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Phytoremediation of Lead Using *Brassica Juncea* and *Vetiveria Zizanioides*

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Abstract: **An engineering based remediation technologies of heavy metals from contaminated soil are time consuming, expensive, create noise to the environment. Accumulation of heavy metals such as Pb, Cd, Hg, Cr, Ni is destructive to not only plants and animals but also to complete ecosystem. Lead contamination keeps on increasing day by day from industrial waste, paints, ceramics, use of biosolids and many more. Metals cannot easily degrade so effective remediations need to be required to reduce the toxicity. Phytoremediation is most promising approach to degrade the contamination, this is environmental friendly approach, cost effective, socially accepted, easy to maintain and it also has long term applicability. It involves the growing hyperaccumulator plant species to detoxify and remove environmental contamination by biological, chemical, physical processes of plants. Hyperaccumulators are plant species that achieve shoot to root metal concentration ratio greater than one. *Brassica juncea* and *Vetiveria zizanioides* grass are well known Pb hyperaccumulator. This review article describes the Lead contamination, effects of Lead toxicity in plants, phytoremediation of lead using *Brassica juncea* and *vetiveria zizanioides*, effects of Reactive Oxygen Species (ROS) in phytoremediation and chelate assisted phytoremediation.**

Keywords: **Phytoremediation, Hyperaccumulator plant species, environment friendly, ROS, Chelate assisted phytoremediation.**

1. INTRODUCTION

The progressive growth of industries and transport, the irrational use of pesticides in agriculture give rise to hazardous effects in the natural landscape and they pollute ground as well surface water and detoriate soil fertility(Gruca et al,2006). Enhanced level of heavy metals in soil poses a threat to human, animals. So it is paramount to develop effective and environmental friendly soil remediation approach. Phytoremediation is green technology which is being used for the removal of toxicity in soil. Phytoremediation can be defined as the use of hyperaccumulator plants species for the removal of heavy metals from the soil,water and municipal waste as well. Hyperaccumulators are able to extract and store extreamely high concentration of metallic elements (Lasat et al 2000). Indian mustard is considered as the most viable hyperaccumulator for the phytoextraction of number of metals such as cadmium, chromium, cesium, lead, nickel, zinc(kumar et al 1995; blaylock et al,1997; zhu et al 1999). *Vetiveria zizanioides* can accumulate high level of metal-tolerance to adverse condition, tolerance to elevated heavy metal concentration, stiff and erect stems forming dense hedges(high shoot biomass) make this plant ideal hyperaccumulator for phytoremediation. Chelate assisted phytoremediation is effective for heavy metals such as lead or cadmium as they are insolouble in a conventional soil environment. Heavy metal accumulation ability in plants can be increased by the addition of chelating agents. Soil can absorb 0.01-0.06% lead in dry weight without chelating agent, while with the addition of chelating agent soil can absorb more than 1% lead dry weight (Huang et al 1996,1997; Blaylock et al,1997). Plants used up for phytoremediation contain enhanced level of contamination in their tissue so they need to be appropriately disposed off, biomass of these plants is nowadays being used to generate heat and electricity. In case of Brassica some research indicated that heavy metals accumulation can stimulate the synthesis of Glucosinolates, it is enzymatically degraded into isothiocyanates which exhibit biocidal properties so after phytoremediation Brassica biomass can be used in biofumigation purposes.

2. LEAD CONTAMINATION

Lead contamination is naturally present in all soil. Pollution can increase soil lead level to several thousands ppm. Lead occurs generally in the range of 15 to 40 parts per million of soil, 15 to 40 mg lead per kilogram of soil. Lead exists in two forms-inorganic and organic lead, organic lead could be more toxic than the inorganic lead because organic lead is more reluctantly absorb by the body. Exposure to small amount of Lead for a long period of time is called as chronic toxicity and chronic exposure of lead in the contaminated residential soil might cause behavioral, social and developmental changes in the childrens. There are many forms of Lead . Basically ionic Lead, Lead Oxide, Lead Hydroxide, Lead Metal Oxanium Complexes, are some forms of Lead which are released into the soil , ground water and the surface water. Whereas pb2+ and Lead Hydroxide complexes are considered as the most stable forms of lead. Lead Sulfide is inspected as the most stable solid from within the soil matrix . it forms under the reducing state when there is a presence of high concentration of sulfide. Ionic form of Pb is appraise as the most reactive form of Lead , which forms mononuclear, polynuclear oxides and hydroxides. . Bioavailability depends on the binding with the soil and solubility. At low pH, Pb is bound less tightly with the soil, more soluble. At neutral or high pH, lead is bound more tightly with the soil and poorly soluble. Many studies have been done that show lead is not reluctantly accumulate in the fruiting part of the vegetable and fruit crop, high concentration of Lead is mostly found in leafy vegetables, and on the surface of root crops.

