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PHYTOREMEDIATION: A NOVEL STRATEGY AND ECO-FRIENDLY GREEN TECHNOLOGY FOR REMOVAL OF TOXIC METALS

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Abstract

Phytoremediation also called as green remediation is the use of plants to remediate selected contaminants in the contaminated soils, sludge, ground water and waste water. Phytoremediation has a number of different methods that can lead to contaminant degradation, remove of metals through accumulation, dissipation and immobilization. The conventional methods existing currently for the remediation of heavy metal contaminated soils are expensive and not necessarily eco – friendly. Phytoremediation is a new 'green technology' which uses plants potential to reinstate the health of the environment. A variety of plants have been identified which are capable of accumulating high concentrations of metals in their aerial parts and roots or stabilizing the metals in soils and thus restricting their translocation to the shoots and removing the metals from the soil through synthesis of volatile compound.

Key words: Contaminants, Green technology, Heavy metals, Conventional methods, Eco-friendly and Phytoremediation.

INTRODUCTION

In the age of industrialization, developmental progress manifest with the hi-tech advancement has raised severe environmental challenges as they by passed the security demands of the natural environment (Bennett et al., 2003). Even though, a number of natural processes (forest fires, volcanoes etc.) discharge different pollutants into the environment, anthropogenic activities are supposed to be the major cause of environmental pollution. The Pollutants of different kinds, mainly heavy metals that arose as a consequence of accidental or process spillage, dumping of raw industrial waste and by sludge application to agricultural soils, have contributed significantly to the deterioration of land and water resources, as is clear from changes in ecosystem processes (Jan *et al.*, 2014). The Metal contamination of soils is everywhere around the earth. Heavy metals enter the soil due to anthropogenic activities such as the use of urban composts, fertilizers, pesticides, sewage sludge, sewage irrigation, burning of municipal waste, auto vehicle exhausts, industrial emissions, metal mining and smelting (Hussain et al., 2006; McGrath et al., 2001; Murtaza et al., 2010). These heavy metals consist of copper (Cu), zinc (Zn), cadmium (Cd), lead (Pb), chromium (Cr), mercury (Hg), iron (Fe), manganese (Mn), and nickel (Ni) (McIntyre, 2003). Metal accumulation is a subject matter of severe concern due to threat to plant growth,

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soil quality, animal and human health (McGrath et al., 2001). Both organic and inorganic pollutants are responsible for the deterioration in the health of soil. Amid organic pollutants, trichloroethylene, herbicides (Burken and Schnoor, 1997), explosives such as trinitrotoluene (Hughes et al., 1997), hydrocarbons (Schnoor et al., 1995) and fossil fuels and methyl tertiary butyl ether are the leading polluting agents (Hong et al., 2001). These xenobiotic noxious compounds persist in the ecosystem due to their non-biodegradable nature, posing a severe threat is to the environment. Though, these noxious compounds can only be altered from one oxidation state to another (Marques et al., 2009) and, without interference, remain in the soil for a long period of time. Generally, these componds exist in distinct states, viz. colloidal state (hydroxides, oxides and silicates) (Lytle et al., 1998), metalloids (As and Se) (Rathinasabapathi and Srivastava, 2006), nutrients, pesticides, herbicides and petroleum derivatives (Macek et al., 2000). These harmful metallic elements possess a high degree of toxicity and thus have a negative impact on the entire ecosystem. Among their distribution along with potential toxic effects on human health, their contamination presents a black picture of a complex industrialized hi-tech society. Further being expensive and non-specific, use of physicochemical techniques for the treatment of soil pollutants has rendered land useless for the growth of plants as the population of microbes connected with diverse biological activities such as nitrogen fixation, are greatly affected during the process of decontamination. Metal pollutants that present a continuing threat to mankind and to its surroundings need to be addressed in order to reverse their effects so as to maintain the balance of the system (Jan et al., 2014). The governments around the world are duty bound to their citizens to advocate a safe environment free from pollution. However, these environmental issues are outweighed by other concerns for the countries' economic, agricultural and industrial development for ever-increasing populations. Thus, prioritization in a particular direction becomes the driving force actually responsible for environmental pollution (Ikhuoria and Okieimen, 2000). Growing plants to clean up the soils is a cost effective and environmentally friendly alternative (Yang et al., 2005). Phytoremediation is a novel 'green technology' which utilizes plants' potential to return the health of the environment. The conclusions drawn on the basis of fundamental and applied research is that the plants possess remarkable potential for eliminating and degrading a range of heavy metal toxicants. The wonderful nature of this technology is in its cost effectiveness, simplicity, sustainability, environmental compatibility and the fact that it is more aesthetically attractive than the conventional classical technologies. It can be implemented in situ to remediate large expanses of contaminated ground or to treat large volumes of dilute wastewater. Plants react differentially to metal contamination in soils and can be classified into different categories, depending upon their responses to metal contamination in their rooting medium. Plants can be classified into accumulators, indicators or excluders depending upon absorption and translocation of metals by the plants to above-ground parts (Baker, 1981). Accumulators can continue to exist by maintaining high concentration of metals in their tissues. Indicator plants are reported to have mechanisms that control translocation of metals from roots to shoots and excluders control the entry of metals into plants at root level (Chaudhry et al., 1998). Plants use diverse adaptive mechanisms to accumulate or exclude metals and thus maintain their growth. Accumulation and tolerance of metals by the plants is a complex phenomenon (Sabir et al., 2014). The movement of metals across the root membrane, loading and translocation of metals through xylem and sequestration and detoxification of metals at the cellular and whole plant levels are important mechanisms adopted by accumulator plants (Lombi et al., 2002). Indicator plants absorb the metals from the soils and then control their movement to the shoots while excluders restrict the entry of metals into the plant roots. Understanding the mechanisms involved in phytoremediation is necessary to effectively use this technique on metal-contaminated soils. Plants have the natural capability to degrade heavy metals by means of different processes such as bioaccumulation and translocation. It has been reported that plants have a greater tolerance to heavy metal pollution without being seriously harmful, signifying that this property of plants can be exploited to detoxify contaminants using novel agricultural and genetic engineering approaches (Pirzadah et al., 2014). Some plants have the natural ability to degrade various recalcitrant xenobiotics and are thus regarded as green livers, acting as an essential sink for environmentally intolerable chemicals (Schwitzguébel, 2000). Nature has bestowed on plants an excellent capacity to reduce the effect of these toxic elements within the growth matrix, may be it soil or water.

