2. REVIEW OF LITERATURE

Previous work pertaining to heavy metal contamination of soil through the application of sewage effluents and its remediation by chemical immobilization complexing agents and growing of metal-extracting, stabilizing and accumulating crop plants (phyto-remediation) has been reviewed under the following heads;

2.1 Metal Contamination of Soil:

2.1.1 Characteristics of Sewage and industrial effluents.

The composition of sewage and industrial effluents, *i.e.* the waste water from urban life and industrial activity, varies with their sources. In general, these effluents are characterized by high BOD, COD and total dissolved salts (Chhonkar *et al.*, 2000 a.b.). Due to lack of treatment and improper mode of disposal, these effluents cause considerable reduction in dissolved oxygen in water and chemical oxygen demand. When such polluted water is used for irrigation, productivity of soil may be seriously affected (Rai and Sharma, 1990). However, now, there is a considerable debate about the application of sewage and industrial effluents to the agricultural land.

Presence of considerable amounts of nitrogen, phosphorus and potassium in sewage effluents is reported to be the cause of long-term sustenance of soil fertility in sewage irrigated soils (Dahatonde *et al.*, 1995). Kemppainen (1987) reported that on an average, sewage effluent contained 4.69% dry matter, 2.01 gL^{-1} total nitrogen, 0.49 gL^{-1} phosphorus, 5.45 gL^{-1}, potassium and 0.65 gL^{-1} calcium. According to Obbard *et al.*, (1993), the addition of sewage effluents to a soil improves its characteristics and acts as a source of nutrients for crops. Ambika and Latha (1996) found that industrial effluents and domestic sewage outlets contribute to high concentrations of phosphorus in the form of inorganic phosphates and polyphosphates in the water.

Sewage effluents generated out of the mixture of domestic and industrial waste water from different cities have many folds higher heavy metal concentration than that of tube well water (Rattan *et al.,* 2002a). For example, sewage effluents emanating from Ludhiana were reported to contain 710, 6412, 6515, 307, 2.3, 53.1, 357 and 132 µg L^{-1} of Zn, Cu, Fe, Mn, Cd, Pb, Co and Ni, respectively. The corresponding values for tube well water were 260 µg L^{-1} for Zn, 70 µg L^{-1} for Cu, 1550 µg L^{-1} for Fe, 140 µg L^{-1} for Mn, 0.55 µg L^{-1} for Cd, 9.3 µg L^{-1} for Pb, 75 µg L^{-1} for Co and 1.5 µg L^{-1} for Ni.

The sewage sludge produced by different cities in India and abroad was heterogeneous in nature and chemical composition. A regional survey of sewage sludge composition was conducted by obtaining more than 250 sewage samples from approximately 150 treatment plants located in 8 states of America. The chemical composition for anerobically digested sludge was as follows ; N,42; P, 3.0; K, 0.3%, Pb, 540; Zn, 1809; Cu, 1000; Ni, 85; and Co, 16mg/kg, and for aerobically treated sludge: N,4.8; P,2.7; K,0.4%; Pb.300; Zn, 1800; Cu, 970; Ni, 31; and Cd, 16 mg/kg (Sommers 1977).

Sewage sludge samples collected from two discharge points, *viz*; Topsia and Pagladanga of Calcutta were determined by Aqua regia APDC-Na-DDTC and HNO_3-H_2O_2 methods. The heavy metal concentration (µg/kg) in respectively, Cr,1010,27.5,3543.0 respectively Mn 382, 2.2, 199 respectively, Pb, 200.0, 11.3, 68.8 respectively Cd, 4.0, 1.0, 10.6 respectively and in Topsia sludge was Cu, 96.3, 64.4, 20.0 respectively, Cr, 101.3, traces, 15.3 respectively Mn 282.5, 2.5, 66.9 respectively Pb, 185.0, 6.3, 175.0 respectively, Cd, 2.0, traces, traces respectively. [Adhikari, *et al.*, 1993]. According to Yassen *et al;*

(2006). The sewage sludge from Cairo city, Egypt had physico-chemical charactersties as pH, 6.95, Ec, 4.01 dSm^{-1}; N, 2.3%, P, 0.35%., K, 0.96%; Pb, 427 ppm., Cd, 34 ppm; Ni,47 ppm; Cr ppml Total contents of trace metals *viz.* Ni, Cu, Zn, Cd and Pb in anaerobically digested sludge obtained from the joint water pollution control plant operated by Los Angels country sanitation Districts reported by Sposito, *et al*, (1982) ranged as Ni, 260-483; Cu, 712-1042; Zn, 1785-3547; Cd, 34-61 and Pb, 1050-1797 µg/g.

2.1.2 Effects of sewage application on soil properties.

The application of the 500 Mg sewage sludge per hectare greatly increased he total metal concentration of Cd, Cu, Ni and Zn in the Ap horizon of the soils (Brallier *et al.* 1996) . In sludge amended soils, Cd, Cu, Ni and Zn content were increased from 0.6 to 35, 10 to 518, 18 to 63 and 71 to 1180 mg kg^{-1} respectively. Most of the metals added in the sludge-amended soils were confined to the 0-15 cm soil layer and that there was very little redistribution of metals down that soil profile (Harrison *et al.* 1994). Rattan *et al.*, (2002a) reported that, under Keshopur Effluent Irrigation System of Delhi (Bakarwala village, Western Delhi), where sewage effluent irrigation had been practiced for the last 23 years, the DTPA extractable Zn, Cu, Fe and Ni were increased by 253, 202, 337 and 153%, respectively.

Data *et al.*, (2000) conducted a comprehensive study on the effect of sewage effluent irrigation in IARI farm, where the soil has been receiving effluent irrigation for more than three decades. They reported that the available P and DTPA extractable Zn, Cu, Fe and Ni contents in soil was increased by 180, 127, 200, 247 and 100 percent, respectively, due to the long-term use of sewage effluents.

Hooda and Alloway (1993) found that metal uptake by ryegrass from the sludge treatments increased in the successive harvests, while that from metal-salt treatment was found to be declining. Increase in plant metal uptake over time coincided with decrease in soil pH in the sludge treatments. Brallier *et al.*, (1996) found that soil pH was reduced from 5.9 to 4.6 by the addition of sludge and consequential enhanced mobilization of the metals occurred.

Moreno *et al.*, (1998) observed that the activity of the protease enzyme was reduced in the compost contaminated with Cd, Ni, Zn and Cu. They reported that the soils amended with the metal-contaminated compost showed lower phosphates activity than those treated with uncontaminated compost. Mitra and Gupta (1999) compared the nutrient and heavy metal status of sewage-irrigated soils from a vegetable growing area close to Kolkota with non-sewage (tube well water) irrigated soils. A high pH and accumulation of salts were observed in sewage-irrigated soil. Moreover, concentration of Pb, Cd, Cu, Zn and Cr were above the permissible limits in sewage irrigated soils.

