Chapter 5
Advances in Plant–Microbe-Based Remediation Approaches for Environmental Cleanup

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Abstract In the present era, one of the most concerning issues is environmental contamination which is endangering human health and the ecosystem, thus the identification and proper implementation of suitable technologies for remediation of contaminated sites is a prerequisite for sustainable development. In this context, several methods have been developed for the mitigation of the adverse impacts of toxic/hazardous contaminants. In the past decade, lot of research have been focused over improving the performance of established remedial technologies with the objective of eliminating the drawbacks and reducing the contaminant concentration to acceptable limits. Plant–microbe interaction has not been extensively studied in agriculture field only but another area in which the partnerships of plants and microbes have been explored is environmental cleanup. Plant–microbe interaction has been found to be a promising approach for in situ remediation of various organic/inorganic pollutants. It offers several ecological and cost-associated benefits. Plant–microbe-assisted phytoremediation could be improved further through genetically modified plants and microbes. The present chapter reviews the role of plant–microbe partnership in removal/detoxification/degradation of different category of contaminants. Additionally, the advancements made in microbe-assisted phytoremediation through the use of transgenic recombinants and integrated nanotechnology are also discussed.

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5.1 Environmental Pollution and Its Effect on Organisms: An Overview

Our earth is getting progressively polluted with different types of inorganic and organic compounds, primarily as a result of anthropogenic activities (Kumar et al. 2017a, 2019). Natural resources (soil, air, and water) have faced a tremendous amount of pressure because of the rising human population and their associated activities. Uncontrolled discharge of effluent from industries in water led to a rapid increase in effluent concentration which alters the nature of ecosystem and adversely affects the health of human beings, plant, and animals (Kumar et al. 2014, 2019; Yadav et al. 2019). There are several ways by which huge amount of toxic compounds enter into the environment.

Industrial processing, facilities for sewage as well as waste water detoxification, accidental oil and chemical spillage, mining processes, military operations, and mobile sources are the pathways for wide range of contaminants through which they enter into different environmental matrices (Kumar et al. 2013, 2014, 2015, 2018a; Singh and Chandra 2019). After getting into the environment, the contaminants can be consumed or inhaled or absorbed by primary consumers that sequentially enter the food chain and get bioaccumulated and then biomagnified at successive trophic levels (Ghavri et al. 2013; Ren et al. 2017). If the adulterants enter the sediments, they could potentially affect the costal bodies and larger water bodies which in turn can have adverse impact on human health (Singh and Chandra 2019). There are some naturally occurring contaminants whose availability and mobility towards food chain may be enhanced due to human activity (Ren et al. 2017).

Contaminants including a wide range of various heavy metals, polyaromatic hydrocarbons (PAHs), persistent organic pollutants (POPs), and polychlorinated biphenyls (PCBs) have already been proven to remain in food chain (Ren et al. 2017; Yadav et al. 2016a, El-Shahawi et al. 2010). Among several toxic contaminants, heavy metal or metalloid, and organic pollutant pose serious threat to plants and animals including humans (Kumar et al. 2014, 2015; Cherian and Oliveira 2005; Yadav et al. 2016b, 2017; Mishra et al. 2018). Heavy metal toxicity in environment is considerably higher due to mining activities (Shahid et al. 2015). Common heavy metals including lead, arsenic, chromium, nickel, zinc, manganese, mercury, aluminum, cadmium, and cobalt occur naturally. As per USEPA (2004) and ATSDR (2012), lead, arsenic, cadmium, and mercury are considered as most significant among top major 20 hazardous adulterants. Human beings are being exposed to the risk of heavy metal toxicity through the uptake of contaminated vegetables, cereals, and pulses (Kumar et al. 2014, 2015; Pierart et al. 2015; Xiong et al. 2016). A lot of serious health disorders viz., nervous system impairment, anemia, cancer, kidney dysfunctions, cognitive impairment, and damage of brain, etc. have been documented as a result of heavy metal toxicity (Jarup 2003).

Generally, inorganic contaminants remain persistent in nature and could pose genotoxic, carcinogenic, mutagenic, and teratogenic effects even at low concentration (Saxena et al. 2019). They also act as endocrine disruptors and persuade developmental as well as neurological disorders, and hence their removal from environment is of prime importance for the betterment of the human society (Saxena et al. 2019). On the other hand, organic pollutants are mostly human generated and have been widely used as industrial solvents, fuel components, and intermediates. Lots of manufactured products such as paints, adhesives, gasoline, and plastics contain harmful organic compounds (Collins et al. 2002; Chandra et al. 2011). Organic compounds like PCBs, TCE, PCE, chloroform, etc. are carcinogenic and neurotoxic in nature (Männistö et al. 2001). Pesticides like atrazine, 2-4 dichloroethane, and hexachlorocyclohexane which are widely used in both agriculture and forestry for pest control have also been reported to have carcinogenic and mutagenic properties (Mauriz et al. 2006).

Soil is the basic requirement for agricultural framework, food security, and environmental sustainability. However, rapid rate of urbanization and imprudent industrialization have rendered this utmost valuable resource contaminated with organic pollutants and heavy metals, debilitating the soil quality, human well-being, and biological systems (Kumar et al. 2017a, 2019). Soil ecosystem degradation may emerge due to buildup of excess heavy metals and organic contaminants within soil. Persistence of the toxicants within soil system is greatly influenced by physical and chemical soil characteristics (Kumar et al. 2018b). Microbial and enzymatic activities within soil may also be hampered due to the accumulation of heavy metals (Kumar et al. 2018b). So, it is indispensable to create an efficient and environment friendly technique to remediate the contaminated soil (Oh et al. 2014). In this regard, several conventional methods like soil excavation and landfilling, soil washing, immobilization, or extraction by physicochemical techniques have been used to clean up the environment, but most of them are costly and require high capital investment.