PHYTOREMEDIATION AND MECHANISMS

Naturally metals are present in the Earth's crust at different levels (Angelone and Bini, 1992). Mining, industry, and agriculture directs to accelerated discharge of metals into ecosystems, causing serious environmental problems (Lantzy and Mackenzie, 1979; Nriagu, 1979; Ross, 1994). Even though many metals are essential for cells (e.g. Cu, Fe, Mn, Ni, Zn), but all metals are toxic at higher concentrations (Marschner, 1995). One reason that metals may become toxic is that they may cause oxidative stress especially transition metals, which can take up or give off an electron (e.g. Fe $^{2+/3+}$, $Cu^{+}/^{2+}$). Another reason why metals may be toxic is because they can replace other essential metals in pigments or enzymes, disrupting the function of these molecules (Rivetta et al.1997; van Assche and Clijsters, 1986). Several metal ions (e.g. Hg^{+} and Cu^{+}) are very reactive to thiol groups and can hinder with protein structure. When released into the environment, some metals occur as free cations (e.g. Zn^{2+}), while others form cations that are bound to organics (e.g. Cu^{2+}) and yet others form oxyanions (e.g. CrO_4^{3-}, MoO_4^{2-}, WO_4^{3-}). Heavy metals degrade soil and water resources and thus cause a severe threat to human and animal health. This threat is further annoyed due to the persistent and non-biodegradable nature of metals (Gisbert et al., 2003). Accumulation of metals in the bodies of animals and humans after entering the food chain has serious implications for health as some metals are known to damage DNA and cause cancer due to their mutagenic abilities (Steinkellner et al., 1998). Remediating the soils polluted with metals is therefore necessary for safe use of such soils and some in situ and ex situ technologies are used for this purpose. Phytoremediation is considered environmentally friendly, non-invasive and cost-effective technology to clean up the metal-contaminated soils. Plants adopt different mechanisms to grow in the metal-contaminated soils without adverse effects on their growth. Some plants eliminate the metals from metabolically active sites by controlled uptake or root to shoot transfer of metals (Küpper et al., 1999). Several other plants can tolerate high metal concentrations in their tissues through binding of metals with organic compounds, metal compartmentalization at cellular and sub-cellular levels and metabolic alterations (Küpper et al., 1999; Peng et al., 2006; Wei et al., 2005). In plants Heavy metals tolerance may be defined as the ability of plants to endure in a soil that is toxic to other plants (Macnair et al., 1999).

Definition and Concept: Phytoremediation can be defined as the practice, which uses green plants for the release, transfer, stabilization or degradation of pollutants from soil (Elekes et al., 2014; USEPA. 2001; Paz- ferreiro et al 2014). Several plant roots can absorb and immobilize metal pollutants, whereas other plant species have the ability of metabolizing or accumulating organic contaminants. Diverse relationships and relations between plants, microbes, soils and contaminants make phytoremediation processes possible. The term phytoremediation consists of two words Phyto derived from the Greek means - plant and remedium derived from Latin means - able to cure or - restore (Vameraali et al., 2010). This thought was first given by Chaney (1983) and then developed through the study of plant species having ability to remove pollutants from environment components. It can be used for a wide range of organic (Cluis et al., 2004) and inorganic contaminants (Vamerali et al., 2010). Phytoremediation processes are most effective wherever contaminants are present at low to medium levels, as high contaminant levels can reduce plant and microbial growth and activity [USEPA, 2010]. Mechanisms involved in the uptake, translocation, and storage of micronutrients are the same involved to translocate and storage heavy metals (Subhashine et al., 2013). On the basis of method by which remediation of metal contaminants is achieved, phytoremediation has been mostly categorized into the following areas:

Phytoextraction: It is the use of metal accumulators to remove metals by absorbing them in the harvestable parts from the soil.

Rhizofiltration: The use of microorganisms that are related with plants to reduce the contaminants from the soil.

Phytostabilization: The process through which environmental pollutants are reduced by stabilizing them through the use of plants.

Phytovolatilization: Transformation of toxic metals and metalloids into less toxic and volatile forms released through foliage by plants into the atmosphere.

