Chapter-2

Review of Literature
LITERATURE REVIEW

To see the effect of heavy metal speciation in soil on the plant metal uptake, which is one of the objectives of the present study, it is important to have a detailed idea about the heavy metal speciation. The following section deals with the review on heavy metal speciation in soils.

Heavy Metal Speciation

Speciation of heavy metals in soils can be defined in many ways. First, it can be defined as the extraction and quantification of a soil phase which is functionally designated in that its element content is, for example, the plant available content. Secondly, speciation can be defined operationally, i.e., by the extraction technique itself (Ure, 1990). In the present study, operational speciation was performed. The term ‘speciation’ is also broadly defined as identification of different, defined species, forms or phases in which an element occurs in the matrix under study (Ure, 1990). Without information on speciation, no meaningful interpretation of either biological or geochemical cycling of a metal can be made (Florence, 1981). It also helps largely to control the potential toxicity of various metals if precise chemical form of a metal is defined and hence, distribution and transformation of metal species in the environment can be ascertained (Bernhard et al., 1986). Speciation analysis of an element yields information on the individual concentrations of various chemical forms of that element in environmental samples. It allows the differentiation not only between oxidation states but also between simple and coordinated ions, polymeric species, as well as between various degrees of homogeneous and heterogeneous associations with natural constituents.

The behavior of heavy metals in changing environmental conditions and their uptake by biota i.e., their bioavailability is a major objective of species differentiation. The main chemically active forms in which the heavy metals can be present are exchangeable, carbonate bound, bound to hydroxides of Fe and Mn and organic matter bound (Forstner and Solomons, 1980). Depending upon the variability in physico-chemical characteristics of metals, their affinities to soil components govern their speciation. Rule (1999) reviewed recent literature on the phase distribution of trace metals in soils and concluded that the highest proportions of most metals are found either in residual, or in Fe-Mn oxide fractions in both natural and contaminated soils. However, some metals like Cd and Zn
occur abundantly in carbonate fractions in some soils. The association of metals with organic matter also has a significant impact on their behavior. The various reactions and interactions between different species of heavy metals in the soil are shown in Figure 2.1.

![Figure 2.1 Reactions of Metals in the Soil](image)

Two approaches exist to resolve species differentiation in modern soil science: Appropriate soil test and analysis and Models, which include kinetic considerations. The models available suggest only the expected trace metal species distribution and results may be different from solid speciation because of the important role of kinetically controlled processes. The design of soil test is based on the concept that metal is divided over several different pools, like "water soluble" "exchangeable", "strongly bound", "occluded by metal oxides, carbonate and phosphates" and "matrix bound" not interacting with each other. Species differentiation can be done either functionally as "plant available species ", "mobile form" and as "exchangeable cation" or operationally by reagents or extractants used to isolate them. The method widely used to assess the fate of heavy metals in soil is the leaching of soil by means of chemical extractants, which attack certain phases in soil, releasing the trace metals. Sequential extraction schemes are used to get an indication of the amount of the metals in various "reservoirs" which could be mobilized by change in soil chemistry like pH, redox potential etc. (Davidson et al.,...
1998). These schemes help in the identification of main binding sites and phase association of trace metals in soil and sediments and hence are useful in understanding the risk assessment and related physico-chemical processes. The procedure consists of a series of successive chemical treatments each being more drastic in action or of a different nature than the previous one (Chao, 1984). Sequential extraction procedure consist of subjecting a solid sample (soil or sediment) to successive attacks with reagents of different chemical properties (acidity, redox potential or complexing properties) and associating a part of the trace metal with each extract.

The total metal concentration obtained after strong acid digestion of a contaminated soil is useful as an overall pollution indicator, but it provides no information on the solubility of specific metals, which depends on their chemical form (Su and Wong, 2003). Several methods have been developed in an attempt to predict phytoavailability of metals. In this context, phytoavailability is often defined through a one step soil extracting procedure. The amount of heavy metals extracted by such methods gives an idea of the size of a pool that might be depleted by a plant during the growth period; however, the extent of extracting methods depends on the soil tested (Gupta et al., 2006). The application of sequential extraction procedure can help to assess how strongly metals are retained in the soil and how easily they may be released into the soil solution for plant availability. Different sequential extraction techniques, such as the five-step procedure of Tessier et al. (1979) is commonly applied to evaluate both the actual and potential mobility of metals in the environment (Cholpecka et al. 1996; Sanchez et al. 1999; Kaasalainen et al. 2003; Gupta et al. 2006). The procedure of Tessier et al. is one of the most thoroughly researched and also widely used procedures to evaluate the efficacy of decontamination treatments (Mao et al., 1997). Indeed, these heavy metal pools may be selectively affected by plant uptake and change in their proportions may give an idea on the mechanism responsible for heavy metal uptake in comparison with chemical pool in soil.

The application of sequential extraction is a subject of controversy concerning issues like non-selectivity of the extractant, proper sample handling and preparation and trace metal redistribution among various phases during stepwise extraction. Sequential extraction schemes are moreover tedious and time-consuming procedures. Despite such shortcomings, sequential extraction procedures are useful for estimating the relative
availability as well as stability of different pools of heavy metals in soils (Sloan et al., 1997). A poor correlation exists between total metal content in the soil and plant uptake. However, better correlation has been observed for extractable forms of metal (Gupta and Chen, 1975; Xian, 1989).

In their study on the uptake of Cd and Cu in rice plants growing near abandoned metal mines Jung et al. (2005) found that all the fractions of Cd in the soil viz. exchangeable, carbonate bound, oxide bound, organically bound and residual or mineral bound metals were positively correlated with the Cd content in polished rice but Cu did not show any correlation with any of the fractions. In another study Wang et al. (2002) reported that the metal (Cr, Ni, Zn, Cu, Pb, Cd) concentrations in the roots of wheat plants showed significant correlation with water-soluble, carbonate bound and exchangeable fraction, while no significant correlation was found with other fractions. This phenomenon was also supported by others (LeClaire et al., 1984; Chlopecka and Adriano, 1996).

Heavy metal speciation in soils gives an idea about the availability of metals. Now, the following section gives an idea why plants take up metals and what for. The section also gives a brief idea about the mechanism of heavy metal uptake by plants.