In recent times, various remedial techniques have been evolved and successfully deployed for environmental cleanup (Kumari et al. 2016; Sabir et al. 2015; Verbruggen et al. 2009). These methods are helpful to ameliorate the available fraction of contaminants within environment as well as can subsequently diminish the rate of bioaccumulation and bio-magnification of environmental contaminants in successive trophic levels (Bhargava et al. 2012). One of these methods that represents an eco-friendly, cost-effective, and eco-sustainable alternative to the conventional methods of treatment is phytoremediation (Pilon-Smits 2005). Certain plants are capable of accumulating inorganic pollutants in their root and shoot systems while degrading the organic pollutants in the surrounding zones. Plant–microbe interaction plays a key role in enhancing the efficiency of phytoremediation in degrading pollutants. Plant–microbe partnership has improved the remediation process to a quite great extent.

Introducing nanotechnology in the transgenic plants is like cherry on the cake. When both these technologies are combined, the contaminants get removed effectively consuming less time and posing no harmful threats on the environment. The present chapter gives a brief overview of existing remedial techniques followed by detailed description of role of plant–microbe partnership in plant-assisted remediation. Further, the impression of transgenic plants and nanotechnology in boosting phytoremediation capacities of plants has been discussed.

5.2 Remediation Strategies for Environmental Cleanup

A systematic approach is required for effective cleanup of contaminated soil and/or ground water. An appropriate remedial action is selected on the basis of contaminants' concentration and the risk to environment likely to be emerged from the consequence of contamination. Site characterization and risk assessment are two important tasks to be done prior to the selection of a particular remedial measure. Both of these actions confirm the actual toxic level of contaminants at a particular environment and their probable risk to environment and human well-being. Depending on the existing risk, suitable remediation strategies are developed. Although remedial measures could not result in absolute cleanup of the contaminated area, however these actions potentially curtail the contaminants' concentration to match the regulatory considerations. This can be achieved through limiting the downward movement of toxicants and/or expelling them. Remediation strategies can be categorized as physicochemical, biological, electro-kinetic, and thermal approaches which are discussed briefly in the following sections.

5.3 Physicochemical Approaches

5.3.1 Replacement and Treatment of Contaminated Soil

This is the simplest technique. The process includes the removal of contaminated soil, disposal of the same, and restoring the area with fresh soil. The evacuated contaminated soil is disposed to landfill site and/or often subjected to soil washing. This approach is apt when the contaminated area is very small (Asquith and Geary 2011).

5.3.2 Soil Washing

This method is appropriate for the soil having inorganic, organic, and radioactive contaminants and lower amount of clay content. If clay content is higher in soil, then a dispersion material is added to break them into fine particles prior to chemical washing of the contaminants. For organic contaminants, a surfactant can be added as a washing agent. During ex situ remediation, the solvent and contaminated soil are mixed up in an extractor vessel (Pavel and Gavrilescu 2008; Balba et al. 1998). The solute and the solvent are treated after separation. Efficiency of the method is increased by using hot water (Wood 2002). It is often applied as a pretreatment approach for soil remediation. In case of in situ soil washing method, solvent or water including additives are injected within the contaminated soil which washes the contaminants. The additives are used for easy release of contaminants.

5.3.3 Solidification and Stabilization

Solidification and stabilization are a source control remediation measure and have already been applied at nearly about 160 superfund sites (Dadrasnia et al. 2013). The process involves the immobilization of toxic contaminants. It can be performed by two ways either through mixing the contaminated soil with a particular additive to make it immobile and insoluble or the contaminated soil converted to insoluble and nonreactive mass prior to solidification. Soil physicochemical characteristics can significantly influence in situ solidification and stabilization action. The ex situ technique includes grinding, dispersing, mixing up with binder material, and disposing off in a landfill. Fly ash, lime, clay, and cement can be used as inorganic binder while resins and bitumen are examples of organic binders (Sun et al. 2016). This method is applicable for remediation of organics, inorganics, semi-volatile, and radioactive contaminants.

5.3.4 Vacuum Extraction

This is a cost-effective in situ remediation measure. Soil and/or ground water contaminated with volatile and semi-volatile organic elements are effectively remediated through vacuum extraction technique. The outline of vacuum extraction technique is shown in Fig. 5.1. The extraction well is fixed at vadose zone (soil water zone). The contaminated soil water and/or volatile compounds are extracted by an injecting medium. Oxygen and nitrogen are commonly used as injecting medium (Reddi and Inyang 2000). Aerobic biodegradation is increased with the use of oxygen as injecting medium. Soil structure and properties of volatile organic components often affect the extraction method. Vacuum extraction is often called as air sparging when air is injected underneath the water table to restore the contaminated groundwater.

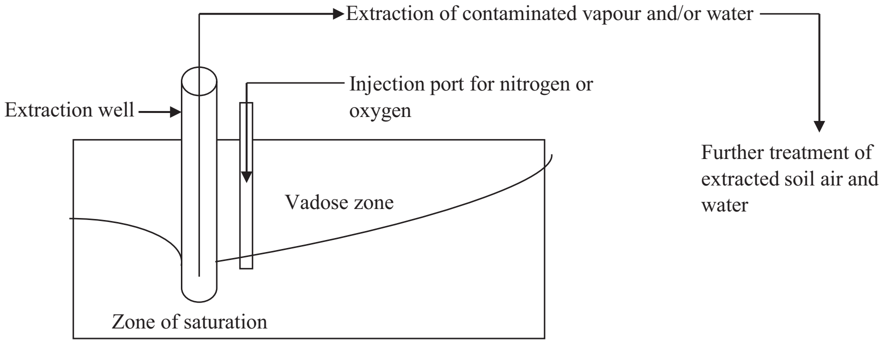

Fig. 5.1 Vacuum extraction technique

5.3.5 Chemical Decontamination

This method is performed to treat the soil having higher concentration of inorganic heavy metals. Selection of extractants is important for sequential extraction of heavy metals from contaminated soil. Oxidizing and reducing agents, electrolytes, acids, etc. can be used as extractants depending upon the heavy metal concentration and soil characteristics. Reduction in heavy metal mobility within the contaminated soil can also be done by introducing immobilizing agents, viz. zeolites, minerals, industrial residues, etc. (Anoduadi et al. 2009). After reducing the mobility of the metals within the soil, contaminated soil can be encapsulated in solid blocks and disposed off to a landfill (Ucaroglu and Talinli 2012). Some additives like concrete, asphalt, or lime are mixed up with the contaminated soil to encapsulate it.

