CHAPTER 2

Literature Review

2.1 The research trends in heavy metals/metalloids pollution and removal

Increasing traffic, industrial production and agricultural practices have led to enhanced release of heavy metals into soil and water. Many metals are essential to organisms and plants in their appropriate concentrations for normal growth and development. However, chronic exposure to even low concentrations of some metals can lead to toxicity [1] and some of them are poisonous such as arsenic (As), lead (Pb) and mercury (Hg). The problem with heavy metals/metalloids is that they do not degrade but get accumulated and transported from one environment to the other [2, 3, 4, 5].

Various laboratory and in-situ techniques have been developed for heavy metals removal such as adsorption [6], sludge activation [7], electro-kinetic [8], electro-osmosis [9], ion exchange [10] and phytoextraction [11, 12, 13, 14] methods.

2.2 Phytoremediation

The term ‘‘phytoremediation’’ is a combination of two words: Greek phyto (meaning plant) and Latin remedium (meaning to correct or remove an evil). Phytoremediation usually refers to the use of plants with or without associated microbes to reduce the concentrations or toxic effects of contaminants in environments [15]. Phytoremediation is a low cost and conventional clean-up technology. Green plants have an enormous ability to uptake pollutants from the environment, and accomplished their detoxification by various mechanisms. Phytoremediation is one such green technology which uses plants to remediate soil, water, sediments and surface with toxic metals, organics and radionuclide [16]. This technique is effective, less expensive and non-intrusive as compared to other prevalent mechanical and chemical approaches [17].

During phytoremediation by live plants, the quality of the process can be affected by physical and chemical properties of the contaminants (water solubility, vapour pressure, molecular weight), environmental characteristics (temperature, pH, organic matter in hydroponics content) and plant characteristics (type of roots, system, enzymes) [18]. There are many mechanisms by which live plants can facilitate/utilize remediation such as phytoextraction (phytoaccumulation), phytopumping, phytostabilization, phytotransformation (degradation), phytovolatilization and rhizodegradation [19].

Phytoremediation technology can successfully be used to remedy of the toxic metal pollutants using different hyperaccumulator plant species. The best-known hyperaccumulators is *Thlaspi caerulescens* accumulated up to 26,000 mg kg^{-1} Zn, without showing injury [20]. *Brassica juncea*, commonly called as Indian mustard, has been found to be a good ability to transport lead from the roots to the shoots [21].

2.2.1 Phytoremediation indexes

Three indexes of heavy metal accumulation were applied (1) Biological Concentration Factor (BCF), calculated as heavy metal concentration ratio of plant root to surrounding substrate [22], (2) Translocation Factor (TF), calculated as heavy metal concentration ratio of plant shoot to root [23, 24] and (3) Biological Accumulation Coefficient (BAC), calculated as heavy metal concentration ratio of plant shoot to surrounding environment [23, 24]. Plants with high BCF, BAC and TF (>1) are suitable for phytoremediation [25].

The ambient metal concentration in water is the major factor influencing the metal uptake efficiency. The appropriateness of a plant for phytoextraction potential is primarily judged by its BCF value. From the view of phytoremediation, a good accumulator should have the ability to concentrate the heavy metal in the tissue, for example, a BCF more than 1000 [26] are generally considered evidence of a useful plant for phytoremediation.

The ability of the plant to accumulate metals with respect to the metal concentration in the substrate is known as the bioconcentration factor (BCF). Zayed et al. [27] reported that BCF can be calculated as follows

BCF = Concentration of metal in plant tissue / Initial Concentration of metal in external solution.

The bioconcentration factor (BCF) is a useful parameter to evaluate plant's potentiality to accumulate metal, it provides the ability index of a plant to accumulate metals with respect to metal concentration in the substrate and it was calculated on a dry weight basis [27].

Plants with both bioconcentration factor and translocation factor greater than one (TF and BCF> 1) have the potential to be used in phytoextraction. Besides, plants with bioconcentration factor greater than one and translocation factor less than one (BCF> 1 and TF< 1) have the potential for phytostabilization [22].

The BAF refers to the ratio of plant metal concentration in roots tissues to the soil or polluted environment [(Metal) root/ (Metal) polluted environment or substrate]. This BAF ratio should be greater than one for inclusion into the hyperaccumulator category [28].

Hyperaccumulating plants are defined by the following characteristics: 1) metal concentrations in the aerial portions are >10,000 mg kg^{-1} dry matter for Zn and Mn; >1,000 mg kg^{-1} for Co, Cu, Ni, As, and Se; and >100 mg kg^{-1} for Cd [29, 30], 2) the bioconcentration factor (BCF) is >1.0, and sometimes reaches 50 to 100 [31] and 3) the translocation factor is >1.0 [32]. This inherent ability to hyperaccumulate metal or metalloids in the above ground parts of plant has been observed in species that grow naturally on metal-rich substrates [29, 33, 34].

Calculation of BAF, EF and TF indices to assess the tolerance categories developed by these species and to evaluate their potential for phytoremediation purposes is another most important objective of this thesis.

2.2.2 Translocation factor

The translocation factors must be invariably higher than one (TF> 1). This indicates an efficient ability to transport metals from roots to shoots and, most likely, the existence of tolerance mechanisms to cope with high concentrations of metals [35].

The translocation factor can be a major criterion in the judgment of plants for phytoremediation. Plants with a higher translocation factor can be considered as a better candidate for phytoremediation [36].

2.3 Mechanisms involved in phytoremediation

A relatively small group of hyperaccumulator plants is capable of sequestering heavy metals in their shoot tissues at high concentrations. In recent years, major scientific progress has been made in understanding the physiological mechanisms of metal/metalloid uptake and transport in these plants. However, relatively little is known about the molecular bases of hyperaccumulation. The current progresses on understanding cellular/molecular mechanisms of metal tolerance/hyperaccumulation by plants are reviewed by researchers [37, 38]. The major processes involved in hyperaccumulation of trace metals from the water to the shoots by hyperaccumulators include: (a) bioactivation of metals in the rhizosphere through root-microbe interaction; (b) enhanced uptake by metal transporters in the plasma membranes; (c) detoxification of metals by distributing to the apoplasts like binding to cell walls and chelation of metals in the cytoplasm with various ligands, such as phytochelatins, metallothioneins, metal-binding proteins; (d) sequestration of metals into the vacuole by tonoplast-located transporters.

Once the rate-limiting steps for uptake, translocation, and detoxification of metals in hyperaccumulating plants are identified, more informed construction of transgenic plants would result in improved applicability of the phytoremediation technology.

Some macrophytes are found to remove different concentrations of arsenic and chromium ions, which make them suitable to act as bio-monitors for metals, and considered as biological filters of the aquatic environment [39, 40, 41]. *Eichhornia crassipes* (family Pontederiaceae) is common macrophyte which is abundant in water logging area. It has a high growth-rate and fibrous root system along with tendency to tolerate high metal concentration, it is considered as an important species to be used in phytoremediation. Many results have been found in relation to the phytoremediation ability of the free-floating *Eichhornia crassipes* for nutrient-rich waters [42, 43, 44, 45].

2.4 Detoxification mechanisms and phytoremediation

Increasing traffic, industrial production and agricultural practices have led to enhanced release of heavy metals into soil and water. Cd, Cu, Al, Cr, Pb, Hg and Zn are the common heavy metals that may be accumulated in plants. Whereas some heavy metals like Cu are essential micronutrients for higher plants by acting as co-factor of several enzymes, plants have developed a wide range of

physiological responses to cope with heavy metal contamination reaching from tolerance to different ways of detoxification. Detailed knowledge on the intracellular localization of elements and their bonding as provided by electron microscopy is essential for understanding bioaccumulation, tolerance and detoxification mechanisms and for obtaining insight into metal toxicity in general.

Toxic metals, once taken up by plants cause deactivation of cell enzymes, consequently affect plant growth. To tolerate high concentrations of metals, plants have a range of potential mechanisms at the cellular level which might be involved in the detoxification of heavy metals and thus tolerance to heavy-metal stress [1]. Such mechanisms involves chelation of the metal cation by high affinity ligands and/or sequestration of metals away from sites and to store accumulated excess metals in organs or subcellular compartments where no sensitive metabolic activities take place [1, 46, 47]. Such accumulation of heavy metals in vacuole and cell walls is probably a potential mechanism of detoxification and thus tolerance to heavy metal stress has already been established [1, 48]. Phytochelatins (PCs) and metallothioneins are two major sulfur-containing classes of metal chelating ligands have been identified in plants and these may play an important role in plant metal tolerance [1, 49].

Valuable information on subcellular localization of different heavy metals in roots and their effects on the ultrastructure of higher plants have been provided particularly by the group of Kottke [50, 51] and in algae by Nassiri et al. [52, 53].

Plants have developed different mechanisms for heavy metal tolerance and detoxification with the central aim of limiting cytoplasmic metal concentrations by storing heavy metals in particular compartments or accumulating them extracellularly in cell walls or in associated mycorrhyzal fungi [47]. Analytical electron microscopy provides an enormous potential for analyzing uptake, transport and storage mechanisms essential for metal accumulating plants and thus further development of phytoremediation.

Accumulation of heavy metals in electron dense granules located at different intracellular compartments or vacuoles represents one possibility of detoxification.

In case of aquatic macrophytes, transversal analysis showed that metal accumulated in a higher proportion on the surface of the roots whereas longitudinal

analysis revealed that this happens at the root caps, their roots of a greater surface area consequently with a higher number of adsorption sites.

It was revealed that physical and chemical processes can occur (adsorption, ionic exchange and chelation) during the fast stage. Free carboxylic groups could probably provide binding sites with metals. These groups are also related to the protein content in tissues; the greater quantity of proteins, the greater the concentrations of carboxylic groups. Proteins as other biological tissues, have atoms of nitrogen, oxygen and sulphur that behave as important ligand atoms and can play an important role in metal sorption [53].

Root cell wall is the important localization site of heavy metals in plants due to its quantities of cation ligands [54]. Plant cell wall is the main composition of apopalsts, which are the "dead" tissues in the plants with lower physiological metabolism activity. The plant cell wall contains protein and polyoses such as cellulose, hemicellulose and lignin, mucilage glue and so on, which have a number of potential ligands such as hydroxyl, carboxyl, amino groups, aldehyde group, phosphate, thiol etc. [54] that can participate in a variety of reactions including ion exchange, adsorption, complexation, precipitation and crystallization, leading to metal sequestration under metal toxicity [55]. When exposed to higher levels of metals, the plant cell can actively secrete calluses which have the ability of chelation to the apoplast parts [56].

2.4.1 Metal tolerance in plants

Strong bindings of Pb to the carboxyl groups of carbohydrate in the cell walls leads to it diminishes transport via apoplast. An electron microscopic study of root tips from tolerant plants reveal the presence of Pb in the cell wall and in the cytoplasm also. Within the cell the major part of Pb is sequestered in the vacuole in the form of complexes. This may represent another mechanism of Pb detoxification in plants. Pinocytosis is observed in leaf cells of many plants treated with Pb salt solution. Through pinocytotic vesicles, Pb particles could be discharged in to the vacuole [57]. Several workers have emphasized the importance of the synthesis of metal chelating compounds such as amino acids like proline to avoid metal toxicity [58].

Plants exposed to Pb and certain other heavy metals like Cd, Zn, Cu and Hg synthesize cysteine-rich low molecular weight peptide called phytochelatins [59].

For practical reasons, the shoot Pb concentration is the most important physiological parameter for evaluating metal concentration or Pb- phytoextraction of the plants [60]. It has been shown that Pb accumulation in roots is slightly higher than in shoots, possibly because of the low Pb translocation from roots to shoots [61, 62].

2.4.2 Metalloids tolerance in plants

Arsenic is widely distributed in the earth's crust and exists in four oxidation states: +5, +3, 0, and −3. However, the most abundant of these states are the pentavalent and the trivalent forms. In aqueous solutions, the pentavalent form occurs in the anion arsenate, H_2AsO^{4-}. Arsenic lies directly below phosphorus in the periodic table, and, from structural and chemical points of view, arsenate is very similar to phosphate. The latter species plays several essential roles in the cell; it is a component of nucleic acids and lipids, regulates processes via phosphorylation of proteins, and transports energy within the cell via ATP. Because of its physico-chemical similarity to phosphate, arsenate, when available inside the cell, can compete with and substitute for phosphate in many reactions. This ability of arsenate raises questions about whether this substitution is harmful for the cell. Previous research has demonstrated that the main toxicity of arsenate is due to its inhibition of the energy- linked reduction of NAD^+, mitochondrial respiration (citric acid cycle) and ATP synthesis. However, under normal environmental conditions, the intracellular levels of phosphate are sufficiently high that arsenate does not directly cause arsenic toxicity.

2.4.2.1 Routes of transport of metalloids through the cell membrane

Because the dominant species of pentavalent arsenic in aqueous solution, H_2AsO^{4-}, is isoelectronic and similar in volume to phosphate, H_2PO^{4-}, phosphate transporters can potentially allow the passage of arsenate. The evidence of arsenate uptake through phosphate transporters in plants is provided by Bienert & Jahn [63]. Upon its entry into the cell, arsenate is reduced to the trivalent arsenite.

As mentioned above, trivalent arsenic is more toxic than the pentavalent state. This finding raises questions concerning why a cell would develop mechanisms to reduce arsenate to arsenite, thus, increasing the toxicity of the element inside the cell.

In contrast to arsenate, the entrance route of pentavalent antimony, or antimonate, into the cells has not yet been identified. The uptake mechanism may be different from that of arsenate because the stable form of antimonate in aqueous solutions, $Sb(OH)^{6-}$ is not isoelectronic with arsenate/phosphate. Nevertheless, once antimonate is inside the cell, it is also reduced to the trivalent antimonite state. The only antimonate reductase known to date is LmACR2 from *Leishmania major*. This enzyme also reduces arsenate and phosphate [64, 65] unlike the arsenate reductases that are selective to As(V) and do not reduce Sb(V). Antimonite appears to be the state in which antimony is extruded from the cell.