2.1.3 Effects of metal contamination on plant growth.

Plant establishment and growth on metal contaminated soils may be inhibited by different factors like acute soil acidity, direct availability of soil metals, restriction of root growth into toxic soil materials and reduction in number of indigenous microbes that cycle nutrients and fix N (Kabata-Pendias and Pendias, 1992). Long term application of sewage sludge can cause phytotoxicity and contamination problems in soil due to the elevated heavy metal content (Mc Grath *et al.*, 1988). For example, the concentrations of Cu exceeded the limits of phytotoxicity in garden spinach, radish, snake cucumber and round guard grown on the dry riverbed of the river Gangas as these soils are inundated by the metal rich waste water. (Rattan *et al.*, 2002a)

Mitra and Gupta (1999) observed that the contents of heavy metals in sewage irrigated radish, guard, spinach and cauliflower around Kolkota were comparatively 2 to 40 times higher than non-sewage irrigated vegetables. Zinc, copper, manganese, nickel and lead contents of crops like chilli, brinjal, carrot, radish, rose *etc.* grown on sewage effluent irrigated soils of IARI farm were below generally accepted critical level of phytotoxicity, whereas, Fe content in most of these plants exceeds phytotoxicity levels (Datta *et al.*, 2000). Although, no toxicity symptoms were observed in plants grown on Keshopur sewage effluent irrigated soils, which has been irrigated with effluents for more than two decades.

The order of toxicity metals are not same for all the soil and plant species. Datta *et al.* (2000) determined the heavy metal content of crops grown on sewage effluent irrigated soils of IARI farm. From the study they reported that the crop species exhibited differential behavior in accumulating metals in their tissues. Nickel was more phytotoxic to lettuce in acid than in calcareous soil (Mitchell *et al.*, 1978). They found that at lower rates of addition, Cu and Cd were more toxic to lettuce grown in calcareous soil than in the acid soil.

Because of difficulties in dealing with metals having different degrees of phytotoxicities, attempts have been made to formulate a general metal equivalent equation. Based on the average relative toxicities as determined from greenhouse pot experiments, Chumbley (1971) proposed the Zn equivalent concept which is based on the assumption that Cu is twice as toxic as Zn and Ni is 8 times toxic as Zn. On the contrary, Mitchell *et al.* (1978) reported that copper and nickel toxicities relative to Zn were much reduced in wheat grown on calcareous soil. Working with a sewage sludge amended soil having a pH of 6.8, Cunningham *et al.* (1975) found the toxicity ratio of Zn:Cu:Ni to be 1.0:1.8:1.0 for rye (*Secale cereal*). In addition, there are other risks like its entry into the food chain, causing health hazards to both human beings and animals.

Lettuce is an ideal test crop for studies on metal uptake because of large biomass production, easy maintenance, tendency to accumulate metals and has a direct relevance to humans (Tambasco *et al.*, 2000), (Sloan *et al.* (1997)_ reported that the concentrations of Cd, Ni, Zn, Cu and Cr in lettuce plant were significantly increased by application of sewage sludge.

2.2 Heavy metal toxicity in plant system

Heavy metals can operate as stress factors in a plant's environment. This can be recognized by adverse reaction shown on the part of the plant. Sensitive or non-tolerant plants are those who die or have reduced growth in a particular metal concentration, at which resistant or tolerant plant show little or no reduction in growth rate (Tomsett and Thurman, 1988). Lambinon and Auquier (1963) have classified plants growing on metal contaminated soils on the basis of degree of restriction imposed on the plants by such soils, which are: metallophytes (fund only on metal contaminated soil) and pseudo metallophytes (occurring on both contaminated and uncontaminated soils). Excessive heavy metal accumulation can be toxic to most plants leading to decrease in seed germination, root elongation and biomass production, inhibition of chlorophyll biosynthesis as well as cell disturbance and chromosome lesion (Balsberg and Pahlsson, 1989; Kumar *et al.*, 1991; Fargasova, 1994; Xiong Zhiting, 1997)

Several workers observed increase heavy metal concentration in plants (Alexander *et al.*, 1978 and Sims and Boswell, 1978) and in soils (Sims and Boswell, 1978) with the application of sewage sludge on the soil.

Agarwala *et al.* (1977) reported the order of decrease in dry matter yield of plants due to various heavy metals as: $Ni^{2+}>Co^{2+}>Zn^{2+}>Mn^{2+}>Cu^{2+}$. In a study on young barley plants in growth medium containing excessive quantities of heavy metals, Zinc was found to cause yellow leaves with brown patches and pale green strips. Nickel toxicity was found to be characterized by longitudinal white strips and pale green strips on leaves (Beckett and Davis, 1978). Petrovic (1978) found that plant dry matter weight of sunflower, bean and maize were significantly increase by low concentration of Ni, but its higher concentration decreased the dry matter yield. Later in 1998 an experiment conducted by Singh and Nayyar had revealed that when Ni was applied at rates of 2.5 mg kg^{-1} soil for rye grass, 15 mg kg^{-1}soil for Egyptiar, clover, significant decline of dry matter of those forage crops were happened.

An increased Cd and Zn content in the harvested leaf, grain and straw fo barley was reported by Chang *et al.* (1982) with the increase being greatest in the straw. Valdares *et al.*(1983) used to types of one was metal rich (Cd, Ni, Cu, Cr and Zn), while the other was relatively poor in heavy metals and reported that metal poor sludge hardly affected the yield of Swiss chard while metal rich sludge reduced the yield drastically in non-calcareous soils after a critical amount of that sludge (1.5%) was added to soils. The long term effect of metal contaminated sewage sludges on soils and crops was studied by Burridge and Berrow (1984) and found considerable increase in the plant content of Zn and Ni by the sludge treatment. Results showed that several metals originally presented in the sludge had not been immobilized by the soil and Ni and Zn, in particular had remained in plant available form.

There are reports which show that the metal concentration in crop and occurrence of metal induced yield reduction depended on soil properties as well as metal loading (Sanders *et al.*, 1987).

2.3 Bio-magnification of heavy metals along the food chain.

Besides the effect on plants, the heavy metals get magnified along th food chain causing toxic manifestation at each and every stage of the food web (Fig.1). The heavy metals get entry into the human and animal food chain through the crops grown on it. Such soils are often used to cultivate leafy vegetables and tuber crops to meet the nearby urban demand. These crops are known for their capacity to accumulate heavy metals in their edible parts. Entry of heavy metals

like Cd, Zn, Pb, Cu, Ni, Mn and Fe to the food chain have been reported through lettuce, beet tubers (John and laerhoven, 1976) and potato (Haan and Lubbers, 1983) consumption.