Lime and concrete are applied to the soil having higher oil and heavy metal concentration while, for hydrocarbon contamination, asphalt coating is generally used (Khalid et al. 2017). Now, silica-based coating of contaminated soil is also popularized as it contains various carboxylic groups which act as effective adsorbent for metal ions (Kuang et al. 2015). Sometimes, permeable reactive barriers are used to decontaminate the ground water. Through the process of adsorption, complexation, and precipitation reaction, the inorganic heavy metals are retained in the packing materials of permeable reactive barriers. Most common materials used in permeable reactive barriers are activated carbon, ferric oxides, resins, zeolites, etc.

5.3.6 Electro-Kinetic Method

Electro-kinetic approach is an emerging popular technique to clean up a contaminated soil especially granular soil through electrical principles. An electric field is established within contaminated soil-water system by inserting two electrodes within it. Low direct electric current is applied which results in ion migration and electro-osmosis. Based on the charges, the ionic contaminants are transported to electrodes which are recovered later (Alshawabkeh 2009). Often complexing agents are used to enhance the movements of toxicants. Heavy metals like mercury and uranium contaminants in soil are recovered commercially through electrokinetic method.

5.3.7 Thermal Methods

Thermal remedial techniques are applicable for the contaminants having higher volatilization potential (Evangelou 1998). In case of thermal desorption process, contaminated soil is heated up at 200–1000 °F temperature to separate the contaminants physically from the soil. Most of the contaminants become vaporized during thermal desorption. To decontaminate the remaining toxic elements, secondary treatment techniques like re-ignition, condensation, catalytic oxidation, etc. are used. This method is often applied for petroleum contaminated area. Thermal stripping is also used for treating the volatile and semi-volatile contaminants. Often hot water is injected within contaminated soil matrix to enhance the volatilization potentiality of the contaminants.

High temperature is required for incineration. Incineration can break down the toxic components to basic components like hydrogen, nitrogen, and carbon which react with oxygen to form water, nitrogen oxides, and carbon dioxide. Evaporative loss of volatile compounds during incineration process sometimes makes it inappropriate (Ezeji et al. 2007). Moreover, it is a costly measure requiring larger area for completing the entire process, and it poses threats to environment by emitting pollutants (Bassam and Battikhi 2005).

5.3.8 Biological Methods

Biological treatments are often applicable for the remediation of organic contaminants present in the soil. Bioaugmentation, biostimulation, biofilters, bioventing, bioreactors, and phytoremediation are most commonly applied biological approach to remediate contaminated soil. These biological techniques can be ex situ or in situ in nature. Examples of ex situ bioremediation approaches include anaerobic digestion, land farming, bioreactors, composting, biosorption, etc. Biostimulation, bioventing, and phytoremediation are examples of in situ bioremediation (Table 5.1).

5.3.8.1 Bioremediation

Bioremediation is one of the feasible and eco-friendly ways to remediate or degrade the pollutant with the help of microorganisms (Bharagava et al. 2017; Chowdhary et al. 2017). Bacteria, fungi, and many types of organisms are found to successfully

Table 5.1 Some remediation strategies for environmental cleanup

Remediation strategies	Explanation	References
Bioremediation **Ex situ**—Biopile, windrow, bioreactor, land farming, composting **In situ**—Bioventing, bioslurping	Encourage the development of microorganisms in the contaminated region for degradation/remediation of target pollutants	Azubuike et al. (2016); Sharma et al. (2019)
Phytoremediation	For the direct use of living green plants for remediation of pollutant in soil, mud, sediment, sludge, surface water and groundwater	Yadav et al. (2018); Ashraf et al. (2019)

degrade the complex form to the simpler forms and incorporating the breakdown products into their metabolism. This process is divided into two types. One is ex situ bioremediation wherein the contaminated material is removed from the contaminated sites and bioremediation process is started off site, for example, biopile, windrow, bioreactor, land farming, and composting. Another is in situ bioremediation wherein bioremediation is initiated in the contaminated zone itself, for example, bio-venting and bio-slurping (Sharma et al. 2019; Azubuike et al. 2016).

Bioremediation process has been used in number of contaminated sites of developed and developing countries (Verma and Kuila 2019; Ying and Wei 2019), which showed variable mark of success. Some of the specific requirements of bioremediation include (1) the condition of contaminated area should be suitable for growth and metabolism of microbial population and (2) the availability of the contaminants should be enough for the growth of microbial population. The environmental factors such as soil types and texture, temperature, pH and EC, moisture content, and the presence of oxygen and nutrients play an important role in the degradation of pollutant by the help of microorganisms.

5.3.8.2 Phytoremediation

In 1991, the word phytoremediation has been introduced which is derived from two words “phyto” means plant and “remediation” means recovery. Phytoremediation is a green technology in which plants (hyperaccumulators) and their associated microbes are used to remediate the contaminated site to safeguard the environment (Saxena et al. 2019). It has great remedial potential especially for those pollutants which remains close to the roots of the plant. Over and above, phytoremediation is an economical tool, as it requires less energy as well as an esthetically pleasing technique for remediating polluted sites (Mojiri et al. 2013).