2.5 Plants selection for phytoremediation: a major challenging work

Identification and selection of locally available plant species for phytoremediation research and implementation is one of the challenges that need to be met and a pre-requisite for successful phytoremediation research. The selection of plants having strong metal-accumulating ability and being compatible with local weather conditions might yield more immediate practical results than that based solely on a high tolerance to the toxic metal [66]. So the current research focused on phytoremediation of heavy metals under hydroponic conditions using adaptable and high biomass content plants common in Assam, India and can easily grow within the laboratory.

Table 2.1 A number of aquatic plants have been tested for the remediation of trace elements from water in literature study

Common name	Scientific name	Trace elements	References
Duckweed	*Lemna gibba* L.	As, U, Zn	[67, 68, 69,70]
Lesser duckweed	*Lemna minor* L.	As, Zn, Cu, Hg	[71, 72, 73, 74]
Water hyacinth	*Eichhornia crassipes*	As, Fe, Cu, Zn, Pb, Cd, Cr, Ni, Hg	[43, 75, 76, 77, 78, 79, 80, 81, 82]
Common reed	*Phragmites australis*	Cr, Cu, Ni, Pb, S, V, Zn, Cd	[83, 84, 85]
Butterfly fern	*Salvinia minima*	As, Pb, Cd, Cr	[86, 87, 88]
Greater duckweed	*Spirodela polyrhiza L.*	As, Hg	[89, 90, 91]
Water spinach	*Ipomoea aquatica*	As, Cd, Pb, Hg, Cu, Zn	[92, 93, 94]
Water fern	*Azolla filiculoides*	As, Hg, Cd	[95, 96,]
Miriophyllum	*Myriophyllum propinquum*	As	[97]

Water lily	*Nymphaea violacea*	Cd, Cu, Pb, Zn	[98]
	Nymphoides germinata	Cd, Cu, Pb, Zn	[98]
Fennel pondweed	*Potamogeton pectinatus*	Cd, Pb, Cr, Ni, Zn, Cu	[99]
Lesser Bulrush	*Typha angustifolia*	Cd, Pb, Cr, Ni, Zn, Cu	[99, 100]
Bulrush	*Typha latifolia*	Cr, As, Zn, Pb, Cd, Cu. Ni	[100, 101, 102, 103, 104, 105]
Water-milfoil	*Myriophyllum spicatum*	Co, Cr, Cu, Pb, Zn, Ni	[78,106, 107]
Water pepper	*Polygonum hydropiper*	As	[97]
Alligatorweed	*Althernanthera philoxeroides*	As, Pb	[108]
Water lettuce	*Pistia stratiotes*	As, Cr, Pb, Ag, Cd, Cu, Hg, Ni, Zn	[109, 110,111])
Floating pondweed	*Potamogeton natans*	Zn, Cu, Cd, Pb	[112, 113]
Watercress	*Lepidium sativum L.*	As	[97]
	Najas indica	Pb	[114]

Depending on the ability of plants used as a phytoremediation medium to absorb, accumulate and tolerate heavy metals, available plants are classified into three categories of hyperaccumulators, indicators and excluders [115]. An important parameter used in environmental toxicology and risk assessment is the bioaccumulation factor [116].

Plants to be used in Phytoremediation should have a) a tolerance to high concentrations of metals, b) high metal accumulation capacity, c) heavy biomass, d) ability to grow fast and e) a profuse root system. The success of phytoextraction depends especially on the plants ability a) to accumulate biomass rapidly, b) to store large quantities of the up taken metal in the shoot tissues [117].

2.5.1 *Eichhornia crassipes* (water hyacinth)

The seven species of water hyacinth comprise the genus *Eichhornia*. Water hyacinths are a free-floating perennial aquatic plant native to tropical and sub-tropical countries of the world.

The water hyacinth which is an aquatic macrophyte has the ability to absorb and accumulate heavy metals from their environment and can withstand trace elements (such as Ag, Pb, Cd, Zn etc) to a great extent [118]. The detoxification mechanisms of the plant have also been reported by various researchers [119, 120, 121]. The ability of water hyacinth to take up and translocate heavy metals was studied under controlled conditions but the number of

such studies to date is still small as compared to field studies [122]. The metal uptake capacity of water hyacinth and other aquatic macrophytes are affected by some biological and non- biological factors via plant species and different organs, season, pH, metal concentration, and exposure time [121].

Table 2.2 Heavy metal removal capacity of the water hyacinth from different sources

Plant	Heavy metal	Source	Adsorption capacity	References
Water hyacinth plant	Fe	Fe rich wastewaters. In constructed wetlands	6707 Fe mg/kg dry weight	[123]
Water hyacinth plant	Al	Al rich waste water in constructed wet lands	Highest phytoremediation efficiency of 63%	[124]
Water hyacinth plant	Mn	Synthetic wastewater in constructed wetlands	Phytoextraction mode of manganese removal	[125]
Water hyacinth plant	Zn and Cr	Aqueous solution	95% of zinc and 84% of chromium	[118]
Water hyacinth plant	Fe, Zn, Cu, Cr and Cd	Aqueous solution	(>90%) of different metals during 15 days experiment	[126]

2.5.1.1 Characteristics favourable for metal accumulation in *Eichhornia crassipes*

1. In terms of biomass, leaves and the epiphytes provide an expanded area to trap particulate matter, sorbs metal ions and accumulate and sequester pollutants [127, 128, 129].

2. Through its profuse root system and rhizomes and leaves can absorb metals.

3. They have voracious feeding habit and unique survival capacity.

4. Water hyacinth being an aquatic macrophyte is stationary and constantly exposed to contaminants such as metals.

2.5.2 *Trapa natans* (water chestnut)

Trapa natans, commonly known as water chestnut is a floating-leaved aquatic angiosperm that populates in natural wetlands. In previous studies, it was shown that the Mn resistance of this plant is linked to induction of chelating phenolics in the floating leaves [130,131]. Leaves are rich in phenolic compounds which include anthocyanin also [130, 131, 132, 133]. Hale et al. [134] suggested that

Anthocyanins may play a role in the mechanisms reducing the toxic effects of the metal. The plant spreads by the rosettes and fruits detaching from the stem and floating to another area on currents or by fruits clinging to objects, birds and animals.

Trapa natans is also recently found to have phytoremediative potential and research works are going in the respective field. *T. natans* are manganese tolerant and is also characterized by manganese hyper accumulation properties [130]. In addition to the well-known ability to bioaccumulate Mn inside the fruit [134, 135] it has been shown that *T. natans* exhibits peculiar Mn bioaccumulation inside specific tissues of the young floating lamina (3000 µg/g [dry weight] [130].

2.5.3 *Monochoria hastata* (L) (Bhat meteka)

Monochoria hastata (L) is a common wetland species that naturally grows in swamps and paddy fields. It is a rapidly growing, high biomass plant with an intensive root system seems to be an ideal plant to clean up water and soil contaminant. The efficacy of *Monochoria hastata* in the absorption and accumulation of As has recently been reported [136,137]. Similarly, two other wetland species of plant of same genera that of *Monochoria hastata* found to have shown the phytoremediation potential namely Cr, Cd and Cu by *Monochoria vaginalis* [138], and Pb by *Monochoria korsakowi* [139].

2.6 Factors affecting metal uptake in plants

There are three categories of factors affecting metal uptake in these aquatic plants:

1. Metals and their effects; characteristics of these aquatic plants and laboratory conditions commonly,
2. Accumulation of a given metal is a function of uptake capacity,
3. Intracellular binding [139].

These conditions are affected by mobilization and uptake metals from water, compartmentalization and sequestration within the root of these aquatic plants, efficiency of metal loading and transportation to stem and leaves, distribution between metal sinks in the aerial parts of the plants, sequestration and storage of metals in leaf cells. This each activity affects absorption, transportation and accumulation of metals in plants [140].

2.7 Metals

2.7.1. Sources of Cd, As, Sb, Pb in the environment

The term 'heavy metal/metalloid' is the used to cover a diverse range of elements which constitute an important class of pollutants. These metals/metalloids are major pollutants in ground water, industrial effluent, and marine water and even treated waste water [141]. The important toxic metals/metalloids (i. e. Cd, Hg, As, Cr, Zn, and Pb) find its way to the water bodies through waste water [142, 143]. Heavy metals/metalloids enter into the environment mainly via three routs: (i) deposition of atmospheric particulates, (ii) disposal of metal and metalloid enriched sewage sledges and sewage effluents, and (iii) by-product from metal mining processes and other processing industries. The other sources of metals and metalloids pollution are by irrigation of agricultural fields and uses of pesticides and fertilizers [144]. Due to the non-biodegradability and persistence nature, these metals and metalloids can enter into the food chain, and thus may pose significant danger to human health, other organisms and plants.

2.7.2 Arsenic (As)

Arsenic exists in the −3, 0, +3 and +5 oxidation states [145]. The various forms of arsenic in the environment include arsenious acids (H_3AsO_3), arsenic acids (H_3AsO_4, $H_2AsO_4^-$, and $HAsO_4^{2-}$), arsenites, arsenates, methylarsenic acid, dimethylarsinic acid, arsine, etc. Arsenic (III) exist a hard acid and preferentially complexes with oxides and nitrogen. Conversely, arsenic (V) behaves like a soft acid, forming complexes with sulfides. The toxicity of As is dependent on its oxidation state, chemical form and solubility in the biological media. The toxicity scale of As decreases in the order: arsine> inorganic As(III)>organic As(III)>organic As(V)> arsonium compounds and elemental Arsenic.

2.7.2.1 Phytoremediation of As

Recent studies have made great effort in developing cost-effective and eco-friendly As phytoremediation by using As hyperaccumulating ferns or macrophytes. Arsenic hyperaccumulator *Pteris vittata* and *Pteris cretica* were found to be very effective in removing As from the water to a level below the guideline value of 10 µg/L [146]. Rootless duckweed *W. globosa* remove almost 50% of As in a hydroponic system [147, 148]. Alvarado et al. [149] also found the water hyacinth

(*E. crassipes*) and lesser duckweed (*L. minor*) had some potential for As bioremediation in waters. A large number of studies have investigated the mechanism of As uptake by different plant species [149, 150, 151].

Arsenate and phosphate share the same transport pathway in plants, where presumably As^{V} is taken up as a H_2PO^{4-} analog [152, 153, 154]. Ma et al. [155] reported As concentrations in the Brake fern, *Pteris vittata*, of up to 22 000 mg/kg^{-1} (2.2%) on a dry-matter basis.

2.7.2.2 As Tolerance and detoxication in plants

Tolerant plants transported a much greater proportion of As to their shoots compared with non-tolerant plants. The main route of As^{V} uptake in plants is through the phosphate transporters as a phosphate analogue [155]. Restricting the influx of As might be an important mechanism to avoid toxicity, and several metallicolous plants, such as *Holcus lanatus* and *Cytisus striatus*, improve As tolerance by constitutive suppression of high-affinity phosphate/As^{V} transport [156, 157]. As part of As detoxification, the majority of As^{V} is reduced to As^{III} by the enzyme arsenate reductase (AR). Plants exposed to As substantially increase the synthesis of glutathione (GSH) and phytochelatins (PCs), the polymers of GSH [158, 159]. The final step of As detoxification in plants involves As sequestration in the vacuoles of root and shoot tissue of the plants.

2.7.3 Cadmium (Cd)

Cadmium is one of the most toxic metals affecting the environment. Mining and metallurgy of cadmium, cadmium electroplating, battery and accumulator manufacturing, pigments and ceramic industries waste waters contain undesired amounts of Cd^{2+} ions [160].

The most common Cd toxicity symptoms due to excessive accumulation of the metal are leaf roll and chlorosis, inhibition of root growth and elongation, reddish veins and petioles, damage to the light- harvesting complex in photosystems and reduction of the chlorophyll biosynthesis. The damage to photosynthesis occurs mainly from an impaired efficiency of the Rubis co activity, a decrease in chlorophyll and an increase in lipid peroxidation within these organelles. Thus, accumulation of Cd in plant may also cause a decrease of mitotic index, inhibits cell division and cell proliferation.

Cadmium is not at present believed to be an essential nutrient for animals or humans. Due to its acute toxicity that has been studied only recently, cadmium has joined lead and mercury in the most toxic "Big Three" category of heavy metals. Cadmium is one of the metals most strongly absorbed by living cells and accumulated along the trophic chain. The cadmium exposure leads to adverse health effects to human viz. renal dysfunction, liver damage, lung problems, bone degradation, hypertension and cancer [161, 162, 163]. It is best known for its association with itai-itai disease [164].

2.7.3.1 Mechanisms of Cd uptake into roots and translocation to shoots

Plant uptake heavy metals (like Cd) from solution through their roots and in submerged condition, whole plant body acted as an active site for absorption [165]. After entry into roots, Cd ions can either be stored in the roots or translocated to the shoots primarily through xylem vessels [166, 167] where they are mostly deposited in vacuoles. Heavy metal sequestration in the vacuole is one of the way to remove excess metal ions from the cytosol, and may reduce their interactions with cellular metabolic processes [168, 169]. Compartmentalization of complex metals in vacuoles is part of the tolerance mechanism in metal hyperaccumulators [170, 171]. The entire mechanism of phytoextraction/phytofiltration of heavy metals has five basic aspects: mobilization of the heavy metals in soil and water, uptake of the metal ions by plant roots, translocation of the accumulated metals from roots to aerial tissues, sequestration of the metal ions in plant tissues and metal tolerance. Mechanisms governing heavy metal tolerance in plant cells are cell wall binding, active transport of ions into the vacuole and chelation through the induction of metal-binding peptides and the formation of metal complexes [172, 173]. The most important peptides/proteins involved in Cd accumulation and tolerance are phytochelatins (PCs) and metallothioneins (MTs). Plant PCs and MTs are rich in cysteine sulfhydryl groups, which bind and sequester heavy metals in very stable complexes [174].

2.7.4 Antimony (Sb)

Antimony (Sb) is a semi-metallic element which can exist in two forms: the metallic form is bright, silvery, hard and brittle; the non metallic form is a grey powder. It is a poor conductor of heat and electricity. Antimony has been known since ancient times. It is sometimes found free in nature, but is usually obtained from the ores stibnite (Sb_2S_3) and valentinite (Sb_2O_3).

