ACCUMULATION AND TOXICITY OF HEAVY METALS, PARTICULARLY CADMIUM, IN LEMNA POLYRRHIZA L. AND AZOLLA PINNATA R. Br.

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TO WHOMSOEVER IT MAY CONCERN

This is to certify that the thesis entitled " ACCUMULATION AND TOXICITY OF HEAVY METALS, PARTICULARLY CADMIUM, IN LEMNA POLYRRHIZA L. AND AZOLLA PINNATA R. BR.", submitted by Mr. Norbert Noraho for the Degree of Doctor of Philosophy of the North-Eastern Hill University, Shillong embodies the record of original investigations carried out by him under my supervision. The thesis presented is worthy of being considered for the award of the Ph. D. Degree. This work has not been submitted for any Degree of any other university.

Y. S. Chauhan
(Y. S. Chauhan)
Supervisor

CONTENTS

PREFACE

In the aftermath of Minamata and Nigata incidents in Japan, a lot of concern has been aroused about the harmful effects of heavy metal pollution in aquatic environment. This led to a surge in studies concerning metal pollution effects on aquatic organisms. Although a good deal of information has been generated on metal effects and accumulation in algae, fungi and bacteria, higher plants, including aquatic macrophytes, have been little explored in this regard. Macrophytes have great relevance in such kinds of investigations because they are important primary producers in ponds and lakes. Tremendous potential exists in using these plants for metal toxicity bioassays, and also for the reclamation of metal-enriched wastewaters. These considerations prompted me to take up this work. Lemna polyrrhiza L. and Azolla pinnata R.Br. which grow abundantly in pools, ponds and lakes in India, were selected as the test organisms in the present study.

The thesis is organised into six chapters with 'Summary' at the end. The Chapter I 'General Introduction' reviews the current status of the subject and gives the reasons for embarking upon this study. The second chapter deals with toxicity and accumulation of Cd, Co, Cr, Cu, Ni, Pb and Zn in test plants. The kinetics of extracellular and intracellular uptake of Cd are described in Chapter III. This is followed by investigations on influence of cations and metals on Cd uptake in Chapter IV. The next chapter (Chapter V) discusses the influence of physico-chemical characteristics of the environment on Cd toxicity and accumulation. Although each chapter includes discussion, a brief 'General Discussion' (Chapter VI) has also been included. A list of papers cited in the text is given at the end in 'Literature Cited'.

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(Norbert Noraho)

GENERAL INTRODUCTION

The term 'heavy metal' although often not rigidly defined, is generally held to those metals having specific gravity greater than 5 g cm^{-3}, about 40 elements in all (Passow et al. 1961). Nieboer and Richardson (1980) suggested the abandonment of the term 'heavy metal', and its replacement with a classification separating metals and metalloids into class A (oxygen-seeking), class B (nitrogen/sulfur-seeking) or borderline (intermediate between A and B) category. This classification is related to atomic properties such as electronegativity and ionic radius, and solution chemistry of metal ions. Class A ions include the alkali metals and alkaline earths, notably the biologically essential K^+, Na^+, Mg^{2+} and Ca^{2+}. Class B ions, in contrast, include Cu^+, Hg^{2+}, Ag^{2+} and Pb^{2+} which are extremely toxic and for the most part non-essential. Borderline ions include Fe^{2+}, Fe^{3+}, Mn^{2+} and Cu^{2+}, which have biological roles. Whitton (1984), however, advocates that the term 'heavy metal' should continue to be used in pollution studies. Tiller (1989) pointed out that 'heavy metal' may be a useful umbrella for metals classed as environmental pollutants. These heavy metals constitute a very heterogenous group of elements which greatly differ in their chemical properties and biological functions. However, high concentrations of all heavy metals are toxic to living organisms.

