ENHANCING MECHANISMS FOR PHYTOREMEDIATION

Dr (Mrs) Jot Sharma
Vice Principal/Associate Professor
Dept. of Biotechnology
Vijayaraje Institute of Science and Management,
NH-75,Turari, Jhansi Road,
Gwalior(M.P) 475001.
INDIA

Abstract

It is no doubt that the contamination of water, air and soil has worsened, and this occurs as a result of the increase in population. However, the need for remediation technologies has to be seriously considered. Phytoremediation is one of the remediation techniques with a relatively slow procedure and low efficiency. This review covers some of the biological, chemical, physical, physico-chemical and genetic methods, which were applied in parallel with phytoremediation, in an attempt to help increase the efficiency in the remediation of air, soil and water.

Key words: Phytoremediation, Molecular approaches, Genetic approaches

1.1. Introduction

Several methodologies are in vogue to enhance the potential of plants for phytoremediation.
Once a tolerant plant species has been selected**, traditional breeding** methods are used to optimize the tolerance of a species to a particular contaminant.

1. **Agricultural methods** such as the application of fertilizers, chelators, and pH adjusters can be utilized to further improve the potential for phytoremediation.

2. **Genetic modification** offers a new hope for phytoremediation as Genetically modified approaches can be used to over express the enzymes involved in the existing plant metabolic pathways or to introduce new pathways into plants.

Each metal may have its own specific mechanism for uptake, translocation and sequestration and hence it is imperative to design suitable strategies for developing transgenic plants specific for each metal.

1.2. Molecular genetic and transgenic strategies for phytoremediation hype

Genetic strategies and transgenic plant and microbe production and field trials will fetch phytoremediaition field applications [1,2,3,4] . Mercury is a world wide problem as a result of its many diverse uses in industry. Mercury has been used in bleaching operations (chlorineproduction, paper, textiles, etc.) as a catalyst, a pigment for paints, for gold mining, as well as a fungicide and antibacterial agent in seeds and bulbs. Elemental mercury, Hg (0), can be a problem because it is oxidized to Hg^{2+} by biological systems and subsequently is leached into wetlands, waterways, and estuaries. Additionally, mercury can accumulate in animals as methyl mercury (CH_3-Hg^+), dimethylmercury ($(CH_3)_2$-Hg) or other organomercury salts. Organic mercury, produced by some anaerobic bacteria, is 1-2 orders of magnitude more toxic in some eukaryotes, is more likely to biomagnify than ionic mercury, and efficiently permeates biological membranes. Monomethyl-Hg is responsible for severe neurological degeneration in birds, cats, and humans.

Certain bacteria are capable of pumping metals out of their cell, and or oxidizing, reducing, or modifying the metal ions to less toxic species. One example is the mer operon. The mer operon contains genes that sense mercury (merB), transport mercury (merT), sequester mercury to the periplasmic space (merP), and reduce mercury (merA). MerB is a subset of the mer operon and is capable of catalyzing the breakdown of various forms of organic mercury to Hg^{2+}. MerB encodes an enzyme, organomercurial lyase, that catalyses the protonolysis of the carbon-mercury bond. One of the products of this reaction is ionic mercury[5,6,7] :

$$Hg` \quad ^{2+}. R\text{-}CH_2\text{-}Hg^{+} \text{ ----merB---->} R\text{-}CH_3 + Hg(II)$$

$$Hg(II) + NADPH \text{ ----merA---->} Hg(0) + NADP^{+} + H^{+}$$

Hg (0) (elemental mercury) can be volatilized by the cell

1.3. Chelator enhanced phytoremediation technology

1. Use of soil amendments such as synthetics (ammonium thiocyanate) and natural zeolites have yielded promising results[8,9.10]. EDTA, NTA, citrate, oxalate, malate, succinate, tartrate, phthalate, salicylate and acetate etc. have been used as chelators for rapid mobility and uptake of metals from contaminated soils by plants. Use of synthetic chelators significantly increased Pb and Cd uptake and translocation from roots to shoots facilitating phytoextraction of the metals from low grade ores. Synthetic cross-linked polyacrylates, hydrogels have protected plant roots from heavy metals toxicity and prevented the entry of toxic metals into roots. Application of low cost the synthetics and natural zeolites on large scale are applied to the soil through irrigation at specific stages of plant growth which might be beneficial to accelerate metal acumulation [11].

