Creating Products and Services in Environmental Biotechnology

4

Calina Petruta Cornea, Catalina Voaides, Oana Alina Boiu Sicuia, Florentina Matei, and Narcisa Babeanu

Abstract

In the process of solving environmental protection problems biotechnology plays an essential role in providing alternative solutions to reducing pollution. The chapter approaches as a green alternative the phytoremediation of polluted environments, complete with microbial and vermiremediation as a clean-up alternative. Special attention is given to natural plant protection products, known as "biopesticides." Another aspect approached is the finding and development of new plants as a biomass source for energy production, which are objectives for start-ups, and have great business potential.

Keywords

Environmental biotechnology · Phytoremediation · Vermiremediation · Biopesticides · Energy crops · Start-up

4.1 Introduction

It is well known that the world is now experiencing the consequences of the overexploitation of natural resources by man and of technological development. The major concerns are related to the loss of biodiversity, to the extinction of many species with an impact on the good functionality of ecosystems, to the deterioration of the soil, water, and air quality, which have major economic implications and significant repercussions for the well-being of human populations (Leitão 2016). For example, contamination of ecosystems by xenobiotic compounds (organic petroleum hydrocarbons, agrochemicals such as pesticides, herbicides or other compounds, pharmaceutical products, heavy metals) causes serious environmental problems. Various measures have been proposed and, sometimes, adopted by governments, for preventing environmental degradation or for the reduction and cleanup of pollution produced by industrial, agricultural, and household waste and accidental spills, but the results are partially satisfactory. For example, in China, where the economy develops continuously and quickly, urbanization and industrialization are promoted, leading to serious environmental problems, a Bioindustry Development Plan was adopted that proposed that "priorities should be given to treating contamination of water, the atmosphere and organic substances, to treat and repair the impaired ecological system, vigorously develop biologically environmental protection materials and biological products with high performance, accelerate demonstration of the whole set of technological processes and equipment for efficient biological supervision, treatment, repair and waste

All authors have made equal contributions.

C. P. Cornea (✉) · C. Voaides · O. A. Boiu Sicuia · F. Matei · N. Babeanu
Faculty of Biotechnologies, University of Agronomic Sciences and Veterinary Medicine of Bucharest, Bucharest, Romania

© Springer Nature Switzerland AG 2019

F. Matei, D. Zirra (eds.), *Introduction to Biotech Entrepreneurship: From Idea to Business*,
https://doi.org/10.1007/978-3-030-22141-6_4

utilization, and expansion of industrial scale" (Wang et al. 2018). Attempts at remediating contaminated sites have usually used conventional but often costly approaches, such as "pump and treat," excavation and removal, soil vapor extraction, and other chemical treatments, but these methods are time-consuming, invasive, disruptive to natural ecosystems, and not always effective (Elekwachi et al. 2014).

Moreover, the most recent strategies in bioeconomy consider that, in addition to the terrestrial ecosystems, the marine environment is rapidly being polluted by human activities, and environmental biotechnology may provide important knowledge and tools that will help to protect the resource base upon which marine-related economic and social activities depend (Kalogerakis et al. 2015).

In a study performed by Gillespie in 2013, it was estimated by the European Environmental Agency (EEA) that in Europe there are over three million sites where potentially polluting activities have occurred, and more than 8% of them need to be remediated as they are highly contaminated with various pollutants. Moreover, it was estimated that the total number of contaminated sites could be increased by more than 50% by 2025 (Gillespie and Philp 2013).

For these reasons, in the process of solving these problems, environment protection biotechnology plays an essential role in providing alternative solutions to reducing pollution (Khan 2016). In this context, bioremediation has proven to be a safe, effective, low-cost, and environmentally friendly alternative for the sustainable remediation of environments contaminated by various pollutants. Bioremediation uses biological processes and naturally occurring catabolic activity realized by microorganisms (bacteria and fungi), green plants, or some animal organisms to eliminate, attenuate or transform contaminants into less hazardous products, such as carbon dioxide, water, inorganic salts, and biomass (Elekwachi et al. 2014).

Naturally occurring bioremediation and phytoremediation have been used empirically for many years. Processes such as desalinization of agricultural land by phytoextraction are applied in different world regions. More recently, the bioremediation technologies using microorganisms were used for treating the contaminated areas at the site (in situ) or after the removal of contaminated materials and their treatment elsewhere (ex situ) (Fig. 4.1).

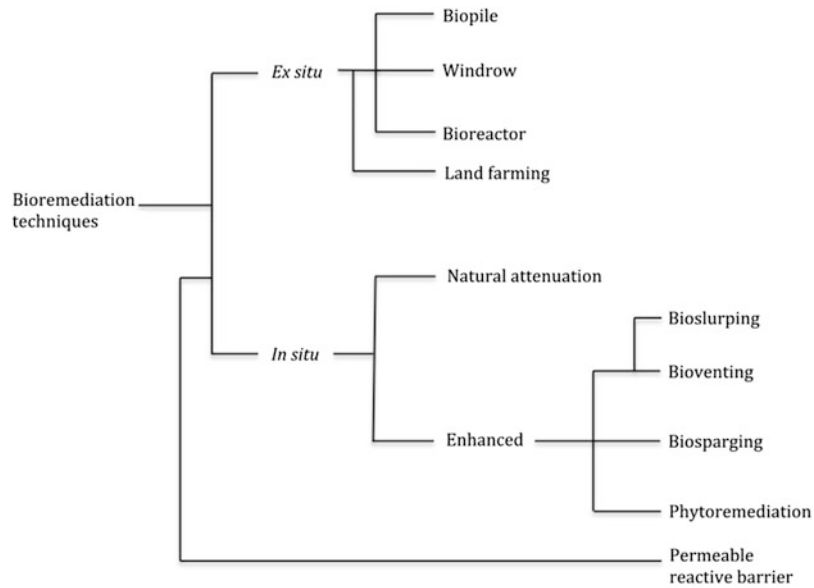

Fig. 4.1 Bioremediation techniques (after Azubuike et al. 2016)

Among the well-developed bioremediation technologies, the following could be mentioned:

- Bioventing is an in situ remediation technology that uses microorganisms to biodegrade organic constituents adsorbed on soils in the unsaturated zone. The technology enhances the activity of soil indigenous bacteria by introducing air/oxygen flow into the soil and, sometimes, by supplemental limited amounts of nutrients, resulting in the stimulation of the microbial biodegradation (http://www.cpeo.org/techtree/ttdescript/bioven.htm).
- Composting is a process that works to speed up the natural decay of organic material by providing the ideal conditions for detritus-eating microorganisms or other soil organisms to develop and act, the end-product of this concentrated decomposition process being nutrient-rich soil) (Ross 2018).
- Bioaugmentation is a process that involves the introduction into a polluted soil of microbial consortia selected from natural ecosystems or developed through successive adaptations under laboratory conditions, to enhance the degradation of toxic compounds (for example, oil spills) (Brown and Ulrich 2014).
- Biostimulation involves the addition in contaminated areas of limited amounts of nutrients to stimulate the growth of indigenous microorganisms and augment their catabolic activity for eliminating polluted compounds, mainly hydrocarbons (Sarkar et al. 2016).
- Phytoremediation is used to solve environmental problems caused by toxic elements by plant activities (Grison et al. 2015).
- Rhizofiltration is the adsorption onto or into plant roots (both terrestrial and aquatic) of various contaminants (heavy metals, radionuclides, etc.) from polluted aqueous sources (effluents discharged from industries and agricultural run-off, acid mine drainage, etc.) that surround the rhizosphere. Rhizofiltration decontaminates polluted water using plants grown in greenhouses in water from the sites instead of soil, acclimatizing the plants to the environment. The plants are then planted on the site of contaminated groundwater where the roots take up the water and contaminants; at the end of the process, when the roots are saturated with the contaminant, the entire plants are harvested (Abdullahi 2015).
- Landfarming is an ex situ waste treatment process that is performed in the upper soil zone or in biotreatment cells, using contaminated soils, sediments or sludges that are transported to the landfarming site, incorporated into the soil surface, and, periodically, tilled to aerate the mixture. The aim of this procedure is the prevention of groundwater pollution by heavy metal, pesticides or other toxic compounds that could contaminate the upper soil layer (https://www.epa.nsw.gov.au/), etc.

In a global survey performed in 2014 by Elekwachi et al. the application of bioremediation technologies was examined in various regions and countries all over the world. It was shown that the use of low-cost in situ bioremediation technologies (such as monitored natural attenuation) (Table 4.1) are prominent in the developed economies (North America and Europe), whereas more expensive technologies, sometimes ex situ, are used in the developing regions.

The development of industries all over the world, the increased waste production, the increased use of pesticides (rodenticides, fungicides, algicides, acaricides or herbicides) for higher agricultural production, mining, petroleum extraction and its transport, are some of the "actors" involved in the increase in contamination by heavy metals, hydrocarbons, and other pollutants of agricultural land and freshwater sources. The consequences of such contamination lead to the erosion of soils, or even the phytotoxicity of soil systems, the migration of pollutants into soil–water systems, and the pollution of rivers, and could reduce the fertility of soils and contaminate agricultural and food products. The polluted resources are used by humans for food production and ultimately accumulate in the food chain (Yadav et al. 2018).

Table 4.1 Bioremediation technologies (adapted from Elekwachi et al. 2014)

Bioremediation technologies	
In situ	Ex situ
Monitored natural attenuation	Bioreactor technique
Bio-stimulation methods: Addition of fertilizers/nutrients; bioventing and air sparging; groundwater treatment and recirculation	Composting
Bio-augmentation methods: Enrichment cultures from the site; pure cultures specifically for the contaminant; commercial cultures/consortia; phytoremediation	Landfarming
	Biopile

4.2 Phytoremediation of Polluted Environments: A Green Alternative

Over time, phytoremediation process has had many definitions. In the first place, the term "phytoremediation" comes from associating two other terms—the Greek prefix *phyto* which means plant, and the Latin suffix *remedium*, meaning restoring balance or to correct or to remove something bad. Phytoremediation represents a group of technologies that use natural or genetically modified abilities of (superior) plants to clean up contaminated sites (Adriano et al. 2004; Pulford and Watson 2003; Robinson et al. 2009), cleaning-up being understood to mean the capacity to remove, degrade, detoxify or transform the contaminant from polluted environments—soil, sediments, groundwater, surface water, and/or atmosphere (Ying 2002). Likewise, phytoremediation has been defined as the employment of science and engineering to study problems and provide solutions involving plants and contaminated environments (Conesa et al. 2012).

This technology has been used to remove heavy metals, such as Hg, Cr, Cd, Cu, Ni, Zn, Pb, As, Mo, Se, Pd (Bolan et al. 2011; Shoji et al. 2008; Ayotamuno et al. 2009; Sampanpanish et al. 2006; Andreazza et al. 2013; January et al. 2008; Meeinkuirta et al. 2016), organic contaminants (alkylated polycyclic aromatic hydrocarbons, fungicides, pesticides, polychlorinated biphenyls) (White et al. 2005; Yavari et al. 2015), crude oil (Ayotamuno et al. 2009), some radioactive isotopes such as Cs, U (Schwitzguébel et al. 2002; Yavari et al. 2015).

Phytoremediation strategies utilize trees, shrubs, crop plants, aquatic macrophytes, and/or grasses from different species for treating contaminated air, soil or water. Some of these "green tools" are presented in Table 4.2.

The option to clean the contaminated environment with plants became more attractive to environmental scientists, as an alternative to the classic methods. These traditional technologies—excavation, chemical soil treatment, thermal treatment—proved to be expensive and destructive to the environment (Wenzel et al. 2004).

To use the most efficient plant for a given pollutant, sound studies are required, owing to the different potential of the plant species in different environments to remediate the problem (Andreazza et al. 2013). Consequently, because the implied factors differ from case to case, a unique phytoremediation scheme is difficult to apply. Each phytoremediation project has to be designed for a specific event, requiring certain approaches (Boroş et al. 2016).

Phytoremediation (Fig. 4.2) is based on different *phytotechnologies*, such as: phytovolatilization, phytoextraction/phytoaccumulation, phytodegradation/phytotransformation, phytofiltration, phytoimmobilization or phytostabilization.

Phytoextraction or *phytoaccumulation* represents the process in which the plant uptake translocates and accumulates the contaminants in harvesting plant parts, which can then be used or disposed of (Trapp and Karlson 2001; Rahman and Hasegawa 2011; Conesa et al. 2012). The aim of this process is to remove the polluted element from the site. The following techniques can be included in this category:

Table 4.2 Examples of plant species used in phytoremediation processes

Plant species	Process	References	Contaminant
Brassica juncea	Phytovolatilization and phytoextraction	Moreno et al. (2005), Ko et al. (2008), de Souza et al. (2000) cited by Bolan et al. (2011)	Heavy metals
	Reduction	Bolan et al. (2003) cited by Bolan et al. (2011)	
	Phytoimmobilization	Bolan et al. (2003) cited by Bolan et al. (2011)	
	Phosphate-induced desorption followed by plant uptake	Neunhauserer et al. (2001) cited by Bolan et al. (2011)	
	Chelation followed by uptake	Quartacci et al. (2006), Duqučne et al. (2009) cited by Bolan et al. (2011)	
	Accumulation	Salt et al. (1994), Kumar et al. (1995) cited by Sampanpanish et al. (2006)	
Brassica napus L.	Chelation followed by uptake	Zeremski-Škoric et al. (2010) cited by Bolan et al. (2011)	Heavy metals
	Phytoextraction	Marchiol et al. (2004) cited by Bolan et al. (2011)	
	Phytoremediation	Shams et al. (2009)	
	Accumulation	Kumar et al. (1995) cited by Sampanpanish et al. (2006)	
	Phytoremediation	Schwitzguébel et al. (2002)	^{137}Cs
Medicago sativa	Chelation followed by uptake	López et al. (2005) cited by Bolan et al. (2011)	Heavy metals
	Phytovolatilization	Duckart et al. (1992) cited by Bolan et al. (2011)	
	Phytoremediation	Xu et al. (2010) cited by Yavari et al. (2015)	Polychlorinated biphenyls
Pome fruit trees	Phosphate-induced desorption followed by plant uptake	Peryea (1991) cited by Bolan et al. (2011)	Heavy metals
Echinochloa crus-galli	Root exudates-enhanced phytoextraction	Kim et al. (2010) cited by Bolan et al. (2011)	Heavy metals
Raphanus sativus	Phytoextraction	Marchiol et al. (2004) cited by Bolan et al. (2011)	Heavy metals
Sedum alfredii	Chelation followed by uptake	Liu et al. (2008) cited by Bolan et al. (2011)	Heavy metals
Brassica rapa	Chelation followed by uptake	Meers et al. (2005) cited by Bolan et al. (2011)	Heavy metals
Cannabis sativa	Chelation followed by uptake	Meers et al. (2005) cited by Bolan et al. (2011)	Heavy metals

