Abstract

The presence of toxic heavy metal(s)/metalloids in aquatic ecosystems, resulting from the discharges of untreated metal containing effluents into water bodies, is one of the most important environmental concerns in the present day content. Heavy metals/metalloids are reported to be toxic and found associated with the occurrence of several health effects. Considering their effects on human beings and other aquatic organisms, appropriate treatment of heavy metals from the waste water is of utmost importance. As heavy metals are non-biodegradable, removal of these metals from the aquatic system is the only remedy available for water decontamination. Different methodologies are used for the removal of different heavy metals viz. electro dialysis, reverse-osmosis and adsorption. All of these methodologies used are quite costly and energy intensive, none of them could claim to treat all the heavy metals in economically feasible manner. Occurrence of toxic metals in pond, ditch and river water affect the lives of local people that depend upon these water sources for their daily requirements. Consumption of such aquatic food stuff enriched with toxic metals may cause serious health hazards through food-chain magnification. Therefore, the research is oriented towards low cost and ecofriendly technology for water purification, which will be beneficial for community.

Phytoremediation is an emerging technology where specially selected plants are used for bioremediation of pollution causing substances. In the present study, some aquatic plants such as *Eichhornia crassipes, Monochoria hastata, Trapa natans, sp.* which have rapid growth and great biomass were used for removal of these toxic metals under hydroponic condition. The study was undertaken to evaluate the potential of these aquatic plants for the Phytoremediation of Lead (Pb), Cadmium (Cd), Arsenic (As) and Antimony (Sb).

The contents of the thesis are integrated and organised into eight different chapters to fulfil the objectives of the present research.

The objectives of the research are:

1. To study the toxic effects of heavy metals/metalloids viz. Pb, Cd, As and Sb in the aquatic plants, *Eichhornia crassipes*, *Monochoria hastata*, *Trapa natans*, etc. under hydroponic condition.
2. To study the phytoremediation potential of these aquatic plants with the help of BCF and TF.
3. To confirm the internalization of these metals in these aquatic plants.
4. To study the cellular distribution and ultrastructural effects of metals/metalloids in these aquatic plants.
5. To evaluate arsenic toxicity and uptake by diatom mats under controlled laboratory conditions.

Hypothesis or research questions considered in this research:

The following hypothesis or questions are considered before starting this study-

1. In this research we hypothesize that aquatic macrophytes and diatoms are potential candidates for remediation of toxic heavy metals/metalloids from aqueous solution. Therefore, extensive research work has been carried out to identify such aquatic macrophytes and diatoms locally available in this part of the world.

2. How efficiently and to what extent these plants can remediate, detoxify or immobilize those toxic metal/metalloids from aqueous solution? And to what extent these metal/metalloids are translocated by these plants, to their harvestable parts?

3. Is there any phytotoxic effects of these metals/metalloids?

4. What possible detoxification and tolerance mechanisms developed by these plants?

5. What possible uptake mechanisms of As in diatom *Navicula* species?

To consider these questions, different chapters were organized in this thesis.

The chapter 1 includes an introductory discussions of Phytoremediation of toxic metals, macrophytes in phytoremediation, overall background, concept and purpose of this study, quantification of phytoremediation efficiency using BCF, TF and BAC (biological absorption coefficient), heavy metals uptake by plant, toxicity, translocation, and tolerance mechanisms, need for removal from waste waters and conventional techniques used for heavy metal removal.

An overview of the current state of knowledge on heavy metal/metalloid phytotoxicity to aquatic macrophytes in a hydroponic experiment and objectives of the present research has also been presented. The subcellular localization and various ultrastructural changes of different parts of macrophytes exposed to heavy metal/metalloid concentrations were also discussed in this chapter. The chapter also summarizes the diatom structure and function and morphology of the diatom frustule, metal toxicity and interaction with cellular surfaces in diatoms, metal accumulation in diatoms.