2.7.4.1 Effects of antimony in the environment and health

Antimony can be found in soils, water and air in very small amounts. Antimony mainly pollutes the soils. Through ground water it can travel great distances towards other locations and surface waters. Laboratory tests with rats, rabbits and guinea pigs have shown us that relatively high levels of antimony may kill small animals. Rats may experience lung, heart, liver and kidney damage prior to death. Animals that breathe in low levels of antimony for a long time may experience eye irritation, hair loss and lung damage. Animals that breathed in low levels of antimony for a couple of months may also experience fertility problems. Especially people that work with antimony can suffer the effects of exposure by breathing in antimony dusts. Human exposure to antimony can take place by breathing air, drinking water and eating foods that contain it and also by skin contact with soil, water and other substances that contain it. Exposure to relatively high concentrations of antimony for a longer period of time can cause irritation of the eyes, skin and lungs.

2.7.4.2 Transport of Sb through the cell membrane

The strong affinity between antimonite and sulfhydryl groups is exploited extensively by plants to neutralize the harmful effects of the metalloids by forming complexes with phytochelatins (PCs) [175]. PCs are thiolate peptides that are synthesized non-translationally from the related tripeptide glutathione by the phytochelatin synthase [176]. The metalloid-thiol complexes are then transported for vacuolar sequestration [177, 178].

2.7.4.3 Mechanisms of Sb uptake and root-to-shoot transfer

Little is known about the mechanisms of Sb uptake by plants. In microorganisms, $Sb(OH)_3$ was found to be taken up through aquaglyceroporins like $As(OH)_3$, which can be attributed to the small size of these two neutral molecules and their similarity in conformation and charge distribution with glycerol [179, 180]. Antimonite may cross cell membranes passively with water through aquaporins. Such transport would be consistent with the observed proportionality between plant and soluble soil Sb concentrations. Aquaporins, however, are not open for anions like antimonate, and cellular uptake of antimonate would require mediation by transporters.

There are two parallel transport pathways for water and solutes through plant tissues: the apoplastic pathway through intercellular spaces including pores in the cell walls and the symplastic pathway from cell to cell. The symplastic pathway is only accessible by crossing a cell membrane. The apoplast of the root cortex is directly accessible to solutes from the external environment, whereas the apoplast of the root stele is separated from the cortex by the Casparian bands, thickenings impregnated with hydrophobic materials, in particular suberin, in the radial and transverse walls of the endodermis [181].

Analogous to arsenic and other elements such as cadmium, antimony in algae and plants is likely to be present as inorganic Sb and tightly bound to phytochelatins or may be present as Sb nano-crystallites [182, 183].

2.7.5 Lead (Pb)

Pb is one of the most common heavy elements. Out of several stable isotopes, it is the most abundant. Lead occurs as Galena (PbS). Early, Pb was used in water pipes and pipe joints. Pb is a very heavy, soft, highly malleable, bluish – grey colour metal contains two oxidation states, +2 and +4 Pb resists corrosion and has a low melting point of 327° C. Solid and liquid sludge wastes contribute more than half of the Pb contamination into environment, mainly through the landfills. The other major lead pollutant is exhaust fumes of cars which cause atmospheric pollution [184].

2.7.5.1 Pb accumulation in plants

Pb uptake and transport within plant; physiological, biochemical and ultrastructural changes due to Pb toxicity; Pb tolerance in plants has been widely studied [185, 186]. Pb uptake studies in plants have demonstrated that roots have an ability to take up significant quantities of Pb whilst simultaneously greatly restricting its translocation to above ground parts [187]. For localization of Pb in the roots it has to bind to ion exchangeable sites on the cell wall and extracellular precipitation, mainly in the form of Pb carbonate deposited in the cell wall. However, addition of synthetic chelates, such as H-EDTA or EDTA, in combination with low pH, effectively prevents cell wall retention of Pb, making it available for translocation to shoots [186]. After being taken up by roots, the localization of Pb is greater in roots than in other parts of the plants. Pb binds strongly to the carboxyl groups of the carbohydrates galacturonic acid and glucuronic acid in the cell wall, which restricts its transportation via apoplast [188]. In general dicots accumulate

significantly higher amounts of Pb in the roots than monocots [189]. Pb moves predominantly into the root apoplast and thereby in a radial manner across the cortex and accumulates near the endodermis. The endodermis acts as a partial barrier to the movement of Pb between the root and shoot. This may in part account for the reports of higher accumulation of Pb in roots compared to shoots [190, 191].

2.7.5.2 Transport mechanism of Pb

After being taken up by roots it has to cross the root-cell plasma membrane and one such likely transport pathway of Pb across the plasma membrane (PM) appears to be through PM cation channels, such as Ca-channels [192, 193, 194]. It has been shown that Pb significantly inhibited voltage gated Ca-channels activity in the PM of wheat roots [189]. The inhibition of the Ca-channel by Pb could arise from Pb blockage of the channel or due to competitive transport of Pb through the Ca-channel. While monitoring Pb entry into isolated cells, Tomsig and Suszkiw [195] observed permeation of Pb through Ca-channels. These workers also found that voltage gated Pb transport was blocked by nifedipine (a Ca-channel blocker) and enhanced by BAY K8644 (a Ca-channel agonist). However, according to Miller and Koeppe [195] *Zea mays* L. plants could translocate and accumulate significant quantities of Pb in the leaves in a concentration dependent manner.

2.7.5.3 Pb Toxicity

The toxicity of Pb in plants depends upon its absorption, transport and cellular localization. Accumulation of Pb depends upon the plant species, plant cultivar, plant parts, the exogenous concentration of Pb and the presence of other metals in the environment. The accumulation in roots and leaves of cv. HT-1, a sensitive variety of *Sesamum indica*, was about 20 times higher than those in the resistant variety of PB-1 [196, 197].

Accumulated Pb content generally increases with the increase of metal of the environment, as reported for maize and pea leaves [198] sesame roots and leaves [196, 197]. Intracellular localization of Pb^{2+} ions is an important determination of its toxicity. Accumulation inside the vacuoles may not cause any deleterious effect. In onion tips the metal was reported as Pb orthophosphate in nucleolus [199]. In *Potamogeton pectinatus*, the metal was found to be bound to cell wall [200].

2.7.5.4 Pb tolerance in plants

Strong bindings of Pb to the carboxyl groups of carbohydrate in the cell walls leads to it diminishes transport via apoplast. An electron microscopic study of root tips from tolerant plants reveal the presence of Pb in the cell wall and in the cytoplasm also. Within the cell the major part of Pb is sequestered in the vacuole in the form of complexes. This may represent another mechanism of Pb detoxification in plants. Pinocytosis is observed in leaf cells of many plants treated with Pb salt solution. Through pinocytotic vesicles, Pb particles could be discharged into the vacuole [201]. Several workers have emphasized the importance of the synthesis of metal chelating compounds such as amino acids like proline to avoid metal toxicity [202].

2.8 Localization of accumulated metals in aquatic plants

Knowledge of the subcellular localization and identification of heavy metals can provide essential information on metal toxicity and bioaccumulation mechanisms. Plants have a range of potential mechanisms at the cellular level that might be involved in the detoxification and thus tolerance to heavy metal stress [1].

The study on sub-cellular localization of heavy metals in tissues and cell compartments, various electron microscope techniques have been used [203, 204]. Accumulation of heavy metals in the cell wall is a known mechanism protecting the plasmalemma and keeping the concentration of toxic ions in the cytoplasm low [205, 171]. The black deposits within the cell wall in plants exposed to Cd might also suggest the apoplastic transport of Cd and translocation within the plant, but Cd deposits accumulated in the cell walls might also negatively affect the enzymes contained in this compartment [206, 207]. Cd accumulated in vacuoles and apoplasts play a significant role in scavenging of free radicals produced in plant cells [204].

Previous studies on the accumulation of various metal ions by aquatic plants have also shown that the deposition of most metals was higher in roots than the other parts of plants. Chandra et al. [208] suggested that the low concentration of metals in the shoot of aquatic macrophytes may be due to the slow mobility of metal transport from root to shoot and also the formation of complex compounds with COOH groups that may inhibit the translocation of metal to shoot.

2.9 Phytoremediation and metal stress in plants

Plants take up metals via their roots and leaves depending on their growth media. In aquatic plants, metal uptake by roots is the main way for metal accumulation. In case of roots, metals are first taken up into the apoplasm, and then transported further by the symplasmic or apoplasmic pathway. The movement of metals from the external solution into the apoplasm is a non-metabolic, passive process. Metals taken up into the apoplasm reach the cytoplasm through the plasma membrane mostly in the form of cations. Heavy metal transport at cell membrane can be mediated by substrate-specific transporters, i.e. HMAs (heavy metal-ATPases), NRAMPs (natural resistance-associated macrophage proteins), CDFs (members of the cation diffusion facilitator family), ZIPs (members of the Zrt-, Irt-like protein family) and cation antiporters [208]. Inside the cytosol, some metals bind to the negative charges of various macromolecules, some soluble molecules, or to sulphur-rich polypeptides which participate in the transport of metals into the vacuole across the tonoplast. The most pronounced effect of heavy metals at the cellular level is the alteration of cell membrane permeability for nutrients and the leakage of ions and solutes [209, 210]. Heavy metals may induce changes to the plasma membrane. Two mechanisms are generally utilized:

i. A direct effect on sulphydryl groups of membrane constituents by binding of heavy metal ions to SH groups [210].

ii. Direct or indirect free radical-mediated lipid peroxidation [211].

Heavy metals are known to have a strong affinity for sulphydryl and carboxyl groups. In higher plants, the H+-ATPase of the plasma membrane is highly affected by heavy metals.

2.10 Diatoms

2.10.1 Introduction

The living world offers many examples of organisms that present order and regularity of shape, with patters that are often like lattice patterns of minerals. Both fresh water and marine ecosystem have plenty of these organisms that synthesize an external inorganic casing (from micro- to macrostructures) and an intercellular nanostructure. Among them, the diatoms have most important

impressive architecture because of their highly elaborate exoskeleton. The highly structured silica nanopore in the skeleton of the diatoms is the target of many researchers. Diatoms are single-celled algae that survive in a diverse range of aquatic environments (marine and freshwater), which range over virtually every body of water on earth [212, 213]. 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 [214].

2.10.2 General morphology of Diatoms

2.10.2.1 Diatom frustules

Diatoms are unicellular algae that occur mostly as single cells but some species form colonies. They have certain features which make them unique amongst the algae. The particular features include the siliceous cell wall (frustule) the possession of unique photosynthetic pigments and specific storage products (oil and chrysolaminarin). There are two groups of diatom common in freshwaters namely the centric diatom species which are in general circular in shape and adapted to live in the water column as part of the phytoplankton and the pennate diatoms that live in benthic habitats but are often temporarily re-suspended in the water column.

The valves exhibit a system of silica ribs (interstriae), which grow out in a circular or linear primary pattern during formation. Some lateral ribs grow out in a circular or linear primary pattern during formation. Some lateral ribs grow out to link these primary ribs and the intervening spaces are filled to form the primary layer of perforated silica. These pores are in the valve depth and are termed as "poroid areolae". Some diatom species have bilayered valves with the chambered pores opening in to the valves. These chambers are known as "loculate valves". According to the arrangement of the valves there are two types of diatoms, centric and pinnate diatoms. In the centric diatoms, the ornamentation of the valves is radiate and concentric. In the pinnate, the ornamentation shows bilateral symmetry and one group possesses a complex longitudinal tissue called the raphe, which is an organ of locomotion [215, 216]. A diatom cell is called a "frustules" which comprises of overlapping halves or valves that are fitted one upon the two like an old fashioned pill-box or a pair of Petri plates.

The edges of the two halves are more or less incurved together forming the girdle. On the back of each valve of most elongated types of diatoms there are

linear perforations forming a longitudinal slit called the "raphe". The chemical constitution of the exoskeleton is made up of silicon. Diverse silicon nanostructures are observed on the diatom exoskeleton. The morphogenesis of the frustules and the peculiar cell wall architecture of diatoms involve biomineralization of silicon forming an array of distinctive structural pattern in a species specific manner.

2.10.3 Basic features of diatom biology

Diatoms are within the class *Bacillariophyceae* in the Heterokont division [217, 218]. Their most well-known characteristic is the presence of a unique type of cell wall (known as frustule), which is constructed from two halves of amorphous polymerized silica taking the form of a box with an overlapping lid. The inner frustule is known as the hypotheca and the outer one is denoted the epitheca. The frustules typically comprise beautiful highly ordered lacework like silica structures Diatom cell walls can therefore be regarded as a paradigm for the controlled production of nanostructured silica, and their ceramic manufacturing capabilities go well beyond current human capabilities, both in terms of miniaturization and complexity [219]. The ecological role of the silica frustule is not yet understood [220]. It has been suggested that it may form a robust first line of defense against grazers [221]. There are two major diatom groups, the centric diatoms and the pennate diatoms, which are distinguished from each other on the basis of differences in cell wall structure. A pennate diatom is elongated and bilaterally symmetrical in face view, whereas centric diatoms are radially symmetrical and often resemble a petridish. In both cases, the silica cell walls are ornamented with species-specific patterns and structures, which has made identification and taxonomic classification over the last century straight forward like other photosynthetic eukaryotes, the photosynthetic apparatus of diatoms is housed within plastids inside the cell [222]. But whereas the plastids of green algae and higher plants are surrounded by two membranes, diatom plastids have four membranes. It is, therefore, believed that diatoms and related algae arose following a secondary endosymbiotic event in which a eukaryotic alga was engulfed by a second eukaryotic phagocyte [223]. In such a scenario the inner two membranes would represent the membranes that normally surround the chloroplast, whereas the third membrane (as counted from the inside) is derived from the

endosymbiont's plasma membrane, and the outer membrane is continuous with the endoplasmic reticulum of the host cell [224]. The brown colour of diatoms is due to the characteristic presence of the carotenoid fucoxanthin, which is utilized together with chlorophyll *a* and *c* for photosynthetic light harvesting. These pigments are bound within the light harvesting antenna complexes by fucoxanthin, chlorophyll *a/c*-binding proteins (FCPs), which are homologous to the chlorophyll *a/b*-binding proteins (CABs) of green algae and higher plants. The FCPs are integral membrane proteins localized on the thylakoid membranes within the plastid, and their primary function is to capture and target light energy to chlorophyll *a* within the photosynthetic reaction centres [225].

