

Phytoremediation of Radionuclides: A Report on the State of the Art

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Abstract Radionuclides mobilization through extraction from ores and processing for various applications has led to the discharge of these harmful elements into the environment. These contaminants pose a great risk to human health and environment. Remediation of radionuclides and toxic heavy metals deserves the proper attention. Conventional remediation methods used for polluted environments have many limitations including high costs, alteration in soil properties, and disruption in soil native microflora. Alternatively, phytoremediation can serve as a prospective method for decontamination and rehabilitation of polluted sites. The term phytoremediation actually refers to a diverse collection of plant-based technologies, i.e. either naturally occurring or genetically engineered plants are used for cleaning the contaminated environment. Phytoremediation techniques are eco-friendly, cost-effective, easy to implement, and offer an aesthetic value and solar-driven processes with better public acceptance. Practicing various agronomic alterations as well as spatial and successful combination of different plant species assures maximal phytoremediation efficiency. Plants and microorganisms can be genetically modified to remediate the contaminated ecosystems at an accelerated rate. We can harvest better results from phytoremediation technologies by learning more about the different biological processes involved. The future of phytoremediation comprises of ongoing research work and has to go through a developmental phase and several technical barriers. Several attempts still need to be performed with multidisciplinary approach for successful future phytoremedial programmes. This report comprehensively reviews the background, techniques, concept and future course in phytoremediation of heavy metals, particularly radionuclides.

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1 Introduction

Scientific and technological progress has occurred with human evolution. New challenges have arisen due to global development, especially in the field of environmental protection and conservation (Bennett et al. 2003). The mobilization of radionuclides through mining, accidents, spills, explosions, weapon fabrication, testing (Madruga et al. 2014), dumping of wastes (Richter 2013) and radioisotopes used in medicines (Frédéric and Yves 2014) has led to the discharge of these elements into the ecosystem. The problem of heavy metal including radionuclide pollution is becoming more and more serious with increasing anthropogenic activities such as industrialization and disturbances of natural biogeochemical cycles (Černe et al. 2010; Fulekar et al. 2010; Wuana and Okieimen 2011; Ali et al. 2013).

²³⁸U, ²³²Th and ⁴⁰K are three long-lived naturally occurring radionuclides present in the earth crust. Generally, two sources of environmental radionuclides

are natural (mainly from the ^{238}U , ^{232}Th series) and artificial (Tawalbeh et al. 2013). U, Th, Cs, Co and Ce are the most common ions found in low-level liquid radioactive wastes (Hafez and Ramadan 2002). A set of radionuclides, including ^3H , ^{14}C , ^{90}Sr , ^{99}Tc , ^{129}I , ^{137}Cs , ^{237}Np , ^{241}Am , as well as several U and Pu isotopes, from the nuclear-related activities, are of special environmental importance due to their abundance, mobility or toxicity (Hu et al. 2010). Metal mining activities and phosphate fertilizer factories produced the waste enriched in radionuclides from the U series including ^{230}Th , ^{226}Ra and ^{210}Pb (SanMiguel et al. 2004). Radioactive isotopes such as ^{14}C , ^{18}O , ^{32}P , ^{35}S , ^{64}Cu and ^{59}Fe are widely used as tracers in plant physiology and biochemistry (Dushenkov et al. 1999). Contamination of soils with typical fission product radionuclides, such as ^{137}Cs and ^{90}Sr , has persisted for far longer (Zhu and Shaw 2000). Nuclear facilities, repository of nuclear waste, tracer and application in the environmental and biological researches release the radionuclides including ^3H , ^{14}C , ^{36}Cl , ^{41}Ca , $^{59,63}\text{Ni}$, $^{89,90}\text{Sr}$, ^{99}Tc , ^{129}I , $^{135,137}\text{Cs}$, ^{210}Pb , $^{226,228}\text{Ra}$, ^{237}Np , ^{241}Am and isotopes of Th, U and Pu (Hou and Roos 2008). Medical radioisotopes cover a wide variety of radionuclides—from short-lived pure gamma emitters such as $^{99\text{m}}\text{Tc}$ and ^{123}I for diagnostic purposes to longer-lived therapeutic isotopes such as ^{131}I , ^7Be , ^{67}Ga , ^{153}Sm and ^{197}Hg (Fischer et al. 2009). It has been estimated that, on average, 79 % of the radiation to which humans are exposed is from natural sources, 19 % from medical application and the remaining 2 % from fallout of weapons testing and the nuclear power industry (Wild 1993).

However, most of the public concern from radionuclides has been due to the global fallout from nuclear weapons testing and the operation of nuclear facilities. Both of these activities have added a substantial amount of radionuclides into the environment and have caused radionuclide contamination worldwide. Radionuclides in soils are taken up by plants and are available for further redistribution within food chains. Radionuclides in the environment can, therefore, eventually be passed through food chains to human beings and represent an environmental threat to the health of human populations (Zhu and Shaw 2000). The migration of radionuclides in the environment depends on many factors, such as physico-chemical, biological, geochemical and microbial influences, soil and water properties, air, flora and specific interactions of radionuclides with vegetation or other organisms where they accumulate (Nollet and Pöschl 2007; Cerne et al. 2010). Radionuclides which have been responsible for major environmental concern are listed in Table 1.

Elevated metal concentrations in the environment also have wide-ranging impacts on animals and plants. For instance, human exposure to a variety of metals causes wide range of medical problems such as heart disease, liver damage, cancer, neurological problems and central nervous system disorders (Roane et al. 1996). Radionuclides can enter human body through ingestion, inhalation and external irradiation. The ingested radionuclides could be concentrated in various parts of the body. ^{238}U accumulates in lungs and kidneys, ^{232}Th in lungs, liver and skeleton tissues and ^{40}K in muscles (Samat and Evans 2011). Depositions of large quantities of these radionuclides in organs affect the health conditions such as