The chapter 2 presents the review of relevant literatures to understand the various phytoremediation technologies used in phytoremediation process, phytoremediation indexes, mechanisms involved in phytoremediation, type and nature of aquatic macrophytes, factors affecting metal uptake in plants, phytoremediation and metal stress in plants, localization of accumulated metals in aquatic plants and sources of Cd, As, Sb, Pb in the environment. The current knowledge of uptake, transport and accumulation of heavy metals/metalloids in plants has been cited here. It gives an overview of the variety of potential mechanism that may be involved in the detoxification and tolerance to heavy metal at the cellular level. The physiological, biochemical and ultrastructural effects of heavy metal in different parts of macrophytes exposed to heavy metal/metalloid concentrations were also reviewed here.

The references regarding general morphology of diatoms, basic features of diatom biology, microalgae and their potential use in metal remediation has been mentioned. The literature showed that diatoms possess characteristics such as abundance, diversity, and high reproductivity, which make their nano-structured frustules (diatom frustules) attractive for a wide range of applications. The Analytical instruments viz. TEM, SEM, FT-IR commonly used by various researchers in similar studies are also highlighted in this chapter.

In chapter 3, Pb uptake, localization within and around root, petiole and leaf of *Eichhornia crassipes* grown hydroponically in a $Pb(NO_3)_2$ solutions were studied by Scanning electron microscopy coupled with elemental X-ray analysis (SEM-EDX) at different increasing concentrations of Pb. Analysis by scanning electron microscopy coupled with the elemental X-ray analysis (SEM-EDX) showed upward Pb transport by root vascular tissues to leaf. The effect of pH and concentrations on uptake capacity of Pb by *Eichhornia crassipes* and their

interplay have been studied. The analysis of residual Pb^{2+} ion was determined using ICP-OES. At minimum initial concentration and low pH (3.0) *Eichhornia crassipes* showed highest bioaccumulation potential. The BCF values decreased with increasing Pb concentration in the growth medium at all the concentrations and all pH. The photosynthetic rate of *Eichhornia crassipes* sharply declined when grown in aquatic medium containing Pb. The chlorophyll content got reduced, with increasing initial concentration of $Pb(NO_3)_2$ over the experimental duration, reflecting probable Pb toxicity.

Phytoremediation of arsenic (As) by water chestnut (*Trapa natans*) in hydroponic system are presented in chapter 4.*T. natans* plants were grown at two concentrations of arsenic, 1.28 mg/L and 10.80 mg/L in a single metal solution. Scanning Electron Microscope-Energy Dispersive X-ray (SEM-EDX) confirmed highest arsenic concentration in the roots, followed by shoots and leaves. SEM-EDX also confirmed internalization of As in *T. natans* and the damage caused due to arsenic (As) exposure. Fourier Transformed Infra-Red Spectroscopy (FT-IR) indicated the binding characteristics of the As ions involved the hydroxyl, amide, amino, and thiol groups in the biomass. Chlorophyll concentration decreased with increasing metal concentration and duration of exposure. But proline content in the plant increases with increasing concentration of As in the aqueous solution. Unhealthy growth and chlorosis were found to be related with As toxicity. From the above studies it is clear that *T. natans* can be used successfully for the removal of arsenic ions by phytoremediation process.