The process of phytoremediation has several mechanisms through which plants accumulate, translocate, and degrade the toxicants like metals, hydrocarbons, pesticides, and chlorinated solvents (Fig. 5.2). This process mainly includes five mechanisms which are as follows:

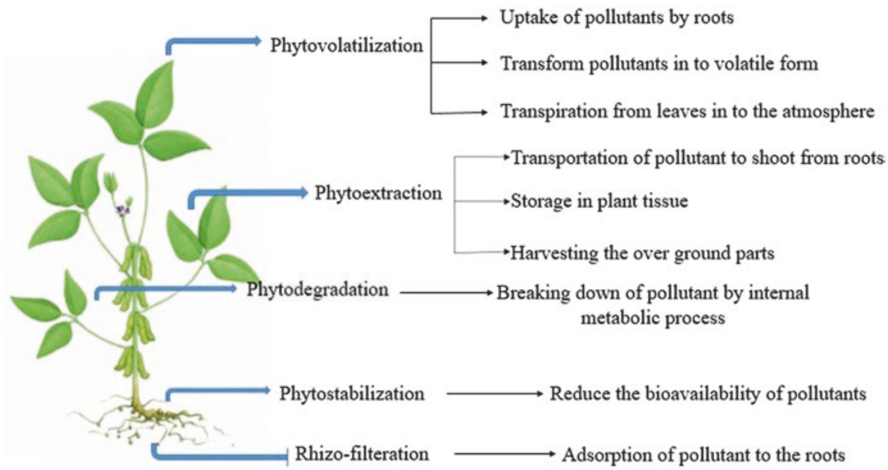

Fig. 5.2 Phytoremediation processes and their mechanisms

(a) **Phytoextraction/Phytoaccumulation:** pollutants in soil, groundwater can be taken up inside plant tissues and accumulated in different plant parts.
(b) **Phytostabilization:** restricts the migration of contaminants in soil and also reduces the bioavailability of pollutants.
(c) **Phytodegradation:** mainly degrades aromatic pollutant (carbon tetrachloride, hexachloroethane, DDT, etc.) by the microorganism in the root zone.
(d) **Phytovolatilization:** converts a contaminant into a volatile form.
(e) **Rhizofiltration:** contaminants adsorbed to the roots (Susarla et al. 2002; Schwarzenbach et al. 2006; Van Aken 2008; Khalid et al. 2017).

5.4 Plant–Microbe Partnership for Improved Remediation of Pollutants

Plant and microbial partnership is a great approach for the removal of several groups of pollutants from the different environmental matrix. There are several reports present in the literature that showed the utility of plants for the removal of inorganic and organic pollutants from contaminated sites (Mishra et al. 2019; He et al. 2019; Navarro-Torre et al. 2017; Barac et al. 2004). Plants give shelter and food/nutrients to their adjacent rhizospheric and endophytic microbe. In exchange of that, the microbes support the plant growth by degrading and detoxifying the contaminants (Arslan et al. 2017; Vangronsveld et al. 2009; Shehzadi et al. 2014). Bacterial and PGPR associations interact with plants and can directly increase the remediation process by altering the metal availability through the production of phytohormones, phytochelors, change of pH, etc. Batty and Dolan (2013). In addition, bacteria associated with plants can degrade catabolic diversity, accumulate, and transform the organic compounds like PCBs, PAHs, pesticides, petroleum hydrocarbon, etc. (Hussain et al. 2018; Ibáñez et al. 2014; Abhilash et al. 2013; Männistö et al. 2001).

Dzantor (2007) reported that the microbial activity associated with plants can enhance the phytoremediation of organic xenobiotic compounds in the rhizosphere and stabilize them into less harmful metabolites. Heavy metals can be degraded up to a remarkable extent by plant-associated microbes such as rhizobacteria, mycorrhizae, and endophytic bacteria (Yousaf et al. 2014). The microbes have the capacity to modify the solubility and bioavailability of the heavy metals and also release some chelating substances that can change the redox potential of the soil. Abou-Shanab et al. (2003) reported that microbes like *Sphingomonas macrogoltabidus*, *Microbacterium liquefaciens*, and *M. arabinogalactanolyticum* reduce the soil pH to enhance the Ni uptake in *Alyssum murale* grown in serpentine soil. There are some other microbes like *Cellulosimicrobium cellulans,* a Cr-tolerant bacterium which has the ability to transform toxic Cr^{6+} to nontoxic Cr^{3+} form and also increased its uptake in the root and shoot parts of green chili (Chatterjee et al. 2009).

Similarly, *Bacillus* sp. and *Geobacillus* sp. isolated from As-contaminated soils have the capacity to biotransform toxic As^{3+} to its lesser toxic form As^{5+} (Majumder et al. 2013). The accumulation of heavy metals in the vacuoles of the plant is also reported such as vacuolar accumulation of Zn, Cu, and Cd was noticed in extraradical mycelium of *Rhizophagus irregularis* (Mishra et al. 2017) and Cd in *R. irregularis* in symbiosis with clover (Yao et al. 2014). It is evident that rhizospheric microbes have their own metabolic pathway that can degrade most of the organic pollutants. Chaudhry et al. (2005) reported that there is a significant decrease in the concentration of dichlorodiphenyldichloroethylene (DDE) in the rhizospheres of lucerne and ryegrass, zucchini, pumpkin, and spinach in the near-root zone as compared to that in bulk soil. Hsu and Bartha (1979) also noticed that organophosphorus insecticides like diazinon and parathion and herbicide 2,4-D not only got accumulated in the rhizosphere of bush bean (*Phaseolus vulgaris*) and sugar cane (*Saccharum officinarum*) but also degraded completely. A degradation study of 17α-ethynylestradiol (EE2) revealed that, when the microbe, *Hyphomicrobium* sp. combined with a hyperaccumulator plant like *Lolium perenne,* the remediation efficacy gets increased greatly (He et al. 2019).