3. EFFECTS OF LEAD TOXICITY IN PLANTS

Several metals are easily absorbed and get bio-accumulated in different organs of the plants(Wang et al 2003; Ashraf et al 2010).They can affect the cell metabolism if present beyond the tolerance limit. Physiological mechanisms that are affected by the heavy metals include water balance, photosynthetic activity, respiration and ATP content, enzymatic activity, structure of protein, cell division and morphogenesis.(Foyer and Noctor 2005;Sharma and dietz 2009;Ahmad et al 2010;Guerra et al2011;Kadukova and Kavulioca 2011). The uptake of the lead in the plants is basically regulated by the ph, solubility, particle size and the cation exchange capability of the soil. High concentration of Pb leans to cause large number of toxic symptoms such as chlorosis, blackening of root system, stunted growth, inhibition of photosynthesis, distortion of chloroplast, inhibition of calvin cycle enzymes ,capable of changing hormonal status, able to upset mineral nutrition and water balance , destructure the structure of membrane, hinders in the permeability of the membrane. The content of the Pb decreases in following order in various plant organs: roots> leaves>stem>inflorescence>seeds. This order can also be varied with plant species. senescing leaves can accumulate maximum Lead than the young leaves(Henry et al, 2000).

4. PHYTOREMEDIATION

It is the most promising approach used for the accumulation or degradation of the contaminants. In phytoremediation plants and their associated microbes are used to eradicate the various contaminants present in polluted soil, sediments, groundwater. It is a low cost , environment friendly approach in order to clean up and the control of environmental pollution (shyama et al,2009). Phytoremediation is ten times cheaper than the engineering based remediation process of contamination. It could be applied over large surface area, as it is cost effective approach and easy to execute and maintain because plants are renewable resources and can be easily available(Marquis et al ,2009).Phytoextraction, evapotranspiration are commonly recongnised phytoremediation technologies. In Phytoextraction, there is removal of heavy metal from soil by means of plant uptake. It is basically based on the potential of roots to absorb, translocate and accumulate toxic metals from the soil to the harvestable part of the plant. Evapotranspiration has also same application like phytoextraction. It is responsible for the movement of contaminants into the plant shoot because there is translocation of contaminants from roots to shoots, which are then harvested, contamination can be eradicated without leaving the soil disturbed.Plants which are used in the process of phytoextraction are termed as hyperaccumulators, those species of plant which can achieve a shoot to root metal concentration ratio greater than one. Few plant species which are known to hyperaccumulation of the lead are *Brassica juncea, Brassica napus, Thalapsi alspres, Thlapsi rotundifolium, Allysum wulfenium.* Indian mustard is considered as the most viable candidate for the phytoextraction of number of metals such as Cadmium, Chromium, Cesium, Lead, Nickel, Zinc. *Brassica juncea* is a Lead and Cadmium accumulator species (kumar et al, 1995; blaylock et al,1997; zhu et al ,1999). Success of phytoremediation is depend on the choice of plant. These plants have to fulfill some criterias: Plants should be able to extract high concentration of heavy metals, must have ability to translocate heavy metals from roots to shoots, should produce large quantity of plant biomass, Bioaccumulation coefficient must have a value greater than one,Plants can be grown easily and should be fully harvestable.