(continued)

Table 4.2 (continued)

Plant species	Process	References	Contaminant
Helianthus annuus	Chelation followed by uptake	Meers et al. (2005) cited by Bolan et al. (2011)	Heavy metals
	Accumulation	Zavoda et al. (2001) cited by Sampanpanish et al. (2006)	
	Hyperaccumulation	January et al. (2008)	Heavy metals
	Phytoremediation	Lotfy and Mostafa (2013) cited by Yavari et al. (2015)	Co
Zea mays L.	Improved metal uptake by plant growth regulators and EDTA	Hadi et al. (2010) cited by Bolan et al. (2011)	Heavy metals
	Phytoextraction	Murakami and Ae (2009) cited by Bolan et al. (2011)	Heavy metals
	Phytoremediation	Shams et al. (2009)	
	Phytoremediation	Ibrahim et al. (2013) cited by Yavari et al. (2015)	Atrazine (pesticide)
Oryza sativa L.	Phytoextraction	Murakami and Ae (2009) cited by Bolan et al. (2011)	Heavy metals
Glycine max [L.] Merr.	Phytoextraction	Murakami and Ae (2009) cited by Bolan et al. (2011)	Heavy metals
Solanum nigrum L.	Improved plant growth and Cd uptake by fungi and citric acid	Gao et al. (2010) cited by Bolan et al. (2011)	Heavy metals
Lolium perenne	Chelation followed by uptake	Duquc̆ne et al. (2009) cited by Bolan et al. (2011)	Heavy metals
Transgenic tobacco	Phytovolatilization	He et al. (2001) cited by Bolan et al. (2011)	Heavy metals
Lycopersicon esculentum	Phytovolatilization	Duckart et al. (1992) cited by Bolan et al. (2011)	Heavy metals
Festuca arundinacea	Phytovolatilization	Duckart et al. (1992) cited by Bolan et al. (2011)	Heavy metals
	Phytoremediation	Huang et al. (2005)	
Pteris vittata	Phytoremediation	Shoji et al. (2008)	Reduce As(V) to As(III)
Urtica dioica L.	Phytoremediation	Shams et al. (2009)	
Vetiveria zizanioides	Phytoremediation	Xia 2004 cited by Ayotamuno et al. (2009)	Heavy metals
Pennisetum purpureum	Phytoremediation		Crude oil
Amaranthus viridis	Phytoremediation	Sampanpanish et al. (2006)	Cr
Brachiaria decumbens	Phytoextraction and phytostabilization	Andreazza et al. (2013)	Cu
Kochia scoparia	Phytoremediation	Schwitzguébel et al. (2002)	^{137}Cs
Juniperus monosperma	Phytoremediation	Ramaswami (2001) cited by Schwitzguébel et al. (2002)	U
Eucalyptus camaldulensis	Phytostabilization	Meeinkuirta et al. (2016)	Cd
Lolium arundinaceum	Phytoremediation	White et al. (2005)	Alkylated polycyclic aromatic hydrocarbons
Cynodon dactylon	phytoremediation	White et al. (2005)	Alkylated polycyclic aromatic hydrocarbons

(continued)

Table 4.2 (continued)

Plant species	Process	References	Contaminant
Callitriche lusitanica	Phytoremediation	Favas et al. (2012) cited by Yavari et al. (2015)	As
Iris pseudacorus	Phytoremediation	Li et al. (2014) cited by Yavari et al. (2015)	Pesticide
Lemna minor and Spirodela polyrhiza	Phytoremediation	Dosnon-Olette et al. (2009) cited by Yavari et al. (2015)	Fungicides
Tagetes patula	Phytoremediation	Patil and Jadhav (2013) cited by Yavari et al. (2015)	Textile dye Reactive Blue 160

EDTA ethylenediaminetetraacetic acid

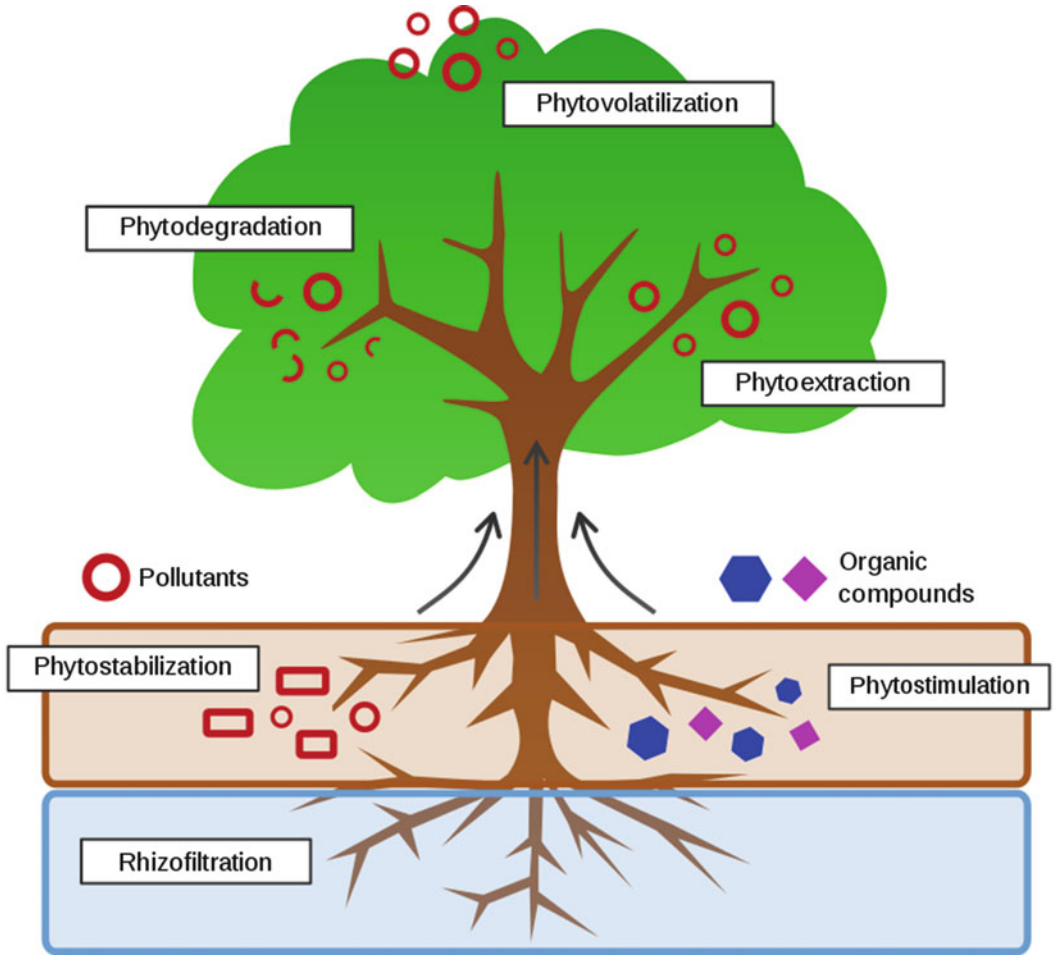

Fig. 4.2 Phytotechnologies (https://commons.wikimedia.org/w/index.php?curid=53861918)

- Bioaugmentation-assisted phytoextraction (including combination with mycorrhiza)
- Phytomining (obtaining economic profit from the metal accumulated by plants)
- Chelated-assisted phytoextraction (implies the adding of different chelants to the soil)

Phytofiltration is the capacity of plants to adsorb and absorb the pollutants from the contaminated environment, into roots or other plant parts. The precipitation of the pollutant in the root area is also a possibility. Usually, this technique is used to extract heavy metals or lipophilic compounds from water and is carried out by aquatic plants (Trapp and Karlson 2001; Rahman and Hasegawa 2011). Phytofiltration includes:

- Biosorption (the pollutant is absorbed or bound in living or non-living plant parts)
- Rhizofiltration (the contaminant is absorbed or bound in the roots)
- Blastofiltration (the toxic compound is absorbed or bound in seedlings) (Conesa et al. 2012)

In *phytodegradation* or *phytotransformation* the organic pollutants are subjected to degradation by plants through their metabolic processes. It can be considered a defense mechanism of the plant to the contaminant (Rahman and Hasegawa 2011), which results in its modification, inactivation, degradation or immobilization. Rhizodegradation represents the transformation of the pollutant in the root area, with or without the implication of the rhizosphere microorganisms.

Phytovolatilization consists in up-taking the pollutants from the contaminated site and their volatilization to the atmosphere (by transpiration) through translocation in the aerial parts of the plant (Trapp and Karlson 2001; Rahman and Hasegawa 2011; Conesa et al. 2012). It should be mentioned that this technique can be applied to those compounds that are volatile or those that can be transformed into volatile forms (chlorobenzene, trichloroethene, organically bound mercury, etc.) (Trapp and Karlson 2001). Transformation of the pollutant into volatile forms and releasing them into the atmosphere only displaces the pollution issue from one medium to another and is therefore seen as an improper process.

Phytostabilization is the process that can be applied to immobilize the pollutant (heavy metals or organic amendments) in soil through ab- or adsorption, accumulation in the roots of the plants or precipitation in the rhizosphere (transformation from a soluble form into a non-soluble one) (Andreazza et al. 2013). In fact, in this way, the mobility and the phytoavailability of the pollutant in the environment are reduced. This process includes:

- Phytoexclusion (use of plants with low metal uptake)
- Assisted phytostabilization (use of amendments to improve the process)
- Hydraulic control (to prevent leaching or movement of pollutants by water pumping)
- Phytorestoration (involves native plant species) (Conesa et al. 2012)

Much research was carried out to *improve the phytoremediation* process, especially the phytoextraction of heavy metals. These improvement efforts include genetic engineering of the plants, the addition of chelating agents or hormones and plant responses to them, formation of mycorrhizae (Vamerali et al. 2010), the exploitation of natural plant diversity, the interactions between plant roots and rhizosphere microorganisms, the use of endophytic bacteria that possess superior capacities for metal accumulation and/or degradation of organic contaminants (Schwitzguébel et al. 2002).

During the last few decades, many studies have emphasized the positive aspects of phytoremediation. Nevertheless, phytoremediation has several disadvantages and limitations as well.

Among the *advantages* of this process, it is worth mentioning the following (Trapp and Karlson 2001; Prasad 2003; Alkorta et al. 2004; Vasavi et al. 2010; Ekta and Modi 2018):

- It can be applied in situ (and ex situ as well)
- The plants can be easily monitored
- It reduces soil disturbance and the spread of pollutants

- The soil remains in place and is accessible for subsequent treatment
- It is solar driven
- It is considered inexpensive
- It costs less than 20% of conventional treatments
- There is no need for expensive equipment or highly specialized personnel
- It is a green tool, environmentally friendly
- It is aesthetically pleasing, socially accepted
- It is a low-tech alternative
- It maintains soil and stimulates soil life
- It can be combined with other methods of treatment
- The transfer of the contaminant is faster than natural remission
- It is considered to have fewer air and water emissions
- It is suitable for a wide variety of inorganic and organic pollutants
- It reduces the amount of waste
- It is possible to recover and re-use valuable metals
- It is easy to implement
- The plants represent a renewable resource, easily available
- It is capable of constantly treating a wide range of pollutants from different kinds of environments

Even if this green alternative has a number of notable advantages, its *limitations and disadvantages* must also be mentioned (Trapp and Karlson 2001; Prasad 2003; Alkorta et al. 2004; Ghosh and Singh 2005; Vasavi et al. 2010; Ali et al. 2013; Stephenson and Black 2014; Ekta and Modi 2018):

- Although faster than natural remission, it requires long time periods (several years)
- Even though it is a lengthy process, the contamination may still not be fully remediated
- It is slower than chemical and conventional treatments
- Only a few uses of the area are possible
- The phytotoxicity, ecotoxicity, and bioavailability of degradation products is unknown
- It is not applicable for all compounds
- It is limited in application to shallow soils, streams, and groundwater
- It is limited by the depth of the roots and both the solubility and the availability of the contaminant
- High concentrations of pollutant materials are toxic, even lethal to plants
- It is considered to be applicable to sites with low to moderate soil contamination over large areas, or to sites with large volumes of groundwater with low levels of contamination because plant growth is not sustained in heavily polluted environments
- Contaminants may accumulate in the groundwater because it is not possible to completely prevent leaching
- It is possible for pollutants to be transferred to another medium, the environment, and/or the food chain in the case of mismanagement and lack of proper care
- It is restricted to sites with low contaminant concentration
- Plant biomass from phytoextraction requires proper disposal as hazardous waste
- It is climate- and season-dependent, because unfavorable conditions can limit plant growth and biomass production, the result being decreased efficiency
- In the case of plant disease or attack by plant pests, effectiveness is lost as well
- The introduction of inappropriate, non-native or invasive plant species can affect biodiversity
- Some amendments and cultivation practices may have negative consequences for pollutant mobility
- Particularly in Europe, the limitation of phytoremediation is also associated with the potential use of genetically modified crops and the risk of their utilization to ecosystems. Consequently, its cost might be increased as sites require greater maintenance, monitoring, and disposal of genetically modified plant materials owing to the strict regulations

Regarding the phytoremediation process as a green alternative, it should be mentioned that

results obtained in the field may be different from those obtained in the laboratory or at a greenhouse level. This is because the field is a real world, a real environment, where different factors act simultaneously. Factors that interfere with phytoremediation in the field also include variations in temperature, nutrients, precipitation and moisture, presence of plant pathogens, uneven distribution of pollutants, soil type, soil pH, and soil structure. Therefore, phytoremediation is an interdisciplinary domain and requires solid background knowledge in soil chemistry, plant biology, ecology, soil microbiology in addition to environmental engineering (Ali et al. 2013).