**Heavy Metals and Plants**

The main sources of heavy metals to plants are their growth media, e.g., nutrient solutions or soils. One of the most important factors that determine the biological availability of a heavy metal is its binding to soil constituents. Plants readily take up the species of heavy metals that are dissolved in the soil solutions- ionic, chelated or complexed forms. In general, metals readily available to plant are those, which are adsorbed on clay minerals, while those fixed by oxides and bound to microorganisms are much less readily available. Some heavy metals are essential for plants (Fe, Mn, Zn, Cu, Mg, Mo, and Ni) which cannot be substituted by others in their specific biochemical roles and that have a distinct influence on the organism so that it can neither grow nor complete some metabolic cycle.

Heavy metals are involved in key metabolic processes such as respiration, photosynthesis and assimilation of major nutrients (e.g. N, S). Transition metals are
known to activate enzymes or to be incorporated into metalloenzymes as electron transfer systems (Cu, Fe, Mn, Zn) and also to catalyse valence changes in the substrate (Cu, Co, Fe, Mo). Metals like Al, Cu, Co, Mo, Mn, and Zn have a particular role in protection mechanism of frost-hardy and drought resistant plants (Shnolnik, 1974; Marutian, 1972). Certain plants also have the ability to accumulate heavy metals, which have no known biological function. These include Cd, Cr, Pb, Co, Ag, Se and Hg (Hanna and Grant, 1962; Baker and Brooks, 1989). However, excessive accumulation of these heavy metals can be toxic to most plants.

**Mechanism of Heavy Metal accumulation in Plants**

According to Salt et al. (1995) living plants can be compared to solar driven pumps, which can extract certain elements like toxic heavy metals and organics from the environment. Some plants have a natural ability to absorb and accumulate heavy metals in very high concentrations in their tissues. They have adaptations that enable them to survive and to reproduce in soils heavily contaminated with Zn, Cu, Pb, Cd, Ni and As (Baker, 1987). Such plant species are mainly of two types: (i) the so-called pseudometallophytes that grow on both contaminated and non-contaminated soils, and (ii) the absolute metallophytes that grow only on metal contaminated and naturally metal rich soils. These plants are particularly important for phytoremediation.

The ability to accumulate heavy metals to unusually high concentrations has evolved both independently and together in a number of different plant species. Heavy metal accumulation in plants occurs by the following three ways:

(i) **Root uptake**: Even in the presence of metal mobilizing soil amendments, a large portion of metal remains sorbed to soil constituents. For plants to accumulate these soil-bound metals they must first mobilize them into the soil solution. This mobilization of soil bound metal can be achieved in a number of different ways. First, metal-chelating molecules (phytosiderophores) can be secreted into the rhizosphere to chelate and solubilize soil-bound metal. These molecules are secreted in response to Fe and Zn deficiency and can mobilize Cu, Zn and Mn from soil. Metal chelating proteins, perhaps related to metallothiones or phytochelatins may also function as phytosiderophores (Robinson et al., 1993). In graminaceous species mugineic acid and avenic acid serve as phytosiderophores. Secondly, roots can reduce soil-bound metal ions by specific plasma membrane bound metal reductase. Thirdly, plant roots can solubilize heavy metals by
acidifying their soil environment with protons extruded from the roots. Solubilized metal ions may enter the root either via the extracellular or intracellular pathways. The absorption of heavy metals by plant roots can be both passive (nonmetabolic) and active (metabolic), but there are some disagreements reported in the literature concerning which method is involved in certain elements. Despite controversies, in each case the rate of heavy metal uptake will positively correlate with its available pool in root surface. When the metal supply is sufficient accumulation and immobilization of metals in roots are also common (Pendias and Pendias, 1989).

(ii) Transport within plants: The transport of metal ions within plant tissues and organs involves many processes such as movement in xylem, movement in phloem, storage accumulation and immobilization. Once metal ions have entered the root they can either be stored or transported to the shoot. Metal transport to the shoot probably takes place in the xylem. However metals may redistribute in the shoot via phloem. According to Tiffin (1972) long distance transport of trace metals in higher plants depends upon the vascular tissues (xylem and phloem) and is partly related to transpiration intensity. Chemical forms of heavy metals in phloem exudates differ for each element. Van Goor and Wiersma (1976), for example reported that Zn was almost all bound to organic compounds, while Mn was partly complexed. The distribution and accumulation patterns of heavy metals vary considerably for each element, kind of plant, and growth season.

(iii) Heavy metal resistance: For a plant to resist the toxic effect of heavy metals, they must limit their cellular uptake (avoidance), detoxify the heavy metals once they enter the cells or develop heavy metal resistance metabolism. The evidence for the avoidance of heavy metal toxicity, by reduced cellular uptake, is very limited. But Taylor (1987) gave a detailed account of how metals could be excluded from symplasm. These mechanisms include:

(a) Immobilization of metals at the cell wall: Metal tolerance could be achieved by preferential accumulation of metals in the cell wall, resulting in the reduced uptake of metals into the symplasm. Metal immobilization at the cell wall is an exchange absorption process with the metals binding to carboxyl groups on pectates (Rathore et al., 1972). The major problem with this hypothesis is the cell
wall gets saturated with metals and its inability to explain the specificity of metal tolerance.

(b) Exudation of chelate ligands or organic acids: Metal uptake would be efficiently reduced if plant roots exude chelates or organic acids, which competed with root transport site for metals. It has been found that uptake of chelated metals is generally less toxic. Exudation of chelates could account for the specificity of tolerance. However, chelate ligands would be vulnerable to microbial attack, hence; continued renewal of the external chelates would be required to maintain low ion activities in the substrate. This would require considerable energy, as a result. It would develop an energy strain on the tolerant plant and may not be feasible at high metal concentrations.

(c) Redox barrier at the plasma membrane: This mechanism is seen in plants growing under reducing conditions. The solubility of metals such as Fe and Mn are strongly dependent on redox potential, hence, plants growing on reduced substrate are often stressed by Fe and Mn toxicity. Some of them establish a gradient of redox potential in the rhizosphere, such that reduced ions in the soil must pass through an oxidized zone before entering the plant. So the solubility and availability of Fe and Mn ions get reduced upon the entry into the oxidized zone, effectively reducing the toxicity of metal. This was observed in the roots of *Oryza sativa*, *Spartina alterniflora*, *Phragmites communis*, *Typha latifolia*, and in a number of other wetland plants. But this process of rhizosphere oxidation may be in competition with reduction by microorganism. Plants growing on nutrient deficient soils showed increased root exudation, increased rhizoflora populations, enhanced reduction of Fe$^{3+}$ and enhanced uptake of Fe. Oxidation of the rhizosphere could inhibit the uptake and accumulation of Cu and possibly other metals also. As some metals bind strongly to Fe hydroxides, thus, accumulation of Fe hydroxides at the root soil interface could also create a sink for some metals (Ponnampuruma, 1972).

