1. INTRODUTION

Pollution of the environment with toxic metals has increased dramatically since the onset of the industrial revolution. Because they are rich sources of plant nutrients, sewage effluents and sludge are commonly used (often untreated) by the farmers for irrigating soils around industrial units and metropolitan cities of India at the cost of heavy metal contamination. However, these sewage effluents carry appreciable amounts of trace toxic metals (Brar et al., 2000; Pescod, 1992; Yadav et al., 2002). Recently, Rattan et al., (2005) reported a significant build up of Zn, Cu, Fe, Ni and Pb in sewage-irrigated soils, as well as an accumulation of heavy metals in vegetable and field crops grown on such soil. Excessive metal concentration in contaminated soils might result in decreased soil microbial activity and soil fertility (over soil quality), yield loss (McGrath, Chaudri, and Giller, 1995) and possible contamination of the food chain (Hann and Lubbers, 1983). Although the cleanup of contaminated sites is necessary, often the application of environmental remediation strategies is very expensive and intrusive (McGrath et al., 1995). Thus, development of a low –cost and environmentally friendly strategy is needed. Recently, the value of metal-accumulating plants for environmental cleanup has been vigorously pursued (Brown et al., 1995; Salt et al., 1995), giving birth to the philosophy of “phytoextraction” within a broader concept of phytoremediation (Kumar et al., 1995). The alarmingly increasing urbanization and industrialization countrywide is generating enormous amount of inorganic and organic wastes posing to serious problem of safe disposal. Nearly 450 cities in India generate around 1200 tonnes of sewage sludge every day, although there exsits a potential to produce 4000 tonnes of sludge per day (Kaul *et al.*, 1989). Sewage sludge contains variable amounts of heavy metals like Pb, Cr, Ni, Cd *etc.* as well as essential plant nutrients like N, P, K, S and Zn *etc.* sewage sludge is generally disposed off or applied in agricultural lands as a source of plant nutrients. Long term application of sewage sludge has been reported to elevate concentrations of heavy metal in soil under peri-urban agriculture around Delhi (Bansal *et al.*, 1992), Calcutta (Adhikari *et al.*, 1993) and Ludhiana (Arora and Chhibba *et al.*, 1992). These metals once mixed into agricultural soils, do not leach appreciably and get accumulated in surface plough layer by adsorption or precipitation phenomenon (Leeper, 1978; Sakal *et al.*, 1992). Thus, a limiting factor in the long term and indiscriminate use of sewage sludge on agricultural lands is the likelihood of excessive accumulation of heavy metals such as Zn, Cd, Pb, Cr, and Ni, in the soil and resultant Phytotoxicity.

High contents of heavy metals may render soils unproductive because of metal toxicity if total metal concentrations exceed 100 to 400 µg kg^{-1} Pb (Kabatapendias, 1984) and 5 mg kg^{-1} Cr (Turner and Rust, 1971). Nickel concentration as low as 2 ppm in nutrient solution has been reported to reduce plant growth (Bradshaw and Mc Nielly, 1981). Chronic exposure even at low concentration of non-essential metals such as Pb, Cr, Ni *etc.* in the environment can prove to be harmful to human health. Amongst the heavy metals chromium is considered as one to the priority pollutant in United State by the U.S. Environment Protection Agency (USEPA, 1975). Following the addition of some contaminents soil, mineralization of organic biomass occurs through a combination of physical, chemical and biological processes operating in the soil. Heavy metals (Cr, Pb, Ni *etc*) present in the biomass get transformed and distributed into the forms of varying solubility and availability to plants. These are water soluble, exchangeable, carbonate-bound, organically bound, Fe and Mn Oxides and hydroxide-bound and residual forms. Only water soluble, exchangeable and partly carbonate-bound forms are considered phytoavailable. Other metal fractions are very slowly available or unavailable to plants during their growth and development period.

Estimating plant availability of micronutrients and heavy metals is an important objective in soil testing procedures. Such estimates are required in amended soil over different levels of metal enrichment. Single extraction of solid phase metals in soil using selective chemical extractants such as strong chelating agent or a mild neutral salt (Hooda *et al.*,1997) has been used to indicate the bioavailability or mobility of heavy metals. Chelating agents such as DTPA (Di-Ethylene tri amine penta acetic acid), EDTA (Ethylene Diamine tetra acetic acid) etc. neutral salts like $CaCl_2$ and dilute mineral acids like $0.1M$ HCl are the most commonly used reagents to assess bioavailable metals in soils (Pitchel and salt, 1998) . However, meager information is available on the usefulness of these chemical extractant in predicting the bioavailable of the metal in amended soils.