Low concentrations of some heavy metals, such as, Cu, Fe, Mn and Zn, are essential for the metabolic machinery of plants. Nevertheless, metals are toxic to microbes, plants and animals at higher concentrations. The essential heavy metals serve as co-factors and activators in enzymatic reactions, e.g., in forming enzyme/substrate/metal complexes (Mildvan 1970), or they exert their catalytic properties as a prosthatic group in metallo-proteins. Most of these essential metals function by valency change (Sändmann and Boger 1983). Copper is an essential element for plants, and is a component of metalloenzymes and respiratory pigments, such as laccase, ascorbate oxidase, plastocyanin, tyrosinase and amine oxidases (NAS 1977). Manganese has an important role in the reactions of some enzymes (e.g., malic dehydrogenase and oxalosuccinic decarboxylase) in the Krebs cycle (Devlin 1979). It is also needed for water-splitting activity of PS II and for superoxide dismutase. Nickel is essential for plant growth as it acts as a co-factor for the urease enzyme system (Dixon et al. 1975, Polacco 1977). It is also needed for the activity of hydrogenase. In plants, Zn is an essential component of several enzymes such as carbonic anhydrase, alcohol dehydrogenase, and glutamic dehydrogenase. Zinc catalyzes oxidation process in plant cells, participates in the formation of carbohydrates and helps in the absorption of water (Lindsay 1972, Farnworth and Kline 1973). Although regarded as non-essential, Cd has been recently found to replace Zn in some of its functions in the marine diatom *Thalassiosira weissflogii* (Price and Morel 1990a). The actual site(s) of replacement remains to be elucidated, but in suspension-cultured tobacco cells, stimulation of biomass production by Cd is correlated with an increase in RNA synthesis (Hirt et al. 1989), suggesting that Cd acts at the level of transcription.

Heavy metal input into aquatic environments occurs from two sources: (i) natural (geologic weathering processes), and (ii) man-made (anthropogenic). One can be misled into believing that metal pollution arises only through anthropogenic means, but geothermal discharges, especially if they are saline or at low pH or high temperature, can contain considerable levels of metals (Smith 1986). Geothermal springs can contaminate natural waterways and algae growing in hot mud pools (Förstner and Wittmann 1981), and submarine geothermal activity is no doubt a source of trace metals in oceanic systems. Volcanic action is a major natural source of Cd (Hutton 1987), and high background levels of As and Hg in streams resulting from weathering of rocks and soil have also been recorded (Förstner and Wittman 1981).

The main sources of metals from a variety of anthropogenic inputs on a global basis are atmospheric fall out, manufacturing processes of metals, chemicals, domestic wastewater, dumping of sewage, sludge, etc. Lead contamination in freshwater and marine systems is unusual in that it arises mainly by atmospheric transport. Organolead comes from volatilization of gasoline additives, but inorganic Pb is a more general problem (Jaworski 1987). An inventory for Lake Erie (Nriagu et al. 1979) revealed that atmospheric inputs accounted for 20 and 50% of Cu and Pb, respectively. Atmospheric dispersion of Hg is probably via Hg^o.

Metal contamination from urban run off and erosion is significant (Jaworski 1987). Organometallic compounds, such as tributyl tin used as antifouling paints on pleasure crafts, can lead to reduced primary productivity in estuaries and marina (e.g., Langston 1990).

Heavy metals in aquatic environments include metals in the sediment, in suspended particulates (>0.45 µM), adsorbed onto oxides and humic colloides, and in true solution. Particles arising from dead biomass are particularly effective in scavenging metals from solution, a high percentage of metals is usually carried by rivers to the oceans in particulate matter (Martin and Whitfield 1983). The enrichment of metals in the dissolved phase (from interaction with sediments and particulate matter) may be more dependent on local natural hydrochemical conditions, than on further anthropogenic inputs. Thus dissolved Cd levels along rivers can fluctuate by as much as a factor of eight depending on local conditions of pH, salinity, chloride ions and alkaline earth elements (Meybeck et al. 1989). Estuaries can be considered to be zones of metal deposition because of reduced flow rates, changes in pH and flocculation of negatively charged particles and colloidal aggregates of Fe oxides and organic materials at the freshwater-seawater interface (Moore and Ramamoorthy 1984). Bryan et al. (1985) listed some of the more common metal species present in aquatic systems, but there is an almost endless array of metal species possible in polluted and unpolluted waters as inorganic complexes, as complexes derived from synthetic and natural chelators present in water, as organometallic compounds of both anthropogenic and natural origins. The biomethylation of Hg, As, and Sn have been shown to occur (e.g., Smith 1986).