2. A major factor influencing the efficiency of phytoextraction is the ability of plants to absorb large quantities of metal in a short period of time. Hyperaccumulators accumulate appreciable quantities of metal in their tissue regardless of the concentration of metal in the soil[12] , as long as the metal in question is present. This property is unlike moderate accumulators now being used for phytoextraction where the quantity of absorbed metal is a reflection of the concentration in the soil. Although the total soil metal content may be high, it is the fraction that is readily available in the soil solution that determines the efficiency of metal absorption by plant roots.

3. To enhance the speed and quantity of metal removal by plants, some researchers advocate the use of various chemicals for increasing the quantity of available metal for plant uptake. Chemicals that are suggested for this purpose include various acidifying agents [13,14,15,16,17,18], fertilizer salts[19] and chelating materials [20]. These chemicals increase the amount of bioavailable metal in the soil solution by either liberating or displacing metal from the solid phase of the soil or by making precipitated metal species more soluble. Research in this area has been moderately successful, but the wisdom of liberating large quantities of toxic metal into soil water is questionable.

4. Soil pH is a major factor influencing the availability of elements in the soil for plant uptake . Under acidic conditions, H^{+}ions displace metal cations from the cation exchange complex (CEC) of soil components and cause metals to be released from sesquioxides and variable-charged clays to which they have been chemisorbed (*i.e*. specific adsorption. The retention of metals to soil organic matter is also weaker at low pH, resulting in more available metal in the soil solution for root absorption. Many metal cations are more soluble and available in the soil solution at low pH (below 5.5) including Cd, Cu, Hg, Ni, Pb, and Zn [16] . It is suggested that the phytoextraction process is enhanced when metal availability to plant roots is facilitated through the addition of acidifying agents to the soil [13,16]. Possible amendments for acidification include NH_4-containing fertilizers, organic and inorganic acids, and elemental indicated that plant roots acidify hydroponic solutions in response to NH_4nutrition and cause solutions to become more alkaline in response to NO_3nutrition. Metal availability in the soil can be manipulated by the proper ratio of $NO_3$to NH_4used for plant fertilization by the effect of these N sources on soil pH, but not much of phytoremediation research has been conducted on this topic to date. The acidification of soil with elemental S is a common agronomic practice, which can be used to mobilize metal cations in soil[13], acidified a Cd- and Zn-contaminated soil with elemental S and observed that accumulation of these metals by plants was greater than when the amendment was not used. Acidifying agents are also used to increase the availability of radioactive elements in the soil for plant

uptake. Huang et al. 1998[15]reported that the addition of citric acid increases uranium(U)accumulation in Indian mustard (*B. juncea*) tissues more than nitric or sulfuric acid although all acids decrease soil pH by the same amount . These authors speculated that citric acid chelates the soil U, thereby enhancing its solubility and availability in the soil solution. The addition of citric acid causes a 1000-fold increase of U in the shoots of *B. juncea* compared to accumulation in the control (no citric acid addition). Despite the promise of some acidifying agents for use in phytoextraction, little research is reported on this subject.

5. The addition of chelating materials to soil, such as EDTA, HEDTA, and EDDHA, is the most effective and controversial means of liberating labile metal-contaminants into the soil solution. Chelates complex the free metal ion in solution, allowing further dissolution of the sorbed or precipitated phases until an equilibrium is reached between the complexed metal, free metal, and insoluble metal fraction . Chelates are used to enhance the phytoextraction of a number of metal contaminants including Cd, Cu, Ni, Pb, and Zn [20,21,22]. Huang et al. 1997a[21] suggested that chelates are able to induce Pb accumulation in agronomic crops such as corn (*Zea mays* L.) and pea (*Pisum sativum*L.). This chelate-assisted accumulation of toxic quantities of metal in a non-accumulator species is termed "chelate-induced hyperaccumulation" [21]. These researchers explained that when chelate-induced hyperaccumulation is the goal, metals on site are initially immobilized to allow for rapid establishment and growth of an agronomic crop such as corn. When the crop accumulates sufficient biomass, chelating materials are applied to the soil to result in the liberation of large quantities of metal into the soil solution. Massive amounts of metal are absorbed by plant roots and are translocated to the shoot tissue where they accumulate to toxic levels.