Plant–microbe association has shown success in case of pharmaceuticals and personal care products (PPCPs) also, which have been categorized as emerging pollutants (Gerhardt et al. 2017); Liu and Wong 2013). For example, carbamazepine could be degraded with an endophytic bacteria isolated from *P. australis*. It has the capacity to degrade carbamazepine concentration by 35–66% from its initial concentration within 1–4 days (Sauvêtre and Schröder 2015). Many other endophytic microbes have also been reported to degrade carbamazepine, remarkably, like *Diaphorobacter nitroreducens*, *Achromobacter mucicolens*, *Chryseobacterium taeanense*, *Rhizobium daejeonense*, and *Pseudomonas moorei* (Nguyen et al. 2019). Another PPCP is ibuprofen (IBU) that can be degraded aerobically via species of family *Flavobacteriaceae, Methylococcaceae* etc. and anaerobically by family *Spirochaetaceae* and genus *Clostridium* (Li et al. 2013). Zhao et al. (2015) reported

90% degradation of Triclosan with three different wetland species, namely emergent *T. angustifolia*, submerged *Hydrilla verticillata*, and floating plant *Salvinia natans* in association with beta-, delta-, and gamma-Proteobacteria, Sphingobacteria, and Cyanobacteria. Several other examples of plant microbe association-mediated remediation are present in the literature, which are listed in Table 5.2.

Table 5.2 Plant-microbe interaction for remediation of pollutant

Pollutant	Microbes used	Plants used for phytoremediation	References
Cd	*Hyphomicrobium* sp. GHH	Ryegrass	He et al. (2019)
As, Cr, Cu, Ni, Pb, Zn	*V. kanaloae*, *Pseudoalteromonas*, *P. prydzensis*, *S. Warneri*, *K. marisflavi*, *M. aloeverae*, *B. vietnamensis*, *H. zincidurans*	*Arthrocnemum macrostachyum*	Navarro-Torre et al. (2017)
Chlorpyrifos	*Mesorhizobium* sp.	Ryegrass (*Lolium multiflorum*)	Jabeen et al. (2016)
Phenol	*Bacillus* spp.	*Vicia sativa*	Ibáñez et al. (2014)
Hexachloro-cyclo-hexane	*Sphingomonas herbicidovorans*	Maize (*Zea mays*)	Abhilash et al. (2013)
Trichloroethylene (TCE)	*Enterobacter* sp.	Poplar (*Populus trichocarpa*)	Kang et al. (2012)
VOCs and toluene	*Burkholderia cepacia*	Yellow lupine (*Lupinus luteus*)	Barac et al. (2004)
PCBs, Trichlorophenol (TCP)	*Herbaspirillum* sp.	Wheat (*Triticum aestivum*)	Männistö et al. (2001)
PCBs	*Pseudomonas fluorescens* sp.	Alfalfa (*Medicago sativa*)	Villacieros et al. (2005)
PCBs	*Pseudomonas putida*	*Arabidopsis*	Narasimhan et al. (2003)
Aroclor compounds (e.g., 1242, 1248, 1254, and 1260)	*Sinorhizobium meliloti*	Alfalfa (*Medicago sativa*)	Mehmannavaz et al. (2002)
Trichloroethylene (TCE) and Ni	*Burkholderia cepacia VM1468*	*Lupinus luteus*	Weyens et al. (2010)
Toluene	*Burkholderia cepacia*	*Zea mays* and *Triticum* sp.	Wang et al. (2010)
2,4,6-Trinitrotoluene	*Consortium*	*Agrostis* sp.	Thijs et al. (2014)
Pentachlorophenol (PCP)	*Sphingobium chlorophenolicum*	*Triticum aestivum*	Dams et al. (2007)
Bisphenol-A and (PCP)	*Coriolus versicolor*	Tobacco (*Nicotiana tabacum*)	Bhatia and Kumar (2011)

5.5 Transgenic Technology for Enhanced Phytoremediation

Although phytoremediation has been an efficient method for mitigation of water as well as soil pollution, the transfer of this technology from lab scale studies to field scale implementation is still a challenge. The introduction of transgenic plants in the field of environmental remediation has enhanced the practical applicability of plant-mediated treatment methods. The transfer of specific traits to transgenic plants not only alleviates the degradation of toxic/hazardous contaminants but also makes the process more time and cost efficient. The whole process of making a transgenic plant includes identification of specific catabolic genes responsible for carrying out degradation/mineralization, their isolation from plants/animals/microbes, and then transferring them to a suitable plant species (Aken et al. 2010).

Initially, transgenic plants were developed to reduce insect and pest damages and to increase crop yield in agriculture (Paul et al. 2017), but later on, they gained attention for environmental cleanup and were subsequently used in remediating contaminated soils (Kawahigashi (2009). The first report on transgenic plants was released in 1984 (Horsch et al. 1984). In 1986, France and the United States conducted the first field trials on tobacco, which was the first genetically engineered plant, aimed to induce resistance against herbicides in plant species. In 1992, China introduced a virus-resistant tobacco plant and became the first country to launch transgenic plant in commercial market. The first transgenic plant commercialized in Europe was a genetically modified tobacco plant which had tolerance against bromoxynil, an herbicide (Schütte et al. 2017). The European Union also gave its consent for its marketing, and subsequently, in 1995, the US Environment Protection Agency (EPA) too approved the Bt-Potato (Agnihotri and Seth 2019).