Several approaches are currently being used for the prevention, control and remediation of soil contaminated with toxic metals. These include, (a) land filling-excavation, transport and deposition of contaminated soil in a permitted land fill site (USEPA, 1991), (b) chemical immobilization of heavy metals by the application of ameliorants like lime, farm yard manure (FYM), phosphates, manganese oxides *etc.* (c) leaching- using acid solutions or complexing leachants (EDTA *etc.*) to desorb and leach metals from a part of soil drawn from the contminated area followed by the return of the soil residues to the site; (d) bioremediation – use of micro-organisms to degrade pollutants in site (since the heavy metals connot be chemically degraded, application of microbial remediation to the insite removal of heavy metals from the contaminated substrates is limited mainly to their immobilization by precipitation or reduction); and (e) Phytoremediation - use of specially selected and engineerd metal-accumulating plants for environment clean-up either by phytoextraction or by phytostabilization. Among these, soil excavation the only method for the total removal of heavy metals from contaminated soil. However, it is not recommended as a viable practice, since it is prohibitively expensive. Thus, chemical immobilization of heavy metals by the application of ameliorants (lime, phosphates, FYM *etc.*), use of complexing extractant/leachants (*e.g.* EDTA.) and phytoremediation appears to be the potential remedial measures to alleviate the heavy metal level in soil.

Metal concentration in crops is mostly not high enough to cause acute toxicity, but in the long run it may provoke chronic damage to health (Adriano, 2001). Due to the heavy metal burden in human nutrition, there is a need for measures to reduce the metal transfer into agricultural plants. In areas where conventional or other remediation technologies are either not feasible or too expensive, other simple but effective approaches may help to reduce the accumulation of heavy metals in the edible parts of crops.

Phosphate addition to contaminated soil has proven to be extremely effective for reducing metal solubility. Experiments involving treatment of metal contaminated soil with rock phosphates (apatite) have shown that formation of metal phosphate precipitates and minerals reduced heave metal solubility. Insoluble and geochemically stable lead pyromorphites such as hydroxypyromorphite [$Pb_5(PO_4)_3OH$] and chloropyromorphite [$Pb_5(PO_4)_3Cl$] have been found to control Pb solubility in apatite-amended contaminated soil (Zhang and Ryan, 1999).

In situ chemical immobilization is a remediation technique that involves addition of chemical to contaminated soil to reduce the solubility of metal through metal sorption and/ or precipitation. Decreased metal solubility and mobility will reduce heave metal transport or transfer from contaminated soils to surface and ground water and also to crop plants. *In situ* chemical immobilization is less expensive than excavation and land filling and may provide a long-term remediation solution through the formation of stable metal minerals and/or precipitates (Vangronsveld and Cunningham, 1998). Metal-phosphate minerals were shown to control metal solubility in soil suspension when soluble phosphorus was added (Santillian Medrano and Jurinak, 1975) and induced the formation of heavy metal phosphate precipitated (Cotter-Howells and Capron, 1996).

Diammonium phosphate and mineral apatite rock phosphate have proved to be effective in 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). Diammonium phosphate is a major water soluble source of P and provides an abundance of solution phosphorous and increases the efficiency of metal phosphate mineral formation (Berti and Cunningham, 1997, Cooper *et al.*, 1998).

The phytoremediation research in Indian is still in its infancy. Very few reports are available on phytoremediation in Indian sub-continent which are primarily confined only to cleaning up of heavy metal contaminated water bodies through aquatic plants (Chandra *et al.*,1997, Ali *et al.,* 1999). Moreover, the mechanisms of metal accumulation involve extracellular and intracellular metal chelation, precipitation, compartmentalization and translocation in the vascular system, which are often very poorly understood. Development of effective phytoremediation strategy is dependent on an understanding of the coupling of root processes in the rhizosphere zone of hyper-accumulator to microbial activity. Thus before phytoremediation strategies can be developed for commercial uses, the behavior of hyper-accumulator species must be more clearly understood. An understanding of the patterns of heavy metal uptake and the specificity of tolerance will enable growers to maximize metal pollutant concentrations in plant shoots.

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

Phytoremediation is the use of crop plants to absorb and remove metal contaminants from the soil. Some crop plants tend to concentrate a specific metal of the heavy metals and allow its removal and safe disposal at the time of harvest. The metal extractability and accumulating ability of crop species is also influenced by the addition or presence of soil amendments and complexing agents.

Phytoremediation of heavy metal contaminated soil is an emerging technology that aims to extract or inactivate merals in soils (Mc Grath, 1998; Salt *et al.*, 1998). Two approaches have been proposed for phytoextraction of heavy metals, namely continuous or natural phytoextraction and chemically enhanced phytoextraction (Salt *et al.*, 1998). The first is based on the use of hyper-accumulator plants with exceptional metal-acumulating capability. These plants have several beneficial characteristics such as the ability to accumulate metals in their shoots and an exceptionally high tolerance to heavy metals (Baker *et al.*, 2000). On the other hand, many hyper-accumulator plants tend to be slow growing and produce low biomass, with the exception of some Ni hyper accumulator species. With the planting materials currently available, years or decades are needed to planting materials currently available, years or decades are needed to clean up a contaminated site. Another problem with the continuous phytoextraction of metals from soils is related to the fact that some metals such as Pb are largely immobile in soil and their extraction rate is limited by solubility and diffusion to root surface.

