INTRODUCTION
1. **Heavy metals in terrestrial and aquatic Ecosystems**

Eco-toxicologists and environmental scientists use the term “heavy metals” to refer to metals that have caused environmental problems. Metals are separated into the essentials and non-essentials in classes A and B, and in a borderline class (Nieboer and Richardson, 1980).

- **A:** Calcium (Ca), Magnesium (Mg), Manganese (Mn), Potassium (K), Sodium (Na), Strontium (Sr)
- **B:** Cadmium (Cd), Copper (Cu), Mercury (Hg), Silver (Ag) and Aluminium (Al)
- **Borderline:** Zinc (Zn), Lead (Pb), Iron (Fe), Chromium (Cr), Cobalt (Co), Nickel (Ni), Arsenic (As), Vanadium (V), Tin (Sn).

### 1.1.1. Heavy Metals in Aquatic Ecosystem

The chemical composition of sea water and freshwater influences to a great extent the speciation of heavy metals. In river water (which mostly is turbid) large proportion of metals is bound to organic and inorganic particulate matter. Other factors which influence speciation are: pH, hardness, and dissolved organic matter (Salomon’s and Forstner, 1984). Large amounts of dissolved organic complexes and particulate matter with heavy metals are transported great distances to end up in the sediments of the estuaries. Some metals, such as Cd, can be released from their organic complexes by increasing Cl (chlorine) concentrations, which form chloride complexes (Elbay-Poulichet *et al.*, 1987). Metal in the aquatic environment are bioaccumulated by organisms either passively from water or by facilitated uptake. Essential metals are maintained by binding to organic molecules at a variety of biochemical sites where they function mainly as catalysts to induce or enhance enzymatic activity (Regan, 1993). Essential metals at high concentrations can have sub lethal toxicity effects to some organisms or lethal consequences to others. Also, metals at deficient concentrations can have again adverse health effects. Thus essential metals can have a double “toxic” threshold (Rainbow, 2007). Metals can be sequestered through storage by metal binding proteins, such as metallothioneins, in cellular vesicles and granules. Some storage mechanisms can be related in some organisms of providing essential metals for future needs (Malins and Ostrander, 1993).
Aquatic plants often grow more vigorously where nutrient loading is high. They are capable of removing water soluble substances from solution and temporarily immobilize them within the system (HO, 1988; Untawale et al., 1980).

1.1.2. Heavy Metals in Terrestrial Ecosystem

There are numerous reports on metal pollution in marine, freshwater and terrestrial ecosystems in the last decades. Reports on metal pollution and effects on marine ecosystems have been published by United Nations Environment Programme (UNEP), GESAMP (Group of Experts on Scientific Aspects of Marine Environmental Pollution, Advisory body, established in 1969, sponsored by UN, FAO, UNESCO-IOC, WMO, IAEA, UNIDO) and environmental specialists (UNEP, 1983; GESAMP, 1990). The terrestrial environment is also an environmental department into which heavy metals are released. The metal content of soils may be strongly influenced by their origins. Soils derived from shales are often rich in Cd, whereas soils derived from serpentine rocks contain elevated concentrations of Co, Ni and Cr (Hopkin, 1989). Environmental pollution by heavy metals in the terrestrial environment include mining, ore smelting, combustion of fossil fuels and certain pesticides containing As and Cu (Hopkin, 2002).

If ecological harm is to be prevented, understanding the behaviour and effects of contaminants in the environment is important and is a central aim in ecotoxicology (Forbes and Kure 1997). The ability to predict this quantitatively is required to avoid harm. In the absence of an objective definition for terrestrial ecological quality (Clarinet, 2001), the assessment of ecological effects and harm is difficult to establish. There is a scarcity of published case study information for terrestrial ecosystems against which to assess the ecological meaning of ‘safe’ concentrations (Posthuma, 1997) and causal relationships between soil quality and their ecosystem functions still need to be demonstrated (Herrick, 2000). Contemporary risk assessment methods do not suggest or identify consequences to a particular ecosystem if exposure exceeds defined ‘safe’ contaminant thresholds, other than that population responses are observed in the field (Posthuma, 1997).
1.2. Consequences of effect of increased concentration of heavy metals on plants and animals

Heavy metals such as aluminum, Cd and Pb are non-essential elements for plants. If plentiful amount are accumulated in the plants, heavy metals will adversely affected the absorption and transport of essential elements, disturb the metabolism, and have an impact on growth and reproduction (Xu and Shi, 2000). The effects of heavy metals on plants are different on different growth stages of plants. In the early stage, Cd inhibits the photosynthesis and growth of rice, and then inhibits the reproductive organs (Wang, 1996). Heavy metals affect the cell division of plants, and the effects are different and depend on the concentration.

Aquatic organisms vary in their physiology so as to maintain internal metal concentrations. This is achieved by reducing uptake and enhanced excretion. Mussels, for example, can regulate internal metal levels more effectively than oysters, although both species have similar feeding preferences (Reidel, 1995). Decapod crustaceans are among the strongest tissue regulators of Cu, Mn and Ag (Bryan, 1968). Certain species, including fish, polychaete worms and bivalve mollusks are also able to regulate the concentrations of essential metals in their tissues (Bryan, 1991; Lewis, 1982). Recent studies found that metals can act as carcinogens through oxidative mechanisms, generating free radicals and reactive oxygen species (ROS), which attack and damage DNA and important enzymatic proteins. The toxicology and carcinogenicity of many heavy metals is another important environmental concern of the scientific community (Chang et al., 1996; Bal and Kasprzak, 2002).

1.3. Marine species in heavy metal toxicity tests

In the last decades after exhaustive testing and repeatability three new prototype tests were launched as Toxkits. The acute marine bioassays with the brine shrimp *Artemia salina* and the rotifer *Brachionus calyciflorus* and *Brachionus plicatilis* (Maltby, Callow, 1989; Van Steertegem and Persoone, 1993).

1.4. Ecological significance of heavy metal pollution

The main goal of toxicological and ecotoxicological studies is to ensure that heavy metal pollution from anthropogenic pollution do not give rise to adverse effects on living organisms. Ultimately, these studies must focus on measuring levels of
pollution that may induce irreversible ecological changes to aquatic ecosystems. Biomonitoring of heavy metals and effect studies on natural populations of organisms must take into account the pollution-induced community tolerance, which is expected from communities that are exposed to particular pollutants for a long time (several generations) (Blanck et al., 1988; Chapman et al., 1998).