(d) pH barrier at the plasma membrane: This is conceptually the same as the redox barrier, except that plant must establish a gradient of pH across which metals must pass.
Heavy metals produce toxicity when they are present in high concentration in plant tissues, which has been stated earlier. Now, the following section deals with the fact that how plants tolerate high metal concentration and what biochemical or metabolic changes occurs.

**Toxicity and Tolerance**

Although plants adapt rather readily to chemical stress, they may also be very sensitive to an excess of a particular trace element. Toxic concentrations in plant tissues are very difficult to establish. Visible symptoms of toxicity vary for each species and individual plants, but most common and nonspecific symptoms of phytotoxicity by metals are chlorotic or brown points of leaves and leaf margins, and brown, stunned coralloid roots. According to Burzynski and Buczek (1989), Sharma and Gaur (1995), Rai et al. (1995), Abdel- Basset et al. (1995), Salt et al. (1995) and Ewais (1997), there is a threshold of tolerance in each plant to the heavy metal accumulation. For a number of environmentally, genetically and physiologically determined reasons this threshold is different among plant species. When this limit is crossed, the toxic effect of the metals in the plants takes it toll. Hypothetical schemes of the reactions of plants to increasing concentrations of the essential heavy metals are presented in figure 2.1 and Figure 2.2.

**Figure 2.1.** Schematic diagram of behavioral plasticity of plants under heavy metal stress; (a) no behavioral change of entirely tolerant species; (b) development of behavioral tolerance; (c) reaction of non-tolerant species leading to damage of organisms followed by no recovery.

Source: Pendias et al., 1989

**Figure 2.2.** Schematic diagram of plant response to stress from deficiency and toxicity of heavy metals. (a) Essential heavy metals; (b) nonessential heavy metals.
There are two basic strategies for metal tolerance: metal exclusion and metal accumulation (Baker, 1981, 1987; Taylor, 1987) in different plant species. The exclusion strategy is usually used by pseudometallophytes comprising avoidance of metal uptake and restriction of metal transport to the shoots (De Vos et al., 1992). Exclusion mechanism or external tolerance mechanisms can also be defined as cases where metals are prevented from entering the organism and reaching sensitive parts (Taylor, 1987). These plants are currently used to revegetate bare soil areas e.g in phytostabilisation technique, i.e where the vegetation is absent due to excessively high metal concentration.

In excluders plant metal concentration in aerial parts are maintained low and constant over a wide range of soil concentrations, up to a critical value above which the exclusion mechanism breaks down, resulting in unrestricted transport and toxicity, plant/soil concentration factors are <<1 (Baker, 1981). In some plants the plant/soil ratio lies between 0.5 and 1. These plants reflect the heavy metal concentration in the soil; and are known as indicator plants. The accumulation strategy consists of strong concentration of metals in plant tissue. In accumulator plants the element is concentrated in the aerial portions as well as in the belowground organs (roots and rhizomes) of the plant and is taken up from the soils with high or low concentrations. The ratio of the concentration of the element in the plant to that in the soil is >1. The concentration of the metal rises steeply as soil concentration increases, until all the sites within the plant are filled; it then levels off (Baker, 1981). There is highly specialized physiology in such species. The plants which are called hyperaccumulator plants, show plant/soil metal ratio >1.5. The first reference of heavy metal hyperaccumulation in plants was made in 1865 when *Thlaspi calaminare* (now *Thlaspi caerulescens*) growing on zinc rich soils near the German-Belgium border was reported to contain 17% of Zn in its ash. Thereafter, interests begin to develop in this subject.

The term 'hyperaccumulator' was first coined by Brooks et al. (1977). He defined it as the plants with Ni concentrations higher than 1000 µg/g dry wt (0.1%). This value was based on the fact that, Ni is a plant micronutrient and is found in the vegetative organs in concentrations of 1-10 µg/g d.w. Toxicity occurs when the concentration is higher than 10-50 µg/g d.w. Surveys showed that very few plants contain between 300 and 1000 Ni µg/g d.w. (Brooks et al., 1977) suggesting that a clear distinction between non-accumulators and hyperaccumulators with the 1000µg/g threshold as a good criterion.
for defining Ni-hyperaccumulation. Later, Baker and Brooks (1989) and Baker et al. (1994) defined hyperaccumulators as higher plant species whose shoots contain more than 100 mg Cd/kg, >1000 mg Ni, Pb and Cu/kg or >10,000 mg Zn and Mn/kg (dry wt.) when grown in metal-rich soils. The use of hyperaccumulators in phytoremediation is limited by some factors (Cunningham, 1995). (a) Hyperaccumulators often take up a specific metal. (b) Most hyperaccumulators grow slowly and have small biomass. (c) The plants often grow in remote areas and are rare; in certain cases, mining and other development activities threaten their habitat. (d) Little is known about their breeding potential, pest management, agronomic characteristics and physiology. Therefore, using wild plants as a seed source is also unreliable.

Approximately 400 species of hyperaccumulators have been reported so far (Assunção, 2003) mainly occurring on metal rich soils in both tropical and temperate zones and belonging to a wide range of unrelated families. There are less than 0.2% of all angiosperms identified as hyperaccumulators (Baker et al., 2000). The list includes hyperaccumulators of Ni, Zn, Cd, Pb, Cu, As, Co and Mn (Baker and Brooks, 1989; Brooks, 1998; Baker et al., 2000; Ma et al., 2001). Majority are Ni hyperaccumulators, almost 317 species (Baker et al., 2000). The genera Assylum and Thlaspi from the Brassicaceae family are reported to be the ones that contain highest number of Ni hyperaccumulations. Other species belong to the genera Phyllanthus (Euphorbiaceae), Homalium (Flacourtiaceae), Geissois (Cunoniaceae) and Hybanthus (Violaceae) (Baker and Brooks, 1989; Reeves, 1992). The list of Zn hyperaccumulators is relatively shorter with only 15 species identified (Baker et al., 1992; Brooks, 1998). Zn hyperaccumulators are limited to the genera Thlaspi (11 species) and Arabidopsis (1 species) (Brassicaceae) and just a few species in other families (Baker and Brooks, 1989). Cd hyperaccumulation was reported only for T. caerulescens and Arabidopsis halleri (Brown et al., 1995) and I. Geisingense (Lombi et al., 2000).