After death, plants are harvested and removed from the site. Chelate-induced hyperaccumulation is in contrast to the normal practice of phytoextraction where plants are given a gradual exposure to non-toxic quantities of metal in solution, and accumulation occurs gradually over time as the plants grow. The controversy surrounding the use of chelates deals with the fate of the residual chelate in the soil after metal absorption occurs [23]. The massive liberation of chelate-bound metals into the soil solution makes them subject to leaching into deeper soil layers. Metals which migrate downward beyond the root zone of plants cannot be recovered through means of phytoremediation and may require the use of more expensive conventional remediation methods. The primary concern is that the liberated metals have the ability to migrate into uncontaminated areas, possibly groundwater reservoirs [24]. The scientific literature lacks appreciable information concerning the appropriate amount of chelate to apply under different levels of contamination and for different plant species. Further research is required to determine the fate of the chelate-metal complex in soil before the use of these amendments are accepted widely for use in phytoextraction. Some positively charged metals and radionuclides may be bound to the soil CEC by weak electrostatic forces and may be displaced by other cations in the soil solution.

As a plant- based technology, the success of phytoextraction is inherently dependent upon proper plant selection. As previously discussed, plants used for phytoextraction must be fast growing and have the ability to accumulate large quantities of environmentally important metal contaminants in their shoot tissue[25,14,20,26] . Many plant species have been screened to determine their usefulness for phytoextraction. Researchers initially applied hyperaccumulators to clean metal polluted soils[27] . At present, there are nearly 400 known hyperaccumulators[28] , but most are not appropriate for phytoextraction because of their slow growth and small size. Several researchers have screened fast-growing, high- biomass accumulating plants, including agronomic crops, for their ability to tolerate and accumulate metals in their shoots[29,2511,21,22,19] . One of the most promising, and perhaps most studied, non- hyperaccumulator plant for the extraction of heavy metals from contaminated sites is Indian Mustard (*B. juncea*). Many hyperaccumulators belong to the Brassica family.

Once it was suspected that known hyperaccumulators were not suited for phytoextraction, researchers looked to other high biomass- accumulating members of the Brassicaceae for plants which accumulated large quantities of toxic metals[29,25] . Kumar et al. 1995a[25] tested many fast growing Brassicas for their ability to tolerate and accumulate metals, including Indian mustard (*B. juncea*), black mustard (*Brassica nigra* Koch), turnip (*Brassica campestris* L.), rape (*Brassica napus* L.), and kale (*Brassica oleracea* L.).

Although all Brassicas accumulated metal, *B. juncea* showed a strong ability to accumulate and translocate Cu, Cr VI, Cd, Ni, Pb, and Zn to the shoots. Kumar et al. 1995a[25] also investigated possible genetic variation of different *B. juncea* accessions in hope of finding some that had more phytoextraction potential than others. The term, accession, refers to seeds that have been gathered from a particular area and are now part of a collection at a seed bank or plant- introduction laboratory/institute. Once in the collection, seeds are assigned a number that identifies the particular accession. Although all Indian mustard accessions are *B. juncea* Czern., they may exhibit different phenotypes as a result of being from different regions where environmental factors may have influenced the natural selection of this species.