From an *economic* point of view, the dedicated literature maintains that phytoremediation will become feasible in the next few years. Approximately 20 years ago, in 2001, the US Environmental Protection Agency (EPA) published data regarding different completed soil remediation projects, a small number of which used phytoremediation (Vangronsveld et al. 2009). The costs varied as follows (Vangronsveld et al. 2009):

- Phytoremediation of a large site (USA) of contaminated soil with heavy metals—USD147–483/m^3
- Phytoremediation of a contaminated soil area with heavy metals (estimation)—USD13–131/m^3
- Phytostabilization (France) for soil contaminated with arsenic—minimum USD54/m^3
- Phytoremediation of a large groundwater site contaminated with heavy metals—USD4.8–6.9/m^3

Over 10 years ago, several commercial companies using phytoremediation technologies had been developed both in the USA and in Europe, for example: Phytotech (USA), Applied Natural Sciences (USA) (http://treemediation.com), Aquaphyte Remediation (Canada), BioPlanta (Germany) (http://www.bionity.com/en/companies/10451/bioplanta-gmbh.html), Consulagri (Italy), Earthcare (USA), Ecolotree (USA) (https://www.ecolotree.com/), Piccoplant (Germany) (https://www.piccoplant.de/en/), PhytoWorks (USA), Plantechno (Italy), Slater (UK) (http://www.slateruklimited.co.uk/), Thomas Consultants (New Zealand) (https://www.thomasconsultants.co.nz/), Verdant Technologies (USA), Viridian Resources (USA) (http://www.viridianresources.com/). As we noticed, some of them no longer exist.

Nowadays, according to Transparency Market Research (https://www.transparencymarketresearch.com/bioremediation-technology-services-market.html), the most important companies in the bioremediation technology and services market are: Altogen Labs (USA) (http://altogenlabs.com/), Aquatech International LLC (USA) (https://www.aquatech.com/about/), Drylet, LLC (USA) (https://www.drylet.com/), InSitu Remediation Services Limited (Canada) (http://irsl.ca/), Ivey International, Inc. (Canada) (https://www.iveyinternational.com/index.php), Probiosphere Inc. (Canada) (https://www.probiosphere.technology/about-us), Regenesis (USA) (https://regenesis.com/en/), Sarva Bio Remed, LLC (USA) (https://sarvabioremed.com/), Sevenson (USA) (https://sevenson.com/), Environmental Services, Inc. (USA) (www.esinc.cc), Soilutions Ltd, (UK) (https://www.soilutions.co.uk/), Sumas Remediation Services Inc. (Canada) (https://sumasrem.com/about-us/), Xylem Inc. (USA) (https://www.xylem.com/en-us/about-xylem/).

In their last report from 2017, the global market for bioremediation technology and services (including phytoremediation) was assessed to be worth USD32.2 billion (2016) and is estimated to reach USD65.7 billion by 2025.

4.3 Microbial and Vermiremediation: Clean-Up Alternatives

4.3.1 Microbial Bioremediation

Microorganisms have biosynthetic and biodegradative abilities, which proved very valuable in finding solutions for maintaining the quality of the environment or repairing the damaged ecosystems.

One of the main applications of microorganisms in environmental protection and bioremediation is the creating of cleaning technologies in oil-contaminated areas, but also in areas contaminated with polychlorinated biphenyl compounds (PCBs), hydrocarbons, dyes, pesticides, esters, heavy metals, or nitrogen-containing chemicals (Table 4.3) (Sharma et al. 2018). Compared with other methods, biological treatment using bacteria, fungi or microalgae is low in cost, highly efficient, and prevents secondary pollution.

4.3.2 Water Bioremediation

An important source of pollution is sewage water created by residences, hospitals, industrial establishments, farms, etc. Conventional sewage treatments are performed in treatment plants, in at least 12 phases, some of them involving aerobic biological processes realized by microorganisms, the technologies being well known and widely applied. For example, in the activated sludge many bacterial genera could be found such as *Pseudomonas*, *Flavobacterium*, *Alcaligenes*, *Bacillus*, *Acinetobacter*, and *Zooglea* spp., primarily involved in the biological treatment of municipal wastewater under aerobic conditions (the presence of organic matter supports the growth of heterotrophic bacteria able to degrade toxic compounds—nitrobenzene, tributyl phosphate, heavy metals, textile dyes, aliphatic and aromatic hydrocarbons, fatty acids, insecticides, etc.) (Shah 2017). In a synthesis performed in 2017, it was shown that the main microorganisms involved in efficient wastewater treatment include *Bacillus*, *Achromobacter*, *Pseudomonas stutzeri*, *P. putida*, *P. mendocina*, *Zooglea ramigera*, *Arthrobacter*, *Alcaligenes faecalis*, *Flavobacterium*, *Micrococcus*, *Rhodococcus species*, and lactic acid bacteria (*Lactobacillus casei*, *L. plantarum*, *Streptococcus* spp., *Rhodopseudomonas*) (Shah 2017).

In a case study carried out in China (Chengnan River) and published in 2018, a microbial product designated as HP-RPe-3 (national patent number: 2017114193785) composed of a large number (more than 100 types) of indigenous microorganisms (isolated from the Tibetan Plateau snow line—altitude 4650 m; species of *Bacillus*, *Micrococcus*, photosynthetic bacteria, nitrifying bacteria, denitrifying bacteria, lactic acid bacteria, yeasts, *Actinomycetes*, *Acetobacter*) and enzymes was used for the degradation of organic and inorganic matter, and toxic substances in water and sediments in the Chengnan River (Gao et al. 2018). The selected microorganisms have important properties (extreme cold resistance, high enzymatic activity, phage-resistant, and presented short cycles of development), but in the experiments microbial accelerating agents (enzymes, vitamins, amino acids, trace elements, and humic acid) were also used to stimulate the proliferation and activity of aerobiotic and facultative aerobic bacteria. The results obtained indicate that bioremediation technology, by adding microbial agents, improves water quality mainly by the degradation of NH3-N and elimination of the black-odor phenomenon of urban rivers (Gao et al. 2018).

4.3.3 Oil Spill Bioremediation

Regarding oil pollution, the accidental large-scale oil spills produced by Exxon Valdez in Alaska in 1989 and the BP Deepwater Horizon spill in the Gulf of Mexico in 2010 are well known: in these two environmental disasters 0.75 and 4.9 million barrels of crude oil were released respectively, which are still affecting some of the most productive and vulnerable marine ecosystems, and are having a high impact on terrestrial ecosystems too (Yavari et al. 2015). Another example is related to the Gulf War that occurred in 1991, when more than 700 oil wells were damaged, forming more than 300 oil lakes, and covering land areas in excess of 49 km^2 (Yateem 2014).

Microbial activity-based bioremediation processes used in situ in the field are classified as natural attenuation, bioaugmentation, and biostimulation (Table 4.4).

To establish a bioremediation technology based on the microbial degradative/biosynthesis activities it is necessary to isolate the

Table 4.3 Biological agents of bioremediation [adapted from Biswas et al. (2015)]

Microorganism		Toxic compounds used	
		Organic pollutants	Heavy metals
Bacteria	*Bacillus* spp.	Cresol, phenols, aromatics, long-chain alkanes, phenol, oil-based paints, textile dye (Remazol Black B), sulfonated di-azo dye Reactive Red HE8B, remazol navy blue dye	Cu, Zn, Cd, Mn
	Pseudomonas spp.	Benzene, anthracene, hydrocarbons, polychlorinated biphenyl compounds	U, Cu, Ni, Cr, Cd, Pb, Zn, As
	P. alcaligenes, *P. mendocina*, *P. putida*, *P. veronii*, *Acinetobacter*, *Achromobacter*, *Flavobacterium*	Petrol and diesel polycyclic aromatic hydrocarbons, toluene	
	Pseudomonas putida	Monocyclic aromatic hydrocarbons, e.g., benzene and xylene	
	Xanthomonas sp.	Hydrocarbons, polycyclic hydrocarbons	
	Nocardia sp.	Hydrocarbons	
	Streptomyces sp.	Phenoxyacetate, halogenated hydrocarbon, diazinon	
	Mycobacterium sp.	Aromatics, branched hydrocarbons benzene, cycloparaffins	
	Alcaligenes odorans, *B. subtilis*, *Corynebacterium propinquum*, *P. aeruginosa*	Phenol	
	Micrococcus luteus, *Listeria denitrificans*, *Nocardia atlantica*	Textile azo dyes	
	Acinetobacter sp., *Pseudomonas* sp., *Enterobacter* sp., *Photobacterium* sp., *Bacillus* spp., *Staphylococcus* spp.	Pesticides (chlorpyrifos, methyl parathion, malathion, endosulfan)	
	Rhodopseudomonas palustris, *Aerococcus* spp.		Pb, Cr, Cd
	Citrobacter sp.		Cd, U, Pb
	Lysinibacillus sphaericus		Co, Cu, Cr, Pb
Fungi	*Coprinellus radians*	Polyaromatic hydrocarbons, methylnaphthalenes, and dibenzofurans	
	Pycnoporus sanguineus, *Phanerochaete chrysosporium*, and *Trametes trogii*	Industrial dyes	
	A. niger, *A. fumigatus*, *F. solani*, and *P. funiculosum*	Hydrocarbons	
	Aspergillus versicolor, *A. fumigatus*, *Paecilomyces* sp., *Trichoderma* sp., *Microsporum* sp., *Cladosporium* sp.		Cd
	Saccharomyces cerevisiae		Pb, Hg, Ni
	Marasmiellus troyanus	Benzo[a]pyrene	
	Gloeophyllum trabeum	1, 1, 1-trichloro-2, 2-bis (4-chlorophenyl) ethane (DDT)	

(continued)

Table 4.3 (continued)

Microorganism		Toxic compounds used	
		Organic pollutants	Heavy metals
	Pleurotus ostreatus	Bisphenol A, hydrocarbons	
	Fomitopsis pinicola	1, 1, 1-trichloro-2, 2-bis (4-chlorophenyl) ethane (DDT)	
	Penicillium simplicissimum	Polyethylene	
	Rhizopus arrhizus		Ag, Hg
	Stereum hirsutum		Cd, Pb
Algae	*Chlamydomonas* sp.	Naphthalene	
	Dunaliella sp.	Naphthalene, DDT	
	Euglena gracilis	DDT, Phenol	
	Selenastrum capricornutum	Benzene, toluene, chlorobenzene, 1, 2-dichlorobenzene, nitrobenzene naphthalene, 2, 6-dinitrotoluene, phenanthrene, di-*n*-butylphthalate, pyrene	
	Chlorella sp.	Toxaphene	Au, Cu, Ni, U, Pb, Hg, Zn, As, Cd, Cr
	Cylindrotheca sp.	DDT	
	Zooglea sp.		Co, Ni, Cd
	Phormidium valderium		Cd, Co, Cu, Ni
	Volvariella volvacea		Cu, Hg, Pb
	Oscillatoria sp.		Ni, Cu, Co, Pb, Zn
	Tetraselmis chuii		Cu
	Spirogyra hyalina		Cd, Hg, Pb, As
	Lyngbya spiralis		Cd, Pb, Hg

DDT dichlorodiphenyltrichloroethane

Table 4.4 Microorganisms involved in oil bioremediation (adapted after Abatenh et al. 2017)

Microorganism	Oil type
Fusarium spp.	Oil
Alcaligenes odorans, *Bacillus subtilis*, *Corynebacterium propinquum*, *Pseudomonas aeruginosa*	Oil
Bacillus cereus	Diesel
Aspergillus niger, *Candida glabrata*, *C. krusei*, *Saccharomyces cerevisiae*	Crude oil
B. brevis, *P. aeruginosa KH6*, *B. licheniformis*, *B. sphaericus*	Crude oil
Pseudomonas aeruginosa, *P. putida*, *Arthrobacter* sp., *Bacillus* sp.	Diesel
Pseudomonas cepacia, *B. cereus*, *B. coagulans*, *Citrobacter koseri*, *Serratia ficaria*	Diesel

microorganisms able to perform, at the highest level, the decontamination of the environment.

The microbiological analysis of polluted sites (oil-contaminated soils or aquatic ecosystems) revealed the presence of a number of heterotrophic oil-utilizing bacteria or fungi (Table 4.4). Most studies revealed the presence in oil-contaminated sites of bacteria belonging to various genera such as: *Acinetobacter*, *Micrococcus*, *Rhodococcus*, *Pseudomonas*, *Bacillus*, *Staphylococcus*, *Kocuria*, etc. (Table 4.4). Moreover, the studies performed in oil-contaminated lakes from Kuwait allowed the identification of extreme halophilic archaea strains belonging to *Halobacterium*, *Haloferax* or *Halococcus* (Yateem 2014).

Recently, research into petroleum hydrocarbon microbial degraders in marine environments was conducted to identify the novel obligate hydrocarbon degraders typical of

marine habitats such *Alcanivorax* and *Cycloclasticus* (Kalogerakis et al. 2015). These data suggest that the search for new efficient oil-degrading bacteria might still be the aim of many studies and could permit the selection of microbial consortia useful in specific technologies.

Microorganisms selected from specific polluted sites, such as consortia or as individual cultures, could be used in cleaning technologies of environments polluted with pesticides, heavy metals, different organic toxic compounds, etc. An example of bacteria useful in such approaches is *Pseudomonas putida*, a soil saprophytic Gram-negative bacteria able to produce a large diversity of enzymes for green chemistry applications and bioremediation. Until now, many strains of *P. putida* able to use aromatic hydrocarbons, trichloroethylene, indole, chlorophenols, nitrotoluenes, etc., as carbon sources were selected and studied from biochemical and genetic points of view. Many bacterial strains useful in bioremediation applications were subjected to legal protection by patent: the first patent for a biological remediation substance was recorded in 1974, and was a strain of *P. putida* capable of degrading petroleum (Biswas et al. 2015).

Moreover, many species of fungi and algae (eukaryotic microorganisms) are involved in biogeochemical transformations in both aquatic and terrestrial habitats. Fungi can mineralize xenobiotic compounds to CO_2 and H_2O generally through their non-specific ligninolytic and highly oxidative enzyme systems, which are also responsible for the degradation and decolorization of a wide range of dyes (Biswas et al. 2015). Various species of eukaryotic algae are able to produce organometallic complexes between algal peptides and heavy metals; the complexes are then included in vacuoles, thus neutralizing or preventing the toxic effects of metals. The transformations performed by them could influence plant productivity, the mobility of toxic elements, with important socio-economic relevance, especially in the mutualistic symbioses, lichens, and mycorrhizas. From a bioremediation point of view, fungal biotransformation has beneficial applications in environmental biotechnology, e.g., in metal leaching, recovery, and detoxification, and xenobiotic and organic pollutant degradation (Table 4.4) (Biswas et al. 2015; Majumder 2016).

The studies regarding the microbial mechanisms involved in the degradation of certain pollutants, such as aliphatic and aromatic hydrocarbons, revealed the presence of specific enzymes that contribute to the transformation of contaminants into less toxic final products, which are integrated into natural biogeochemical cycles (Peixoto et al. 2011). The efficiency of bioremediation depends on many factors such as: the chemical nature and concentration of pollutants, the physicochemical characteristics of the environment (type of soil, temperature, pH, the presence of oxygen or other electron acceptors, nutrients), the availability of xenobiotics to the microorganism, and the diversity of microbial consortia.