Heavy metals accumulating in the food chain, pose risk for health of animals and humans, who are less sensitive to metal toxicity than plants, but can concentrate heavy metals in certain tissues and organs (Mantovi et al., 2003). Cytotoxicity of heavy metals in plants has been well documented (Delhaize and Ryan, 1995; Marienfeld et al., 2000). Cadmium (Cd) is a toxic heavy metal, causing phytotoxicity, and its uptake and accumulation in plants pose a potential threat to human health (Shah and Dubey, 1997). Its accumulation causes reductions in photosynthesis, diminishes water and nutrient uptake (Sanità di Toppi and Gabbrielli, 1999), and results in visible symptoms of injury in plants, such as chlorosis, growth inhibition, browning of root tips, and finally death (Kahle, 1993).

1.5. Heavy metal content in terrestrial and aquatic ecosystem the content and increase over the threshold levels

Aluminum occurs ubiquitously in natural waters as a result of the weathering of aluminum containing rocks and minerals. Of the known geochemical responses to environmental acidification, the best documented is the mobilization of aluminum from terrestrial to aquatic environments (Campbell et al., 1992). This mobilization of aluminum is often episodic in nature and is associated with pH depressions (acidification) occurring during the spring snowmelt (in temperate regions) or associated with erosion from specific storm events (Rosseland et al., 1990).

Heavy metals can create adverse effects on environmental and human health due to their toxicity and their bioaccumulation in various environmental compartments. A number of studies have been carried out to assess the behavior of these pollutants in the environment (e.g., review by Pacyna et al. 1993).

1.5.1. Terrestrial ecosystem

Background levels of heavy metals in the soil vary regionally, and are primarily affected by the geology of local bedrock. The anthropogenic contribution to
heavy metal contamination through long range atmospheric fallout (from precipitation and dry deposition) exceeds the natural component (from degassing of the earth’s crust) (Pacyna, 1994). In the 1950s and 1960s, the use of heavy metals in agricultural chemicals (the use of alkyl mercury fungicides as seed dressings in particular) resulted in intoxication of the terrestrial food chain. Industrial sources of contamination, so-called ‘hot spots,’ are generally localized; deposition rates are markedly elevated nearest the source and decrease rapidly with distance. Most heavy metals deposited in soil-water systems are rapidly bound by organic particulate matter. The availability of these metals to terrestrial microorganisms depends largely on the oxidation state, the organic content of the soil, and pH. In the Arctic, soil acidification due to acid rain is an important factor contributing to heavy metal bioaccumulation, as most metals are more readily accumulated as divalent cations, and acidification generally enhances the concentration of divalent cations in solution. Mercury behaves in the opposite way: decreasing pH enhances the absorption of Hg onto organic matter (Bergkvist 1986; Lodenius 1990). At pH 4.8 Bergholm et al. (1985) found the following binding strength sequence based on the solubility of metals: Fe > Al > Cu > Pb > Zn > Mn = Cd.

The increase in concentration of a given metal, measured in a certain reference material, such as crustal rocks or soils, in relation to a certain reference metal, such as Al, and Ti, can be defined as the enrichment factor of this metal (EF). Most often, metals are enriched on a local scale, but some are enriched on regional and global scales. Regional scale is often defined as continental (1000-2000 km), whereas global scale is usually regarded as intercontinental, e.g., Northern Hemisphere. Episodes of long-range transport of pollutants within air masses result in the enrichment of metal concentrations far from source regions; the Arctic is a receptor of such transport (Pacyna and Winchester 1990).

Wetlands can be seriously destabilised by deposition and accumulation of heavy metals in sediments. Metals may bioaccumulate in living organisms and be transferred into the food chain. Predators of contaminated organisms can be chronically or acutely intoxicated (Crommentuijn et al., 1995), and this may lead to drastic changes in species composition and biodiversity in contaminated areas. Based on the knowledge about metal transfer from the soil through the food chain, new and more relevant tolerance limits can be drafted for contaminated ecosystems and
vulnerable and tolerant links of the food chain can be identified (Butovsky and Van Straalen, 1995). Total soil metal concentrations indicate the degree of pollution, but do not provide information about bioavailability and toxicity with respect to specific biotic components. These are determined among other factors by the specific physicochemical form of the metals. Often sequential extraction procedures are applied to differentiate between reactive metal pools in an attempt to explain the bioavailability and the uptake of metals by organisms living in the affected soils or sediments (Ma and Rao, 1997). Many Flemish rivers and their sediments are more or less contaminated with heavy metals. Total metal contents and fractionation patterns in the sediments of these ecosystems could be determined and related to metal transfer in wolf spider. Wolf spider is a frequently occurring soil dwelling predator on saltings and in turn is a source of food for many vertebrates such as birds, mammals and amphibians. As bio-accumulators they can play an important role in the transfer of heavy metals into the food chain.

1.5.2. Aquatic ecosystems

A complete understanding of chemical speciation is essential for gaining a comprehensive understanding of the chemical status of aquatic ecosystems. This is in turn is essential for evaluating the risk to the health of the ecosystems and individuals within them as a result of exposure to metals, and for being able to predict how changes in environmental parameters will influence bioavailability, bioaccumulation, and the toxic effects of metals.

Studies on the total ecosystem effects involving not only macrophytes but also sediment and other biota are necessary to provide a complete picture of effect of heavy metals on aquatic ecosystem. Significant differences obtained for the heavy metal concentration between macrophyte species suggest that the interaction and behavior of heavy metals with macrophytes are different for each species (Prasad and Frietas, 2003).

1.6. Strategies for decontaminating the polluted sites

Due to their immutable nature, metals are a group of pollutants of much concern. As a result of human activities such as mining and smelting of metalliferous ores, electroplating, gas exhaust, energy and fuel production, fertilizer and pesticide
application, etc., metal pollution has become one of the most serious environmental problems today. Phytoremediation, an emerging cost-effective, non-intrusive, and aesthetically pleasing technology, that uses the remarkable ability of plants to concentrate elements and compounds from the environment and to metabolize various molecules in their tissues, appears very promising for the removal of pollutants from the environment. Within this field of phytoremediation, the utilization of plants to transport and concentrate metals from the soil into the harvestable parts of roots and above-ground shoots, i.e., phytoextraction may be, at present, approaching commercialization. Improvement of the capacity of plants to tolerate and accumulate metals by genetic engineering should open up new possibilities for phytoremediation. The lack of understanding pertaining to metal uptake and translocation mechanisms, enhancement amendments, and external effects of phytoremediation is hindering its full scale application. Due to its great potential as a viable alternative to traditional contaminated land remediation methods, phytoremediation is currently an exciting area of active research (Alkorta et al., 2004).