In chapter 5, the distribution and subcellular localization of cadmium in roots, shoots and leaves of *M. hastata* were evaluated to understand structural and ultrastructural changes caused by the metal. Several visual toxic symptoms such as withering, chlorosis and failing of leaves appeared in *M. hastata*, especially at 15mg/L of Cd. Accumulation and transfer of metals were evaluated as a biological concentration factor (BCF), translocation factor (TF) and bio-accumulation coefficient (BAC). Analysis of Cd concentrations by ICP-OES in plant organs showed that Cd concentrations in root were significantly higher than those in shoot and found to be in the order: root > stem > leaf. In the present study the translocation factor (TF) of metals in shoots/roots ratio of *M. hastata* in all three concentration was <1. But it has quite considerable extent of BCF value suggesting that *M. hastata* is a moderate accumulator and the plant may potentially be useful for removal of Cd from Cd containing wastewater. SEM provides strong evidence of closing of stomata and changes of the vascular cell of the leaf due to Cd-induced stress. The results of TEM showed the deposition of electron-dense material in vacuoles, cell wall, chloroplasts and mitochondria. Besides several significant ultrastructural changes namely changes of the shapes of the chloroplasts, reduction of the number of cristae, high vacuolization in cytoplasm, decrease in the intercellular spaces, shrinkage of vascular bundle and loss of cell shape were observed in the TEM micrograph study. FT-IR technique was helpful in elucidating the characteristic functional groups of *M. hastata* biomass which are responsible for binding with Cd ions.

Chapter 6 reports the distribution and subcellular localization of Antimony (Sb) in roots, shoots and leaves of *Trapa natans* and *Eichhornia crassipes* grown in hydroponic condition. Several visual toxic symptoms such as withering, chlorosis and failing of leaves appeared in *T. natans* and *E. crassipes* especially at 7.47 mg/L Sb level. Increasing Sb concentration in the nutrient medium led to a significant reduction in chlorophyll content and an increase in root Sb concentration. After 10 days of harvesting, the percentage removal of Sb from the Sb treatments solutions reached up to 78.91% at 5.17 mg/L in case of *T. natans* and 50.0% at 0.18 mg/L in case of *E. crassipes*. This indicates that the both plants possess a tolerance to Sb and found to show a good application potential in the removal of Sb from the aqueous solution. Sb accumulation followed the pattern of roots> shoots>leaves, in case of *E. crassipes*, a typical behaviour of metal excluders; but it was found as roots> leaves> shoots in case of *T. natans*, due to the morphology of the plant. In the present study the translocation factor (TF) that is shoots/roots ratio of *T. natans* in all 3 concentration of Sb was >1 or =1 and in case of *E. crassipes* TF values was <1 in all concentrations. In *T. natans* the BCF value was 742.69 in 0.18 mg/L Sb concentration suggested that the plant has the ability to take up Sb from solution and is considered as potential accumulator of Sb. In *E. crassipes* TF of Sb for all concentration was less than the critical value (1.0), which indicates that this plant is not very effective to transfer Sb from roots to shoots and can be categorized as tolerant plants. Analysis by SEM-EDX showed a partial upward movement of Sb transport by root vascular tissues.

The toxic effects of Sb were avoided to a certain extent by absorption of Sb in root hair of the plants. The results of TEM showed the deposition of electron-dense material in vacuoles, cell wall and in chloroplasts and showed that Sb induced several significant ultrastructural changes in the shapes of the chloroplasts, mitochondria and cell wall of both *T. natans* and *E.crassipes*.

In the chapter 7 a microcosm study was undertaken to examine the effects of dissolved arsenic (As) at various concentrations (3mg/L, 8 mg/L, 14 mg/L and 55 mg/L) on accumulation and assemblages of diatom *Navicula* sp. The bioaccumulation of As into a freshwater diatom, *Navicula* sp. was examined using various analytical techniques: Fourier-transform infrared (FT-IR) spectroscopy and scanning electron microscopy (SEM) with energy dispersive X-ray spectroscopy (EDX). A laboratory experiment was developed with these As concentrations with 3 replications and control. The effect of As treatment on algae was evaluated for its toxicity on the diatom species and for its bioremediation perspectives also. The FT-IR analyses showed that the main chemical groups involved in the biosorption were silicon–silicon bond and carboxylic groups etc.

The chapter 8 discusses the findings obtained from the results of the laboratory tests in hydroponic condition carried out on aquatic plants and diatoms. The chapter discusses findings about making a case for phytoremediation using aquatic plants, metal absorption by aquatic plants, effects of metals, accumulation of metals in plants and diatoms, ultrastructural localization of these metals in these aquatic plants.