The biodegradation may occur under aerobic or anaerobic conditions, the processes being best studied in the case of hydrocarbons. Microbial strains involved in aerobic degradation are able to produce oxygenase enzymes that introduce oxygen atoms into hydrocarbons: for example, monooxygenases introduce one oxygen atom to a substrate whereas dioxygenases introduce two. Under anaerobic conditions, the hydrocarbon degradation is produced mainly by sulphate-reducing bacteria, by using different terminal electron acceptors (nitrate, sulphate, or Fe (III)). Similar studies were performed to establish the mechanisms involved in other microbial bioremediation processes, and more than 1000 different enzymes were described (Whiteley and Lee 2006).

Based on the results obtained in experiments performed with microorganisms and/or microbial enzymes, various products useful in practical applications, mainly in oil spill bioremediation, were developed. The US EPA has defined bioremediation agents (bioaugmentation agents or biostimulation agents) as "microbiological cultures, enzyme additives, or nutrient additives that significantly increase the rate of biodegradation to mitigate the effects of the discharge" (Zhu

et al. 2004). Numerous bioremediation products (microbial cultures, enzyme additives, and nutrient additives) have been proposed and promoted by the manufacturers or vendors (Table 4.5).

In conclusion, the advantages of microbial bioremediation are related to the use of natural processes that cause less damage to ecosystems and take place underground, as additives and microbial cultures are introduced underground to clean up contaminants in ground water and soil. Bioremediation technologies are generally cheaper than most cleanup methods, and they does not require special equipment or labor. In a survey from 2012, it was encountered that bioremediation has been used to clean up more than 100 Superfund sites around the USA (https://www.investopedia.com/terms/b/bioremediation.asp).

The bioremediation could be considered a business domain, the large number of companies founded and the numerous products and technologies developed and commercialized being a proof of success. According to a study performed in 2018 by Transparency Market Research, the global market for bioremediation technology and services market was valued at USD32.2 billion in 2016 and is estimated to reach USD65.7 billion by 2025 at a compound annual growth rate of 8.3% from 2017 to 2025.

Based on technology developed, the bioremediation technology and services market is classified into phytoremediation, biostimulation, bioaugmentation, bioreactors, fungal remediation, and land-based treatments. Among these technologies, fungal remediation represents a major segment of the bioremediation technology and services market, as the use of mycelium to disintegrate contaminants from waterways, soil or even radioactive contaminated areas has increased. It was estimated that the use of fungi for the treatment of soils polluted by mercury and other heavy metals will increase by 2025.

Regarding the services, the market is divided into soil remediation, wastewater remediation, oilfield remediation, and others. Among the services offered by companies, wastewater remediation was accounted to hold the largest market share in 2016, whereas soil remediation services are likely to expand from 2017 to 2025. Owing to rapid industrialization, an increase in the disposal of pharmaceutical products, and a rise in the use of harmful insecticides, pesticides, petroleum hydrocarbons, chlorinated solvents, etc., were encountered, which is the reason why bioremediation technologies must be developed.

In the study it was shown that, at present, the major players in the bioremediation technology and services market are: Altogen Labs, Aquatech International LLC, Drylet LLC, InSitu Remediation Services Limited, Ivey International Inc, PROBIOSPHERE Inc, REGENESIS, Sarva Bio Remed LLC, Sevenson, Environmental Services Inc, Soilutions Ltd, Sumas Remediation Services Inc, and Xylem Inc.

Table 4.5 Bioremediation agents useful in oil spill bioremediation (adapted from Zhu et al. 2004)

Name of the product	Type of the product	Manufacturer
Bet Biopetro	Microbial culture	BioEnviro Tech, Tomball, TX
Inipol Eap 22	Nutrient additive	Societe, CECA SA, France
Land and Sea Restoration	Nutrient additive	Land and Sea Restoration LLC, San Antonio, TX
Oil Spill Eater II	Nutrient additive/enzyme additive	Oil Spill Eater International, Corporation, Dallas, TX
Oppenheimer Formula	Microbial culture	Oppenheimer Biotechnology, Inc, Austin, TX
Step one	Microbial culture	B & S Research, Inc, Embarrass, MN
Biosolve Pinkwater	Nutrient additive	The BioSolve Company, Lexington, MA, USA
Remediact™	Microbial culture	Chemtex, Inc., Cumberland, RI, USA
EcoPondSweep™	Microbial culture	Confluence Energy, Kremmling, CO, USA

4.3.4 Vermiremediation

Vermiremediation or vermicomposting is an effective, low-cost technology dedicated to recycling agricultural waste, city garbage, kitchen waste or even sewage sludge, by the activity of earthworms able to convert the organic waste materials into compost (Khan 2016). The beneficial role of earthworms (*Eisenia fetida*, *Lumbricus terrestris*, *Aporrectodea caliginosa, A. nocturna*, *Pheretima hawayana*, *Pontoscolex corethrurus*, *Dendrobaena veneta,* etc.) in the physical, chemical, and biological properties of soil (increasing soil fertility) is well known, but the use of these organisms in bioremediation technologies has been examined over the past decades. In this respect, the effect of earthworms on the removal of various contaminants, such as oil, PAHs, PCBs, pesticides, and heavy metals has been reported by many authors, both from scientific and practical points of view (in the laboratory or outdoors) (Rodriguez-Campos et al. 2014; Rorat et al. 2017). The results obtained demonstrated that more experiments are required so that the practical application of vermiremediation can be demonstrated on a large scale (soil remediation in extended areas). Additionally, the costs of the technologies may be too high to remediate large contaminated areas, because of the conditions needed for the survival and activity of the earthworms.

However, presently many products of the vermicomposting process are available on the market. Such products are obtained both in small-scale or home systems (from mixtures of fruit and vegetable waste, coffee grounds and filters, grains such as bread, crackers, and cereals, eggshells, leaves and grass, newspapers, paper) and in large-scale systems (generally using dairy cow or pig manure, brewery waste, other industrial and agricultural waste, grass clippings and wood, etc.) (Adhikary 2013).

In a report presented in 2019 it was shown that the vermicompost industry is very fragmented, manufacturers are mostly in the India and Southeast Asia, and products are manufactured and commercialized all over the world. The key manufacturers in the vermicompost market include: MyNOKE (the world leading manufacturer in global vermicompost market with the market share of 8.79% in 2015), NutriSoil, Davo's Worm Farms, Earthworm, Wormpower, Kahariam Farms, SAOSIS, Sri Gayathri Biotec, Jialiming, Dirt Dynasty, SLO County Worm Farm, Agrilife, and Suman Vermi Compost. Regarding the benefits, it was shown that compared with 2014, the vermicompost market managed to increase sales by 24.89% to USD38.09 million worldwide in 2015, which allows the conclusion that overall, the vermicompost performance is positive, despite the weak economic environment. For example, among the manufacturers, production in India accounted for less than 9.50% of the total value of global vermicompost in 2015 (http://www.qyresearchglobal.com/goods-1814370.html; http://www.marketsnresearch.com/global-vermicompost-market-report-2019-industry-analysis-size.html).

4.3.5 Microbial Biofertilizers for Bioremediation

Biofertilizers are microbially enriched products, containing latent or living cells of selected beneficial microorganisms that are able to improve soil quality and promote plant growth, mainly by increasing the uptake of nutrients. Biofertilizers accelerate certain bioconversion processes in the growing substrate and increase the bioavailability of nutrients for plants. They can be applied to the soil, seed or plant surface, enriching the microbial communities of the rhizosphere and colonizing the inner and external parts of the plants.

In sustainable agriculture, biofertilizers are cost-efficient supplements of plant nutrients that increase the efficacy of chemical fertilizers or reduce their application requirements.

Among the beneficial microorganisms used as biofertilizers are: mycorrhiza, several soil- and plant-inhabiting fungi, most of the plant growth-promoting bacteria, and some blue-green algae (Santra et al. 2015). Based on their function, they can be classified as nitrogen fixers, phos-

phorus solubilizers, phytohormone and enzyme producers, and others.

The nitrogen-fixing microorganisms can convert atmospheric nitrogen (unavailable for direct plant nutrition) into organic nitrogen compounds, which are available for plants. Such biofertilizers can substitute nitrogen fertilization in some cultivated plants. The microorganisms used as nitrogen-fixing biofertilizers include symbiotic bacteria and free-living or non-symbiotic microorganisms (bacteria, actinomycetes, and blue-green algae). Among the symbiotic bacteria, *Rhizobium* and related genera (*Azorhizobium*, *Bradyrhizobium*, *Sinorhizobium*, *Ensifer,* etc.) are able to fix nitrogen in leguminous plants, producing nodules on their roots. Worldwide, there are various commercialized biofertilizers based on such microorganisms, such as Rhizolife^{BJ}, Polarhizo, Effect Grow, Biobium, Rhizo-Enrich, Nitragin™ Gold, Biodoz™, Optimize™, Cell-Tech™, GlyciMax™, Nitrofix®, etc. Various other nitrogen-fixing microorganisms were found, such as both symbiotic and free-living bacteria and actinomycetes. In such cases *Acetobacter*, *Azotobacter*, *Azospirillum*, *Paenibacillus*, and *Frankia* were found. Commercially available biofertilizers based on such nitrogen-fixing bacteria are: Power Grain Booster, GreenAzoto, Azomax (containing *Azotobacter* strains), Sugar-Plus (containing endophytic *Acetobacter*), Abtech Azospirillum, and Azostim F9 PTS (containing *Azospirillum* strains).

Some biofertilizers contain mixed cultures of beneficial microorganisms such as Rhizodyne (containing *Azospirillum*, *Azotobacter*, *Rhizobium*, *Acetobacter* as NPK and Zn providers), Micosat (containing *Glomus mosseae* and *G. intraradices* mycorrhizal fungi and plant growth-promoting bacteria *Pseudomonas fluorescens* and *Bacillus subtilis*), BIO-NPK and Bharpur (containing a consortium of various bacterial strains providing NPK-balanced nutrition), and TagTeam™ LCO (which combines *Rhizobium* and phosphate-solubilizing inoculants with lipo-chitooligosaccharide molecules).

Among other beneficial microorganisms used as biofertilizers are phosphorus-solubilizing microorganisms, which increase phosphorus uptake from phytic acid and phytate organic phosphorus and improve the solubility of inorganic phosphates. Available commercial biofertilizers with phosphorus-solubilizing activity are VICI Routz GR soil probiotics, Rich Paddy biofertilizer, Rhizocell GC, and Rhizocell C. Some bioproducts are based on bacterial strains, such as BIOPHOS and GET-PHOS containing *Bacillus megaterium* var. *phosphaticum*, and others such as JumpStart® contain the soil fungus *Penicillium bilaii*.

Other biofertilizers found on the market are used to increase potassium accumulation (BioPotash and Potash-Cure based on *Frateuria aurantia*), sulfur solubilization (BIOSULF and SULF-CURE based on *Thiobacillus thiooxidans*), zinc solubilization (BIOZINC and ZINC-CURE), or silica (such as BioSilica and Silica-Cure containing strains of *Bacillus* spp.).

Mycorrhizal fungi are also very good soil fertilizers. Mostly, they are efficient in phosphorus uptake from insoluble sources, but because of their capacity for colonization, they improve plant nutrition with several other nutrients from sources generally unavailable to host plants. Moreover, they positively influence soil aggregation and water dynamics (Piotrowski et al. 2004). HPM Gold, Myco-Rise, Mycoxol, NutriVAM, BioVam, VAM Riches, Myconox, MycoStim, PlantSuccess, Myco-Win, Ecomax, Root care, and Glow Raja are some of the commercially available biofertilizers.

Regarding bioremediation with microbial biofertilizers, it has been noticed that bio-augmentation of hydrocarbon-polluted soils with nitrogen-fixing bacteria improves the soil decontamination process (Huesemann and Moore 1993). Several other authors maintain the fact that improved substrate fertilization stimulates contaminant degradation by microorganisms (Perez-Vargas et al. 2000; Santra et al. 2015).

4.4 Natural Plant Protection Products ("Biopesticides")

Plant protection products (PPPs) are mainly used to protect plants from harmful organisms such as pests, diseases or weeds. However, some plant

protection products regulate plant growth by means other than nutrients or influence the shelf life of the harvest. PPPs contain one or more active substances responsible for the purposes mentioned. These active ingredients could be either chemical or natural. The latter category is low risk and includes microorganisms, insect pheromones, and plant extracts.

In the European Union (EU), the active ingredients of PPPs must be approved by the European Commission and the final product must be authorized before being marketed. Nowadays, around 25% of the active substances approved are natural products. This means that more than 70 natural active substances have been approved on the EU market (http://ec.europa.eu).

Among the microbial strains approved as plant protection products (Table 4.6), most have fungicidal and bactericidal activity (52.7%) and insecticidal effects (29.1%), the rest (18.2%) being elicitors, nematicides, and virus inoculants.

4.4.1 Microbial Biocontrol of Plant Pathogens

The spectrum of microorganisms used as biological control agents is relatively wide. In the EU, 22 species of fungi and bacteria have been approved for use as pesticide active substances, according to the data available in February 2019 (Table 4.7). However, worldwide, the spectrum of microbial biocontrol agents is much wider, especially in the USA, China, India, and the South American countries.

When searching for new microbial biological control agents (MBCAs), in addition to the efficacy and spectrum of activity, some other traits are also considered important for selection. As MBCAs are intended to be formulated, the preferred microorganisms are spore-forming fungi and bacteria, owing to their increased resistance and viability. Usually, in microbially based PPPs, in addition to the plant protective effects of the active ingredients, the self-replicating capacity of the formulated microorganisms is also exploited (Chattopadhyay et al. 2017). Other important issues are the adaptability and colonization capacity of the microorganisms. However, only strains that are neutral to non-target organisms, and safe for the environment, have been approved.

4.4.2 Entomopathogenic Fungi

The entomopathogenic fungi are highly efficient, as they produce insect-infecting spores that can induce pest death in 4–10 days, depending on the fungal strain. Moreover, the fungus continues its growth and sporulation on the body of the dead insect, continuing its biological control activity in the area of application. The main groups of entomopathogenic fungi occur in the phylum Zygomycota (mostly fungi of Entomophthorales order) and the phylum Ascomycotina, where the most efficient anamorphic genera known to have entomopathogenic activity are *Beauveria*, *Isaria*, *Metarhizium*, *Lecanicillium*, and *Purpureocillium.*

Most of these biological control agents are mass produced and formulated as PPPs. They are formulated as solid-state, emulsifiable suspension, oil dispersion, liquid suspension, dry flowable, wettable powder or water dispersible granules. Spore concentration depends on the formulation type. For example, powdery formulation has a lower concentration of colony-forming units (CFU)/g than liquid suspensions, in which the concentration is usually 10 times higher than in CFU/ml.