5. *BRASSICA JUNCEA* A POTENTIAL HYPERACCUMULATOR

In the brassicacea family ,genus *Brassica* is economically important. Various species and types of Brassicas are vegetable crops, forage crops, oilseeds crops, and are used in the preparation of condiments such as mustard (*Brassica juncea*). To test fast growing Brassica taxa, for the ability to tolerance and accumulation of metals , an experiment was conducted by (Kumar et al,1995), He included mustard*(Brassica junce*a),black mustard (*Brassica nigra*),turnip(*Brassica campstrus*), rape (*Brassica napus*), kale(*Brassica oleracea*). Among all the Brassica taxas were found with the accumulation of heavy metal but highest capacity to accumulate heavy metals in roots and translocated metals(Copper, Chromium, Lead, Nickel, Cadmium, Zinc) for accumulation was found in the *Brassica juncea* (Kumar et al ,1995 ; Jiang et al,2000). Some calculations indicated that *Brassica juncea* is capable of removing 1550 kilogram of lead per acre. 1.7 is the phytoextraction coefficient of *Brassica juncea*. It has also been found that concentration of Lead 500miligram per Litre is not phytotoxic to Brassica species (Henry et al,2000). Some research indicated that in Cruciferous plants, accumulation of heavy metals can trigger the synthesis of Glucosinolates(GLS), GLS are organic compounds containing sulphur. The products of their enzymatic degradation i.e isothiocyanates manifest biocidal activity, can be used for biofumigation. Degradation of Glucosinolates lead to the formation of Isothiocyanates(ITC) in presence of an enzyme Myrosinase(Kirkegaard et al, 1998; Fahey et al, 2001). Isothiocyanates have biocidal properties; wide spectrum activity against Fungi (Smolinska et al, 2003), Herbivores (Lord et al, 2011), and Bacteria (Lin et al, 2000). These pests cause serious loss in agriculture and so do reduce crop yields. All the Brassicas are not equal, species of the family Brassica have different level of GLS, so their hydroysed product have also different biocidal activity (Clarke et al,2010). In the plants GLS are localized in the vacuoles, whereas myrosinase is located in myrosin cells. As there is a attack of pest, structure of cell is disrupted; GLS and myrosinase come in contact as a result hydrolysis occurs. Thiohydroxymate-O-Sulfonate is formed as intermediate product , it then can be converted into isothiocyanates(ITC), thiocyanates, epithilionitriles in the presence of reaction conditions such as pH, Heavy metal concentration, presence of specifier protein and coenzymes. At low concentration ITC are beneficial to human health. At high concentration they are considered as general biocides that behave as commercial pesticides. In studies it has been shown that Brassica plays a role in controlling Rhizoctonia(canker and black scurf), common scab(*Streptomyces scabies*), powdery scab((*Spongospora subterranean*), and verticillium wilt (*Verticillium dahliae*). Populations of nematodes, Heterodera (cyst), and Meloidogyne species (*M. chitwoodi* and *M. hapla*) (Griffiths et al,2011).

6. *VETIVERIA ZIZANIUIDES* A HYPERACCUMULATOR

Vetiver grass(*Vetiveria zizanioides*) belong to the grass family, same as the sorghum,lemongrass, sugarcane, maize. Vetiver grass is a native of south and south east asia, where it has been growing for 100 of years for roof thaching, fodders,for the cosmetic and perfumery industry. There are various uses of vetiver grass, known worldwide. In Australia ,vetiver grass was used to remediate heavy metals such as As, Cd, Cr, Ni, Pb, Hg, Cu from the landfills and industrial waste contaminated with heavy metals (Brennan et al,1999). During 1980 , in India vetiver grass was promoted by the world bank for soil and water conservation(Dalton et al,1996). It is a fast growing, high biomass perennial grass, has a high C4 photosynthetic efficiency (Mucciarelli et al,1998). It grows 2 meter above and 3 meter deep in the ground. Vetiver has dense complex root system which could be able to penetrate into deep layer of the soil (Dalton et al,1996; Troung,2000; Pichai et al,2000). It grows in hydrophilic as well as xerophitic conditions. The leaves emerges from the bottom and each blade of the leaf is long,narrow and coarse. Dimensions of the leaf:45cm -100cm long,6-12 cm wide. Leaves produce dry matter yield about 15,000-30,000 kg per hectare, while grass roots produce dry matter 1428-2142.9 kg per hectare. (Marchiol et al ,2004). Vetiver has tolerance to extreame climatic variations such as flood, prolonged drought, fire, frost, submergence, heat waves. It also has tolerance to wide range of soil acidity, alkalinity, salinity, elevated level of heavy metals such as As, Cr, Ni, Pb, Zn, Hg, Se, Cu in soil, low concentration of plant nutrients. Vetiver grass roots are fibrous roots, penetrate deep into the soil; expand vertically. Horizontal expansion of the leaf limits upto 50cm but the vertical penetration extends upto 5 meter. An experiment was conducted by Vo Van Minh,2009 on Vetiver grass for the removal of Cadmium and Lead, He concluded that cadmium accumulation rate of Vetiver shoot/root was fairly low (<13.32%), while Lead accumulation rate of Vetiver shoot/root was very high (9.72%-88.14%) (Vo Van Minh et al,2009).

