et al. 2014a, b; Ma et al. 2016). Oxalic and citric acid components of root exudates of *Echinochloa crus-galli* can function as effective chelating agents which promote effective translocation and bioavailability of heavy metals (Pb, Cu, and Cd), signifying their importance in phytoextraction (Kim et al. 2010). The formation of metal complexes was observed during the release of oxalate (a low molecular weight organic acid) by mycorrhizal interactions with Scots pine seedlings which facilitated metal immobilization (Johansson et al. 2008). However, not all organic chemicals of the plant root exudates exert effects on the bioavailability or translocation of metals within the rhizosphere (Zhao et al. 2001).

Some endophytes are heavy metal resistant and effective degraders of contaminants. The use of endophyte-facilitated phytoremediation has long been reported as a viable technology for in situ restoration of contaminated soils. The effectiveness of the endophytes in aiding phytoremediation is attributed to their resistance to heavy metal toxicity, their ability to alter metal uptake and accumulation, and also their growth-promoting capability (Li et al. 2012). Organic pollutants such as the TNT explosives are successfully degraded by naturally occurring bacterial endophytes (Van Aken et al. 2004). For example, a genetic construct of an endophytic strain of *Burkholderia cepacia* bearing the plasmid pTOM from a strain of the same species, known for colonizing plant roots, was able to successfully degrade toluene. The genetically modified endophyte neutralized toluene phytotoxicity with less amount evatranspirated (Barac et al. 2004). Similarly, mineralization of toluene occurred when *Populus trichocarpa* and *Populus deltoides* were treated with separate toluene-degrading strains of *B. cepacia* (Taghavi et al. 2005). Phytoremediation has been applied in constructed wetlands (water-logged soils in basins) for the management of organic contaminants (Vymazal 2011), as well as for the elimination of pollutants from groundwater (Seeger et al. 2011). Phytoremediation in comparison with other remediation approaches confers advantages such as low cost of establishment and maintenance and less or no negative impact on the environment, enabling carbon sequestration and its utilization for biofuel production as well as providing the ecstatic beauty of green technology (Gerhardt et al. 2009; Van Aken 2008). However, the technology is faced with drawbacks such as the slow rate of degradation and the inability of plants to achieve complete metabolism for lack of established biochemical machineries required for mineralization of pollutants (McCutcheon et al. 1995). Because plants are autotrophic in nature, phytoremediation is capable of ensuring the return of accumulated chemical contaminants into the ecosystem upon death of the plant that can be evaporated into the atmosphere or be transferred down the food chain putting stress on the health of man and the ecosystem (Arthur et al. 2005; Eapen et al. 2007; Pilon-Smits 2005). The time length required for a plant to attain maturity also forms an important drawback to the phytoremediation technology (McCutcheon et al. 1995).

4.10 Relevance of Plant-Microbe Interactions to Agroecosystem

Plant growth-promoting microorganisms (PGPMs) confer better advantages over chemical conditioning for phytoremediation. This is because the metabolites they produce are easily biodegradable and of less toxicity (Rajkumar et al. 2012). Metal-resistant PGPMs have been evaluated for tendencies toward enhanced plant growth and development, reduced metal toxicity, as well as immobilization, mobilization, and transformation of metal contaminants in the soil (Rajkumar et al. 2012). Arbuscular mycorrhizal fungi (AMF) occurring in a heavy metal-contaminated environment have demonstrated the ability to enhance plant growth (Orłowska et al. 2013) and modification of the soil pH to affect metal availability (Rajkumar et al. 2012), improve nutrient and mineral uptake (Guo et al. 2013), influence metal translocation (Jianfeng et al. 2009), and affect induced metal toxicity (Meier et al. 2011). The AMF are important ecological organisms found in obligate symbiotic interactions with the roots of plants in the terrestrial ecosystems. During interactions, plant supplies the needed carbon for fungal cell development as well as benefit from enhanced nutrient uptake and resistance to abiotic stress factors and phytopathogens (Smith and Read 2010). AMF possess the ability to influence ecological community processes and plant community dynamics through the distribution of essential resources such as carbon (C), nitrogen (N), and phosphorus (P) required for effective interactions (Rashid et al. 2016; Smith et al. 2011). In P-deficient soils, AMF are able to facilitate a large supply of P for uptake by the plant roots (Cavagnaro et al. 2015) and liberate other essential (macro and micro) nutrients such as N, magnesium (Mg), potassium (K), zinc (Zn), and copper (Cu) from the ores or less soluble forms (Smith and Read 2010). AMF external hyphae have also been shown to liberate about 10% of K, 25% of N and Zn, and 60% of Cu (Hodge and Storer, 2015). Certain AMF members such as *Glomus mosseae*, *G. caledonium*, and *G. intraradices* enhance the performance of plant under drought and salt conditions (Hashem et al. 2015; Ortiz et al. 2015) and help to remedy heavy metal-induced stress (Zhipeng et al. 2016; Zong et al. 2015).

Plant growth-promoting rhizobacteria (PGPR) and mycorrhizal fungi are effective at promoting plant growth and development even in stressed environments (Bach et al. 2016; Nadeem et al. 2014; Singh 2015; Prasad et al. 2015). During interactions with their plant counterparts, the PGPR play three fundamental roles: (1) synthesizing essential compounds, (2) enhancing plant nutrient uptake, and (3) promoting plant defense against disease and its etiological agents (Hayat et al. 2010). The growth promotion and development of plants can occur through direct or indirect mechanisms based on the interactions involved. PGPR can also initiate indirect inhibition against phytopathogens by producing cell wall-degrading enzymes (β-1,3-glucanases and chitinases) that act against fungi and can also produce hydrogen cyanide for toxicity against intruding pathogens. The direct plant growth promotion mechanisms can be mediated by plant-associated PGPR via the production of plant growth hormones (such as gibberellins, auxins, cytokinins, abscisic acid, and ethylene), indole-3-acetic acids (IAA), or indole-3-ethanol. Some PGPR are able to hydrolyze the ethylene precursor 1-aminocyclopropane-1-carboxylate (ACC) into ammonia and α-ketoglutarate which enhance root development by regulating the concentration of ethylene in the rhizosphere. They can also facilitate organic phosphates and nutrient mineralization, improve soil aggregation and structure, and elevate the organic matter content of the soil (Bhattacharyya and Jha 2012; Hayat et al. 2010; Kurepin et al. 2015; Ma et al. 2016; Nadeem et al. 2014). The soil aggregation and structure are also improved by AMF due to the production of glomalin, an insoluble glycoprotein (Gadkar and Rillig 2006) which functions to stabilize the soil (Rillig et al. 2003; Sharma et al. 2017).

4.11 Plant-Microbe Interactions in Management of Phytopathogens

Plants basically defend themselves from pathogen invasion through the production of antimicrobials, phytoalexins, hydrolytic enzymes, hypersensitive reactions, and defense barriers such as lignin and suberin polymers (González-Teuber et al. 2010). Other defense mechanisms against pathogens include production of certain defense proteins and secondary metabolites (Ashry and Mohamed 2012; Castro and Fontes 2005). The phytohormone auxin apparently plays an important role in defense against phytopathogenic bacteria (Spaepen and Vanderleyden 2011). In the event of attack by phytopathogens, the plant immune system is elicited by pathogen-derived molecules produced by certain functional membrane receptors. Immune response can also be elicited through direct or indirect pathogen effector protein molecules when they interact with the nucleotide oligomerization domain (NOD)-like cytoplasmic receptors of a plant host. Similarly, the reorganization of the host skeleton and secretory functions is another principal approach to plant immune response (Spanu and Panstruga 2017). The microbial partners of the association are also vital at ensuring plant health and viability. Other than promoting plant growth through phosphate solubilization, nitrogen fixation, and production of ACC deaminase and phytohormones, the microbial partners facilitate antagonistic response through the production of substances such as siderophores, hydrolytic enzymes, and a spectrum of antibiotics (Bach et al. 2016; da Costa et al. 2014), outcompeting the phytopathogen or its physical displacement (Glick and Bashan 1997). The bacterial species such as *Paenibacillus riograndensis*, *Bacillus cepacia*, and *B. mycoides* extensively demonstrate defining biocontrol features such as motility, root colonization, production of biosurfactants, antifungal metabolites, and hydrolytic enzymes (which degrade cell walls of invading phytopathogens) (Bach et al. 2016). For the management of phytopathogens, mycorrhizal fungi play principal role in maintaining ecological balance for improved ecosystem viability. Plant-AMF interactions have been beneficial in the reduction of soil-borne phytopathogens. The AMF, *G. intraradices* and *G. mosseae*, enhanced nutrient uptake in wheat plant, resulting in improved tolerance against pathogens (Bach et al. 2016). Besides, the high presence of fungal biomass colonizing plant roots is highly beneficial in the aspect of competition with phytopathogens. These interactions have been perceived as the mechanism by which phytopathogens abundance is abated by AMF in the rhizosphere (Vimal et al. 2017).

Plant growth hormones are essential ingredients of plant-microbe interactions toward pathogen management (Chagnon and Bradley 2015). The organic compounds exuded from the rhizosphere of tomato AMF (involving *G. intraradices* and *G. mosseae*) were reported to be possibly modified by AMF to inhibit the phytopathogen, *Phytophthora nicotianae* (Lioussanne et al. 2009). The application of the AMF, *G. mosseae*, for biocontrol activity against nematodes has been reported (Vos et al. 2012). The fungus demonstrated systemic resistance to two nematode species *Pratylenchus penetrans* and *Meloidogyne incognita* which were found in association with the tomato *Lycopersicon esculentum* (Table 4.3). The

Table 4.3 Studies on plant-microbe interaction for the management of phytopathogens

Microbe	Plant host	Pathogen	Effect	Reference
G. mosseae, *G. intraradices*	*Lycopersicon esculentum*	*Phytophthora nicotianae*	Enhanced plant resistance via modification of exuded root substances	Lioussanne et al. (2009)
T. harzianum, *G. mosseae*	*Nicotiana tabacum*	*R. solanacearum*	Improved systemic resistance, nutrient uptake, and biomass	Saifei Yuan et al. 2016
G. mosseae	*Lycopersicon esculentum*	*Pratylenchus penetrans*, *Meloidogyne incognita*	Inhibited phytopathogenic nematodes infection	Vos et al. (2012)
G. mosseae, *G. intraradices*, *G. clarum*, *Gigaspora gigantea*, *G. margarita*	*Phaseolus vulgaris*	*Fusarium solani*	Enhanced nutrients uptake, phenolic content, and activities of defense-related enzymes resulting in decreased	Al-Askar and Rashad (2010)
G. mosseae, *G. intraradices*, *G. claroideum*, *G. geosporum*, *G. etunicatum*	*Senecio vernalis*, *Senecio inaequidens*, *Inula conyza*, *Conyza Canadensis*, *Solidago virgaurea*, *Solidago gigantea*	*Pythium ultimum*	Promotion of plant growth and pathogens inhibition	Del Fabbro and Prati (2014)
G. clarum, *T. harzianum*	*Helianthus tuberosus*	*Sclerotium rolfsii*	Reduced incidence of the disease, southern stem rot	Sennoi et al. (2013)
G. mosseae	*Hordeum vulgare*	*Gaeumannomyces graminis*	Formation of high mycorrhizal colonization network that inhibited root infection	Khaosaad et al. (2007)
G. monosporus, *G. clarum*, *G. deserticola*	*Phoenix dactylifera*	*Fusarium oxysporum*	Reduced incidence of disease, improved plant growth, and alters defense-related enzymes activity	Jaiti et al. (2007)

resistance induced by the mycorrhizal fungus was determined by the significant depreciation of the nematode species around the roots, 45% in the case of *Meloidogyne incognita* and 87% for *Pratylenchus penetrans* (Vos et al. 2012). Similarly, when *T. harzianum*-amended bioorganic fertilizer (BOF) or the AMF *G. mosseae* were separately applied in the rhizosphere of the plant, remarkable decrease occurred in the incidence of tobacco bacterial wilt (TBW) caused by *Ralstonia solanacearum* (Saifei Yuan et al. 2016). However, combined application of the mycorrhiza gave the highest inhibition of the pathogen. This could infer that the greater the mycorrhizal complexity, the more robust the benefits derived by the plant host in terms of systemic resistance, improved nutrient, and biomass yield. See Table 4.3 for some other important reports on the impact of plant-fungal association against phytopathogens.

4.12 Plant-Microbe Interactions for Enhanced Phytoremediation of Contaminants

Over the years, soil pollution by heavy metals has been tremendously amplified through increased anthropogenic activities such as urbanization, industrialization, and exploration. These activities are often characterized by inadequate waste disposals resulting in heavy metal pollution of agriculture soils and distortion in the functioning of the ecosystem and its food chain, effects on human and animal health through possible bio-magnification (McMichael et al. 2015; Zhipeng et al. 2016). Plants which grow in soil with high levels of metal contamination are naturally endowed with diverse microbial partners that tolerate metal contamination and also remedy the soil environment for plant growth (Rajkumar et al. 2012). Most vascular plants enter into a beneficial mutual relationship with mycorrhizal fungi for increased nutrient yield and its uptake (Hashem et al. 2015), a relationship which benefits the agroecosystem in the following ways: (1) enhanced nitrogen fixation in the rhizosphere (Krapp 2015), (2) osmoregulation of the rhizosphere environment for improved productivity, (3) production of bioactive secondary metabolites (Goicoechea et al. 1997), (4) increased phosphatase enzyme activity (Liu et al. 2015a, b), (5) enhanced photosynthesis (Hashem et al. 2015; Ruíz-Sánchez et al., 2011), (6) elevated resistance against stress factors (biotic and abiotic) (Del Fabbro and Prati 2014; Saifei Yuan et al. 2016), and (7) an improved metal detoxification (Amir et al. 2013; Nadeem et al. 2014; Zong et al. 2015). However, the effectiveness of these mechanisms remains a function of plant-AMF interactions and the plant and soil factors (Nadeem et al. 2014). Mycorrhizae-assisted phytoremediation has been applied for the restitution of contaminated soils for agriculture production purposes. Hyper-accumulating plant AMF-assisted phytoremediation via phytodegradation, phytoextraction, phytostabilization, phytovolatilization, and rhizofiltration (see Fig. 4.1) has been employed for efficient restoration of contaminated soils based on their unique abilities (Mohammad Miransari 2011). Fungal cell wall consists of certain free radicals, amino acids, and other functional groups with free binding sites to adsorb certain trace elements. The microbe-metal interaction enables the plant host to survive metal-contaminated soils (Vimal et al. 2017; Zhipeng et al. 2016). Liu et al. (2015a, b) observed an increased P uptake and growth in the cadmium (Cd) hyper-accumulator, *Solanum nigrum*, even under high Cd concentrations when *G. versiforme* was inoculated, suggesting AMF enhancement of plant growth, resistance to heavy metal toxicity, metal bioavailability, and uptake by plants. Moreover, *G. claroideum* obtained from Cu-contaminated soil has been suggested for the remediation of contaminated soils due to its ability to alleviate Cu toxicity (Meier et al. 2012a, b). For ECM, the formation of fungal mantle and Hartig net is essential for interaction with plant. The mycelia of the ECM fungus, *Tricholoma vaccinum*, form a structure called the Hartig net on the root apoplast of the host, *Picea abies*, which serves as the interface for the exchange of nutrients between the plant and the fungus (Henke et al. 2015). Nutrients for uptake are transported via the Hartig net and translocated unto the roots where they are released for uptake through the aid of plant metal transporters (Luo et al. 2014a, b). ECMs play critical role in the sequestration and detoxification of heavy metals from contaminated soils (Henke et al. 2015), as well as the organic compounds exuded from plant roots (Meier et al. 2012a, b). Similarly, the growth and performance of the Japanese red pine, *Pinus densiflora*, and the oak, *Quercus variabilis*, on copper mine tailings were attributed to effective nutrient uptake when inoculated with ECM fungi, *Pisolithus* spp. and *Cenococcum geophilum* (Zong et al. 2015). Thus, plant-microbe interactions are essential for the biogeochemical cycling of metal contaminants and can be applied in phytoremediation (Ma et al. 2016). Metal-stressed agricultural plants are capable of producing certain compounds, LMWOAs such as malic, succinic, citric, and oxalic acids which are essential at neutralizing metal phytotoxicity (Meier et al. 2012a, b; Songhu Yuan et al. 2007). In the ecosystem, plants strive for adaptation, uptake of nutrients, and growth when confronted with the challenge of pollution. The myriads of organic compounds produced in interactions serve as essential drivers for enhanced tolerance and resistance to metals or organic pollutants in agroecosystems. Some of these organic compounds that can be harnessed and applied to sustain a balance in the rhizosphere environment include organic acids, siderophores, metal chelators, and bacterial biosurfactants.

4.12.1 Organic Acids

Organic acids are carbohydrate-based natural compounds which are usually identified by the presence of carboxyl groups (Jones and Edwards 1998). Organic acids form complexes with metal ions in the soil in order to make them bioavailable for uptake by plants. The endophytic diazotroph *Gluconacetobacter diazotrophicus* produced 5-ketogluconic acid, a gluconic acid derivative which enhanced effective solubility of zinc compounds [ZnO, $ZnCO_3$, and $Zn_3(PO_4)_2$] (Saravanan et al. 2007). Similarly, airborne bacteria isolated from the tannery surrounding air, such as a strain of *P. aeruginosa*, effectively solubilized both zinc oxide (ZnO) and zinc phosphate [$Zn_3(PO_4)_2$] in the presence of glucose carbon source. The solubilization of zinc compound by the bacterium was attributed to its ability to produce

Table 4.4 Microbial metabolites and their significance on heavy metals activity in contaminated ecosystem

Metabolites of microbial origin	Microbe	Effects on metal transformation/ assimilation by plant	References
Organic acids			
2-Ketogluconic acid	*P. aeruginosa*	Enhanced solubility of ZnO and $Zn_3(PO_4)_2$	Fasim et al. (2002)
5-Ketogluconic acid	*G. diazotrophicus*	Solubilize zinc compounds ZnO, $ZnCO_3$ and $Zn_3(PO_4)_2$	Saravanan et al. (2007)
Gluconic acids and exopolymeric compounds	*B. caribensis*	Phosphorus mobilization	Delvasto et al. (2009)
Malic and citric acids	*O. maius*	Solubilize zinc compounds [ZnO, $ZnCO_3$ and $Zn_3(PO_4)_2$]	Martino et al. (2003)
Oxalic	*B. bassiana*	Solubilize $Zn_3(PO_4)_2$	Fomina et al. (2004)
Biosurfactants			
Lipopeptide	*Bacillus* spp.	Elevated uptake of Cd into above ground plant tissues	Sheng et al. (2008)
Rhamnolipids	*P. aeruginosa*	Mobilization of Cu for uptake by plants	Venkatesh and Vedaraman (2012)
Siderophore			
Pyochelin and pyoverdine	*P. aeruginosa*	Enhanced Pb and Cr bioavailability and assimilation	Braud et al. (2009)
Catecholate	*S. luteus*, *R. luteolus*, and *S. verrucosm*	Production of various forms of metal chelators for enhanced metal bioavailability	Machuca et al. (2007)
Desferrioxamine B and C and coelichelin	*S. tendae* F4	Facilitated increased Cu uptake	Dimkpa et al. (2009a, b)

2-ketogluconic acid (Fasim et al. 2002). Delvasto et al. (2009) observed that *Burkholderia caribensis* isolated from high-phosphorus iron ore demonstrated high level of phosphate mobilization from the phosphate-rich ores. The mobilization of phosphate by *B. caribensis* was possible due to its formation of dense biofilm and production of gluconic acid and exopolymeric compounds (Table 4.4). Similarly, rhizobacteria obtained from the rhizosphere of *Sedum alfredii*, a Cd/Zn hyper-accumulator, were able to solubilize Zn and Cd. The mobilization of these metals showed positive correlations with the production of organic acids such as tartaric acid, formic acid, oxalic acid, acetic acid, and succinic acids (Li et al. 2010). Mycorrhizal fungi are also able to secrete organic metal chelators into the rhizosphere for enhanced metal mobilization (Martino et al. 2003). The mycorrhizal strains of *Oidiodendron maius* were able to solubilize zinc compounds via the production of malic and citric acids. Similarly, the soilborne entomopathogen, *Beauveria bassiana*, produced oxalic acid molecules which enhanced the dissolution of pyromorphite and $Zn_3(PO_4)_2$ during acidolysis (Fomina et al. 2004). In addition, the fungus, *A. brasiliensis*, effectively mobilized large volumes of lead (Pb) and P from pyromorphite due to its elaborate production of organic acids, making these metals bioavailable for plant uptake. This feature substantiates the potential for the application of *A. brasiliensis* in phytoextraction for ecosystem restitution. Also, plant-associated microbes are known to secrete essential organic acids which promote plant root absorption of metal ions including Zn and Cd (Li et al. 2010; Rajkumar et al. 2013), Cu (Chen et al. 2005), and Pb (Sheng et al. 2008).

Nevertheless, organic acids can either be neutral or negative in effects against the mobilization of metals in contaminated environments (Rajkumar et al. 2012). For instance, no significant mobilization of metals (including Cu and Pb) occurred in a contaminated agricultural soil bio-augmented with the efficient organic acid-producing strain of *B. subtilis* (Braud et al. 2006). As well, LMWOAs (tartaric, oxalic, and citric acids) failed to facilitate the phytoextraction of Pb from contaminated soil even when applied in high amounts (Evangelou et al. 2006). The high rate of its biodegradation vis-à-vis the low mobility and bioavailability of Pb may explain its failure.

Most plant-associated microbes produce siderophores, the iron chelator molecules secreted in response to stress factors encountered in the rhizosphere (Das et al. 2007). Iron is an essential nutrient required in almost all forms of life. Other than few species of lactobacilli, all other microbes require iron as an essential nutrient for growth activity (Neilands 1995). Iron basically occurs as Fe^{3+} under aerobic conditions and as such can easily form insoluble hydroxides and oxyhydroxides, making it less accessible to both microbes and plants (Rajkumar et al. 2010). Bacteria secrete siderophores chelators which possess high affinity constants for complexing iron molecules. Other than iron, siderophores are capable of forming stable complexes with other heavy metals such as Cd, Al, Ga, In, Zn, and Pb and also with radionuclides including Np and U (Kiss and Farkas 1998; Neubauer et al. 2000). Siderophores have been classified based on solubility in water and functional groups. Based on solubility in water, siderophores are classed into extracellular and intracellular siderophores (Khan et al. 2009). As regards functional groups, siderophores are classified into three groups, namely, the hydroxamates (e.g., desferrioxamine B and C, ferrichrome, ornibactin, rhodoturolic acid, etc.), catecholates (e.g., enterobactin, bacillibactin, and vibriobactin), and (α-hydroxy) carboxylate (e.g., aerobactin) (Dimkpa et al. 2009a, b; Rajkumar et al. 2010). In both Gram-positive and Gram-negative bacteria, the iron (Fe^{3+}) component of Fe^{3+} siderophore complexed on the membrane is reduced to Fe^{2+} for onward delivery into the cell through a gating mechanism that connects both the outer and inner membranes. The reduction process can result in the siderophore being destroyed or recycled (Ahemad and Kibret 2014; Rajkumar et al. 2010). Because siderophores possess the ability to enhance metal solubility from their ores, microbes inhabiting the rhizosphere are believed to impact greatly on the phytoextraction of heavy metals (Rajkumar et al. 2010). The siderophores, pyochelin and pyoverdine, produced by *P. aeruginosa* enhanced the bioavailability of Pb and Cr in the rhizosphere for easy uptake by maize plant (Braud et al. 2009), while siderophores from a strain of

Streptomyces tendae F4 significantly elevated the uptake of Cd by the sunflower plant (Table 4.4) (Dimkpa et al. 2009a, b). This implies that bacterial siderophores are essential organic substances that are capable of alleviating stress factors including heavy metals from contaminated soils (Ahemad and Kibret 2014). Siderophores have also been produced by mycorrhizal fungi (Goodell et al. 1997; Machuca et al. 2007). For example, hydroxamate and catecholate siderophores were produced by the ECM fungi *Suillus luteus*, *Rhizopogon luteolus*, and *Scleroderma verrucosum* isolated from *Pinus radiata* fruiting bodies (Machuca et al. 2007).

However, plants utilize different mechanisms for the assimilation of microbial siderophores. These mechanisms include the direct uptake of siderophore-Fe complexes, chelate and release of iron, or ligand exchange reaction (Ahemad and Kibret 2014; Schmidt 1999; Das et al. 2007). For instance, Crowley and Kraemer (2007) described an iron transport process in oats that is siderophore mediated and deduced that siderophores originating from rhizospheric microbes effectively deliver iron to oats, which possess the mechanisms for Fe-siderophore complex utilization in iron-deficient soil conditions. Additionally, the formation of Fe-pyoverdine complex by *P. fluorescens* strain C7 was successfully assimilated by *A. thaliana* resulting in elevated iron accumulation in the plant tissues and growth (Vansuyt et al. 2007). Furthermore, Sharma et al. (2003) assessed the role of siderophores produced by *Pseudomonas* strain GRP_3 on the nutrition of *Vigna radiata* and observed a reduced chlorotic symptoms and an elevated level of chlorophyll in the plant after 45 days. However, chlorophyll a, chlorophyll b, and total chlorophyll content increased significantly compared to the control. Nevertheless, there are emerging arguments that the mobilization and uptake of metals in the rhizosphere are reduced by the presence of siderophore-producing microbes (Rajkumar et al. 2012). For example, Sinha and Mukherjee (2008) reported that the efficient siderophore-producing *P. aeruginosa* strain KUCd1 caused a reduction in Cd assimilation in the tissues of *Brassica juncea* and *Cucurbita pepo*. Moreover, Tank and Saraf (2009) observed a reduced Ni uptake when a species of a Ni-resistant siderophore-producing species of *Pseudomonas* was applied on chickpea plants. In addition, it has been observed that siderophore-producing microbes do not facilitate any increased assimilation of heavy metals by plants (Kuffner et al. 2010; Kuffner et al. 2008). The existing contrasts on the role of siderophores on metal uptake by plants may be attributed to variation in plants' ability to effectively assimilate heavy metals, which indirectly depends on the bioavailability of the metal, the plant type, and the system of heavy metal transport to their tissues (Dakora and Phillips 2002; Jones et al. 2003).

4.12.2 Biosurfactants

Biosurfactants are amphiphilic molecules containing a hydrophilic head and a hydrophobic tail. The hydrophilic moiety consists of mono-, oligo-, or polysaccharides, peptides, or proteins, whereas the hydrophobic group consists of saturated, unsaturated, and hydroxylated fatty alcohols or fatty acids (Rajkumar et al. 2012). Microbial biosurfactants can undergo complexation with heavy metals and their ions on the surface of the soil. The formed complex enables metal desorption from the soil matrix into readily soluble and bioavailable forms for plant uptake. Evidence shows that biosurfactant-producing microbes are capable of elevating metal mobilization in contaminated soils (Juwarkar and Jambhulkar 2008; Sheng et al. 2008). Biosurfactant rhamnolipids from *P. aeruginosa* have been shown to efficiently remove 71% and 74% of Cu from contaminated soil with initial Cu concentration of 474 and 4484 mg kg^{-1}, respectively, when applied at 2% (Venkatesh and Vedaraman 2012). Thus, biosurfactants can be applied as a cost-effective, environment-friendly, and specific metal bioremediation alternative to conventional chemicals. Also, live cells of biosurfactant-producing strain of *Bacillus* sp. significantly enhanced the mobilization and uptake of Cd from the contaminated soil compared to the control soil with dead bacterial cells (Sheng et al. 2008). Although existing studies reveal the significance of microbial biosurfactants on metal bioremediation and uptake by plants, a more elaborate understanding of the chemistry between plants and their biosurfactant-producing microbial partners is desirable.

The application of plant-microbe interactions for ecosystem management is a complex phenomenon. Both partners in the relationship employ diverse mechanisms for adaptation, resistance, and persistence in the face of stress factors. Plants are known to demonstrate resistance to metal contamination in agroecosystems through various mechanisms, such as (1) active efflux pump system, (2) metal sequestration, (3) biosorption and precipitation of metals, (4) metal chelate exclusion, and (5) enzyme-catalyzed redox reaction (Ma et al. 2016). Other ways by which plant-microbe interactions can be applied for the management of contaminated agroecosystems include bioaccumulation/biosorption, bioexclusion, and bioleaching.

4.12.3 Bioaccumulation/Biosorption

The role of the microbial partner in the plant-microbe interactions is enormous. Bioaccumulation refers to the phenomenon of intracellular accumulation of metals (Ma et al. 2016), whereas biosorption is defined as the adsorption of metals by microbial cells through passive, metabolism-independent and active metabolic processes (Ma et al. 2011). The bioaccumulation of metals is one significant way by which associated microbes contribute to metal resistance. Two major mechanisms through which these occur include biosorption (toxic metals being concentrated in the biomass of nonliving microbial cell) and bioaccumulation (concentration of poisonous compounds in the living microbial cell) (Ma et al. 2011; Rajkumar et al. 2012). The process of bioaccumulation involves two principal stages, viz., metabolism-dependent biosorption (e.g., metal ion exchange, physical and chemical adsorption, surface complexation, chelation, coordination, and micro-precipitation) and metabolism-dependent active bioaccumulation (e.g., endocytosis, carrier-dependent ion pumps, and metal assimilation and complex permeation) (Chojnacka 2010). Bioaccumulation processes in various microbes have been shown to reduce metal uptake and toxicity on their plant partners (Ma et al. 2011; Mishra and Malik 2013). More complex processes are required in bioaccumulation than in biosorption. These involve metabolic processes of living cells such as extracellular precipitation, intracellular sequestration, metal accumulation, and formation of complexes (Gadd 2004). Biosorption and bioaccumulation of heavy metals (Fe, As, Cr, Co, and Hg) by living cells of *B. sphaericus* and biosorption by dead cells of the bacterium showed that the living cells had higher degrees of biosorption and accumulation of these metals than the dead cells (Velásquez and Dussan 2009). The disparity in the biosorption and bioaccumulation levels of these metals was attributed to the lack of active metabolic machineries in the dead cells. It has been shown that biosorption of metals by microbial cells reduced uptake in plant. For example, inoculation of *Burkholderia* sp. and *Magnaporthe oryzae* caused a reduction in the accumulation of Cd and Ni in tomato plant (Madhaiyan et al. 2007). Similarly, reduction of Zn accumulation occurred when a strain of *Brevibacillus* sp. was introduced in *Trifolium repens*. The reduction in Zn concentration in the plant was traced to the biosorption capability of the bacterium (Vivas et al. 2006).

Recently, Ma et al. (2015) reported that the *Bacillus* sp. strain SC2b demonstrated extensive resistance to heavy metals such as cadmium (Cd), zinc (Zn), and lead (Pb) by mobilizing high concentration of the metals from the soil through different biosorption processes. Other than mobilization and biosorption of heavy metals, the strain expressed some PGP features such as P solubilization, production of siderophore and IAA, and utilization of 1-aminocyclopropane-1-carboxylate. However, no specific correlations exist between tolerance and biosorption of the metals, chromium (Cr) and Cd, among the filamentous fungi, *Rhizopus* and *Aspergillus*, isolates from metal-contaminated soil (Zafar et al. 2007). The mycelial network of mycorrhizae can function to effectively inhibit heavy metal translocation to plant tissues. A marked reduction has been observed in the translocation of Zn, Cd, and Pb by the mycelia of the ECMF, *Lactarius rufus*, *Amanita muscaria*, and *Scleroderma citrinum* with pine seedlings (Krupa and Kozdrój 2007). Metal biosorption by the fungal mycelia was reflected in the reduction of metal concentrations. Although plants and their associated microbial symbionts may vary in their tendencies to enhance metal bioavailability and uptake, the proliferation, survival, and colonization of the rhizosphere greatly affect the abundance of heavy metals in an environment and its accumulation in plants growing on such a soil. This is because biological processes in the rhizosphere are capable of causing such alterations (Rajkumar et al. 2012).

4.12.4 Bioexclusion

Bioexclusion mechanisms in microbes include the efflux pump system and active transport process. The efflux pump system and the active transport mechanisms responsible for ejecting toxic materials from microbial cytoplasmic enclosures are critical components of resistance to metals (Ma et al. 2016). The exclusion of inorganic metal ions through the microbial efflux pump system is a function of certain membrane proteins and the activity of ATPases, whereas ATPase efflux system forms an essential component of active transport of some required metal ions (Bruins et al. 2000).

4.12.5 Bioleaching

The solubilization of metals from ores in acid environments by certain group of microorganisms is termed bioleaching. Acidophilic microbes are mainly responsible for the bioleaching of metals from their ores most often resulting in acid mine drainage (AMD) which causes severe negative environmental effects. Usually, trapping of metal ions by metal chaperones and the efflux pump system are recognized mechanisms employed by bacteria to resist high levels of metals in contaminated environments (Navarro et al. 2013). Microbes, such as the iron-oxidizing bacteria (*Acidithiobacillus ferrooxidans* and *Leptospirillum*) and sulfur-oxidizing bacteria (*A. thiooxidans*, *A. albertis*, and *A. caldus*) (Wong et al. 2004), thermophiles (e.g., *Sulfobacillus thermosulfooxidans*, *Archaeans* sp., *S. brierleyi*, *S. ambivalens*, and *Thiobacter subterraneus*) (Kletzin 2007), heterotrophs (*Arthrobacter*, *Acetobacter*, *Pseudomonas*, and *Acidophilum*), as well as fungi (*Fusarium*, *Aspergillus*, *Trichoderma*, and *Penicillium*) (Mulligan and Galvez-Cloutier 2003), are capable of bioleaching metals from their ores in sediments, soils, and sludge. These microorganisms neutralize the ore's phytotoxic effects on plants through direct or indirect metabolic processes such as complexation, oxidation, adsorption, dissolution, and reduction, respectively (Pathak et al. 2009). The endowed potential of microbes to bioleach heavy metals is species dependent. However, acidophiles are more efficient at bioleaching of metals from their ores than their neutrophilic counterparts (Navarro et al. 2013). Heavy metals such as Cd, Cu, Fe, Cr, Zn, and Pb were successfully bioleached by the acidophilic bacteria, *A. thiooxidans*, under acidic conditions (Kumar and Nagendran 2009).

4.12.6 Oxidation and Reduction of Metals

The redox reaction pathway has been successfully exploited by some microbial symbionts of plant to influence the bioavailability and mobility of heavy metals in agricultural fields. The phytoextraction of metals from contaminated rhizosphere is often a function of microbial metal oxidation. For example, sulfur-oxidizing bacteria in the rhizosphere enable the mobilization of Cu and its uptake by the plant tissues from a contaminated soil (Shi et al. 2011). This reflects the ability of the bacteria to reduce the ambient pH within the rhizosphere by way of converting the reduced sulfur into sulfate, to make the Cu ions bioavailable for uptake by plants (Rajkumar et al. 2012). One other mechanism adopted by microbial plant symbionts is to immobilize metals in the rhizosphere through reduction process (Rajkumar et al. 2012). For instance, metal-resistant strain of *Cellulosimicrobium cellulans* isolated from a waste canal harboring industrial effluents exhibited remarkable reduction of

Cr under aerobic conditions. The bacterial strain reduced greatly the uptake of this heavy metal by the chili test plant, through reducing the phytotoxic Cr (VI) to a nontoxic Cr (III) within the rhizosphere (Chatterjee et al. 2009). Similarly, selenite-resistant bacterium, *Stenotrophomonas maltophilia*, from the selenium hyper-accumulator legume, *Astragalus bisulcatus*, efficiently reduced the toxic selenite (IV) into the nontoxic elemental form Se (0) (Di Gregorio et al. 2005). These features explain the processes utilized by microbes in the rhizosphere to either mobilize, immobilize, or make bioavailable heavy metals that ordinarily could be of high phytotoxicity. In addition, microbes in synergistic interactions have been jointly applied to ameliorate heavy metal-contaminated agro-soils. The utilization of Fe-reducing and Fe-/S-oxidizing bacterial consortia enhanced greater heavy metal solubility than when separately applied for the same metal treatment (Beolchini et al. 2009).

4.13 Conclusion

Chemical contamination of soils and water is a serious environmental problem. The application of physical and chemical remedial methods is limited by their high cost, damages to microflora and microfauna in the soil, and potential creation of secondary pollution in the ecosystem. Thus, the need to consider solar-driven, eco-friendly phytoremediation technology derives from the interaction of plant roots and microbes to remedy hazardous chemical contaminations in agroecosystem. Bacterial roots and symbiotic mycorrhizal interactions modify soil pH; affect metal availability; improve nutrient and mineral uptake within the rhizosphere and plant growth-promoting microorganisms (PGPMs), particularly metal-resistant PGPMs; and produce easily biodegradable metabolites important for nutrient and mineral uptake that supports plant growth. Also, microbes contribute remarkably to phytoextraction of metals from contaminated rhizosphere through microbial metal oxidation and extraction of heavy metals, removing and detoxifying the contaminants in the soil. As well, biosurfactant-producing microbes and bacteria-secreting siderophore chelators are particularly involved in bioleaching, redox reaction and solubilization, bioavailability, and heavy metal mobility.

Plant exudates enhance root-microbes association and contribute to pathogen management. Besides, the association of plant and arbuscular mycorrhizal fungi boosts plant growth, resistance to heavy metal toxicity, and metal bioavailability and uptake by plants as well as encourages soil phytoremediation via phytostimulation, phytodegradation, phytoextraction, phytostabilization, phytovolatilization, and rhizofiltration. Although phytoremediation is considered a relatively cheap, eco-friendly technology for the restitution of contaminated fields, it is still challenged by the difficulty surrounding its extensive large-scale applications. Hence, further work is required for better understanding of the relationships among plant root microbes, soil types, chemicals, and heavy metal contaminants within the rhizosphere, so as to fully exploit the potential in phytoremediation of agroecosystems. Therefore, it is important to develop phyto-hyper-accumulators and super microbial solubilizers for various soil types either by conventional breeding techniques, other methods of hybridization (e.g., spheroplast fusion), or genetic modifications (transgenic plants), to improve on desirable plant traits (such as appropriate root exudates, efficient metal uptake, translocation, sequestration, and high tolerance) and enhance their soil remedying capabilities.

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5

Phytoremediation for the Elimination of Metals, Pesticides, PAHs, and Other Pollutants from Wastewater and Soil

Hussein I. Abdel-Shafy and Mona S. M. Mansour

Abstract

Phytoremediation is an important process that uses plants, green vegetation, trees, aquatic plants, and grasses to remove, stabilize, transfer, and/or destroy toxic pollutants from surface water, groundwater, wastewater, sediments, soils, and/or external atmosphere. The phytoremediation mechanisms include phytoextraction (i.e., phytoaccumulation), enhanced rhizosphere biodegradation, phytostabilization, and phytodegradation. Certain plant species have the tendency and the ability to accumulate and store pollutants such as metals and organic contaminants in their roots. The remediation of pollutants includes translocation, accumulation, transpiration, and possibly metabolization of the organic contaminants to plant tissue or CO_2. They also prevent the flow of groundwater from transferring pollutants away from the site to the deeper.

Many countries have been successfully remediated several million acres of contaminated soil and land by employing soil phytoremediation technology. The biodegradability of given pollutants is affected by several factors, including the physical-chemical properties of the contaminants and how the soil can influence and affect its chemical state. Nevertheless, the high costs of the conventional physical and chemical strategies hindered these efforts. Thus, the use of higher plants, bacteria, microalgae, and fungi is feasible for degrading persistent contaminants. Phytostimulation process is known as “rhizosphere degradation,” in which degradation of the pollutants is achieved by organisms that are associated with the plant roots.

Phytoextraction or phytoaccumulation is the uptake, accumulation, and concentration of pollutants from the contaminated environment by the roots of plant.

H. I. Abdel-Shafy (✉)
Water Research and Polluted Control Department, National Research Centre, Cairo, Egypt

M. S. M. Mansour
Analysis & Evaluation Department, Egyptian Petroleum Research Institute, 1 Ahmed El-Zomor Street, Nasr City, Cairo, Egypt

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The pollutants are then translocated/accumulated into the plant biomass. Phytoabsorption, phytosequestration, or phytoaccumulation involves the absorption of pollutants by the plant roots followed by translocation and accumulation in the aerial parts. This took place mainly in the uptake of heavy metals as well as organic compounds. In addition, the hyper-accumulator plants have the tendency to store reasonable concentrations of certain metals in their tissues. On the other hand, the phenomenon of producing chemical compounds by plant to immobilize pollutants at the interface of soil and roots is described as “phytostabilization.” However, the phytovolatilization technique relies and depends on the ability of certain plants to volatilize and absorb some metals/metalloids. Rhizofiltration is the process through which plants concentrate, absorb, and/or precipitate pollutants, such as heavy metals and/or radioactive elements, from an aqueous medium.

The importance of the phytoremediation process is that it is efficient for the removal of toxic organic aromatic pollutants, polycyclic aromatic hydrocarbons (PAHs), explosives (RDX, TNT, HMX), pesticide, landfill leachates, as well as herbicide contamination. Phytoremediation process is also efficient for wastewater and improving quality of water. Thus, employing phytoremediation in constructed wetland (CW) technology offers a low-cost treatment system for wastewater. Thus, CWs are perfect for the decentralized treatment of wastewater for offering great potential for the phytoremediation of contaminants and removal of pathogens and toxic substances. In conclusion, phytoremediation is an emerging “green bioengineering technology” that uses plants to remediate environmental problems. Green plants (*both aquatic and terrestrial*) have the wonderful properties of environmental restoration, such as decontamination of polluted soil and water. In general, the phytoremediation technology has several advantages and disadvantages that should be considered when applying such process. The low cost is one of the most important advantages. However, the time needed to observe the necessary achievement can be long. The concentration of contaminants should also be considered.

5.1 Introduction

Phytoremediation is an important process that uses plants, green vegetation, trees, aquatic plants, and grasses, to remove, stabilize, transfer, and/or destroy toxic pollutants from surface water, groundwater, wastewater, sediments, soils, and/or external atmosphere. The phytoremediation mechanisms include phytoextraction (i.e., phytoaccumulation), enhanced rhizosphere biodegradation, phytostabilization, and phytodegradation (Singh and Jain 2003).

Phytoremediation is applicable for the uptake and remediating metals, hazard organic pollutants (i.e., pesticides, PAHs, crude oil), explosives, solvents, and landfill leachates. Certain plant species have the tendency and the ability to accumulate and store pollutants such as metals in their roots. These plant species can be transplanted to filter metals and pollutants from contaminated water or wastewater. Once the roots become saturated with contaminants and/or metals, they should be harvested. Usually, the hyper-accumulator plants are able to remove, accumulate, and store remarkable amount of the metallic pollutants. Trees are under examination, currently, to investigate their ability in removing the organic pollutants from groundwater. The study includes the translocation, accumulation, transpiration, and possibly metabolization of the organic contaminants to plant tissue or CO_2. Meanwhile, plants help prevent wind, dust, and rain. They also prevent the flow of groundwater from transferring pollutants away from the site to deeper underground and/or the surrounding areas.

5.2 Phytoremediation of Soil

As the anthropogenic activities increase around the world, contamination of soil and remediation of the polluted sites become a worldwide concern with the environmental threat (Bundschuh et al. 2012). In Europe, the contaminated sites are about 340,000. About 15% out of them have been remediated (EEA 2014). It was reported by the Office of Solid Waste and Emergency Response (OSWER), of the United States of America, that they have been successfully remediated over 540,000 sites and 23 million acres of contaminated soil and land (Treasury Board of Canada Secretariat 2014). In Australia, agricultural activities and industrial processing including mineral mining, petroleum refinery, and chemical manufacturing have caused soil contamination with pesticides, nutrient elements, heavy metals, mineral salts, hydrocarbons, particulates, etc. The estimated contaminated sites across that country were 80,000 according to DECA (2010). In China, in 2014, the Ministry of Land and Resources and the Chinese Ministry of Environmental Protection released their first bulletin survey concerning the nationwide soil pollution. They estimated about 20 million hectares of land were contaminated with heavy metals. This contaminated land may cause reduction in food supplies exceeding 10 million tons/year in China (Wei and Chen 2001).

The biodegradability of given pollutants is affected by several factors, including the physical-chemical properties of the contaminants and how the soil can influence and affect its chemical state. Diverse soils, generally, vary in their ability to overcome the contamination. This may be due to the behavior of insoluble organic pollutants. There are an extensive efforts and enormous developing strategies for the remediation of environmental pollutants. Nevertheless, the high costs of the physical and chemical strategies hindered these efforts. Thus, the use of biological strategies is a more feasible and applicable. These employed biological methods are using different biological agents such as higher plants, bacteria, microalgae, and fungi to degrade persistent contaminants. The process of soil bioremediation is to utilize the metabolic activities of microorganisms to remove and clean up the hazard contaminants. The main aim is to convert the organic pollutants, completely to harmless compounds, such as water and CO_2. The time factor is also an important factor. It is important that microorganisms are capable to achieve the desired degradation within a reasonable period of time (Fu and Secundo 2016).

5.3 Soil Phytoremediation Process

The technical processes of the phytoremediation are varied widely. According to Schnoor (1997), this variation depends on the properties and the chemical nature of the pollutants (if it is subject to degradation in the soil or in the plant or it is volatile or inert) and the characteristics of plant as illustrated in Figs. 5.1 and 5.2.

5.3.1 Phytostimulation (Biodegradation of Enhanced Rhizosphere)

Phytostimulation process is known as rhizosphere degradation, and it is also known as “Rhizo degradation”, in which enhancement of soil microbial activity for contaminants degradation. Such degradation of the pollutants is achieved by organisms that are associated with the plant roots. Enhanced rhizosphere biodegradation is carried out in the soils that are immediately surrounding plant roots. The microorganisms are supplied by nutrients via the natural substances released by plant roots. Meanwhile, the microorganisms enhance the biological activities of the plant roots; the latter also loosen the soil and then die, leaving paths for transporting of aeration and water. This process also causes to pull water to the surface zone of the soil and tends to dry the lower saturated zones. Plant roots, thus, stimulate soil microbial activity for the degradation of pollutants in the rhizosphere or soil root

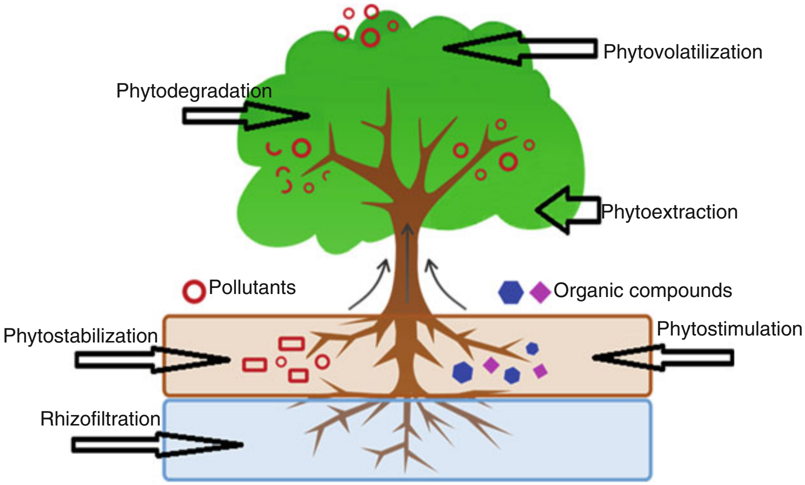


Fig. 5.1 Schematic representation of phytoremediation techniques. (Schnoor 1997)

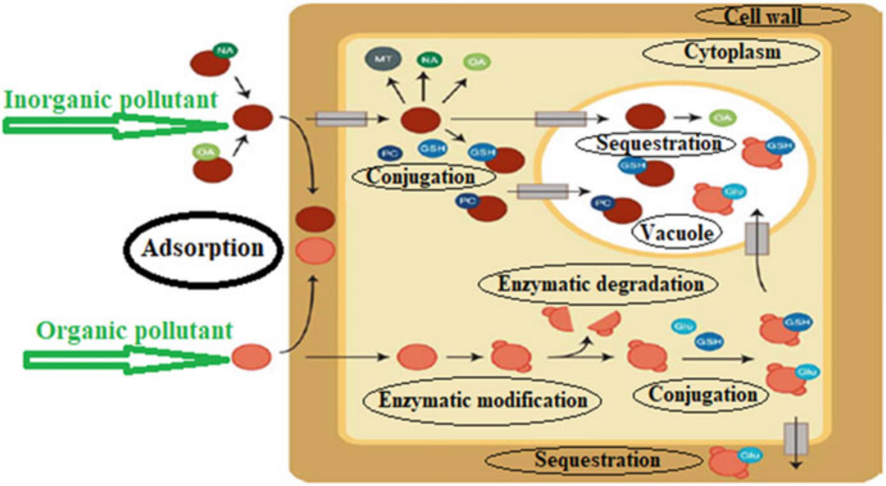


Fig. 5.2 Pathway of pollutants inside the plant cell wall. (Schnoor 1997)

zone. According to Dowling et al. (2009), plant roots stimulate microbial activity in the following different ways:

1. They enhanced aerobic transformations through oxygenate rhizosphere.
2. They increase the bioavailability of organic carbon.
3. Root exudates possess enzymes, carbohydrates, sugars, amino acids, etc. that enrich the microbes.
4. Degradation of the organic contaminants by the mycorrhizae fungi that grow within rhizosphere.
5. An ideal habitat is provided by the roots to increase the microbe populations. This process is applicable in the containment of organic pollutants from soil like aromatics, poly-aromatic hydrocarbons (PAHs), and pesticides.

In addition, the growing roots promote and enhance the proliferation of degrading rhizosphere microorganisms. The latter utilize metabolites and exudates of plants as a source of energy and carbon. Plants, however, may exude biodegrading enzymes themselves. The phytostimulation application is limited to organic pollutants only (Prasad 2004). In the rhizosphere, the microbial community is heterogeneous due to the different spatial distribution of nutrients. Nevertheless, the genus *Pseudomonas* species are the predominant organisms, and they are associated with roots (Ali et al. 2013).

Besides, phytostimulation can involve aquatic plants to support active populations of the microbial degraders. Similarly, the stimulation of atrazine degradation was carried out by hornwort (Rupassara et al. 2002). In the phytoremediation projects, the most commonly used flora is poplar trees. This is primarily because the trees are fast growing and they can survive in a very broad range of climates.

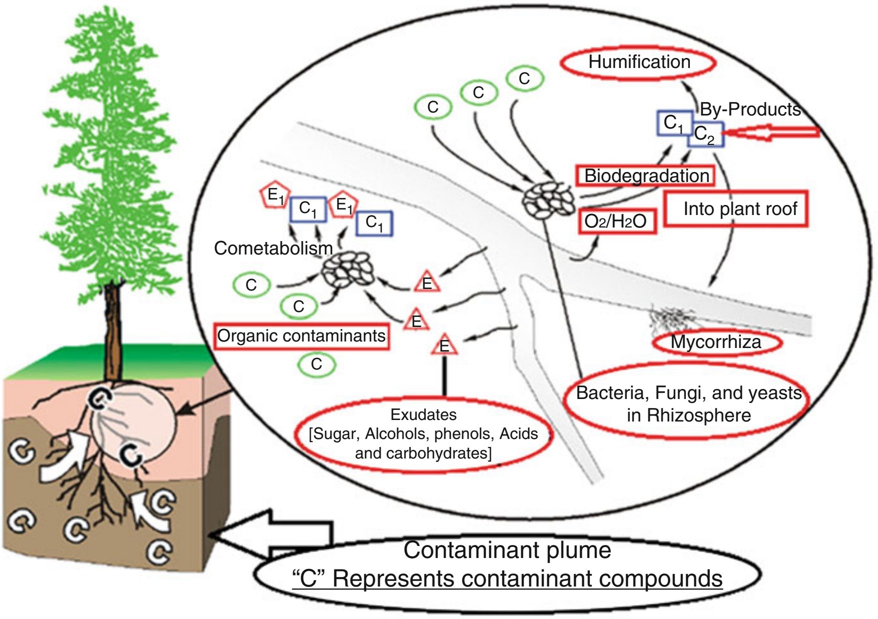


Fig. 5.3 Rhizo-degradation of organic compounds from contaminated media. (ITRC 2009)

Relative to other plant species, poplar trees can draw large amounts of water, mainly because water passes directly from an aquifer or through soil. These trees may draw greater quantities of dissolved contaminants from polluted media. They reduce and consume the amount of waters that are originally from an aquifer or pass through soil. Therefore, these trees are reducing the amount of pollutants that pass though the soil or out of aquifer (ITRC 2009) as shown in Fig. 5.3.

5.3.2 Phytoextraction or Phytoaccumulation

Phytoextraction is the uptake, accumulation, and concentration of pollutants from the contaminated environment by the roots of plant. The pollutants are then translocated/accumulated into the plant biomass (i.e., shoots and leaves). Phytoabsorption, phytosequestration, or phytoaccumulation involves the absorption of pollutants by the plant roots followed by translocation and accumulation in the shoots and leaves (i.e., aerial parts). This took place mainly in the uptake of heavy metals such as Cd, Ni, Cu, Zn, and Pb (Abdel-Shafy et al. 1986). Other heavy metals including Se, As, Ni-56, and Co and organic compounds can also be eliminated (Abo-El-Souad et al. 1994). The hyper-accumulator plants are used to concentrate specific metals in their aerial parts. These hyper-accumulator plants have the tendency to store reasonable concentrations of certain metals in their tissues (i.e., 0.01–1% dry weights). The amount of accumulated metals varied from one metal

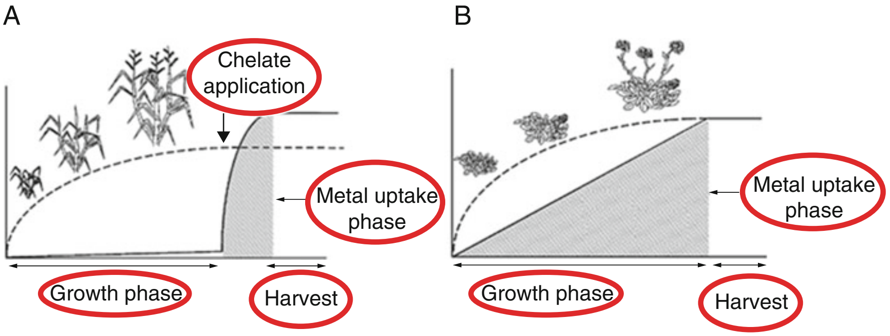


Fig. 5.4 (**a**) Schematic presentation of chelate-assisted phytoremediation of metals, (**b**) continuous phytoremediation and uptake of metals. Metal concentration is presented by solid line and shoot biomass is presented by dashed line. (Taiz and Zeiger 2010)

to the other (Abdel-Shafy and Farghaly 1995). *Pteris vittata*, *Elsholtzia splendens*, *Thlaspi caerulescens*, and *Alyssum bertolonii* are hyper-accumulator plants. They are known for the uptake of Cu, Ni, Zn/Cd, and As (Vander Ent et al. 2013).

The aerial parts of plants (i.e., above ground) can be harvested and burnt to obtain energy and regain metals from ash as recycling. This technique has been extensively used for bioremediation and elimination of heavy metals including Pb, Zn, Cu, Ni, and Cd using plants such as *Thlaspi caerulescens*, sunflower (*Helianthus* spp.), Indian mustard (*Brassica juncea*), and vascular plants (Abdel-Shafy et al. 1994a, b). Effective phytoextraction and elimination of certain pollutants require hyper-accumulator plants. These plants are able to accumulate metals at the rate of more than 100 times as compared to non-accumulator plants. In this respect, nickel hyper-accumulator *Berkheya coddii* was utilized to remove Ni from land near the Rustenburg smelter, South Africa in 1990s (Vatamaniuk 2001). In addition, sunflower (*Helianthus annuus*) or bracken fern, as hyper-accumulators, was employed to clean up arsenic. The leaves of bracken fern are able to store arsenic as much as 200 times as compared to soil. Similarly, lead was phytoextracted by hemp dogbane, ragweed, or Indian mustard (Fig. 5.4) (Taiz and Zeiger 2010).

In the root zone, phytochemical complexation reduces the fraction of the bio-available organic pollutants. Transport protein inhibition, located on the root membrane, prevents organic pollutants from entering the plant. Vacuolar storage in the root cells, as phytoaccumulation of organic compounds, in which the organic pollutants can be sequestered into the vacuoles of root cells (ITRC 2009) as shown in (Fig. 5.5).

5.3.3 Phytodegradation (Phytotransformation)

The metabolism of pollutants within plant tissues is known as phytodegradation. Enzymes including oxygenase and dehalogenase are produced by plants. These

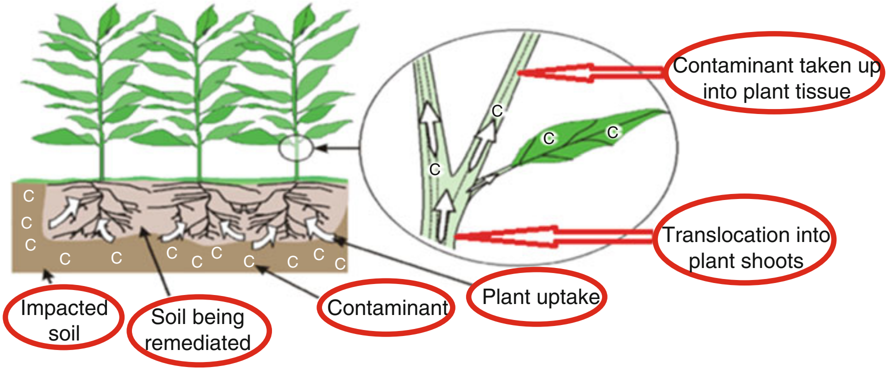


Fig. 5.5 Phytoaccumulation of organic contaminants. (ITRC 2009)

enzymes help catalyze the degradation process. Several researches are proceeding to investigate if both chlorinated aliphatic and aromatic organic compounds are amenable to phytodegradation by plants. Organic pollutants are degraded, mineralized, or metabolized inside plant cells via specific enzymes. These enzymes are laccases (degradation of anilines), dehalogenases (degradation of chlorinated solvents and pesticides), and nitro-reductases (degradation of nitro-aromatic compounds). Plants such as *Myriophyllum spicatum* and *Populus* species are examples that have these enzymatic systems. It was reported by Rylott and Bruce (2008) that chemical modification of environmental substances resulted from plant metabolism and often induces the degradation (phytodegradation), inactivation, or immobilization (phytostabilization) as shown in Fig. 5.6.

In the case of organic contaminants, certain plants, such as *Canna*, render some pollutants including industrial chemicals, pesticides, solvents, explosives, and other xenobiotic substances, as nontoxic by their metabolism (Kvesitadze et al. 2006). In other cases, however, microorganisms that live and associate with plant roots may metabolize such contaminants in soil or water. The plant tissue metabolism of these recalcitrant compounds and contaminants cannot be broken down to basic molecules (carbon dioxide, water, etc.). Thus, the term phytotransformation is representing the change that took place in the chemical structure of pollutants without complete breakdown of their compound structure.

5.3.4 Phytostabilization (Phytoimmobilization)

The phenomenon of producing chemical compounds by plant to immobilize pollutants at the interface of soil and roots is described as phytostabilization. This phenomenon of phytostabilization reduces the mobility of substances in the environment. This can be achieved by limiting the leaching of substances from soil. Inorganic or organic contaminants are incorporated into humus or into the lignin of

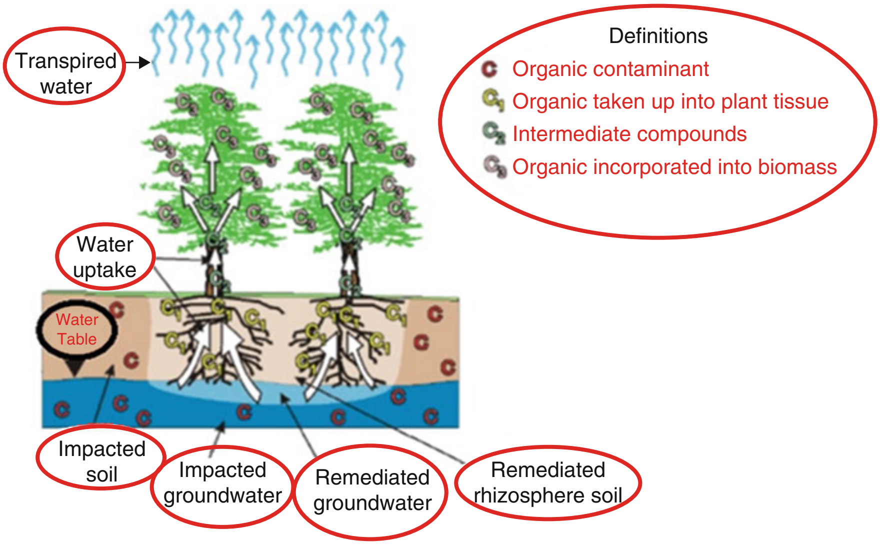


Fig. 5.6 Phytodegradation of organic compounds by plant. (ITRC 2009)

the cell wall of roots. By the direct action of root exudates, metals are precipitated as insoluble forms; subsequently metals are trapped in the soil matrix.

The main aim is to avoid the mobilization of the pollutants and to limit their presence in soluble form as well as to avoid their diffusion in the soil. It was reported by Ali et al. (2013) and Berti and Cunningham (2000) that some genera species including *Alyssum*, *Haumaniastrum*, *Eragrostis*, *Gladiolus*, and *Ascolepis* are examples of plants that are cultivated for this purpose. The study focused on the elimination of contaminants in soil adjacent to the roots for the purpose of reducing their bioavailability. The pollutants are rendered insoluble, immobile, and less toxic through accumulation and adsorption by the roots. Thus, they precipitate or exudate within the soil adjacent to the roots and root zone. Phreatophytic trees that are characterized with fibrous roots are extensively used for hydraulic control and for soil erosion control. These trees are being used also for the uptake of heavy metals such as Cd, Pb, Zn, Cu, As, U, and Se. Besides, they are utilized for the removal of hydrophobic non-biodegradable organic compounds (Fig. 5.7).

5.3.5 Phytovolatilization

The phytovolatilization technique relies and depends on the ability of certain plants to volatilize and absorb some metals/metalloids. Certain elements such as Hg, Se, As, and others from groups IIB, VA, and VIA of the periodic table can be absorbed by the roots, converted into nontoxic forms, and finally released into the atmosphere air. Similarly, selenium can be absorbed by the species of *Stanleya pinnata* and *Astragalus bisulcatus* and/or transgenic plants (with bacterial genes) of *Arabidopsis*

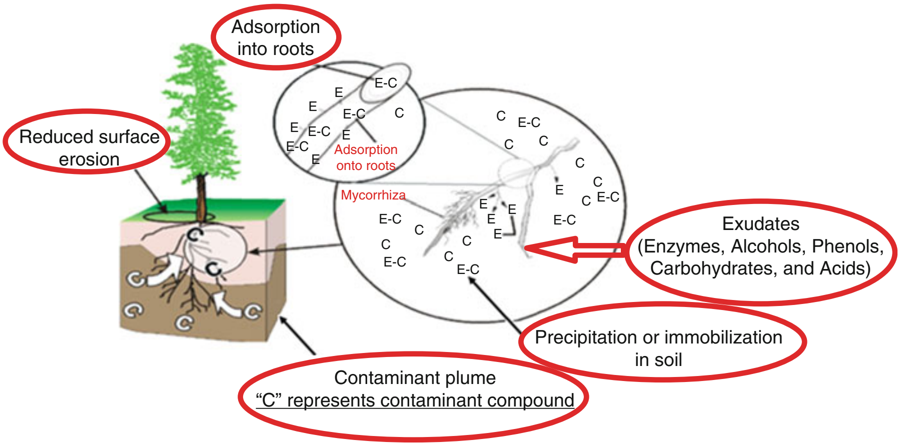


Fig. 5.7 Phytostabilization of organic and inorganic compounds. (ITRC 2009)

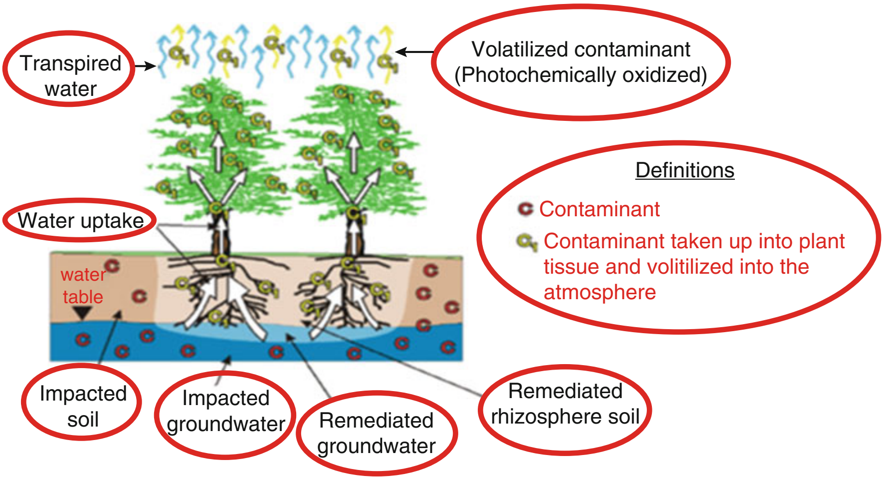


Fig. 5.8 Phytovolatilization of organic contaminants by plant. (ITRC 2009)

thaliana. Meanwhile, Hg can be absorbed by *Brassica napus*, *Liriodendron tulipifera*, or *Nicotiana tabacum*. The same technique can also be employed for the absorbance of organic compounds (Pilon-Smits and Le Duc 2009).

Contaminants are, thus, absorbed from soil or water to be released into the air to less polluting and/or volatile substances as a result of phytotransformation. In this respect, the contaminants are uptaken and/or absorbed by the plant roots and translocated to the leaves to be volatilized through plant stomata (i.e., they are the sites for gaseous exchange as the plant transpiration). These sites are used for the containment of volatile organic compounds. Phreatophytic trees are used for capturing of organic compounds from groundwater (Fig. 5.8). Similarly, Fig. 5.9

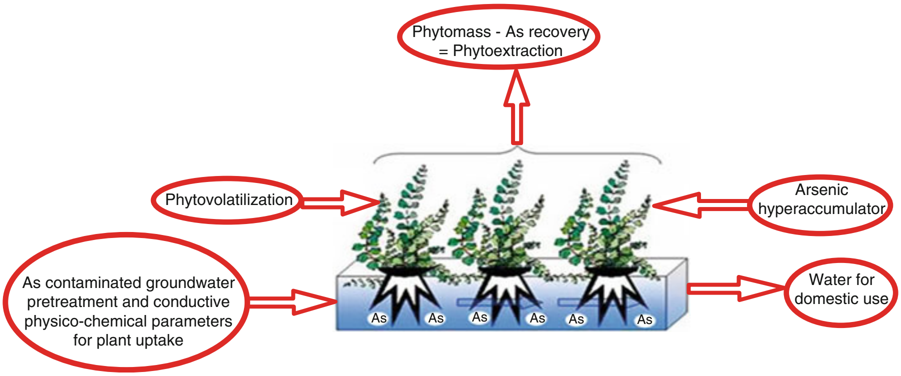


Fig. 5.9 Illustration of phytovolatilization of heavy metals as plant uptake. (ITRC 2009)

represents the uptake of Hg, Se, and As by *Brassica juncea* in the wetland treatment system (ITRC 2009).

5.3.6 Rhizofiltration (Phytofiltration)

Rhizofiltration is the process through which plants concentrate, absorb, and/or precipitate pollutants, such as heavy metals and/or radioactive elements, from an aqueous medium. This process is achieved through plant root system and other submerged organs. The contaminants remain adsorbed on or absorbed in the roots. Rhizofiltration of plants is also the process where mass of plant roots could filter groundwater to remove toxic substances and contaminants. It is employed to reclaim groundwater rather than soil remediation. Here how plants are prepared: firstly, the plants are allowed to grow in clean water to achieve a large root system. After developing a mass of sizeable root system, contaminated water is supplied to the plant root to adapt them. Plants are, then, placed in contaminated area where the root mass system accumulates and uptakes pollutants from water. Once the root mass become saturated with the contaminants, the plants are to be harvested and disposed in a safe way. The plants, thus, are kept in a hydroponic system. The effluents or the aqueous media pass and are “filtered” through the roots and/or other organs. The latter is to absorb and concentrate the pollutants (Dhote and Dixit 2009). It is worth to mention that plants with more accumulation capacity, high absorption surface, high root biomass, (aquatic hyper-accumulators) and/or tolerance to pollutants achieve the best accumulation results. The best promising results were achieved by *Fontinalis antipyretica*, *Helianthus annuus*, *Phragmites australis*, *Brassica juncea*, and several species of *Callitriche*, *Salix*, *Lemna*, and *Populus* (Favas et al. 2012). It was reported that *Phragmites australis* was very effective in the removal of contaminants from wastewater (Abdel-Shafy et al. 2017). In addition, water hyacinths proved to be a promising plant for the uptake of heavy metals from the aquatic system (Abdel-Shafy et al. 2016; Fayed and Abdel-Shafy 1985).

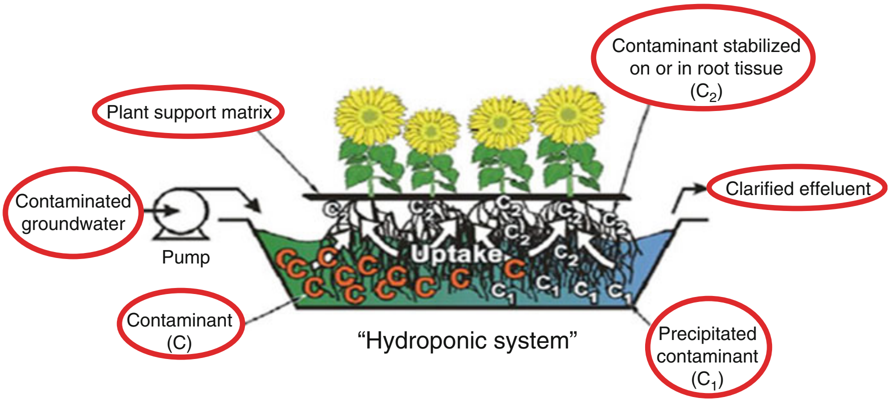


Fig. 5.10 Rhizofiltration process of the sunflower in a hydroponic system. (Paz-Alberto and Sigua 2013)

Water is filtered through a mass of roots to remove the toxic contaminants or excess nutrients (Favas et al. 2012; Fayed and Abdel-Shafy 1986). It was reported that aquatic plants, submergent plants (algae, *Hydrilla* spp., stonewort), and emergent plants (coontail, bullrush) are used for the bioremediation of heavy metals including Cd, Pb, Zn, and Cu (Fayed and Abdel-Shafy 1985; Abdel-Shafy and Farghaly 1995). These plants are also used for the bioremediation of hydrophobic organics and radionuclides. The sunflower roots (*Helianthus annuus*) proved to be good candidate as rhizofilter for the uptake of pollutants including heavy metals, namely, Pb, Cu, Zn, and Cr (Abdel-Shafy and Dewedar 2012). Several common plants are known for being a good accumulator of pollutants and are considered by researchers as remediation. These plants include ragweed (*Ambrosia artemisiifolia*), sea pink or sea thrift (Armeria maritima), Indian mustard (*Brassica juncea*), turnip (*Brassica napus*), rape, rutabaga, cabbage, broccoli (*Brassica oleracea*), and flowering/ornamental kale. Similarly, other plants are considered as good phytofiltration such as corn (*Zea mays*), blue sheep fescue (*Festuca ovina*), wheat (scout) (*Triticum aestivum*), pennycress (Thlaspi rotundifolium), and sunflower (*Helianthus annuus*), (Paz-Alberto and Sigua 2013). Figure 5.10 represents the rhizofiltration process of the sunflower in a hydroponic system.

5.4 Phytoremediation of Soil: Advantages and Limitations

The phytoremediation technology has several advantages and disadvantages that should be considered when applying such process. The low cost is one of the most important advantages. However, the time needed to observe the necessary achievement can be long. The concentration of pollutants as well as the presence of other contaminants should also be considered. Meanwhile, selecting plant species that could be efficient is not easy and could limit the process. These limitations could hinder the advantages of this technology. The possibility of these plants to enter the food chains should be considered carefully and should be taken into account (Burken et al. 2011).

5.4.1 Advantages of Phytoremediation Process

- The technique can be implemented in situ as well as ex situ.
- Sunrays are the energy for the phytoremediation process.
- The process reduces the diverse impact of the environment.
- It contributes to the landscape view.
- The phytoremediation view is highly accepted by the public.
- The system provides good habitat for the animal life.
- Protect the concern area from contaminated winds and the dispersion of dust.
- Reducing the surface runoff.
- Reduces the mobilization and/or leaching of pollutants to the soil.
- Harvesting of the organs or plants that accumulated pollutants can easily be achieved.
- The harvested organs or biomass can be economically used.
- Plant phytoremediation process is easy to be controlled than employing the microorganisms for remediation.
- It is possible to reuse and recover any valuable metals by the "phytomining" companies that are specialized in this field.
- The phytoremediation is an eco-friendly process as it protects the environment in a more natural state.
- Monitoring of plants to follow up the phytoremediation process can be easily achieved.

5.4.2 Limitations of the Phytoremediation Technology

- The mass of plant root is mostly at variable depths in which the treatment zone is determined as phytoremediation. In most cases, however, pollutants are localized at the surface (<5 m).
- High concentrations of the contaminants are mostly hazardous and toxic to plants.
- Mass transfer through the phytoremediation process is limited similar to other bio-treatments.
- This process may be limited to seasonal variation according to the location.
- In some cases the phytoremediation transfers pollutants from soil to air.
- The process is not efficient to eliminate the strong sorbet (such as PCBs).
- The bioavailability and toxicity of biodegradation products cannot always be predicted.
- Bioaccumulation of the products by animals or mobilization into groundwater is possible.

- The technology is still in the developing and demonstrating stages.
- The phytoremediation technology is still unfamiliar to regulators.
- Generally, plants are selective in terms of metal uptake in the phytoremediation process.
- The phytoremediation process is much slower than the conventional physico-chemical techniques.
- At contaminated sites, the phytoremediation plants may not always adapt to the environmental and climatic conditions.
- To allow the application of phytoremediation techniques in a contaminated area, this area should be large enough for cultivation.
- The degradation products and the bioavailability of the contaminants remain, mostly unknown.
- Efficiency of the phytoremediation is limited only down to the depth occupied by the roots.
- The growth of plants is slow, and the formation of the biomass requires a long-term commitment.
- Preventing the leaching of pollutants into the groundwater completely is not possible.
- Survival and efficiency of the plants are greatly affected by the condition of the soil and the toxicity of the pollutants.
- The bioaccumulation of the pollutants by plants requires a careful and safe disposal of these plant materials to protect the environment.

5.5 Phytoremediation for the Removal of Heavy Metals: Concept and Application

Heavy metals, in the environment, form an important polluting group. These metals naturally occur at low concentrations in the environment. They rarely occur naturally at toxic level. Heavy metals, mostly, come from the industrial local sources such as iron and steel, nonferrous industries, chemical industries, and power plants. Soil contamination with heavy metals is a critical and environmental concern due to their toxic and potential adverse effects to the ecology. Due to their widespread occurrence, heavy metals are considered hazard soil contaminants as they possess chronic and acute toxicity. Heavy metals are strongly persistent in the environment. They are non-biodegradable pollutants and non-thermo-degradable. Unlike organic pollutants they persisted and cannot be degraded into smaller constitutes. They cannot be broken down; thus, they can be accumulated to toxic levels. They cannot also be degraded to harmless or small molecules. The long-term effect might be toxic to the biosphere. Heavy metals are known for having toxic effect (Abdel-Shafy and El-Saharty 2015). Besides, they tend to accumulate in all living organisms with a permanent toxic and carcinogenic effect (Ben Chekroun and Baghour 2013).

Furthermore, heavy metal pollutants are difficult to remediate and/or remove from water, soil, and air. Toxic heavy metals such as and zinc, copper, lead, cadmium, arsenic, and mercury are immutable through all biochemical reactions (Kramer and Chardonnens 2001).

On the other hand, the use of wastewater for irrigation as well as dumping sewage sludge and solid wastes on soils has been a widespread practice in agricultural areas (Abdel-Shafy et al. 2003). The wastewater includes industrial, municipal, and household liquid waste. The source of heavy metals to the agriculture area comes from irrigation with polluted water, use of sewage sludge, contaminated manure, pesticides containing heavy metals, as well as the use of mineral fertilizers especially phosphates (Abdel-Shafy et al. 2005). Other sources of heavy metals are burning of fossil fuels, waste incineration, and road traffic. Several investigators studied the impact of using sewage effluent, drainage water and industrial wastewater in irrigation, and their diverse effect on water, soil, and plants related to heavy metal contamination (Abdel-Sabour et al. 2001; El-Bahy et al. 2005). Their study included the accumulation of heavy metals in soil, in plant, in animals, and lastly in the food product (Khan et al. 2010).

Heavy metals and metalloids, including mercury, cadmium, arsenic, selenium, and lead, are released into the environment by human activities such as industry, mining, and agriculture. These activities are threatening the environment as well as human health (Danik et al. 2006; Kamal et al. 2010). In the United States alone, there are more than 50,000 sites contaminated with heavy metals. Many of these sites are under Superfund, and they should be remediated (Bennett et al. 2003). There is a stinging need to adapt low-cost, efficient, and sustainable technology to detoxify or remove such acute and toxic contaminants from the environment. The phytoremediation process as plant-based approaches is the most convenient. These processes are performed in situ, are relatively inexpensive, and are solar-driven (LeDuc and Terry 2005).

Most researches on the phytoremediation practically aim to extract and remove the pollutants from wastewater, soil, and sediment. The pollutants are to be transported aboveground and to be concentrated by orders of magnitude. Similarly, plants can also assist with the cleanup of surface water polluted with elemental contaminants. In this term, phytoremediation has important advantages over the traditional physical remediation processes. The latter relocates capping or postpones the problem. Plants have the ability to enhance the activity of rhizosphere and extract the contaminants aboveground. Thus, plants could both support and amend fungal and bacterial remediation schemes for metal pollutants. In addition, plants can be applied to the bioremediation of various airborne pollutants. This is mainly due to their natural capacity to extract nutrients including nitrogen, carbon dioxide, and sulfur from air (Meagher and Heaton 2005).

The plant employed in the phytoremediation process must be selected carefully. They should have a considerable capacity of heavy metal absorption and accumulation. The time is an important factor for decontamination and removal of pollutants from the ecosystem (Mudgal et al. 2010). Plants proved to be able to uptake and accumulate different heavy metals from the environment (Baghour et al. 2002;

Abdel-Shafy et al. 1994a, b). Tolerance of heavy metal in plants could be referring to their immobilization in the plant cell wall (Davis et al. 2003). It may be also referring to their conferred by compartmentalization in the vacuoles (Ben Chekroun and Baghour 2013). Some algae exhibit high capacity for the accumulation of heavy metals (Abdel-Shafy and Farghaly 1995). This capacity is the result of the tolerance mechanisms. Many algae synthesize metallothioneins and phytochelatins that can form strong complexes with metals and, thus, translocate them into the vacuoles (Abdel-Shafy and Farghaly 1995; Ben Chekroun and Baghour 2013). So far, the main success for phytoremediation application is the removal of toxic metals and trace elements from soil (Chaudhry et al. 2005).

5.6 Origin and Source of Heavy Metals to Aquatic Ecosystems

Aquatic ecosystems, in many countries, have been subjected to the discharge of industrial wastewater. Domestic, sewage sludge, and agricultural drainage have been discharged also to several waterways in many countries. These wastes are important source of inorganic and organic contaminants including heavy metals and pesticides. These contaminations lead to widespread of pollutants to both groundwater and surface water by runoff. Meanwhile, weathering of rocks and soil from volcanic eruptions introduces metals to the aquatic ecosystems. Varieties of human activities including industrial processing and mining are important sources of heavy metals to the environment (Jain 2004). The input of these metals may also be derived from remobilization of natural soils as a result of the changes in local environmental redox conditions. Additional source of heavy metals is the corrosion of metallic subsurface engineering structures that resulted from prolonged submergence under acidic groundwater (Leung and Jiao 2006).

González et al. (2007) studied the origin and bioavailability of heavy metals from the Nador Lagoon sediments (Morocco). They found that the most important trace element anomalies including Mn, Zn, Cu, Co, Pb, Cd, and As were mainly found around old mining and industrial activities. Such industrial activities are the main contributors of very high concentrations of heavy metal into the environment. Their contributions are between 100- and 1000-fold higher than those in the Earth’s crust (Ben Chekroun and Baghour 2013).

In a study by Ben Chekroun and Baghour (2013) on a river polluted by base metal mining, they found that cadmium was potentially bioavailable and the most mobile metal. They also reported that Cd was primarily scavenged by organic matter, non-detrital carbonate minerals, and iron-manganese oxide minerals. On the other hand, mercury is always present in the environment as naturally occurring metal at very low concentrations. Global human activity, however, has led to a remarkable increase of this metal in land and aquatic environment and is released into the atmosphere (Dietz et al. 2009). Wang et al. (2004) mentioned that the most important anthropogenic sources of Hg contamination in aquatic environment are mining, the atmospheric deposition, agricultural material runoff, urban discharges, industrial discharges, fossil fuel use, pharmaceutical production, and burning of coal. It was reported by Mohiuddin et al. (2010) that heavy elements may be immobilized in the stream sediments. Thus, these trace elements could be involved in complex formation, coprecipitation, and absorption (Okafor and Opuene 2007). Trace elements, sometimes, are co-adsorbed along with other elements in the form of hydroxides or oxides of Fe and Mn. They may, also, occur in particulate form (Mwiganga and Kansiime 2005). However, treatment of the metal-contaminated wastewaters before their discharge into the environment is essential. This way protection of the ecosystem can be achieved to control the levels of heavy metal in the environment.

5.7 Mechanism of Phytoremediation for the Uptake of Heavy Metals

Mechanism of the phytoremediation of trace elements/metalloids in soils is illustrated in Fig. 5.11. Plants accumulate metals from different media such as water, wastewater, and soil. These media act as bioavailable pool of trace metals as well as plant nutrients. Factors including pH, competitive cations, root exudates, microbial biomass, and organic matter can affect the availability of trace metals in environment (Sarwar et al. 2010). A specific trace elements once accumulated by plant roots may be either uptaken in root tissues (phytoimmobilization). Metals may also be translocated to the aerial shoot parts of plant through xylem vessels. The latter is achieved via symplastic and/or apoplastic pathways. Generally, metals in the

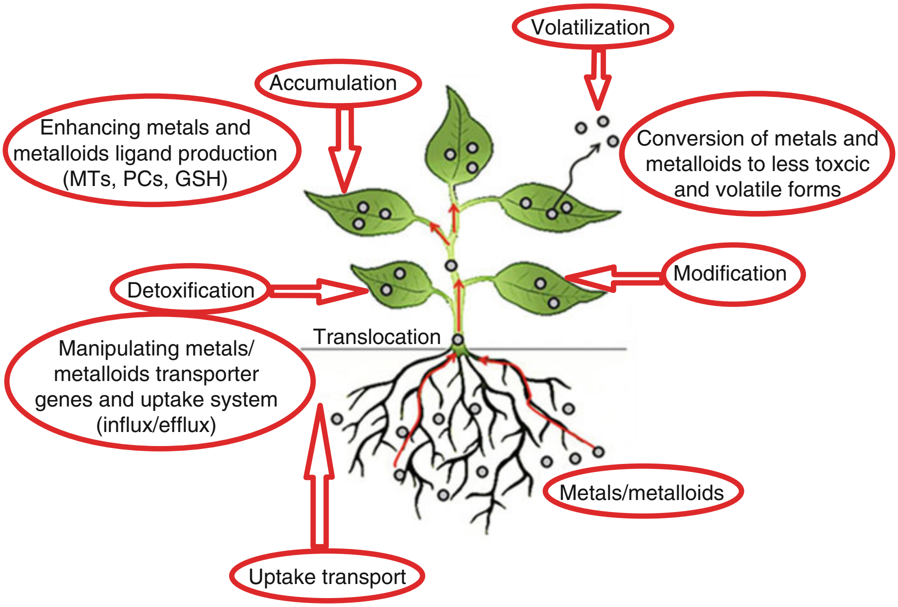


Fig. 5.11 Mechanisms involved in phytoremediation of trace metals/metalloids in soils. (Memon and Schreoder 2009)

aerial part are accumulated in vacuoles (cellular organelles with low metabolic activity). In the hyper-accumulator plants, this might be an important tolerance mechanism. The purpose is to keep deleterious metals away from important cellular metabolic processes. The mechanism of the phytoextraction has the following five major steps: metal mobilization in rhizosphere, metal uptake by plant roots, translocation toward aerial plant parts, metal sequestration in plant tissues, and trace metal tolerance (Ali et al. 2013). Tolerance of trace metals is a prerequisite for the process of phytoremediation. As the more the plant is tolerant to trace metal stress, the more will be the metal accumulation in plant tissues. This will result with minimum adverse effects on plant health. Metal tolerance potential of a plant depends on mechanisms like active transport of metal ion into the vacuoles, cell wall metal binding, complex formation, and chelation of metal ions with proteins and peptides (Memon and Schreoder 2009).

5.8 Role of Algae in Phytoremediation and Heavy Metal Removal

Phytoremediation is considered the most favorable technology in which plants are used for removal of environmental contaminants and/or detoxify these pollutants to make them harmless (Ben Chekroun and Baghour 2013). Several living organisms are able to concentrate and accumulate certain toxicants to their body to level much higher than their environments (Fayed and Abdel-Shafy 1986; Kord et al. 2010). As a result, the use of plants for the removal of heavy metals has become attracted because of the various problems associated with the removal of pollutants using traditional methods. Bioremediation including phytoremediation strategies has been used as an attractive alternative simple technology due to their low cost, low energy, and high efficiency (Abdel-Shafy et al. 2017; Mejare and Bulow 2001).

Recently, there has been great interest in using algae for determining the eutrophication through the biomonitoring of organic and inorganic contaminants. It was possible to estimate the total nitrogen content spectrophotometrically in water of the aquatic systems (Ben Chekroun and Baghour 2013). This was achieved by determining the chlorophyll formation by the algae (Abe et al. 2004). This can give us clear idea about the levels on eutrophication. The high biomass production by algae is one of the advantages of using it in phytoremediation. These species have high tendency to absorb and accumulate heavy metals (Baghour et al. 2002).

It was reported that removal of heavy metal by the conventional treatment involves variable mechanisms which include increasing the pH, flocculation, settling, sedimentation, precipitation, cation and anion exchange, oxidation/reduction, complexation, adsorption, and microbiological activity/microalgae (Abdel-Shafy 2015). The latter can remove heavy metals from contaminated water via two major mechanisms. The first mechanism is the metabolism of metal uptake into their cells at low concentrations. The second mechanism is the bio-sorption of metals which is a non-active adsorption process (Fayed et al. 1983; Ben Chekroun and Baghour 2013).

The algae are ideal candidates for concentrating and removing heavy metals from the environment. They have many features to make them so ideal including large surface area/volume ratios, high tolerance to heavy metals, phytochelatin and phototaxy expression, potential for genetic manipulation, and ability to grow both autotrophically and heterotrophically (Ben Chekroun and Baghour 2013). Macroalgae have been used extensively to determine the environmental contamination and marine pollution with heavy metal in marine ecosystem locations in the world (Abdel-Shafy 2015). Recently, green algal species, namely, *Cladophora* and/or *Enteromorpha*, have been used to determine the level of heavy metals in different parts of the world (Al-Homaidan et al. 2011; Abdel-Shafy and Farghaly 1995). Due to their efficiency in accumulating heavy metals and concentrating them in their tissues, the macroalgae have become widespread, used as biomonitors of metal in aquatic and marine ecosystems (Gosavi et al. 2004, Abdel-Shafy and Farghaly 1995). It was reported that Cyanophyta and Chlorophyta are hyper-accumulators and hyper-absorbents for boron and arsenic. They can accumulate and absorb these elements from the environment to concentrate them in their bodies (Ben Chekroun and Baghour 2013). Thus, these algae are considered hyper-phytoremediators. On the other hand, the Phaeophyta as brown algae are efficient in accumulating heavy metals. This is mainly due to the high levels of alginates and sulfated polysaccharides within their cell walls. Thus, heavy metals show a strong affinity to these Phaeophyta brown algae (Davis et al. 2003). Nielsen et al. (2005), afterward, proposed that brown algae including fucus spp. mostly dominate the heavy metal-contaminated vegetation habitats.

5.9 Phytoremediation for the Removal of Toxic Organic Aromatic Pollutants

Poly-aromatic hydrocarbons (PAHs) are identified as one of the most hazardous chemical pollutants in the environment. These chemicals have carcinogenic, toxic, and mutagenic effects on living body (Abdel-Shafy and Mansour 2016). Beside their carcinogenic activity, these polyaromatic hydrocarbons (PAHs) are recalcitrant, ubiquitous occurring and have bioaccumulating potential. The diesel and all hydrocarbon fuels composed of excessive quantity of PAHs. Thus, they cause abundant ecosphere pollution of the PAHs (Ruma et al. 2007). The level of PAHs in the environment varies widely depending on the mode of PAHs transported in the environment, the level of industrial pollution, and the nature of the contaminated sites. It was reported by Kanaly and Harayama (2000) that the concentrations of PAHs in sediment and soil vary from 1 μg/kg to over 300 g/kg. The PAHs are colorless, white/pale yellow solids, and low soluble in water and have high boiling and melting points with low vapor pressure.

The remediation of contaminated sites with PAHs has become the first priority for society due to the increase in the awareness of environmental issues as well as the quality of life standards. During the past few decades, there are increasing interest to develope the in situ strategies to remediate the increasing amount of environmental pollution. For this purpose, several tools of the biological remediation have become more interesting for the removal of these persistent contaminants. Particular interest of employing such biological remediation tools is the high economic cost of the physicochemical processes.

A specific type of phytoremediation is the rhizoremediation. It involves both plants and their associated rhizospheric microbes. The latter is the creative biotechnological approach as an important tool of the phytoremediation technique. Thus, the rhizoremediation of PAHs has advantages over other bioremediation strategies for being bioaugmentating, natural attenuating, and phytoremediating. It was reported that various bacterial species are active in degrading different types of PAHs (Bisht et al. 2015). Most of these bacterial species are isolated from contaminated soil. Nevertheless, few of these bacteria can be also isolated from non-contaminated soil. *Pseudomonas aeruginosa*, *Mycobacterium* spp., *Pseudomonas fluorescens*, *Rhodococcus* spp., *Paenibacillus* spp., and *Haemophilus* spp. are some of these bacteria that were commonly studied for the degradation of PAHs (Bisht et al. 2010). Moreover, investigating the molecular communication between microbes and plants to discover such communication is important to achieve better results for the purpose of pollutant elimination. This area of research could be a fascinating point of research for future perspective.

5.10 Phytoremediation: Remedial Strategy for Polycyclic Aromatic Hydrocarbons (PAHs)

A diverse range of hazardous contaminants can be treated efficiently via phytoremediation process. This process can be employed in much larger scale to clean up the pollution. It is easy to implement to contaminated soil, and it is an environmental friendly eco-technology. Besides, it can be visually attractive sites and sustainable technology for the polluted sites. Earlier, different studies investigated the effects of plants on removal of contaminants from spiked water or soil from polluted sites (Huang et al. 2004; Abdel-Shafy et al. 1986). Most of these studies provided valuable results concerning the insights of phytoremediation mechanisms for the removal of organic contaminants and heavy metals (Reed and Glick 2005; Abdel-Shafy and Farghaly 1995). Organic contaminants that have been successfully removed by phytoremediation include herbicides (atrazine), organic solvents such as trichloroethylene (TCE), polychlorinated biphenyls (PCBs), explosives such as trinitrotoluene (TNT), PHC, the fuel additive as methyl tertiary butyl ether (MTBE), PAHs, and BTEX – mono-aromatic hydrocarbons (Pilon-Smits 2005). In addition, the phytoremediated inorganic pollutants include phosphate, nitrate, radioactive isotopes, and plant macronutrients (Harvey et al. 2002). The finding of their researches and experiments allowed the scientists to explore processes for overcoming pollutant stress, without the effects of environmental condition including nutrient limitation and weather. However, it was concluded by Harvey et al. (2002) that the only problem of phytoremediation lies in the removal of high level of pollutants. The latter tends to inhibit the growth of plant, including their root, as well as the oxidative stress. These two factors limit the rate of removal and the efficiency of the phytoremediation (Huang et al. 2005).