Table 1 Sources of radionuclides in the environment

Radionuclide	Sources	Reference
Uranium ($^{235}, ^{238}\text{U}$)	Natural, mining, milling, nuclear waste disposal	Chabalala and Chirwa (2010)
Thorium (^{232}Th)	Natural, mining, milling and processing, phosphate fertilizer production, tin processing, industrial boilers, military operations	Atwood (2010), Tawalbeh et al. (2013)
Radium ($^{226}, ^{228}\text{Ra}$)	Uranium decay product from mill tailing	Madrua et al. (2001), Cerne et al. (2010)
Cobalt (^{60}Co)	Car, truck and airplane exhausts, burning coal and oil, industrial processes, nuclear medicines	Simeonov and Sargsyan (2008)
Iodine (^{131}I)	Nuclear test (underground), fuel reprocessing, spent nuclear fuel	Hu et al. (2010)
Strontium (^{90}Sr)	Spent nuclear fuel, nuclear accidents, nuclear fallout, nuclear fission, nuclear power plants, radioactive tracer in medical and agricultural studies	Hu et al. (2010), ATSDR (2004)
Caesium (^{137}Cs)	Nuclear power stations	Stohl et al. (2012)
Carbon (^{14}C)	Natural and nuclear reactor	Zhu and Shaw (2000)
Potassium (^{40}K)	Natural	Zhu and Shaw (2000)
Plutonium (^{239}Pu)	Nuclear reactor	Zhu and Shaw (2000)

weakening the immune system induces various types of diseases and the increase in mortality rate. Metal toxicity in plants can cause stunted growth, leaf scorch, nutrient deficiency and increased vulnerability to insect attack (Roane et al. 1996). The carcinogenic nature and long half-lives of many radionuclides make them a potential threat to human health. Plant uptake of radionuclides into the human food chain is one of many vectors used for calculating exposure rates and performing risk assessment (Rosén et al. 1995). Geras'kin et al. (2007) performed long-term radioecological investigations and concluded that adverse somatic and genetic effects are possible in plants and animals due to radium and uranium–radium contamination in the environment.

The removal of radioisotopes from soil is theoretically simple to achieve. Soil is moved offsite for leaching/chelating treatments and then returned to its previous location. However, in practice, the movement of large quantities of soil for decontamination is environmentally destructive and costly due to transportation. It also increases the risk of releasing potentially harmful radionuclides into the atmosphere as particulate matter (Entry et al. 1996). Safe and cost-effective methods are needed for removing radionuclides and heavy metals from the contaminated soils (Phillips et al. 1995). All the conventional remediation methods used for radionuclide-polluted environments have serious limitations. Over the past decade, there has been increasing interest for the development of plant-based

remediation technologies, which have the potential to be environmentally sound, a concept called phytoremediation (Laroche et al. 2005; Jagetiya and Purohit 2006; Jagetiya and Sharma 2009; Roongtanakiat et al. 2010; Borghei et al. 2011; Jagetiya et al. 2011). The concept of phytoremediation was suggested by Chaney (1983). It is an aesthetically pleasing mechanism that can reduce remedial costs, restore habitats and clean up contamination in place rather than entombing it in place or transporting the problem to another site (Bulak et al. 2014; Kamran et al. 2014). Phytoremediation can cost as less than as 5 % of alternative clean-up methods (Prasad 2003). The thriving plants display efficiency for remediation; they act as natural vacuum cleaners sucking pollutants out of the soil and depositing them in various plant parts (Rajalakshmi et al. 2011).

2 Sources of Radionuclides in the Environment

Radionuclides make their way in the environment from natural and anthropogenic sources. The most common natural sources are weathering of minerals, erosion and volcanic eruptions, while anthropogenic sources include nuclear weapons production and reprocessing, nuclear weapons' testing, uranium mining and milling, commercial fuel reprocessing, geological repository of high-level nuclear wastes and nuclear accidents. The other potential sources are coal combustion, cement production, phosphate fertilizers production and its use in agriculture management (Nollet and Pöschl 2007).

Nuclear weapons production and reprocessing programs produce high-level waste liquid and sludge. Fissile isotopes such as ^{235}U , ^{239}Pu and ^{238}U are used together with the radionuclide ^3H and are separated from fission products in spent nuclear reactor fuels to produce weapons-grade fuel (Hu et al. 2010).

Nuclear weapons testing has released considerable amount of radionuclides in the environment. Choppin (2003) reported that over 2×10^8 TBq of radioactivity has been released into the atmosphere from worldwide nuclear weapons' tests. In terms of radioactivity, ^3H , ^{90}Sr , ^{137}Cs , ^{241}Am and Pu isotopes are currently the radionuclides of great importance. Long-lived ^{14}C , ^{36}Cl , ^{99}Tc , ^{129}I , ^{237}Np , as well as several U and Pu, isotopes are important.

Nuclear power plants produce 200 radionuclides during the operation of a typical nuclear reactor in which radionuclides decay to low levels within a few decades (Crowley 1997). A number of radionuclides are emitted from normal operation of nuclear reactor. Based on combined worldwide operable nuclear reactors of 3.72×10^5 MWe (World Nuclear Association 2007), the annual discharge of ^{14}C worldwide is about 60 TBq Y^{-1} .

The U mining and the milling processes of raw material containing uranium and thorium are one of the main causes of discharging of radionuclides into the environment, mainly from the tailings. The radionuclides in uranium mill tailings includes ^{238}U , ^{235}U , ^{234}U , ^{230}Th , ^{226}Ra and ^{222}Rn . ^{238}U and ^{230}Th are long-lived α -emitters, whereas ^{222}Rn is an inert radioactive gas with a short half-life, which

has been identified as an important carcinogen. In addition to radioactivity, uranium mill tailings are associated with elevated concentrations of highly toxic heavy metals. Oxidation of high-sulphide content in uranium tailings generates acidic waters and increases the release of radioactive and hazardous elements (Abdelouas 2006).

Commercial fuel reprocessing results into the discharge of ^{99}Tc and ^{129}I (liquid and gaseous) into the sea and atmosphere from the nuclear fuel reprocessing plants (Hu et al. 2010). In addition to environmental contamination, a principal concern with fuel reprocessing has always been the possibility of the diversion of fissile material, mainly ^{235}U and ^{239}Pu , for weapons production. However, other fissile nuclides, such as ^{237}Np and Am , may be separated during reprocessing (Ewing 2004).

Geological repository of high-level nuclear wastes Nuclear energy production and research facilities create waste in the form of spent nuclear fuel. Spent nuclear fuel remains highly radioactive for thousands of years. Separating this waste from people and the environment has been a challenging issue for all countries with nuclear power (Hu et al. 2010). High-level waste makes up around 3 % of the world's total volume, but it has approximately 95 % of the radioactivity (low- and high-level wastes combined). Countries with high-level radioactive waste and spent nuclear fuel must dispose off these materials in a geologic disposal facility called as repository (Witherspoon and Bodvarsson 2001).