Metal hyperaccumulator plants possess additional detoxification mechanisms. For example, in T. geisingense, a Ni hyperaccumulator, high tolerance was due to Ni complexation by histidine which rendered the metal inactive (Krämer et al., 1997; Krämer et al., 1996). Sequestration in the vacuole has been suggested to be responsible for Zn tolerance in the shoots of the Zn-hyperaccumulator T. caerulescens (Lasat et al., 1996; Lasat et al., 1998). Several mechanisms have been proposed to account for Zn
inactivation in the vacuole including precipitation as Zn-phytate (Van Steveninck et al., 1990), and binding to low molecular weight organic acids (Mathys, 1977; Tolrà et al., 1996; Salt et al., 1999). Complexation to low molecular weight organic compounds (<10 kD) was also shown to play a role in tolerance to Ni (Lee et al., 1977). Cadmium is detoxified by binding to phytochelatins (Wagner 1984; Steffens, 1990; Cobbett and Goldsbrough, 1999), a family of thiol (SH)-rich peptides (Rauser, 1990). Metallothioneins (MT), identified in plants and bacteria (Kagi, 1991), are also compounds (proteins) with heavy metal-binding properties (Tomsett et al., 1992). Probably metal hyper accumulation evolved as an adaptive trait, offering a new niche to plants. Current view is that the trait is an extension of the metal tolerance trait as found in many species and it has evolved independently in various genera. The most plausible explanation is that metal hyperaccumulation is a way to reduce damage by herbivory and parasitism (Boyd and Martens, 1994).

Padinha et al. (2000) reported high thiolic protein (which binds metals) and low photosynthetic efficiency in Spartina maritima growing in polluted sites. These indices, reflecting toxic responses, were considered to be useful for quantifying the health of the plants and could potentially be used as biomarkers. Mendelssohn et al. (2001) also examined physiological indicators in Typha domingensis and Spartina alterniflora in response to increasing levels of cadmium. Leaf expansion and the regrowth after initial harvest were significantly reduced with increased cadmium. Of the physiological indicators (photosynthesis, chlorophyll fluorescence, AEC ratio and leaf reflectance) only photosynthesis and AEC ratio responded to cadmium before damage was visible, and were thus the two most sensitive indices. However, decrease in protein content in plants due to heavy metal stress has also been reported by many authors (Jana et al., 1987; Gupta and Chandra, 1996; Sinha et al., 1996). This may be due to increased activity of protease or other catabolic enzymes, which are activated by heavy metals and destroy protein. Increase in heavy metal concentration in soil or culture medium can result in significant decrease in nutrient (N, P, K) concentration in test plants (Trivedi and Erdei, 1992; Antosiewicz, 1993; Gupta and Chandra, 1996). As for example, Srivasatava and Jaiswal, (1989) reported low P content in Spirodela polyrhiza due to increased activity of phosphatase triggered by high heavy metal concentration. Decrease in K content was also reported by Sela et al. (1989) for Azolla filiculoides in the presence of heavy metals.
Several studies on different aquatic plants and algae show that the changes in the concentration of chlorophyll a and b and particularly changes in their ratio is an equally important parameter, which should always be taken into consideration when estimating the effect of an environmental parameter (as heavy metal contaminated soil or water) in plants. Ewais (1997) used *Cyperus difformis* L, Sharma and Gaur, (1995) used *Lemna polyrrhiza*, Abdel-Basset et al. (1995) used two algal species *Chlorella fusca* and *Kirchneriella hungaris*, Sen et al. (1987) and Rai et al. (1995) used *Pistia stratiotes*, Gupta et al. (1996, 1998) used *Hydrilla verticillata* and *Vallisneria spiralis* to evaluate the effect of heavy metals on total chlorophyll concentration. All of them agreed that heavy metal accumulation responsible for the reduction of total chlorophyll concentration had a similar negative effect on the ratio of chlorophyll ‘a’ and ‘b’. This occurs due to faster hydrolysis of ‘chlorophyll a’ compared with ‘chlorophyll b’ when plants are under heavy metal stress (Schoch and Brown 1987; Drazkiewicz, 1994). Meachannisms directly concerned with the reduction of photosynthetic pigments include inhibition of enzymes involved in chlorophyll biosynthesis including \( \delta \)-aminolaevulinic acid (ALA)-dehydratase (Van Assche and Clijsters, 1990) and protochlorophyllide reductase (De Fillipis and Pallaghy, 1994). Substitution of the central magnesium ion by heavy metals can also be an important damage mechanism, where substitution prevents photosynthetic light harvesting and results in chlorophyll decay and breakdown in photosynthetic activity (Prasad and Strazalka, 1999). Manios et al. (2003) reported the effect of heavy metals on the chlorophyll concentration of *Typha latifolia* growing on a substrate containing sewage sludge compost and watered with metaliferus water. In this study the total chlorophyll concentration increased with time in the leaves of plants which were watered with low metal containing water. However, plants watered with high metal containing water showed a decrease in total chlorophyll content. However, statistically there was no significant difference between the mean chlorophyll values of the two different treatments, concluding that no significant toxic action was imposed on the plants by the metals. However, when similar statistical analysis was implemented for the ratios of ‘chl a’ and ‘chl b’, there was significant reduction of the ratios in plants watered with high metal containing water, suggesting some increase in chlorophyll hydrolysis due to the metals accumulation (toxic effect) in comparison with the other groups.
Metabolism of heavy metal compounds in higher plants takes place through a number of mechanisms including oxidation, hydrolysis, conjugation and occasionally reduction (Higashi, 1998; Mumma et al., 1990). Oxidation reactions are most common and crucial (Dohn et al., 1981). Heavy metal metabolism in aquatic macrophytes is poorly understood. Peroxidases, which are ubiquitous in the plant kingdom, are one of the principal enzymes oxidizing heavy metals in plants with a wide spectrum of substrate specificity. The formation of free radical species, which may be initiated directly or indirectly by metals, can cause severe damage to different cell components, particularly biological membranes (Van Assche and Clijsters, 1990; Dietz et al., 1999). Metals such as Cu, Pb and Zn are efficient generators of active oxygen species and therefore, an important factor in the metals toxicity is the generation of oxidative stress (Aust et al., 1985). To avoid the accumulation of these toxic intermediates (hydrogen peroxide, super oxide radical and hydroxyl radical), plant tissues have a series of de-toxifying antioxidants involving both non-enzymatic and enzymatic mechanisms. Peroxidases catalyze quenching reactions of dehydrogenation with transfer of hydrogen from an antioxidant donor to hydrogen peroxide (Vangronsveld and Clijsters, 1994). In consequence, the increase in peroxidase activity on exposure to heavy metals in the cell may play an important cellular defense mechanism against metal toxicity. Peroxidase activity levels are significantly correlated in the tissues of several plant species with levels of Cu (Mocquot et al., 1996), and Zn (Van Asshe et al., 1998). Macfarlane et al. (2001) reported an increase in peroxidase activity and a decrease in chlorophyll content in Avicennia marina (Forsk.) Vierh with increasing heavy metal content in sediments.