5.10.1 Rhizosphere Technology: For Bioremediation of Polycyclic Aromatic Hydrocarbons (PAHs)

In the rhizosphere technique, the microbial communities are used for biodegradation of contaminants. It was reported, earlier, that the endophytic and rhizospheric bacteria were used for rhizoremediation of PAHs in the environment by employing *Populus* sp. The latter species, namely, *Populus* sp., was used in the soil as an inoculation system (Bisht et al. 2010; Bisht et al. 2014). In correlation with the bioaugmentation, nevertheless, *Populus* sp. in rhizosphere bacteria assisted phytoremediation technology. The rhizoremediation is effective for the degradation and/or removal of organic pollutants from contaminated soils. Particular efficiency can be achieved when the rhizoremediation is used in combination with appropriate agronomic techniques (Zhuang et al. 2007). This is mainly due to the fact that the chemical condition of the rhizosphere greatly differs from bulk soil. Consequently, various processes are induced by the roots of plant along with the rhizobacteria (Marschner 2001).

It is greatly agreeable that the contribution of microbes is of great importance for the degradation of contaminants. The phytoremediation alone, without the microbial contribution, is not an efficient technology for many PAH degradation (Chaudhry et al. 2005). Rhizoremediation term describes the combined beneficial interaction of both the plant and the rhizobacteria. The combined interaction of microbial-plant in the rhizosphere offers very useful tools for PAH environmental remediation (Chaudhry et al. 2005). The flavonoids and other compounds are released naturally by roots to stimulate the activity and growth of the degrading bacteria of PAHs (i.e., rhizoremediation occurs naturally in the environment) (Leigh et al. 2006). However, there are several other factors that can improve the efficiency of the rhizosphere process. As mentioned previously, depending on plant species, the composition of root exudates changes as the plant stage developed. These variations, thus, exert obvious effects on the community of the rhizospheric (Garbeva et al. 2004). In many phytorhizoremediation investigation, *Salix* supplants are used as they produce salicylic acid and other related compounds. According to De Cárcer et al. (2007), these produced compounds assist in the degradation of both PCBs and PAHs.

5.11 Phytoremediation of Explosives, Pesticide, and Herbicide Contamination

5.11.1 Phytoremediation of Explosives (RDX, TNT, HMX)

There is a large-scale production of munitions worldwide. This has led to severe environmental contamination in different parts of the world. These explosive compounds include RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine), TNT (2,4,6-trinitrotoluene), and HMX (octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine). Explosives consist of these mutagenic and toxic xenobiotics. They are recalcitrant to remediation and stable in the environment. Thus, special technologies can be used including adsorption, incineration, chemical reduction, advanced oxidations processes, etc.). These technologies are very expensive to implement. Besides, they cause additional environmental problems. The most popular technologies, recently, are the biotechnological methods, including the phytoremediation. The latter is relatively low cost, eco- friendly, low energy, and a highly accepted solution by administration and society. The usage of genetically modified plants, in combination with the ability of bacterial genes, is a very promising technology. This combination is able to detoxify compounds via the phytoremediation-modified plants (Panz and Miksch 2012). Effective phytoremediation could also be achieved through transgenic plants with the expression and introduction of bacterial cytochrome p450 genes and nitro-reductases. Meanwhile, effective phytoremediation could be enhanced by introducing pnrA gene from *Pseudomonas putida* "superbug", encoding nitro-reductase into rapidly growing aspen tree (Aken 2009).

According to Clark and Boopathy (2007), the concentrations of RDX and HMX in soil samples are generally lower than that of TNT. Concentration of RDX and HMX in soil ranges from 800 to 1900 mg.kg^{-1} and 600–900 mg.kg^{-1}, respectively. The maximum detected concentrations were 74,000 mg.kg^{-1} and 5700 mg.kg^{-1}, successively (Clark and Boopathy 2007). It is worth mentioning that these compounds are, generally, less toxic than trinitrotoluene. The earthworm studies by Robidoux et al. (2002) at concentrations lower than 711 mg.kg^{-1} for HMX and 167.3 mg.kg^{-1} for RDX in the forest soil showed that oligochaete survived and no change in biomass was observed. Nevertheless, low concentrations of HMX (15.6 mg.kg^{-1}) and RDX (46.7 mg.kg^{-1}) exhibited a significant decline in earthworm reproduction, particularly cocoon and juvenile production (Robidoux et al. 2002).

5.11.2 Phytoremediation for the Removal of TNT

The typical concentration of trinitrotoluene (TNT) in soil is 4000 and 10,000 mg kg^{-1}, and the highest recorded concentration was 87,000 mg kg^{-1} (Vila et al. 2008). The TNT compound and its transformation products are known for being toxic. Acute toxicity test was carried out by an earthworm using forest soil that was spiked with TNT. The studied assay by Lachance et al. (2004) proved that the TNT at concentration of 143 mg kg^{-1} is the lethal (L_{50}) for half of the oligochaete population. The compound 2, 4, 6-trinitrotolune is known by the United States Environmental Protection Agency (US EPA 2014) as a potential human carcinogen (class C). It was confirmed by Vila et al. (2007a) that a direct contact with this chemical compound can cause skin irritation, liver disorder, immune system damage, or anemia.

The aquatic and terrestrial plants proved to be able to uptake the trinitrotoluene in hydroponic culture (Wang et al. 2003). Short-term experimental studies were conducted with TNT-contaminated soil. The results showed that the aquatic plants can absorb great percentage of the TNT from the contaminated water. This study showed that the aquatic plant species could remove the concentration of 681 mg L^{-1} (0.003 mM) trinitrotoluene in groundwater as initial concentration, to 94–100% removal during a period of 10 days. The plant used in most of the previous research is “Parrot feather water milfoil” (*Myriophyllum aquaticum*) (Wang et al. 2003). This plant was able to perform efficiently for decreasing the concentration of TNT even when the initial concentration was 0.11 mM (Wang et al. 2003). Meanwhile, vetiver grass was efficient in removing the TNT from contaminated soil. At TNT initial concentration of 40 mg kg^{-1}, it was reported that vetiver grass was able to uptake 97% of trinitrotoluene from the contaminated soil after only 3 days of incubation (Das et al. 2010).

5.11.3 Phytoremediation of RDX

RDX is most widely used as military explosive. Using the transgenic plants, the RDX could be phytoremediated. *Arabidopsis thaliana* plants were reported of being genetically manipulated to express bacterial gene, XplA, that encodes a RDX degrading fused flavodoxin-cytochromeP450-like enzyme. In a laboratory bench-scale study, pure cultures of *Rhodococcus rhodochrous* strain 11Y demonstrated around 30% mineralization of radiolabelled RDX. These pure cultures of *Rhodococcus rhodochrous* strain 11Y are the donors for the abovementioned gene and are isolated from RDX-polluted areas. By denitrification, the bacterium could degrade RDX followed by ring cleavage and the release of small aliphatic metabolites. The liquid cultures of *A. thaliana* expressing XplA were able to remove between 32% and 100% of RDX. By non-transgenic plants, however, only less than 10% was removed. It was suggested by Aken (2009) that transgenic plants were competent of efficient phytoremediation of RDX pollutant. Previous study was conducted by Vila et al. (2007a) to determine the ability of soybean (*Glycine max*), corn (*Zea mays*), rice (*Oryza sativa*), and wheat (*Triticum aestivum*) for the uptake RDX from contaminated soil (138 mg.kg^{-1}). Further investigation was carried out by Vila et al. (2007b), on the efficiency of rice for the uptake of RDX from the soil at high concentrations (2000 mg.kg^{-1}). They found that the uptake level of RDX in the studied rice rose from 0 to 31.5 mg.g^{-1} to 0–1000 mg.kg^{-1} as dry weight, respectively. Later study by Chen et al. (2011) confirmed the founding obtained earlier by Vila et al. (2007a). Both investigations studied the abilities of sorghum, wheat, corn, and soybean on the accumulation and uptake of RDX. The most effective plant for the uptake of RDX was wheat seedling. The soybean, on the other hand, could not survive in the contaminated soil.

5.11.4 Phytoremediation of HMX

The efficiency of ryegrass (*L. perenne*) for the uptake of heterocyclic nitramines from contaminated soil was studied by Rocheleau et al. (2007). The decrease in the concentration of HMX concentration in the contaminated soil was less than 10%. The study showed that the HMX compound was translocated in the plant from the root to the shoots and concentrated in the aerial parts in an unchanged form. A 3-m-tall tropical plant known as kenaf plant (*Hibiscus cannabinus*) assimilated HMX from the soil to the plant. According to study on the plant mass balance, for the uptake of octogen from soil, about 9% of the initial concentration was taken by the plants and was accumulated mainly in the aerial parts. The highest efficiency for the uptake of HMX was recorded when dendroremediation (phytoremediation by trees) was employed. In a study on the uptake of octogen from solution, 44.58% was removed by poplar seedlings (*Populus deltoides* x nigra, DN-34) according to Yoon et al. (2002). After a 65-day period of incubation, the studied plants were examined by radiochromatographic methods. This examination revealed that 70% of the assimilated HMX were translocated to the leaves, without any HMX transformation products.

5.12 Phytoremediation of Pesticide Residues

Environmental threat and serious environmental risk are arising due to the presence of pesticide residues in water and soil. Thus, pollution with pesticides becomes one of the most serious concerns of scientists and environmentalists. Pesticides could be accumulated by plants, soil, and organisms. Such pollutants may be distributed into the water, food chain, and soil. In addition, pesticide could induce reduction in the productivity of the polluted soil. The point and nonpoint pollution are the immediate sources of contamination to soil and the environment. The point source pollution of pesticides is originated from certain agriculture activities, spills, formulation facilities, manufacturer, and agrochemical dealerships. On the other hand, the nonpoint source of pollution is considered, mostly, as the essential source of pesticide contamination. Several studies demonstrated that several plants can increase the dissipation rate of the investigated pesticides from contaminated soil through accumulation into the plant tissue. Additionally, plant vegetation in the contaminated soil may reduce leaching by decreasing the rate of distribution and movement of the organic pollutants through the column of such soil (Belden et al. 2004).

During the last few years, significant investigations were carried out on the interaction between plants in the rhizosphere and microorganisms in terms of the potential to implement this for the remediation of contaminated media with pesticide (Tripathy et al. 2014). Additional studies were conducted using *Kochia* sp. concentrated on the phytoremediation of pesticide from contaminated groundwater and soils (Erdei et al. 2005). Results indicated that plants show strong evidence of an intrinsic ability to detoxify certain xenobiotic compounds. However, these plants require catabolic pathways, generally, to execute mineralization/degradation. The transfer of genes, therefore, involves in catabolism of xenobiotics from eukaryotes or microbes to plants. This will, further, augment their potential for remediation of the contaminants (Eapen et al. 2007). Tobacco, the transgenic plant, is capable to remove pentachlorophenol through bringing into being laccase and secreting into the rhizosphere. For the purpose of decontamination and removal of organ phosphorus pesticide, a bacterial organophosphorus hydrolase (OPH) gene in the plants of tobacco has been executed. The study showed that transgenic plants after 14 days of growth were able to degrade more than 99% of methyl parathion. A multiplicity of plant enzymes, including peroxidase, peroxygenases, glutathione-S-transferases, cytochrome P450, carboxylesterases, O- and N- malonyltransferases, and O- and N- glucosyltransferases, are involved in the phytotransformation of xenobiotics in the cells of plant (Karavangeli et al. 2005). It was further reported that P450-dependent monooxygenase activity of the transgenic potato plants was found to range from 3.5 to 4.2 times higher than those of the control plants (Tripathy et al. 2014).

5.13 Phytoremediation of Herbicide

Herbicide is classified as one of the serious pollutants in the environment. However, herbicide plays vital and important roles in agriculture. For enhanced phytoremediation, development of herbicide-tolerant transgenic plants is under investigation recently. For removal of atrazine, a herbicide, cytochrome P450 (CYP) was introduced into rice plant. Similarly, for efficient removal of alachlor, g-glutathione synthetase gene was introduced into poplar. Developing transgenic plants, for remediation, can be achieved by increasing root mass bacterial 1-aminocyclopropane-1-carboxylate deaminase, and this can be implemented via introducing bacterial gene atrazine chlorohydrolase.

Cytochromes P450 (CYP) enzymes, from heme proteins superfamily, are involved in the metabolism and the removal of xenobiotics, including substances of abuse and therapeutic drugs, herbicides, and industrial contaminants, as illustrated in Fig. 5.12. They are responsible for safe clearance of around 80% of marketed drugs that is achieved in the liver by conversion into relatively hydrophilic compounds. Due to their broad substrate specificity, CYP enzymes are being used as biocatalysts for remediation. *Herbicides atzZ* (Paz Alberto and Sigua 2013).

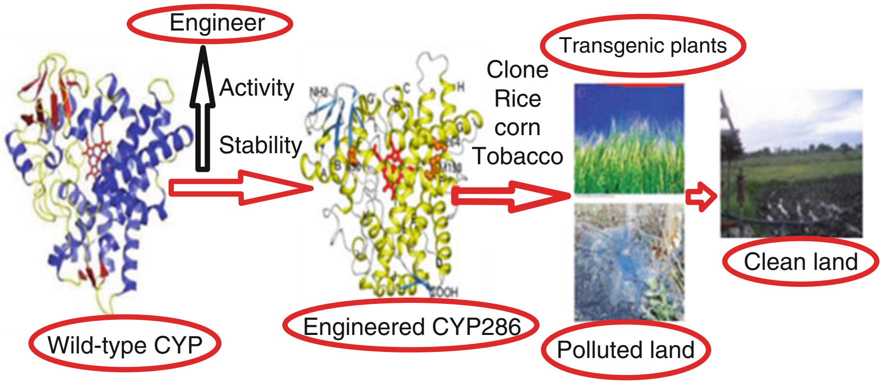


Fig. 5.12 Genetic manipulation of CYP enzymes for overexpression and better activity and stability and cloning of engineered CYP enzymes (e.g., CYP2B6) in plants. This is followed by growth of transgenic plants in contaminated areas which has the potential to detoxify the lands. (Paz Alberto and Sigua 2013)

5.14 Applications of Phytoremediation for Wastewater and Improving Quality of Water

Increasing urbanization, industrialization, and population in Benin City, Nigeria, are responsible for the huge and various discharges of enormous forms of industrial, human, and animal wastewaters into the local nearby surface waters including lakes, rivers, streams, and ponds (Aisien et al. 2010). Such discharged wastewaters contain enormous and various amount of different pollutants that are threatening the environment. Besides, these wastewaters have serious health hazard impact on man, animals, plants, and microorganisms. Thus, it leads to severe environmental challenges (Aisien et al. 2009). One of these wastewater is that produced from abattoir processes including both white and red meat. The slaughter animal's blood is the main organic pollutants from abattoir wastewater (Osibajo and Adie 2007). The white meat is poultry, and the red meat is beef, mutton, and pork. The abattoir wastewater consists of dissolved pollutants, such as blood and urine, as well as high concentration of total suspended solids, including pieces of flesh tissue, grease, fat, feathers, hair, grit, manure, and undigested feeds. Such insoluble and slowly biodegradable pollutants represented 50% of the contamination load.

Abattoir wastewater contains millions bacterial colonies of total coliform, fecal coliform, and *Streptococcus* groups. Most of the time, wastewater contains pathogens, such as *Salmonella* and *Shigella* bacteria, amoebic cysts, and parasite eggs. The continuous discharge of untreated abattoir wastewater into the nearby surface waters induces increase in algal growth, leads to eutrophication, and reduces aquatic plant and animal growth. It also increases odor of water, color, foaming, temperature, and electric conductivity as well as increases level of heavy metal. Thus, the impact of wastewater discharge to water bodies will contribute to deteriorate the quality of the surface water (Osibajo and Adie 2007). Many physical, chemical, and biological techniques have been studied concerning the degradation, transformation, and consumption of several organic matter and nutrient elements of plants within the constructed wetland treatment system (Aisien et al. 2010). The reuse of treated effluent in non-potable purposes including aquaculture/agriculture is highly encouraged to minimize the demands of freshwater.

Erdei et al. (2005) conducted a study to the potential of plants that can be employed in the phytoremediation of trace metals in Nueva Ecija, Philippines. Samples of water and plant were taken near the discharge sites, about 500 m distance away from the creek. Analysis of water samples indicated that the dumpsite and Panlasian Creek were slightly contaminated with phosphate at considerable amount. Analysis of plant samples showed that Hydrocharitaceae (*Ottelia alismoides* L.) and kangkong (*Ipomea aquatica*) were both able to uptake Pb in the phytoremediation process. The study showed also that the concentrations of Pb in Hydrocharitaceae (*Ottelia alismoides* L.) and morning glory (*Ipomea violacea* L.) were about 210% more than the concentration of Pb in the water (Erdei et al. 2005).

The potential of water hyacinth plant (*Eichhornia crassipes*) for the removal of phosphorus pesticide ethion was conducted by Xia and Ma (2006). The results showed that disappearance rate constants of ethion in culture solutions were as follows: −0.01059 for the non-sterile planted, −0.00930 for sterile planted, −0.00294 for non-sterile unplanted, and −0.00201 for sterile unplanted treatment. Furthermore, the uptake of ethion in live water hyacinth plant decreased by 74–81% in roots and 55–91% in shoots after the plant grows for 1 week in ethion-free culture solutions. The results suggested that the plant accumulation and phytodegradation are the most dominant processes for ethion uptake by the water hyacinth. According to this promising finding of this investigation, water hyacinth plant could be employed as an economical, efficient, and eco-friendly alternative to accelerate the degradation and treatment of agro-industrial wastewater contaminated with ethion.

In addition, earlier investigators (Fayed and Abdel-Shafy 1985) confirmed that water hyacinth has the potential to accumulate toxic trace metals including Cd, Zn, Co, and Pb within the plant tissues. The investigators concluded that the water hyacinth plant can be a biological indicator for heavy metal contamination in the environment. This conclusion was also confirmed by other scientists (Abdel-Haleem et al. 1992). According to other investigators, the reported accumulation of metals by water hyacinth was in the following ranges: 63–277 for Fe, 220–280 for Mn, 55–60 for Zn, 5–10 for Cu, and 0–<5 mg/l for Pb (Abdel-Shafy et al. 1994a, b).

Recently, water hyacinth samples were collected from the Nile River, Egypt. The plants were portioned into leaves, stems, and roots and dried. General analyses including crude protein, ash, crude fat, fibers, nitrogen, and free extract of sugars and carbohydrates were determined in the dried plant samples. In addition, level of heavy metals in these samples was also investigated (Abdel-Shafy et al. 2016). Stem of the plants showed maximum reducing sugar content. Leaves showed the highest level of both nonreducing and total sugars. The analysis revealed that metals such as Na, K, Mg, and Ca were found in relatively higher concentration. Trace metals, namely, Fe, Zn, Mn, Cu, Pb, and Cd, were mostly accumulated in the plant roots. The overall results indicated that water hyacinth plant contains considerable amount of nutrient elements. It was then confirmed that water hyacinth has the potential of accumulating metals from the surrounding environment. The level of trace metals in the plant depends on their concentration in the surrounding aquatic media (Abdel-Shafy et al. 2016).

Letachowicz et al. (2006) investigated the phytoremediation capacity in terms of trace metal accumulation by different parts of *Typha latifolia* L. plant. The plant samples were collected from different seven water bodies in the Nysa region in Poland. Trace metals including Fe, Mn, Zn, Ni, Cu, Pb, and Cd were detected in the *Typha latifolia*. The results concluded that *Typha latifolia* species have the potential of absorbing trace metals. Thus, this plant can be utilized as bioindicator of pollution. This plant is linked with nutritious water as well as organic or inorganic contents in the bottom sediments. In addition, *Typha latifolia* plant is a strongly expansive species. It can control water space because of their intensive growth of rhizomes; besides they often create mono-species group. Meanwhile, this plant can also be found in numerous groups of rushes.

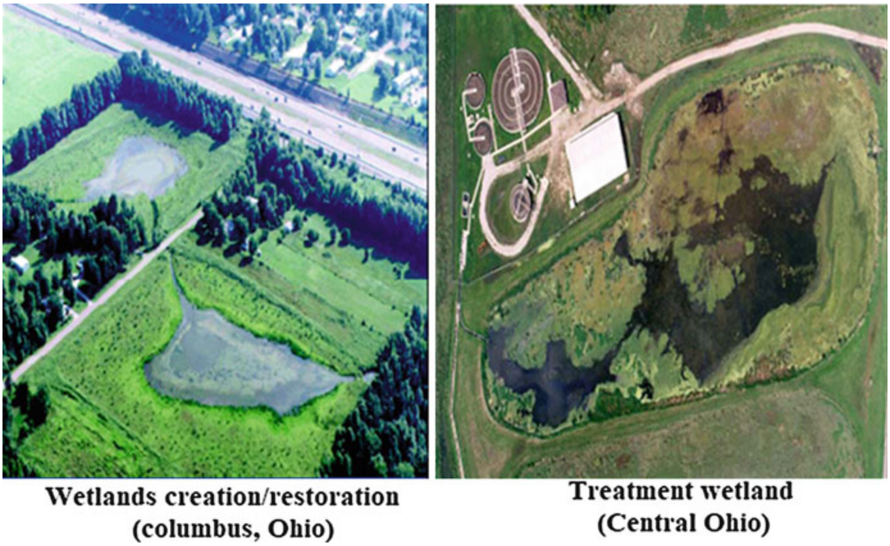
5.15 Phytoremediation for Tertiary Treatment of Sewage

5.15.1 Treatment of Wastewater via Constructed Wetland

Constructed wetlands (CW) offer a low-cost system for wastewater treatment (Masi et al. 2010). Besides, the CWs have low consumption in energy, low-cost operation and maintenance, and low investment and are eco-friendly and simple in construction using phytoremediation (Abdel-Shafy et al. 2017). The operation and maintenance of constructed wetlands do not need high-skilled personnel. A study of CWs showed that the pollution parameters can be removed efficiently including pathogens. Additional advantage of CWs over the traditional treatment systems is that they can be implemented in the same place where the wastewater is produced, maintained by relatively untrained personnel (EPA 2000; Abdel-Shafy et al. 2009). Thus, CWs are perfect for the decentralized treatment of wastewater (Abdel-Shafy and El-Khateeb 2013).

Wetlands offer great potential for the phytoremediation of contaminants and toxic substances. Constructed wetlands are shallow manmade systems (typically between 0.6 and 1.0 m) or bodies of slow-moving wastewater (Fig. 5.13). The CW is planted with dense and tolerant vascular plants including *Phragmites australis*, cattails, elephant grass, bulrushes, or reed plants. These bodies are artificially created, and they are usually long, narrow channels or trenches (Bennett et al. 2003).

There are several designs for constructing wetlands. These designs can be categorized according to their topology as free water surface (FWS), horizontal subsurface flow (HF or SSF), vertical flow (VF), and the hybrid wetland system (Abdel-Shafy et al. 2017). The main factors of designing are type of wastewater, the required quality of the treated effluent, and the available land area for construction



Wetlands creation/restoration (columbus, Ohio)

Treatment wetland (Central Ohio)

Fig. 5.13 Wastewater treatment via constructed wetlands. (ITRC 2009)

(Masi et al. 2010). The wetland hybrid system is mainly combinations of two or three designs: HF, VF, and FWS (Masi and Martinuzz 2007). Each design of construction (HF, VF, or FWS systems) specifies certain function treatment efficiencies (i.e., the selected design is an important controlling factor). In fact, the hybrid wetland design is able to combine the advantages of each constructed topology. Thus, it minimizes the drawback of single design system. The hybrid wetland system could be one of the following combinations (EPA 2000; Abdel-Shafy et al. 2017):

- HF + VF: Horizontal subsurface flow has the efficiency of eliminating most of organic load as well as the suspended solids. The subsurface vertical flow ensures the oxidation process and acts for an efficient nitrification of the wastewater without any clogging problems. Through this design, recycling the effluent was carried out to flow again at the beginning of the system for the purpose of more efficient denitrification.
- VF + HF: Vertical subsurface flow followed by horizontal subsurface flow system. This combined design is aiming to obtain effluent with more efficient denitrification through the vertical treatment system.
- HF + VF + HF + FWS: The horizontal subsurface systems followed by vertical, horizontal, and finally free water flow system, in which removal of suspended solids and denitrification are efficiently enhanced in the wastewater. The free water surface is able to remove almost all the nitrogen compounds and the entire microbial load.

The aquatic plants present main catalytic role in the purification process of the wetlands. This role is a combination of microbial, biological, and physical activities.

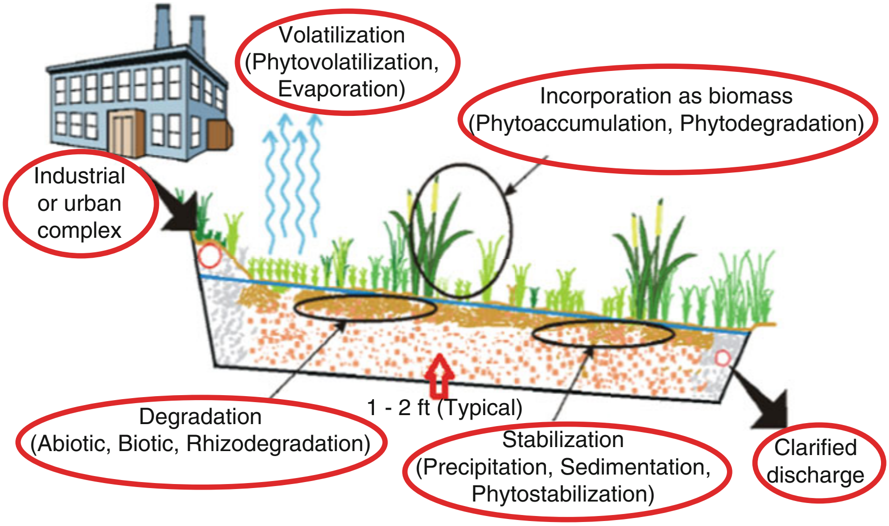


Fig. 5.14 Wastewater treatment via constructed wetland depicting the various methods of phytoremediation. (ITRC 2009)

The aquatic plants (Fig. 5.14) contribute in the removal of N, P, nutrient elements, and organic matters as well as accumulation of heavy metals (Abdel-Shafy et al. 1994a, b). Furthermore, the attached aerobic bacterial colonies and their continuous building up on the plant rhizomes offer an efficient degradation of the organic pollutants in the wastewater. Meanwhile, there is the convection mechanism that is responsible to pump air from the leaves to the root zone (Masi and Martinuzz 2007; Abdel-Shafy et al. 2017) (Fig. 5.15).

5.16 Conclusions

Phytoremediation is an important "green bioengineering technology." It uses green plants for remediating environmental contaminants.

Both aquatic and terrestrial green plants have wonderful characteristics for environmental restoration, including eliminating contaminants from polluted water and soil.

The use of higher plants, bacteria, microalgae, and fungi is feasible for degrading persistent contaminants.

Phytoremediation technology is eco-friendly, solar energy driven, green in nature, cost-effective, nonintrusive, and a safe alternative for removal of environmental contaminants.

This green technology is capable to accumulate, absorb, tolerate, transfer, assimilate, degrade, and stabilize highly toxic materials from the polluted soil and water.

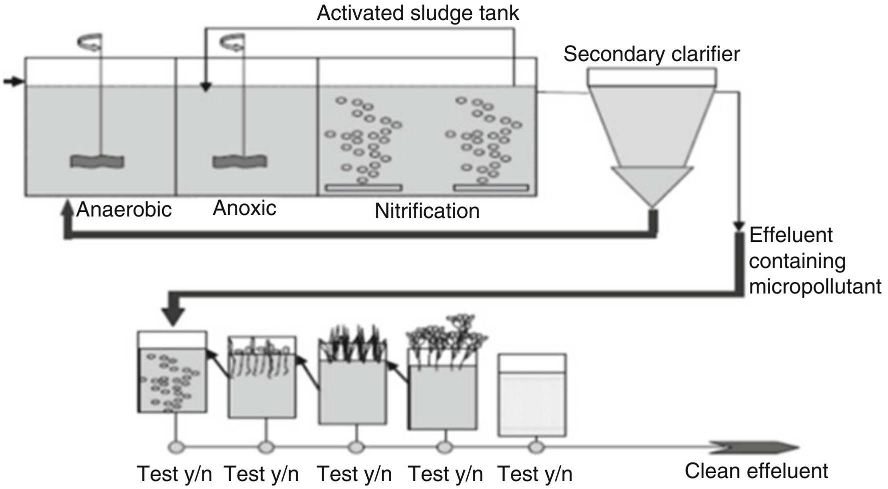


Fig. 5.15 A proposed step for wastewater treatment using phytoremediation and AOP modules

These toxic materials are heavy metals, polycyclic aromatic hydrocarbons, pesticides, explosives, crude oil, and organics such as solvents.

The aquatic plants, microorganisms, and algae have the ability to remove organic and inorganic matter, nutrients, pathogens, heavy metals, and other pollutants from wastewater in an eco-friendly natural way. These plant species include reeds, bulrushes, cattails, *Phragmites australis*, and aquatic plants such as water hyacinths, duckweed, and pennywort.

The importance of the phytoremediation process is that it is efficient for the removal of toxic organic aromatic pollutants, polycyclic aromatic hydrocarbons (PAHs), explosives (RDX, TNT, HMX), pesticide, landfill leachates, as well as herbicide contamination.

Thus, employing phytoremediation in constructed wetland (CW) technology offers a low-cost, eco-friendly treatment system for wastewater.

The phytoremediation technology has several advantages and disadvantages. The low cost is one of the most important advantages. However, the time needed to observe the necessary achievement can be long.

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Bioremediation of Contaminants

6

Mona S. Zaki and Mostafa F. Abdel Zaher

Abstract

The idea of bioremediation is with the nature itself. Owing to contamination in a particular region, some organisms may die; growth of few others might on the contaminants by metabolizing it. Bioremediation would thrive well on the contaminants by metabolizing it. Bioremediation would involve identification of such organisms and fostering their growth, naturally or by inoculation, so as to breakdown the contaminants into less harmful metabolites. This technology being cheaper and nature friendly is certainly a technology for the future. But, like other technologies, this too is not a panacea to all the maladies of environmental contaminants; toxic metals like cadmium obliterate complete flora and fauna of the contaminated area, and hence, it is not possible to use biological agents to treat them. Microbes require oxygen as an electron acceptor hence in aqueous phase; oxygen concentration below 1 mg/1 restricts the process of bioremediation.

6.1 Introduction

Even if we travel to the Mars, send rockets to the new planet, manufacture the super computers cloning the human beings, and do all unimaginable things, still we have many difficulties to clean the soil, air, and water we use. In many parts of the globe, the accessibility of water is a critical and vital issue and, even more so, the clean hygiene water. The environmental pollution is the most terrible and nasty ecological catastrophe that man is facing today. The environmental pollution is a global threat, and it is increasing day by day and also becomes a today's world's scare word. Rapid increase in human populations fueled by technological advancements in agriculture

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M. S. Zaki (✉) · M. F. Abdel Zaher
Department of Hydrobiology, National Research Centre, Dokki, Egypt

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and health has led to speedy enhancement in the environmental pollution. The extraordinary and unmatched population growth and industrial development during the twenty-first century have not only increased usual liquid and solid waste pollutants to a hazardous level but also generated a range of earlier unknown chemical pollution problems for which human society was unsuspecting. Growth of world population, the installation of numerous industries, and the wide application of chemical fertilizers and pesticides in modern agriculture have burdened not only the clean water resources but also the ambiance and the soil with toxic pollutants.

6.2 Bioremediation of Petroleum Contaminants

Petroleum and its components drive the present civilization and are the major energy sources. But, where there is use, there is a chance for abuse too. Hence, being the prime source of energy, petroleum is also a major environment pollutant. Since 1992, there have been 21 major oil spills causing huge economic and immeasurable noneconomic losses (Sarkar et al. 2005. http:/www.endgame.org/oilspills.htm). Petroleum contamination is quite harmful for the higher organisms (Cheong et al. 2011; Janjua et al. 2006; Lyons et al. 1999), but it is fortunate that microorganisms can thrive on it and assimilate (Atlas 1995; de Oliveira et al. 2012). Soon after major oil spill incident is reported, the efforts are concentrated at physical removal of oil, but they rarely achieve complete clean up. As per Office of Technology Assessment (OTA; USA), such mechanical methods are efficient at removing no more than 10–15% of oil after a major spill. In such cases, bioremediation has a major role to play in neutralizing the harmful effects of oil in the open environment. The basic principle is to use organisms that can use petroleum as carbon source and, hence, break them down to harmless end products.

Like any other technology that uses biological agents, success of bioremediation of petroleum contamination also depends on establishing and maintaining conditions that favor proliferation of petroleum scavenging microorganisms. Bio augmentation and bio stimulation are the two main approaches followed in this regard. Bio augmentation refers to inoculating the affected area with degrading microorganisms, while bio stimulation would require favoring growth of such microorganism through addition of nutrients or by providing other growth-limiting substrates (e.g., oxygen, surf washing, etc.). As petroleum is hydrophobic in nature, its bioavailability becomes a major constraint in the process of bioremediation. The use of biosurfactants is a common approach to increase the bioavailability. Requirements of a successful bioremediation process of petroleum contamination are as follows.

The very first requirement is the availability of microorganisms that can utilize oil as a metabolic substrate. Finding and transplanting such an organism to the site of contamination would be the first approach. Jones et al. in 1983 reported for the first time biodegraded petroleum by-products in marine sediments (Das and Chandran 2011). Enzymatic degradation of petroleum can be achieved by bacteria, algae, or fungi. Different organisms have varied degradation capabilities and act on different substrates. As petroleum is an assortment of different components, it is advisable to use a cocktail of organisms to affect remediation. Bacteria are the most efficient of all organisms that can degrade hydrocarbons (Rahman et al. 2003; Brooijmans et al. 2009). Floodgate (1984) mentioned 25 genera of hydrocarbon-degrading bacteria and 25 genera of hydrocarbon-degrading fungi which were isolated from marine environment.

Some of the bacteria recognized as hydrocarbon degrading are *Arthrobacter*, *Burkholderia*, *Mycobacterium*, *Pseudomonas*, *Sphingomonas*, *Rhodococciis*, *Pseudomonas fluorescens*, *P. aeruginosa*, *Bacillus subtilis*, *Bacillus* sp., *Alcaligenes* sp., *Acinetobacter lwoffii*, *Flavobacterium* sp., *Micrococcus roseus*, and *Corynebacterium* sp. (Jones et al. 1983; Adebusoye et al. 2007). Some fungal genera utilized for this purpose are *Amorphoteca*, *Neosartorya*, *Tal arontyces*, *Graphium*, *Candida lipolytica*, *Yarrotvia*, *Pichia*, *Aspergillus*, *Cephalosporium*, *Rhodotorula mucilaginosa*, *Geotrichum* sp., *Trichosporon mucoides*, and *Penicillium* (Boguslawska Was and Dabrowski 2001; Chaillan et al. 2004; Singh 2006). After the potential scavengers have been identified, the conditions for their survival and proliferation have to be ascertained.

Among the physical factors, temperature is most important one determining the survival of microorganisms and composition of the hydrocarbons (Das and Chandran 2011). At higher temperature, some fraction may get evaporated and the oil would tend to spread, while in low temperature, the slick would be more viscous, and retention of otherwise volatile fractions thereby delays the bioremediation process. For freshwater, bioremediation process 20–30 °C is the ideal temperature, while for marine 15–20 ° C is recommended. For high molecular weight polycyclic hydrocarbons, which are otherwise difficult to degrade, higher temperatures may be required (Bartha and Bossert 1984; Cooney 1984). As temperature has effect on enzymatic turnover rate "Q^{10}" hence, higher temperature would favor bioremediation. It was reported that the rate of hydrocarbon remediation was maximum in the range of 30–40 °C in general, and above this, the membrane toxicity effect of hydrocarbons was found to inhibit the survival of microorganisms (Bartha and Bossert 1984).As there is a close relationship between temperature and oil bioremediation, it is easy to understand why an oil leak disaster would be dangerous in polar regions.

The first step in degrading hydrocarbons is action of oxygenase which microbial communities are in very high salinities. However, some bacteria like *Streptomyces albaxialis* (Kuznetsov et al. 1992) for crude oil degradation and *Halobacterium* spp. (Kulichevskaya et al. 1992) for degradation of n-alkanes (C10–C30) have been identified. Kapley et al. (1999) cloned *E. coli* pro U operon, which is responsible for osmoregulation, into some bacterial consortium which can attack various fractions of crude oil making them salinity tolerant up to 6% NaCl.

pH also had an implication on biodegradation rates. The rates were found to be highest at neutral pH (Leahy and Colwell 1990). Lower pH at around 5.0 (Patrick and DeLaune 1977) as seen in salt marshes reduces oil mineralization, but the rates were satisfactory at pH above 6.5 (Hambrick et al. 1980). Octadecane mineralization improved further at pH 8.0 (Leahy and Colwell 1990).

Bioavailability of petroleum is a major problem that limits the rate of biodegradation. In order to enhance bioavailability, it is a must that solubilization be increased. Such a task is accomplished by certain microorganisms that secrete surfactants which are a group of surface active chemicals that increase the bioavailability of petroleum floating on the water column by increasing their solubilization (Das and Chandran 2011). Biosurfactants increase the oil surface area and, hence, the amount of oil that is actually available for degradation to the bacteria. Due to this property of enhancing biodegradation of oil, such surfactant-producing bacteria have potential to be used in bioremediation (Cameotra and Singh 2008). A consortium of bacteria was used for evaluation of surfactants and their composition by Camoetra and Singh (2008). The surfactant was found to be a conglomerate of 11 rhamnolipid family members and found that crude biosurfactant addition to the oil contamination was very effective in degradation process. Genus *Pseudomonas* is widely known for efficient surfactant production properties (Rhaman et al. 2007; Cameotra and 2008; Beal and Betts 2000; Pornsunthorntawee et al. 2008).

6.3 Bioremediation of Pesticides

Today, intensification of agriculture has increased the risk of losses due to improper crop health making agriculture sector heavily dependent upon the use of pesticides to prevent losses from pests. Pesticides are usually applied as a spray over the crop in aqueous or some nonpolar solvent medium of which only 5% is estimated to be utilized for the intended purpose, and the rest remains in the environment as residues. These residues may get washed off and either seep into the groundwater or reach water bodies along with the runoff. Once reaching the water bodies, the process of biomagnification begins. Vaccari et al. (2006) estimated that pesticide dichlorodiphenyldichloroethane (DDD) may get accumulated 85,000 times more in a predatory fish than at concentration it enters in water.

Some pesticides may get decomposed sooner after they are dissolved in a solvent, but the most commonly used organochlorines have a very long half-life making them threatening to the ecosystem and human beings. Pesticides may get accumulated in the human adipose tissue which enter the system orally, through inhalation, and some are even absorbed dermally. In humans, pesticides may cause irritation, affect mental health, affect digestion, and even cause carcinosis (Green and Hoffnagle 2004). Concern of this chapter would only be limited to persistent organic pesticides which have a very long half-life and are recalcitrant. UNEP's (United Nations Environment Programme) list of persistent organic pollutants includes aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, hexachlorobenzenes, mirex, and toxaphene.

Sometimes the pesticide used may be less toxic than the degraded product that is produced from it. Hence, an effective bioremediation technique would be one that acts fast so as to prevent the degradation process, and the end product that results from bioremediation is either nontoxic or less toxic. Bioremediation of metal contaminants or hydrocarbon contaminants is easier as the organisms that can survive in excess of metals and hydrocarbons can be naturally found, but this is not the case with pesticide as these are artificial chemicals intended to kill. Hence, identification of organisms that may help in bioremediation process is crucial. Usually, four remediation technologies are followed at the pesticide-contaminated regions – low temperature desorption, incineration, bioremediation, and phytoremediation. All these techniques have their own advantages and disadvantages. While incineration and low temperature desorption are faster technologies, they are usually very expensive. Bioremediation and phytoremediation on the other hand are very efficient and cheaper technologies but the time taken for remediation.

6.4 Conclusion

Industrial revolution and increased handling of heavy metals, POPs, and similar hazardous chemicals have raised the chances of accidents. Nuclear disasters at Fukushima and other previous similar incidents have raised lot of questions about the usage of such hazardous chemicals. One thing is clear that with rising population and rising demand for food and energy will certainly rule out utilization of hazardous metals and other chemicals, but we can certainly develop technologies that can help in mopping them up so that impact on biodiversity and on human population can be minimized. Bioremediation is one such method which is eco-friendly, cost-effective, and cleans up the contaminants to quite an extent efficiently. But, side pace and threat from genetically modified organisms to biodiversity may be deterrents to this technology. Bioremediation by itself may not be a complete solution to the problem of contamination, but mixing physical and chemical remediation techniques with bioremediation may be an answer to complete remediation of natural resources.

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Modeling Applications in Environmental Bioremediation Studies

7

Mahmoud Nasr

Abstract

Bioremediation and biodegradation processes in environmental studies involve a high degree of nonlinearity owing to the multiple and complex physical, biological, and chemical reactions. This chapter attempted to represent different modeling and statistical techniques that have been recently employed for describing the environmental systems that cover carbonaceous removal, nitrification, denitrification, and other microorganism activities. Activated sludge models (ASMs), viz., ASM1, ASM2, ASM2d, and ASM3, were used for an adequate description of biological treatment processes including nitrogen and phosphorus removals, as well as the degradation of organic carbons. In addition, Langmuir, Freundlich, Dubinin-Radushkevich, and Temkin models were developed to demonstrate the adsorption of metal ions from aqueous solutions onto solid materials. Moreover, statistical analysis, e.g., principal component analysis, clustering, dendrogram, and decision trees, were used for assessment of water quality in aquatic environments. Furthermore, the chapter included artificial intelligence methods such as artificial neural network and fuzzy inference system for simulation, prediction, and control of the treatment processes and environmental systems. These modeling tools were supported with literature cases that employed innovative methods within the field of bioremediation and biodegradation.

M. Nasr (✉)
Sanitary Engineering Department, Faculty of Engineering,
Alexandria University, Alexandria, Egypt

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7.1 Introduction

Increased concern about environmental issues has encouraged specialists to focus their efforts toward developing modeling tools that can describe bioremediation and biodegradation processes (Nasr and Ismail 2015). These methods involve a high degree of nonlinearity regarding physical, biological, and chemical reactions, as well as a large number of microorganisms (Gernaey et al. 2004). In addition, some aquatic systems are subjected to significant variations of wastewater flow rate, concentration, and composition, which require highly complex models to define them (Nasr et al. 2012). The biological treatment of wastewater is also modeled to carry out multiple activities of carbonaceous removal, nitrification, and denitrification (Henze et al. 1987). The term "modeling" is used to express a particular system using mathematical language containing a set of equations and variables (Nasr et al. 2017a). The developed models should simulate, predict, and control the complex environmental systems, as well as the mechanisms of microbial processes with reasonable accuracy (Jeppsson 1996). In addition, modeling of the treatment process is used to maintain the effluent quality within regulation-specified limits. Hence, modeling of environmental systems is considered an attractive and essential point of the study.

Any environmental system can be described by either a white-box model (also known as deterministic models) or a black-box model (i.e., due to lacking process knowledge) (Dreiseitl and Ohno-Machado 2002). For example, a white-box system can be used to describe activated sludge models, viz., ASM1, ASM2, ASM2d, and ASM3, in which the process variables are translated into a set of differential equations (Gernaey et al. 2004). However, this type of model has some limitations concerning the validity of assumptions, availability of data for calibration, and prediction accuracy (Dreiseitl and Ohno-Machado 2002). Black-box models use only input-output data when process knowledge is insufficient to develop a white-box model (Yurtsever et al. 2015). For instance, artificial intelligence (AI) such as an artificial neural network (ANN) and fuzzy inference systems (FISs) can be used as a black-box model to provide accurate predictive tools for nonlinear and nonstationary processes (Fawzy et al. 2016). Practically, AI methods and white-box models can be combined in a hybrid scheme to provide a more reliable description of an individual system. AI tools can maximize the information obtained from data and operator experience, and then this knowledge is applied to enhance the system performance. For example, ANN was employed to model the errors between simulated responses from the white-box model and the corresponding experimental data (Cote et al. 1995). Multivariate analysis forms another appropriate black-box modeling tool that is used as a statistical technique for process monitoring, assessment, recognition, and isolation (Molaie et al. 2014).

Other modeling methodologies such as stochastic gray-box and hybrid models are useful in bioremediation applications for estimation of biomass activities and prediction of effluent quality parameters (Hijosa-Valsero et al. 2011). In addition, Box-Jenkins models were employed for the prediction of primary settler performance in a WWTP (El-Din and Smith 2002). Multiple models such as hydraulic model, oxygen transfer model, and clarifier model can interact with the process knowledge included in white-box models to provide an accurate description of a full-scale WWTP. Several simulator environments, viz., AQUASIM, EFOR, GPS-X, MATLAB/Simulink, SIMBA, STOAT, and WEST, can be used to allow for a reliable simulation of a wastewater treatment process (Nasr et al. 2011).

An accurate model can be constructed by following these steps: objective identification (e.g., design, simulation, control, etc.), model selection (activated sludge model, hydraulic model, settler model, etc.), data collection and processing, calibration of the model parameters, model validation, and scenario evaluations (Khataee et al. 2010a, b). An incomplete understanding of the system behavior can be related to a multiple sources including influent and effluent data, physical properties, operational settings, system performance, and model structure (Jeppsson 1996). Model calibration is an iterative step employed to represent an acceptable explanation of a certain set of data (i.e., improve fit) by adjustment of the model parameters (Alves et al. 2014). The default parameters from the literature can be set as a starting point for calibration. The model learning is affected by several factors including the composition of influent wastewater, the degree of inhibition by toxic compounds, operational conditions, plant configuration, and population dynamics (Nasr and Ismail 2015). The number of inputs and outputs and initial conditions for state variables should be defined as they might influence the model parameters during calibration. In validation, a different data set (i.e., that is not used for calibration) is compared with the responses of the developed model to obtain reliable predictive solutions (Alves et al. 2014).

Hence, the objective of this chapter was to cover different white-box and black-box models that have been recently employed for environmental bioremediation and biodegradation. Activated sludge models and adsorption isotherm studies were presented as white-box systems. On the other side, multivariate statistical analysis and artificial intelligence were introduced as black-box models.

7.2 Biological Activity in Environmental Bioremediation

An adequate model of a biological treatment process is essential to provide a predictive tool that can minimize the operation costs and sustain the environmental balance (Gernaey et al. 2004). In biological treatment processes, microorganisms, mainly bacteria, are adapted to consume organics (substrate) in wastewater for their nutrition and metabolism (Eq. 7.1).

$$\begin{aligned}\text{Substrate} + \text{microbial cells} \rightarrow{} & \text{biomass growth} + \text{energy production} \\ & + \text{byproducts}\end{aligned} \tag{7.1}$$

7.3 Growth of Bacterial Population

Figure 7.1 shows a batch processing system that contains an initial substrate concentration, S_o, and a biomass concentration, X. The system is operated under a completely mixed and aerobic condition, and thus, the dissolved oxygen (DO) level is not a limiting factor for microbial growth. Microbial cells utilize substrate for biomass growth, energy production, and by-product formation (Nasr et al. 2014a). Hence, during the course of an experiment, the substrate decreases (negative dS/dt) along with an increase in the biomass concentration (positive dX/dt).

As displayed in Fig. 7.2, a plot of biomass concentration against time results in a growth curve that is composed of five distinct phases. These stages can be defined as follows (Nasr et al. 2017b):

1. The lag phase that occurs directly after inoculation of bacteria, and it remains until the cells are adapted (acclimated) to the new environment.

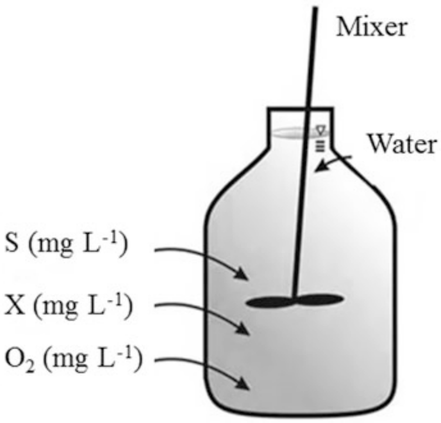

Fig. 7.1 Substrate utilization and biomass growth in a completely mixed batch reactor supplied with oxygen

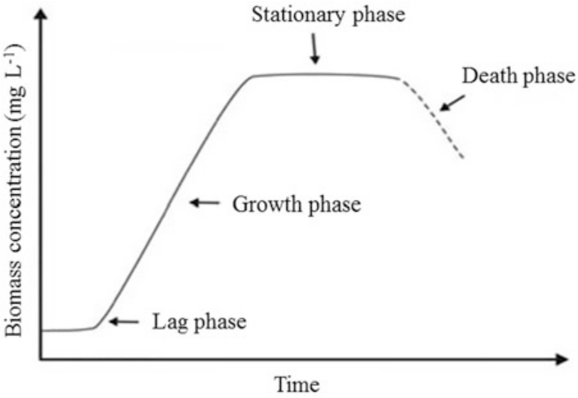

Fig. 7.2 Typical growth curve for a batch system

2. The exponential phase, in which the biomass concentration increased steadily describing the period of the optimum growth state.
3. The stationary phase that happens when essential substrates, nutrients, or dissolved oxygen are depleted to certain limits, and thus, the population is neither growing nor declining.
4. The death phase, where some cells are destroyed due to lysis, and thus, the net growth of biomass becomes negative.

7.4 Monod-Type Expression

Monod equation (Eq. 7.2) is a kinetic model used to simulate the microbial growth as a functional correlation between the specific growth rate and substrate concentration (Rieger et al. 2001). The model can also be employed to predict the amount of substrate removal in treatment plants.

$$\mu = \mu_{\max}\left(\frac{S}{S + K_S}\right) \tag{7.2}$$

where μ is the specific growth rate constant (d^{-1}), $\mu_{\max}$ is the maximum specific growth rate (d^{-1}), S is the limiting substrate concentration (mg L^{-1}), and K_S is the half saturation constant given at $\mu = 0.5\ \mu_{\max}$ (mg L^{-1}). Once the relationship between μ and S is determined, the bio-kinetic growth constants (i.e., $\mu_{\max}$ and K_S) can be computed statistically or graphically.

As seen in Eq. 7.3, the Monod formula can also be employed to estimate the bacterial growth rate (Gujer et al. 1999).

$$\frac{\mathrm{dX}}{\mathrm{dt}} = \mu X \tag{7.3}$$

where dX/dt is the biomass growth rate (mg L^{-1} d^{-1}) and X is the concentration of mixed liquor volatile suspended solids (mg L^{-1}).

The stoichiometry between the utilized substrate and generated biomass can be expressed in Eq. 7.4 (Nasr and Ismail 2015).

$$\frac{\mathrm{dX}}{\mathrm{dt}} = Y\frac{\mathrm{dS}}{\mathrm{dt}} - k_d X \tag{7.4}$$

where Y is the cell yield coefficient (dimensionless) and k_d is the endogenous decay rate (d^{-1}).

As seen in Eq. 7.5, a plot of μ versus U should give a linear line with a slope of Y and an intercept of k_d.

$$\mu = Y \times U - k_d \tag{7.5}$$

where U is the specific substrate utilization rate (d^{-1}).

7.5 Activated Sludge Models

In activated sludge processes, aerobic microorganisms are used to convert a significant portion of organic compounds into inorganic components, carbon dioxide, nitrogen, and new cells (Henze et al. 1999). The biomass consumes organic matters as a substrate in the presence of oxygen or other types of electron acceptors (e.g., nitrate). A portion of the precipitated suspended solids in the secondary settler is recycled to the bioreactor, while the remaining percentage is disposed of as waste sludge. An earlier study by Jeppsson (1996) has presented a review of the historical development of the activated sludge system.

7.5.1 Activated Sludge Model No. 1

Activated sludge model no. 1 (ASM1) was established as a simple mathematical tool to simulate, predict, and control the biological activities in wastewater treatment systems (Henze et al. 1987). The model includes the processes of carbon oxidation, nitrification, and denitrification, and it can be used to present a good description of the sludge production. The carbonaceous organic matter in ASM1 is defined as COD, and it is classified into biodegradable, non-biodegradable, and biomass (Gujer et al. 1999).

The biodegradable COD is divided into a readily biodegradable substrate (S_S) and slowly biodegradable substrate (X_S). The microorganisms can directly metabolize the soluble molecules of S_S for cellular growth and maintenance. However, the complex organic molecules of X_S involve enzymatic breakdown before assimilation and utilization (Cote et al. 1995). Practically, the X_S may be soluble although it is incorporated into the model as a colloidal component.

The non-biodegradable COD is divided into soluble inert COD (S_I) and particulate inert COD (X_I). These components are deemed to be unaffected by the biological activities of microorganisms. The S_I escapes from the biological system as untreated effluent, whereas the X_I is enmeshed in the biomass and then disposed of the system as excess sludge.

The active biomass is divided into heterotrophic biomass ($X_{B,H}$) and autotrophic biomass ($\mathrm{X_{B,A}}$). Based on the death-regeneration model, the products resulting from microorganism decay are termed as inert particulate (X_P).

Hence, the total COD balance of ASM1 can be presented as Eq. 7.6.

$$\mathrm{COD_{tot}} = S_I + S_S + X_I + X_S + X_{B,H} + X_{B,A} + X_P \tag{7.6}$$

The nitrogen components in ASM1 are divided into nitrate and nitrite (S_{NO}), ammonia nitrogen (S_{NH}), soluble biodegradable organic nitrogen (S_{ND}), and particulate biodegradable organic nitrogen (X_{ND}). Dissolved oxygen (S_O) and alkalinity (S_{ALK}) are other components described in ASM1. $X_{B,A}$ consumes S_O (i.e., during their aerobic growth) for the oxidation of S_{NH} to S_{NO}, whereas $X_{B,H}$ can grow under both aerobic and anoxic conditions. The aerobic and anoxic conditions are modeled through the uptake of S_O and S_{NO} as electron acceptors, respectively.

7.5.2 Activated Sludge Models No. 2 and 2d

Activated sludge model no. 2 (ASM2) was established for the improvement of ASM1 by adding the phosphorus removal mechanism (Henze et al. 1995). ASM2 added a new group of biomass known as phosphorus-accumulating organisms (PAOs), which are capable of accumulating and storing phosphorus in their cells. Hence, the model can be used to simulate the performance of biological nutrient removal in activated sludge systems. Activated sludge model no. 2d (ASM2d) is based on ASM2, but it addresses the ability of X_{PAO} to utilize the products of internal cell organic storage for denitrification (Henze et al. 1999). Hence, ASM2d allows for a better explanation of the dynamics of phosphate and nitrate, and it can be successfully used to simulate the biological phosphorus removal with a simultaneous nitrification-denitrification process.

7.5.3 Activated Sludge Model No. 3

Activated sludge model no. 3 (ASM3) added some updates to ASM1, such as the inclusion of storage polymers in the heterotrophic-activated sludge conversions, and the use of the growth-endogenous respiration model to represent the biomass decay (Gujer et al. 1999). ASM3 assumes that the readily biodegradable substrate (S_S) is stored into an internal cell structure as, X_{STO}, followed by biomass growth. ASM3 can predict oxygen consumption, nitrification, denitrification, and sludge production of activated sludge systems. In addition, the ASM3 model can be extended for simulation of biological phosphorus removal (Rieger et al. 2001).

7.6 Adsorption in Environmental Bioremediation

Adsorption is related to physicochemical studies that use a solid material to uptake metal ions from aqueous solution. The adsorption process can be described by the following models:

7.7 Langmuir Adsorption Isotherm

Langmuir isotherm is used to quantitatively describe the transfer of metal ions between the solid phase and liquid phase at an equilibrium state. Langmuir equation has been formulated based on the following assumptions (Langmuir and Waugh 1940): (1) maximum monolayer coverage, i.e., a single layer of adsorbate is formed on the outer surface of the adsorbent; (2) the surface of solid phase contains a finite number of vacant sites, and each site occupies only one molecule; (3) the surface of solid is homogeneous, i.e., adsorption sites are identical with equal size and shape, and they have similar affinity for adsorbate molecules; (4) after adsorption, no transmigration of adsorbate in the plane of the surface, and no interaction between adsorbate atoms; and (5) coverage-independent binding energy, i.e., the heat of adsorption is the same (uniform) for each site.

As seen in Eq. 7.7, a linear plot of C_e/q_e versus C_e provides a slope of $1/q_m$ and an intercept of $1/(K_L q_m)$.

$$\frac{C_e}{q_e} = \left(\frac{1}{q_m}\right) C_e + \frac{1}{K_L q_m} \tag{7.7}$$

where C_e is the equilibrium concentration of adsorbate (mg L^{-1}), q_e is the amount of metal adsorbed per gram of the adsorbent at equilibrium (mg g^{-1}), q_m is the maximum monolayer coverage capacity (mg g^{-1}), and K_L is the Langmuir isotherm constant (L mg^{-1}).

Based on the Langmuir-type adsorption process, the isotherm shape can be classified by a dimensionless constant separation factor (Eq. 7.8)

$$r = \frac{1}{1 + K_L C_o} \tag{7.8}$$

where r is a dimensionless separation factor, K_L is the Langmuir constant (L mg^{-1}), and C_o is the initial concentration (mg L^{-1}).

The factor "r" describes the shape of the isotherm according to the following classification: $r > 1$ "unfavorable," $r = 1$ "linear," $0 < r < 1$ "favorable," and $r = 0$ "irreversible."

7.8 Freundlich Adsorption Isotherm

Freundlich model is based on the adsorption process for a single solute system on heterogeneous surfaces (Freundlich 1906). The model describes the distribution of a solute between the solid phase and aqueous phase at equilibrium (Ng et al. 2002). The model presumes an exponential variation in site energies, and that the surface adsorption is not a rate-limiting step (Mattson and Mark 1971). In addition, Freundlich model does not follow Henry's Law of ideal dilute solutions. As seen in Eq. 7.9, a plot of log(q_e) versus log(C_e) gives a linear form with a slope of (1/n) and an intercept of log(K_F).

$$\log(q_e) = \left(\frac{1}{n}\right)\log(C_e) + \log(K_F) \tag{7.9}$$

where K_F is Freundlich's constant corresponded to the adsorption capacity (mg g^{-1}) and 1/n describes the adsorption intensity or surface heterogeneity (L g^{-1}).

The Freundlich exponent "1/n" indicates the type of isotherm; i.e., the isotherm is favorable at $0 < 1/n < 1$, irreversible at $1/n = 0$, and unfavorable at $1/n > 1$ (Saruchi and Kumar 2016). In addition, the value $1/n < 1$ implies the chemisorption process, whereas $1/n > 1$ indicates a cooperative process. Moreover, if $1/n$ is close to zero, the sorbent surface is more heterogeneous; otherwise, the more homogeneous the surface, the closer $1/n$ value is to unity.

7.9 Dubinin-Radushkevich (D–R) Isotherm Model

The D–R isotherm corresponds to the Gaussian energy distribution multiplied by the first power of the adsorption energy (Rudziński et al. 1974). The model expresses overall adsorption isotherm in the sub-monolayer coverage region for a variety of heterogeneous surfaces. This isotherm is temperature dependent, and it is valid for physical adsorption processes involving van der Waals forces (Boparai et al. 2011). As observed in Eq. 7.10, a linear plot of ln(q_e) vs. ε^2 gives a slope of -β and an intercept of ln(q_o).

$$\ln(q_e) = \ln(q_o) - \beta\varepsilon^2 \tag{7.10}$$

where q_o is the D–R constant related to the saturation capacity (mg g^{-1}), β is the activity coefficient (mol^2 kJ^{-2}), and ε is the Polanyi potential (dimensionless).

The value of ε is calculated from Eq. 7.11.

$$\varepsilon = \mathrm{RT}\ln\left[1 + \frac{1}{C_e}\right] \tag{7.11}$$

where R is the gas constant equivalent to 8.314 J mol^{-1} K^{-1} and T is the temperature (K).

The value of β is used to determine the mean sorption energy, as seen in Eq. 7.12.

$$E = \frac{1}{\sqrt{2\beta}} \tag{7.12}$$

where, E is the mean sorption energy (kJ mol^{-1}).

7.10 Temkin Isotherm Model

The Temkin isotherm model describes the interaction effect of adsorbent–adsorbate (Temkin 1941). The Temkin isotherm presumes that the heat of adsorption would decrease linearly rather than logarithmically while ignoring extremely low and very high concentration values (Aljeboree et al. 2014). In addition, the model suggests that adsorption is characterized by a uniform distribution of binding energies, up to a maximum binding energy. As seen in Eq. 7.13, the linear plot of qe vs. $\ln(C_e)$ obtains a slope of RT/b and an intercept (RT/b)ln(A) (Boparai et al. 2011).

$$q_e = \frac{\mathrm{RT}}{b}\ln(A) + \frac{\mathrm{RT}}{b}\ln(C_e) \tag{7.13}$$

where b is the Temkin constant corresponded to the heat of adsorption (J mol^{-1}) and A is the equilibrium constant associated with the maximum binding energy (L g^{-1}).

7.11 Pseudo-First-Order Kinetic

The formula of Eq. 7.14 expresses the linear curve of the pseudo-first-order model. A plot of $\ln(q_e - q_t)$ against t gives a straight line with a slope of k_1 and an intercept of ln (q_e) (Ho and McKay 1999).

$$\ln(q_e - q_t) = -k_1 \times t + \ln(q_e) \tag{7.14}$$

where q_e and q_t are the amounts of adsorbed solute at equilibrium and at time t, respectively, and k_1 is the pseudo-first-order rate constant.

7.12 Pseudo-Second-Order Kinetic

The applicability of pseudo-second-order model designates that the adsorption process follows a chemical interaction, also known as chemisorption (Atkins 1995). The linear form of the pseudo-second-order model can be expressed as Eq. 7.15 (Fawzy et al. 2016). The values of t/q_t were computed from the kinetic data and plotted against time, which will then provide a straight line with a slope and an intercept of $1/q_e$ and $1/(k_2.q_e{}^2)$, respectively.

$$\frac{t}{q_t} = \frac{t}{q_e} + \frac{1}{k_2 \cdot q_e^2} \tag{7.15}$$

where k_2 is the pseudo-second-order kinetic rate constant.

7.13 Statistical Analysis in Environmental Bioremediation

7.13.1 Design of Experiments

The design of experiments is a statistical technique used to determine the effects of several input factors on a response. Experimental design can be classified into three approaches, namely, one-factor-at-a-time, full factorial, and fractional factorial (Khataee et al. 2010a, b). In one-factor-at-a-time, only one factor or variable varies with time while holding other inputs constant. On the contrary, a factorial design is performed to examine two or multiple factors simultaneously. The factorial design is developed for an optimization purpose, as it describes the effect of each factor on the response as well as the interaction effects between factors (Nasr et al. 2017a). Hence, this method has the ability to reduce the number of experiments, save time and cost, and obtain accurate outputs.

A full factorial design with n-factors and m-levels for each factor is noted as a m^n factorial experiment (Elhalil et al. 2016). For example, a full factorial design denoted as 2^3 identifies three number of factors, and each factor has two levels; i.e., hence the number of experiments is $2^3 = 8$. Similarly, a 3^2 factorial design has two-factors, each with three-levels, and $3^2 = 9$ experimental runs. In this context, a full factorial design describes all possible combinations of factors in a single experiment, and it can build a response surface.

The fractional factorial experiment includes the most relevant combinations of the variables (Cristóvão et al. 2015). This approach is useful when the number of factors is large; i.e., it allows to obtain information about all main effects and interactions while finding the minimum number of experiments for the purpose. The design of experiment can also be performed using central composite, Box-Behnken, Plackett-Burman, and Taguchi.

Results from the design of experiment can be graphed using a box plot, which describes each variable by four components as follows (Nasr et al. 2012): (1) a central line in each box is the sample median that represents 50th percentile of the data; (2) a box representing variability around the average, where the tops and bottoms of each box are the 25th and 75th percentiles of the sample, respectively; (3) the whiskers are lines situated above and below each box, which express the range of the variable; and (4) outliers of a + sign extended beyond the top or bottom of the whisker length, where its value is over 1.5-fold the interquartile range.

Cristóvão et al. (2015) employed a 3^2 factorial design to determine the effect of hydraulic retention time (HRT) and initial dissolved organic carbon (DOC_i) on DOC removal by activated sludge treatment. The input factor of HRT has three levels of 4.20 h (−1), 6.15 h (0), and 8.10 h (+1), whereas the three levels of DOCi were 200 mg L^{-1} (−1), 500 mg L^{-1} (0), and 800 mg L^{-1} (+1). Results from the factorial design revealed that the optimum HRT and DOCi were 6.42 h and 406.2 mg L^{-1}, respectively, which achieved DOC removal of 88.0%. The proposed model achieved r^2-value: 0.98463 and adj-r^2: 0.95902.

Elhalil et al. (2016) developed a 2^4 full factorial experimental design to estimate effects of four-factors, viz., malachite green dye (10 and 20 mg L^{-1}), Fe^{2+} (5 and

10 mm), H_2O_2 (25.6 and 51.2 mm), and temperature (27 and 40 °C), on the degradation of dye by a Fenton process. Results from the factorial design indicated that the optimum malachite green dye, Fe^{2+}, H_2O_2, and temperature were 10 mg L^{-1}, 10 mm, 25.6 mm, and 40 °C, respectively, which attained a removal efficiency of 93.83%. The model validity and practicability were confirmed by computing r^2-value: 0.986 and adj-r^2: 0.889.

A study by Khataee et al. (2010a, b) developed a central composite design (CCD) to determine the influences of operational factors on biological dye removal in the presence of *Chara* culture. Their study indicated that the optimum condition that achieved the maximum decolorization efficiency was initial pH 6.8, dye concentration 9.7 mg L^{-1}, algae weight 3.9 g, and contact time 75 min. The predicted results were in good agreement with experimental data (r^2: 0.982 and adj-r^2: 0.966).

7.14 Clustering

Clustering analysis attempts to classify a set of records into a number of important groups (Ferati et al. 2015). A reliable cluster result produces a high similarity between the observation inside the same cluster and a small similarity between the data in different groups. Kohonen networks, also known as self-organizing feature maps (SOFMs), are used to realize maximum separation between the data in different clusters using nonlinear activation functions (Kuo et al. 2005). K-means clustering is another algorithm used to split data points into a fixed number (k) of clusters based on the centroid of each cluster. Recently, clustering analysis has been employed for monitoring and assessment of environmental studies including natural resource management (e.g., agriculture, fisheries, and forests) and pollution risks to living organisms.

Kamble and Vijay (2011) applied cluster analysis for assessment of water quality in the coastal region of Mumbai, India. Six water quality parameters, i.e., turbidity, DO, BOD, NH_3-N, PO_4, and FC, were measured at 17 sampling sites during post-monsoon, winter, and pre-monsoon. The sampling areas were classified into three major groups, viz., cluster-I "less polluted sites," cluster-II "moderately polluted sites," and cluster-III "highly polluted sites." Based on seawater standards, Mahim was the worst-affected beach because of an incoming organic load from the Mithi River, and thus, it was grouped in cluster-III.

Ferati et al. (2015) applied cluster analysis to handle a large data of eight heavy metals, i.e., As, Cd, Cr, Co, Cu, Ni, Pb, and Zn, collected from six locations at Trepça and Sitnica rivers during April – July 2014. Water and sediment samples were collected from each site and analyzed for heavy metal concentrations. Cluster analysis specified two major distinct clusters with three groups suggesting that the metal contamination resulted from anthropogenic sources.

7.15 Decision Trees

The decision tree is a hierarchical model used to represent classifications from a set of independent variables (called attributes) by following a structure of nodes, branches (links), and leaves (D'heygere et al. 2003). Decision trees are also flexible in handling both categorical and numerical data by finding the set of decision rules. A decision tree signifies each input attribute as a node, where the branches going downward from each node are the possible values that the data can be assigned (Hijosa-Valsero et al. 2011). A root node is the topmost decision node in the tree, which has no incoming edges. Leaf nodes, also known as terminal nodes, are situated at the bottom of the tree, and they predict outcomes expressed as class labels.

D'heygere et al. (2003) built a decision tree model to predict the absence or presence of benthic macroinvertebrate taxa in the non-navigable watercourses of Flanders using independent variables of 15 physical-chemical, structural, and eco-toxicological variables. The study found that conductivity and dissolved oxygen were the most relevant variables in the input attributes by placing them at the top of the tree.

Hijosa-Valsero et al. (2011) applied a decision tree method to predict the removal efficiency of pharmaceuticals and personal care products (PPCPs) and organics from wastewater using constructed wetlands. Their study indicated that DO, temperature, pH, conductivity, and redox potential influenced the removal of the investigated matters.

7.16 Artificial Intelligence in Environmental Bioremediation

7.16.1 Artificial Neural Network

Artificial neural network (ANN) is a computer-based system proposed to mimic the learning process of nerve cells in the human brain (Nasr and Zahran 2014). ANN is quite robust since it can achieve a high degree of prediction accuracy even when it receives erroneous and noisy datasets. It can also be employed to organize, classify, and summarize data sets, owing to its ability to solve nonlinear functions and to capture complex relationships (Yurtsever et al. 2015). ANN contains a large number of interconnected neurons (also called nodes), which is organized in layers including first (input) layer, hidden layers, and last (output) layer. Adaptive weights, biases, and transfer functions are used to interconnect the neurons and layers within a network (Yurtsever et al. 2015). The predicted output is obtained by examining the ANN through multiple steps of training, validation, and test. The optimal number of neurons, layers, and adjusted network parameter (weights and biases) is computed according to the minimum mean squared error (MSE) of the training and validation sets (Nasr et al. 2012).

Recently, several articles have attempted to apply ANNs for modeling the biological reactions in the area of environmental engineering. For example, Khataee et al. (2009) used an ANN model to predict the removal efficiency of BG4 dye using inputs of dye concentration (2.5–15.0 mg L^{-1}), temperature (5–45 °C), pH (2–11), reaction time (0–420 min), and algal concentration (1.5×10^6–9.0×10^6 mg L^{-1}). The study used a three-layered feed forward back propagation NN with a structure of 5 – 16 – 1. The proposed ANN provided a reasonable predictive performance with a coefficient of determination of r^2: 0.979.

Khataee et al. (2010a, b) developed a three-layer ANN with a back-propagation algorithm to predict the biological degradation efficiency of Malachite Green (MG). The input factors were reaction time (0–420 min), pH (1.5–8.5), temperature (5–45 °C), dye concentration (2.5–17.5 mg L^{-1}), and algae dosage (0.5–6.0 g), whereas the output was dye removal efficiency (0–100%). Their study found that the network (5 – 12 – 1) succeeded to attain a coefficient of determination r^2: 0.970, indicating that the model was reliable to predict the dye removal efficiency.

Prakash et al. (2008) applied ANN to predict the biosorption efficiency of sawdust for the removal of Cu(II)-ions. The input attributes were Cu(II)-ion concentration, 50–80 mg L^{-1}; pH, 3–6; temperature, 25–40 °C; and particle size, 50–200 μm, while the output parameter was the percent of sorption efficiency. The network used a simple back-propagation recurrent algorithm with three hidden layers, equivalent to a structure of 4 – 50 – 40 – 27 – 1. The ANN model notably tracked the experimental data and achieved average MSE of 0.002139579.

Yurtsever et al. (2015) employed a fast ANN to predict Cd(II)-ion adsorption rate using six inputs: initial pH, temperature, agitation speed, particle size, Cd (II) concentration, and reaction time. The model architecture was composed of four layers (6 – 25 – 5 – 1), viz., an input layer, first hidden layer with 25 neurons, second hidden layer with five neurons, and an output layer. The resulting model achieved an accurate prediction of Cd(II)-ion removal with r^2-value of 0.999. In addition, the proposed ANN model was found to be more promising for modeling the Cd(II) adsorption when compared to conventional isotherm and kinetic studies.

7.17 Fuzzy Inference System

A fuzzy inference system (FIS) is used to represent a nonlinear relation mapping of an input space to an output space (Zadeh 1997). A FIS is composed of four major parts: i.e., fuzzification, "If-then" rules, inference engine, and defuzzification. The engine that handles these components can be Mamdani fuzzy inference, Sugeno fuzzy inference, or Tsukamoto fuzzy inference (Alalm et al. 2016). Figure 7.3 displays the general architecture and components of the FIS, which can be illustrated as follows (Nasr et al. 2014b):

A crisp set of input data is collected, prepared, and further converted into a fuzzy set using linguistic variables and terms and membership functions. These steps are recognized as fuzzification, in which a crisp (numeric) value is converted into a fuzzy input (Giusti and Marsili-Libelli 2010). For example, a fuzzy variable (e.g., temperature) can be defined in terms of linguistic concepts such as low, medium,

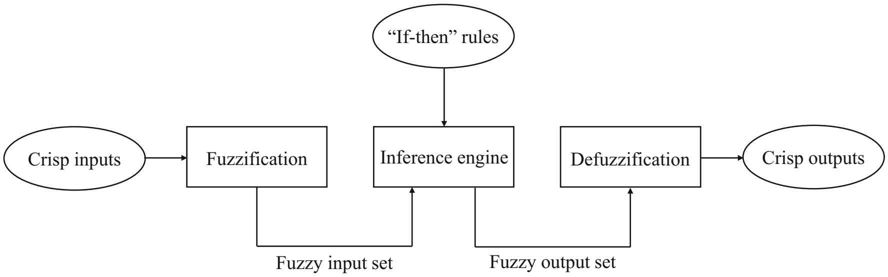

Fig. 7.3 A fuzzy inference system

high, etc. Each linguistic concept is expressed graphically as a membership function, like linear, Gaussian, and trapezoidal fuzzy sets.

Subsequently, an inference engine is organized using a set of "If-then" rules. A single fuzzy "If-then" rule has the form "If x is A Then y is B." Assume x and y are the variables "temperature" and "dissolved oxygen," respectively, and A and B are linguistic variables "low" and "high," respectively, then the "If-then" rule will have the form "If Temperature is Low Then Dissolved Oxygen is High." Note that aggregation of rules may be used when the rule-based system contains more than one "If-then" rule (Fawzy et al. 2016).

Finally, defuzzification step is used to convert the resulting fuzzy output into a single crisp number. There exist several defuzzification methods available in the literature, such as Max-membership, center of gravity, weighted average, mean-max, and center of sums (Gupta et al. 2017).

Giusti and Marsili-Libelli (2010) developed a Sugeno fuzzy model to control the maximum in cycle temperature (output) by adjusting airflow (input) in a composting process. The model consisted of three fuzzy rules corresponding to three possible in cycle temperature trends (varied between mesophilic and thermophilic phases). Results from their study revealed that the proposed model could elucidate 95.46% of the observed variance, indicating a good agreement with the studied cycles.

Gupta et al. (2017) employed a FIS to predict the extraction yields of lipids, carbohydrates, and proteins from microalgae using input factors of specific growth rate, carrying capacity, and physiological health. The study used Sugeno-type FIS, and eight IF-THEN rules, in addition, the membership functions of input and output variables were Gaussian and linear, respectively. The fuzzy model provided a high coefficient of determination r^2-values >0.98, and it was concluded that an increase in microalgae concentrations resulted in higher lipid and carbohydrate extractions but lower protein yields.

Nasr et al. (2014b) employed a fuzzy logic control (FLC) to maintain the dissolved oxygen level in the last aerobic tank of Benchmark model.1 that comprises anoxic/aerobic tanks at the level of 2 mg L^{-1}. The study used a fuzzy inference system with Mamdani's method that was constructed based on five IF-THEN rules. The input variable of soluble oxygen had five fuzzy linguistic sets, viz., very low, low, medium, high, and very high. The model output (i.e., oxygen transfer coefficient) was classified into close-fast, close-slow, no-change, open-slow, and open-fast. Results revealed that the fuzzy model was able to handle variations in the influent wastewater characteristics. In addition, FLC was able to self-adapt the aeration supply, and hence, low aeration energy was consumed. The effluent wastewater characteristics were within the allowable limits of BOD ≤ 10 mg L^{-1}, COD ≤ 100 mg L^{-1}, ammonium ≤ 4 mg L^{-1}, and total nitrogen ≤ 18 mg L^{-1}.

7.18 Conclusions

This chapter presented several modeling and statistical methods that have been recently applied in environmental engineering studies. Activated sludge models (i.e., ASM1, ASM2, ASM2d, and ASM3) used for prediction of biological nitrogen and phosphorus removals, besides degradation of organic carbon matters, were covered. The application of adsorption isotherm models such as Langmuir, Freundlich, Dubinin-Radushkevich, and Temkin for the removal of metal ions from aqueous solutions was also demonstrated. In addition, this chapter included multivariate analysis, viz., principal component analysis, clustering, dendrogram, and decision trees, for evaluation and assessment of water quality in aquatic environments. Artificial intelligence such as ANN and fuzzy logic was also introduced as a black-box model for prediction of the treatment performance. Finally, this work included literature studies that have performed innovative methods within the field of modeling environmental processes. Future studies should be focused on the applications of stochastic gray-box and hybrid models for prediction of the treatment and bioremediation performances, as well as for estimation of microorganism activities.

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Development of Field Platforms for Bioremediation of Heavy Metal-Contaminated Site

8

Shazia Iram

Abstract

An irrigation system in areas near urban periphery is partial or totally relies on untreated sewage effluents. There is very less data available about heavy metal status in raw sewage used for soil irrigation in Pakistan. On the other hand, soil of arid areas and semiarid areas is rich in metals like nickel, zinc, copper, and lead. The bioavailability of these heavy metals is affected largely by physical and chemical characteristics of soil and partially affected by characteristics of plants. This issue is a major concern for the health of humans and animals. Therefore, in order to prevent the possible health hazards of metals in agrarian land monitoring of soil, water and plant quality is essential. Heavy metal-contaminated soils need to be remediated. In Pakistan as a developing country, soil reclamation methods include physical and chemical management that cannot be brought into action because of expensive technologies involved. Phytoremediation, in general, phytoextraction, and microbial remediation in particular offer a promising alternative to conventional engineering-based technologies. Phytoremediation is an emerging technology that may be used to clean up contaminated soil in which plants are used for removing pollutants from the contaminated soils. Phytoextraction remediation technique has two strategies such as natural phytoextraction and chemically enhanced phytoextraction. In one study (Rawalpindi, Pakistan), tolerance potential of plants (*Zea mays*, sorghum, *Helianthus*, *Brassica*) was assessed against deleterious effects of heavy metals (Pb, Cd, Cr, Cu) on plant growth, and role of chelator (EDTA, DTPA, and NTA) and tolerant fungal strains was also checked to increase the tolerance index. By 3 years of research, it was assessed that heavy metal uptake and their translocation in biomass of plant enhanced the phytoremediation process from contaminated soil. Phytoremediation research in field can provide capacity building to youth and farmer community. By the bioremediation of soil and water, it is possible to

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S. Iram (✉)
Department of Environmental Sciences, Fatima Jinnah Women University, Rawalpindi, Pakistan

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produce biofuel, biomass, and gasification for energy production. Bioremediation techniques will provide training and capacity building to youth and serve an important role at field level for technology transfer and as a broker of emerging technologies.

8.1 Introduction

The agriculture sustainability largely depends on two natural resources: water and land; agricultural production is affected badly if one of them is limited. On Earth for existence and survival of life, water is a very important factor. It is being used for agricultural, domestic, industrial, and recreational purposes, though agricultural sector is using 90% of water (Dara 1993).

There is shortage of irrigation water in Pakistan because surface water does not fulfill the water requirements for crops. This water shortage is being fulfilled by combined use of groundwater and wastewater (domestic and industrial) of urban areas. This mixed water is being used for vegetable and crop growth in peri-urban agricultural areas (Lone 1995). In Pakistan it is estimated that in big cities like Lahore, Karachi, Multan, Peshawar, Faisalabad, Hyderabad, Kasur, Quetta, Sukkur, and Islamabad/Rawalpindi, sewage is being produced 116,590 million gallons per day, and 32,000 hectares of land is being irrigated with this water. Big cities have no proper disposal and management systems but produce constantly huge volumes of wastewater. In Pakistan only 2% of cities are using wastewater treatment plants (Clemett and Ensink 2006). So, 90% of untreated wastewater is being used in agricultural activities in more than 80% of cities of Pakistan (Ensink et al. 2004).

Currently 0.3 million hectares of agricultural land is irrigated with wastewater. The use of wastewater and its disposal ultimately boost agricultural production and minimize the threats of environmental contamination. This wastewater is used for irrigation as it is a rich source of nutrients which are beneficial for plant growth. There are various types of industries situated in and around industrial cities. These industries discharge their untreated effluent which will ultimately mix with urban wastewater and contain exceeding quantity of heavy metals such as chromium, cadmium, copper, lead, etc. This industrial wastewater may be poured directly into water courses without pretreatment, while on the other hand, farmers use this contaminated water in their fields (Malik et al. 2009). Soil acts as filter of toxic chemicals it may adsorb and retains heavy metals from wastewater (Rattan et al. 2005), but when the capacity of soil to retain toxic metals is reduced, then these toxic metals are released into groundwater. These toxic metals may enter the plants and whole food chain making it poisonous for human beings. Thus, environmental and human life quality simultaneously is under threat by increasing soil pollution (Zia et al. 2008).

8.2 Effects of Heavy Metals on Environment

Soil contamination with heavy metals is a common practice and difficult to treat because soil is a source and also sink for the heavy metals. With concern to human health, heavy metal assessment in the soil is a very important issue, because they are toxic in nature and their degradation is difficult. Therefore, these heavy metals remain persistent in the soil and in the ecosystem. Bioconcentration in different levels of food chain threatens all the living beings (Aragay et al. 2011). Soil contaminated with metals is a primary route of toxic metal exposure to humans causing cytotoxic, mutagenic, and carcinogenic effects in animals. Heavy metals could enter into the body of human beings when they consumed food contaminated with heavy metals. Dietary crops and vegetables grown in heavy metal-contaminated soils pose serious health problems (Zia et al. 2008). Heavy metal pollution increases in biological and ecological systems and exerts harmful effects. Even a very low level of these heavy metals causes serious health disorders. Heavy metals can persist in the soil for many thousands of years and dangerous for the higher organisms. These heavy metals also affect the plant growth and soil microbiota. It is a well-known fact that heavy metals cannot be degraded chemically, so they are physically removed and can be transformed into less toxic and nontoxic forms (Ghani 2010). Contamination of soil with heavy metals poses serious effects on living organisms and the ecosystem. Prolonged human exposure to heavy metals causes renal dysfunction disease – tubular proteinuria. Similarly inhaling dust and fumes having high metal concentrations can cause destructive lung disease, that is, pneumonitis. Cadmium pneumonitis can be identified as pain in the chest, with reddish sputum and an ultimately destroyed inner layer of tissues of the lungs due to excessive watery fluid accumulation. Excessive metal exposure may cause pulmonary edema which can lead to death. Contamination of soil with heavy metals needs the implementation of suitable remedial techniques (Vaxevanidou et al. 2008). Hence, there is a need to maintain the soil quality that is not a one-time course rather a continuous process.

8.3 Field Platform for Bioremediation

A variety of treatment technologies has been developed for the remediation of metal-contaminated soils, and bioremediation by plants (phytoremediation) is an economically feasible technique. It is more acceptable publically because overall it increases the aesthetic beauty of the contaminated area and also has potential to clean the environment (Chen and Cutright 2002). Phytoremediation is a plant-based remediation strategy which uses the plants for environmental remediation (Rauf et al. 2009).

Phytoextraction of heavy metals is a technique under phytoremediation plants act as a solar energy driven pumps and extract and accumulate different elements from the soil and environment (Luo et al. 2005). Heavy metals, for example, cadmium, lead, copper, nickel, and zinc, can be extracted through this technique. Among the phytoremediation categories, phytoextraction is used to extract heavy metals from the soil system by plants because some metals like Mn, Fe, Mg, Mo, Ni, Zn, etc. are essential plant nutrients. But for Pb removal, it is a commercially available technique. Phytoremediation efficiency depends on different factors like climate, time period, soil type, and root depth. Vassilev et al. (2002) reported the phytoextraction metal protocol that follows four main strategies:

1. Plant cultivation on heavy metal-contaminated soil
2. Harvest of metal-rich plant biomass
3. Postharvest treatments and successive plant biomass disposal as hazardous or toxic waste
4. Recovery of metals from metal-loaded plant biomass

Phytoremediation is an affordable and effective technical solution for heavy metal extraction from soil. Phytoremediation is an economical and environmental-friendly technique. Plant root's ability of uptake of heavy metals is being used in this process of phytoremediation along with the transformation, accumulation, and biodegradation capability of the whole plant body (Tangahu et al. 2011). Efficiency of remediation depends on different factors such as soil type, nutrient status of soil, and plant tolerance against heavy metals. For the management of contaminated agricultural land, the use of heavy metal-tolerant crops for remediation of heavy metals is a new emerging technique. It is indicated from the recent studies that different high-biomass-producing crop varieties have potential for heavy metal accumulation such as oat (*Avena sativa*), Indian mustard (*Brassica juncea*), sunflower (*Helianthus annuus*), maize (*Zea mays*), ryegrass (*Lolium perenne*), and barley (*Hordeum vulgare*) (Meers et al. 2005). In this technique by using high-biomass-producing crops, with better management of soil and improvement of plant husbandry, an alternative strategy could be developed for remediation of heavy metal-polluted soils (Evangelou et al. 2007). Effectiveness of phytoremediation depends on root zone of the plants. This may be from few centimeters to many meters. This phytoremediation technique is a long-term strategy, and it is more beneficial than other physical and chemical technologies. Hyper-accumulators are those plants which have 50–500 times greater capability to absorb metals than average plants (Lasat 2000). Hyper-accumulators have greater than one bioconcentration factor; sometimes it reaches 50–100 (McGrath and Zhao 2003). Hyper-accumulator plants are ideal model organisms for scientists and have acquired attention all over the world for their use in phytoremediation technology.

8.4 Native Plant Species of Pakistan for Bioremediation

Escalating heavy metal contamination in the environment is stated in most of the developing countries including Pakistan (Jamali et al. 2007; Kausar et al. 2012). In Pakistan tremendously higher concentration of mercury (Hg) has been noticed in marine and riverine ecosystems (Mubeen et al. 2010). Soil and water of Pakistan are most probably in direct use of fertilizers, herbicides, and pesticides in agrarian sector.

Soil heavy metal contamination became a very grave environmental concern in Pakistan. The instant increment in population together with untreated effluent disposal from textile and tannery industrial sector enhances the threat of soil pollution (Khan 2001). The decontamination and removal of heavy metal-contaminated soil are very rare in Pakistan.

Various hyper-accumulative species of plants have been broadly explored that led to the considerable advancement in this field. Plants with BCF (bioconcentration factor) greater than 1000 are hyper-accumulators, whereas those with less than 1000 and more than 1 BCF are accumulators. BCF targets the plants efficiency to uptake metals from soil. There are total of 17 biomes in the world, while Pakistan has 9 out of them, so it shows the unique geographical landscape of Pakistan, and it has over 6000 higher plant species. Currently over 400 plant species of angiosperms had been examined and characterized as hyper-accumulators all over the world (Freeman et al. 2004). In Pakistan however almost 50 plant species were identified and characterized as metal accumulators of soil and water.

Mubeen et al. (2010) stated that utilizing locally accessible wild plants for uptake of Cu from industrial wastewater city Lahore has proved a succesful technique. *Calotropis procera* roots were used as biosorbent for removal of heavy metals from wastewater. The synthetic chelators prepared increased the heavy metal uptake and translocation in plant biomass which speed up the phytoremediation process of nickel and lead.

Substantial progress has been made in the field of metal remediation by plants, and various number of plant species have been widely investigated all over the world to date. However, for indigenous flora of Pakistan, inadequate information is available. About 400 species of plants from angiosperms have been inspected and identified as hyper-accumulators from all over the world. However, limited data is available about the use of flora of Pakistan, and about 30 reported studies have been carried out for investigation of potential flora for phytoremediation from Pakistan. Still there is a large figure of plant species present in diverse localities of the country that needed to be tested for phytoremediation process.

Among those unfamiliar species of plants, most of them are used for the purpose of phytoremediation all over the world, e.g., *Brassica juncea* used for remediation of Pb, Zn, and Cu from soils by many researchers (Zaidi et al. 2006). This species is found in different regions of Pakistan (Rawalpindi, Islamabad, Quetta, Karachi, and Lahore). Different studies stated the heavy metal assessment in contaminated soils and water from Pakistan. Younas et al. (1998) and Malik et al. (2010) investigated that soil samples from industrial areas of Rawalpindi, Lahore, and Islamabad have been heavily contaminated with Ni, Cd, Cu, Pb, and Zn. It could be useful for remediation of the mentioned *Eschhornia crassipes* which has been sighted in industrial and urban regions of the province of Punjab. Although this is not very common to use for remediation, it has been cited as heavy metal accumulator (Zn, Cr, Cd, Ni, Hg, Pb, P, pesticides) from various parts of the world (Xia and Ma 2006; Odjegba and Fasidi 2007; Mishra and Tripathi 2009).

8.5 Enhanced Bioremediation for Field

Many chemical and biological treatments, such as inoculums of fungi and bacteria, EDTA, DTPA, NTA, and other organic compounds, have been used in pot and field experiments to facilitate the heavy metal extraction and to acquire the higher phytoextraction efficiency (Ke et al. 2006; Wu et al. 2006). It is known that microbial populations affect the solubilization of heavy metals and their availability to plants, through acidification, releasing chelaters, and reduction-oxidation changes (Peer et al. 2006). It is reported that presence of microbes in the rhizosphere increases the levels of Zn, Cu, Pb, Ni, and Cd in plants. Heavy metal tolerance and production of biomass could be enhanced by improved interaction among the plants and rhizosphere microbes. It is also considered as an important phytoremediation technology factor (Whiting et al. 2003).

8.6 Chemically Enhanced Bioremediation

The bioremediation effectiveness becomes limited often because of low solubility of metals and their sorption on surfaces of soil particles; however metal solubilization could be increased by adding complexing/chelating agents with time (Pivetz 2001). Several chelating agents have been reported which enhance the rate of phytoextraction. However EDTA and DTPA have been investigated widely, and they have high chelating ability toward most of the metals, like Cd, Cu, Cr, and Pb, which ultimately leads to increased translocation of metals from soil to plant (Wong et al. 2004).

From the literature it is seen that chelating agents may pose potential risk when they are applied in situ, because during extending period of time, chelating agents leech down to the groundwater. However, some studies showed that ammonium application to soil might promote the heavy metal phyto-availability from polluted soils (Wenzel 2009). A lot of researches on phytoextraction are based on greenhouse experiments; few tested the hyper-accumulator plants in the field and actually determined their heavy metal accumulation potential (Zhuang et al. 2007).

Different chelates such as DTPA, EDTA, and EDDS have shown enhancement in the uptake of heavy metals by solubilizing them from the soil solid phase because of the formation of water-soluble complexes (Lestan et al. 2008). Primarily, heavy metals are distributed mainly in two phases which are reversible and irreversible. The chelates try to extract metal ion from reversible phase first and then from irreversible phase. Based on these criteria, ability of chelates is analyzed. If a chelate dissolves metals more from irreversible phase, it is more efficient. Recently NTA and EDDS are used because of their ability to be biodegraded as compared to the EDTA (Evangelou et al. 2007).

