Chapter 6
Mechanisms and Importance of Phytoremediation

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6.1 Introduction

Sustainability principles are essential to be respected when human beings interact with different environs; otherwise, anthropogenic actions can cause damage to living forms and also pollute air, water, and soil with organic and/or inorganic pollutants product of mine exploitation; industrial wastes from the production of fabrics, medicines, and paints; exploitation of offshore oil/petroleum; pesticides' use; etc. (Jafari et al. 2013; Bhat et al. 2017; Barrios-Estrada et al. 2018; Carpenter 2018; Bilal et al. 2019; Mendes et al. 2019; Pesantes et al. 2019; Pu et al. 2019; Rosculete et al. 2019; Vázquez-Luna and Cuevas-Díaz 2019; Zhang et al. 2019).

Some of these contaminants, known as persistent contaminants, are even more damaging due to the fact that they may last for a very long time without transformations on contaminated environs, posing as threats for generations (Bhat et al. 2018; Mushtaq et al. 2018). Aromatic molecules, for example, are hardly ever easily degraded, persisting on the polluted sites (Parrilli et al. 2010). Others can bioaccumulate in some organisms, reaching high levels and presenting high risk of causing chronic poisoning to the organism or to an organism that eats the first one. Pharmaceuticals, for example, when thrown in water, such as in rivers, can bioaccumulate in zooplankton (Xie et al. 2017), algae (Vannini et al. 2011), mussels (Maruya et al. 2014), fish (Du et al. 2012), among others. There are also pollutants

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that can do both. Heavy metals, for example, are highly toxic (being able to poison, induce cancer, cause death) and persistent contaminants that may accumulate in living organisms, performing trophic level transfer in food chain (Jaishankar et al. 2014; Jacob et al. 2018; Ali et al. 2019; Rashid et al. 2019; Mehmood et al. 2019).

Therefore, it is necessary that strategies capable of restoring polluted environs be developed. Polluted water causes concern worldwide once it is essential that water of good quality can be offered to human beings to live healthy. However, conventional methods of water treatment (such as reverse osmosis) might present some important disadvantages to be performed in large scale as elevated costs and generation of toxic waste (Dasgupta et al. 2015; Albering et al. 2016; Bilal et al. 2018).

Among the new strategies developed are the ones related to bioremediation: methods that use biological agents to clean up contaminated environs (Strong and Burgess 2008). The use of plants in these strategies originated the phytoremediation, and there are also researchers that consider the microbes associated to plants as part of this remediation's concept (He et al. 2020). Phytoremediation can be performed through different mechanisms, depending on plant, polluted environ, and contaminant characteristics (Panesar et al. 2019).

This chapter will address the phytoremediation as a strategic method to deal with polluted environs in an attempt to restore them, also highlighting the mechanisms by which plant species can remediate.

6.2 Phytoremediation of Polluted Environs

Phytoremediation is a remedial approach that allows in situ recovery of polluted environs in a sustained way, and some mechanisms (such as rhizofiltration) also allow ex situ remediation (Panesar et al. 2019; Bhat et al. 2018). There are some plant species that can remediate various types of pollutants, inorganic (such as radioactive isotopes, phosphates, heavy metals, nitrates) or organic (such as dyes, pesticides, fuels) (Dushenkov 2000; Horne 2000; Nwoko and Egunjobi 2002; Nwoko et al. 2004; Okeke et al. 2004; Singh et al. 2018); *Eichhornia crassipes* (water hyacinth), for example, can remediate dyes, phosphate, heavy metals, and radioactive isotopes (Jayaweera and Kasturiarachchi 2004; Nie et al. 2015; Thapa et al. 2016; Priya and Selvan 2017).

Phytoremediation can be performed through different mechanisms, depending on plant and contaminant characteristics. Phytostabilization, phytovolatilization, and mechanisms involving pollutant sequestration are commonly the ones used by plants to remediate inorganic pollutants, while organic ones are commonly phytoremediated by phyto- and rhizodegradation (Nwoko 2010).