Wetland plants are generally good accumulators of metal and show good tolerance towards them. As the present study is on wetland plants, so it is necessary to understand how wetland plants interact with heavy metals in soils and within the plant body. The following section gives an idea on relation of wetland plants with heavy metals. Brief review on studies carried out on these aspects in abroad and India are also given in the following segment.

Wetland Plants and Heavy Metals:

Aquatic macrophytes and wetland plants have been studied for a long time for heavy metal uptake potential, the pathways and mechanism of uptake in heavy metal contaminated natural water bodies, constructed wetlands or in solutions spiked with
heavy metals. An aquatic ecosystem consists of three reservoirs of metals and heavy metals viz. water, sediment and biota. However, metal levels in these reservoirs are determined by a complex equilibrium of various physical, chemical and biological factors. Macrophytes and algae must be considered while assessing the fate and transport of metals in aquatic systems. The uptake of metals through root system and release of metal due to decomposition of plant material represents a pathway of trace metal cycling in aquatic system (Mudroch et al., 1979). From the viewpoint of phytoremediation also many aquatic macrophytes or wetland plants are important for their uptake potential of heavy metals.

Wetland plants such as Eichhornia crassipes, Hydrocotyle umbellata, Lemna minor, Scirpus lacustris, Phragmites karka, Bacopa monnieri, Azolla piñata, Schoenoplectus lacustris, Iris pseudacorus and Typha latifolia are effective accumulators of heavy metals and they have been found efficient for heavy metal removal from aquatic systems (Sadwosky 1999; Mungur et al. 1997; Zayed et al., 1998).

Relations of Wetland Plants with Soil

Wetland sediments are generally considered a sink for metals and, in the anoxic zone, may contain very high concentrations of metals in a reduced state. As such, the bioavailability of the metals is low compared to terrestrial systems with oxidized soils. Different forms of metals have different availability: water-soluble metals and exchangeable metals are the most available, metals precipitated as inorganic compounds, metals complexed with large molecular weight humic materials and metals adsorbed to hydrous oxides are potentially available, and metals precipitated as insoluble sulfide and metals bound within the crystalline lattice of minerals are essentially unavailable (Gambrell, 1994). Because of these reducing conditions, the depth to which plant roots can penetrate is limited and this restricts the uptake of contaminants and rhizosphere actions to shallower levels (Williams, 2002).

Wetland plants can oxidize the soil in the root zone through the movement of oxygen downwards through aerenchyma tissue (Moorhead and Reddy, 1988), and this oxidation can remobilize the metal contaminants, thus increasing the otherwise low availability of metals in wetland soils. Avicennia species of mangroves were found to oxidize the rhizosphere, thus reducing the sulfides concentration and enhancing metal
concentrations in the exchangeable form (De Lacerda et al., 1993). Metal remobilization may also result from the acidification of the rhizosphere by plant exudates (Doyle and Otte, 1997).

Wright and Otte (1999) found that *Typha latifolia* oxidized the rhizosphere, but this did not increase pore water metal concentrations. It caused decreased pH within 1 cm of the roots and increased the concentration of soluble zinc near and beneath the roots, while another plant, *Glyceria fluviatilis*, had little effect on sediment chemistry. Greater uptake and availability of Cd was seen in a number of wetland species under dry (more oxidized) than under flooded (reduced) conditions (Gambrell, 1994). The larger root system and increased number of fine roots of *S. alterniflora* compared with *P. australis* (Ravit et al., 2003) may indicate that the former species will oxidize the rhizosphere to a greater extent, increasing the availability of metals. By oxidizing the soil in the immediate vicinity of the rhizosphere, plants can alter the distribution of metals in wetland soils. Doyle and Otte (1997) found that the concentrations of several metals were higher in vegetated soils than in unvegetated ones, and were particularly high in soils in the immediate vicinity of the plant roots. In a study of cadmium and zinc concentrations in sediment cores from areas under *S. alterniflora* compared with adjacent mud flats without plant cover, the metal concentrations at 5–15 cm depth (corresponding to maximum root biomass) were higher by a factor of 4 under the vegetation (De Lacerda et al., 1997). Plant activity (metal mobilization by oxidation of the root zone and movement into the rhizosphere) was considered responsible for the increase. Similar results were obtained at sites with *S. maritima* by Cacador et al. (1996) who concluded that the roots influence metal concentrations in the marsh sediments as a result of producing complex organic compounds and oxidizing the rhizosphere.

A striking feature of roots of some wetland plants is the presence of metal-rich rhizoconcretions or plaque on the roots (Mendelssohn and Postek, 1982; Vale et al., 1990). These structures are composed mostly of iron hydroxides and other metals such as manganese that are mobilized and precipitated on the root surface. The metals are mobilized from the reduced anoxic sediments and concentrated in the oxidized microenvironment around the roots. Their concentrations can reach 5–10 times the concentrations seen in the surrounding sediments (Sundby et al., 1998). There have been conflicting reports as to whether the presence of the plaque reduces or increases the
uptake of metal by the plants. The presence of these concretions appeared to reduce the amount of zinc taken up by Aster tripolium (Otte et al., 1989) and the amount of manganese taken up by P. australis (Batty et al., 2000). The mechanism may have been through the plaque acting as a physical barrier, although the barrier was not effective at low pH. At higher pH the presence of plaque enhanced Cu uptake into roots. However, in T. latifolia (cattail), the presence of iron plaque did not reduce uptake of toxic metals (Ye et al., 1998). Iron plaque increased zinc uptake by rice (O. sativa) and movement into shoots (Zhang et al., 1998). In contrast, Al was not adsorbed onto the iron or the manganese plaque but formed a separate phosphate deposit that resembled the iron and manganese plaques (Batty et al., 2002). They attributed the precipitate to the leakage of oxygen from the roots, and suggested that the phosphorus might be immobilized at the root surface. The discrepancies in effects of plaque on metal uptake need to be resolved by further study. Different metals, environmental conditions or physiologies may account for these differences.