2.10.4 Diatom ecology

The world's oceans cover two-thirds of the earth's surface. As such they are an essential component of the global ecosystem. For example, approximately 50% of global primary productivity is derived from marine sources. Marine phytoplankton are at the bottom of marine food webs and thus determine the well-being (or not) of the whole marine ecosystem. We know very little about the biology of these organisms, partly because it has not yet been possible to establish representative model organisms for laboratory studies from the different algal groups. Phytoplankton is comprised of photoautotrophic organisms from 12 taxonomic divisions spanning three Kingdoms [226]. It includes photosynthetic bacterioplankton such as prochlorophytes (e.g. *Prochlorococcus*) and cyanobacteria (e.g. the *Synechococcus* genus), and eukaryotic microalgae such as heterokontophytes (brown algae), rhodophytes (red algae) and chlorophytes (green algae) [227]. Diatoms are the most important group of eukaryotic phytoplankton, responsible for close to 40% of marine primary productivity. There are well over 250 genera of living diatoms, with perhaps as many as 100,000 species, making them the most diverse group of photosynthetic organisms after the angiosperms. Diatom abundance is generally highest at the beginning of spring and in the autumn, when nutrients are not limiting and when light intensity and day length are optimal for diatom photosynthesis. More unusual adaptations have also evolved [228]. For example, in nutrient-depleted conditions such as permanently stratified oligotrophic regions of the ocean, solitary or mat-forming diatoms (e.g., *Rhizosolenia*) can sometimes be successful. This is thought to be due to vertical migration or through symbioses with nitrogen-fixing azotrophs. Diatoms are also

an important component of phytoplankton in fresh waters. In some regions of the oceans, the annual production of fixed carbon can be up to 2000 g^{-2} (equivalent to a cereal or corn crop). Furthermore, algal blooms are often caused by diatoms. These blooms can sometimes be harmful, producing biofouling mucilage and/or toxins such as domoic acid (one of the causes of amnesic shellfish poisoning), which can have negative impacts on the local ecosystem, as well as on fishing and aquaculture activities. The most distinctive characteristic of diatoms is their siliceous cell wall that resembles a glass (or more accurately quartz) box. Ecologically the diatom requirement for silica means that they are a critical component of global biogeochemical silica cycling. The fossil deposits from past geological periods are now used as diatomaceous earth, which is used in filters, deodorants and decoloring agents, and as an abrasive in tooth-paste, amongst other things. Furthermore, the invention of dynamite was made possible by adsorbing nitroglycerin onto diatomite.

2.10.5 Microalgae and their potential use in metal remediation

Diatoms are photosynthetic Microalgae, in terms of biomass, they form the world's largest group of primary producers responsible for at least 32% of global photosynthesis [229]. The use of algae in toxicity bioassays can be justified because they are known to be part of the food chain and primary producers of organic substances upon which many other components are dependents. They are also important suppliers of O_2 and thus contribute to the aerobic decomposition processes. Their presence in natural water is therefore indispensable and their exclusion usually influences unfavourably the biological processes [230]. With the implementation of the European Water Frame Work Directive (2000/60/EC), there is a need to take into account priority substances such as heavy metals, studies are necessary for the improvement of diatom monitoring of these pollution.

Heavy metals have been suspected to be a significant factor in the apparition of diatom deformities [231, 232, 233, 234]. During an experiment conducted in freshwater microcosms exposed to high cadmium concentrations (100 µg·L-1), up to 30% aberrant frustules were enumerated, whereas almost no deformed valves were observed in the control (non-contaminated) microcosms [235, 236]. Few laboratory experiments have investigated the uptake of Ba by diatoms. Dehairs et al. [237] as well as Fisher et al. [238] observed Ba

accumulation in the cells and proposed that most of it, if not all, was associated with the frustules.

In our study, we want to set up an in vitro experiment to describe diatom development under metal contamination. In this Microcosm study, diatom growth was quantified through dry weight, ash-free dry mass and chlorophyll a measurements in order to point out arsenic effects.

Table 2.3 Toxicity of Cd. Cu and Zn for different microalgae according to different authors

Microalgae species	Metal toxicity ($mg.L^{-1}$)			Exposure time (h)	References
	Cd	Cu	Zn		.
Planothidium lanceolatum	0.25	0.62	0.35	72	[239]
Micractinium pusillum			0.34	72	[240]
Phaeodactylum tricornutum		0.008		72	[241]
Thalassiosira pseudonana	0.0078			96	[242]
Nitschia palea	0.0276			120	[243].

2.11 Analytical instruments commonly used in the study

Analytical electron microscopy provides an enormous potential for analyzing uptake, transport and storage mechanisms essential for metal accumulating plants and thus further development of phytoremediation.

Analytical instruments commonly used to study the elemental or metal concentrations of plant tissues include inductively coupled plasma-mass spectroscopy system, atomic absorption spectrometry and other procedures designated for specific elements. Scanning Electron Microscopy (SEM) with EDX and Transmission electron microscopy (TEM) have the qualitative, quantitative, imaging and element mapping capabilities with minimum sample preparation, time and sample size.

2.11.1 Transmission electron microscopy (TEM)

The TEM is used in this research study to ascertain the location of accumulated metals in the roots, stems and leaves of the used aquatic plants. For this purpose, cross sections of the plants were prepared to determine the location of the accumulated heavy metals. The TEM operates on the same basic principle as the light microscope but it uses electrons instead of light.

Transmission electron microscopy (TEM) observations confirm the results obtained by SEM analysis [244]. TEM provides the exact localization of heavy metals as electron dense depositions and X-ray microanalysis provides more specific signals of metal occurrence. I am doing my works on remediation of some heavy metals by using some aquatic plants and the usefulness of TEM is to study the exact localization and sequestration of the particular metal in that particular plant. TEM has sufficient resolution to study the spatial relationship between cells and reduction products, as well as chemistry of the cell [245]. Scanning electron microscopy is utilized for characterizing surface microstructures, porosity and fundamental physical properties of different adsorbents.

Many other techniques have been used to study the metal bindings to biomolecules. The techniques which are generally used to investigate the cellular distribution of heavy metals in plant tissues include particle- induced X-ray emission (micro-PIXE) [246, 247] nuclear micro- probe technique (NMP) [248] electron energy loss spectroscopy (EELS) [249] and transmission electron microscopy [250].

TEM is a useful tool for analyzing metal and metal- composite specimens. The study on sub-cellular localization of heavy metals in tissues and cell compartments, various electron microscope techniques have been used [250, 251]. The sub-cellular localization of Pb and Cd reflects their chemical properties and/or the role in plant's physiology and ecology. Pb and Cd uptake and accumulation through roots of higher plants have been expounded in some species of plants [252]. Accumulation and location of Pb and Cd occurred as electron dense granules in the roots of some plants [250, 253, 254]. However, the mechanism of uptake, translocation and accumulation of Pb and Cd in plants should be better understood. Using this method, previous studies have evaluated the subcellular distribution of heavy metals in plants [255, 256, 257]. The microlocalization characters of copper in *Elsholtzia splendens* was described by using transmission electron microscope (TEM) and energy dispersive analysis of X-rays (EDX) [258].

2.11.2 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR has been widely used to determine the interactions of functional groups on the plant biomass with the metal ions. The functional group is one of the keys to understand the mechanism of metal binding on the plant surface. The

characterization of plant biomass showed presence of acidic groups in higher quantities.

The infrared region of the electromagnetic spectrum includes radiation with wave numbers ranging from approximately 12,800 to 10 cm^{-1}. This is divided into near-, mid- and far infrared with wavenumbers 12800-4000 cm^{-1},4000- 200 cm^{-1} and 200-10 cm^{-1} respectively. Infrared spectra arise from various changes in energy brought about by transitions of molecules from one vibrational or rotational energy state to another. The energy at which any peak in a spectrum appears corresponds to the frequency of a vibration of a particular part of a sample molecule [259].

Vibrational energy levels are quantised, and for most molecules the energy differences between quantum states correspond to the mid-infrared region. Relatively discrete closely spaced lines are normally observed for the infrared spectrum of a gas, while broadened vibrational peaks are noticed for solid and liquid samples [260].

FTIR of the sample was obtained by KBr pellet method using Perkin Elmer FTIR spectrophotometer SPECTRUM 100 of Perkin- Elmer.

FTIR analysis was done to confirm the identity of functional groups involved in the binding of various heavy metals to plant biomass. Numerous chemical groups responsible for metal bindings in plants include carboxyl, amino, sulphonate and hydroxyl and their importance in metal binding depends on factors such as the quantity of sites, their accessibility and the affinity between the sites and the metal [261]. Fourier Transform Infrared Spectroscopy (FTIR) one such technique, which may be used to assess the interaction of the surface functional groups with metal ions. FTIR offers excellent information on the nature of the bonds present on the surface of plants and algae.

Several intense characteristic bands in the IR spectra can be attributed to functional groups present in the plant proteins and in polysaccharides. For example, bands at approximately 1740, 1640, 1420 and 1240 cm^{-1} can be attributed to various carboxyl stretches (free C=O, chelate/asymmetri C=O, symmetric C=O and C-O respectively, while polysaccharide ether and hydroxyl groups exhibit stretches of around 1160 and 1030 cm^{-1} respectively. On the other hand, algae proteins exhibit –NH stretches at approximately 1540 cm^{-1}. Changes in band intensity and frequency after metal binding can be used to identify the

functionalities involving in binding. Earlier, metal binding through negatively charged ligands such as carboxyl groups of cell wall, oxygen atoms, positively charged ligands such as amino groups, or through ligand exchange, ion exchange, or reduction mechanisms has been reported in several plant groups [262, 263]. Moreover, involvement of functional groups viz. O-H, N-H stretching, C-H stretching and S O stretching in binding heavy metals to plant biomass of *Atriplex canescens* has been reported earlier [263].

2.11.3 SEM/EDX

FTIR analysis in Chapter 4, chapter 5 and chapter 7 gave an idea of the functional groups responsible for metal/metalloid bindings. In SEM, the surface of a solid sample is scanned in a raster pattern with a beam of energetic electrons. Several types of signals are produced from the surface in this process, including backscattered, secondary and Auger electrons: X-ray fluorescence photons; and other photons of various energies [264]. Scanning electron microscope has often been used to localize metals in plants tissues [244, 265, 266] studied the different toxic effects of mercury on the cellular structure in leaves of *P. vittata* and *N. exaltata* with the help of SEM micrograph.

The two most common are 1. Backscattered and 2. Secondary electrons which serve as the basis for SEM.

An EDX instrument can be attached to an SEM to provide supplementary information. As the SEM electron beam strikes the sample surface X-ray produced. An X-ray photon impinging on the surface of the EDX detector produces electron hole pairs which are detected as a single pulse by the liquid nitrogen cooled preamplifier. The pulse energy is determined by the X-ray energy which in turn is determined by the element being examined.

The EDX analyser produces a spectrum of the elements present in targeted areas of the sample allowing detectable elements to be quantified or mapped.

2.11.3.1 Usefulness of SEM-EDX to study heavy metal uptake and elemental composition in plant tissues

Analytical instruments commonly used to study the elemental concentrations of plant tissues include inductively coupled plasma-mass spectrometry system, (ICP-MS), atomic absorption spectrophotometry (AAS), and other procedures designated for specific elements. SEM-EDX technology offers a new approach to

instantaneous analyses of elemental composition of various plant tissues and organs. The microanalyser (EDX) has qualitative, quantitative imaging and elemental mapping capabilities.

Sahi et al. [267] have done their work on accumulation, speciation and cellular localization of copper in *Sesbania drummondii* by SEM-EDX. They found that the distribution of Cu within seedlings tissues predominantly accumulated in the corticular and vascular (xylem) regions of root tissues. In the stem, most of the Cu was found within the xylem tissue. However, the deposition of Cu within the leaf tissues was in the parenchyma.

In another experiment, Hu, P.-J., et al. [268] done their experiment to study the tolerance, accumulation and distribution of zinc and cadmium in hyperaccumulator plant *Potentilla griffithii*. In roots, SEM-EDX confirmed that highest Zn concentration was found in xylem parenchyma cells and epidermal cells, while for Cd, a gradient was observed with the highest Cd concentration in rhizodermal and cortex cells, followed by central cylinder.

SEM allows for the observation of samples in a dry or wet state, at high magnifications and good field depth with a minimum preparation, and the possibility to combine structural and analytical information by energy-dispersive x-ray microanalysis and digital images. Although, energy-dispersive x-ray microanalysis has relatively low detection limits, it is useful in establishing distribution maps of potentially toxic elements inside cells and tissues.

2.11.4. Inductively Coupled Plasma- Optical Emission Spectrometric (ICP-OES)

Inductively coupled plasma optical emission spectrometry (ICP-OES) is a multi-element technique that can be used to accurately measure the concentration of over 80 elements in a variety of sample matrices. Its analytical potential combines the features of both ICP emission and graphite furnace atomic absorption. The ELAN software provides the operator with a wide array of options for optimizing system performance and simplifying operation while performing ICP-OES determinations.

ICP-OES manual working principle is that heavy metals would be detected by detector after through the mass Spectrometer. Mass Spectrometer accepts ions through it after generate by induction plasma twin ICP (Inductively Coupled Plasma). Mass Spectrometer also function as to separate and ensure only atoms that need only would pass through it before to the detector.

During the past decade a number of analytical approaches based on the combination of a separation technique, usually chromatography, with sensitive, element-specific detection, for instance by inductively coupled plasma mass spectrometry (ICP-MS), have been developed. The advantage of these techniques is that the information obtained concerns the metal complex and not the biological ligand.

