# Dharmendra Kumar Gupta Clemens Walther *Editors*

# Radionuclide Contamination and Remediation Through Plants



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### Preface

Atomic nuclei which are not stable but decay by emission of highly energetic radiation are called radionuclides. They are omnipresent in nature, some of them with half-lives exceeding the age of the solar system. Amongst these are, e.g., potassium-40, uranium-238, uranium-235, and thorium-232. Uranium is found in many types of soil and rocks (concentrations ranging from 0.003 ppm in meteorites to 120 ppm in phosphate rock). In addition, there are shorter lived radionuclides produced by natural processes such as interaction of cosmic radiation with the earth's atmosphere. Carbon-14, beryllium-10, and tritium are examples. Human activities such as nuclear weapon's testing in the 1960s, accidents involving nuclear material (military and peaceful use of nuclear power), lost and orphan sources from, e.g. medical use add to the radioactive inventory. Further sources are mining activities. Any matter originating from deep underground may contain considerable amounts of natural radioactive matter (NORM). For instance, the production of oil, gas, or phosphate fertilizers goes hand in hand with the release of considerable amounts of uranium and decay products. Enhanced radiation levels from tailing of these uses are called TENORM (technically enhanced NORM). Also, regenerative energies are not free from radiation risks. Geothermal water used for energy production may contain high levels of radium from the uranium and thorium decay series' accumulating in filters and scales.

Once radionuclides are deposited on the soil surface, they eventually are incorporated into the soil structure, taken up by plants and, via the food chain, enter animals, and also humans. A 75 kg human contains approximately 9,000 Bq of natural radioactivity in his body, mainly due to K-40 and C-14. Some organs such as the thyroid gland and also certain plants may enrich radionuclides, as is known for seaweed, enriching iodine by a factor >10.000 L Kg<sup>-1</sup>. During the course of evolution cells learned to repair damages caused by the ionizing radiation emitted from radioactive decay (alpha, beta or gamma radiation) and the damages caused by secondary species generated from ionizing radiation such as free oxygen radicals (ROS). As a rule of thumb organisms are the more sensitive to radiation the higher their DNA content is. However, at too high radiation levels even simple organisms and cells will suffer and finally the total organism will be damaged or die. While damage from the ionizing radiation to the cells DNA is

most important at high dose the chemical toxicity of many radioactive isotopes plays an important role. Uranium, thorium, plutonium, and lead, to name just a few, are heavy metals. In these cases, stress to cells due to chemical toxicity adds to effects of ionizing radiation.

Like heavy metals, radionuclides cannot be naturally or synthetically degraded. Therefore, radionuclides become a risk factor to public health when exposed and/ or deposited in soil and water.

Being sessile in nature, plants are exposed to radionuclides which are released and disseminated into the environment as dry or wet deposition on soil or water. Both routine and accidental incorporation of nuclear wastes in the environment cause radionuclides swallowing, where soil to plant transfer of such materials take place. However, uptake of the radionuclides by plants depends upon several factors including mode of interaction with the materials and physiological characteristics of the species and factors like concentrations, bioavailability, and mobility of radionuclides in surface and subsurface geologic systems. The concentration, mobility, and bioavailability of radionuclides depend upon the quality, quantity, and the rate of release of radionuclides present at the source; different hydrological factors, such as dispersion, advection, and dilution; and geochemical processes, like complexation at aqueous phase, pH, solid/liquid distribution coefficient, reduction/oxidation (redox), adsorption/desorption and ion exchange, precipitation/dissolution, diffusion, colloid-facilitated transport, exchangeable potassium ion distribution, anion exclusion and organic matter contents. Absorption and distribution of the contamination in plants may take place either through direct (exposures at aerial organs) or indirect (through root systems in soil related contamination) routes, which varies considerably in different plant species especially in case of long-lived radionuclides. Furthermore, biological activity or physical changes in the soil properties/texture (like drying and subsequent cracking of soils) and colloid-facilitated transport may augment the mobility and/or affectivity of certain radionuclides Plant tolerance to metals depends largely on plant efficiency in uptake, translocation, and further sequestration of metals in specialized tissues or in trichomes and cell organelles. Metals which are complexed and sequestered in cellular structures become unavailable for translocation to the shoot. Metal binding to the cell wall is not the only plant mechanism responsible for metal immobilization into roots and subsequent inhibition of ion translocation to the shoot. The vacuole is generally considered to be the main storage site for metals in veast and plant cells and there is evidence that phytochelatin-metal complexes are pumped into the vacuole in plants.