Although in the EU member states only 11 strains of entomopathogenic fungi have been approved, worldwide a wider spectrum of species and strains are used for insect, mite, and nematode control (Table 4.8).

4.4.3 Entomopathogenic Bacteria

Entomopathogenic bacteria are able to produce various toxins and virulence factors causing insect death after ingestion. Therefore, the insect must ingest the bio-pesticide, by feeding, which is less efficient than the entomopathogenic fungi, which act by contact. Moreover, entomopathogenic bacteria have a restrictive

Table 4.6 Selected microbial strains approved in the EU as active substances of plant protection products (http://ec.europa.eu)

No.	Active substance	Active substance ID	Category	Approval date	Expiration of approval	Rapporteur Member State (RMS)	Countries where authorized
1.	*Ampelomyces quisqualis* strain AQ10	959	FU	01/08/2018	31/07/2033	FR	BE, CY, DE, DK, EL, ES, FR, IE, IT, LU, NL, SI, SK, UK
2.	*Aureobasidium pullulans* (strains DSM 14940 and DSM 14941)	973	FU, BA	01/02/2014	31/01/2024	AT	AT, BE, DE, EL, ES, FR, HU, IT, NL, PL, PT, SI, SK
3.	*Bacillus amyloliquefaciens* (former *B. subtilis*) strain QST 713	986	BA, FU	01/02/2007	30/04/2020	DE	BE, CY, CZ, DE, DK, EE, EL, ES, FI, FR, IE, IT, LT, LU, LV, NL, PL, PT, SE, SI, SK, UK
4.	*Bacillus amyloliquefaciens* MBI 600	2325	FU	16/09/2016	16/09/2026	FR	
5.	*Bacillus amyloliquefaciens* strain FZB24	2324	FU	01/06/2017	01/06/2032	FR	
6.	*Bacillus amyloliquefaciens* subsp. *plantarum* D747	2252	FU	01/04/2015	31/03/2025	DE	BE, CY, EL, ES, FR, IT, SI, UK
7.	*Bacillus firmus* I-1582	2248	NE	01/10/2013	30/09/2023	FR	DK, EL, ES, FR, IT, NL, PT, SE, UK
8.	*Bacillus pumilus* QST 2808	2253	FU	01/09/2014	31/08/2024	IT	FR
9.	*Bacillus thuringiensis* subsp. *aizawai* strains ABTS-1857 and GC-91	988	IN	01/05/2009	30/04/2020	NL	AT, BE, CY, DE, DK, EL, ES, FI, FR, IT, LU, NL, PL, PT, SE, SI, UK
10.		989	IN	01/05/2009	30/04/2020	SE	

(continued)

Table 4.6 (continued)

No.	Active substance	Active substance ID	Category	Approval date	Expiration of approval	Rapporteur Member State (RMS)	Countries where authorized
	Bacillus thuringiensis subsp. *israeliensis* (serotype H-14) strain AM65-52						AT, DE, DK, ES, NL, UK
11.	*Bacillus thuringiensis* subsp. *kurstaki* strains ABTS 351, PB 54, SA 11, SA12 and EG 2348	990	IN	01/05/2009	30/04/2020	DK	AT, BE, BG, CY, CZ, DE, DK, EL, ES, FR, HR, HU, IE, IT, LT, LU, NL, PL, PT, RO, SE, SI, SK, UK
12.	*Bacillus thuringiensis* subsp. *tenebrionis* strain NB 176 (TM 14 1)	991	IN	01/05/2009	30/04/2019	IT	AT, DE, ES, FR, HR, HU, PL
13.	*Beauveria bassiana* IMI389521	2388	IN	19/02/2019	19/02/2029	NL	
14.	*Beauveria bassiana* PPRI 5339	2387	IN	20/02/2019	20/02/2029	NL	AT
15.	*Beauveria bassiana* strain 147	2311	IN	06/06/2017	06/06/2027	FR	FR
16.	*Beauveria bassiana* strain NPP111B005	2312	IN	07/06/2017	07/06/2027	FR	
17.	*Beauveria bassiana* strains ATCC 74040 and GHA	997	IN	01/05/2009	30/04/2020	DE	AT, BE, CY, DE, DK, EL, ES, FR, HU, IE, IT, NL, SI, UK
18.	*Candida oleophila* strain O	1074	FU	01/10/2013	30/09/2023	SI	AT, FR, NL, UK
19.	*Clonostachys rosea* strain J1446 (*Gliocladium catenulatum* strain J1446)	1435	FU	01/04/2005	31/07/2019	HU	AT, BE, CY, DE, EE, ES, FI, FR, IE, NL, PL, SE, SI, UK
20.		1156	FU	01/08/2017	31/07/2032	NL	

(continued)

Table 4.6 (continued)

No.	Active substance	Active substance ID	Category	Approval date	Expiration of approval	Rapporteur Member State (RMS)	Countries where authorized
	Coniothyrium minitans Strain CON/M/91-08 (DSM 9660)						AT, BE, CZ, DE, DK, EL, ES, FR, HU, IE, IT, LU, NL, PL, PT, SE, SK, UK
21.	*Cydia pomonella* granulovirus (CpGV)	1178	IN	01/05/2009	30/04/2020	DE	AT, BE, BG, CZ, DE, DK, EL, ES, FI, FR, HR, HU, IT, NL, PL, PT, RO, SE, SI, SK, UK
22.	*Fusarium* sp. L13	2389	FU	Pending	–	FR	
23.	*Isaria fumosorosea* Apopka strain 97 (formerly *Paecilomyces fumosoroseus*)	1653	IN	01/01/2016	31/12/2030	BE	BE, FI, FR, NL, SE
24.	*Lecanicillium muscarium* (formerly *Verticillium lecanii*) strain Ve6	1515	IN	01/05/2009	30/04/2020	NL	BE, DK, ES, FI, FR, NL, UK
25.	*Metarhizium anisopliae* var. *anisopliae* strain BIPESCO 5/F52	1559	IN	01/05/2009	30/04/2020	NL	AT, BE, DE, DK, EL, FR, IE, IT, LU, NL, PT, UK
26.	*Metschnikowia fructicola*	2457	FU	27/12/2018	27/12/2028	FR	
27.	*Paecilomyces fumosoroseus* strain Fe9901	1654	IN	01/10/2013	30/09/2023	PL	BE, ES, IT
28.	*Paecilomyces lilacinus* strain 251	1655	NE	01/08/2008	31/07/2019	HU	BG, CY, EL, ES, IT
29.	*Pasteuria nishizawae* Pn1	2460	NE	14/10/2018	14/10/2033	DK	
30.	*Phlebiopsis gigantea* (several strains)	1698	FU	01/05/2009	30/04/2020	EE	DK, EE, FI, FR, LT, LV,

(continued)

Table 4.6 (continued)

No.	Active substance	Active substance ID	Category	Approval date	Expiration of approval	Rapporteur Member State (RMS)	Countries where authorized
							PL, SE, UK
31.	*Pseudomonas chlororaphis* strain MA342	1786	FU	01/10/2004	30/04/2020	NL	AT, BE, DE, DK, ES, FI, FR, IT, LT, LU, NL, PT, SE, UK
32.	*Pseudomonas* sp. strain DSMZ 13134	1787	FU	01/02/2014	31/01/2024	NL	AT, BE, CY, CZ, DE, EL, FR, HR, IE, IT, NL, RO, SE, SI, SK
33.	*Purpureocillium lilacinum* PL 11	2391	NE	Pending	–	UK	
34.	*Pythium oligandrum* M1	1810	FU	01/05/2009	30/04/2020	SE	CZ, FR, HU, IT, PL, SK, UK
35.	*Saccharomyces cerevisiae* strain LAS02	2323	FU	06/07/2016	06/07/2031	FR	
36.	*Streptomyces* K61 (formerly *S. griseoviridis*)	1895	FU	01/05/2009	30/04/2020	EE	BE, CY, EE, FI, FR, HU, IT, LT, LV, NL, SE, UK
37.	*Streptomyces lydicus* WYEC 108	2256	FU, BA	01/01/2015	31/12/2024	NL	
38.	*Trichoderma asperellum* (formerly *T. harzianum*) strains ICC012, T25 and TV1	1979	FU	01/05/2009	30/04/2020	SE	DE, EL, ES, FR, IT, PL, PT
39.	*Trichoderma asperellum* (strain T34)	2066	FU	01/06/2013	31/05/2023	SE	BE, FR, IE, IT, NL, PT, RO, UK
40.	*Trichoderma atroviride* (formerly *T. harzianum*) strains IMI 206040 and T11	1980	FU	01/05/2009	30/04/2020	SE	EL, IT, SE
41.		2532	FU	Pending	–	FR	

(continued)

Table 4.6 (continued)

No.	Active substance	Active substance ID	Category	Approval date	Expiration of approval	Rapporteur Member State (RMS)	Countries where authorized
	Trichoderma atroviride AGR2						
42.	*Trichoderma atroviride* strain I-1237	1981	FU	01/06/2013	31/05/2023	IT	FR
43.	*Trichoderma atroviride* strain SC1	2329	FU	06/07/2016	06/07/2031	FR	AT, BE, FR, LU, PT
44.	*Trichoderma gamsii* (formerly *T. viride*) strain ICC080	1982	FU	01/05/2009	30/04/2020	SE	DE, EL, ES, FR, IT, NL, PL, PT
45.	*Trichoderma harzianum* strains T-22 and ITEM 908	1983	FU	01/05/2009	30/04/2020	SE	BE, DK, EL, ES, FR, HU, IE, IT, NL, PL, SE, UK
46.	*Trichoderma polysporum* strain IMI 206039	1984	FU	01/05/2009	30/04/2019	SE	SE
47.	*Verticillium albo-atrum* (formerly *Verticillium dahliae*) strain WCS850	2008	FU	01/05/2009	30/04/2020	SE	DE, DK, NL, SE, UK

spectrum of activity, usually being active against a reduced number of susceptible pest species.

Most common bacterial insecticides are based on *Bacillus thuringiensis (Bt)*. In the EU, several products are registered, all having as an active ingredient *Bt* ssp. *aizawai*, *israeliensis*, *kurstaki* or *tenebrionis*. *Bt* ssp. *aizawai* and *kurstaki* have restricted efficacy against lepidopteran larvae (caterpillars), *Bt* ssp. *israeliensis* is effective against some mosquito species, black flies, and a range of filter flies, and *Bt* ssp. *tenebrionis* is restricted to susceptible species of foliar feeding beetle larvae of Coleoptera.

Worldwide, other species have also been identified to act against insects, mites, and nematodes. Commercially available entomopathogenic bacteria include *Bacillus firmus* against cyst, lance, lesion, root-knot, sheath, spiral, sting, and stunt nematodes, *Lysinibacillus sphaericus* (sin. *Bacillus sphaericus*) for mosquito control (El-Bendary 2006; Glare et al. 2017), *Clostridium bifermentans* against mosquitos (Qureshi et al. 2014), *Paenibacillus popilliae* to control Japanese beetles (Kaya et al. 2008; Glare et al. 2017), *Pasteuria nishizawae* registered as a biological nematicide parasitic on cyst nematodes of genera *Heterodera* and *Globodera*, *Saccharopolyspora spinosa* against two-spotted spider mites (Sparks et al. 2012), *Streptomyces avermitilis* against Colorado beetles (Wang et al. 2011a, b), and Gram-negative bacteria such as *Pseudomonas alcaligenes* against locusts and grasshoppers (Ruffner et al. 2015), or bacteria in the genus *Serratia* to control beetle larvae.

The most successful bacterial pesticides to date are spore-forming *Bacillus* species, owing to their long-term stability and storage compared

Table 4.7 Commercial microbial plant protection products used as natural fungicides and bactericides (Woo Sheridan et al. 2014; Fravel et al. 1998; EFSA documents)

Active ingredients	Commercial names	Formulation type
Ampelomyces quisqualis	AQ10 Biofungicide	Water dispersible granules
Aureobasidium pullulans several strains	Botector, Blossom Protect	Wettable granules
Bacillus amyloliquefaciens several strains (some *formerly B. subtilis)*	CX 9030, TAE-022 WDG, TAEGRO, TAE-022 Technical, Subtilex®, BUEXP1780S, QST 713 Technical	Water dispersible granules, wettable powder
Bacillus pumilus	Ballad Plus, Sonata, Sonata® ASO, QST 2808 AS organic, QRD288 ASO, QST 2808 MUP, BAY2100	Suspension concentrate
Bacillus subtilis	Epic, Kodiak	Dry powder
Candida oleophila	Aspire	Wettable powder
Clonostachys rosea (formerly *Gliocladium catenulatum*)	Primastop biofungicide, Prestop Mix	Powder
Coniothyrium minitans	Contans	Aqueous biomass suspension
Fusarium oxysporum non-pathogenic	Biofox C, Fusaclean	Dust or alginate prill, microgranules
Gliocladium virens	GlioGard 12G	Granule
Metschnikowia fructicola	SHEMER	Water-dispersible granule
Phlebiopsis gigantea (several strains)	Rotstop	Spores in inert powder
Pseudomonas chlororaphis	Cerall	Flowable concentrate for seed treatment
Pseudomonas fluorescens	BlightBan A506, Conquer/Victus	Wettable powder, aqueous biomass suspension
Pseudomonas syringae	Bio-Safe 10, Bio-Safe 11	Wettable powder
Pythium oligandrum	Polygandron	Granule, powder
Saccharomyces cerevisiae	ALD1202	Water dispersible granule
Streptomyces griseoviridis	Mycostop	Powder
Streptomyces lydicus	Actinovate, Actinovate Soluble, Actinovate BioFungicid	Solid, water soluble powder
Trichoderma asperellum (several strains formulated as individual or mix cultures)	Ecohope, Ecohope-Dry, Quality WG, Trichodermax EC, Trichotech WP	Suspension, wettable powder, water-dispersible granules, emulsifiable concentrate
Trichoderma atroviride	Esquive WP, Trichopel, Trichodry, Trichospray, Vinevax Bio-dowel, Sentinel, Tenet	Wettable powder, pellet for soil incorporation
Trichoderma gamsii	Remedier WP	Wettable powder
Trichoderma harzianum (several strains)	TRIANUM-P, TRIANUM-G, Binab T wettable powder biorational fungicide, Rootshield WP biological fungicide, T-22 HC, T-22 WP, T-22 Granules, T-22 Planter Box, T-22 technical, T-Gro, Floragard, Trichodex, Supresivit	Wettable powder, granule, dust
Trichoderma polysporum	BINAB TF WP	Wettable powder
Trichoderma virens (several strains)	G-41 Technical, BW240 G, BW240 WPBiological Fungicide, Biocure F, Bio-Shield, Bioveer	Wettable powder, granules
Verticillium albo-atrum	Dutch Trig	Ultralow volume suspension