Nuclear accidents It was estimated that 1.2×10^7 TBq of radioactivity was released in the Chernobyl accident (UNSCEAR 2000). Eikenberg et al. (2004) compared the total atmospheric release of long-lived fission radionuclides and actinides from the atomic bomb tests and the Chernobyl reactor explosion. In comparison with the sum of all previously performed tests, the values for ^{90}Sr , ^{137}Cs and $^{239+240}\text{Pu}$ from the Chernobyl accident were in the order of 10 % and much higher for ^{238}Pu and ^{241}Am . Fallout of hot particles caused a considerable contamination of the soil surface, with ^{137}Cs up to 106 Bq m^{-2} , and 116,000 people were evacuated within a zone of 30 km distance from the reactor (Balonov 2007). Six artificial radionuclides (^{131}I , ^{134}Cs , ^{137}Cs , ^{129}mTe , ^{95}Nb and ^{136}Cs) were detected in soil samples around Fukushima Nuclear Power Plant (Taira et al. 2012). Nuclear energy sources are also utilized in some spacecraft, satellites and deep sea acoustic signal transmitters for heat or electricity generation, the two common types of nuclear energy sources are radioisotope thermoelectric generators (RTGs) and nuclear reactors. Due to the radiotoxicity and long half-life, some radionuclides are of particular concern in the radiological dispersion devices (RDD): ^{241}Am , ^{252}Cf , ^{60}Co , ^{137}Cs , ^{90}Sr , ^{192}Ir and ^{238}Pu . Commercial radioactive sources for potential RDD include RTG (^{90}Sr), teletherapy and irradiators (^{60}Co and ^{137}Cs), industrial radiography (^{60}Co and ^{192}Ir), logging and moisture detectors (^{137}Cs , ^{241}Am and ^{252}Cf) (Hu et al. 2010).

3 Conventional Versus Phytoremediation Clean-up

The conventional remediation technologies, which are used for metal-polluted environments are in situ vitrification, soil incineration, excavation and landfill, soil washing, soil flushing, solidification, reburial of soil, stabilization of electrokinetic systems as well as pump and treat systems for water. When high radionuclide concentrations in soils pose risk to the environment, then two traditional soil treatments are usually used. Soil excavation is the first method, which removes the soil with radionuclides in its present state or after stabilization in concrete or glass matrices. However, this method is expensive as it requires packaging, transporting and disposal of contaminants (Ensley 2000; Negri and Hinchman 2000). This method only relocates the problem in the same proportion to a new location. The bulk density, soil compaction as well as aeration and water-holding capacity are affected due to heavy equipment's, which are used in soil excavation (Entry et al. 1997). Extra restoration applications are required to establish vegetation on such altered site (Huang et al. 1998). Another method involves soil washing, soil removal and chemical manipulations. Soil which is brought back after washing does not contain radionuclides, but is not thoroughly sterile with detergents, surfactants and chelating agents. If these chemicals leach into the ground water, they could pose more environmental problems (Entry et al. 1997). These technologies are too expensive, unsafe and inadequate and have a risk of releasing potentially harmful radionuclides into the atmosphere. Effectiveness and costs are also important for alternate remediation methods after ensuring public and ecosystem health. Environmental Protection Agency (EPA) requires, in order of preference suggests, that the nine criteria may be used to evaluate alternatives for remediation (Fig. 1).

Removal of toxic substances from the environment (soils) by using accumulator plants is the goal of phytoremediation. When decontamination strategies are impractical because of the size of the contaminated area, phytoremediation is advantageous. Due to the proven efficiency of phytoremediation, it draws great deal of interest from site owners, managers, consultants and contractors, in applying this technology to private, superfund and brown field sites. The success of phytoremediation depends upon the ability of a plant to uptake and translocate the contaminants (Chen et al. 2003). The ability of different plants to absorb radionuclides also depends on the environment and the soil properties (Entry et al. 1999). Recent studies have led to progressive insights into phytoremediation. The selection of an appropriate plant species is a crucial step (Huang et al. 1998), and screening of the suitable species involves complex studies (Mkandawire and Dudel 2005). The use of plant species for environmental clean-up of trace elements is based on their ability to concentrate element or radionuclide in their tissue (Zhu and Shaw 2000; Pratas et al. 2006). Successful utilization of phytoremediation technology involves analysis of factors governing the uptake, transportation and accumulation of metals in various plant parts (Diwan et al. 2010). High growth rate and biomass production are the desirable qualities for this process (Soudek et al.



Fig. 1 Suggestions of Environmental Protection Agency (EPA) in order of preference that the nine criteria may be used to evaluate alternatives for remediation

2004; Cerne et al. 2010). Increasing metal accumulation in high-yielding crop plants without diminishing their yield is the most feasible strategy in the development of phytoremediation (Evangelou et al. 2007).

Willey and his colleagues (Broadley and Willey 1997; Willey and Martin 1997) have obtained relative radiocaesium uptake values in about 200 species and found that the highest values are all in the Chenopodiaceae or closely related families. Lasat et al. (1998) identified that red root pigweed (*Amaranthus retroflexus*) is an effective accumulator of radiocaesium which is capable of combining a high uptake of ^{137}Cs with high shoot biomass yield.

Hung et al. (2010) assessed the efficiency of vetiver grass for uranium accumulation and reported higher accumulation in lower fertile soils and more accumulation in roots in comparison with shoots. Štok and Smodiš (2010) collected samples of plants from a uranium mill tailings waste pile containing ^{201}Pb , ^{226}Ra

and ^{238}U and found that all radionuclides were highly accumulated in foliage, followed by shoots and wood, whereas Rodríguez et al. (2009) reported more U accumulated in leaves than fruits of some plant samples growing on a uranium mine. Sunflower (*Helianthus annuus* L.) and Indian mustard (*Brassica juncea* Czern.) are the most promising terrestrial candidates for metal (uranium) removal in water (Prasad and Freitas 2003). As discussed above, different plant species have different abilities to accumulate radionuclides from soil. While this variation has particular relevance in terms of being able to reduce the transfer of radionuclides from soil to food chains, it can also be exploited for the purpose of phytoremediation. However, with the present knowledge of plant uptake of radionuclides from soils, phytoremediation takes excessively long time. To speed up the process selection of suitable plant taxa, a special plant-breeding programme assisted by molecular biotechnology may be useful (Zhu and Shaw 2000).

4 Phytoremediation Techniques

The application of plants for environmental remediation requires the evaluation of a number of practical issues that have been divided into pre-harvest and post-harvest plans or strategies. Pre-harvest plan include the selection, design, implementation and maintenance of phytoremediation applications, whereas post-harvest strategies involve the disposal of plant and contaminant residues, which must also be taken into account fully during the design phase (Fig. 2). There are different techniques of phytoremediation (Table 2; Fig. 3) of toxic heavy metals and radionuclides from soil, groundwater, wastewater, sediments and brownfields (Zhu and Chen 2009; Sarma 2011; Ali et al. 2013).

4.1 Phytoaccumulation

It is also called as phytoextraction, phytoabsorption and phytosequestration. It involves the uptake and translocation of metal contaminants from the soil by plant roots into the above ground parts of the plants (Chou et al. 2005; Eapen et al. 2006; Singh et al. 2009). Metal translocation to shoots is desirable in an effective process because generally the root biomass is not feasible (Singh et al. 2009; Tangahu et al. 2011). Certain plants called hyperaccumulators absorb unusually large amounts of metals in comparison with other plants. After the plants have been allowed to grow for several weeks or months, they are harvested and either incinerated or composted to recycle the metals. This procedure may be repeated as necessary to bring soil contaminant levels down to allowable limits (Horník et al. 2005).