1.7. Importance of phytoremediation as a viable strategy for reclaiming polluted sites

Phytoremediation, also referred as botanical bioremediation (Chaney et al., 1997), involves the use of green plants to decontaminate soils, water and air. It is an emerging technology that can be applied to both organic and inorganic pollutants present in the soil, water or air (Salt et al., 1998). However, the ability to accumulate heavy metals varies significantly between species and among cultivars within species, as different mechanisms of ion uptake are operative in each species, based on their genetic, morphological, physiological and anatomical characteristics. There are different categories of phytoremediation, including phytoextraction, phytofiltration, phytostabilization, phytovolatization and phytodegradation, depending on the mechanisms of remediation. Phytoextraction involves the use of plants to remove contaminants from soil. The metal ion accumulated in the aerial parts can later be removed to dispose or burnt to recover metals. Phytofiltration involves the plant roots or seedling for removal of metals from aqueous wastes. In phytostabilization, the plant roots absorb the pollutants from the soil and keep them in the rhizosphere, rendering them harmless by preventing them from leaching. Phytovolatization involves the use of plants to volatilize pollutants from their foliage such as Se and Hg.
Phytodegradation means the use of plants and associated microorganisms to degrade organic pollutants (Garbisu and Alkorta, 2001). Some plants may have one function whereas others can involve two or more functions of phytoremediation.

1.7.1. Phytoremediation of polluted water

Rhizofiltration is the removal of pollutants from the contaminated waters by accumulation into plant biomass. Several aquatic species have been identified and tested for the phytoremediation of heavy metals from the polluted water. These include sharp dock (*Polygonum amphibium*), duck weed (*Lemna minor*), water hyacinth (*Eichhornia crassipes*), water lettuce (*P. stratiotes*), water dropwort (*Oenanthe javanica*), calamus (*Lepironia articulata*), pennywort (*Hydrocotyle umbellate*), (Prasad and Freitas, 2003). The roots of Indian mustard are found to be effective in the removal of Cd, Cr, Cu, Ni, Pb and Zn, and sunflower can remove Pb, U, Cs-137 and Sr-90 from hydroponic solutions (Zaranyika and Ndapwadza, 1995; Wang *et al*., 2002; Prasad and Freitas, 2003).

1.7.2. Phytoremediation of heavy metal contaminated soils

Early research indicates that phytoremediation is a promising clean-up solution for a wide variety of contaminated sites, although it has its restrictions. Many of the limitations and advantages of phytoremediation are a direct result of the biological aspect of this type of treatment system (Singh *et al*., 2003). The fact that phytoremediation is carried out in situ contributes to its cost-effectiveness and may reduce exposure of the polluted substrate to humans, wildlife, and the environment (Pilon-Smits, 2005). Plants have a range of potential mechanisms at the cellular level that might be involved in the detoxification and tolerance to heavy metal stress. These all appear to be involved primarily in avoiding the build-up of toxic concentrations at sensitive sites within the cell, thus preventing the damaging effects (Hall, 2002).

Various phytoremediation strategies are possible for the remediation of heavy metal contaminated soils (Salt *et al*., 1998). Different phytotechnologies make use of different plant properties (Pilon-Smits, 2005). The main treatment streamlines are described below:
Phytovolatilization: contaminants taken up by the roots pass through the plants to the leaves and are volatized through stomata, where gas exchange occurs (Vroblesky et al., 1999).

Phytostabilization: plants are used to reduce the mobility and bioavailability of environmental pollutants (Vangronsveld et al., 1995). Phytoextraction: plant roots take up contaminants and store them in stems. And leaves (harvestable regions) (Kumar et al., 1995).

1.8. Phytovolatilization of heavy metals

The chemical conversion of toxic elements into less toxic and volatile compounds is a possible strategy for detoxification of metal ion contaminants, resulting in the removal of specific harmful volatile elements (e.g., Hg, Se and Aluminum) from soil and plant foliage to the atmosphere (Raskin et al., 1997).

1.9. Phytoextraction of heavy metals

The term “phytoextraction” mainly concerns the removal of heavy metals from soil by means of plant uptake. This technology is based on the capacity of the roots of plants to absorb, translocate, and concentrate toxic metals from soil to the aboveground harvestable plant tissues. The concentration process results in a reduction of the contaminated mass and also in the transfer of the metal from an aluminosilicate-based matrix (soil) to a carbon-based matrix (plants). The carbon in the plant material can be oxidized to carbon dioxide, further decreasing (and concentrating) the mass of material to be treated, disposed, or recycled (Blaylock and Huang, 2000).

1.10. Phytostabilization of heavy metals

An alternative means of decreasing the environmental risk posed by these metal-contaminated soils may be the use of plants to stabilize the surface, thus reducing erosion and leaching to the soil deeper layers. This option is called phytostabilization, and considers the use of metal-tolerant plant species to immobilize heavy metals belowground, decreasing metal mobility and reducing the likelihood of metals entering into the food chain (Wong, 2003).
1.11. Factors affecting phytoremediation

The success of phytoremediation as an environmental clean up technology depends on several factors including bioavailability of metals in soil, plant’s ability to uptake, translocate and accumulate metals in shoots and plant-microbe interactions. To dismay, the underlying biological mechanisms of plant decontamination processes are poorly understood and much still remains to be known.

1.12. Future

Since last decade phytoremediation has gained acceptance as a technology and has been acknowledged as an important area of research aimed at better understanding of the interactive roles among plants roots and microbes will help scientists to utilize their integrative capacity for soil decontamination. Further, genetic evaluation of hyper accumulators growing in metal contaminated soil and associated microbes would provide the researchers with a gene pool to be used in genetic manipulation of other non accumulators and production of transgenics. As more results demonstrating the effectiveness of phytoremediation become available, its use may continue to grow, reducing clean up costs and enabling the clean up of more sites with the limited funds available (Rock, 2003). Currently a great deal of research is in progress in this direction and its impact will soon be felt in phytoremediation market.