Synthetic chelants, such as EDDS, EDTA, and NTA, have been used in facilitating the heavy metal solubility from the soil system and their uptake and translocation in the shoot parts of plants (Shen et al. 2002). For example, Cu metal could be toxic for many plant species. The recommended threshold limit of Cu metal for the plants is 30 mg per kg of plant dry matter. In multimetal-polluted soil system, Cu toxicity for the plants might be a constraint in the phytoremediation process (Lombiet al. 2001).

Blaylock et al. (1997) reported the results of his study that concentration of Cu in *Brassica juncea* shoots in Cu-contaminated soil containing 200 mg kg^{-1} of Cu reached 1000 mg kg^{-1} dry matter 1 week after 2.5 mm application of EDTA. Chelant concentration is also an important factor to develop an effective model for remediation of metal-polluted soils. As reported (Greman et al. 2003), EDDS enhanced the phytoremediation and increased Cd, Pb, and Cu solubilization. Tandy et al. (2004) reported that chelate application rate affects the metal extraction efficiency. However, a single conclusion cannot be drawn from their concentrations; it varies in different conditions (Nowack 2002). Chelants have high affinity for different metals; chelate metal ratio is important. Consequently, concentration of chelating agent should be higher than metals for optimum extraction (Kim et al. 2003). For soil conservation, lower chelate concentration was more favorable (Lim et al. 2005).

Another important factor governing the solubilization of metal ions in the soil is shaking time. Stability of the complex in soil determines the ability of chelate to sustain the metals in soluble form. As reported by the Kim et al. (2003), a continuous steady-state condition between Pb and EDTA was not achieved within 1 day in Pb-contaminated soil. So by monitoring the time factor, we can predict the persistent and availability of the chelating agent in the soil matrix.

Incubation time is another important factor, which should be considered while evaluating the solubilization efficiency of chelating agent. Chaney et al. (1997) demonstrated that oxidation of metal ion and formation of metal-chelate complex may take different time period for different heavy metals. Therefore, incubation period should not be ignored while evaluating other environmental factors. In addition to this, incubation period helps in determining the biodegradation period of chelants.

8.7 Biologically Enhanced Bioremediation

Soil upper layer is an interface of plant and soil, and the process of bioremediation takes place in this layer. Microorganisms in soil horizons release different organic compounds and make the contaminated metals present in the rhizosphere available for the plants through phytoremediation. Soil microorganisms play very effective roles in different processes and have effects on human beings. Soil microbes involve in different nutrient availability for the plants and develop symbiotic relationships with plant roots; however these processes are needed to fully explore.

Fungi play a very important role in solubilization and fixation of heavy metal ions and change the availability of these ions for the plants. Different soil and plant factors affect the phytoremediation process, and these also include the soil fungi. There is a need of information about symbiotic relationships between soil microbes (bacteria and fungi) and roots of plants. Heavy metals are also in compound forms in soil which also affects the metal behavior in their solubilization and uptake processes

(Boruvka and Drabek 2004). Different types of components present in the fungal cell walls like carboxyl, hydroxyl, amino, and other functional groups. Through these functional groups, fungi can bind with toxic heavy metals such as Pb, Cu, Cd, Ni etc.

A large number of filamentous fungi may absorb heavy metal ions and used commercially. Protein present in the fungal cell wall has potential to sorb the heavy metal ions; this is in accordance with those fungi that can tolerate with the toxic heavy metals (Prasad 2017, 2018). Gonzalez-Chavez et al. (2004) reported that hyphae of the arbuscular mycorrhizal fungi consist of glomalin which can sequester the heavy metal ions. Fungi play a very important role in the phytostabilization of toxic heavy metals in the contaminated soils by sequestration and ultimately help mycorrhizal plants survive in contaminated soils.

The phytoremediation efficiency depends mainly on plant characteristics like growth rate, biomass production, harvesting ease, metal resistance, and shoots' ability of heavy metal accumulation (Heet al. 2005). Due to some constraints like large-scale cropping technique deficiency, less biomass and slow rate of plant growth researches are directed to using amendments like addition of chelates which enhanced biomass production of agronomic crops, and their yield also increased (Neugschwandtner et al. 2008).

Many research studies have been conducted, and very few species of plants have been used for bioremediation or phytodegradation. These few plant species depend on their rhizosphere microorganisms (fungi) for heavy metal remediation of soils. Crops used in the laboratory experiments and greenhouse experiments (maize, sorghum, wheat, mustard, canola, sunflower, etc.) could be used for multitasking like for the management of heavy metal-polluted soils (phytoremediation) as well as for biomass production which would be ultimately used for biogas and biofuel production. Based on preliminary data, inoculums of best fungal species would be used to enhance the process of metal uptake by the pretested plants in the field, and in addition to these, pretested fungal inoculums would be prepared, and their effect on biomass production of tested crops may be evaluated.

8.8 Bioremediation of Wastewater

In Pakistan first time bio-treatment ponds were constructed to understand the reuse of treated water for agriculture and aquaculture purpose. Environmental assessment studies on the reuse of wastewater for agriculture and aquaculture were conducted by the National Agricultural Research Center (NARC), Islamabad, Pakistan (Fig. 8.1). The bio-treatment pond performance was assessed after 1 year at NARC (National Agriculture Research Centre, Islamabad). The physical and chemical parameters including color, pH, EC, TDS, turbidity, Zn, Cu, Ni, Cd, Mn, Pb, and Fe were all within defined limits that were not sublethal for rearing fish. *Lemna* accumulated large amount of heavy metals and suggested as best for phytoremediation of wastewater. The treated water is now used for rearing fish and for plant and vegetable cultivation (Iram et al. 2012).

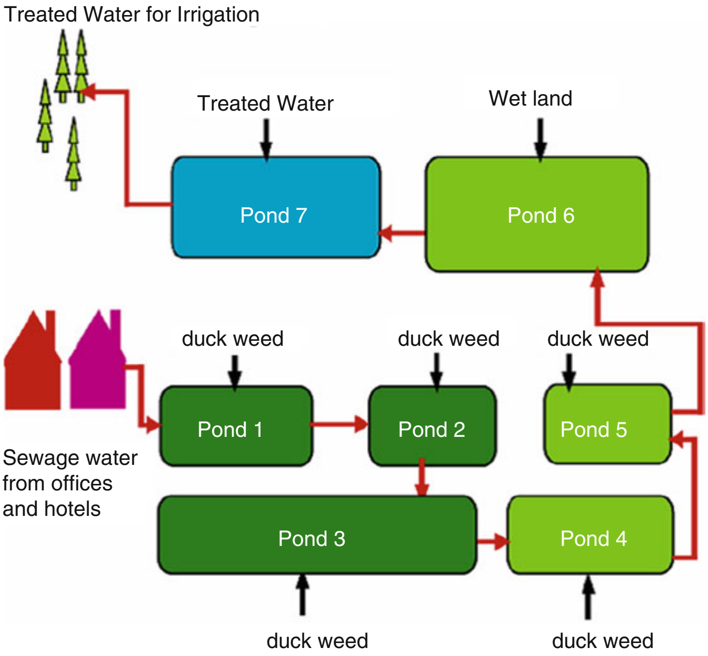

Fig. 8.1 Treatment of wastewater by plants (bioremediation ponds)

8.9 Bioremediation at Contaminated Field

In present time for rapid progress, industries are keys for achieving development. In major cities of Pakistan, industrial estates are established. Besides contributing a major share of the economy to the country's GDP, these industries are creating pollution problem. The increase level of contamination is making land useless for better yield production. Heavy metal contamination on lands is a threatening issue. Many local and conventional technologies seem to be expensive and environmentally unfriendly. Thus, bioremediation is an uprising technology to clean the environment and is a cost-effective and noninvasive alternative technique. The use of plants and tolerant microbes (bacterial and fungal strains) to remove, contain, inactivate, or degrade harmful environmental contaminants and to revitalize contaminated sites is gaining more and more attention in the world. The main purpose of proposed research is to provide the experience of the use of plants, chelates, and fungi for the remediation of contaminated soils.

From a 2012 to 2015 (Akhtar 2015) study, soils contaminated with heavy metals were considered, and remediation with fungi and plants was carried out because these technologies were untested in Pakistan and were beneficial in the economic aspects, uses, and processing of the biomass. Furthermore, development of a model of plant-contaminant-soil interaction was used in remediation technology for rapid and successful remediation of polluted peri-urban arable soils. The study helped in developing new cost-effective strategy for heavy metal-contaminated agrarian land by selecting an appropriate indigenous plant to extract metals and harvest the biomass as valuable wealth. The generated biomass could be either subjected to biomethanation or composting to reduce the volume and then processed for recycling of heavy metals. Ethanol would be then extracted and used as a biofuel in the future. The use of plants on different sites would serve to restore wetlands and other habitats, create natural parks and other green areas, and resolve the pollution problems. This research would help the farmers in selection of best germinated seeds on contaminated agricultural lands. This further promoted the research and development in the future about implementing phytoremediation which makes use of local plants to extract, transfer, and stabilize potentially toxic metals from contaminated soil. This study also advocated the development of a model of plant-contaminant-soil interaction and future remediation programs for rapid and successful remediation of polluted peri-urban soil.

8.10 Conclusions

In the field it is possible to test and accelerate the implementation of bioremediation technologies and enhance biofuel production. Much of the investigation was conducted, and very few species of plants have been discovered as phytodegrading plants. These few plants species depend on their rhizosphere microorganisms (fungi) for heavy metal remediation of soils. Crops used in laboratory experiments and greenhouse experiments (maize, sorghum, wheat, mustard, canola, sunflower, etc.) could be used for multitasking like for the management of heavy metal-polluted soils (phytoremediation) as well as for biomass production which could be ultimately used for biogas and biofuel production. Plant species, maize, mustard, sunflower, sorghum etc., have the ability to remove metals from soils; they also have potential as a bioenergy crop in Cu-, Cd-, Cr-, and Pb-contaminated land. Also sorghum proved to be good for bioethanol production. Thus, resource conservation and their sustainability are very crucial goals; hence the prospect of higher plants for pollution cleanup also served as source of biofuel in a useful prospect. The innovation is to develop a complex technique which will cover the whole value chain from setting the heavy metal-degraded soil management target through successful crop production and biofuel feedstock preparation up to conversion to energy in a local small-scale gasification installation.

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Seasonal Variations and Prevalence of Some External Parasites Affecting Freshwater Fishes Reared at Upper Egypt

9

Mahmoud A. El-Seify, Mona S. Zaki, Abdel Razek Y. Desouky,
Hossam H. Abbas, Osman K. Abdel Hady, and Attia A. Abou Zaid

Abstract

This study was carried out to detect prevalence and seasonal variation of external parasites affecting freshwater fishes. Three hundred and thirty *Oreochromis niloticus* and 140 *Clarias gariepinus* were collected from three different ecosystems at Kafr El Sheikh province. Obtained results revealed that, the highest infection rate was recorded among *O. niloticus* followed by *C. gariepinus.* Also, seasonal dynamics among the examined *O. niloticus* were recorded.The isolated ectoparasites among examined fishes were *Cichlidogyrus tilapiae, Cichlidogyrus aegypticus, Cichlidogyrus cirratus, Quadricanthus aegypticus, Macrogyrodactylus clarii, Trichodina centrostrigeata, Trichodina rectinucinata, Chillodinella hexastica, Ichthyophthirius multifillis, Henneuguya branchialis, Lamproglena monody, Ergasilus sarsi* and *Copepodit stage* (2nd stage) of *Lernea cyprinacea.*

9.1 Introduction

Fish is one of our most valuable sources of protein food. Worldwide, people obtain about 25% of their animal protein from fish and shell fish.

By the increasing intensification of fish production and lack of health management measures have lead to many disease problems of bacterial, viral, fungal and

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M. A. El-Seify · A. R. Y. Desouky
Faculty of Veterinary Medicine, Kafrelsheikh University, Kafr El Sheikh, Egypt

M. S. Zaki (✉) · H. H. Abbas · O. K. Abdel Hady
Department of Hydrobiology, National Research Centre, Dokki, Egypt

A. A. Abou Zaid
Animal Health Research Institute Kafrelsheikh, Kafr El Sheikh, Egypt

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parasitic origin. About 80% of fish diseases are parasitic especially in warm water fish (Eissa 2002). Ecto-parasites are the most dangerous group that causes severe mortalities (Shalaby and Ibrahim 1988). In Egypt there are a long periods of optimum warm weather that enable external parasites for more production and cause bad effects on fish. The majority of the monogenitic trematodes of fishes are ectoparasites, Monogeneans (flatworms) are among the most host-specific of parasites in general and may be the most host-specific of all fish parasites. Monogenitic trematodes usually don't cause any problems in the natural environment unless the host is continually reinvested so that massive numbers of worms build up on the fish (Woo 1995).

The most identified protozoa are belonging to ciliates. They can easily spread among most of the fish hosts. Uncontrollable or recurrent infection with ciliated protozoans is indicative of unhygienic husbandry problems (Al-Rasheid et al. 2000).

Parasitic crustaceans are increasingly serious problem in cultured fish. Most Parasitic crustacean of freshwater fish can be seen by the naked eyes as they attach to the gills, body and fins of the host and it spent a large part of their life on fish, possessing an adhesive organs and mouth parts adapted for piercing and sucking fish blood (El Moghazy 2008).

9.2 Materials and Methods Fish Samples

A total number of 470 (330 *Oreochromus niloticus* and 140 *Clarias gariepinus*) freshwater fish were collected alive from three different ecosystem in Kafr El-Shiekh governorate River Nile Branch (Bahr Nashart), Drainage canal (Damroo Drainage canal) and Fish farm supplied water from damroo Drainage canal by the aid of fisher man and then transported alive to the laboratory of parasitology department-Faculty of Vetrinary Medicine-Kafr El Sheikh university where they examined immediately (Table 9.1).

9.2.1 Parasitological Examination

Parasitological examination was carried out for the detection and identification of the external parasites on the skin, gills and the accessory respiratory organs of the samples.

Table 9.1 Number of fish species examined from different localities

	Locality			
	River Nile Branch	Drainage canal	Fish farm	
Fish spp.	Examined number			Total fish spp.
Oreochromus niloticus	117	100	113	330
Clarias gariepinus	80	60	–	140
Total	197	160	113	470

9.3 Collection and Preparation of the Detected Ecto-parasites

Monogenea: Monogenea were collected under binocular dissecting microscopic by means of small pipette in small Petri-dish and cleared several times with water to remove the attached mucous and debris.

The worms were then left in refrigerator at 4C till complete relaxation. Then, they were fixed in 5% formalin for permanent preparation, worms were washed carefully in water to get red of formalin traces and stained with Semichon's acetocarmine stain for about 5–10 min till reaching staining, the specimens were passed through ascending grades of ethyl alcohol (30, 50, 70, 90% and absolute) for dehydration. Then, cleared in clove oil, xylene and mounted in canda balsam (Pritchard and Kruse 1982), while the unstained Monogeneas were mounted in glycerin jelly (Abd EL-Hady 1998).

9.4 Protozoa

Some of the positive slides were stained according to Klein's dry silver impregnation method in which the slides were air –dried, covered with 2% aqueous solution of silver nitrate ($AgNO_3$) for 8 min, rinse thoroughly in distilled water and exposed to UV light for 20–30 min or to direct sun light for 1–2 h. The slides were allowed to dry and mount with neutral *Canada balsam*. This method is indispensable technique for staining *Trichodina* (Ali 1992).

Other positive slides were also air-dried, fixed with absolute methanol and stained with 10% Giemsa stain for 20–30 min to detect the other protozoa. (Ali 1992).

9.5 Crustacea

The detected crustacean parasites were carefully collected by a fine brush and special needle, and transferred into Petri-dish for cleaning by using preserved and cleared in lacto phenol then mounting with polyvenylalcohol (Raef et al. 2000).

9.6 Results

As shown in (Table 9.2); from 330 examined *O. niloticus* taken from different three localities, the total infected number was 226 (68.5%), While the rates of infection in the River Nile branch, the drainage canal and the fish farm were 71.8% (84/117), 69% (69/100) and 64.6% (73/113) respectively. In addition; the total infection rate among *Clarias gariepinus* was 58.6% (82/140). While the rates of infection in the River Nile branch and the drainage canal was 53.7% (43/80) and 65% (39/60) respectively.

As described in (Table 9.3); in *O. nilotica* the percentage of infection by monogenetic trematodes was higher in drainage canal than that of River Nile branch and

Table 9.2 Prevalence of ecto-parasites in examined fish spp. In different localities

	Locality											
	River Nile Branch			Drainage canal			Fish farm			Total		
Fish spp.	No ex.	No inf.	% of inf.	No ex.	No inf.	% of inf.	No ex.	No inf.	% of inf.	No ex.	No inf.	% of inf.
O. niloticus	117	84	71.8	100	69	69	113	73	64.6	330	226	68.5
Clarias garipienus	80	43	53.7	60	39	65	–	–	–	140	82	58.6

Table 9.3 Prevalence of different ecto-parasites in examined fish species in different localities

Parasites	Locality												Total			
	River Nile branch				Drainage canal				Fish farm							
	O. Niloticus		*C. gariepinus*		*O. Niloticus*		*C. gariepinus*		*O. niloticus*		*C. gariepinus*		*O. niloticus*		*C. gariepinus*	
	no = 117		no = 80		no = 1oo		no = 60		no = 113		no = 0		no = 330		no = 140	
	No inf.	%	No inf.	%	No inf.	%	No inf.	%	No inf.	%	No inf.	%	No inf.	%	No inf.	%
Monogenea	27	23	23	28.7	41	41	36	60	43	38	–	–	111	33.6	59	42
Protozoa	76	65	29	36.3	48	48	12	20	53	46.9	–	–	177	53.6	41	29.3
Crustacean parasites	16	13.7	–	–	36	36	–	–	39	34.5	–	–	91	27.6	–	–

fish farm, in case of infection by protozoa; it was higher in River Nile branch than that of drainage canal and fish farm, while the percentage of infection by crustacea was higher in drainage canal than that of fish farm and River Nile branch.

In case of *Cl. Gariepinus*, the percentage of infection by monogenetic trematodes was higher in drainage canal than that of River Nile branch and the infection was not detected in fish farm branch, protozoal infection among *Cl. Gariepinus* was higher in River Nile than that of drainage canal and not detected in fish farm locality. Parasitic crustacean was not detected among *Cl. Gariepinus* in all localities.

Concerning the seasonal dynamics in the examined *O. niloticus* Table (9.4) revealed that the highest seasonal prevalence of ecto-parasites in examined *O. niloticus* was recorded in spring followed by summer then autumn and finally in winter. In The River Nile branch the highest prevalence of ecto-parasites was recorded in spring then winter followed by summer and autumn. But the highest prevalence of ecto-parasites in the drainage canal was recorded in summer followed by autumn then spring and winter, while in the fish farm the highest prevalence of ecto-parasites was recorded in spring then summer followed by winter finally in autumn.

Table (9.5) showed the peak of seasonal dynamic of Monogenea in total examined *O. niloticus* was during autumn followed by summer then winter and spring. While parasitic Protozoans recorded highest infection during spring followed by summer then winter and autumn. The highest seasonal prevalence of Crustaceans among total examined *O. niloticus* was recorded during summer then spring followed by autumn and finally in winter.

9.7 Discussion

The present investigation revealed that *Monogenetic trematodes* recorded an incidence of (33.6%) which is nearly similar to those obtained by Abd El-Maged (2009) among examined *O. niloticus* was infected on the other hand higher value (80.76) was recorded by Abd El-Gawad (2004) which may be due to different of sample collection and changes in water quality in different localities.

In total examined *Clarias gariepinus*, our study revealed (42%) prevalence of *Monogenetic trematodes* which is considered higher than obtained by Ramadan (2000) 36.28%. and lower than recorded by Abd El-Maged (2009) (51.7%).

Parasitic protozoa recorded an incidence of (55.5%) among total examined *O. niloticus*. This result is found higher than that recorded by Abd El-Maged (2009) who recorded an infection rate of (6.3%).The prevalence of parasitic protozoa among total examined *Clarias gariepinus* reached (29%). This result was in contrary with Abd El-hady (1998) who did not detect parasitic protozoans among *Clarias gariepinus* in River Nile and other water branches. This result may be related to different localities of sample collection.

The prevalence of Parasitic crustaceans in this study was (27%) in total examined *O. niloticus*. This result is higher than obtained by Abd El-Khalek (1998) who recorded that the prevalence was (24.73%), while being lower than that recorded

Table 9.4 Seasonal prevalence of ecto-parasites in examined *O. niloticus* in different localities

	Locality											
	River Nile branch			Drainage canal			Fish farm			Total		
Season	No. ex.	No. inf	%	No. ex.	No. inf.	%	No. ex	No. inf	%	No. ex.	No. inf	%
Autumn	25	16	64	25	19	76	23	14	60.9	73	49	67
Winter	25	17	68	30	17	56.6	37	23	62	92	57	62
Spring	31	27	87	20	13	65	26	19	73	77	59	76.9
Summer	36	24	66.6	25	20	80	27	17	63	88	61	69

Table 9.5 Seasonal dynamics of different ectoparasites among examined *O. niloticus*

	Parasites					
	Monogenea		Protozoa		Crustacea	
Season	No. infected	%	No. infected	%	No. infected	%
Autumn ($N = 73$)	28	38.4	32	43.8	19	26
Winter ($N = 92$)	29	31.5	46	50	21	22.8
Spring ($N = 77$)	22	28.6	51	66	22	28.6
Summer ($N = 88$)	32	36.4	48	54.7	29	33

N Number examined

by El-Moghazy (2008) who mentioned that the prevalence was (80%) While parasitic crustaceans not recorded is among *Clarias gariepinus*, being coincided with Abd El-Hady (1998). This is may be due to differences in localities and water quality in these localities.

With regard to the effect of the seasonal variation on the prevalence of *Monogenetic trematodes* in the present study, the highest rate of infection was during autumn. This result agreed with Ramadan (2000) and Abd El-Gawad (2004) Mean while, this result was in contrary with Abd El-Maged (2009) who recorded the lowest infection rate was obtained during autumn.

Regarding the seasonal dynamics of external protozoa, the highest infection rate was in spring. This result was in agreement with El-Sayed (1993) stated that the seasonal incidence of protozoal infection was high in spring.

Concerning the seasonal dynamics of crustacean's infection the maximum rate of infection was during summer. This result agreed El-Moghazy (2008) mentioned that the highest incidence was recorded during summer. But this result did not agree with Hassan (1992) who detected the crustacean during winter.

These differences in the rates and seasonal dynamics of infection between the different localities may be attributed to the differences in environmental conditions, fish species, and the differences in the degree of water pollution as well as number of examined samples.

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10

Biocontrol Agents as Strategy of Agro-ecosystem Management to Restitution of Productive Soils for Food Production

K. Nathiely Ramírez-Guzmán, Cristian Torres-León,
Salvador Saldaña-Mendoza, Gloria Martinez-Medina, Marie Tranier,
Sevastianos Roussos, Reynaldo De la Cruz-Quiroz,
Roberto Parra-Saldívar, Adriana C. Flores-Gallegos,
Raúl Rodríguez-Herrera, and Cristóbal N. Aguilar

Abstract

This chapter analyzes and describes the importance and application of biocontrol agents as an alternative of managing agroecosystem for restitution of productive soils for food production. Also, the ethics, benchmarks, biosafety rules, and the various approaches and explicit features of controlling the production of food in conventional ways and emerging trends for the conservation of foods are included. Biocontrol has an important impact on the maintenance, safeguarding, and security provision on ecological and environmental aspects toward promoting the biosafety for food production. The term biocontrol incorporates the maintenance, conservation, and care of fauna and flora, as well as the native habitat on this earth. The precautionary courses include all those things where biological security must be guaranteed for all forms of life; consequently, the damages and hazards instigated should be minimalized or diminished. In any course or progression where we are employing physicochemical and biological

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K. Nathiely Ramírez-Guzmán · C. Torres-León · S. Saldaña-Mendoza · G. Martinez-Medina · A. C. Flores-Gallegos · R. Rodríguez-Herrera · C. N. Aguilar (✉)
Food Research Department, School of Chemistry, Universidad Autónoma de Coahuila Saltillo, Saltillo, Coahuila, Mexico
e-mail: cristobal.aguilar@uadec.edu.mx

M. Tranier
Toulouse White Biotechnology (TWB). Aix-Marseille Université, Ramonville-Saint-Agne, Midi-Pyrénées, France

S. Roussos
IMEP- IRD-Biotrans, Facultés des Sciences et Techniques St Jérôme, Aix-Marseille Université, Marseille, France

R. De la Cruz-Quiroz · R. Parra-Saldívar
Group of Bioprocesses, Tecnologico de Monterrey, Monterrey, Mexico

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agents, usually there are chances of hidden risk; therefore it becomes very significant for us to understand and look for the preventive measures. Recently, the use of microbial sources as biological agents has become one of the greatest challenges that have captured the attention of everyone globally. This is chiefly owing to the intensified application of biological agents in numerous industries all over the globe, for example, food and feed production, agricultural products, value-added compound production, etc.

10.1 Introduction

Currently, the world population is over 7.5 billion, and according to a United Nations report, it could exceed 10 billion this century. This considerable demographic increase has alarming consequences directly impacting a medium environment by the over production of necessary inputs for daily life, the increase of urbanized zones, the contamination of soil and water air, as well as the exhaustion of natural resources (Al 2017).

This imminent population growth demands a higher production of food, since the rates of soil degradation are higher than the reconstruction of the formation of this. The factors influence the quality and the quantity of raw materials (Wang et al. 2015).

A soil should be considered as productive or fertile implies that can give life to another type of vegetation depending on the characteristics of the region as temperature and humidity, in addition to others such as pH, a balance in the composition of sands, clays and silts among them are soil organisms known as soil-endemic biota (Blaser et al. 2017; Torquebiau 2000). These microbes are particularly an essential part for the reconstruction of the soilfor the reconstruction of the soil and some have the ability to degrade organic matter, such as animal waste and plants being able to use as their food, also helps the plant that excess food such as Nitrogen phosphorus (Santamaría-Romero et al. 2001). The organic matter decomposition is a natural biological progression, and the speed with which it happens is mainly by the nature of organic matter. The quality of organic matter, the physical environment and the composition of soil organisms helps in building up of fertile land (FAO 2006).

The main energy-recycling organisms in the soil are invertebrate grids such as worms and insects and microorganisms; in the part of microorganisms, there is a wide variety such as bacteria, fungi, protozoa, nematodes, viruses, and algae (Sivila de Cary and Angulo 2006). Each microbe fulfill a specific function and complement each other as is the example of the bacteria that help by decomposing simple substrates in contrast the fungi decompose more resistant organic matter like proteins and the protozoans are those that contribute greater nitrogen production releasing it in ammonia to be more digestible for plants, to remark a few (Tokpah et al. 2016).

However, the need to eradicate pathogenic pests affecting crops and products has focused on the use of herbicides, pesticides, and pesticides of synthetic origin of broad-spectrum dating from 1940 in the so-called era of synthetic products which carries consequences (Plenge-Tellechea et al. 2007), as well as environmental damages due to its soil residual capacity and contamination of aquifers, without the damages in natural biota. Pesticides can enter the food chains accumulate successively until they reach a lethal concentration for some organism (Gutiérrez et al. 2013).

For all this, agriculture demands the application of new technologies and strategies to reconstruct the agroecological soil balance where the introduction of alternatives of chemical control is implemented under two approaches: integrated pest management (IPM) consisting in the growth of healthy crops, which disrupts agricultural ecosystems as little as possible and promotes natural pest control mechanisms such as good agricultural practices, biological control, and the rationalization of natural resources, and ecological pest management (MEP), which is the use of biological control agents and organic agriculture (Ripa et al. 2008).

10.2 Damage in Soils

The soil is an invaluable natural resource; it constitutes the support of the plants which provides the necessary conditions that are required to live, and this is where microorganisms that develop some important biochemical cycles live, necessary for the preservation and development of the ecosystems (Lal 2014).

After social human evolution and their transition to sedentary lifestyle humans have developed activities such as agriculture and cattle raising in order to supply themselves with food, and with the passage of time have become a fundamental support for the preservation and development of the societies, starting from the need to supply themselves of resources and turn it later into sources of real economic development (Suding and Hobbs 2009).

However, as it is well known, the misuse of resources most of the time ends by deteriorate or exhausting them in the worst case and this is not the exception, bad practices and lack of planning and setting long-term and medium-term developed over the years have made the soil affected in a way that it is damaging its quality to the degree to leave it in some cases eroded and infertile (Dubovyk 2017). In this way they are incapacitated to continue the agricultural production as well as livestock because they lose the properties that allow to develop the agriculture and the characteristics necessary to keep livestock animals, representing on this way, a real impact not only ecological but also economic (López Reyes 2001).

The wear of the soil is made present indiscriminately in all parts of the world; the poor state of the land is derived from pollution, physical and biological degradation (Suding and Hobbs 2009). Activities such as cattle raising produce considerable damage to this resource due to their inadequate and careless implementation, leading to a deterioration of soil, derived from an animal overpopulation, which by pasturage and compacting the land generated by the passage of livestock, ends with great percentage of natural vegetation of the lands (Pietola et al. 2005). Such is the case of

Sonora, Mexico, where wear is such that practically all the state suffers this condition, despite the restoration activities implemented (López Reyes 2001).

On the other hand, the role of agriculture is not left behind. In the province of Cienfuegos, Cuba, studies have been carried out to determinate through the use of a technique based on the analysis of the tracer radius "cesio 137" areas with the highest erosion rate obtaining as a result that there were those destined to agriculture. Similarly, other regions of the country have been assessed by the same technique as the Pinar del Rio obtaining similar results, indicating a larger cup of erosion in agricultural production lands than those with topographical characteristics who naturally favor it (Gil et al. 2009).

Another example we have is southern Uruguay, a country with significant 30.1% erosion derived from activities such as pasturage, burning that causes soil infertility by volatilizing essential nutrients and root damage, and deforestation, among others (Ananda and Herath 2003). Horticultural production is a common activity that is unfortunately carried out without a well-defined planning, causing soil wear and thus almost 87% of the erosion, affecting not only the land but also the economy of the producers because with nutrient losses, they have to invest even larger amounts in the introduction of inputs and irrigations in their systems, to maintain the conditions to continue with the required production, while the cost of the products, on the other hand, remain without rising in the market, representing in this way a huge problem, when it means getting lower income for their families. Fortunately the research carried out this role, proving that it was possible to decrease erosion by up to 60% by adding pastures to crops incorporating also green manures, what represents a palpable hope (Ananda and Herath 2003).

On the other hand, urbanization is also a factor of environmental deterioration derived of the topographical modification involving the growth of a city, as the construction of works such as roads and bridges that make it difficult to recharge the mantle underground aquifers and complicates soil regeneration (Lal 2014). It is also worth mentioning the participation of forest fires that can easily change ecosystems eliminating the protection provided by vegetation as well as the nutrients and organic matter that are the source of enrichment of the soil, and that together with other factors are able to generate different levels of runoff and erosion (Zemke 2016).

Erosion constitutes only one of the types of damage that the soil can suffer. Water is a process that is characterized by the loss of layers of soil committing its productivity; it is considered with the major importance because of its irreversible nature, and it is generated by the impact of rain on the earth with a posterior runoff of water; it causes the drag of organic matter and minerals being intensified by the presence of slopes (Honorato et al. 2001). In the same way, the climatic conditions like the strong currents of wind favor to a great extent its development. The absence of plant protection activities, carrying out agricultural activities or stemming from problems such as wildfires are also considered predisposing factors (Zemke 2016).

It is very important to evaluate soil erosion, to have an idea of the pace of wear, identifying the factors that produce it and thus the magnitude of the problem, as well as to find a concrete way to fight it, using techniques such as analysis of radio tracers or by using oxides of rare terrestrial elements that can determine the level of erosion of an area (Gil et al. 2009). Through analysis, it has been possible to develop software such as “la Ecuación Universal de Pérdida de Suelo” (Universal Soil Loss Equation) USLE (Honorato et al. 2001). This allows to identify and analyze the factors that produce erosion under a wide range of series conditions. From this have been developed reviews to be able to work on efficient way according to the conditions where is wanted to implement it, as the case with its revised version “RUSLE” has also served as the basis for the generation of new programs that constantly seek to overcome their limitations.

It is clear that there is a need to find effective ways to combat the deterioration of soils, whether for ecological or productive purposes; fortunately the efforts aimed at research and development of new technologies for their control and treatment in addition to the implementation of new methodologies that seek to avoid this condition are promising panorama for the conservation and restoration of this precious resource (Aziz et al. 2013; Langdale et al. 1992).

10.3 Pesticides and Synthetic Fertilizers

The pesticides are organic chemicals which are man-made and are being employed in the agricultural sector to control different types of pests (Valavanidis and Vlachogianni 2010) and promote crop yields (Ren et al. 2013). Pesticide use has experienced a dramatic increase worldwide (Zhang et al. 2015). To feed the growing world population, countries have made irresponsible use of pesticides. For example, the use of pesticides in China has increased sharply, from 0.76 million tons in 1991 to 1.8 million tons in 2011, amounting to an average annual growth rate of 4.9% (Li et al. 2017). This has caused China to apply 1.5 and 4 times more pesticides than the world average (Zhang et al. 2015).

The extensive use of synthetic chemicals for pest control is recognized as a major threat to ecosystem integrity, besides produced about 14% of the world’s total greenhouse gas emissions (Oertel et al. 2016). Pesticides also have adverse health effects. Farmers who spray more pesticides are more likely to have headache, nausea, and skin problems; pesticides have significant invisible impact on farmers’ liver and neurological and kidney systems Nicolopoulou-Stamati et al. 2016).

Pesticides include different organic-based chemicals, which are used to inhibit the growth of weeds, insects, fungi, nematodes, and rodents. Commonly used pesticides are customarily herbicides (to kill unwanted herbs), amounting to 44 % around the globe, insecticides (to kill unwanted insects) around 28%, and fungicides around 26 % (to kill pathogenic fungi) (Valavanidis and Vlachogianni 2010). DDT was an organochlorine-based pesticide, widely employed for agricultural and household pest control between the 1950s and 1980s (Zhang et al. 2015). Subsequently, pesticides having pyrethroid (PYR) and organophosphate (OP) bases have become appealing replacements owing to their comparatively less persistence and environmental toxicity (Li et al. 2017). Though, recently, scientific research has reported that still chemical insecticides classified as lesser lethal may also lead to chronic

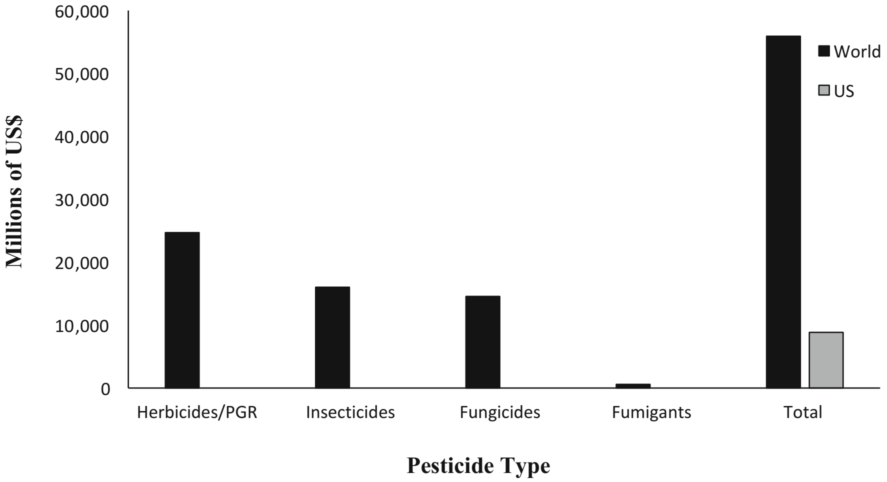

Fig. 10.1 The world market for pesticides in 2012. (Source: Phillips McDougall, AgriService (2008–2012). (http://phillipsmcdougall.co.uk/agriservice/). Modified from: EPA (2017))

diseases and that few of these chemicals are even fatal for human beings having continual disclosure (Wang et al. 2012).

According to the US Environmental Protection Agency (EPA), the global market for chemical pesticides has increased considerably from 2.7 billion $ in 1970s, 18.5 billion $ in 1990s, $ 32.7 billion in 2001 to $56 billion in 2012 (see Fig. 10.1).

Table 10.1 shows the expenditure on different pesticides during 2008–2012

10.3.1 Synthetic Pesticides: Human Health Risks

Pesticides refer to any substance addressed to stopping, averting, abolishing, repelling, attracting, or controlling any type of pest, during production process, storing, transporting, distributing, and processing of food or animal feeds or any agricultural commodity (FAO WHO 2001); pesticides can be divided according to their chemical composition, and the most widely used are the synthetic or organic pesticides, synthetized by combination from elements like carbon, hydrogen, and oxygen which can be enumerated and subdivided into organochlorines, organophosphates, peroxyacetic acids, carbamates, and synthetic pyrethroids (Hough 2014).

Developing countries need to enhance agriculture production, and therefore, the use of agrochemicals allows higher crop yields, being synthetic pesticides the first option due to their low costs; however the indiscriminate use of this kind of substances results in environmental damage and high health risks (Peshin 2014).

Pesticides comprise a group of toxic and bioactive substances that can interact not only with their target and can disturb soil productivity and ecosystem quality; they tend to persist much longer and affect soil microflora and soil health, specifically

Table 10.1 The world and US pesticide expenditures at the producer level by pesticide type, 2008–2012 estimates

Year and type of pesticide	Global market		USA market		US percentage of global market
	Million $	%	Millions of $	%	
2012					
Herbicide/PGR	24,727	44	5,115	58	21
Insecticides	16,023	29	2,184	25	14
Fungicides	14,565	26	1,430	16	10
Fumigants	606	1	137	2	23
Total	55,921		8,866		16
2011					
Herbicides/PGR	23,322	44	4,904	58	21
Insecticides	15,055	28	2,125	25	14
Fungicides	13,898	26	1,348	16	10
Fumigants	554	1	145	2	26
Total	52,829		8,522		16
2010					
Herbicides/PGR	21,131	45	4,755	58	23
Insecticides	13,356	28	2,038	25	15
Fungicides	12,106	26	1,232	15	10
Fumigants	578	1	138	2	24
Total	47,171		8,163		17
2009					
Herbicides/PGR	21,376	46	5,058	59	24
Insecticides	12,382	27	2,009	23	16
Fungicides	11,692	25	1,166	14	10
Fumigants	557	1	122	1	22
Total	46,007		8,355		18
2008					
Herbicides/PGR	23,516	48	5,364	63	23
Insecticides	12,486	26	1,882	22	15
Fungicides	12,249	25	1,186	14	10
Fumigants	591	1	123	1	21
Total	48,842		8,555		18

Source: Phillips McDougall, AgriService (2008–2012) (http://phillipsmcdougall.co.uk/agriservice/)
Taken from: EPA (2017)

nutrient contents, soil organic carbon, pH, moisture, soil enzymes, and others (Prashar and Shah 2013).

In terms of environmental damage, these molecules may enter groundwaters, provoking lethal effects in aquatic organisms and increasing water temperature due to these chemicals' presence, and the fact that the molecules are most easily concentrated in aquatic organism is concerning (Hough 2014). Pesticides as water contaminants can promote a set of side effects in water living organisms, like cancer or tumors, reproductive failure, immune suppression, disruption of endocrine system, cellular and DNA damage, and teratogenic effects; thus ecological effects can be considered as a primary warning indicator for potential human health impact (Ongley 1996), and the most concerning preoccupation is the damage that this chemical molecules can exhibit in human health through contaminated water, air, occupational exposure, or traces in food.

There exists a high prevalence of pesticides found in food purchased in markets, where approximately from 8 fruits to 12 vegetables, 73% contain pesticide residues (Baker et al. 2002), and wide evidence has presented that most common issues in health effects linked to pesticides include endocrine disruption (Song et al. 2017), cancer, diabetes, asthma, cognitive effects, or sperm damage (Kim et al. 2017).

10.4 Biological Control Agents as an Alternative for Soil Restoration

10.4.1 Definition and Generalities

All over the globe, people are using several definitions of the term biopesticide; however, the EPA has the most managed definition in the field of agricultural science that includes particular types of pesticides obtained from such natural substances as plant, animal, plants, fungal and bacterial species, and some minerals (EPA 2017). Biopesticides are also called as biological pesticides; these are a specific group which considers three main classes: (1) biochemical pesticides, (2) plant-incorporated protectants (PIPs), and (3) microbial pesticides (Moshi and Matoju 2017). *Biochemical pesticides* are innately occurring chemical substances or unnaturally derived counterparts to a naturally happening chemical substance that controls pests by action of nontoxic means. Biochemical pesticides use enzymatic or protein mediation or natural antimicrobial substances which inhibit or kill the desired organism and also have a plus point in that they are degraded in short times and are not persistent in the environment (Leahy et al. 2014; Matthews 2014). *Plant-incorporated protectants (PIPs)* are defined as a substance that is incorporated through genetic engineering to the plants in order to induce the expression of genes encoding insecticidal toxins. That way, plants can produce their own pesticide providing itself the protectant effect. The active ingredient could be inert substances or genetic substance which is required for specific protectant substance, produced by a plant (Matthews 2014; Sarwar 2015). *Microbial pesticides* (MPs) consist of a naturally occurring or genetically altered microorganism (e.g., a bacterium, fungus, virus, or protozoan) as the active ingredient. MPs are intended to prevent, repel, mitigate, or destroy many different kinds of pests (Matthews 2014). MPs can be either naturally occurring or genetically engineered. Modes of action of microbial pesticides include ecological competition, growth inhibition, direct toxicity, and parasitism using the pest biomass as a substrate (Gasic and Tanovic 2013). MPs are the largest section of the biopesticide group, including 53 registered products in the USA, 22 in Canada, and 21 in the European Union (Koul 2011). That is because of the advantages shown by most MPs such as safety (wildlife, humans, and other organisms), specific toxic action (beneficial insect is not affected), and compatibility with synthetic chemical pesticides. In some cases, the pest control is subsequent for generations and seasons, and most of the microorganisms used as MP are an enhancer of roots and plant growth as well as benefiting the microflora of the soil (Usta 2013). However, due to the biological origin of MPs, they also have limits, such as specificity and selectivity because most of the products might handle only a fraction of different pests existing in that area. Other important issues include the high sensitivity to heat, desiccation, and ultraviolet exposure that reduces the viability and effectiveness of the microbial products. Also, it is necessary to consider the specific proceedings for application, formulation, and storage (Usta 2013).

10.4.2 Bacteria-Based Biopesticides (BBBs)

This category is the most common and inexpensive form of MPs, because of the facilities and high-scale production. BBBs are mainly focused on the control and elimination of insect pests such as moths, butterflies, beetles, flies, and mosquitoes (Mnif and Ghribi 2015). In this particular case, BBBs need to be ingested by the insect to be effective and cause death. These products are subclassified into four sections: (1) crystalliferous spore formers, (2) obligate pathogens, (3) potential pathogens, and (4) facultative pathogens (Koul 2011). The spore formers are the most studied, produced, and commercialized due to their high effectiveness and safety. The species of *Bacillus* can be found everywhere around the world in seawater, soil, etc. *Bacillus* appears as the main bacterial strain used as biopesticide (Melo et al. 2016). The high biocontrol potential is because of the production of a crystalline protein highly toxic and responsible for the gut cells lysis after consuming by insects, which ultimately leads to its death (Hung et al. 2016). Besides *Bacillus*, other important bacterial genera such as *Clostridium*, *Saccharopolyspora*, *Streptomyces*, *Pseudomonas*, *Agrobacterium*, *Xanthomonas*, *Ralstonia*, and *Serratia* are also popular as biopesticides (Chattopadhyay et al. 2017; Montesinos 2003).

10.4.3 Fungal-Based Biopesticides (FBBs)

This section also is composed of four kinds of products including herbicides (*Chondrostereum purpureum*), fungicides (*Trichoderma harzianum*, *Coniothyrium minitans*), nematicides (*Paecilomyces lilacinus*), and insecticides (Balasubramanian and Tyagi 2016; Gupta and Dikshit 2010). This last subsection of FBBs is the most commercialized product around the world integrating important fungal genera such as *Beauveria* and *Metarhizium* as insecticides (Mohammadbeigi and Port 2013). These entomopathogenic fungi are mainly focused on the control of insects such as spittlebugs, locust, and grasshopper (Chandler et al. 2011). The adhesion and germination of the spores from entomopathogenic fungi are very crucial steps in order to kill insect pests. After that, a combination of a physical and enzyme activities allows fungi to penetrate the insect and proliferate inside of it (Fernandes et al. 2012). The last step is the production of toxic metabolites, mainly destruxins (Muñiz-Paredes et al. 2017). However, the activity of the fungal entomopathogenic continues until it develops propagules (spores) over the dead body for a posterior dispersion (Beris et al. 2013) (Table 10.2).

10.4.4 Yeast-Based Biopesticides (YBBs)

There are a few commercialized bioproducts containing yeasts as a biopesticide. However, several investigations have been carried out in order to select effective yeast strains against important pests. Among the most studied yeast, it is possible to find species such as *R. mucilaginosa*, *R. glutinis*, *Trichosporon pullulans*, *Metschnikowia pulcherrima*, *Cryptococcus flavescens*, and *Cryptococcus laurentii* as biopesticides against phytopathogenic fungus like *Botrytis cinerea*, *Fusarium graminearum*, and *Penicillium expansum* (Qin et al. 2003; Rong et al. 2016; Spadaro et al. 2010; Zapata et al. 2016). Insecticidal yeast is also reported including strains of *Pichia onychis*, *Pichia pastoris*, and *Debaryomyces hansenii* with high activity against *Drosophila suzukii*, *Mamestra brassicae*, and *Acyrthosiphon pisum* (Hinchliffe et al. 2010; Murphy et al. 2016). The mechanisms of action reported for yeasts are similar to those reported for bacterial biopesticides such as production of antibiotic substances and severe competition for space and nutrients, as well as host resistance induction, biofilm formation, quorum sensing, and competition for iron (Droby et al. 2016).

10.4.5 Nematodes as Biopesticides (NBs)

The use of nematodes as biopesticides is a section integrated mainly by two genera, *Steinernema* and *Heterorhabditis* (Chavarría-Hernández et al. 2014). Both nematodes infect only insects living as endoparasite inside of them until the insect dies, including butterflies, moths, beetles, flies, crickets, and grasshoppers (Atwa 2014; Shields 2015). There are 61 species of *Steinernema* and 14 species of *Heterorhabditis* proved as bioinsecticides; however *S. glaseri*, *S. carpocapsae*, *S. rarum*, *S. feltiae*, *S. kraussei*, *H. bacteriophora*, *H. indica*, *H. megidis*, and *H. amazoenzis* were found as the most efficient species (De Brida et al. 2017; Del Valle et al. 2017; Guy et al. 2017; Heve et al. 2017; Matadamas-Ortiz et al. 2014; McGraw and Schlossberg 2017). Among the insect with more sensitivity against these nematodes, it is possible to find *Dacus ciliatus*, *Spodoptera cilium*, *Thaumatotibia leucotreta*, *Diloboderus abderus*, *Anasthrepa suspensa*, *Gromphadorhina portentosa*, *Nauphoeta cinerea*, and *Blaptica dubia*, among others (Cutler et al. 2017; Gulcu et al. 2014; Kamali et al. 2013; Manrakhan et al. 2014). Several nematodes showed synergistic activities as symbiote with bacteria like *Xenorhabdus* or *Photorhabdus* killing insects in 24–48 h; in addition, NBs are safe for animals, plants, and nontarget organisms (Shields 2015).

Table 10.2 Bacterial- and fungal-based biopesticides registered by EPA (2017)

Microbial agent	Target pest
Trichoderma harzianum T-39	*Botrytis cinerea*
T. asperellum ICC 012	Soil-borne plant pathogens on various plants including different vegetables, fruits, turfs, orchards, aromatic plants, and legumes
T. gamsii ICC 080	
T. hamatum 382	
Bacillus firmus	Plant-parasitic nematodes
B. thuringiensis var. *aizawai*	
PS811	Larvae of lepidopteran (moth)
NB200	
B. pumilus	
QST2808	*Rhizoctonia* and *Fusarium*, as well as molds, mildews, blights, and rusts
GB34	
B. licheniformis SB3086	Fungal species, especially those causing leaf spot and blight diseases
B. subtilis	
Amyloliquefaciens FZB24	*Rhizoctonia* and *Fusarium*
Israelensis EG2215	Mosquito larvae
Kurstaki M-200	Lepidopterous pests of tree fruits and vegetables
QST713	Sour rot disease, powdery mildew, early leaf spot, scab, bacterial spot, downy mildew, walnut blight diseases, early blight and late blight diseases
Pasteuria usgae	*Belonolaimus longicaudatus*
Candida oleophila O	*B. cinerea* and *P. expansum*
Beauveria bassiana	
HF23 (70787-1)	House flies in chicken manure
447	Fire ants and other ants found indoors
Pythium oligandrum DV 74	20 soil-borne pathogenic fungi
Colletotrichum gloeosporioides f.sp. *aeschynomene* (82681-1)	*Aeschynomene virginica*
Pantoea agglomerans	
C9-1 (71368-45)	*Erwinia amylovora*
E325 (71975-1)	
Chondrostereum purpureum	
HQ1	Hardwood trees such as red alder, Sitka alder, speckled alder, and trembling aspen
PFC 2139	
Paecilomyces lilacinus 251	Plant-parasitic nematodes in soil
P. fumosoroseus Apopka 97	Whiteflies, thrips, aphids, and spider mites
Alternaria destruens 059	*Cuscuta* spp., known as dodder, swamp dodder, large seed dodder, small seed dodder, and field dodder
Muscodor albus QST 20799	Root rot, damping off, and wilt disease-producing fungi and bacteria
Aspergillus flavus	
NRRL 21882	*A. flavus* that produce aflatoxin
AF36	
Metarhizium anisopliae F52	Various ticks and beetles; root weevils, flies, gnats, thrips

(continued)

Table 10.2 (continued)

Microbial agent	Target pest
Puccinia thlaspeos	*Isatis tinctoria*
Pseudozyma flocculosa PF-A22 UL	Powdery mildew on roses and cucumbers
C. minitans CON/M/91-08	*Sclerotinia sclerotiorum* and *S. minor*
Pseudomonas chlororaphis 63-28	Certain fungal species which infect plant roots and induce wilt diseases also cause root and stem rots
P. aureofaciens Tx-1	*Sclerotinia homoeocarpa*, *Colletotrichum graminicola*, *Pythium aphanidermatum*, *Microdochium nivale*
Reynoutria sachalinensis	Powdery mildew and gray mold
Agrobacterium radiobacter K1026	*Agrobacterium tumefaciens* and *A. rhizogenes*
Gliocladium catenulatum J1446	Fungi that cause damping off disease, seed, stem and root rot, and also wilt disease
Burkholderia cepacia J82	Certain diseases of seedlings and for controlling nematodes that attack the roots of crops

10.4.6 Insects as Biopesticides (IB)

Several insects are a concern worldwide because of the damage they cause in a huge number of plants and fruits. The phytopathogenic insects can cause damage to the plant in three ways such as mainly (1) direct affection on the plant surface, (2) transmission of different viral diseases, and (3) contamination of leaves and fruits through the secretion of some kind of gum called honeydew (Peng et al. 2017; Wang et al. 2016). The most dangerous insect pests are shown in Table 10.3. Insects have an incredible capacity to generate resistance against the chemical pesticides; therefore in many cases, the application of chemical pesticides is done in an excessive way (Rodríguez-Álvarez et al. 2017). One of the principal management strategy to control the population of insect pests is the increase of the population of their natural insect enemies including the genera of *Neoseiulus*, *Amblyseius*, *Stratiolaelaps*, *Hypoaspis*, *Encarsia*, and *Eretmocerus*, among other important ones (Fernández et al. 2017; Seiedy et al. 2017; Wu et al. 2017). It is relevant to mention that the use of this kind of treatment is more effective when is applied in greenhouse conditions, helping to keep the biopesticides in contact with the pest (Rakha et al. 2017). Also, warm regions are more feasible in order to accelerate the biopesticide multiplication and development, increasing the effectively (Fernández et al. 2017). The group of insects used as biopesticides is more commonly named as parasitoids because its larval stage feeds on the host until to death and taking advantage to oviposit on the body to generate more individuals. In some cases of compatibility, it is possible to apply in a synergistic way with some fungal entomopathogens, such as *Beauveria bassiana* or *Metarhizium anisopliae*, generating a better impact in the control of pests (Wu et al. 2017).

Table 10.3 Most dangerous insect pests and their natural enemies used as biopesticides

Pest insect	Entomopathogenic insect	Reference
Bemisia tabaci (whitefly)	*Encarsia formosa*	Bonato et al. (2011), Hanafi et al. (2007), and He et al. (2017)
	Eretmocerus californicus	
	Macrolophus caliginosus	
Thysanoptera spp. (thrips)	*Neoseiulus barkeri*	Kakkar et al. (2016), Wu et al. (2017), and Otieno et al. (2017)
	Amblyseius cucumeris	
Tetranychus cinnabarinus (carmine spider mite)	*Phytoseiulus persimilis*	Moghadasi et al. (2016)
Aphidoidea (plant lice)	*Aphidoletes aphidimyza*	Barbosa et al. (2017) and de Azevedo et al. (2017)
	Chrysoperla carnea	
	Aphidius colemani	
Spodoptera spp. (armyworms)	*Trichogramma* spp.	Leite et al. (2017) and Takada et al. (2000)
Phyllocnistis citrella (citrus leaf miner)	*Ageniaspis citricola*	de Morais et al. (2016) and Goane et al. (2015)

10.5 Traditional Strategies for Food Control and Security

Food security is a term that sometimes may be indistinctly used with food safety; however, the best known and widely used term was that coined by FAO et al. (2015), which states that food security is “a situation that exist when each person, in all time, have social, physical and economically approach to enough, nutritious and safe food to meet its daily need and preference to live a healthy and an active life.” Defra (2009) used a similar definition for food safety: “ensuring access to, availability of, inexpensive or affordable, nutritious and safe food sufficient for and active life-style, for each, at all times.” While food safety is related with problems in raw materials, food processing, and pathogens and cross contamination, food security deals more with food utilization, access, and availability. Food utilization deals with good health indicators, clean water, food quality and safety, sanitation, and nutritious food, whereas food access refers more to equitable distribution, affordability, transport, purchasing power, and marketing; finally, food availability is related to processing, water and soil management, production, and trade and stockpiling. Although, there is a relation between both terms, food safety and security; external conditions such as poverty and climate change may affect both food safety and food security. Another term is *biosecurity*, which is defined as “the protection of the environment, economy and health of living beings from various diseases, bioterrorism and pests.”

Table 10.4 Food security risk of different countries according to the food security index 2013

Low	Middle	High	Extreme
USA, Canada, Chile	Latin America	India	Somalia, Congo
Europe	South of Africa	Most African countries	Haiti, Chad, Ethiopia
Australia, Japan	North Asia	Pakistan	Afghanistan
	Oceania (except Australia)	Guatemala	Sudan, Burundi, Eritrea

According to FAO, food safety management systems should also be based on risk analysis approach and also endorse the use of Codex Alimentarius Commission (CAC) which is comprised of three components which are interlinked: (1) risk assessment, (2) risk management, and (3) risk communication (FAO/WHO 2004, 2010).

Although the impressive improvements have been seen in agriculture, agrochemicals, food technology, soils, fertilizers, irrigation, food processing and storing techniques in recent years, but inspite of this development, everybody do not have guarantee of the safe and healthy food for today and tomorrow. Food security risk of different countries is measured taking into account different parameters such as food production, accessibility, transportation, etc. In Table 10.4 are shown different countries according the food security risk (Global Food Security Index 2013).

Currently, hunger in the world is a big problem, and it is estimated that 795 million people are malnourished (FAO et al. 2015) and 9 out of 10 live in developing countries. Causes of this hunger are poverty (896 million of impoverished people live with less than 2 US dollars per day), adverse economic systems, political conflicts, population growth, wrong food and agricultural policies, and climate change. In addition, there are some threats to food security such as evolution of pest and pathogens, genetic contamination of landrace plant cultivars by transgenic crops, high production costs, and less nutritious food. In addition to hunger, there are mineral deficiencies prevalent in the human diet, especially in countries with low income, which currently affect about 3000 million of persons (Peleg et al. 2008), referred to as hidden hunger (Bohra et al. 2015). Some causes of mineral deficiencies are diet based on cereals and no diversified diet which is poor in nutrients (Mulualem 2015).

If food supply is compromised, there would be economic, physical, political, and psychologic consequences. But also, if eatable food is conceded with harmful chemicals or pathogenic bioagents, it may lead to indirect result of hunger, morbidity, and mortality. For this reason, providing safe food is the responsibility of the central government, many federal organizations as well as the local and state counterparts, professional agencies, and food processing units (Bruemmer 2003).

The preventive policies are the first line of defense against potential hazards; however, controlling chemical and biological agents and rapid detection of pollutants is vital as well as essential to food safety. In this sense, there is a scheme or method which assures safety of foods at producer's level. The technique "Hazard

Analysis Critical Control Points" (HACCP) identifies technical means and approaches in process of food production which is able to eradicate chemical, physical, and biological hazards. In this technique the food producer has to ascertain or determine limits or ranges of certain critical control points (CCPs), their monitoring methods, and steps for correction. Another system that sets processing rules and eliminates the risk of occurrence of a harmful food is "Good Manufacturing Practice" (GMP). These two systems, HACCP and GMP, follow up and are complementary to each other (Steinhauserova and Borilova 2015).

In 2015, the WHO assessed that around 2 million people die annually, owing to unsafe food consumption all over the world. One major risk for food security is evolution of plant pest and pathogens which day by day is more difficult to control and have improved genes for pathogenicity. It has been reported that around 200 diseases may be spread by consuming contaminated or polluted water or food stuff. On the other hand, the foodborne infections or disorders may also be augmented by enhanced international food transportation and people's movement. Waterborne and foodborne diseases are mainly caused by pathogenic microbes (bacteria, fungi, viruses, and potential parasites) and harmful chemicals (heavy metals, pesticides, allergen, toxins, mycotoxins), and the severity of disease ranges from mild gastroenteritis to life-frightening (Fusco et al. 2015). In case of plant pathogens, one of the more difficult to control is *Rhizoctonia solani* Kühn teleomorph fungus [*Thanatephorus cucumeris* (Frank) Donk] which damages different potato parts: root, shoots, stems, stolons, and tubers (*Solanum tuberosum* L.) (Carling et al. 2002). Its incidence during potato cultivation causes losses in production that vary between 7% and 64% and up to 100% in quality aspects (Hernández et al. 2001).

Management of this kind of pathogens depends mainly on application of synthetic fungicides through all crop season. In some regions, there are more than ten synthetic fungicide applications through the whole crop season in order to manage of regulate plant pest and pathogens. However, the indiscriminate use of these synthetic fungicides has consequences since some fungal isolates have showed resistance to the active ingredients of synthetic fungicides (Hernández et al. 2005). In addition, there is also important evidence of serious environmental contamination derived from application of synthetic fungicides, which not only affects flora and fauna but also contributes to quality deterioration of air, water, soil, and food, in addition to health of humans (Albert 2004). With the intention to maximize the efficiency of plant pests and disease control at field level, studies on the monitoring of pest and pathogen populations to sensitivity of the main fungicides used in crop commercial production are necessary. These studies are a determinant of the behavior and the degree of sensitivity of these pest and pathogen populations in the field. The necessity to find mechanisms that increase crop productivity has promoted development of new strategies for pest and pathogen control which should be efficient alternatives to chemical control and also reduce environmental and health risk without risking human health (Gallegos et al. 2004) such as biological control based on organisms antagonist to pest and pathogens, use of extracts, genetic engineering, and so on.

The expected growth of human population by 2050 signifies a great challenge to state-of-the-art agricultural system. For this reason, agroecology has been highlighted by the United Nations for sustainable farming practices to produce sufficient food without causing injurious effects on the ecosystem (De Schutter 2010). Agroecology is defined as "the integrated study of ecology of entire food systems, including the economic, ecological and social aspects" (Francis et al. 2003). Thus, attention has been paid to the influence of dissimilar agriculture procedures on biodiversity.

In the soil ecosystems exist various types of microbes; these include fungi, bacteria, actinomycetes, protozoans, etc. Its distribution in the adjacent areas of the plant rhizosphere is higher and is more active physiologically (Guetsky et al. 2001). The great difference in biological diversity between a natural ecosystem and an agroecosystem is caused by the decline of biomass in the latter. This reduction is usually caused by a reduction in content of soil organic matter and by the loss of diversity in the plants given by monoculture. The microbial communities that occur naturally in the rhizosphere play a role in the healing of the radical system. This interrelation between the root tissue and the microbial community of the soil is significantly more intense than that found in the plant aerial part (Vilich and Sikora 1998). Different microorganisms that usually act in plant rhizosphere have been reported as effective biocontrol agents. One of the major contrasts of biocontrol for root pathogens is that a biocontrol agent may not provide a good effect, particularly if the biocontrol agent is not adapted to plant rhizosphere. Antagonism operates in a variety of ways: antibiosis, competition, prefiguration, or parasitism. The latter involves of several hydrolytic enzymes production that biodegrade the pathogen cell walls. Some of these hydrolytic enzymes are B-1,3-glucanase and chitinase, which are products of several fungal and bacterial species (Russell et al. 1994).

10.6 Commercial and Market Opportunities of Biological Products

Currently, the global population is increasing breathtakingly fast, and it is expected to reach 9.7 billion by the year 2050 (United Nations 2015). All those people will need to be fed; therefore this close situation will impact in the necessity to increase the production and quality of food. On this expected scenario crop protection, it's a serious issue and should be the main focus of all nations in the world (Moshi and Matoju 2017). The traditional way of ensuring crop protection has been the application of conventional chemical pesticides; however, they are facing important challenges in the present (Maute et al. 2017; Miro Specos et al. 2017). Chief restrictions for application of the chemical pesticides are because of (1) the generation of resistant pests, (2) the death of nontarget organisms, and (3) the residual chemicals on the food and soil (Arora et al. 2016; Eski et al. 2017; Pavela et al. 2017). In short, this puts at risk the human health and ecological balance (Leahy et al. 2014; Matthews 2014). The information mentioned above has been very well known, but the laws have not been stricter until now, since most of the countries are changing its political rules in order to limit the excessive employment of harmful chemical-based pesticides (Desai et al. 2016; Goñi et al. 2017; Hazra et al. 2014).

10.6.1 Market Overview

In the present, the food processors and supermarkets are in the same line with the intention to offer better and healthy products. In terms of economic feasibility, biological products are gaining market, because the development of traditional chemicals (pesticide, fertilizers, and plant growth stimulators) has become harder and more expensive (Thakore 2006). For example, $250 million USD are required to carry out a new agrochemical at commercial level spending 9–10 years for development and regulatory approval, while a biological product only needs $10 million USD and only 3–4 years to put it on the market (Olson 2015; Pucci 2014). All these factors mentioned above plus the demand for sustainable alternatives are driving the trend about research and development of biological products. Analyzing the facts, it is possible to see more increase on the development of biological products, as well as the consolidation of small enterprises and the creation of several start-up companies (Duke et al. 2014). That is a reason to explain the growth of the global agricultural biological market which represents only about 4–5% of the total agroscience market but valued at $5.11 billion USD in 2015 with a growth rate of 13.5% (Technavio 2017). In this segment, it is expected an increase in the market size of $10.05 billion USD for 2020 (Duke et al. 2014; Olson 2015). The segments of the global agricultural biological market are integrated by 45.58% of biopesticides, 38.82% of biostimulants, and 21.60% of biofertilizers (Technavio 2017). In the segment of biopesticides are included bioinsecticides, biofungicides, bioherbicides, bionematicides, and biochemical products (Sudakin 2003). The adjuvants, plant growth regulators, and inoculants in order to enhance the health of crops are classified as biostimulants (du Jardin 2015; Yakhin et al. 2017). In the third section are the biofertilizers that comprise the products for nitrogen fixation, potassium mobilization, and the solubilization of phosphate and other compounds (Kulasooriya and Magana-Arachchi 2016; Sharma et al. 2013). The revenues of each segmentation products are shown in Table 10.5.

Table 10.5 Revenue of products by segmentation of the agricultural biological market

	2016		2020	
Segment	Revenue ($ billion USD)	Growth rate (%)	Revenue ($ billion USD)	Growth rate (%)
Biopesticides	2.65	13.98	4.68	16.04
Biostimulants	1.90	13.57	3.31	15.62
Biofertilizers	1.24	12.40	2.06	14.18

10.6.2 Commercial Leaders on Biological Products

Important brands have been established selling this kind of products, from companies such as Agrinos AS, Camson, Vertis USA, Koppert BV, Marrone Bio Innovations, T. Stanes & Company Limited, and Valent BioSciences (Technavio 2017). However, BASF, Bayer CropScience, and Novozymes are the giants of the market who are dominating as the leader players representing 11.60%, 10.10%, and 1.90% in the global agricultural biological market, respectively (Table 10.6). Currently, the key market players are offering different options of environmental friendly products in order to make a sustainable process of crop protection enhancing the production and quality through the combat of weeds, pests, and diseases of crops by the use of bacteria, fungi, and viruses. A very marked tendency is the synergy and acquisition of small companies by the market leaders. This activity has become common in the last 10–15 years, and it is possible to see it easily on the news or the Internet, such as the integration of Becker Underwood with BASF SE, the purchase of Prophyta by Bayer, or the partnership of Monsanto and Novozymes A/S (Seiber et al. 2014; Olson 2015). These movements among companies give them important advantages, in order to make a faster development of biological products, offering more and better options, as well as providing major geographical availability, among others.

10.6.3 Impact of Biological Products on the Health of Soil

The majority of the companies dedicated to the agroscience are continually searching for more attractive biological solutions for pest control, through the area of research and development. As mentioned before, most of their products are focused on biopesticides, biostimulants, and biofertilizers, but also all biological products have a positive effect on the microbial soil populations. In the recent years, the investigations in terms of the soil microbiota incorporating the improvement on crop productivity and the lessening of the hostile and antagonistic consequences of change in climate, among other factors, have attracted more attention. This includes the environmentally friendly agronomic techniques, which can improve yields in agricultural production in a process that is directly linked to soil microbial load. The diversity of the soil microbiota is a very important factor in order to maintain a healthy soil and therefore the development of quality crops. High microbial diversity has a trascendental impact in several beneficial ways, such as (1) the inhibition of pathogens or invader organisms, (2) the high levels of CO_2 trapping, (3) the release of plant growth stimulators, and (4) the improvement in the amount of nutrients in the soil by lytic enzymes production (Li et al. 2017; Sathya et al. 2016; van Elsas et al. 2012; Vishwakarma et al. 2016; Vukicevich et al. 2016). Application of traditional herbicides disrupts the natural nutrients decomposition process, because herbicides also kills the beneficial organisms in the soil, such as earthworms, fungi, and bacteria (Andersen et al. 2013; Vukicevich et al. 2016).

Table 10.6 Business, geographical, and type of products by agrobiological key leaders

Segmentation		BASF SE	Bayer CropScience AG	Isagro SPA	Novozymes A/S
Business		Fungicides	Crop protection/seeds	Fungicides	Crop production
		Herbicides		Stimulants	Microbial solutions
		Insecticides	Environmental science		
		Functional crop care			
Geographical	America	33%	25%	33%	43%
	Europe	35%	30%	37%	38%
	Asia-Pacific	10%	15%	24%	18%
	Rest of the world	23%	29%	6%	–
Products	Biopesticides	Millennium	Serenade	Bio-Tam	Met52 EC
		Nemasys	Sonata	Bioten	
		Vault HP	Requiem	Radix	
		Tricho Plus		Remedier	
		Green Muscle		Tenet	
		Greenguard			
		PL Gold Broadbrand			
		Beta-pro			
		Nogall			
	Biostimulants	BioGain WSP	–	HYT A	Ratchet
				HYT B	RhizoMyco
		BioGain WSP/Sprint 330		HYT C	RhizoMyx
					RhizoPlex
		VigaROOT			Torque

BASF (2017), Bayer CropScience (2017), Isagro SPA (2017), Novozymes A/S (2017), and Technavio (2017)

10.6.4 Current Researches and Future Perspectives

The beneficial effects mentioned in the last section have been supported by several authors, such as Bernard et al. (2014), who reported an increment of the microbial community on a potato field through the combination of three different sustainable disease management practices: (1) compost amendment improving the chemical, biological, and physical characteristics of cultivable soil; (2) biocontrol organisms using *Bacillus subtilis*, *Trichoderma virens*, and *Rhizoctonia solani* Rhs1A1 as biocontrol agents; and (3) disease-suppressive rotation. Crop rotation and composting are a good complements in order to increase the microbial populations. Commonly crop rotation is used to increase the nitrogen-fixing bacteria that have been used for biocontrol of pathogens infecting plants, like *Fusarium* species (Yang et al. 2017). Conversely, the employment of compost in agriculture and horticulture is important because is a natural nutrient as well as energy and carbon source for the microorganism's development. Moroever, the compost also contributes in the recycling of organic waste and minimizing the application of chemical fertilizers Since, compost is a natural source of biological control microbiota, several beneficial microorganisms have been isolated from this material (López-González et al. 2015). In the case of Suárez-Estrella et al. (2013), they reported more than 126 different effective strains against important phytopathogens such as *F. oxysporum*, *Fusarium melonis*, *R. solani*, *P. ultimum*, *Pectobacterium carotovorum* subsp. *carotovorum*, *Pseudomonas syringae* subsp. *syringae*, and *Xanthomonas campestris*. Trichoderma spp. is one of the potential biocontrol agents that proliferate by compost application, the commonly applied efficient species are *Trichoderma harzianum* and *Trichoderma asperellum*. These fungal genera have shown great effectiveness against pathogens of plants such as *Phytophthora nicotianae*, *R. solani*, *Sclerotinia sclerotium*, *B. cinerea*, and *Alternaria alternata,* among others (De la Cruz Quiroz et al. 2015; Ros et al. 2017). Despite the improvement of soil health depending on several factors, the addition or fortification of beneficial microbial populations is the fastest way to obtain better results (Larkin and Tavantzis 2013; Lenc et al. 2015).

The trends are very clear; the market is growing by leaps and bounds including its three sections; biopesticides, biostimulants, and biofertilizers. Despite the presence of four big established companies in agroscience, there are still challenges to attend to, such as the high or sufficient production, the widespread distribution, the maintenance of long shelf life of bioproducts, and the overhead costs. Therefore, it is a great opportunity for start-ups to reach to the places less attended with this type of products, as well as generate technological development in the weak points of the current products, such as the high variability in efficiency of biopesticides, the tracking of adverse effects of fungicides on biopesticides, and a more deep exploration with the aim to offer less limited results from bioassays. In the case of soil amendment, all kinds of biological products have shown improving activities mainly with the increase of diversity in the microbiota; however an integrated pest management is highly recommended integrating practices of preventive cultures, such as monitoring crop fields, mechanical controls, and responsible and low use of chemical pesticides, among others.

10.7 Future Perspectives

Biocontrol is very significant and imperative in the present situation of crop production. This is a compelling alternative to increase soil productivity and reduce the consequences of climate change. However, its wide benefits are not being fully exploited; new scientific research should be proposed to assess the effects of biocontrol on the crop produce where it has not yet been evaluated and the effect of the use of endemic microorganisms in the increase of the fertility of the soil.

Governments must also articulate clear strategies to strengthen the commercialization of bioagents. Since the biocontrol products that are marketed have not been used efficiently by the farmers due to the lack of information on their use. There is also an urgent need to mass produce biological agents, to evaluate their synergistic effect with other alternatives such as compost, to understand the mechanisms of action, to evaluate environmental factors that favor the growth of biocontrol agents, and to evaluate symbiotic microorganisms in environments where they were found.

Countries should generate government alternatives for small farmers to receive a bonus in exchange for contributing to the reduction of greenhouse gases using good production practices. This alternative guarantees stability in the prices of the products and improves the eco-efficiency in the production.

10.8 Conclusion and Final Remarks

Biological control agents contained into microbial biopesticides offer a very wide spectrum of opportunities in order to control or inhibit huge insect pests. Despite most of them are so specific in the action against the pest. They also offer the possibilities to use with different combinations of agricultural products, which increases its efficeincy to controls pests and also enhance the health of the agricultural soils. Also, a number of papers founded about yeasts and nematodes as biopesticides are scarce or limited only to a few species. However taking into account the results published by some authors, they also have a high potential to offer to the agro-industries a very deep strategy to control more efficiently.

The use of biopesticides could enhance the results on soil amendment, if they are integrated into a well-studied strategy, such as the integrated pest management where it is included the monitoring and the application of physical controls. Also, it is important that the main objective is not destroying the whole population of pests but the reduction of the damages caused by them, creating a balance.

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11

Heavy Metals, Polycyclic Aromatic Hydrocarbons (PAHs), Radioactive Materials, Xenobiotic, Pesticides, Hazardous Chemicals and Dyes Bioremediation

Ihsan Flayyih Hasan Al-Jawhari

Abstract

Rapid growth, development and coveted desire to progress at any cost, man has produced various problems in the form of toxic pollutants in the ecosystem. Industrialization and modernization in the last few decades resulted in imbalance of the ecosystem by adding different chemical compounds, such as heavy metals, polycyclic aromatic hydrocarbons (PAHs), radioactive materials, xenobiotic, pesticides, hazardous chemicals and dyes. These compounds badly influenced the biological systems of plants, animals, microorganisms and human being. The high toxicity of some of these polluted compounds also negatively influence the normal function of the body. On the other hand, the presence of toxicants in chemical fertilizers, pesticides and sewage also contaminate the soil and potable water. There are several approaches which can solve the problem to certain extent and these are physical and chemical methods. These methods have some limitations, and not therefore, not that successful, on the other hand are not cost effective and also causes interference with natural water bodies' composition. Moreover, these physico-chemical methods also leads to different toxins and other compounds in the ecosystem. In spite of this, biological methods are now a days available which are eco-friendly and to some extent completely mineralize the organic pollutants. Another positive aspects of the these methods are that they are cost effective and does not generate toxic wastes. These biological approaches employs the potential microorganisms such as bacteria, algae and fungi under aerobic or anaerobic conditions. The use of microbial sources has several advantages mentioned above and have and long term applicability.

I. F. H. Al-Jawhari (✉)
Marshes Research Centre, Thi-qar University, Nasiriyah, Iraq

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11.1 Introduction

The sources of chemical pollutants are generally of two types, natural and industrials, such as originating from human activity. Natural contamination may result from elemental processes such as the emission of poisonous gasses during a volcanic eruption, as well as the microbial activity in rocks, soil and water which produces toxic substances. Compared to the natural contamination, the human contribution to environmental contamination is much higher which disturbed the natural ecosystem (Kvesitadze et al. 2006).

In the different regions from the world, these toxic chemicals are produced periodically or on regular basis. These harmful substances or toxic large amount of secondary products of their incomplete transformations are accumulated in the ecosystem, and affecting the ecological balance. In the plant kingdom, various microorganisms and plants having deep and adventitious root system, alone or in synergistic way can effectively contribute to environmental remediation and finally balancing the ecosystem (Korte et al. 2000).

11.2 Heavy Metals

Heavy metals are the chemical elements having higher density compared to water. Among 35 commonly occurring metals, 23 are the heavy elements or heavy metals, the common heavy metals are – Ag, As, Au, Bi, Cd, Ce, Cr, Co, Cu, Fe, Ga, Hg, Mn, Ni, Pb, Pt, Te, Th, Sn, U, V and Zn (Glanze 1996). The presence and toxicity of heavy metals causes adverse effects on microorganisms and plants, moreover, also affecting the health of animals and humans. The higher toxicity of these elements, if ingested may influence the normal human function of the body parts and may leads to cancer. The occurrence of heavy elements such as arsenic, lead, mercury and cadmium were, if present in excess causes serious ecological problems, therefore these four heavy metals are considered a serious threat to the environment. Let's discuss these metals in detail.

11.3 Arsenic

This metal is highly toxic in any form to the environment and the main sources of environmental contamination by arsenic are emissions from industry. Aquatic and land-living plants and all types of animals exhibit a varied variety of sensitivities to various chemical forms of the arsenic metal. The sensitivity of arsenic is changed both by natural or biological features and also by their chemical and physical environmental adjoining. Generally, the inorganic form of As is more toxic to ecosystem compared to organic or carbonaceous forms. Amongst the inorganic forms, arsenite is more lethal compared to the arsenate form. This difference could be because the mode in which the numerous As forms are taken up by body varies and once it is taken up by the body, metal act in diverse ways in body. The chief reason that why arsenite is more toxic is considered to be since it binds to a specific chemical group, the sulfhydryl group, which is found on the proteins. On the other hand, the arsenate, influences the important energy generating process in all the cells (Sinha et al. 2009).

11.4 Lead

It is a delicate and soft metal, is widely known for many uses over the years has been employed extensively since 5000 BC. This metal has been used in cable, metal products, pipe lines and also in pesticides and paints. Lead is considered as the most damaging metal having adverse effects on human being health. Lead can make entry in human body by food uptake (65%), air (15%) and water (20%). Foodstuffs such as vegetables, fruits, grains, meats, seafood, various drinks and wine might have substantial amounts of Pb. Person smoking cigarette may inhale lead as smoke of cigarette do contains little lead amounts. Through pipes corrosion, lead can enter the potable water and this is more possible to occur when water is somewhat acidic in nature. Owing to this reason, now a days, the public water management systems needs to carry out pH-adjustments of potable water. To the best of our knowledge, Pb does not fulfils any significant function in human body, rather, it can only harm the person after getting from water, food or air (Rusin 1988).

There are several strains of fungi and bacteria which are resistant to lead and their compounds. Therefore, such potential microbes may be employed as bioremediators for lead contamination. Al-Jawhari (2014) showed that lead acetate was utilized by fungal strains such as *Aspergillus niger, Rhizopus stolinifer*, *Trichoderma harzianum* and *Fusarium solani* in solid media. In the same experiment it was also observed that the dry weight of *A. niger* was increased with addition of all concentrations of lead acetate liquid media.

11.5 Mercury

Mercury is found in the soil in the form of cinnabar (HgS), which is a relatively harmless substance. Conversely, the natural processes and manmade activities have led to the buildup of mercury in the soil and oceans. The chief sources of the mercury are fossil fuel-based electric power plants, industrial wastes, pesticides containing mercury, organic compounds synthesis (Goyer 1996). Consumption of methyl mercury is very harmful to animals and human body, large concentration of it may leads to damage in body normal function (Roberts 1999). Three utmost normal forms of mercury are; inorganic, elemental, and methyl mercury, all these can produce adversative health effects if consumed at adequately high doses. The United State Environmental Protection Agency has ascertained that mercury polluted fish eating is the chief reason of exposure to mercury metal for most of the people.

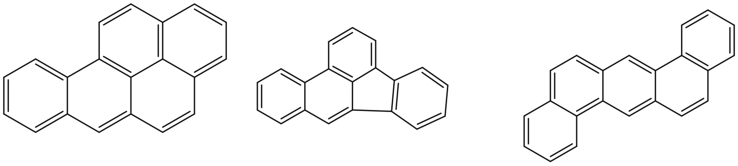
11.6 Cadmium

Cadmium metal is chiefly used in the pigment production, accumulator's production, laboratory chemicals, electric cables, motor radiators, chemical fertilizers etc. Cadmium has been reported to contaminate the water and soil from fertilizer plants and industrial waste (Satarug et al. 2010). Cadmium can easily penetrate into the body of organisms by contact and precipitation from the atmosphere and penetrates into plant leaves. Reports showed that excess of Cadmium can adversely affect the growth of plants and there are many fungal species which can accumulate high amount of cadmium in mycelium (Satarug et al. 2010). Also, there are some fungi which can tolerate and easily grow in high concentrations of cadmium chloride (AI-Jawhari 2000).

11.7 Polycyclic Aromatic Hydrocarbons (PAHs)

Polycyclic aromatic hydrocarbons (PAHs) are carbonaceous compounds which are primarily white, colorless, or pale yellow solids. These are a universal group of scores of chemically correlated compounds, persist in environment with several arrangements and structures with diverse toxicity. PAHs have lethal effects on organs and organisms via various actions. In general, the PAHs make an entry in the ecosystem across several ways and are generally originate as a amalgam containing several complexes, e.g. soot. Some polycyclic aromatic hydrocarbons are manmade produced. The toxicity mechanism is considered to interfere with cellular membranes function along with cellular membrane enzyme systems. As per many reports that polycyclic aromatic hydrocarbons can cause mutagenic and carcinogenic effects and are powerful immune system suppressants (Sinha et al. 2009). Figure 11.1 shows the molecular structure of commonly found polycyclic aromatic hydrocarbons (PAHs). Excess amount of these compounds in the environment may leads to many problems including soil, water and air contamination (Curfs et al. 2003).

Carcinogenicity caused by polycyclic aromatic hydrocarbons in mice has been reported (Curfs et al. 2003). After entry of polycyclic aromatic hydrocarbons into the organism's body, the enzymes form epoxy compounds. These epoxy compounds react with Guanine and block the synthesis of DNA. Therefore, induces impairment of the transcription processes, which leads to mutations, and might stimulate cancer.



3,4-Benzopyrene 3,4-Benzofluoranthene 1,2,5,6-Dibenzanthracene

Fig. 11.1 Structures of various polycyclic aromatic hydrocarbons (PAHs)

There are substantial information in the literature, which indicates the capabilities of microbes and plants alone and in synergism to biodegrade the PAHs, which is later utilized as routine cellular metabolites (Trenk and Sandermann 1978; Trust et al. 1995; Selifonov et al. 1996).

11.8 Radioactive Materials

The nuclear industry is very significant in modern age, since it provides several products and these products are very stable and also used in medicine (imaging and diagnostic purposes). On the other hand, the nuclear energy is also one of the major energy sources for the electricity generation to meet the world's energy needs. Yet, after some horrifying tragedies in Nuclear power plants all over the globe, people have become further conscious and responsive that, when nuclear material not treated prudently, the nuclear power may pose somewhat a noteworthy hazard to our normal way of existence and living. There also have been health and safety issues related to storing of the radioactive (nuclear) waste material. Fortunately, although, in contemporary years the safety issues and precautions of the running and functioning nuclear power plants have become extra and more firm and stringent, therefore, these plants are now unbelievably and really safe. Nevertheless, they still produce huge amount of very perilous and lethal waste material every year, and this material is very difficult to manage and shift. Improper handling of nuclear material and their wastes may leads to environmental contaminated which will in turn leads to harmful effects on human beings, plants and animals (Rao et al. 2009).

There are different approaches used in the treatment and removal of radionuclide from the environment and these methods are bioremediation, biosorption, accumulation and translocation.

11.9 Bioremediation

The bioremediation methods depend on using potential bacteria, fungi and algae under aerobic conditions. These potential microbes can remove toxic pollutants from the environment by excretion of extracellular enzymes and also by producing mild acids (Francis 1994; Prasad 2017, 2018). Removal of contaminants from the aqueous solution includes many means and methods, such as biosorption, accumulation, precipitation, solubilization, reduction and also phytoremediation with the help of plant roots (Gadd 1993; Lovley 1995).

11.9.1 Biosorption and Accumulation

Biosorption approach depends on microbial uptake of nuclear pollutants and its type. The contaminated material may be soluble or insoluble, the microbe absorb it by applying physcio-chemical process. The ability of a microbe to biosorb the aggregates nuclear wastes is performed by several bacteria, fungi and algae. The nuclear wastes was absorbed and transported or biosorption took place on the microbial cell walls (Geesey and Jang 1990). However, chitin is an imperative component of the fungal cell walls, and proved as an effective bio-sorbent for nuclear wastes (Rhodes 2014).

11.9.2 Reduction and Precipitation

Reduction is one of the part of required chemical transformations reactions which is being catalyzed by microorganisms, resulting in the solubilization of the nuclear-waste under anaerobic conditions (Prakash et al. 2013).

11.9.3 Solubilization

Microorganisms, *Thiobacillus ferroxidans* and plants have capability to removal nuclear wastes under aerobic conditions (Francis 1990). Heterotrophic bacteria also produce a sizable amount of chelating agents such as dicarboxylic acids (Lloyd and Macaskie 2000).

11.9.4 Phytoremediation

Phytoremediation is a technology, which involves the plants and this approach should be considered for remediation of polluted sites owing to its economic efficacy. This technology can be applied for the treatment of radio-metal contaminants by phyto-transformation, phyto-stabilization, phyto-extraction and rhizospheric bioremediation (Schnoor 1998).

11.10 Hazardous Chemicals and Dyes Bioremediation

11.10.1 Textile Dyes

At industrial level, there are many chemicals which are used for synthetic dyes synthesis, the composition of these chemical dyes vary from inorganic to organic, depending upon the chemical substrates used. There are more than 100,000 synthetic dyes all over the globe, commercially available and periodically produced. The waste water discharged from dye industries dyes badly affect and cause severe damage to the environment. The color of dyes should be removed from the dye wastewater before discharging to the terrestrial and aquatic ecosystem. There are different industries which are involved in discharging colored and toxic waste waters having unused dyes directly into the aquatic environment leading to severe damage to the ecosystem (Zollinger 1987). These colored dyes effluents discharge owing to toxicity also causes detrimental effect on the life of diverse flora and fauna in the ecosystem (Dong et al. 2011). Treatment of dye industry wastewater is very essential before being discharged into the ecosystem, since the untreated wastewater of dye industries is toxic (Hazrat 2010). There are various approaches to treat the dye waste water such as commonly applied physical and chemical methods. The use of these methods are limited and not also successful, since the treated water interfere with the flora and fauna of water bodies and also this method is not cost effective (Banat et al. 1996). However, the resultant products of physical and chemical methods also adds various toxins and other chemical compounds to the aquatic bodies (Du et al. 2011). To overcome the problem of physical and chemical methods, biological methods are available, which uses the microbial sources and also are eco-friendly, and entirely mineralize the toxic contaminants (Pandey et al. 2007).

These biological approaches are cost effective and in expensive and also environment friendly (Forgacs et al. 2004). Some dyes such as Malachite green (MG), it is a water soluble triphenyl methane, it is used to color various fabrics (Zho et al. 2015). This dye is also used in medical and food industries (Chowdhury et al. 2011). There are reports that, this dye causes lethal effects on terrestrial and aquatic animals and also causes damaging effects on mammalian liver (Srivastava et al. 2004). On the contrary, the Congo red dye ($C_{32}H_{22}N_6Na_2O_6S_2$), is highly miscible in water and persist for long time, once it is released into the native ecosystem (Tapalad et al. 2008; Jalandoni-Buan et al. 2009; Tang et al. 2011). The application of potential fungal strains in dye bioremediation is a assuring technology, since the higher biomass of fungi greatly help in absorption and remediation (Fu and Viraraghavan 2001; Dos Santos et al. 2004). Many potential fungi are capable to bioremediate and decolorize various chemical dyes by extracellular enzymes excretion, one of the common and potent excreted enzyme is laccase (Dos Santos et al. 2004). Studies carried out on non-basidiomycetes fungi that biodegrade dyes showed that these fungi are also very effective and competent for removal of a wide range of chemical compounds (Cha et al. 2001). *Aspergillus* species (EI-Rahim and Moawad 2003; Jin et al. 2007) *Cunninghamella elegans* (Ambrosio and Campos-Takaki 2004); *Fusarium solani* and *Penicillium funigulosum* (Al-Jawhari 2015a) are some examples showing the bioremediation of synthetic dyes. AI-Jawhari (2015b) exhibited that fungal strains *A. niger*, *A. fumigatus*, *Rhizopus stolinifer*, *Fusarium solani* and *P. funigulosum* are capable to remediate and decolorized Methylene blue and Crystal violet, but *Fusarium solani* and *Penicillium funigulosum* showed more decolorizing activity.

11.11 Xenobiotic Compounds

Manmade chemical compounds which are recalcitrant in nature at high concentrations polluting the environment is recognized as Xenobiotic compounds. These chemical compounds are consisted of dissimilar organic substances such as pesticides and petrochemical products. There are some fungal, bacterial and algal strains which have the capability of breaking down the xenobiotics to other less toxic compounds. There are several xenobiotic compounds which are non-degradable, this is because of their recalcitrant nature. However, these recalcitrant xenobiotic compounds are causing severe damage to the environment (Singh 2017).

11.12 Halocarbons

These chemical compounds are composed of halogen group in their structure. These compounds are chiefly employed in pesticides, medicines and solvents. The compounds are found in insecticides, organochlorine compounds, pesticides, organobromine compounds etc. These halocarbons are also widely used in refrigeration system. The mishandling of halocarbons leads their leaching into terrestrial and aerial ecosystem where they accumulate and result in biomagnification as well as greenhouse effect.

11.13 Bioremediation of Xenobiotic Compounds

Xenobiotic compounds, owing to their recalcitrant nature and chemical complexity, is hard to degrade or break down into simpler form. Some microbial enzymes such as oxygenase, peroxidase and laccase play significant role in break down of these compounds into the intermediate substances. These intermediates produced were used as carbon source and energy for the microorganisms.

11.13.1 Bioremediation

Some microbes on their constant and unceasing exposure to xenobiotic compounds develop the capability to degrade these components as a result of adaptations in that particular environment or mutations. Mutations in microbes, sometimes resulted in gene modification so that the modified enzymes or active site of enzymes is modified to show enhanced affinity to xenobiotic compounds. Some mutations also leads to development of novel enzymatic pathways for xenobiotic compound bioremediation.

11.13.2 Pesticides

The pesticides are a group of chemicals which are used for prevention and control of various pests such as bacteria, viruses, fungi, insect, nematodes, weeds and unwanted flora and fauna. Depending on the classes of pests they are broadly classified as:

Fungicides	These chemical kills fungi, main classes include dithiocarbamates, copper, mercurials, etc.
Herbicides	Kill weeds and other un wanted plants. Classes include carbamates, triazines, phenylureas, phenoxy acetic acid, etc.
Insecticides	Exterminate diverse types of insects under dissimilar conditions
Nematocides	These chemicals eradicate nematodes in soil and plants
Rodenticides	These chemicals exterminate rodents like mice
Algicides	These chemicals prevent the growth of algae infestations or kills the algae in various water bodies
Biocides	Kill various types of microbes, such as bacteria, fungi etc.
Acaricides or Miticides	Kill various types of mites and ticks

Sinha et al. (2009) and Varsha et al. (2011)

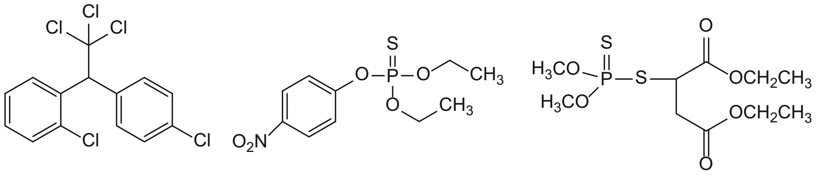
Commonly used pesticides are highly soluble in water. However, they attached very strongly to soil. Many process are used to biodegrade pesticides and to diminish their poisoning effect to the nature. Sometimes in the environment, due to the microbial activity, some non-toxic chemical compounds are converted or degraded into poisonous components. The main process are employed for biodegradation of pesticides are as:

- Photodegradation: This process depends on the breakdown of pesticides by the action of sun light.
- Bioremediation: This process depends on the ability of bacteria, fungi and algae to break down pesticides.

11.13.3 Insecticides

These compounds are used in agriculture and disease control. These compounds are classified to:

(a) Organochlorine: -These chemical components are having organic hydrogen, carbon and chlorine.
(b) Diphenyl aliphatics components: Like DDT (dichlorodiphenyltrichloro ethane) and hexachlorocyclohexane.
(c) Cyclodienes compounds: -such as Heptachlor, Aldrin and Dieldrin, are employed to control termites in the soil.
(d) Organophosphate: -All these insecticides contains phosphorus as one of the component. These compounds are most poisonous to vertebrates, however, they are unstable or non-persistent such as Malathion, Diazinon and Ethyl Parathion (Fig. 11.2).
(e) Organosulphurs
(f) Carbamates
(g) Formamidines
(h) Dinitrophenol
(i) Organotins and several others.