According to Prakash, 1980[30], the oldest reference to *B. juncea* in Sanskrit literature is by the name `Rajika', and carbonized seeds of this species have been found in the ancient sites of the Harappan civilization (2300-1750 B.C)[31,32,33]. Zaurov et al.1999(34)reported that biomass accumulation *of B. juncea* was greatest when plants in soil are supplied with 200 kg N, 100 kg P_2O_5, and 66 kg K_2O per hectare. However, Cd concentration in the tissue was greatest when no N was supplied. Indian mustard is given considerable attention by present day researchers, geneticists, and plant breeders in particular, because of its unique polyploid genome. Several accessions of *B. junceahave* been identified as moderate accumulators of metallic elements . The benefit of *using B. juncea* seed from the plant introduction station is that the genetic integrity of the accessions is preserved through appropriate breeding techniques. Experiments that utilize these seeds have more precision than those conducted with seeds from commercially available sources. Precision is also greater, because future researchers can obtain the same accessions for their experiments. The USDA-ARS Plant Introduction Station maintains a world-wide collection of *B. juncea* accessions that are known metal-accumulators, and the seeds are distributed to public and private research institutions at no cost.

1.4. Increasing the bioavailability of heavy metals

One of the most critical points in phytoremediation is the phytoavailability of heavy metals in the soil[35] . Based on the uptake by plants, heavy metals in soil could be classified into three groups which include “available” fractions (easily absorbable forms including free ions and chelating ions), “exchangeable” fractions (bound to organic matter, carbonates or Fe-Mn oxides), and “unavailable” fractions (residual forms which are most difficult to be absorbed [36,37]. Nevertheless, several methods are employed to increase the bioavailability of heavy metals such as decreasing the pH by adding sulphuric acid or organic fertilizers [38,39] or by using chelating agents. SappinDider et al.,(2005)[40] showed the increase in the accumulation of Cd in transgenic tobacco as pH decreased, whereas Singer et al., (2007)[41] proved an increase in the Ni concentrations of *Alyssum lesbiacum* which was paralleled with the increase in pH. The latter case showed different situations on the accumulation of metal.

Synthetic chelating agents are shown to have the potential to increase the bioavailability of unavailable and exchangeable heavy metal fractions [42]. The use of non-biodegradable or the least biodegradable chelating agents, such as EDTA,(Ethylene diamine tetra acetic acid) can leach metals into the ground water [43] and create a new source of pollution by this residual chelating reagent[36].

1.5. Alteration in biomass:

Biomass of known hyperaccumulators can be altered by the introduction of genes which effect phytochrome synthesis resulting in enhanced biomass. Recently, biosynthetic pathways have been elucidated for most of the plant hormone classes and genes encoding many of the enzymes have been cloned. These advances offer new opportunities to manipulate hormone content and regulate their biosynthesis [44]. Increased gibberellins biosynthesis in transgenic trees were shown to promote growth and biomass production[45] . Appropriate application of fertilizers (N,P,K) and irrigation also have beneficial effects [36]; for instance, Jankong et al., (2007)[46] found an increase in the biomass and accumulation of arsenic in silverback ferns (*Pityrogramma calomelanos*) after application with phosphorous. Meanwhile, Barrutia et al.,(2009)[47] observed an increase in the mean plant biomass and tolerance when treated with fertilizers in Pb, Cd and Zn contaminated soils. Nitrate fertilizers could also be used to enhance the biomass of shoot and stimulate the accumulation of Zn. In spite of the presence of positive effect of fertilizers on metal accumulation, Marques et al (2008a)[48] showed a reduction in the accumulation of Zn in *Solanum nigrum* when the contaminated soil is amended with manure. However, in spite of these few controversial reports, little work has been carried out in this area for improving the biomass of plants for phytoremediation

1.6. Genetically engineered approaches

Higher efficiency in the remediation by plants is achieved mostly by an increase in the tolerance and/or accumulation capacity of transgenic plants (see review[49] . Hseih et al.,2009[50] found an increase in mercury accumulation and tolerance of *Arabidopsis thaliana* when mercuric ion binding protein (Mer P) originated from transposon TnMER1 of TnMERI1 *Bacillus megaterium* strain MB1, was expressed in the transgenic plants. Genetically modified plants usually contain some beneficial enzymes like ACC(1-aminocyclopropane-1-carboxylic acid) deaminase[51] and γ-glutamylcysteine synthetase [52], which in turn improve the tolerance of plants to stress and increase the ratio between plant growth and shoot/root. Grichko et al (2000)[53] has reported overexpression of ACC deaminase may lead to enhanced accumulation of variety of metals. Similarily, overexpression of glutathione S transferase and peroxidase leads to enhanced aluminium tolerance [54]. Transgenic plants with selected genes have also been shown to express higher abilities to biodegrade organic contaminants in their tissues [55].