Though radionuclide uptake into plants and consequently into the food chain is generally undesired. A very effective and even selective uptake of certain elements by plants can, however, be even helpful in remediating contaminated soils. This concept is known as phytoremediation. Phytoremediation of radionuclides has many advantages over the traditional treatments. Firstly, in phytoremediation the soil is treated in situ, which does not cause further disruption to the soil dynamics. Secondly, once plants are established, they remain for consecutive harvests to continually remove the contaminants. Lastly but not least, phytoremediation reduces the time workers are exposed to the radionuclides. Finally, phytoremediation can be used as a long term treatment that can provide an affordable way to restore radionuclide contaminated areas. For phytoremediation of radionuclides to be successful, a few criteria have to be met. The most important is that the radionuclides be spread throughout a huge area and be present in very low-level concentrations. The radionuclides must be bioavailable in water/soil solution for plants to up take them into roots. The plants themselves must also be tolerant of the radionuclides when they are accumulated into their biomass. The best plants for phytoremediation are those that have an extensive root system and adequate above-ground biomass.

When plants are exposed to ionizing radiation, molecular and cellular effects are induced directly through energy transfers to macromolecules or indirectly through a water radiolytic reaction producing reactive oxygen species (ROS). By energy transfer from the radiation field to plant tissue, ionizing radiation can directly induce DNA strand breaks, lipid oxidation, or enzyme denaturation. Besides directly damaging macromolecules, potentially toxic ROS can be generated during radiolysis of water, indirectly inducing cellular damage. As ROS are also produced under natural metabolism and also function as signalling molecules regulating normal growth, development, and stress responses, plants also possess an antioxidative defense system comprising enzymes (e.g., superoxide dismutase (SOD) and catalase (CAT)) and metabolites (e.g., ascorbate and glutathione) to regulate the amount of ROS in cells. Plant tolerance mechanisms require the coordination of complex physiological and biochemical processes, including changes in global gene expression. Plants employ various strategies to cope with the toxic effects of radionuclides like metals or metalloids.

Resistance to radionuclides stress can be achieved by "avoidance" when plants are able to restrict metal uptake, or by "tolerance" when plants survive in the presence of high internal metal concentration. Avoidance involves reducing the concentration of metal entering the cell by extracellular precipitation, biosorption to cell walls, reduced uptake, or increased efflux. In a second type of situation, radionuclides are intracellularly chelated through the synthesis of amino acids, organic acids, glutathione (GSH), or metal-binding ligands such as metallothioneins (MTs), phytochelatins (PCs), compartmentation within vacuoles, and upregulation of the antioxidant defense and glyoxalase systems to counter the deleterious effects caused by ROS.

It is an intriguing question whether the toxicity effect induced by heavy metals was the result (at least partially) of signalling pathways evolving the action of the formed substances, or parallel direct metal action and signalling pathways. The molecular mechanisms of signal transduction pathways in higher plant cells are essential to vital processes such as hormone and light perception, growth, development, stress resistance, and nutrient uptake from soil and water. Heavy metals interfere with cell signalling pathways. In fact, it might be hypothesized that metals-induced deregulation of signaling events significantly participates in the metal toxicity response, as well as in damage development. The main purpose of the present book is to focus on the mechanistic (microscopic) understanding of radionuclide uptake by plants from contaminated soils, both, in order to understand the risks originating from plant uptake and the benefits by potential use for phytoremediation.

The key features of the book are related to the radionuclide toxicity in plants and how the radioactive materials are taken up by plants and cope up from their toxic responses. Some chapters deal with how soil classification affects the radionuclide uptake in plants. Other chapters focus on natural plant selection, speciation of actinides, kinetic modeling, and some case studies on cesium and strontium after radiation accident. Overall, the information compiled in this book will bring in-depth knowledge and advancement in the field of radionuclide toxicity and their remediation through plants in recent years.

Dr. Dharmendra K. Gupta and Prof. Clemens Walther personally thank the authors for contributing with their valuable time, knowledge, and enthusiasm to bring this book into its present shape.

Hannover, Germany

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

Bhagawatilal Jagetiya, Anubha Sharma, Akash Soni and Umesh Kumar Khatik

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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**Keywords** Phytoremediation • Radionuclides • Phytoaccumulation • Metal tolerance • Hyperaccumulator • Chelators

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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).