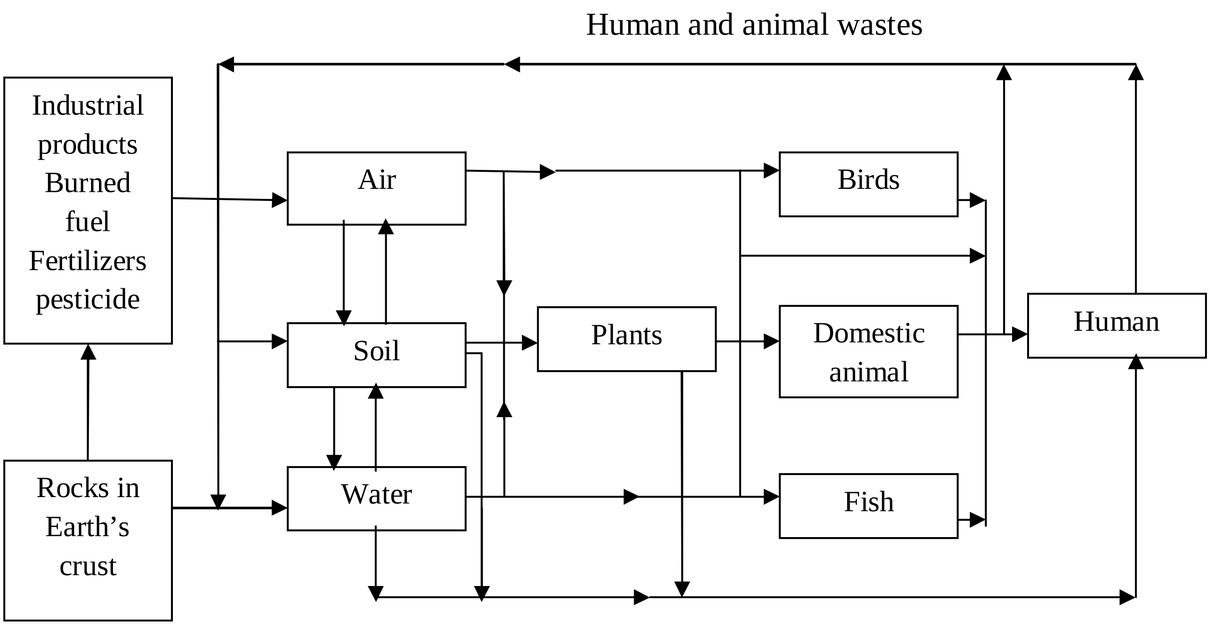

Fig. 1. Bio-magnification of heavy metals along the food chain.

In Indian context, Rattan and co-workers (2001) observed an increasing accumulation of Zn, Ni, Cu and Fe in different fields, vegetable and fruit crops like maize, mustard, rice, jowar, spinach, cauliflower, brinjal, radish, guava, citrus etc which were grown under sewage irrigation from Keshopur Effluent Irrigation System in Western Delhi. Agricultural sustainability of such production system depends to a large extent upon the maintenance or enhancement of soil quality that is rapidly deterioratin due to disposal of untreated effluents to soil.

2.4 Heavy metal toxicity to animals and humans system.

Different doses of heavy metals may cause undetectable, therapeutic, toxic or lethal effects (Fraser, 1986). Selenium, Cu and Zn often become toxic as the dose and exposure increase. Through the food chain these get entry to the body of livestock as well as human being. Acute Zn toxicity in animals is uncommon and is first observed at Zn intake of several grams (Sadstrom, 1995). Zinc toxicity is manifested as gastrointestinal distress, decreased food consumption, anorexia, haemoglobinuria, anemia, poor bone mineralization and arthritis (Gupta and Gupta, 1998).

Lead poisoning is the most frequently diagnosed toxicological condition in veterinary medicine (Fraser, 1986). Its occurrence has been reported in all domestic species and most commonly in cats and dogs because of their indiscriminate eating habits and relative susceptibility to Pb. Wild ducks are frequently poisoned by ingested Pb pellets (Gupta and Gupta, 1998)

Although many trace elements are required by humans for normal metabolism, most may also be toxic in levels above the very low body requirements, ingestion of Zn in large amounts has caused vomiting and diarrhea and neurological damage. A single dose of 0.1 to 0.2 mg Cu kg^{-1} body weight can elicit gastrointestinal disturbances in sensitive persons (Bosshard and Zimmerli, 1994). Wilson’s disease is an autosomal recessive disorder in which the inherited metabolic defect is associatd with the gradual and progressive accumulation of Cu in the liver (Randolph and Rotter, 1989).Toxicity symptoms of Cu include hemolusis, hepatic necrosis, and renal damage (Gupta and Gupta, 1998). In adults, excessive exposure of Pb (blood concentration greater than 80 to 100 μg dL^{-1}) is accompanied by pallor gingival lead line, anemia and a variety of neurological symptoms (Langston and Irwin, 1989). Blood Pb concentrations greater than 25 μg dL^{-1} would alert suspicion of toxic exposure. In Bangkok, Thailand a study conducted in 1990 showed that by age seven the average child had lost sic points in IQ tests because of Pb poisoning from the air (Gupta and Gupta, 1998). Average blood Pb levels in Thailand were 40-45 μg dL^{-1} which is ten times the U.S. standard.

2.5 Chemical remediation of the metal-contaminated Soil :

Remediation refers to processes or methods for treating contaminants in soil or water such that they are contained, removed, degraded, or rendered less harmful (Pierzinsk *et al.*, 2000). Use of chemical amendments is one approach for immobilization of the natural or added metals. Lime, FYM and phosphatic fertilizers have been used extensively for detoxification of the dreaded heavy metals in soil plant system. A brief review on each is presented below:

2.5.1 Lime as an amendment

Lime has been used for centuries to increase pH and thus decrease metal uptake by crops (Knox *et al.* 2001). Repeated application (every 2-5 years 2-10 t ha^{-1}) is necessary to maintain metal immobilization and therefore large quantities are necessary compared to other inorganic amendments (Knox *et al.,* 2001). Application of lime increase pH and thus decreases availability of metals. Lime was found to reduce the transfer of metals into crops.

Liming the soils offers a means of minimizing the risk of food chain contamination by reducing the uptake of heavy metals (Smith, 1994). Brallier *et al.* (1996) observed reduction in soluble and exchangeable Cd, Ni and Zn upon liming. Lime application also reduced the uptake of Cd, Ni and Zn in most of the crops but was without any change on Cu uptake. Application of alkaline biosolids to acidic soils was reported to be more effective in reduction of plant uptake of Cd, Cu, Ni and Zn (Brown and Bush, 1992). However, application of alkaline biosolids on non-acidic soils enhanced the Cu content in barley (Lu and Christie, 1998). According to Singh *et al.* (1989), even in alkaline soils, application of $CaCO_3$ reduced the amount of DTPA-extractable Cd as well as tissue Cd concentration of wheat. Kukier and Chaney (2000) observed that liming reduced Ni concentration by 80, 35-50 and 35% in redbeet, wheat and oat shoots respectively, and lleviated the symptoms specific to Ni phytotoxicity in oats (banded chlorosis). Studies conducted by Brallier *et al.* (1996) indicated that soluble and plant available soil fractions of Cd, Ni and Zn were significantly reduced by liming, with an equivalent amount of metal entering highly insoluble fractions which could only be removed by the strong extractants. Thus, several studies indicate that liming might not always have a significant effect; the effectiveness of liming could also vary depending on the soils, metals, pH values of limed soil and crop species (Hooda *et al.*, 1997).