2.11.5. X-**Ray diffraction study**

The powder X-ray diffraction (XRD) of sample was determined by using Rigaku D/max-2000 diffractometer in the range of diffraction angle (2Ɵ) between 10^0 to 80^0. X-ray diffraction techniques.

X-ray diffraction yields the atomic structure of materials and is based on the elastic scattering of X-rays from the electron clouds of the individual atoms in the system. The most comprehensive description of scattering from crystals is given by the dynamical theory of diffraction.

• Single -crystal X-ray diffraction is a technique used to solve the complete structure of crystalline materials, ranging from simple inorganic solids to complex macromolecules, such as proteins.

• Powder diffraction (XRD) is a technique used to characterize the crystallographic structure, crystallite size (grain size), and preferred orientation in polycrystalline or powdered solid samples. Powder diffraction is commonly used to identify unknown substances, by comparing diffraction data against a database maintained by the International Centre for Diffraction Data. It may also be used to characterize heterogeneous solid mixtures to determine relative abundance of crystalline compounds and, when coupled with lattice refinement techniques, such as Rietveld refinement, can provide structural information on unknown materials. Powder diffraction is also a common method for determining strains in crystalline materials. An effect of the finite crystallite sizes is seen as a broadening of the peaks in an X-ray diffraction as is explained by the Scherrer Equation.

• Thin film diffraction and grazing incidence X-ray diffraction may be used to characterize the crystallographic structure and preferred orientation of substrate-anchored thin films.

• High-resolution X-ray diffraction is used to characterize thickness, crystallographic structure, and strain in thin epitaxial films. It employs parallel-beam optics.

• X-ray pole figure analysis enables one to analyze and determine the distribution of crystalline orientations within a crystalline thin-film sample.

• X-ray rocking curve analysis is used to quantify grain size and mosaic spread in crystalline materials.

X-ray diffraction yields the atomic structure of materials and is based on the elastic scattering of X-rays from the electron clouds of the individual atoms in the system. The most comprehensive description of scattering from crystals is given by the dynamical theory of diffraction. Powder diffraction (XRD) is a technique used to characterize the crystallographic structure, crystallite size (grain size), and preferred orientation in polycrystalline or powdered solid samples. Powder diffraction is commonly used to identify unknown substances, by comparing diffraction data against a database maintained by the International Centre for Diffraction Data. It may also be used to characterize heterogeneous solid mixtures to determine relative abundance of crystalline compounds and, when coupled with lattice refinement techniques, such as Rietveld refinement, can provide structural information on unknown materials. Powder diffraction is also a common method for determining strains in crystalline materials. An effect of the finite crystallite sizes is seen as a broadening of the peaks in an X-ray diffraction as is explained by the Scherrer Equation. Thin film diffraction and grazing incidence X-ray diffraction may be used to characterize the crystallographic structure and preferred orientation of substrate-anchored thin films.

2.11.6 Zeta Potential Analysis

The development of a net charge at the particle surface affects the distribution of ions in the surrounding interfacial region, resulting in an increased concentration of counter ions (ions of opposite charge to that of the particle) close to the surface. Thus an *"electrical double layer"* exists around each particle. The liquid layer surrounding the particle exists as two parts; an inner region, called the *"Stern layer",* where the ions are strongly bound and an outer, diffuse, region where they are less firmly attached. Within the diffuse layer there is a notional boundary inside which the ions and particles form a stable entity. When a particle moves

(e.g. due to gravity), ions within the boundary move with it, but any ions beyond the boundary do not travel with the particle. This boundary is called the surface of hydrodynamic shear or slipping plane. The potential that exists at this boundary is known as the *"Zeta potential"*.

References

1. Hall, J. L. Cellular mechanisms for heavy metal detoxification and tolerance, *J. Exp. Bot*. **53**, 1--11, 2002.
2. Nriagu, J. O. A silent epidemic of environmental metal poisoning, *Environ. Pollut.* **50**, 139 -- 161, 1988.
3. Nriagu, J. O. A global assessment of natural sources of atmospheric trace metals, *Nature* **338**, 47 -- 49, 1989.
4. Long, E. R., et al. Incidence of adverse biological effects within ranges of chemical concentrations in marine and estuarine sediments, *Environ. Manage*. **19**, 81-- 97, 1995.
5. Ho, Y., & El-khaiary, M. I. Metal research trends in the environmental field, in Wang et al (eds) 2009, *'Heavy metals in the environment*', London, CRC Press, Taylor and Francis Group, 2009.
6. Babel, S., & Kurniawan, T. A. Low cost adsorbents for heavy metals uptake from contaminated water. A review, *J. Hazard. Matter*. **97**, 219 -- 243, 2003.
7. Brown, M. J., & Lester, J. N. Metal removal in activated sludge – Role of bacterial extracellular polymers, *Water Res*. **13**, 817 --837, 1979.
8. Pamukcu, S., & Wittle, J. K. Electrokinetic removal of selected heavy metals from soil, *Environ. Progr*. **11**, 241--250, 1992.
9. Acar, Y. B., et al. Electrokinetic remediation: basic and technology status, *J. Hazard. Mater*. **40**, 117--137, 1995.
10. Ma et al, et al. Effect of aqueous Al, Cd, Cu, Fe(II), Ni, and Zn on Pb immobilization by hydroxyapatite, *Environ. Sci. Technol.* **28**, 1219--1228, 1994.
11. Baker, A. J. M., et al. The possibility of in-situ heavy metal decontamination of polluted soils using crops of metal accumulating plants, *Resoures. Conserv. Recycling* **11**, 41--49, 1994.

12. Dushenkov, V., et al. Rhizofiltration – the use of plants to remove heavy – metals from aqueous streams, *Eviron. Sci. Technol*. **29**, 1239--1245, 1995.
13. Ebbs, S. D., et al. Phytoextraction of Cadmium and Zinc from a contaminated soil, *J. Environ. Qual*. **26**, 1424--1430, 1997.
14. Lasat, M. M. Phytoextraction of toxic metals: A review of biological mechanisms, *J. Environ. Qual*. **31**, 109--120, 2002.
15. Greipsson, S. Phytoremediation, *Nat. Educ. Knowl*. **2**, 7, 2011.
16. Pradhan, S. P., et al. Potential of phytoremediation treatment of PAHs in soil at MGP sites, *J. Soil Contam*. **7**, 467 --480, 1998.
17. Bollag, J. M., et al. Role of microorganisms in soil remediation, In: Anderson, T. A., Coats, J. R. (eds), *Bioremediation through rhizosphere technology*, ACS Symp. Ser. 563. Am. Chem. Soc., York, PA: Maple Press, 1994.
18. Susarla, S., et al. Phytoremediation: An ecological solution to organic chemical contamination, *Ecol. Eng*. **18**, 647--658, 2002.
19. Shammas, N. K. Management and removal of heavy metals from contaminated soil, as chapter 13 in Wang et al, 2009, Heavy metals in the environment, CRC Press, 2009.
20. Brown, S. L., et al. Phytoremediation Potential of *Thlaspi caerulescens* and Bladder Campion for Zinc- and Cadmium-Contaminated Soil, *J. Environ. Qual*. **23**, 1151--1157, 1994.
21. Henry, J.R. In An Overview of Phytoremediation of Lead and Mercury, NNEMS, Report. Washington, D.C., 3-9, 2000.
22. Yoon, J., et al. Accumulation of Pb, Cu, and Zn in native plants growing on a contaminated Florida site, *Sci. Total Environ*. **368**, 456--464, 2006.
23. Cui, S., Zhou, Q., & Chao, L. Potential hyperaccumulation of Pb, Zn, Cu and Cd in endurant plants distributed in an old smeltery, northeast China, *Environ. Geol*. **51**, 1043--1048, 2007.
24. Li, M.S., et al. Heavy metal concentrations in soils and plant accumulation in a restored manganese mine land in Guangxi, South China, *Environ. Pollut*. **147**, 168--175, 2007.
25. Vamerali, T., et al. Field crops for phytoremediation of metal-contaminated land- are view, *Environmental Chemistry Letters* **8**, 1--17, 2010.

26. Wolverton, B.C. *Water hyacinth and alligator weeds for filtration of sewage*, NASA. Tech. Memo. No. TM-X-72721, National Space Technol. Lab. Bay. St. Louis, Miss. 8, 1975.
27. Zayed, A., et al. Phytoaccumulation of trace elements by wetland plants: I. Duckweed, *J. Environ. Qual*. **27**, 715--721, 1998.
28. Badr, N., et al. Phytoremediation: an economical solution to heavy-metal-polluted soil and evaluation of plant removal ability, *World Appl. Sci. J.* **16**, 1292--1301, 2012.
29. Baker, A.J.M., & Brooks, R. R. Terrestrial higher plants which hyperaccumulate metallic elements—a review of their distribution, ecology and phytochemistry, *Biorecovery* 1, 81--126, 1989.
30. Ma, L.Q., et al. A fern that hyperaccumulates arsenic, *Nature* **409**, 579, 2001.
31. Cluis, C. Junk-greedy greens: phytoremediation as a new option for soil decontamination, *BioTech J*. **2**, 61--67, 2004.
32. Wei, S. H., et al. A newly-discovered Cd hyperaccumulator *Solanum nigrum* L. *Chin. Sci. Bulletin* **49**(24), 2568--2573, 2004.
33. Brooks, R. R., et al. Detection of nickeliferous rocks by analysis of herbarium specimens of indicator plants, *J. Geochem. Explor*. **7**, 49--77, 1977.
34. Wenzel, W.W., & Jockwer, F. Accumulation of heavy metals in plants grown on mineralized soils of the Austrian Alps, *Environ. Pollut*. **104**, 145--155, 1999.
35. McGrath, S. P., & Zhao, F. G. Phytoextraction of metals and metalloids from contaminated soils, *Curr. Opinion Biotechnol. J.* **14**(3), 277--282, 2003.
36. Mishra, V.K., et al. Phytoremediation of mercury and arsenic from tropical opencast coalmine effluent through naturally occurring aquatic macrophytes, *Water Air Soil Pollut*. **192**, 303--314, 2008.
37. Prasad, M. N. V., et al. Knowledge explosion in phytotechnologies for environmental solutions, *Environ. Pollut.* **158**, 18--23, 2010.
38. Prasad, M.N.V. Aquatic plants for phytotechnology. *In Environmental Bioremediation Technologies,* Eds S.N. Singh and R.D. Tripathi. 259-274, 2007b.

39. Fayiga, A. O., et al. Effects of heavy metals on growth and arsenic accumulation in the arsenic hyperaccumulator *Pteris vittata* L, *Environ. Pollut*.**132**, 289--296, 2004.
40. Huang, J.W., et al. Phytofiltration of arsenic from drinking water using arsenic-hyperaccumulating ferns, *Environ. Sci. Technol*. **38**, 3412--3417, 2004.
41. Mishra, V. K., et al. Phytoremediation of mercury and arsenic from tropical opencast coalmine effluent through naturally occurring aquatic macrophytes, *Water Air Soil Pollut.* **192**, 303--314, 2008.
42. Alvarado, S., et al. Arsenic removal from waters by bioremediation with the aquatic plants Water Hyacinth (*Eichhornia crassipes*) and Lesser Duckweed (*Lemna minor*), *Bioresour. Technol*. **99**(17), 8436--8440, 2008.
43. Misbahuddin, M., & Fariduddin, A. Water hyacinth removes arsenic from arsenic-contaminated drinking water, *Arch. Environ. Health* **57** (6), 516--519, 2002.
44. Snyder, K.V.W. Removal of Arsenic from Drinking Water by Water Hyacinths (*Eichhornia crassipes*), *J. U.S. S. J. W. P.* **1**, 2006.
45. Low, K.S., & Lee, C. K. Removal of arsenic from solution by water hyacinth (*Eichhornia crassipes* (Mart) Solms), *Pertanika* **13**(1), 129--131, 1990.
46. Rascioa, N. Heavy metal hyperaccumulating plants: How and why do they do it? And what makes them so interesting? *Plant Science* **180**, 169--181, 2011.
47. Salt, D. E; et al. *Annu. Rev. Plant Physiol. Plant Mol. Biol*. **49**, 643--668, 1998,
48. Rauser, W. E. *Cell Biochemistry and Biophysics*, **31**, 19--28, 1999.
49. Baker, A. J. M. *In Phytoremediation of Contaminated Soil and Water*, Terry, N.; Banuelos, G.Eds. Lewis: Boca Raton, 2000, 85-107.
50. Liu, D., & Kottke, I. Subcellular localization of cadmium in the root cells of *Allium cepa* by electron energy loss spectroscopy and cytochemistry, *J. Biosci*. **29**, 329--335, 2004a.
51. Liu, D., & Kottke, I. Subcellular localization of copper in the root cells of *Allium sativum* by electron energy loss spectroscopy (EELS), *Bioresour. Technol*. **94**, 153--158, 2004b.
52. Nassiri, Y., et al. Cadmium bioaccumulation in *Tetraselmis suecica*: an electron energy loss spectroscopy (EELS) study, *Arch. Environ. Contam. Toxicol*. **33** 156--161, 1997a.