Industrial and mining effluents can contain extremely high concentrations of metals, which can persist despite remedial works to stabilize spoil heaps. For example, in Australia, the concentrations of Zn in the river immediately below a mine were still very high (up to 25.3 mg l^{-1}) twenty years after the mine had closed (Kelly 1988). Heavily contaminated sites such as these are generally impoverished or completely denuded in fauna. Resulting changes in species abundances of both algae and fauna have been described at length by Kelly (1988).

Upon exposure to high levels of heavy metals, algae and higher plants accumulate these pollutants to a dangerous extent. A great deal of information has been generated on algae, whereas higher plants, including macrophytes, have been little explored in this regard. Lemna trisulca could concentrate Cd in its tissues upto 24,800 times in comparison to the growth medium (Huebert and Shay 1991). Lemna valdiviana accumulated Cu more than 10,000 times the concentration in the culture solution, and the concentration appeared to increase at the higher levels of metal in solution (Hutchinson and Czyrska 1975). The water hyacinth can accumulate upto 500 ppm of Cd, Pb and Hg (Chigbo et al. 1982). Azolla filiculoides accumulated Cd, Cu, Ni and Zn at concentrations 500 to 1,000 fold higher than in the growth medium (Sela et al. 1989). Agrostis grown in culture solution, containing 6 ppm Cu accumulated 3,500 ppm metal in roots (Wu et al. 1975). In freshwater plants the concentration of Cd ranges between 0.15 and 342 $\mu g\ g^{-1}$ (Moore and Ramamurthy 1984).

In higher plants, roots are the first organs to come in contact with the toxic metals, and roots usually accumulate significantly higher metal levels than the aboveground plant parts (Breckle 1989). Koeppe (1977) observed that roots generally contain two-fold cadmium concentration in comparison to the top portion. Accumulation of As in the root of *Silene vulgaris* is much higher than the shoot (Paliouris and Hutchinson 1991). In *Eichhornia crassipes* and *Pistia straliotes* the concentration of As in the roots was an order higher than that in the leaves (Lee et al. 1991). High quantities of Cd, Cu and U were localized within the cell wall in the shoot and root of *Azolla* (Sela et al. 1988), and as much as 70% of the total content of Cd taken up by beans was stored in the cytoplasmic fraction in roots (Weigel and Jager 1980). All these reports tend to indicate that roots can immobilize heavy metals and raise a very efficient barrier against heavy metal translocation within the plant. Leita et al. (1991) also indicated the existence of a physiological barrier for Cd in roots and stems of *Phaseolus vulgaris*.

It would be tempting to assume that because primary producers are at the bottom of the trophic ladder, herbivory would lead to the biomagnification of metals within higher trophic levels. Although biomagnification, the process whereby a substance is found at higher total body tissue concentrations at successively higher trophic levels, may be true for certain organic pollutants. The case of heavy metals points to the reverse (Moore and Ramamoorthy 1984). In general, with the possible exception of Hg and As (e.g., Kneip and Laver 1973, Forstner and Wittmann 1981, Langston and Bryan 1984, Prahalad and Seenayya 1988), heavy metals are not biomagnified from algae because algae contain higher concentrations in polluted and unpolluted situations than the next member in the food chain (Kneip and Laver 1973, Forstner and Wittmann 1981). Some recent examples which have largely confirmed the absence of biomagnification from algae include studies on As, Cr, Mn, Fe, Cd, Cu, Pb and Zn (Moore and Ramamoorthy 1984, Tateda et al. 1985, Romeo and Nicolas 1986, Prahalad and Seenayya 1986, Seenayya and Prahalad 1987, Sanders et al. 1989).