The effectiveness of liming may be different for each metal. Singh and Narwal (1984) reported that lime application reduces the uptake of Zn and Ni more than that of Cd. Metal uptake in response to liming may also very among plant species. Reduced Cd accumulation induced by liming was more pronounced for lettuce and carrot as for potatoes and peanuts (Chaney *et al.,* 1987). Likewise, liming reduced Zn concentrations in soyabean seeds to a greater extent than in corn grains or cotton seeds.

Successful reports on liming are available for pot and for filed studies. In pot experiments, Brune *et al.* (1984) and Lombi *et al.* (2002) found a significant reduction of Cd and Zn concentrations Tlustos *et al.* (1995) observed a decrease of Cd and Zn concentration in spinach by 80% and 75% respectively. In the following the effect of liming in field studies is presented. Brallier *et al.* (1996) reported a study about the effect of liming on the availability of heavy metals in a soil amended with sewage sludge 16 year previously. The soil pH was adjusted from 4.6 to 5.8, 6.5 and 6.9. The most consistent trends in plant concentrations in this study were found for Cd, Ni and Zn. The concentrations of these elements in Cabbage and tomato fruit were significantly reduced at liming rates of 15 and 22 t/ha. (pH 6.5 and 6.9, respectively). Oliver *et al.* (1996) have observed that Cd concentration in wheat and barley grain can be decreased when pH increased from 4.0 to 5.0 by liming. Liming could reduce the Zn concentration in oat grains by 54% (Brune *et al*., 1984). Monicke *et al.* (1999) reported a reduction of Cd transfer by 50% caused by liming, however the effect was lower on highly contaminated soils compared to soils with lower Cd levels (*i.e.* 1.7 mg Cd/kg). Rietz *et al.* (1983) reported that lime application of 3 t/ha reduced the uptake of Cd in winter wheat by 33% in straw and 36% in grain. In their study, only a slight reduction of Zn was found and levels of Pb remained unchanged, which is probably because Pb accumubtion was mainly due to air pollution.

Adding to much lime to the soil can lead to the immobilization of essential nutrients and the mobilization of harmful anions (As, Mo) (Conyers 2002). Additionally, it should be noted that in some cases liming could not decrease the uptake of Cd due to the highly buffer capacity of the soil (Tiller *et al.,* 1997). Also in saline soils, the effect of the limestone application may be negligible (Mench 1998).Therefore soil characteristics have to be evaluated to estimate the effects of the lime application before addition to the soils.

2.5.2 Phosphates as an amendment

Mench *et al.* (1994) reported that the addition of thomas phosphate basic slag lowered the bioavailable lead content in the metal-contaminated soils. Phosphate and phosphate-containing minerals have been shown to reduce bioavailable Pb in soils. The phosphate rock (PR) also effectively immobilized Pb from both aqueous Pb solutions and Pb-contaminated soils (Ma *et al.*, 1995). However, the effectiveness of PR in immobilizing Pb from contaminated soils primarily depends on its ability to provide soluble P in soil solution. According to Ma and Rao (1998), even though the effectiveness of PR was determined by the soil pH and extent of contamination, phosphate rocks significantly immobilized Pb in contaminated soils with aqueous Pb reduction ranging from 21.8 to 100%. The primary mechanism of Pb immobilization in these systems was through the dissolution of Hydroxyapatite or phosphate rock and subsequent precipitation of a pyromorphite like mineral (Ma *et al.*, 1995).

Ruby *et al.* (1994) contended that lead was converted to insoluble lead phosphate in a metal-contaminated soil containing both phosphorus and lead, and formation of pyromorphite like mineral significantly reduced the Pb bioavailability. XiaoBing *et al.*, (1997) the apatite was able to reduce the metal (Zn, Pb) concentrations in the leachates to below US EPA maximum allowable levels, suggesting that apatite could be used as a cost-effective option for remediating metal-contaminated soils. The use of phosphate compounds (apatite or hydroxyapatite) for the immobilization of metals was in the focus of many studies. Knox *et al.* (2001) found that hydroxyapatite from North Carolina (25 and 50 g P/kg) effectively immobilized Pb, Zn and Cd. Chemical immobilization research using phosphate addition has included mineral apatite and synthetic hydroxyl apatite materials. These materials have proven to be effective at reducing the solubility and bioavailability of heavy metals through the formation of metal phosphate minerals. (Chen *et al.*, 1997; Ma *et al.*, 1995; Ma and Rao, 1997).

Soluble phosphate sources could provide an abundance of solution phosphorus and increase the efficiency of metal-phosphate mineral formation (Berti and Cunningham, 1997; Cooper *et al.*, 1998; Hettiarachchi *et al.*, 1997; Ma *et al.*, 1993), metal-phosphate minerals were shown to control metal solubility in soil suspensions when soluble phosphorus was added (Santillian-Medrano and Jurnak, 1975) and induced the formation of heavy metal phosphate precipitates (Cotter-Howells and Capron, 1996). Investigation of soluble phosphate fertilizers, monoammonium phosphate (MAP) and diaammonium phosphate (DAP) showed that MAP decreased and DAP increased the amount of Cd sorbed by the soil (Levi-Minzi and Petruzzelli, 1984). However Pierzynski and Schwab (1993) found that DAP increased Cd and Zn bioavailability to soyabean (*Glycine Max (L) merr.).*

2.5.3 Farmyard manure as an amendment

Metal-organic associations in both solution and solid phases by way of complexation and specific adsorption are the important meachanisms responsible for rendering the indigenous and applied metals less available for absorption by the plants (Karapanagiotis *et al.*, 1991). De Villarroel *et al.* (1993) demonstrated that phytoavailability of Cd and Zn in sewage sludge-treated soil was most likely to be affected by the kinetics of solid-phase metal dissolution. Addition of lime and FYM together in alkaline soils resulted in a significant decrease in the DTPA-extractable Cd in soil and Cd content in wheat (Singh *et al.*, 1989). However, individual application of FYM significantly increased the extractable Cd in soil and Cd content in plant. The predominant role of organic matter for Cu adsorption in soils has been reported by Sposito *et al.* (1982). Application of FYM is found to decrease the concentration of Zn, Cu and Ni in wheat grain and straw (Singh, 1994; Gupta *et al.*, 1989).

Sims and Kline (1991), during their speciation studies of biosolids amended soils, found most of Cu to be associated with organic fraction. During their investigation on the effect of organic matter on the adsorption of Zn and Cu, Borah *et al.* (1992) found that maximum decrease in adsorption capacities with removal of organic matter was obtained for Cu followed by Zn. However, Miller *et al.* (1986) clearly showed that the oxide fraction of soil Cu was nearly as large as organic fraction. Singh and Oste (2001) indicted that metal adsorption depended on the presence of clay and organic matter, and as a result the soils containing high amount of clay and organic matter showed the highest adsorption for heavy metals.