53. Nassiri, Y., et al. Ultrastructural and electron energy loss spectroscopy studies of sequestration mechanisms of Cd and Cu in the marine diatom *Skeletonema costatum*; *Arch. Environ. Contam. Toxicol*. **33**, 147--155, 1997b.
54. Sune, N. Cadmium and chromium removal kinetics from solution by two aquatic macrophytes, *Environ. Poll.* **145**, 467--473, 2007.
55. Hayens, R.J. Ion exchange properties of roots and ionic interactions within the root POPLsm: Their role in ion accumulation by plants, *Bot. Rev*. **46**, 75--99, 1980.
56. Mullen, M.D., et al. Sorption of heavy metala by soil fungi *Aspergillus niger* and *Mucor ruoxii*, *Soil Biol. Biochem*. **24**, 129--135, 1992.
57. Wissenmeier, A. H., et al. Aluminum induced callose synthesis in roots of soybean (*Glycine max* L.), *J. Plant Physiol*. **129**, 487--492, 1987.
58. Wierzbicka, M., & Antosiewicz, D. How lead can easily enter the food chain - a study of plant roots, *Sci. Total Environ*. **1**, 423--429, 1993.
59. Alia, P., & Saradhi, P. Proline accumulation under heavy metal stress, *J. Plant Physiol*. **138**, 554--558, 1991.
60. Cobbett, C.S. Phytochelatin biosynthesis and function in heavy-metal detoxification, *Curr. Opin. Plant Biol*. **3**, 211--216, 2000.
61. Huang, J.W., & Cunningham, S.D. Lead phytoextraction: species variation in lead uptake and translocation, *New Phytol*. **134**(1), 75--84, 1996.
62. Cunningham, S.D., et al. Phytoremediation of contaminated soils, *Trends Biotech.* **13**, 393--397, 1995.
63. Verma, S., & Dubey, R. S. Lead toxicity induces lipid peroxidation and alters the activities of antioxidant enzymes in growing rice plants, *Plant Sci.* **164**, 645--655, 2003.
64. Bienert, G. P., & Jahn, T. P. Major intrinsic proteins and arsenic transport in plants: new players and their potential role, *Adv. Exp. Med. Biol*. **679**, 111--125, 2010.
65. Zhou, Y., et al. Leishmania major LmACR2 is a pentavalent antimony reductase that confers sensitivity to the drug pentostam, *J. Biol. Chem*. **279**, 37445--37451, 2004.

66. Zhou, Y., et al. Bifunctional role of the leishmanial antimonate reductase LmACR2 as a protein tyrosine phosphatase, *Mol. Biochem. Parasit.* **148**,161--168, 2006.
67. Murakami, M., & Ae, N. Potential of phytoextraction of copper, lead and zinc by rice (*Oryza sativa* L.), soybean (*Glycine max* [L.] Merr.), and maize (*Zea mays* L.), *J. Hazard. Mater.* **162**, 1185--1192, 2009.
68. Fritioff, A., & Greger, M. Aquatic and terrestrial plant species with potential to remove heavy metals from storm water, *Int. J. Phytorem*. **5**, 211--224, 2003.
69. Mkandawire, M., et al. Toxicity of arsenic species to *Lemna gibba* L. and the influence of phosphate on arsenic bioavailability, *Environ. Toxicol*. **19**, 26--35, 2004a.
70. Mkandawire, M., et al. Capacity of Lemna gibba L. (Duckweed) for uranium and arsenic phytoremediation in mine tailing waters, *Int. J. Phytorem*. **6**, 347--362, 2004b.
71. Mkandawire, M., & Dudel, E.G. Accumulation of arsenic in *Lemna gibba* L. (duckweed) in tailing waters of two abandoned uranium mining sites in Saxony, Germany, *Sci. Total Environ*. **336**, 81--89, 2005.
72. Kara, Y. Bioaccumulation of copper from contaminated wastewater by using Lemna minor, *Bull. Environ. Contam. Toxicol*. **72**, 467--471, 2004.
73. Miretzky, P., et al. Aquatic macrophytes potential for the simultaneous removal of heavy metals (Buenos Aires, Argentina), *Chemosphere* **57**, 997--1005, 2004.
74. Mishra, V., et al. Phytoremediation of mercury and arsenic from tropical opencast coalmine effluent through naturally occurring aquatic macrophytes, *Water Air Soil Pollut*. **192**, 303--314, 2008.
75. Alvarado, S., et al. Arsenic removal from waters by bioremediation with the aquatic plants water hyacinth (*Eichhornia crassipes*) and lesser duckweed (*Lemna minor*), *Bioresour. Technol.* **99**, 8436--8440, 2008.
76. Wolverton, B.C., & McDonald, R.C. Bioaccumulation and detection of trace levels of cadmium in aquatic systems by *Eichhornia crassipes*, *Environ. Health Persp*. **27**, 161--164, 1978.
77. Delgado, M., et al. Uptake of Zn, Cr and Cd by water hyacinths, *Water Res.* **27**, 269--272, 1993.

78. Vesk, P. A., et al. Metal localization in water hyacinth roots from an urban wetland, *Plant Cell Environ*. **22**, 149--158, 1999.
79. Chandra, P., & Kulshreshtha, K. Chromium accumulation and toxicity in aquatic vascular plants, *Bot. Rev*. **70**, 313--327, 2004.
80. Odjegba, V. J., & Fasidi, I.O. Accumulation of trace elements by Pistia stratiotes: implications for phytoremediation, *Ecotoxicology* **13**, 637--646, 2004.
81. Espinoza-Quiñones, F., et al. Chromium ions phytoaccumulation by three floating aquatic macrophytes from a nutrient medium, *World J. Microbiol. Biotechnol*. **24**, 3063--3070, 2008.
82. Junior, A.C.G., et al. Removal of toxic heavy metals cadmium, lead and chromium from swine biofertilizer, using an aquatic macrophyte (*Eichhornia crassipes*) as a bioindicator, *Acta Sci. Technol*. **30**, 9--14, 2008.
83. Dixit, S., & Dhote, S. Evaluation of uptake rate of heavy metals by *Eichhornia crassipes* and *Hydrilla verticillata*, *Environ. Monit. Assess*. **169**, 367--374, 2010.
84. Deng, H., et al. Accumulation of lead, zinc, copper and cadmium by 12 wetland plant species thriving in metal-contaminated sites in China, *Environ. Pollut*. **132**, 29--40, 2004.
85. Ghassemzadeh, F., et al. Arsenic phytoremediation by *Phragmites australis*: green technology, *Int. J. Environ. Studies* **65**, 587--594, 2008.
86. Baldantoni, D., et al. Macro- and trace-element concentrations in leaves and roots of *Phragmites australis* in a volcanic lake in Southern Italy, *J. Geochem. Explor*. **101**, 166--174, 2009.
87. Olguin, E., et al. Productivity, protein content and nutrient removal from anaerobic effluents of coffee wastewater in *Salvinia minima* ponds, under subtropical conditions, *Acta Biotechnol*. **23**, 259--270, 2003.
88. Hoffmann, T., et al. Capacity of Salvinia minima Baker to tolerate and accumulate As and Pb, *Eng. Life Sci*. **4**, 61--65, 2004.
89. Sanchez-Galvan, G., et al. Assessment of the hyperaccumulating lead capacity of Salvinia minima using bioadsorption and intracellular accumulation factors, *Water Air Soil Pollut.* **194**, 77--90, 2008.

90. Rahman, M. A., et al. Arsenic accumulation in duckweed (*Spirodela polyrhiza* L.): a good option for phytoremediation, *Chemosphere* **69**, 493--499, 2007.
91. Rahman, M. A., et al. Arsenic uptake by aquatic macrophyte *Spirodela polyrhiza* L.: interactions with phosphate and iron, *J. Hazard. Mater*. **160**, 356--361, 2008a.
92. Lee, C. K., et al. Accumulation of arsenic by aquatic plants, *Sci. Total Environ.* **103**, 215--227, 1991.
93. Hu, M. H., et al. Treating eutrophic water for nutrient reduction using an aquatic macrophyte (*Ipomoea aquatica* Forsskal) in a deep flow technique system, *Agric. Water Manage*. **95**, 607--615, 2008.
94. Wang, K. S., et al. Phytoextraction of cadmium by Ipomoea aquatica (water spinach) in hydroponic solution: effects of cadmium speciation, *Chemosphere* **72**, 666--672, 2008.
95. Rahman, M.A., et al. The effects of phosphorous on the accumulation of arsenic in water fern (Azolla pinnata L.), *J. Ecotechnol. Res*. **14**, 21--24, 2008b.
96. Rai, P. K. Phytoremediation of Hg and Cd from industrial effluents using an aquatic free floating macrophyte *Azolla pinnata*, *Int. J. Phytorem*. **10**, 430--439, 2008.
97. Rai, P.K., & Tripathi, B.D. Comparative assessment of Azolla pinnata and Vallisneria spiralis in Hg removal from G.B. Pant Sagar of Singrauli industrial region, India, *Environ. Monit. Assess*. **148**, 75--84, 2009.
98. Robinson, B., Marchetti, M., Moni, C., Schroeter, L., van den Dijssel, C., Milne, G., Bolan, N., Mahimairaja, S., 2005. Arsenic accumulation by aquatic and terrestrial plants. In: Naidu, R., Smith, E., Owens, G., Bhattacharya, P., Nadebaum, P. (Eds.), *Managing Arsenic in the Environment: From Soil to Human Health*. CSIRO, Collingwood, Victoria, 235–247.
99. Cardwell, A. J., et al. Metal accumulation in aquatic macrophytes from southeast Queensland, Australia, *Chemosphere* **48**, 653--663, 2002.
100. Demirezen, D., & Aksoy, A. Accumulation of heavy metals in *Typha angustifolia* (L.) and *Potamogeton pectinatus* (L.) living in Sultan Marsh (Kayseri, Turkey), *Chemosphere* **56**, 685--696, 2004.
101. Chandra, P., & Kulshreshtha, K. Chromium accumulation and toxicity in aquatic vascular plants, *Bot. Rev*. **70**, 313--327, 2004.

102. Ye, Z.H., et al. Copper and nickel uptake, accumulation and tolerance in Typha latifolia with and without iron plaque on the root surface, *New Phytol*. **136**, 481--488, 1997.

103. Ye, Z., et al. Zinc, lead and cadmium accumulation and tolerance in Typha latifolia as affected by iron plaque on the root surface, *Aquat. Bot*. **61**, 55--67, 1998.

104. Blute, N.K., et al. Arsenic sequestration by ferric iron plaque on cattail roots, *Environ. Sci. Technol*. **38**, 6074--6077, 2004.

105. Deng, H., et al. Accumulation of lead, zinc, copper and cadmium by 12 wetland plant species thriving in metal-contaminated sites in China, *Environ. Pollut.* **132**, 29--40, 2004.

106. Pratas, J., et al. Arsenic accumulation in aquatic plants (Central Portugal). In: The 6th WSEAS International Conference. World Science and Engineering Academy and Society (WSEAS), Arcachon, France, 73–76, 2007.

107. Keskinkan, O., et al. Heavy metal adsorption characteristics of a submerged aquatic plant (*Myriophyllum spicatum*), *Process Biochem*. **39**, 179--183, 2003.

108. Lesage, E., et al. Sorption of Co, Cu, Ni and Zn from industrial effluents by the submerged aquatic macrophyte *Myriophyllum spicatum* L, *Ecol. Eng.* **30**, 320--325, 2007.

109. Elayan, N.M.S. Phytoremediation of Arsenic and Lead from Contaminated Waters by the Emergent Aquatic Macrophyte Althernanthera philoxeroides (alligator weed), Southern University, Louisiana, USA, 1999.

110. Maine, M. A., et al. Chromium bioaccumulation: comparison of the capacity of two floating aquatic macrophytes, *Water Res*. **38**, 1494--1501, 2004.

111. Espinoza-Quiñones, F., et al. Chromium ions phytoaccumulation by three floating aquatic macrophytes from a nutrient medium, *World J. Microbiol. Biotechnol*. **24**, 3063--3070, 2008.

112. Espinoza-Quiñones, F., et al. Kinetics of lead bioaccumulation from a hydroponic medium by aquatic macrophytes *Pistia stratiotes*, *Water Air Soil Pollut*. **203**, 29--37, 2009.

113. Fritioff, A., & Greger, M. Aquatic and terrestrial plant species with potential to remove heavy metals from storm water, *Int. J. Phytorem*. **5**, 211--224, 2003.

114. Fritioff, A., & Greger, M. Uptake and distribution of Zn, Cu, Cd, and Pb in an aquatic plant *Potamogeton natans*, *Chemosphere* **63**, 220--227, 2006.

115. Singh, R., et al. Lead bioaccumulation potential of an aquatic macrophyte *Najas indica* are related to antioxidant system, *Bioresour. Technol*. **101**, 3025--3032, 2010.

116. Ghosh, M., & Singh, S.P. A comparative study of cadmium phytoextraction by accumulator and weed species, *Environ. Pollut.* **133**, 365--371, 2005.

117. Badr, N., Fawzy, M., & Al-Qahtani, K. M. Phytoremediation: An Ecological Solution to Heavy-Metal-Polluted Soil and Evaluation of Plant Removal Ability, *World Appl. Sci. J*. **16** (9), 1292--1301, 2012.

118. Mcgrath, S.P. Phytoextraction for soil remediation. In: Brooks, R.R. ed. *Plants that hyperaccumulate heavy metals: their role in phytoremediation, microbiology, archaeology, mineral exploration and phytomining*, New York, CAB International, 1998, 261-288.

119. Mishra, V. K., & Tripathi, B.D. Accumulation of chromium and zinc from aqueous solutions using water hyacinth (*Eichhornia crassipes*), *J. Hazard. Mater*. **164**, 1059--1063, 2009.

120. Gupta, M., & Chandra, P. Bioaccumulation and toxicity of mercury in rooted submerged macrophyte *Vallisneria spiralis*, *Environ. Pollut.* **103**, 327--332, 1998.

121. Tokunaga, T.N., et al. Accumulation of Cadmium in *Eichhornia crassipes* Solms, *J. Hyg. Chem.* **22**, 234--239, 1976.

122. Xiaomel, L., et al. Removal of cadmium and zinc by water hyacinth, *Eichhornia crassipes*, *Sci. Asia* **30**(1), 93--103, 2004.

123. Jana, S., et al. Accumulation of Hg and Cr by three aquatic species and subsequent changes in several physiological and biochemical plant parameters, *Water Air Soil Pollut*. **38**,105--109, 1988.

124. Jayaweera, M.W., et al. Contribution of water hyacinth (*Eichhornia crassipes* (Mart.) Solms) grown under different nutrient conditions to Fe-removal mechanisms in constructed wetlands, *J. Environ. Manage*. **87** (3), 450--460, 2008.