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

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are natural (mainly from the <sup>238</sup>U, <sup>232</sup>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 <sup>3</sup>H. <sup>14</sup>C. <sup>90</sup>Sr. <sup>99</sup>Tc, <sup>129</sup>I. <sup>137</sup>Cs, <sup>237</sup>Np, <sup>241</sup>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 <sup>230</sup>Th, <sup>226</sup>Ra and <sup>210</sup>Pb (SanMiguel et al. 2004). Radioactive isotopes such as <sup>14</sup>C, <sup>18</sup>O, <sup>32</sup>P, <sup>35</sup>S, <sup>64</sup>Cu and <sup>59</sup>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 <sup>137</sup>Cs and <sup>90</sup>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 <sup>3</sup>H, <sup>14</sup>C, <sup>36</sup>Cl, <sup>41</sup>Ca, <sup>59,63</sup>Ni, <sup>89,90</sup>Sr, <sup>99</sup>Tc, <sup>129</sup>I, <sup>135,137</sup>Cs, <sup>210</sup>Pb, <sup>226,228</sup>Ra, <sup>237</sup>Np, <sup>241</sup>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 <sup>99m</sup>Tc and <sup>123</sup>I for diagnostic purposes to longer-lived therapeutic isotopes such as <sup>131</sup>I, <sup>7</sup>Be, <sup>67</sup>Ga, <sup>153</sup>Sm and <sup>197</sup>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 physicochemical, 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. <sup>238</sup>U accumulates in lungs and kidneys, <sup>232</sup>Th in lungs, liver and skeleton tissues and <sup>40</sup>K in muscles (Samat and Evans 2011). Depositions of large quantities of these radionuclides in organs affect the health conditions such as

Radionuclide	Sources	Reference
Uranium ( <sup>235, 238</sup> U)	Natural, mining, milling, nuclear waste disposal	Chabalala and Chirwa (2010)
Thorium ( <sup>232</sup> Th)	Natural, mining, milling and processing, phosphate fertilizer production, tin processing, industrial boilers, military operations	Atwood (2010), Tawalbeh et al. (2013)
Radium ( <sup>226, 228</sup> Ra)	Uranium decay product from mill tailing	Madruga et al. (2001), Cerne et al. (2010)
Cobalt ( <sup>60</sup> Co)	Car, truck and airplane exhausts, burning coal and oil, industrial processes, nuclear medicines	Simeonov and Sargsyan (2008)
Iodine ( <sup>131</sup> I)	Nuclear test (underground), fuel reprocessing, spent nuclear fuel	Hu et al. (2010)
Strontium ( <sup>90</sup> 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 ( <sup>137</sup> Cs)	Nuclear power stations	Stohl et al. (2012)
Carbon ( <sup>14</sup> C)	Natural and nuclear reactor	Zhu and Shaw (2000)
Potassium ( <sup>40</sup> K)	Natural	Zhu and Shaw (2000)
Plutonium ( <sup>239</sup> Pu)	Nuclear reactor	Zhu and Shaw (2000)

Table 1 Sources of radionuclides in the environment

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 <sup>235</sup>U, <sup>239</sup>Pu and <sup>238</sup>U are used together with the radionuclide <sup>3</sup>H 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  $2 \times 10^8$  TBq of radioactivity has been released into the atmosphere from worldwide nuclear weapons' tests. In terms of radioactivity, <sup>3</sup>H, <sup>90</sup>Sr, <sup>137</sup>Cs, <sup>241</sup>Am and Pu isotopes are currently the radionuclides of great importance. Long-lived <sup>14</sup>C, <sup>36</sup>Cl, <sup>99</sup>Tc, <sup>129</sup>I, <sup>237</sup>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  $3.72 \times 10^5$  MWe (World Nuclear Association 2007), the annual discharge of <sup>14</sup>C worldwide is about 60 TBq Y<sup>-1</sup>.

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  $\alpha$ -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 <sup>99</sup>Tc and <sup>129</sup>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 <sup>235</sup>U and <sup>239</sup>Pu, for weapons production. However, other fissile nuclides, such as <sup>237</sup>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  $1.2 \times 10^7$  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 <sup>90</sup>Sr, <sup>137</sup>Cs and <sup>239+240</sup>Pu from the Chernobyl accident were in the order of 10 % and much higher for <sup>238</sup>Pu and <sup>241</sup>Am. Fallout of hot particles caused a considerable contamination of the soil surface, with <sup>137</sup>Cs up to 106 Bq m<sup>-2</sup>, and 116,000 people were evacuated within a zone of 30 km distance from the reactor (Balonov 2007). Six artificial radionuclides (<sup>131</sup>I, <sup>134</sup>Cs, <sup>137</sup>Cs, <sup>129</sup>mTe, <sup>95</sup>Nb and <sup>136</sup>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): <sup>241</sup>Am, <sup>252</sup>Cf, <sup>60</sup>Co, <sup>137</sup>Cs, <sup>90</sup>Sr, <sup>192</sup>Ir and <sup>238</sup>Pu. Commercial radioactive sources for potential RDD include RTG (<sup>90</sup>Sr), teletherapy and irradiators (<sup>60</sup>Co and <sup>137</sup>Cs), industrial radiography (<sup>60</sup>Co and <sup>192</sup>Ir), logging and moisture detectors (<sup>137</sup>Cs, <sup>241</sup>Am and <sup>252</sup>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.