Phytoextraction: Over the preceding few decades, remediation of sites polluted with metal pollutants by plants has gained much interest over the microbial-based remediation strategies (Kramer, 2005; Pilon-Smits, 2005; Doty, 2008). Though not restricted, different phytoremediation strategies can be used simultaneously in order to achieve remediation against the particular pollutant at a given time. However, for any remediation strategy to be efficient in eliminating the toxic pollutant, basic criteria settle on the type and form of the contaminant present at that site. Despite the fact that soluble fractions of different pollutants are usually present as ions or unionized organometallic complexes, heavy metals also exist in colloidal, ionic, particulate and dissolved phases, with high affinity for humic acids, organic clays and oxides coated with organic matter (Connell and Miller, 1984; Elliot et al., 1986). In the rhizospheric region, bioavailability of metals is affected up to a greater degree by various plant and/or microbial activities that affect their uptake by plants via changing their mobility and bioavailability (Ma et al., 2011; Miransari, 2011; Aafi et al., 2012; Yang et al., 2012). Occurrence of lipophilic compounds in plant exudates or lysates has an effect either in increasing their solubility in water or in promoting growth of biosurfactant producing microbial populations. Moreover contributing to plant growth, microbial processes and/or activities in the rhizosphere soils increase the effectiveness of phytoremediation either by: (1) enhancing metal translocation (facilitate phytoextraction) or by reducing metal bioavailability in the rhizosphere (phytostabilization) and (2) by indirect promotion of phytoremediation achieved either by conferring resistance to plants and/or enhancing their biomass production so as to achieve remediation of pollutants up to a greater extent (Glick, 2010; Kuffner et al., 2010; Rajkumar et al., 2010; Babu and Reddy, 2011). Amongst the different approaches that are used to achieve phytoremediation, phytoextraction of metals and metalloids is considered to be the most challenging due to the high quantity of pollutants present. The use of plants to remove metals from the soil is known as phytoextraction. Plants absorb metals from the soils, transport and concentrate them in the above-ground parts of plants. The above-ground are harvested and can be carefully processed for dumping or recycling of metals (Ali et al., 2013; Garbisu and Alkorta, 2001). The plants used for phytoextraction should not only be metal tolerant but, must be fast growing with the potential to produce high biomass. Though, most of the metal-accumulating plants are slow growing with low biomass production (Evangelou et al., 2007). These characteristics of metal-accumulating plants have made the process of phytoextraction of metals very slow. Such metal-accumulator plants having ability to accumulate 100 mg kg^{-1} of cadmium (Cd), 1000 mg kg^{-1} of arsenic (As), cobalt (Co), copper (Cu), lead (Pb) or nickel (Ni) or > 10,000 mg kg^{-1} of manganese (Mn) and zinc (Zn), are classified as hyper - accumulator plants. Hyper accumulation of heavy metals by plants depends upon numerous steps, including assimilation and transportation of metals across the membranes of root cells, loading of metals into xylem and translocation to the shoots and sequestration and detoxification of metals within plant tissues (Yang et al., 2005). Epidermis, trichomes and cuticle are the ideal sites of metal detoxification, and in various cases subsidiary and stomatal cells are protected against metal toxicity (Rascio and Navari-Izzo, 2011). Metal detoxification or sequestration traits are restricted by expression of genes encoding the protein responsible for exclusion of metals from cytoplasm and transfer across tonoplast and plasma membranes. Cation Diffusion Facilitator (CDF) family members like metal transporter proteins there in the tonoplast are over expressed in Zn and Ni hyper accumulators and these transporters are also reported to be concerned in Ni accumulation by Ni hyper accumulators (Gustin et al., 2009; Hammond et al., 2006; Persans et al., 2001; Rascio and Navari-Izzo, 2011). About 400 plants have been known as hyper accumulators which comprises only < 0.2% of higher plants (McGrath and Zhao, 2003). However, these species have an essentially low ability to absorb metals but can accumulate higher concentrations of metals if grown in the soils treated with chemical amendments to increase metal phytoavailability and plant uptake (Meers et al., 2005). Researchers all over the earth target the new hyper accumulating plant species and attempt to understand their biological pathways. Various plant families are reported to accumulate high concentrations of heavy metals which consist of Asteraceae, Brassicaceae, Euphorbiaceae, Fabaceae, Flacourtiaceae and Violaceae (Kumar et al., 1995). Amongst the Brasicaceae family, some species possess vast potential to scavenge heavy metal ions and are hence considered as potential candidates for phytoextraction of metals. Usually, this family is identified to scavenge metals like Pb, Cd, Zn and Ni (Robinson et al., 2009). While *B. juncea* that belongs to the family Brassicaceae possess one-third of the concentration of zinc in its tissues, it is more capable to remediate zinc than Thlaspi caerulescens (known zinc hyperaccumulator) and the cause behind this fact is that the biomass production of *B. juncea* is about 10-times more than *T. caerulescens* (Ebbs and Kochian, 1997). Experimental study was carried out in different Brassica species in order to verify their ability to resist and accumulate heavy metals. These consist of: Indian mustard (*B. juncea* L.), kale (*B. oleracea* L.), turnip (*B. campestris* L.), black mustard (*B. nigra* Koch) and rape (*B. napus* L.) (Kumar et al., 1995). Among different Brassica species, Indian mustard (*B. juncea*) is the primary candidate crop to remediate numerous heavy metals from the soil which include: cadmium (Cd), chromium-IV (Cr IV), caesium-137 (137Cs) copper (Cu), nickel (Ni), lead (Pb), uranium (U) and zinc (Zn) (Jiang et al., 2000). Several plant species also have the ability to decontaminate the soil from radionuclides and the best example is Sunflower (*H. annuss*). Experimental study revealed that the level of radionuclides such as caesium-137 and strontium-90 in water declines by 90% within a 2-week time period; moreover, the utmost concentration of these radionuclides was found in roots (8000 times) relatively than water when flowers were planted as a demonstration of phytoremediation in a pond contaminated with these radioactive elements (Chernobyl nuclear disaster in the Ukraine). Now very few plants are known for phytoextraction though they possess few desirable traits, but with the help of biotechnological approaches transgenic plants can be developed which possess all the traits important for phytoextraction technology. Phytoextraction is mostly categorized into: (i) chelate-assisted phytoextraction or induced phytoextraction – this approach involves the use of artificial chelates in order to improve the mobility of heavy metal ions so that they become amenable to plants; (ii) continuous phytoextraction – this approach involves the natural capability of plants to uptake and scavenge the toxicants (Salt et al., 1997 and Ayyappan et al., 2016).