The possibility of employing higher plants as monitors of metal pollution is dependent upon an understanding of the metabolic processes which enable plants to acquire needed nutrients and tolerate increasing levels of toxic elements. Plant breeding studies have led to the realization that many aspects of mineral nutrition are under genetic regulation and therefore governed by selection. Genetic control has been shown to govern the initial absorption of ions (Foy and Barber, S. A, 1958; Wutscher et al., 1970) the oxidation-reduction of Fe (Brown, 1967), compartmentalization of ions within the root (Munns et al., 1964), transfer from root to xylem (Pinkas et al., 1966), and metabolic utilization (Shea et al., 1968). Similarly, tolerance of plants to high levels of both nutrient and non nutrient elements appears to be genetically controlled (Epstein, 1964; Vose, 1962). Tolerance of plants to individual metals, although forming the functional basis for the behavior of "indicator" or "accumulator" plants, is often misconceived. In the case of accumulator species, their adaptation of geographical areas containing high concentrations of endogenous metals involved genotypic evolution and selection over a
period of time induced by the specific habitat. Mechanisms for exclusion have been shown to include low root cation exchange capacities limiting uptake of Al and Mn (Vose and Randall, 1962), sorption of Zn to cell walls (Turner and Gregory, 1967), and precipitation of Al by hydroxyl ions at the root surface (Clarkson, 1967; Foy et al., 1965). The major mechanisms appear to be compartmentalization, complexation and metabolic adaptation. Compartmentalization would provide a means of limiting the presence of toxic metals at cellular locations where toxicity is initiated. For example, Al effects are most pronounced on processes of cell division and respiration; and compartmentalization or exclusion from these metabolic sites, provides, an effective protective mechanism (Clarkson, 1965). A plant adaptation which may play a role to tolerance is the alteration of metabolic sequences to allow an organism to function in an apparently normal manner in the presence of large amounts of heavy metals (Turner, 1969).

Heavy metals accumulating in the food chain, pose risk for health of animals and humans, who are less sensitive to metal toxicity than plants, but can concentrate heavy metals in certain tissues and organs (Mantovi et al., 2003). Cytotoxicity of heavy metals in plants has been well documented (Delhaize and Ryan, 1995; Marienfeld et al., 2000). Aluminum (Al) toxicity is a major agricultural problem in acid soil, and has been intensively studied in plants. Plants grown in acid soils due to Al solubility at low pH have undeveloped root system and exhibit a variety of nutrient-deficiency symptoms, consequently decrease in yield. Al interferes with uptake, transport and utilization of essential nutrients including Ca, Mg, K, P, Cu, Fe, Mn and Zn (Foy, 1984; Guo et al., 2003). Soil acidification could bring about many other changes of physical and chemical properties in soil, which in turn affect plant growth and development (Chen et al., 2000).

Generally, aluminum is not bioaccumulated to a significant extent. However, certain plants can accumulate high concentrations of aluminum. For example, tea leaves may contain very high concentrations of aluminum, >5,000 mg/kg in old leaves (Dong et al. 1999). Other plants that may contain high levels of aluminum include Lycopodium (Lycopodiaceae), a few ferns, Symplocos (Symplocaceae), and Orites (Proteaceae) (Jansen et al., 2002). Aluminum does not appear to accumulate to any significant degree in cow’s milk or beef tissue and is, therefore, not expected to undergo biomagnification in terrestrial food chains (DOE, 1984). Similarly, because
of its toxicity to many aquatic organisms, including fish, aluminum does not bioconcentrate in aquatic organisms to any significant degree (Rosseland et al., 1990).

Plant have mechanisms for detoxification of Al, though micronutrients such as Zn, Mn, Ni and Cu are essential for plant growth and development, yet high intracellular concentrations of these ions can be toxic. To deal with this potential stress, common non accumulator plants have evolved several mechanisms to control the homeostasis of intracellular ions. Such mechanisms include regulation of ion influx (stimulation of transporter activity at low intracellular ion supply, and inhibition at high concentrations), and extrusion of intracellular ions back into the external solution. Metal hyper accumulator species, capable of taking up metals in the thousands of ppm, possess additional detoxification mechanisms. For example, research has shown that in Thlaspi goesingense, a Ni hyper accumulator, high tolerance was due to Ni complexation by histidine which rendered the metal inactive (Kramer et al., 1997; Kramer et al., 1996).

Interest in phytoremediation has grown significantly following the identification of metal hyper accumulator plant species. Hyper accumulators are conventionally defined as species capable of accumulating metals at levels 100-fold greater than those typically measured in common non accumulator plants. Thus, a hyper accumulator will concentrate more than 10 ppm.

Possibly, the best-known metal hyper accumulator is Thlaspi caerulescens (alpine pennycress). While most plants show toxicity symptoms at Zn accumulation of about 100 ppm, T. caerulescens was shown to accumulate up to 26,000 ppm without showing any injury (Brown et al., 1995). Possibly, hyper accumulator plants may have a higher requirement for metals such as Zn than non-accumulator species (Hajar, 1997).

The techniques in Bioremediation/Phytoremediation include the application of appropriate plants for in-situ risk reduction through contaminant removal, detoxification or containment in contaminated soil, sediments, and ground water. This strategy/approach can be used along with or, in some cases, in place of mechanical cleanup methods. Cleanup can be accomplished to certain level within the reach of plants’ roots. Such sites need to be maintained and monitored (watered, fertilized, and monitored).
1.12.1. Plant Species for Phytoremediation

To identify plant populations with the ability to accumulate heavy metals, 300 accessions of 30 plant species were tested by Ebbs et al. (1997) in hydroponics for 4 weeks, having moderate levels of Cd, Cu and Zn. The results indicate that many Brassica spp. such as B. juncea, B. juncea, B. napus and B. rapa. exhibited moderately enhanced Zn and Cd accumulation. They were also found to be most effective in removing Zn from the contaminated soils. To date, more than 400 plant species have been identified as metal hyper accumulators, representing less than 0.2% of all angiosperms (Brooks, 1998; Baker et al., 2000). The plant species that have been identified for remediation of soil include either high biomass plants such as willow (Landberg and Greger, 1996) or those that have low biomass but high hyper accumulating characteristics such as Thlaspi and Arabidopsis species. On worldwide basis, number of species has been identified to have ability to accumulate one or more metals (Reeves, 2003).