The process of heavy metal uptake by organisms is very complex, and dependent on the metal ion and the biological system in question. In aquatic plants the uptake occurs in two stages, an initial rapid uptake (passive uptake) followed by a much slower uptake (active uptake). During the passive uptake the metal ions adsorb onto the surface of the cells within a relatively short span of time (few seconds or minutes). This includes the physical adsorption, ion exchange and chemosorption. In the second stage, the metal ions are transported across the cell membrane into the cytoplasm. The surface adsorption does not involve any metabolic process or require any expenditure of energy by the cells, while the membrane transport is dependent on cell energetics and metabolism. Two phases can also be distinguished in the process of Cd uptake by algae. The first fast phase is Cd adsorption on the cell surface and the second slow phase is the energy-dependent transport (Skowronski 1984a and 1984b). Similarly, Skipnes et al. (1975) have shown that the uptake of Zn in Ascophyllum nodosum occurs through a fast reversible process and a relatively slow irreversible process. In water hyacinth, Turnquist et al. (1990) found a rapid initial Ni uptake extending through approximately the first 4 h at lower concentration, but of some what shorter duration at higher concentrations. The uptake of Ni was increased by an increase in the root mass. The uptake of Cd by Chlorella also follows two phases, the initial was not affected by temperature or by light, whereas the following phase was light- and temperature-dependent, and the absorbed Cd was firmly bound to the cells (Sakaguchi et al. 1979, Gipps and Coller 1980a). Thus, the initial phase may be considered as adsorption at the cell surface or penetration into the free space. The following phase has characteristics of a carrier-mediated transport and may represent an uptake into the cells with binding to cell membrane or intracellular components (Parry and Hayward 1973, Sakaguchi et al. 1979, Stacey and Klaassen 1980).

Adsorption and uptake of metal ions will, however, depend upon the nature and chemical composition of cell surface in direct contact with metal ions. Cell surfaces are known to consist of a mosaic of interspersed cationic and anionic exchange sites, with the net charge on the cell wall being dependent on the extent to which these sites are occupied by the anions or cations (Davies 1978). Phytoplankton cells exhibit large surface areas containing various functional groups, such as, carboxylic, amino, thio, hydroxo, and hydroxy-carboxylic, that can interact coordinately with heavy metals (Crist et al. 1981). Among the metal binding chemical groups present in the bacterial cell wall (carboxyl, phosphate, amine and hydroxyl), the carboxyl groups seems to be of great importance (Bauda and Block 1990). The alteration of carboxyl groups in cell wall of gram positive bacteria and *E. coli* severely limited binding of a large number of metals (Doyle et al. 1980, Bauda and Block 1990). It seems that the carboxyl-metal interaction is directly influenced by the electric charge. Introduction of positive charges into the cell walls results in a decrease of the metal binding. Decreased pH diminishes the affinity of the wall for metal, suggesting that metallic cations and protons compete for the same binding site (Doyle et al. 1980).

Heavy metals disrupt many physiological processes in plants. Metals like Cd, Ag, Pb are phytotoxic even in very small amounts (Sandmann and Böger 1983). These metals have a strong affinity for acidic and thiol groups of proteins and nucleotides, and thus interfere with the function of these biologically-important macromolecules.