DDT Ethyl parathion Malathion

Fig. 11.2 Structures of various insecticides

11.13.3.1 DDT

These chemical compounds comes under the classes of compounds popularly called diphenyl aliphatics. Many microorganisms (bacteria and fungi) can biodegrade DDT, DDD (dichloro diphenyl dichloro ethane), and DDE (dichloro diphenyl ethane). Phyto-remediation approaches have been employed to handle and manage chlorinated aromatic compounds, such as DDT and atrazine when they contaminate the natural ecosystem (terrestrial and aquatic). However, the DDT chemical might not be straight forward damaging to human beings, but the main damage is caused when this compound is accumulate in organisms such as crustaceans, fish and birds etc., this phenomenon known as biomagnification (Fig. 11.3).

11.13.3.2 Carbamates

Carbamates are the chemicals which are used to control various insects. They are no longer persistent in the environment as the organochlorines, such as propoxur, carbaryl and carbofuran, and (Fig. 11.4).

11.13.3.3 Carbaryl

This compound can used and removed by some species of bacteria such as *Micrococcus,* which uses the carbaryl compound as principal carbon and energy source. Other microorganisms also consume naphthalene, carbofuran, and numerous other aromatic chemical compounds as growth substances (Magan et al. 2010).

11.13.3.4 Aldicarb

This chemical compound is highly soluble in aquatic ecosystem such as groundwater. Some microorganisms can remove this compound from the contaminated environment (Singh and Walker 2006).

11.13.3.5 Organophosphates

The organophosphate compounds include all major insecticides, which contains phosphorus as major component. These pesticides were first time manufactured in Germany in the form of TEPP (Tetraethyl pyrophosphate) during the period of World War II. The TEPP was the byproduct of nerve gas, which was extensively produced to overcome enemies. These compounds are most chemical poisoning

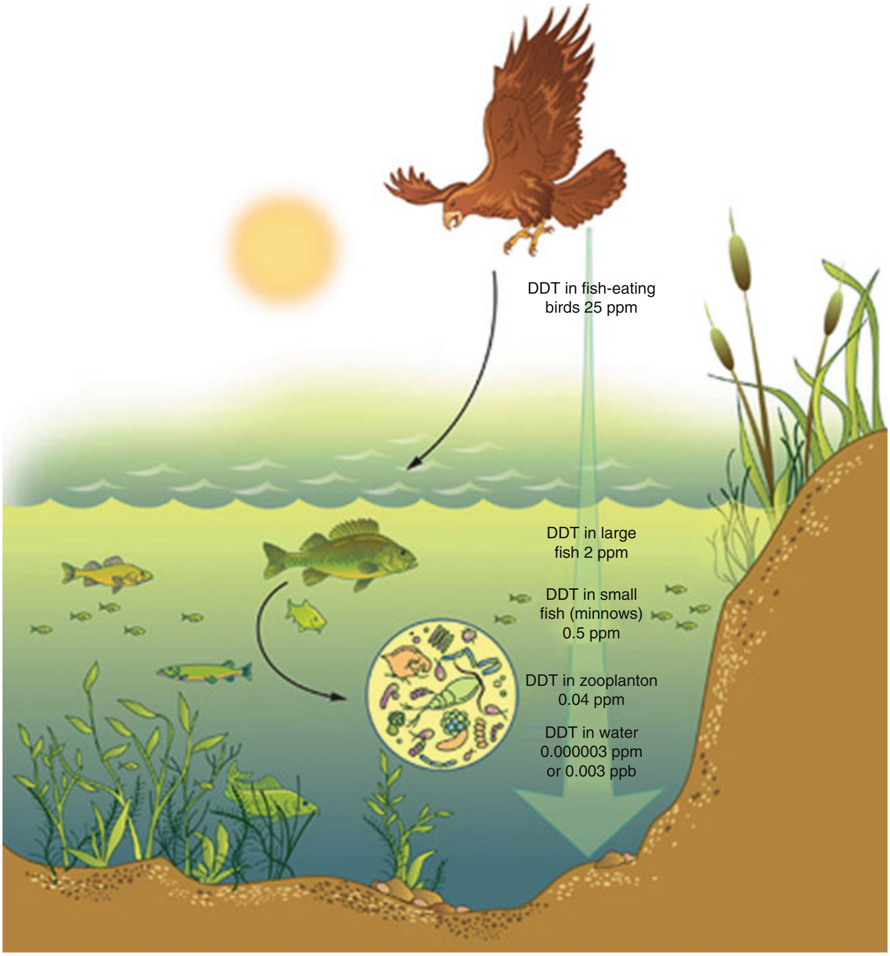


Fig. 11.3 Bio magnification in nature

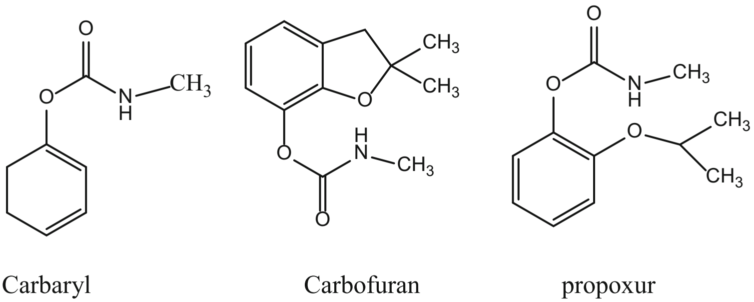


Fig. 11.4 Structures of various carbamates

agents of all known pesticides related to vertebrates, however, the plus point with components is that they are unstable or less-persistent. The common organophosphates are malathion, ethyl parathion and diazinon. The enzyme acethylcholine esterase has capability to biodegrade pesticides like diazinon, malathion and methylparathion effectively. In the course of hydrolysis process, the aromatic ring of the pesticide is being used as source of carbon by microorganisms. However, AI-Jawhari (2001) exhibited that the fungal strains such as *Aspergillus niger*, *Rhizopus stolinifer* and *Trichoderma harzianum* can degrade and converted Diazinon to other compounds and use this pesticide as an energy and carbon source, but *Rhizoctonia solani* was not able convert this pesticide to other compounds. Also in the same time AI-Jawhari (2015b) demonstrated that bacterium *Pseudomonas aeruginosa* was able to convert the Diazinon to other compounds in laboratory conditions and the bacterium used this insecticide as energy and carbon source.

11.13.4 Fungicides

Fungicide are chemical compounds that used to kill fungi. These compounds are mainly categorized into two main types:-

(a) The Preventive fungicides:

These are the chemical constituents that stop or preclude the occurring of fungal infections in a plant or plant part. These chemicals include the main components such as benzimides, sulfur, dichloro-carbamates, pthalimides, and organometallics.

(b) The Curative fungicides:

These are the chemical substances (Tebuconazole, Cyproconazole etc.) that move from one place to another place where the infection has been caused and action of fungicide prevent the additional progress of the fungal pathogen. These chemicals include the components actinides, sterol inhibitors, dicarboxymides and many others. Some bacteria and fungi have ability to break down these compounds such as pentachlorophenol (PCP).

11.13.5 Herbicide

These chemical compounds are used to kill unwanted plants or weeds. These chemicals have higher level of toxicity and noxiousness, moreover also have longer half-life. *Pseudomonas* sp. was able to entirely biodegrade the herbicide Mecoprop (Phenoxyalkyl carbonic acid), but was not able to biodegrade herbicides such as Isoproturon (Phenylurea), Terbuthylazine, and Metamitron (Triazine herbicide) (Topp 2001).

In an interesting experiment conducted by AI-Jawhari (1998) demonstrated that the fungal strains such as *Aspergillus niger*, *Rhizopus stolinifer*, *Trichoderma harzianum*, *Penicillium* sp., bacterial strains such as *Pseudomonas aeruginosa*, *Escherchia coli*, *Klebsiella pneumonia*, and aquatic fungi *Saprolegnia* sp., *Achyla prolifera* were able to biodegrade the potential herbicide propanil to 3,4-Dichloroaniline and 3,3,4,4- tetrachloroazobenzene in rice field in Iraq.

11.14 Conclusions

The distributions, managing, incorporation, application of contaminated residues to land and contaminated byproducts constituents into the ecosystem can lead to harm the human beings, animals, wild flora and fauna, cultivated crops, or innate plants. Contaminants of insecticides, carbon based compounds, and heavy metals might mount up in flora, soils, water and sediments. By using the process of composting, substantial mitigation of perilous and harmful wastes or polluted soil, plants, and deposits may be achieved. Raw substrates which can be composted (feedstocks) might inhabit metabolizable carbonaceous products, which will augment microbe's activity and diversity during the process of composting. This will also encourage biodegradation of recalcitrant organic substances, for instance various types of pesticides, polyaromatic hydrocarbons, and polychlorinated biphenyles.

As a result, the biodegradation of recalcitrant organic compounds during the process of composting is quicker compared to untreated soils on the location. The heavy metallic contaminants are not degraded owing to their inorganic nature, but during the process of composting they might be transformed or changed into less bioavailable organic components. The biodegradation of carbonaceous pollutants in soils and sediments is assisted and expedited by adding the composted or crude organic substance, thus enhancing the substrate levels for co-metabolism of pollutants. Difficult to biodegrade compounds, for example; organo-chlorines, might not undertake the process of biodegradation in soils, sediments or composts. Therefore, the outcomes of organic complexes formation with heavy metals contaminants might be short lived or temporary. The overall inference or conclusion is, that composting process with the help of potential microbe's biodegrades or attaches contaminants to inoffensive stages or into harmless components and has considerable and ample possibilities for bioremediation of contaminated substances.

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Rhizospheric Microbe-Plant Exudate Formulation for Enhanced Restoration of Contaminated Agricultural Soil

12

Maryam L. Riskuwa-Shehu and Udeme Josiah Joshua Ijah

Abstract

Industrial and human activities add a lot of chemicals to the soil environment. In the oil-producing areas like Nigeria, hydrocarbon contamination has been the major problem. The hydrocarbon contaminants upset the soil ecological balance, including important microbial processes. In the rhizosphere, microbial interactions occur in a dynamic manner, resulting in the production of microbial products of ecological importance. The plant associates exude compounds that benefit the microorganisms. The overall interactions of the plant-microbe products contribute greatly to the reclamation of the contaminated soil. Thus, current emphasis should be placed on the formulation of products that can be effective in soil bioremediation. The rhizospheric microbes and plant exudates should be extensively studied. Microbe-plant exudate formulations have comparative advantages over chemicals applied for reclamation of contaminated soil. Soil formulations are ecologically friendly and cost-effective. This chapter deals with microbial activities and plant exudation in the rhizosphere which help contaminated soil to recover. The prospects of microbe-plant exudate formulation are highlighted.

M. L. Riskuwa-Shehu (✉)
Department of Microbiology, Faculty of Science, Usmanu Danfodiyo University Sokoto, Sokoto, Sokoto State, Nigeria

U. J. J. Ijah
Department of Microbiology, Faculty of Science, Federal University of Technology Minna, Minna, Niger State, Nigeria

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12.1 Introduction

Overdependence on fossil fuels have led to widespread problems (especially in oil-producing areas) associated with their use, and this has attracted public awareness to different investigators. Exploration activities pose threat which tends to be severe to the environment principally agricultural farmlands because of the persistent nature of the chemicals in food chain. Committee on Oil in the Sea, USA, estimated that 600,000 tonnes (±200,000) of crude oil have been lost per year worldwide (Rohrbacher and St-Arnaud 2016). Similarly, Haferburg and Kothe (2010) reported that about 10,000–600,000 mt (ib.) of metals (As, Cd, Cu, Ni, Pb, and Zn) resulting from ore processing are released as tailings. The main sources of these anthropogenic substances (including halogenated aromatic compounds, PAHs, BTEX, and pesticides) are oil refining, gas stations, agrochemicals, waste dump sites, and other chemical industries (Mrozik and Piotrowska-Seget 2010; Rohrbacher and St-Arnaud 2016).

The harmful effects of oil spill on the environment are many, the most devastating of which include their properties of being toxic, carcinogenic, and mutagenic (Ling et al. 2015). Assessment of the effect of PAH contamination of agricultural soil by various researchers (Merkl et al. 2004; Smith et al. 2006; Somtrakoon et al. 2014) indicated that PAHs adversely affect plant growth through reduction of seed germination, crop yield, biomass accumulation, inhibition of photosynthesis, and disruption of mineral transport by plant root. Health hazards associated with metal contamination of soil include but are not limited to soil erosion, land degradation, and acid mine drainage leading to continuous toxicity to plants and animals (Haferburg and Kothe 2010). In addition, the continuous application of pesticides on agricultural farmlands as pest control has its own negative consequences especially on the health of humans and animals as well as biodiversity (Gavrilescu 2005; Hussain et al. 2009). Therefore, the wide application rate and duration of these xenobiotic chemicals in soil either directly or indirectly leads to increasing demand for removal of these contaminants from the environment.

In most cases, remediation techniques (such as landfilling, pyrolysis, recycling, and incineration) that have been traditionally applied for environmental cleanup are expensive and have also been indicated (Debarati et al. 2005; Porto et al. 2011) to adversely affect the environment, by forming toxic intermediates. Moreover, execution of such methods may sometimes be difficult, especially in extensive agricultural areas with difficult terrain (Porto et al. 2011). A promising remediation strategy that is cost-effective and ecologically friendly and that has received recent attention is the exploitation of biological techniques of remediation also known as bioremediation. Several bioremediation techniques have been employed to restore contaminated soils, and one of such technologies is phytoremediation. Phytoremediation has been described by Cunningham et al. (1996) as the use of plants and their associated microorganisms to degrade, contain, or render contaminants harmless in soil.

Phytoremediation is applied for decontamination of soil, water, and sediments of petroleum hydrocarbons, metals, radionuclides, and pesticides successfully (Pilon-Smith 2005). Merkl et al. (2006) also referred to phytoremediation as plant-assisted bioremediation since it is presumed to be facilitated by the activity of microorganisms in the rhizosphere. Plants selectively modify their rhizosphere microbiome by releasing substances through their roots referred to as root exudates into their surrounding which influence the type and composition of microbial species (Haichar et al. 2008; Berg 2009). Plant root exudates therefore serve as the connecting link in the plants-microorganism-pollutant system, playing significant role in altering or regulating the soil environment (Merkl et al. 2006; Muratova et al. 2009). Studies demonstrating the effect of root exudates on phytoremediation of PAH-contaminated soils by Joner et al. (2004) showed that phytoremediation may be enhanced by stimulation of PAH degrading microbes in the rhizosphere. Therefore, most studies have focused their attention on the root-associated microbial community which has been perceived to strongly influence the degradation of contaminants and plant health in the rhizosphere.

Rhizospheric microbes have been documented (Ibrahim et al. 2009; Díaz-Ramírez et al. 2010; Shukla et al. 2013; Ibrahim and Ijah 2014) to degrade pollutants, and this ability will have to be sustained by providing nutrients in the form of root exudates to the microorganisms, deprivation of which leads to degradation process being stopped. In the assessment of the degradation ability by microorganisms in the rhizosphere, Rajaei et al. (2013) have shown that beneficial root effects and long-term petroleum contamination can develop a very specific highly potent microbial community capable of detoxifying pollutants. As a result, the rhizosphere was found to compose up to 100 times more microorganism biomass but poorer in diversity than bulk soil (Lynch and Whipps 1990; Martin et al. 2014; Rohbacher and St-Arnaud 2016) and predominantly associated with gram-negative bacteria (Curl and Truelove 1986). Though Jussila et al. (2007) found that in oil-contaminated rhizosphere, Gram-positive bacteria dominated, but the best m-toluate degraders, however, belonged to the genus *Pseudomonas*. Consequently, these activities have provided avenue for harnessing the rhizo-microbiome interaction toward a better beneficial approach to management of environmental contaminants.

In the rhizosphere, some microorganisms confer several advantages to plant health and development by directly or indirectly enhancing growth and health through several mechanisms (Richardson et al. 2009; Vacheron et al. 2013). Previous works have indicated the biodegradation capability of rhizosphere microorganisms including their conservation within the rhizosphere microenvironment to be essential for efficient removal of contaminants in rhizoremediation (Shukla et al. 2013). In most cases, known degrading microorganisms previously isolated from similar contaminant spilled environments or engineered in the laboratory are used as inoculants for remediation of contaminated soil. However, some studies are of the opinion that cometabolism may be a better option for hydrocarbon remediation. For example, Mrozik and Piotrowska-Seget (2010) and Vacheron et al. (2013) have shown that it is more advantageous to utilize a consortium of hydrocarbon-degrading bacteria than each individual strain in hydrocarbon rhizoremediation. This fact has been debated by other investigators (Britton 1984; Al-Wasify and Hamid 2014) that while not all organisms have complete degradation enzymes to utilize a mixture of compounds such as crude oil, others are of the opinion that intermediate metabolic products of one strain can be utilized by another with the right catabolic pathway for that substrate. Besides, microorganisms have been reported to live as a community interacting with each other rather than individually in the rhizosphere. In a study on bioremediation of oil sludge using crude biosurfactants, Cameotra and Singh (2008) demonstrated that the use of microbial consortium which consisted of two isolates of *Pseudomonas aeruginosa* and one isolate of *Rhodococcus erythropolis* from soil contaminated with oily sludge indicated that the consortium degraded 90% of hydrocarbons in 6 weeks in liquid culture.

Root exudation has largely been identified as the driving force for microbial interaction in the rhizosphere by recruiting PGPR, arbuscular mycorrhizal fungi, or other functional groups of microorganisms such as nitrogen-fixing bacteria and phosphate-solubilizing bacteria. It may be suggested that formulations of root exudate and beneficial microorganisms from the rhizosphere may be an effective means of restoring agricultural soils contaminated with different xenobiotics. Previously, Ghiglione et al. (2012) hypothesized that application of a combination of live microorganisms with substances that are organic in nature in specific proportions to plants could lead to synergistic interaction. Therefore, this review discusses the recent applications of PGPR together with root exudates in the rhizoremediation of various contaminants in agricultural ecosystems.

12.2 Rhizosphere

The rhizosphere was described by Prof. Lorenz Hiltner (1862–1923) in 1904 as the region of soil close to the roots that provide nutrients or initiate diseases in plants (Tiedje Lab-Projects 2006; Hartmann et al. 2008). It is the portion of soil where microorganisms interact with plant roots at the root soil interface (Prasad et al. 2015). The rhizosphere has been classified into two as the endo-rhizosphere which include the root cortex and epidermal and root hairs and the ecto-rhizosphere which involves root-associated soil compartments up to a distance of 5 mm (Tiedje Lab-Projects 2006). Observation of the rhizosphere revealed two types of interactions: (1) those involving dead plant materials (organic matter) and identified to influence the flow of energy by providing nutrients and (2) those found to be associated with living plant roots (Barea et al. 2005). Similarly, Kennedy (1998) reported that there are three individual components that make up the rhizosphere which interact with each other, these include the rhizosphere (soil), the rhizoplane (root surface), and the actual root.

According to Yang and Crowley (1999) colonization of the plant rhizosphere by microorganisms is an important process that may be influenced by several parameters some of which include structure and diversity of the microbial communities that inhabit the roots. Grayston et al. (1998) assessed the selective influence of plant species on microbial diversity in the rhizosphere and observed that diversity depended on the plant species, soil type (Campbell et al. 1997), and cultural practices, such as crop rotation or tillage (Lupwayi et al. 1998). Despite these, however, it has been reported by Giri et al. (2005) that different species of microorganism can initiate significant ecological role within the community.

The microbial population in the rhizosphere has been indicated to be higher than those in the bulk soil as a result of the rhizosphere content of nutrients secreted by the plants (Siciliano and Germida 1998). This stimulation of microorganisms in root-soil compartment is termed the "rhizosphere effect" (Atlas and Bartha 1998; Whipps 2001) and has been demonstrated to be responsible for enhanced phytoremediation of organic pollutants in the rhizosphere. Gunther et al. (1996) while studying the effects of ryegrass on biodegradation of hydrocarbons in soil, observed higher population and activities of microorganisms which also correlated with enhanced degradation in contaminated soil planted to ryegrass in comparison to unplanted soil. Similarly, Kirk et al. (2004) observed changes in microbial population in rhizosphere soil of perennial ryegrass and/or alfalfa compared to the non-rhizosphere and concluded that changes were plant specific thus suggesting that different mechanism may be employed by both plants and could lead to increased degradation of contaminants in the rhizosphere of the plants.

12.3 Rhizospheric Interactions in the Rhizosphere

Rhizosphere environment serve as an important niche where significant interactions take place between the different components of the ecosystem. Plant's root, which forms part of the niche, provides support mechanically to microorganisms by adhering to these structures. In the course of this, however, a myriad of interactions between microorganisms and plants are formed (Doornbos et al. 2012). These interactions have been speculated to be responsible for enhancing growth and development of plants or on the other hand antagonistic against their host plant (Hartmann et al. 2008). Examples of the former are the class termed the plant growth-promoting rhizobacteria (PGPR) and the latter being fungal and bacterial antagonists of root pathogens.

12.3.1 The Mutualistic Symbionts

Symbiotic N_2 fixation is one of the mutual cooperations occurring in the rhizosphere, and it is exclusively driven by bacteria rhizobia. It is the only organism capable of synthesizing nitrogenase enzyme that reduces atmospheric nitrogen within the root nodules to compounds that can be assimilated by plants such as ammonia (Leigh 2002; Shrivastava et al. 2014). Vance (2001) opined that a significant process involved in the cycling of nitrogen is atmospheric nitrogen fixation which plays important role in nitrogen acquisition for plant development (Vance 2001). Rhizobia, to which *Rhizobium, Sinorhizobium, Bradyrhizobium, Mesorhizobium*, and A*zorhizobium* belong, has been identified as the main organism responsible for fixing atmospheric nitrogen. These organisms form infectious nodules on legume roots that activate the formation of N_2 fixation (Sprent 2002). Besides nitrogen fixation, *Azospirillum* are reported to enhance plant growth by releasing several plant hormones including auxins, cytokinins, and gibberellins which aid in promoting plant development (Doornbos et al. 2012). Of these hormones, auxins are the most abundant compounds produced in large quantity by *Azospirillum* and have also been implicated in improving the development of the entire plant by facilitating root growth (Steenhoudt and Vanderleyden 2000). In addition to rhizobia, *Frankia* which belongs to the actinomycetes also plays a significant role of ecological importance by their involvement in the formation of infectious root hairs in *Frankia*-actinomycete root interactions (Vessey et al. 2004).

Rhizosphere microorganisms also exhibit other significant roles important to their host plants by helping the plants to induce heavy metal resistance and other abiotic stress (Yang et al. 2009). For example, the secretion of 1-aminocyclopropane-1-carboxylate (ACC) deaminase enzyme by PGPR acts as stress signals (Glick et al. 2007). Several investigations (Lavania et al. 2006; Shaw et al. 2006; Dardanelli et al. 2010; Chamam et al. 2013) have shown the effect of inoculating PGPR on metabolomics of plants, by assessing the products of metabolism in root exudates and tissues of plants under normal or conditions of stress (Vacheron et al. 2013). For example, a study by Shaw et al. (2006) have shown that PGPR are capable of altering the activities of root enzymes involved in the production of metabolites, specifically flavonoids, which might also lead to changes in root exudation pattern. These beneficial activities have also been indicated to be advantageous to degradation of xenobiotic compounds in contaminated soils, there by having the potential for improving phytoremediation (David and Sharon 2009; Shukla et al. 2013). Indeed several important functions of flavonoids in the plant rhizosphere have been identified including (Rohrbacher and St-Arnaud 2016) being a chemoattractant of rhizobia that enables its movement to the root surface (Neal et al. 2012); inducing the expression of *nod* genes, enzymes involved in the synthesis of Nod factors or lipochitin oligosaccharides (LCOs) (Mandal et al. 2010); and initiation of nodulation during fixation of atmospheric nitrogen for plants (Abdel-Lateif et al. 2012).

Besides bacteria, fungi have also been implicated in forming mutual associations with plants. Fungi establishes a mycorrhizal relationship by colonizing the root cortex of vascular plants and then forms an external mycelium which is a bridge connecting the root with the surrounding soil microhabitats (Barea et al. 2005). Mycorrhizal symbioses aid in improving plant health and soil quality through several identified processes as reported by Brundrett and Abbott (2002).

12.3.2 Synergistic Interactions

The development of relationship between PSB and arbuscular mycorrhiza (AM) leads to establishment of a synergistic interaction that leads to improvement of phosphate utilization by plants (Barea et al. 2005). The AM fungi contribute to phosphorus (P) capture and supply, through the external mycelium formed that links the roots with the surrounding soil ecosystem, hence influencing geochemical cycling rates and patterns of phosphorous on both agricultural farmlands and Preston soils (Whitelaw 2000; Jeffries and Barea 2001).

Phosphate-solubilizing bacteria (PSB) have been identified, and their functional role in the soil-plant system has been documented by several investigators (Rodríguez et al. 1999; Sharma et al. 2013; Gupta et al. 2016). Rodríguez et al. (1999) have shown the effectiveness of *Aspergillus niger* and *Glomus deserticola* as inoculants for phosphate solubilization, as highest increase on alfalfa growth was observed when cultivated in soil amended with rock phosphate and red beet residues. Similarly, experiments conducted by Saxena et al. (2015) revealed that consortium of *Bacillus* sp. RM2 and *Aspergillus niger* S36 with AMF supplemented with tricalcium phosphate enhanced the growth of chickpea. Additionally, Wahid et al. (2016) have shown that the use of a combination of arbuscular mycorrhizal fungi and phosphate-solubilizing bacterial strains with rock phosphate had significant ($p \leq 0.05$) influence on the vegetative growth of maize in comparison with other singular or non-inoculated treatments.

12.3.3 Microbial Antagonism

The importance of plant defenses has been reported to influence the number of bacteria in the rhizosphere through the recruitment of various species of beneficial bacteria or preventing pathogenic microorganisms inhabiting the soil from proliferation (Doornbos et al. 2012). Diverse soils have been indicated (Whipps 1997) to be inherently suppressive to some soilborne plant pathogens of fungal origin which include *Fusarium*, *Gaeumannomyces*, *Rhizoctonia*, *Pythium*, and *Phytophthora.* The researcher also attributed the suppression to abiotic components of the environment as well as microbiological properties of the soil. Several mechanisms of antagonism have been reported by researchers (Choudhary and Johri 2009; Neal et al. 2012; Rohrbacher and St-Arnaud 2016) which include secretion of root exudates, production of antibiotics, release of exoenzymes, production of volatiles and other compounds, competitiveness for iron and colonization sites by production of siderophores for the former, and, lastly, nutrients obtained from plant seeds and roots (Whipps 1997; Wen et al. 2007; De-La-Pena et al. 2008; Rohrbacher and St-Arnaud 2016). De-La-Pena et al. (2008) studied the interaction between plant roots and microbes from protein secreted by the microorganisms and found that the interaction between *A. thaliana* and *Pseudomonas syringae* DC3000 strongly influenced the production of several proteins of plant origin that are responsible for defense included are peroxidases, glycosyl hydrolase family 17, and chitinase and glycosyl hydrolase family 18.

The groups of microorganisms with antagonistic properties toward plant pathogens are diverse, including plant-associated prokaryotes and eukaryotes (Barea et al. 2005). For more comprehensive mechanisms of microbial antagonism and organisms involved in antagonistic interactions, the reader is directed to the reviews of Whipps (1997, 2001). Also Barea et al. (2005) reported that *Pseudomonas* spp., including *P. aeruginosa* and *P. fluorescens*, are the most efficient bacteria used as biocontrol agents and perhaps play the most significant role in the colonization of plant root. More so, other class of microbes (the eukaryotes) involved in antagonistic behavior is the fungi. These group of organisms have also gained application in biocontrol; however, the fungus well known for its involvement in biocontrol is *Gaeumannomyces graminis* var. *tritici* (Ggt), the causative agent of take-all disease of wheat (Kwak et al. 2009) as well as the ubiquitous *Trichoderma* species (Barea et al. 2005).

Experiments conducted by Thomashow and Weller (1988) revealed that phenazine antibiotic production by florescent *Pseudomonas* spp. correlated with its biocontrol activity against take-all disease of wheat. Similarly, studies by Cronin et al. (1997) have indicated that production of the antibiotic 2,4-diacetylphloroglucinol (DAPG) by *Pseudomonas fluorescens* (Trevisan) Migula F113 was capable of controlling *Erwinia carotovora* pigment. Further, during the experiment, Powell et al. (2000) showed that phenanzine-1-carboxylic acid (PCA) from *Pseudomonas aureofaciens* Kluyver TX-1 can be inoculated directly as a field treatment for the control of dollar spot on creeping bentgrass.

12.4 Plant Exudate in the Rhizosphere

A number of studies have reported the capacity of plants to modify their rhizosphere microniche due to the selective influence of root exudates secreted by plants on the microbial community (Wu et al. 2015). Plant roots release exudates to their surrounding because of the selective effect it exerts on its rhizosphere colonizers, such as alteration of the chemical components of the soil in the region of the plant roots and in addition serving as source of growth substrate for soil microorganisms (Yang and Crowley 1999; Bais et al. 2006; Doornbos et al. 2012). It has been shown (Badri and Vivanco 2009, 2013) that root exudates are classified as low molecular weight compounds which consists of acids in the form of organic, amino and fatty acids, carbohydrates, vitamins, nucleotides, and phenolic compounds as well as other by-products of metabolism and high molecular weight compounds which include polysaccharides and proteins.

Several mechanisms of root exudation by plants have long been recognized and regarded as either a passive (diffusion, ion channels and transport vesicles) or an active (secretion) process (Doornbos et al. 2012; Huang et al. 2014). The type of exudates released by a particular plant root determines the variety and structure of the associated rhizosphere organisms which in turn impact significantly in the formation of the rhizosphere microenvironment (Bertin et al. 2003; Doornbos et al. 2012). For example, experiments conducted by Teplitski et al. (2000) revealed that root secretions from pea (*Pisum sativum*) contained bioactive compounds that mimic N-acyl homoserine lactones (AHLs) which are communications that stimulate AHL regulated behaviors in certain strains of bacteria and inhibits in others. Badri et al. (2013) demonstrated that the presence of phenolic compounds in a root exudate correlated positively with higher population of operational taxonomic units (OTUs) not commonly found in comparison to other phytochemicals present in the exudates, including carbohydrates, alcohols, and amino acids. Similarly, changes in the population and diversity of rhizospheric microorganisms were observed following exogenous supply of p-coumaric acid and vanillic components of cucumber root exudates (Zhou and Wu 2012, 2013). In addition, the secretion of root exudates, in the form of sugars and amino acids, was shown to be chemoattractive to PGPR (Huang et al. 2014), and the composition varied accordingly. Weston and Mathesius (2013, 2014) provided insight into the mechanism of production, transport, and specific roles of two phenolic compounds (flavonoids and long-chain hydroquinones or phenolic lipids) released into the rhizosphere through passive exudation by *Sorghum* spp. plant.

Microorganisms on the other hand determine the constituents and properties of various root exudates through their influence on root cell efflux, cell viability, and plant nourishment (Yang and Crowley 1999; Bowen and Rovira 1999; Barea 2000). For instance, Qu et al. (2008) detected two phenolic acids (phenol 2,4-di-tert-butylphenol and vanillic acid) in soybean root exudates and found that when these acids were added to soil, they significantly influenced the microbial associates as well as ameliorated other problems of soybean monoculture. Similarly, Zhou et al. (2012) found that root exudates in a monoculture regime did not influence phytotoxicity on the development of cucumber directly but indirectly effected changes by alteration of the microbial associates in the soil. More recently, Lu et al. (2017) found that addition of root exudates altered soil microbial community specifically the taxonomic structure thereby influencing the dissipation of pyrene.

12.5 Contamination of Agricultural Soils

Huang et al. (2011) cited in Mahdavi et al. (2014) defined agricultural pollution as a by-product of biotic and abiotic substances emanating from modern farming activities that lead to contamination or degradation of the environment with substances which could be hazardous to humans and other life forms. Soils have been identified as the major receptor for a myriad of chemicals which include petroleum hydrocarbons (PHCs), PAHs, halogenated hydrocarbons, solvents, heavy metals, fertilizers, and pesticides released into the environment (Canadian Council of Ministers of the Environment (CCME) 2001) by aforementioned anthropogenic procedures.

12.6 Sources and Effects of Contamination of Agricultural Soil

Agricultural pollution may arise from different sources which could be regarded as point source or nonpoint source pollution. The United States Environmental Protection Agency (USEPA) cited in defined point source pollution "as any single identifiable source of pollution from which pollutants are discharged such as a pipe, ditch, ship or factory smokestack". In farmlands, nonpoint sources of pollution include more diffuse, landscape-level causes such as the discharge of heavy metals and metalloids from emissions of industrial activities, mine tailings, disposal of high metal wastes, leaded gasoline and paints, land application of fertilizers, animal manures, sewage sludge, pesticides, wastewater irrigation, coal combustion residues, spillage of petrochemicals, and atmospheric deposition (Khan et al. 2008).

12.6.1 Abiotic Sources

12.6.1.1 Organic Contaminants

Organic contaminants are usually generated from industrial activities and management of wastes of this nature constitutes global challenge due to their effect on soil ecosystem. These pollutants which are usually derived from different sources as mentioned earlier are usually recalcitrant. Manures and biosolids most of the time are introduced into the soil because of their nutrient content and serve as substrates for animals and humans consumption. Application of these waste products to farmlands provides opportunity for geochemical cycling of minerals in soil. One of the provocative problems associated with manures and biosolids is that they not only consist of nutrients such as carbon, nitrogen, and phosphorus but may likely be composed of other pollutants from drugs and chemicals. The ecological impact of oil pollution may be thought of in terms of direct and indirect effects. The direct effects are those caused by coating and asphyxiation and those that result from oil's toxicity, while the indirect effects are not as apparent as the direct effects and, therefore, are more difficult to assess (Stokes and Seager 1976).

Animals and plants may be affected by the physical properties of floating oil which prevent respiration, photosynthesis, or feeding. Many water soluble components of crude oil and refined products are toxic to marine organisms. In addition to killing such organisms, oil spillage appears to have more subtle effects by disrupting the chemoreception of such organisms leading to elimination of many species from the spilled area even when the oil concentration is far below the lethal level, since feeding and mating responses largely depend on chemoreception.

The magnitude of any chemical pollution may be measured by its toxicity and the amount discharged. When deposited into water bodies, these contaminants have been shown to cause acute toxicity (death) to aquatic life, whereas at lower concentration they are capable of causing chronic effects, such as stunted growth, reduced offspring, nervous system disorders, or likely concentrate in tissues. However, toxicity and recalcitrance is determined by the nature of the fraction of petroleum. For example, while some petroleum fractions tend to be volatile and do not persist in the environment (Frick et al. 1999), others like the polynuclear aromatic compounds are recalcitrant. Effects of oil spill have also been reported (Amund et al. 1993; Ijah and Antai 2005) on the physicochemical properties of the soil.

12.6.1.2 Heavy Metals

Environmental contamination due to heavy metals is a growing worldwide concern. However, Zarcinas et al. (2004) opined that the most area of concern from heavy metal pollution is that of agricultural soil pollution, because of the problems associated with heavy metal contamination such as biomagnification which may occur at each trophic level (Chojnacka 2010; Dixit and Singh 2013). Soil contamination by heavy metals can be caused by industrial activities which include mining and smelting, application of fertilizers, disposal of metal scrap wastes, gasoline spills, atmospheric deposition from industrial produce, paints, animal manures, sewage sludge, pesticides, discharged wastewater for irrigation, coal residues from combustion of coal, and petroleum hydrocarbon spillage (Kamal et al. 2010). However, removal of these metal contaminants has been shown to be impossible except through recovery and reuse mechanism (Gupter and Rastogy 2008). Several mechanisms of remediation of metal contaminated soil have been shown in various studies with bioremediation been of utmost concern because of the advantages associated with the process. Phytoremediation has been given more attention although a number of studies (Zhuang et al. 2009; Khan et al. 2008; Wauna and Okieimen 2011) have indicated the limitations of this process. To mitigate these challenges, PGPR have been developed for inoculation with the plants to enhance phytoremediation process (Glick 2010). Huang et al. (2013) evaluated a *Burkholderia* sp. LD-11 that was resistant to multiple heavy metals and antibiotics for its plant growth promoting properties and heavy metal accumulation in plant tissues. The authors concluded that combined action of the plant used and the bacterial strain LD-11 enhanced phytoextraction process.

12.6.1.3 Pesticides

Pesticides are broad range chemical compounds added to farmlands by farmers to control pests before and after crop production. However, indiscriminate use of these compounds leads to soil contamination which may persist and accumulate in food chain. Several effects associated with pesticide use in agriculture have been well documented (Martin et al. 2003; Aktar et al. 2009; Gullan and Cranston 2010). The fate and transport of pesticides in environmental media are determined by the chemical nature of the compound which also affects its sorption dynamics (Environmental Protection Agency (EPA) 2006; Gosh et al. 2010). For instance, Martin et al. (2003) assessed pesticides in agricultural areas and urban areas in which the result indicated that over 90% of samples from streams of agricultural areas and 75% of streams sampled in urban areas contained atrazine, whereas metolachlor was found to be higher (80%) in agricultural streams than urban streams (50%).

12.6.1.4 Fertilizers

Historically, agriculture was the first major influence on the soil (Scragg 2006 cited in Wauna and Okeieimen 2011). Evidence has shown that for plants to grow adequately, nutrients in the form of macronutrients (N, P, K, S, Ca, and Mg) and essential micronutrients (Co, Cu, Fe, Mn, Mo, Ni and Zn) must be supplied (Lasat 2000). However, high application rates of nitrogen-containing fertilizers has been indicated to combine with high water soluble nitrate leading to increased runoff into surface waters as well as leaching into groundwater thereby causing pollution (FAO/ECE 1991). The excessive use of nitrogen-containing fertilizers (be they synthetic or natural) is particularly damaging, as much of the nitrogen that is not taken up by plants is transformed into nitrate which is easily leached. Nitrate levels above 10 mg/L (10 ppm) in groundwater can cause "blue baby syndrome" (acquired methemoglobinemia). The nutrients, especially nitrates, in fertilizers can cause problems for natural habitats and for human health if they are washed off soil into watercourses or leached through soil into groundwater.

12.7 Restoration of Contaminated Soil Using Microbe-Exudate Interaction/Formulation

Phytoremediation, though a promising technique for in situ bioremediation of petroleum hydrocarbons, has been described as a relatively slow process that is capable of being prolonged in the cause of reducing the level of a particular contaminant in the environment (Rohrbacher and St-Arnaud 2016). However, to augment these constraints, Gerhardt et al. (2009) are of the opinion that the process may be enhanced by exploiting the rhizosphere in such a way that suitable pair of plants and their associated microorganisms are used. These can be either combinations of plants and beneficial microbes or blending phytoremediation plants with contaminant-degrading microbes and/or their products such as enzymes and biosurfactants. Through the use of known degrading microbes, for example, Kuiper et al. (2004) reported that combined effect of grass and efficient naphthalene-degrading bacteria provided protection to the grass seeds by reducing naphthalene toxicity. In this process the growing roots aided the movement of the naphthalene-degrading bacteria deep into the soil in which penetration would have been near impossible for the bacteria to undergo without the roots. Co-evolvement of microbes associated with a plant in form of parasitic or symbiotic relationship has shown that they may have a considerable influence on their host (Zilber-Rosenberg and Ronsenberg 2008). According to Shukla et al. (2013), synergistic relationship between plant roots and rhizosphere microorganisms such as the release of root exudates, synthesis of siderophores, HCN, plant hormones, and phosphate-solubilizing enzymes by PGPR may be efficient for rehabilitation of polluted sites. Various studies (Tak et al. 2013; Vacheron et al. 2013; Rohrbacher and St-Arnaud 2016) have shown the effectiveness of PGPR as a promising approach for improving the success of phytoremediation of contaminated soils. Microorganisms can be selected based on previous history of degradation capability, isolation from sites polluted with the same type of contaminants, or by preadaptation in vitro (Mrozik and Piotrowska-seget 2010). Therefore, from the various actions of PGPR highlighted above, it may be suggested that they may likely improve phytoremediation processes albeit several mechanisms of activity (Gerhardt et al. 2009).

12.7.1 Hydrocarbon Contaminated Soil

It has been observed that plants established under the influence of contamination more often than not need to devise means of surviving the harsh conditions of both nutrient deficiency and the toxicity from the chemicals. For instance, a prosperous approach for overcoming this limitation has been indicated to be the use of PGPR that expresses 1-aminocyclopropane-1-carboxylic acid deaminase (Glick 2003; Gerhardt et al. 2009). Narasimhan et al. (2003) showed that quite a number of the rhizobacteria (e.g., *Pseudomonas* spp.) are capable of enhancing growth of plants and capability to degrade contaminants.

Several researchers (Germida et al. 2002; Gao et al. 2011; Phillips et al. 2012; Martin et al. 2014) demonstrated the contribution of root secretions in improving hydrocarbon rhizoremediation. The most important functions of root exudates in biodegradation is by serving as the source of energy and nutrients which supports development and activity of the plants (Kuiper et al. 2004). Since the release of exudates was shown to mediate rhizospheric interactions by recruiting PGPR (Vicre et al. 2005; Rudrappa et al. 2008; Badri et al. 2013), one phytoremediation strategy is to inoculate a hydrocarbon degrader PGPR together with root exudate that stimulates the PGPR, thereby inducing a nutritional bias. For example, Vicrè et al. (2005) demonstrated that some cell surface glycoproteins called arabinogalactan proteins secreted by *Arabidopsis* aid rhizobia in identifying and adsorbing to the root surface.

Efficient colonization of degrading microbes or PGPR on growing roots has been shown to be significant in a successful rhizoremediation process (Lugtenberg et al. 2001; Gerhardt et al. 2009). Chemotaxis toward specific root exudate is critical for efficient root colonization, although the chemotactic response can be elicited by different compounds depending on the colonizing species (Kuiper et al. 2004). Indeed, Fabaceae have been reported to exude flavonoids from their roots which serve as chemoattractant to rhizobia and are also important in hydrocarbon rhizoremediation (Hall et al. 2011). Dandanelli et al. (2010) reported that on soybean root, the PGPR *Chryseobacterium balustinum* Aur9 impacted flavonoids released and may also play significant role in affecting flavonoid secretion by Fabaceae roots. Complex aromatic compounds like the coumarins have also been implicated in aiding root colonization (Gerhardt et al. 2009). It is fortuitous that these compounds just like the flavonoids have structural analogy to many organic contaminants including polychlorinated biphenyls (PCBs), PAHs, and PHC, thereby opening avenues for exploiting natural processes in remediation of contaminants in the rhizosphere (Chaudhry et al. 2005).

The efficiency of any phytoremediation system depends on the bioavailability of the targeted pollutant and root – microbial modifications of their solubility and chemical speciation in the rhizosphere (Wenzel 2009). Plant root exudates have also been used to enhance the bioavailability of organic contaminants by the release of biosurfactants which increases the solubility of hydrocarbons (Gao et al. 2010; LeFevre et al. 2013). Root exudate amendment on contaminated soils with low molecular weight organic acids has been reported to enhance the desorption of hydrocarbons and/or compete for soil adsorption sites from the soil matrix such as clay surfaces (Huang et al. 2011). More recently, Lu et al. (2017) studied the role of artificial root exudate components, glucose, organic acids, and serine, in facilitating the degradation of pyrene in soil and found out that glucose could significantly facilitate the removal of pyrene in soil through promoting dehydrogenase activity, as well as enhanced the amount of *Mycobacterium* markedly.

In addition to the use of root exudates as biosurfactants, microbial production of these biomolecules and the use thereof in increasing the bioavailability of organic contaminants have been documented in literature (Cameotra and Singh 2009; Banat et al. 2010; Kumar et al. 2013; Ibrahim et al. 2013; Santos et al. 2016). The exudation of biosurfactants by plant roots or the use of species of microorganisms that are known biosurfactant producers as inoculants may help to overcome the challenges of limited pollutant bioavailability experienced in hydrocarbon contaminated soils. In some instances, exogenous supplementation of biosurfactants may improve substrate preference for a normal organism with right appetite for hydrocarbons species; therefore, remediation outcome becomes challenging (Franzetti et al. 2008).

In addition to the production of biosurfactants, chemotaxis, the targeted movement of microorganisms in response to chemical gradients, with the aim of finding ideal conditions for growth and survival has been reported (Pandey and Jain 2002; Strobel et al. 2011; Nisenbaum et al. 2013) to be important for microbial exploitation of PHCs in soil and water. For example, the capability of bacteria to sense and swim toward n-hexadecane have been documented (Nisenbaum et al. 2013). The attraction of competent bacteria to the root zone may improve bioavailability and increase PAH degradation in the rhizosphere (Shukla et al. 2013). Thus combining chemotactic bacteria with biosurfactants could be a good strategy for increasing bioavailability of hydrophobic organic contaminants.

Horizontal gene transfer is another mechanism employed by microorganisms to aid them in adaptation to new ecological stress (Dong et al. 1998; Thijs et al. 2016). For example, legume symbionts have evolved via horizontal transfer of symbiotic plasmids. And because root exudates can stimulate gene transfer (Kroer et al. 1998; Mølbak et al. 2007), possibility of enhancing this process can be achieved by combining root exudates (such as flavonoids) that are known to trigger signals to initiate the symbiotic process with the plasmid carrying organisms. Various researchers (Van Elsas et al. 2003; Taghavi et al. 2005; Jussila et al. 2007; Wang et al. 2014; Wei et al. 2014) have identified the plant-soil interface as a hot spot for horizontal gene transfer involving both endophytic and rhizospheric interactions. Taghavi et al. (2005) reported improved phytoremediation of toluene through horizontal gene transfer to endogenous endophytic bacteria *Burkholderia cepacia* VM1468 containing the pTOM-Bu61 plasmid coding from *poplar*. This improved the mineralization of toluene and trichloroethylene that would have otherwise been volatilized. More recently, Ling et al. (2016) demonstrated that plant nodulation inducers enhance horizontal gene transfer of *Azorhizobium caulinodans* symbiosis island. The authors found that the symbiosis island of the *S. rostrata* symbiont, *A. caulinodans*, is an 87.6-kb integrative and conjugative element (ICE^{Ac}) that is able to excise, form a circular DNA, and conjugatively transfer to a specific site of the gly-tRNA gene of various bacterial species.

12.8 Conclusion and Future Prospects

Today, rhizosphere microbiology has become very important because of the intense and beneficial interactions that take place in the rhizosphere. The interactions are mainly microbe-microbe and microbe-plant interactions. In both interactions, the products including amino acid and plant exudates are produced. Various formulations for restoration of contaminated soil can be obtained. Unfortunately this is lacking or is minimally developed, probably because of the huge resource investment it might require. Besides, modern facilities for the study of microbes in their natural environments are just beginning to emerge. These problems can be overcome by institutional support. It is now the time to start exploring the rhizospheric microbe-plant exudate formulation for the restoration of contaminated agricultural soil. Thus, apart from rehabilitating contaminated soil, it will enrich soil for more rewarding agricultural productivity.

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Rhizoremediation of Azodyes by Constructed Wetland Technology using *Typha latifolia*

13

Anju Anil Kumar, Dipu Sukumaran,
and Salom Gnana Thanga Vincent

Abstract

Synthetic azodyes used in textile industries generate hazardous waste and adverse effect on soil and water, probably affects the whole environment. The traditional treatment technologies used for synthetic dyes are costly and adversely affect the biota. Phytoremediation, an arising new technology that generally uses the aquatic macrophytes to reduce, stabilize and also remove toxic pollutants in an ecofriendly and cost effective way. However, the stress on plants results in slow growth and low biomass and its rhizospheric bacteria will enhance the degradation potential and reduce the stress on plants. The chapter describes the bioremediation of azodyes by the combination of plant and root associated bacteria of *Typha latifolia* in constructed wetland system.

13.1 Introduction

Textile industries are one of the major users of synthetic dyes in the world and release huge quantities of dyes, which are generally discharged out from these industries, causing serious damage to the environment related to the bleaching and dying process. In India, in the year 2010 water consumed by the textile industry was around 1900 mm^3 (Million cubic meters) and effluent water generated was approximately 75% of its total intake. The color in water for the most part restrains the entrance of sunrays in to the water body and also it decreases the level of photosynthetic and dissolved oxygen in water (Shehzadi et al. 2014). Furthermore, dye waste water is extremely hard to treat since it regularly changes its high pH, COD, colour

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A. A. Kumar · S. G. T. Vincent (✉)
Department of Environmental Sciences, University of Kerala, Thiruvananthapuram, Kerala, India

D. Sukumaran
Centre for Water Resources Development and Management, Kozhikode, Kerala, India

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and its degradation intermediates contain high organized polymers (Neppolian et al. 1998). However different treatment technologies such as coagulation, flocculation, adsorption on activated carbons, oxidation, ion exchange etc have been used for treating dye wastewater, but these processes are with high cost and also having environmental impacts (Kharub 2012). The introduction of phytotechnology is an emerging field that implements solutions to scientific and engineering problem by using plants and its associated microorganism by the construction of artificial wetlands. Now a days plant-based technology have become alternatively used in cleanup strategies generally in light of their low expenses, high success rates, low upkeep requirements and also its elegant nature. Phytoremediation technologies generally incorporate with a wide a scope of innovations that utilized different types of plants to expel, minimize, degenerate, and cripple natural toxins from soil and water and then restore the contaminated goals to a generally perfect, non-lethal environment.

Phytoremediation is a naturally occurring process, in which plants removes inorganic and organic pollutants, through certain process such as degradation, sequestration, or transformation (Pilon-Smits and John 2006). Phytoremediation innovations are arranged into various classes, for example, phytodegradation or rhizodegradation (degradation of toxins by rhizosphere microorganisms), phytoextraction, phytostabilisation and phytostimulation. The most conventional treatment technology is the combination of plants and its associated microorganism in constructed wetland technology. Developed constructed wetland, is an outlined simulated wetland, gives off an impression of being a most encouraging for the treatment of color wastewater. This chapter generally features the role of constructed wetland by using macrophytic plants and its associated rhizosphere bacteria in the degradation of azodyes.

13.2 Toxicity of Textile Dyes in Water

Dyes can be generally classified according to its different structural varieties like acidic, basic, disperse, azo, anthraquinone, phthalocyanine, triaryl methane, diaryl methane, indigo dyes, ketazines, oxazine, aldazines, Leuco, phthalein dyes, indamines dyes, hydroxyl ketonestilbene and sulphur dye compounds (Shah 2014). Azo compounds are the most vital textile colours which are classified, as indicated by their solvency, into soluble and insoluble azo dyes and azo pigments. These are generally synthesized from arylamines by diazotation and coupling (Platzek et al. 1999). Azo dyes are considered as carcinogenic and they are generally produced by the dye manufacturing industries causing serious defilement to water bodies. The disposal of these dyes is very much essential due to its hazardous effect to the whole environment. Synthetic dyes, which were used in the textile industries, have the ability to produce strong covalent bond to textile fibers. Exposure of these synthetic dyes may cause potential health hazards and it is mutagenic and carcinogenic and are either toxic to flora and fauna (Nilsson et al. 1993). Various technologies are been introduced to treat wastewater; however, multifunctional treatment technologies are reviewed as a productive treatment technology for the proper removal of synthetic dye from textile dye industry by a single treatment (Saba et al. 2015).

Textile dye industries are considered to be the largest water consuming and waste water generating industries by its different dyeing and finishing processes. Effluent water produced from textile industries are rich in color content, containing high deposits of residual matter, toxic chemicals, and complex components with suspended solid (Wang et al. 2011). These textile wastes are extremely dangerous to organisms in the water body that also exhibit genetical disorder and change in its physiological condition. Different treatment technologies were available for the treatment of textile effluent wastewater but they are not suggested because of its high maintenance cost and also the generation of secondary pollutants due to the use of chemicals (Pundlik et al. 2013). By observing these views development of economical and feasible technology to restrict the textile effluent pollution and tremendous water utilization is exceptionally fundamental.

Toxic textile dyes in water bodies that are released from the textile industry decrease the dispersal of light, which consequently, reduces the photosynthetic rate of algae and other aquatic vegetation (Khandare and Govindwar 2015). These activities generally lower the dissolved oxygen concentration and increase the concentration of inorganic salts and acid, which will lead to long-term adverse effects in the aquatic environment. These colored synthetic compounds have complex structure, are xenobiotic and after absorption, they are distributed in the whole body and force to change some kind of action (Chequer et al. 2011).

These complex structures of synthetic dyes especially amino azo derivatives may lead to health hazard and this may increase the risk of malignancy (Garg et al. 2002). According to the study of IARC has listed benzene derivatives dyes to the great degree of cancer causing agent to human beings (IARC 1982). Sharma et al. (2007) studied the toxicity of textile wastewater in swiss albino rats. Malachite green is a water soluble cationic dye and its metabolite lecuomalachite may cause malignancy or tumor in rats and this malachite green colors will cause hepatic and cellular damages in certain fishes (NTPC 2005; Khandare and Govindwar 2015). Srivastava et al. (1995) reported that malachite green can cause reduction in calcium and protein and also increase cholesterol level in blood of *Heteropneustes fossilis*.

13.3 Constructed Wetland Technology with Aquatic Plants

Constructed wetland treatment system involves the use of a well-designed engineered system, which is generally being utilized as a natural process. These designed systems generally mimic natural wetland system by using wetland plants, soil and also its associated microorganisms to minimize contaminants from wastewater. Generally, common types of constructed wetlands are the Free Water Surface (FWS) systems, the Subsurface Flow (SSF) systems including horizontal or vertical flow. FWS act as like natural wetlands in which, the wastewater flows horizontally over the sediment. In SSF, effluent water flows horizontally or vertically through different material or substance, which is mainly layered by clay, sandy soil, gravel or with other substrates (Zhang et al. 2009). Aquatic macrophytes are the major component of constructed wetlands. They absorb contaminants in their plant parts and the plant enzymes which may act as a catalyst for the degradation of pollutants. The diversity rhizosphere bacteria in the root zone area will also help the plants to improve their efficiency in the removal contaminants (Jenssen et al. 1993; Hadad et al. 2006).

Treatment performance in constructed wetlands is a combination of aerated and non aerated treatment mechanisms that store, change, and expel organic matter and its related toxins (Wallance et al. 2008). The plants utilized in constructed wetlands were planned for wastewater treatment ought to be tolerant to highly natural and nutrient loadings and these are effectively degraded under anoxic and anaerobic conditions (Vymazal and Lenkropfelova 2008; Kvet et al. 1999). Macrophytic plants that were growing in constructed wetlands showed a couple of properties in connection with the treatment technique. These findings make these plants to hold a major part in the plan of constructed wetlands in relation to its physical effects such purification; control erosion etc. and also its root associated microorganisms influence the plant growth and also maintain the hydraulic properties of the substrate (Haberl et al. 2003).

Anjana and Salom Gnana Thanga (2011) studied the decolourization of synthetic dyes using aquatic macrophytes. In 2012, Dorota and Krzyszt of reported the decolourization of dye acid orange using different aquatic macrophytes in constructed wetlands. Aquatic macrophyte *Phragmites* showed a maximum growth in textile wastewater than its control, Lemna and spirodela are been strongly recommended for the toxicity assessment study of textile dye effluents (Sharma et al. 2005). Water hyacinth is a nuisance aquatic weed showed a maximum biosorption capacity to reduce the concentration of dye stuff, heavy metals and also minimize the COD, BOD, TDS in textile wastewater (Sanmuga Priya and Senthamil Selvan 2017). This can also been used in water bodies contaminated with dye wastewater (Tan et al. 2016). *Leucaena leucocephala* plant remediates the dye contaminated soil and also act as a biodegradable organic phytostabiliser for dyes (Jayanthy et al. 2014). The role of adventitious roots of *Ipomoea hederifolia* along with endosymbiont *Cladosporium cladosporioides* decolorized synthetic dye; such synergistic approaches increase the efficiency of phytoremediation (Patil et al. 2016). Plant species like *Pistia stratiotes*, *Eichhornia crassipes* and *Dichanthium annulatum* plays a pivotal role in remediation of toxins from textile effluents (Yasar et al. 2013).

13.4 *Typha latifolia* and Dye Remediation

Typha latifolia plant is one of the most commonly available and widely used macrophyte in constructed wetland. These narrow leaved cattails wetland plants have an efficiency to decolorize and degrade textile effluent and also have the ability to survive in dye effluents (Nilratnisakorn et al. 2008). *Typha latifolia* species has thick and broad leaf cattail, which may survive in any rough condition and these roots release oxygen and which plays a major role in the degradation of contaminants in the wetlands (Li et al. 2010). These plants also have the dye removal efficiency because of its large biomass rapid propagation and complex metabolism (Chandanshivea et al. 2017). Some of the limiting factors which influence the performance of *Typha latifolia* based constructed wetland remediation are water, oxygen and nutrients. Sánchez-Orozco et al. in 2018 studied that the *Typha latifolia* plant parts showed a high efficiency in the removal of methylene blue. These plant parts are used to prepare activated carbon which act as an adsorbant for the removal of dyes (Jaya Santha Kumari et al. 2015).

13.5 Rhizosphere Bacteria and Degradation of Dyes

Rhizosphere was defined by Hiltner in 1904 as the portion of soil influenced by plant root. This part is the focal point of vigorous organic action, because of its rich nutritional supply; gas exchange and enhanced release of root exudates promote the growth of plants (Prasad et al. 2015). Rhizosphere bacteria occupy a narrow zone of soil immediately adjacent to plant roots which represents a biological niche with in the soil environment. This plays a vital role in the plant growth by mobilizing different enzymes, controlling plant pathogens and also plays a major role in the degradation of different contaminants. These plant-rhizosphere interactions enhance the degradation of different pollutants by uptake, translocation mechanisms and also by tolerance mechanisms (Pilon 2005). Rhizosphere bioremediation is also known as rhizodegradation or plant-assisted bioremediation. This remediation process is an active process that interact both plants and its rhizosphere microbes (Nwoko 2010). In this rhizosphere zone the pollutants can be fixed or debased by the plant roots (Pilon 2005). Exudates are perplexing emissions acquired from the plant roots, which “bolster” the microorganisms by giving carbohydrates, additionally contain characteristic chelating agents that make the ions of both nutrients and contaminants more mobile in the soil, which then results in a more competent degradation of pollutants. On the other hand beneficial microorganisms increment the bioavailability of the toxins to the plants or decrease the harmfulness of the contaminations helping the plants to survive and increment the rate of remediation. The roots of aquatic plants generally require oxygen for their growth and development, so with the help of their developed lacunar system encourages the flow of oxygen from shoot to root (Sculthorpe 1967; Armstrong 1979). By this process radical oxygen loss will occur this lossed oxygen may consumed by the root zone microbes which may fasten the degradation process.

Chaudery et al. in (2005) explained the synergistic action of rhizosphere and plant. Typha showed a maximum growth in textile effluents and also showed the potential to host the maximum endophytic bacteria in its root and shoot; these interactions will increase the efficiency of the constructed wetland (Shehzadi et al. 2015). Khandare et al. in (2011) explored plant and its bacterial synergism and also determine the role of bacterial enzyme in the degradation of Remazol Black B dye.

Introduction of endophytic microbes in Typha species diminished the toxic effects and also it showed an upgradation in plant and also decreases in its genotoxicity (Shehzadi et al. 2014). Bacteria which are isolated from the rhizosphere of plant exhibited 60–100% of decolorization capacity (Shafqat et al. 2017). The endophytic bacteria isolated from mangrove plants showed the degradation of dyes and also act as a potential candidate for dye degrading enzymes (Gayathri et al. 2010). Studies conducted by researchers to detect the role of bacterial consortia and also fungal-bacterial consortia and its efficiency in dye colorization and also its dye degradation (Khandare et al. 2012). Watharkar et al. (2013) reported the role of rhizospheric bacterial isolate *Bacillus pumilus* strain in *Petunia grandiflora* to decolorize dye reactive navy blue. Hairy roots of *Tagetes patula* (Marigold plant) which was induced by *Agrobacterium rhizogenes* showed the ability to decolorize different textile dyes (Patil et al. 2009).

13.6 Advantages of Plant-Microbe Interaction in Textile Dyes

Plant- microbe interaction is an economical technology in dye remediation. Plant and its associated bacteria, such as endophytic bacteria and rhizosphere bacteria play a major part in the degradation of poisonous chemicals in its contaminated site through traditional treatment technology. Phytoremediation is considered as a traditional treatment technology using plants, but the major disadvantage of this technology was its slow process, but the introduction of dye degrading bacteria in the root zone area will increases the growth of plants, enzyme activity, degradation efficiency. This treatment technology looks truly encouraging being a sun powered and profitable in view of low support and irrelevant prerequisites (Cluis 2004; Ma et al. 2011). By several plant – microbe interaction studies proved that these technology will provide better results which definitely help to improve the waste land area in to beautiful avenues (Khandare et al. 2013). *Typha latifolia* plants and other aquatic macrophyte *Eichhornia crassipus* have showed efficiency in the removal of metals in the water bodies with textile effluents (Dipu et al. 2011). So the combination of plant – microbe process showed an extremely high sensible achievability to discover the application in the disposal of synthetic dyes.

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Some Investigations Observed in Cultured Seabass (*Dicentrarchus labrax* L.) Infested with *Lernanthropus kroyeri* and *Nerocila orbignyi* and Exposed to Cadmium Pollution During Different Seasons at Dammaitte Province

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AE Noor El-Deen, Mona S. Zaki, and IS Shalaby

Abstract

This study was conducted to investigate the effect of crustaceans' copepoda and isopoda on cultured marine seabass (*Dicentrarchus labrax* L.) to cadmium pollution. A total of 400 adult infested fish collected seasonally from Damietta province were clinically examined for isolation and identification of infested parasites. Results obtained revealed some clinical pictures as bulging of opercula; sluggish movement; emaciation, severe erosion, and hemorrhages of gills; and mortality. The detected parasites were identified as copepoda (*Lernanthropus kroyeri*) and isopoda (*Nerocila orbignyi*). The morphological characteristics of *Lernanthropus kroyeri* van Beneden, 1851, and *Nerocila orbignyi* Guerin-Maneville, 1832, were studied by light microscopy. The total prevalence was 16%, and the summer displayed the highest seasonal prevalence. The relation between fish body weights and lengths and infestation rate was studied. Besides, the relation between heavy metal pollution and parasitic infestation was discussed. The present study concluded that there was inversely proportional relationship between cadmium concentration pollution in aquaculture and the prevalence of *Lernanthropus kroyeri* and *Nerocila orbignyi* infestation during European seabass summer and spring seasons, while infestation disappeared during autumn and winter seasons.

A. Noor El-Deen · M. S. Zaki (✉)
Department of Hydrobiology, National Research Centre, Dokki, Egypt

I. Shalaby
Department of Reproduction, Veterinary Division, National Research Centre, Dokki, Egypt

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14.1 Introduction

European seabass fish products have reached to 19,027 tons in Egypt (GAFRD 2011). The decrease of this production of fish resulted in serious parasitic diseases in Egypt where intensive aquaculture is practiced (Noor El Deen et al. 2011). Thus, focus has been placed on parasitic fish diseases in these enterprises and their economic and ecological impact. Parasitic infestations represent the majority of the known infectious diseases affecting fish (Noga 2010). In recent years, crustacean parasitic diseases are becoming more frequent in the aquacultures and considered the more parasitic problem on cultured marine fish (Tansel and Fatih 2012). The major groups of crustacean fish parasites are Isopoda and Copepoda (Öktener and Sezgin 2000). *Lernanthropus kroyeri* van Beneden, 1851, has been recorded from Suez Canal area in seabass *Morone labrax* (Eissa et al. 2012). Also, *Nerocila* is a large genus of *Cymothoidae*. *Nerocila orbignyi* attaches to the gills of infested *Dicentrarchus labrax* (Mladineo 2003). Commonly distribution areas of *Nerocila orbignyi* are Mediterranean, Northwest Africa, Red Sea, and Egypt (Trilles 1994). The more prevalent parasites that infested cultured seabass were *Lernanthropus kroyeri* (Copepoda) and *Nerocila orbignyi* (Isopoda) in marine seabass (Alas et al. 2008; Eissa et al. 2012). Many fish species are infested by isopods which are found in various parts of the fish body, including gill cavity, and cause gill damage (Toksen 2007). They provide portals of entry for other pathogens in fish (Horton and Okamura 2001). *Lernanthropus* is the most common genus of parasitic copepods affecting only *Dicentrarchus labrax*, which inhabit in warmer waters. Some species of *Lernanthropus* are strictly specific, but many are parasitic on several species of fish belonging to one genus or on several genera of one family (Sharp et al. 2003).

Pollutants may affect an intermediate or alternate hosts in parasite life cycle and on free-living life cycle stages of parasite invasion (Sindermann 1990). Pollution stress can influence the prevalence of parasites directly or indirectly by impairing the host's immune response, or the parasite infestation may decrease the host resistance to toxic pollutants (Khan and Thulin 1991). Cadmium is potentially harmful to most organisms even in very low concentrations (Kaoud and El-Dahshan 2010) and present in aquatic environment and gills which increased due to domestic and industrial mining (El-Seify et al. 2011). Fish accumulate cadmium to concentration many times higher than present in water (Yilmaz 2005; Noor El Deen et al. 2011). The relationship between parasitism and pollution is not simple and in essence involves a double-edged phenomenon in which parasitism may increase host susceptibility to toxic pollutants, or pollutants may result in an increase or decrease in the prevalence of certain parasites (Kuperman 1992).

The present study was directed toward further understanding of marine seabass fish in Azbat El Borg marine fish farms in Damietta region. The objectives were decided to throw the light on the clinical picture, total and seasonal prevalence of the crustacean parasitic diseases affecting *Dicentrarchus labrax*. Besides, the infestation rates in relation to body weights and lengths in Damietta province. Moreover, the effect of cadmium pollution on fish parasitism in different seasons.

14.2 Materials and Methods

14.2.1 Fish for Examination

During the years 2011–2012, a total of 400 specimens of European seabass, *Dicentrarchus labrax*, were collected which were cultured in marine fish farm in Azbat El Borg area (Damietta province corresponding to Mediterranean Sea). Their body weights and lengths were ranged from less than 60 up to 500 g and 18 to 40 cm, respectively. The fish were obtained seasonally (each 50 fish) from private fish farms, then transported to the laboratory alive in fiberglass tank containing 2/3 of its volume the same farm and supply with aerator to oxygen.

14.2.2 Water and Tissue Samples for Heavy Metal Measurements

Water and fish samples were collected during the years 2011–2012. Eight water samples were collected in different times from the same area, kept in refrigerator, and transferred cold to the laboratory for analysis according to APHA (1992). Parasitized fish specimens were dissected freshly to obtain gills and then frozen until ready for acid digestion using Conc. H_2SO_4 according to the method outlined by EOSQC (1993). Each 0.5 g of collected gill fish samples were well digested using Conc. H_2SO_4 according to the method outlined by Cottenie (1980). The levels of Cd were determined at central lab of National Research Centre using atomic absorption spectrophotometer (Model Thermo, AA spectrometer, S series, type s4).

14.2.3 Clinical Pictures

The collected European seabass were examined clinically according to the methods described by Noga (2010) paying an attention to the seabass behaviors in the earthen ponds. Fish specimens under investigation were grossly examined for determination of any clinical abnormalities and any external parasite on all fish according to Woo (2006).

14.3 Parasitological Examination

14.3.1 Macroscopic Examination

Macroscopic examination was done for detection of any abnormalities in different parts of the fish body by naked eyes and hand lens. Skin, fins, gills, eyes, and opercula were dissected and examined for the presence of parasitic crustaceans.

14.3.2 Microscopic Examinations

The microscopic parasites were collected by a fine brush, special needle, or eye dropper, and washed for several times in fresh water until the specimens had died and left in refrigerator at 4° C to completely relax. The crustaceans are then fixed in 70% alcohol glycerin, passed through ascending grades of alcohol (70, 80, 90, 95%, and absolute) cleared in xylol, mounted in Canada balsam or by clearing in lactophenol, and mounted in glycerin-gelatin (Lucky 1977). Crustacean parasites were identified according to Kabata (1979), Badawy (1994), and Al-Zubaidy and Mhaisen (2013).

14.4 Statistical Analysis

Data were analyzed by analysis of variance using the SAS program. Duncan's multiple range test (1955) was used to verify significance of the mean differences among treatments.

14.5 Results

14.5.1 Clinical Examination

The main clinical signs of naturally infested European seabass showed in the form of respiratory distress, surface swimming, bulging of opercula, sluggish movement, and emaciation, severe erosion, and hemorrhages of gills, and grayish dots with inverted V-shaped egg sacs were obvious even macroscopically, and Isopoda was found in between the operculum and pectoral fin on one side, and weak tissue damage was noticed on the host fish (Plate 14.1 (1 and 2)).

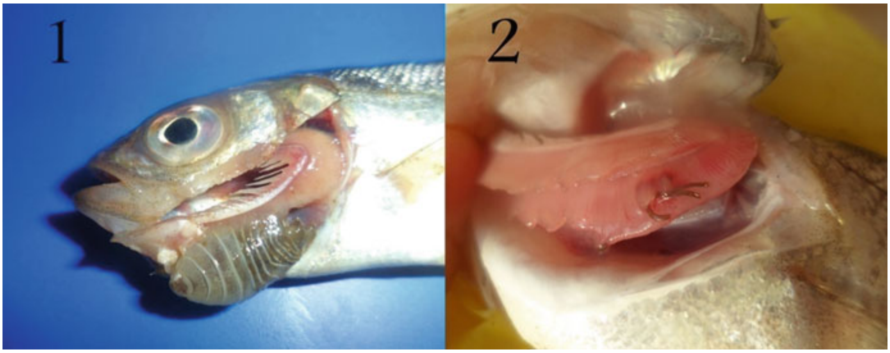

Plate 14.1 Showing infested seabass with isopoda embedded in the gill filament and arch (1). Emaciated seabass infested with heavy infested with copepoda (2)

14.5.2 Parasitological Examination

A crustacean parasite was collected from gills of European seabass, *Dicentrarchus labrax*. The male body is slender in shape. The mandible has seven denticles. The first maxilla consists of three segments, the terminal is conical, and the basal segment has two distal broad spines. The terminal segment of the second maxilla is provided of two rows of blunt teeth and blunt spines on the inner margins. The third segment has a single distal spine. The exopod has five short distal spines, and the caudal rami are short, while the endopod has slender bristled seta. The female is somewhat cylindrical and width at the middle of the body. The head is separated by a constriction from the rest of the body. The first thoracic leg is biramous, and the exopod of the first segment bears blunt distal spines, while the endopod bears an elongated distal spine. A tiny papilla-like process is located at the base of the endopod. The egg strings are elongated and uniseriate, strongly flattened eggs (Plate 14.2 (1–8)). Based on the morphological characteristics, such crustaceans belonged to Lernanthropidae, *Lernanthropus kroyeri*.

Another crustacean parasite was attached to the base of the gill arch and operculum of European seabass, *Dicentrarchus labrax*. The parasite was found in between the operculum and pectoral fin on one side of the infested seabass, and weak tissue damage was noticed on the host fish (Plate 14.3 (1, 2)). It was dorsoventrally flattened crustaceans with symmetrical body and measured up to 2.4 mm. The mouthparts are often styliform. The head is not embedded in first segment of the peraeon. The pleon (abdomen) is markedly narrower and shorter than peraeon and consists of six segments; each of the first five segments carries a pair of biramous natatory limbs (pleopods). The sixth segment is called the pleotelson, which is flanked by the biramous uropods. Both appeared without marginal setation. Uropods with exopods tilted so as not to be fully seen in dorsal aspect, slight to deep notch often present on medial margin. The peraeon, the largest part of the body, is composed of the cephalothorax, where the head is unsegmented and bears two pairs of antennae as well as two large black eyes. It consists of seven segments; each carries a pair of appendages (peraeopods). These can be prehensile or ambulatory. Such leg-bearing segments are clearly separate from each other. Based on the morphological characteristics, these crustaceans are related to Cymothoidae, *Nerocila orbignyi.*

14.5.3 Prevalence of Crustacean Infestation in Seabass Fish

Table 14.1 shows total and seasonal prevalence of crustacean parasites in examined seabass fish. Tables 14.2 and 14.3 show the prevalence in relation to body weights and lengths.

As shown in Table 14.1, there is a significant difference ($P < 0.05$) among the total and seasonal prevalence of crustacean parasites in examined seabass fish. The total prevalence of infestation was 16%, and seasonal prevalence of *Lernanthropus kroyeri* in examined seabass fish in spring and summer seasons were 4% and 6%,

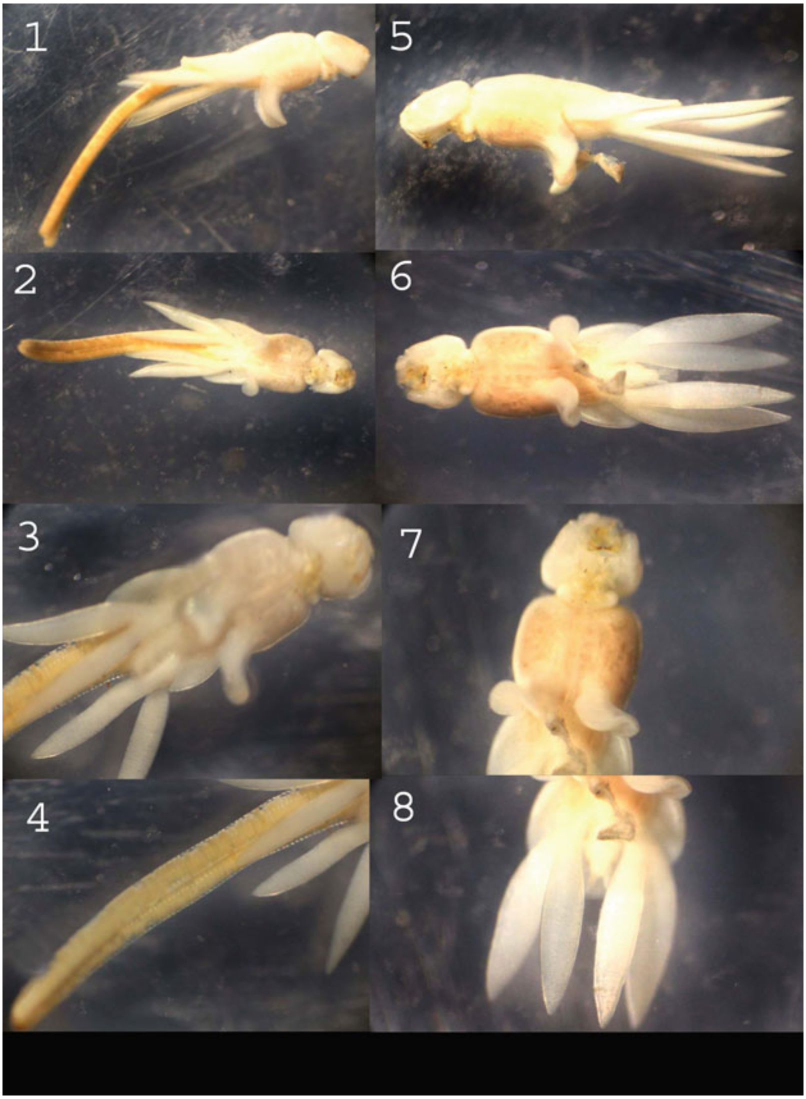

Plate 14.2 Light photomicrograph of female *Lernanthropus kroyeri* (lateral view, 1, ventral view, 2, and anterior part, 3, posterior part, 4). Light photomicrograph of male (lateral view, 5, ventral view, 6, and anterior part, 7, posterior part, 8)

respectively, while the seasonal prevalence of *Nerocila orbignyi* in examined seabass fish showed significant differences ($P < 0.05$) in spring and summer seasons as its prevalence were 2% and 4%, respectively. Tables 14.2 and 14.3 show the significant differences ($P < 0.05$) of parasitic prevalence in relation to body weights,

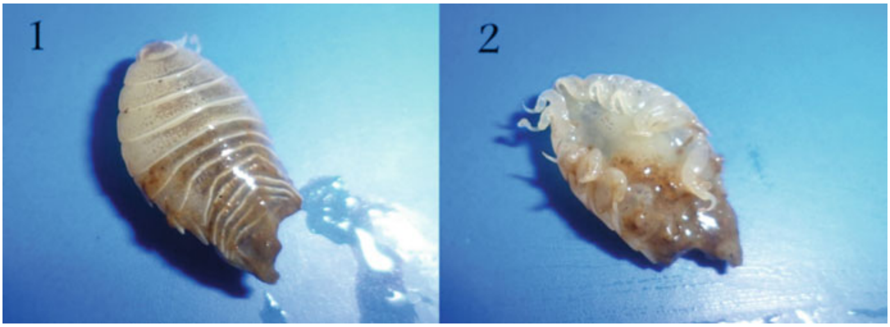

Plate 14.3 Light photomicrograph of *Nerocila orbignyi* (dorsal view, 1, and venteral view, 2)

Table 14.1 Showing prevalence and intensity of crustacean parasites in seabass (*Dicentrarchus labrax*)

	Prevalence (%)*				Intensity**		
Parasite species	Season	No. of examined fish	No. of infested fish	%	Season	Average S. D	Sn-1
Lernanthropus kroyeri	Spring	50	8	4	Spring	A 5.42 ± 5.6	5.82
	Summer	50	12	6	Summer	A 4.58 ± 4.3	4.6
	Autumn	50	0	0	Autumn	C 0	0
	Winter	50	0	0	Winter	C 0	0
Nerocila orbignyi	Spring	50	4	2	Spring	B 1.55 ± 1.55	1.50
	Summer	50	8	4	Summer	A 4.45 ± 3 0.49	4.51
	Autumn	50	0	0	Autumn	C 0	0
	Winter	50	0	0	Winter	C 0	0

Chi^2 = $^{(14.56)**}$; ** = significant at ($P < 0.01$)
**Means within the same column of different letters are significantly different at ($P < 0.05$)

and its lengths were 4% and 6% at body length 20–25 and 25–30 cm and body weight 100–140 and 150–210 g of seabass, respectively.

Table 14.2 Seasonal prevalence of crustacean infestations among seabass

Body length (cm)	No. of examined	Crustacean infestation			
		Lernanthropus kroyeri		*Nerocila orbignyi*	
		No. of infected	%	No. of infected	%
15–20	50	0	0	0	0
20–25	50	8	4	4	2
25–30	50	12**	6	8**	4
30–35	50	0	0	0	0
Total	200	20	10	12	6

$\mathrm{Chi}^2 = (6.88)$**; ** = significant at ($P < 0.01$)

Table 14.3 Seasonal prevalence of crustacean infestations among seabass

Body weight (g)	No. of examined	Crustacean infestation			
		Lernanthropus kroyeri		*Nerocila orbignyi*	
		No. of infected	%	No. of infected	%
60–100	50	0	0	0	0
100–140	50	8	4	4	2
150–210	50	12**	6	8**	4
220–350	50	0	0	0	0
Total	200	20	10	12	6

$\mathrm{Chi}^2 = (7.99)$**; ** = significant at ($P < 0.01$)

Table 14.4 Mean heavy metal concentration (ppm) in gills of infested fish in different seasons

Heavy metal	Permissible limit (mg/Kg)	Seasonal variation in gills (mg/Kg)			
		Au	Wi	Sp	Su
Cadmium in gills	0.05[a]	8.73	8.77	5.75	4.71
	0.1[b]				
Cadmium in water	0.01[c]	0.0028	0.0031	0.0030	0.0027

B *Pb* lead, *Zn* zinc, *Cu* copper, *Cd* cadmium, *Au* autumn, *Wi* winter, *Sp* spring, *Su* summer

[a] FAO/WHO (1992)

[b] EOSQC (1993) and FAO/WHO (1984)

[c] Permissible limit according to USEPA (1986)

14.5.4 Heavy Metal Residues and Seasonal Variations

Table 14.4 shows the residues and concentration of cadmium in gills of examined fish samples in different seasons. Contamination levels of heavy metal in gills were 8.73, 8.77, 5.75, and 4.71 and 0.0028, 0.0031, 0.0030, and 0.0027 in autumn, winter, spring, and summer seasons, respectively.

14.6 Discussion

The main clinical signs showed in naturally infested European seabass *Dicentrarchus labrax* with crustacean infestations were respiratory distress, surface swimming, bulging of opercula, sluggish movement, and emaciation, severe erosion, and hemorrhages of gills. In addition to hemorrhagic areas on gill cover and in late stages are external ulcers located in the gill filaments. These results may be attributed to the low respired oxygen of destructed gill epithelium which is caused by feeding activity, attachment, fixation, and locomotion of crustaceans causing massive destruction of respiratory epithelial cells. These results are in agreement with those reported by Eissa et al. (2012). Emaciation recorded in infested seabass may be due to crustacean infestation which reduces fish appetite and became off food; this agreed with Nagasawa (2004). Crustaceans reduce growth rates, and this result agreed with that recorded by Costello (2009).

Regarding the postmortem examination showed that marbling appearance of gills. This result may be attributed to destruction of the efferent vessels which may happen in copepod crustaceans and isopoda, where the blood pressure is low, no extensive hemorrhages were caused, the very short clotting time of blood brings about rapid occlusions of the vessel, and then thrombus is formed resulting in ischemia, which in turn leads to necrosis. This result agreed with that recorded by Noor El-Deen (2007). This result may be attributed to its parasite attached to the gill filaments using antennae and third legs leading to pathological effects such as erosion, desquamation of tissue, necrosis in branchial epithelial tissue, and the severe irritation caused by movement, feeding activity, and their claw fixation to such crustaceans which result in asphyxia and then death. These results were similar to that recorded by Toksen et al. (2008).

The second parasite *Nerocila orbignyi* under discussion is isolated from gills and feeds on gill tissues of their hosts, which can lead to many problems especially emaciation for small fish. These results may be attributed to the adult isopods that are hematophagous (feed on blood) and cause anemia. In addition the stationary parasites attaching especially on gills can seriously reduce the respiratory surface by causing atrophy of the gills on which they press. This result was similar to that recorded by Horton and Okamura (2001). The crustacean parasites were isolated from gills of *Dicentrarchus labrax*. This result agrees with Manera and Dezfuli (2003), Mladineo (2003), and Toksen et al. (2008) regarding the same genus isolated from the same host and site and disagrees with Kayış and Ceylan (2011) who obtained the same species from gills of *Solea solea*.

The measurements and morphological characteristics of *Lernanthropus kroyeri* were nearly similar to that obtained by Ravichandran et al. (2009), while *Nerocila orbignyi* was nearly similar to that obtained by Özak (2006).

The total prevalence of crustacean infestation of *Lernanthropus kroyeri* was 10%. This result is lower than that obtained by Eissa et al. (2012) who recorded the prevalence within the Red Sea seabass (*Dicentrarchus labrax*) being 47%. This difference may be attributed to the type of breeding of seabass that wild fish in Red Sea and locality from which fish samples are obtained, while the total prevalence of isopoda was 6%. This result is lower than that obtained by Horton and Okamura (2001) who recorded the prevalence within the Turkish seabass (*Dicentrarchus labrax*) farm being 66%. This difference may be attributed to the age of seabass and locality from which fish samples are obtained.

Concerning the seasonal prevalence of crustacean infestation, the peak was highest in summer (10%), followed by spring (6%), and absent in autumn and winter. This disagrees with results obtained by Eissa et al. (2012) in which they recorded the summer season as the season of the highest infestation rate (19%), followed by autumn (17%), spring (7%), and winter season (4%) which was the lowest. These results may be attributed to cultured fish less infested than wild fish and the difference of geographical distribution of hosts and parasites.

Heavy metals concentrated in gills of *seabass* were higher in autumn and winter seasons than that in spring and summer seasons. These results may be attributed to increase of water flow in summer and spring seasons than that in autumn which leads to solution of pollution and salinity. These results may agree with that recorded with Al-Weher (2008) who recorded that certain environmental conditions such as salinity could play an important factor in heavy metal accumulation in the living organisms up to toxic concentrations and cause ecological damage.

Gills were higher of all tested of cadmium pollution than water surrounds. These results showed an agreement with that of Saeed and Abdel-Mageed (2008). This may be due to the metallothionein proteins which are synthesized in gill tissues when fishes are exposed to cadmium and detoxify them. Moreover, seasonal variation showed higher residual values in winter, spring, and autumn than in summer in both tissues and water. These results are in agreement with these of Saeed (2007) and El-Seify et al. (2011) and disagree with that recorded by Noor El Deen et al. (2011) who recorded that cadmium concentration in gills of examined *Tilapia zilli* was increased gradually from winter season (0.33 ± 0.0004 ppm) and autumn season (0.43 ± 0.004 ppm) to (0.53 ± 0.003 ppm) spring season and (0.63 ± 0.006 ppm) summer season. These differences of concentrations and seasons may be attributed to difference of area, water, and type of fish.

Finally, the relationship between heavy metal, abundance of parasites, and environmental change due to pollutants can influence parasitic-host interaction (Khan and Thulin (1991) and Khan (2012)). In this study, crustacean infestations were found to be negatively related to heavy metal concentrations in different seasons. This may be attributed to the toxic effect of the heavy metals on the crustaceans which may cut its life cycle (El-Seify et al. 2011). On the other hand, no infestation was detected in liver or musculature with high accumulated heavy metals in different seasons. These results are in agreement with that of Lafferty and Kuris (1999) who recorded that pollutants may kill sensitive free-living stages of the parasite or reduce survival of free-living cercariae and miracidia, leading to a lower prevalence of parasitic larvae.

The present study concluded that there was inversely proportional relationship between cadmium concentration pollution in aquaculture and the prevalence of gill crustacean infestation during spring and summer seasons, while infestation disappeared during autumn and winter seasons. Also, there was a relationship between cadmium residues in *Dicentrarchus labrax* gills and its concentration in the water, and the obtained results showed that the cadmium concentration in the gills was higher than that in the water.

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Bioremediation of Polyaromatic Hydrocarbons: Current Status and Recent Advances

15

Simran Bhatia, Moyna Kalia, and Baljinder Singh

Abstract

Environmental pollutants like polyaromatic hydrocarbons (PAHs) due to their persistence, carcinogenicity, and toxicity have created havoc. Benzopyrene, naphthalene, anthracene, and phenanthrene are main aromatic hydrocarbons which are ubiquitous and recalcitrant causing majority of the problems associated with PAHs. Bioremediation using various microorganisms or active consortia can effectively degrade these hydrocarbons. This chapter enlists the properties and the hazards associated with these PAHs. The overall procedure of bioremediation including the factors affecting is also discussed charting the main bacterial and fungal species involved in biodegradation of these hydrocarbons. Through the works of many researchers, many new species or an actively respiring consortium of species, namely, *Mycobacterium*, *Sphingobacterium*, *Sphingomonas*, *Pseudomonas*, *Alteromonas*, *Streptomyces*, and *fungi* like *Irpex lacteus* and *Aspergillus fumigatus*, have emerged as potential PAH degraders. The biochemical basis as well as the impact, various techniques used, and efficiency of these species for each of the above four hydrocarbons in contaminated soil samples, tidal sample, petrochemical refinery, and mangrove soils has been individually discussed.

15.1 Introduction

Environment pollution has been one of the great concerns to the science and the society. The physical and chemical properties of pollutants determine their persistence and toxicity in the soil environment and therefore affect the activity of

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Authors Simran Bhatia and Moyna Kalia have been equally contributed to this chapter.

S. Bhatia · M. Kalia · B. Singh (✉)
Department of Biotechnology, Panjab University, Chandigarh, India
e-mail: sbaljinder@pu.ac.in

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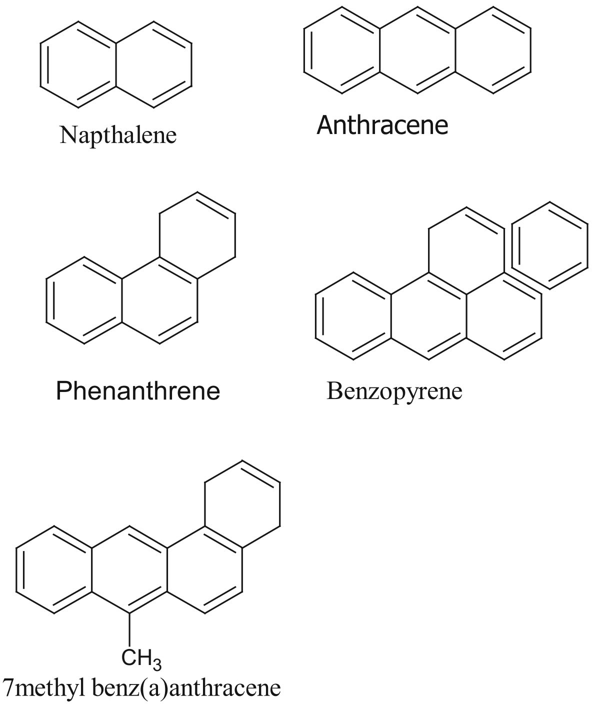

Fig. 15.1 The above figure shows some of hydrocarbons like naphthalene, anthracene, phenanthrene, and benzopyrene and some of their derivatives in their active as well as inactive form

microorganism in a soil ecosystem (Singh et al. 2012, 2014; Singh and Singh 2016a). Polyaromatic hydrocarbons (PAHs) belong to the category of organic compounds that primarily contain fused benzene or pentacyclic rings that are arranged in variety of structural conformations. The hydrophobic effects with low solubility in water are the major reasons for their recalcitrant nature. Natural sources include forest and oil seeps, volcanic eruptions, land fires, and tree exudates. Some other sources of PAHs include burning of wood, fossil fuel, used lubricating oils, garbage, and oil filters and petroleum spills and discharge. They are ubiquitous molecules which also exhibit toxicity, mutagenicity, and carcinogenicity. These hydrocarbons were the first to be recognized as environmental carcinogens. Degradation is difficult under natural conditions. Persistence has a directly proportional relationship with the molecular weight. However they are the primary air pollutants, soil acts as the eventual reservoir of these molecules. There are various fate of PAHs in environment such as leaching, volatilization, adsorption on soil particles, chemical oxidation, photooxidation, and microbial degradation (Haritash and Kaushik 2009). Some of the polyaromatic hydrocarbons in their active and inactive form have been shown in Fig. 15.1.

The point sources of PAH pollution are from human activity. However the areas of contamination are comparatively small in size, the chemical concentration of the contaminant is often high and is mostly associated with co-contaminants such as ethylene, xylene toluene, and benzene compounds, aliphatic hydrocarbons, and heavy metals that can decrease the efficiency of bioremediation efforts (Bamforth and Singleton 2005).

15.2 Properties and Persistence of PAHs

PAHs contain pentacyclic rings or fused benzene arranged in angular, cluster, or linear fashion. PAHs have been broadly classified into two classes based on number of rings in the structure, high molecular weight (HMW, including four or more rings), and low molecular weight (LMW, including three or fewer rings). The persistence of PAHs is mainly contributed by high hydrophobicity and chemical stability. With increasing molecular mass, the hydrophobicity also increases, along with decrease in aqueous solubility for LMW PAHs (low mg/l) and HMW PAHs (1 μg/l). Due to hydrophobic effects, low volatility, and higher affinity for sediment particles, PAHs are readily absorbed in soil and aquatic environments and get consistently circulated through air. Several PAHs have mainly A, B, Bay, K, and L regions that can be metabolized yielding highly reactive epoxides. Figure 15.2 shows active regions of PAHs. Carcinogenicity has been found in some of these epoxides.

PAHs do not often come across alone in the environment, and various interactions take place within a mixture of PAHs enhancing both the carcinogenicity and the genotoxicity. The bioavailability, dispersion, and physical and chemical properties are various factors that determine their higher persistence in the external environment. Generally the longer environmental persistence of PAHs leads to higher toxicity. For example, the average half-life ranges from 16 to 126 days in soil for tricyclic phenanthrene and 229 to 1500 days for the five-ringed HMW PAH benzo[a]pyrene, naphthalene, anthracene, phenanthrene, fluoranthene, and pyrene. Biotic factors also hold a lot of importance because the soil microorganisms can metabolize a variety of PAHs. The dwelling time for PAHs in the environment is also affected by many factors such as microbial-degraded PAH metabolites, the presence of co-substrates, and metabolic repression. The persistence of these hydrocarbons is also regulated by physicochemical parameters like soil properties and structure, water levels, optimum temperature, pH, oxygen, and nutrient (Chauhan et al. 2008).