Tolerance of plants to heavy metals could be attained in three ways: pumping out of heavy metals at the plasma membrane; thorough chelating of heavy metals and binding the heavy metals to various thiol compounds in the cytosol; and sequestering them into vacuoles.

Genetic manipulation of genes involved in phytochelatin synthesis has contributed to higher tolerance and accumulation of heavy metals in various plants, this includes Genes such as γ-glutamyl cysteine synthetase, glutathione synthetase, cystothionine synthetase, ATP sulfurylase, serine acety-l transferase, glutathione reductase, and phytochelatin synthase. Recent studies have documented the role of glyoxylase pathway in heavy metal stress tolerance by maintaining glutathione redox ratio. Singla – Pareekh et al 2006[56] has also reported the suitability of engineering (manipulation of glyoxylate pathway) strategy for improved metal tolerance in transgenic tobacco[57].

1.7. Multi functional approaches:

As each described method has its own advantages and disadvantages, new approaches have been focusing on multi functional improvement methods. Lin et al., (2009)[58] found a better efficiency of the low dose EDTA with a medium soil nutrient level on the accumulation of lead in sunflower. In the same way,Vaxevanidou *et al*. (2008)[59] showed approximately10% increase in the extraction of lead with bacteria and EDTA as compared to the amendment of EDTA alone. Similarly Di Gregorio et al., 2006[60], showed a 56% increase in the efficiency of EDTA-led phytoextraction by *B.juncea*, along with application of triton X 100 Sinorhizobium inoculums. Multifunctional proceeses such as volatilization, photooxidation and microbial remediation are currently in use for the removal of organic contaminants.

ACKNOWLEDGEMENT

*I sincerely thank chairperson VISM Group**Smt Saroj Rathore,** Chairman VISM Group*
***Dr Sunil kumar Singh Rathore**, Director VISM Group **Dr Pragya Singh** for providing refined atmosphere and constant encouragement to pursue my work single mindedly.*

REFRENCES

1. Misra S, Gedamu L(1989) Heavy metal tolerant transgenic *Brassica napus* L. and *Nicotiana tabacum* L. plants. *Theoretical and Applied Genetics*. vol. 78, p. 161-168.
2. Stomp AM,Han KH, Wilbert S et al(1994) Genetic strategies for enhancing phytoremediation. *Annals of New York Academy of Sciences*, 1994. vol. 721, p. 481-491

