CHAPTER 1

Introduction

1.1 Context of the thesis
Water, soil and air pollution are the greatest concern of environment as some of the most toxic metal/metalloids (Pb, Cd, As, Sb) have no known biological functions [1] not only affects natural resources but also causes a major strain on ecosystems. The removal of health hazard pollutants from water and soil has been long researched in the fields of environmental science and engineering to find ways of eliminating this serious problem in both the developed and developing world. Many different extraction methods have been developed empirically for commercial usage. Hence there is a need to develop and apply alternative, environmentally sound technologies. One of the effective and affordable technological solutions is the application of a process known as phytoremediation. Phytoremediation is the application of plants for in situ or ex situ treatment/removal of contaminated soils, sediments and water. The green plants degrade, assimilate, metabolize, or detoxify inorganic and organic pollutants from the environment or render them harmless. It is a cost effective ‘green’ technology based on the use of specially selected metal-accumulating plants to remove toxic metals. This research thesis addresses water pollution through heavy and toxic metals/metalloids in detail and applies prevailing scientific knowledge and the laboratory experimentation to assess the abilities of some aquatic plants and diatoms to extract soluble and toxic heavy metals from water grown hydroponically.

1.2 Research overview
Environmental science provides the scope of studying various components of chemical processes related to water, soil and plants. This branch of science when interlinked with engineering applications, produce useful techniques and processes that can lead researchers to new manufacturing and control techniques for example, pollution control systems. This research is divided into segments of heavy metal pollution, some aquatic plant characteristics, heavy metal/metalloid removal methods in hydroponic conditions and application of algae in metal remediation.

With the ecological problems in view, therefore the motivation of this research is to evaluate the species of aquatic plant for waste water quality
improvement. Phytoremediation using aquatic plants is evolving as a cost-effective alternative to high-energy, high-cost conventional method, thus considered to be a “Green Method” in the clean-up technologies. Phytoremediation for the cleanup of the metal contaminated and polluted ecosystems is emerging as a cutting edge area of research gaining commercial significance in the contemporary field of environmental biotechnology. Several microbes, including mycorrhizal and non-mycorrhizal fungi, agricultural and vegetable crops, ornamentals, and wild metal hyperaccumulating plants are being tested both in lab and field conditions for decontaminating the metalliferous substrates in the environment.

The objectives of the research are:

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

Some algae and macrophytes accumulate high concentrations of potential contaminants such as metals in their roots, stems and other tissues [2]. This ability has led to their common use in traditional wastewater treatment systems such as trickling filters and sewage stabilization ponds to improve the quality of municipal and industrial effluents prior to discharge to receiving waters.

**1.3 Hypothesis or research questions considered in this research**

The following hypothesis or questions were considered before starting this study

1. In this research we hypothesize that aquatic macrophytes and diatoms are potential candidates for remediation of toxic heavy metals/metalloids from aqueous solution. Therefore, extensive research work has been carried out to identify such aquatic macrophytes and diatoms locally available in this part of the world.
2. How efficiently and to what extent these plants can remediate, detoxify or immobilize those toxic metal/metalloids from aqueous solution? And to what extent these metal/metalloids are translocated by these plants, to their harvestable parts?
3. Is there any phytotoxic effects of these metals/metalloids?
4. What possible detoxification and tolerance mechanisms developed by these plants?
5. What possible uptake mechanisms of As in diatom *Navicula* species?

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

### 1.4 Conventional Techniques Used for Heavy Metal Removal

A variety of conventional treatment technologies, based on the principle of precipitation, ion exchange, electrolysis, solvent extraction, reverse osmosis, aeration, membrane and biosorption process have been proposed and is tested for removal efficiency of different pollutants from potable water as well as industrial effluent [3, 4, 5, 6, 7]. Each technique provides a different and unique approach and perhaps provides certain advantages over others for a particular situation. But these process are not very popular because one or more disadvantage. However, when large volumes of water containing toxic elements are to be treated, it would, be of great advantage if the method would provide reliable results without involving much cost and working efforts.

### 1.5 The Need for Novel Technology

The increasing concern about the contamination of water bodies by heavy metals has stimulated a large number of researches to find possible ways to remove these toxic substances from the environment. To overcome some of the limitations of physicochemical treatments, there is a need for inexpensive and efficient technology for the treatment of metal containing wastes so that metal concentration can be reduced to environmentally acceptable levels [8]. Use of biomass for metal removal/recovery is considered to be a viable alternative to conventional methods. Aquatic macrophytes treatment system (AMATS) is a well-established environment protective technique as a phytoremediation procedure for removing pollutants. Some fresh water macrophytes including *Potamogeton lucens, Salvinia hergozi, Eichhornia crassipes, Myriophyllum spicatum, Cabomba* sp., and *Cratophyllum*
demersum have been investigated for their potential in heavy-metal removal. Their mechanisms of metal removal by biosorption can be classified as extracellular accumulation/precipitation, cell surface sorption/precipitation, and intracellular accumulation [9].