Phytostabilization: The next way of achievement to be taken in the case of phytoremediation of soil contaminated with heavy metals is phytostabilisation. Some plant species are used to immobilize the contaminants in the soil by absorption and accumulation by roots, adsorption on the surface of roots, or precipitation in the zone adjoining to the plant roots (Anna Małachowska Jutsz, 2015). The phytostabilisation uses abilities of exudates of several plant roots for decrease in the bio-availability of toxic substances (Cheraghi et al. 2011) as the main reason of phytoremediation to prevent the migration of metals in the environment. Plants used in phytostabilisation must have an extensive root system and a low degree of metal translocation from roots to shoots. Phytostabilization (also called phytorestoration) is a plant-based remediation technique that is aimed at reducing the risk of metal pollutants by stabilizing them through formation of a vegetative cap at the plant rhizosphere, where sequestration (binding and sorption) processes immobilize metals so as to make them unavailable for livestock, wildlife and human exposure (Munshower, 1994; Cunningham et al., 1995; Wong, 2003). Contrasting other phytoremediative techniques, the aim of phytostabilization is not to remove metal contaminants from a site, but moderately to stabilize them and decrease the risk to human health and the environment. Being less expensive, less environmentally evasive and simple to execute, phytostabilization is considered to be more beneficial than other soil-remediation practices (Berti and Cunningham, 2000). The main goal of this technique is not to restore sites from toxicants but rather to stabilize them and decrease the risks to the ecosystem. It is generally meant for those soils contaminated with Zn/As/Cr/Cd/Pb/ Cu (Malik et al., 2014). Various advantages related with this technology are that the dumping of hazardous material/biomass is not necessary (United States Environmental Protection Agency, 2000) and it is very efficient when rapid immobilization is desired to preserve ground and surface water. The occurrence of plants also reduces soil erosion and decreases the amount of water available in the system (United States Protection Agency, 2000). Phytostabilization has been used to treat contaminated land areas affected by mining activities and Superfund sites. Normally the role of plants in this technique is to lessen the amount of water percolating through the soil matrix that will finally lead to the formation of toxicants (hazardous leachates) and avoid soil erosion and transport of toxic metals to distinctive areas. The plants selected for this technology should follow certain criteria: (1) effectiveness of translocation of heavy metals from root-to-shoot system should be low; (2) they must have a fast growth rate and resistance to heavy metals; and (3) there should be cost-effective management. Basically, this technique is not only applicable at sites with high organic load and porosity but is also efficient for a wide range of surface contamination sites (Berti and Cunningham, 2000). One of the drawbacks of this technique is that it is not applicable to those areas which are heavily contaminated because such conditions become an obstacle in plant growth and development (Berti and Cunningham, 2000).

