

## CHAPTER 8

### CONCLUSION

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

In this thesis, five problems have been addressed: 1. Phytoremediation of Pb by *Eichhornia crassipes* plant, 2. Bioaccumulation of As by *Trapa natans* plant, 3. Removal of Cd from aqueous solution using *Monochoria hastata* plant, 4. Sb removal by *T. natans* and *E. crassipes* plants and 5. As accumulation study in diatom *Navicula* sp.

#### 8.1 Phytoremediation of metals using aquatic plants

Phytoremediation is cleanup alternative where plants are used to degrade, extract, contain or immobilize contaminants from soils and water. For effective phytoremediation of metals they must be translocated and accumulated in the aerial parts of the plant.

The investigation in this thesis has applied 'phytoextraction or phytoaccumulation' technique from a set of phytoremediation techniques. It is found that it is possible to utilize aquatic plants such as *E. crassipes*, *T. natans* and *M. hastata* as seen from the metal uptake tables in the previous chapter.

In Chapter 3 *Eichhornia crassipes* used in 'Eco-technology' for phytoextraction and phytofiltration of Pb are the best-developed subsets for removal of toxic metal from environment. Nutrient culture is an efficient method for screening heavy metal ions tolerant for free floating plants of *Eichhornia crassipes* in hydroponic culture. The high removal efficiency and more accumulation capacity of Pb<sup>2+</sup> ions make *Eichhornia crassipes* an excellent choice for phytoremediation processes.

In Chapter 4, the characteristics of accumulation and transportation of As in wetland plant, *T. natans* are summarized as follows: 1) The distribution of As in the root is greater than in shoot; after uptake of As by roots, most of the As was

stored in root tissues, and less transported in to the shoots, 2) Plaque can act as a buffer area for the uptake of toxic metals (As) into root tissues, but it does not block the As transport into root tissues. FTIR spectra confirmed the interaction of As ions with the hydroxyl, amide, thiol and amino groups present on the *T. natans* biomass.

Our results are in agreement with earlier observations with other types of aquatic plants showing high metal accumulation and binding capacities on aquatic plants.

In chapter 5 the shoot/root ratios of Cd is less than one and it has quite considerable extent of BCF value suggesting that *M. hastata* is moderate accumulator. TEM microanalysis showed that Cd is accumulated in the vacuoles and cell wall and thereby prevent it entering plant cells. Analysis of Cd concentrations in plant organs showed that Cd concentrations in roots were higher than in the aerial part. Similarly the FTIR spectra confirmed the interaction of Cd<sup>2+</sup> ions with the hydroxyl, carboxyl and amino groups present on the *M. hastata* biomass.

The mechanism of uptake, translocation and detoxification of As and Cd<sup>2+</sup> ions are well understood in plant root cells of *T. natans* and *M. hastata*.

Although the role of cell walls in heavy metal binding and storage in plants is controversial, the present results showed that the cell wall seemed to be a site of preferential Cd binding in root cells of *M. hastata* in Chapter 5. It was agreed with other studies, which suggested that despite its rapid uptake by roots, divalent cadmium has a strong tendency to adsorb on the cation binding sites in cell walls.

In Chapter 6 accumulation of Sb in the plant organs was highest in roots in case of *E. crassipes* in all the sets of experiments. In *T. natans* Sb accumulation was more in leaves than shoots. It is thought that the direct sorption of Sb happens through the leaves; according to the morphology of this plant, leaves are in contact with the solution having large surface area.

In Chapter 7 the metal uptake was ascertained by adopting various characterization methods like ICP-OES, SEM-EDX, XRD and FTIR of the biomass before and after treatment. XRD analysis showed that the diatom microparticles mostly consist of amorphous silica. In case of the freshwater diatom, *Navicula* FTIR results also shows that due to the presence of arsenic, there is a shifting of the peak for carboxyl stretching of fatty acids and amino acids as it

gives a peak at  $1638.4\text{ cm}^{-1}$  where as in normal media, it is  $1635.96\text{ cm}^{-1}$ . FTIR analysis also shows the shifting of vibrational stretching of OH bond at  $3457\text{ cm}^{-1}$  where as in normal media; it is  $3420\text{ cm}^{-1}$ .