125. Jayaweera, M.W., et al. Removal of aluminium by constructed wetlands with water hyacinth (*Eichhornia crassipes* (Mart.) Solms) grown under different

nutritional conditions, *J. Environ. Sci. Health A Tox. Hazard. Subst. Environ. Eng.* **42** (2), 185--193, 2007.

126. Kularatne, R. K., et al. Mechanisms of manganese removal from wastewaters in constructed wetlands comprising water hyacinth (*Eichhornia crassipes* (Mart.) Solms) grown under different nutrient conditions, *Water Environ. Res*. **81**(2) 165--172, 2009.
127. Mishra, V. K., & Tripathi, B. D. Concurrent removal and accumulation of heavy metals by the three aquatic macrophytes, *Bioresour. Technol*. **99**(15) 7091--7097, 2008.
128. Ward, T.J. Temporal variation of metals in the seagrass *Posidonia australis* and its potential as a sentinel accumulator near a lead smelter, *Mar. Biol*. **95**, 315--321, 1987.
129. Bishop, P. L., & De Waters, J. Biotechnology for Degradation of Toxic Chemicals in Hazardous Wastes. – Noyes Data Corp, Park Ridge, NJ, 1988.
130. Levine, S. N., et al. Pollution dynamics as influenced by seagrass beds: experiments with tributyltin in *Thalassia microcosms*, *Mar. Environ. Res*. **30**, 297--322, 1990.
131. Baldisserotto, C., et al. Specific intra-tissue responses to manganese in the floating lamina of *Trapa natans* L, *Plant Biol*. **6**, 578--589, 2004.
132. Baldisserotto, C., et al. Responses of *Trapa natans* L. floating laminae to high concentrations of manganese, *Protoplasma* **231**, 65--82, 2007.
133. Hoque, A., & Arima, S. Overcoming phenolic accumulation during callus induction and in vitro organogenesis in water chestnut (*Trapa japonica* Flerov), *In Vitro Cell. Dev. Biol. Plant* **38**(4), 342, 2002.
134. Hale, K. L., et al. Molybdenum Sequestration in *Brassica* Species. A Role for Anthocyanins? *Plant Physiol*. **126**(4), 1391--1402, 2001.
135. Maljuga, D. P. Chemical composition of soils and plants as indicators of prospecting for metals, Izv Akad Nauk SSSR, *Ser. Geogr. Geofiz*. **11**, 135--138, 1947.
136. Rai, U. N., & Sinha, S. Distribution of metals in aquatic edible plants: *Trapa natans* (Roxb.) Makino and *Ipomoea aquatica* Forsk, *Environ. Monit. Assess*. **70**, 214--252, 2001.

137. Islam, Md. S., et al. Phytoaccumulation of arsenic from arsenic contaminated soils by *Eichhornia crassipes* L., *Echinochloa crusgalli* L. and *Monochoria hastata* L. in Bangladesh, *Int. J. Environ. Protect*. **3**(4), 17--27, 2013.

138. Talukdar, T., & Talukdar, D. Heavy metal accumulation as phytoremediation potential of aquatic macrophyte, *Monochoria vaginalis* (Burm.F.)K. Presl Ex Kunth, *Int. J. Appl. Sci. Biotechnol.* **3**(1), 9--15, 2015.

139. Kim, In Sung., et al. Effects of Lead on Bioaccumulation Patterns and the Ecophysiological Response in *Monochoria korsakowi*, *Plant Bio*. **51**(4), 284--290, 2008.

140. Clemens, S., et al. A long way ahead: understanding and engineering plant metal accumualtion, *Trends Plant Sci*. **7**, 309--315, 2002.

141. Vymazal, J., et al. Trace metals in *Phragmites australis* and *Phalaris arundinacea* growing in constructed and natural wetlands, *Sci*. *Total Environ*. **380**, 154--162, 2007.

142. Hogan, C.M., *Heavy metal*. In: Encyclopaedia of Earth. Eds. Cutler J. Cleveland, Washington, D.C.: Environmental Information Coalition, National Council for Science and the Environment, 2012.

143. Agarwal, S. K. *Heavy metal pollution*. APH publishing, 1-270, 2009.

144. Merrill, J.C., Morton, J.J.P. & Soileau, S.D. Metals. In A. W. Hayes. *Principles and methods of toxicology*, 5th edition. CRC Press, 2007.

145. Hani, A., & Pazira, E. Heavy metals assessment and identification of their sources in agricultural soils of Southern Tehran, Iran, *Environ. Monit. Assess.* **176**(1-4), 677--691, 2011.

146. Smedley, P. L., et al. Hydrogeochemistry of arsenic and other inorganic constituents in ground waters from La Pampa, Argentina, *Appl. Geochem*. **17**(3), 259--284, 2002.

147. Huang, Z.C., et al. Difference of toxicity and accumulation of methylated and inorganic arsenic in arsenic-hyperaccumulating and -hypertolerant plants, *Environ. Sci. Technol*. **42**, 5106--5111, 2008.

148. Zhang, W.D., et al. Toxicity and accumulation of arsenic in wheat (*Triticum aestivum* L.) varieties of China, *FYTON* **78**, 147--154, 2009.

149. Alvarado, S., et al. Arsenic removal from waters by bioremediation with the aquatic plants water hyacinth (*Eichhornia crassipes*) and lesser duckweed (*Lemna minor*), *Bioresour. Technol*. **99**, 8436--8440, 2008.

150. Asher, C.J., & Reay, P.F. Arsenic uptake by barley seedlings, *Aust. J. Plant Physiol*. **6**, 459--466, 1979.

151. Meharg, A.A., & Macnair, M.R. Suppression of the high-affinity phosphate uptake system: a mechanism of arsenate tolerance in *Holcus lanatus* L, *J. Exp. Bot.* **43**, 519--524, 1992.

152. Meharg, A.A., et al. Phosphorus nutrition of arsenate tolerant and nontolerant phenotypes of velvetgrass, *J. Environ. Qual.* **23**, 234--238, 1994.

153. Khattak, R. A., et al. Accumulation and interactions of arsenic, selenium, molybdenum and phosphorus in alfalfa, *J. Environ. Qual*. **20**, 165--168, 1991.

154. Pickering, I. J., et al. Reduction and coordination of arsenic in Indian mustard, *Plant Physiol*. **122**, 1171--1177, 2000.

155. Ma, L. Q., et al. A fern that hyperaccumulates arsenic, *Nature* (London) **409**, 579, 2001.

156. Asher, C. J., & Reay, P. F. Arsenic uptake by barley seedlings, *Aust. J. Plant Physiol*. **6**, 459--466, 1979.

157. Meharg, A.A., & Macnair, M.R. Suppression of the high-affinity phosphate uptake system: a mechanism of arsenate tolerance in *Holcus lanatus* L, *J. Experi. Bot*. **43**, 519--524, 1992.

158. Bleeker, P.M., et al. Mechanisms of arsenate tolerance in *Cytisus striatus*, *New Phytol*. **157**, 33--38, 2003.

159. Grill, E. S., et al. Role of phytochelatins in phytoremediation of heavy metals. In: Environmental bioremediation technologies (Eds.: S.N. Singh and R.D. Tripathi). Springer, Heidelberg, 101-145, 2006.

160. Gupta, D. K., et al. Changes in the levels of phytochelatins and related metal-binding peptides in chickpea seedlings exposed to arsenic and different heavy metal ions, *J. Plant Res*. **117**, 253--256, 2004.

161. Pino, G.H., et al. Biosorption of cadmium by green coconut shell powder, *Min. Eng*. **19**, 380--387, 2006.

162. Arisawa, K., et al. Cadmium-induced renal dysfunction and mortality in two cohorts: disappearance of the association in a generation born later, *Toxicol. Lett*. **169**(3), 214--221, 2007.

163. Menke, A., et al. Cadmium levels in urine and mortality among U.S. adults, *Environ. Health Perspect*. **117**(2), 190--196, 2009.

164. Nishijo, M., et al. Causes of death and renal tubular dysfunction in residents exposed to cadmium in the environment, *Occup. Environ. Med*. **63**(8), 545--550, 2006.

165. Yoshida, F., et al. Itai-Itai disease and the countermeasures against cadmium pollution by the Kamioka mine, *Environ. Econ. Pol. Stud.* **2**, 215--229, 1999.

166. Rahman, M.A., & Hasegawa, H. Review on Aquatic arsenic: Phytoremediation using floating macrophytes, *Chemosphere* **83**, 633--646, 2011.

167. Prasad, M.N.V. Phytoremediation of metals in the environment for sustainable development, *Proc. Indian Natl Sci Acad Part B* **70**, 71--98, 2004.

168. Jabeen, R., et al. Phytoremediation of heavy metals: physiological and molecular mechanisms, *Bot. Rev.* **75**, 339--364, 2009.

169. Assuncao, A.G.L., et al. *Thlaspi caerulescens*, an attractive model species to study heavy metal hyperaccumulation in plants, *New Phytol*. **159**, 351--360, 2003.

170. Sheoran, V., et al. Role of hyperaccumulators in phytoextraction of metals from contaminated mining sites: a review, *Crit. Rev. Environ. Sci*. *Technol*. **41**, 168--214, 2011.

171. Cluis, C. Junk-greedy greens: phytoremediation as a new option for soil decontamination, *BioTech J.* **2**, 61--67, 2004.

172. Tong, Y.P., et al. Vacuolar compartmentalization: a second generation approach to engineering plants for phytoremediation, *Trends Plant Sci*. **9**, 7--9, 2004.

173. Mejare, M., & Bulow, L. Metal-binding proteins and peptides in bioremediation and phytoremediation of heavy metals, *Trends Biotechnol*. **19**, 67--73, 2001.

174. Memon, A.R., & Schroder, P. Implications of metal accumulation mechanisms to phytoremediation, *Environ. Sci. Pollut. Res.* **16**, 162--175, 2009.

175. Karenlampi, S., et al. Genetic engineering in the improvement of plants for phytoremediation of metal polluted soils, *Environ. Pollut*. **107**, 225--231, 2000.

176. Cobbett, C. S. Phytochelatins and their roles in heavy metal detoxification, *Plant Physiol.* **123**, 825--832, 2000.

177. Vatamaniuk, O. K., et al. Worms take the 'phyto' out of 'phytochelatins', *Trends Biotechnol*. **20**, 61--64, 2002.

178. Schmöger, M. E., et al. Detoxification of arsenic by phytochelatins in plants, *Plant Physiol.* **122**, 793--801, 2000.

179. Hartley-Whitaker, J., et al. Phytochelatins are involved in differential arsenate tolerance in *Holcus lanatus*, *Plant Physiol*. **126**, 299--306, 2001.

180. Filella, M., et al. Antimony in the environment: a review focused on natural waters. III. Microbiota relevant interactions, *Earth-Science Reviews* **80**, 195--217, 2007.

181. Porquet, A., & Filella, M. Structural evidence of the similarity of $Sb(OH)_3$ and $As(OH)_3$ with glycerol: implications for their uptake, *Chem. Res. Toxicol*. **20**, 1269--1276, 2007.

182. Waisel, Y., Eshel, A., & Kafkafi, U. *Plant Roots - The Hidden Half*, 2nd Ed. New York, Basel, Hong Kong: Marcel Dekker, Inc.; 1996.

183. Scarano, G., & Morelli, E. Properties of phytochelatin-coated CdS nanocrystallites formed in a marine phytoplanktonic alga (*Phaeodactylum tricornutum*, Bohlin) in response to Cd, *Plant Sci*. **165**, 803--810, 2003.

184. Williams, P., et al. Production of cadmium sulphide microcrystallites in batch cultivation by *Schizosaccharomyces pombe*, *J. Biotechnol*.**48**, 259--267, 1996.

185. Naja, G. M., & Volesky, B. Toxicity and sources of Pb, Cd, Hg, Cr, As and radionuclides in the environment in *Heavy Metals in the Environment*, Wang, L. K., et al. Eds., 16–18, CRC Press, Taylor & Francis Group, Boca Ratón, Fla, USA, 2009.

186. Sharma, P., & Dubey, R.S. Lead toxicity in plants, *Braz. J. Plant Physiol*. **17**, 35--52, 2005.

187. Javis, M.D., & Leung, D.W.M. Chelated lead transport in *Pinus radiata*: an ultrastructural study, *Environ. Exp. Bot*. **48**, 21--32, 2002.

188. Lane, S.D., & Martin, E.S. A histochemical investigation of lead uptake in *Raphanus sativus*, *New Phytol.* **79**, 281--286, 1977.

189. Rudakova, E.V., et al. The role of plant cell walls in uptake and accumulation of metal ions, *Fiziol. Biochim. Kult. Rast*. **20**(1), 3--12, 1988.

190. Huang, J.W., & Cunningham, S. D. Lead phytoextraction: species variation in lead uptake and translocation, *New Phytot*. **134**, 75--84, 1996.

191. Jones, L.H.P., et al. Lead uptake from solution by perennial ryegrass and its transport from roots to shoots, *Plant Soil* **38**, 403--414, 1973.

192. Verma, S., & Dubey, R.S. Lead toxicity induces lipid peroxidation and alters the activities of antioxidant enzymes in growing rice plants, *Plant Sci.* **164**, 645--655, 2003.

193. Marshall, J., et al. Membrane potential-dependent Ca^{2+} transport in right-side-out plasma membrane vesicles from *Zea mays* L. roots, *Plant J.* **5**, 683--694, 1994.

194. Huang, J.W., et al. Voltage dependent Ca^{2+} influx into right-side-out plasma membrane vesicles isolated from wheat roots: characteristic of a putative Ca^{2+} channel. Proceeding of the National Academy of Science USA 91: 3473-3477, 1994.

195. Tomsig, J.L., & Suszkiw, J.B. Permeation of Pb^{2+} through calcium channels: fura-2 measurements of voltage- and dihydropyridine- sensitive Pb^{2+} entry in isolated bovine chromaffin cells', *Biochimica et Biophysica Acta* **1069**, 197--200, 1991.

196. Miller, R. J., & Koeppe, D. E. Accumulation and physiological effects of lead in corn. Proc. Univ. Mo. 4th Ann. Conf. Trace Substances Environ. Health. 1971, 186-193.