2.6 Solid – solution equilibria of heavy metals in soils

Bioavailability of metals in soil, to a large extent is governed by the solid-solution phase equilibria. The influence of various soil properties on metal retention was evaluated by

Hooda and Alloway (1998). Soil pH, $CaCO_3$ and organic matter contents had positive and significant correlations with metal retention. Their study also indicated that light textured soils with neutral to alkaline pH or with appreciable amounts of $CaCO_3$ (>10%) and organic matter would be the most suitable for disposal of Cd and Pb containing wastes,. Generally, metal retention capacity of soils is positively influenced by organic carbon content of soils (Mandal and Hazra, 1997). Like organic carbon, addition of lime also enhanced the metal adsorption capacity of soils (Singh and Oste, 2001).

Sloan *et al.* (1997) found that the sludge application increased the percentages of Ni and Zn in the exchangeable and specifically adsorbed fractions. They also observed that the biosolids-applied Ni had the greatest effect on the more easily extractable forms *i.e.* exchangeable and specifically adsorbed fractions of soil. McGrath and Cegarra (1992) reported that sludge application increased the percentage of Zn in an exchangeable form relative to unamended soils. In the sludge-amended soils, the relative bioavailability of metals followed the order. Cd>>Zn>Ni>Cu>>Cr>Pb. Zinc and nickel concentrations in lettuce were highly correlated to metal concentrations in the exchangeable and specifically adsorbed soil chemical fractions.

Leckie and James (1974) found that there was a shift toward high affinity adsorption with increased pH in heavy metal sorption. Kuo and Baker (1980) could obtain a direct correlation between soil pH and metal retention. Borah *et al.* (1992) observed that adsorption of trace metals in the soil resulted in release of protons. According to McBride (1994), electronegativity is an important factor in determining which of the trace metals chemisorb with the highest preference, and on this basis the predicted order of preference would be Cu>Ni>Co>Pb>Cd>Zn. But Gomes (2001) found that the metal cations having same valency did not exactly follow the order of electronegativity. They observed that the most frequently occurring heavy metal cation selectivity sequences were Pb>Cu>Cd>Zn>Ni and Cr>Cu>Cd>Ni>Zn. Harter (1983) reported that the amounts of Pb, Cu, Zn or Ni that could be retained by any soil was strongly influenced by the soil pH, but the response did vary widely with soil and metal.

2.7 Extraction of metals from contaminated soils.

Chemical extraction procedures have been widely used to predict availability of metals to plants. Different chemicals can remove metal associated with different fractions of the soil. Calcium chloride presumably extracts metal found in soluble and exhchangeable fractions of soil (McLaren and Crawford 1973, Iyengar *et al.*, 1981). DTPA is capable of extracting the exchangeable and organically bound trace metals (O'Connor, 1988) and also dissolves metal precipitates (Schalscha *et al.*, 1982).

Numerious studies have demonstrated that the effectiveness of an extractant to correlate with plant uptake is actually dependent upon the metal and is also specific to plant species (Taylor *et al.*, 1992). Chemical extractants such as dilute acids, chelating agents, and salts have been successfully employed for predicting uptake of metals by plants (Tambasco *et al.*, 2000). Hooda and Alloway (1993) found that the concentrations of Cd an Pb extracted by DTPA from contaminated soils failed to predict the changes in metal uptake by ryegrass. Hooda *et al.*, (1997) compared the concentrations of trace metals in the edible parts of carrots, wheat and spinach with the concentrations of the metals extracted from soil with four different chemical reagents such as 0.005 *M* DTPA, 0.05 *M* EDTA $(Na)_2$, *1M* NH_4NO_3 and 0.05 *M* $CaCl_2$. The results showed that 0.05 *M* EDTA was a reliable test for predicting metal availability to carrot, spinach and wheat grown on the sludge amended soils. But, in general, the ability of the extractants to predict the plant available metals varied with crop species, the nature and amount of metal and the extractant used.

Pitchel and Salt (1998) correlated the heavy metals extracted by five different chemical extractants, *viz.* Mehlich 1, 0.1 *M* HCI, 0.005 *M* DTPA, 0.005 *M* EDTA and 0.005 *M* NTA (nitrilo triacetic acid) with plant tissue accumulation. Mehlich 1 and HCl extractable-Zn poorly reflected Zn concentration in plants. Of all the extractants they tried, DTPA appeared to predict the increased uptake of Zn from metal contaminated soil. None of the extractants reflected the uptake of Ni by the plants. They could not fit any acceptable linear parametric models correlating Cu, Zn or Ni in plant tissue with extractable metals.

Misra and Pande (1974) reported a significant association between Ni in the sorghum tissue and that extracted by 0.1 *M* HCl in calcareous soils. Cooper concentrations in lettuce were best predicted by DTPA, HCl or H_2O extractable Cu, where as total HCl extractable and free Zn^{2+} were the best predictors of lettuce Zn concentrations. Also, they observed that some of the individual extraction procedures were good predictors of Cu and Zn but none was satisfactory in predicting metal concentration in lettuce. However, some of these promising extractants are yet to be evaluated for their suitability to predict the bioavailability of metals in contaminated soils amended with lime, manure and phosphates.

2.8 Phytoremediation of heavy metals polluted soil

Phytoremediation is define as the use of green plants to remove pollutants from the environment or to render them harmless. The basic idea that plants can be used for environmental remediation is very old and can not be traced to any particular source.

However, a series of fascinating scientific discoveries combined with an interdisciplinary research approach have allowed the development of this idea into a promising, cost-effective and environmental friendly technology. Phytoremediation can be practiced for both organic and inorganic pollutants, present in solid substrates (*e.g.* soil), liquid substrates (*e.g.* water) and the air. Phyto-remediation is currently divided into the following areas.

- Phytoextraction: the use of pollutant-accumulating plants to remove metals or organics from soil by concentrating them in the harvestable parts;
- Phytodegradation: the use of plants and associated microorganisms to degrade organic pollutants;
- Rhizofiltration: the use of plant roots to absorb and absorb pollutants, mainly metals, from water and aqueous water streams.
- Phytostabilization: the use of plants to reduce the bioavailability of pollutants in the environment;
- Phytovolatilization: the use of plants to volatilize pollutants;

(Salt *et al.*, 1998)

The use of specially selected and engineered metal accumulating plants for environmental clean-up is an emerging technology called phytoremediation (Salt *et al.*, 1995). It is a process in which plant exclude the metals, extract and store them in their body or convert them into volatile form that can be released into air. The primary objective of phytoremediation is to maximize the transfer of contaminants to the plant shoots so that the greater amount of these are removed by each cropping.