Remediation promoted by plant species can suffer interference by the presence of other plant species and microorganism (especially in phytodegradation, a process in which microorganisms associated to plant's roots take part). *Eupatorium* *odoratum* extract at low concentration, for example, can attenuate formaldehyde toxic effects over *E. crassipes*, enhancing phytoremediation (Gong et al. 2018). Especially when dealing with mixed pollutants, phytoremediation can also be improved by the participation of plant growth-promoting rhizobacteria and plant endophytes (including bacteria and fungi) in a process known as microbe-assisted phytoremediation (He et al. 2020).

It is also important to mention that not only microorganism and the presence of other plant species can affect the phytoremediation efficiency. The pH of the area to be remediated, the composition regarding pollutants (if it is mixed in contaminants that need to be remediated), the presence of fertilizers if it is related to soil remediation, and the presence of chelators can impact the results obtained (Rostami and Azhdarpoor 2019; Xu et al. 2019, Yu et al. 2019; He et al. 2020).

Especially when it comes to remediating heavy metals, biochar is an interesting tool. It is produced from various kinds of biomass through pyrolysis and carbon sequestration (Woolf et al. 2010), also regulating soil pH and reducing plants' stress caused by contaminants (Kiran and Prasad 2019). Biochars could, for example, increase *Ricinus communis* Pb tolerance to sustain phytoremediation, helping the plant to deal with oxidative stress (producing more antioxidant enzymes) and improve nutrient intake as same as plant growth (Kiran ans Prasad 2019).

Phytoremediation presents itself as an advantageous strategy to restore environs from pollution once it presents low initial investment requisition and is an auto-sustained process well accepted as a feasible and safe remediation strategy (Muthusaravanan et al. 2018).

There are various mechanisms of phytoremediation and various plant species available to be used in this kind of procedure. Depending on the type of pollutant and the type of environment that is to be recovered through remediation, certain species and mechanisms are more suitable.

6.3 Mechanisms of Phytoremediation

Phytoremediation of contaminated environs can occur by different mechanisms (Fig. 6.1 and 6.2) and involving different plant species (Table 6.1). Each mechanism of phytoremediation possesses its peculiarities regarding the type of environment in which they can remedy more efficiently and which is the main type of pollutant they can deal with and involving different plant species that receive or not help from microorganisms during the process.

The main types of mechanisms of phytoremediation – phytohydraulic control, phytoaccumulation, phytodegradation, phytostabilization, phytovolatilization, rhizofiltration, and rhizodegradation – are revisited in this chapter.