Another factor than can affect the accumulation of metals in wetland plants is the presence of microbial symbionts such as rhizosphere bacteria. De Souza et al. (1999) found that, when bacteria were inhibited with antibiotics, plants (Scirpus robustus and Polypogon monspeliensis) accumulated lower concentrations of Se and Hg. Likewise, axenically grown plants that had bacteria added to them accumulated more of these metals than axenic controls, indicating an important role of these symbionts for efficient metal uptake. Mycorrhizae (symbiotic fungi associated with roots) provide an interface between the roots and the soil increasing the absorptive surface area of root hairs and are effective at assimilating metals that may be present at toxic concentrations in the soil (Meharg and Cairney, 2000).

Heavy Metal Uptake and Translocation in Wetland Plants

Heavy metals are absorbed by roots and translocated to the aerial parts of the plant. Most of the studies have observed that the heavy metal concentration in the roots of wetland plants is high and there is little translocation from the root to shoot or leaves. Metal concentrations analyzed in roots may reflect some proportion of metals that are merely adsorbed onto the root surface rather than within the root tissue. However, Vesk et al. (1999) analyzed the localization of metals within roots of the water hyacinth (Eichhornia crassipes) using energy dispersive X-ray microanalysis, and found that
copper, lead and zinc were not localized at the root surface, but were more highly concentrated in the inner root tissues. Levels were highest within cells of the stele (the vascular bundles), in electron-dense granules. Significant amounts were also seen in the cell walls. In the roots of seedlings of the grey mangrove, *Avicennia marina*, metals (Cu, Pb and Zn) were concentrated predominantly in cell walls (MacFarlane and Burchett, 2000). The root epidermis served as a barrier to transport of lead to aboveground tissues, but not the other metals. The endodermal casparian strip provided a barrier to the movement of all three metals into the stele. Once in the leaves, however, metals were highest in the xylem, followed by the mesophyll and then hypodermal tissue. Concentrations of metals in the cell walls were also higher than in intracellular locations.

When wetland plants translocate metals from the root tissue to aerial tissue, these are accumulated in leaves and stems. The degree of upward translocation depends on the species of plant, the particular metal and a number of environmental conditions. Fitzgerald et al. (2003) found that copper accumulated primarily in the roots of monocots and dicots, while lead accumulated mainly in the roots of monocots but in the shoots of dicots. *A. marina*, accumulated metals in roots proportional to the concentrations in sediments. However, the degree of accumulation in leaves varied according to the metal. Accumulation of copper in leaves followed a linear relationship at lower concentrations then leveled off, indicating an exclusion mechanism operating at higher sediment levels (MacFarlane et al., 2003). Zinc accumulation in leaves correlated with sediment concentrations even when they were high, indicating the greatest translocation to aerial portions of the plant. However, lead levels in leaves remained quite low at all levels of sediment lead. Lower pH resulted in increased zinc accumulation, and higher levels of lead and zinc in the sediments resulted in greater lead accumulation in roots and shoots.

Heavy metal uptake and translocation by wetland plants are studied by several group of scientist throughout the world. Mudroch et al. (1979) reported on the uptake of heavy metals by aquatic macrophytes from mine effluent in Moria river basin, Canada. The fluvial sediments were contaminated with high concentrations of Ni, Co, Cu and As (upto 1740μg/g, 3570 μg/g, 345μg/g and 1695μg/g dry wt. respectively). Concentrations of these metal were low in water (Co<1μg/l, Ni 3-24μg/l, Cu 1-3μg/l), except As (6-250μg/l). The highest concentration of Ni, Co, Pb, Zn, Cr and As were found in *Myriophyllum verticillatum* (104μg/g, 860μg/g, 37μg/g, 31μg/g, 92μg/g, 6.9μg/g and 1200μg/g respectively) and in *Elodea Canadensis* (72μg/g, 66μg/g, 25μg/g, 17μg/g, 17μg/g,
3.2µg/g and 430µg/g respectively). *Nymphaea odoratae* and *Pontederia cordata* took up metals to a much lesser extent.

In another study (Welsh et al., 1980), examined the Pb and Cu uptake by nine submerged aquatic macrophytes of Ullswater and Conistonwater lakes in the English Lake District. The source of these metals was the mineralization of rocks in that area. Results showed high concentration of lead and copper in shoots, roots and in lake sediments. Positive correlation between concentration of heavy metals in submerged shoots and underlying sediments were found, indicating hyperaccumulation. No correlation was found with water samples.