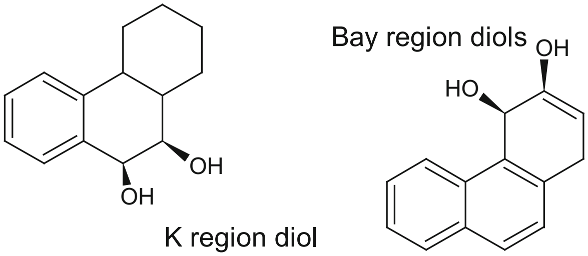

Fig. 15.2 A,B, Bay, K, and L regions of PAHs involved in the fermentation of metabolically active epoxides

15.3 Toxicity of PAHs

From a very long time, PAHs are found to have hazardous effects to human health. PAHs were recognized with highly selective toxicity by several organizations including the US Environmental Protection Agency (US EPA), Joint FAO/WHO Expert Committee on Food Additives (JECFA), Scientific Committee on Food (SCF), International Agency for Research on Cancer (IARC), and European Food Safety Authority (EFSA). PAHs affect all organs of the body displaying properties of acute carcinogenicity, mutagenicity, and teratogenicity. PAHs are absorbed by practically all routes including the gastrointestinal tract, mucous membranes, skin, and lungs because of their high lipophilicity. Benzopyrene is found to be the most potent carcinogen of all the compounds. A deadly consequence of the rapid absorption of PAHs is their biological magnification in the food chain. Generally, the greater toxicity of the PAHs results from the greater number of benzene rings. The relative toxic effects of PAHs can be accurately calculated using LD_{50} values. The enzymatically activated form of PAHs such as epoxides and quinones is considered to be genotoxic. It is also reported that epoxides and quinones can combine with DNA to give covalent adducts with DNA. Mutations of the DNA can arise from these adducts resulting in the formation of tumor (Selina M Bamforth et al.). Degradation metabolism of some of the PAHs is shown in Fig. 15.3.

15.4 The Solution: Bioremediation

Bioremediation, also known as biorestoration and bioreclamation, can be defined as the method through which pollutants are degraded biologically under engineered process. The underlying principle behind this technique is to either reduce the

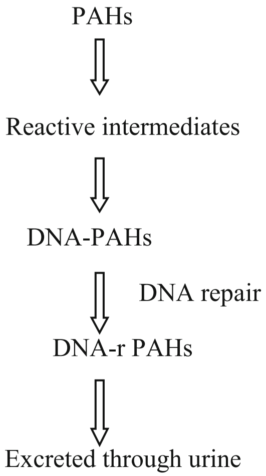

Fig. 15.3 The above figure shows degradation metabolism of PAHs in the body and formation of reactive metabolites from these compounds

toxicity or remove pollutants from the natural environment using the inhabitant microbes of the polluted environment (Prasad 2017, 2018).

Biodegradation approach is developed to increase the microbial metabolism of pollutants, by maintaining the oxygen, water, and nutrient supply (Singh et al. 2013; Singh and Singh 2016b). This can be achieved by either biostimulation (nutrients and oxygen are applied for stimulating the existing bacteria) or bioaugmentation (addition of cultured microbes into the subsurface for solving the purpose of biodegradation) of the polluted environment. Biodegradation of PAH-polluted water and soils can be achieved by in situ treatment or ex situ methods including composting and biopiling. Bioreactors can also be used to treat waste but the major disadvantage being the cost factor as compared to in situ technologies. It is therefore utmost essential for bioremediation to match the physical as well as chemical treatments like soil washing, incineration, and landfilling in terms of cost and success. The application of bioremediation can vary and depends on site conditions, and a detailed knowledge of sites will yield more effective results. From a more commercial point of view, sites having high amounts of PAHs are not suitable for the biodegradation of PAH-polluted soils. This is due to the presence of more than four rings that leads to higher molecular weight which results in economic unviability.

15.5 Metabolism of PAHs by Microbes

The microbial degradation of PAHs under aerobic conditions includes three fundamental mechanisms which are mainly bacteria and ligninolytic and non-ligninolytic fungi. All these three mechanisms involve the initial oxidation of the aromatic ring, followed by the sequential breakdown of PAH metabolites or complete mineralization with release of carbon dioxide, etc. The microbial metabolism of PAHs under anaerobic conditions occurs via the hydrogenation process involving the aromatic ring.

15.6 Factors that Affect the Bioremediation of PAHs

There are many applications of bioremediation of PAHs using methods like composting and biopiling. There are many factors that affect bioremediation of PAHs. These include temperature, pH, and nutrient availability under laboratory and natural conditions. However, it is apparent that environmental conditions can considerably affect the process of bioremediation by curbing the growth of the pollutant-degrading microorganisms. The major environmental conditions that could affect the efficacy of bioremediation as a process are as follows.

15.6.1 Temperature

Temperature affects the growth of microorganism in PAH-contaminated sites, and in general, an optimum temperature balance is impossible to maintain every season throughout the year. The increase in temperature increases the solubility and bioavailability of the PAH molecules. The metabolic activity of aerobic microorganisms is affected by its solubility as solubility of oxygen decreases with increasing temperature. Biodegradation of PAHs mainly occurs on mesophilic temperatures, but it is also seen that certain microorganisms have capability to degrade PAHs at severe temperatures.

15.6.2 pH

The biodegradation of PAHs in the soil is also affected by pH, and the majority of the PAH sites are not at the optimal pH for biodegradation. The majority of PAHs present at contaminated sites give rise to acidic environments due to their oxidation (oxidation of coal and sulfides). Therefore increase in the pH of soil by adding chemical such as CaO results in increase in biodegradation rate of PAHs. However optimum pH for bioremediation of PAHs is 5.5–7. Studies shed further light that future research can be benefitted from isolating and characterizing the PAH-digesting microbes from both acidic and alkaline conditions.

15.6.3 Oxygen

The microbial bioremediation of PAHs occurred under both aerobic and anaerobic conditions, but majority of microbes degrade PAHs under strict aerobic conditions, the reason for this being the ease with which aerobic microorganisms can be cultured. The aerobic biodegradation of PAHs involves the activity of mono- and dioxygenase enzymes followed by ring cleavage. However, the presence of oxygen is limited in natural conditions because oxygen is consumed in the aerobic biodegradation processes. This decrease in oxygen results in the reduction of electron acceptors such as sulfate iron or nitrate. Hydrogen peroxide, sodium nitrate, and perchlorate have been primarily used. However, under denitrifying condition anaerobic biodegradation rate of PAHs was almost similar to aerobic conditions as compared to normal conditions where aerobic degradation was higher than anaerobic degradation. The number of anaerobes capable of degrading PAHs is relatively scarce. The sulfate-reducing bacteria are involved in the biodegradation of PAHs under anaerobic conditions. The reductive pathways are involved in the degradation of PAHs under anaerobic conditions.

15.6.4 Availability of Nutrient

The carbon, nitrogen, phosphate, and energy sources are required for the growth of microorganism. The PAH-contaminated sites decrease the bioavailability of nutrient to microbes, and the nature of pollutants determines the chemical complexity in soil. The increase in aromatic ring of PAHs decreases the microbial metabolism of PAHs in soil. Therefore addition of nutrient is generally required to enhance the bioremediation activity of microbes. The amounts of N and P required for optimal microbial growth and hence bioremediation can be estimated from the C/N/P ratio analysis in microbial biomass.

15.7 Pyrene and Benzo[a]pyrene

Bacteria are primarily the most important class of microbes used for bioremediation. A variety of bacterial species are known to metabolize aromatic hydrocarbons. Mostly, they are isolated from contaminated soil or sediments. Long-standing petrochemical waste discharge is a site that harbors an actively respiring culture of those bacteria that can degrade these hydrocarbons to a substantial extent. Among the various aromatic hydrocarbons in petrochemical waste, benzo[a]pyrene is considered as the PAH with the highest carcinogenicity and toxicity. Subsequent studies have also revealed that bacteria are capable of degrading benzopyrene while alternatively growing on a carbon source in a liquid culture media experimentation. A 5% decrease in BaP concentration was observed by Ye et al. after a time period of 168 h when incubating with *Sphingomonas paucimobilis* strain EPA 505. Organisms charted out at least included three species of *Pseudomonas* and of *Agrobacterium*, *Bacillus*, and *Sphingomonas* species. Benzopyrene is also known to be metabolized by some other bacteria which include *Rhodococcus* sp. and *Mycobacterium* and also some cultures of *Pseudomonas* and *Flavobacterium* species which act as mixed cultures. A bacterial isolate which was capable of mineralizing pyrene was described by Heitkamp et al. Romero et al. isolated *Pseudomonas aeruginosa* from a stream which was extensively polluted by a petroleum refinery. Rehmann et al. isolated a *Mycobacterium* spp. strain KR2 from a soil contaminated with aromatic hydrocarbons of a gaswork plant, which was capable of utilizing pyrene as solitary and primary source of carbon as well as energy. The isolate metabolized up to 60% of the pyrene added (0.5 $\text{mgml}-1$) within 8 days at 20 °C. Cis-4,5-pyrenedihydrodiol, 4-5-phenanthrenedicarboxylic acid, 1-hydroxy-2-naphthoic acid, 2-carboxybenzaldehyde, phthalic acid, and protocatechuic acid were identified as the degradation products, and subsequently a degradation pathway for pyrene was also identified (Haritash and Kaushik 2009).Metabolism of pyrene degradation is shown in Fig. 15.4.

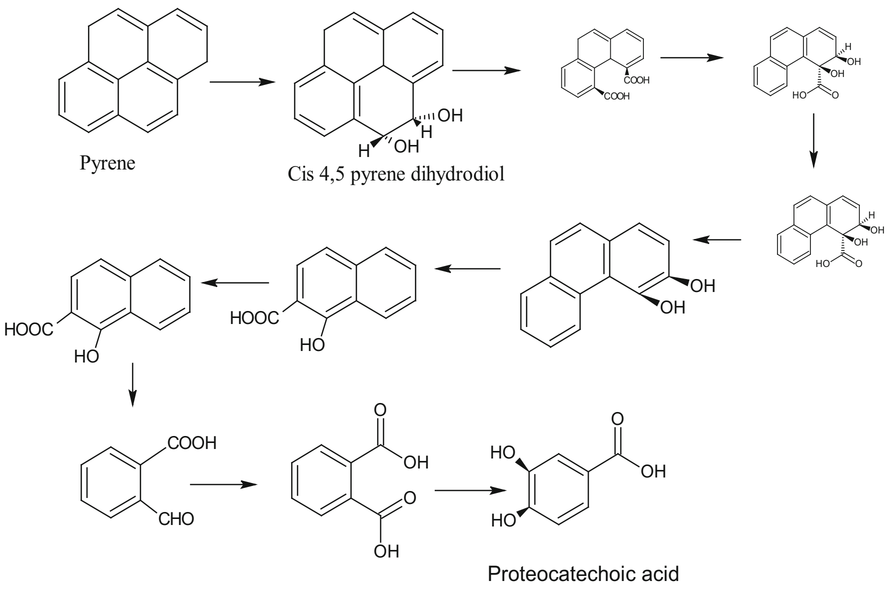

Fig. 15.4 Proposed degradation of pyrene by *Mycobacterium* sp.

15.7.1 Degradation of Benzo[a]pyrene by Mitosporic Fungi and Extracellular Oxidative Enzymes

The degradation of benzopyrene is mainly performed by three mitotic fungi (*Deuteromycetes*), which involved extracellular oxidative enzymes – laccase, lignin peroxidase, and manganese-dependent peroxidase, mainly a high-molecular-weight polycyclic. These fungal strains in study were found to have different capabilities of degrading benzo[a]pyrene. After several studies it was found that relative degradation percentages per unit biomass for *Fusarium solani*, *Trichoderma viride*, and *Fusarium oxysporum* were approximately 39, 17, and 8, respectively (Verdin et al. 2004).

15.8 Phenanthrene Bioremediation

Biodegradation of PAHs in contaminated sediment is a prominent bioremediation technique, and its achievement depends on kinetics and the optimal state for the PAH-degrading microbes; however, knowledge on this feature still remains scarce (Table 15.1).

Various microorganisms were under study to study the effects of various factors on biodegradation of microorganisms. One such study was carried using bacteria *Sphingomonas* in contaminated sediment slurry. It was found that the most significant factors were salinity and inoculum size, whereas the effect of phenanthrene

Table 15.1 Different PAHs along with some of microorganisms which degrade them and the metabolic pathway of their degradation

PAH	Microorganism	Isolation	Biodegradation pathway	%age degradation	Technique	References
Benzo[a] pyrene	*Mycobacterium* sp. strain KR2	Contaminated soil of a gaswork plant	Utilized pyrene as a sole carbon and energy source	60%		Haritash and Kaushik (2009)
Benzo[a] pyrene	Consortium of *Sphingobacterium* and *Mycobacterium*	Contaminated soil	Metabolic conversion of BaP to $^{14}CO_2$	65%	Radiocarbon mass balance analysis and PCR	Haritash and Kaushik (2009)
Phenanthrene	*Achromobacter insolitus* MHF ENV IV	Petrochemical refinery field	Intermediates were formed like salicylic acid, catechol, and salicylaldehyde	100%, 56.9%, and 25.8% at concentrations 100, 250, and 500 mg/l, respectively	GC-MS	Haritash and Kaushik (2009)
Naphthalene	*Alteromonas* sp., SN2	Coastal areas	Gentisate pathway	Efficiency is 20-fold higher in winter season than in summer season	Detected using 16s rRNA-specific PCR primers and GC-MS	Jin et al. (2012)
Naphthalene	*Neptunomonas napthovorans*	Marine bacteria	Degrade 1-methylnapthalene and 2-methylnaphthalene	Highly efficient new strain	16s ribosomal-based phylogenetic analysis of different strains	Hedlund et al. (1999)
Anthracene	*Aspergillus fumigatus*		Electron oxidative pathway forming anthraquinones as intermediate	60% efficiency after 5 days of experiment	Experimental degradation with pH between 5 and 7.5 and 30-degree temperature	Ye et al. (2011)
Anthracene	*Pseudomonas* and *Beijerinckia*	Variable	Hydroxylation reactions forming dihydroxy dihydroanthracene	Variable		

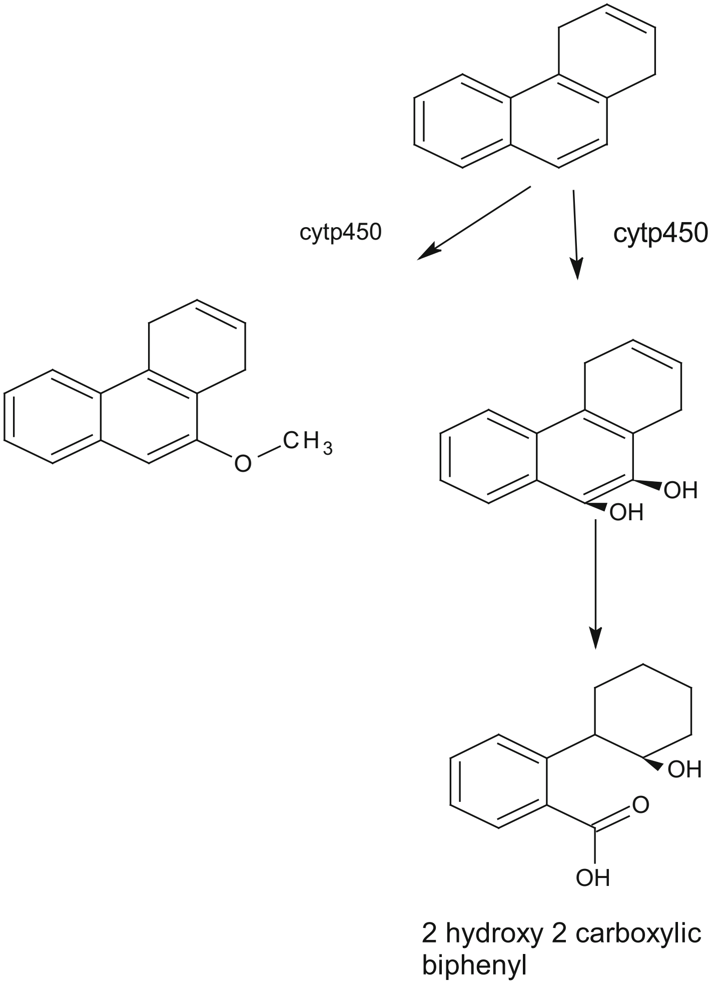

Fig. 15.5 Degradation of phenanthrene using cytochrome p450 forming 2-hydroxy-2-carboxy biphenyl

concentrations, nutrient addition, and temperatures was comparatively low. Also the most favorable conditions for biodegradation in contaminated mangrove sediment slurry were seen at a temperature of nearly 30 °C, 15 ppt salinity, a carbon/nitrogen ratio of 100:1 (the background ratio in sediment), and an inoculum size of 106 most probable number gram 1 sediment. Apart from bacteria, fungi can also be used for degrading phenanthrene. The fungus *Irpex lacteus* is mainly used, and the pathway is shown in Fig. 15.5 (Chen et al. 2008).

In the developing countries like India, a lot of industrial areas are present which discharge their effluent containing an enormous amount of polyaromatic hydrocarbon causing a detrimental effect on the water-soil environment. Based on the results of various biochemical tests and analyses of the 16S rDNA gene sequence, the conglomerate was identified to consist of microorganisms like *Sphingobacterium* sp. and *Bacillus cereus* and a novel bacterium *Achromobacter insolitus* with a very effectual phenanthrene-metabolizing ability. The statistics of phenanthrene biodegradation indicates about 100%, 56.9%, and 25.8% degradation at concentrations of 100 mg/l, 250 mg/l, and 500 mg/l, respectively, in the time period of 14 days.

Another bacterium *Achromobacter insolitus* has been reported for the very first time to mineralize phenanthrene effectively which was further confirmed by GC-MS analysis and by further detecting the intermediates like salicylic acid, salicylaldehyde, and catechol. All the results showed that the microbial conglomerate has a hopeful purpose in biodegradation of petrochemical-contaminated environments and could be of great use for the study of PAH degradation (Janbandhu and Fulekar 2011).

15.8.1 Phenanthrene Degradation by Biosurfactant-Producing Bacteria

Studies revealed the degradation of phenanthrene by phenanthrene-degrading and biosurfactant-producing bacteria. For example, in one of the studies, the bioavailability of phenanthrene was investigated in phenanthrene-degrading bacteria *Pseudomonas* strain R and an isolate P5-2 in the presence of biosurfactant-producing bacteria *Pseudomonas aeruginosa* ATCC 9027 and biosurfactant rhamnolipid. It was observed that *Pseudomonas* strain R mineralized PHE more than that of isolate P5-2. Also by the addition of biosurfactant rhamnolipid, the PHE degradation was increased by *Pseudomonas* strain R, whereas no effect was seen in the case of isolate P5-2. Similar results were seen in the case of other soil samples like sandy loam soil. The effect of rhamnolipid was seen only if it was added above a critical micelle concentration. These results showed that microorganisms degrading hydrocarbons in sorbed conditions might be interacting with biosurfactant-producing bacteria in an unknown manner which enhances the biodegradation of hydrocarbons (Dean et al. 2001).

15.9 Metabolism and Degradation of Naphthalene

Simple PAHs like naphthalene and its derivatives have been found to be increasingly toxic in their crude form. They have been extensively found in water-soluble fractions of water and crude oil. The ability of bacteria and fungi to degrade polyaromatic hydrocarbons has been known for years. A large number of bacteria have been discovered in the recent years for their activity of degrading hydrocarbons. Some of them include *Mycobacterium* sp., *Pseudomonas* sp., *Alcaligenes*, *Sphingomonas*, *Rhodococcus*, and *Nocardia*. Among these naphthalene-degrading bacteria includes *Mycobacterium* sp., *Alcaligenes* *denitrificans*, *Pseudomonas fluorescens*, *Pseudomonas putida*, *P. paucimobilis*, *P. vesicularis*, *Rhodococcus* sp., *P. cepacia*, *Bacillus cereus*, *Corynebacterium renale*, *Streptomyces* sp., *Vibrio* sp., etc. (Samanta et al. 2001).

15.9.1 Mechanism of Bacterial Oxidation of Naphthalene

Bacteria's ability to use naphthalene as sole source of carbon and energy has been reported early in 1927 (Phale et al. 1997). Many years later salicylic acid was isolated from cultures of *Pseudomonas aeruginosa* grown on naphthalene (Phale et al. 1997). From then onward salicylic acid is believed to be an intermediate in metabolic pathway for degradation of naphthalene. The sequential degradation of naphthalene was first described by Davies and Evans.

The degradation pathway of naphthalene involves formation of dihydrodiol intermediates formed by double hydroxylation of its aromatic rings. Trans-naphthalene dihydrolipoyl has been found to be present in naphthalene-metabolizing strains of *Pseudomonas* and *Nocardia*. NMR spectroscopic studies of dihydrolipoyl proved that it is a cis-naphthalene, and reduction of this with palladium forms hydroxy 1,2,3,4 tetrahydronaphthalene which gave the exact structure of bacterial naphthalene (Phale et al. 1997). The enzymatic conversion of cis-naphthalene dihydrolipoyl from naphthalene is catalyzed by the enzyme naphthalene dioxygenase (Phale et al. 1997). The enzyme is found to have three basic components as follows:

1. NADH-ferredoxin reductase. It accepts electrons from NADPH and is an iron-sulfur flavoprotein. It functions as NAD(P)H oxidoreductase in naphthalene dioxygenase system.
2. It is ferredoxin-type component and is an intermediate in electron transport system.
3. Oxygenase component. It binds naphthalene and takes part in terminal step of naphthalene oxidation. In the degradation pathway for naphthalene, catechols formed from salicylate are further subjected to meta or the ortho pathway forming 2-hydroxy-muconic semialdehyde or cis,cis-muconic acid, respectively. In the oxidation process of naphthalene, all the strains used were found to have genes of meta pathway possessing activities of enzymes for both ortho and meta pathways of catechol oxidation: catechol 1,2 dioxygenase and catechol 2,3 dioxygenase (Filonov et al. 2000).

15.9.2 Degradation of Naphthalene in *Pseudomonas putida*

The metabolic pathway for degradation of naphthalene in *P. putida* is explained diagrammatically in Fig. 15.6 (Filonov et al. 2000). The degradation of naphthalene has also been studied in *Streptomyces griseus* NRRL 8090 strain. It produced

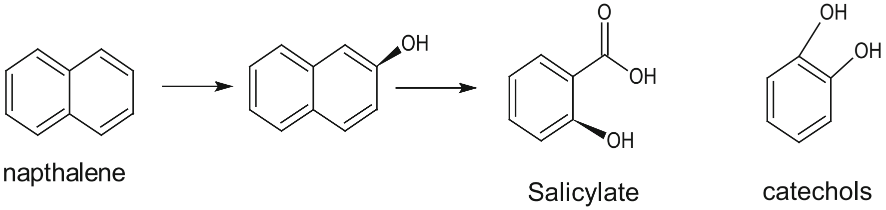

Fig. 15.6 Pathway of degradation of naphthalene in *Pseudomonas putida*. Salicylate is involved as an intermediate in the degradation pathway

4-hydroxy-1-tetralone in a good amount. *S. griseus* was not found to catalyze the biotransformation of tetralin and 1,4-naphthaquinone (Gopishetty et al. 2007).

15.9.3 Degradation of Naphthalene in Fungi

A wide range of taxonomically different fungi have been known to metabolize naphthalene. Ligninolytic and non-ligninolytic fungi both can metabolize naphthalene. Fungi do it via cytochrome monooxygenase pathway. They incorporate one atom of molecular oxygen to form naphthalene-1,2-oxide. The enzyme catalyzing the oxidation is cytochrome p450 monooxygenase. The arene oxide generated is highly unstable and is broken down into 1-napthol (major) and 2-napthol (minor) by rearrangements. Another pathway for further oxidation is via enzymatic hydration of arene oxide catalyzed by epoxy hydrolase to form trans-dihydroxy 1,2-dihydronaphthalene pathway for degradation is (Phale et al. 1997). Figure 15.7 shows the degradation mechanism of naphthalene in fungi:

15.9.4 Degradation of Naphthalene by *Alteromonas* Species in Crude Oil-Contaminated Coastal Sediment

In the 2007 tidal spills of South Korea, some of the microorganisms were found to affect the environmental fate of these PAHs. Enrichment cultures were established using naphthalene as carbon source. The microbes were profiled using 16S rRNA-DGGE sequencing, and selected bands indicated that this particular bacterium was active. *Alteromonas* sp., SN2, was isolated which degraded naphthalene, anthracene, phenanthrene, and pyrene. Following this, PCR analysis was done which shows the presence of naphthalene dioxygenase genes of two species: *Alteromonas* and *Cycloclasticus*. These two species have gentisol and catechol metabolic pathways, respectively. Analysis by GC-MS shows degradation of naphthalene by gentisate pathway, in tidal flat samples. From this data it was concluded that SN2 species and its relatives can act on broad range on PAHs and are most active during winter season (Jin et al. 2012).

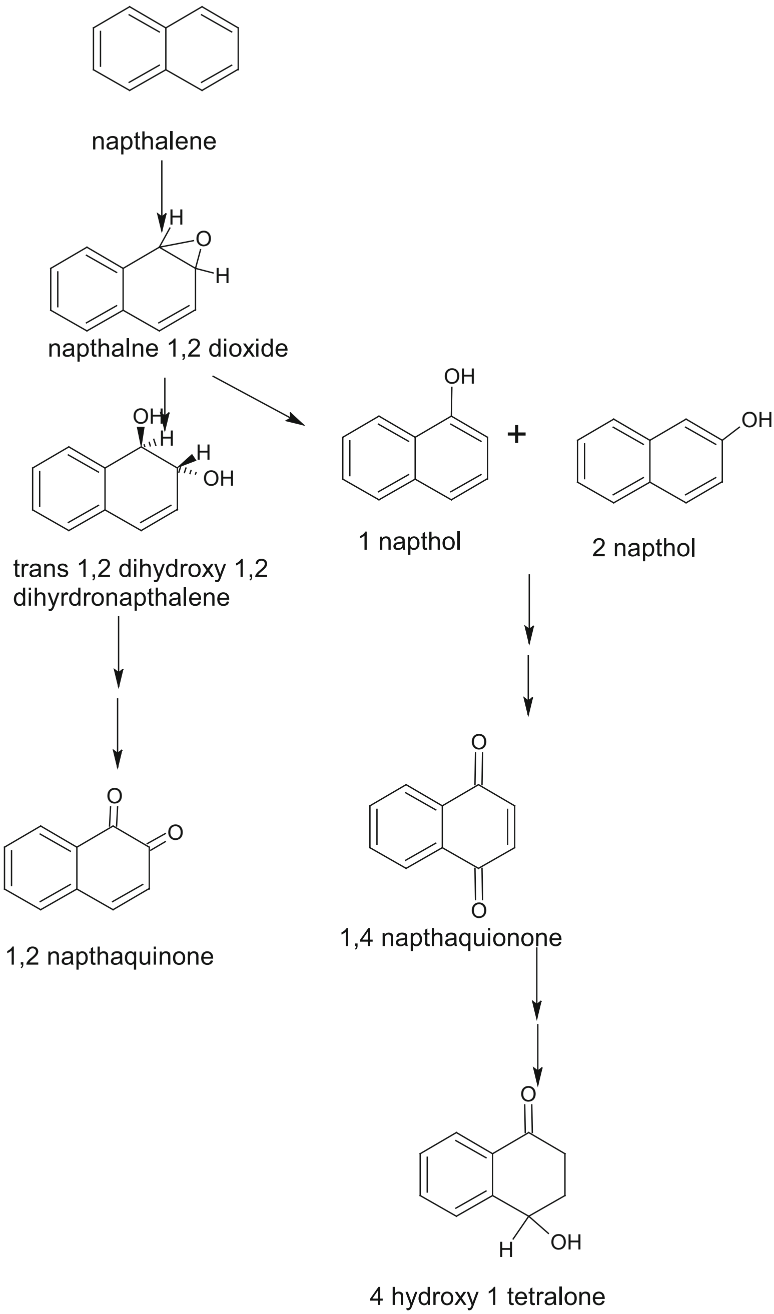

Fig. 15.7 Degradation of naphthalene by non-ligninolytic fungi. 1-Napthol and 2-napthol are formed as major and minor products, respectively

15.9.5 Degradation by Marine Bacteria *Neptunomonas naphthovorans*

Two strains of bacteria were isolated based on their ability to utilize naphthalene as sole source of carbon. Both the strains were found to degrade 2-methylnaphthalene and 1-methylnaphthalene when incubated with polyaromatic hydrocarbons. In addition another strain NAG-2N-113 also degrades 2,6-dimethylnaphthalene and phenanthrene. Naphthalene dioxygenase iron-sulfur protein (ISP) gene was isolated using PCR. These bacteria were placed in gamma-3 subgroup of *Proteobacteria*, closely related to *Oceanospirillum* (Hedlund et al. 1999).

15.10 Metabolism and Degradation of Anthracene

Anthracene and its metabolites are not directly found to be carcinogenic, but their presence in toxic metabolites specifies the need for their degradation. Pure microbial cultures from marine and fresh water have been found to degrade anthracene and its compounds. The decomposition of anthracene comes out to be a total decomposition into end product anthraquinone. The degradative pathway is one electron oxidative pathway giving rise to anthrol and anthrone. The oxidation of anthracene by nucleophilic attack at ninth and tenth position is via one electron oxidative pathway. This results in the formation of 9,10-anthraquinone by spontaneous nonenzymatic rearrangements (Collins et al. 1996). The oxidation product of anthracene is phthalic acid and that of fluoranthene is 4-hydroxy-9-fluorenone.

15.10.1 Degradation by Fungi

Aspergillus fumigatus shows a great range for degradation of anthracene. The optimal temperature for degradation is 30 °C, and optimal pH is between 5 and 7.5 (Ye et al. 2011). Very little is known about anthracene degradation in fungal systems. Cerniglia and his colleagues in 1982 found that *Cunninghamella elegans* oxidizes anthracene to trans-1,2-dihydroxy-1,2-dihydroanthracene and 1-anthryl sulfate. The degradation pathway is shown in Fig. 15.8 (Phale et al. 1997).

15.10.2 Degradation by Bacteria

The degradation of anthracene in bacteria is performed via hydroxylation. The initial steps in hydroxylation of anthracene involve oxidation of anthracene to form cis-anthracene dihydrodiol. Double hydroxylation of rings gives rise to trans-1,2-dihydroxy-1,2-dihydroanthracene. *Pseudomonas putida* and *Beijerinckia* both show double hydroxylation reaction. Hydroxy naphthalene, naphthoic acid, salicylic acid, and catechols are found to be the reaction intermediates. The metabolism of 2-hydroxybenzoate and its role in anthracene metabolism are yet to be discovered.

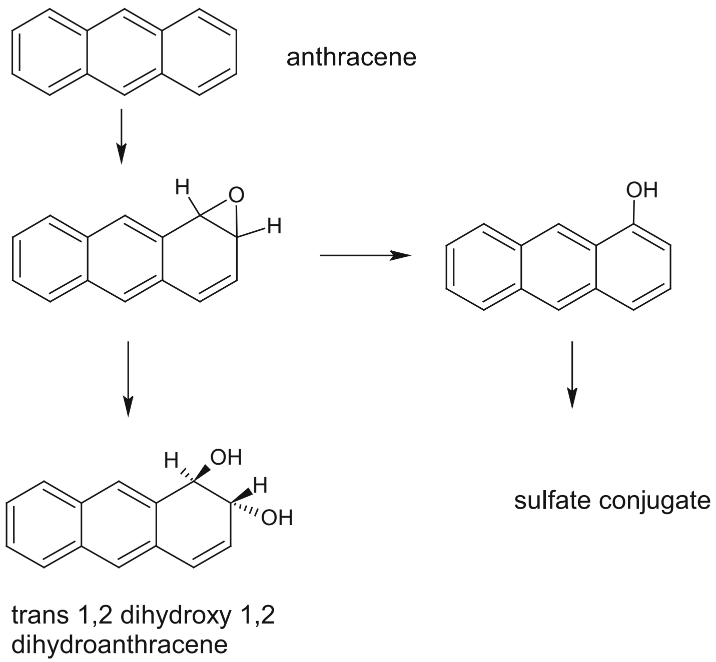

Fig. 15.8 Degradation of anthracene by fungi

The pathway for anthracene degradation in bacteria is described in Fig. 15.9 (Phale et al. 1997).

15.11 Principles of Degradation of Hydrocarbons in Soil

PAHs are dangerous to human health. The ubiquitous distribution and high persistence in the soil make them easy targets for biodegradation mechanisms. PAHs are highly hydrophobic and interact with nonaqueous phases and soil organic matter which makes its degradation difficult by microorganisms. Since bacteria are known to degrade chemicals only in their aqueous phase, PAHs of high molecular weight which are insoluble in water are of greater environmental concern. Therefore conditions must be optimized for degradation of PAHs by making them bioavailable to microbes (Johnsen et al. 2005). Surfactins are compounds which are found to increase the bioavailability of PAHs. Cyclodextrins are natural compounds that are found to increase the bioavailability by forming complexes with hydrophobic molecules (Bardi et al. 2000).

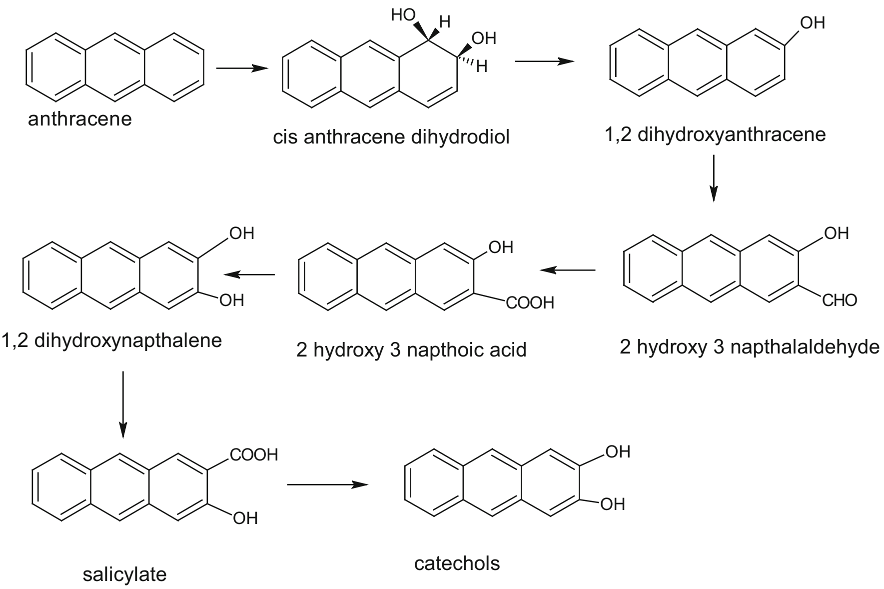

Fig. 15.9 Anthracene degradation in bacteria. Catechols are formed as end products which are processed further

15.12 Conclusion

In the last 20 years, our knowledge about bacterial and fungal degradation of PAHs has advanced leaps and bounds. The PAH's contamination has been as malaise and its treatment medicine. Bioremediation is a safety measure for a safe and secure environment in the near future. A lot has already been done to degrade toxic hydrocarbons, but there is still a long way to go. A lot of work is needed to be done to isolate the newer bacterial and fungal strains which degrade hydrocarbons efficiently. Also recombinant strains and their associated plasmids needed to be studied. The metabolic pathway of phenanthrene, benzo-pyrene, naphthalene, and anthracene is to be studied in detail. The mechanism of the key enzymes and all other regulatory pathway of the hydrocarbons is needed to be still worked upon. The overall process of bioremediation is a multidisciplinary technique with the thrust pertaining to microbiological perspectives. No doubt total process of bioremediation is a difficult process, but it is one of the most cost-effective.

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Microbial Assisted Phytoremediation for Heavy Metal Contaminated Soils

16

M. L. Dotaniya, S. Rajendiran, C. K. Dotaniya, Praveen Solanki, V. D. Meena, J. K. Saha, and A. K. Patra

Abstract

Increasing water crisis across the globe, farmers are forced to use marginal quality water for agricultural activities mainly for crop production. Marginal quality water contains lots of contamination load, i.e. microbial population, heavy metals; and caused a range of diseases through food chain contamination. The long- term application of contaminated water accumulate significant amount of heavy metals mostly in industrial regions as well as peri-urban area in developing countries. Use of various phytoremediation technologies for the removal of organic and inorganic pollutant from soil and water are used across the earth boundaries. Among all, bioremediation is a cheaper and more viable technology for the removal of contaminants from contaminated sites. Phytoremediation is a viable, low cost and green technology having a slow process of metal remediation and affecting by the climatic conditions of a particular region. In this regards, use of soil microbial biomass for the decontamination of heavy metals and other contaminated load from soils. The plant-microbe- modulated phytoremediation enhancing the heavy metal remediation, detoxification and mediated the plant nutrient dynamics in a sustainable manner. The soil organic matter decomposition and biogeochemical cycles of plant nutrients are mainly governed by the rhizospheric biomass of the soil. Microbial assisted phytoremediation is a holistic novel approach for the remediation of contaminants. It can use for the location specific contaminant, easy to operate, eco-friendly in nature. In this chapter,

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M. L. Dotaniya (✉) · S. Rajendiran · V. D. Meena · J. K. Saha · A. K. Patra
ICAR-Indian Institute of Soil Science, Bhopal, India

C. K. Dotaniya
College of Agriculture, SKRAU, Bikaner, India

P. Solanki
Department of Environmental Science, GBPUA&T, Pantnagar, India

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described the role and interaction effect of plant assisted microbes in heavy metal removal from contaminated soils.

16.1 Introduction

Increasing the production from limited land is forced the farmers to over exploitation of natural resources. These resources, natural resources, i.e. land, water are having its optimum productivity levels and also observed decline rate in agricultural productivity. It is a clear indication of over exploitation of natural resources (Budnuka et al. 2015; Singh et al. 2017). Most of the developing countries are using poor quality of resources with quantum growth in population (Dotaniya et al. 2016a, 2017c). This situation will be worst in the coming year. A huge volume of industrial and household effluent is generating and contaminating water bodies and land (Saha et al. 2017a). Poor quality water is used for the cultivation of crops; accumulated significant amount of metals in soil (Bingham et al. 1986; Dotaniya et al. 2014g; Singh et al. 2016b) and reached to human and animal body via food chain contamination (Hapke 1996; Fischer 2005; Rajendiran et al. 2015; Coumar et al. 2016a, b). Heavy metals are toxic in nature and having an atomic density greater than 5 g cc^{-1} or atomic number more than calcium (Singh 2002; Emamverdian et al. 2015; Rajendiran et al. 2018). In most of the cases trace metals (chromium-Cr, cadmium-Cd, arsenic-As, lead-Pb, mercury-Hg, selenium-Se, aluminum-Al; and essential plant nutrients zinc-Zn, copper-Cu, manganese-Mn) are causing various types of malfunctions in biological system and extreme side caused death (Hapke 1991; Dotaniya et al. 2014d; Lenka et al. 2016; Saha et al. 2017b). The toxicity depends on the type and concentration present in ecosystems (Tchounwou et al. 2012; Saha et al. 2017c). As per the guidelines issued by the Commission of the European Community regarding the heavy metal permissible limit in dry agricultural soils, i.e. Hg (1–1.5 mg kg^{-1}), Pb (50–300 mg kg^{-1}) and Zn(150–300 mg kg^{-1}) (CEC 1986). In plants, poor growth with toxicity symptoms and in soil reduced the soil biological diversity by the heavy metals (Singh et al. 2011). Application of Cr more than 20 mg kg^{-1} reduced the germination, root and shoot growth in wheat (Dotaniya et al. 2014a) and pigeonpea (Dotaniya et al. 2014c). Increasing concentration of Cr reduced the C mineralization rate and enzymatic activities in Vertisol of central India (Dotaniya et al. 2017d). The enzymatic activities are showing the good bioindicators against reflecting the human disturbance in soil ecology (Hinojosa et al. 2004). It is easy to measure soil quality via soil enzymatic activities in cheaper cost (Khan et al. 2007). These toxicity symptoms are well acknowledged by various researchers in different ecosystems (Malley et al. 2006; Oliviera and Pampulha 2006; Wang et al. 2008; Saha et al. 2017c). Many researchers were described the heavy metal toxicity in term of ED_{50} value, means the metal concentration that inhibited 50% reaction rate of enzymes (Huang and Shindo 2000).

Remediation of heavy metals from the soil for the sustainable crop production is a demand of the present situation to combat food shortage for a burgeoning population (Dotaniya et al. 2018c). Most of the countries across the world are more focusing on safe utilization of poor resources for mitigating the food, fodder and related demand (Emamverdian et al. 2015; Saha et al. 2017a). Use of different remediation methods, i.e. physical, chemical and biological; among all, biological method is cheaper and eco-friendly (Dotaniya et al. 2014d). Phytoremedition process using green plants for removing heavy metals from soil water bodies. In which, plant performed various process metabolic and physiological process to decontaminate or removal of process (Singh and Fulekar 2012). Plant secreted a range of low molecular organic acids, which degraded the toxic compounds, immobilized, convert toxic to non- toxic, enhanced uptake are pattern of metals (Dotaniya et al. 2013a, b, d; Dotaniya and Meena 2013). The plant also converted few metals into volatile compounds and release into the environment (Razzaq 2017). In this line, plant associated micro-organisms are also performing a valuable place in remediation of metals (Mandal et al. 2017). Plant secreted organic compounds are the source of food for the microbial population of soil. It enhances the microbial count and diversity in soil and accelerates the remediation process (Dotaniya and Meena 2017). It also secreted various types of plant growth promoting substances, and enhanced the growth of the plant in adverse conditions. Plant secreted phytosiderophores are also enhancing the Fe and Zn concentration in soil under deficiency conditions (Dotaniya et al. 2013a). These situations are more favorable for the biological remediation of metals from soil and water bodies. Microbes reduce the toxicity of metal by decomposition or immobilizing the metals from the soil (Abou-Shanab et al. 2003; Seshadri et al. 2015). In this chapter, most of the microbial assisted phytoremedition mechanisms are described for remediation of metal to enhance the sustainable crop production.

16.2 Heavy Metals Toxicity and the Environment

Heavy metals are metal and metalloid having the high atomic density and trace concentration caused adverse effects on plant, animal and human system (Table 16.1). In recent years, metal toxicity pays more attention towards public health and its remediation from ecosystems. The metal toxicity rose due to geogenic and anthropogenic origin and the second one playing more drastic effect on soil and water contamination (SrinivasaGowd et al. 2010). The point source of metal toxicity is from mining and extracting of metals, smelting, industrial use and foundries (Fergusson 1990; Bradl 2002; He et al. 2005). Several ways contaminants affected the soil-plant-human continuum on earth and caused toxicity symptoms. Environmental contaminations deteriorate the quality of the environment and affect the ecological process and services (Wenzel et al. 1999). Most of the contamination due to atmospheric dry and wet depositions, soil erosion and leaching of heavy metal ions, evaporation of metals via volatilization compounds, trace metal corrosion, automobile exhausts, sewage sludge application and direct contribution from the geogenic origin of metals (Nriagu 1989; Khan et al. 2007; Yang et al. 2002, 2006; Kamal et al.

Table 16.1 Source and effect of heavy metals on human health

Metals	Major source	Effect on human health
As	Geogenic process, smelting operations, thermal power plants, agricultural inputs (pesticides, fungicides)	It is having chemical structure similar to phosphorus and affect the cell activities, mediated ATP process, bronchitis, skin allergy, poisoning
Cd	Zn smelting, paint industries, e-waste, welding, electroplating, pesticides, fertilizers, batteries industries etc	Carcinogenic, renal dysfunction, mutagenic, Ca imbalance, long-term anemia, lung cancer, kidney damage, gastrointestinal disorder, enzymatic disorder
Pb	Lead acid batteries, paints industrial effluent,coal based thermal power plants, automobile industries ceramics, bangle industries, agricultural chemicals	More toxic to infants, poor development of mental in children, damage nerve system, long exposure caused liver, kidney, gastrointestinal cancer, cardio-vascular disease
Hg	Chlor-alkali industries effluent, pesticides, fluorescent lamps, batteries, medical waste, paper industry, electrical appliances.	Fatigue, hair fall, tremors, memory loss, damage kidney and lungs, damage to nervous system, protoplasm poisoning
Cr	Leather industries, industrial coolants, mining, wooden industries	Hair fall, vomiting, fatigue, skin irritation, damage to the nervous system, eye irritation, long exposure caused cancer
Zn	Agriculture fertilizers, sewage sludge, smelting, electroplating, brass manufacture, plumbing	Vomiting, damage to nerve system, skin irritation, weakness
Cu	Cu mining, pesticide formulations, sulphuric acid plant, chemical industry, metal piping, smelting operations	Brain, liver and kidney damage, chronic anemia, stomach irritation, fatigue

2010). Increasing the application of metal contaminants in soil or water bodies enhanced the metal concentration in a system. Chromium is the twenty-first most abundant element in the earth's crust, and one of the toxic metals in the environment (Eliopoulos et al. 2013). Land and water pollution by Cr is a worldwide issue. In Western Europe, 1,400,000 sites were affected by heavy metals, of which, over 300,000 were contaminated, and the estimated total number in Europe could be much larger, as pollution problems increasingly occurred in Central and Eastern European countries specially Cr pollution. In the USA, there are 600,000 brown fields which are contaminated with heavy metals and need reclamation (Bahafid et al. 2013). In India, Cr pollution emerged as a challenge to remediate it. It mainly occurs in tannery and paint industries locations. It occurs in nature in bound forms that constitute 0.1–0.3 mg kg^{-1} of the earth's crust (Dotaniya et al. 2014d). It has several oxidation states ranging from Cr (−II) to Cr (+VI). It exists predominantly in the Cr^{+3} and Cr^{+6} oxidation states (Dotaniya et al. 2017c). The most stable oxidation state of Cr is +III, and under most prevailing environmental conditions Cr (VI) is rapidly reduced to Cr (III) (Dotaniya et al. 2014d). The intermediate states of +IV and +V are metastable and rarely encountered (Lokhande et al. 2011). Application of tannery industrial effluent for crop production accumulated Cr concentration 25–30 times more compared to tube well irrigated fields (Dotaniya et al. 2014g). Such types of studies showing the metal toxicity due to anthropogenic activities and its toxicity effect on soil and plant system. Immobilization of Cr in the plant vacuole of plant roots are the main reasons of Cr concentration higher in plant root than shoot (Oliveira 2012; Nematshahi et al. 2012). Similar type higher concentration of Ni in soil reduced the uptake mechanism of Fe and Zn; and showed chlorosis symptoms on leaves (Khan and Khan 2010). In plant, metal toxicity affects the plant nutrient mineralization rate and release kinetics in soil and ultimately reduced the plant growth (Singh et al. 2016a). Crop plant looks like brushes and crop yield decline drastically. Heavy metal contamination reduced the soil enzymatic activities and carbon mineralization rate (Dotaniya et al. 2017d). Increasing concentration of Cd (2 mg kg^{-1}), in more than 100 mg kg^{-1} Cr contaminated soil; reduce the Cr uptake in spinach crop (Dotaniya et al. 2017a). Zinc toxicity caused induces chlorosis due to deficiency of Fe and Mn in plant (Sivasankar et al. 2012). The deficiency and toxicity of a metal also affected by soil texture, organic matter, soil pH, and concentration of other metals in soil (Bucher and Schenk 2000; Broadley et al. 2007; Aref 2011; Dotaniya et al. 2014d, 2017a). Most of cationic heavy metals are more available in lower pH conditions (Dotaniya and Meena 2013). Higher concentration (150–300 mg kg^{-1}) of plant essential Zn behaves like toxic metal and reduced the plant growth (Yadav 2010).

16.3 Mechanism of Metal Tolerance in Plants

Application of heavy metals produced stress on plant and plant react adversely to counter the negative effect of heavy metal. In this condition secreted various types of secondary metabolites and avoid the harmful effects. It is difficult to measure the signal transduction effect of a plant under stress conditions. Heavy metal toxicity affected the plant physiological and biochemical process and reduces the growth and yield. Singh et al. (2016b) described the toxicity of metals and the plant responds in following ways:

1. Sensing of external stress stimuli.
2. Signal transduction and transmission of a signal information into the cell.
3. Triggering suitable precaution measures for counter the adverse effect.

Metal toxicity reduces the plant mitosis and root elongation process (Hossain et al. 2012a, b; Thounaojam et al. 2012). Some of the metals are analogs of plant essential nutrients, and plant uptake mechanism cannot identify the metal and reach into plant parts (Sivasankar et al. 2012). To avoid the toxicity, the plant having self mechanism and survive in contaminated soils in following ways:

16.3.1 Physical Barrier

A sophisticated and inter-related network of self defense mechanism in plant playing a vital role to avoid metal negative effect under stress. Physical barriers are the first line on defense mechanism, in which cell wall, trichomes, and various types of plant-microbial associations are reducing the metal toxicity in plants (Hall 2002; Wong et al. 2004; Harada et al. 2010). Most of the cases trichomes accumulated the heavy metal orproducing the many types of secondary metabolites to detoxification of metals in plants (Lee et al. 2002; Hauser 2014). If the metal pass through the physical barrier and reached to a cell site, than biosynthesis of different cellular biomolecules are acting as a potential heavy metal neutralizer. During the metal entry into the root, and transfer to the shoot part (mostly in hyperaccumulators) and avoid the metal toxicity by deposing metals in vacuoles (Fahr et al. 2013).

16.3.2 Uptake by Hyperaccumulators

Hyperaccumulator are those plants having higher capacity of metal absorption without affecting growth activities (Ma et al. 2001). It has extraordinary capacity to absorb the metal ion concentration from contaminated sites (Yang et al. 2002). Nowadays plant based metal removal is not in practice due to slow process and limited bioavailability of metals and greatly influenced by the climatic conditions of the regions (Mandal et al. 2016). Hyperaccumulator plants are not accumulated higher amount of metal in different part due to novel genes, but due to differential expression of genes (Verbruggen et al. 2009). A complete mechanism of heavy metal uptake by hyperaccumulator and non-hyperaccumulator are described in Fig. 16.1. Most of the cases plant interact with the heavy metals and affected due to, (1) absorption of plant nutrient, for example, some of the heavy metals are analogs of essential plant nutrients As for P, and Cd for Zn; (2) direct interaction with functional protein groups, i.e. sulfhydryl group (-SH); (3) generation of reactive oxygen species (ROS), it damages the plant cell (DalCorso et al. 2013). Sundaramoorthy et al. (2010) reported that Cr (VI) extended the cell cycle, and leads inhibitor effect on cell division and reduced the growth of the paddy plant. Later on, Yuan et al. (2013) evaluate the toxic effect of Cd and found that, it affected the cell elongation and meristem zones by modifying the auxin distribution via protein and reduce the primary root elongation process. Most of the cases toxic metals affected the functions of each other metals in harmful ways in biological systems.

16.3.3 Role of Metal Analog and Protein

Some of the metals having similar type of physico-chemical properties and plant could not identify the essential plant nutrients or competitive environment reduce the metal uptake by plants. Increasing the application of sulfur (S) reduced the uptake

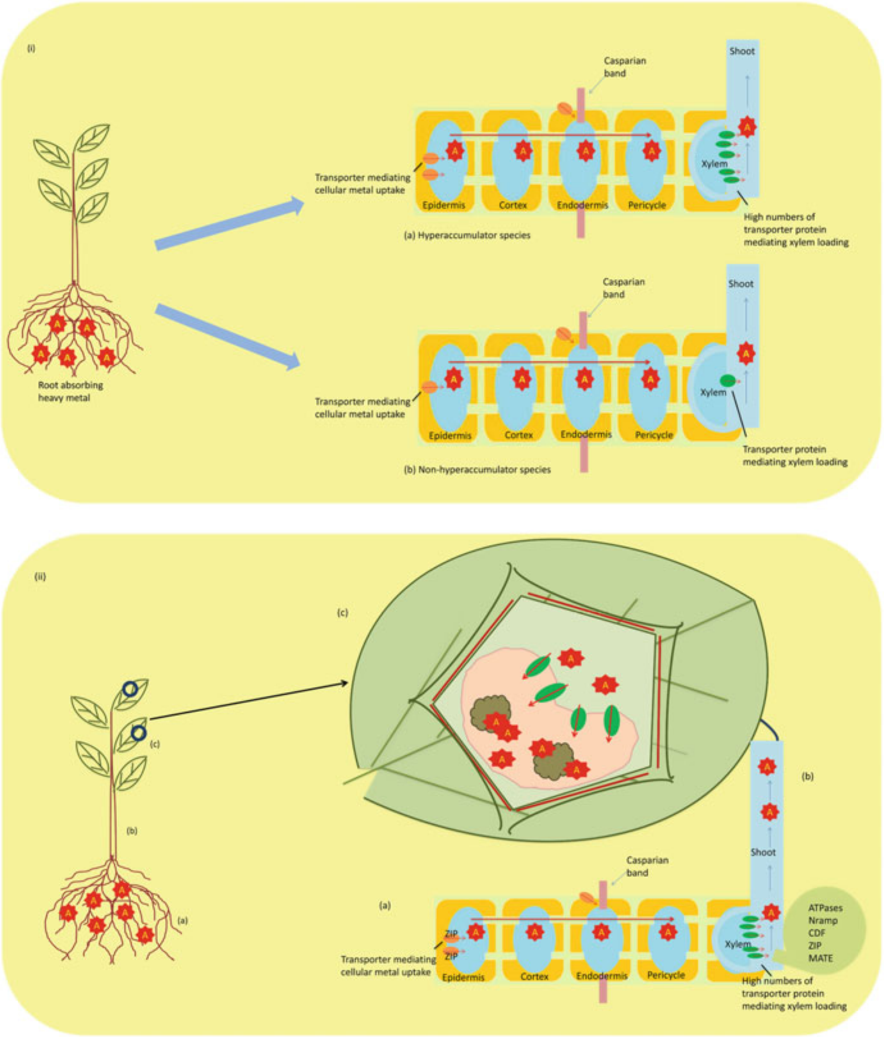

Fig. 16.1 Mechanism of heavy metal uptake and defense mechanisms. (Adopted from Singh et al. 2016b)

pattern of Se in Brassicaceae family plants (Galeas et al. 2007). Increasing concentration of Zn reduces the uptake of Fe and Mn (Sivasankar et al. 2012); Ni toxicity induces Zn deficiency in plants (Khan and Khan 2010). Increasing concentration of Zn reduced the Cd uptake in *T. caerulescens*, due to control of ZIP gene on Zn (Assuncao et al. 2010). The heavy metal uptake from the soil and transported to plant parts via xylem with the help of various proteins, i.e. Heavy metal transporting ATPases (or CPx-type, P1B-type), Natural resistance-associated macrophage proteins (Nramp), Cation diffusion facilitator (CDF) family proteins, Zn–

Fepermease (ZIP) family proteins, and MATE (Multidrug And Toxin Efflux) protein family. All the proteins are having a specific role in metal tolerance by various plants. The CPx-type ATPases involved in transportation of metal such as Cu, Zn, Cd and Pb (Williams et al. 2000); whereas, P1B ATPases type protein regulates metal tolerance and homeostasis in hyperaccumulators (Axelsen and Palmgren 1998). Similar type other protein like CDF involved in regulating cytoplasmic cation activities (Maser et al. 2001).

16.3.4 Soil Enzyme Mechanism

Soil enzymes are the key factors for the determination of any anthropogenic disturbance in soil fertility. These are more sensitive to metal toxicity and affect the plant nutrient dynamics in soil. Soil enzymes make a temporary complex with metal and enhance the availability to plants (Segel 1975). Most of the cases in the soil, enzyme inhibitor (reduce the reaction rate) and activators (increasing the reaction rate) are found and determined the level of soil enzymes. The effect of metals on enzyme functions are a complex mechanism and may also affected by soil (type of soil, pH, EC, organic matter, texture), plant (metal bioaccumulation, transfer ratio, plant type and cultivar), metal (type concentration, mode of action) and also by enzymatic properties (type and sensitivity, structural inhibition) (Tabatabai 1994; Karaca et al. 2009, 2010, 2011; Dotaniya et al. 2017d, 2018a). The soil enzyme reaction rate controlling by the inhibitor and activator is described in Fig. 16.2 (Voet and Voet 1995). Most of the cases, metal characteristics are playing a crucial role; however, Cd affected more negatively than Pb due to its greater mobility and lower affinity with soil colloids (Khan et al. 2007). Another study conducted by Shen et al. (2005) found a negative correlation between Zn and Cd metals due to sorption and exchange sites in the soil.

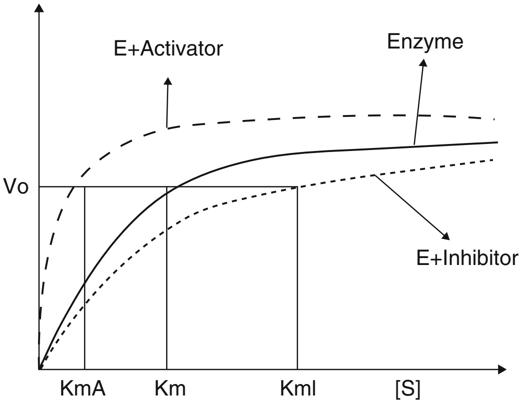

Fig. 16.2 Effect of metal toxicity on enzyme activity

16.4 Mechanism of Metal Tolerance in Microbes

Microbes are the scavenger of contaminants and reduce the metal toxicity in soil towards plants (Saha et al. 2017a). It is responsible for the nutrient dynamics in soil-plant system that affects the soil to soil solution and also solution to plant-roots movement/availability (Dotaniya et al. 2013c, 2014b, e, 2015, 2016a; Dotaniya and Datta 2014). These are also responsible for plant growth regulators/promoters secretion in plants under metal stress condition to protect them from the stress (Dotaniya et al. 2016a). Immobilization of nutrients and metals from soil by microbes is also widely accepted (Dotaniya et al. 2014f, 2016c). The researchers are isolated specific type of soil microorganism for the degradation of toxic metals. Microorganisms are omnipresent in nature and engage in nearly all biological processes of life (Singh et al. 2016a). Metal toxicity occurs in any ecosystem by either natural or anthropogenic enrichment or by both the means. Higher amount of metals in the environment is harmful to microbes, plant, animal and human. Due to increasing and enlarging area under urbanization and industrial activities, proportion of metals use tremendously increased nowadays and resulted in higher accumulation of metals in ecological habitats (Rajkumar et al. 2012). Occupation of metals in native binding sites of microbial cells that is specifically for essential nutrients or metals and through ligand interactions result in metal toxicity in microbes (Bruins et al. 2000). For instances, Hg^{2+}, Cd^{2+} and $Ag^{2+}$are likely to bind with SH groups of some sensitive enzymes and hinter the function of the enzymes (Nies 1999). But at higher concentration whether be a essential or non-essential metals can damage the membranes of microbial cell wall and interrupt the function of the cells by damaging DNA structure and altering enzyme specificity (Bruins et al. 2000). However, some of heavy metal resistant microbes are adaptive to heavy metal rich environments. The possible mechanisms of metal resistance systems in microbes are identified and are elimination through permeability barrier; enzymatic reduction; capturing and sequestering in the cell (either intra- or extra-cellular means); active efflux pumps; and diminution in the sensitive cellular targets to metal ions (Bruins et al. 2000; Nies 1999; Rensing et al. 1999). These mechanisms responsible for microorganisms to overcome metal toxicity and help them function well enough in contaminated environments (Dotaniya et al. 2018d). The energy-dependent active efflux of toxic metal ions is mostly recognized in the largest group of metal resistance microbes. Further, many plasmids and chromosomal responsible metal tolerance mechanisms in bacteria have also been documented.

Biosorption of metals by the bacterial cells is mostly characterized by non-enzymatic process such as, adsorption. Increasing the amount of crop residue in the soil, provide the food material to soil biota that enhance the microbial population and their diversity as well as activity and improve nutrient bio-availability in the soil (Rajendiran et al. 2012; Dotaniya 2013; Dotaniya and Kushwah 2013; Dotaniya et al. 2013b). Mineralization and release of various types of C substrate during the decomposition of crop residue act as a biosorption for metals in soil (Kushwah et al. 2014; Prajapati et al. 2014, 2016) and siliceous material also provide immunity to crop plant (Meena et al. 2013). Polysaccharides and proteins associated with cell surface or extracellular surfaces are involved in adsorption of metals and this is a non-specific binding process (Rajkumar et al. 2010). However, it can be, depending upon the microbial species, either active or passive process and/or both. In addition to chitosan and glucans, chitin present in the cell wall of microbes believed to be effective biosorbent. For instances, the cell walls of fungi, yeasts, and algae, are also reported to be an efficient metal biosorbents. Bioaccumulation of metals in microbial cells is an metabolic energy dependent active process (Martino et al. 2003). Potential metal bioaccumulation mechanisms in the bacterial cell membranes include carrier mediated transport, ion pumps and channels, complex permeation, endocytosis, and lipid permeation. These mechanisms are generally involved in transport of metals like Hg, Pb, Ag, Cd and Ni. The bacterial detoxification of arsenic is often carried out through the chemiosmotic gradient and the intracellular As concentration can be reduced by the active export mechanism through simple As^{3+} efflux systems (Rensing et al. 1999). However this system is not involves in transport of As^{5+}. Therefore, As^{5+} is converted to As^{3+} by the arsenate reductase enzymes which enables microbes to detoxify both the As species. Similarly, Pb resistance also based on metal ion efflux system, i.e. through zinc and cadmium specific pumps in bacterial cells and also Pb-phosphate precipitation within the cells of metal tolerant bacterial species (Nies 1999; Rensing et al. 1999). Microbial transformation of metals through oxidation and reduction and methylation and demethylation also considered as important resistance mechanisms in microbes. For example, microbes can acquire energy through oxidation of Fe, S, Mn and As (Santini et al. 2000). On the other hand, microbes during anaerobic respiration can convert the metals into its reduced state/form through dissimilatory reduction. With this process metal can act as a terminal electron acceptor. Oxyanions of As, Cr, Se and U are the terminal electron acceptors used by the microbes during anaerobic respiration process (Turpeinen et al. 2002). Moreover, reduction process performed by the microbes is not mainly linked to respiration, but to impart metal resistance. Aerobic and anaerobic reduction of Cr^{6+} to Cr^{3+}, $Se^{6+}$to Se^{0}, $U^{6+}$to U^{4+} and $Hg^{2+}$to Hg^{0} are generally carried out by the microbes to detoxify them.

Biomethylation of metals becomes resulted in formation of volatile compound of metal. In case of mercury, Hg(II) can be transformed into methylmercury by different group of bacterial species (e.g. *Bacillus* sp., *Clostridium* sp., *Escherichia* sp. and *Pseudomonas* sp.) and methyl mercury is volatile in nature, easily absorbed and accumulated an also highly toxic Hg species. In the same way, As is transformed into arsines, selenium is converted to to dimethyl selenide and Pb to dimethyl Pb (Gao and Burau 1997; Pongratz and Heumann 1999; Dungan and Frankenberger 2000). In addition to above phenomenons, high concentrations of As, Cd, Cu, Co, Ni and Zn are leached out from contaminated areas by acidophilic iron- and sulfur-oxidizing bacteria (Groudev et al. 2001). Moreover, sulfate-reducing bacteria, on the other hand, can precipitate metals into sparingly soluble metal sulfide compounds through metabolic processes (Lloyd and Lovely 2001). In another study, the resistance against copper by *Pseudomonas syringe* has been reported and it is mainly due to the Cu accrual and compartmentalization in the cell's outer membrane and the periplasm (Cooksey 1993). Either microbs can increase the bioavailability of metals by solubilizing and mobilizing the insoluble metals become potentially toxic or reduce their bioavailability by immobilization processes. These kinds of bio-transformation processes of metals are major components of the metals biogeochemical cycles to maintain the proper ecosystem functioning. The microbes involved in metal decontamination processes can be further exploited in remediation of contaminated environments.

16.5 Microbe-Plant-Metal Interaction

Plants and microbes are generally coexisting in nature at any given ecosystems and they may symbiotic or compete with one another for their survival. Microbes and plant root exudates play a major role in functioning of rhizosphere ecology and influence the bioavailability of metals and nutrients in rhizosphere soil. Microbes help in stimulating plant root exudation and the root exudates are generally rich in carbon can be used as food and energy sources by microbes. Cohesive plant-microbe associations can play a very important role in adapting to metal rich environments can be focused further to advance microbe-mediated phyto-remediation. The metal mobility and availability can be influenced and phytoremediation efficiency of plant enhanced by root exudates through (1) proton (H^+) release mediated change in soil pH or formation of organo-metal complexes; (2) binding compounds present in the cell (e.g., organic acids, phytochelatins, and amino acids); (3) influencing redox potential of rhizosphere soil through enzyme mediated e^- transfer and (4) enhanced microbial activity in the rhizosphere (Sessitsch et al. 2013). In this connection, Kim et al. (2010) have reported that translocation and bioaccumulation of metals are significantly enhanced by citric and oxalic acid and suggesting that these acids can be used as natural chelating agents for better phytoextraction. Further, microorganisms particularly growth promoters (PGPMs) like some beneficial fungi and bacteria can involve in reducing phytotoxicity of metal by indirectly improving plant growth through stimulating defence mechanisms in opposition to phytopathogens and directly through generation of growth promoting substances, enzyme secretion and mineral nutrients solubilization of (N, P, K, Fe, etc.). Microbes induce or enhance phytoremediation of plant by improving its biomass growth and influencing metal availability and facilitate for bioaccumulation from soil -root and translocation from root-shoot (Ma et al. 2013).

To recruit the beneficial microorganisms and to make better plant-microbe interrelationship plant roots selectively exudates plant metabolites (organic compounds) that are effectively signals bacteria and fungi for its association. Each plant species has its characteristic group of associated microbes and able to link up with them by selection from surrounding soil environments for creating its own root microflora (Hartmann et al. 2009). This mechanism is directly associated with the type and amount of root exudates produced as well as rhizosphere soil features. In rhizospheric zone, microbes can establish proficient symbiosis with plants through triggering host functional signals (chemotaxis and colonization) and plants can well communicate with their adjoining soil microorganisms by signals or root exudates (Bulgarelli et al. 2013). Besides chemotaxis, rhizo-bacterial colonization can be initiated by electric gradients (electrotaxis) created by plant roots believed to be a possible mechanism (Lugtenberg and Kamilova 2009).

Presently, results from number of studies have revealed that beneficial microbes mediate plants to acquire sufficient mineral nutrients (such as N, Ca, Fe, Mg, and P) in metal contaminated soils, thus, establishment of highly developed and thriving root system in the initial crop growth stages is highly advantageous in phytoremediation of metal polluted soils (Ahemad and Kibret 2014). For instance, N fixing bacterial groups like *Rhizobium*, free living bacterial species in rhizosphere and endophytic bacteria can improve the soil fertility polluted areas resulted in enhancing plant growth and N concentration in roots and shoots (Wani et al. 2007). Similarly, in zinc/lead mine tailings arbascular mycorrhizal fungi that absorb and mobilize nutrients helped plant growth and nutrient uptake by leguminous trees grown on the contaminated tailings (Harris and Lottermoser 2006). Moreover, microbes transform highly insoluble metal sulphides to readily available form that enables the hyper-accumulator plants to remove toxic metals from soil solution. This will provide additional attractive options for microbes to improve metal resistance ability per se (Sharma et al. 2000). Furthermore, siderophores and H^+ are specifically generated by soil microorganisms under iron (Fe) deficiency conditions. Of late the role of siderophore producing microbes (SPMs) such as bacteria and fungi involved in Fe acquisition of different plant species and related mechanisms behind their promotion of Fe acquisition has been extensively studied (Gaonkar and Bhosle 2013). Even under stress conditions, phytohormones produced by plant associated microbes such as IAA, cytokinins, GA, ABA and others, can govern the hormonal balance in plants as a response to stress (Ullah et al. 2015; Ma et al. 2016). Also, Arbascular mycorrhizal fungi colonization in the plant root zones also has constructive effects on plant cell growth and division because of fungal hormones production (Yao et al. 2005). The alteration in endogenous phytohormones levels are also accountable for morphological changes encouraged by AMF inoculation.

Apart from the above beneficial mechanisms, soil microbes involve in initiation of synthesis of ethylene inhibitors to support plant growth under stress conditions, (Glick 2014), antimicrobial enzymes (Saima et al. 2013) and polysaccharides (Naseem and Bano 2014). These play a major role and enable plants to overcome or copeup with the negative impact of both biotic (fungi or harmful insects) and abiotic stresses (such as waterlogging, drought, salt stress, and metals toxicity; Fig. 16.3). Production of ACC deaminase by plant growth promoting bacteria is one among the key traits which hydrolyses ACC, plant ethylene precursor, to NH_3 and ketobutrate (Glick 2014). In spite of above, plant growth and biomass improvement through root modification by inoculating efficient fungal and bacterial species under compatible environment of plant-microbe-site combinations. This can be envisaged through advanced biotechnological applications in phytoremediation. In general, plant associated microorganisms can promote plant growth and development by resorting to any one or more of the above mechanisms. For that reason, PGPM can be effective utilized in stressful environments for phytoremediation of

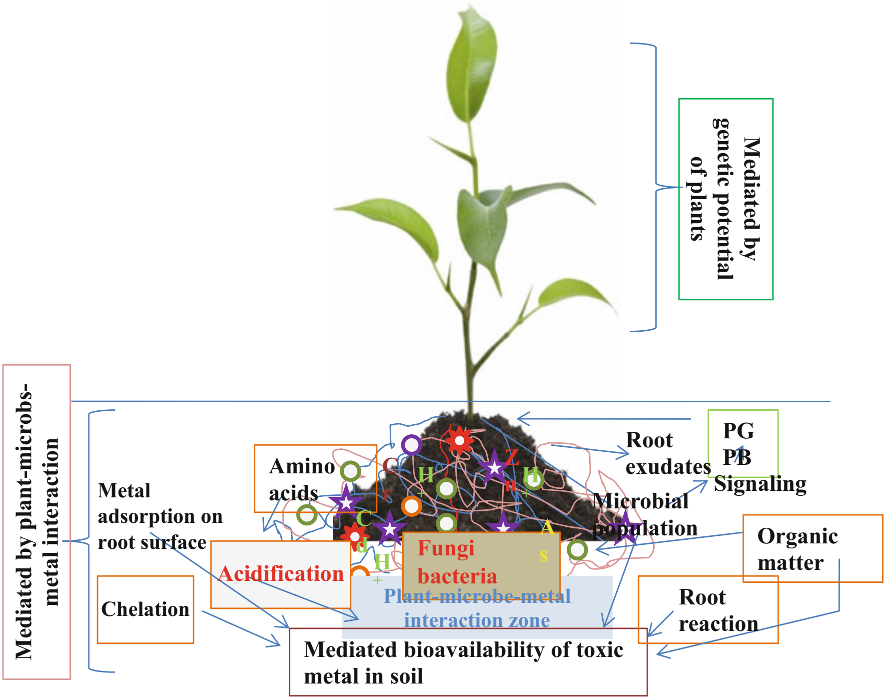

Fig. 16.3 Pictorial outline of the plant-microbe-metal interactions for heavy metal decontamination of polluted soils

metals. Apart from inherent capabilities, the usefulness of PGPM for higher plant growth is mainly associated with intimate interaction with host plant and soil characteristics. However, in future, contribution of genes in relation to phyto-beneficial traits and occurrence of preferential symbiosis needs to be studied in-depth to harness the benefit of plant-microbe interactions.

16.6 Can Heavy Metal Uptake Mediated by Climate Change?

Climate change effect on crop productivity and water use efficiency are clearly observed by the various researchers (Amrawat et al. 2013; Jajoria et al. 2014; Meena et al. 2016, 2017b). In major crops, increasing temperature enhances the respiration rate and reduces the crop yield (Dotaniya 2015; Dotaniya et al. 2018b). This situation is more pathetic in tropical and sub-tropical countries like India, Sri Lanka etc. (Kundu et al. 2013; Meena and Dotaniya 2017). Increasing the greenhouse gases concentration in the atmosphere and elevated the temperature due to more absorbance of shortwave radiations, generate global warming effect (Dotaniya et al. 2017b; Meena et al. 2017a). Increasing the temperature slightly enhanced the photosynthesis activity in temperate regions where temperature acts as a limiting factor (Bharti et al. 2017). Direct effects of climate change on heavy metal uptake by the plant are even sparser (Wijngaard et al. 2017). Heavy metal update pattern indirectly affected by the climate change effect. Increasing the root exudates as low molecular organic acids mediated the availability of heavy metals in soil (Dotaniya et al. 2016b). However, increasing the root exudates enhance the microbial count and diversity in the soil. Increasing the microbe's population accelerates the decontamination process in the soil and reduces the toxicity. The soil enzyme activities in soil, increase and decrease by the metal type, speciation, availability and toxicity (Shen et al. 2005; Yang et al. 2006; Khan et al. 2007; Karaca et al. 2010). Extracellular enzyme like phosphatases and dehydrogenase enzyme activities accelerated the decomposition of organic matter (Bell et al. 2010; Dotaniya 2015) and enhanced the metal availability in soil solution or appear toxicity in plants. Dhillon et al. (1996a, b) reported that under elevated CO_2 concentration extracellular enzymatic activities increased due to microbial demand for N and P. Rate of microbial immobilization can also increase (Mikan et al. 2000) or decrease (Berntson and Bazzaz 1997) with elevated CO_2concentration. Increasing global precipitation accelerated the metal mobility via biogeochemical cycles and also enhances the metal availability and uptake by plants (Carillo-Gonzáles et al. 2006; Reeder et al. 2006). These processes also accumulate heavy metals in soil and sediment, due to sorption mechanisms (Foster and Charlesworth 1996). Increasing the precipitation rate can dissolve the heavy metals from contaminated area and transported into a new region; it acts as a base for metal uptake. Larger amount of heavy metals moved from various parts of contaminated sites to uncontaminated areas and accumulate in the upper soil furrow (Rozemeijer and Broers 2007; Bonten et al. 2012). Use of marginal quality water is an alternative water management strategy for combating the adverse effect of climate change. Increasing wastewater use in water scarceareas for the cultivation of crops in the developing countries is more prone to contamination of toxic metals via food chain contamination (Meena et al. 2015). The repeated irrigation with poor quality water accumulated more amounts of heavy metals in soil and enhances the metal uptake by plants.

16.7 Future Line of Research

- Effect of climate change on heavy metal dynamics with respect to plant metabolites.
- The interaction effect of various metals on each other dynamics and its toxicity effect on soil microbial population.
- Genetic engineering assisted phytoremedition and its effect on root exudations.
- Plant metal uptake pattern in various crops with respect to water and soil conditions.
- Soil organic carbon dynamics and its effect with root exudates on metal dynamics.

16.8 Conclusions

Soil water pollution is the challenging task to remediate for the sustainable agricultural crop production. Increasing population forced per hectare more food grain, which is limited by the potential capacity of natural resources. Increasing use of poor quality natural resources, enhance the metal toxicity in human beings via food chain contamination. Most of the peri-urban areas of metropolitan cities are using industrial or sewage water for cultivation of crops specially vegetable production. Repeated application of metal contaminated effluent for agriculture purpose accumulated huge amount of heavy metal in the field. The Bioremediation is a one of the low cost technology for the heavy metal remediation. In which, bio-agent (plant or microbe) are using for minimizing of metal toxicity from the soil and plant environments. Microbes secreted various types of organic acids, reduced or convert toxic metal to non toxic. However, it improved the soil physico-chemical properties and increases the crop sustainability in contaminated soils. Use microbial assisted phytoremediation can reduce the contamination level in soil; it is a low cost, eco-friendly and more viable than phytoremediation techniques.

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Implementation of *Trichoderma* spp. for Conservation of Soil Health

17

Manish Kumar, Shabbir Ashraf, Rizwan Ali Ansari, Mohd Zuhaib, Arshi Jamil, Naresh Dhakar, Rafeeq Kasim, and Amber Rizvi

Abstract

Applications of extensive use of fungicides for the protection of crops from diseases have serious consequences on the environment and consumers. Disease suppression, through biocontrol agents (BCAs), is outcome interaction among the plants, pathogens and the microbial community. Soil microbes are capable of influencing the productivity, composition and diversity of plant communities directly or indirectly. *Trichoderma* spp. have potential to keep safe plants from pathogen populations under distinct soil conditions. *Trichoderma* spp. produce biologically active compounds, antibiotics, hydrolytic enzymes including cell wall-degrading enzymes and secondary metabolites which act against pathogen and promote growth of plants. It also releases metabolites helping resistance against biotic stress. BCAs *Trichoderma* spp. have been studied broadly and commercially marketed as biofertilizers, biopesticides and soil amendments. In the rhizosphere region, *Trichoderma* spp. act against soilborne pathogens and have potential to conserve soil health by replacing harmful chemicals in the near future.

M. Kumar (✉) · S. Ashraf · M. Zuhaib · A. Jamil · N. Dhakar · R. Kasim · A. Rizvi
Department of Plant Protection, Faculty of Agricultural Sciences, Aligarh Muslim University,
Aligarh, Uttar Pradesh, India

R. A. Ansari
Section of Plant Pathology and Nematology, Department of Botany, Aligarh Muslim University,
Aligarh, Uttar Pradesh, India

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17.1 Introduction

Applications of fungicides not only put a burden on growers but also exert harmful impact on ecosystem. Soil microbes are capable of influencing the productivity, composition and diversity of plant communities both directly and indirectly (Barea et al. 2002; Fitzsimons and Miller 2010; Lau and Lennon 2011; van der Heijden et al. 2006, 2008). A significant reduction of chemical synthetic fertilisers in agriculture is highly desirable. Biocontrol agents (BCAs) like *Trichoderma* spp. are the promising means that can replenish nutrient demands of the plants through several ways. For management of plant diseases, integrated approach of biocontrol agents with reduced doses of chemicals has been suggested to manage plant pathogens which reduced bad impact of chemicals on the ecosystem (Chet and Inbar 1994; Harman and Kubicek 1998). Dubey et al. (2007) reported integration of *Trichoderma harzianum* and carboxin enhanced seed germination grain yield and reduce wilt incidence of chickpea. *Trichoderma* spp. exhibit various biocontrol activities through mechanism such as mycoparasitism, competition and production of growth enhancer molecules which promote plant development and growth (Chadha et al. 2014). *Trichoderma* spp. are very much successful against soilborne pathogen and enhance the activity of normal plant developing process.

17.2 Soil Health

Promotion of soil microbial diversity maximise the coadaptation between plants and microbes. Small changes in the environment resulting from environmental stress or natural perturbations may be monitored through soils which are used as indicator (Sharma et al. 2010). Richness of species within the soil microbiome help in producing high functional inclusion allowing it to quickly recover through stress (Nannipieri et al. 2003; Yin et al. 2000).The microbial diversity of soil also endowed with protection against soilborne pathogens (Brussaard et al. 2007; Garbeva et al. 2004; Mendes et al. 2011; Nannipieri et al. 2003). The capability of the soil microbiome also plays an important role in antagonism against soilborne pathogens and increases plant productivity (Janvier et al. 2007). More than hundred species of fungi have been reported which are antagonist to plant pathogens they help in trap and prey on nematodes (Jatala 1986) as well as hyperparasites other fungi (Adams 1990). *Trichoderma* spp. secrete lytic enzymes which act against cell wall of fungal pathogens (Sivan and Chet 1989). Mendes et al. (2011) reported that diversity of soil microbiome as a whole involved different taxa or groups of microbes which convey the disease suppressive proficiency of the soil.

17.3 *Trichoderma*–Plant Interaction

The potential fungi *Trichoderma* is associated with plants to mitigate biotic and abiotic stresses, with matter of organic composition in the context of receptivity of the soil (Simon and Sivasithamparam 1989; Wakelin et al. 1999). Composts depict an optimal substrate for BCAs, for establishment into the environment of soil (Hoitink et al. 2006; Leandro et al. 2007). Krause et al. (2001) exhibited that inoculation of *T. hamatum* in pot mix with organic matter, supported high populations of BCAs, significantly diminished the severity of *Rhizoctonia* spp. The activity of *Trichoderma* is best in high organic matter which promotes plant growth and development.

17.4 *Trichoderma* Against Soilborne Pathogens

Pathogens associated with soil have a wide host range which persists for longer period as resting-resistant structures. Soilborne pathogens are controlled by chemicals, but there are also adverse effects on environment. *Trichoderma* spp. are used as fungal bioagents which are effective against soilborne pathogens (Chet and Baker 1981; Papavizas 1985; Chet 1987; Kumar et al. 1996). Roberts et al. (2005) reported that *Rhizoctonia solani*, *Pythium ultimum*, *and Meloidogyne incognita* are soilborne pathogens that cause severe economic loss. Isolates of *Trichoderma virens* are found to be most effective against *Rhizoctonia solani* causing damping off. It also improved the plant health of cucumber by suppressing *M. incognita* and *P. ultimum.* They also produce antifungal phenolic compounds which inhibit plant pathogen (Amin et al. 2010). The various mechanisms involve antagonism either through mycoparasitism, competition or antibiosis.

17.4.1 Mycoparasitism

Mycoparasitism is the greatest and direct form of hostility or antagonism in ecosystem (Pal and McSpadden Gardener 2006). It is one of the main mechanisms against the target organism by the action of coiling and dissolution of target pathogens cell wall through enzymatic activity (Tiwari 1996; Sharma 1996). *Trichoderma* release lytic enzymes like β-1, 3-glucanase, chitinase and proteases which act against plant pathogens (Haran et al. 1996). Lewis and Papavizas (1987) reported that *Trichoderma* spp. with alginate pellets stored for 6 weeks at temperature of 25 °C in the greenhouse were found effective against *Rhizoctonia solani* causing damping off.

17.4.2 Competition

Interactions between pathogens and bioagents compete for the space and nutrients. Siderophore chelate Fe(II) ions and the membrane-bound protein receptors specifically recognise and take up the siderophore-Fe complex (Mukhopadhyay and Mukherjee 1998). It makes iron unavailable to the pathogens, which produces less siderophores with lower binding power and causes less pathogenic infection. The substances act as stimulant to overcome their dormancy and exert competition and help in reducing their disease-causing ability. BCAs are more efficient in the nutrient utilisation and compete with the pathogens (Nelson 1990).

17.4.3 Antibiosis

Antibiosis involves antimicrobial compound to suppress fungal pathogens by disturbing their metabolic activity and stimulation of plant defence system (Corley et al. 1994; Horvath et al. 1995). Dubey et al. (2007) conducted dual culture experiment of *Trichoderma* spp. against *Fusarium oxysporum* f. sp. *ciceris* and found production of volatile and non-volatile compound-inhibited pathogen causing Fusarium wilt of chickpea.

17.5 Mass Production

For commercial development of biocontrol agents (BCAs), the products should be stable, potent under field conditions and above all should be economically viable (Fravel et al. 1999). Different types of grains are considered as best source of nutritive media for growing different BCAs; commonly used grains are bajra (*Pennisetum typhoides*) and jowar (*Sorghum bicolor L*). For delivery of *T. harzianum* preparations, medium of wheat bran and peat (1:1, v/v) is extensively used (Sivan et al. 1984). The pH should remain constant and low (5.5) during entire growth period, which prevent from contamination of bacterial agents. The shelf life of BCAs decreases after 1–2 years as well as number of colony-forming units (CFUs) also reduced as the time increases (Chet 1987; Elad and Chet 1995; Sivan and Chet 1992). Formulation of *Trichoderma* is prepared by the amalgamation of oils, water-soluble adjutants, stickers and different emulsions help to enhance their performance. One of the most important reasons for the limited commercialisation is the high cost of substrate, low biomass productivity and high cost of production (Rhodes 1996). The Table 17.1 shows the *Trichoderma* spp. and their application against various soilborne pathogens.

Table 17.1 *Trichoderma* spp. and their application against soilborne plant pathogens

Host plant	BCAs (*Trichoderma* spp.)	Causative agent	References
Brassica oleracea (cauliflower)	*T. viride*, *T. harzianum*	*R. solani*, *P. aphanidermatum*	Ahuja et al. (2012) and Sharma and Dureja (2004)
Vigna mungo (black gram)	*T. viride*, *T. harzianum*	*Macrophomina phaseolina*, *Alternaria alternata*	Mishra et al. (2011) and Dubey and Patel (2001)
Agaricus bisporus (mushroom)	*Trichoderma viride*	*Rhizopus stolonifer*, *Fusarium oxysporum*	Rawal et al. (2013)
Citrus	*T. viride*, *T. harzianum*	*Fusarium solani*	Kalita et al. (1996) and Singh et al. (2000)
Brassica oleracea (cauliflower)	*T. viride*, *T. harzianum*	*R. solani*, *P. aphanidermatum*	Ahuja et al. (2012), Sharma and Dureja (2004), and Sharma and Sain (2005)
Capsicum annuum L. (chilli)	*Trichoderma viride*, *Trichoderma harzianum*	*S. rolfsii*, *Fusarium oxysporum*, *Pythium* spp.	Kapoor (2008), Rini and Sulochana (2006) and Vasanthakumari and Shivanna (2013)
Cicer arietinum (chickpea)	*Trichoderma harzianum*, *Trichoderma viride*	*Fusarium oxysporum*, *Rhizoctonia solani*, *Macrophomina phaseolina*	Mukherjee et al. (1997), Pandey et al. (2003) and Poddar et al. (2004)
Solanum melongena L. (brinjal)	*Trichoderma viride*, *Trichoderma harzianum*	*Fusarium solani*, *Fusarium oxysporum*	Jadon (2009) and Balaji and Ahir (2011)
Gossypium hirsutum (cotton)	*T. viride*, *T. harzianum*	*R. solani*, *S. rolfsii*, *P. aphanidermatum*	Gaur et al. (2005)

17.6 Role of *Trichoderma* as Bioremediator

The fungi most potent used widely for bioremediation is *Trichoderma* spp. The BCAs help in promoting growth of the plants, as well as improvement of soil fertility, diseases suppression and composting (Contreras-Cornejo et al. 2009; Lorito et al. 2010). *Trichoderma* spp. are the producer of organic acids, which help in reducing soil pH and promoting dissolution of macro- and micronutrients such as iron, manganese and magnesium, which are necessary for plant metabolism. *Trichoderma* are capable to degrade chemical pesticides, chlordane, lindane and DDT which resides in soil and make contaminant-free sites (Ezzi and Lynch 2005). Table 17.2 shows the bioremediation of various contaminants by different *Trichoderma* spp.

Table 17.2 Mycoremediation of soil contaminants using various *Trichoderma* spp.

S. No.	Soil pollutants	*Trichoderma* spp.	References
01.	Cadmium, lead, manganese, nickel and zinc	*Trichoderma harzianum*	Adams et al. (2007)
02.	Poly resistance of pesticides	Spp. of *Trichoderma*	Hatvani et al. (2006)
03.	Water and soil contaminants	Spp. of *Trichoderma*	Harman et al. (2004)
04.	Various types of heavy metals from mud sludge	*Trichoderma atroviride*	Errasquin and Vazquez (2003)
05.	Phytoextraction in cadmium- and nickel-polluted soils	*Trichoderma atroviride*	Cao et al. (2008)
06.	Heavy metals	*Trichoderma*	Hajieghrari (2010)
07.	Agrochemicals pollutants DDT, dieldrin, pentachlorophenol endosulfan and pentachloronitrobenzene	*Trichoderma harzianum*	Katayama and Matsumura (1993)

17.7 *Trichoderma*-Induced Plant Health

Trichoderma genes enhance plant resistance by expressing broad range of stress, and tolerance in the plant genome efforts is in progress. Lorito et al. (1998) demonstrated that genes of *Trichoderma* spp. expressed functional enzymes to control plant diseases. Gene endochitinase chit42 of *Trichoderma harzianum* were obtained from different plant tissues, which show no effect on plant growth and development but work against plant pathogen. In transgenic cotton plants, endochitinase gene Tv-ech1 of *Trichoderma virens* showed significant resistance against *A. alternata* and *R. solani* (Emani et al. 2003; Kumar et al. 2009).

17.8 Conclusion

The success of genus *Trichoderma* as bioagents is based upon the complicated interactions between advantageous microbes which establish with plants and pathogens in the soil ecosystem. Fungicides control pathogens effectively, but the accumulation and persistence of chemicals pollute soil which effect human health. For the protection of plants and their crop yield, *Trichoderma* spp. are the best and safer option. Advancement of modern techniques like proteomics and metabolomics could provide best knowledge about the complex tripartite interaction of *Trichoderma* with environment and plant microbial community (Vinale et al. 2008). Further experiments should be conducted to understand the mechanisms of *Trichoderma* secondary metabolites and their possible synergisms with other compounds used in agriculture. For marketability enhancement of *Trichoderma* spp. as BCAs, commercial production should be improved.

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Water Quality Assessment and Treatment of Pharmaceutical Industry Wastewater: A Case Study of Pharmacity Selaqui, Dehradun of Uttarakhand State, India

18

Sanjay Gupta, Rajendra Dobhal, Ashulekha Gupta, Usha Rani, and Vivek Kumar

Abstract

Undesirable antrhopogenic activities leads to pollution of river waters which is mainly caused by many factors such as every industry including pharmaceutical, agricultural sector and human generated sewage sludge and water, is a matter of great apprehension. Because societal progress together with population progression and environment change, therefore, it could cause a large-scale opposing impacts on the surface quality of water. On the other hand, the medicinal compounds are characteristically prepared in an industry through a series of processes which lead towards the occurrence of varied types of compounds in its effluents, which are produced in dissimilar processes. Moreover, abundant amount of aqua are utilized for cleaning of equipment and rock-solid material cake or taking out of potent chemicals from raw materials. The occurrence of pharmaceutical complexes in potable water chiefly derived from two diverse origins: the manufacturing process of medicinal industries and every day or usual use of pharmaceutical products, which results in their occurrence in metropolitan and rural effluents. Therefore, the effluents produced in various methods during the preparation of drugs and pharmaceuticals products comprise of a different variety of compounds. These compounds in natural environment also cause resistance among sensitive microbes, which may further transfer the resistant genome to sensitive microbes. Additionally, recycling of wastewater after contaminant removal, whether pharmaceutical or other industries, is generally wanted by the manufacturing industry. Keeping in mind the shortage of water

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S. Gupta (✉) · U. Rani · V. Kumar
Himalayan School of Biosciences, Swami Rama Himalayan University, Dehradun, Uttarakhand, India; vivekkumar@srhu.edu.in

R. Dobhal
Uttarakhand Council for Science and Technology, Dehradun, India

A. Gupta
School of Management Studies, IMS Unison University, Dehradun, India

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reserves, it has become absolutely essential to comprehend and prepare sophisticated procedures for the cure of pharmaceutical effluents as part of crucial water administration system. In this chapter, the several origins of effluents from the drug industry are detected along with the types of contaminants and the best existing methods and technologies to eliminate them from the ecosystem.

18.1 Introduction

The use and application of water quality indices (WQI) abridge the performance of the results of any type of analysis which is associated with a general aqua body, as it précises in one understandable value or concept a series of analysed considerations and parameters. The water quality indices are usually pondered as measuring the means of understanding the different types of water quality parameters into a very simple and easy index. Nonetheless, the indices and indicators are very valuable and advantageous to transfer the data and report about the quality of water to the general public, which in turn gives a satisfactory impression of the ongoing water quality trend changing over an interval of time and moreover also allows the relationship and assessment between various waterways and channels or diverse places along the same path.

18.1.1 Water Quality

Quality of raw water from different sources, i.e. surface, subsurface and ground, is being polluted by various factors, which include industrial wastewater, domestic wastewater, agriculture runoff and many other pollution factors. It is therefore, very important to regulate and maintain the water quality of different sources. Pollution in water is the leading cause of death and disease across the globe, and it accounts for thousands of human deaths daily. It is estimated that in India, 580 people die every day because of water pollution-related disease (Kaur and Kaur 2014). Basically water quality is a combination of physiological and biochemical characteristics of water. It depends on the condition of water relative to the needs and is termed as drinking water quality, irrigation water quality, etc.