7. ROLE OF REACTIVE OXYGEN SPECIES (ROS)

Excess presence of heavy metals lead to the formation of reactive oxygen species (Dat et al 2000). ROS act as the signals for activation of stress response and defense pathways(knight and knight 2001).Under biotic and abiotic stresses amount of ROS may lead to the disruption of cell equilibrium,ion leakage, cleavage of DNA strands,dismantling of membranes, lipid peroxidation, programmed cell death. Decrease in protein content was observed in the *Brassica juncea* when it was exposed to the high concentration of Cadmium and Lead(Pb) and it was suggested that decrease in protein concentration was result of the increase in protease activity(Palma et al,2002).

Via haber weiss and fenton reaction, mostly redox active metals are directly involved in the formation of O2- , H2O2 and highly reactive OH while redox non-active metals cause oxidative stress in plants by getting interacted with the membrane lipids and proteins, antioxidative enzymes, elements of electron transport chain (Hall 2002; Romero purtas et al 2007). There are different intrinsic defense systems available in the nature; cells have been evolved with Superoxide Dismutase (SOD), Catalase (CAT), Ascorbate Peroxidase (APX) which control the production and elimination of ROS in plants. There is direct quenching of ROS by some low molecular weight compounds include Ascorbate (ASA), Glutathione(GSH), Phenolic compounds, alkaloids, alpha tocopherol, non protein amino acids(Foyer and Noctor 2005; Sharma and Dietz 2009;Ahmad el al 2010; Guerra el al 2011).some oxidative enzymes APX, GPX, GST or GR regulate the expression of proteins which are involved in oxidative stress. In response to Cd, Fe, Pb an increase in CAT activity was observed in *Nicotiana plumbaginofolia*, *Pisum sativum*, *Brassica juncea*(Minglin et al 2005; Romero Puertas et al,2007). Most important low molecular weight thiols which show high affinity for toxic metals are glutathione and cysteine. Glutathione is a substrate for phytochelatins. Enzyme phytochelatin synthase is responsible for the synthesis of phytochelatins (Grill et al 1989). Glutathione is a Sulphur containing tripeptide; formula-γ-Glutamate-Cysteine-Glycine. Phytochelatins are Cysteine rich peptides with formula [(γ-Glu-Cys)n-Gly)]; n=2-11(Yadav el al, 2010).

8. CHELATE ASSISTED PHYTOREMEDIATION

Chelating agents are biodegradable physicochemical factors which stimulate the heavy metal-uptake capacity of the plants by increasing the bioavailability of heavy metals. The faster uptake of heavy metals shortens the remediation period (Van Ginneken et al,2007). In chelate assisted phytormediation such as NTA, EDTA, HEDTA, DTPA are synthetic chelating agents which are added to enhance the accumulative potential of plants. Several studies reported that chelating agents such as EDTA, HEDTA and citric acid could be used to increase metal mobility, thereby increase phytoextraction (Elless & blaylock et al,2000; Chen and Cutright et al,2001; Chen et al,2003). Increasing order of effectiveness of chelates in increasing Pb desorption from the soil was found to be EDTA>HEDTA>DTPA>EGTA>EDDHA (Jianwei et al,1997). Plants exposure to EDTA for longer period (2 weeks) can improve metal translocation in plant tissues. The application of synthetic chelating agent EDTA at 5 mmol/kg was found with positive results (Roy et al,2005). It has also been found that high concentration of EDTA was found to be necrotic for the plants and also causes leaf shedding (Ahuja et al,2012).

9. CONCLUSION

Increasing contamination of Lead by industrial and natural processes is a major concern of pollution, so heavy metals need to be degraded. Phytoremediation is a low cost, environmental friendly approach to eradicate the contaminants. This review showed that *Brassica juncea* and *Vetiveria zizanioides* have remediatory effects on removal from contaminated soil. The ability of *Brassica juncea* and *Vetiveria zizanioides* being hyperaccumulators, to bioaccumulate heavy metal like Pb can be used to eradicate metallic contaminants in the soil. Brassica juncea has antimicrobial activity as well so it can be used for the biofumigation purpose.

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