3. Pence NS, Iarsen PB, Ebbs SD et al(2000).The molecular physiology of heavy metal transport in the Zn/Cd hyperaccumulator *Thlaspi caerulescens*. *Proceedings of the National Academy of Sciences of the United States of America*. vol. 97, no. 9, p. 4956-4960.
4. Palmer EF, Warwick F , Keller W(2001) Brassicaceae (Cruciferae) family, plant biotechnology and phytoremediation. *International Journal of Phytoremediation*. vol 3, no. 3, p. 245-287.
5. Rugh CL, Wilde HD, Stacks NM et al(1996) Mercuric ion reduction and resistance in transgenic *Arabidopsis thaliana* plants expressing a modified bacterial merA gene. *Proceedings of the National Academy of Sciences of the United States of America*. vol. 93, no. 8, p. 3182-3187.
6. Heaton ACP, Rugh CL, Wang N et al (1998) Phytoremediation of mercury - and methylmercury - polluted soils 5.Rugh CL, Wilde HD, Stacks NM et al(1996) Mercuric ion reduction and resistance in transgenic *Arabidopsis thaliana* plants expressing a modified bacterial merA gene. *Proceedings of the National Academy of Sciences of the United States of America*. vol. 93, no. 8, p. 3182-3187. using genetically engineered plants. *Journal of Soil Contamination*.vol. 7, no. 4, p. 497-510.
7. Pilon-Smits EAH, Pilon M (2000) Breeding mercury-breathing plants for environmental cleanup. *Trends in Plant Science*. vol. 5, no. 6, p. 235-236.
8. Huang JW, Chen J, Berti WR et al (1997) Phytoremediation of lead contaminated soils-Role of synthetic chelates in lead phytoextraction. – Environmental Science and Technology, 31; 800-806
9. Anderson CWN, Brooks RR, Stewart RB, Simcock R (1998) Harvesting a crop of gold in plants, *Nature*. vol. 395, no. 6702, p. 553-554.
10. Zorpas AA (1999) constantinides, T.; vlyssides, A.G.; ARALAMBOUS, I. and LOIZIDOU, M. Heavy metal uptake by natural zeolite and metals partitioning in sewage sludge compost. *Bioresource Technology*. vol. 71, no. 2, p. 113-119.
11. Blaylock, MJ, Salt DE, Dushenkov S etal(1997) Enhanced accumulation of Pb in Indian mustard by soil-applied chelating agents. *Environmental Science and Technology*.vol. 31, no. 3, p.860- 865
12. Baker AJM (1981)Accumulators and excluders - strategies in the response of plants to heavy metals. *Journal of Plant Nutrition*. vol. 3, p. 643-654.
13. Brown SL, Chaney RL ,Angle JS et al(1994) Phytoremediation potential of *Thlaspi caerulescens* and bladder campion for zinc and cadmium-contaminated soil. *Journal of Environmental Quality*. vol. 23, no. 6, p. 1151-1157.
14. Cunningham SD, Ow DW(1996) Promises andprospects of phytoremediation. *Plant Physiology*. vol.110, no. 3, p. 715-719
15. Huang JW, Blaylock MJ, Kapulnik Y et al (1998) Phytoremediation of Uranium-Contaminated Soils: role of organic acids in triggering uranium hyperaccumulation in plants. *Environmental Science and Technology*, 1998, vol. 32, no. 13, p. 2004-2008.
16. Blaylock MJ, Huang JW (2000) Phytoextraction of metals. In: Raskin, I., Ensley, B.D. (Eds.), Phytoremediation of Toxic Metals: Using Plants to Clean up the Environment. – John Wiley and Sons, New York; pp. 53–70.
17. Chen HM, Zheng CR,Tu C, Shen ZG (2000) Chemical methods andphytoremediation of soil contaminated with heavy metals. *Chemosphere*.vol. 41, no. 1-2, p. 229-234
18. Chen H, Cutright T(2001) EDTA and HEDTA effects on Cd, Cr, and Ni uptake by *Helianthus annuus*. *Chemosphere*.vol. 45, no. 1, p. 21-28.
19. Lasat MM, Fuhrmann M, Ebbs SD et al (1998) Phytoremediation of a radiocesium contaminated soil: evaluation of cesium- 137 bioaccumulation in the shoots of three plant species. *Journal of Environmental Quality*, 1998, vol. 27, no. 1, p. 165-168.
20. Blaylock MJ, Salt DE,Dushenkov S et al(1997)Enhanced accumulation of Pb in Indian mustard by soil-applied chelating agents.*Environmental Science and Technology*. vol. 31, no.3, p.860- 865.
21. Huang JW, Chen J, Berti WR. et al (1997a) Phytoremediation of lead contaminated soil: role of synthetic chelates in leadphytoextraction. *Environmental Science and Technology*. vol. 31, no. 3, p. 800-805.
22. Huang JW, Chen J , Cunningham SD(1997 b)Phytoextraction of lead from contaminated soils. In:KRUGER, E.L.; ANDERSON, T.A. and COATS, J.R.(ed)*Phytoremediation of soil and water contaminants*.Washington, DC, American Chemical Society. p. 283-298
23. BROOKS RR (1998a)General Introduction. In: R.R. Brooks ed. *Plants that hyperaccumulate heavy metals:their role in phytoremediation, microbiology, archaeology, mineral exploration and phytomining*. New York, CAB International. p. 1-14.