In India, CPCB (Central Pollution Control Board) has established a concept for the use of water for different purposes depending on its quality. According to this, various applications of water obtained from specific water resource, the highest to lowest quality is designated and categorized into five types of quality of water. This classification as shown in Table 18.1 helps policy makers, managers and planners involved in water quality monitoring and management to set quality targets and design suitable processes and strategies to restore and preserve various water bodies.

One can determine the water quality of different sources by comparing it with standard value of parameter(s) which is set by different regulating agencies of different countries. In India, BIS has recommended the defined water quality parameters for various uses (Table 18.2).

Table 18.1 Water quality parameters for different activities as per CPCB

Water use	Grades	Parameters
Potable water by nonconventional and disinfectant treatment	a	1. TC, MPN/100 ml = <50
		2. Dissolved oxygen = >6 ppm
		3. Biological oxygen demand 5 days 20 °C = <2 ppm
		4. pH 6.5–8
Organized out-of-doors bathing	b	1. TC, most probable no./100 ml = <500
		2. Dissolved oxygen = >5 ppm
		3. pH 6.5–8
		4. Biological oxygen demand 5 days 20 °C = <3 ppm
Potable water by conventional and disinfectant treatment	c	1. TC, MPN/100 ml or less
		2. pH 6–9
		3. DO = >4 ppm
		4. Biological oxygen demand 5 days 20 °C, = <3 ppm
Wild life and fishery	d	1. pH 6.5–8
		2. Dissolved oxygen = >4 ppm
		3. Free NH_3 (as nitrogen)
		4. Biological oxygen demand 5 days 20 °C = <2 ppm
Watering, industrial air-conditioning, authorized waste disposal	e	1. pH 6–8.5
		2. EC in 25 °C micro mhos/cm, maximum 2250
		3. SAR maximum 26
		4. Boron(B) maximum 2 ppm
Uncategorized	Below e	None of the criteria of a, b, c, d and e

Source: Mareddy (2017); (http://cpcb.nic.in/water-quality-criteria/)

The quality of water from different sources is affected by a number of factors, among which industries are one of the major polluters. The industries release the wastewater either untreated or partially/improperly treated. This wastewater contains a large number of pollutants, and this varies with the type of industry.

18.2 Types of Industrial Wastewater

There are several types of industrial wastewater based upon different industries, and each industry produces its own particular combination of pollutants (Table 18.3).

Table 18.2 Standards (BIS 2296:1992) for water quality in India

	Best use				
Parameters	A	B	C	D	E
DO ppm, minimum	6	5	4	4	–
BOD ppm, maximum	2	3	3	–	–
TC, MPN/100 ml, maximum	50	500	5000	–	–
pH	6.5–8.5	6.5–8.5	6.0–9.0	6.5–8.5	6.0–8.5
Colour, Hazen units, maximum	10	300	300	–	–
Odour	Unobjectionable		–	–	
Taste	Tasteless	–	–	–	–
TDS, ppm, maximum	500	–	1500	–	2100
Total hardness as $CaCO_3$, ppm	200	–	–	–	–
Calcium hardness as $CaCO_3$, ppm	200	–	–	–	–
Magnesium hardness, ppm, maximum	200	–	–	–	–
Copper (Cu), ppm, maximum	1.5	–	1.5	–	–
Iron (Fe), ppm, max	0.3	–	0.5	–	–
Manganese (Mn), ppm, maximum	0.5	–	–	–	–
Chlorides (Cl), ppm, maximum	250	–	600	–	600
Sulphates (SO_4), ppm, maximum	400	–	400	–	1000
Nitrates (NO_3), ppm, maximum	20	–	50	–	–
Fluorides (F), ppm, maximum	1.5	1.5	1.5	–	–
Phenolic compounds (C_2H_5OH) ppm max	0.002	0.005	0.005	–	–
Mercury (Hg), ppm, maximum	0.001	–	–	–	–
Cadmium (Cd), ppm, max	0.01	–	0.01	–	–
Selenium (Se), ppm, maximum	0.01	–	0.05	–	–
Arsenic (As), ppm, maximum	0.05	0.2	0.2	–	–

(continued)

Table 18.2 (continued)

Parameters	Best use				
	A	B	C	D	E
Cyanide (CN), ppm, maximum	0.05	0.05	0.05	–	–
Lead (Pb), ppm, maximum	0.1	–	0.1	–	–
Zinc (Zn) ppm, maximum	15	–	15	–	–
Chromium (Cr^{6+}), ppm, maximum	0.05	–	0.05	–	–
Anionic detergents (MBAS), ppm, maximum	0.2	1	1	–	–
Barium (Ba), ppm, maximum	1	–	–	–	–
Free Ammonia (N), ppm, maximum	–	–	–	1.2	–
EC, micromhos/cm, maximum	–	–	–	–	2250
SAR, maximum	–	–	–	–	26
Boron, ppm, maximum	–	–	–	–	2

Source: Mareddy (2017)

Table 18.3 Different water pollutants produced by different industries

Type of industry	Pollutants contained in wastewater
Pulp and paper	COD, BOD, solids, chlorinated organic compounds
Iron and steel	COD, BOD, oil and grease, heavy metals, acids, cyanides and phenols
Textile and leather	BOD, chromium, solids and sulphate
Chemicals	COD, heavy metals, suspended solids, cyanides and organic chemicals
Petrochemicals and refineries	COD, BOD, mineral oils, acids, chromium and phenols
Microelectronics	COD and organic chemicals
Mining	Suspended solids, heavy metals, acids and salts
Non-ferrous metals	Fluorine and suspended solids

18.2.1 Characteristic of Industrial Wastewater

Broadly physical and chemical characteristics are responsible for pollution of wastewater effluent from industries and are provided below:

18.2.2 Physical Characteristic of Industrial Wastewater

The physical characteristics of industrial wastewater include solids, colour, odour and temperature.

18.2.3 Total Solids

Solid materials in wastewater may be present in dissolved, suspended or settleable form. Amount of dissolved particlulate matters in wastewater measures in term of total dissolve solids (filterable residue), total suspended solids (non-filterable residue) which is a measure of suspended solids present in wastewater and electrical conductivity reflects salinity of wastewater caused by dissolved materials.

18.2.4 Colour

It is a qualitative feature to evaluate the usual case of effluents.

Straw tint of wastewater reflects the age of wastewater, which is below than 6 h old.

Dove grey colour reflects that the wastewater have gone through some degree of deterioration or have been in the system and accumulated for some time.

Dark grey or black wastewater reflects the extreme bacterial decomposition under anaerobic condition.

Black colour wastewater reflects the formation of various sulphides.

18.2.5 Temperature

The assessment of temperature is important because most wastewater treatment process includes biological system that is temperature dependent. The temperature of wastewater generally fluctuates from season to season and also with geographical locations. In cold areas the temperature will vary from about 7 to 18 °C, while in warmer areas the temperature alters from 13 to 24 °C.

18.3 Chemical Characteristic of Industrial Wastewater

18.3.1 Inorganic Chemicals

The principal chemical assessment includes free ammonia, organic nitrogen, nitrates, nitrites, inorganic phosphorus and organic phosphorus. Phosphorus and nitrogen are important because these nutrients are responsible for the growth of aquatic plants. Other assessment, such as pH, sulphate, chloride and alkalinity, are performed to evaluate the suitability of reusing treated industrial wastewater and in regulating the various treatment processes. Trace elements are not determined generally because of the variation in requirement of trace elements by different organisms, i.e. zinc, copper, iron and cobalt, for proper growth. Heavy metals (other than trace element) can also produce toxic effects; therefore, assessment of the concentrations of heavy metals is especially important, where the further use of treated wastewater is to be evaluated. Some metals are also classified as priority pollutants such as cadmium, chromium, lead, mercury, arsenic, etc.

18.3.2 Organic Chemicals

Most aquatic habitat requires a critical threshold, i.e. minimum amount of dissolved oxygen for the survival of organisms. Dissolved oxygen concentration is measured directly in wastewater, but the oxygen demand depends on the oxygen requirement by different chemicals in the wastewater. The oxygen demand is estimated with respect to chemical oxygen demand (COD) and biochemical oxygen demand (BOD). The COD and BOD measure the relative oxygen depletion due to pollutants in wastewater. The BOD gives a value of oxygen demand of biodegradable pollutants, and COD governs the oxygen demand for oxidation of both biodegradable and non-biodegradable pollutants. Generally there is no correlation between COD and BOD, but it is feasible to initiate a correlation for a precise waste contaminants in a well-defined wastewater runlet, but such correlation is not to be found for any different wastewater streams.

18.3.3 Standards for Industrial Wastewater

In India, to check and regulate pollution, CPCB, a government body, has framed standards for discharge of industrial wastewaters, as summarized in Table 18.4.

18.4 Role of Industries in Water Sources Pollution

In India, almost trine of entire water contamination is through industrial solid scarps, wastewaters and unsafe wastes. Industrialized effluents present a possible risk to the regular water ecosystem (Obasi et al. 2014; Rana et al. 2014a; Deepali 2012; Kansal et al. 2011; Lokhande et al. 2011a, b; Modak et al. 1990). This industrial waste incorporates diversity of inorganic and organic materials, which are poisonous to different kinds of life of ecological systems (Ashfaq and Khatoon 2014; Rana et al. 2014b; Spina et al. 2012). The increasing tons of contaminants from industrial waste runlets created great damage to the rivers, causing threat to majority of health issues on both direct drinking and bathing in the natural water resources (Seth et al. 2013; Bala 2011; Semwal and Akolkar 2006).

Water contamination created by industrial wastewater consequently gives harmful effects on the normal healthcare of the people, as well as the general population,

Table 18.4 Standards for discharge of pollutants from pharmaceutical industry wastewaters (www.cpcb.nic.in)

S. No.	Parameters	Standards			
		Inland surface water	Public sewers	Land/irrigation	Marine/coastal areas
1.	Colour/odour	–	–	–	–
2.	pH	5.5–9.0	5.5–9.0	5.5–9.0	5.5–9.0
3.	Suspended solids, ppm	100	600	200	a. Process wastewater 100 b. Cooling water effluent 10% above TDS influent
4.	Suspended solids	850 μ IS sieve	–	–	a. Floatable solids, maximum 3 mm b. Settleable solids, max 856 μ
5.	Temperature	Not above 5 °C than the receiving water temperature	–	–	Not above 5 °C than the receiving water temperature
6.	Oil and grease, ppm	10	20	10	20
7.	Ammonia (N), ppm	50	50	–	50
8.	Total nitrogen (N), ppm	100	–	–	100
9.	Free ammonia (NH3), ppm	5.0	–	–	5.0
10.	Total residual chlorine, ppm	1.0	–	–	1.0
11.	COD, ppm	250	–	–	250
12.	BOD, ppm	30	350	100	100
13.	Lead, ppm	0.1	0.1	–	2.0
14.	Arsenic (As), ppm	0.2	0.2	0.2	0.2
15.	Mercury, ppm	0.01	0.01	–	0.01

16.	Hexavalent chromium as (Cr^{+6}), ppm	0.1	2.0	–	1.0
17.	Total chromium (Cr), ppm	2.0	2.0	2.0	2.0
18.	Cadmium (Cd), ppm	2.0	1.0	–	2.0
19.	Zinc (Zn), ppm	5	15	–	15
20.	Copper (Cu), ppm	3.0	3.0	–	30
21.	Nickel (Ni), ppm	3.0	3.0	–	50
22.	Selenium (Se), ppm	0.05	0.05	–	0.05
23.	Cyanide (CN), ppm	0.2	2.0	0.2	0.2
24.	Dissolved phosphates (P), ppm	5.0	–	–	–
25.	Fluoride (F), ppm	2.0	15	–	15
26.	Phenolic compounds (C6H5OH), ppm	1.0	5.0	–	5.0
27.	Sulphide (S), ppm	2.0	–	–	5.0
28.	Nitrate (N), ppm	10	–	–	20
29.	Vanadium (V), ppm	0.2	0.2	–	0.2
30.	Iron (Fe) ppm	3.0	3.0	3.0	3.0
31.	Bio-assay test	90% survival fish after 96 h in 100% effluent	90% survival of fish after 96 h in 100% effluent	90% survival of fish after 96 h in100% effluent	90% survival of fish 96 h in 100% effluent
32.	Radioactive materials				
	a. α emitters micro curie, ppm	10^{-7}	10^{-7}	10^{-8}	10^{-7}
	b. β emitters micro curie, ppm	10^{-6}	10^{-6}	10^{-7}	10^{-6}

Source: Water Science and Technology Library (2012), (http://cpcb.nic.in/water-quality-criteria/)

who reside in the vicinity of the allied and chemistry industries, and workers along with cultivators (Asamudo et al. 2005). The surface water bodies are the principal source for disposal of wastewater by the industries (Kar et al. 2008). Unprocessed/improper processed industrial wastewaters have increased the pollution grade of groundwater up to several points above the secure/permissible grade in many vital contaminated regions of the India. It has been observed in India that the stretches of almost all rivers are polluted by industrial effluents/wastewater (Lokhande et al. 2011a, b; Prabha and Selvapathy 1997; Modak et al. 1990). The pollution grade due to industrial effluent contamination differs from one sector to another sector industry which relies on the nature of operations and the scale of the industries (Garcia et al. 1995).

In a country like India, there are enough proofs accessibly linked with the inefficient management of industrialized wastewater (Lokhande et al. 2011a, b; Singare et al. 2010a, b; Rajaram and Das 2008). Previous workers have reported the presence of high level of pollutants especially organic and inorganic in industrial wastewater of Dehradun (Singh et al. 2012) and Haridwar district (Kumar and Bharti 2012) in the state of Uttarakhand, India.

18.4.1 Pharmaceutical Industrial Wastewater

In the present work, emphasis is given to wastewater generated by pharmaceutical industries; therefore, their effect on our ecosystem needs proper evaluation. Different types of pharmaceutical industries and their characteristic wastewater, standards parameters for discharge of their wastewater provided by Indian regulatory agency and a review on assessment of pharmaceutical industries wastewater are provided hereunder.

18.4.2 Pharmaceutical Industries Pollution and Their Impact on Human Health, Environment and Flora and Fauna

The amount of unprocessed or improperly processed medicinal sector effluent is minute, but it involves a critically top-level grade of contaminants due to the existence of non-bio decomposable organic materials (reproductive hormones), antimicrobials and their analogues, different pharmaceuticals, dope based on flora and fauna, non-prescription drugs, beta-interferons, anti-depressants, anti-inflammatory drug, painkillers, regulatory, chemotherapeutic agents, daily concern goods, soaps and cleanser metabolites, fire retardants, oil byproducts, combustion and another routinely utilized chemical compounds, i.e. chemical process remnant, residual and utilized solvents, filter bed, heavy metals (chromium, arsenic, zinc, iron, sodium, potassium, mercury, lead, manganese, magnesium, nickel, cadmium, etc.) and other non-specified pollutants (Ashfaq and Khatoon 2014; Obasi et al. 2014; Rana et al. 2014a, c; Ramola and Singh 2013; Vuppala et al. 2012; Chelliapan et al. 2011).

Effects of effluents on plants and animals change extensively due to the alteration in the features of the effluents (chemical synthesis, formulation unit and fermentation section contain waters with spent solvents, catalyst, reactants; fermentation unit contains wastewater having spent fermentation broth, sugar, nutrients; and natural product extraction unit contains wastewater with equipment washings, spills, leaks, spent solvents). The impact of pharmaceutical metabolites on human health and environment required a much needed impetus not just due to their crucial poisonousness but their mutagenic, genotoxic plus carcinogenic influence. These pharmaceutically bioactive contaminants when released onto the surface or in aqua reserves accumulate in environmental structure over the food cycle which consequently influence the human well-being and other microflora and fauna (Nadal et al. 2004).

18.4.3 Types of Pharmaceutical Industries Wastewater

Sources of wastewater from different pharmaceutical processes are mentioned in detail below (Table 18.5):

18.4.3.1 Pharmaceutical Process Wastewater Sources

Process wastewater is referred to as a form of water which is generated using different pharmaceutical manufacturing processes, directly or indirectly, by the utilization of any crude material, in-between product, after-effect, finished product or waste material.

Wastewater is produced in pharmaceutical manufacturing processes as follows:

- *Reaction unit water*
- *Process solvent*
- *Process washings*
- *Product washings*
- *Condensed water steams*
- *Spent caustic/acid streams*

Other sources of wastewater/effluent from pharmaceutical operations include:

- *Air pollution unit scrubber blow down*
- *Ancillary equipment and floor washes*
- *Pumps/seal water*

18.4.3.2 Other Wastewater Sources

In addition to above, other types of wastewater generate during pharmaceutical operations. This includes sanitation wastewater; noncontact cooling, i.e. heat

Table 18.5 Sum up of distinctive material intakes and contamination end product in the pharmaceutical sector

Processes		Materials	Effluents	Residual wastes
Chemical synthesis	Chemical reaction unit	Reactants, catalysts, solvents like $CHCl_3$, C_6H_6 toluene, ethylene, CH_2, xylenes, methanol, glycol, Cl-, hydrochloric acid, etc.	Wastewater with different levels of solvents, catalyst, chemical reactants, wet scrubber water, pump seal waters, instrument washings. This water contains high, chemical oxygen demand, biological oxygen demand, TSS, pH ranges 1–11	Reaction byproducts and vessel bottom wastes
	Separation	Solvents like methanol, toluene, acetone and hexanes	Instrument washings, leaks, spills, spent solvents	Separation residues
	Purification	Solvents, i.e. methanol, toluene, acetone hexane	Spills, instrument washings, spent solvents, leaks	Purification leftovers
	Drying	Active drugs and intermediates	Instrument washings, leaks, spills, spent solvents	–
Normal product separation units		Flora and fauna tissues, solvents, e.g. NH_3, $CHCl_3$ and C_6H_5OH	Equipment cleaning, spills, leaks, spent solvents. Water should have low chemical oxygen demand, biological oxygen demand, TSS and pH ranges 6–8	Unused crude materials (roots, plants, etc.)
Fermenting unit		Starter culture, sugar contents, starch, PO_4^{3-}, nutrient, solvents like C_6H_6O, $C_5H_{11}OH$, CH_3OH, C_3H_6O and MIBK etc.	Spent broth, effluents having sugar, starch nutrient, etc. Effluents should have higher biological oxygen demand, chemical oxygen demand, TSS and pH ranges 4–8	Filter cakes, fermented leftovers
Construction unit		Active pharmaceuticals, binding-agents, sugar syrups, starches and more	Leaks, equipment washings, spills, spent solvents. Wastewater should contain lower biological oxygen demand, chemical oxygen demand, TSS and pH ranges 6–8	Waste packaging, particulates, rejected capsules, tablets, etc.

Source: US EPA (2015), https://www.epa.gov/wqs-tech/final-rulemaking-update-national-water-quality-standards-regulation exchanger waters; other noncontact ancillary water sources such as boiler blowdown and bottle washing; and wastewater from storm water runoff.

Entire drug industries in India engaged under the hard-and-fast norms of the regulating agency, Central Pollution Control Board (CPCB). However, the environmental pollution status is far from satisfactory.

18.5 Pharmaceutical Industrial Wastewater Quality Standards

Monitoring and management of effluent is necessary to analyse the character of effluents generated from industries. Central Pollution Control Board (CPCB) in India has set a prerequisite as their standards and their restricting values for emission of contaminants from medicinal industries (Table 18.6). These standards help in the evaluation and assessment of the quality of PIWW.

18.5.1 Assessment of Pharmaceutical Industrial Wastewater

The wastewater generated during the pharmaceutical process operations varies according to the crude materials, equipment's utilized, manufacturing process, combining and preparation operation used (Mayabhate et al. 1988).

Summary of assessment of PIWW is provided below in Table 18.7a, b.

Table 18.6 Standard values for liberation of contaminants from pharmaceutical industries

Effluent standards	Limiting concentration (ppm), except for pH
Mandatory parameters	
pH	6.0–8.5
Oil and grease	10
BOD (3 days at 27 °C)	30
COD	250
Total suspended solids (TSS)	100
Total dissolved solids (TDS)	–
Bioassay test	90% survival after 96 h in 100% effluent
Additional parameters[a]	
Mercury (Hg)	0.01
Arsenic (As)	0.2
Chromium (hexavalent)	0.1
Lead (Pb)	0.1
Cyanide (CN)	0.1
Phenolics (C_6H_5OH)	1.0
Sulphides	2.0
Phosphate	5.0

[a] "Additional Parameters" shall be depends on the process and product. Source: (www.cpcb.nic.in); (envirotrends.net) Rana et al. (2014a, b)

Table 18.7a Assessment of physicochemical parameters of pharmaceutical industries wastewater

Parameters (all parameter values are in ppm, except pH, temp., turbidity and EC)	Reference								
	Gome and Upadhyay (2013)	Choudhary and Parmar (2013)	Wei et al. (2012)	Lokhande et al. (2011a)	Saleem (2007)	Idris et al. (2013)	Ileri et al. (2003)	Das et al. (2012)	Oktem et al. (2007)
pH	6.9	5.8–7.8	7.2–8.5	3.69–6.77	6.2–7.0	5.65–6.89	6.4–6.8	–	7–8
TSS	370	230–830	48–145	280–1113	690–930	29.67–123.03	900	24–84	0.6–0.7
TDS	1550	650–1250	–	1770–4009	600–1300	136.33–193.05	–	484–1452	–
Total solids	1920	880–2040	–	2135–4934	–	–	–	–	–
BOD	120	20–620	480–1000	995–1097	1300–1800	–	90–130	–	–
COD	490	128–960	2000–3500	2268–3185	2500–3200	–	200–300	1257.9–1542.9	40,000–60,000
Biodegradability	0.259	–	0.20–0.39	–	–	–	–	–	–
Alkalinity	–	130–564	–	–	90–180	–	–	–	900–1000
Total nitrogen	–	–	80–164	–	–	–	–	–	–
Total kjeldahl nitrogen	–	–	–	–	–	–	–	–	800–900
Ammonium nitrogen	–	–	74–116	–	–	–	–	–	–
Ammonia							26		–
Total phosphate	–	–	18–47	–	–	–	8.5	–	3–6
Turbidity (NTU)	–	–	76–138	–	2.2–3.0	17.22–28.78	–	–	–
Chloride	–	–	–	205–261	–	–	–	–	–
Sulphate	–	–	–	–	–	–	–	44–1527	–
Oil and grease	–	–	–	0.5–2.9	–	–	–	–	–
Phenol	–	–	–	–	95–125	–	–	–	–
EC (µS/cm)	–	–	–	–	–	157–673	–	–	–
Temp. (°C)	–	–	–	–	–	32–46	20	–	–

Source and reference: (doras.duc.ie), Rana et al. (2014a, b)

Table 18.7b Assessment of heavy metals and physicochemical parameters in PIWW

Parameters (all parameter values are in mg/L, except, EC, pH and colour)	Reference							
	Ramola and Singh 2013	Rohit and Ponmurugan (2013)	Rao et al. (2004)	Mayabhate et al. (1988)	Vanerkar et al. (2013)	Sirtori et al. (2009)	Madukasi et al. (2010)	Imran (2005)
Iron	8.5–10.8	–	–	–	–	–	2.1	–
Chromium	0.12–0.31	0.01	–	–	0.057–1.11	–	–	–
Lead	0.158–0.262	0.03	–	–	0.559–6.53	–	–	–
Cadmium	0.16–0.56	–	–	–	0.036–0.484	–	–	–
Nickel	0.05–0.12	0.02	–	–	0.892–2.35	–	–	–
Zinc	1–1.3	0.20	–	–	0.583–0.608	–	0.056	–
Dissolved organic carbon	–	–	–	–	–	775	–	–
Copper	–	0.02	–	–	0.649–1.67	–	0.022	–
Selenium		–	–	–	0.428–0.666	–	–	–
Arsenic	–	–	–	–	0.0049–0.0076	–	–	–
Manganese	–	–	–	–	6.41–8.47	–	0.605	–
Sodium	–	–	–	–	155–266	2000	–	–
Potassium	–	–	–	–	128–140	–	–	–
Oil and grease	–	10.27	–	–	140–182	–	–	1925–3964
Calcium		–				20	–	–
BOD	–	410	7200	1200–1700	11,200–15,660	–	146.7	263–330
COD	–	548	25,000	2000–3000	21,960–26,000	3420	–	2565–28,640
Dissolve phosphate	–	–	–	–	–	10	–	–
Nitrogen	–	185	–	–	389–498	–	533.7	–
TDS	–	622	20,000	–	2564–3660	–	1600	1443–3788
TSS	–	110	7500	300–400	5460–7370	407	425	761–1202
Total solids	–	–	–	–	8024–11,030	–	–	–
EC (μS/cm)	–	945	–	–	–	–	–	–

(continued)

Table 18.7b (continued)

Parameters (all parameter values are in mg/L, except, EC, pH and colour)	Reference							
	Ramola and Singh 2013	Rohit and Ponmurugan (2013)	Rao et al. (2004)	Mayabhate et al. (1988)	Vanerkar et al. (2013)	Sirtori et al. (2009)	Madukasi et al. (2010)	Imran (2005)
Temp. (°C)	–	–	–	–	–	–	–	31–34
pH	–	6.01	7.5	6.5–7.0	3.9–4.0	–	–	5.8–6.9
Phosphate	–	–	100	–	260–280	–	–	–
Sulphide	–	–	100	–	42–54	–	–	–
Sulphate	–	–	360	–	82–88	160	–	–
Nalidixic acid	–	–	–	–	–	45	–	–
Colour	–	White	Orange	–	Dark yellow	–	–	–
Chloride	–	–	200	–	–	2800	–	–
Alkalinity	–	–	2500	50–100	–	–	–	–
VFA	–	–	6000		–	–	–	–
Phenols	–	–	–	65–72	–	–	–	–
Volatile acids	–	–	–	50–80	–	–	–	–
Total acidity	–	–	–	–	3000	–	–	–
Acetic acid	–	–	–	–	–	–	422.7	–
Propionic acid	–	–	–	–	–	–	201.3	–
Butyric acid	–	–	–	–	–	–	304.5	–

Reference: Rana et al. (2014a, b)

18.6 Treatment Options of Pharmaceutical Industrial Wastewater

Thus keeping in view the above-mentioned facts, it is necessary to control the environmental pollution generated by pharmaceutical industries, and thus, there is a need of a suitable treatment strategy for the regulation of polluted wastewater. The aim of the wastewater treatment should be to covert waste material present in wastewater into stable oxidizable end products that can be readily disposed without any detrimental effects.

Pharmaceutical industries incorporate a mixture of remedial strategies that include basic, i.e. biochemical and physicochemical process, auxiliary biotic process and tertiary advanced oxidation processes (Gadipelly et al. 2014; Ashfaq and Khatoon 2014; Rana et al. 2014a; Vanerkar et al. 2013; Deegan et al. 2011).

Such treatment methods include anaerobic and aerobic moving bed biofilm reactors (MBBR) process (Xing et al. 2014), membrane bioreactor (Lefebvre et al. 2014), ozone (Xing et al. 2014), membrane-aerated biofilm reactor (MABFR) (Wei et al. 2012), anaerobic and aerobic sequencing batch reactor (ASBR) (Lefebvre et al. 2014; Patil et al. 2013), Fenton coagulation/flocculation, electrocoagulation (Dixit and Parmar 2013), fungal treatment (Spina et al. 2012), photo-electrocoagulation, membrane separation, UV irradiation, peroxi-electrocoagulation, reverse osmosis, chlorination, bacterial treatments (Madukasi et al. 2010), peroxi-photo-electrocoagulation, adsorption, distillation, solar photo-Fenton, neutralization/pH adjustment, sedimentation sand filtration (Saleem 2007), algal treatment, phyto-remediation, activated sludge (Mayabhate et al. 1988) and anaerobic fixed film reactor (Rao et al. 2004), O_3/H_2O_2 and O_3/UV processes (Balcioglu and Otker 2004), etc. Some important methods of PIWW treatment are discussed hereunder.

18.6.1 Biological Treatment (Bioremediation) Process

Bioremediation strategies involving organisms have been utilized frequently for the cure of medicinal industry effluent. The processes are divided into aerophilic and anaerobic methods of treatment. Aerophilic strategies involve activated sludge, sequence batch reactors (SBR) and membrane batch reactors (MBR) (Chen et al. 2008; Chang et al. 2008; LaPara et al. 2002). Anaerobic processes include anaerobic sludge blanket reactors (ASBR), anaerobic filters and anaerobic membrane reactors (Sreekanth et al. 2009; Oktem et al. 2007; Enright et al. 2005).

Treatment process, which includes activated sludge, is not appropriate for the treatment of effluents which contain COD level greater than 4000 ppm. Activated sludge with a large hydraulic retention time (HRT) is found to be suitable for the bioremediation of medicinal industry effluent (El-Gohary et al. 1995; Oz et al. 2004). Parameters affecting the performance of activated sludge processes for the treatment of PIWW are hydraulic retention time (HRT), temperature, pH, organic load, dissolved oxygen (DO) in wastewater, microbial population and presence of poisonous or recalcitrant compounds (LaPara et al. 2002).

18.6.2 Anaerobic Treatment

Anaerobic treatment processes earlier are used for the treatment of effluents of a variety of industries such as pulp and paper, distilleries, leather industries, yarn and clothing industries and food treatment having variation of highly durable waste to low durable waste. Different types of reactors, i.e. anaerobic contact reactor, up-flow anaerobic sludge blanket (UASB), fluidized bed reactor (FBR) and anaerobic fixed film reactor (AFFR), are prepared to treat the effluent from a variety of industrial sectors. These reactors have different functional constraints, though being used broadly for different processes (Rao et al. 2004). Anaerobic remedies encounter high biodegradable content in industrial effluent decomposing into CH_3 and CO_2 with the support of microorganism especially acetogenic and methanogenic bacteria. Anaerobic treatment strategies for industrial wastewater offer several advantages as follows:

1. It creates very minute sludge.
2. It needs less energy.
3. It operates at high organic loading rate (OLR).
4. It requires a low nutrient uptake for biogas generation for yielding energy and in treatment process (Nandy et al. 2002).

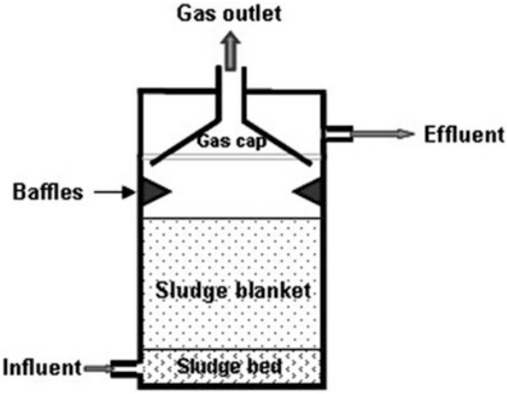
Substances used for inoculation and feed pretreatment are the principal phases, which influence the treatment efficacy. Because of the low pH, sluggish growth rate and lengthier hydraulic retention time (HRT) observed in the process, high-pace configurations were prepared which are used to cure industrial effluent at relatively shorter HRT (Patel and Madamwar 2000). Enright et al. (2005) studied the anaerobic organic treatment of pharmaceutical effluent and reached 60–70% COD removal ability.

18.6.3 Up-Flow Anaerobic Sludge Blanket (UASB) Reactors

Up-flow anaerobic slurry blanket (UASB) reactors have been majorly utilized for the treatment of industrial effluent (Awaleh and Soubaneh 2014; Toloti et al. 2011). The principal components of this reactor are shown in Fig. 18.1; it shows four major parts:

1. Microbial slurry bed
2. Slurry blanket
3. Separator for air-sludge liquid
4. Settling chambers

Fig. 18.1 UASB reactor



This reactor was used for the high-strength anaerobic effluent remedy. The process was successful using this UASB reactor because of the formation of bioactive chippings (Fang et al. 1994). These chippings are of self-locked-in, compacted aggregate of living beings leading to an efficient holding time of microbes in the reactor (Akunna and Clark 2000).

The benefits of UASB reactor are:

1. No mechanized blending.
2. Reutilization of active slurry biomass.
3. Capacity to deal with disturbances by high organic upload rates.
4. Up-flow anaerobic slurry blanket (UASB) bioreactor can be effectively used for the treatment of effluents in psychrophilic condition (Fig. 18.1).

18.6.4 Anaerobic Fixed Film Reactor (AFFR)

The anaerobic fixed film reactor (AFFR) (Fig. 18.2) involves a biofilm nutriment for the adherence of biomass. This bioreactor offers many advantages which some of them are:

1. Ease of development
2. No mechanized blending
3. Higher constancy
4. High resistance to toxic impact upload

The fermentor can be recharged instantly after a phase of semi-deprivation stage (Rajeshwari et al. 2000). In this fermentor, various types of packing material can be used such as polyurethane foam, glass bead, waste rubber, red drain clay, polyacryl nitrile acrylamide, splintered glass, shredded plastic, coconut chippings, charcoal

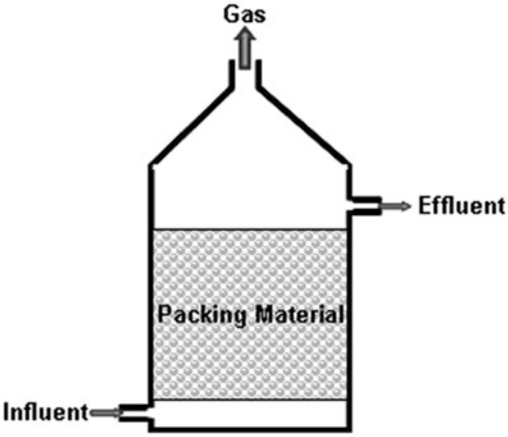


Fig. 18.2 Anaerobic fixed film reactor

and nylon fibre as a helper media, for the remedy of industrial effluents, which greatly enhance the performance of the reactor (Acharya et al. 2008).

18.6.5 Aerophilic Treatment

Aerophilic methods usually involve the use of aerobic sequencing batch reactor (ASBR) and activated slurry process for the processing of industrial effluents. The main components of the reactor are:

- Settling tank
- Holding trough
- Storing tanks
- Aerophilic tank

18.6.6 Microbial Treatment

18.6.6.1 Fungus Treatment

There are many fungous strains, which have a pivotal part in the processing of industrial effluents and also at the same time have limitations due to the extended development cycle and spore development of fungi cells. Spina et al. (2012) reported the fungus processing methods under the conventional activated slurry treatment for medicinal industry effluent.

Strains of *Aspergillus* like *Aspergillus niger*, *Aspergillus fumigatus*, and *Aspergillus niveus* show a decrease of COD in watered-down industrial wastewater at varied concentrations. Further, a cluster of fungi known as *Ascomycetes* also plays a vital role in the processing of industrial effluents such as *Penicillium decumbens* and *Penicillium lignorum* by significantly reducing chemical oxygen demand (COD), C_6H_5OH (phenol) and colour (Mohammad et al. 2006).

18.6.7 Phytoremediation

Phytoremediation is a unique process involving the various kinds of plants to remove, stabilize and transfer pollutants from the effluents. It is a promising cheap method for eradication of heavy metals from industrial effluents. This process is still in an infancy stage. However, for some heavy metals like Cd and Pb, phytoremediation offers a better treatment option than microbial adsorption processes because of their ability to bio-accumulate these toxins in natural or transgenic plants (Amin et al. 2013). In water, flora possesses a remarkable capability for minimization of the concentration of harmful heavy metals, organic solids and biological oxygen demand (BOD) from the effluents. Billore et al. (2001) reported the use of *Typha latifolia* and *Phragmites karka* flora for the processing of industrial effluent leading to substantial decrease in chemical oxygen demand (COD), biological oxygen demand (BOD), total solids and phosphorus content of wastewater. Workers also indicated the removal of C_6H_5OH (phenol) by phytoremediation approach in industrial effluent by peroxidase enzyme from crown gall cultures of tomato.

18.6.8 Membrane Bioreactor

The membrane bioreactor is used at a large-scale industrial application for the processing of industrial effluents, municipal effluents and domestic effluents. The process is utilized for the decomposition of different components of effluents by primary segregation of biomass and subsequent treatment of water by porous membrane filtration (Fig. 18.3).

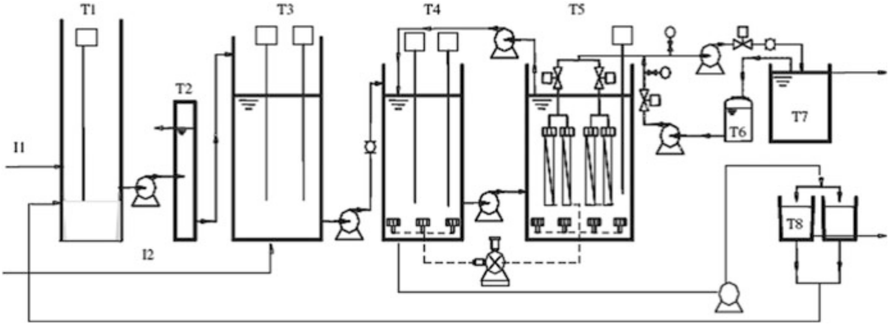


Fig. 18.3 MBR system for pharmaceutical industry effluent treatment. I1: influent from manufacturing unit, I2: influent of septic tank; T1: wet well; T2: solvent–liquid separation; T3: equalization unit; T4: biological separator; T5: membrane bioreactor; T6: backwash effluent unit; T7: effluent tank; T8: sludge drying unit

Advantages

1. Removal of total suspended solids (TSS)
2. Packed-in plant unit
3. Increased degradation rate
4. Ease of operation
5. Low slurry production
6. Decontamination and scent control
7. Increased holding time of microorganism
8. Mineralization of toxic and recalcitrant pollutants

Disadvantages

1. Membrane clogging and fouling
2. High capital input for erection and commissioning
3. Full-scale treatment is not dependable, depending on the applications
4. Process complexity

18.6.9 Bacterial Treatment

During the past few years, bacterial culture was used for bioremediation of industrial wastewater. Strains like *Pseudomonas*, *Enterobacter*, *Streptomonas*, *Aeromonas*, *Acinetobacter* and *Klebsiella* showed maximum COD reduction up to 44% (Ghosh et al. 2004). *Escherichia coli* and methane-producing bacterial pool described to be used for the exclusion of deadly contaminants like alternated phenols/pentachlorophenol which are further unsafe and poisonous and may lead to malignancy and mutations. Numerous other bacteria recognized to decompose phenolic and complicated biological compounds belong to the genus *Arthrobacter*, *Comamonas*, *Rhodococcus* and *Ralstonia*. Some *Clostridium* species were shown to degrade resorcinol. *Pseudomonas* predominantly present in rhizosphere soil possess significant phenolic decomposition capability. Reported phenol/cresol decomposition by the heat-resistant bacteria, *Bacillus thermoglucosidasius* A7, which mineralize phenol at 65 °C by means of the meta-cleavage route. The kinetics of the biodegradation of phenol/catechol by *Pseudomonas putida* MTCC 1194 is properly documented. The preadapted cultures of *P. putida* completely reduce the basic phenol and catechol strength of 100 ppm and 500 ppm in 162 and 94 h, consecutively. The potential of bioremediating phenol/chlorophenols has also been presented by *P. fluorescence*, a soil bacteria (Agarry and Solomon 2008).

18.6.10 Packed Bed Bioreactor

The first rational arrangement of a packed cell bioreactor was proposed by Pasteur, in which *Acetobacter* cells were restricted on wood chips. These cell immobilized bed bioreactors are still used for the production vinegar and wastewater treatment. Packed bed bioreactors are the most common among all these types of bioreactors though, alternatively, fixed bed bioreactor types, rotating biological contactors, trickling filter and parallel flow sheet bioreactors are also utilized for restriction of cell processes. Further, plug flow packed bed bioreactors are also being used extensively in immobilized cell-based bioprocesses (Branyik et al. 2000; Mamo and Gessesse 2000). PBRs operated offer high rates of treatment. On contrary due to low liquid velocities, these reactors have relatively less operating volume and poor heat transfer coefficients. Reactors where recycling was involved show an improved volume, heat transfer and control of process. The variable range of dynamics affect the suitability of kind of reactor for a specific process. These factors involve the procedure of packed bed, characteristic features of the matrix, character of the substrate, inhibitive reaction on process and mechanical properties of the fluids plus economics of the process.

Packed bed reactor has numerous merits and demerits, plus the suitability of the defined fermentor for a procedure is related with operational process parameters and the type of microbe(s) involved. The dimensions of micro-bearer are of prime attention in PBR, as it influences both internal diffusion resistance and pressure drop. The dimension allocation within the bed is always uniform since the pressure depends on the packed bed porosity.

18.7 Bioremediation of the Pharmaceutical Industry Wastewater of Selaqui, Dehradun, and Bhagwanpur, Haridwar, India

In Garhwal region of Uttarakhand, industries are located at two major industrial zones, namely, (1) Selaqui, Dehradun and (2) Roshanabad, Haridwar.

Complex industrial wastewater containing toxic chemicals as effluents is released by most of the industries due to the lack of regular monitoring and checking system from government machinery. The following types of industries are established in the referred two industrial zones:

1. Pharmaceutical industries
2. Chemical industries
3. Paint and varnish industries
4. Synthetic resin manufacturing units
5. Dye synthesis units
6. Metal plating industries
7. Textile industries
8. Automobile units
9. Liquefaction processes
10. Plastic industries
11. Fibre glass units

The earlier methods used for the treatment of pharmaceutical industry effluent include physical, chemical and thermal methods. But these treatment methods have disadvantages, which include high maintenance cost, huge labour requirement, low efficiency and huge equipments. Therefore, it becomes necessary to develop new effective and cost-effective technologies for the processing of pharmaceutical effluent since each available method is no more being practiced by the pharmaceutical industrial units in Uttarakhand as well as other parts of the country. Bioremediation is an important area in which we use living organism to clean up toxic waste in water and soil. The method is helpful in protecting public health, recycle and recovery of valuable components and compliances of legal standards and consent conditions placed on discharge. Bioremediation process has almost no waste byproduct, good treatment efficiency, less sludge production and potential of being cheaper and can be adopted by poor masses at household level.

Selaqui, Dehradun, and Bhagwanpur, Haridwar, are the two major pharmaceutical industrial areas of Garhwal region of Uttarakhand, which release the complex wastewater containing toxic chemicals as wastewater without proper treatment due to the lack of regular monitoring and checking system from government machinery. Many biotic and abiotic constituents are generally involved in the complex wastewater of each pharmaceutical cluster of these industrial sites as determined through preliminary investigations.

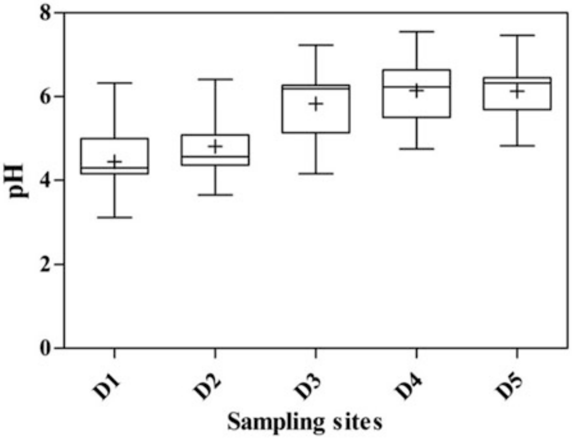
18.8 Physicochemical Wastewater Quality Analysis

The five pharmaceutical industries of Pharma City, Selaqui, Dehradun of Uttarakhand state, which are designated as D1–D5 were monitored each month for the duration of 2 years from January 2012 to December 2013. The overall physicochemical wastewater quality data of 15 parameters are, namely, (1) pH, (2) total suspended solids, (3) total dissolved solids, (4) electrical conductivity, (5) oil and grease, (6) chloride, (7) sulphate, (8) phenols, (9) biological oxygen demand, (10) chemical oxygen demand, (11) arsenic, (12) chromium-hexavalent, (13) lead, (14) sodium absorption ratio and (15) boron of these five industries.

18.8.1 Assessment of pH

For any given water sample, alkalinity or acidity is a measure of the pH, though the pH is the simplest of all the parameters but is of enormous importance as majority of the chemical reactions occurring within any aquatic habitat are regulated by the difference in its value. Strongly acidic or alkaline conditions would destroy aquatic existence as these individuals are prone to change in alkalinity. Thus, any biological processing strategies should always be focused on alkalinity control or its regulation. Further, the harmful level of heavy metals increases drastically at particular pH. Therefore, alkalinity is of prime concern in concluding the condition of industrial wastewater.

Fig. 18.4 Box and Whisker plot of pH analysed from wastewater of five pharma industrial sites (D1–D5) of Dehradun district during 2012 and 2013



The pH value of 6.0–8.5, standard permissible limit for discharge of environmental pollutant from pharmaceutical industry, has been suggested by CPCB. In the present research work, the pH values of wastewater samples for Dehradun district ranged from 3.11 to 7.55 in all five sampling sites as presented in Table 18.4.

The Box and Whisker plot represented in Fig. 18.4 shows the highest pH value as 7.55, which was found for wastewater sample of D4 sampling site during overall study period, and the lowest pH value 3.11 was found for wastewater sample of D1 sampling site.

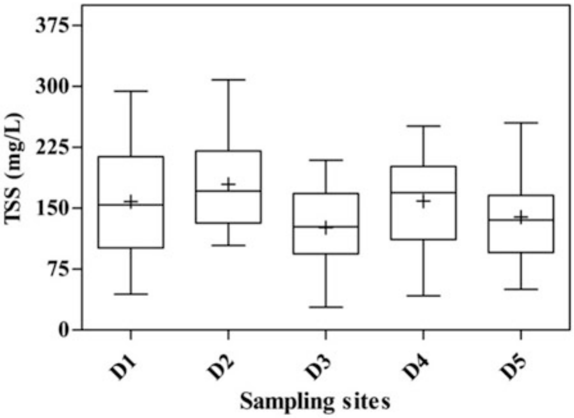
It was found that the pH values of all sampling sites ranged from 4.44 to 6.14. The average pH values of D1 (4.44), D2 (4.80) and D3 (5.82) sampling sites were found below the standard permissible limit, and pH value of D4 (6.14) and D5 (6.12) sampling sites were found within the standard permissible limit as shown in Table 5.1(1). The minimum pH values for D1, D2, D3, D4 and D5 were observed as 3.11, 3.65, 4.16, 4.75 and 4.82, respectively. The maximum pH values for the above-mentioned sequence of sampling sites were found as 6.32, 6.41, 7.23, 7.55 and 7.46, respectively.

From the pH analysis of Dehradun district, it is clear that two pharmaceutical industries (D4 and D5) discharge their wastewater with proper treatment with respect to pH, and D3 sampling site needs a little bit and D1 and D2 sampling sites need a great improvement in their treatment strategy to control the pH level in their wastewater.

18.8.2 Assessment of TSS

The average TSS values of all sampling sites of Dehradun district provided in Table 18.7a were found above the standard permissible limit (100 mg^{-L}) for discharge of environmental pollutant from pharmaceutical industry as promulgated by CPCB. The TSS values fluctuated from 28 to 308 (mg^{-L}). The average highest

Fig. 18.5 Box and Whisker plot of TSS analysed from wastewater of five pharma industrial sites (D1–D5) of Dehradun district during 2012 and 2013



value (180 mg^{-L}) was seen in D2 sampling site, and minimum average value (126 mg^{-L}) was observed for D3 sampling site. The variation in TSS values of wastewater at all sampling sites located in Dehradun district throughout the study is shown in Fig. 18.5. The maximum TSS value (308 mg^{-L}) for all sampling sites was found for D2 sampling site, and the minimum TSS value (28 mg/L) was found for D3 sampling site.

From the comparison of average values of TSS for D1, D2, D3, D4 and D5 as 158, 180, 126, 158 and 139 mg/L with standard limit of 100 mg/L, it is clear that all five pharmaceutical industries of Dehradun district (D1–D5) discharge their wastewater with improper treatment with respect to TSS and need improvement in their treatment strategy to control the higher TSS level in their wastewater.

18.8.3 Assessment of TDS

The TDS values in wastewater samples of the study area of Dehradun district varied from 740 to 4456 mg/L as presented in Table 3.1(2). The TDS value of 2100 mg/L as standard permissible limit has been designated by CPCB for discharge of this environmental pollutant from pharmaceutical industry. The average values for all sampling sites ranged from 1623 to 2476 mg/L. The value for D5 (2476 mg/L) was found above the standard permissible limit, whereas the average values for D1 (2020 mg/L), D2 (1697 mg/L), D3 (1623 mg/L) and D4 (1951 mg/L) were observed below the permissible limit of TDS (Fig. 18.6).

The range of TDS values for all sampling sites of Dehradun district is shown in Fig. 18.6. The maximum TDS value (4456 mg/L) for all sampling sites was found for D5 sampling site, and the minimum TSS value (740 mg/L) was found for D3 sampling site. From the TDS analysis, it is clear that four pharmaceutical industries (D1–D4) discharge their wastewater with proper treatment with respect to TDS and

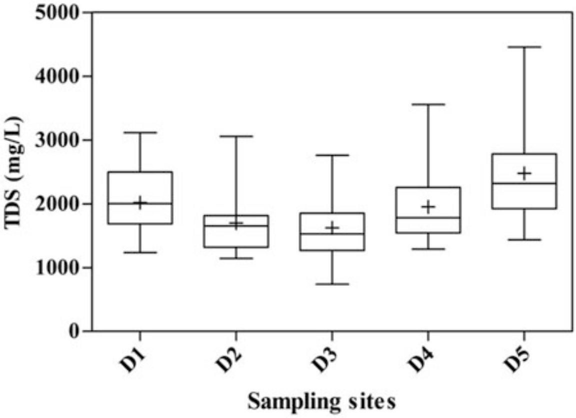


Fig. 18.6 Box and Whisker plot of TDS analysed from wastewater of five pharma industrial sites (D1–D5) of Dehradun district during 2012 and 2013

D5 sampling site requires updating of treatment unit to control the excess TDS level in its wastewater.

18.8.4 Assessment of Electrical Conductivity

The electrical conductivity values of five sites (D1–D5) of Dehradun fluctuated from 1132 to 6778 μS/cm (Table 3.1(2)).The standard permissible limit for discharge of environmental pollutant from pharmaceutical industry as per CPCB for electrical conductivity is 2250 μS/cm. The average values for all sampling sites ranged from 2473 to 3781 μS/cm, which were above the standard permissible limit in all the five sampling sites. In this range highest average electrical conductivity was observed in D5, whereas the lowest value was at D3 sampling site.

Figure 18.7 describes the variation of electrical conductivity during the whole monitoring of 2 years (2012–2013) of pharmaceutical industrial wastewater of Dehradun district. The figure shows that minimum electrical conductivity (1132 μS/cm) was observed in wastewater of D3 sampling site and maximum value (6778) in wastewater of D5 sampling site.

From the average values of (D1, 3078; D2, 2588; D3, 2473; D4, 2974 and D5, 3781 μS/cm) EC, it is clear that all pharmaceutical industries of Dehradun district (D1–D5) discharges their wastewater without proper treatment with respect to EC and updated treatment protocol is required at these sampling sites to control the observed EC levels in their wastewater.

18.8.5 Assessment of Oil and Grease

The oil and grease values in wastewater samples of the study area of Dehradun district range from 0.27 to 6.2 mg^{-L}. The oil and grease value accepted is 10 mg^{-L}

Fig. 18.7 Box and Whisker plot of EC analysed from wastewater of five pharma industrial sites (D1–D5) of Dehradun district during 2012 and 2013

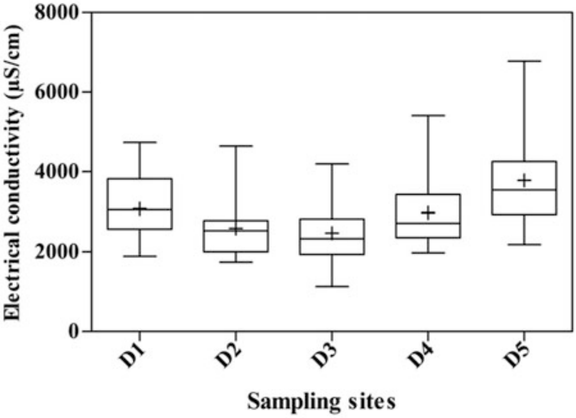
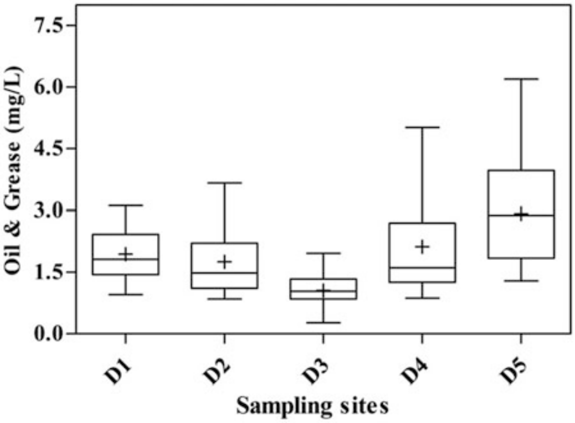


Fig. 18.8 Box and Whisker plot of oil and grease analysed from wastewater of five pharma industrial sites (D1–D5) of Dehradun district during 2012 and 2013

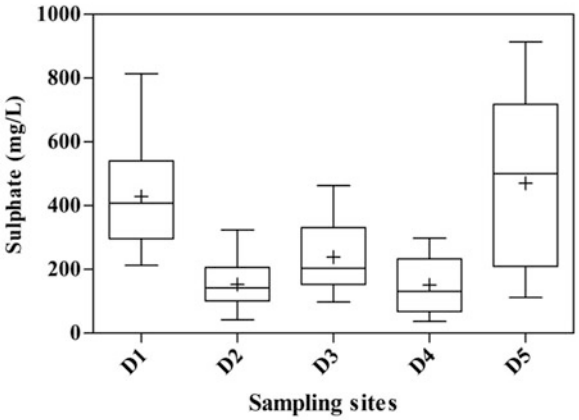


as standard permissible limit for discharge of environmental pollutant from pharmaceutical industry has been suggested by CPCB. The average values for all sampling sites ranged from 1.05 to 2.92 mg^{-L} for all sampling sites (D1–D5) which were well below the standard permissible limit for oil and grease.

The variation in oil and grease values of wastewater at all the sampling sites of Dehradun district throughout the study is shown in Fig. 18.8. The maximum oil and grease value (6.2 mg/L) for all sampling sites was found for D5 sampling site, and the minimum oil and grease value (0.27 mg/L) was found for D3 sampling site.

From the oil and grease analysis, it is clear that all pharmaceutical industries of Dehradun district (D1–D5) discharge their wastewater with proper treatment with respect to oil and grease. Though in present studies oil and grease were within limit, some researchers have previously found the oil and grease value for pharmaceutical industrial wastewater above the standard permissible limit for discharging of pharmaceutical industrial wastewater into inland surface water.

Fig. 18.9 Box and Whisker plot of sulphate analysed from wastewater of five pharma industrial sites (D1–D5) of Dehradun district during 2012 and 2013



18.8.6 Assessment of Sulphate

The sulphate values in wastewater samples of the study area of Dehradun district varied from 37 to 914 mg/L. The CPCB has prescribed sulphate value of 1000 mg/L as standard permissible limit for discharge of environmental pollutant from pharmaceutical industry. The average values for all sampling sites for sulphate ranged from 151 to 470 mg/L. The average values for all sampling sites were found below the standard permissible limit. The range of sulphate values for all sampling sites of Dehradun district are shown in Fig. 18.9. The maximum sulphate value (914 mg/L) was found for D5 sampling site, and the minimum sulphate value (37 mg/L) was found for D4 sampling site.

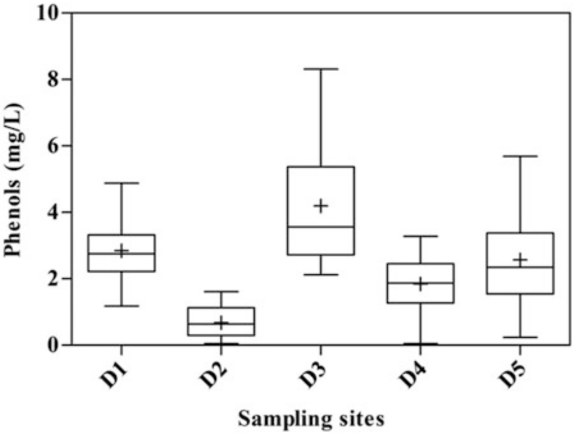
From the sulphate analysis, it is clear that all pharmaceutical industries of Dehradun district (D1–D5) discharge their wastewater with proper treatment with respect to sulphate.

18.8.7 Assessment of Phenols

The average phenol value of D2 sampling sites of Dehradun district was found below the standard permissible limit (1.0 mg^{-L}) for discharge of environmental pollutant from pharmaceutical industry as per CPCB, and it was found above the standard limit for D1, D3, D4 and D5 sampling sites. The phenol values fluctuated from 0.043 to 8.31 mg^{-L}. The average highest value (4.19 mg^{-L}) was noticed at D3 sampling site, and minimum average value (0.67 mg^{-L}) was observed for D2 sampling site.

The range of phenol values for all sampling sites of Dehradun district is provided under Fig. 18.10. The maximum phenol value (8.31 mg^{-L}) was found for D3 sampling site, and the minimum phenol value (0.043 mg^{-L}) was found for D2 sampling site.

Fig. 18.10 Box and Whisker plot of phenols analysed from wastewater of five pharma industrial sites (D1–D5) of Dehradun district during 2012 and 2013



From the phenol analysis, it is clear that D1, D3, D4 and D5 pharmaceutical industries of Dehradun district need improvement and D2 sampling site fulfils all requirements to control the phenol level in their wastewater.

18.8.8 Assessment of BOD

The BOD values in wastewater samples of the study area of Dehradun district range from 52 to 378 mg^{-L}. The BOD value of 100 mg^{-L} as standard permissible limit for discharge of environmental pollutant from pharmaceutical industry has been suggested by CPCB. The mean value for entire sampling locations ranged from 107 to 229 mg^{-L}. The mean values for entire sampling locations were found above the standard permissible limit of CPCB.

The range of BOD values for all sampling sites of Dehradun district is shown in Fig. 18.11. The maximum BOD value (378 mg/L) among all sampling sites was found for D1 sampling site, and the minimum BOD value (52 mg/L) was found for D2 sampling site.

From the BOD analysis, it is clear that all pharmaceutical industries of Dehradun district (D1–D5) discharge their wastewater without proper treatment with respect to BOD and these need strong improvement in their treatment strategy to control the exceeded BOD level in their wastewater.

18.8.9 Assessment of COD

The average COD values for all sampling sites of Dehradun district were found above the standard permissible limit (250 mg/L) for discharge of environmental pollutant from pharmaceutical industry, of CPCB. The average COD values fluctuated from 562 to 1504 mg^{-L}. The average highest value (1504 mg^{-L}) was

Fig. 18.11 Box and Whisker plot of BOD analysed from wastewater of five pharma industrial sites (D1–D5) of Dehradun district during 2012 and 2013

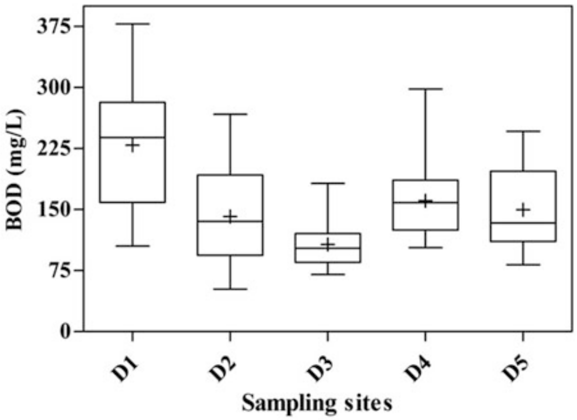
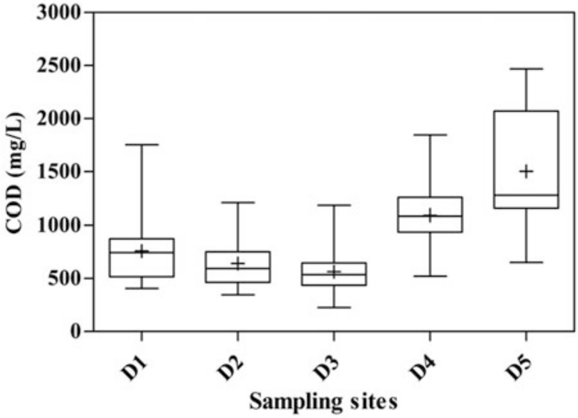


Fig. 18.12 Box and Whisker plot of COD analysed from wastewater of five pharma industrial sites (D1–D5) of Dehradun district during 2012 and 2013



noticed at D5 sampling site, and minimum average value (562 mg^{-L}) was observed for D3 sampling site.

The variation in COD values of wastewater at all the sampling sites of Dehradun district throughout the study is shown in Fig. 18.12. The maximum COD value (2469 mg^{-L}) for all sampling sites was found for D5 sampling site, and the minimum COD value (226 mg/L) was found for D3 sampling site.

From the COD analysis, it is clear that all pharmaceutical industries of Dehradun district (D1–D5) discharge their wastewater without proper treatment with respect to COD and improvement to control very high COD level in their wastewater is required.

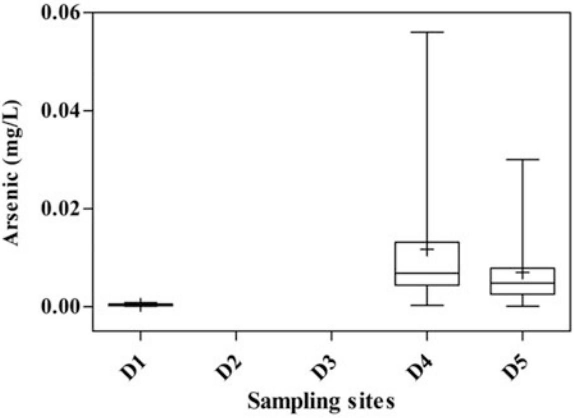


Fig. 18.13 Box and Whisker plot of arsenic analysed from wastewater of five pharma industrial sites (D1–D5) of Dehradun district during 2012 and 2013

18.8.10 Assessment of Arsenic

The arsenic values in wastewater samples of the study area of Dehradun district range from 0.0001 to 0.056 mg^{-L}. The arsenic value accepted is 0.2 mg^{-L} as permissible limit for discharge of environmental pollutant from pharmaceutical industry has been suggested by CPCB. The average values for all sampling sites vary from 0.0004 to 0.0117 mg^{-L}. The average worth for all sampling sites was found below the standard permissible limit.

The range of arsenic values for all sampling sites of Dehradun district is shown in Fig. 18.13. The maximum arsenic value (0.056 mg/L) for all sampling sites was found for D4 sampling site, and the minimum arsenic value (0.0001 mg/L) was found for D1 and D5 sampling sites, and for D2 and D3 sampling sites, the concentration of arsenic in wastewater was not detected (ND). From the arsenic analysis, it is clear that all pharmaceutical industries of Dehradun district (D1–D5) discharge their wastewater with proper treatment with respect to arsenic.

18.8.11 Assessment of Hexavalent Chromium

The hexavalent chromium value of 0.1 mg/L as standard permissible limit for discharge of environmental pollutant from pharmaceutical industry has been suggested by CPCB. The hexavalent chromium values of wastewater samples for Dehradun ranged from 0.001–0.078 mg^{-L} at all five sampling sites (D1–D5) in Dehradun during 2012 and 2013.

The Box and Whisker plot (Fig. 18.14) shows the highest hexavalent chromium values as 0.078 mg^{-L}, which is found for wastewater sample of D4 sampling site during overall study period, and lowest hexavalent chromium value 0.001 mg^{-L} which was found for wastewater sample of D2 and D3 sampling sites. The average hexavalent chromium values of D1 (0.022 mg^{-L}), D2 (0.005 mg^{-L}), D3 (0.004 mg^{-L}),

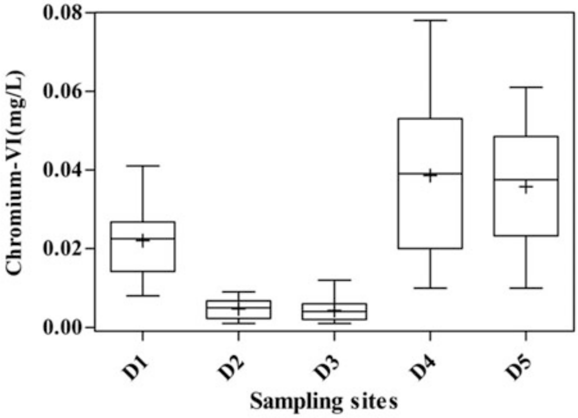


Fig. 18.14 Box and Whisker plot of chromium analysed from wastewater of five pharma industrial sites (D1–D5) of Dehradun district during 2012 and 2013

D4 (0.039 mg^{-L}) and D5 (0.0358 mg^{-L}) sampling sites were found below the standard permissible limit.

From the chromium-hexavalent analysis, it is clear that all pharmaceutical industries of Dehradun district (D1–D5) discharge their wastewater with proper treatment with respect to chromium-hexavalent.

18.8.12 Assessment of Lead

The average lead values for all sampling sites of Dehradun district were found below the standard permissible limit (0.1 mg^{-L}) for discharge of environmental pollutant from pharmaceutical industry, given by CPCB. The average lead values fluctuated from 0.0003 to 0.0158 mg^{-L}. The average highest value (0.0158 mg^{-L}) was noted in D3 sampling site, and minimum average value (0.0003 mg^{-L}) was observed for D1 sampling site.

The range of lead values for all sampling sites of Dehradun district is shown in Fig. 18.15. The maximum lead value (0.0347 mg^{-L}) for all sampling sites was found for D3 sampling site, and the minimum lead value (0.0001 mg^{-L}) was found for D1 and D5 sampling sites.

From the lead analysis, it may be inferred that all pharmaceutical industries of Dehradun district (D1–D5) discharge their wastewater with proper treatment with respect to lead and do not require further measures for controlling lead (Fig. 18.15).

18.8.13 Assessment of Sodium Absorption Ratio (SAR)

The SAR values in wastewater samples of the study area of Dehradun district varied from 0.35 to 13.98. The SAR value of 26 as water quality standard of water use in irrigation and industrial cooling has been promulgated by Bureau of Indian Standard

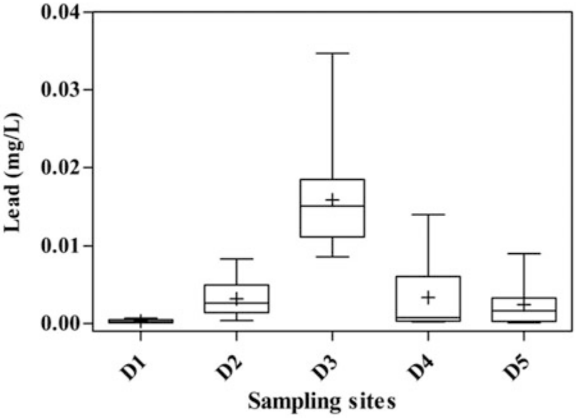


Fig. 18.15 Box and Whisker plot of lead analysed from wastewater of five pharma industrial sites (D1–D5) of Dehradun district during 2012 and 2013

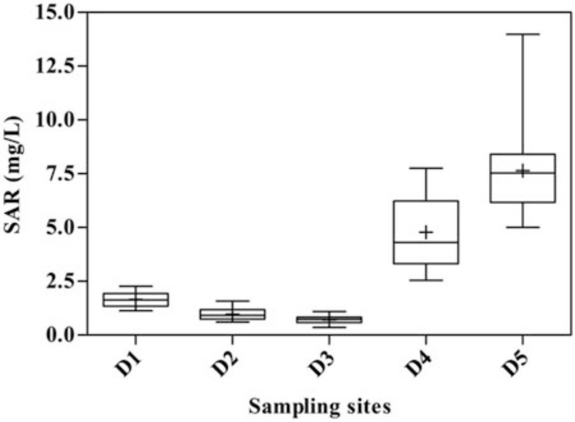


Fig. 18.16 Box and Whisker plot of SAR analysed from wastewater of five pharma industrial sites (D1–D5) of Dehradun district during 2012 and 2013

(Source IS 2296:1992). The mean values for entire sampling location ranged from 0.71 to 7.64, and the mean values for entire sampling locations were found below the standard permissible limit.

The variation in SAR values of wastewater at all the sampling sites of Dehradun district throughout the study is shown in Fig. 18.16. The maximum SAR value (13.98) for all sampling sites was found for D5 sampling site, and the minimum SAR value (0.35) was found for D3 sampling site.

From the SAR analysis, it is clear that all pharmaceutical industries of Dehradun district (D1–D5) discharge their wastewater with proper treatment with respect to SAR and for these sampling sites, no additional measures are required to control the SAR level in their wastewater.

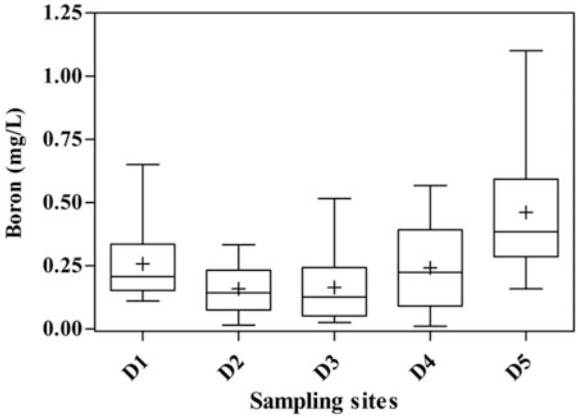


Fig. 18.17 Box and Whisker plot of boron analysed from wastewater of five pharma industrial sites (D1–D5) of Dehradun district during 2012 and 2013

18.8.14 Assessment of Boron

The boron value of 2.0 mg/L as standard permissible limits for release of industrial pollutants into internal surface has been provided by BIS (IS: 2490, Part-I-1981). The average boron values for all sampling sites of Dehradun district were found below the standard permissible limit. The average boron values fluctuated from 0.158 to 0.460 mg^{-L}. The average highest value (0.460 mg^{-L}) was seen in D5 sampling site, and minimum average value (0.158 mg^{-L}) was observed for D2 sampling site.

The range of boron values for all sampling sites of Dehradun district is shown in Fig. 18.17. The maximum boron value (1.1 mg/L) for all sampling sites was found for D5 sampling site, and the minimum boron value (0.011 mg/L) was found for D4 sampling sites.

From the boron analysis, it is clear that all pharmaceutical industries of Dehradun district (D1–D5) discharge their wastewater with proper treatment with respect to boron and these sampling sites do need any special improvement treatment procedure to control the boron level in their wastewater.

18.9 Problem Parameters Selected for Bioremediation

The study of PIWW of five sampling sites of Dehradun during 2012 and 2013 has led to the seven problem parameters (Table 18.8), which are more than their prescribed standard limits in wastewater. Out of these seven parameters, five parameters, namely, TDS, EC, phenols, BOD and COD, have been selected for bioremediation process for reduction in PIWW so that they can meet the requirement of wastewater for agriculture reuse.

Table 18.8 The problem parameters found for PIWW of Dehradun during 2012 and 2013

S. N.	Name of parameter	Std. limit	Average value more than or less than Std. limit found	Site code
1.	pH[a]	6.0–8.5	4.4	D1
			4.80	D2
			5.82	D3
2.	TSS[a]	100 mg^{-L}	158 mg^{-L}	D1
			180 mg^{-L}	D2
			126 mg^{-L}	D3
			158 mg^{-L}	D4
			139 mg^{-L}	D5
3.	TDS	2100 mg^{-L}	2476 mg^{-L}	D5
4.	EC	2250 μS/cm	3078 μS/cm	D1
			2588 μS/cm	D2
			2473 μS/cm	D3
			2974 μS/cm	D4
			3781 μS/cm	D5
5.	Phenols	1.0 mg^{-L}	2.85 mg^{-L}	D1
			4.19 mg^{-L}	D3
			1.85 mg^{-L}	D4
			2.57 mg^{-L}	D5
6.	BOD	100 mg^{-L}	229 mg^{-L}	D1
			141 mg^{-L}	D2
			107 mg^{-L}	D3
			161 mg^{-L}	D4
			150 mg^{-L}	D5
7.	COD	250 mg^{-L}	758 mg^{-L}	D1
			641 mg^{-L}	D2
			562 mg^{-L}	D3
			1095 mg^{-L}	D4
			1504 mg^{-L}	D5

Sources: Rana et al. (2014a, b)

[a] Not considered for bioremediation process

Among seven parameters, pH was maintained during bioremediation process and hence was not considered for reduction or treatment. Similarly, TSS was removed by filtration using sieve of relevant/varying mesh sizes before bioremediation and hence not considered for further reduction.

18.10 Isolation, Screening and Identification of Microorganisms

Different soil samples as well as wastewater samples from pharmaceutical industrial sites were serially diluted and spread on nutrient agar medium. After 24 h six isolates, which showed the growth on solidified medium, were isolated and purified on agar medium by streak plating method. These isolated microbial colonies were studied for their morphological and biochemical characterization. The six bacterial isolates showing maximum growth capability in pharmaceutical wastewater and six bacterial strains procured from Institute of Microbial Technology (IMTECH), Chandigarh, showing the degradation capability of pollution load were selected for the further study. The six selected bacterial isolates showed the less to moderate degradation capability in terms of COD, BOD and other pollutant characteristics. However, the bacterial strains procured from the IMTECH showed enhanced capabilities of biodegradation of different pollutants present in pharmaceutical industrial wastewater (PIWW). Among them, *Xanthomonas campestris* showed the maximum degradation capability of 75.09% in terms of COD and other pollutants in terms of 70–77%.

18.11 Reuse of Treated Pharmaceutical Industries Wastewater in Agriculture

Agriculture uses the majority of water of lower quality than domestic and industrial use. Therefore, seeing the need of time, it is unavoidable to look towards irrigated agriculture for the entire solution for the challenge for disposal of effluents. Such wastewaters have hazardous pollutants and impurities which need a careful consideration for the feasible long-duration influence on soils and plants. The trace elements, nutritious elements and salinity that takes place usually are controllable; if related challenges with these pollutants and contamination are understood, then approvals should create for them after proper treatment.

The purpose of this overall research is to assess the qualitative and quantitative presence of pollutants and remove these contaminants from pharmaceutical wastewater by biological system so that the treated wastewater meets the acceptable quality standards for its use in agriculture as per the norms of Indian standards. BIS (Bureau of Indian Standards) have given water quality standards for irrigation water as shown in Table 18.9.

18.12 Development of Bacterial Consortia

An increased degree of biodegradation/mineralization was observed, when co-metabolic activities within a diverse microbial population explored. In such a “microbial consortium”, the organisms can act synergistically on a reducing capability of pollutants in wastewater. Single organism may be capable of initiating a biotransformation of the wastewater components, which subsequently renders it

Table 18.9 Water quality parameters for water utilization for irrigation (Source IS 2296:1992)

Characteristics (all parameters in mg/L, max except pH, EC, SAR)	For use in irrigation (class E)
pH	6.0–8.5
TDS	2100
Chlorides	600
Sulphates	1000
EC (μS/cm), max	2250
SAR, max	26
Boron	2

Source: Mareddy (2017)

Table 18.10 Development of bacterial consortia in PIWW

Bacterial consortia	TDS	EC	COD	BOD	Phenols
	Before treatment analysis				
	2713 mg/L	4136 mg/L	1443 mg/L	216 mg/L	1.93 mg/L
	After treatment analysis (% reduction)				
B1, 2, 3 (C1)	71.02%	70.82%	70.02%	72.25%	75.2%
B1, 2, 3, 4 (C2)	73.12%	73.00%	65.00%	71.20%	76.02%
B1, 2, 3, 4, 5(C3)	76.25%	76.08%	66.01%	72.03%	77.2%
B1, 2, 3, 4, 5, 6 (C4)	74.00%	74.51%	72.05%	75.02%	73.0%
B2, 3, 4, 5, 6 (C5)	93.14%	92.90%	95.02%	91.05%	94.1%
B2, 3, 4, 5 (C6)	75.05%	75.02%	70.01%	73.09%	74.05%
B2, 3, 4 (C7)	71.05%	71.40%	70.06%	70.04%	70.02%
B1, 3, 4, 5, 6 (C8)	75.12%	74.20%	60.02%	75.09%	79.02%
B3, 4, 5, 6 (C9)	84.14%	84.00%	75.04%	79.14%	80.0%
B3, 4, 5 (C10)	76.14%	76.02%	70.11%	78.16%	76.03%
B4, 5, 6 (C11)	85.20%	85.65%	78.09%	80.00%	80.45%

more easily accessible to another organism that otherwise would be unable to attack the pollutants of wastewater. This approach for reduction capability of pollutants in wastewater by mixed culture led us to develop a consortia containing at least four distinct microbial strains for the decomposition of the complex drug industry effluent. In this evaluation, a total of 11 consortia were developed using combinations of 6 procured bacterial strains from Microbial Type Culture Collection, Chandigarh: *Bacillus subtilis (B1)*, *Bacillus stearothermophilus (B2)*, *Bacillus pumilus (B3)*, *Micrococcus luteus (B4)*, *Pseudomonas putida (B5) and Xanthomonas campestris (B6)*. These 11 consortia were tested for its TDS, EC, COD, BOD and phenol reduction ability.

For bacterial consortia, selection of bacterial culture was based upon their maximum degradation capabilities towards complex pharmaceutical industrial wastewater at previously optimized performable conditions. In this study, 11 bacterial consortia were used in shake flask studies for reduction of complex industrial wastewater, and results obtained are shown in Table 18.10. This table clearly reflects that consortia no. 5 (C5) showed maximum degradation (91–95%) capabilities for all five parameters selected for study. In all the studies, five bacteria culture (B2, B3, B4, B5 and B6) used in selected bacterial consortia (C5) were chosen for further bioprocess development studies at previously optimized conditions in laboratory.

18.13 Bioprocess Development for Pharmaceutical Wastewater Treatment

Degradation of pollutants generally does not take place in highly anaerobic conditions which shows that a minimum critical amount of oxygen present in the facultative anaerobic condition, i.e. static culture, is needed for the bacterial consortium to maintain their cellular and metabolic activity for effective reduction of pharmaceutical industry wastewater. The performance of consortium C5 (*Bacillus stearothermophilus (B2)+ Bacillus pumilus (B3)+ Micrococcus luteus (B4)+ Pseudomonas putida (B5)* + *Xanthomonas campestris (B6)*) shows the better performance than the individual isolates showing more than 85% reduction for all selected parameters. The process was further carried out in indigenously designed glass bioreactor (Fig. 18.18a, b).

18.14 Wastewater Treatment Studies with Cell-Free Matrices

The cell-free calcium alginate and sawdust micro-carrier matrix performed only 2.0% and 2.9% of total treatment. Beside these matrices, micro-carriers, namely, activated charcoal and fly ash, had a higher adsorption capability which resulted in 8.5% and 9.43% reduction, respectively, for complex pharmaceutical wastewater product. These results showed that the degradation contributed by matrix adsorption of wastewater is negligible for calcium alginate matrix and sawdust in comparison to fly ash and activated charcoal micro-carrier matrices in a fixed bed process. The low wastewater removal capability of the matrices can be attributed due to low wastewater treatment capability of the matrices.

18.15 Degradation Capability of Bacterial Consortia Immobilized with Different Carrier in Packed Bed Bioreactor for PIWW Treatment

The maximum percentage reduction (81%) in terms of all parameters was achieved by bacterial consortia immobilized with fly ash, while bacterial consortia immobilized with sawdust, calcium alginate and activated charcoal showed 74%, 74.2% and 67% reduction, respectively, in terms of all parameter (Table 18.11, Fig. 18.19).

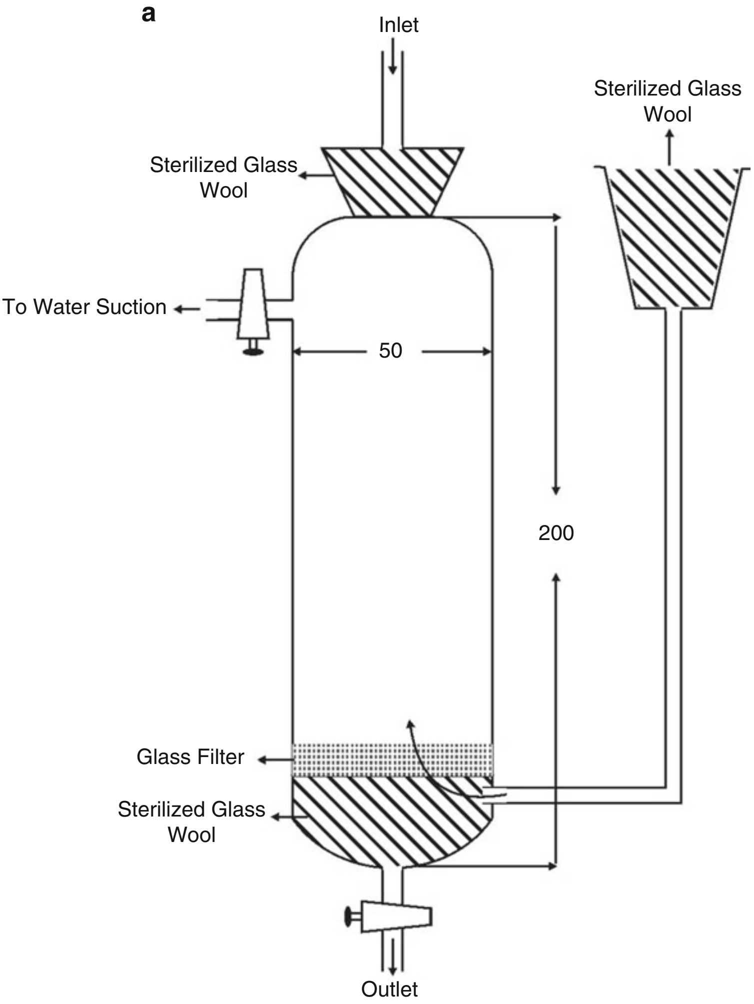


Fig. 18.18 (**a**) Single-stage bioreactor. (**b**) Two-stage bioreactor

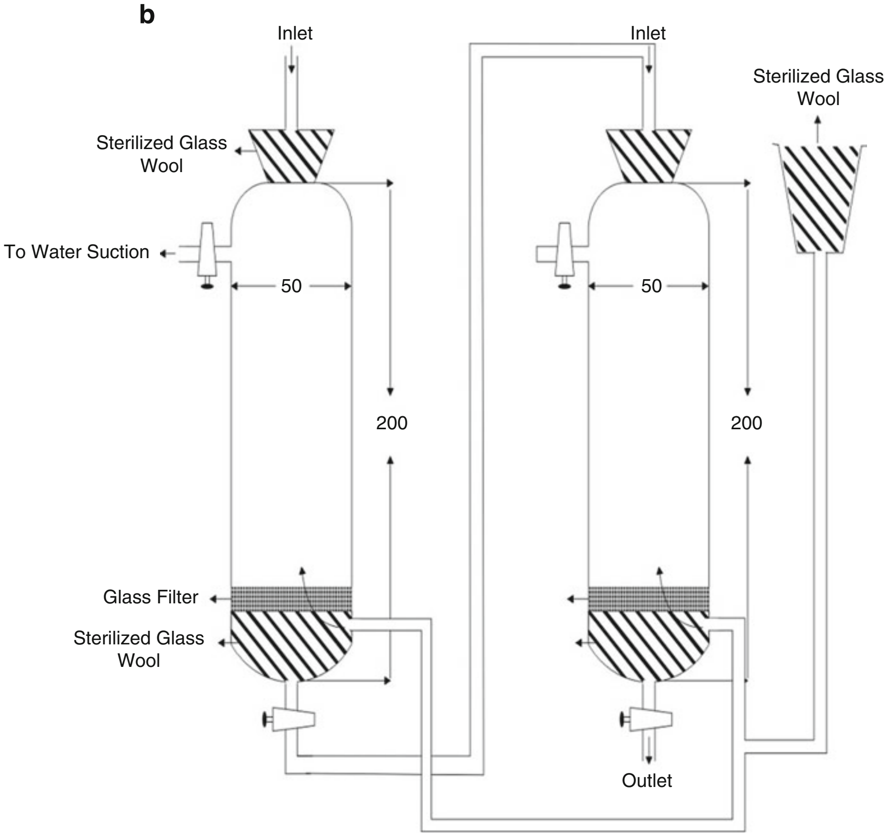


Fig. 18.18 (continued)

18.16 Wastewater Treatment in Sawdust Packed Bed Bioreactor

After determining the optimum conditions for degradation of wastewater-supplemented minimal growth medium by the batch process, further development of the continuous bioprocess was performed. Degradation of wastewater for continuous treatment was carried out in a bioreactor under conditions optimized by batch process treatment (single-stage bioreactor process). In the study of the time course for degradation of wastewater in the single-stage bioreactor process, during 30 h and 42 h, the culture eventually attained the maximal degradation in sawdust and calcium alginate immobilized bioreactors, respectively. This period could be taken as the acclimatization period. For the maintenance of culture in the acclimatization period for sawdust and calcium alginate, immobilized bioreactors were 24 and 36 h, respectively. After, late log phase during batch treatment process, continuous

Table 18.11 Degradation efficiency of different carriers in packed bed bioreactor

	Fly ash				Sawdust				Activated charcoal				Calcium alginate			
Time (h) reduction (%)	6	12	18	24	6	12	18	24	6	12	18	24	6	12	18	24
TDS	20	30	49	78	9	14	23	70	19	28	45	62	9	13	23	75
EC	21	31	48	78	9	15	24	71	20	29	46	63	10	13	24	76
COD	23	39	56	89	12	21	30	80	23	39	48	70	11	19	25	77
BOD	12	30	56	77	9	13	22	78	12	30	48	70	8	11	19	78
Phenols	10	22	41	84	30	25	36	74	31	30	33	74	25	29	30	65

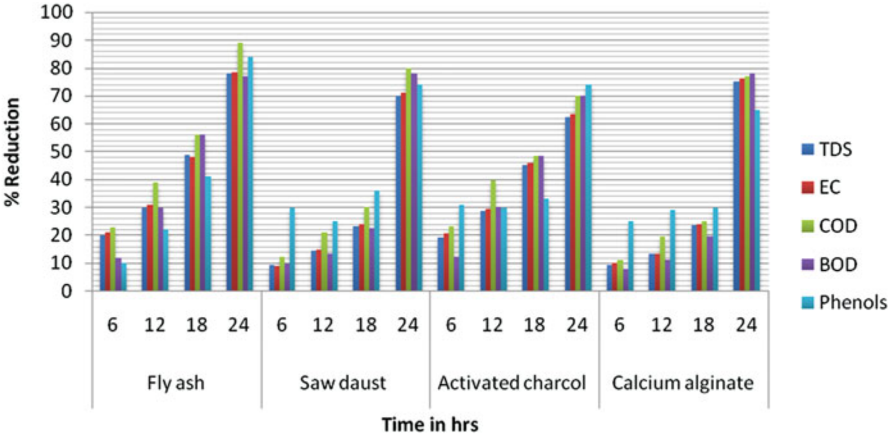


Fig. 18.19 Degradation potential of bacterial consortia immobilized on different inert micro-carriers in packed bed bioreactor (PBR) (Source: www.docstoc.com)

feeding of wastewater (100 mL/h) suspended in minimal growth medium was started through an inlet at a previously determined desired flow rate to give sufficient residence point (time for the medium to flow through the inlet port of the reactor to the outlet port) for the treatment to complete. The wastewater was collected continuously and analysed every 6 h. To further validate the techno-economic feasibility of the bioreactor at the scaled-up level, the sawdust as an immobilize carrier seems to be promising and can be exploited because of its availability as a cheap waste product of wood-processing industry.

The optimum wastewater loading rate was determined with feeding among the range from 50 to 400 ml of wastewater. The results shown in Fig. 18.20 showed that optimum wastewater loading rate required for the maximal sustainable degradation was up to 100 ml/h.; above this loading rate, the degradation capability of the strain drops drastically. This may be correlated to the fact that at higher concentration, the toxicity of the wastewater affected the growth of biomass.

The optimum conditions for the maximum wastewater degradation in a continuous treatment are summarized in Table 18.12. Using optimum conditions, the culture stability was determined by monitoring the degradation potential six hourly over 20 days from the start of continuous feed of the wastewater into the bioreactor.

18.17 Phytotoxicity Study

Plant growth pot trial studies were carried out by *Phaseolus mungo* irrigated with unprocessed wastewater and processed waste aqua (Table 18.13). The untreated wastewater showed poor growth as compared to plants watered with tap water and treated wastewater. The flora irrigated with unprocessed effluent showed senescence

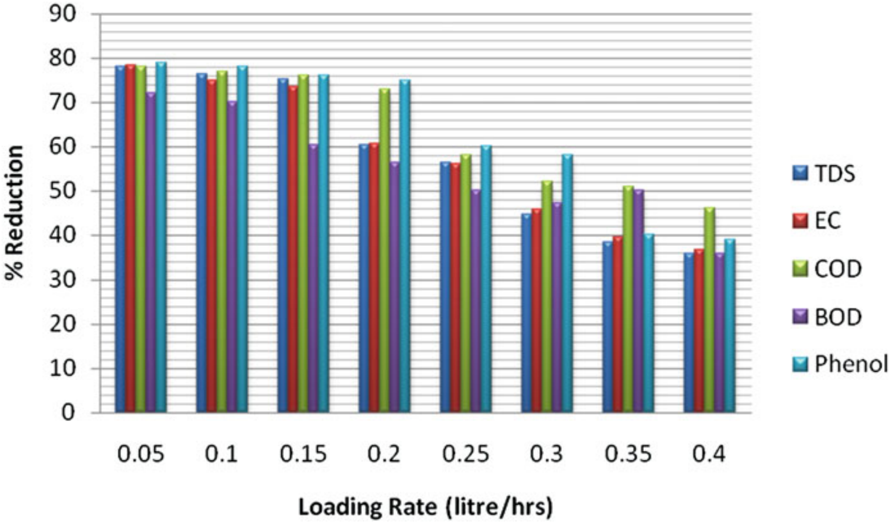


Fig. 18.20 Wastewater loading rate in (litre/h) sawdust in packed bed reactor

Table 18.12 Optimization for the continuous treatment process of PIWW with sawdust immobilized bacterial consortia in packed bed bioreactor

S. No.	Parameters	Values
1.	Wastewater loading rate	100 mL/h
2.	pH	7.0
3.	Temperature	35 °C
4.	Aeration	Static condition
5.	HRT	24–30 h

Table 18.13 Mean value of germination %, root length, shoot length and wet weight in *Phaseolus mungo* on 15-day field trial

Parameters	Group A (watered with tap water)	Group B (watered with PIWW)	Group C (watered with treated wastewater)
Germination %	80	30	70
Root length (cm)	9.56	3.22	9.13
Shoot length (cm)	17.23	6.40	16.18
Wet weight of plant (g)	2.30	0.73	2.17

and wilting. However, the flora irrigated with processed effluent by the bacterial consortia immobilized in sawdust showed increment in germination %, root length, shoot length and plant fresh weight. The plants watered with treated wastewater showed best results as compared to untreated wastewater, and there was 133% enhancement in germination % and 251% enhancement in fresh plant weight. The degradation products of the wastewater pollutants of pharmaceutical industry

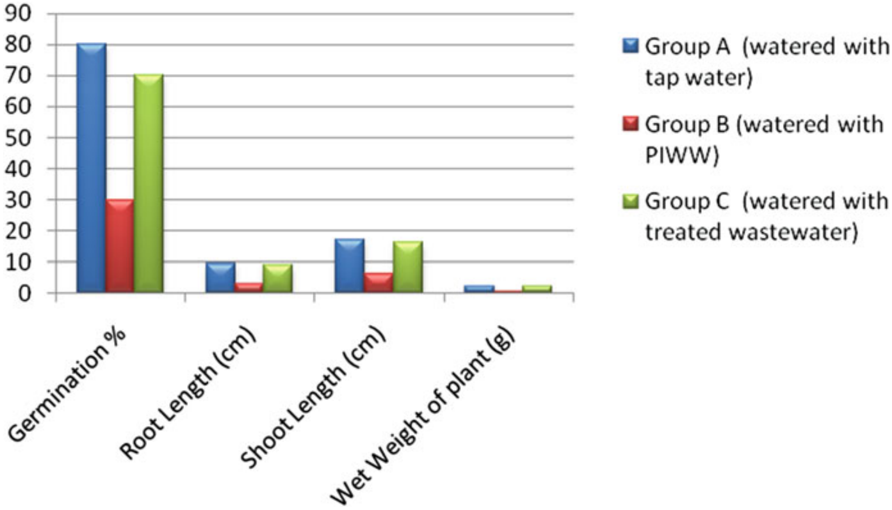


Fig. 18.21 Phytotoxicity study of treated wastewater

wastewater significantly affected the length of root and shoot which indicates less toxicity of the degradation product by the microbial consortia (Fig. 18.21).