Chemically enhanced phytoextraction has been developed to overcome these problems (Huang and Cunningham, 1996; Blaylock *et al.*, 1997; Huang *et al.*, 1997; Blaylock, 2000). This approach makes use of high biomass crops that are induced to take up large amounts of metals when their mobility in soil is enhanced by chemical treatments. Several chelating agents, such as citric acid, EDTA, CDTA, DTPA, EGTA, EDDHA and NTA, have been studied for their ability to mobilize metals and increase metal accumulation in different plant Species (Huang *et al.*, 1997; Cooper *et al.*, 1999). Though different metals such as Pb (Blaylock *et al.*, 1997; Huang *et al.*, 1997), U (Huang *et al.*, 1998), Cs^{137} (Lasat *et al.*, 1998), and Au (Anderson *et al.*, 1998) have been targeted, the most promising application of this technology at the moment is for thr remediation of Pb contaminated soils using Indian mustard [*Brasicca juncea* (L.) Czern] in combination with EDTA (*e.g.* Blaylock, 2000). Despite the success of this technology, some concern has been expressed regarding the enhanced mobility of metals in soil and their potential risk of leaching to ground water (Cooper *et al.*, 1999). However, no detailed studies regarding the persistence of metal- EDTA complexes in contaminated soils have been conducted.

Thlaspi caerulescen (Brown *et al.,* 1994) and Brassica juneca (Kumar *et al.,* 1995; Ebbs *et al.,* 1997) have been widely cited to be hyper-accumulators for several heavy metals (Zn, Pb, Cd, Se, Ni, Cu etc.). These have also been commercially used for the purpose of phytoremediation in developed contries mainly under temperate climate (Watanabe, 1997). Different members of Brassica family with special reference to Indian mustard have been reported to accumulate several heavy metals in its aboveground biomass and act as ideal hyper-accumulator in phytoremediation. However, various species of Brassica have not been explored in the light of their use in phytoremediation studies in Indian condition. These species being widely cultivated and adapted in Indo-Gangetic alluvial plain of India, have enormous promise to clean up the contaminated soils engaged in periurban agriculture around Delhi and in similar agro-ecological region.

A few measures are suggested for the prevention and control of heavy metal contamination in agricultural soils, which includes

i) Excavation of soil followed by soil washing and subsequent disposal of treated soils (USEPA, 1991).

ii) Use of plants which are hyper accumulators of metals like Indian grass (*Brassica juneca L*) for phytoremediation of polluted soil and

iii) Chemical immobilization of heavy metals by the application of ameliorants like lime, farm yard manure (FYM), phosphates, iron oxides, manganese oxides, zeolites etc. among these, soil excavation is the only method for the total removal of heavy metals from contaminated soil. However, it can not be recommended as a viable practice, since it is prohibitively expensive. The phytoremediation also takes a long period to remove a substantial quantity of heavy metals from contaminated soils. Thus, a logical and rational remediation process appears to be to render the metals immobile by using different amendments.

A common method for immobilization of metals in soil is to apply lime, phosphates or organic matter residues to the contaminated sits (Impens *et. al.,*1991). Addition of other natural or synthetic chemical additives , like zeolites, beringite, hydrous oxides of Al, Fe and Mn are also know to enhance metal immobility in soils (Gwarek,1992). However, the application of lime, phosphates and FYM is found to be a promising option for developing countries, as for as the economic constraint of marginal farmers is considered. Soil pH is reported to be an important factor affecting the mobility and bioavailability of the heavy metals in soil (Evans *et. al.* 1995). Hence, application of agricultural lime is appeared to be a universal treatment for reducing the metal uptake and alleviating the toxicity in different crop species (Kukier and chancy,2000). Phosphatic substances like basic slag and rock phosphate are also used as an effective amendment for decreasing the phytoavailabilty of heavy metals (Mench *et. Al.* 1994). Soil organic matter is widely discussed in contaminated soils (Chang *et. al.* 1997; Hyun *et. al.*1998).

Keeping in view the above consideration, the investigation entitled as “Remediation of Heavy metal contaminated soils using different amendment and different *species of Brassica*.” was undertaken with the following objectives.

Objectives:

1. To study the effect of lime, FYM and phosphate on *Zn, Cu* and *Ni* adsorption in soils.
2. To screen the possible hyper-accumulators (*Brassica Sp.*) for decontamination of heavy metal-polluted soils.
3. To study the influence of different hyper-accumulators (*Brassica Sp.*) on decontamination of heavy metal polluted soils.