- **Hyper-accumulator-use of different plant species**

Phytoremediation is an environment friendly technology that is still in its infancy. However, the recent development of methods to enhance Pb accumulation by high biomass crop plants (Blaylock *et al.,* 1997. Huang *et al.*, 1997; Smith *et al.*, 1999) suggests that phytoremediation of metal contaminated soil will soon be a viable alternative to most conventional cleanup technologies. Initial phytoremediation research suggested that this could be achieved with hyper-accumulator plant species such as *Thlaspi caerulescens* having higher accumulation of Zn and Cd (Baker *et al*., 1994, Brown *el al*., 1994, 1995, Escarre *et al* ., 2000) Some researchers have suggested that the small size and slow growth of this species may limit its utility for phytoremediation (Black *et al*., 1995, Brown *et al*., 1995) Recent evidence suggests that moderate accumulating high biomass species such as Indian mustard (*Brassica juncea*) may accumulate four times more Zn than *T. caerulescens* (kumar *et al*., 1995; Salt *et al*., 1995; Ebbs *et al*., 1997). This was primarily due to the fact that *B. juncea* produced 10 times more biomass that *T. caerulescens*. Indian mustard can grow normally in soil contaminated with 250 mg Cu/kg 500 mg Pb /kg or 500 mg Zn/kg soil (Jiang *et al*., 2000). Indian mustard (*B. juncea*), for example, has been shown to reduce soil Se concentration to non-toxic levels (Banuelos and Meek, 1990; Banuelos *et al*., 1993, 1997). Other *Brassica sp*., namely *B. napus*. and *B. rapa* have shown a similar tendency to accumulate moderate levels of heavy metals. It was reported that the above *Brassica sp.* was more effective at removing Zn from nutrient solution that Cu (Ebbs *et al*., I997). Rape, kenaf and tall fescue reduced the total soil Se between (pre-plant and the final harvest by 47%, 23% and 2l%, respectively (*Banuelos et al*. 1997). In a hydroponic experiment it was observed that Indian mustard (*B. juncea*) was more tolerant to Cd than rape (*B. rapa*) and *B. napus* (Ebbs and Kochian, 1997). Among the thirty six plant species examined to select higher accumulation of Cr, it was reported that Indian mustard (*B. juncea* cv. 426308) and sun□ower (*Helianthus annus L.*) accumulated more Cr than other plant species (Shahandeh and Hossner, 2000). Indian mustard had a greater capacity for uptake of Pb, Zn and Cd than *Brassica carinata* (Rio *et al*.. 2000). There is also evidence that grass species such as corn. barley and rye grass have varieties that display significant heavy metal tolerance (Baker, l987). Several of these grass species also produce high biomass. The possibility thus exists that such species may be as effective as *B.*

juncea in phytoextracting heavy metals. There are many reports on the hyper accumulating potential of different species of plants. The reports have been compiled in Table 1.

Ebbs *et al*. (l997) could conclude from their experiment that in case of Zn extraction, *Brassica juncea* is better when compared to *B. nigm*, *B. campestris* and *B. carinata* in nutrient culture. The pot study conducted as a part of the same experiment could indicate that *Brassica sp*. were more effective in removing Zn from soil than *Thlaspi caerulescens* but Cd removal was comparable for both.

- **Content and uptake of heavy metals in Hyper-accumulators**

Hyper-accumulator species are defined as those whose leaves contain >100 mg Cd kg^{-1}, 1000mg Ni and Cu kg^{-1}, or >10,000 mg Zn or Mn kg^{-1} (dry weight) when gown in metal rich soils (Baker and Brooks.l989; Baker *et al.,* 1994). Hyper-accumulator species also accumulate metals when grown in conventional potting media (Reeves and Brooks. 1983). Possibly, hyper-accumulator plants have a higher requirement for metals such as Zn. Which are essential micronutrients, and show positive response to increased soil or solution concentrations of these elements (Hajar. 1987).

Chaney (1983) reported that some ecotypes of *Thlaspi caerulescens* can tolerate as much as 40,000 ppm Zn per dry weight in the shoots. *Brassica-juncea* was reported to be a superior hyper-accumulator to *Thlaspi caerulescens* it produces at least 10 times more biomass than *T. Caerulescens.* Under filed conditions (Salt *et al,* 1995). *Thlaspi caerulescens* can accumulate Zn up to 4 percent in leaf dry matter (Tolra *et al.,*. 1996) and up to 3 per cent in shoot (Brown *et al.,* 1994)

Ebbs and Kochain (1997) could notice that the shoot Cr concentration in the hyper-accumulator *Brassica juncea* was more than three times greater than the mean Cu concentration in the shoots of the control plants. In a nutrient culture experiment performed by Begonia *et al.,* (1998), the application of 100, 250, 500 μg Pb/ml to the growth medium resulted in the shoot uptake of 72, 149 and 258 μg Pb/g dry mass of *Brassica juncea,* respectively. Ebbs *et al.,* (1997) reported a Zn content between 500 and 600 mg/kg dry weight for all three species of Brassica (*B.juncea. B. rapa and B. napus*) studied under their experiment. Begonia *et al.,* (1998) reported that Indian mustard (*B. juncea*) plants grown in soil contaminated with 100, 250, 500 μg/mg Pb showed Pb uptake 494, 1482 and 2675 μg Pb/g dry biomass, respectively. When averaged across treatments, accumulation of Pb in roots was almost ten times more than in the shoots.

Recently, Brewer *et al.,*(1999) produced several somatic hybrids between *T. caerulescens* and the high biomass crop oilseed rape (*Brassica napus*). These hybrids produced a large biomass than *T. caerulescens* and had an erect growth habit that is suitable for mechanical harvesting. The hybrids were able to accumulate and tolerate Zn and Cd at levels that are toxic to *B. napus*, although their ability to accumulate metals appeared to be lower than *T. Caerulescens.*

Certain well known hyper-accumulators of Ni are in the genus Alyssum (*Brassicaceae)* (Baker *et al.2000,*) although the most remarkable example is perhaps *Sebertia accuminata* (Sapotaceae), a new Caledonian tree than can be grown to a hight of about 10m. A mature tree of *Sebertia accuminata* was estimated to contain 37 kg Ni (Sagner *et al.*, 1998). The other species that has recently received attention is *Berkheya coddil,* which can accumulate Ni to more than 1 per cent of its weight and is tall, fast-growing (Morrey *et al.*, 1989).

- **Changes in mental concentration in soil**

Hyper accumulator species have the capacity to take up heavy metals in excess, thus reducing the heavy metal concentration in soil. Brown *et al.*, (1994) reported reduced concentration of water-extractable Zn in pots containing *T. caerulescens* (7.2 mg kg^{-1}) as compared to blader compion (10.7 mg kg^{-1}) and tomato (9.3 mg kg^{-1}) . McGrath *et al.* (1997) reported that the concentrations of mobile Zn in both rhizosphere and non-rhizosphere soils decreased compared with the initial values before *T. caerulescens* and *T.Ocheoleucum* were planted. Lombi *et al.* (2001) have reported that the concentration of Cd, Ni and Zn in the pore waters from UK soil and of Cd from French soil appeared to increase with cropping of *T.caerulescens.* Banuelos *et al.* (1997) have reported a decrease in total selenium in an experiment using *B. napus* and *Hibicus cannabinus.* They could note a successive decrease in total Se from pre-plant to subsequent harvests. The extractable Se was found to follow a reverse terend in the rhizospheres of both the plants. Zhao *et al.* (2001) have studied the ability of root exudates from *Thlaspi caerulescens, B. napus* and wheat to mobilize heavy metals from a soil or from a metal loaded resin. The mobilizing ability was significantly higher in wheat rhizosphere over others. In a comparison study by Salt *et al.* (2000), non-hyper accumulator was found to produce large amounts of histidine thus reducing Ni uptake and toxicity over a hyper accumulator species.