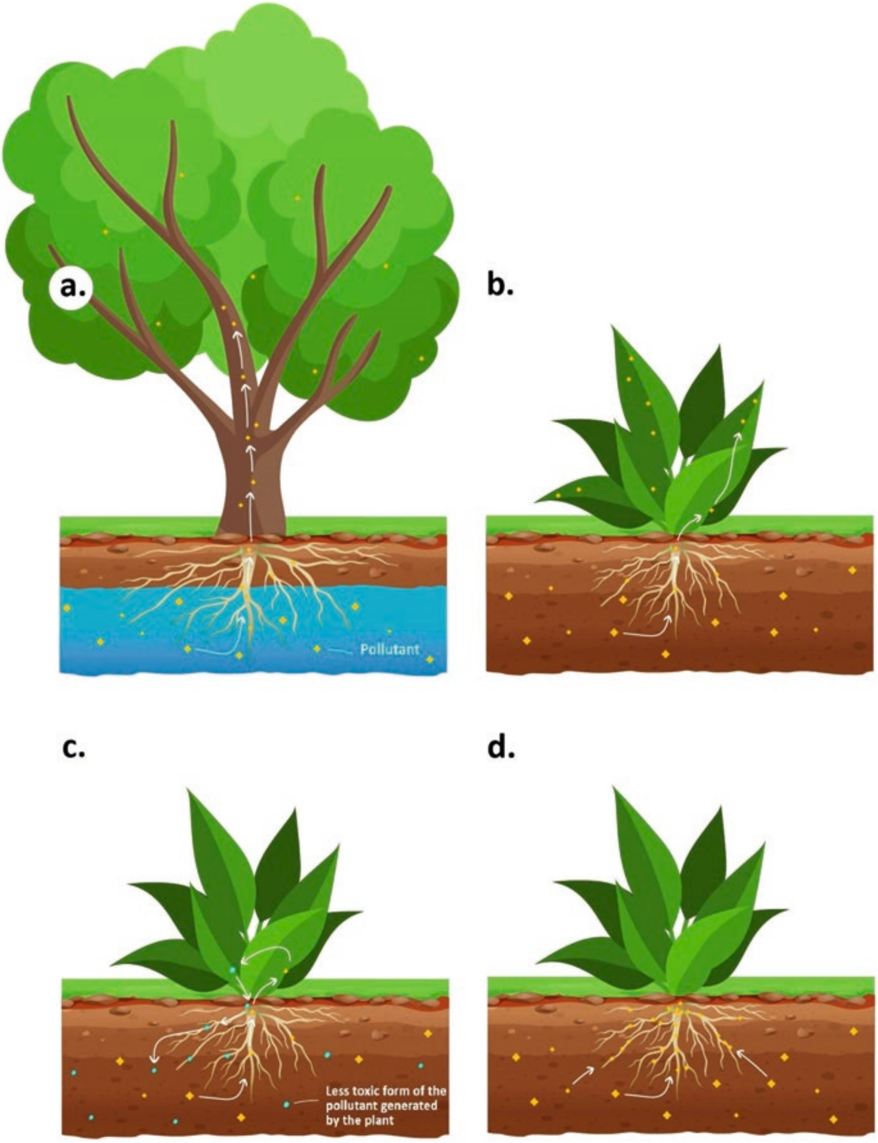

Fig. 6.1 Phytoremediation mechanisms: (**a**) phytohydraulic control; (**b**) phytoaccumulation; (**c**) phytodegradation; (**d**) phytostabilization

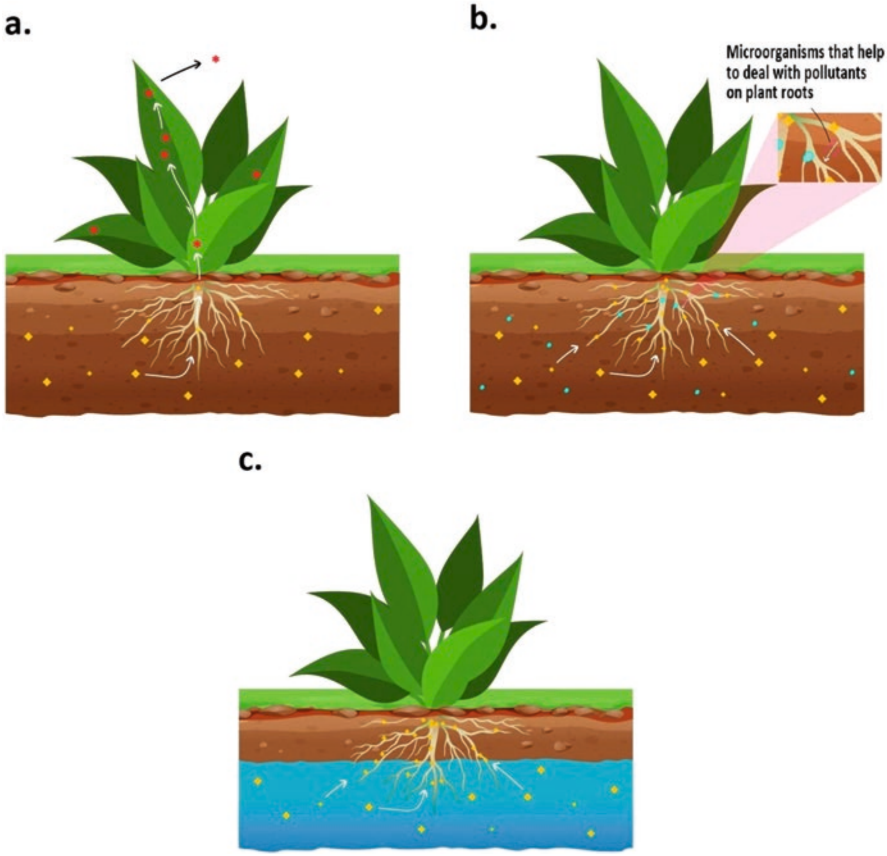

Fig. 6.2 Phytoremediation mechanisms: (**a**) phytovolatilization; (**b**) rhizodegradation; and (**c**) rhizofiltration