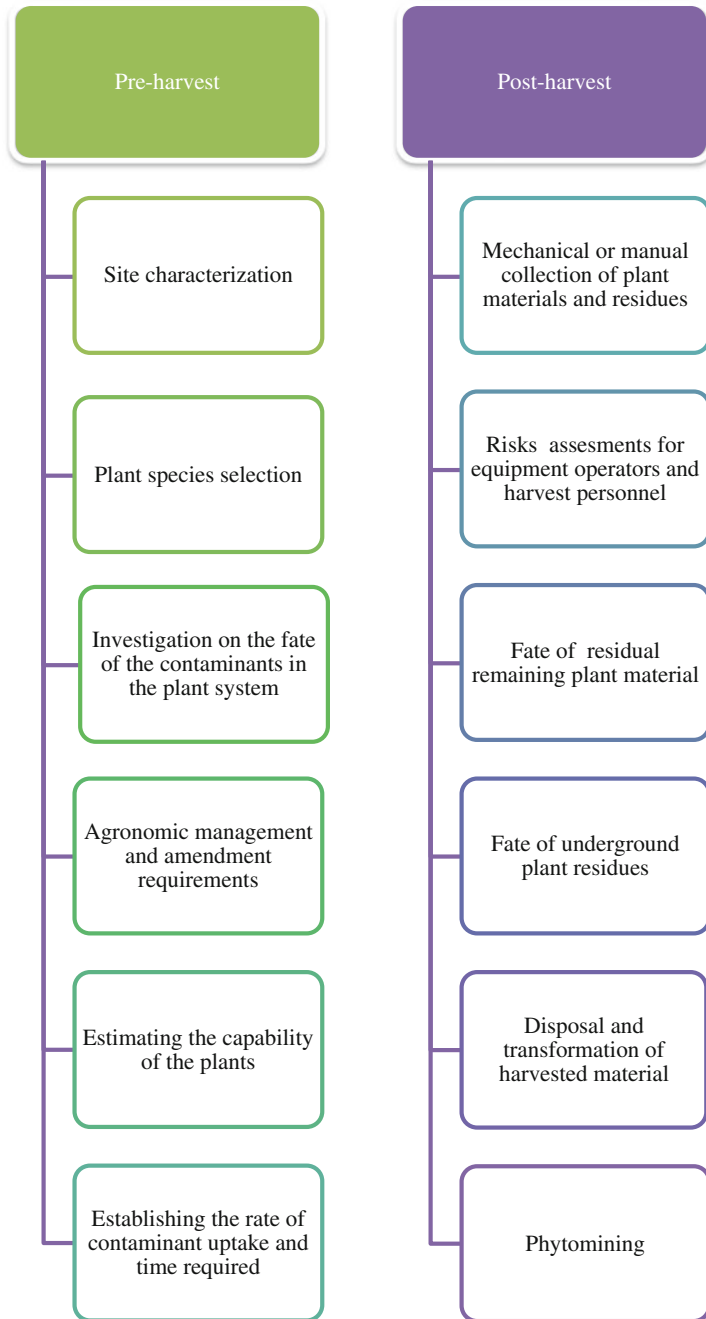


Fig. 2 Pre-harvest planning, implementation, maintenance issues and post-harvest strategies for effective phytoremediation

Table 2 Phytoremediation techniques and mechanisms for various heavy metals and radionuclides

Technique	Mechanism	Substrate	Heavy metals/radionuclides	Typical plant species
Phytoaccumulation	Hyperaccumulation	Soil, sediments	Cd, Cu, Ni, Pb, Zn, U, Cs, Sr	Indian mustard, rape seed, sunflower, Amaranthus, barley, maize
Phytofiltration	Root accumulation	Waste water, ground water	Cd, Cu, Ni, Pb, Zn Some radionuclide such as Cs, Sr, U and organic compounds	Algal spp., stonewort, hydrilla, water lens, catintail, pondweed (Potamogeton, Chara)
Phytostabilization	Absorption/adsorption/precipitation/complexation	Soil, sediments	As, Cd, Cu, Zn, Cr, Pb, Se, U Hydrophobic organics	Phreatophyte trees, members of Poaceae with fibrous roots
Phytodegradation	Degradation in the plant	Soil, waste water, ground water	Herbicides, aromatics, chlorinated aliphatics, ammunition wastes	Poplar, willow, cottonwood, sorghum, rye, cowpea, alfalfa
Rhizodegradation	Breakdown by plant roots through microbial activity	Soil, sediments, waste water	Pesticides, aromatic hydrocarbons, polynuclear aromatic hydrocarbons	Apple, mulberry, rye fescue, Bermuda grass
Phytovolatilization	Volatilization by above ground parts	Soil, sediments, ground water	As, Hg, Se, tritium	Chinese brake, Indian mustard, poplar, canola, tobacco