197. Kumar, G., et al. Nitrate assimilation and biomass production in *Sesamum indicum* L. seedlings in lead enriched environment, *Water Air Soil Pollut.* **66**, 163--177, 1993.

198. Bharti, N., & Singh, R. P. Growth and nitrate reduction by *Sesamum indicum* cv PB-1 respond differentially to lead, *Phytochemistry* **33**, 531--534, 1993.

199. Sinha, N. Organ and cell fusions in the *adherent1* mutant in maize, *Int. J. Plant Sci.* **159**, 702--715, 1998.

200. Tandler, C. J., & Alberto, J. S. Nucleolar Orthophosphate Ions, *Electron Microscope and Diffraction Studies* 1969.

201. Sharp, V., & Denny, P. Electron Microscope Studies on the Absorption and Localization of Lead in the Leaf Tissue of *Potamogeton pectinatus* L, *J. Exp. Bot.* **27**(6), 1155--1162, 1976.

202. Wierzbicka, M., & Antosiewicz, D. 'How lead can easily enter the food chain - a study of plant roots,' *Sci. Total Environ.* **1**, 423--429, 1993.

203. Alia, P. S. P. Proline accumulation under heavy metal stress, *J. Plant Physiol*. **138**, 554--558, 1991.

204. Heumann, H. G. Ultrastructural localization of zinc in zinc-tolerant *Armeria maritime* ssp. halleri by autometallography, *J. Plant Physiol*. **159**(2), 191--203, 2002.

205. Sridhar, B.B.M., et al. Anatomical changes due to uptake and accumulation of Zn and Cd in Indian mustard (*Brassica juncea*), *Environ*. *Experi. Bot*. **54**, 131--141, 2005.

206. Białon´ska, D., et al. Phenolic compounds and cell structure in bilberry leaves affected by emissions from a Zn–Pb smelter, *Water Air Soil Pollut.* **181**(1–4):123–131, 2007.

207. Ernst, W.H.O. Effects of heavy metals in plants at the cellular and organismic level ecotoxicology. In: Gerrit S, Bernd M (eds) Bioaccumulation and biological effects of chemicals. Wiley and Spektrum Akademischer, New York, 1998, 587–620.

208. Chandra, P., et al., Bioremediation of chromium form waster and soil by vascular aquatic plants, E. Kruger, T.A. Anderson, J.R. Coats (Eds.), *Phytoremediation of Soil and Water*, American Chemical Society, Washington, 274–282, 1997.

209. Hall, J. L., & Williams, L. E. Transition metal transporters in plants, *J. Exp. Bot*. **54**, 2601--2613, 2003.

210. Ros, R.O.C., et al. Effects of the herbicide MCPA and the heavy metals, cadmium and nickel on the lipid composition, Mg^{2+}-ATPase activity and fluidity of plasma membranes from rice, *Oryza sativa* (cv. Bahia) shoots, *J. Exper. Bot*. **41**, 457--462, 1990.

211. De Vos, C. H. R., et al. Increased resistance to copper-induced damage of the root cell plasmalemma in copper tolerant *Silene cucubalus*, *Physiologia Plantarum* **82**, 523--528, 1991.

212. Shi, X., et al. Generation of free radicals from hydrogen peroxide and lipid hydroperoxides in the presence of Cr(III), *Biochimica et Biophysica Acta* **302**, 294--299, 1993.

213. Branco, D., et al. Sensitivity of biochemical markers to evaluate cadmium stress in the freshwater diatom *Nitzschia palea* (Kutzing) W. Smith, *Aquat. Toxicol.* **99**, 109--117, 2010.

214. Taylor, J.C. Harding, W.R. and Archibald, C.G.M. *A Methods Manual for the Collection, Preparation and Analysis of Diatom Samples*, Version 1.0, WRC Report TT 281/07, January 2007.

215. Sandhage, K.H., et al. Novel, bioclastic route to self-assembled, 3D, chemically tailored meso/nanostructures: Shape-preserving reactive conversion of biosilica (Diatom) microshells, *Adv.Mater*. **14**, 429--433, 2002.

216. Stefano, D. L., et al. Marine diatoms as optical chemical sensors, *Appl. Phys. Lett*. **87**, 233--902, 2005.

217. Round, F. E., Crawford, R.M., and Mann, D. G. The diatoms biology and morphology of the genera, Cambridge University Press, Cambridge, 1990.

218. Goertzen, L. R., & Theriot, E. C. Effect of taxon sampling, character weighting, and combined data on the interpretation of relationships among the heterokont algae, *J. Phycol*. **39**, 423--439, 2003.

219. Guillou, L., et al. Bolidomonas: a new genus with two species belonging to a new algal class, Bolidophyceae (Heterokonta), *J. Phycol*. **35**, 368--381, 1999.

220. Fischer, H., et al. Targeting and covalent modification of cell wall and membrane proteins heterologously expressed in the diatom, *Mar. Biotechnol.* **1**, 239--251, 1999.

221. Borowitzka, M.A., et al. Ultrastructure of a chain-forming diatom *Phaeodactylum tricornutum*, *J. Phycol*. **13**, 162--170, 1977.

222. Falciatore, A., et al. Perception of environmental signals by a marine diatom, *Science* **288**, 2363--2366, 2000.

223. Gibbs, S.P. The chloroplast endoplasmic reticulum: structure, function, and evolutionary significance, *Int. Rev. Cytol*. **72**, 49--99, 1981.

224. Edlund, M. B., & Stoermer, E. F. Ecological, evolutionary, and systematic significance of diatom life histories, *J. Phycol*. **33**, 897--918, 1997.

225. Bhattacharya, D., et al. Algae containing chlorophylls a + c are paraphyletic: molecular evolutionary analysis of the Chromophyta, *Evolution* **46**, 1808--1817, 1992.

226. Bhaya, D., & Grossman, A. Targeting proteins to diatom plastids involves transport through an endoplasmic reticulum, *Mol. Gen. Genet*. **229**, 400--404, 1991.

227. Medlin, L. K., et al. Evolution of the Diatoms (Bacillariophyta). II. Nuclear-Encoded Small-Subunit rRNA Sequence Comparisons Confirm a Paraphyletic Origin for the Centric Diatoms, *Mol. Biol. Evol.* **13**(1), 67--75, 1996.

228. Medlin, L. K., et al. The evolution of the diatoms (Bacillariophyta). I. Origin of the group and assessment of the monophyly of its major divisions, *Eur. J. Phycol*. **28**, 261--275, 1993.

229. Qiu, Y.L., & Palmer, J.D. Phylogeny of early land plants: insights from genes and genomes, *Trends Plant Sci*. **4**, 26--30, 1999.

230. Priyadarshani, I., et al. Microalgal bioremediation: Current practices and perspectives, *J. Biochem. Tech*. **3**(3), 299--304, 2011.

231. Matulová, D. The application of algae as test organisms, *Arch. Protistenkd*. **139**, 279--289, 1991.

232. McFarland, B. H., et al. Abnormal *Fragilaria* spp. (Bacillariophyceae) in streams impacted by mine drainage, *J. Freshwat. Ecol*. **12**, 141--9, 1997.

233. Dickman, M. D. Benthic marine diatom deformities associated with contaminated sediments in Hong Kong, *Environ. Int*. **24**, 749--59, 1998.

234. Ruggiu, D., et al. Paleoecological evidence for diatom response to metal pollution in Lake Orta (N. Italy), *J. Paleolimnol*. **20**, 333--45, 1998.

235. Nunes, M. L., Da Silva, E. F., & De Almeida, S.F.P. Assessment of water quality in the Caima and Mau River basins (Portugal) using geochemical and biological indices, *Water Air Soil. Pollut*. **149**, 227--250, 2003.

236. Morin, S., et al. Cadmium toxicity and bioaccumulation in freshwater biofilms, *Arch. Environ. Contam. Toxicol*. **54**, 173--186, 2008b.

237. Dehairs, F., Chesselet, R., & Jedwab, J. Discrete suspended particles of barite and the barium cycle in the open ocean, *Earth Planet. Sci. Lett*. **49**, 528--550, 1980.

238. Fisher, N. S., Guillard, R. L., & Bankston, D. C. The accumulation of barium by marine phytoplankton grown in culture, *J. Mar. Res*. **49**, 339--354, 1991.

239. Sbihi, K. Accumulation and toxicological effects of cadmium, copper and zinc on the growth and photosynthesis of the freshwater diatom *Planothidium*

lanceolatum (Brébisson) Lange-Bertalot: A laboratory study, *J. Mater. Environ. Sci.* **3** (3), 497--506, 2012.

240. Toumi, A., et al. Effect and Bioaccumulation of Heavy Metals (Zn, Cd) on *Micractinium pusillum* Alga A, *Environ. Technol.* **28**, 19--23, 2007.
241. Strasser, B.J., & Strasser, R.J. Measuring fast fluorescence transients to address environmental questions: The JIP test. In Mathis, P. (ed.) Photosynthesis: From Light to Biosphere, Kluwer Academic, the Netherlands, Vol 5: 977–980, 1995.
242. Levy, J.L., et al. Uptake and internalization of copper by three marine microalgae: Comparison of copper-sensitive and copper-tolerant species, *Aquat. Toxicol.* **89**, 82--93, 2008.
243. Wang, M.J., & Wang, W.X. Cadmium in three marine phytoplankton: Accumulation, subcellular fate and thiol induction, *Aquat. Toxicol.* **95**, 99--107, 2009.
244. Solis-Domingues, F.A., et al. Accumulation and localization of cadmium in *Eichnochola polystachya* grown within a hydroponic system, *J. Hazard. Mater.* **141**, 630--636, 2007.
245. Mullen, L. D., et al. Bacterial sorption of heavy metal, *Appl. Environ. Microbiol.* **55**, 3143--3149, 1989.
246. Ager, F. J., et al. Cadmium localization and quantification in the plant *Arabidopsis thaliana* using micro-PIXE, *Nuclear Instruments and Methods B* **189**, 494--498, 2002.
247. Vogel-Mikus, K., et al. Comparison of essential and non-essential element distribution in leaves of the Cd/ Zn hyperaccumulator *Thlaspi praecox* as revealed by micro-PIXE, *Plant Cell Environ.* **31**, 1484--1496, 2008.
248. Ager, F. J., et al. Nuclear micro-probe analysis of Arabidopsis thaliana leaves, *Nuclear Instruments and Methods B* **210**, 401--406, 2003.
249. Liu, D., & Kottke, I. Subcellular localization of cadmium in the root cells of *Allium cepa* by electron energy loss spectroscopy and cytochemistry, *J. Biosciences* **29**, 329--335, 2004.
250. Sridhar, B. B. M., et al. Anatomical change due to uptake and accumulation of Zn and Cd in Indian mustard (*Brassica juncea*), *Environ. Exper. Bot.* **54**, 131--141, 2005.

251. He-man, H.G. Ultrastructural localization of zinc in zinc tolerant *Armeria maritime* sp. halleri by autometallography, *J. Plant Physiol*. **159**, 191--203, 2002.

252. Salt, D.E., Smith, R.D., & Raskin, I. Phytoremediation. Annu. Rev. Plant Physiol, *Plant Mol. Biol*. **49**, 643--668, 1998.

253. Han, Y.L., et al. Cadmium tolerance and accumulation by two species of *Iris*, *Ecotoxicology* **16**, 557--563, 2007.

254. Han, Y.L., et al. Tolerance and accumulation of lead by species of *Iris* L, *Ecotoxicology* **17**,853--859, 2008.

255. Ke, W.S., et al. Accumulation, subcellular localization and ecophysiological responses to copper stress in two *Daucus carota* L. popularions, *Plant Soil* **292**, 291--304, 2007.

256. Wu, H., & Yang, M. Reduction in vacuolar volume in the tapetal cells coincides with conclusion of the tetrad stage in *Arabidopsis thaliana*, *Sex. Plant Reprod.* **18**, 173--178, 2005.

257. Chen, M.H. Effects of calreticulin on viral cell-to-cell movement, *Plant Physiol*. **138** 1866--1876, 2005.

258. Ni, C. A., et al. Subcellular localization of copper in tolerant and non-tolerant plant, *J. Environ. Sci*. **17**(3), 452-456, 2005.

259. Chalmers J. M., & Griffiths P. R. *Handbook of Vibrational Spectroscopy*, Chichester, Wiley, 2002.

260. Stewart, D. Fourier-Transform infrared microscopy of plant tissues, *Appl. Spectrosc*. **50**, 357, 1996.

261. Wase, D.A.J. & Forster, C. F. *Biosorbents for metal ions*, Taylor & Francis Ltd (Editors), London, United Kingdom, 1997.

262. Gardea-Torresdey J.L., et al. Phytofilteration of hazardous cadmium, chromium, lead, and zinc ions by biomass of Medicago *sativa* (alfalfa), *J. Hazard. Mater*. **57**, 29--39, 1998.

263. Sawalha, M.F., et al. Thermodynamic and isotherm studies of the biosorption of Cu(II), Pb(II) and Zn(II) by leaves of saltbush (*Atriplex canescens*), *J. Chem. Thermodynamics* **39**, 488--492, 2007.

264. Skoog, D. A., & Leary, J. J. *Principles of Instrumental Analysis*, 4th ed. Saunders College Publishing, Orlando, USA, 1992.

265. Fernando, D.R. et al. Manganese accumulation in the leaf mesophyll of four tree species: a PIXE/ EDXA localization study, *New Phytol*. **171**, 751--758, 2006a.

266. Turnau, K., et al. Metal uptake and detoxification mechanisms in *Erica andevalensis* growing in a pyrite mine tailing, *Environ. Exp. Bot*. **61**,117--23, 2007.

267. Sahi, S.V., et al. Characterization of a lead hyperaccumulator shrub *Sesbania drummondii*, *Environ. Sci. Technol*. **36**, 4676--4680, 2002.

268. Hu, P.-J., et al. Tolerance, accumulation and distribution of zinc and cadmium in hyperaccumulator *Potentilla griffithii*, *Environ. Exp. Bot*. **66**, 317--325, 2009.