The SEM-EDX studies reveal the morphological changes with respect to shape and size of these aquatic plants after accumulation of  $\text{Pb}^{2+}$ , arsenic (III),  $\text{Cd}^{2+}$  and  $\text{Sb}^{2+}$  ions with these plant cells.

Both the process represents a cost-effective, efficient and easy to use plant and algae (diatom) based technology for the removal of metals from the water environment and have great potential for future applications.

The reduction of chlorophyll content in these plants may be attributed to inhibition of chlorophyll synthesis which results in the loss of photosynthetic activity due to the disruption of chloroplast phosphorylation as has been observed in the study of submerged aquatic angiosperms and other aquatic vascular plants

In the plant cell, the whole process of photosynthesis is completed in the chloroplasts. Therefore, the normal performing of plant photosynthesis depends on the integrity of chloroplast ultrastructure. Our experimental results demonstrated that after the plant Cd treatment, chloroplast was swollen, the membrane of chloroplast was damaged, Lamellae structure of thylakoid was loose and disorderly, and the orderly arrangement of grana was also destroyed. To study Cd compartmentalisation at the subcellular level in the leaves, shoots and roots of the *Monochoria* plant, high doses of Cd were supplied to the medium, so that sufficient quantities of this metal would accumulate in the different subcellular compartments and be detectable by scanning electron microscopy (SEM) in conjunction with energy-dispersive X-ray microanalysis (EDXMA). In addition, the structural alteration of chloroplasts by Cd was investigated using transmission electron microscopy (TEM). Cd was mainly localised in the root cell walls; it also was detected inside the cells vacuoles (not in cytoplasm). The retention of Cd by the cell walls suggests it is transported apoplastically in the root.

The highest of both BCF and TF was shown for both the plants in *T. natans* and *E. crassipes*, which indicated that these species are an excellent candidate for the remediation of Sb pollution. For BCF, harvesting of above ground part of plants with high BCF and TF is very important in the view of toxicology. Harvesting will prevent the accumulated heavy metals get into food chain and thus avoid negative potential risk to environment.

## 8.2 Validity of the research findings

The reliability of any research finding is a measure of consistency of experimental findings in repeated accordingly. For example, other researchers look for accuracy of measurement and replicability of findings for same investigations in different research contexts. Whereas validity of the findings refers to measurements applied which in turn should predict, correlate and contribute to what they are measuring collectively. Therefore, the main validity checks are: is the methodology applied are reliable and relevant to the task; how novel the argument in the findings or proposition is; does refuting the existing literature by the researcher have any evidence; how much similarity or differentiation is there between existing and new findings.

Therefore, following **Table 8.1** shows the support from the literature for materials and methods applied in this study which increases its validity. A variety of methods have been used by researchers to localize heavy metals in plant tissues (**Table 8.1**). These include Scanning electron microscopy (SEM), Transmission electron microscopy (TEM) and Energy-dispersive X-ray microanalysis (EDX).

**Table 8.1 Validity of the methodology applied**

Metal	Species	Tissue	Method	Supporting research done by
Cadmium (Cd)	<i>Echinochloa polystachya</i>	Leaves and stem	TEM, SEM, EDX	[1]
Cadmium (Cd)	<i>Agrostis gigantea</i> and <i>Zea mays</i>	Root	TEM	[2]
Copper (Cu)	<i>Eichhornia crassipes</i>	Root	STEM and X-ray microanalysis	[3]
Copper (Cu)	<i>Allium sativum</i>	Root	TEM	[4]
Lead (Pb)	<i>Brachiaria decumbens</i>	Root	TEM	[5]
Lead (Pb)	<i>Chloris gayana</i>	Root	TEM	[5]
Lead (Pb)	<i>Iris pseudacorus</i>	Root	TEM	[6]
Lead (Pb)	<i>Eichhornia crassipes</i>	Root	STEM and X-ray microanalysis	[3]
Arsenic (As)	<i>Pteris vittata</i>	Upper and lower epidermis of the pinna	Energy dispersive X-ray analysis (EDXA)	[7]

**Table 8.2** shows findings of this research study and their support from the existing academic literature for similarity which again confirms the validity of these findings and further recommendations based on these findings.