Cadmium ions at low concentrations can uncouple mitochondrial phosphorylation. Cadmium has a tendency to replace

Zn in certain enzymes, altering their stereostructures, and impairing their catalytic functions. It also has an affinity for sulfur and carboxylate sites (Carty et al. 1976). Cadmium interacts with phospholipid monolayer, and this may affect the biological membranes (Wong et al. 1980). Simpson (1981) reported that sublethal concentrations of cadmium suppress photosynthesis in marine algae by uncoupling the photosystem II, electron transport system. Cadmium is also known to disrupt normal cell division process (Nakamo et al., 1978, De Fillipis et al. 1981). Chromium is considered to be toxic to plants interfering with the uptake by root of some essential elements, e.g., Ca, K, P and their translocation (NRC 1976). Excess of copper results in an inhibition of photosynthetic electron transport (Shioi et al. 1978, Bohner et al. 1980). When present at comparatively high levels within the chloroplast, the redoxactive copper ions compete with catalase for hydrogen peroxide and other peroxo compounds. Lead inhibits photosynthesis and ATP synthesis (Silverberg 1975). Green house experiments have shown that lead decreased Ca, Mg, K and P uptake by corn plants, and also reduced their growth (Walker et al. 1977). Chlorosis at excessive levels of Zn, Cu, Ni and Cd appears to be due to a direct or an indirect interaction with foliar Fe (Chaney et al. 1975). Tyler (1981) reported that inactivation of enzymes by heavy metals could be a result of masking of active groups, protein denaturation, effect on enzyme conformation and competition with activating cations involved in the formation of enzyme-substrate complexes.

Heavy metals are known to interfere with acquisition and assimilation of certain nutrients. Alkaline phosphate, an ecto-enzyme needed for utilizing dissolved organic phosphorus, has been shown to be inhibited by Cu (Rueter 1983). Inhibition occurs at concentrations that do not inhibit the growth rate of the organism (Brand et al. 1986), when orthophosphate is the phosphorus source. Certain inorganic metal complexes interfere with the transport and assimilation of major nutrients. Arsenate, a structural analogue of PO_4^{3-}, competitively inhibits P transport in a marine yeast (Button et al. 1973), and inhibits growth and P uptake of phytoplankton (Planas and Healey 1978). On the other hand, growths of some As-resistant phytoplankton cultured under P limitation are actually stimulated by arsenate addition (Creed et al. 1990). This perhaps represents As substitution for P in certain metabolic functions. In light of the two contrasting responses, the importance of As/P interactions seems to be difficult to predict at this stage.

The transport and metabolism of essential heavy metals may be inhibited by high concentrations of other heavy metals. A high concentration of Cu exerts its toxic effect by interfering with Mn metabolism (Sunda et al. 1981). As Cu concentration increases the intracellular Mn level decreases and the growth rate is reduced. Cadmium exerts its toxic effects on Thalassiosira weissflogii by inhibiting Fe transport and by interfering with Fe metabolism (Harrison and Morel 1983). At low levels of ferric ions, Fe uptake rates are competitively inhibited by Cd, resulting in decreased intracellular Fe concentration and growth. It has been further pointed out by Harrison and Morel (1983) that Cd blocks or interferes with Fe assimilation and creates a condition of Fe deficiency in these cells in spite of high intracellular Fe levels.

The order of toxicity of heavy metals has been found to vary from organism to organism. Among the most toxic heavy metals are Hg, Cd and Ag, whereas Pb and Zn are the least toxic (Hutchinson 1973, Rosko and Rachlin 1975, Gächter 1976, Rai et al. 1981a, Fisher et al. 1984, Kapur and Chopra 1989, Sela et al. 1989, Wong and Chang 1991).

Plants are known to synthesize metal-binding proteins, popularly known as phytochelatins, in response to heavy metal stress. These polypeptides are composed of the repeating dipeptide units of gamma-glutamylcysteinyl with a single carboxyl terminal glycine residue - $(\gamma\text{-}EC)_nG$ (Robinson 1989). Grill et al. (1985, 1987, 1988) found that phytochelatins (oliogo-peptides capable of binding heavy metal ions via thiolate coordination) are the principal metal binding components of plants. Low molecular weight, cysteine-rich, soluble, metal-binding proteins, similar to the metallothioneins studied extensively in animals (Webb 1975) have been found in resistant plant cells (see Steffen 1990). In Datura innoxia cells that are resistant to Cd, such a metal binding protein has been found and de novo syntesis of this protein has been found to be induced by Cd (Delhaize et al. 1989). A similar Cu-binding metallothionein-like protein has been found in the roots of Mimulus gattatus