About 25% genera of the *Brassicaceae* family comprising of around 90 species have been identified as efficient hyperaccumulators for various heavy metals. *Brassicaceae* can accumulate selenium up to 100 times more if it is grown in seleniferous soils (Pilon-Smits and Quinn 2010). Brooks et al. (1998) mentioned that *Brassicaceae* family includes a large number of Ni-accumulating plants as well. Similarly, Nouairi et al. (2006) reported that *B. juncea* and *B. napus* can accumulate 1450 and 555 μg Cd/g dry wt., respectively. Species of genera like *Brassica, Arabidopsis, Alyssum,* and *Noccaea* are known to accumulate, remove, sequester, transform, and/or detoxify majority of heavy metals (Agnihotri and Seth 2019). The high capacity of plants belonging to *Brassicaceae* family towards metal accumulation indicate that the development of transgenic plants from *Brassicaceae* family having high rate of heavy metal accumulation such as *Noccaea caerulescens, Arabidopsis halleri*, and *Populus trichocarpa*, along with fast rate of growth and high biomass could be a solution for remediation of heavy metal contaminated soil in a much effective and time-efficient manner.

Apart from members of *Brassicaceae* family, there are other plant species also, which have been used successfully for the development of transgenic plants having improved phytoremediation potential. For instance, yeast cadmium factor (YCF1) protein which is known for its high tolerance towards Pb and Cd was transferred from yeast to *Arabidopsis thaliana* and was studied for the removal of Pb and Cd from contaminated soil (Bhuiyan et al. 2011). The results revealed that the YCF1 active plants have relatively high tolerance capacity against Pb and Cd, suggesting that these transgenic plants could be a potential tool for phytoremediation of other heavy metals also (Chen et al. (2013). It was further observed that the efficiency of YCF1 protein get enhanced up to 9 times when it was inserted into a vector named as YCF1-deletion mutant DTY167 (Bhuiyan et al. 2011). Transgenic *Arabidopsis* and tobacco plants were reported to overexpress the nicotinamine synthase gene, which is responsible for increased synthesis of nicotinamine in host plant.

The alleviated levels of nicotinamine subsequently enhances detoxification of metals particularly nickel (Kim et al. 2005). Another gene phytochelatin synthases gene (PCS) is known for regulating metal tolerance in plants. Zhang et al. (2018) isolated PCS (VsPCS1) from *Vicia sativa* for checking its role in regulating Cd tolerance in *Arabidopsis thaliana* and found a positive correlation between Cd tolerance and phytochelatin content in plants. Laccase (LAC) belonging to ceruloplasmin oxidase family regulates oxidation of monolignols to higher order lignins which are significant for plant development as well as metal tolerance. Liu et al. (2017) reported that OsLAC10 increases the process of root lignification in *Arabidopsis*, inhibiting excessive absorption of Cu, thereby improving the overall tolerance of the plant against Cu.

Transgenic plants have not shown success for heavy metal removal only, but organic contaminants have also been reported to degrade using transgenic methods. One of the genes, which has been reported to show tolerance against wide group of herbicides and pesticides, is cytochrome P450 monooxygenase (Zhang et al. 2015; Hussain et al. 2018). For instance, bispyribac sodium herbicide responsible for inhibiting the activity of acetolactate synthase could be detoxified using CYP72A31 present in indica variety of transgenic *Oryza Sativa* (Saika et al. 2014). Similarly, CYP72A31 present in transgenic *Arabidopsis* has also shown tolerance against bensulfuron methyl, an herbicide (Saika et al. 2014).

Genetically transformed tobacco plants having mammalian cytochrome P450 2E1 have been reported to degrade wide range of halogenated organic compounds like trichloroethylene, ethylene dibromide, carbon tetrachloride, chloroform, etc. (Doty et al. 2000). Singh et al. (2011) demonstrated enhanced tolerance of transgenic tobacco plants having CYP 2E1, against an organichlorine pesticide, Lindane in soil and hydroponic solution. Germaine and coworkers (2006) successfully degrade 2,4-dichlorophenoxyacetic acid (2,4-D) by the insertion of *Pseudomonas putida* (endophytic bacterium) strain VM1450 in to pea plant. Table 5.3 enlists different kinds of transgenes derived from source species and then inserted into target plant species to enhance phytoremediation capability of plant species. There are several other studies available in the literature which suggests that development of transgenic plants through recombinant DNA technology is a promising approach for improving the phytoremediation potential of plants.

Table 5.3 Different transgenes obtained from source species and inserted into a target plant to enhance phytoremediation potential

Source species	Target species	Transgenes used	Remarks	References
Thaliana	*N. tabacum*	Nicotianamine synthase (NAS1)	Increased Fe level in leaves of adult plants, enhanced accumulation of Zn and Mo, increased Ni tolerance	Douchkov et al. (2005)
E. coli	*B. juncea*		Accumulate more Se in their leaves.	Banuelos et al. (2007)
Arabidopsis	*N. tabacum*	Phytochelatin synthase (AtPCS1)	Cd^{2+} tolerance was increased, twofold increase in Cd^{2+} accumulation was observed in roots and shoots at seedling stage	Pomponi et al. (2006)
B. campestris	*A. thaliana*	Metallothioneins (MtTs) two MT genes, *BcMT1* and *BcMT2*	Improved Cd and Cu tolerance in transgenic plant	Lv et al. (2013)
A. thaliana	*N. tabacum*	AtMt2b	Increased transport of Cd and Zn to shoot	Grispen et al. (2011)
S. cerevisiae	*A. thaliana*	Protein YCF1, member of the ATP-binding cassette (ABC)	Enhanced tolerance towards Pb and Cd	Song et al. (2003)
Arabidopsis	*A. thaliana*	xcd2-D, codes for a zinc-finger transcription factor called as ZAT6	Increased Cd accumulation	Chen et al. (2016)
Bacillus megaterium	*A. thaliana*	Protein (MerP)	Higher tolerance and accumulation potential for Hg, Cd, and Pb	Hsieh et al. (2009)
Arabidopsis thaliana	*Oryza sativa*	AtPCS	Enhanced tolerance towards Cd stress	Venkataramaiah et al. (2011)
Cajanus cajan	*Arabidopsis thaliana*	Metallothioneins MT1	Better tolerance and accumulation against Cu and Cd	Sekhar et al. (2011)
Enterobacter cloacae	*B. napus*	ACC deaminase	Boosted tolerance and accumulation against As(V)	Nie et al. (2002)
Pseudomonas putida	*B. napus*	ACC deaminase	Enhanced tolerance and accumulation against Ni	Agnihotri and Seth (2019)