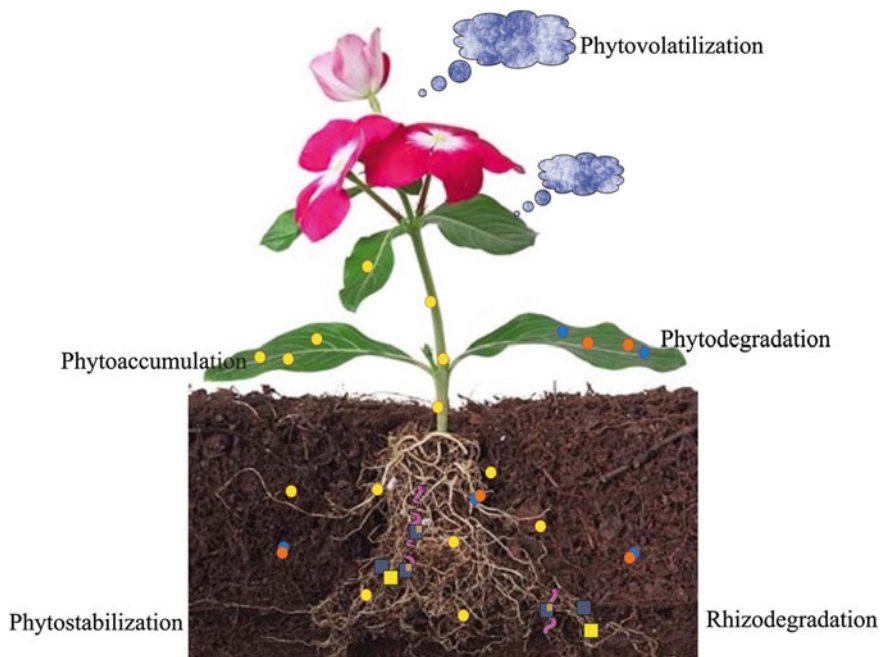


Fig. 3 Conceptual model showing various phytoremediation techniques

4.2 *Phytofiltration*

It is the exclusion of pollutants from contaminated surface waters or waste waters through plants. Phytofiltration may be categorized as blastofiltration (seedlings), caulofiltration (plant shoots) and rhizofiltration (plant roots) depending upon application of plant organ (Ali et al. 2013). During this process, absorption or adsorption of contaminants occurs, which minimizes their movement to underground waters (Ali et al. 2013). Rhizofiltration is the adsorption or precipitation on to plants roots or absorption of contaminants into the roots that are in solution surrounding the root zone. The plants to be used for clean-up are raised in green houses with their roots in water rather than in soil. To acclimatize the plants once a large root system has been developed, contaminated water is collected from a waste site and brought to the plants where it is substituted for their water source. The plants are then planted in the contaminated area where the roots take up the water and the contaminants along with it. As the roots become saturated with contaminants, they are harvested and either incinerated or composted to recycle the contaminants (Singh et al. 2009; Pratas et al. 2012).

4.3 Phytostabilization

It exploits certain plant species to immobilize contaminants in the soil and ground water through absorption and accumulation by roots, adsorption on to roots or precipitation within the root zone, complexation within rhizosphere (Wuana and Okieimen 2011; Singh 2012). Extended and abundant root system is a must to keep the translocation of metals from roots to shoots as low as possible (Mendez and Maiter 2008). This process reduces the mobility of the contaminant and prevents migration of contaminants to the ground water or air, and it reduces bioavailability for entry into the food chain (Erakhrumen 2007). This technique can be used to re-establish a vegetative cover at sites where natural vegetation is lacking due to high metal concentration in surface soils or physical disturbances to surficial materials. Tolerant species can be used to restore vegetation to the sites, thus decreasing the potential migration of contamination through wind erosion, leaching of soil and contamination of ground water (Dary et al. 2010; Manousaki and Kalogerakis 2011). By secreting certain redox enzymes, plants convert hazardous metals to a relatively less toxic state and decrease possible stress and damage (Ali et al. 2013).

4.4 Phytodegradation

It is also called as phytotransformation, which is the breakdown of organic contaminants or pollutants with the help of certain enzymes, e.g. dehalogenase and oxygenase. Phytodegradation is independent of rhizospheric microorganisms (Vishnoi and Srivastava 2008). Plants can uptake organic xenobiotics from contaminated environments and detoxify them through their metabolic activities. Phytodegradation is restricted to the removal of organic contaminants and cannot be applicable to heavy metals as they are non-biodegradable (Ali et al. 2013).

4.5 Rhizodegradation

It is also known as enhanced rhizosphere biodegradation, phytostimulation or plant-assisted bioremediation/degradation, which is the breakdown of contaminants in the soil through microbial activity in the presence of the rhizosphere (Mukhopadhyay and Maiti 2010). It is a much slower process than phytodegradation. Natural substances released by the plant roots—sugars, alcohols and acids—contain organic carbon, amino acids, flavonoids, that provides carbon and nitrogen sources for soil microorganisms, and creates a nutrient-rich environment. Certain microorganisms can digest organic substances such as fuels or solvents that are hazardous to humans and break down them into harmless products through

biodegradation. Certain microorganisms can facilitate the oxidation of Fe^{2+} to Fe^{3+} . The Fe^{3+} ion, in turn, can convert insoluble uranium dioxide to soluble $(\text{UO}_2)^{2+}$ ions. This reaction enhances the mobility of uranium in soil from mining and milling wastes (Jagetiya and Sharma 2009).

4.6 Phytovolatilization

It is the uptake and transpiration of contaminants by plants, their conversion to volatile form with release of the contaminants or a modified form of the contaminant into the atmosphere. It does not remove the pollutant thoroughly; therefore, there are chances of its redeposition. Several controversies are there with this technique (Padmavathiamma and Li 2007). This process is used for removal of organic pollutants and heavy metals such as Se and Hg (Ali et al. 2013).

5 Plant Categorization According to Heavy Metals or Radionuclides Response

Plants show avoidance and tolerance strategies towards contaminants and based on this plants may be classified as indicators, excluders, accumulators and hyperaccumulators.

5.1 Indicators

Plants in which uptake and translocations reflect soil metal concentration with visible toxic symptoms are known as indicators. These plants generally reflect heavy metal/radionuclide concentration in the substrate. Metal indicators are species characteristic for soil contamination with specific metals. *Tradescantia bracteata* indicate radionuclides presence in the substrate (Prasad 2004).

5.2 Excluders

Plants that restrict the uptake of toxic metals into above ground biomass are known as excluder. Excluder plant has high levels of heavy metals in the roots and shoot/root ratio are less than one. These plants have low potential for extraction but are useful for phytostabilization purposes to avoid further contamination (Lasat 2002). According to Burger et al. (2013) *Plantago major* is an excluder plant particularly for U.

5.3 Accumulators

Accumulator plants reflect background metal concentrations by uptake and translocation of contaminants without showing visible toxicity signs. Metals are sequestered into the leaf epidermis, old leaves, epidermal secretory cells, in vacuoles and cell walls. Examples of accumulator plants are *Brassica campestris*, *Picea mariana* for U and *Festuca arundinacea* for ^{137}Cs and ^{90}Sr (Entry et al. 1997; Negri and Hinchman 2000; McCutcheon and Schnoor 2003).

5.4 Hyperaccumulators

The standard for hyperaccumulator has not been defined scientifically; however, hyperaccumulators species are capable of accumulating metals at levels 100-fold greater than those measured in common plants. The term ‘hyperaccumulator’ was first coined by Brooks et al. (1977). More than 500 plant species have been reported for their ability of heavy metal hyperaccumulation (Sarma 2011; Bulak et al. 2014), which includes members of the Asteraceae, Brassicaceae, Caryophyllaceae, Cyperaceae, Cunouniaceae, Fabaceae, Flacourtiaceae and Lamiaceae families (Padmavathiamma and Li 2007). Literature shows that about 75 % of the species are Ni-hyperaccumulators (Prasad 2005). Some plants have natural ability of hyperaccumulation for certain heavy metals; these are known as natural hyperaccumulators, while the accumulation capacity of various plant species can be enhanced through soil amendments and genetic modification. Huang et al. (1998) reported that *Brassica juncea*, *Brassica narinosa*, *Brassica chinensis* and *Amaranth* sp. had more than 1,000-fold citric acid-triggered U hyperaccumulation. Members of family Brassicaceae, *Thlaspi caerulescens* and *Amaranth retroflexus* are found as hyperaccumulators of Co and Sr (McCutcheon and Schnoor 2003). Li et al. (2011) performed studies for the analysis of concentrations of U, Th, Ba, Ni, Sr and Pb in plant species collected from uranium mill tailings. The removal capability of a plant for a target element was assessed. Out of the five plant species, *Phragmites australis* had the greatest removal capabilities for uranium (820 μg), thorium (103 μg) and lead (1,870 μg). Eapen et al. (2006) designate *Calotropis gigantea* (giant milky weed) as a potential candidate to remove ^{137}Cs and ^{90}Sr from soils as well as solutions.