*Typha latifolia* was reported to tolerate elevated levels of Zn (5000µg/g), Pb (435 µg/g) and Cd (73µg/g) (McNaughton et al., 1974). In another study, in the wetlands of Sudbury (Ontario, Canada) Taylor et al. (1983) showed that despite high levels of Cu and Ni in the soil sediment, these metals were largely excluded from the aboveground tissues of *Typha latifolia*. The patterns of uptake of the metals Cu, Ni, Zn, Fe, Mn and Mg were similar. Roots had higher concentration than the rhizomes and aboveground parts and leaf bases had lower concentrations than leaf tips. Zn, Mg and Ca accumulation in tissues lacked correlation with soil sediment concentration. But the Cu and Ni concentrations were significantly correlated between belowground and reproductive tissues and soil sediment concentration Fe and Mn had significant correlations between soil sediment and virtually all plant tissues. Correlations between soil-sediment pH, Eh and organic carbon and plant heavy metal uptake were also calculated. They showed inconclusive result and failed to exhibit any pattern. However, *Typha latifolia* when grown in solution culture spiked with 100µg/g Cu, 150µg/g Ni and 100µg/g Fe, showed a linear relationship with concentrations of metals in nutrient solution. Leaves accumulated 127 ± 28 µg/g Cu, 467± 50µg/g Ni and 672±-130µg/g Fe, in a 20-day study. Under similar conditions, roots accumulated 2364±209µg/g Cu, 1024± 62 µg/g Ni and 6578± 1106 µg/g Fe, respectively. In 1983, Babcock et al. showed that the common cattail, *Typha latifolia* could take up metals such as As, Cd, Cr, Cu, Fe, Mn, Mo, Ni, Se and Zn from fly ash basin. In another study Gersberg et al. (1986) reported 97% Cu, Zn and Cd removal from metal amended sewage effluent in gravel-based trenches planted with *Scirpus sp* and *Typha* in a 10-month experiment.
Eichhornia one of the world's most noxious, prolific and productive weeds (Tochobanolous, 1987) with a developed fibrous root system and large biomass has been investigated for its wastewater treatment potential by many researchers (Wolverton and McDonald, 1975; Reddy, 1983; Hauser, 1984; Boutin, 1987; Brix 1989). The ability of Eichhornia to absorb metals like Cu (Nor et al., 1986; Lee and Hardy, 1987), Cd (Cooley et al., 1980; O'Keeffe et al., 1984) has been demonstrated. Bioconcentration factor of this plant is reported as high as 2150 for Cd, 1283 for Cr and 595 for Cu (Zhu et al., 1999). It is also very useful for rhizofiltration. It can also readily reduce the levels of heavy metals in acid mine water while exhibiting few signs of toxicity (Falbo et al., 1990; O'Keefe et al. 1996).

Otte et al. (1991) reported that Scirpus maritimus and Spartina anglica growing in a salt marsh contaminated with Zn, Cu and Cd in Dutch coast accumulated high levels of these metals. Sinicrope et al. (1992) reported that Scirpus californicus grown in a wetland mesocosm was able to remove some amount of heavy metals from wastewater, when the wetland mesocosms are subjected to four hydroperiods. After one year of metal additions fine roots accumulated 35% of the added Cd, 6% of Cu and 13% of the Zn. The shoots, rhizomes and coarse roots each accumulated about 1% or less of the added metals. Most of the metals were retained by the soil. Kadelec and Knight (1996) also reported that a subsurface flow wetland planted with Scirpus sp., removed 63%, 86% and 79% of Cu, Pb and Zn respectively.

Phragmites australis, Typha latifolia, Schoenoplectus lacustris, Iris pseudacorus were also found to accumulate varying amounts of Cu, Pb and Zn in roots in an experiment carried out in a constructed wetland (Mungur et al., 1997).

Duckweed (Lemma minor) was a good accumulator of Cd, Se and Cu, a moderate accumulator of Cr, and a poor accumulator of Ni and Pb (Zayed et al., 1998). However, other wetland species such as Vallisneria spiralis L. and Alternathera sessilis accumulated these metals to a lesser extent. Another study carried out in Taiwan reported that Najas graminea Del., Myriophyllum scabratum and Myriophyllum elantinaides had promising potential for metal removal from contaminated aquatic systems (Lee et al., 1998).
Qian et al. (1999) studied twelve wetland species to determine the phytoaccumulation capability of ten heavy metals viz As, B, Cd, Cr, Cu, Pb, Mn, Hg, Ni and Se. Except B, highest tissue concentration of heavy metals were found in roots than in shoots. Highest tissue concentrations in shoot (mg/kg dry weight) were found in *Cyperus alternifolius* L. for Mn (198), and Cr (44); *Wedelia trilobata* Hichc. For Cd (148) and Ni (80); *Polygonum hydropiperoides* Michx for Cu (95) and Pb (64); *Pistia stratoiotites* L. for Hg (92), As (34) and Se (39) and *Hippuris vulgaris* L. for B (1132). Highest concentrations in roots were in *Baumea rubiginosa* for Mn (1683); *Myriophyllum brasiliense* Camb for Cd (1426) and Ni (1077), *Pistia stratoiotites* L. for Cu (1038), Hg (92), and As (177); *Polygonum hydropiperoides* Michx for Cr (2980) and Pb (1882); *Hippuris vulgaris* L. for B (1277) and *Mimulus guttatus* Fisch. for Se (384).

Groudeva et al. (2001) reported removal of heavy metals from a constructed wetland using *Typha latifolia*, *Typha angustifolia*, *Phragmites communis*, *Scirpus lacustris*, *Juncus sp.* and different algae. Initially the concentration of heavy metals in wastewater was two to eight times higher than the permissible levels, but after treatment heavy metal concentrations decreased below the permissible limit.

Shu et al. (2002) reported the ability to tolerate and accumulate Pb, Zn and Cu in populations of *Paspalum distichum* and *Cynodon dactylon* under laboratory conditions. Both the species were found to grow naturally on mine tailings with high levels of Pb, Zn and Cu concentrations. *Paspalum distichum* was more tolerant and it accumulated all the three metals more than *Cynodon dactylon*.

Kamal et al. (2004) examined phytoaccumulation of heavy metals by three aquatic plants viz. parrot feather (*Myriophyllum aquaticum*), creeping primrose (*Ludwigina palustris*), and water mint (*Mentha aquatic*). The average efficiency of the three species for removal of heavy metals from municipal wastewater was 99.8%, 76.7%, 41.62%, and 33.9% of Hg, Fe, Cu, and Zn, respectively. Parrot feather showed greater tolerance to toxicity followed by water mint and creeping primrose. The growth of creeping primrose was significantly affected by heavy metal toxicity. The selectivity of heavy metals for the three plant species was the same (Hg>Fe>Cu>Zn).
Deng et al. (2006) reported Pb and Zn tolerance of six wetland species viz. *Alternanthera philoxeroides, Oenanthe javanica, Beckmannia syzigachne, Lessia hexandra, Neyraudia reymudiana* and *Polypogon fugax* in China. All the species exceeded the threshold limit for toxicity of Pb and Zn in plants. The higher-than-toxic level of metal concentrations in plants, such as Pb (990 mg kg/l) and Zn (2345 mg kg/l) in shoots of *A. philoxeroides*, and Zn in shoots of *L. hexandra* (592 mg kg/l) and *O. javanica* (503 mg kg/l), indicated that some internal detoxification mechanisms for metal tolerance might be involved.