**Table 8.2 Validity of the findings**

Conceptual proposition of this study	Findings supporting the conceptual proposition in this study	Literature supporting the findings of this study
Phytoremediation characteristic of <i>T. natans</i> plants utilised to removal of As in hydroponic condition	<i>T.natans</i> plant roots absorb more metal then stem and leaf.	[8, 9]
Cd accumulation and ultrastructural distribution in <i>M. hastata</i>	Cd was mainly localized in the root cell walls; it also was detected inside the cells vacuoles	[10, 11]
Sb accumulation and ultrastructural changes in <i>E. crassipes</i> and <i>T. natans</i> plants	Sb treated plastids show a disturbed shape with a wavy appearance of the grana and stroma thylakoids and the intrathylakoidal space swollen but the envelope intact	[12, 13, 14]
As accumulation and effect of As on diatom <i>Navicula</i> sp.	Total abundance of diatoms decrease with respect to increasing As concentration	[15, 16]

Aquatic plants have been demonstrated to possess the ability to accumulate Cd and play a significant role in phytoremediation of metal-polluted aquatic ecosystems. Based on the TEM observation, we suggested that the ultrastructural damage was one of the key reasons for the Cd<sup>2+</sup> toxic effect on plants.

### 8.3 Contributions of this research

This thesis makes a theoretical contribution in terms of literature analyses, materials and methodologies, research findings and creating further research streams.

The first contribution is in terms of literature analyses about various toxic and heavy metals/metalloids removal methods and making a case for phytoremediation technology. These aquatic plants can be used on large scale water pollution removal based on their tested and proven phytoremediation characteristic in this thesis. This is both a theoretical and practical contribution. Secondly, it found that Diatoms are also capable for As removal.

#### 8.4 Future scope of research

As discussed in previous chapters, environmental pollution of water is a serious problem not only in industrialized countries but worldwide. New methods of remediation are needed to combat this problem but must be sympathetic to the environment and also be within the infrastructural capabilities of those countries. The research has shown that these aquatic plants like *E. crassipes*, *T. natans* and *M. hastata* and diatom *Navicula* sp. can be used as a metal sink and, as these are ubiquitous plants. These findings may be useful for other researchers for the future work and can develop other research methodology.

Proper disposal of the harvested plant parts is the final and most important step in any kind of plant-based remediation technology. The higher BCF and TF values of tested species enable them to accumulate large amount of hazardous metals in their harvested parts and if not disposed properly; the accumulated heavy metals may back to the system or can enter into the food chain through browsing animals. At the end of the growth period, plant biomass must be harvested, dried or incinerated, and the contaminant-enriched material should be deposited in a special dump or added into a suitable smelter. Recovery of Cd from harvested plant parts is another technological option. However, the cost benefit analysis of the recovery process should be conducted in order to evaluate the feasibility of various disposal techniques.

Phytoremediation is one new cleanup concept that involves the use of plants to clean contaminated environments. An interdisciplinary technology can benefit from many different approaches that used aquatic plants are suitable for wastewater treatment because they have tremendous capacity of absorbing nutrients and removes heavy metals from wastewater and hence bring the pollution load down. This study showed that aquatic plants such as *E. crassipes*, *T. natans*, and *M. hastata* can have remediatry effects on metal removal from hydroponic metal solutions. Therefore, aquatic plants uptakes on heavy metals are varied based on their species to species as well as metal to metal.

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Chapter 8

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