Table 4.8 Commercial biological acaricide, insecticides, and nematicides based on entomopathogenic fungi (Ruiu 2018; European Food Safety Authority, EFSA, documents)

Active ingredients	Commercial names	Formulation type
Beauveria bassiana	balEnce™ Fly Spray, BioCeres WP, Broadband, Bio-Power, BotaniGard® (22WP, ES, MAXX), Mycotrol® (ESO, WPO), Daman, Naturalis-L, Nagestra, Green Beauveria, Beauvitech-WP, Myco-B2, DuPont Benevia, Bb-Protec, Racer, Velifer	Liquid suspension, wettable powder, oil dispersion, emulsifiable suspension
Beauveria brongniartii	Bas-Eco	Liquid formulation, wettable powder
Hirsutella thompsonii	No-Mite, Hirsutellin	Liquid formulation, aqueous suspension, wettable powder
Isaria fumosorosea (formerly *Paecilomyces fumosoroseus*)	Preferal, Bioact WG, No-Fly-WP, Paecilomite	Water-dispersible granules, wettable powder
Lecanicillium muscarium (sin. *Lecanicillium lecanii* formerly *Verticillium lecanii*)	Mycotal, Bio Fire, VertiSoft, Vertiguard, Peak Victor, Verticon, Verti-Q, Vertici Power, Lecatech-WP, Varunastra, Bio-Catch, Mealikil, Bioline/Verti-Star	Wettable powder, liquid formulation
Mix of *Arthrobotrys oligospora*, *Hirsutella rhossiliensis*, and *Acremonium butyri*	Custon NC	Liquid formulation
Metarhizium anisopliae	Devastra, Kalichakra, Bio-Magic, Bio King, Metar-Q, Meta Power, Bio Storm, Emrald Dakshan, Biomet/ Ankush, Novacrid, Met52/BIO1020 granular, Pacer	Liquid, granular, and powder formulations, emulsifiable concentrate
Metarhizium brunneum	Attracap	Granular formulation
Myrothecium verrucaria	DiTera	Dry flowable
Paecilomyces lilacinus (current name *Purpureocillium lilacinum*)	Bio-Nematon, Nematofree, MeloCon, BIONICONEMA, Mytech-WP, Paecilo, Wellpacilo, Agronema, Green Nemagon, Ecopal	Wettable powder, wettable granules, liquid formulation

with Gram-negative bacteria (Chattopadhyay et al. 2017).

Current goals include the identification and development of novel pathogens/strains and toxins that increase efficacy and extend the activity range.

Among other types of biological insecticides are entomopathogenic viruses such as *Spodoptera littoralis* nucleopolyhedrovirus, *Helicoverpa armigera* nucleopolyhedrovirus (HearNPV), and *Cydia pomonella* granulovirus (CpGV).

4.4.4 Plant Extracts Used in Plant Protection

Several plant extracts, decoctions, oils, and powders have been demonstrated to act as PPPs, being approved as fungicides, insecticides, acaricides, repellents or plant growth regulators.

When such natural products are approved as active substances in PPPs, the qualified presumption that they are safe is considered if these botanicals or botanical extracts are used as

ingredients in food supplements or traditional herbal medicine.

Equisetum arvense L. is used as a natural PPP, prepared as a decoction in the water of dried edible sterile aerial stems. Its application as a fungicide is used to control foliar pathogens such as *Venturia inaequalis* and *Podosphaera leucotricha* in apple trees; *Taphrina deformans* in peach trees; downy and powdery mildew in grapevine; *Podosphaera xhanthii*, *Pythium* spp., *Alternaria solani*, and *Septoria lycopersici* in legumes (SANCO/12386/2013–rev. 5/2014).

Mustard seed powder of *Sinapis alba (Brassica alba)*, *Brassica juncea*, and *Brassica nigra* is approved in plant protection as a water-dispersible powder for slurry seed treatment. It is used against common bunt *Tilletia caries* and *Tilletia foetida* in different wheat species. It is recommended as a mix of 1.5 kg of mustard seed powder with 4.5 L water to create a slurry for treating 100 kg of seeds (SANTE/11309/2017–rev. 2/2017).

Salix spp. cortex is used as a natural PPP prepared as a water infusion. Its application as a fungicide is used to control peach leaf curl, foliar scab disease, and powdery mildews in apple trees, or powdery and downy mildews in grapevine (SANCO/12173/2014–rev. 4/2015).

Urtica dioica and *Urtica urens* extract is used as natural a PPP, prepared as a complex mixture for spray applications or soil-covering (mulch). Its function in plant protection is insecticidal, fungicidal, and acaricidal. As an insecticide, *Urtica* spp. Extract is used against a wide number of aphid species on fruit trees, bean, potato, leaf vegetables, and woody ornamental plants, against flea beetle and diamondback moths on cabbage, rapeseed, and radish, or against codling moth on apple and pear trees. Its acaricidal activity is against the two-spotted spider mite and red spider mite on beans and grapevine. Against fungi, *Urtica* spp. extract is used to prevent and control *Alternaria* sp. on Brassicaceae and Cucurbitaceae species and several fruit trees, powdery mildew in cucumber, brown rot blossom blight, gray mold and black bread mold of fruit trees, downy mildew of grapevine and potato blight (SANTE/11809/2016–rev.0.1/2017).

Several plant oils have also been approved as natural PPPs. Plant oils such as Citronella oil, clove oil, spear mint oil, and rapeseed oil have been approved for different purposes, such as herbicides, acaricides, insecticides, or repellants. Sunflower oil is used as a fungicide against tomato powdery mildew (SANTE/10875/2016), and onion oil is used as an insecticide against carrot root fly (SANTE/10615/2018–rev.1/2018).

4.5 Energy Crops in Europe: Feasible Resources for Biofuel Production

In the past decades, biofuels have attracted a lot of attention owing to the increasing demand on energy resources in addition to increasing concerns about greenhouse gas emissions due to the use of fossil fuels. Based on the type of the feedstock used, biofuels are classified into four generations. First-generation biofuels make use of edible biomass, which has caused controversy because it competes with global food needs. The second-generation biofuels are based on non-edible biomass, but there are some limitations related to the cost-effectiveness when scaling-up the production to a commercial level. The third-generation biofuels use the microorganisms as feedstock, while in the case of the fourth-generation biofuels the focus is on genetically modifying microorganisms able to achieve a preferable yield in the ratio hydrogen/carbon to eliminate or minimize carbon emissions (EC 2016).

Despite European efforts in the past decade, the USA is still the leader on the biofuel market with a target of substituting 20% of transportation fossil fuels with biofuel by 2022 (Saladini et al. 2016). The European Union 2020 Climate and Energy Package has committed to a 20% reduction of greenhouse gases, in addition to a target of a 20% renewable share in the energy market and a 20% increase in energy efficiency by 2020. Future ambitious targets are set for 2030 under the Climate and Energy Framework; these targets consist of a 40% reduction in emissions, with a 27% renewable share of the energy mix (Krol et al. 2019).

In the following paragraphs we are going to analyze the economic potential of the *second-generation biofuels*, which are based on renewable alternatives by utilizing inedible lignocellulosic biomass such as annual or perennial plants. We are not aiming to present here the drawbacks of the technical conversion into biofuel (thermal, biological, enzymatic or chemical processes), but we do focus on the non-edible feedstock production as a resource for the economic income of the farmers, because this biomass is considered to be an inexpensive, attractive biofuel resource (Westensee et al. 2018). Bioethanol can be produced from lignocellulosic biomass through hydrolysis and subsequent fermentation; this is why in *bioethanol* production the use of fermentative microorganisms is a must. Such examples of microorganisms can be yeast (*Saccharomyces*), bacteria (*Zymomonas*) or even molds.

Plants have been traditionally used for food, fiber, and feed applications. Their utilization for biofuels may require the breeding of novel phenotypes, or entirely new species. Scientists have provided different strategies for the genetic selection of plants as sources of biomass for biofuel production. Genetic modification of plants provides a wide range of options for improving the composition of biomass and for plant modifications to assist the fabrication of biofuels (Furtado et al. 2014). More references and solutions are provided in a special chapter included in this handbook, "Creating products and services in plant biotechnology".

In the past decades, most of the *biodiesel* was made from soybean, rapeseed, sunflower, and palm oils, whereas soybean oil was commonly used in the USA, about 80% of the EU's total biofuel production came from rapeseed and sunflower seeds (Demirbas et al. 2016). Because of socio-economic issues, nowadays biodiesel produced from edible vegetable oils is considered non-feasible and solutions have been proposed. Apart from different forms of agricultural waste, a wide variety of plants can be used as lignocellulosic biomass for biofuel production such as poplar trees, willow and eucalyptus, miscanthus, switchgrass, reed canary grass, camelina, Jatropha jojoba oil, etc.

The EU is one of the few global biofuel markets that explicitly addresses the sustainability impacts of biofuels; models of potential future biofuel systems have permanently focused on the resources available within certain EU regions and national areas (Tomei and Helliwell 2015).

When making the choice of which energy crop should be cultivated, apart from the plants' adaptability to different European climatic areas, it is important to have an already well-defined cultivation and harvesting technology. Generally, it is recognized that grass-plants (non-woody) are preferable in terms of cultivation technology because they can be employed in common agricultural techniques, which are not bringing complications to farmers. Still, some of the farmers consider that perennial crops are more simple to cultivate and harvest, being more profitable; in the latter case, high costs are involved only during the first year, when setting up the perennial plantation; costs are assumed to be 1.5 to 3 times higher than comparable costs of annual planting/seeding (OECD 2004), which is why incentives/subsidies from the governments are required.

In Europe, over the last few years, different trials have been conducted to establish feasible technologies for the cultivation and harvesting of suitable and *non-edible energy crops*, such as sorrel, red canary grass, camelina, miscanthus, ready to be implemented on a large scale by the farmers. The largest areas of energy crops reported in 2009 by Intelligent Energy Europe were found in Finland (reed canary grass), the UK (mainly willow and miscanthus), Sweden (reed canary grass, willow), Spain and Italy (miscanthus, poplar), and Germany (miscanthus, willow). According to the EU Energy Reference Scenario 2013, about 10.6% of land uses will change across the EU28 during the period 2010–2050. The largest countries producing energy crops are expected to be Poland, France, Germany, Spain, Romania and the UK, together accounting for about 83% of the total European acreage (Perpiña Castillo et al. 2015). In the following, some technological solutions reported in different EU countries are presented.

Among *annual herbaceous plants* cultivated in Europe for energy, the most frequently studied in the past few years, proving high potential, is camelina (*Camelina sativa* L. Crantz).

Camelina (*Camelina sativa*)

Camelina (*Camelina sativa* L. Crantz) is an annual plant member of the Brassicaceae family and returned to the attention of farmers relatively recently. The main advantage of the culture is that camelina is adaptable to many different environmental conditions and the only real limitations are heavy clay and organic soils. Camelina is a short-season crop (85–100 days) that is well adapted to production in the temperate climatic zone, germinating at a low temperature.

Camelina Company España (CCE) is the European reference company for the production of camelina. CCE develops camelina plantations for the production of camelina oil and meal. In cooperation with UASMV Bucharest, CCE has delivered a simple camelina cultivation technology for farmers with lands in a continental climate. It is recommended to plant camelina on plots that are sufficiently fertile and free of weeds, especially broad leaf weeds. Land with flooding or crusting problems must be avoided, in addition to shallow soils. The presence of residual herbicides affecting the Brassicaceae family must be avoided. The seeding rate is 6–8 kg/ha and the seeding depth is less than 1 cm. The seeding moment differs; it can be early autumn or spring. Regarding fertilization, the proposed scheme is (in fertilizing units/ha): N 50–60; P 30–40; the amount of the fertilizer is distributed between background and dressing fertilization. The optimal time for harvesting is when the crop becomes a yellow-cream color (Fig. 4.3). The yields vary according to environmental conditions and vary from 1200 to 2100 kg/ha in conditioned seeds.

It has been demonstrated that camelina can be cultivated as a double crop in Romania if the following requirements are met: sowing must be done at least 3–4 weeks prior to the sowing date of this trial; fertilization is a must, in addition to watering (Dobre et al. 2014).

A simple efficiency cost calculation has been proposed in Romania by CBM Biotehgen. To set up and harvest camelina seeds from a hectare, the estimated costs are 1800 Romanian Leu (RON)/ha (about 360 Euro/ha). If technology is respected, an average of 2 tonnes of dried seeds are obtained per hectare, the source of 600 l pressed oil. The costs of pressing 1 tonne of seeds is about 170 RON (34 Euro). The final cost for 1 l of pressed camelina oil is 3 RON (0.6 Euro/l).

A Case Study for Biojet Production: Camelina

An important example of scaling-up the biojet production on a European level was the FP7 funded project "Initiative towards sustainable kerosene for aviation" (ITAKA), coordinated by SENASA (Spain). The project has demonstrated the feasibility of using the biojet mixed into conventional airport fuel systems during conventional operation of the airport. Since the project at the end of 2015, all airplanes departing from

Fig. 4.3 Camelina culture during full-flowering (left) and at full maturity, ready for harvesting (right)

Gardermoen Oslo airport are partially using biojet fuel (below 3%), which would account for about 60,000 flights and about 6 million passengers, according to Avinor statistics from January to June 2016. Such results have been possible thanks to 100% EU-made biojet fuel, based on camelina oil produced in Spain and Romania and later refined in Finland. An important conclusion from ITAKA is related to the feedstock; camelina may be cultivated in a fallow rotation scheme (in dry land from Spain) or on polluted lands (demonstrated in Romania), bringing environmental and socio-economic benefits. It is estimated that in Eastern European countries there about 900,000 ha of polluted lands are available, ready to be used for the production of feedstock for biofuel (ITAKA sources). Meanwhile, in the ITAKA project, the camelina's cultivation protocol for cropping the plant under different European climate conditions has been developed and delivered to the farmers. During the project implementation, the consortium has learnt that even on this small scale, the availability of sustainable feedstock is a clear bottleneck: new crops, such as *Camelina sativa*, require a long time to expand and become significant.

As a consequence, UASMV Bucharest, in cooperation with CBM Biotehgen Romania, has been developing a new camelina variety adapted to heavy winters and resistant to strong winds (Matei et al. 2014); after 3 years of trials, the variety was patented in Romania under the name "Madalina" (Sauca et al. 2018).