- **Mechanisms involved in metal detoxification**

Hyper-accumulation is an eco-physiological adaptation to metalliferous soils. The mechanisms of metal accumulation, which involve extra cellular and intracellular metal chelation, precipitation, compartmentalization and translocation in vascular system, are poorly understood (Raskin *et al.*, 1994). Phytochelatins like low molecular weight gramm-

Glu-Cyspeptides with high affinity for certain metals are assumed to be involved in accumulation, detoxification and metabolism of mental ions such as Cd, Zu, Cu, Pb, Hg in plant cells (Maywald and Weigel. 1997). Two plant-based biotechnologies have been recently developed which take advantage of the ability of plant roots to absorb or secrete various substances (Gleba *et al.,* 1999). Among the organic acids. Citric acid was most effective in enhancing uranium accumulation in Indian mustard (Huang *et al.*,1998). Plants can provide carbon substrates and nutrients, as well as increase solubility of contaminants. These biochemical mechanisms increase the degradative capacity of plants, or Induce the phytotoxicity of the contaminated soil (Siciliano and Germida. 1998). Roots of *T. Caerulescens* stored virtually all of their Cd in the wall fraction for the first 7 to l0 days. It is suggested that this delay in transmembrane uptake may represent an important defensive strategy against Cd-poisoning allowing time for activation of intracellular mechanisms for heavy metal detoxification (Nedelkoska and Doran, 2000). Lyble *et al.* (1998) found that *Eichhomia crasspes,* water hyacinth have the capacity to reduce Cr (VI) to Cr (III) thus making possible *in situ* detoxification.

Siciliano Germida (1998) studied plant-bacteria interactions that increase the degradation of hazardous organic compounds in soil. They reported that plants and bacteria can form both specific and non specific associations in which the soil microbial community is stimulated with specific plant derived carbon sources and normal plant processes, respectively. These biochemical mechanisms could increase the degradative activity of bacteria associated with plant roots and in return the bacteria augment the degradative capacity of plants, or reduce the phytotoxicity of the contaminated soil. In recent year, knowledge of the physiological and molecular mechanisms of phytoremediation have begun to emerge, together with biological and engineering strategies designed to optimize and improve phytoremediation (Salt *et al.*, 1998).

- **Phytoremediation studies in India**

There is lack of scientific studies on phytoremediation of heavy metal contaminated soils in India. The brassica sp. Which is widely cultivated over Indo-Gangetic plants is found to have hyper accumulating capacity. The stage specific accumulation of heavy metals in plants also seems to be an area which needs further exploration. Brassica being the widely grown crop of North India, can be effectively used for remediating the soil with no change in the agriculture systems followed by farmers. For the effective adoption of this, the accumulating potential of different species of Brassica should be studied in detail. However the acceptance of phytoremediation is not going to be immediate. The concerned stakeholders are to be convinced about the potential danger associated with continuous loading of heavy metals and about the potential use of this green technology in remediating them.

- **Prospects and potentials of the frontier technology of phytoremediation**

Recent research and development activities have resulted in the development of novel technologies as saviours of mankind. Many more laboratories are joining this area of research of phytoremediation and many agriculturally important species still await the discovery of its hyper accumulating potential so as to tackle the issue of environmental clean up. Phytoremediation is cost effective and can remediate a site without dramatically disturbing the landscape. It can fulfill the need of physical removal of heavy metals from the contaminated site, which is impossible through other alternatives. The advantages of this technology over other approaches of decontamination are

(i) Aesthetically pleasing and accepted by the public

(ii) Relatively low cost

(iii) Less disruptive of the remediation site

(iv) Creates a beneficial habitat for wild life even

(v) Plant roots and shoot can take up heavy metals thus effecting the physical removal of toxicity

(vi) No accumulation in the edible parts of hyper accumulation thus making the position of consumer safe

Results of the experiment conducted by Chen *et al.* (2000) showed that treatments with CC, SS and FS decreased Cd uptake by wetland rice, Chinese cabbage and wheat by 23-95% compared with the unamended control. The phytoremediation of heavy metal contaminated soil with vetiver grass was also studied in a field plot experiment in China. The concentrations of Zn, Pb and Cd in the shoots of vetiver grass were 42-67, 500-1200 and 120-260% higher in contaminated plots than in control, respectively, Cadmium accumulation by vetiver shoots was 218 q Cd/ha at a soil Cd concentration of 0.33 mg Cd/kg. thus from the experiment, Chen *et al.* (2000) could estrablish the superiority of phytoremediation over the conventional chemical methods of decontamination. It is suggested that heavy metal contaminated soil could be remediated with a combination of chemical treatments and plants.

Although phytoremediation is time consuming and may require several years before contaminant concentrations are significantly lowered, the vegetation technology can reduce the total contaminant concentration with minimum cost. The scope of this technology can extended to phytomining to extract metals from soils or ores that are subeconomic for conventional mining (Brooks *et al.,* 1999). Improvements such as microcomputer software for design and implementation of phytoremediation have revolutionized the green cure technology (Fleisher *et al.,* 1997). Moreover, it is a fact that many of the remediation techniques currently in use will lose economic favour and public acceptance in the near future. Therefore, new technologies like phytoremediation based on ecofriendly and low cost processes will be a need of the biosphere, for ecosystem sustainability. Now we are in an era wherein plants ranging from pennycress to poplar trees are proving their worth as clean up tools. Thus it is clear that utilization of the remarkable potential of green plants to accumulate elements and compound from the environment is going to serve as the green cure technology for the agro-ecosystem in future

2.9 Phytoremediation case studies

- **Phytoextraction**

This technology involves the extraction of metals by plant roots and the translocation thereof to shoots. The roots and shoots are subsequently harvested to remove the contaminants from the soil. Salt *et al.* (1995a) reported that the costs involved in phytoextraction would be more than ten times less per hectare compared to conventional soil remediation techniques. Phytoextraction also has environmental benefits because it is considered a low impact technology. Furthermore, during the phytoextraction procedure, plants cover the soil and erosion and leaching will thus be reduced with successive cropping and harvesting, the levels of contaminants in the soil can be reduced (Vandenhove *et al.,* 2001). Researchers at the University of Florida have discovered the ability of the Chinese brake fern, P. vittata to hyperaccumulate arsenic. In a field test, the ferns were planted at a wood preserving site containing soil contaminated with from 18.8 to 1,603 parts per million arsenic, and the accumulated from 3,280 to 4,980 parts per million arsenic in their tissues (Ma *et al.*,2001). Sunflower, *H,annus* have proven effective in the remediation of radionuclides and certain other heavy metals. The flowers were planted as a demonstration of phytoremediation in a pond contaminated with radioactive cesium 137 and strontium- 90 as a result of the Chernoby1 nuclear disaster in the Ukraine. The concentration of radionuclides in the water decreased by 90% in a two-week period. According to the demonstration, the radionuclide concentration in the roots was 8000 times than that in the water. In a demonstration study performed by Phytotech for the Department of Energy, *H annus* reduced the uranium concentration at the site from 350 parts per billion to 5 parts per billion, achieving a 95% reduction in 24 h (Schnoor, 1997).