18.18 Conclusion

Submerged, surface and pseudo-immobilized system and immobilized system tried to treat pharmaceutical wastewater. The results obtained with the various systems of treatment clearly indicate that there is a great potential for free, pseudo-immobilized and whole cell-immobilized cultures to be used in bioreactor for the degradation of pharmaceutical industry wastewater. The degradation with immobilized cells was slightly lower than free cells and pseudo-immobilized cells. However, the important aspect of the process and whole cell immobilization was the possibility of reusing the same biomass, in subsequent treatment cycles. Our result showed that the cumulative half-life of the pseudo-immobilized replacement batch cycle was 24 days while in the case of whole cell was 30 days. Further, research will, however, be required to develop it into a commercial reality.

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Phytobionts of Wastewater and Restitution

19

Praveen Solanki, Maitreyie Narayan, Shiv Singh Meena,
R. K. Srivastava, M. L. Dotaniya, and C. K. Dotaniya

Abstract

Wastewater generation cannot even reduce without compromising economic and urban development; it originates mainly from point sources of water pollution and contains high concentrations of agricultural nutrients, i.e., N, P, K, S, B, etc., as well as OM, COD, BOD, and different heavy metals. These nutrients as energy source boost up the growth of phytobionts and result in enhanced phytoremediation and bioremediation of wastewater. In addition to this, nutrients and organic matter present in wastewater show tremendous potential for application in agriculture as “fertigation.” Furthermore, the huge generating wastewater needs more advanced treatment methods using phytobionts as the catalyst in the future. This chapter mainly focuses on wastewater crisis, its economic reuse for fertigation, other non-potable purposes, and bio-treatment through phytobiont-mediated phytoremediation and bioremediation to overcome the scarcity of freshwater. Later in the chapter, recycling of nutrients, metals, and organic matter through bioremediation and phytoremediation is also discussed.

P. Solanki (✉) · M. Narayan · R. K. Srivastava
Department of Environmental Science, GBPUA&T, Pantnagar, India

S. S. Meena
Department of Soil Science, GBPUA&T, Pantnagar, India

M. L. Dotaniya
ICAR-Indian Institute of Soil Science, Bhopal, India
e-mail: Mohan.Dotaniya@icar.gov.in

C. K. Dotaniya
College of Agriculture, SKRAU, Bikaner, India

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19.1 Introduction

As the population doubled after half of the nineteenth century, the water requirement has reached tripled accordingly; consequently, the population explosion has resulted in the increased quantity of wastewater and its pollution load by incorporating human, industrial, and agricultural wastes (Ward 2002; Chan et al. 2009). There are many numbers of natural and anthropogenic waste compounds present in the aquatic environment, which are mainly contribution of surface runoff and disposal of domestic and industrial wastewaters (Kolpin et al. 2002; Kuemmerer 2010). These compounds include natural toxins (Hoerger et al. 2009; Schenzel et al. 2010), pesticides through runoff from agricultural field (Stoob et al. 2005), and waste from industries based on beauty care products and pharmaceuticals (Oulton et al. 2010).

In India, approximately 1.2 billion people had access to only 1820 m^3 of water per capita per year in 2014, which was 5177 m^3 per person per year in 1951 (Bhati et al. 2015), indicating the water crisis of the present and future. Furthermore, only 68% of Indian population are drinking safe water, and the problem is becoming worst due to population explosion and rapid industrialization. India's rank (141th) in Environmental Performance Index (EPI) for the year 2016 shows an alarming situation for the protection of human and environmental health on world map (Hsu et al. 2016). The Central Pollution Control Board (CPCB 2015) realized the big gap between sewage generation and treatment capacity for urban India in 2015 and reported that only a small fraction of about 23,277 MLD (37.54% of total wastewater) out of 62,000 MLD (million liters per day) is treated through 816 STPs across the country. Water demand of industries is also under pressure, and many industries have been forced to adopt wastewater recycling systems and its reuse for different industrial purposes (WWTI 2010; Grant 2011). Hence, there is an urgent need to develop low-cost and eco-friendly technologies for wastewater treatment and also to focus on the reuse of this treated water for non-potable purposes such as fertigation, flushing the toilet, thermal cooling tower, iron industries, etc.

Phytobionts are photosynthetic in nature such as algae; still only little is known about phytobionts and their association with other microorganisms and plants. Since phytobionts significantly affect plant growth, they enhance the phytoremediation efficiency of plants for efficient accumulation and extraction of several metals and pollutants. Phytoremediation is a plant-based technology (Solanki et al. 2017a), viz., phytoextraction, rhizofiltration, rhizodegradation, phytovolatilization, phytostabilization, etc. Bioremediation of wastewater is a microbial-mediated process, in which more efficient indigenous microorganisms are inoculated. Mostly industrial and municipal effluents serve as a source of significant amount of phytobionts, and harvesting of these for bioremediation of wastewater is an emerging and eco-friendly way of wastewater treatment. Phytobionts play a significant role in recycling of metals and nutrients, since they use nutrients like P, K, N, S, Ca, and so forth as the energy source for their growth.

The eco-friendly management of wastewater can also be done by the process called phytoremediation, in which wastewater is used as irrigation source, and up to some extent, it is also called as "fertigation" (Shankhwar and Srivastava 2014) as it contains several essential nutrients as well as organic matter which is required for healthy plant growth (Matheyarasu et al. 2016; Plaza et al. 2007). Most part of available freshwater, approximately 79%, is used in irrigation; hence for sustainable use of water resources and to reduce extreme water scarcity in the future, it is very necessary to reuse the domestic and industrial wastewater for irrigation or fertigation purpose after certain treatment (Zaidi 2007); however the water crisis in the future can also be compensated by rainwater harvesting, since it is the most significant method for recharge of freshwater resources (Yenmis et al. 2016).

19.2 Need of Wastewater Treatment in Present Situation

Water is one of the most crucial elements, which is required every day by human beings as well as the entire living biosphere for their survival. Without the adequate availability of hygienic water, the existence of the entire biosphere would be under crisis and will lead to the collapse of several living and nonliving ecosystems. The global scarcity of freshwater is a strong challenge for sustainable development. In India also freshwater scarcity has been found in several states, as groundwater continues to exhaust more than its recharging rate (Mandal and Priti 2015). As per Indian Standards (IS:1172–1963), i.e., 135 l per person per day (LPD) is required for various domestic purposes throughout the day; however, the daily wastewater generation is around 64% of 135 LPD (Shankhwar et al. 2016). Besides this, population explosion is boosting up wastewater generation in manifold ratio. Globally in 2013, the unsafe drinking water leads to approximately 2% (~1.24 million) of deaths, and access to freshwater has been drastically decreased by approximately half from 960 million in 2000 to 550 million people in 2016 (Hsu et al. 2016). Thus, the reuse of this huge wastewater for irrigation purpose, such as "fertigation" (Shankhwar and Srivastava 2014), is an emerging issue to manage the increasing freshwater demand for human consumption. The sources of wastewater generation, particularly from the nonpoint sources of wastewater, are multiple in nature as well as it is very tedious to assess the exact point from where the pollutants are entering into the water body (Wagener et al. 2010; Destouni et al. 2013). Quantitatively wastewater generation is also very tedious to assess, as it completely depends upon human, industrial, socioeconomic, and cultural activities at different times (Zhoua et al. 2000). Wastewater generation varies from season to season, day to day, and even it is changing on an hourly basis also (Henze et al. 2000), which creates hurdle in the preparation of wastewater treatment strategy. Due to the lack of wastewater treatment facility, e.g., only 60% of industrial and approximately 26% of domestic wastewater are currently treated in India (Bhati et al. 2015). As well as due to inactive strong policies, most of the domestic wastewater and industrial effluent water are directly discharged into the vicinity rivers and other water bodies, resulting in approximately 75% contamination of all freshwater sources across the country (CPHEEO 2012). Human interference for unsustainable instant benefits and inappropriate use of water leads to more pollution load. Hence, it is very necessary to develop low-cost and eco-friendly, phytobiont-based wastewater treatment methodologies which will be more efficient and less time-consuming in managing and reusing this huge quantity of wastewater.

19.3 Manurial Values and Toxic Metal in Wastewater

Wastewater comprises two types of water, i.e., gray water and black water, with approximately 7:3 ratio of gray and black water, respectively (Pandey et al. 2014). Gray water comes from bathroom, kitchen, washing, and so on, whereas the black water comes from the toilet. The nutritional value of sewage water at Gangneung City, South Korea, has been reported by Choi and Lee (2015) as total phosphorus (9.24 mg/l), PO_4 (8.19 mg/l), and total nitrogen (40.02 mg/l), which shows the agricultural potential of sewage water. The manurial potential of gray water was also assessed at Pantnagar Agriculture University, where the total aboveground biomass (AGB) production of *Eucalyptus* hybrid (clone K-413) revealed 34.19 kg/tree in gray water-treated plots as compared to 25.69 kg/tree in control plots (irrigated with well water) (Shankhwar et al. 2016). More presence of microorganisms indicates the higher nutrient level of water sewage from residential areas and biodegradable waste from industrial discharge has shown relatively higher microbial biomass (Kumar and Pal 2015). In India, 75% effluents are discharged from paper and pulp mill emerging as wastewater (Kumar et al. 2014) and widely used for irrigation. Furthermore, this wastewater contains several essential plant nutrients, viz., nitrogen (N), potassium (K), sulfur (S), phosphorus (P), calcium (Ca), etc. (Jais et al. 2017; Hultberg and Bodin 2017). Wastewater can be the best option to irrigate turfgrass of golf courses as an alternative to freshwater since it contains some essential nutrients; however, the electrical conductivity can be a limiting one (Beltrao et al. 2014). Globally it is also observed that rapid increase in the concentration of phosphorus (P) and nitrogen (N) in wastewater leads to accelerating eutrophication of receiving water bodies (Conley et al. 2009).

In recent era of rapid industrialization, over 30,000 chemicals are produced and used in simple as well as in their complex form (EPA 2014). Approximately 3300 chemical compounds are essential for drug and human health care (UNEP 2013). It is surprising to know that, in developing countries, most of these chemicals are discharged into the environment without proper and safe disposal (Cooper Smith and Rundle 1998). Many of these chemicals have extremely long environmental persistence (Popile and Sankowska 2011; Arnot 2009). Natural geochemical and bio-physiological processes produce huge organic as well as inorganic chemicals, which are harvested and consumed by several microorganisms as their energy source (Fantroussi et al. 2006). However, thousands of toxic compounds are produced artificially by different industries, e.g., leather, plastic, electroplating, agricultural chemicals, etc. (Teodorescu and Gaidau 2008). Many chlorinated acids are discharged as waste from the paper and pulp industries; in addition to this, several heavy metals such as Cr, Ni, Hg, Pb, etc. are also discarded during manufacturing activities. The effluents of tannery industries are the major sources of chromium (Cr) in wastewater (Dotaniya et al. 2016a). Continuous application of Cr-contaminated effluents as irrigation into the fields by farmers resulted in relatively more accumulation of Cr in soil by 28–30% as compared to freshwater irrigation (Dotaniya et al. 2014). Most of the irrigation water contains certain toxic ions. Concentrations beyond the maximum permissible limits can cause toxicity in plants, which leads to impaired growth, degrade the quality of yield, etc. The most frequently observed phytotoxic ions in wastewater are boron (B), sodium (Na), chloride (Cl^-), etc. Hence, before using any wastewater or industrial effluent water for irrigation, the presence of phytotoxic ions should be assessed for suitability of wastewater for agricultural production.

19.4 Characterization of Wastewater Based on Properties

19.4.1 Physicochemical Properties

19.4.1.1 pH

The pH shows the acidity or alkalinity of any solution, and usually, pH value lies between 1 and 14, with 7 being neutral (Bagshaw et al. 1972). The pH is one of the most important parameters of any wastewater, as it measures the acidity/alkalinity or hydrogen ion concentrations and also affects most of the other parameters of wastewater such as availability of heavy metals, the activity of ions, chemical oxygen demand, nutrient level, etc. It also shows the source of wastewater, e.g., most of the effluents from dye industries are acidic in nature. Oxidation of sulfides and iron pyrite by bacteria leads to discharge of their acidic leachates into rivers and other water bodies. These leachates also contain dissolved ferric iron that results in sufficient increase in the pH of stream water for the precipitation of iron into ferric hydroxides. It leads to blanketing the surface of water bodies and wiping out several sensitive plants and animals due to insufficient sunlight and oxygen. When the pH of water is below 7.0 (acidic pH), or slightly alkaline, this water would have a property of corrosiveness, as the acid has the potential to rapidly dissolve metals.

19.4.1.2 Electrical Conductivity

The electrical conductivity (EC) of wastewater is measured as “the ionic compounds dissolved in water” (Ali et al. 2011). Similar to pH, EC of wastewater is also an important parameter for water quality standards and measured in siemens per meter (S/m). In developed countries like the USA and Australia, wastewater reclamation is very common, and for that purpose, the EC is known as an important parameter (EPA 1999). The EC is a key parameter for total cations and salinity determination (APHA 2005). It is also used to determine total ionic concentrations as well as ionic salinity (Zinabu et al. 2002).

19.4.1.3 Chemical Oxygen Demand

The chemical oxygen demand (COD) is the measure of total organic and inorganic compounds present in wastewater. The COD determination is more preferable, and unlike the BOD test, it is easier and less time-consuming, i.e., only 2-h reflux at 150 °C for complete digestion by potassium dichromate ($K_2Cr_2O_7$) as strong oxidizing agent, silver sulfate ($AgSO_4$) as catalyst, and mercuric sulfate ($HgSO_4$) to avoid interference of chlorides with COD reaction (Yintao et al. 2004). The value of COD is usually expressed in milligram per liter (mg/l), which is proportional to the consumed potassium dichromate during digestion. If the wastewater contains only organic or easily biodegradable matter, the COD and BOD are supposed to have approximately equal values. For domestic wastewater, the ratio of COD/BOD is 2:1 (Liu et al. 2016), while for wet market wastewater, it is 5:1 (Zulkifli et al. 2012); however, if this ratio is high (e.g., >8:1), it shows increased presence of toxic compounds, resulting in death of bacteria which has to lower the BOD value (Wright 1987).

19.4.1.4 Total Solids

The runoff from agricultural fields and overflow of urban sewage water are major sources of solids in wastewater. The total solids (TS) consist of total dissolved solids (TDS) and total suspended solids (TSS). Total solids are added by natural disasters, viz., floods, typhoons, earthquakes, cyclones, etc. similarly by anthropogenic activities like coal mining, stone cruising, direct throwing of waste into water drain, industrial effluents, etc. (Pettersson and Lavieille 2007). Sewage water or domestic wastewater consists of about 99% water, which comes from the kitchen, bathing, urine, night soil, laundry, and so on. A small portion of solid wastes degrade into the solution, and the rest remain as such in colloidal as well as in true suspension form; besides these solids, salts by cooking, bathing, laundry, urine, detergents, etc. are also added (CPHEEO 2012).

19.4.1.5 Total Dissolved Solids

The total dissolved solids (TDS) are the solids that remain as suspension or completely in dissolved form in the water, and do not settle down easily; however, they are filterable in nature. The presence of significant amount of dissolved solids leads to reduced aesthetic value of the water body; also the taste, odor, and available oxygen of the water are reduced. The TDS of the sample is proportional to the hardness. Some of the dissolved solids are carcinogenic in nature and have several adverse impacts on human and aquatic animals. High concentration such as 3000 mg/l of TDS can cause distress in animals, scaling in boilers, and corrosion of many industrial instruments. These solids also act as an obstacle for oxygen, as they prevent oxygen to reach up to the bottom of the water body, resulting in the depletion of photosynthesis by phytoplankton deep layers (Klausmeier et al. 2004).

19.4.1.6 Total Suspended Solids

These are non-filterable in nature and are defined as the residues left over filter paper (2.0 μm or smaller pore size) after complete evaporation at the controlled temperature of 179–181 °C. However, the difference between TS and TDS also gives the value of TSS for the same water sample. Suspended solids, those that are biologically active in nature, also are known disease-causing organisms and cause many diseases in human beings.

19.4.1.7 Heavy Metals

Fast industrialization and rapid growth in intensive agriculture have enhanced heavy metal concentration in the water-plant-soil system. Anthropogenic materials, viz., fertilizers, paints, cosmetic materials, etc. contain several heavy metals like cadmium (Cd), lead (Pb), arsenic (As), chromium (Cr), and so on (Yetis 2008). These metals are very sensitive to leaching and runoff due to excessive irrigation and finally reached the water body, where they remain unchanged for many years based on their persistence in the environment. These metals are in very trace amounts, still extremely toxic to the aquatic animals, plants, and human being as well. They also have the tendency to impair the entire biological system of the living organisms. Most toxic metal for plant and soil microorganisms is chromium (Dotaniya et al. 2016b), which exists in two forms, Cr^{3+} (less toxic) and Cr^{6+} (more toxic). Cadmium is another toxic element whose long-term application through Cd-contaminated fertilizers and wastewater results in deterioration in soil fertility and crop quality. Sources of arsenic (As) in wastewater are As-bearing rocks, rat-killing poisons, fungicides, and weedicides. The consumption of this As-contaminated water affects the soil health, crops, and human beings, which is more commonly observed in West Bengal of India and in Bangladesh. In sewage water and sludge, selenium (Se) is found in the elemental and selenite form, which is bound with hydrous iron oxides and trimethyl selenonium salts (Dhillon and Dhillon 1997).

19.4.2 Biological Properties

19.4.2.1 Biological Oxygen Demand

The BOD (biological oxygen demand) test is one of the most significant tests to assess the efficiency of any wastewater treatment method, which is simply equal to the amount of oxygen required by the microorganism to degrade the organic compound in the specific incubation period of 5 days and at controlled temperature of 20 ± 1 °C. The oxygen saturation level of normal water is very low, i.e., about 10 ppm; however the typical value of BOD for wastewater may be 20 times of saturation level; hence, discharge of this high BOD wastewater into water bodies results in further depletion of available oxygen that is necessary for aquatic organisms.

Organic pollutant loading of wastewater depends on flow rate and total pollutant concentration; therefore variations in wastewater generation significantly affect the total pollution load. The organic pollutant loading in wastewater can be calculated by the following formula (EPA 1997):

$$\mathrm{OL}\ (\mathrm{kg/day}) = \frac{\mathrm{Q}(\ \mathrm{m3/day}) \times \mathrm{BOD5}\ (\mathrm{mg/l})}{1000}$$

where, OL is organic loading, Q daily flow, and BOD5 biological oxygen demand of wastewater for the incubation period of 5 days at the controlled temperature of 20 ± 1 °C. Figure 19.1 represents the consumption of oxygen during the incubation period.

The wastewater with high BOD in order to degrade itself requires more oxygen. Enrichment of organic compounds into water bodies results in explosive growth of microorganisms and algal bloom. This way the oxygen again depletes and leads to death the entire aquatic ecosystem due to insufficient oxygen. The value of BOD for domestic wastewater ranges between 200 and 600, whereas for industrial whey it ranges between 40,000 and 50,000 (Choi and Lee 2015). The BOD test is the most significant parameter in designing of the sewage treatment plants (STP), since the total contribution of BOD is approximately 36 g per capita per day (g/capita/day) by

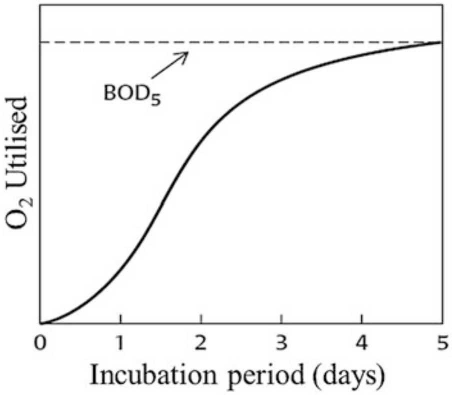

Fig. 19.1 Oxygen utilization curve during incubation period

generated volume of sewage. For example, if a town has a population of 550,000 and the volume of generated sewage is 55 MLD than the design for raw sewage, BOD can be calculated by the following formula (CPHEEO 2012):

$$\mathrm{BOD}\ (\mathrm{mg/l}) = \frac{\mathrm{Population\ (no.)} \times 36 \times 1000}{\mathrm{Generated\ wastewater\ (liter)}}$$
$$\mathrm{BOD}\ (\mathrm{mg/l}) = \frac{550000 \times 36 \times 1000}{55 \times 1000000}$$
$$\mathrm{BOD}\ (\mathrm{mg/l}) = 360$$

19.4.2.2 Total *E. coli*

The *Escherichia coli* (or *E. coli*) is probably the most found bacterium in wastewater, which is responsible to cause diarrheal diseases in the human being (WHO 2011). The presence of *E. coli* as indicator organism is one of the most significant tests of potable water. The coagulation process through aluminum sulfate (alum) and ferric sulfate is the most appropriate method to remove these coliforms from wastewater (Pritchard et al. 2016). The EPA (Environmental Protection Agency) standards for safe drinking water are based on the presence of coliforms, which are Gram-negative and gas-producing bacteria, viz., *Escherichia coli*, *Enterobacter*, and *Citrobacter*. The presence of *E. coli* in any water sample can be determined based on the lactose fermenting potential of coliform bacteria, which can be assessed by most probable number (MPN), membrane filter method (more direct method), etc. (Brejova et al. 2004).

19.5 Challenges in Wastewater Treatment

19.5.1 Excess Generation

The excessive generation of wastewater is a big challenge in treatment and recycling; even the unequal generation of wastewater from morning to evening is also an important factor in designing and construction of treatment units (Ahmed 2007).

Table 19.1 Wastewater generation and percentage of treatment capacity in India (By courtesy of ITF 2011)

Period	Wastewater generation (MLD)	Wastewater treatment (MLD)	Percentage of treated water (%)
2004–2005	26,254	7044	26.83
2005–2006	29,129	6190	21.25
2007–2008	33,000	7044	21.34
2008–2009	38,254	11,787	30.81
2009–2010	41,131	13,066	31.76
2010–2011	51,232	14,484	28.27

Moreover, the pollution load of undesirable substances is also increasing steadily due to the use of excessive fertilizers and agrochemicals for intensive agriculture, urbanization, and industrialization in the past three to four decades (Lang et al. 2013). However, the quantity of wastewater is greatly affected by human activities (WWTI 2010). Table 19.1 shows the quantity of wastewater generation and percentage of treatment capacity in India from 2004 to 2011 which is 51,232 MLD and 28.27%, respectively, in the year 2010–2011. Although, despite excess generation and less treatment of wastewater, the total quantity of water in the global hydrological cycle is unchanged over millions of years (Peixoto 1995).

19.6 Lack of Awareness

Water pollution control mainly depends on “three R” fundamental principles, i.e., reduce pollution at source point, recycle of wastewater, and reuse of wastewater. Among these three, reducing pollution at the source from where the pollutant is entering into the water body plays a vital role in the prevention of water pollution, and it mostly depends on public awareness. Lack of awareness among public for water crisis and relatively very less freshwater resources results in the excess generation of wastewater from households and overburden of pollutant load in the water which is collectively hindering the wastewater management. Still, in India, where people are throwing many worship materials as well as idol immersion, mass ritual bathing in the vicinity and holy rivers and traditional cremation areas at riverbank play a significant role in increasing pollution load of many rivers in India. The Ganges River is considered as India’s holiest river as well as the source of spiritual purification of the Hindus. However, due to pressures of rapid industrialization and direct discharge of municipal wastewater, the river is now the world’s most polluted one. It is surprising to know that 75% of wastewater pollution load is due to untreated municipal sewage, and the remaining 25% is by various industrial sectors (Helmer and Hespanhol 1997). Hence, public awareness is the first and foremost step for the prevention of water pollution, which is well said as “prevention is better than cure.”

19.7 Level of Pollutant Concentration

Prevention of water pollution through industrial and municipal wastes is another strong challenge for policy makers. However, by enforcing policies and by spreading awareness to the public, the load of pollution can be reduced up to a certain level and further reduced by treatment techniques. The first decade of twenty-first century has shown the tremendous growth in industrial sectors that resulted in manifold increase in the pollution level of rivers. Many agrochemicals and fertilizers are very sensitive to leaching and are getting easily washed out from the field with rainwater. Most of these chemicals are having long persistence in the environment and high resistant property to easy degradation (Agarwal and Joshi 2010). An alerting example is the discharge of mercury (Hg) by paper and thermometer manufacturers. Metallic mercury is allowed to discharge as waste and supposed to remain in sediments and later converted into its soluble form of methyl mercury which is consumed by fishes; finally this mercury enters the human body by consumption of contaminated fish in their diet, and this leads to Minimata disease (Carrier et al. 2001). Rural wastewater and runoff from agricultural fields often contain comparatively more amount of nitrogen, phosphorus, synthetic detergents, and so forth. It results in several diseases like blue baby syndrome in infants, as well as complete death of aquatic bodies by the process called eutrophication (Bashan and Bashan 2004). The Central Ground Water Board (2013) conducted a survey on groundwater for the assessment of contamination level of water in the different states of India, and the board observed that maximum arsenic (As) pollution was seen in West Bengal (56% of total groundwater sources were affected) followed by Bihar (32%) and Jharkhand (6%). In contrast to arsenic, maximum fluoride (F) pollution was found in Rajasthan (34%) followed by Andhra Pradesh (14%) and Karnataka (12%); similarly maximum nitrate (NO_3^-) pollution was observed in Maharashtra (40%) followed by Rajasthan (25%) and Gujarat (20%). Furthermore, both chemical and biological contaminations of water have been widespread, for example, earlier high concentration of arsenic (As) and fluoride (Fl) was traced only in the state of Uttar Pradesh and West Bengal, which has now extended in the states of Assam, Jharkhand, Maharashtra, Bihar, Chhattisgarh, etc. (CPCB 2015). Due to lack of sewage treatment plants in India, untreated domestic sewage water is being directly discharged into vicinity rivers and other water bodies resulting in bacterial contamination; similarly, runoff from agricultural fields adds pesticide, herbicides, and fertilizers to the water (Mandal and Priti 2015). To reduce the overburden of pollutants in dischargeable wastewater, rivers, and other water bodies and in order to protect human and environmental health, the Ministry of Environment, Forest and Climate Change (MoEFCC), Government of India, has made standards to be followed by all private as well as government bodies for discharging of their wastewater on inlands, water bodies, and marine coastal area and for irrigation purposes. These standards are presented in Table 19.2.

Table 19.2 Discharge standards for treated sewage water in mg/l (Developed from CPHEEO 2012)

Sl. No.	Parameters	Inland surface water	Public sewer	Land for irrigation	Marine coastal area
1	SS (suspended solids)	100	600	200	100
2	TDS (total dissolved solids)	2100	2100		2100
3	pH	5.5–9.0			
4	Temperature °C	[a]			[a]
5	Oil and grease	10	20	10	20
6	Total residual chlorine	1			1
7	Ammonia nitrogen as N	50	50		50
8	Total Kjeldahl nitrogen as N	100			100
9	Free ammonia	5			5
10	BOD (biological oxygen demand)	30	350	100	100
10	COD (biochemical oxygen demand)	250			250
11	Dissolved phosphorous as P	5			
12	Nitrate nitrogen as N	10			20
Fecal coliform courtesy of NRCD (2010)					
		Discharge onto land		Discharge onto water	
		Desirable	Max permissible	Desirable	Max permissible
13	Fecal coliform (MPN/100 ml)	1000	10,000	1000	10,000

[a] The temperature of receiving water body shall not exceed above 5 °C

19.8 Less Implementation of Policies to Ground Level

The central as well as all state governments in India enacted many acts and regulation for the prevention of water pollution; however, their implementations for ground-level action are very weak. Furthermore, currently, there is no particular act, regulation, or guideline which deals with safe disposal of wastewater at ground level. Since 1974, when India's first environmental act was enacted, i.e., the Water (Prevention and Control of Pollution) Act 1974, several acts and their amendments were done time to time for water pollution prevention across the country by the CPCB and MoEF&CC at central level and by State Pollution Control Board (SPCB) at state level (Bhati et al. 2015). However, in India, there is a norm which stipulates no more than 50 houses establishment without a wastewater treatment plant (WWTI 2010), but in real condition, only limited cities have working wastewater treatment plants, probably due to no direct economic return to the local authorities by STPs (Trivedy and Nakate 2001). The National Water Policy 2002 of India has focused on sustainable use of water resource for various purposes, viz., drinking, agricultural and nonagricultural industries, irrigation, hydropower, and so forth (WCR 2012). The CPCB (2015) made the following rules to maintain both the quantity and quality of wastewater: (a) all SPCB of the respective state shall make it mandatory to set up sewerage system for collection, treatment, and safe disposals of wastewater, (b) the board shall issue directives to all municipalities and other authorities to regularly assess the standards of STPs, (c) sewage at the secondary level of treatment should be used for non-potable activities instead of using freshwater, (d) each new housing construction should be enforced to install the dual piping system for reuse of treated sewage for flushing proposes, etc.

19.9 Phytobionts of Wastewater

Phytobionts are photosynthetic in nature such as algae. Several types of algae, viz., microalgae, macroalgae, blue-green algae, etc., are found in wastewater; however, the algae are also used for phycoremediation of wastewater since they degrade pollutants and nutrients efficiently and effectively. Algae are primary producers and contribute about half of the world's net primary productivity (Field et al. 1998) and live in planktonic aquatic habitat along with cyanobacteria, which are usually known as phytoplankton (Buchan et al. 2014). Application of phytobionts for wastewater treatment has been found to be superior to the conventional treatment methods, since it adds no further pollutant and supposed to be one of the most significant and eco-friendly methods at the present and future time (Clarens et al. 2010; Wijffels and Barbosa 2010). The microalgae also proved the potential for treatment of brewery effluents (Mata et al. 2012). Previous studies on wastewater treatment also demonstrated the pollutant removing potential of microalgae (Olguin 2003; Rawat et al. 2011).

19.10 Recognition of Wastewater's Phytobionts

Compared to mycobionts, only little is known about phytobionts and their association with other microorganisms and plants, as the diversity of phytobionts along with their associated habitats is studied by little research only. Beck et al. (2002) reported that the species of green algae could be isolated from highly diverse "pool of locally available algae," by fluorescence and light microscopy. The culture of phytobionts can be identified from thallus fragments of the lichens, viz., *Ramalina gracilis*, *Cladina confuse*, and *R. complanata* (Yamamoto et al. 1985). Phytobiont of the green fragments remaining on the filter paper of 150 μm pore size is later pick up using sterile needle and transferred to Sabouraud-2% sucrose agar-salted medium (Stocker 2002); after 6–8 weeks, the cultured lichen phytobiont could be transferred to slants using inorganic Bold's basal medium-2% with agar as medium (Bischoff and Bold 1963). Phytobionts can be isolated from the lichens using the method developed by Green and Smith (1974) and further examination into the distilled water.

19.11 Enhancement of Phytoremediation Through Phytobionts

Since the phytobionts significantly affect plant growth, it can also enhance the phytoremediation efficiency of plants for efficient accumulation and extraction of several metals and pollutants. A scientifically enhanced phytobiont-phytoremediation approach is greatly needed for the reclamation of more polluted wastewater; furthermore, it is a cost-effective green technology (Cay 2016; Solanki et al. 2017b). *Trebouxia* (sensu lato) is a type of phytobiont found in about 20% of lichens (Rambold and Triebel 1992), which consist of the symbiotic algae for 50–70% lichen species (Ahmadjian 1982).

19.12 Phytoremedial Plants

Phytoremediation is a plant-based group of technology, viz., phytoextraction, rhizofiltration, rhizodegradation, phytovolatilization, phytostabilization, etc., which is used to remove, degrade, reduce, and immobilize environmental toxins and pollutants with the aim of restoring contaminated sites. Since it is low-tech, low-cost, and eco-friendly in nature, phytoremediation is the most emerging cleanup method for contaminated wastewater, groundwater, and soils. The selection and indigenous availability of suitable plant species according to types and concentration of the pollutant to be removed are very crucial (Solanki et al. 2017c). Plants use several mechanisms, i.e., extraction, sedimentation, absorption, flocculation, cation and anion exchange, complexation, oxidation/reduction, microbiological activity, precipitation, and uptake for removal of toxic substances, while microalgae use two different mechanisms: metabolism-dependent uptake into cells at the low concentration of pollutants and biosorption of pollutants. Table 19.3 shows different plant species used for phytoremediation of different contaminants.

19.13 Hyperaccumulator Plants

Hyperaccumulator plants are very efficient in rapid accumulation (more than 1 mg/g of dry weight) (Verbruggen et al. 2009) of several toxic ions and pollutants into their biomass through particular physiological mechanisms as shown in Fig. 19.2; however very little is known about the genetics of hyperaccumulator plant (Tong et al. 2004; Aken et al. 2009). The first case of hyperaccumulation property was observed in the members of the Fabaceae and Brassicaceae families. Currently, approximately 400 plant species are reported which hyperaccumulate metals and toxic ions (McIntyre 2003). In addition to this, some species have the capacity for the accumulation of two or more metals in their biomass (Yang et al. 2004). Interactions

Table 19.3 Examples of selected plants for phytoremediation of different contaminants

Plant species	Contaminant	Reference
Salix alba	Cd	Weyens et al. (2013)
Sedum alfredii	Zn	Chen et al. (2014)
Brassica juncea	Ni and Cr	Kumar et al. (2009)
Ricinus communis	Ni, Cu and Zn	Rajkumar and Freitas (2008)
Brassica napus	Pb and Cd	Sheng et al. (2008)
Pisum sativum	2,4-D	Germaine et al. (2004)
Arabidopsis thaliana	Atrazine	Wang et al. (2005)
Populus deltoides	Hg	Che et al. (2003)
Nicotiana glauca	Cd, Pb, Cu, and B	Martinez et al. (2006)

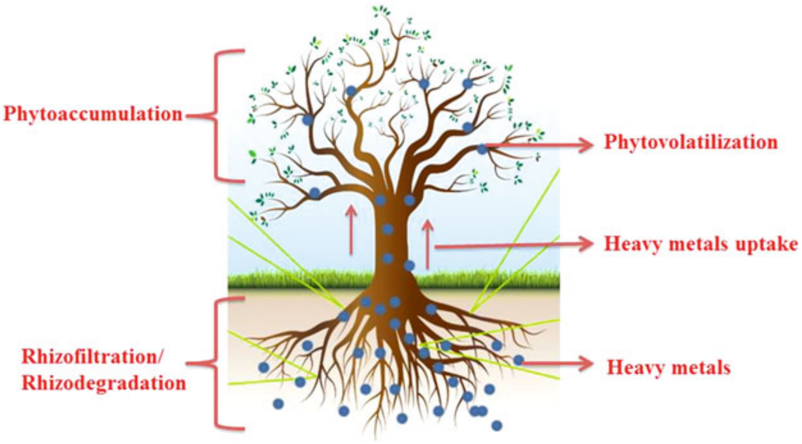

Fig. 19.2 Mechanisms of hyperaccumulator plant for detoxification of contaminants

between phytobionts and plants are very crucial and play a significant role to enhance the metal accumulation potential of plants. The close association was observed among endolichenic fungi and algal phytobionts which lead to enhance pollutant accumulation rate of plant.

19.14 Boost Up Plant's Phytoremedial Efficiency Through Phytobionts

Phytobionts show the tremendous potential to enhance the phytoremedial efficiency of plant since they break down the complex toxic substance into a simple form. The consortium of algae-bacteria enhances phytoremediation efficiency through metal bioremediation (Boivin et al. 2007) as well as deformation of organic pollutants (Tang et al. 2010). Many studies have shown that the bacteria and algae are significantly involved in the remediation of organic pollutants, i.e., black oil, phenol, naphthalene, acetonitrile, azo compounds, etc. (Mahdavi et al. 2015).

19.15 Bioremediation and Phytobionts

Bioremediation of wastewater is a microbial-mediated process in which more efficient indigenous microorganisms are inoculated; such microorganisms are bacteria, fungi, algae, protozoa, etc. but not viruses (Mueller et al. 1996). In this process, toxic substances are converted into less toxic form by several assimilations and transformation processes. Since both bioremediation and phytobiont approach are microorganisms based, they are interlinked with each other. Phytobiont cultivar such as macroalgae and bioremediation of wastewater can be clumped together for further enhancement of bioremediation potential of phytobionts. Being photosynthetic in nature, phytobionts consume adequate nutrients from wastewater environment. The process of wastewater treatment through phytobionts has exhibited high potential to remove pollutants by the process of assimilation, biosorption, and bioaccumulation of pollutants (Hultberg et al. 2013). Therefore, wastewater as nutritive habitat produces adequate algal biomass which has several utilities in aquaculture, human nutrition, pharmaceuticals, etc. (Bala et al. 2016). In many algae high potential is observed to absorb metals by binding them at the cell surface as well as to intracellular ligands. The bioremediation potential depends on a number of phytobionts; therefore, factors affecting the growth of phytobionts are also affecting the bioremediation of wastewater.

19.16 Interactions Between Bioremediation and Phytobionts

It is widely observed that phytobionts such as different forms of algae, bacteria, cyanobacteria, etc. are found in almost every niche of the planet Earth (Thomas and Dieckmann 2002). Phycospheric interactions between algae and bacteria and particular habitat interaction among algae, bacteria, and other organisms are two major interactions (Bell and Mitchell 1972). Previous researches indicate that a particular type of bacteria is associated with most algae, which are found in the unique habitat; furthermore, this association enhances algal growth and bioremediation process through mutualism (Ramanan et al. 2015). Researchers also studied the mutualistic interaction among *Azospirillum* and *Mesorhizobium* and algal growth (Gonzalez and Bashan 2000; Hernandez et al. 2009). Particularly in the oligotrophic (nutrient-lacking) environment, the growth of algae is supplemented by bacteria by establishing algae-bacteria mutualistic interactions. In contrast to the mutualism, some bacteria also have a negative effect on the algae, which encourages for more scientific study on microalgae, cyanobacteria, and their antagonisms (Wang et al. 2010). Moreover, some of the known algae, i.e., red algae, also act as a parasite against their own counterparts and other organisms (Sachs and Wilcox 2006).

Approximately 10% of the total identified red algae are known for their parasitic behavior (Hancock et al. 2010).

19.17 Isolation of Phytobionts from Effluents for Potential Use in Bioremediation of Wastewater

Mostly industrial and municipal effluents serve as the source for significant amount of phytobionts, and harvesting of these for bioremediation of wastewater is an emerging and eco-friendly way of wastewater treatment since it requires no chemical and toxic product addition. These microorganisms have enough capabilities to compete with toxic metals by several mechanisms, viz., adsorption, uptake, oxidation, reduction, methylation, and so forth. The most frequently served genera for wastewater treatment, e.g., *Scenedesmus*, *Phormidium*, and *Oscillatoria*, are mediated with microalgae, whose application resulted in the progressive reduction in COD and BOD. Cyanobacteria are the most isolated one and account for about 27% of the total soil microbial biomass (TSMB) (McCann and Cullimore 1979).

19.18 Nutrient's Recycling and Heavy Metal's Recovery Through Phytobionts

Phytobionts play a significant role in recycling of metals and nutrients, since they use these nutrients (P, K, N, S, Ca, etc.) as the energy source for their growth. Addition of the algal biomass to the land as biofertilizer leads to recycling of nutrients and organic matter. By this way, essential nutrients and organic matter (OM) are added to the soil resulting in a lower cost of cultivation and also reduction in the freshwater application in agriculture. The photoautotrophic (consume energy from atmospheric CO_2) nature of phytobionts leads to their faster and healthy growth and more recycling of nutrients. Additionally, phytobionts utilize the enriched atmospheric CO_2 and deposit it on land as biomass leading to decreased global warming (Pittman et al. 2011; Kim et al. 2010).

19.19 Future Research Perspectives

The generation of wastewater cannot be reduced without compromising the urbanization and economic development of any country. However, the pollution loads can be significantly reduced by awaking the people, enforcing the policies, and using phytobiont-based eco-friendly, low-cost wastewater treatment methodologies. In India, about 18% gross domestic product (GDP) is contributed by the agriculture, which provides the livelihood for about 65–70% Indian population; however nearly 60% of Indian agricultural production is driven by rains of seasonal monsoon. In the future, it is necessary to develop additional sources of irrigation rather than to depend on seasonal rainfall. Therefore fertigation of agricultural production through wastewater and reuse of wastewater for various industrial activities could be the best option for the management of this huge quantity of wastewater as well as to keep the freshwater safe for drinking.

19.20 Conclusion

Increased concern about pollution-free environment encourages enough for wastewater treatment and its reuse for different non-potable purposes to reduce the freshwater crisis. Since the generation of wastewater is huge with high pollution load, therefore, more efficient, advanced, cost-effective as well as eco-friendly technologies are required for its treatment. In this regard, phytobiont-mediated bioremediation and phytoremediation could be the most significant prospect for the bio-treatment of wastewater. Phytobionts are mainly algae-based microorganisms which enhance wastewater treatment through various mechanisms like phytotransformation, phytovolatilization, biodegradation, etc. and recycle the nutrients in the water-plant-soil ecosystem. The excessive and deliberate applications of agrochemicals and fertilizers in modern intensive agriculture have necessitated the demand for eco-friendly and more economically viable alternatives; hence the application of wastewater as fertigation leads to its economic and sustainable management as well as reduced cost of cultivation for better yield.

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Role of Fungi in Dye Removal

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Rajeev Kumar, Nikita Dhiman, Sushma Negi, I. B. Prasher,
and Chander Prakash

Abstract

Rapid urbanization and industrialization result in the discharge of harmful and toxic waste into the water bodies which are not easy to degrade thereby causing environmental pollution. Out of so many waste discharges, dye waste is noxious for aquatic life and for human as well; therefore, removal of these toxic compounds from water is one of the major environmental concerns today. The reported methodology like chemical and physical process is often costly, requires higher energy, and is not eco-friendly. In today's world, biological methods are trying to minimize pollution by environment-friendly way. Mycoremediation is one of the techniques which is effective and affordable for degradation and decolorization of dye-bearing effluents. The chapter concludes the potential of mycoremediation in dye removal, its mechanism, and optimizing the conditions for efficient removal of dyes.

20.1 Introduction

The earth has been called a blue planet due to abundant availability of water on its surface which approximately covers 70% of the earth's surface. About 97% of all water on earth is in oceans, sea, and bays; it means that only 3% of all water on earth is considered to be fresh. But that remaining water is not completely available for us to

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R. Kumar (✉) · N. Dhiman · S. Negi
Department of Environment Studies, Panjab University, Chandigarh, India
e-mail: rajeev@pu.ac.in

I. B. Prasher
Department of Botany, Panjab University, Chandigarh, India

C. Prakash
Department of Chemistry, M.L.S.M. College, Sunder Nagar, Himachal Pradesh, India

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use as 2/3 of it, which is 2%, is present in the form of ice and glaciers, so only 0.62% of freshwater is available which is present in lakes, rivers, and groundwater. Reconsidering the environmental pollution from the last decade has shown that waste such as dyes, paints, medicines, disinfectants, pesticides, laundry detergents, and food additive released by various chemical industries serves as a major threat to the environment and human health (Michael 1993). More and more organic and inorganic compounds get accumulated due to the development of these chemical technologies; as earth's population continues to grow, pressure on the water resources also increases. Now the efforts are made for the efficient and judicious use of water. Pollution explosion increases a large amount of wastewater and makes it crucial to develop such kind of cost-effective technologies for wastewater treatment. Especially the textile sector produces large volume of wastewater which contain dye and other chemicals that cause severe water pollution. Therefore, it becomes vitally important to treat the contaminated water before discharging into the environment. Physical and chemical properties of water bodies get altered due to the presence of large amount of certain chemicals, bleaches and salts, which ultimately lead to eutrophication. Theses dyes and chemicals obstruct the light penetration which affects the water ecosystem. Because of the multiplex structure and synthetic origin, colorants are very strenuous to degrade (Fernandez et al. 2010). So these dyes remain persistent in the aquatic environment that affect the water ecosystem by biomagnification. As the water resources (rivers, lake, and groundwater) get contaminated, there will be a growing need to remediate this issue (Fernandez et al. 2010). Utilization of treated waste water in agriculture will be a step towards treated wastewater best use. Although various physiochemical procedures have been studied for the removal of such compounds, they are not environment-friendly and also produce hazardous by-products which need further step for removal. On the other hand, biological methods are nontoxic, cheap, and environment-friendly (Gomes 2009; Srinivasan et al. 2001). But fungi are recognized as superior from any other microorganism as they produce different types of extracellular enzyme, precursor, and a wide range of metabolites which are non-specific in nature. Due to the presence of such a wide range of applicable metabolites, fungi are attracting a considerable attention in the transmutation of different dyes.

20.2 Brief Introduction to Fungi

Fungi are non-photosynthetic organisms that include molds, mushrooms, rusts, and yeast which are globally estimated to be approx. 1.5–5.1 million (Tedersoo et al. 2014). The cell of a fungus is surrounded by a nucleus having chromosome with the genomic material, for example, deoxyribonucleic acid (DNA) organelles which are bounded by the membrane-like mitrochondria and a cell wall made out of chitin and glucans. Growths are fundamentally heterotrophic in nature implying that these organisms obtain their nutrition from nonliving natural resources, for example, the saprophytic fungi which nourish on lifeless or rotting natural ingredients. Very less number of fungi do exist as unicellular individuals, such as yeast; this organism develops into barrel-shaped string-like structures, having a size of 2–10 cm recognized as hyphae. These hyphae might be either septate or nonseptate. The hyphae are the fundamental structure

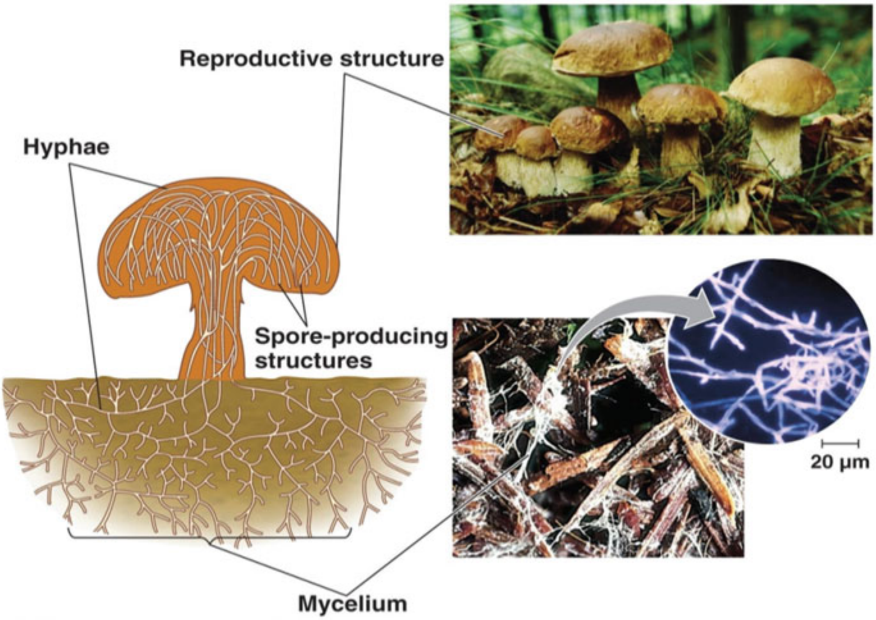

Fig. 20.1 Structure of fungus. (Pearson Education Inc.; Publishing as Pearson Benjamin Cummings 2008)

of fungi and constitute a mycelium. Finely fanned mycelium covers very huge surface zone in the soil and produces a scope of compounds for its growth and development. The fungi can procreate both sexually and asexually, e.g., through different types of spores, and abiogenetically by the process of binary fission or budding. These organisms are exceptionally different and play an extensive variety of parts in their encompassing condition, for example, potential decomposers, plant mutualists, and beneficial endophytes of plants, and also act as harmful pathogens and significant predators. The fungal hyphae are the essential component of earth's food networks since these establish a nourishment hotspot for soil micro- as well as macrobiota, whereas the fungal sporocarp provides sustenance to bigger organisms (Fig. 20.1).

Classification of fungus includes seven different phyla: *Chytridiomycota*, *Ascomycota*, *Glomeromycota*, *Blastocladiomycota*, *Neocallimastigomycota*, *Basidiomycota*, *Zygomycota*. *Chytridiomycota* is a division of zoosporic organisms that may degrade chitin and keratin. *Blastocladiomycota* contrast from the *Chytridiomycota* in generation since they display diverse types of meiosis, they have motile spores and gametes, and they can survive in water and soil. *Glomeromycota* live in close cooperative connection with the underlying foundations of plants and trees show a few highlights indistinguishable to bring down growths, e.g., they have coenocytic without septate mycelia, and the greater part of these provides no information about recognized sexual stages. They duplicate across huge thick-walled abiogenetic spores, generally recovered from soils. The higher fungal phylum has a unique appearance having two perfect cores in a hyphal cell called dikaryon. The biggest fungal phylum is *Ascomycota*, and this phylum has a distinctive saclike structure, which bears spores, called asci; these are produced in large amount during the process of sexual reproduction. *Neocallimastigomycota* is anaerobic fungi present in the gut of animals related to core chytrids. *Basidiomycota* is a filamentous fungus. *Zygomycota* is also called as conjugation fungi; they include molds, which grow over bread and different food items and form zygospores during their sexual generation phase and do not bear hyphal cell wall except in propagative fungal structures (Fig. 20.2).

20.3 Role of Fungi

Fungi are considered as an important part of biosphere as they act as a recycler of nutrients in different terrestrial habitats because fungi are decomposers which break down the complex components into simpler ones. Fungi are efficient in degrading natural substances like plant polymers, cellulose, waxes, insect cuticle, animal flesh, and a wide variety of waste generated by anthropogenic activities like pharmaceutical waste, hydrocarbon, polyaromatic hydrocarbon, metals, pesticides, and synthetic dye from industries. These compounds are recalcitrant in nature (Pointing 2001). Synthetic dyes are considered toxic for the environment, and due to their complex chemical structure, they are not easy to degrade (Lu et al. 2009). These dyes are used in wide varieties of industries like paper, color photography, textile, food, beauty products, and leather industries (Rafi et al. 1990; Kuhad et al. 2004; Couto 2009). Approximately 280,000 L has been discharged every year (Jin et al. 2007a, b). For decolorization a wide range of dye is achieved by various microorganisms which include algae; filamentous freshwater species of *Spirogyra* (Gupta et al. 2006); various species of *Chlorella* (Acuner and Dilek 2004); bacteria *Pseudomonas luteola* (Chang et al. 2001), *Staphylococcus aureus*, and *E. coli* (Kalyanaraman and Vaithilingam 2015); *E. coli* NO_3 (Chang et al. 2000); and fungi *A. niger* (Fu and Viraraghavan 2002), *Aspergillus lentulus*, and *Saccharomyces cerevisiae* (Phugare et al. 2011).

20.4 Textile Dye

The compounds which add color to textile are called “dye.” By different processes like absorption or dispersion, these compounds get incorporated into the fiber. Dyes with chemical and physical properties act differently at times; they show resistance to sunlight, washing, alkalies, etc. They show affinity to different fibers: their reaction to various cleaning materials, water solubility, and the methods of application to fibers (Fig. 20.3).

20.4.1 Pigment Dyes

Pigment dyes are one of the most widely used colorants. They are insoluble and nonionic in nature, and they do not undergo any physical and chemical changes

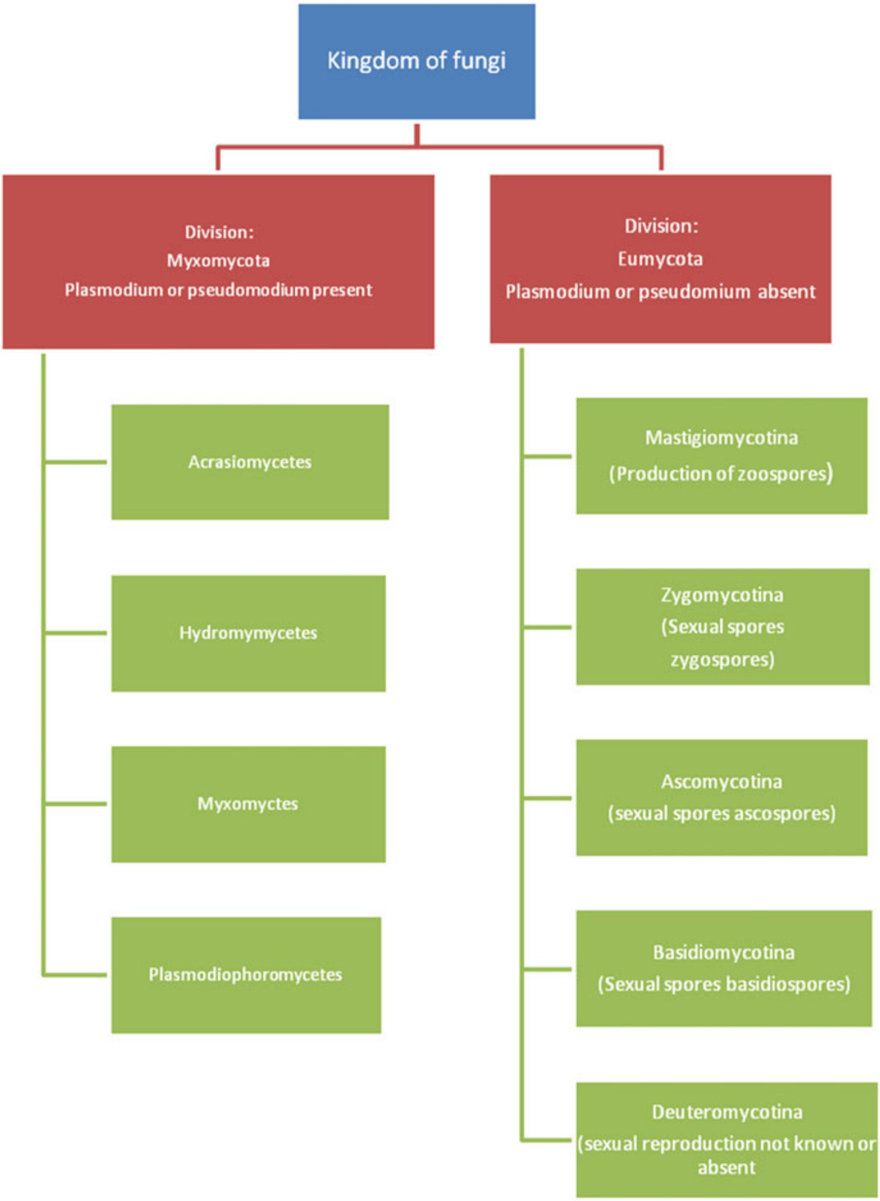

Fig. 20.2 Division of fungi on the basis of plasmodium presence or absence: Ainsworth (1973) is commonly followed

throughout the application. Pigment dyes are attained from the dispersed solution, so they require dispersing agents. Most of the pigment dyes include yellow-colored dye, acetoacetic acid and anilide pigments; red-colored dye, azoic pigments; and

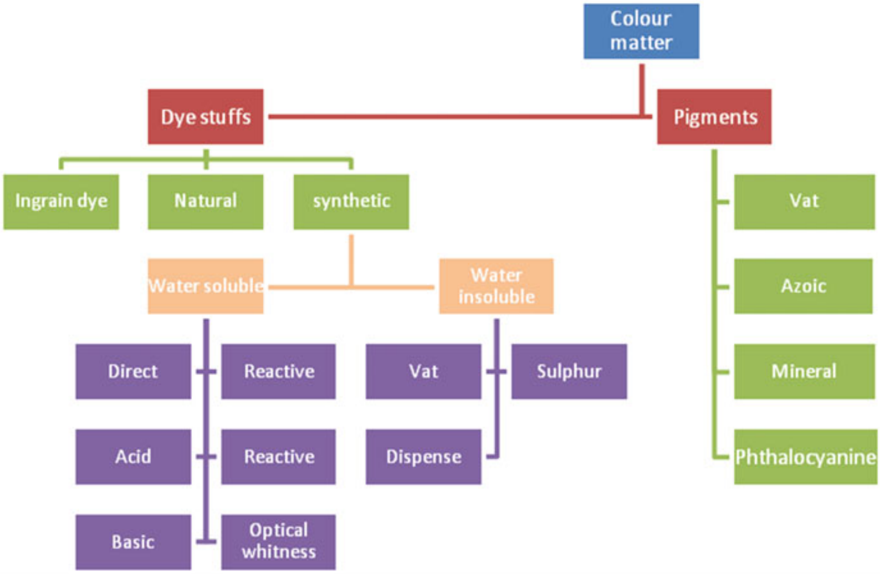

Fig. 20.3 Classification of dyes: according to method of applications. (Singh RL Applied Environmental Biotechnology for Sustainable Future 2017)

green- or blue-colored dye, phthalocyanine pigments. Moreover, the anthraquinone and quinacridone pigment dyes are also used for dyeing different varieties of fibers (Robinson et al. 2001).

20.4.2 Sulfur Dyes

Sulfur dyes are mostly used for different kind of cellulosic materials. These dyes are cheap and easy to apply and generally have good washfastness. Sulfur dyes are water-insoluble in nature. Alkali treatment around 80 °C is given to make them soluble as the bond between the dyes breaks down into simpler compounds; hence, they can be absorbed by the fabric. Sulfur dyes are absorbed by the cotton fibers and are oxidized by suitable oxidizing agents and get converted into insoluble parent dye, which gives good colorfastness. They are mainly used in dyeing cellulose fibers, viscose, and cotton (O'Neill et al.1999; Teli et al. 2001).

20.4.3 Solvent Dyes

A solvent dye is a very versatile dye. They are used for different organic solvents like waxes, hydrocarbon fuels, oil, paraffins, and other aliphatic and aromatic hydrocarbons. Their molecules are typically nonpolar in nature or little polar, and they do not form any ions therefore insoluble or very little soluble in water. Solvent dyes can also be used for marking inks, glass coloration, and inkjet inks (O’Neill et al. 1999; Dixit and Patel 2010).

20.4.4 Azoic and Ingrain

The synthetic dyes having insoluble azo group (-N=N-) are called azoic dye. These dyes are produced by the coupling of two components (usually naphthols, phenols, and acetoacetyl amides), and a diazotized aromatic amine in suitable and proper environment and the final color are controlled by components coupling. These dyes are used for silk, crayons, cellulose acetate, paints, and polyester (O’Neill et al. 1999).

20.4.5 Vat Dyes

They are considered as the superior dyes. When it comes to washing and fastness to light, these dyes are incapable of dyeing fibers as they are insoluble in water. Vatting is the process by which insoluble dye is converted into soluble dye. Eighty percent of the vat dyes belong to the group of anthraquinones and indigoid compounds (Slokar and Marechal 1997; Teli et al. 2001).

20.4.6 Reactive Dyes

In 1956 reactive dyes were prepared commercially and used for dyeing different fabric materials. The presence of reactive group helps them to form a chemical bond with the fiber. Because of the presence of reactive groups, they form covalent bond with carbon atoms of dye molecule and different functional groups in fibers. It is considered to be as a most permanent of all dye types. These dyes can be used for dyeing nylon and woolen fibers. They are concluded as the second largest dye class in Colour Index. This class of dye contains metal complex of different azo compounds (O’Neill et al. 1999).

20.4.7 Basic or Cationic Dyes

Basic dyes are positively charged cationic colorants. These are water-soluble in nature and can be used for a variety of fibers like silk, wool, cotton, and modified acrylic fibers. Sometimes organic compounds are used in the dye bath as it can take up the dye. This type of dye is just fair when it comes to fastness to light and washing. Diarylmethane, triarylmethane, anthraquinone, and azo compounds are some of the common dyes used for dyeing purposes (O’Neill et al. 1999).

20.4.8 Acid Dyes

Acid dyes work best when applied to acid bath. These dyes have a wide variety of color and have better lightfastness than basic dyes. They are highly soluble in nature and used for a wide variety of fibers such as silk, mohair, linen, and leather. Azo, anthraquinone, and triarylmethane are the three main groups of acid dyes (Kaushik and Malik 2010).

20.4.9 Direct Dyes

Direct dyes contain wide range of color and are easy to use; these dyes are not fast to washing, and with the help of other treatments, its fastness can be improved. These dyes are bound by Van der Waals forces to the cotton fiber; alkaline or neutral conditions are maintained for the dyeing bath, by adding certain salts. They are mostly used on protein fibers, viscose rayon, leather, synthetic fiber, and nylon. Direct dyes contain multiple azo, phthalocyanine, stilbene, and oxazine (De las Marias 1976).

20.4.10 Disperse Dyes

Disperse dye is a kind of an organic substance which does not contain any free ionizing group. They are water-insoluble in nature. These dyes are finely grounded with dispersing agent and mostly in the form of a paste. Disperse dyes are mostly used to dye polyester but can also be used for dyeing nylon, Vilene, synthetic velvet, paintings, and PVC. In some cases, high temperature up to 130 °C is used for dyeing, their fine-sized particles provide a large surface area which allows dye to adhere to the fiber, and dyeing rate is dependent upon the choice of dispersing agents (Slokar and Marechal 1997).

20.4.11 Mordant Dyes

Mordant dyes are the substances used to set dyes on fabrics as they form complex structure which help in attachment with the fiber; these dyes are used for dyeing only when they are combined with different chemicals (chromium complexes, dichromates, iron, and tin). These dyes are mostly used for navy and black shades of colors. Mordant helps in fixing the dye to the fiber. It is very important to choose the right mordant because they can significantly affect the final color (Ingamells 1993) (Fig. 20.4).

20.5 Dyeing Process: Figure 20.4 Explains the Dyeing Process

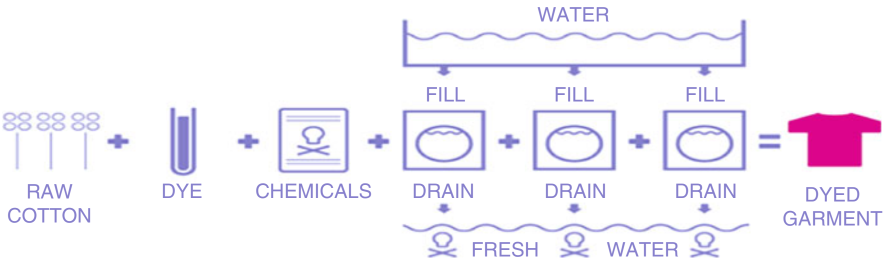

Fig. 20.4 Dyeing process of textile industry. (Dyeing Environmental Impact http://www.colorzen.com/dyeing-environmental-impact)

20.6 Toxicity of Dyes and Their Environmental Impacts

Wastewater from dye industries is very difficult to treat. Textile dye that gets dumped into the nearest water body without being treated can cause many problems like:

1. Today's environmental concern with the dyes is their absorptive nature, and they reflect back the sunlight entering the water surface, hence reducing the photosynthetic activity which adversely affects the food chain.
2. When dyes break down, they produce harmful products which are carcinogenic in nature and reprotoxic (Novotny et al. 2006; Mathur and Bhatnagar 2007).
3. Dyes cause various diseases like cancers and also effect the renal and urinary system of the dye workers (Puvaneswari et al. 2006).
4. Benzidine dyes also cause various dermal and immunological diseases. The workers who get exposed to such dyes have urinary system problems (Van der Zee et al. 2002; Golka et al. 2004).
5. Dyes also affect the transparency of water bodies and cause damage to the aquatic ecosystem (Rocha 1992).
6. The highly toxic dye effect, penetration of light rays, cause deficiency of oxygen and limit the downstream beneficial uses such as recreation, irrigation and drinking water (Van der Zee et al. 2002; Golka et al. 2004).
7. Azo dyes are hazardous in nature, when they enter the body and get metabolized by microorganisms causing DNA damage (Van der Zee et al. 2002; Golka et al. 2004).

20.7 Removal Process

Various methods of treatment are available for the dye removal from the wastewater. Many chemical, biological, and physical methods are generally used to remove dyes from the industrial effluent.

20.7.1 Physicochemical Techniques

Different methods are used for the removal of dye (Lin and Liu 1994). And many physicochemical techniques include ozonation, ion exchange, adsorption, membrane filtration, precipitation, electrokinetic coagulation, ultrasonic mineralization, electrolysis, and chemical reduction, but these processes alone are not sufficient for the removal of toxic waste. In several studies, many techniques have not been able to achieve decolorization because of their expensive nature, large energy requirements, limited lifetime, formation of unwanted by-products, foaming, etc. And the by-products formed by these removal processes are harmful and recalcitrant. Thus, the extent of the mineralization in waste decolorization should be evaluated (Fu and Viraraghavan 2002).

20.7.2 Biological Decolorization Methods

There are numerous classes of microbes which are involved in disintegration of various synthetic colors and also cleaning mechanized wastewater. It has been reported that application of potential microbes such as bacterial, fungal, algal strains, actinomycete, microbial diverse cultures, or using the enzymes of microbes which take part in decolorization of synthetic colors (Thummar and Ramani 2014). A wide range of microflora are being used for the removal of various classes of synthetic dyes; it includes some efficient bacterial strains such as various species of *Escherichia coli*, *P. luteola* (Chang et al. 2001), *A. hydrophila* (Chen et al. 2003), and various species of *Kurthia* (Saini and Banerjee 1997); algae also play a role in dye remediation, of which commonly used are species of *Spirogyra* (Gupta et al. 2006) and *C. vulgaris* (Acuner and Dilek 2004); common fungal species used are *A. niger* (Fu and Viraraghavan 2002), *A. terricola*, *P. chrysosporium* (Saikia and Gopal 2004), and *P. chrysosporium* (Fouriner et al. 2004); various species of yeasts have also been used such as *Candida tropicalis*, *C. lipolytica*, and *S. cerevisiae* (Aksu and Donmez 2003).

20.7.3 Degradation by Fungi

Bioremediation process includes brown-rot and white-rot fungal species for the removal of dye and other xenobiotics and is termed as “mycoremediation” (Prasad 2017, 2018). Bioremediation of such complex compounds relies upon the extracellular enzyme production by the fungi; these enzymes includes hydrolases and oxidoreductases (Makela et al. 2013). Oxidation and reduction reaction is formed by the oxidoreductase enzyme secreted by fungi that break down the chemical bond which attached to the water molecule. Lignin-modifying enzymes are also secreted by the fungi and are referred as oxidative enzyme. Since they are non-specific in nature, they degrade a wide range of xenobiotic compounds by using wide varieties of enzymes (Harms et al. 2011; Tuomela and Hatakka 2011; Winquist and Steenland

2014). Dye-decolorizing peroxidases are glycoproteins which require H_2O_2 for all enzyme reactions. They are named so because they oxidize by different classes of dyes like anthraquinone, and these are not properly oxidized by peroxidase enzyme (Kim and Shoda 1999; Passardi et al. 2005). A significant characteristic of dye peroxidase is having free position for the H_2O_2 binding. Due to this characteristic, they offer a wide range of dye degradation like 2′azinobis-3-ethylbenzothiazoline-6-sulfonate, polymeric, triphenylmethane, azo, phthalocyanine, and heterocyclic colors and other phenolic mixes (Petrides and Nauseef 2000). The extracellular ligninolytic enzymes produced by white-rot fungi are a standout among other techniques for color degradation. The ligninolytic enzymes found in organisms which include potential fungal strains such as *T. rubrum*, *P. chrysosporium*, various species of *Ganoderma*, *Irpex lacteus*, *Funalia trogii*, *T. versicolor*, and many more have been broadly utilized for the dye effluent treatment. White-rot fungi (WRF) have such enzymes which can degrade lignin easily.

Decolorization by the fungi is interceded by biosorption and additionally biodegradation tool. *Lentinus sajor-caju* parasitic stain is reported for the expulsion of removal of material color responsible for red (at 800 mg/l). The yeast *Saccharomyces cerevisiae* and unwanted biomass of yeast have been recorded for color removal and eradication of various types of industrial dyes (Phugare et al. 2011). *Coriolus versicolor* species were recorded for reduction of wide varieties of colors from five unique factories, and degradation rate is up to 36% every 5 days. Different species of *Penicillium* are also known for color removal; cotton blue color (50 mg/l) has been decolorized by *Penicillium ochrochloron* in 2.5 h; moreover, efficient fungal strain of *Penicillium* sp. decolorized azo dye effectively under laboratory conditions (Gou et al. 2009). Fungal strains have been considered as highly efficient group of microorganism for synthetic dye biodegradation. On the other hand, the white-rot fungal species have been proved to play a significant role in lignin expulsion (Kubilay 2009). Ligninolytic microorganisms were the conceivable option examined for dye decolorization and degradation. Interestingly the WRF were the first organisms reported for removal of dye (Kubilay 2009); however, few non-ligninolytic fungal species such as various species of *Aspergillus* and few species of *Penicillium* have also been documented to remediate and decolorize various synthetic dye effluents (Winquist and Steenland 2014).

Biological remediation of amaranth color by species of *Ganoderma* has been accounted for the removal of different dyes like triphenylmethane, bromophenol blue, and malachite green (Revenkar and Lele 2007). *Trametes versicolor* strain was reported for the removal of two different benzidine-based dyes: Direct blue 1 and Direct red 128. Fungal strains like *Funalia trogii*, *Coriolus versicolor*, and *Pleurotus ostreatus* have been reported in remediation and decolorization of dye Drimaren Blue CL-BR (DB) and Remazol Brilliant Blue Royal (RBBR) within 48 h. *Pyricularia oryzae* produce laccase that has the capability to decolorize phenolic azo dyes, while *P. chrysosporium* contain enzymes which degrade lignin and also have the capability to decolorize the azo-triphenylmethane dyes into harmless products (Kubilay 2009). A few different compounds like manganese peroxidase and lignin peroxidase from *P. chrysosporium* effectively decolorize wastewater from factories. Also, *Trametes* sp. is considered for the decolorization of other different dyes (Harms et al. 2011).

20.8 Mechanism of Dye Decolorization

Physical and enzymatic two steps of mechanisms are used for the degradation of dye (Knapp and Newby 1999). Surface of microbial cell provides a platform for the dye adsorption which is the primary step in the removal of dye. Various extracellular and intercellular enzymes produced by the fungal hyphae and other physical adsorption techniques are used for the color removal (Conneely et al. 2002; Chen et al. 2005, Singh et al. 2006; Chander and Arora 2007; Diwanian et al. 2010). Due to complexity in the structure of dye and its transformation mechanism make the demonstration of pathways a difficult task. Fungi produce various efficient enzymes such as laccase, lignin peroxidase, and manganese peroxidase for biodegradation of lignin, which participate in synthetic dye decolorization. In one report, Conneely et al. (2002) examined that in case of phthalocyanine dye degradation, lac and MnP are involved. Also in the case of *P. chrysosporium*, LiP act as a main decolorizing agent. Abadulla et al. (2000) observed that dyes with different chemical structure were degraded by different enzymes and they have different removal rates. Kirby et al. (2000) demonstrated that *P. tremellosa* produce laccases which efficiently remove textile dyes, but they also examined that there was a certain process which participates in the removal of remaining color which was observed in the absence of detectable level of these enzymes. Wesenberg et al. (2002) examined that lignin-modifying enzyme helps in the decolorization of industrial effluent. T azo dyes are removed by peroxidases which are responsible in the removal of phenolic group and further break down the phenyl diazine and oxidize it by one-electron reaction generating N_2 (Paszczynski et al. 1991; Spadaro et al. 1994; Paszczynski et al. 1992) (Table 20.1).

20.9 Factors Affecting Adsorption of Dye

Factors which affect the dye adsorption include temperature, pH, and initial dye concentration. Thus, for the large-scale removal of dye, the optimization of above parameters will be necessary.

20.9.1 Effect of pH

pH is considered as the crucial factor in the growth of fungus. Initial pH solution affects complexity of dye molecule and fungal biomass (Fu and Viraraghavan 2002). With the increase of pH the biosorption capacity of fungi also changes it get decreased for basic dye and get increased in case of acidic dye (Mahony et al. 2002). At pH 2 higher biosorption capacity (95 mg/g) of *Rhizopus arrhizus* for acidic

Table 20.1 Dye degradation by various fungi

Fungi	Dye	Incubation period	Percentage removal	References
Ganoderma sp.	Cibacron brilliant	8 h	96%	Revankar and Lele (2007)
Datronia sp.	Reactive black, reactive blue	5 days	86%	Pilanee et al. (2010)
Aspergillus lentulus	Acid magenta, orange HF, acid navy blue, acid sulfone blue, fast red	7 days	56% 78% 82% 70% 62%	Kaushisk and Malik (2010)
Penicillium sp.	Reactive brilliant red X 3B	5 days	70%	Gou et al. (2009)
Saccharomyces cerevisiae	Textile effluents	2 days	78%	Phugare et al. (2011)
Ganoderma sp.	Reactive orange system	5 days	95%	Lima et al. (2014)
Ganoderma lucidum	Acid orange 7	7 days	77%	Chang et al. (2015)
Aspergillus niger, Aspergillus terreus	Procion Red max-5B	7 days	98%	Almeida and Corso (2014)
Phanerochaete	Amido black	3 days	98%	Senthil et al. (2011)
Aspergillus niger	Azo dye	5 days	74%	Arumugam et al. 2011)
Trichoderma sp.	Orange G			
Agaricus bisporus	Acid red 44	30 min	75%	Tamer et al. (2016)
Agaricus bisporus	Reactive blue 49	90 min	90%	Sibel et al. (2009)
Trametes sp.	Azo dyes	5 days	69%	Yang et al. (2009)
Coriolus versicolor	Industrial effluents	3 days	84.4%	Muhammad Asgher et al. (2009)
A. niger, Spirogyra	Reactive dye	18 h	88% 85%	Mahmoud and Khalaf (2008)
Trametes versicolor	Direct red 128, Direct blue 1	7 days	79%	Gulay et al. (2007)

group is observed as compared to pH 10 (30 mg/g), and the reason behind this change is the protonation of weak base group at lower pH. Thus base group acquire positive charge and bind themselves with chemical species of acidic group that caries negative charge (anionic group). Similarly Iqbal and Saeed (2007) studied that uptake of acidic dye 100 mg/l Remazol Brilliant Blue R by *Phanerochaete chrysosporium* at pH 2 was 53.46%, but for cationic dyes Maurya et al. (2006) reported that pH increased from 3 to 11.This is due to the fact that there was an increase in electronegativity of biosorbent because of deprotonation of functional group.

20.9.2 Effect of Temperature

Temperature also affects the dye absorption rate. By examining various studies, it is concluded that the biosorption capacity of fungus maximizes when temperature increases. Annadurai et al. (1999) used chitin as a biosorbent as they observed that Verofix Red is 28 mg/g at 30 °C and at 60 °C is 38 mg/g. As with the increase in temperature, the structure of chitin get swelled up which enables the dye molecule to get incorporated in the structure. It was observed that with the increase in the temperature, the biosorption capabilities of *Trametes versicolor* also speed up from 5 to 35 °C for Direct Blue 128 and Direct Blue 1, as the kinetic energy and surface activity of dye molecule get increased. In another study Iqbal and Saeed (2007) examined that Remazol Brilliant Blue R dye biosorption by *P. chrysosporium* increases with rise in temperature, i.e., 70% at 30 °C, but with constant increase in temperature (60% at 50 °C), it gets decreased.

20.9.3 Ionic Strength Effect

Textile dye is not properly treated before being disposed of into the environment, so they contain various amounts of different chemical impurities; due to the presence of such impurities, ion concentration of the solution which effects the dye removal increases. *Phellinus igniarius* is used for the biosorption of rhodamine; the biosorption rate decreased to 7% as the concentration of dye increases from 0.00001 mmol/l to 0.1 mol/l, and in case of methylene blue due to higher ionic strength, dye removal rate decreases. This suggests there is a probability that there is a competition between the ions N^+ and positively charged dye ions (Maurya et al. 2006).

20.9.4 Dye Concentration Effect

The dye concentration is a very important factor in adsorption process. Adsorption of dyes concentration determines the adsorption capacity of adsorbent. If initial concentration of dyes is high, the time required for the degradation is also increases, as in the case of biosorption capacity of *R. stolonifer* for bromophenol dye increases from 190 to 700 mg with the increase in the dye concentration also increases at pH 3. But there is an opposite trend for the percentage removal of dye concentration which lessened to 55% from 80% (Zeroual et al. 2006). In case of *Rhizopus nigricans* and *Saccharomyces cerevisiae* (85–30% and 90–30%, respectively), the initial concentration of dye increased from 500 to 200 mg/l (Kumari and Abraham 2007).

20.9.5 Physical and Chemical Parameters

Physical and chemical properties play major role in the biosorption. Fu and Viaraghvan (2002) determined the various absorption sites and chemically modified the particular ionic group for different dyes. They examined that two different functional groups including carboxylic and amino group were very effective in binding with cationic dye. And other functional group including phosphate and amino carboxylic bind themselves with anionic dye Congo red. This implies electrostatic force is not the only reason. Certain other chemical and physical treatments like drying and autoclaving organic and inorganic chemicals can also be used so as to increase the biosorption (Aksu 2005).

20.10 Conclusion

Wastewater contamination with dye and various kinds of colored compounds is becoming a problem worldwide. Further, addition of effluents from smelting and electroplating industries containing toxic and hazardous chemicals leads to various environmental troubles. Color removal from dye-containing wastewater using different methods is still a challenging task. Taking in view above facts and issues, there is great need to use such methods which are technically sound, feasible, and cost-effective. Among various physical and chemical treatments, the biological treatment appears to be the most efficient and promising choice available for the decolorization of wastewater. Mycoremediation is considered to be the best method to achieve the highest dye removal as they contain non-specific enzyme system which is capable of degrading different classes of dyes. It is believed that the microbial enzyme-based development systems will be the future technologies owing to its simplicity and green effect. In this book chapter, we are throwing light on the fungi and its functioning as dye removal agent. The very first portion deals with the fungi classification and kingdom; further we are discussing about the various dye materials available in the market and their chemical and mechanical methods for removal. In the last section, the emphasis is given in the mycoremediation for the dye removal and its efficiency to be a good biological medium.

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Does Mycoremediation Reduce the Soil Toxicant?

21

Manish Kumar, Rizwan Ali Ansari, and Shabbir Ashraf

Abstract

The soil polluted with heavy metals (HMs) is a matter of concern in current scenario. In agriculture, contaminants have negative effects on both crop quality and their yields. Mycoremediation is a new technology for the reduction of petroleum hydrocarbons and HMs through fungal strains. The efficient fungal strains play a significant role in the decomposition of contaminants and keep environment clean. They are good decomposers which degrade the cellulose and lignin of plants. It also helps in breaking down various toxic substances and helps to sustain soil health. Fungi like mushroom, *Trichoderma* spp., help to concentrate and absorb HMs which act as hyperaccumulator. Mushrooms are fungi which secrete certain enzymes or biocatalysts and are able to biodegrade a varied variety of agro-industrial wastes into products and transform industrial waste and environmentally persistent pollutants. In addition, the potential fungal species are also known to improve and boost plant yield, development, and growth.

21.1 Introduction

Remediation deals with the elimination and exclusion of pollutants from polluted soil and supports conservation of natural resources. Secretion of certain enzymes from certain fungi helps in the decomposition of hazardous chemicals into nontoxic compounds (Barry and Austa 1994). Environmental pollution is a global issue

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M. Kumar (✉) · S. Ashraf
Department of Plant Protection, Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh, Uttar Pradesh, India

R. A. Ansari
Section of Plant Pathology and Nematology, Department of Botany, Aligarh Muslim University, Aligarh, Uttar Pradesh, India

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wherein heavy metals and organic contaminants continue to be major pollutants (Xiezhi et al. 2005). Metal toxicity inhibits metabolic activity of the plants and lowers down the yield of various agricultural crops. Fungi can rapidly ramify through substrates due to the presence of mycelium (Kulshreshtha et al. 2014). There are certain species of mushroom which are involved in production of extra-cellular enzymes such as oxidases, cellulases, peroxidases, ligninase, pectinases, and xylanases (Nyanhongo et al. 2007). Mushrooms are fungi which secrete their enzymes that help to biodegrade wide and varied types of agro-industrial wastes into useful products and transform industrial waste and environmentally persistent pollutants. These enzymes also help in the degradation of non-polymeric, man-made xenobiotic contaminants such as nitrotoluene (VanAcken et al. 1999), polyaromatic hydrocarbons (Hammel et al. 1991; Johannes et al. 1996), pentachlorophenol (Lin et al. 1990), synthetic dyes, and organic compounds (Ollikka et al. 1993; Heinfling et al. 1998). Mycoremediation helps in the improvement of local soil condition by encouraging the biological activity and the subsequent degradation toxic substances.

21.2 Mycoremediation

Mycoremediation is a form of bioremediation. This term was coined by Stamets (2005). Chemical pesticides and fertilizers are used by the agriculturist to increase crop production. The heavy metals associated with contaminants are also dumped in the soil which pollute environment. Fungi-secreted enzymes can penetrate through hypha and support degradation of chemical pesticides. The mycelium of fungi provides large surface area which maximizes mechanical and enzymatic action on contaminants to make environment clean (Singh 2006; Prasad 2017, 2018). The presence of *organic* compounds, pesticides, and petroleum products can be degraded by certain fungi. Enzymes secreted by the mycelium of fungi are able to remove chlorine atom from larger molecules (Johnston 2010). Oxidoreductase constitutes an important class of enzymes that help in the decomposition of lignin in soil followed by humification of phenolic compound (Park et al. 2006). In the diffusion of enzymatic molecules by hyphae of fungi, they extract metals from soil and build up in the mycelium or tissues of fruiting bodies of mushroom (Mai et al. 2004).

21.3 Role of *Trichoderma* spp. in Mycoremediation

One of the most potent groups of fungi used widely for mycoremediation is *Trichoderma* spp. In addition, they are also known to improve and enhance plant development and growth. It acts as biocontrol agent and plant growth promoter and helps in the improvement of soil fertility, disease suppression, and composting (Contreras-Cornejo et al. 2009; Lorito et al. 2010). *Trichoderma* spp. are generally known as biocontrol agent and also known to produce various organic acids such as citric acid, gluconic acid, and fumaric acid, which help to lessen the pH of soil and also lead to solubilization of insoluble phosphate. These fungi also play a significant role in solubilization and transport of macro- and micronutrients such as Fe, Cu, Mn, and Mg, which are vital for normal plant cellular metabolism. *Trichoderma* spp. exhibit resistance to many agrochemicals; thus, it is used as a tool for integrated pest management. It is a potent producer of important enzymes, which are required in industrial as well as for hydrolytic activities. The common enzymes produced by *Trichoderma* spp. are cellulases from *Trichoderma reesei* (Ahamed and Vermette 2009), amylase from *Trichoderma harzianum* (Harman et al. 2004a), and 1,3-β-glucanases from *Trichoderma harzianum* and *Trichoderma koningii* (Monteiro and Ulhoa 2006). Meanwhile chitinases are being produced using *Trichoderma harzianum* and *Trichoderma atroviride*. *Trichoderma* spp. are also being reported to resist varied types of hazardous materials such as organometallic complexes, heavy metals, and detrimental chemicals such as cyanide (Ezzi and Lynch 2005). Moreover, it can adsorb, eliminate, and assemble the common toxic heavy metals, viz., lead, cadmium, copper, zinc, and nickel (Yazdani et al. 2009; Srivastava et al. 2011). Species of *Trichoderma* have mechanisms to facilitate metal stress tolerance and hyperaccumulator of toxicants in plants and help to enhance root biomass production (Arriagada et al. 2009; Mastouri et al. 2010). Ezzi and Lynch (2005) reported the biodegrdation potential of *Trichoderma* for pesticides residue remains such as lindane, DDT, and chlordane in soil.

21.3.1 Mycoremediation of Inorganic Pollutants by *Trichoderma* spp.

Contaminants like HMs from sewage sludge which cannot be destroyed easily live in soil for a long period of time. It makes the soil infertile which makes it difficult for farmers to grow crops. HMs like cadmium, mercury, copper, zinc, and arsenic are increasingly released into the environment due to the use of pesticides, fertilizers, and other activities of human beings (Errasquin and Vazquez 2003; Tripathi et al. 2007). Fungi like *Trichoderma* spp. play an important role in degrading and detoxifying toxic substances. There are several mechanisms which help for metal resistance that includes metal efflux of fungal cell membrane, intracellular chelation by proteins which are involved in metal capture, peptides derived from glutathione known as phytochelatins (Tripathi et al. 2007), and metal compartmentalization in cell vacuoles. Kredics et al. (2001) reported that out of 13 *Trichoderma* soil isolates, 4 isolates tested against 3 heavy metals, nickel, arsenic, and zinc, and exhibited an effective colonization of soil and also demonstrated higher rate of biodegradation (Harman et al. 2004a, b; Lorito et al. 2010).

21.3.2 Mycoremediation of Organic Pollutants by *Trichoderma* spp.

Organic chemicals such as polycyclic aromatic hydrocarbons (PAHs) are strong environmental contaminants which are consisted of fused benzene rings (three or more in numbers) in a linear fashion. PAHs are sparingly soluble, hydrophobic, and

Table 21.1 Mycoremediation of various contaminants using *Trichoderma* spp.

S. No.	Pollutant	*Trichoderma* spp.	References
1	Metal-contaminated soil including cadmium, lead, manganese, nickel, and zinc	*Trichoderma harzianum*	Adams et al. (2007)
2	Various heavy metal isolated from sewage mud	*Trichoderma atroviride*	Errasquin and Vazquez (2003)
3	Agrochemical pollutants endosulfan, DDT, pentachlorophenol, dieldrin, pentachloronitrobenzene	*Trichoderma harzianum*	Katayama and Matsumura (1993)
4	Solvents with organic base	*Trichoderma* spp.	Oros et al. (2011)
5	Pesticide poly-resistance	*Trichoderma* spp.	Hatvani et al. (2006)
6	Soil contaminated with diesel	*Trichoderma*	Gestel et al. (2003)
7	Metal-contaminated soil Pb, Cu, and Zn	*Trichoderma* spp.	Hajieghrari (2010)
8	Cadmium- and nickel-polluted soils	*Trichoderma atroviride*	Cao et al. (2008)
9	Pollutants in water and soil	*Trichoderma* spp.	Harman et al. (2004b)
10	Pesticides with organophosphate base dichlorvos	*Trichoderma atroviride*	Tang et al. (2010)

strongly bound to soil particles. They damage genetic materials and change the structure of cells (Pashin and Bakhitova 1979). The techniques such as biostimulation, bioaugmentation, aeration, and turning or combining these practices help in bioremediation. The substrate of bioremediator acts upon biowaste and contaminated soil (Alexander 1994; Gestel et al. 2003). The response of 25 *Trichoderma* spp. is reported against PAHs. Among them *Trichoderma longibractum* proved more tolerant than other strains (Oros et al. 2011). The fungus *Trichoderma* was identified as the highest prevailing species in diesel-contaminated sites, which has the capability to colonize and help in biodegradation of diesel-polluted soils (Gestel et al. 2003; Hajieghrari 2010). Mishra and Nautiyal (2009) demonstrated that *Trichoderma reesei* have prospective of promoting plant development and growth in soil with diesel as pollutant (Table 21.1).

21.4 Role of Mushroom

Mushroom can be effectively and magnificently employed in the process of mycoremediation, and their enzymatic machinery helps in the degradation of contaminants (Purnomo et al. 2013; Kulshreshtha et al. 2013a, b). Degradation of pollutants by mushroom involves the process of biodegradation and biosorption followed by bioconversion process (Akinyele et al. 2012; Kulshreshtha et al. 2013a;

Kumhomkul and Panich-pat 2013; Lamrood and Ralegankar 2013). A large number of mushroom species like *Lepista nuda*, *Agaricus bisporus*, *Calvatiaexci puliformis*, *Boletus edulis*, *Pleurotus platypus*, *Lepiota rhacodes*, *Pleurotus sajor-caju*, *Pleurotus ostreatus*, *Calocybe indica*, *Hygrophorus virgineus*, and *Psalliota campestris* have been proven to mycoremediate HMs (Mejstrik and Lepsova 1992). Mushrooms have the ability to accumulate agrochemical wastes and persistent pollutants and transform industrial wastes.

21.4.1 Biodegradation

The term biodegradation is used for the degradation and recycling of complex molecules into simple molecules and makes the environment clean. Complex compounds after mineralization transform the molecules into carbon dioxide, water, nitrate, and other inorganic components by oxidative activities of living beings. Mushroom produces enzymes like oxidase, pectinase, peroxidase, ligninase, cellulase, and xylanase (Nyanhongo et al. 2007). Mushroom also produces enzymes radicals of hydroxyl and the higher levels of $H_2O_{2,}$ also ligninolytic enzymes interactions with cytochrome P 450 monooxygenases for the biodegradation of complex compounds. They help to oxidize the recalcitrant contaminants by enzymatic activity on substrate like nitrotoluene (VanAcken et al. 1999), polyaromatic hydrocarbons (Hammel et al. 1991; Johannes et al. 1996), synthetic and organic dyes (Ollikka et al. 1993; Heinfling et al. 1998), and pentachlorophenol (Lin et al. 1990).

21.4.2 Biosorption

Mushroom uses a combination of two processes for the uptake of pollutants which are bioaccumulation and biosorption. Bioaccumulation is an active metabolic process that transports metallic ions into the microbial cell and later segregates them into intracellular components, while biosorption is the involvement of ions to the biomass without requiring metabolic energy (Kapoor and Viraraghavan 1995) (Table 21.2).

21.5 Mycoremediation of Agrochemicals

Agrochemical accumulation in the environment is a major concern for growers as well as environmentalist. Repeated use of pesticides in a frequent manner makes field unfit for agricultural practices. Unused pesticide solution having organic compounds that run off directly into the soil is becoming a worrying situation. These xenobiotics exert harmful consequences to human health. Moreover, these chemicals are responsible for decreasing population of microbiome. The microbiome in the soil help fight against pathogens and promote growth and development of plants. Removal of pesticides has become cumbersome for scientific

Table 21.2 Role of mushroom spp. in biodegradation of contaminants

S. No	Mushroom spp.	Contaminants	Citations
1	*Agaricus bisporus*, *Lactarius piperatus*	Cadmium(II) ions	Nagy et al. (2014)
2	*Pleurotus pulmonarius*	Radioactive waste	Eskander et al. (2012)
3	*Flammulina velutipes*	Copper	Luo et al. (2013)
4	*Pleurotus ostreatus*	Cadmium	Tay et al. (2011)
5	*Lentinula edodes*	2,4-dichlorophenol	Tsujiyama et al. (2013)
6	*Pleurotus tuber-regium*	Heavy metals	Oyetayo et al. (2012)
7	*Pleurotus sajor-caju*	Heavy metals	Jibran and Milsee Mol (2011)
8	*Pleurotus platypus*, *Calocybe indica*, *Agaricus bisporus*	Cadmium, lead, zinc, copper, iron	Lamrood and Ralegankar (2013)
9	*Coriolus versicolor* MKACC 52492	Polycyclic aromatic hydrocarbons (PAHs)	Jang et al. (2009)

community. Conventional treatment appears inefficient (Badawy et al. 2006). *Trichoderma* are able to biodegrade toxic pollutant efficiently (Harman et al. 2004b; Cao et al. 2008). Extracellular enzyme system of the fungi and their catalytic reaction help to degrade toxic aromatic compounds. They also have the capability to degrade a variety of chemical pesticide compounds which remains in the soil like DDT, lindane, and chlordane (Ezzi and Lynch 2005; Zhou et al. 2007). Combined or integrated management strategy having *Trichoderma* spp. that is merged with less amount of pesticides helps in the remediation of contaminated sites and reduction of the chemical load to make the environment clean. *Trichoderma viride* has been described as the most effective and competent among other tested fungal species for biodegradation of common pesticides such as photodieldrin and chlorpyrifos (Tabet and Lichtenstein 1976; Katayama and Matsumura 1993; Mukherjee and Gopal 1996).