Mechanisms of phytostabilization: Plants seize the metals in the rhizosphere through adsorption and precipitation of metals into less soluble forms like carbonates and sulphides of metals, metal complexes with organic compounds, metal adsorption on root surfaces and metal accumulation in root tissues (Mendez and Maier, 2008; Wong, 2003). The occurance of plants in metal-contaminated soils promotes heterotrophic microbial communities which may in turn, promote plant growth and take part in metal stabilization. The Metal-tolerant plants having the capacity to keep the metals away from the metabolic sites (shoots) are the best candidates for phytostabilization. However, such plants have developed mechanisms to restrict the metals in the roots, still the concentration of metals in shoots should be monitored (Mendez and Maier, 2008). The best accumulator of as in roots was found to be *cynodon dectylon* and hence a promising candidate for phytostabilization and have wide adaptations in Pb- and Zn-contaminated soils (Leung et al., 2007). Mycorrhizae play a vital role in stabilization in binding the metals with hyphae and a few mycorrhizae like ericoid and Ectomycorrhizal fungi colonizing in *Cynodon dactylon* can modify the rhizosphere by excreting organic acids and hence stabilizing metals in the rhizosphere (Meharg, 2003). Hyphae of Mycorrhizal fungi have polyphosphate which can bind heavy metals up to saturation and greater than 60% metals are reported to be retained in apoplast cell walls (Bücking and Heyser, 1999, 2000; Yang et al., 2005). Some of the plants can detoxify the metals in the roots by releasing organic acids therefore tendering the metals less available (Brunner et al., 2008; Qin et al., 2007). Another process for the detoxification of metals is immobilization of metals within fine roots through binding with pectins in the cell walls and to the negatively charged cytoplasm-membrane surfaces owing to their strong electrochemical potential (Kochian et al., 2005; Rengel and Zhang, 2003). Several plants have the capability to reduce the valence of metals by releasing redox enzymes and thus toxic metals are transformed into less toxic forms (Ali et al., 2013). Conversion of tetravalent chromium (more toxic) to trivalent chromium (less toxic) is the best-studied example of this strategy being adopted by the plants (Bluskov et al., 2005). Phytostabilization has extremely promising results for stabilization of chromium and lead in soils. Hexavalent chromium (Cr^{6+}) is highly toxic and is changed into less soluble and less toxic trivalent chromium (Cr^{3+}) by deep-rooted plants (Chaney et al., 1997; James, 1996). Lead (Pb) is present in the soil in various species which are mostly bioavailable. Plants which can live in metal-contaminated soils without affecting growth and retain low concentrations of metals in aerial parts, although concentration of metals is very high in the roots, is known as metal excluder plants (Baker, 1981; Krämer, 2010; Wei et al., 2005). Several plants with the potential to exclude metals from aerial parts have been known. These include Ni-excluders such as *Silene vulgaris*, *Zea mays*, Cu excluder Hyparrhenia *hirta* and Co excluder *Armeria maritima* (Brewin et al., 2003; Poschenrieder et al., 2001; Seregin et al., 2003; Wenzel et al., 2003a). Although, excluder plants can grow in metal-contaminated soils without affecting their growth and keeping metal concentration in aerial parts at minimum levels (Wei et al., 2005). Plants use different strategies to exclude metals from the tissues and these may include the role of mycorrizae, cell walls and plasma membranes (Hall, 2002). Mycorrhizae in general adopt the same mechanisms as those are adopted by higher plants like binding to extracellular materials or sequestration in the vacuolar compartment (Hall, 2002; Tam, 1995). Various hypotheses have been recommended about the mechanisms of metal exclusion. These consist of metal binding in cell walls, exudation of metal-chelating ligands and formation of redox and pH barriers at the plasma membrane (Taylor, 1987). There are conflicting reports about the role of the cell wall in metal tolerance of plants (Hall, 2002). Some researchers found that the cell wall plays a very small role in metal tolerance, while others have found heavy metals accumulated in the cell wall as bound with protein or as silicates (Bringezu et al., 1999). Metal-tolerant plants select for homeostasis to maintain the high concentration of metals due to their inability to tolerate reactive oxygen species or free radicals (Dietz et al., 1999; Sharma and Dietz, 2009; Panda et al., 2003). Aluminum tolerance in wheat is activated by extracellular chelation of Al with citrate and malate (Delhaize and Ryan, 1995) and discharge of organic acids from roots has also been reported in Al-resistant Arabidopsis (Larsen et al., 1998). Phytostabilization is considered a very good choice for those soils which cannot be immediately remediated through phytoextraction.

Phytovolatilization: Conversion of toxic metals and metalloids like mercury (Hg), selenium (Se) and arsenic (As) into less toxic and volatile forms released through foliage by plants into the atmosphere is known as phytovolatilization (Malik and Biswas,2012; Marques et al.,2009). This technology is usually applied to remediate groundwater, soil, sediments and sludges. Previouly only microorganisms were known to play this role (Karlson and Frankenberger, 1989), but recently it has been discovered that plants (*B. juncea*, *B. napus*) also possess an excellent ability to carry out the same function (Terry et al., 1992). Few aquatic plant species, viz. Azolla, rabbit foot grass, rice, (*Typha latifolia* L.) and pickle weed, also have the ability to act as the best volatilizers of selenium (Zayed et al., 2000). Even though this remediation approach has added benefits of minimal site disturbance, less erosion and no need to arrange of contaminated plant material, it is still considered as the most controversial of all phytoremediation technologies as discharge of mercury into the environment is likely to be recycled by precipitation and then redeposit back into the ecosystem (Henry, 2000). *Brassica Juncea* has revealed to volatilize Se into the atmosphere through assimilation of Se from the soil into organic seleno-amino acids, seleno-cysteine and seleno-methionine which later on can be biomethylated to form the volatile compound dimethylselenide (Banuelos et al., 1993; Banuelos and Meek, 1990; Terry et al., 2000). A gene responsible for reducing mercuric ion into elemental mercury through enzyme mercury reductase has been introduced into Arabidopsis thaliana which finally volatilizes large amounts of Hg into the atmosphere (Rugh et al., 1996). Recently, a bacterial Hg ion reductase gene was incorporated into plants to make a transgenic plant which shows excellent mercury volatilization. Further, it has also been reported that bacterial organomecurial lyase (merB) and mercuric reductase (merA) genes were incorporated into model plants such as A. thaliana and *N. tabacum*; the resulting transgenic plants have the potential to absorb elemental mercury (II) as well as methyl mercury from the soil and convert it into a volatile form (Hg^0) (Heaton et al., 1998). Transgenic yellow poplar (*Liriodendron tulipifera*) plantlets were produced which exhibited resistance to and grew well in normally toxic levels of ionic mercury, and the altered plantlets volatilized about 10-times more elemental mercury than non-transgenic plantlets (Rugh et al., 1998). Though this technology is a promising tool for the remediation of selenium and mercury, at the mean time it has some demerits like loss of control of volatile compounds over their migration to other areas. Presently by means of this technology,tritium (3H), a radioactive isotope of hydrogen is decayed to stable helium with a half-life of about 12 years as reported Dushenkov et al. (1995).