- **Phytostabilization**

Phytostabilization, also referred to as inplace inactivation, is primarily used for the remediation of soil, sediment, and sludge (United States Protection Agency, 2000). It is the use of plant roots to limit contaminant mobility and bioavailability in the soil. The plants primary purposes are to (1) decrease the amount of water percolating through the soil matrix, which may result in the formation of a hazardous leachate, (2) act as a barrier to prevent direct contact with the contaminated soil and (3) prevent soil erosion and the distribution of toxic metal to other areas (Raskin and Ensley, 2000). Phytostabilization can occur through the sorption, precipitation, complexation or metal valence reduction. It is useful for the treatment of lead (Pb) as well as arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu) and zinc (Zn). Some of the advantages associated with this technology are that the disposal of hazardous material/biomass is not required (United States Protection Agency, 2000) and it is very effective when rapid immobilization is needed to preserve ground and surface waters. The presence of plants also reduces soil erosion and decrease 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. The experiment on phytostabilization by Jadia and Fulekar (2008) was conducted in a greenhouse, using sorghum (fibrous root grass) to remediate soil contaminated by heavy metals and the developed vermicompost was amended in contaminated soil as a natural fertilizer. They reported that growth was adversely affected by heavy metals at the higher concentration of 40 and 50 ppm, while lower concentrations (5 to 20 ppm) stimulated shoot growth and increased plant biomass. Further, heavy metals were efficiently taken up mainly by roots of sorghum plant at all the evaluated concentrations of 5, 10, 20, 40 and 50 ppm. The order of uptake of heavy metals was: Zn>Cu>Cd>Ni>Pb. The large surface area of fibrous roots of sorghum and intensive penetration of roots into the soil reduces leaching *via* stabilization of soil and capable of immobilizing and concentrating heavy metals in the roots.

- **Rhizofiltration**

Rhizofiltration is primarily used to remediate extracted groundwater, surface water and wastewater with low contaminant concentrations (Ensley, 2000). It is defined as the use of plants, both terrestrial and aquatic, to absorb, concentrate and precipitate contaminants from polluted aqueous sources in their roots. Rhizofiltration can be used for Pb, Cd, Cu, Ni, Zn and Cr, which are primarily retained within the roots (United States Protection Agency, 2000). Sunflower, Indian mustard, tobacco, rye, spinach and corn have been studied for their ability to remove Pb from water with sunflower having the greatest ability. Indian mustard has a bioaccumulation coefficient of 563 for lead and has also proven to be effective in removing a wide concentration range of lead (4mg/L -500 mg/L) (Raskin and Ensley, 2000; United States Protection Agency, 2000). The advantages associated with rhizofiltration are the ability to use both terrestrial and aquatic plants for either in situ or ex situ applications. Another advantage is that contaminants do not have to be translocated to the shoots. Thus species other then hyperaccumulators may be used. Terrestrial plants are preferred because they have a fibrous and much longer root system, increasing the amount of root area (Raskin and Ensley, 2000). Sunflower (*Asteracaea spp.*) have successfully been implemented for rhizfiltration at Chernobyl to remediate uranium contamination. Dushendov *et al.* (1995) observed that root of many hydroponivally grown terrestrial plants such as Indian Mustard (*B.juncea (L.) Czem*) and sunflower (*H. annus L.*) effectively removed the potentially toxic metals, Cu, Cd, Cr, Ni, Pb and Zn, from aqueous solutions.

An experiment on rhizofilteration by Karkhanis *et al.* (2005) was conducted in a greenhouse, using pistia, duckweed and water hyacinth (*Eichornia crassipes*) to remediate aquatic environment contaminated by coal ash containing heavy metals. Rhizofiltration of coal ash starting from 0, 5, 10, 20, 30, 40 % Simultaneously the physicochemical parameters of leachate have been analyzed and studied to understand the leachability. The results showed that pistia has high potential capacity of uptake of the heavy metals (Zn, Cr, and Cu) and duckweed also showed good potential for uptake of these metals next to pistia.

- **Phytovolatilization**

Phytovolatilization involves the use of plants to take up contaminants from the soil, transforming them into volatile forms and transpiring them into the atmosphere (United States Protection Agency, 2000). Mercuric mercury is the primary metal contaminant that this process has been used for. The advantage of this method is that contaminant, mercuric ion, may be transformed into a less toxic substance (that is, elemental Hg). The disadvantage to this is that the mercury released into the atmosphere is likely to be recycled by precipitation and then redeposited back into lakes and oceans, repeating the production of methyl-mercury by anaerobic bacteria. In laboratory experiments, tobacco (N.tabacum) and a small model plant (Arabidopsis thaliana) that had been genetically modified to include a gene for mercuric reductase coverted ionic mercury [Hg(II)] to the less toxic metallic mercury (Hg(0)). And volatilized it (Meagher *et al.*, 2000). Similarly transformed yellow poplar (*Liriodendron tulipifera*) plantlets had resistance to, grew well in, normally toxic concentration to, ionic mercury. The transformed plantlets volatilized about ten times more elemental mercury then did untransformed plantlets (Rough *et al.,* 1998). Indian mustard and canola (*Brassica napus*) may be effective for phytovolatilization of selenium and in addition accumulate the selenium (Banuelos *et al.,* 1997).

- **Plant-metal uptake**

Plants extract and accumulate metals from soil solution. Before the metal can move from the soil solution into the plant, it must pass the surface of the root, this can either be a passive process, with metal ions moving through the porous cell wall of the root cells or an active process by which metal ions move symplastically through the cells of the root. This letter process requires that the metal ions traverse the plasma lemma, a selectively permeable barrier that surrounds cells (Pilon-Smits, 2005). Special plant membrane proteins recognize the chemical structure of essential metals; these proteins bind the metals and are then ready for uptake and transport. Numerous protein transporters exist in plants. For example, the model plant thale cress (*A. thaliana*) contains 150 different cation transporters (Axelsen and

Palmgren,2001) and even more than one transporter for some metals (Howkesford, 2003). Some of the essential, nonessential and toxic metals however are analogous in chemical structure so that these proteins regard them as the same. For example arsenate is taken up by P transporters. Abedin *et at.* (2002) studied the uptake kinetics of as species, arsenite and arsenate, in rice plants and found that arsenate uptake was strongly suppressed in the presence of arsenite, Clarkson and luttge (1989) reported that Cu and Zn, Ni and Cd compete for the same membrane carriers. For root to shoot transport these elements are transported *via* the vascular system to the above-soil biomass (shoots). The shoots are harvested, incinerated to reduce volume disposed of as hazardous waste or precious metals can be recycled (phytomining). Different chelators may be involved in the translocation of metal cations through the xylem, such as organic acid chelators (malate, citrate, histidine (Salt *et al.*, 1995b;vonWiren *et al.*1999). or nicotianamine (Stephen *et al*1996; von Wiren *et al.*,1999) Since the metal is complexed within a chelate it can be translocated upwards in the xylem without being absorbed by the high cation exchange capacity of the xylem (von Wiren *et al.*,1999).