1.12.2. Transgenic plants in phytoremediation

The plant species currently being developed for phytoremediation seem capable of effective bioaccumulation of targeted contaminant, but efficiency might be improved through the use of transgenic (genetically engineered) plants. Naturally occurring plant species that can be genetically engineered for improved phytoremediation include Brassica juncea for phytoremediation of heavy metals from soil (Dushenkov et al., 1995), Helianthus annulus (Dushenkov et al., 1995) and Chenopodium amaranticolor (Eapen et al., 2003) for rhizofiltration of uranium. The increase in metal accumulation as the result of these genetic engineering approaches is typically two to threefold more metal per plant, which potentially enhances phytoremediation efficiency by the same factor. It is not yet clear how applicable these transgenics are for environmental cleanup, since no field studies have been reported except one using transgenic Indian mustard plant that over expresses enzymes involved in sulfate/ selenate reduction (Pilon Smits et al., 1999; Zhu et al., 1999 a, b).

Classic genetic studies have shown that only a few genes (up to three) are responsible for metal tolerance (Macnair et al., 2000). The possible areas of genetic manipulation are as follows:
Metallothioneins: The transfer of human metallothionein gene in tobacco resulted in plants with enhanced Cd tolerance (Misra and Gedamu, 1989), and pea metallothionein gene transfer to Arabidopsis thaliana resulted in increased Cu accumulation (Evans et al., 1992).

Phytochelatins: Transgenic Brassica juncea over expressing different enzymes involved in phytochelatin synthesis were shown to extract more Cd, Cr, Cu, Pb, and Zn than wild plants (Zhu et al., 1999a, b).

Organic acids: The over expression of citrate synthase has shown to promote enhanced Al tolerance.

Alteration of oxidative stress mechanisms: Over expression of glutathione-S-transferase and peroxidase in Arabidopsis plants resulted in enhanced Al tolerance (Ezaki et al., 2000).

1.13. Effect of heavy metals on the plant growth

Heavy metals such as Cu, Aluminum and Zn are essential for normal plant growth and development since they are constituents of many enzymes and other proteins. However, elevated concentrations of both essential and nonessential heavy metals in the soil can lead to toxicity symptoms and growth inhibition in most plants (Hall, 2002).

Plentiful amount are accumulated in the plant, heavy metals will adversely affect the absorption and transport of essential elements, disturb the metabolism, and have an impact on growth and reproduction (Xu and Shi, 2000). The effect of heavy metal on plants is different in different growth stages of plants.

Aluminum toxicity is a major agricultural problem, and is intensively studied in plant systems. Cytotoxicity of Al has been well documented in plants (Delhaize and Ryan, 1995; Horst et al., 1999; Kollmeier et al., 2000; Marienfeld et al., 2000). It is generally known that plants grown in acid soils due to Al solubility at low pH have reduced root systems and exhibit a variety of nutrient-deficiency symptoms, with a consequent decrease in yield. Inhibition of root and shoot growth is a visible symptom of Al toxicity. The earliest symptoms concern roots. Shoots in contrast to the situation observed for Mn toxicity are less affected (Chang et al., 1999). Root stunting is a consequence of Al-induced inhibition of root elongation. Roots are usually stubby and
brittle and root tips and lateral roots become thick and may turn brown (Mossor-Pietraszewska et al., 1997). Such roots are inefficient in absorbing both nutrients and water. Young seedlings are more susceptible than older plants. Al apparently does not interfere with seed germination, but does impair the growth of new roots and seedling establishment (Nosko et al., 1988). The common responses of shoots to Al include: cellular and ultrastructural changes in leaves, increased rates of diffusion resistance, reduction of stomatal aperture, decreased photosynthetic activity leading to chlorosis and necrosis of leaves, total decrease in leaf number and size, and a decrease in shoot biomass (Thornton et al., 1986).

Blancaflor et al. (1998) have studied Al-induced effects on microtubules and actin microfilaments in elongating cells of maize root apices, and related the Al-induced growth inhibition to stabilization of microtubules in the central elongation zone. With respect to growth determinants (auxin, gibberelic acid and ethylene), Al apparently interacts directly and/or indirectly with the factors that influence organization of the cytoskeleton, such as cytosolic levels of Ca$^{2+}$ (Jones et al., 1998), Mg$^{2+}$ and calmodulin (Grabski et al., 1998), cell-surface electrical potential (Takabatake and Shimmen, 1997), callose formation (Horst et al., 1997), and lipid composition of the plasma membrane (Zhang et al., 1997).

1.13.1. Effect of heavy metals on pigment content of plant

Heavy metals affected the function of PS I and PS II, and it was stronger with the latter (Yang et al., 1989). The chlorophyll proteins, which took protons for photosynthesis in PS II, were decomposed and decreased under Cd stress. The Sub-microstructure of chloroplast was changed and membrane system was destroyed. Therefore, the capacity of taking protons decline and the photosynthesis function was influenced (Peng and Wang, 1991). Thus the photosynthetic yield would be one of the indicators for heavy metal pollution.

Excess Cu, Zn and Al cause numerous toxic effects in plants. All three metals have been shown to cause reduction in chlorophyll content and the rate of photosynthesis while Al and Cu inhibit respiration in some plants (Sarkunan et al., 1984; Fernandes and Hendriques, 1991; Doncheva, 2001; Lim et al., 2006). Toxic concentration of Cu, Zn and Al through their impact on chlorophyll content and nitrae
reductase activity. The tolerance index and bioaccumulation factor of this plant are also determined to assess its phytoremediatory property.

Detailed studies indicate that heavy metals have effects on chlorophyll content in plants. Heavy metals are known to interfere with chlorophyll synthesis either through direct inhibition of an enzymatic step or by inducing deficiency of an essential nutrient (van Assche and Clíjsters, 1990).

1.13.2. Effect of heavy metals on the proline content of plant

Proline accumulation, accepted as an indicator of environmental stress, is also considered to have important protective roles. Heavy metal stress leads to proline accumulation (Alía and Saradhi, 1991). Proline accumulation in plant tissues has been suggested to result from:

(a) A decrease in proline degradation,
(b) An increase in proline biosynthesis,
(c) A decrease in protein synthesis or proline utilization,
(d) Hydrolysis of proteins (Charest and Phan, 1990).