Rhizofiltration: Rhizofiltration is a type of phytoremediation that uses plant roots to absorb, concentrate and precipitate contaminants present in the soil through the plant root system into the harvestable parts of the roots and above-ground shoots (Verma et al. 2006, Lee et al. 2010). The plants are raised hydroponically and transplanted into metal-polluted water where they absorb and concentrate the metals in their roots and shoots. Root exudates and changes pH in rhizosphere may also cause metals to precipitate on root surfaces. Roots or entire plants are harvested for disposal after they become saturated with the metal contaminants (Padmavathiamma and Li 2007). Rhizofiltration can be used for Pb, Cd, Cu, Ni, Zn, and Cr, which are mostly retained within the roots (United States Environmental Protection Agency, 2000). Sunflower, Indian mustard, tobacco, rye, spinach, and corn have been studied for their capacity to eliminate lead from water, with sunflower having the maximum capability. Indian mustard have a bioaccumulation coefficient of 563 for lead and has also verified to be effective in removing a wide concentration range of lead (4 mg/L -500 mg/L) (Raskin and Ensley, 2000; United States Environmental Protection Agency, 2000). It has also been confirmed that several chemicals ooze out of the roots that lead to a change of pH in rhizosphere which finally causes metals to precipitate on the root surface. Once roots get saturated with these toxicants, either only the roots or the entire plants are harvested for more processing (Zhu et al., 1999b). The advantages related with rhizofiltration is the capability to use both terrestrial and aquatic plants for either in situ or ex situ applications. Another advantage of rhizofiltration is that contaminants do not have to be translocated to the shoots. Therefore, species other than hyper accumulators may be used. Terrestrial plants are ideal as they have a tough and much longer root system, increasing the amount of root area (Raskin and Ensley, 2000). Sunflower (Asteracaea spp.) has effectively been implemented for rhizofiltration at Chernobyl to remediate uranium contamination. This process is not possible in those areas where metal concentration is high in water because the contaminants must be in the solution form in order to be sorbed into the plant system. Other complications related with this technique involve drying, composting or incineration. For efficient deletion of contaminants from the site, plants should possess efficient and fast-growing root systems with the ability to scavenge these toxicants and hence neutralize their harmful effects. Low maintenance costs, easy handling and resistance of plants to heavy metals are other central criteria for effective removal of heavy metals from a particular area. A number of aquatic plant species show a enhanced response in the removal of toxicants from water. These include: water hyacinth (*Eichhornia crassipes* Mart.; Zhu et al., 1999c), pennywort (*Hydrocotyle umbellata* L; Dierberg et al., 1987) and duckweed (*Lemna minor* L; Mo et al., 1989). Though, there are certain limits to these plants as they have limited potential for rhizofiltration, because of their small, slow-growing root systems (Dushenkov et al., 1995). Zhu et al. (1999c) reported that water hyacinth is a potential candidate for the deletion of trace elements from waste streams. Experimental evidences demonstrate that terrestrial plants with dense and fibrous root systems are suitable for this technique as they have greater metal-absorbing powers, and the chief examples include Sunflower (*Helianthus annuus* L.) and Indian mustard (*Brassica juncea* Czern.). Indian mustard is known to eradicate a wide concentration of Pb (4–500 mg l^{-1}) (Raskin and Ensley, 2000). These terrestrial plants are also being used to remove various metals such as Cd, Zn, Cu, Ni and Cr (Dushenkov et al., 1995), uranium (Dushenkov et al., 1997a) and Sr (Dushenkov et al., 1997b) from hydroponic solutions. The technique of using young plant seedlings in order to remove toxicants (heavy metals) from water is called as blastofiltration (blasto is 'seedling' in Greek) and is considered to be second-generation, plant-based water treatment technology. In this technique, there is a remarkable improvement in surface to volume ratio that typically occurs after germination and some germinating seedlings also adsorb huge quantities of toxic metal ions; this is why young seedlings are suitable for restoring water quality. Indian mustard is considered to be a potential crop for blastofiltration as it is effective in sorbing divalent cations of toxic metals (Salt et al., 1997) due to some unique features of Indian mustard such as fast growth rate and resistance to heavy metals or microbial infection. Through data analysis, it has been reported that for a few metals, blastofiltration techniques are more effective and economical than rhizofiltration; but the major advantage of rhizofiltration is that it can be utilized both in situ as well as ex situ and species other than hyper accumulators can also be taken into account.