(Salt et al. 1989). In *Scenedesmus actutiformis* and *C. fusca* the synthesis of (gamma EC)$_n$G has been shown to increase following the exposure to Cd, Pb, Zn, Ag, Cu and Hg (Gekeler et al. 1988). On the basis of the results obtained, Leita et al. (1991) confirmed that the synthesis of Cd-associated polypeptides with low molecular weight was induced in leaves, stems and roots. In addition roots were able to synthesize another specific protein fraction with higher apparent molecular weight, and this can contribute to elucidate the higher ability of roots to retain Cd. They also showed that Cd ions can induce the biosyntesis of both metallothionein and phytochelatin in roots of bushbean. Various metal binding substances have been isolated from fungi, including the Cd and Cu-metallothioneins from *Saccharomyces cerevisiae*, animal-like Cu-metallothioneins from *Neurospora crassa* (Lerch 1980) and *Agaricus bisporus* (Münger and Lerch 1985), and also Cd-cadystins (phytochelatins) induced by Cd^{2+} and other metal ions (Grill et al. 1985, Robinson and Jackson 1986, Reese and Wagner 1987).

Phytochelatins are distinctive in that heavy metals are the primary inducers. Cadmium, Pb, Zn, Sb, Ag, Ni, Hg, Cu, Sn, Au, Bi, Te, and W induced phytochelatins (Grill et al. 1987). Among the common metals, Cd is the strongest inducer, while Zn appears to be the weakest, requiring very high levels for induction (Steffens 1990), and phytochelatin biosynthesis is tightly regulated by the availability of metal ions. Biochemical analysis of tissue samples showed that acclimation to Cd by

Salvinia minima parents led to increases of phytochelatins and thiols in daughter ramets (Outridge and Hutchinson 1991). Increased accumulation of (gamma EC)$_n$G occurs very rapidly following exposure to elevated concentrations of metals (Grill et al. 1986). In one higher plant cell line increased accumulation of (gamma EC)$_n$G was detected as early as 5 min after exposure to Cd (Robinson et al. 1988).

The phytochelatin response or synthesis of heavy metal-binding polypeptides, in plants is an adaptive response. The extent to which this response accounts for the differential tolerance is not clear (Steffens 1990). However, it is evident that phytochelatins play a major role in the detoxification of excess metals. Phytochelatins are also involved in trace metal homeostasis, and their participation in detoxification of excess metals may be a consequence of this homeostasis.

Exclusion is yet another mechanism adopted by plants for resisting heavy metals. The mechanism(s) responsible for the exclusion of the metalloid As from the shoots of tolerant Silene vulgaris individuals is perhaps located in the roots and it may be any one of the following: Cell wall binding, complexation with organic acids, and complexation with metal binding proteins (Paliouris and Hutchinson 1991). In Plectonema boryanum, Jensen et al. (1982a) have found that a range of heavy metals, including Zn, are taken up and sequestered in sectors of the cell with polyphosphate bodies.