Madejon et al. (2006) reported 0.25mg/kg Cd and 61 mg/kg Zn accumulation in tubers of *Scirpus maritimus* L. form Donana marshes (South Spain). However, concentrations of As, Pb, Cu, Zn and Ti were below toxic limits in the tubers.

**Studies in India:**

In India several studies have been done on the heavy metal uptake by wetland plants. I Sen et al. (1987) reported accumulation of Cr (VI) from culture medium by *Pistia stratiotes*. 90% removal of Cr (VI) was noted below 5-ppm concentration. Cr (VI) was accumulated mainly in the roots. At 5 ppm and 10 ppm, Cr (VI) promoted senescence of *Pistia stratiotes* plants by decreasing chlorophylls, protein, RNA, Hill activity. The activities of protease, catalase, alkaline phosphatase showed reverse trend. Sen et al. (1989) found *Salvania natanas* very efficient (about 90%) in removing Cu (II) from the wastewater of Hindusthan Copper Limited, Ghatksila, Bihar, India. Jain et al. (1989) showed *Azolla piñata* and *Lemna minor* removed lead and zinc effectively from the water containing 1, 2, 4 and 8mg/l of Pb and Zn in a 14 days experiment.

Increase in Ni accumualtaion in *Salvania natanas* with increasing time was reported by Sen and Bhattacharya (1994). Maximum accumulation was noted in day one in a 6-day experiment. Roots and shoots accumulated upto 14.75 µg/ml and 5.25µg/ml of Ni. Ni > 10 µg/ml promoted senescence of *Salvinia* plants by decreasing chlorophylls, protein, amino acid, Hill activity, dry wt and by inducing necrosis. Gupta et al. (1994) reported lead uptake potential of two aquatic macrophytes *Vallisneria spiralis* and *Hydrilla verticulata*. Both the plants showed sufficient accumulation of lead, though it
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was insignificant in lower concentration. But Pb concentration 100\(\mu\)M showed inhibition in chlorophyll protein content and in vivo nitrate reductase activity.

Rai et al. (1995) reported appreciable amounts of Cd accumulation from aqueous solution in the roots and leaves of *Pistia stratiotes* L. The accumulation of Cd was accompanied by a decline in cellular levels of glutathione, which was more evident in roots at different treatments durations. 2.5 \(\mu\)M and 10\(\mu\)M concentrations of Cd enhanced cysteine content, accumulation of acid soluble thiols and induced synthesis of phytochelatins. Lower concentrations of Cd induced low molecular weight proteins while higher concentration completely low molecular weight proteins and reduced concentrations of other high molecular proteins in the SDS-Gel electrophoresis patterns. In another study Rai et al., (1995) reported heavy metal (Cu, Cr, Fe, Mn, Cd, Pb) removal potential of *Hydrodictyon reticulatum*, *Spirodela polyrhiza*, *Chara corollina*, *Ceratophyllum demersum*, *Vallisneria spiralis*, *bacopa mannieri*, *alternanthera sessilis* and *Hygrophila aristata* under laboratory conditions. Results showed a decrease in Cr level from 4.866\(\mu\)M to below maximum permissible limit by *C. demersum*, *H. reticulatum*and *S. polyrrhiza* within 15 day. Similarly, elevated levels of Fe were reduced to below permissible limit by *C. demersum* and *H. reticulatum* after 15 day. Manganese concentration (6.63 \(\mu\)M) were reduced to 1.63\(\mu\)M by *C. demersum* and *H. reticulatum* in 7 day. *Bacopa monnieri* and *H. aristata* decreased Cd levels from 0.155 \(\mu\)M to 0.009 \(\mu\)M whereas *S. polyrrhiza* and *H. reticulatum* reduced levels to 0.036 \(\mu\)M after 15 day of treatment. More than 70% Pb was removed by *C. demersum*, *H. aristata* and *H. reticulatum* within 15 day. Results suggest the use of these plants for metal abatement in dilute wastewaters.

Gupta et al. (1996), reported that *Hydrilla verticillata* is capable of accumulating Hg efficiently. No significant phytotoxicity was observed at lower (0.5\(\mu\)M and 1.0\(\mu\)M) ambient Hg concentration. Reduction in chlorophyll content, protein, cystein and N', P, K was observed above 1.0\(\mu\)M concentrations. The same author reported Hg accumulating potential of another aquatic macrophyte *Vallisneria spiralis* in 1998. Plants were subjected to different concentrations (0.5, 1.0, 5.0, 10, 20 \(\mu\)M) of Hg for 24, 48, 96 and 168 hours. Plants showed Hg accumulation of 0.25\(\mu\)M /g dry wt in leaf and 1.12 \(\mu\)M/g
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dry wt. in roots, respectively, at 20μm Hg after 168 hr. in this case also the chlorophyll, protein, N, P, K content decreased with increasing Hg concentration.

Ali et al. (1999) reported high metal accumulation in the roots of Salix growing in Lake Nainital, India. In 2000, Mehra et al. reported high metal removal capacity of Eichornia crassipes grown on fly ash slurry of Indraprastha Power Station, New Delhi and water hyacinth in the banks of river Yamuna.

Rupainwar et al. (2004) also reported Zn removal from wastewater by Eichornia crassipes. The study showed that the growth was significant at low metal concentrations, i.e 2.0 and 4.0 mg/l of Zn. At higher metal concentration growth was inhibited. The metal concentration increased in different plant parts as the metal concentration increased in water but the accumulation was more in roots than top.

Bhattacharya et al. (2006) reported that Scirpus littoralis accumulated Mn, Ni, Cu, Zn and Pb up to a maximum of 494.92, 56.37, 144.98, 207.95 and 93.08 ppm dry wt respectively in belowground organs, from fly ash and metal spiked soils under waterlogged conditions. However, there was little translocation from roots to shoots.

Most of the studies carried on wetland plants dealt with heavy metal accumulation capacity and biochemical or metabolic changes caused by heavy metals. Most of them were either short term or field studies, and analyzed the change in total metal concentration or only the available fractions of heavy metals in soils. Few studies were done relating heavy metal speciation and their uptake by wetland plants. Moreover, to my knowledge no study has been done in India relating metal speciation in soil, growth and metal accumulation in wetland plants. So the present study was focused on these aspects involving two wetland plants namely Paspalum distichum and Scirpus littoralis which again has not been studied in India except (study of Bhattacharya et al., 2006 on Scirpus sp).