6.4 Phytohydraulic Control

The hydraulic control is a kind of indirect phytoremediation strategy especially regarding to groundwater and involving tree species (Fig. 6.1a) (Khalifa and Alkhalf 2018). It consists in a method by which plants act as natural pumps contributing to regulate groundwater's cycle/movement: roots take up water from groundwater, plants can use this or return it into the environment (through transpiration), and then this water can be condensed to return to the groundwater after raining. If, however, the groundwater contains pollutants, the hydraulic control can help to control, limit, and diminish migration or even clean up the area, removing contaminants among the water mass taken up and sometimes phytotransforming them (Ahlfeld and Heidari 1994; Muthusaravanan et al. 2018). The most interesting species to perform this kind of remediation are plants with large root mass and that can release a considerable part of the water taken up into the environment, in other words, species

Table 6.1 Examples of plant species suitable for phytoremediation

Plant species	Favorite remediation mechanism	Pollutant	Reference
Polygonum punctatum	Hydraulic control	Perchlorates	Susarla et al. (2000)
Nymphaea odorata	Hydraulic control	Perchlorates	Susarla et al. (2000)
Allenrolfea occidentalis	Hydraulic control	Perchlorates	Susarla et al. (2000)
Vetiveria zizanioides	Phytoaccumulation	Polycyclic aromatic hydrocarbons	Un Nisa and Rashid (2015)
Cyperus rotundus	Phytoaccumulation	Cadmium and chromium	Subhasini and Swamy (2014)
Brassica juncea	Phytoaccumulation	Copper, lead, and nickel	Singh and Sinha (2005)
Centella asiatica	Phytoaccumulation	Copper	Mokhtar et al. (2011)
Eichhornia crassipes	Phytoaccumulation	Copper	Mokhtar et al. (2011)
Arabidopsis thaliana	Phytoaccumulation	Cesium	Adams et al. (2017)
Chromolaena odorata	Phytodegradation	Sodium dodecyl sulfate	Gong et al. (2019)
Eichhornia crassipes	Phytodegradation	Malathion, ethion	Xia and Ma (2006)
Scirpus grossus	Phytodegradation	Petroleum hydrocarbon	Al-Baldawi et al. (2015)
Myriophyllum aquaticum	Phytodegradation	Trinitrotoluene	Rajakaruna et al. (2006)
Senna multijuga and peat	Phytostabilization	Copper	De Marco et al. (2017)
Festuca rubra	Phytostabilization	Copper	Radziemska et al. (2017)
Osmanthus fragrans, Ligustrum vicaryi, Cinnamomum camphora, Loropetalum chinense, and *Euonymus japonicus*	Phytostabilization	Cadmium	Zeng et al. (2018)
Typha latifolia	Phytovolatilization	Selenium	LeDuc and Terry (2005)
Brassica juncea	Phytovolatilization	Mercury	Moreno et al. (2008)
Populus deltoides and *Populus nigra*	Phytovolatilization	Perchloroethylene	James et al. (2009)
Pistia stratiotes	Rhizofiltration	Iron, chromium, lead, and copper	Galal et al. (2018)
Arundo donax	Rhizofiltration	Copper	Oustriere et al. (2017)

(continued)

Table 6.1 (continued)

Plant species	Favorite remediation mechanism	Pollutant	Reference
Phragmites australis	Rhizofiltration	Uranium	Wang and Dudel (2017) and Wang and Dudel (2018)
Phleum pratense	Rhizofiltration	137 cesium	Mikheev et al. (2017)
Sesbania cannabina	Rhizodegradation	Petroleum hydrocarbons	Maqbool et al. (2012)
Medicago sativa	Rhizodegradation	Hydrocarbons from oily sludge	Bano et al. (2015)
Sorghum x drummondii	Rhizodegradation	Polycyclic aromatic hydrocarbons	Dominguez et al. (2019)

that can transpire large volumes of water, influencing the existing water balance. These species remove a great amount of polluted groundwater and reduce infiltration/leaching of contaminants back to the water table (Etim 2012). In *Populus* gender there are species that are considered high-transpiring ones (Hirsh et al. 2003). Some genotypes from the species *Populus nigra* L., for example, are suitable to perform this kind of remediation. The Spanish genotype can generate organisms with low water use efficiency and high capacity to use hydraulic control to remediate, possessing a high transpiration rate and a large root system (Bogeat-Triboulot et al. 2019).