6 Improved Phytoremediation

In order to increase the efficiency of phytoremediation technologies, it is important that we must learn more about different biological processes involved. These include plant–microbe interactions, rhizosphere processes, plant uptake,

translocation mechanisms, tolerance mechanisms and plant chelators involved in storage and transport. Research on the movement of contaminants within the ecosystems via soil–water–plant system to higher trophic levels is also necessary (Pilon-Smits 2005).

Several approaches may be applied to further enhance the efficiency of metal phytoremediation. All of the above, a screening study may be performed to identify the most suitable plant species for remediation. Second, agronomic practices may be optimized for a selected species to maximize biomass production and metal uptake (Chaney et al. 2000). Amendments such as organic acids or synthetic chelators may be added to soil to accelerate and increase metal uptake (Blaylock and Huang 2000). Spatial and successful combination of different plant species assures maximal phytoremediation efficiency (Horne 2000).

Agronomic practices such as fertilization, addition of vermicompost and plant clipping may also affect plant metal uptake by influencing microbial density and composition of the root zone. Further breeding of selected species can be done for the desired property, either through classic breeding or via genetic engineering. Considerable progress had been made in unrevealing the genetic secrets of metal-eating plants. Metal hyperaccumulator genes have been marked and cloned (Moffat 1999; Macek et al. 2008). These will identify new non-conventional crops, metalocrops that can decontaminate metals in the environment (Ebbs and Kochian 1998).

6.1 Chemically Induced Phytoremediation

Chemically induced phytoremediation makes use of natural and synthetic chelators that enhance the mobility of metals by adding them in soil (Marques et al. 2009; Marchiol and Fellet 2011). In late 1980s and early 1990s, ethylenediaminetetraacetic acid (EDTA) was suggested as a chelating agent for the assistance of phytoaccumulation. The influence of EDTA has ranged from non-significant to over 100-fold enhanced accumulation of heavy metals (Grčman et al. 2001). Nitrioltriacetic acid (NTA) is a chelating agent, which has been used in the last 50 years primarily in detergents. The influence of addition of NTA on the mobilization and uptake of heavy metals was observed in various studies (Chiu et al. 2005; Quartacci et al. 2005). Natural low molecular weight organic acids (NLMWOAs), such as citric acid (CA), oxalic acid (OA) or malic acid, because of their complexing properties, are of particular importance and play a significant role in heavy metal solubility, plant uptake and accumulation (Qu et al. 2011; Jagetiya and Sharma 2013). Both synthetic and natural chelators can desorb metals from the soil matrix to form water-soluble metal complexes into the soil solution (Quartacci et al. 2005; Saifullah et al. 2010). There are few limitations to the use of complexing agents. Many synthetic chelators, such as EDTA, Ethylenediamine-N,N'-disuccinic acid (EDDS) have low degree of biodegradability (Jiang et al. 2003; Wu et al. 2005; Bianchi et al. 2008; Dermont et al. 2008). This problem may be

overcome by usage of low phytotoxic and easily biodegradable compounds such as NTA and NLMWOAs (Chen et al. 2003; Wenger et al. 2003), which are more effective in increasing the metal solubility (Vamerali et al. 2010; Rahman and Hasegawa 2011).

Radionuclides existing in soil can be dissolved in solution, complexed with soil organics, precipitate as pure or mixed solids and ion-exchanged in reaction (Gavrilescu et al. 2009). For moderately polluted soils, in situ phytoremediation (Behera 2014) is an eco-friendly but time-requiring solution (Evangelou et al. 2007; Jensen et al. 2009). The order for complexation of heavy metals with different complexing agents in soils occurs in the following order, EDTA and related synthetic chelators > NTA > citric acid > oxalic acid > acetic acid, which was shown by many comparative experiments (Krishnamurti et al. 1997; Wenger et al. 1998; Jagetiya and Sharma 2013). Enhanced uranium accumulation through EDTA has also been reported by (Hong et al. 1999; Sun et al. 2001). Huang et al. (1998) proposed that citric acid was the most effective of some organic acids (acetic acid, citric acid and malic acid) tested in enhancing uranium accumulation in plants. Shoot uranium concentration of *B. juncea* and *B. chinensis* grown in uranium-contaminated soil (total soil uranium, 750 mg kg⁻¹) increased from 5 to more than 5,000 mg kg⁻¹ in citric acid-treated soils. This is the highest shoot uranium reported for plants grown on uranium-contaminated soils.

Applications of chelating agents, such as citric acid, oxalic acid, EDTA, cyclohexylene dinitrilo tetraacetic acid (CDTA), diethylene triamine pentaacetic acid (DTPA), and NTA, have been tested by many researchers (Sun et al. 2011; Jagetiya and Sharma 2013; Oh et al. 2014). Synthetic chelators are non-biodegradable and can leach into underground water supplies making an additional environmental problem. Furthermore, synthetic chelators can be toxic to plants at higher concentrations. Therefore, proper measures should be followed while practicing induced phytoextraction (Marques et al. 2009; Zhuang et al. 2009; Zhao et al. 2011; Song et al. 2012). However, use of citric acid as a chelating agent could be promising because it has a natural origin and is easily biodegraded in soil. Its non-toxic nature does not hamper plant growth (Smolinska and Krol 2012; Ali et al. 2013).

6.2 Phytoremediation Through Microorganisms

Among the microorganisms, algae are of predominant interest of the ecological engineer as they can live under many extreme environments. Once induced to grow in waste waters, they would provide a simple and long-term means to remove radionuclides from the mining effluents. According to a study performed by Kalin et al. (2004), some algal forms possess the quality to sequester U from the contaminated sites. Fukuda et al. (2014) examined 188 strains from microalgae, aquatic plants and unidentified algal species that can accumulate high levels of radioactive Cs, Sr and I from the medium.

In order to understand the radionuclide cycling and dispersal, the effects of bioaccumulation by bacteria or fungi must be acknowledged. The symbiotic relationships can lead to radionuclide uptake by the vascular plant hosts (Shaw and Bell 1994). In the experiments performed by Horak et al. (2006), a new bio-sorption material, called biocer, was used, which consists of a combination of a biological component with ceramic material. The bacterial strain used for this purpose was *Bacillus sphaerius*, which is known for its excellent sorption capacity of U and other heavy metals.