Harding and Whitton (1981) reported genetic adaptation to high zinc levels of a natural population of <u>Anthoxanthum odoratum.</u> They found that filamentous green algae from high Zn site were more tolerant than those from low zinc site. Heavy metals affect the number, species diversity and productivity of microbiota in aquatic ecosystems. Decreased growth of a natural phytoplankton community occurred in estuarine waters supplemented with 10 μM Hg or 100 μM Cd, Pb or Zn (Hollibaugh et al. 1980). Blue green and diatoms appear to be less tolerant than green algae, and metal-contaminated waters favour filamentous green algae (Whitton 1970). Shifts in the species of phytoplankton and decreases in the numbers of species were evident in lake waters amended with combinations of Hg, Cu, Cd, Zn and Pb (Gächter and Mares 1979). Primary productivity of natural communities is also affected by heavy metal contaminants. Mercury, Cu or Cd at 10 ppb inhibited photosynthesis of a phytoplankton community collected in the west Caspian Sea, but 10 ppb Zn stimulated photosynthesis by 15% (Babich and Stotzky 1985). Williams and Mount (1965) determined that 9 mg Zn l^{-1} causes shift from predominantly autotrophic to heterotrophic communities of epilithon. Colwell et al. (1989) also found changes in epilithon community dosed with 1 mg Zn l^{-1}. Kumari et al. (1991) reported a direct relationship of phytoplankters with Fe, Mn, Co and an inverse relationship with Zn, Cu, Pb and Ni. Aquatic plants growing in pools contaminated with mine debris can partially reflect the concentration of metals in the water (Lee et al. 1991).

The physico-chemical characteristics of an environment into which heavy metals are deposited determine the chemical speciation forms and hence the bioavailability and toxicity of heavy metals to indigenous biota (Babich and Stotzky 1985). The interactive effect of environmental factors on the toxicity of heavy metals is therefore extremely important for realistic interpretations. Rates of uptake, translocation, accumulation and concentration or retention of heavy metals could be influenced by temperature, light, pH and ionic nature of the metal, type of toxicant combinations, level of metals in the medium, existence of competing metal chelators and the physiological state and type of the organism (Ting et al. 1989). Accumulation of zinc and copper is temperature-dependent in *Fucus serratus*, but temperature-independent in *Potella vulgata* (Miramand and Bentley 1992). Cadmium transport into *Stichococcus bacillaris* greatly depended on temperature and pH (Skowroński 1986b). The uptake of Cd is also affected by temperature in *Lemna minor* (Kwan and Smith 1991). It is generally observed that free ions predominates at low pH in solutions (Darimont and Frenay 1990). At high pH, the complexes such as carbonates, oxides, hydroxides, and silicates are more stable and thus prevail. Soeder et al. (1978) also demonstrated an increase in the uptake of Cd by algal cells at lower pH. The interaction intensities are pH-dependent for Cd in oxidative medium; in alkaline conditions insoluble hydroxides are formed and become unavailable to test plants (Kwan and Smith 1991). In mixture of two or more metal species in solutions the synergistic or antagonistic interactions occurring between the metal ions may affect the uptake of individual metals. The response of organisms to mixtures of metals may show antagonistic or synergistic interactions. The most logical reason for antagonistic action was claimed to be the competition for adsorption sites on the cells and competititon for transport across the membrane with the more efficient competitor preventing the uptake of the other metal. Another mechanism of antagonism between heavy metals may involve the sorption of one heavy metal to the amorphous complex of the other metal. Synergistic effect of two metals on organisms may result from the adsorption of both metals on the surface of the cell, with the adsorption of one metal increasing the permeability to the second metal.

A perusal of literature suggests that effect of metals on floating aquatic plants has not been adequately studied, although some preliminary reports have appeared during the last five years (Wang 1986a, Charpentier et al. 1987, Sela et al. 1988, Sela et al. 1989, Outridge and Hutchinson 1990, Wang 1990). Not much is known about the effects of heavy metals on the physiology of aquatic vascular plants (Porter and Francko 1991). On the other hand, the corpus of information on algae seems quite impressive (Rai et al. 1981a, Davies 1983, Stokes 1983, Whitton 1984, Vymazal 1987, Gadd 1988).

Algae and aquatic plants growing in metal-enriched wastewaters tend to concentrate metals to exceptionally high levels. A direct relationship between metal content in milieu and organisms has been reported, and suggestions have been put forth to use metal content of plants for biomonitoring of metals in aquatic environments. In order to make most effective use of metal accumulation in a particular species as a means of monitoring aqueous metal concentration, the following should be known: relationship between concentration in plants and water; influence of environmental factors on this relationship; rate of loss following environmental downshift in metal concentration or uptake when there is an upshift.