In Europe, as energy crops are the preferred *perennial species*, woody or non-woody. The most frequently studied and successful species are sorrel, reed canary grass, willow, and miscanthus.

Sorrel (*Rumex* sp.)

Sorrel is a perennial herb with aerial parts of about half a meter and with roots that run deep into the ground. It can be propagated by seeds. As an energy crop a hybrid sorrel (*Rumex patientia* × *Rumex tianschanicus*) is actually used, known as sorrel of Uteush. Long-term trials have confirmed that the hybrid sorrel is one of the perennial energy crops with potential suitable for fuel biomass cultivation as a renewable source of energy in the European temperate climate (Ustak and Ustaková 2004). The studies have revealed that it is a plant with a high ecological plasticity, cold and winter resistance, and ia tolerant to salt stress and increased humidity (Kosakivska et al. 2008). According to Zhuang et al. (2005), hybrid sorrel has also been proven to be tolerant to heavy metals, and has potential in the phytoremediation of soils contaminated with heavy metals.

Nielsen (2008) has conducted trials in Norway on hybrid sorrel. The proposed technology for small parcels (for trials) was the following: sowing into rows30 cm apart, with a rate of 11 kg/ha; mineral fertilization with P, K, and 65 kg nitrogen/ha/year for the first 2 years, and then no fertilization in the 3rd year. On large-scale production, the seeding rate is recommended to be 5 kg/ha and the output is about 500 kg/ha of seeds.

Sorrel cultivation has been also tested in the Czech Republic as a pilot plot for commercial use; tests were done with the fodder sorrel of the variety Rumex OK 2, a hybrid of *Rumex patientia L.* and *Rumex tianschanicus A.Los*. REPROMO partnership (2003), coordinated by ENVIROS (Czech Republic) has drawn some conclusions. Fodder sorrel is productive for 10–12 years; the sowing rate is 5 kg/ha; it is highly tolerant to agrometeorological conditions and has low requirements for fertilization; it can be harvested in early July; the ripe biomass has low humidity (in dry weather 15–20%), so there is no need for additional drying; the dry biomass is 10–14 tonnes/ha since the 3rd year of cultivation.

Reed Canary Grass (*Phalaris arundinacea*)

Reed canary grass is a rhizomatous, perennial grass species that can be cultivated on the low-value areas and on marginal lands, which are in use for food production. It is mostly grown as a fodder crop in Europe, especially in the northern part, such as Norway, Denmark, or Sweden. The first crop can be harvested 2 years after sowing.

In a trial conducted by Nielsen (2008) in Norway, the culture was fertilized with 85 kg

nitrogen per hectare per year in the form of mineral fertilizer also containing phosphorus and potassium. The culture was reported to have some problems because of the presence of weeds. The yield varied among the testing years from 6100 g/ha to 9000 kg/ha.

In Finland and Sweden reed canary grass is typically harvested in the spring, after the snow melts, when the water content of the biomass has decreased to the level enabling storage without additional drying. In the spring-harvested crop, the ash content is lower and the ash melting point higher than in an autumn-harvested crop (Intelligent Energy Europe 2009).

An example of good practice of the use of reed canary grass for biofuel production is the power plant of Kokkolan Voima Ltd. from Finland, which was commissioned in 2001.

Miscanthus (*Miscanthus* sp.)

Miscanthus is a perennial tropical plant, successfully adapted to temperate areas by European researchers. Its stems can be used as a heating biomass source or can be transformed into other useful products such as biogas, bioethanol or biodiesel. The sterile hybrid genotype *Miscanthus* × *giganteus*, obtained from *Miscanthus sacchariflorus* and *Miscanthus sinensis*, has attracted attention and is widely used in Europe. It has had perennial growth for 10–15 years, is considered a non-invasive plant, and the roots can reach up to 6 m deep to find water.

In Romania, a cultivation technology has been proposed by INMA Bucharest. The planting materials are rhizomes with a minimum of 3–4 viable buds; the optimal planting depth is 80–100 cm; and fertilization is compulsory only during the first year of cultivation. Harvesting starts in the 3rd year of cultivation by using harvesters or balers; every year before harvesting, the plants should be cut (in March–April) and the cuts should remain on the soil.

Harvestable *Miscanthus* yields (dry matter) have been reported to range from 15 to 44 tonnes/ha in Europe; higher values can be achieved in irrigated areas of southern Europe than in northern Europe owing to its higher average temperature and high accumulation of solar radiation (Brosse et al. 2012).

An example of cost calculation per hectare is provided by ARGE Mischantus Romania: the culture establishment cost is around 2400 Euros/ha of which about 90% is the cost of the rhizomes and the rest agricultural work); harvesting costs are about 50 Euros/ha; considering the lifetime of the culture is 25 years, of which 22 years will have full productivity, the total costs/year are 3600 Euros/ha; for an average yield of 20 tonnes of dry matter/ha/year after the 3rd year, the cost of a tonne of dry material is 7.95 Euros; if bracketing, additional costs of 20 Euros/tonne are considered.

Willow (*Salix* spp.)

Energetic willow (tree and shrub species) is characterized by a short rotation cycle and vegetative regeneration; it has rapid growth of up to 3–3.5 cm/day and a lifespan of 20–25 years. In 2–3 years, it can reach a shoots height of 6–7 m, with a shoots base diameter of 6–8 cm. Beginning with the 2nd/3rd year, a yield of at least 35 tonnes/ha/year (wet) biomass can be obtained in the form of raw biomass—bales, chips, or straight rods and pellets or briquettes. Clones of *Salix viminalis* are mainly used in energy forestry. Other species, such as *Salix dasyclados*, have also been cultivated, but to a much more limited extent.

Willow plantations are established from cuttings during spring. Planting material should be bought from a well-known source to ensure a good starting quality. Willow is usually supplied as 2- to 3-m long branches that are cut between December and March when the buds are fully dormant. The branches can be planted immediately or stored in cool conditions (−2 to −4 °C) until they are used. It is compulsory to protect the planting material from moisture loss prior to planting. Weed control during the first year is very important. The plantations are very demanding of water and nutrients, generally requiring 3–5 mm of water per day during the growing season. The demand for nutrients varies according to age of the plantation and the stage

of crop development. For example, no nitrogen fertilization is recommended in Sweden during the year of establishment, but 45 kg nitrogen per hectare should be applied during the second year (i.e., the first harvest) and 100–150 kg nitrogen during the 3rd and 4th years (Intelligent Energy Europe 2009). Willow is better harvested in the winter when the ground is frozen and the moisture content in the biomass is lowest. A constant biomass quantity can be harvested for 20–25 years. By applying adequate technology, yields of over 60 tonnes/ha can be obtained. Without irrigation, over last the 5–6 years, average yields reached at the most 30–35 tonnes/ha/year on well-administered plantations (Jovicic 2016).

An example of cost breakdown for willow cultivation (Table 4.9) is proposed by Jovicic (2016) after information provided by REBINA Group Romania (group of German–Austrian–Romanian companies promoting non-conventional and renewable energy sources).

According to an Intelligent Energy Europe report (2009), a *good practice case* is the multifunctional willow plantation in Enköping (Sweden), which is an example of large-scale energy farming. The biomass of willow is supplied in chips to the ENA Energy's CHP plant in Enköping. The original concept comprises about 80 ha of willow plantation, an irrigation system, and three ponds connected to the municipal wastewater treatment plant.

Another example of good practice in the willow sector is SalixEnergi Europa, which is a privately owned incorporated company with its head office located in southern Sweden. They have conducted plant breeding for over 25 years and delivered new willow varieties that provide superior yields and characteristics. The company is offering planting project management, from concept development, planning and preparation to site management. Planting projects of 2–2000 ha have been implemented across Europe, for private and public investors and end users.

Table 4.9 Cost breakdown (Euros/ha) for willow cultivation (after Jovicic 2016)

Action	Estimated costs
Planting material (cuttings)	1400
Soil preparation	1000
Irrigation system	1000
Harvest, maintenance, land lease/ annually	300

References

Abatenh E, Gizaw B, Tsegaye Z, Wassie M (2017) The role of microorganisms in bioremediation – a review. Open J Environ Biol 2(1):038–046

Abdullahi MS (2015) Soil contamination, remediation and plants, Chapter 18. In: Hakeem KR, Sabir M, Öztürk M, Mermut AR (eds) Soil remediation and plants. Academic, Cambridge, MA

Adhikary SD (2013) Vermicompost, the story of organic gold: a review. Agric Sci 3(7):905–917

Adriano DC, Wenzel WW, Vangronsveld J, Bolan NS (2004) Role of assisted natural remediation in environmental cleanup. Geoderma 122:121–142

Ali H, Khan E, Sajad MA (2013) Phytoremediation of heavy metals–concepts and applications. Chemosphere 91:869–881

Alkorta I, Hernandez-Allica J, Becerril JM, Amezaga I, Albizu I, Garbisu C (2004) Recent findings on the phytoremediation of soils contaminated with environmentally toxic heavy metals and metalloids such as zinc, cadmium, lead, and arsenic. Rev Environ Sci Bio-Technol 3:71–90

Andreazza R, Bortolon L, Pieniz S, Camargo FAO, Bortolon ESO (2013) Copper phytoextraction and phytostabilization by *Brachiaria decumbens* Stapf. in vineyard soils and a copper mining waste. Open J Soil Sci 3(6):273–282. https://doi.org/10.4236/ojss.2013.36032

AVINOR statistics, January–June 2016

Ayotamuno JM, Kogbara RB, Agoro OS (2009) Biostimulation supplemented with phytoremediation in the reclamation of a petroleum contaminated soil. World J Microbiol Biotechnol 25:1567–1572. https://doi.org/10.1007/s11274-009-0045-z

Azubuike CC, Chikere CB, Okpokwasili GC (2016) Bioremediation techniques–classification based on site of application: principles, advantages, limitations and prospects. World J Microbiol Biotechnol 32(11):180

Biswas B, Sarkar B, Rusmin R, Naidu R (2015) Bioremediation of PAHs and VOCs: Advances in clay mineral–microbial interaction. Environ Int 85:168–181. https://doi.org/10.1016/j.envint.2015.09.017

Bolan NS, Park JH, Robinson B, Naidu R, Huh KY (2011) Phytostabilization: a green approach to contaminant containment. Adv Agron 112:145–204. https://doi.org/10.1016/B978-0-12-385538-1.00004-4

Boroş MN, Micle V, Flămînd L, Sur IM (2016) Phytoremediation planning in the case of former industrial sites. Studia Ubb Ambientum LXI(1–2):5–14

Brosse N, Dufour A, Meng X, Sun Q, Ragauskas A (2012) Miscanthus: a fast-growing crop for biofuels and chemicals production. Biofuels Bioprod Biorefin. https://doi.org/10.1002/bbb

Brown LD, Ulrich AC (2014) Bioremediation of oil spills on land. In: Handbook of oil spill science and technology. Gulf, Houston, TX, pp 395–406

Chattopadhyay P, Banerjee G, Mukherjee S (2017) Recent trends of modern bacterial insecticides for pest control practice in integrated crop management system. 3 Biotech 7:60. https://doi.org/10.1007/s13205-017-0717-6

Conesa HM, Evangelou MWH, Robinson BH, Schulin R (2012) A critical view of current state of phytotechnologies to remediate soils: still a promising tool? Sci World J. https://doi.org/10.1100/2012/173829

Demirbas A, Bafail A, Ahmad W, Sheikh M (2016) Biodiesel production from non-edible plant oils. Energy Explor Exploit 34(2):290–318

Dobre P, Jurcoane S, Matei F, Cristea S, Farcas N, Moraru AC (2014) *Camelina sativa* as a double crop using the minimal tillage system. Roman Biotechnol Lett 19 (2):9190–9195

EC (2016). https://ec.europa.eu/clima/news/articles/news_2016100401_en

EFSA (European Food Safety Authority) (2017) Technical report on the outcome of the consultation with Member States and EFSA on the basic substance application for mustard seeds powder from *Sinapis alba* (*Brassica alba*), *Brassica juncea* and *Brassica nigra* for use in plant protection as fungicide. EFSA supporting publication 2017:EN-1169. 35 p. doi:https://doi.org/10.2903/sp.efsa.2017.EN-1169

Ekta P, Modi NR (2018) A review of phytoremediation. J Pharmacogn Phytochem 7(4):1485–1489

El-Bendary MA (2006) *Bacillus thuringiensis* and *Bacillus sphaericus* biopesticides production. J Basic Microbiol 46:158–170

Elekwachi CO, Andresen J, Hodgman TC (2014) Global use of bioremediation technologies for decontamination of ecosystems. J Bioremed Biodeg 5:225. https://doi.org/10.4172/2155-6199.1000225

EU Energy, Transport and GHG Emissions – Trends to 2050. http://ec.europa.eu/energy/observatory/trends_2030/index_en

Fravel DR, Connick WJ, Lewis JA (1998) Formulation of microorganisms to control plant diseases, Chapter 5. In: Burges HD (ed) Formulation of microbial biopesticides, 1st edn. Springer Science+Business Media, Dordrecht, pp 187–202. https://doi.org/10.1007/978-94-011-4926-6

Furtado A, Lupoi JS, Hoang NV, Healey A, Singh S, Simmons BA, Henry RJ (2014) Modifying plants for biofuel and biomaterial production. Plant Biotechnol J 12:1246–1258

Gao H, Xie Y, Hashim S, Akhtar Khan A, Wang X, Xu H (2018) Application of microbial technology used in bioremediation of urban polluted river: a case study of Chengnan River, China. Water 10(5):643

Ghosh M, Singh SP (2005) A review on phytoremediation of heavy metals and utilization of its byproducts. Appl Ecol Environ Res 3(1):1–18

Gillespie IM, Philp JC (2013) Bioremediation, an environmental remediation technology for the bioeconomy. Trends Biotechnol 31(6):329–332

Glare TR, Jurat-Fuentes J-L, O'Callaghan M (2017) Basic and applied research: entomopathogenic bacteria, Chapter 4. In: Microbial control of insect and mite pests from theory to practice. Academic, Cambridge, MA, pp 47–67. https://doi.org/10.1016/B978-0-12-803527-6.00004-4

Grison C, Escande V, Biton J (2015) Ecocatalysis – new integrated approach to scientific ecology. ISTE Press Elsevier, London

http://ec.europa.eu/assets/sante/food/plants/pesticides/lop/index.html#inline_content

http://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=activesubstance.selection&language=EN

http://www.cpeo.org/techtree/ttdescript/bioven.htm

https://www.certiseurope.co.uk/products/insecticides/

https://www.epa.nsw.gov.au/~/media/EPA/Corporate%20Site/resources/clm/140323landfarmbpn.ashx