1.5.1 Laboratory Test Method
The laboratory methods include main research application, procedures and experiments required for this study. For example, ‘phytoremediation’ process to extract toxic metals from polluted water using aquatic plants and diatoms is a research application. However, using hydroponic culture whilst determining location, quantity of metal uptake are laboratory tests required to use such an application. Use of various apparatus such as hydroponic culture, scanning electron microscope (SEM) with EDX, transmission electron microscopy (TEM), FTIR, ICP-OES, XRD are integral to laboratory experiments which are main phases of this research study.

1.6 Phytoremediation of toxic metals
Phytoremediation, a plant based green technology, has received increasing attention after the discovery of hyperaccumulating plants which are able to accumulate, translocate, and concentrate high amount of certain toxic elements in their above-ground/harvestable parts. Phytoremediation of the toxic contaminants can be readily achieved by aquatic macrophytes or by other floating plants since the process involves biosorption and bioaccumulation of the soluble and bioavailable contaminants from water [10]. Phytoremediation includes several processes namely, phytoextraction, phytodegradation, rhizofiltration, phytostabilization and phytovolatilization. Both terrestrial and aquatic plants have been tested to remediate contaminated soils and waters, respectively. BCF is a more important measure than shoot metal concentration when considering the potential of a given candidate species for phytoextraction [11]. Translocation factor value greater than 1 indicates the translocation of the metal from root to above-ground part [12]. According to Yoon et al. [13] only plant species with both BCF and TF greater than 1 have the potential to be used for phytoextraction.

Metal accumulation is expressed by the metal biological absorption coefficient (BAC), i.e., the plant (harvestable)-to-soil/water metal concentration ratio [10]. Besides convenient BAC, both the high bioconcentration factor (BCF, root-to-soil/water metal concentration ratio) and the translocation factor (TF, shoot-
to-root metal concentration ratio) can positively affect phytoextraction. Tolerant plant species tend to restrict soil-root and root-shoot transfers, and therefore, have much less accumulation in biomass, whereas hyperaccumulators actively take up and translocate metals into above-ground tissues. Plants with high BAC (greater than 1) are suitable for phytoextraction; those with high BCF (higher than 1) and low TF (lower than 1) have potential for phytostabilisation [14].

The bioconcentration factor (BCF) is the most widely used ratio to describe the capacity of the plants to concentrate metals from an aqueous solution (Table 1.1).

### Table 1.1 BCF and TF used to assess metal uptake in various macrophytes

<table>
<thead>
<tr>
<th>Factor</th>
<th>Plant Species</th>
<th>Metals/metalloids</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCF</td>
<td><em>Ipomea aquatic</em>, <em>Elodea nuttallii</em>, <em>Lemna gibba</em>, <em>Azolla caroliniana</em>, <em>Eichhornia crassipes</em></td>
<td>Cd, Zn, As</td>
<td>[15, 16, 17, 18, 19]</td>
</tr>
<tr>
<td>TF</td>
<td><em>Typha domingensis</em>, <em>Pistia stratiotes</em>, <em>Limnocharis flava</em> (L.) Buchenau, <em>Hydrilla verticillata</em> (L.f.) Royle</td>
<td>Al, Cd, As</td>
<td>[20, 21, 22, 23]</td>
</tr>
</tbody>
</table>

### 1.7 Metallophytes

Metallophytes are plants that are specifically adapted to and thrive in heavy metal-rich substrate [24, 25]. Metallophytes are divided into three categories: metal excluders, metal indicators and metal hyperaccumulators.

#### 1.7.1 Metal excluders

Metal excluders plants accumulate heavy metals from substrate into their roots but restrict their transport and entry into their aerial parts [25, 26]. Such plants have a low potential for metal extraction but may be efficient for phytostabilization purposes [27, 28].

#### 1.7.2 Metal indicators

This categories of plants accumulate heavy metals in their aerial parts. As the name indicates, these plants generally reflect heavy metal concentrations in the substrate [25].

#### 1.7.3 Metal hyperaccumulators

Hyperaccumulators are plants, which can concentrate heavy metals in their aboveground tissues to levels far exceeding those present in the soil or in the nearby
Growing non-accumulating plants [29, 30]. They are regarded as hypertolerant to the metals, which they accumulate in the shoots [31]. Generally, hyperaccumulators achieve 100-fold higher shoot metal concentration (without yield reduction) compared to crop plants or common nonaccumulator plants [27, 32]. Hyperaccumulators achieve a shoot-to-root metal concentration ratio (called translocation factor, TF) of greater than 1 [33, 34]. However, TF cannot be used alone to define hyperaccumulation although it is a useful measure in supporting other evidence of hyperaccumulation [35].