6.5 Phytoaccumulation

This mechanism of phytoremediation is also called phytoextraction (Fig. 6.1b). It consists in removing contaminants, especially inorganic ones, without destroying them, from polluted spots, primarily from soil (Ahalya and Ramachandra 2006), and concentrating them in plant parts (commonly shoots or leaves) (Rashid et al. 2014; Muthusaravanan et al. 2018). The pollutants, such as heavy metals (Kamal et al. 2004), are captured by the plant among water and nutrients from polluted spots and stored by plants. When it comes to heavy metals, for example, plants' capacity to store them in a large amount depends upon the rate through which roots can uptake the metal, rate of its internal translocation to shoots, how well plant cells can tolerate increasing concentration of the pollutant without experiencing cytotoxicity, and bioavailability of the heavy metal in rhizosphere (Etim 2012). There are organisms, known as hyperaccumulators, that store high amounts of pollutants, being suitable to be used to remediate environs. When it comes to copper, for example, these organisms can store more than 1000 μg from this metal to 1 g of dry plant. The hyperaccumulator *Calendula officinalis* could tolerate high soil concentrations of copper, and at 300 mg/kg of this metal in soil samples, the plant species could accumulate this pollutant in leaf at 4675 μg/g and in root at 3995 μg/g. Antioxidant enzymes such as superoxide dismutase and catalase helped the plant to deal with Cu stress, making high accumulation rates possible (Goswami and Das 2016).

6.6 Phytodegradation

Through this mechanism, also known as phytotransformation (Fig. 6.1c), plants can not only uptake especially organic pollutants but also promote their breakdown into less complex substances by plant's metabolic pathways or by special enzymes produced by plants to perform this role (Newman and Reynolds 2004; Trap et al. 2005). Pollutant's characteristics such as hydrophobicity influence on the remediation efficiency once it interferes on uptake efficiency, as same as contaminants' concentration on the spot and plant phytochemical properties (Etim 2012). Pharmaceuticals are an increasingly concerning problem when it comes to environs' pollution by persistent and biologically active contaminants. In this way, phytoremediation strategies are being developed to deal with this challenging issue. *Phragmites australis*, a wetland plant, could uptake and transform Ibuprofen using enzymes such as cytochrome P450 monooxygenase; the pollutant was degraded in hydroxy-, 1,2-dihydroxy-, carboxy-, and glucopyranosyloxy-hydroxy-Ibuprofen versions (He et al. 2017).

6.7 Phytostabilization

Contaminants can be immobilized, having their soil migration limited, by this remediation mechanism, reducing their bioavailability and consequently their damaging risks. Through this strategy (Fig. 6.1d), the pollutants do not enter vegetative parts of plants, being kept in rhizosphere (Berti and Cunningham 2000; Mendez and Maier 2008; Muthusaravanan et al. 2018). It is a very useful remediation technique to ecological restoration of polluted spots such as mine areas. *Erica australis* could efficiently sequestrate in root cortex toxic elements such as Cd, Cu, Pb, and S and could also tolerate extreme acidic condition, being suitable for remediation of areas containing mine wastes. This plant species also presented interesting characteristic of favoring the reestablishment of vegetation, improving the survival capacity of plant species less tolerant, such as *Nerium oleander* (Monaci et al. 2019). If inorganic soil amendments are added to the sample to be remediated, it can improve the capacity of some plant species to promote remediation; limestone could improve Cu, Ni, and Cd phytostabilization performed by *Festuca rubra* (Radziemska et al. 2019). The presence of organic matter such as manure-based compost or biomass product after pyrolysis (known as biochar) (Saletnik et al. 2019) could help to immobilize the pollutants such as metallic ones (Cd and Zn) by corn plants (Sigua et al. 2019). Microorganisms can also improve plants' ability to promote phytostabilization of pollutants. The fungi *Funneliformis mosseae* could improve *Robinia pseudoacacia* Pb phytostabilization by helping to immobilize the pollutant and could also attenuate its toxicity to roots (Huang et al. 2019).