Tsuruta (2004) examined the cell-associated adsorption of Th and U from the solution by using various microorganisms. Those with high Th adsorption abilities were exhibited by strains of the gram-positive bacteria *Arthrobacter nicotianae* IAM12342, *Bacillus subtilis* IAM1026, *Bacillus megaterium* IAM1166, *Micrococcus luteus* IAM1056, *Rhodococcus erythropolis* IAM1399 and *Streptomyces levoris* HUT6156, and high U adsorption abilities were noticed in some gram-positive bacterial strains *S. albus* HUT6047, *S. levoris* HUT6156 and *A. nicotianae* IAM12342.

Lichens can occur in extreme metalliferous environments and can accumulate high amounts of potentially toxic metals (Richardson 1995). They can be used for biomonitoring U discharge from mining activities and radionuclide fallout from nuclear weapon testing and nuclear accidents (Feige et al. 1990). McLean et al. (1998) suggested U adsorption to melanin-like pigments in the outer apothecial wall of the lichen *Trapelia involuta*. The relationships between U, Cu and Fe and the melanin-like pigments in fungal hyphae suggest that the pigments in the exciple and epithecium have a high probability related to the metal accumulation (Takeshi et al. 2003).

Arbuscular mycorrhiza (AM), protect host roots from pathogens, assist in uptake of heavy metals and radionuclides (Selvaraj et al. 2004, 2005). The assistance of AM fungi and the soil's nature to hold the radionuclide to prevent the expression of radioactivity provides greater chances for the vegetation's to survive in the disturbed ecosystem in a better way. Selvaraj et al. (2004) hold a view that due to strong circumstantial evidence, AM fungi would enhance uptake and recycling of radionuclides particularly ^{137}Cs and ^{90}Sr . According to Declerck et al. (2003), mycorrhizal fungi have also been observed to enhance acquisition of ^{137}Cs and Entry et al. (1999) observed the same for ^{90}Sr . In a study performed by Chen et al. (2005), the effects of the mycorrhizal fungus *Glomus intraradices* on U uptake and accumulation by *Medicago truncatula* L. were studied and it was found that such mycorrhiza-induced retention of U in plant roots may contribute to the phytostabilization of uranium-contaminated environments.

Excellent biosorption ability in fungi and yeast are from genera of *Aspergillus*, *Rhizopus*, *Streptovorticillum* and *Sacchromyces* (Akhtar et al. 2013). Plant growth promotion and detoxification of hazardous compounds occur in rhizosphere (Epelde et al. 2010). The cooperation between plants and beneficial rhizosphere microorganisms can upgrade the tolerance of the plants to heavy metals, thus making the microorganisms an important component of phytoremediation technology (Melo et al. 2011).

Microorganisms may directly reduce many highly toxic metals (e.g. Cr, Hg and U) via detoxification pathways. Microbial reduction of certain metals to a lower redox state along with other metal precipitation mechanisms may result in reduced mobility and toxicity (Gadd 2008; Violante et al. 2010). Bioremediation technology utilizes various microorganisms or enzymes for the abolition of heavy metals from polluted sites (Gaur et al. 2014).

6.3 Phytoremediation Through Transgenic Plants

Genetic engineering can be implemented in improving phytoremediation capacity of plants (Wani et al. 2012). Transgenic approaches successfully employed to promote phytoextraction of metals (mainly Cd, Pb and Cu) and metalloids (As and Se) from soil by their accumulation in the aboveground biomass involves implementation of metal transporters, improved production of enzymes of sulphur metabolism and production of metal-detoxifying chelators. Phytovolatilization of Se compounds was promoted in plants overexpressing genes encoding enzymes involved in production of gas methylselenide species (Kotrba et al. 2009).

Genetic studies on hyperaccumulators have been underway for many years (Whiting et al. 2004). Most of the studies have been carried out on the identification of genes involved in the process of hyperaccumulation, uptake, transport and sequestration (Rutherford et al. 2004). Van Huysen et al. (2003, 2004) have described transgenic plants with the ability to take up and volatilize Se.

Genetic engineering has provided new gateways in phytoremediation technology by offering the opportunity for direct gene transfer (Bhargava et al. 2014). This approach of the development of transgenic having increased uptake, accumulation and tolerance can be considered as a good alternative. Engineered plants and microbes are used to treat efficiently low to moderate levels of contamination (Behera 2014). The selection of ideal plant species for phytoremediation engineering is based upon production of high biomass, accumulation, tolerance and competitive and a good phytoremediation capacity (Doty 2008). The genes involved in metabolism, uptake or transport of specific pollutants can enhance the effectiveness of phytoremediation in transgenic plants (Cherian and Oliveira 2005; Eapen et al. 2006; Aken 2008). *Populus angustifolia*, *Nicotiana tabacum* and *Silene cucubalis* have been genetically engineered to overexpress glutamylcysteine synthetase and thus provide enhanced heavy metal accumulation as compared to a corresponding wild-type plant (Fulekar et al. 2009). At the same time, ecological, social and legal objections persist to the practical application of genetically modified organisms in the field. Thus, genetic strategies, transgenic plants, microbe production and field trials will fetch phytoremediation field applications (Pence et al. 2000; Krämer and Chardonnens 2001; Ali et al. 2013).

7 Metal Uptake, Translocation and Accumulation

The main steps during accumulation of metals in plants involve mobilization of metals, uptake from soil, compartmentation and sequestration, xylem loading, distribution in aerial parts and storage in leaf cells (Dalvi and Bhalerao 2013). At each step, concentration, selectivity of transport activities and affinities of chelating molecules affect metal accumulation (Clemens et al. 2002).

Root exudates of natural hyperaccumulators solubilize metals, which causes acidification of rhizosphere (Mahmood 2010) and leads to metal chelation by secretion of mugenic and aveic acid (Dalvi and Bhalerao 2013). The complete mechanism of whole process is unclear. Metal enters in plant either through inter-cellular spaces (apoplastic pathway) or by crossing plasma membrane (symplastic pathway) (Peer et al. 2006; Saifullah et al. 2009). Ghosh and Singh (2005) stated that inward movement of metals during symplastic pathway takes place due to strong electrochemical gradient.

The fate of metal after entry into roots can be either storage in the roots or translocation to the shoots primarily through xylem vessels (Jabeen et al. 2009) where they are stored in vacuoles as they possess low metabolic activities (Denton 2007). Sequestration in the vacuole removes excess metal ions from the cytosol and reduces their interactions with cellular metabolic processes (Sheoran et al. 2011).