Jennet et al. (1977) have expressed the possibility of using algae to glean heavy metals from metalliferous effluents. However, outdoor cultivation and harvesting of algae present formidable difficulties (Benemann et al. 1977). Aquatic plants may serve the purpose provided they can accumulate high concentrations of heavy metals. However, not much has been done in evolving a macrophyte-based system for the biological treatment of metalliferous wastewaters. In order to achieve this objective a thorough investigation about the mechanism of metal uptake and accumulation by floating plant species should be conducted. It is also necessary to study the role of environmental factors and nutrient ions on metal accumulation by aquatic plants.

Present Study

It aims at examining the interaction of heavy metals, particularly Cd, with Lemna polyrrhiza and Azolla pinnata. The species of Lemna, commonly known as duckweeds, are small free-floating plants capable of fast growth under wide ranging environmental conditions, and these could be employed for stripping nutrients from wastewaters (Oron et al. 1984), and are ideal for toxicological studies (Huebert and Shay 1991). Azolla, a widely-distributed water fern commonly occurring in paddy fields, stagnant waters or ponds and at wide-range of altitudes, has tremendous capacity for vegetative multiplication (Jamir 1982). Water hyacinth (Eichhornia crassipes) can accumulate very high levels of heavy metals. However, it has not been included in the present study due to its following characteristics: inability to grow in cold climate, high rate of transpiration and loss of huge amounts of water, porous foliage providing excellent conditions for mosquito larvae development, and notoriety for creating serious ecological problems (Oron et al. 1984). If species of Lemna and Azolla could take up and accumulate high concentrations of heavy metals, it may become possible to use them for removing metals from polluted waters. The recovery of precious metals (like, Ag) may also become feasible once we know the mechanism of their uptake and accumulation by these organisms. Metal toxicity bioassays often use algae as the test organisms despite the fact that the culturing of algae requires sophisticated laboratory facilities. Floating plants are relatively easier to handle and have all important characteristics needed to become ideal test organisms for toxicity bioassays. Their routine application in laboratory bioassays would, however, require a more thorough understanding of the effects of heavy metals on them.

In the present study greater emphasis has been laid upon Cd as it is one of the most toxic and widely distributed heavy metals. However, other metals, such as Co, Cr, Cu, Ni, Pb and Zn were also briefly studied with regard to their toxicity and bioaccumulation. Generally speaking, Cd is a non-essential element which enters into aquatic environments from mines, smelters and industries involved in the manufacture of alloys, paints, batteries, and from burning of fossil fuels (Reeder et al. 1979). On a global scale, the total anthropogenic input of Cd into the aquatic environments ranges between $2.1-17 \times 10^6$ kg yr^{-1} (Nriagu and Pacyna 1988). Of which, manufacturing processes contribute $0.5-1.8 \times 10^6$ kg yr^{-1}, whereas atmospheric fall out contributes $0.9-3.6 \times 10^6$ kg yr^{-1}.

As mentioned earlier, *Lemna polyrrhiza* L. and *Azolla pinnata* R. Br. were used as test plants, and the following aspects have been investigated:

(i) Toxic effects of Cd, Co, Cu, Cr, Ni, Pb and Zn on growth and pigment levels of test plants.

(ii) Extent of metal bioaccumulation and changes in the levels of essential cations (Ca^{2+}, K^+ and Mg^{2+}) in test plants.

(iii) Time course study on Cd uptake.

(iv) Mechanisms of extracellular and intracellular uptake of Cd in test plants.

(v) Interactive effects of metal combinations on Cd uptake in test plants.

(vi) The uptake of Cd as influenced by Ca, Mg, K, and Na.

(vii) Effects of environmental factors, namely, pH, temperature and light on the accumulation and toxicity of Cd.