6.8 Phytovolatilization

This mechanism of phytoremediation consists in plants uptaking contaminants from polluted areas, generally converting them to a less toxic form, and then releasing them to the atmosphere as volatile products from leaves or stems, in a process known as direct phytovolatilization (Fig. 6.2a), or from roots – the indirect type of phytovolatilization (Limmer and Burken 2016). However, it is also possible that the form released by plants to the atmosphere still possesses a concerning toxicity to living forms, so it is essential to first analyze if a certain pollutant would be suitable for phytovolatilization without provoking additional damage. This mechanism of phytoremediation should be avoided to remediate, for example, samples polluted with arsenic and mercury by *Pteris vittata*, once the final products (dimethyl selenide and mercuric oxide) generated by the plant are also toxic (Sakakibara et al. 2010). Species from *Populus* and *Salix* gender are commonly used in this kind of strategy due to their characteristic of uptaking contaminants very well and also *Brassica juncea* and *Arabidopsis thaliana* that possess the ability to convert heavy metal pollutants into volatile forms (Pulford and Watson, 2003; Ghosh and Singh 2005). Trichloroethylene could be remediated by *Eucalyptus sideroxylon* through phytovolatilization from the leaves and roots (soil) in Travis and Fairchild Air Force Bases, California (Doucette et al. 2013).

6.9 Rhizodegradation

Through this mechanism (Fig. 6.2b), plant species can degrade organic contaminants working together with microorganisms associated to plant's roots, which have their development/growth stimulated by metabolites (e.g., amino acids and growth factors) released by these plants in rhizosphere (Dominguez et al. 2019). *Hylotelephium spectabile* could help to deal with petroleum hydrocarbons' pollution mainly through rhizodegradation (maximum rate of 53.3%), which was optimized by inducing the presence of microorganism with capacity to degrade these pollutants and with high salt tolerance (*Alcanivorax* and *Bacteroidetes*). Compared to the control group, the plants involved in promoting remediation increased their gene copy number of genes related to petroleum hydrocarbons' degradation up to 14.44 times (Cheng et al. 2019).

6.10 Rhizofiltration

This mechanism of phytoremediation (Fig. 6.2c) is similar to phytoaccumulation/phytoextraction: pollutants, through biotic or abiotic process, are absorbed by plant's roots or hold on tightly to this plant part (Panesar et al. 2019). However, rhizofiltration focus on remediating polluted groundwater (mainly with metallic contaminants), while phytoaccumulation/phytoextraction is applied mainly to polluted soil (Ahalya and Ramachandra 2006). Plants that will be used to perform this type of remediation are grown hydroponically and not in soil and can perform in situ or ex situ remediation (Mikheev et al. 2017; Tiwari et al. 2019). Within 24 h of experiment, *Helianthus annuus* could remove, through rhizofiltration, 80% of the uranium present in contaminated water from groundwater and laboratory solution samples, offering final concentrations that were under the drinking water limit; *Phaseolus vulgaris* presented the capacity of removing 60–80% of the contaminant, and both species offered a root's capacity of removing this contaminant superior than 500 mg/kg (Lee and Yang 2010).

6.11 GMPs and Phytoremediation

As previously discussed in this chapter, there are plant species naturally capable of promoting environs' remediation. However, depending upon pollutant concentration and toxicity mechanism, the stress induced in the vegetal can limit the success of the phytoremediation or even induce plant's death (Tiwari and Lata 2018).

Genetic manipulation arises as a tool to allow DNA manipulation, for example, to improve plant's resistance to pollutants to phytoremediate them, to improve the efficiency of plants' previous ability to remediate, and to convert a vegetal that was not able to remediate into an organism that can perform this process (Prasad 2019).

To generate a transgenic plant, the most popular methodology involves the use of organisms from *Agrobacterium* gender (*Agrobacterium rhizogenes* or more commonly *Agrobacterium tumefaciens* (Cunningham et al. 2018)).

It is common, for example, strategies that use genes from plants that possess a high capacity to deal well with pollutants, to generate a genetically modified plant (GMP) with an improved phytoremediation potential. The gene that codifies a selenocysteine methyltransferase could be obtained from *Astragalus bisulcatus* (a plant species that can hyperaccumulate selenium) and inserted into *Brassica juncea*'s DNA to enable it to remediate the pollution caused by this element (LeDuc et al. 2004). The MT2 coding sequence from *Sedum alfredii* could reprogram *N. tabacum* to tolerate better and accumulate larger amounts of the pollutant copper (Zhang et al. 2014).