Genetic Engineering Approaches for Phytoremediation: For any plant to be used in the practice of phytoremediation, it desires to fulfill the basic criteria of being tolerant to various metal pollutants. In order to increase the metal accumulation property of plants, existing remediation strategies are mostly aimed at enhancing the metal accumulation property subsequent its transport from the soil to the roots and translocation to shoots in plants. For any particular approach to be efficient, it is essential to have a systematic understanding of the mechanisms that are using within the hyper accumulators that are employed in acquisition and translocation, followed by accumulation so as to attain remediation of polluted sites (Jan et al., 2014). Genetic engineering is one of the essential approaches that can be employed as an alternative to enhance the phytoremdiation potential of plant species with high biomass production. Biotechnological approaches (genetic Engineering) are used to introduce more effective accumulator genes into other plants, and these methods have been suggested by various authors (Chaney et al., 2000). This approach of inserting many effective accumulator genes from taller plants into other natural plants increases the final biomass. (Malik et al., 2014). Genetic engineering approach has effectively facilitated to change the biological functions of plants through modification of primary and secondary metabolism and by adding new phenotypic and genotypic characters to plants with the aspire of understanding and improving their phytoremediation properties (Davison, 2005). Zhu et al. (1999a) studied the transgenic *Brassica juncea* for rate-limiting factors while inserting gshl-gene from *Escherichia coli* for glutathione and phytochelatin production. There are numerous factors that limit the achievement of genetic engineering sand one of the limiting factors is the anatomical restraint (Ow, 1996). Commercial use of phytoremediation can be improved by transforming metal-scavenging properties of hyper accumulating plants, such as *Thlaspi caerulescens*, to high-biomass generating species, such as Indian mustard (*Brassica juncea*) or maize (*Zea mays*) (Brown et al., 1995). Currently, genetic engineering is successfully utilized to manipulate metal uptake and stress resistance properties in various species. For instance, enhanced metal resistance in tobacco (*Nicotiana tabacum*) was achieved by expressing the mammalian metallothionein metal binding proteins (Maiti et al., 1991). At present, genetic engineering plays a essential role in the production of transgenic plant species that have remarkable potential to remediate soil contaminated with methyl mercury (a neurotoxic agent). Examples are transgenic Tobacco and Arabidopsis which express bacterial genes *merB* and *merA*, and have the ability to remove mercury from the soil. It has been recognized after a number of detailed genetic studies, that the adaptive metal tolerance is governed by a small number of major genes and possibly contribution of some minor modifier genes (Schat et al., 2002). Perhaps it is this adaptive metal tolerance that gears a plant species for hyper accumulation. For instance, Zn-hyperaccumulator *Arabidopsis halleri* and the nonaccumulator *Arabidopsis petrea* that are Zn tolerant are controlled by a single major gene (McNair et al., 2000). Hence the desired characters for phytoremediation can be enhanced by identifying candidate protein, metal chelators, and transporter genes for transfer and /or over expression of particular gene. Through genetic engineering modification of physiological and molecular mechanisms of plants heavy metal uptake and resistance is successfully achieved by implanting bacterial gene or mutant cells on the basis of desired phenotype in plant genome which enhances the very process of uptake of metals (M.H. Fulekar, 2009) Metallothioneins (MT) a metal binding protein, cysteine-richand having low-molecular-weight endowed with a ample range of functional abilities in a biosystem (Hamer, 1986;Sousa et al.,1998). They possess high metal content (mostly Zn, Cu or Cd), bound by sulphur atoms in thiolate clusters, further having highly conserved cysteine residues (18–23) that bind metal ions and seize them in the biologically inactive form (Vasak, 2005; Bell and Vallee, 2009).

While establishing its role in metal detoxification, plant metallothioneins are grouped into four subfamilies, MT1, MT2, MT3, MT4, with different expressions for satisfying different functions during development in plant tissues (Cobbett and Goldsbrough, 2002). It was found that expression of MT1a and MT1b in roots of non-accumulator plants, like *A. thaliana* and *poplar* were high during exposure to Cd, Cu and Zn (Garcia-Hernandez et al., 1988; Zhou and Goldsbrough, 1994; Kohler et al., 2004), where as in *Thlaspi caerulescens,* level of expression of MT1 mRNA were constitutively higher in leaves than in roots (Roosens et al., 2005). Though MT2b is not considered as the major competitor in the process of metal tolerance, increased expression of MT2b was found to be connected with Cu tolerance and increased as translocation from root to shoot in *A. thaliana* (Schat et al., 1996; Grispen et al., 2009). In non-accumulator plants, expression of MT3 genes increases on exposure to Cu and as such is known to play an important role in the upholding of Cu homeo-stasis under conditions of high Cd and Zn in the cytoplasm (Guo et al., 2003; Kohler et al., 2004; Roosens et al., 2004). This suggestion is supported by the discovery of a small cavity that is adapted for Cd chelation in the MT3 protein by *A. thaliana* as compared with the analogous protein from *T. caerulescens* (Roosens et al., 2004, 2005). In *A. thaliana*, it is reported that MT4 plays a vital role in metal homeostasis during seed development and seed germination slightly than in metal decontamination (Guo et al., 2003).

In genetic engineering of plants, a foreign piece of DNA is steadily inserted into the genome of a cell, which is regenerated into a mature transgenic plant. The piece of DNA can approach from any organism, from bacteria to mammals. The foreign DNA frequently contains two genes, one a resistance gene used for selection after transformation, the other the gene of interest. Each gene is attached to a plant promoter, ensuring the development of the gene product (typically a protein) in the plant. While the transformed plant is propagated, the foreign gene is inherited by its offspring. The foreign stretch of DNA may be transferred to the plant either via a particle gun, for which the DNA is coated onto metal particles and turn into the plant tissue, or via Agrobacterium, a soil bacterium that makes living by inserting part of its DNA (called T-DNA) into a plant cell and feeding off of the gene products formed by the plant. The Agrobacterium T-DNA genes can be replaced by genes of interest, which are then inserted into the plant by Agrobacterium infection (Hoekema et al., 1983). For various plant species (e.g. Arabidopsis thaliana, conversion merely involves plunging the flowers in an Agrobacterium suspension. Most plants needs to be grown in tissue culture as undifferentiated callus in order to be transformed. After the transformation, mature plants are regenerated from the tissue culture by means of shoot inducing plant hormones (Horsch et al., 1985). The gene product can be targeted to certain cellular compartments (e.g. chloroplast, vacuole, mitochondrion, or apoplast) by adding specific targeting information in the gene construct.