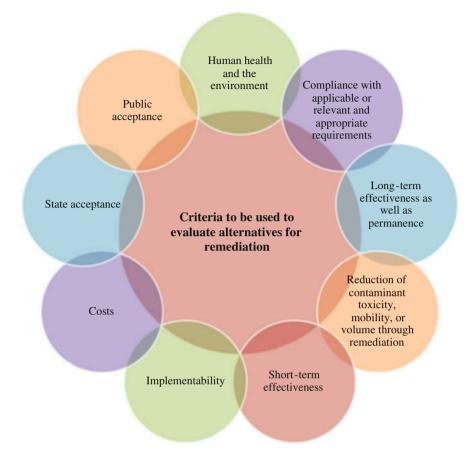


Fig. 1 Suggestions of Environmental Protection Agency (*EPA*) in order of preference that the nine criteria may be used to evaluate alternatives for remediation

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 <sup>137</sup>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. Štrok and Smodiš (2010) collected samples of plants from a uranium mill tailings waste pile containing <sup>201</sup>Pb, <sup>226</sup>Ra and <sup>238</sup>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* Czem.) 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 phytosequesteration. 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).

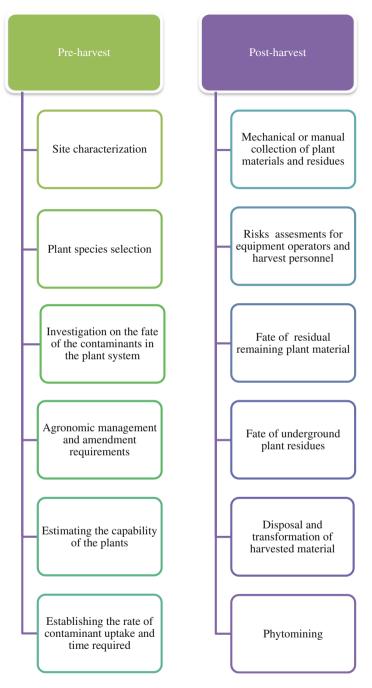


Fig. 2 Pre-harvest planning, implementation, maintenance issues and post-harvest strategies for effective phytoremediation

Table 2 Phytoreme	ediation techniques and mech	nanisms for various	Table 2 Phytoremediation techniques and mechanisms for various heavy metals and radionuclides	
Technique	Mechanism	Substrate	Heavy metals/radionuclides	Typical plant species
Phytoaccumulation	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, catninetail, pondweed (Potamogeton, Chara)
Phytostabilization	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	Phytodegradation Degradation in the plant	Soil, waste water, ground water	Herbicides, aromatics, chlorinated aliphatics, ammunition wastes	Poplar, willow, cottonwood, sorghum, rye, cowpea, alfalfa
Rhizodegradation	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	Phytovolatilization Volatilization by above ground parts	Soil, sediments, ground water	As, Hg, Se, tritium	Chinese brake, Indian mustard, poplar, canola, tobacco

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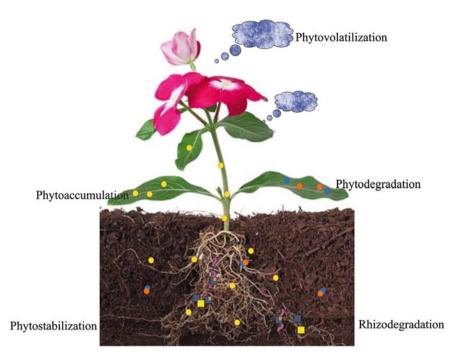


Fig. 3 Conceptual model showing various phytoremediation techniques

#### 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). Rhizofilteration 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  $(UO_2)^{2+}$  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 <sup>137</sup>Cs and <sup>90</sup>Sr (Entry et al. 1997; Negri and Hinchman 2000; McCutcheon and Schnoor 2003).

#### 5.4 Hyperaccmulators

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 Brassicace, 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 australies 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 <sup>137</sup>Cs and <sup>90</sup>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, metallocrops 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). Nitrilotriacetic 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<sup>-1</sup>) increased from 5 to more than 5,000 mg kg<sup>-1</sup> 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-biode-gradable 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 biosorption 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 grampositive 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 <sup>137</sup>Cs and <sup>90</sup>Sr. According to Declerck et al. (2003), mycorrhizal fungi have also been observed to enhance acquisition of <sup>137</sup>Cs and Entry et al. (1999) observed the same for <sup>90</sup>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*, *Streptoverticullum* 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. Phytovolatization 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 solubalize 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 intercellular 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