Bacterial genes can also be used in the generation of a GMP. The *atzA* gene from *Pseudomonas* sp. after being inserted in *Medicago sativa* and *N. tabacum* (tobacco plant) could allow the production of the enzyme atrazine chlorohydrolase, allowing the transgenic to efficiently remediate the pollution caused by the pesticide atrazine (Wang et al. 2005). *Enterobacter cloacae NfsI* gene when inserted into tobacco plants' DNA led to the production of a nitroreductase that allowed remediation of sample polluted with 2,4,6-trinitrotoluene (TNT) (Hannink et al. 2007). The gene of a nitroreductase from *Escherichia coli* allowed a similar effect on the genetically modified *Arabidopsis thaliana* (Kurumata et al. 2005). Other gene from *E. cloacae* (*Onr* gene) allowed nitroglycerin's remediation to be performed by transgenic *N. tabacum* (French et al., 1999). *Rhodococcus rhodochorus*' *XplA* and *XplB* genes allowed *Arabidopsis thaliana* to remediate 1,3,5-trinitro-1,3,5-triazine (Jackson et al. 2007). Transgenic tobacco plants expressing *Pseudomonas putida* genes *CzcB* or *CzcA* could efficiently accumulate more Cd in the roots than wild-type plants (Nesler et al., 2017). The gene *copC* from *Pseudomonas fluorescens* was used to generate transgenic tobacco hairy roots, converting these organisms into copper hyperaccumulators that could deal well with the stress induced by the heavy metal (Pérez-Palacios et al. 2017). Genes *nfsI*, *xplA*, and *xplB* from bacteria allowed *Pascopyrum smithii* to remediate, respectively, the explosives 2, 4, 6-trinitrotoluene and hexahydro-1, 3, 5-trinitro-1, 3, 5-triazine (*xplA* and *xplB*) (Zhang et al. 2018).

Human genes such as *CYP2E1* could also be used to generate transgenic *Arabidopsis thaliana*, capable of remediating samples polluted with trichloroethylene and ethylene dibromide (Doty et al., 2000). *CYP1A1*, *CYP2B6*, and *CYP2C19* genes allowed *Oryza sativa* to phytoremediate the pesticides atrazine, metolachlor, and simazine (Kawahigashi et al. 2006).

The genes used to generate transgenic plants to perform phytoremediation can also be sequences from the same species to generate autotransgenic organisms that will overexpress the transgene. Autotransgenic potato designed to overexpress StDREB transcription factors could deal more efficiently with Cd pollution (Charfeddine et al. 2017).

6.12 Conclusion

Phytoremediation is an interesting strategy to remediate polluted environs containing pollutants from diverse chemical nature, organic or inorganic. It can be performed through different mechanisms such as phytohydraulic control, phytoaccumulation, phytodegradation, phytostabilization, phytovolatilization, rhizofiltration, and rhizodegradation. The process' efficiency depends upon some plants characteristics, the area to be remediated, and the contaminant to be remediated. Strategies such as using biochar and microorganisms can favor the aimed results to be achieved. Besides presenting advantageous characteristics intrinsic to the remediation involving plants, phytoremediation may also benefit from generation of genetically modified plants with enhanced remediation capacity, converting even plants that normally cannot remediate in organisms able to clean up contaminated environments.

6.13 Futures Perspectives

It is expected that bioremediation protocols continue to be proposed involving the use of plant species and also strategies to improve the remediation capacity. However, when these strategies involve recombinant DNA technology, it is also expected a special concern regarding safety of these modified organisms' use in the field.

It is also expected that phytoremediation limitations, such as incomplete remediation; roots' size that does not allow cleaning deep inside aquifers; species that can hyperaccumulate but do not degrade pollutants, presenting a risk of food chain contamination with high level of pollutants; the necessity of large areas under controlled situation for field studies; and the risk of converting into air pollution the contaminants remediated through phytovolatilization, can be successfully surpassed.

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