Proline is thought to play a cardinal role as an osmoregulatory solute in plants subjected to hyper osmotic stresses, primarily drought and soil salinity. Indeed, the accumulation of this imino acid may be part of a general adaptation to adverse environmental conditions, having been documented in response to several stresses including exposure to Al. Proline stabilizes cellular structures as well as scavenges free radicals (Hare and Cress, 1997).

Metal toxicity in plants is very complex and depends on plant species, especially metal concentration, pH of soil and soil composition. Contamination by heavy metals such as lead, harmful effects on the growth and metabolism of plant leaves. Proline accumulation reduced damage to membranes and proteins. Proline concentration increase during stress may indicate a possible role of these amino acids in osmoregulation (Martin et al., 1993). Kao (1981) reported that in mature leaves decreased protein degradation and increased concentration of free amino acids such as proline.
1.13.3. Effect of heavy metals on the protein content of plant

Heavy metal Toxicity may result from the binding of metals to sulphydryl groups in proteins, leading to inhibition of activity or disruption of structure, or from displacement of an essential element, resulting in deficiency effects (Van Assche and Clijsters, 1990). In addition, a heavy metal excess may stimulate the formation of free radicals and reactive oxygen species, perhaps resulting in oxidative stress (Dietz et al., 1999).

The possibility that Al is detoxified by formation of stable metal–protein complexes has been raised. Many authors showed inducible synthesis of a cytosolic Al binding protein (Basu et al., 1999; Snowden et al., 1995; Somers and Gustafson, 1995; Wu et al., 2000).

Small molecular weight organic acid can chelate heavy metals (Yang et al., 2000), and metal-binding proteins or metallothione are perfect for chelating heavy metals (Zhang et al., 1999). Metal-binding proteins were also identified and purified in rice, bean, broccoli, tobacco, with the molecular weight being from 3.1 to 33.1 KD (Li and Yu 1990).

1.13.4. Effect of heavy metals on sugar content of plants

Growth reduction and altered levels of major biochemical constituents such as chlorophyll, protein, free amino acids, starch, and soluble sugars, that play a major role in plant metabolism, It is interesting, that the sites of the most abundant reduction of metal salts to nanoparticles were chloroplasts, regions of high reducing sugar (glucose and fructose) content. Growth reduction and altered levels of major biochemical constituents such as chlorophyll, protein, free amino acids, starch, and soluble sugars, that play a major role in plant metabolism, were observed in response to varying concentrations of Cd$^{2+}$ in the nutrient medium.

1.13.5. Effect of heavy metals on enzyme activity of plant

Al is reported to interfere with cell division in root tips and lateral roots, increase cell wall rigidity by cross linking pectins, reduce DNA replication by increasing the rigidity of the double helix, fix P in less available forms in soils and on plant root surfaces, decrease root respiration, interfere with a number of enzymes, decrease deposition of cell wall polysaccharides, decrease production and transport of cytokinins, modify structure and function of plasma membranes, reduce water uptake,
and interfere with the uptake, transport, and metabolism of several essential nutrients. (Taylor et al., 1998). Al stress results in a decrease in the total adenine nucleotide level and the adenylate pool sizes. This may lead to a change of energy state (Lorenc-Plucińska and Ziegler, 1996). Hamilton et al. (2001) described an induction of vacuolar ATPase and mitochondrial ATP synthase by Al in an Al-resistant cultivar of wheat. These enzymes were reported to play a role in Al resistance.

Superoxide dismutase (SOD), peroxide dismutase (POD) and catalyse (CAT) are important enzymes for plants adapted to environmental stress; they are called the plant protective enzymatic system. The harmonium interaction of the three enzymes make the balance of free radical production and elimination, and keep the level of free radical in plants low to prevent the injury of cells by free radical, DNase, RNase (Duan and Wang, 1992), Proteinase (Chen and Gong, 1996) and nitrate reductase (Xu and Shi, 2000). All these findings suggest that heavy metals inhibit the nitrogen metabolism, respiration and nucleic acid metabolism, and show toxicity to plants.

1.13.6. Phytoaccumulation of heavy metals in root, stem and leaf

Phytoaccumulation involves the uptake of contaminants by plant roots, followed by their translocation through the xylem and accumulation in the shoots and leaves. While some contaminants, such as selenium, mercury and volatile organics, can be released through the leaves into the atmosphere. Phytoaccumulation has normally been applied to polluted soils. The method relies on the identification, cultivation and harvesting of known contaminant tolerant plants. For the process to be economically viable, a cultivated plant must hyper accumulate the contaminant (s) and produce large biomass. Other factors such as growth rate, element selectivity, resistance to disease, method of harvesting and disposal, are also important (Cunningham and Ow, 1996; Baker et al., 1994).

Phytoaccumulation refers to plant uptake of toxicants, which is known to occur and has been studied in the storm water and mine water wetland context (Kadlec, 1999). However, in many cases the contaminant is selectively bound up in below ground tissues, roots and rhizomes, and is not readily harvested. For example, metals are taken up by plants, and in many cases stored preferentially in the roots and rhizomes (Sinicrope et al., 1992). Rhizofiltration is the adsorption or precipitation onto plant roots (or absorption into the roots) of contaminants that are in solution.
surrounding the root zone (Neate, 2003). It is based on a combination of principle of phytoextraction and phytostabilization specially suited to remove metals and radio nuclides from polluted water. Contaminants are absorbed and concentrated by plant roots, then precipitated as their carbonates and phosphates (Salt et al., 1995). The accumulation of pollutants (nutrients and heavy metals) show that the contribution of macrophytes in the sense of the uptake of pollutants are significant in this study, apart from providing a large surface area for attached microbial growth, supplying reduced carbon through root exudates and micro aerobic environment and a via root oxygen release in the rhizosphere, and stabilizing the surface of the bed (Gersberg et al., 1986; Tanner, 2001; Gagnon et al., 2006).