24. Cunningham SD, Shann JR, Crowley DE et al(1997). and ANDERSON, T.A. Phytoremediation of contaminated water and soil. In: KRUGER, E.L.; ANDERSON, T.A. and COATS, J.R. eds. Phytoremediation of soil and water contaminants. ACS symposium series 664. Washington, DC, American Chemical Society. p. 2-19.
25. Kumar PBAN, Dushenkov V, Motto H etal (1995a)Phytoextraction: The use of plants to remove heavy metals from soils. *Environmental Science and Technology*. vol. 29, no. 5, p. 1232-1238.
26. Mcgrath SP (1998) Phytoextraction for soil remediation. In:BROOKS, R.R., (ed). *Plants that hyperaccumulate heavymetals: their role in phytoremediation, microbiology, archaeology, mineral exploration and phytomining*. New York, CAB International. p. 261-288.
27. Chaney RL(1983) Plant uptake of inorganic waste constitutes.In: PARR, J.F.; MARSH, P.B. and KLA, J.M. eds. *Landtreatment of hazardous wastes*. Park Ridge, NJ, Noyes Data Corp. p. 50-76.
28. Salt DE, Kramer U (2000) Mechanisms of metalhyperaccumulation in plants. In: RASKIN, I. and ENSLEY,B.D., eds. *Phytoremediation of toxic metals: using plants to clean-up the environment*. John Wiley & Sons, Inc., NewYork. p. 231-246.
29. Dushenkov V, Kumar PBAN, Motto H et al(1995) Rhizofiltration: the use of plants to remove heavy metals from aqueous streams. *Environmental Science and Technology*, 1995, vol. 29, p. 1239-1245.
30. PRAKASH S (1980) Cruciferous oilseeds in India. In:TSUNODA, S.; HINATA, K. and GÓMEZ-CAMPO,C*Brassica crops and wild allies: biology and breeding*. Tokyo, Japan Scientific Societies Press, 1980, p. 151-166.
31. Thakral SK, Singh BP, Faroda AS et al (1995) Fatty acids composition in seeds of Brassica species as affected by moisture and fertility levels.*Crop Research*. vol. 10, no. 2, p. 137-140.
32. GURJAR, B.S. and CHAUHAN, D.V.S. Yield attribute and seed yield of Indian mustard (*Brassica juncea*) as influenced by varieties, fertility levels and spacing in Harsi Command area. *Indian Journal of Agronomy*, 1997, vol. 42, no. 1, p. 142-144.
33. Tomar TS, Singh S, Kumar S. , Tomar S.(1997Response of Indian mustard (*Brassica juncea*) to nitrogen,phosphorus and sulfur fertilization. *Indian Journal of Agronomy*. vol. 42, no. 1, p. 148-151.
34. Zaurov DE, Perdomo P, Raskin I(1999)Optimizing soil fertility and pH to maximize cadmium removed by Indian mustard from contaminated soils. *Journal of Plant Nutrition*. vol. 22, no. 6, p. 977-986.
35. Lombi, Zhao FJ, Dunham SJ et al(2001) Phytoremediation of heavy metal contaminated soils: natural hyperaccumulation versus chemically enhanced phytoextraction. Journal of Environmental Quality 30: 1919-1926.
36. Wei H, Teixeira DA, Silva JA et al (2008) Agro-improving method of phytoextracting heavy metal contaminated soil. Journal of Hazardous Materials 150:662-668.
37. Zhou QX, SongYF (2004) Remediation of contaminated soils. Principles and methods. Beijing Sciences press, China.
38. Roy S, Singh SB (2006) Effect of soil type, soil pH and microbial activity on persistence of clodinafop herbicide. Bulletin of Environmental Contamination and Toxicology 77: 260-266.
39. Karami A, Shamsuddin ZHJ(2010) Phytoremediation of heavy metals with several efficiency enhancer methods. African Journal of Biotechnology 9 (25):3689-3698.
40. Sappin-Didier V, Vansuyts G, Mench M et al (2005) Cadmium availability at different soil pH to transgenic tobacco overexpressing ferritin. Plant and So44. Sappin-Didier V,
41. Singer A., Bell T, Heywood CA etal. (2007) Phytoremediation of mixed contaminated soil using the hyperaccumulator plant Alyssum lesbiacum: evidence of histidine as a measure of phytoextraction of nickel. Environmental pollution 147:74-82.
42. Komarek M, Tlustos P, Szakova J et al (2007).The use of maize and poplar in chelate enhanced phytoextraction of lead from contaminated agricultural soils. Chemosphere 67:640-651.
43. Santosh FS, Hernandez- Allica J, Becerril JM etal (2006) Chelate-induced phytoextraction of metal polluted soils with Brachiaria decumdens, Chemosphere 65: 43-50.
44. Hedden P, Phillips AL (2000) Manipulation of hormone biosynthetic genes in transgenic plants Current Opinion In Biotechnology 1(2): 130-137.
45. Eriksson ME, Israelsson M, Olsson O et al (2000) Increased gibberellins biosynthesis in transgenic trees promotes growth, biomass production and xylem fibre length. Nature Biotechnology 18: 784-788.
46. Jangkong P, Bisoottiviseth P, Khokiattiwong S (2007). Enhanced phytoremediation of arsenic contaminated land. Chemosphere, 68:1906-1912.