5.6 Nanotechnology to Enhance the Efficiency of Phyto-bio Remediation

Nanotechnology is a wide area focused on materials occurring on a very small scale. It deals with a structure which is having at least one dimension is in the range of nanoscale, i.e., 1–100 nm (Yadav et al. 2018). Nanoparticles (NPs) can be broadly divided into two parts: organic NPs; carbon NPs, i.e., fullerenes; inorganic NPs: magnetic NPs, metal NPs (Au, Ag), semiconductor (TiO_2, ZnO) (Lin and Xing 2007). Srivastava et al. (2018) reported that NPs are of three types: natural (e.g., volcanic or lunar dust, mineral composites), incidental (resulting from anthropogenic activity, e.g., diesel exhaust, coal combustion, welding fumes), and engineered (quantum dots, nanogold, nano zinc, nano aluminum, TiO_2, ZnO, and Al_2O_3). Nanoparticles exhibit a number of special properties such as high surface to-volume ratio, small enough to generate quantum effects, unique physicochemical properties, etc. and that is the reason nanoparticles are getting increasing interest in the field of science, engineering, cosmetics, pharmaceuticals, drug delivery, and also in environmental cleanup Das et al. (2015). In the area of environmental remediation, several types of nanoparticles such as metals (Zn, Fe, Ni, Pd, etc.), metal oxides (TiO_2, ZnO, Fe_2O_3, Fe_3O_4, MnO_2, etc.), and bimetallic (Pd/Fe, Fe/Ag, Cu/Ni, etc.) nanoparticles have been used successfully for the removal/degradation of wide array of inorganic and organic contaminants. The mechanism of nanoparticle-mediated remediation generally includes oxidation-reduction, ab/adsorption, precipitation, co-precipitation, catalytic degradation, etc. (Crane and Scott 2012; Singh and Misra 2016).

Basically cleanup process of contaminants is called as remediation. If a biological agent is involved in the removal of the pollutant/s, then it is called as bioremediation, whereas if it is done with the involvement of a plant species, then it will be referred as phytoremediation. The integration of nanotechnology with either of these methods has been proved as an effective alternative to the existing conventional methods of remediation. For instance, Jiamjitrpanich et al. (2013) combined nanotechnology and phytotechnology for remediation of trinitrotoluene (TNT) contaminated soil. When nanoscale zero-valent iron (nZVI) was added at different concentrations to the TNT-contaminated soil, the uptake of TNT by the roots of *Panicum maximum* was comparatively increased than those without nanoparticles. The removal efficiency was observed to be higher when the TNT-nZVI ratio was kept at 1:10. Another study also demonstrated that nZVI effectively improved the efficiency of different plant species like *Alpinia calcarata, Cymbopogan citratus*, and *Ocium sanctum* against the removal of an organochlorine pesticide, endosulphan (Pillai and Kottekottil 2016).

Similar kind of enhanced remedial efficiency has been reported in another study wherein Ag nanoparticles enhanced the accumulation of Pd, Ni, and Cd in maize inoculated with *Pseudomonas and Bacillus cereus*, respectively (Khan and Bano 2016). The increased efficiency of integrated method could be ascribed to nanoparticle-induced alleviated production of phytochromes [abscisic acid (ABA),

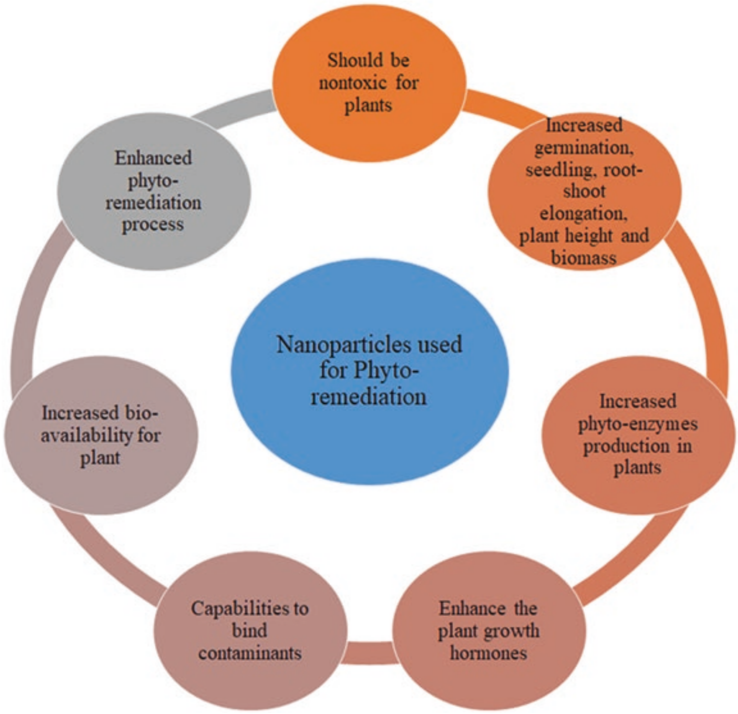

Fig. 5.3 Characteristics of nanoparticles suitable for phytoremediation

indole acetic acid (IAA), and Gibberellin (GA)] and proline in PGPR along with reduced oxidative stress. Salicylic acid nanoparticles were reported to enhance As tolerance in *Isatis cappadocica* (Souri et al. 2017). Similarly, nZVI showed positive effect on stabilization in sunflower rhizosphere (Vitkova et al. 2018). The major characteristics that should be present in nanoparticles to be used for enhancing phytoremediation potential of plant are presented in Fig. 5.3.