1.13.7. The effect of heavy metal on soil and its removal

This technology is based on the capacity of the roots of plants to absorb, translocate, and concentrate toxic metals from soil to the aboveground harvestable plant tissues. The concentration process results in a reduction of the contaminated mass and also in the transfer of the metal from an aluminosilicate-based matrix (soil) to a carbon-based matrix (plants). The carbon in the plant material can be oxidized to carbon dioxide, further decreasing (and concentrating) the mass of material to be treated, disposed, or recycled (Blaylock and Huang, 2000). Plant species vary significantly in the ability of accumulating metals from contaminated soils, as a balance between the uptake of essential metal ions to maintain growth and development and the ability to protect sensitive cellular activity and structures from excessive levels of essential and non-essential metals is required (Garbisu and Alkorta, 2001). Generally, metals enter the plants primarily via absorption of the available metal ions from the soil solution into the root symplasm, driven by the electrical chemical potential gradient across the plasma membrane of root cells (Blaylock and Huang, 2000). Once inside the plant, most metals are too insoluble to move freely in the vascular system, so they usually form phosphate, sulphate, or carbonate precipitates.

1.13.7.1. Effect of heavy metals on dehydrogenase activity of soil

Heavy Metals are natural constituents of soil. They persist in soils and have a very slow leaching rate; hence they tend to accumulate in soils. Trace amount of some heavy metals are required by living beings but in excess they are detrimental. The
ecotoxicological risks of metal contamination bears potential harm for plants, animals, humans beings, and microorganisms. Heavy metal pollution can suppress or even kill sensitive parts of plant and soil microbial communities and lead to a shift in their functional diversity and structure. Once they are accumulated in the food chain, their effect gets adverse with tropic levels due to biomagnification. On the other hand, heavy metals like Cu, Fe, Mn, Ni, and Zn are essential for plant growth and are important constituents of many enzymes. In addition, metals like Al, As, Cd, Cr, Hg, Pb, Sb, Se, among others are nonessential and toxic above certain threshold levels (Panda and Choudhury, 2005).

Soils contaminated with heavy metals are poor in nutrients and microbial diversity and contribute to sub-optimal plant biomass accumulation as well as impeded rates of remediation (White et al., 2006). A number of biological properties in soil are influenced by heavy metals and changes in these properties may act as sensible indicators of soil quality, since they are more dynamic and often more sensitive than physical or chemical parameters. One of such biological properties is the microbial and soil enzyme activity that is frequently used for determining the influence of various pollutants on the living system. As high contaminant levels inhibit plant and microbial activity, therefore an effective phytoremediation is realized where contaminants are present at low to medium levels. Recent research have shown stunning effects with the amendments of biofertilizer and biosludge to the contaminated lands, plants were able to grow and survive in extreme higher levels of heavy metal contamination (Juwarkar et al., 2008). Soil enzyme activity is a key feature of plant nutrients and cycling processes, thus measurement of specific enzyme activities is found useful in determining soil biological activity which in turn is an index of soil fertility (Perucci, 1992). Soil enzymes are believed to be primarily of microbial origin (Ladd, 1978) but also originate from plants and animals (Tabatabai, 1994).

Dehydrogenase activity is used as an active soil biomass measurement indicator and is related to the overall microbial activity in soil that reflects their total range of oxidative activity (An and Kim, 2009). Dehydrogenase enzyme is known to oxidize soil organic matter by transferring protons and electrons from substrates to acceptors (Makoi and Ndakidemi, 2008). Dehydrogenase assay measures the total activity of a soil sample which is due to active microorganisms and enzymes
stabilized in the soil matrix (Knight and Dick, 2004). This can also be used as a method to describe the biological activity in thermophilic and mesophilic stages of composting (Barrena et al., 2008). Because it is difficult to extract intact enzymes from soil, activity rather than mass is measured. These processes are a part of respiration pathways of soil microorganisms and are influenced by environmental factors. Their activity not only increases in well-irrigated soil but also on addition of nutrients to soil but decreases with soil depth (Brzezinska et al., 2001). The activity is enhanced only if the rate of sludge addition is limited (Obbard et al., 1994). Dehydrogenase is sensitive to heavy metal pollution; hence it is used to access the side effects of chemicals on microorganisms (Nweke et al., 2007). The activity also varies with soil type and season. It is found that higher dehydrogenase activity in the rhizosphere during the dry seasons than in moist winters.

1.13.7.2. Effect of heavy metals on organic carbon, nitrate, ammonium and available phosphorus

Dissolved organic carbon (DOC) consists of several types of low molecular weight organic compounds, such as polyphenols, simple aliphatic acids, amino acids and sugar acids (Fox and Comerfield, 1990). DOC stays dissolved in the soil solution under natural conditions and it has been found that it may be responsible for the dissolution equilibria of metals in the soil solution especially at neutral pH values (Harter and Naidu, 1995). DOC also has a unique role in the chemistry of heavy metals in soils; it reduces metal adsorption onto soil surfaces by either competing more effectively for the free metal ion or forming soluble organometallic complexes or being preferentially adsorbed onto the surfaces instead of the metals it is competing with (Guisquiani et al., 1998).

Plants need substantially more nitrogen (N) than phosphorus. In agricultural systems N comes from the mineralisation of organic matter, the fixing of nitrogen from the air by legumes, and the application of fertilizers. Fertilizer N interacts with soil components through biological and chemical processes, so that soon after their application most N compounds (apart from nitrate) cannot be found in the soil in the form in which they were applied.

On farmed land most soil N is in organic matter. The top 15 cm of soil frequently contains 500 to 5000 kg N/ha in highly complex, organic compounds.
In this form the N is not normally available to plants. The soil inorganic N is the N immediately available to plants and consists of ammonium (NH$_4^+$) and nitrate (NO$_3^-$). The amount of inorganic N varies greatly depending on seasonal conditions, past fertilizer treatment, history of growing legumes and soil properties, but it is seldom greater than about 100 kg N/ha. In a soil that has not received any N fertilizer nor grown any legumes it is usually less than 50 kg N/ha.

How nitrogen reacts and moves in the soil

1.13.7.3. N undergoes several reactions in the soil:

- **Mineralization**: microorganisms convert the N from organic matter or urea fertilizer to inorganic forms (ammonium and nitrate) that plants can use.

- **Immobilization**: plants and microorganisms take up inorganic forms of N (mainly nitrate from fertilizers and other sources) and convert them to organic matter, which can be recovered after breakdown of plant residues. This is the reverse process to mineralization.