47. Barrutia O,Epelde , Garcia-Plazaola JI et al (2009)Phytoextraction potential of two Rumex acetosa L. accessions collected from metalliferous and non-metalliferous sites: Effect of fertilization. Chemosphere 74: 259-264.
48. Marques APGC, Oliveira RS, Rangel AOSS etal (2008a)Application of manure and compost to contaminated soils and its effect on zinc accumulation by Solanum nigrum inoculated with arbuscular mycorrhizal fungi. Environmental Pollution 151:608-620
49. Eapen S, D'Souza SF(2005) Prospects of genetic engineering of plants for phytoremediation of toxic metals. Biotechnology Advances 23: 97-114.
50. Hseish JL, Che CY, Chiu MH et al(2009). Expressing a bacterial mercuric ion binding protein in plant for phytoremediation of heavy metals. Journal of hazardous materials 161:920-925.
51. Nie L, Shah S, Burd Get al(2002) Phytoremediation of arsenate contaminated soil by transgenic canola and the plant growth promoting bacterium, Enterobacter clocae CAL2. Plant Physiology and Biochemistry 40: 355-361
52. Han KH,Meilan R, Ma C,et al (2006) . An Agrobacterium tumefaciens transformation protocol effective on a variety of cotton wood hybrids (genus Populus). Plant Cell Reports 19: 315-320.
53. Grichko VP,Filby B,Glick BR (2000) Increased ability of transgenic plants expressing the bacterial enzyme ACC deaminase to accumulate Cd, Co, Cu, Ni, pb and zinc. Journal of Biotechnology 81: 45-53.
54. Ezaki B, Gardner RC,Ezaki Yet al (2000). Expression of aluminium induced genes in transgenic Arabidopsis plants can ameliorate aluminium stress and or oxidative stress. Plant Physiology 122: 657-665.
55. Kawahigashi H, Hirose S, Ohkawa H et al (2003) Transgenic rice plants expressing human CYP 1A1 exude herbicide metabolites from their roots. Plant Science 165:373-381.
56. Singla- Pareek, SL, Yadav SK, Pareek A etal (2006). Transgenic tobacco over expressing glyoxylase pathway enzymes grow and set viable seeds in zinc-spiked soils. Plant Physiolgy 140:613-623
57. Yadav SK(2010) Heavy metals toxicity in plants: An overview on the role of glutathione and phytochelatins in heavy metal stress tolerance of plants. South African Journal of Botany 76 : 167-179.
58. Lin C, Liu J, Liu L (2009) Soil amendment application frequency contributes to phytoextraction of lead by sunflower at different nutrient levels. Environmental and Experimental Botany 2(3) 410-416.
59. Vaxevanidou K, Papassiopi N, Paspaliaris I (2008) Removal of heavy metals and arsenic from contaminated soils using bioremediation and chelant extraction techniques. Chemosphere 70: 1329-1337.
60. DI Gregario S, Barbafieri M, Lampis S et al(2006). Combined application of Triton X-100 and Sinorhizobium sp. Pb002 inoculum for the improvement of lead phytoextraction by Brassica juncea in EDTA amended soil. Chemosphere 63:293-299.