El-Kassas et al. (2016) biologically synthesized Fe_3O_4 NPs using two seaweeds, namely *Padina pavonica* (Linnaeus) *Thivy* and *Sargassum acinarium* (Linnaeus) and compared their Pb removal capacity. The findings revealed that Fe_3O_4 NPs entrapped alginate beads prepared from *P. pavonica* were smaller in size and have relatively higher Pb removal capacity (91%) than that of *S. acinarium* (78%). Liang et al. (2017) reported that nano-hydroxyapatite elevates Pb accumulation capacity (up to 46.55%) in Ryegrass plant species. In another phytoremediation study, the impact of different concentrations of nZVI (0, 100, 200, 500, 1000, 2000 mg/kg) on Pb accumulation capacity of Ryegrass species was studied for 45 days. Maximum Pb accumulation was noticed at nZVI concentration of 100 mg/kg. It was further noticed that lower nZVI concentration boosts Pd accumulation, but as the concentration goes up, the remedial efficacy of the plant gets reduced (Ding et al. (2017). The probable reason for decreased removal could be attributed to the critical oxidative stress caused by the higher concentration of nanoparticles in the plant (Huang et al. 2018).

The experimental study by Vasantharaj et al. (2019) is mainly focused on synthesizing eco-friendly and nontoxic metallic nanoparticles. The plant extracts of *R. tuberosa* was used to produce FeO NPs that were reported to be effectively used for bioremediation application. It showed potential antimicrobial activity against different Gram-positive and Gram-negative pathogens and also promoted successful degradation of synthetic dye like crystal violet. Table 5.4 enlists more examples of nano-phytoremediation below. Though nanotechnology has been evolved as a promising approach in the integrated phytoremediation study, the effect of nanoparticles on the non-target species is yet to be explored. There is a need for further research in this field to fully investigate the fate of nanoparticles in the environment.

5.7 Conclusions

Pollutants are ever increasing in the environmental ecosystem due to rapid industrialization, urbanization, non-mechanized agricultural practices, vehicular emissions, etc. In natural ecosystem, the speedy cleanup of the environment and stabilization of pollutant is extremely needed to support the sustainability of environmental ecosystems. Phytoremediation is a widely used remediation technique over the period of time, but the need of faster contaminant removal, consuming less time is a bit difficult to achieve with this technique leading further research in this domain. The presence of living plants and its association with native microbes in polluted region assist the phytoremediation technology making it more efficient in remediating organic and inorganic pollutants. Plant–microbial partnership could be further enhanced through genetic engineering which led the way for the development of transgenic plants. These plants are quite superior to the plants that naturally degrade the toxicants in terms of efficiency and time consumption. Besides, transgenic plants also facilitate the plant–microbe interaction and improve the microbial activities at rhizosphere for increased pollutant uptake and their removal from environmental systems. Though transgenic plant systems are much hyped removal technology to the date, the biochemical activities and transport mechanism inside the plant are still not fully explored and need further research to make them more pollutant selective and transferring it to target cell type without interfering in other cell functions. Integrated nano-phytoremediation technology also holds great promises towards environmental cleanup; however, uncertain fate of nanoparticles in the environment and toxicity towards non-targeted species is not fully understood and yet to be explored further.

Table 5.4 List of species and nanomaterials used for contaminant removal

Species	Nanomaterials	Contaminants	Remarks	References
Alpinia calcarata, Ocimum sanctum, Cymbopogon citratus	nZVIs	Endosulfan	Removal efficiency is ≈100 (%), 76.28 (%), 86.16 (%) respectively	Srivastava et al. (2018)
Panicum (*Panicum maximum Jacq*.)	nZVI	Trinitrotoluene (TNT)	The removal efficiency of TNT increased from 85.7 to 100% after 120 days	Jiamjitrpanich et al. (2013)
Maize (*Zea mays L.*)	Silver nanoparticles	Cd, Pb, and Ni	Enhanced accumulation of Cd, Pb, and Ni in shoot	Khan and Bano (2016)
Ryegrass (*Lolium perenne L.*)	Nano hydroxyapatite	Pb	Removal efficiency was increased from 11.67 to 21.97% under Pb stress of 800 mg/kg	Jin et al. (2016)
Sunflower (*Helianthus annuus L.*) and ryegrass (*Lolium perenne L.*)	nZVI	As, Cd, Pb, and Zn	The concentrations of As, Cd, Pb, and Zn in roots and shoots decreased by 50–60% as compared to the control sample	Vitkova et al. (2018)
Collard greens (*Brassica oleracea L.*)	Multiwall carbon nanotubes	Carbamazepine	The functionalization of carbon nanotubes enhanced carbamazepine translocation	Jin et al. (2016)
Zucchini (*Cucurbita pepo L.*) and soya bean [*Glycine max (L.) Merr.*]	Ag nanoparticles	p,p’-DDE	Decreased the uptake and accumulation of p,p’-DDE in both the plants	Jiamjitrpanich et al. (2013)
Ramied seed (*Boehmeria nivea*)	Starch stabilized nZVI	Cd	Increase Cd-accumulation in roots, stems and leaves by 16–50%, 29–52%, 31–73% respectively in Ramied seed	Gong et al. (2017)
Soya bean plant (*Glycine max*)	TiO_2 NPs	Cd	Accumulation of Cd in the roots, stems, and leaves of soya bean increased by 2.5, 2.6, and 3.3 times, respectively	Singh and Lee (2016)
Eastern cottonwood (*Populus deltoids*)	Fullerene NPs	Trichloroethylene	Uptake increased from 26 to 82%	Ma and Wang et al. (2010)

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