Uranium uptake and accumulation were investigated in twenty different plant species by Soudek et al. (2011). They used hydroponically cultivated plants, which were grown on uranium-containing medium. *Zea mays* were found to have highest uptake, while *Arabidopsis thaliana* had the lowest. The amount of accumulated U was strongly influenced by U concentrate in the cultivation medium. U accumulated mainly in the roots.

Viehweger and Geipel (2010) conducted a comparative study of U accumulation and tolerance in terrestrial versus laboratory trials on *A. halleri*, which grew on U mining site. In the native habitat, the plant sequesters high amount of U in roots than shoots, but in hydroponic trails, roots accumulated 100-fold more and shoots accumulated tenfold more U. This drastic increase in U accumulation could be attributed to iron deficiency in hydroponic trials.

Due to the similar oxidation states and ionic radii, non-essential heavy metals compete and enter roots through the same transmembrane transporters used by essential heavy metals (Alford et al. 2010). Seth (2012) suggests that the relative lack of selectivity in ion transport may explain the reason of the entry of such metals.

8 Advantages and Limitations of Phytoremediation

Phytoremediation, which is also called as green remediation, botano-remediation, agroremediation or vegetative remediation is an emerging group of technologies utilizing green plants to clean up the environment from contaminants and has been

offered as a simple and non-invasive alternative to the conventional engineering-based remediation methods (El-Gendy 2008; Vandenhove et al. 2009; Sevostianova et al. 2010; Hoseinizadeh et al. 2011). Soil is the ultimate and most important sink of chemical components in the terrestrial environment (Roy et al. 2010). Some of the advantages listed till date are (Negri and Hinchman 2000; Doty 2008; Lone et al. 2008) as follows:

- It is economically viable, aesthetically pleasing and easy to implement.
 - It has the potential to treat sites polluted with more than one type of pollutant.
 - During the whole process, plants serve as stabilizers, thus contaminants cannot escape into the neighbourhoods.
 - The plants also provide the soil nutrients and stabilization by reducing wind and water erosion.
 - Reduces the exposure time to the radionuclides.
 - Easy monitoring of the sites with wildlife enrichment.
 - No harm to the soil dynamics as the soil is treated in situ. A special advantage of phytoremediation is that soil functioning is maintained and life of soil is reactivated.
 - Once plants are established, they remain for consecutive harvests to continually remove the contaminants.
 - It has lower side effects than physical and chemical approaches.
- Despite of the above-mentioned advantages, it holds some limitations (Wu et al. 2005; Singh et al. 2007; Ali et al. 2013).
- It is applicable to sites with low to moderate levels of metal contamination because plant growth is not sustained in heavily polluted soils.
 - Slow growth rate and low biomass of hyperaccumulators.
 - It requires lot of time.
 - Limited tolerance of the plant species.
 - Tightly bound fraction of metal ions cannot be removed from soil due to limited bioavailability.
 - Sometimes, the agro-climatic and hydrological conditions may limit the plant growth and there are chances of entering of the contaminants in food chain through animals/insects feeding on plant material loaded with contaminants.
 - Lower efficiency over other non-biological remediation techniques and the limitations when the contaminated soil layer occasionally extends to the deeper profile.

9 Future Prospects of Phytoremediation

Phytoremediation is used for removal of variety of toxic metals and radionuclides, while minimal environmental disturbance and larger public acceptance (Liu et al. 2000; Tangahu et al. 2011; Fukuda et al. 2014). An easy handling of this

technology shows its strong ability as a natural, solar energy-driven remediation approach for multiple pollutants (Singh et al. 2007). Phytoextraction using a combination of high biomass with hyperaccumulator mechanisms will successfully remove contaminants from the environment (Ali et al. 2013).

Phytoremediation field projects, proposed for the forthcoming time, should benefit from collaboration between research groups and industry so that they can be designed to address hypotheses and obtain scientific knowledge for clean-up standards. Phytoremediation is expected to be commercialized and used as a vital tool in sustainable management of contaminated soils, especially in developing countries which cannot afford sophisticated technologies due to their vast populations (Mirza et al. 2014). Bioavailable fraction of the pollutant should be the focal point in order to reduce the costs and enable the clean-up of sites with the limited funds (Pilon-Smits 2005).

As the mixtures of organic as well as inorganic pollutants occur in 64 % of polluted sites (Ensley 2000), phytoremediation can be helped by more collaborative studies by teams of researchers from different backgrounds. In general, the advantages and limitations of phytoremediation must be accessed for a particular project to determine whether this type of remediation is the most appropriate for the task. Several hyperaccumulator plants remain to be discovered or recognized, and we need to acquire more information about their physiology (Raskin et al. 1994).

Further, research on easily biodegradable chemicals in phytoremediation process is still required before the safe adoption of this technology in fields (Luo et al. 2006). The use of easily biodegradable chelating agents (Tandy et al. 2006) enhances the process of phytoextraction (Evangelou et al. 2007) and reduces the remediation time period. There is a necessity of optimization of the process, proper understanding of the mechanism of uptake and proper disposal of biomass produced. Several methods regarding plant disposal have been described but data are scarce (Ghosh and Singh 2005).

In-depth research of cellular mechanisms involved in heavy metal avoidance, uptake, transport and accumulation is essential (Dalvi 2013). Investigations are being done to identify and characterize several proteins involved in cross-membrane transport and vacuole sequestration of heavy metals. Molecular advancement and achievements in such studies will greatly help in unrevealing the mechanism as well as enhancing the efficiency of phytoremediation (Ali et al. 2013).

In times to come, mining of genomic sequences from *A. thaliana* and rice and availability of new genetic technologies should lead to the identification of novel genes important for pollutant remediation and tissue specific transporters. Challenging issues such as biosafety assessment and genetic pollution involved in adopting the new initiatives for cleaning up the polluted ecosystems must not be ignored, from both ecological and greener point of view (Mani and Kumar 2013). Laboratory studies on the potential of transgenic plants and/or microbes to remediate organic and inorganic contaminants are to be further explored (Doty 2008).

The future of phytoremediation consists of ongoing research work and has to pass through a development phase, and there are several technical barriers, which need to be addressed. We still need to completely understand the ecological complexities of the plant–soil interactions. We should soon be able to shed light on some of the poorly understood phenomena related to the extensive field of phytoremediation. This area of research deserves multidisciplinary (soil chemistry, plant biology, ecology and soil microbiology as well as environmental engineering) investigations using molecular, biochemical and physiological techniques (Khan 2006). Heavy metal detoxification can be achieved by optimization of plants through multidisciplinary approach. Phytoremediation of multiple contaminated sites is urgently required along with increasing its scope and efficiency (Oh et al. 2014). We need to optimize the agronomic practices, better plant-microbe combinations and plant genetic abilities in order to develop commercially useful practice (Jagetiya and Sharma 2009; Oh et al. 2014).

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