Huang XD, El-Alawi Y, Gurska J, Glick BR, Greenberg BM (2005) A multi-process phytoremediation system for decontamination of persistent total petroleum hydrocarbons (TPHs) from soils. Microchem J 81:139–147

Huesemann MH, Moore KO (1993) Compositional changes during landfarming of weathered Michigan crude oil-contaminated soil. J Soil Contam 2:245–246

Intelligent Energy Europe (2009) Energy from field energy crops – a handbook for energy producers. Jyväskylä Innovation Oy & MTT Agrifood Research Finland

January MC, Cutright TJ, Van Keulen H, Wei R (2008) Hydroponic phytoremediation of Cd, Cr, Ni, As, and Fe: Can *Helianthus annuus* hyperaccumulate multiple heavy metals? Chemosphere 70:531–537

Jovicic B (2016) Energy willow *Salix viminalis* – biomass where you want it. Blakan Green Energy News. 26 Apr 2016

Kalogerakis N, Arff J, Banat IM, Broch OJ, Daffonchio D, Edvardsen T et al (2015) The role of environmental biotechnology in exploring, exploiting, monitoring, preserving, protecting and decontaminating the marine environment. New Biotechnol 32(1): 157–167

Kaya HK, Klein MG, Burlando TM (2008) Impact of *Bacillus popilliae*, *Rickettsiella popilliae* and entomopathogenic nematodes on a population of the scarabaeid, *Cyclocephala hirta*. Biocontrol Sci Tech 3:443–453

Khan FA (2016) Biotechnology fundamentals, 2nd edn. CRC, Boca Raton, FL

Kosakivska D, Klymchuk V, Negretzky D, Bluma D, Ustinova A (2008) Stress proteins and ultrastructural characteristics of leaf cells of plants with different types of ecological strategies. Gen Appl Plant Physiol 34(3–4):405–418

Krol DJ, Jones MB, Williams M, Ni Choncubhair O, Lanigan GJ (2019) The effect of land use change from grassland to bioenergy crops Miscanthus and reed canary grass on nitrous oxide emissions. Biomass Bioenergy 120:396–403

Leitão A (2016) Bioeconomy: the challenge in the management of natural resources in the 21st century. Open J Soc Sci 4:26–42. https://doi.org/10.4236/jss.2016.411002

Majumder DR (2016) A multifaceted bioremediation of xenobiotics using fungi. In: Sangeetha J, Thangadurai D, David M, Abdullah MA (eds) Environmental biotechnology: biodegradation, bioremediation, and bioconversion of xenobiotics for sustainable development. CRC, Boca Raton, FL

Matei F, Sauca F, Dobre P, Jurcoane S (2014) Breeding low temperature resistant *Camelina sativa* for biofuel production. New Biotechnol 31:S93

Meeinkuirta W, Kruatrachueb M, Pichtelc J, Phusantisampand T, Saengwilaib P (2016) Influence of organic amendments on phytostabilization of Cd-contaminated soil by *Eucalyptus camaldulensis*. Sci Asia 42:83–91

Nielsen HK (2008) Sorrel and reed canary grass in southern Norway. Asp Appl Biol Biomass Energy Crops III 90:75–79

OECD (2004) Biomass and agriculture sustainability, markets and policies. doi:https://doi.org/10.1787/9789264105546-en

Peixoto RS, Vermelho AB, Rosado AS (2011) Petroleum-degrading enzymes: bioremediation and new prospects. Enzyme Res. doi:https://doi.org/10.4061/2011/475193

Perez-Vargas J, Poggi-Varaldo HM, Calva-Calva G, Rios-leal E, Rodriguez-Varquez R, Ferrera-Cerralto R, Esparza-Garcia F (2000) Nitrogen-fixing bacteria capable of utilizing kerosene hydrocarbons as a sole carbon source. Water Sci Technol 42:407–410

Perpiña Castillo C, Baranzelli C, Maes J, Zulian G, Lopes Barbosa A, Vandecasteele I, Mari Rivero I, Vallecillo Rodriguez S, Batista E Silva F, Jacobs C, Lavalle C (2015) An assessment of dedicated energy crops in Europe under the EU Energy Reference Scenario 2013 Application of the LUISA modelling platform – Updated Configuration 2014. EUR 27644. doi:https://doi.org/10.2788/64726

Piotrowski J, Denich T, Klironomos JM, Graham J, Rillig M (2004) The effects of arbuscular mycorrhizas on soil aggregation depend on the interaction between plant and fungal species. New Phytol 164:365–373. doi: https://doi.org/10.1111/j.1469-8137.2004.01181.x

Prasad MNV (2003) Phytoremediation of metal-polluted ecosystems: hype for commercialization. Russ J Plant Physiol 50(5):686–700

Pulford ID, Watson C (2003) Phytoremediation of heavy metal-contaminated land by trees – a review. Environ Int 29(4):529–540

Qureshi N, Chawla S, Likitvivatanavong S, Lee HL, Gill SS (2014) The Cry toxin operon of *Clostridium bifermentans* subsp. malaysia is highly toxic to *Aedes* larval mosquitoes. Appl Environ Microbiol 80:5689–5697

Rahman MA, Hasegawa H (2011) Aquatic arsenic: phytoremediation using floating macrophytes. Chemosphere 83:633–646

Robinson BH, Banuelos G, Conesa HM, Evangelou MWH, Schulin R (2009) The phytomanagement of trace elements in soil. Crit Rev Plant Sci 28:240–266

Rodriguez-Campos J, Dendooven L, Alvarez-Bernal D, Contreras-Ramos SM (2014) Potential of earthworms to accelerate removal of organic contaminants from soil: a review. Appl Soil Ecol 79:10–25

Rorat A, Wloka D, Grobelak A, Grosser A, Sosnecka A, Milczarek M et al (2017) Vermiremediation of polycyclic aromatic hydrocarbons and heavy metals in sewage sludge composting process. J Environ Manag 187:347–353

Ross R (2018) The science behind composting. https://www.livescience.com/63559-composting.html

Ruffner B, Péchy-Tarr M, Höfte M, Bloemberg G, Grunder J, Keel C, Maurhofer M (2015) Evolutionary patchwork of an insecticidal toxin shared between plant-associated pseudomonads and the insect pathogens Photorhabdus and Xenorhabdus. BMC Genomics 16:609

Ruiu L (2018) Microbial biopesticides in agroecosystems. Agronomy 8:235. https://doi.org/10.3390/agronomy8110235

Saladini F, Patrizi N, Pulselli FM, Marchettini N, Bastianoni S (2016) Guidelines for energy evaluation of first, second and third generation biofuels. Renew Sustain Energy Rev 66:221–227

Sampanpanish P, Pongsapich W, Khaodhiar S, Khan E (2006) Chromium removal from soil by phytoremediation with weed plant species in Thailand. Water Air Soil Pollut Focus 6:191–206. https://doi.org/10.1007/S11267-005-9006-1

SANCO/12173/2014–rev. 4/2015. Final review report of the Standing Committee on Plants, Animals Food and Feed at its meeting on 29 May 2015 in view of the approval of *Salix* spp. cortex as basic substance in accordance with Regulation (EC) No 1107/2009

SANCO/12386/2013–rev. 5/2014. Final review report of the Standing Committee on the Food Chain and Animal Health at its meeting on 20 March 2014 in view of the approval of *Equisetum arvense* L. as basic substance in accordance with Regulation (EC) No 1107/2009

SANTE/10615/2018–rev.1/2018. Final review report of the Standing Committee on Plants, Animals, Food and Feed at its meeting on 20 July 2018 in view of the approval of onion oil as basic substance in accordance with Regulation (EC) No 1107/2009

SANTE/10875/2016. Final review report of the Standing Committee on Plants, Animals, Food and Feed at its

meeting on 7 October 2016 in view of the approval of sunflower oil as basic substance in accordance with Regulation (EC) No 1107/2009

SANTE/11309/2017–rev. 2/2017. Final review report of the Standing Committee on Plants, Animals, Food and Feed at its meeting on 6 October 2017 in view of the approval of mustard seeds powder as basic substance in accordance with Regulation (EC) No 1107/20091

SANTE/11809/2016–rev. 0.1/2017. Final Review report of the Standing Committee on Plants, Animals, Food and Feed at its meeting on 24 January 2017 in view of the approval of *Urtica* spp. as basic substance in accordance with Regulation (EC) No 1107/2009

Santra SC, Mallick A, Samal AC (2015) Biofertilizer for bioremediation. In: Pati BR, Mandal SM (eds) Recent trends in biofertilizers. I K International Publishing House Pvt. Ltd., pp 205–234

Sarkar J, Kazy SK, Gupta A, Dutta A, Mohapatra B, Roy A, Sar P (2016) Biostimulation of indigenous microbial community for bioremediation of petroleum refinery sludge. Front Microbiol 7:1407

Sauca F, Jurcoane S, Dobre P, Matei F, Podgoreanu E, Moraru A, Cristea S (2018) Patent ISTIS 504/2018. New variety of *Camelina sativa* – Mădălina

Schwitzguébel JP, van der Lelie D, Baker A, Glass DJ, Vangronsveld J (2002) Phytoremediation: European and American trends successes, obstacles and needs. J Soils Sediments. https://doi.org/10.1065/jss2002.03.37

Shah MP (2017) Waste water pollution. J Appl Biomater Biomech 3(1):262–263

Shams KM, Tichy G, Fischer A, Sager M, Peer T, Bashar A, Filip K (2009) Aspects of phytoremediation for chromium contaminated sites using common plants *Urtica dioica*, *Brassica napus* and *Zea mays*. Plant Soil. https://doi.org/10.1007/s11104-009-0095-x

Sharma B, Dangi AK, Shukla P (2018) Contemporary enzyme based technologies for bioremediation: a review. J Environ Manag 210:10–22

Shoji R, Yajima R, Yano Y (2008) Arsenic speciation for the phytoremediation by the Chinese brake fern, *Pteris vittata*. J Environ Sci 20:1463–1468

Sparks T, Dripps J, Watson G, Paroonagian D (2012) Resistance and cross-resistance to the spinosyns – a review and analysis. Pestic Biochem Physiol 102:1–10

Stephenson C, Black CR (2014) One step forward, two steps back: the evolution of phytoremediation into commercial technologies. Biosci Horiz. https://doi.org/10.1093/biohorizons/hzu009

Tomei J, Helliwell R (2015) Food versus fuel? Going beyond biofuels. Land Use Policy 56:320–326

Trapp S, Karlson U (2001) Aspects of phytoremediation of organic pollutants. J Soil Sediment I:37–43

Ustak S, Ustaková M (2004) Potential for agricultural biomass to produce bioenergy in the Czech Republic. In: Parris K (ed) Biomass and agriculture: sustainability, markets and policies. OECD, Paris, pp 229–239

Vamerali T, Bandiera M, Mosca G (2010) Field crops for phytoremediation of metal-contaminated land. A review. Environ Chem Lett 8:1–17. https://doi.org/10.1007/s10311-009-0268-0

Vangronsveld J, Herzig R, Weyens N, Boulet J, Adriaensen K, Ruttens A, Thewys T, Vassilev A, Meers E, Nehnevajova E, van der Lelie D, Mench M (2009) Phytoremediation of contaminated soils and groundwater: lessons from the field. Environ Sci Pollut Res 16:765–794. https://doi.org/10.1007/s11356-009-0213-6

Vasavi M, Usha R, Swamy PM (2010) Phytoremediation – an overview review. J Ind Pollut Control 26(1):83–88

Wang XJ, Wang JJ, Wang JD, Zhang J, Xu MD, Xiang WS (2011a) Two new doramectin analogs from *Streptomyces avermitilis* NEAU1069: fermentation, isolation and structure elucidation. J Antibiot 64: 591–594

Wang XJ, Zhang J, Wang JD, Huang SX, Chen YH, Liu CX, Xiang WS (2011b) Four new doramectin congeners with acaricidal and insecticidal activity from *Streptomyces avermitilis* NEAU1069. Chem Biodivers 8:2117–2125

Wang R, Cao Q, Zhao Q, Li Y (2018) Bioindustry in China: an overview and perspective. New Biotechnol 40:46–51

Wenzel WW, Lombi E, Adriano DC (2004) Biogeochemical processes in the rhizosphere: role in phytoremediation of metal-polluted soils. In: Prasad M, Hagemeyer J (eds) Heavy metal stress in plants: from biomolecules to ecosystems. Springer, Berlin, pp 273–303

Westensee DK, Rumbold K, Harding KG, Sheridan CM, Van Dyk LD, Simate GS, Postma F (2018) The availability of second generation feedstocks for the treatment of acid mine drainage and to improve South Africa's bio-based economy. Sci Total Environ 637:132–136

White Jr PM, Wolf DC, Thoma GJ, Reynolds CM (2005) Phytoremediation of alkylated polycyclic aromatic hydrocarbons in a crude oil-contaminated soil. Water Air Soil Pollut 169: 207–220

Whiteley CG, Lee DJ (2006) Enzyme technology and biological remediation. Enzym Microb Technol 38 (3–4):291–316

Woo SL, Ruocco M, Vinale F, Nigro M, Marra R, Lombardi N, Pascale A, Lanzuise S, Manganiello G, Lorito M (2014) *Trichoderma*-based products and their widespread use in agriculture. Open Mycol J 8(Suppl-1, M4):71–126

Yadav KK, Gupta N, Kumar A, Reece LM, Singh N, Rezania S, Khan SA (2018) Mechanistic understanding and holistic approach of phytoremediation: a review on application and future prospects. Ecol Eng 120:274–298

Yateem A (2014) Bioremediation strategies for the clean-up of oil contamination in an arid environment: an insight on the Kuwaiti experience. In: Velazquez-Fernandez JB, Muniz-Hernandez S (eds) Bioremediation: processes, challenges and future prospects. Nova, Hauppauge, NY, pp 269–284

Yavari S, Malakahmad A, Sapari NB (2015) A review on phytoremediation of crude oil spills. Water Air Soil Pollut 226(8):226–279

Ying OY (2002) Phytoremediation: modeling plant uptake and contaminant transport in the soil-plant-atmosphere continuum. J Hydrol 266(1–2):66–82

Zhu X, Venosa AD, Suidan MT (2004) Literature review on the use of commercial bioremediation agents for cleanup of oil-contaminated estuarine environments EPA/600/R-04/075

Zhuang P, Ye ZH, Lan CY, Xie ZW, Shu WS (2005) Chemically assisted phytoextraction of heavy metal contaminated soils using three plant species. Plant Soil 276:153–162