Examples of genetically engineered plants

Phytoreduction of mercury: Mercury and mercurial compounds are harmful to all biological organisms. Bacteria have developed mechanisms for colonizing mercury contaminated environments and an operon of mercury resistance (mer) genes encoding for transporters and enzymes for biochemical detoxification (Summers, 1986). Mer^{+} bacteria convert organic and ionic mercury compounds to the volatile and less toxic elemental form, Hg (O) which quickly evaporates through cell surface. Genetically engineered plants with mer A and mer B genes were produced in three plant species *Arabdopsis thaliana (*Bizily et al., 2000), *N. tabacum* and *Liriodendron tulipifera* L. (Rugh et al., 1998) and have confirmed that transgenic plants could grow in the occurrence of toxic levels of mercury (Rugh et al., 2000). In plants to improve the expression of mer genes mer A DNA sequence was modified, by reducing the GC content in a 9% block of the protein coding region and adding plant regulatory elements (Rugh et al., 1996). When transferred to *A. thaliana* and *N. tobacco*, the new gene construct (mer A) conferred resistance to 50 mm Hg (II) suggesting that mer A plants enzymatically reduce Hg (II) and evaporate away Hg (0). Three modified mer A constructs were used for conversion of yellow poplar proembryogenic masses, each having different amounts of altered coding sequences (Rugh et al., 1998). Each of these constructs was shown to confer Hg (II) resistance (Rugh et al., 2000). Transgenic *Populus deltoids* overexpressing mer A9 and mer A18 gene when exposed to Hg (II) evolved 2- to 4-fold Hg (0) relative to wild plant (Che et al., 2003). If phytovolatilization is inappropriate due to the hazards of releasing Hg (0), alternate methods should be explored. One alternative is to develop plants that sequester high mercury loads in harvestable tissues. The strategy for mercury sequestration may be further improved by root specific expression of mer A and mer B genes to detoxify charged mercurials prior to transport to shoots.

Tolerance to selenium: Selenium is one of the major environmental pollutant and larger doses above the Se requirement can result in toxic effects (Wilber, 1983). The oxidised form of Se i.e. selenate or selenite are greatly soluble and are easily removed by plants whereas inorganic forms such as selenide or elemental Se are less bioavailable. Selenium and sulphur are nutrients having similar chemical properties and their uptake and assimilation proceeds through common pathways. The assimilation of sulfate and selenate is activated by ATP sulfurylase. Selenate is converted into adenosine phosphoselenate (ADP-Se) which is then reduced to selenite (DeSouza et al., 2000). Transgenic plants, which overexpresses ATP sulfurylase gene (APS), had 4-fold higher APS enzymatic activity and accumulated three times as much Se per plant than the wild type. The APS transgenics were more tolerant to Se, growing at higher rates than wild type (Pilon-Smits et al., 1999). The Indian mustard plant was also transformed with the help a bacterial glutathione reductase in the cytoplasm and also in the chloroplast. Both types of transgenic plants grew better than wild type seedlings on agar medium spiked with toxic concentrations of selenate or selenite (DeSouza et al., 2000)

Tolerance to arsenic: Arsenic is an exceptionally toxic metalloid pollutant which is harmful to human health (Chen et al., 1992; Kaiser, 1998). Dhankher et al. (2002) developed transgenic Arabidopsis plants, which can transport oxyanion arsenate to aboveground, reduce to arsenite and sequester it in thiol peptide complexes. Ars C gene of E. coli encoding arsenate reductase (Ars C) that catalyzes the glutathione (GSH) coupled electrochemical reduction of arsenate to the more toxic arsenite. The transformation of *Arabidopsis* plants with Ars C gene that have been expressed from a light induced soybean rubisco promoter (SRSIp) strongly expressed Ars C protein in leaves, but not in roots and were hypersensitive to arsenate. Plants expressing SRSIp/ArsC and ACT 2p/g-ECS mutually showed higher tolerance to arsenic. These transgenic plants accumulated 4- to 17-fold greater fresh shoot weight and accumulated 2- to 3-fold more arsenic per gram of tissue than wild plants or transgenic plants expressing-ECS or Ars C alone.

Iron uptake: The Soils contain insoluble Fe (III) oxides and hydroxides. The proteins which were extruded by ATP driven proton pump, promotes Fe (III) solubility and reduction to Fe (II) by plasma bound reductase. FRO 2 genes isolated from Fe deficient roots of *A. thaliana* encoding a ferric chelate reductase was capable of restoring ferric chelate reductase activity in an Arabidopsis mutant deficient in this enzyme (Robinson et al., 1999). The same gene also restores a mutant (frd 1-1) with deficient copper chelate reduction. Two Fe (III) reductase FRE 1 and FRE 2 have been isolated from S. cerevisae (Dancis et al., 1990; Georgatsou and Alexandra, 1994) and transferred to tobacco (Samuelsen et al., 1998).

CONCLUSIONS

Metal contamination of soils is a common problem around the world with changeable intensities and magnitudes in different regions. Metals are the most common forms of contamination found at waste sites, and their remediation in soils and sediments are among the technically difficult one. An idea of eradication through a utilization technique should be developed. Future strategies should be developed in such a way to help restore soil health. Nowadays phytoremediation has gained universal attention due to the distinctive feature of plants growing on metalliferous soils and accumulating heavy metal concentration. Phytoremediation is considered to be environmentally friendly, non-disruptive and low in cost. At the same time, approval of phytoremediation technology on a commercial scale warrants serious consideration of issues of being slow and time consuming and the fate of the plants being used. A variety of plants have been identified which are capable of accumulating high concentrations of metals in their aerial parts (phytoextraction), retaining the metals in roots or stabilizing the metals in soils and thus restricting their translocation to the shoots (phytostabilization) and removing the metals from the soil through synthesis of volatile compounds (phytovolatilization). The choice of phytoremediation technology to be employed for remediation of metal-contaminated sites depends on soil type, metal type, degree and extent of contamination and environmental disturbance involved. In spite of being a promising alternative in the management and remediation of contaminated sediments, phytoremediation still requires further development and optimization in order to improve traits that will lead to improvement in its applicability in the restoration of polluted sites.

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