- **Nitrification**: microorganisms use oxygen to convert ammonium from fertilizers and other sources to nitrite and nitrate.

- **Denitrification**: Bacteria convert nitrite or nitrate to nitrous oxide (N$_2$O) and elemental nitrogen (N$_2$), which can be lost to the atmosphere.

- **Nitrogen Fixation**: microorganisms associated with the nodules on legume root systems convert N gas from the atmosphere to ammonium and organic N, which is stored in nodules. In contrast to P, N as nitrate is very mobile in soils. It is readily leached to groundwater and drainage waters that may end up in surface waters. It is also easily dissolved in run-off.

Nitrate is currently one of the most hazardous pollutants (Awasthi and Rai; 2005, Johnson and Kross, 1990; USEPA, 1987). Nitrate reductase (NR) activity is the limiting factor when considering the growth and protein production of algae (Lau et al., 1998). There is substantial concern about the growing levels of heavy metals in the environment and the detrimental effects of these elements on living organisms.

Since heavy metals and nitrate are both harmful pollutant and often occur together, it is necessary to know the effect of heavy metals on the enzyme activity.
more so when NR in immobilized algae can be used to treat the nutrient pollution. The biological treatment system will definitely be affected when heavy metals are present besides NO$_3^-$.

Although some reports are available regarding the nitrate reductase activity of algal cells in the free state however only scant information is available about the influence of these metals on the immobilized state. 

The solubility of heavy metals in the polluted soils can be increased by using organic and inorganic agents, thus enhancing the phytoextraction capabilities of many plant species. Ebbs et al. (1997) amended the contaminated soil with Grower-Power, a commercial soil amendment that improves soil structure and fertility, and the removal of Zn by plant shoots was doubled to more than 30 000 mg Zn/pot (4.5 kg). Other applied enhancement materials include ethylene diamine tetra acetic acid (EDTA), citric acid, elemental sulfur or ammonium sulfate. Increases greater than 100 folds in Pb concentration in the biomass of crops were reported when EDTA was applied to the contaminated soils (Cunningham and Berti, 2000).

Phosphorus is a major nutrient, and plants respond favorably to the application of P fertilizer by increasing biomass production. The addition of P fertilizer, however, can also inhibit the uptake of some major metal contaminants, such as Pb, due to metal precipitation as pyromorphite and chloro-pyromorphite (Chaney et al., 2000). This underlines the importance of finding new approaches for P application. Such an alternative may be foliage application. This method may lead to improvement of plant P status without inhibiting Pb mobility in soil.

Small amounts of P are held in organic matter. Inorganic P and other nutrients that plants can use are released when the organic matter is broken down by microbial activity. However, generally this is inadequate for agricultural production and P in the form of fertilizer must be added to most soils to meet plant requirements where factors such as moisture or other nutrients are not limiting growth. P is usually available to plant roots only at the surface and when soil is moist.

A range of organic and inorganic compounds (Adriano et al., 2004), such as lime, phosphate, and other low economical value organic materials like biosolids, litter, compost, and manure, can be used. Liming has been considered as an important management tool in reducing the toxicity of metals in soils (Gray et al., 2006). There is conclusive evidence for the mitigative value of both water-soluble (e.g.,
diammonium phosphate) and water-insoluble (e.g., patite) phosphate to immobilize some metals in soils, thereby reducing their bioavailability for plant uptake (Brown et al., 1995). Phosphate enhances the immobilization of metals in soils through various processes, including direct metal adsorption, phosphate anion-induced metal adsorption, and precipitation of metals with solution phosphate as metal phosphates (Adriano et al., 2004). In fact, Bolan et al. (2003) reported that the sole application of lime or phosphate is effective in reducing Cd in contaminated soils.

1.13.7.4. Heavy metal remaining in the soil

The toxicity and potential mutagenicity of heavy metals such as arsenic, lead, chromium, cadmium, copper, nickel and zinc in the environment have been well established. According to the United States Environmental Protection Agency (USEPA), there are over 200,000 heavy-metal-contaminated sites in the United States that require urgent cleanup to protect public health and the environment. Among the physical and biological processes in the subsurface soils, geochemistry plays a major role in the distribution, speciation, as well as the remediation potential of heavy metals. Fine-grained soil particles are geochemically active, with the soil-mineral surface sites possessing negative or positive charge or being electrically neutral (Evans 1989).

Many studies have been conducted on heavy metal contamination in soils from various anthropogenic sources such as industrial waste (Gibson and Farmer, 1983), automobile emissions (Garcia-Miragaya, 1984) and agriculture practice (Colbourn and Thornton, 1978).

Soil pollution by heavy metals has become one of the chief topics of discussion of all environmental crises today. Heavy metals exist in colloidal, ionic, particulate, and dissolved phases. They are present in soil as free metal ions, soluble metal complexes, exchangeable metal ions, organically bound metals, precipitated or insoluble compounds like oxides, carbonates, and hydroxides, or a part of silicate materials (Leyval et al., 1997). Metals are natural constituents of soil. They persist in soils and have a very slow leaching rate; hence they tend to accumulate in soils. Heavy metals indirectly affect soil enzymatic activities by altering the microbial communities that synthesize enzymes and their mode varies with the enzyme type (Moreno et al., 2003). Soil enzyme inhibition by heavy metals depends on the nature
and concentration of the metals, and its extent varies from one enzyme to another and at certain concentration some heavy metals can also stimulate the activity of an enzyme. Metal ions may inhibit enzyme reactions by complexing the substrate, reacting with the protein-active groups of enzymes, enzyme-substrate complex (Mikanova, 2006) and sulphydral group of enzymes (Shaw and Raval, 1961).

**Objectives**

- Removal of aluminium from polluted sites/soils by selected plants
- To study the effect of heavy metal on physiological parameters like Photosynthetic pigments, proline, protein.
- To assess the Phytoremediation potential by treating hyper accumulator plant *Pedilanthus tithymaloides* L., *variegates* and *Pedilanthus tithymaloides* L. *tithymaloides* and *V. unguiculata* L. with heavy metal.
- Plant samples and soil samples are digested in microwave for qualitative and quantitative analysis of Heavy metal (Aluminum) in ICP-OES.
- pH, Electrical conductivity, Nitrogen, Phosphorus, Organic carbon and dehydrogenase activity of soil are estimated.