21.6 Conclusions

For environment cleaning, there is an availability of numerous technologies each having its own benefits, pluses, and restrictions for treating a specific chemical pollutant. Mycoremediation is an innovative approach having a prospective to diminish several ecological contaminant hindrances. The fungi are a well-known tool being used in mycoremediation. The *Trichoderma* are diverse species which are resistant to a wide range of refractory contaminants involving various HMs, harmful pesticides, and PAHs. Besides, mushroom is used as food due to their protein richness. Cultivation of mushroom will alleviate major problem of the world waste accumulation and supply protein as food simultaneously. Activity of microbial species to address a variety of wastes which foregoing generated and enter into the soil is a major concern for today. Further research are needed to exploit natural resources such as fungi to enhance mycoremediation.

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The Importance of Plant-Microbe Interaction for the Bioremediation of Dyes and Heavy Metals

22

Varsha Dogra, Gurpreet Kaur, Rajeev Kumar, and Chander Prakash

Abstract

Heavy metals and dyes are released from different industries which cause adverse effects on the environment. It is a persistent problem because metals are not biodegradable. Conventional treatment of heavy metals and dyes is not cost-effective and also produces large amounts of hazardous waste and mud. Plant-microbe synergism is an essential portion of our earthly bionetwork; recently many researchers have explored this field to understand the plant-microbe-heavy metal/dye interactions. These interactions have many applications in the field of phytoremediation technology. The technique rhizorestitution is a particular type of phytoremediation that can solve the problems of sites contaminated with heavy metals and dyes. Rhizospheric and endophytic microbiome connected with plant system have the potential of biodegrading the organic compounds in the contaminated site. Potential metabolites such as 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase, indole-3-acetic acid (IAA), organic acids, some volatiles, etc. are synthesized by plant-associated microorganisms (e.g., mycorrhizae, bacteria); these metabolites are involved in many biogeochemical progressions which operate in rhizoplane and rhizospheric zone. Plant-associated microbes have acidifying reduction and chelating power. Plant-microbe interactions enhance the uptake of heavy metals using many biological and geochemical processes, which mainly includes uptake, translocation,

V. Dogra · R. Kumar (✉)
Department of Environment Studies, Panjab University, Chandigarh, India
e-mail: rajeev@pu.ac.in

G. Kaur (✉)
Department of Chemistry and Centre of Advanced studies in Chemistry, Panjab University, Chandigarh, India
e-mail: gurpreet14@pu.ac.in

C. Prakash
Department of Chemistry, M.L.S.M. College, Sunder Nagar, Himachal Pradesh, India

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immobilization, chelation, precipitation, solubilization, volatilization, and complex formation of heavy metals, and finally lead to phytorestitution. In general, the plant-microbe interaction increases the effectiveness of phytoremediation process by altering the heavy metal gathering or accumulation and dye in plant tissue parts. In this chapter, we are focusing on the different types of plant-microbe interactions for the bioremediation of synthetic dyes and different types of heavy metals. Focus will be on different plant-microbe interaction-based bioremediation methods to eliminate the dyes and heavy metals in polluted sites.

22.1 Introduction

With increasing industrialization, there have been increased production and release of synthetic dyes and heavy metals in the ecosystem. Commonly used heavy metals (HMs) such as Pb, Hg, Cd, Cu, As, Zn, and Ni are utilized as a part of mining industries and agriculture, whereas man-made dyes are being employed in several fields such as textile industry, leather tanning industry, paper industry, food technology (Slampova et al. 2001), research related to agriculture, photochemical cells (Wrobel et al. 2001), light harvesting arrays, fur or hair colors, etc. Many HMs and dyes are noxious or poisonous even at very less concentration and cause a threat to flora and fauna, as they are cytotoxic, mutagenic, and carcinogenic in nature (Salem et al. 2000). They have detrimental consequences on the natural ecosystem and soil ecosystem and also influence health of human beings because of their persistence, solubility, and mobility (Kabata-Pendias 1992). Some organic compounds stay for a long time in the nature, enter the food chain, and can harm the plant and animal life (Saleh et al. 2004). Dyes are complex structures, and many techniques are utilized for upgrading the dye properties with the goal that they give enhanced conveyance to textures of fabric, are fade resistant, and have expanded assortment of shades. These properties make them biological nondegradable (Togo et al. 2008). Many physicochemical strategies, for instance, adsorption, flocculation, coagulation, membrane processes, filtration, photodegradation, reverse osmosis, and chemical oxidation, are powerful for dye removal, but these have many limitations such as less cost-effective, low effectiveness, auxiliary problems of pollution, and difficulty in applying to an extensive range of synthetic dyes (Tamboli et al. 2010; Kurade et al. 2011). The noxious and lethal consequences of dyes on flora and fauna are well documented (Karaca et al. 2008). Industrial effluents raise major concerns due to its non-biodegradable attributes. Disposal of untreated industrial effluent into the receiving water body causes harm to aquatic life by the mutagenic and carcinogenic effects. The release of such effluents is stressing for both toxicological and ecological reasons (Baskaralingam et al. 2006; Tor and Cengeloglu 2006).

With the aim of controlling or decontaminating the soil from industrial effluents, many physicochemical (chemical and physical methods) and biotic (biological) approaches have been exercised. Biological methods have more advantages than the other physical and chemical restitution techniques owing to their numerous advantages such as they are cost-effective, appropriate, and easy to operate and thorough biodegradation of chemicals and cause no harm to native plants and animals (Timmis and Pieper 1999). A biological method such as bioremediation makes use of organisms to breakdown the hazardous chemicals into less toxic or nontoxic forms, by using them as a source of food and energy (Gianfreda and Rao 2004). It is an alternative biological way to manage or neutralize the waste.

With the help of rhizobacteria, bioremediation of heavy metals and dyes can also be done. Plant growth-encouraging rhizobacteria (PGERs) are effective soil bacterial species that lead to colonization of the plant roots and augment the growth of plants by providing resources (phosphorus, nitrogen, and minerals) (Kloepper and Schroth 1978). According to their association with plants, these bacterial species are of two types: free-living bacteria (*Azotobacter*) and symbiotic (*Rhizobium*) species (Khan 2005). Many researchers have worked on the PGPR-plant relationships to know about the principles, means, and mechanisms behind the symbiotic association, which is extensively established as rhizospheric effects (Compant et al. 2005; Glick 1995, 2003; Hall 2002; Hallmann et al. 1997; Lucy et al. 2004; Prasad et al. 2015; Sturz et al. 2000; Welbaum et al. 2004). Rhizobacteria play their role in different ways (Glick 1995, 2003).

In general, secretion of beneficial components by bacterial strains in the rhizosphere leads to enhancement of the nutrient uptake from the soil (Cakmakçi et al. 2006; García et al. 2004; Lucas Garcia et al. 2004; Siddiqui and Mahmood 2001) and thereby lessens or averts the effects of different plant pathogens on plant growth and development and leads to improvement in different types of biological control operators (Guo et al. 2004; Jetiyanon and Kloepper 2002; Raj et al. 2003; Saravanakumar et al. 2007). The plant roots spread the microscopic organisms through the soil system and help infiltrate generally impervious layers of soil. Plant root exudates help in the bacterial survival and activity (Kabra et al. 2013). Microscopic organisms increase the percentage of bioavailability of the heavy metals and dyes to the plants. Consequently, such a synergistic framework can enhance the productivity of phytoremediation (Whiting et al. 2001). Plants play a significant but an unintended or secondary character as they provide protection and carbonaceous compounds and other beneficial nutrients to the rhizospheric microbiome, which play a vital role in the uptake, adsorption, and decontamination of pollutants (Gerhardt et al. 2009).

22.2 Plant-Microbiome Synergism

These are the positive or negative synergism amid the plant and the diverse communities of the microorganisms (fungi, bacteria, and archaea). This type of interaction can be friendly or hostile. There are two types of plant-microorganism interaction:

1. Positive or symbiotic interaction
2. Negative or pathogen-plant interaction

Plant-associated microbes include a plant that acts as its host and its associative microbiome, either synergistic or pathogenic, which influences plant growth (Jones and Dangl 2006; Oldroyd 2013). Nonetheless, the host plants are encompassed by various microorganisms in indigenous habitats, particularly in soil system; here a different variety of bacteria exists (Gans et al. 2005). These microorganisms augment the plant development and growth by providing mineral nutrients and fixed nitrogen in exchange of carbon. Microorganism associated with the plants supports them to deal with biotic and abiotic stresses and to produce phytohormones, siderophores, and inhibitory allelochemicals (Bulgarelli et al. 2013; Arshad et al. 2007; Weyens et al. 2009a, b). Plant-microbe interaction also plays a significant part in the phytoremediation by degrading, detoxifying, or sequestrating the pollutant and enhancing the plant progress and growth (Weyens et al. 2009a, b).

22.3 Significance

- Plant-associated microbes have importance in agriculture either because of nitrogen fixation and biocontrol significance or harmful impacts such as pathogenesis.
- Negative interaction of plants such as pathogenic microbe and host communications leads to the development of disease plant or constructive interaction with the advantageous soil microorganisms for increasing plant progress and growth, handling abiotic and biotic stresses, and helping the plant to decontaminate the polluted soil (Abhilash et al. 2012).
- Plant and microbe’s association can also alter the biochemical, physical, and chemical soil features and properties.
- Plant root exudates also provide various nutrients and carbonaceous compounds in the form of polymerized carbohydrates, essential amino acids, various organic acids, and other nutrients in to the soil.
- Secondary metabolites produced by the root exudates help in the communication of plants and microorganisms.
- Phytosiderophores secreted by the plants help in the sequestration of heavy metals and dyes from the soil. However, these plant-microbe interactions are very complex, and it is very difficult to interpret at dissimilar boundaries such as in phytosphere, phyllosphere, rhizosphere, and also endosphere.
- Plant-associated microbes assist in the agriculture, relieve the impacts of climate change, increase the bioenergy production, aid in ecosystem restoration, and enhance the biomass production (Saleem and Moe 2014).

The three different types of plant-microbe interactions are discussed below.

22.4 Plant-Bacteria Interaction

Diverse communities of bacteria are found in the soil. *Actinobacteria*, *Proteobacteria*, nonsporulating rods, and *Pseudomonas* are the most dominating bacterial population (Sylvia et al. 2005). Roots of plants realized metabolites which act as chemical signals for bacteria to obtain nutrients from surface of roots (Nihorimbere et al. 2011). In addition, plant growth-encouraging rhizobacteria (PGERs) inhabit roots very efficiently to augment plant physical condition and development. Moreover, symbiotic relationships between the plant and the PGPR give an advantage to both the plant and the rhizobacteria, such as encouragement of plant root development and improved nutrient uptake (Bonkowski et al. 2009).

There are different types of symbiotic bacteria which help in the degradation of heavy metals and dyes such as the following.

22.4.1 Rhizobacteria

Rhizobia are Gram-negative soil bacterium that forms nodulation with roots of many leguminous plants. Rhizobacteria help in the nitrogen fixation inside the root nodules of the leguminous plants (Fabaceae). This bacterium converts the atmospheric nitrogen into the ammonia and then into nitrates and nitrites. Glutamine or ureides are the nitrogenous compounds which are provided to the plants by the rhizobia bacterium after conversion of atmospheric nitrogen. In return, the plant provides photosynthesis product to the bacteria (Sawada et al. 2003). Rhizobia release into the soil from the root nodule once the legumes die and reinfect the new legume (Herridge 2013). Some reports suggest that PGERs augment the plant development and growth directly by various means and mechanisms such as fixation of atmospheric nitrogen (Zakry et al. 2012), siderophore production, phytohormone synthesis, and mineral solubilization such as phosphorous. Low-nitrogen soil can be improved by nitrogen-fixing systems as they play a noteworthy and substantial role in augmenting the soil potency, fertility, and production (Zahran 1999). Rhizobacteria include *Pseudomonas*, *Bacillus*, *Azospirillum*, *Azotobacter*, *Achromobacter*, *Arthrobacter*, *Enterobacter*, *Serratia*, and *Streptomyces* species. Plant growth-encouraging bacteria (PGEB) have gained immense attention among the rhizosphere microorganisms. Plant-associated bacterial species travel from soil to plant rhizoplane or rhizosphere and may also move inside and colonize; these microbes are rhizobacteria (Kloepper and Schroth 1978; Kapulnik 1991). They have positive consequences on many plants in heavy metal-polluted soil (Smith 2005; Tokala et al. 2002; Dimkpa et al. 2008a, b, 2009a, b). The growth of the plant relies on different strains of the bacteria and on the secondary metabolites produced by these friendly microbial species. Presence of different microbial strains depends indirectly or directly on the plant development enhancing hormones, i.e., phytohormones such as cytokinins, auxin, ethylene, and gibberellins and together with secondary metabolites of bacteria (Forchetti et al. 2007; Perrig et al. 2007; Ryu et al. 2005; Aslantas et al. 2007; Dimkpa et al. 2009a). Rhizobacteria produced many additional favorable components for the plant such as amino acids, beneficial enzymes, carbohydrates, siderophores, nitric oxide, mild organic acids, biosurfactants, osmolytes, and antimicrobial compounds which help in the repression of pathogens, enhancement in mineral uptake, fixation of biological nitrogen, release of phytohormones, and abiotic stress tolerance (Chakraborty et al. 2006; Sikora et al. 2007; Dobbelaere et al. 2003; Vessey 2003; Sziderics et al. 2007; Belimov et al. 2009; Dimkpa et al. 2009a).

22.4.2 *Frankia*

This is a filamentous bacterium having a synergetic and cooperative association with family of Leguminosae plants; this type of symbiosis helps in atmospheric nitrogen fixation. *Frankia* bacteria are found in leguminous plant root nodules, and this type of association has been found around more than 200 plant species all over the globe (Benson and Silvester 1993). The first strain of *Frankia* was isolated by Pommer in 1956, but this strain later got lost (Pommer 1959). *Frankia* nodulates a broad variety of perennial woody plants, which are dicotyledonous in nature (Mishra et al. 2010).

22.4.3 *Azospirillum*

Bacterial species of the *Azospirillum* genus are Gram-negative, rod-shaped, nitrogen-fixing associative, and microaerobic in nature, and they come under *Proteobacteria* and belong to k-subclass (Okon 1994), and five species of *Azospirillum* have been described as *Azospirillum halopraeferens* (Reinhold et al. 1987), *Azospirillum brasilense*, *Azospirillum lipoferum* (Tarrand et al. 1978), *Azospirillum irakense* (Khammas et al. 1989), and *Azospirillum amazonense* (Magalhaes et al. 1983). Azospirilla are *Azospirillum* species that are found to be in symbiosis with corn, oilseeds, wheat, fodder crops, rice, vegetables, sorghum, and other important agricultural crops (Zhang et al. 1997). According to some studies, azospirilla can increase the crop yield, plant development, growth, and nitrogen amount (Zhang et al. 1997; Boddey et al. 1986). *Azospirillum* is rhizosphere bacteria and shows adaptable carbon and nitrogen metabolism in the root of plants. The nitrogen sources for plants are ammonium, nitrite, nitrate, amino acids, and molecular nitrogen (Hartmann and Zimmer 1994). Under unfavorable conditions, such as nutrient limitation and extreme environment conditions, azospirilla changes its morphology and convert into cyst-like form (Lamm and Neyra 1981; Sadasivan and Neyra 1985, 1987). In the stress- and nutrient-constraint conditions, cyst gets carbon energy source from the outer covering of polysaccharides and from the poly-L-hydroxybutyrate granules (Tal and Okon 1985; Tal et al. 1990). Azospirilla bacteria bear flagella and therefore are greatly motile such as *Azospirillum irakense*, *Azospirillum lipoferum*, and *Azospirillum brasilense* (Hall and Krieg 1984; Moens et al. 1995). Bacteria of the *Azospirillum* genus have chemotaxis behavior toward amino acids, sugars, mild organic acids, few aromatic compounds, and root exudates also (Barak et al. 1983; Reinhold et al. 1985; Zhulin and Armitage 1993; Lopez-de-Victoria and Lovell 1993; Lopez-de-Victoria et al. 1994; Heinrich and Hess 1985). *Azospirillum* also displays an interesting phenomenon, i.e., movement toward optimum concentration of oxygen, known as aerotaxis (Barak et al. 1982).

22.5 Plant-Fungi Interaction

Symbiotic and pathogenic fungi both interact with rhizosphere; there are many factors affecting the interactions such as soil type and root exudates. In particular, root exudate is a significant component for rhizospheric fungal species (Buée et al. 2009). Zygomycetes and deuteromycetes are the fastest to metabolize the simple sugars in the rhizosphere (Sylvia et al. 2005). *Aphanomyces* and *Pythium* are pathogenic fungi that cause plant root diseases. *Gibberella* is a genus of fungi that develops a synergetic or cooperative association with plant roots and enhances their growth. *Armillaria* is a parasitic fungus that infects the plant root and causes white rot root disease (Fox 2000).

Mycorrhizal fungal species develop a cooperative association with roots of higher plant species. More than 80% of the terrestrial plant have a mutualistic relationship with different types of mycorrhizal fungi. Mycorrhizal fungi inhabit the roots of plant and forms a synergetic association called mycorrhiza. Plant relation with the mycorrhizal fungi helps in increasing the root length by hundred fold or more and also in augmenting the outside area for nutrient uptake and transport and also captures water molecules and solubilizes insoluble nutrients such as phosphorus by the plants. Plants in return provide a fixed amount of carbon to the fungi (Das et al. 2006; Goltapeh et al. 2008; Sylvia et al. 2005). There are dissimilar kinds of mycorrhizal fungi which help in the bioremediation.

22.5.1 Orchid Mycorrhizal Fungi

Orchid mycorrhiza is a mutual association amid plant roots of family Orchidaceae and different diversity of fungal species. This type of symbiotic association is important for orchid germination as it obtains carbon from the mycobiont (McCormick et al. 2012). The orchids are obligate mycoheterotrophic and need nutrients acquired from fungal strains for the plant growth. This symbiotic association remains from short duration to the complete cycle of life (Rasmussen 1995; Rasmussen and Whigham 1998) and usually needs particular mycorrhizal fungal species (Taylor et al. 2002; McCormick et al. 2004).

22.5.2 Ectomycorrhizae

This is the synergetic and reciprocal association amid fungal species and roots of different plant species. Ectomycorrhizae form with around 2% of plant species including woody plant species such as birch, beech, willow, pine, myrtle, and rose families (Tedersoo et al. 2010; Smith and Read 2010). This fungus is different from other fungi species as it forms a dense hyphal sheath around the root surface known as the mantle (Hock 2012). Root sheath can be 40 μm thick, and fungal hyphae can extend several centimeters into the soil to explore water and various micro- and macronutrients (Smith and Read 2010). In return, fungi obtain carbohydrates from the plant. Ectomycorrhizal fungi do not penetrate the root cell wall; instead highly branched hyphae form an intercellular interface between epidermal and cortical root cells known as Hartig net (Hock 2012).

22.5.3 Arbuscular Mycorrhizae

Arbuscular mycorrhizae are also known as endomycorrhizal because it enters the plant roots' cortical cells and moves deep inside. This symbiotic relationship allows the nutrient exchange and carbon amid plant and the fungal species very efficiently. Mycorrhizae fungi include *Glomus tenue* and *Scutellospora* (Sylvia et al. 2005). Symbiont forms unique structures such as arbuscular and vesicles by fungi of the phylum *Glomeromycota*. Arbuscular mycorrhizae played significant and imperative part in the evolution of vascular plants (Brundrett 2002). This type of mutual relationship exists between 80% vascular plant families and helps in plant development, growth, and health (Schuessler et al. 2001). Fungal mycelium holds the soil together, prevents soil erosion, and helps in improving the soil health. Mycobiont part of this symbiont secretes a sticky substance, i.e., glomalin, which grips the soil particles, increases the stability of the soil, and further helps in the proficient uptake of phosphorous as arbuscular mycorrhizae upsurge the cover area of the roots of plant (Gianinazzi et al. 2010).

22.6 Plant-Archaea Interaction

Archaea constitute a kingdom of single-celled microorganisms and were classified as an extremophile, but now they are found in different environments such as soil, oceans, and human skin also. They play an essential role in universal progressions, for example, ammonification process in rhizosphere (Leininger et al. 2006), nitrification (Wuchter et al. 2006), and production of methane gas (Erkel et al. 2006). They are very important for transformation of organically bound nitrogen from microbial/ plant biomass to ammonia (Buée et al. 2009). In addition, archaea play a crucial part in biogeochemical cycles, for example, recycling of nitrogen and carbonaceous compounds.

22.7 Plant-Microbe Interaction Mechanism

The mechanism behind the plant and microbe interaction has been of great interest from the last two decades. In plants, there are two layers of defenses for their protection from different kinds of pathogens. The principal or first layer of immunity is pathogen-associated molecular pattern-triggered immunity (PAMP-PTI) which gets activated upon the identification of pathogens by the plant pattern recognition receptors (PRR) at the cell membrane of plants (Dodds and Rathjen 2010). The second layer of defense is activated when effector proteins produced by the pathogen are recognized by the receptor proteins also called resistance proteins (Jones and Dangl 2006). Resistance proteins recognize pathogen effector proteins, i.e., avirulence factors (Avr), and provide effector-triggered immunity (ETI) to the plants which are specific to pathogens; it is also known as R gene-mediated resistance against the modified pathogens having Avr genes (Jones and Dangl 2006). Pattern-triggered immunity plays a key part in fungal-plant communications to inhibit entry of pathogens inside the plant cells. Effector-triggered immunity in contrast to PTI is activated after the invasion of pathogens to constrain or impede the development and proliferation of adapted plant pathogen that resist the pattern-triggered immunity (Dodds and Rathjen 2010; Jones and Dangl 2006; Chisholm et al. 2006).

"Zigzag" model was given by Jones and Dangl in 2006 to explain the four stages of plant-microbe co-advancement (Jones and Dangl 2006). In stage I of this model, plant pattern-recognition receptors (PRRs) present on plant cell surface detect and recognize the pathogen-associated molecular patterns (PAMPs) of invading microbes to activate immunity to prevent the invasion. In stage II, attacking microbes transport effectors into the plant cells to repress pathogen-triggered immunity (PTI) ensuing effector-triggered susceptibility (ETS) to establish the plant-microbe relationship. In stage III, the plant develops intracellular immune receptors (R proteins) that identify specific effectors to stimulate the effector-triggered immunity (ETI); this immunity often ends up in hypersensitive response (HR), i.e., programmed cell death at contagion sites. In stage IV, effector perceived by R protein may experience changes such that the pathogen evades effector-triggered immunity (ETI) or new effectors may develop in the pathogen to suppress ETI, leading to ETS gain. Consequently, ETI and ETS happen as an outcome of the plant-pathogen co-development.

22.8 Rhizosphere Interaction

The rhizosphere is a narrow soil zone which is in nearby vicinity of plant roots. This microecological zone is influenced by root exudes and symbiotic microorganisms. Rhizosphere is considered to be the most biodiverse and metabolically active habitat on earth (Hinsinger et al. 2009; Srivastava et al. 2014). Rhizobacteria, mycorrhizae, and endophytes (fungal and bacteria that reside inside the plant cells) are the main organisms that directly interact with the rhizosphere of plants. The key applications of microbes in the rhizosphere of plants are enhanced phosphorus uptake, nitrogen fixation, and defense against disease-causing microorganisms. Plants also in return provide carbon (synthesized with the process of photosynthesis) to the organisms; they provide 15–20% carbon to root nodules, 15% to rhizoplane, and 4–20% to the mycorrhizae (Kiers and Denison 2008). Many different kinds of interactions take place in the rhizosphere of plants, ranging from valuable symbiotic relationships (i.e., beneficial for the plant) to harmful pathogenic interactions (Sylvia et al. 2005). Contrariwise, plant-microbe interaction could also lead to microbes who help less than the others in sustaining plant health. These types of microorganisms are called cheaters, and according to recent studies, plants choose microbial interaction ensuring mutual cooperation. Recognition signal is given by the plant to know the potential microbes and microbes that cheat (Kiers and Denison 2008).

22.9 The Role of Microorganisms in Metal Phase Transformation and Uptake

Plant growth-encouraging bacterial species help in the reduction of heavy metal phytotoxicity by dissimilar means and procedures such as biosorption and bioaccumulation. Bacterial cells have a tremendously great ratio of volume to surface area that helps in the adsorption of heavy metal by metabolic rate-independent less active process or by a metabolic rate-dependent active or less passive process than the components of soil (e.g., vermiculite, perlite, kaolinite, etc.) (Ledin et al. 1996; Khan et al. 2007).

Many researchers have found that heavy metal adsorption mechanisms such as biosorption and bioaccumulation along with features that enhance the pant growth including ACC deaminase enzyme and plant development-encouraging hormones contribute to enhancement of plant development in heavy metal-polluted site (Zaidi et al. 2006; Madhaiyan et al. 2007; Kumar et al. 2009).

Heavy metal movement and accessibility to different plants are affected by soil microorganisms through the process of acidification, by generating proteinaceous compound siderophores, which are iron chelators and required for iron accessibility, and solubilizing the insoluble metal phosphates and redox changes (Abou-Shanab et al. 2003; Burd et al. 2000; Guan et al. 2001) (Fig. 22.1). The existing phase of heavy metal is an additional problematic issue that influences the uptake of metals. In soil, the heavy metal is bound to different inorganic and organic soil components or exists as precipitates which are insoluble, and this situation makes the metal inaccessible for uptake by plant roots. Researchers have studied different parameters to upsurge the accessibility of heavy metals to the plant roots (Ernst 1996; Kukier et al. 2004). In one interesting study conducted by Abou-Shanab and co-workers, they observed the consequence of rhizobacterial species on uptake of Ni metal (Abou-Shanab et al. 2006). They observed that rhizobacteria assist in discharge of metal nickel from insoluble soil phases, therefore increasing accessibility of nickel to soluble and removable form.

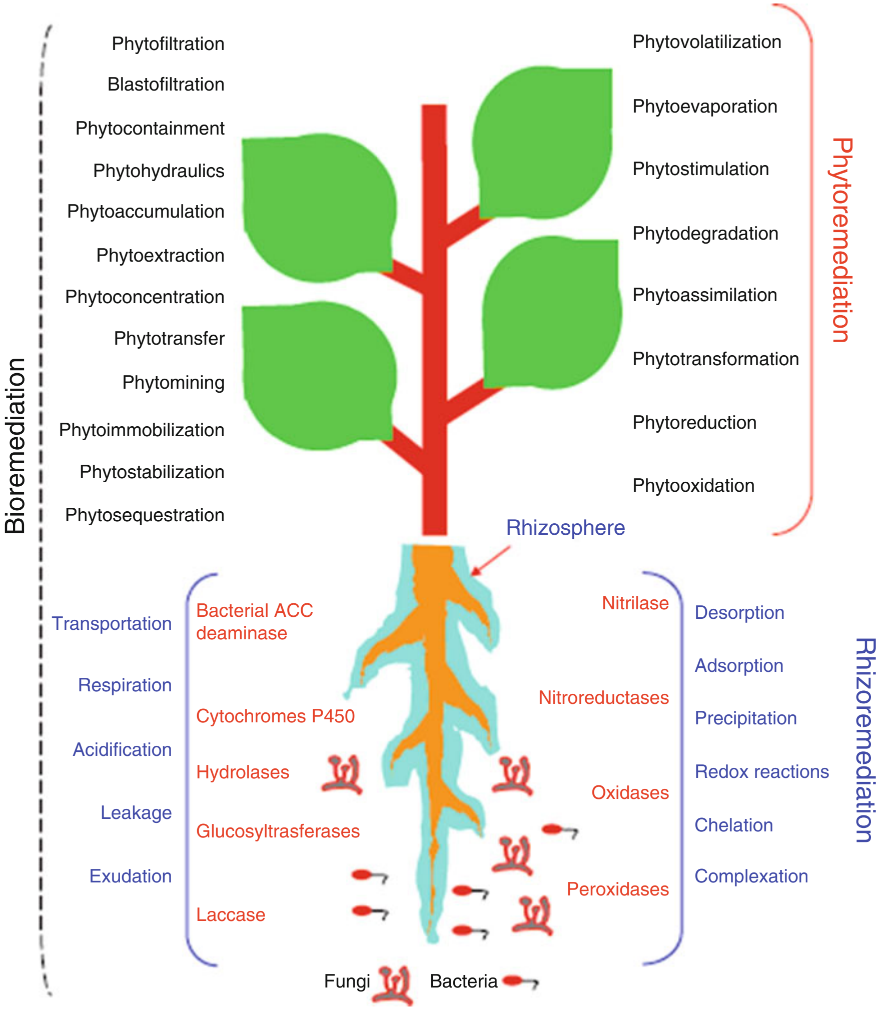

Fig. 22.1 Plant-soil and microbiome communication importance in biodegradation of heavy metals and dyes. (Ma et al. 2011)

22.10 Bioremediation Approaches

The plant and the microbe symbiosis assure the bioremediation of the land and the wastewater (Glick 2010). For the remediation of contamination, the combinatorial framework of plants and the root-associated microbes could be the more successful approach (Glick 2010; Chaudhry et al. 2005). Plants provide favorable conditions to the communities of microbiome existing in rhizospheric zone to degrade the contaminants (Doran 2009). Advantage of this type of remediation method is low cost, habitat restoration, degradation of pollutants at their site, eco-friendly approach, neutral for carbon, and inert tactic for decontaminating ecosystem environment (Schröder et al. 2002; Prasad et al. 2010). Other than these, a number of soil bacterial species help the plant in improving the shoot and root development (Glick 2010). There are many microbes which interact with the plants and help in the bioremediation of contaminants as given in Table 22.1.

22.11 Degradation of Heavy Metals

22.11.1 By Plant-Fungi-Bacteria Association

A number of plants show the good capability of removing heavy metals from the soil system and their uptake and transport in plant root structure, restricting the translocation to the shoot system. Heavy metal sequestration inside the plant roots and rhizosphere helps in stabilization of the contaminated soil. "Plant-fungi-bacteria" association in the rhizosphere system provides the main contribution to the stabilization of the soil. Enzymes secreted by the fungi, bacteria, and root exudates help in the formation of highly stable biotic complexes which integrate with organic matter and activate the biological process that supports the complexation and chelation of heavy metals and the organic content (Guarino and Sciarrillo 2017).

Nitrogen and phosphorus promote the precipitation of small amount of metals; HPO_4^{2-} phosphate ion forms the stable molecules by involving in the precipitation reaction and converts the bioavailable portion into the residual, in this manner insoluble and inaccessible to organic associations (Cao et al. 2003). Fungal and bacterial communities degrade the heavy metal and encourage the disintegration of the complexes and uptake and transport of biologically available metal by plant system. This type of integrated biodegradation scheme is also called bio-factory which permits the uninterrupted exclusion of contaminants from the soil.

22.11.2 By Plant-Bacteria Interaction

From many types of research, it was found out that different species of *Pseudomonas* help in the removal of diverse kind of contamination such as *P. putida* Flav1-1 and *P. putida* PML2 species in association with *Arabidopsis* plant which helps in the removal of polychlorinated biphenyls (PCBs) (Narasimhan et al. 2003). Researchers used "rhizosphere metabolomics" approach to show that plant's secondary metabolites are secreted in adequate quantities to ascertain a plant-microbe association that is capable of metabolizing phenylpropanoids. These phenylpropanoids using microbes are more competitive and grow very fast, and better colonization of the phenylpropanoid-utilizing strain on the roots of flavonoid-producing plants leads to nearly 90% removal of PCBs.

Pseudomonas fluorescens F113 strain is an exceptional colonizer of various plant rhizospheres, including alfalfa. This type of association of plants and microbial

Table 22.1 Examples of bioremediation of contaminants by plant-microbe interaction

Microbes	Plants	Contamination	Role and beneficial features of plant-microbe interaction	Reference
Pseudomonas putida Flav1-1	*Arabidopsis*	PCBs	Utilized plant secondary metabolites, direct biodegradation of PCBs	Narasimhan et al. (2003)
P. putida PML2				
Pseudomonas fluorescens F113	*Alfalfa*	Polychlorinated biphenyls (PCBs)	More effectively metabolized PCBs with bph gene cloned	Villacieros et al. (2005)
Pseudomonas fluorescens 2–79	Wheat	Trichloroethylene (TCE)	Biodegraded TCE with enzyme toluene o-monooxygenase	Yee et al. (1998)
Microbacterium oxydans AY509223 (RS)	*Alyssum murale*	Nickel	Ni mobilization	Abou-Shanab et al. (2006)
Bacillus sp. Ba32 (RS), *Pseudomonas* sp. PsA	*Brassica juncea*	Chromium	ACC production, siderophore production, IAA excretion	Rajkumar et al. (2006)
			Solubilization of phosphate	
B. subtilis SJ-101 (RS)	*Brassica juncea*	Nickle	IAA production, phosphate solubilization	Zaidi et al. (2006)
			Ni bioaccumulation	
Bacillus sp. SN9 (RS), *Psychrobacter* sp. SRS8, *Pseudomonas* sp. SRI2	*Brassica juncea* L. (Mustard)	Nickel	ACCD, siderophore production, IAA excretion	Ma et al. (2009)
			Solubilization of phosphate, mobilization of nickel	
P. fluorescens ACC9, *P. tolaasii* ACC23 and *Mycobacterium* sp. ACC14 (RS)	*B. napus*	Copper, nickel, cadmium	ACCD, siderophore, IAA	Dell'Amico et al. (2008)
Burkholderia sp. J62 (RS)	*Zea mays*	Heavy metals	ACCD, siderophore production, excretion of IAA, solubilization of phosphate	Jiang et al. (2008)

(continued)

Table 22.1 (continued)

Microbes	Plants	Contamination	Role and beneficial features of plant-microbe interaction	Reference
Pseudomonas putida UW3, *Azospirillum brasilense* Cd, *Enterobacter cloacae* CAL 2	Tall fescue	Poly cyclic aromatic hydrocarbons (PAHs)	Increased plant tolerance to PAHs	Huang et al. (2004)
			Endorsed plant development and growth under various stress conditions	
Pseudomonas monteilii ANK	*Glandularia pulchella* (Sweet) Tronc.	Dye Scarlet RR (SRR)	Removal of dye	Kabra et al. (2011)
Rhizospheric bacteria *Exiguobacterium Aestuarii* ZaK	*Zinnia angustifolia*	Dye Remazol Black B	Removal of dye	Khandare et al. (2011)
Pseudomonas veronii	*S. alfredii*	Zinc	Production of IAA, lessening soil pH, make available phosphate and iron	Long et al. (2013)
Bacillus megaterium HKP-1, *Azotobacter chroococcum* HKN-5, and *Bacillus mucilaginosun* HKK-1	*B. Juncea*	Lead and zinc	IAA, gibberellins	Wu et al. (2006)

strain helps in the metabolism of PCBs very effectively with bph gene cloned (Villacieros et al. 2005). Yee et al. noticed that association of recombinant *Pseudomonas fluorescens* 2–79 with wheat plant facilitates the degradation of trichloroethylene with enzyme toluene o-monooxygenase (Yee et al. 1998). From soil in wheat rhizosphere, organic compound trichloroethylene (TCE) was eliminated by using a recombinant *Pseudomonas fluorescens* strain which was capable of degrading TCE.

Beneficial association of *Bacillus* sp. Ba32 and *Pseudomonas* sp. PsA4 with Indian mustard (*Brassica juncea* L.) plant was investigated by Rajkumar et al. in 2006. These microbial species were assessed in different concentrations of Cr6+ in soil, and it was observed that these microbes enhance the growth of the plant and the tolerance capacity to the chromium. In PsA4 and Ba32 strains, siderophore production and solubilization of phosphate were observed. Indole acetic acid was also produced in strain PsA4. Both strains inhibit the effects of chromium on plant growth most likely owing to the siderophore and indole-3-acetic acid production and also solubilization of phosphate (Rajkumar et al. 2006). Ma et al. studied the interaction of three different bacterial species: *Bacillus* sp. SN9 (RS), *Pseudomonas* sp. SRI2, and *Psychrobacter* sp. SRS8 with *Brassica oxyrrhina* and *Brassica juncea* L. This investigation assesses the effects of nickel mobilizing bacterial species on plant growth and development and nickel uptake by *Brassica oxyrrhina* and *Brassica juncea* L. It was observed that nickel-mobilizing strains produce siderophores and phytohormone (IAA) by utilizing ACC as only nitrogen resource and solubilizing phosphatic compounds (Ma et al. 2009).

22.11.3 Use of Genetically Engineered Rhizobacteria

For the bioremediation of polluted soil, rhizosphere provides the promising environment. Many microorganisms have a limited role in the remediation as they show partial capability in biodegrading the carbon-based contamination. With technology development, genetically engineered (GE) rhizobacterial species are developed having pollutant-biodegrading genes for bioremediation of the polluted site. For the development of genetically engineered (GE) rhizobacteria, the following norms should be considered: (1) stability of strain after cloning, (2) the target gene must have elevated expression in bacterial cell, (3) bacterial strain should be tolerant toward contaminants, and (4) a few strains require precise plant rhizospheric zone for survival (Brazil et al. 1995; Yee et al. 1998).

22.12 Degradation of Dye

Among manufacturing industries, the textile industry is the main problematic industry as it releases various pollutants to the water bodies. The chief ingredients and elements of textile dye effluents are the huge amount of obstinate or hard-to-degrade compounds which contaminate the soil system and natural water resources causing harmful impact on every aspect of living systems. Dyes released from dye manufacturing plants and textile industries (Cripps et al. 1990) are the most undesirable substance as they do not degrade easily (Daneshvar et al. 2007; El-Rahim 2006). Textile dyes are toxic, carcinogenic, and mutagenic (Hu et al. 2009). Industrial effluent released from dyeing units contains various organic compounds, heavy metals (Sekhar et al. 2006; Soares et al. 2006), dyes, and chemicals. All these compounds are toxic to aquatic life, hence requiring environment-friendly biological method for the degradation. Dye removal from soil is also done by different synergistic systems of plant and microbes. Many researchers investigate the symbiotic relationships of microbes and plants for the remediation of dyes.

22.12.1 By Plant-Bacteria Association

Synthetic dyes such as diazo and highly sulfonated reactive compounds can be degraded by plant-bacteria synergism, for example, Remazol Black B dye was degraded by the plant *Zinnia angustifolia* Kunth and its root system connected with *Exiguobacterium aestuarii* ZaK (Khandare et al. 2012). During the dye decolorization, increase in the enzyme activity such as azoreductase (73%), DCIP reductase (161%), and veratryl alcohol oxidase (223%) was confirmed in *E. aestuarii*, whereas enzyme activity of DCIP reductase (106%), laccase (120%), LiP (282%), and tyrosinase (82%) was confirmed to be enhanced in *Z. angustifolia* roots (Khandare et al. 2012). Plant and bacteria enzymes work synergistically to increase the degradation of dye efficiently (Khandare et al. 2012). Kabra et al. (2011) studied the *Glandularia pulchella* (Sweet) Troncoso association with *Pseudomonas monteilii* ANK and observed color removal in dye mixture and dye scarlet RR. Consortium of P. *monteilii* and *G. pulchella* exhibited the better possibilities of color removal in effluents of textile dyes. Enzymes of both plant and microbe worked together in the removal of dyes resulting into efficient and faster method (Kabra et al. 2013).

Khandare et al. (2012) studied the role of *Zinnia angustifolia* plant and *Exiguobacterium aestuarii* strain consortia in Remazol Black B dye removal. He found out that the consortium ZE was more proficient than plant and bacteria individually. Significant enhancement in the enzyme activities present in roots of *Z. angustifolia*, for example, lignin peroxidase, laccase, tyrosinase, and DCIP reductase, was observed during the process of dye decolorization. The researcher found out that consortium ZE was more proficient and faster in the biodegradation of dye Remazol Black B (RBB) than biodegradation by *Z. angustifolia* and *E. aestuarii* separately.

22.12.2 By Plant-Fungi Association

In plant-fungi interaction, phycobiont supports the mycobiont part by providing the nutrients and fixed carbon (20% of photosynthesis product is provided to the mycorrhizae); in return mycobiont part provides nitrogen and phosphates to the plants (Sharma et al. 2015). This type of interaction helps in the bioremediation of contaminants present in the soil by mineralization and detoxification. The biodegradation of dyes is very efficient and promising approach to remove the contaminations such as dyes. Azo dyes are widely employed in various textile industrial sectors (Saroj et al. 2014) and get discharged in the effluent. These azo dyes get deposited in the environment and also enter the food chain. Microorganisms break down the dyes and convert them into stable complex (Butani et al. 2013). In plant rhizospheric system, bioremediation or conversion of harmful contaminants by plant root-connected bacterial and fungal species occurs under the stimulus of selected species of plants. The plants can upsurge or reduce the proliferation and survival of advantageous and favorable bacterial and fungal species in polluted soil system using its rhizospheric influence. This concept was validated and authenticated by Olson et al. (2003). This group observed the greater microbiome biomass and more activity in rhizospheric zone.

Lichen is an organism which shows the symbiosis amid fungi and algae which have the bioremediation potential (Kulkarni et al. 2014). Mycobiont part of lichen belongs to the division of *Ascomycota* and also rarely to the *Basidiomycota* that live with the symbiosis of photobiont part (Lisci et al. 2003). Lichens are able to tolerate the extremities of harsh environmental conditions and exhibit the wider distribution in different geographical regions. They grow on the surface of the rocks, tree trunks, and also on the ground but very rarely. They grow very slowly and have high absorptivity. Algae or photobiont through photosynthesis process produces carbohydrates and provides it to the mycobiont or fungi for the growth; in return fungi provide moisture and nutrients from the environment to the algae, and both gets benefitted (Nash 2008). The earliest description about the capability of lichens for color removal of textile synthetic dyes was provided by Kulkarni et al. (2014). According to the investigation, the use of lichen *Permelia perlata* confirmed the efficacy for color removal, biodegradation, and detoxification of dye Solvent Red 24 (SR24). Lichen *Permelia perlata* removed color of different dyes (50 mg L^{-1}) such as Navy Blue 3G, Navy Blue HE2R, Brown 3REL, Remazol Red, Green HE4B, and Rubin GFL, up to 93, 74, 87, 54, 39, and 46%, respectively. The degradation potential of lichen was assessed with various structurally different dyes (Saratale et al. 2009). Application of lichen has also been employed in heavy metal detoxification. Lichens accumulate and retain high concentrations of heavy metals by sequestrating metals extracellularly as crystals of oxalate or making a complex with acid of lichens. Significant quantities of heavy metals are also restrained by the cell wall of lichens, and the chief procedures of transportation of heavy metals across the cell membrane of lichens for maintaining metal homeostasis include chelation and sequestration. On the dry mass basis, 16% of Cu was accumulated by the *Acarospora rugulosa* (Chisholm et al. 1987). Detoxification mechanism includes the exclusion of heavy metals, and this mechanism has been the most examined by using different lichen cells. The cell walls of both symbiotic parts (algae and fungi) are efficient in metal segregation, though the intact cells of lichens exhibit other means and approaches which involve production of lichen secondary metabolites and organic acids. The most effectual and successful extracellular approaches of detoxification of heavy metals consider oxalates, as it is extensively distributed in lichens. In some copper-tolerating lichen species, copper oxalate has been reported (Chisholm et al. 1987; Purvis 1984). Sarret et al. (1998) investigated the job of extracellularly produced oxalates for zinc and lead immobilization by *Diploschistes muscorum* lichen, while Pawlik-Skowrońska et al. (2006) explored the function of various acids such as oxalic, malic, and citric in *Lecanora polytropa*.

22.13 Conclusions

Heavy metals and dyes are a worldwide problem as they contaminate the soil through industrial means and have harmful effects on different environmental aspects and health of human beings. An alternative method of incineration and excavation methods of cleaning the contaminated sites result in the environment-friendly methods of bioremediation. Microbial enzymes help in the biodegradation of contaminants. Plant-microbe interaction with enhanced phytoremediation seems to be an assuring methodology for heavy metals, synthetic dyes, and noxious textile effluent treatment. Analysis of plant-microbe interaction offers a new understanding of the mechanism behind the remediation methods. Environmental factors like temperature, light, salinity, soil pH, multiple heavy metals, and dyes may affect the metal uptake. This plant-microbe method of bioremediation is environment-friendly, promising, and cost-effective, restores the contaminated site, and is an alternative to other methods which harm the environment.

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Bioremediation of Textile Dyes: Appraisal of Conventional and Biological Approaches

23

Sweety

Abstract

The world is facing numerous environmental changes and challenges, which include generation of huge amounts of wastewater owing to several man-made activities. Use of man-made dyes has increased in the clothing and textile industries because of their bright colors and cost effectiveness in manufacturing. On the other hand, the use of natural dyes is not cost effective, and their preparation is a cumbersome process. Therefore, people are using synthetic colors and textile dye industries are producing huge volumes of wastewater, creating aquatic pollution all over the globe. Man-made dyes or imitation dyes are broadly employed in plastic toys, textile staining, printing of paper, food, various plastic wares, dye cinematography, pharmaceuticals, cosmetics, and other important industries. During the process of fabric dyeing, a huge quantity of colored wastewater is generated as polluted effluent.

23.1 Introduction

Textile industries generate huge amounts of contaminated water, which can lead to serious complications of environmental issues if not treated properly (Kunz et al. 2002; Hemapriya et al. 2010). Every textile industry uses harmful dyes and pigments to color its products, which include fabric, tannery, colored food, paper and pulp, printing, carpet, and mineral dispensation products (Asamudo et al. 2005).

Many studies have clearly demonstrated that the use of synthetic dyes has increased in textile industries due to their bright and stable colors, consequently

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Sweety (🖂)
Division of Biosciences, College of Applied Education and Health Sciences, Meerut, Uttar Pradesh, India

Department of Bioscience and Biotechnology, Banasthali University, Banasthali, Rajasthan, India

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also increasing the price of clothes. As a result, huge discharges of contaminated colored effluent are released into the ecosystem by different industries. Studies have reported that color can be noticeable in water at a concentration higher than 1 mg/L (Neill et al. 1999).

Wang et al. (2002) have stated that around 10–15% dyes are dumped into the environment during coloring practices. The effluent from textile industries thus contains a large number of dyes and other chemicals, which are added during the tinting process. Colored textile effluent can easily enter natural aquifers and pollute natural and pure water bodies or areas (Ezeronye and Ubalua 2005). The discharge of colored effluent into water bodies and the environment is detrimental not only because of the complexity of the color but also because of the breakdown products of those dyes, which are toxic and carcinogenic to life systems. These dyes contain mutagenic agents such as benzidine, naphthalene, and other aromatic compounds (Suteu et al. 2009). Without proper treatment, these dyes can persist in the environment for a greatly extended period of time. For example, the half-life of hydrolyzed Reactive Blue 19 is about 46 years at a pH of 7 and a temperature of 25 °C (Hao et al. 2000; Wijannarong et al. 2013; Gupta et al. 2014a). Certain nonbiodegradable dyes utilized by textile industries create serious environmental hazards. The color of wastewater becomes unappealing and unfriendly to aquatic life-forms, hampering the oxygenation of water; moreover, it also disturbs the whole aquatic ecosystem (Xu et al. 2005) and the food chain. Textile effluent comprises different dyes; therefore, it is becoming essential to encourage combination of existing techniques with new techniques to decolorize the mixture of dyes. Dyes are categorized on the basis of their uses and chemical structure. Basically, dyes comprise a group of atoms called chromophores, which are accountable for the color of the dye. These chromophore-comprising centers are made up of varied functional groups: azo, methine, nitro, anthraquinone, aril methane, carbonyl, and many other groups. There are certain electrons removing and adding substituents which change the stain of the chromophores; known as auxochromes. Commonly used auxochromes are amine, carboxyl, sulfonate, and hydroxyl (Christie 2001; Santos et al. 2007; Prasad and Rao 2010).

Numerous studies have suggested that microbes are proficient in biodegradation of lethal components (Aksu 2005). The bioremediation approach proposes several benefits in that it can be carried out on-site, it is cost effective and causes minimal problems, and it can be used in combination with physical and chemical approaches (Boopathy 2000; Dias 2000).

23.2 Textile Dyes and Their Types

On the basis of their usage or application, dyes are classified according to the Colour Index (CI) scheme, compiled by Zollinger (1987).

23.2.1 Reactive Dyes

Under appropriate conditions, these dyes bond to fibers by forming covalent chemical bonds, such as ether or ester bonds. Reactive dyes encompass azo groups that consist of metallized azo, triphendioxazine, phthalocyanine, formazan, and anthraquinone. The molecular arrangements of these dyes are different from and structurally simpler than those of direct dyes. They also yield brighter, more cheerful shades than direct dyes. Reactive colorants are principally used for staining and printing of cotton fibers.

23.2.2 Direct Dyes

These dyes are anionic and water soluble in the presence of electrolytes. They bind effectively to cellulose fibers. Dyes in this group mostly comprise polyazo compounds, as well as some stilbenes, phthalocyanines, and oxazines. Improvement in wash processing is regularly achieved by chelation with metallic group (such as copper and chromium) salts. They are also used with formaldehyde or a cationic dye–complexing resin.

23.2.3 Disperse Dyes

Dyes in this group are substantively water impermeable and are used to color polyester, polyamide, polyacrylonitrile, and polypropylene fibers. Such dyes are not used for dyeing of nylon, cellulose acetate, or acrylic fibers. Chemically, these dyes are chiefly prepared from azo and anthraquinonoid groups, with groups that support formation of stable aqueous solutions.

23.2.4 Sulfur Dyes

These dyes are not dissolved in water and are useful for dyeing cotton cloth using sodium salts by a reduction process. The chemical used for this process is sodium sulfide, which is used as a reducing agent under highly basic conditions. Owing to the low cost of these dyes, they are commonly used in industries.

23.2.5 Vat Dyes

Vat dyes are water insoluble. They are applied mainly to cellulose fiber by altering them to their leuco compounds. The leuco compound is formed by reduction and solubilization with $Na_2S_2O_4$ and NaOH solution, which is called a “vatting process.” The chief chemical/structural groups of vat dyes are anthraquinones.

23.2.6 Cationic (Basic) Dyes

The dyes in this group are cationic and soluble in water. They are used for dyeing paper, polyacrylonitrile, modified nylons, and modified polyesters. These dyes are also used to apply to silk fiber, wool, and tannin-mordant cotton when bright shades are more necessary than fastness to light and washing.

23.2.7 Acid Dyes

Dyes in this group are water soluble anionic dyes and are used to color nylon, wool, silk, and modified acrylics. Furthermore, they are used in inkjet printing and in the dyeing of paper, leather, food, greasepaints, and cosmetics.

23.2.8 Solvent Dyes

These dyes are not soluble in water but do dissolve in certain specific solvents. Their molecules are generally nonpolar or sometime polar; moreover, they do not undergo a process of ionization. They are used for coloring plastics, gasoline, oils, and waxes.

23.2.9 Mordant Dyes

These dyes are capable of bonding strongly with metal residuals by forming covalent and coordinate bonds. A chelate compound is also involved. The salts of Al, Cr, Cu, Co, Ni, Fe, and Sn are used as fixatives of metallic salts. In the field of chemistry, chromophores and auxochromes are the principal constituents of dye molecules. Stain comprises an unsaturated group, basically accountable for color, and a known chromophore. Auxochromes ("auxo" means augment) are the distinguishing groups that strengthen color and/or develop the affinity of the dye for the substrate (Rangnekar and Singh 1980).

Table 23.1 lists common chromophores and auxogroups (Rangnekar and Singh 1980).

Table 23.1 Chromophore and auxochrome groups of dyes

Chromophore groups	Names	Auxogroups	Names
$-N=N-$	Azo	$-NH_2$	Amino
$-N=N^+-O^-$	Azoxy	$-NHCH_3$	Methyl amino
$-N=N-NH$	Azoamino	$-N(CH_3)_2$	Dimethyl amino
$-N=O$, $N-OH$	Nitroso	$-SO_3H$	Sulfonic acid
$>C=O$	Carbonyl	$-OH$	Hydroxy
$>C=C<$	Ethenyl	$-COOH$	Carboxylic acid
$>C=S$	Thio	$-Cl$	Chloro
$-NO_2$	Nitro	$-CH_3$	Methyl
$>C=NH$, $>C=N-$	Azomethine	$-OCH_3$	Methoxy

23.3 Bioremediation Methods

23.3.1 Ex Situ and In Situ Approaches

Bioremediation methods are broadly classified as in situ and ex situ. Ex situ methods are treatments that involve physical elimination of the polluted material for treatment processes, whereas in situ technologies involve the removal of the contaminants at the site itself. Details of in situ and ex situ bioremediation are given below:

1. *Land-dwelling*: A solid-phase treatment scheme for polluted soils may be used in both techniques.
2. *Bioreactors*: Biodegradation in a big reactor or container may be used to treat liquids or slurries.
3. *Composting*: This is an anaerobic and thermophilic treatment process in which contaminated substances are mixed with a bulking agent. This can be done using stationary piles or aerated piles.
4. *Bioventing*: This method treats contaminated soil by passing oxygen through it to stimulate microbial activity.
5. *Biofilters*: In this method, microbial stripping columns are used to treat air emissions.

23.4 Bioremediation of Dyes

Dyes are chemical substances that, when applied to a substrate, are responsible for providing such constituents with extensive coloring capability. Mostly they are used in the textile, plastics, therapeutics, food, cosmetics, graphics, and paper and pulp industries (Zollinger 1987;Carneiro et al. 2007). Dyes can stick to compatible surfaces by solution, with the help of formation of covalent bonds or complexes through salts or metals, by physical adsorption, or by mechanical retention (Kirk-Othmer Encyclopedia of Chemical Technology 2004; Bafana et al. 2011). Mauveine, the first artificial dye, was discovered by an Englishman, William Henry Perkin, in 1856. Rangnekar and Singh (1980) classified dyes into two groups on the basis of their structures, mainly consisting of chromophoric groups. Dyes can also be classified on the basis of their applications.

23.4.1 Dye Removal Techniques

Reports have described many approaches for the elimination of waste products from effluent. These approaches can be grouped into three classes: organic (biological), chemical, and physical (Robinson et al. 2001). Different methods have their own advantages and drawbacks.

Because of high costs and disposal problems, certain conventional approaches are not used to treat dye wastewater widely in the textile and paper and pulp industries.

Presently, no single process is sufficient to treat the effluent, because of its complex structure (Pereira et al. 2003; Marco et al. 1997). Nowadays, a combination of different approaches is frequently used to achieve the desired water quality in a less expensive way. A literature survey reported that research has been continuously carried out to study combined adsorption–biological treatments for biodegradation of dyestuffs and reduced sludge production. The different dye elimination approaches are discussed in the following sections.

23.4.1.1 Physical Methods

Various physical techniques are used worldwide—for example, membrane filtration processes (nanofiltration, reverse osmosis, and electrodialysis) and adsorption systems. The major shortcoming of membrane filtration methods is that they have a shorter life-span before fouling of the membrane occurs, and the cost of membrane replacement is too high. Liquid-phase adsorption is considered the most popular method to remove pollutants from wastewater. Adsorption is a popular equilibrium separation process and an effective technique for treatment of wastewater (Dabrowski 2001). Physical methods are also considered superior to other methods in terms of water reuse at low cost, flexibility, simplicity of design, and ease of operation. Adsorption also does not lead to formation of toxic or harmful substances. Physical methods include various processes, which are discussed in the following subsections.

23.4.1.2 Adsorption

Among all physical methods, adsorption is the method best suited to removal of pollutants that are very stable and not removed by any other method (Le Marechal 1998). Adsorption has an advantage over other techniques in that the pH of the discharged wastewater is not affected and no additional sludge is produced during the wastewater treatment. However, it also has some limitations (Weber et al. 1970).

23.4.1.3 Activated Carbon

For the removal of dyestuffs, activated carbon is the most widely used adsorbent (Nasser and El-Geundi 1991) and gives good results in adsorbing mordant, cationic, and acid dyes on a large scale. It has slightly poorer potential for removal of dyes such as disperse, direct, vat, pigment, and reactive dyes (Raghavacharya 1997; Rao et al. 1994).

23.4.1.4 Peat

Peat is considered a good choice for wastewater removal because of its cellular structure. It is highly effective in adsorbing transition metals (along with organic compounds with a polar nature) from textile mill effluent (Poots et al. 1976).

23.4.1.5 Wood Chips

The effectiveness of wood chips is limited to removal of acidic dyes.

23.4.1.6 Fly Ash and Coal Mixture

This method is not so effective, hence it is rarely used. In the mixture of fly ash and coal, if the concentration of fly ash increases, the efficiency of the adsorption increases. This is due to the availability of a large surface area for adsorption (Gupta et al. 1990).

23.4.1.7 Silica Gel

This method is limited to the removal of basic dyes but has good potential.

23.4.1.8 Other Materials

In this method, natural clay, corncobs, rice hulls, etc., are used to remove dye from wastewater. This method has the advantage of being inexpensive, and the compounds used in this technique are readily available (Nawar and Doma 1989).

23.4.1.9 Membrane Filtration

This method is effective for continuous removal of color from dye effluent. It also concentrates the coloring molecules and finally separate them (Mishra and Tripathy 1993).

23.4.1.10 Ion Exchange

This method is not used on a commercial scale, as it has certain limitations and eliminates only those dye molecules that have charges (cations or anions) present on them. As maximum dyes have no charge, they cannot be eradicated by means of an ion exchange method (Slokar and Le Marechal 1997).

23.4.1.11 Irradiation

This method also has limitations, as it can be used only at the laboratory level. It removes only some dyes and organic compounds such as phenols (Hosono et al. 1993).

23.4.2 Chemical Methods

These approaches comprise coagulation or flocculation combined with filtration and flotation. Precipitation–flocculation is done through iron(II)/calcium hydroxide and conventional oxidation approaches by oxidizing agents (ozone), electrokinetic coagulation, irradiation, or electrochemical methods. These technologies are generally costly, and removal of colorants by these methods leaves concentrated sludge, which creates problems in its removal. These techniques also lead to secondary pollution due to the use of excessive chemicals. The big limitations of these methods are their high cost and high consumption of electric energy and chemical reagents. Chemical oxidation is achieved by strong oxidizing agents, leading to ring opening of aromatic dyes, which thus can be easily removed from wastewater (Raghavacharya 1997). Certain chemical methods are discussed in the following subsections.

23.4.2.1 Hydrogen Peroxide Iron(II) Salts (Fenton's Reagent)

H_2O_2_ Fe(II), or Fenton's reagent, is useful to remove dyes from wastewater where biological and physical treatments cannot be used because they show toxicity to living biomass (Slokar and Le Marechal 1997). For the removal of dissolved dyes from effluent, a chemical separation method is used, which involves bonding of dye molecules with chemicals. This method can also be used to remove insoluble dyes (Pak and Chang 1999).

23.4.2.2 Ozonation

Ozone is used as an oxidizing agent. This method is effective for the degradation of compounds of hydrogen and carbon with halogens and hydroxyl groups or without any substitutes (Lin and Lin 1993; Xu and Lebrun 1999). The amount of ozone to be used for the treatment depends upon the percentage of dyes present in the wastewater (Ince and Gönenç 1997). This method does not lead to any pollution (Gahr et al. 1994). Ozonation completely removes colors from effluent and produces less chemical oxygen demand (COD); hence, treated water can be directly discharged into water bodies (Xu and Lebrun 1999).

23.4.2.3 Photochemical Removal

This method uses ultraviolet light (UV) along with H_2O_2 to degrade dye molecules. During the degradation, water and carbon dioxide are formed as nontoxic by-products. In the reaction, the production of hydroxyl free radicals takes place, in turn causing the oxidation of dye molecules (Yang and Wyatt 1998; Peralto-Zamora et al. 1999).

$$\mathrm{H_2O_2} + \mathrm{h}\upsilon \rightarrow 2OH^{\circ}$$

23.4.2.4 Sodium Hypochlorite

This technique involve the breakdown of azo bonds and destruction of amino groups in dye molecules by the action of NaOCl, thereby aiding removal of dyes from effluent (Bannat et al. 1996).

23.4.2.5 Cucurbituril

Cucurbituril is a cyclic compound obtained from glycoluril and formaldehyde (HCHO) (Karcher et al. 1999). It has the ability to form complexes with compounds of azo groups with aromatic chains to remove dye molecules from textile effluent (Mock 1995).

23.4.2.6 Electrokinetic Coagulation

This is the least expensive method for the removal of dyes from textile effluent. It has limitations in that it can be used only for direct dyes. The removal of dyes is done by the use of Fe_2SO_4 and $FeCl_3$ (Gahr et al. 1994).

23.4.2.7 Electrochemical Destruction

This technique is economical and effective for dye decolorization. This approach can be used to degrade a wide range of dyes, even those with organic compounds that are resistant to degradation (Ogutveren and Kaparal 1994; Pelegrini et al. 1999).

23.4.2.8 Physicochemical Treatments

In addition to physical and chemical methods, there are numerous physicochemical approaches that are used for removal of colorants from textile effluent (Robinson et al. 2001). Physicochemical treatments transfer the contaminants present in effluent from one stage to another without eliminating them (Erswell et al. 1988), whereas biological method eliminate a variety of colors by aerobic and anaerobic microbial degradation (Aksu 2005). Advanced oxidation processes lead to the formation of reactive free radicals for oxidation of complex dye molecules present in effluent. This oxidation converts complex toxic contaminants to simpler and nontoxic degraded products. Generation of highly reactive free radicals can be attained by using UV, Fe + 2/H_2O_2, TiO_2/H_2O_2, UV/O_3, UV/H_2O_2, etc. (Kestioglu et al. 2005). However, the use of physical or chemical techniques has certain shortcomings: it is economically unfeasible, as it requires more energy consumption and chemical use; it is incapable of degrading azo dyes and certain chemical compounds completely; and it leads to secondary pollution problems due to the release of large amounts of sludge (Zhang et al. 2004).

23.4.3 Biological Treatments

This method is the best alternative to physical and chemical processes used for bioremediation, as it is cost effective and eco-friendly. Biological degradation includes fungal decolorization, bacterial degradation, and adsorption of dyes by (dead and living) microbiological biomass and detoxification systems. This method can be used to degrade dyes present in textile effluent, as microbes such as yeasts, bacteria, fungi, and algae are able to accumulate in the effluent (Banat et al. 1996; McMullan et al. 2001). However, this treatment has certain limitations due to its technical constraints. Microbes such as fungi (Barr and Aust 1994) and bacteria are difficult to handle, as there is a risk of contamination, though their culturing is simple (Banat et al. 1997). Biological degradation is an economical procedure and is attracting wide attention (Van Der Zee and Villaverde 2005). Biological treatment can involve anaerobic and aerobic degradation of effluent. Anaerobic treatment of textile effluent utilizes numerous anaerobic bacteria, which produce powerful enzymes such as azoreductase. Van Der Zee (2005) have studied combined sequential or integrated anaerobic–aerobic treatment of textile effluent. The use of bacteria in degradation and decolorization of dyes has been studied by Santos et al. (2007). Aksu (2005) has reviewed the use of some algae for decolorization. Dye degradation by fungi has been widely investigated and studied (Brar et al. 2006; Kaushik and Malik 2009).

Among all microorganisms, fungi have been considered the most suitable microbes for removal of dyes from industrial effluent. Fungal mycelium has an advantage over unicellular organisms as it solubilizes insoluble substrates by means of extracellular enzymes. Fungi have a greater cell to surface ratio; hence, they show better physical and enzymatic interactions with the environment. They also have a greater ability to degrade high concentrations of toxins. Biological treatment has limitations, as it needs a huge area and it is affected by day-to-day variations (Bhattacharya et al. 2003). Biological treatment sometimes becomes ineffective for color removal with the present conventional biodegradation processes (Robinson et al. 2001). Furthermore, numerous biological components are degraded and several others are recalcitrant because of their structural complexity (Ravi Kumar et al. 1998). Specifically, azo dyes are not completely degraded by biological methods, because of their xenobiotic nature. Textile effluent is difficult to treat; hence, combinations of biological treatment and physicochemical techniques have been used to decolorize textile effluent (Kannan and Sundaram 2001; Rai et al. 2005; Solmaz et al. 2009; Wojnarovits and Takacs 2008; Ince and Tezcanh 1999; Chaudhari et al. 2011).

23.4.3.1 Roles of Fungi in Bioremediation

Application of fungi has been mentioned in many reports on dye degradation and decolorization using various potential fungal strains.

The most effective and widely used fungi for removal of colorants are ligninolytic fungi (Bumpus 2004). Apart from them, fungi such as *Schizophyllum commune* IBL-062 (Asgher et al. 2013), *Aspergillus allahabadii* (30), *Aspergillus niger* (30), *Aspergillus sulphureus* (Namdhari et al. 2012), and white-rot fungi (e.g., *Pleurotus florida* (Krishnaveni 2011) and *P. eryngii* F032 (Hadibarata et al. 2013)) have been shown to be capable of degrading textile dyes in effluent. With regard to the potential of these dyes, numerous publications have shown that these fungi are able to oxidize nonphenolic, phenolic, soluble, and insoluble dyes (Padmanaban et al. 2013).

Fungi produce enzymes that degrade harmful dyes present in textile effluent or wastewater; for example, white-rot fungi produce enzymes such as lignin peroxidase, manganese peroxidase, and laccase, which degrade various aromatic compounds because of their nonspecificity (Toh et al. 2003) and can be used to degrade synthetic dyes (Reghukumar et al. 1996; Fu et al. 2001). The lignin peroxidase produced by *Phanerochaete chrysosporium* plays a crucial role in the degradation of azo dyes (Ollikka et al. 1993). However, the use of white-rot fungi for dye decolorization of wastewater has many problems, such as production of large amounts of biomass, the structure of the synthetic dyes involved, and management of the biomass (Stolz 2001).

Sathiya Moorthi et al. (2007) reported that two species of white-rot fungi have the capability to detoxify Blue CA, Black B133, and Corazol Violet SR dyes. *P. florida* and *Trametes hirsuta* show great potential in dye degradation. Laccase is the ligninolytic enzyme released by these fungi. Their decolorization activity was tested using several different concentrations of dyes such as 25, 50, and 75 mg/L. The maximum decolorization was seen with Blue CA and Corazol Violet SR dyes.

Wastewater from textile industries has been treated by using *T. hirsuta* and *P. florida* with different concentrations of glucose (1% and 2%) as an additional carbon source. Effective results were exhibited by *P. florida* with 2% of glucose.

Paszczynski et al. (1992) studied the comparative effectiveness of the soil actinomycete culture *Streptomyces chromofuscus* with *P. chrysosporium* and found that bacteria present in the soil were able to decolorize dyes present in effluent but to a lesser degree than white-rot fungi. Some actinomycete strains have shown the ability to degrade Cu-based azo dyes such as formazan (Zhou and Zimmermann 1993). Some other actinomycetes have the ability to degrade reactive dyes such as phthalocyanine, anthraquinone, and azo dyes through adsorption of colorants by the cellular biomass without their degradation.

The application of color removal of azo dyes by means of the white-rot fungus *P. chrysosporium* was first described by Cripps et al. (1990). It was shown that the colors of certain azo dyes such as Tropaeolin O, Orange II, and Congo Red could be removed by *P. chrysosporium*. Some other white-rot fungi such as *Bjerkandera adusta* and *Trametes versicolor* have also shown high efficiency in degrading azo dyes (Heinfling et al. 1997). Several other white-rot fungi such as *P. chrysosporium*, *P. tremellia*, *P. cinnabarinus*, *T. versicolor*, *Ceriporiopsis subvermispora*, *Cyathus stercoreus*, *P. ostreatus*, *Pleurotus oxysporum*, and *Phellinus pini* have also been tested for dye decolorization, and it was concluded that these fungi also degrade synthetic dyes efficiently (Cao 2000).

Vijaykumar et al. (2006) isolated a novel fungus, *Cladosporium cladosporioides*, from coal and assessed its efficiency in decolorization of five different azo and triphenylmethane dyes—Acid Black 210, Acid Blue 193, Crystal Violet, Reactive B \Black B(S), and Reactive Black BL/LPR—on solid media as well as in liquid media.

The effects of different factors such as the inoculum size, temperature, nitrogen source, and carbon source were investigated in degradation of industrial effluent using *P. chrysosporium*. The results showed that the efficiency of these fungi in decolorizing the industrial effluent was up to 97% (Shahvali et al. 2000).

White-rot fungi are considered superior dye decolorizers to prokaryotes. Decolorization efficiency depends on the components present in the medium and on the combination of the dye and microorganisms. Certain physical factors also influence dye decolorization, such as the temperature, pH, dye concentration, and use of agitators. Lignin-modifying enzymes also play an important role in dye decolorization by the action of white-rot fungus (McMullan et al. 2001).

It is possible to degrade Solar Brilliant Red 80 to the extent of 84.8% by using *S. commune* IBL-06 maintained at a pH of 4.5 and a temperature of 30 °C for 7 days (Asgher et al. 2013).

Namdhari et al. (2012) reported that *A. allahabadii*, *A. niger*, and *A. sulphureus* were capable of degrading Reactive Blue MR by up to 95.13% at a temperature of 25 °C maintained for 10 days.

23.4.3.2 Roles of Algae in Bioremediation

Only limited studies on the role of algae in bioremediation have been carried out, though they showed great potential for removing dyes and other complex organic compounds (Semple et al. 1999). Certain species of *Chlorella* and *Oscillatoria* are sufficiently capable of degrading azo dyes into their aromatic amines and also breaking down the aromatic amines into simpler organic compounds. A few algae can even utilize azo dyes as core sources of nitrogen and carbon. The ability of such algae to remove aromatic amines means they can be used to stabilize ponds. Algae have a high surface area to volume ratio; hence, they have significant potential for adsorption (Rice and Sikka 1973; Tikoo et al. 1997).

Experimental results have shown that *Spirogyra* is efficient in degrading azo dye compounds. The decolorization rate depends upon the initial inoculum of the algae, the concentration of the dye, and its type. Algae take time to acclimatize to a new environment; hence, the initial elimination of dye is low but later on increases quickly prior to attainment of saturation (APHA 1995).

Hanan (2012) worked on 11 strains of green algae; his studies revealed that Cyanobacteria and diatoms were efficient for decolorizing a wide range of structurally different dyes. Selected isolates studied by him decolorized dyes either completely or to some extent. Maximum decolorization was achieved with lower concentrations of dye. The maximum degradation rate was observed in the first 3 days, after which the degradation rate until 6 days was slower. The decolorization percentage varied from 100% to only 13% after 6 days of incubation. Omar (2008) reported that variations in structural and functional groups in a monoazo dye (tartrazine) and a diazo dye (Ponceau) affected the decolorization process. The maximum degradation was observed at 5 parts per million (ppm) of tartrazine with *S. bijugatus* (68%) and *Nostoc muscorum* after 6 days of incubation.

23.4.3.3 Roles of Bacteria in Bioremediation

Studies have revealed that bacterial dye decolorization is generally nonspecific and rapid (McMullan et al. 2001). Various bacteria such as *Pseudomonas putida* (Tripathi and Srivastava 2011), *Bacillus subtilis* (Milikli and Ramachandra Rao 2012), *Bacillus* sp. (Abraham et al. 2014), *P. putida* (Wang et al. 2012), *Pseudomonas* spp. (Shah et al. 2013), *Tsukamurella* sp. J8025 (Wen-Tung and Jean 2012), *B. subtilis* SPR42 (Saharan and Ranga 2011), *P. fluorescens* (Saleh Al-Garni and Kubli 2013), *Georgenia* sp. CC-NMPT-T3 (Sahasrabudhe and Pathade 2013), and *Bacillus cereus* (Vidhyakalarani and Premaraj 2013) have the ability to degrade textile dyes. Some of these are aerobic bacteria and some are anaerobic. Bacterial decolorization is effective for anthraquinone and azo dyes, resulting in the manufacture of biogas. Sometimes the rate of decolorization becomes very slow (Bumpus 2004). Complete and effective dye decolorization has certain specific requirements such as specific oxygen-catalyzed enzymes and carbon and nitrogen sources (Hadibarata et al. 2013). Some strains of aerobic bacteria are able to utilize dyes as their basic sources of carbon and nitrogen, and some are capable of reducing azo dyes. Many researchers have reported that aerobic bacteria have the ability to mineralize azo dyes but not completely; they are just degraded into intermediate compounds. Complete degradation occurs only under coupled aerobic–anaerobic degradation (Sudha et al. 2014). In aerobic conditions, azo dyes are converted to their aromatic amines and mineralized by nonspecific enzymes through ring cleavage. Consequently, it has been suggested that for effective decolorization, couple treatment is used, followed by aerobic degradation. Feigel and Knackmuss (1993) and Mubarak Ali et al. (2011) found that appropriate growth of bacterial species took place in aerobic or agitation conditions, but the rate of decolorization was maximized in anaerobic culture.

23.4.3.4 Dye Decolorization by Microbial Consortia and Biofilms

Industrial effluent carries a diverse range of azo dyes. No single bacterial strain is capable enough to degrade aromatic amines. The difficulty of maintaining purity is the shortcoming that limits the application of pure strains in degradation of textile dyes. Pure cultures are considered less effective in dye decolorization and degradation; hence, use of a bacterial consortium may provide a good solution to this problem. Several studies have revealed that a consortium of microbes has an extra effect in removing colors from effluent as compared with pure cultures (Khouni et al. 2012; Aruna et al. 2015; Ndasi et al. 2011). In a consortium, higher efficiency of degradation is achieved because different bacteria attack different sites on dye particles and the by-products obtained after degradation can be used by other microbes as an energy source (Chang et al. 2004; Saratale et al. 2009).

23.4.3.5 Dye Decolorization by Molds

Filamentous fungi or multicellular molds have the capability to clean the environment. Their diversity of hyphal structures, large cell to surface ratios, presence of extracellular or intracellular enzymes, mixtures of secondary metabolites, and different modes of reproduction provide good scope for decolorization. Many researchers have studied the degradation efficiency of molds in decolorization of lignin-containing effluent from the paper and pulp industry. Two white-rot fungi (*P. chrysosporium* and *Tinctosporia* sp.) were studied and reported in 1980 (Eaton et al. 1980; Fukuzumi 1980); both organisms were capable of degrading polymeric lignin molecules. The mechanism behind the degradation of dyes includes enzymes such as lignin peroxidase and Mn-dependent peroxidase or laccase enzymes (Michel Jr et al. 1991). In all of the examples it was observed that limitation of nitrogen increased the ligninolytic activities of Mn-dependent peroxidase and lignin peroxidase, enabling efficient removal of color from wastewater (Perie and Gold 1991). However, Chao and Lee (1994) found that the rate of decolorization was higher when microbes were pregrown in a nitrogen-rich medium. Degradation of xenobiotic compounds was achieved in nonligninolytic conditions by laccase enzyme activity (Dhawale et al. 1992).

23.4.3.6 Roles of Yeasts in Dye Decolorization

Fewer studies have been done on removal of azo dyes by yeasts, as opposed to bacterial and mold species. In comparison with fungi and bacteria, molds have many advantages. Unlike bacteria, they do not have a rapid growth rate, but like filamentous fungi, they are able to grow in unfavorable conditions (Zhisheng and Xianghua 2005). Some yeasts have the capability to treat organic effluent such as wastewater and food industry effluent (Yang et al. 2003). Marco et al. (2005) isolated a phenolic acid–assimilating yeast, *Candida oleophila*, which could completely degrade up to 200 mg of Reactive Black 5 within 24 h of aerobic incubation. Zhisheng and Xianghua (2005) isolated two other species, *Pseudozyma rugulosa* and *Candida krusei*, which had the capability to decolorize the azo dye Reactive Brilliant Red K-2BP.

Certain yeast species are also able to degrade anthraquinone dyes and other dyes. Studies by Yang et al. (2003) revealed that the manganese-dependent peroxidase (MnP) activity of the yeasts *Debaryomyces polymorphus* and *Candida tropicalis* enabled them to efficiently remove Reactive Black B and other azo dyes such as anthraquinone dye within 48 h. Martorell et al. (2012) reported that removal of dyes by yeasts had additional benefits in comparison with the use of fungi because of their fast growth and survival in harsh conditions. Reports have shown that for many yeasts, dye decolorization depends upon enzymatic action on the chromophore structure (Ramalho et al. 2004).

23.5 Mechanisms Behind Bioremediation of Textile Dyes

23.5.1 Biosorption

The accumulation of chemical substances present in dye molecules in microorganisms is known as biosorption. Biosorption can occur in both living and dead microbes. Dead microbes have the capability to adsorb compounds that contain the natural polysaccharide chitin or its derivative chitosan, which is present in the cell walls of fungi and shows great affinity for numerous groups of dyes because of its unique molecular structure (Joshi et al. 2004).

23.5.2 Biodegradation

The term "biodegradation" refers to a process in which complex organic compounds are decomposed into simpler substances by living microorganisms. This is a natural process performed by microbes. Biodegradation can be achieved by the action of enzymes secreted by microbes. Studies by many researchers have revealed that most white-rot fungi produce nonspecific enzymes such as lignin peroxidase (LiP), manganese peroxidase (MnP), and laccase. These enzymes enable generation of free radicals during the biodegradation process (Pointing 2001; Knapp et al. 2001). The fungus *Flavodon flavus* releases laccase, which has the ability to decolorize synthetic dyes such as Azure B and Brilliant Blue R in scarcity of water (Soares et al. 2002).

Enayatizamir et al. (2011) reported that Azo Black Reactive 5 dye was degraded by up to 92% by *P. chrysosporium* within 3 days. Similarly, *P. chrysosporium*

URM6181 and *Curvularia lunata* URM6179 strains were shown to degrade indigo dye present in textile effluent by up to 95% within 10 days. Laccase obtained from the fungus *T. hirsuta* has the ability to degrade triarylmethane, indigo, azo, and anthraquinonic dyes present in industrial effluent (Annibale et al. 2000).

Sathiya Moorthi et al. (2007) reported that extracellular laccase was produced by *T. hirsuta* and *P. florida*. The oxidase activity of these microbes decolorized Blue CA, Corazol Violet SR and, to a lesser extent, Black B133. The degree of color elimination is not consistent for all dyes. The decolorization depends upon the amount of laccase produced, the type of media used, and the class of dye.

23.5.3 Mineralization

The action of fungi on mineralization or decolorization of textile dyes depends upon the chemical structure of the dyes. The rate of decolorization is higher in dyes with chains of aromatic rings than in those with unbranched rings. Better results have been obtained in media with a low nitrogen concentration. Studies have shown that that the dyes are used as carbon sources in certain cases, through cleavage of the bonds of the dye molecule, leaving the chromophore group unaffected. Mineralization occurs mainly in consortia, not in pure cultures (Knapp et al. 2001; Singh 2006).

23.5.4 Bioaccumulation

Many studies have been done on the use of living cells in bioaccumulation processes. Bioaccumulation of dyes can be done efficiently by growing cells with sufficient carbon and nitrogen sources. Use of living biomass for bioaccumulation has an advantage over use of dead biomass in that cell growth and bioaccumulation occur simultaneously, so there is no need to allow separate times for growth and bioaccumulation. However, this method has major limitations in that the microbes need nutrients for their growth and high dye concentrations can affect their growth rate (Aksu and Donmez 2005).

23.6 Factors Affecting Biodegradation of Dyes

There are several factors that affect the growth of microbes, such as certain harmful gases, pH, the presence of oxygen, temperature, metals, salts, and chemical compounds. Optimization of these abiotic factors is helpful in the development of industrial-scale bioreactors for bioremediation. Other than these abiotic factors, several factors such as the type of dye, the composition of the effluent, operating costs, environmental thrift, and handling of the produced sludge determine the technical and economic practicability of the methods used for treatment.

23.6.1 pH

Decolorization and biodegradation by fungi generate better results at either an acidic or neutral pH, whereas bacteria show optimal activity at either a neutral or basic pH. All microorganisms degrade textile effluent at their specific pH. Commonly, the optimal pH for decolorization of microbes is pH 6–10 (Ramachandran et al. 2013). At a neutral or slight basic/acidic pH the decolorization rate is high, but a strong acidic/basic pH decreases the percentage of decolorization. Nearly all fungal dye decolorization studies have found that the best results are obtained at an acidic pH. With a change in the pH range to 7–9.5, no variation or just a slight variation is observed in the decolorization rate (Ramachandran et al. 2013).

23.6.2 Temperature

Temperature also acts as an imperative factor that affects the dye decolorization activity of microbes. The rate of biodegradation increases with rises in the initial temperature (Ramachandran et al. 2013). The optimal temperatures for dye decolorization have been found to be 35–45 °C in the case of bacteria and 23–35 °C in the case of fungus. The degradation efficiency of microbes can be decreased by slow growth, their reproduction rate, secretion of enzymes, etc. (Adinew 2012).

23.6.3 Initial Dye Concentration

The dye concentration has a strong impact on the extent of dye decolorization and also influences the toxicity of dye molecules. In the case of bacteria, the decolorization rate increases with time irrespective of the initial dye concentration. An increase in the dye concentration has an adverse effect on the growth of bacteria; hence, the decolorization rate decreases. Growth of fungi is also affected by increased dye concentrations. Kapdan and Kargi (2002) reported that a 1200 mg/L dye concentration of Everzol Turquoise Blue G, a phthalocyanine-type reactive dyestuff, was degraded by *Coriolus versicolor*. The dye removal affinity was increased if the dye concentration was lower: a concentration of 100–250 mg/L was degraded within 3–5 days, and a concentration of 700–1200 mg/L required up to 9 days of incubation. Other researchers have also shown that decolorization of dye decreases with increasing dye concentrations (Parshetti et al. 2006).

23.6.4 Effects of Azo Dye Structure

Biodegradation of azo dyes by the action of enzymes is greatly influenced by the dye structure (Sawhney and Kumar 2011). Azo dyes comprising electron-withdrawing groups are more easily degraded than those consisting of electron-releasing groups (e.g., –NH–triazine). Therefore, it has been concluded that dyes with additional electron-withdrawing groups perform better decolorization.

23.6.5 Effects of Nutrients (Carbon and Nitrogen Sources)

Sudhakar et al. (2002) reported that nutrients play significant roles in dye removal processes; larger amounts of nutrients significantly increase the growth of microorganisms and hence improve the rate of degradation. *Pseudomonas* sp. BSP-4 isolated from azo dye–polluted soil has the ability to decolorize and degrade the azo dye Black E by consuming it as a nitrogen source at up to 300 ppm within 36 h. The nutrient constituents of the culture medium play an important role in color removal. Natural supplements have been shown to have a positive impact on dye decolorization by the fungus *Aspergillus fumigatus* XC6; supplementation with high carbon and nitrogen sources, predominantly in the form of salts such as ammonium sulfate, had a significant effect on effluent degradation (Jinqi and Houtian 1992).

23.6.6 Effects of Biological Structures Involved in Decolorization of Azo Dyes

Various microbial systems have been tested for decolorization and degradation of azo dyes, including algae, yeasts, molds, filamentous fungi, actinomycetes, and bacteria. All microbes have their own mechanisms and act differently in dye decolorization. The mechanisms involved are biosorption, enzymatic degradation, bioaccumulation, etc.

23.6.7 Effects of Electron Donors

Studies have revealed that addition of an electron donor (e.g., glucose or acetate ions) leads to the breakdown of azo bonds. The cleavage depends upon the type and availability of the electron donor, which plays an important role in attaining optimal dye decolorization in fermenters working under anaerobic situations (Razia Khan et al. 2013).

23.6.8 Effects of Redox Mediators

Razia Khan et al. (2013) have reported that redox mediators (RMs) improve the degradation processes of azo dyes under anaerobic conditions.

23.6.9 Effects of Agitation

Rohilla et al. (2012) reported that agitation favored the decolorization of certain dyes such as Orange M2R, with a maximum decolorization rate of 89.3% being achieved by a fungal consortium, whereas *Aspergillus flavus* and *A. niger* degraded dye concentrations by up to 60–70% in stationary conditions within 10 days. It was observed that shaking of the medium suppressed the functioning of ligninolytic enzymes in *P. chrysosporium* (Swamy and Ramsay 1999). Usually, better results were achieved under shaking conditions than under stationary conditions because of increases in mass and the oxygen transfer rate between cells and the medium. Jarosz Wilkołazka et al. (2002) concluded that shaking had a positive effect on dye decolorization. Shaking of cultures of various strains such as *Bjerkandera fumosa*, *Stropharia rugosoannulata*, and *Kuehneromyces mutabilis* promoted greater dye removal than that achieved in stationary cultures (Kirby et al. 2000). Yet, certain microbes such as *Phlebia tremellosa* gave better results in stationary conditions (Rohilla et al. 2012).

23.6.10 Effects of Aeration

Aeration means supply of oxygen to microbes growing in culture media. This is an important factor for the growth of microorganisms. The limitation of aeration is that oxygen has low water solubility (8 mg/L at 20 °C). To overcome this problem and make oxygen more soluble in water, aeration and agitation are required. This may affect the morphology of fungi and may lead to a decrease in the production of enzymes (Žnidaršič and Pavko 2001). Nowadays, bioreactors are designed to have static and agitated configurations to provide plenty of oxygen. Selection of the bioreactor depends on the specific system; however, proper agitation provides better results than static conditions (Knapp et al. 2001).

23.7 Other Technical Factors Affecting Bioremediation

There are several other factors that affect the biodegradation of textile dyes. These factors are categorized as environmental, microbial, substrate, mass transfer limitation, aerobic and aerobic processes, etc., as listed in Fig. 23.1.

Fig. 23.1 Factors affecting bioremediation

23.8 Environmental Concerns

Environmental issues related to the residual dye content of textile effluent from fabric industries are considered a big challenge. Direct discharge of effluent directly into water bodies contaminates them (Zaharia et al. 2011). It is not known exactly what amounts of dyes are produced worldwide, nor what amounts are discharged into the environment (Forgacs et al. 2004). As dyes are used on a large scale, their discharge into the environment remains high, which in turn leads to serious health risk concerns. The growing impact of environmental protection on industrial development is resulting in the introduction of new eco-friendly technologies (Forgacs et al. 2004).

Robinson et al. (2001) reported that most dye effluent was not completely degraded by conventional wastewater treatment, because of its high stability to light, temperature, water, chemicals, soap, detergent, and certain additional parameters. Water is essential for life and is required in various household activities. Water is also used as a chief component in all industries. It is the most essential natural resource, but unfortunately it is the most polluted for reasons such as increased population, urbanization, lavish lifestyles, and industrialization.

23.8.1 Harmful Effects to Living Bodies

The deadly effects of textile dyes may be due to the presence of azo dyes or aryl amine derivatives produced during breakdown of azo bonds (Rajaguru et al. 1999). These dyes enter organisms by consumption and are converted into aromatic amines by the enzymes present in the intestinal tract. Nitro dyes are metabolized by nitroreductases produced by the same microorganisms (Umbuzeiro et al. 2005). Mammalian liver enzymes catalyze the reductive breakdown of azo bonds and nitroreduction of nitro groups. In both cases, if N-hydroxyl amines are produced, they can cause destruction of DNA (Umbuzeiro et al. 2005; Arlt et al. 2002). Moreover, the effects produced by other contaminants greatly disturb the quality and transparency of water bodies such as ponds, rivers, and lakes, damaging aquatic life (Ibrahim et al. 1996; Wijetunga et al. 2010).

As noted by Hassani et al. (2008), textile effluent consists of a blend of contaminating substances comprising dyes and organic substances. Ninety-three percent of water intake comes out contaminated by dyes containing large amounts of organic complexes and heavy metals (Wijannarong et al. 2013; Gupta et al. 2014b). The nonbiodegradable nature of some dyes and organic compounds leads to serious ecological hazards. Colored wastewater is unhealthy for aquatic bodies, reducing the oxygenation ability of the water and disturbing the life of the aquatic ecosystem and the food chain (Xu et al. 2005).

23.8.2 Effects on Humans

Research in 1992 found that occupational exposure to some azo dyes with aromatic amines—principally benzidine, 2-naphthylamine, and 4-aminobiphenyl—increased the risk of bladder cancer (Chequer et al. 2011;Puvaneswari et al. 2006). These dyes are toxic and also contain chemical compounds that are carcinogenic, mutagenic, or teratogenic to humans (Novotný et al. 2006; Mathur and Bhatnagar 2007). Carcinogenicity caused by the presence of azo dyes in textile effluent is well known (Weisburger 2002;Umbuzeiro et al. 2005). Studies done by Srivastava et al. (2004) have shown that Malachite Green not only destroys the human immune system and reproductive systems but also acts as a probable genotoxic and cancer-causing agent. The dye CI Disperse Blue leads to frameshift mutations and base pair substitutions in *Salmonella* (Umbuzeiro et al. 2005). The genotoxic and cytotoxic effects of CI Disperse Blue on human cells was studied by Tsuboy et al. (2007).

23.8.3 Effects on Water Bodies

Chen (2006) has reported that nitro and azo dyes are degraded into harmful carcinogenic amines and deposited in water bodies. These derivatives lead to water pollution and affect aquatic life.

Table 23.2 shows the various advantages and disadvantages of different conventional and bioremediation processes.

Table 23.2 Advantages and disadvantages of conventional and bioremediation processes

Methods	Advantages	Disadvantages
Fenton's reagent	High-grade removal of both soluble and insoluble dyes	Huge sludge production
Ozonation	Applied in a gaseous state: no alteration of volume	Short half-life (20 min)
Photochemical	No sludge production	Generation of toxic chemical compounds as by-products (e.g., NaOCl)
Cucurbituril	Good sorption potential for a large variety of dyes	Economically unfavorable
Electrochemical destruction	Decomposition products are nontoxic	High cost of electricity
Activated carbon	High-grade potential to adsorb a wide variety of dyes	Very expensive
Peat	Efficient absorbent	Less effective than activated carbon
Wood chip/ wood sawdust	Good sorption capacity for acid dyes; effective adsorbent because of cellular structure; good adsorption capacity for acid dyes	Requires long retention times
Silica gel	Applied successfully for basic dye removal	Less suitable on a commercial scale because of side reactions
Membrane filtration	Good potential to remove all dye types	Concentrated sludge generation
Ion exchange	Regeneration: no adsorbent loss	Less effective
Irradiation	Effective oxidation at the lab scale	Requires a lot of dissolved O_2
Electrokinetic coagulation	Good economic feasibility	Sludge production
Sonication	Simple to use; very effective in integrated systems	Relatively new method, awaiting full-scale application
Enzymatic treatment	Effective for specifically selected compounds	Enzyme isolation and purification is tedious
Photocatalysis	Process carried out in ambient conditions; inputs are nontoxic and inexpensive; complete mineralization with shorter detention times	Effective for only small amounts of colored compounds; expensive process
Single cell (fungal, algal, and bacterial)	Good removal efficiency for low volumes and concentrations; very effective for specific color removal	Culture maintenance is cost intensive; cannot cope with large volumes of wastewater

Data sources: Anjaneyulu et al. (2005), Babu et al. (2007), Robinson et al. (2001), and Joshni and Subramaniam (2011)

23.9 Conclusions

Various physical and chemical approaches have been used for degrading dye effluent. These approaches commonly have many limitations, such as high costs, low efficiency, and inadequate resources; they also release secondary pollution (sludge, etc.). On the other hand, bioremediation is an economical, efficient, eco-friendly, and biologically benign technique for textile dye and wastewater treatment. Microbial degradation of textile effluent includes use of bacteria, fungi, yeasts, algae, and plants. Studies have shown that diverse microbes are proficient in detoxifying various dyes. Use of microbes for the elimination of synthetic dyes from textile effluent is an alternative to make remediation processes economically viable. Thus, bioremediation is considered the method best suited to treatment of textile effluent.

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