1.8 Macrophytes in phytoremediation

Freshwater as well as seawater resources are being contaminated by various toxic elements through anthropogenic activities and from natural sources. Phytoremediation of the toxic contaminants can be readily achieved by aquatic macrophytes since the process involves biosorption and bioaccumulation of the soluble and bioavailable contaminants from water [10]. In aquatic phytoremediation systems, aquatic plants can be either floating on the water surface or submerged into the water. The floating aquatic hyperaccumulating plants absorb or accumulate contaminants by its roots while the submerged plants accumulate metals by their whole body. Macrophytes play prominent role in nutrient and heavy metal recycling of many aquatic eco-system [36]. Among various plant groups used for phytoremediation, aquatic macrophytes attain the most important position. Several species of aquatic macrophytes such as free floating, water hyacinth (*Eichhornia sp.*), duck weeds (*Lemna sp.*), *Spirodella sp.*, small water fern (*Azolla sp.*) and water lettuce (*Pistia sp.*); submerged macrophytes such as *Hydrilla, Egeria* and emergent macrophytes like water chestnut (*Trapa natans*), have been used for the removal of heavy metals from waste water. They have tremendous capacity of absorbing nutrients and other substances from the water [37] and hence bring the pollution load down. So these plants are suitable for phytoremediation of heavy metals from waste water.

The main route of heavy metal uptake in aquatic plants is through the roots in the case of emergent and surface floating plants, whereas in submerged plants, roots as well as leaves take part in removing heavy metals [38].
In recent years, many aquatic plants, usually those found in polluted water bodies have been suggested for waste water treatment, i.e., they have the ability to accumulate unusually high concentration of heavy metals, without impact on their growth and development.

Some freshwater macrophytes including *Potamogeton lucens*, *Salvinia hergozi*, *Eichhornia crassipes*, *Myriophyllum spicatum*, *Cabomba* sp., and *Cratophyllum demersum* have been investigated for their potential in heavy metal and colour removal. Their mechanisms of metal and colour removal by biosorption can be classified as extracellular accumulation/precipitation, cell surface sorption/precipitation and intracellular accumulation [39]. Among the various plants species group, aquatic macrophytes attain greatest interest in the field of phytoremediation because of their great potential to accumulate heavy metals inside their plant bodies. These plants can accumulate heavy metals up to 100,000 times greater than the amount in the associated water. Therefore, these aquatic macrophytes have been used for heavy metal removal from a variety of sources [40]. Zhu et al. [41] have studied the ability of water hyacinth to take up and translocate six elements namely As(V), Cd(II), Cr(VI), Cu(II), Ni(II) and Se(VI) under controlled laboratory conditions. Cd, Cr, Cu, Ni and As were more highly accumulated in roots than shoots whereas Se was accumulated more in shoots than in roots at the most external concentration.

Gupta et al. [42] compared the phytoremediation efficiency of three aquatic macrophytes water hyacinth, water lettuce and vetiver grass. Aquatic macrophytes were found to absorb nutrients with their effective root system. They are widely used to remove nutrients and heavy metals in the form of constructive wetlands or retention ponds because of their fast growth rate and ability to accumulate toxic materials. It was concluded that many researchers have used water hyacinth, water lettuce and vetiver grass for the removal of water contaminants but their treatment capabilities depend on different factors like climate, contaminants concentrations, temperature, etc.

A plant-based phytoremediation approach to remove heavy metals uses plant roots to extract, the vascular system to transport and the leaves as a sink to concentrate the elements above ground for harvest and processing of metals [43, 44, 45]. Phytoremediation is considered a clean, cost-effective and non-environmentally disruptive technology, as opposed to mechanical clean-up methods such as soil
excavation or pumping polluted groundwater. Over the past two decades, this technology has become increasingly popular and has been employed in situ in soil and water, contaminated with lead, uranium, and arsenic. However, one major disadvantage of phytoremediation is that, it requires a long-term commitment, as the process depends on plant growth, tolerance to toxicity, and bioaccumulation capacity [46, 47]. The use of hydroponic culture treatment has been considered as a means of assessing the plant tolerance to the toxic elements or its efficiency in mineral utilization. The bio removal process using aquatic plants contains two uptake processes such as (i) biosorption which is an initial fast, reversible, and metal-binding process and (ii) bioaccumulation which is a slow, irreversible, and ion-sequestration step. At the end of the 19th century, *Thlaspi caerulescens* and *Viola calaminaria* were the first plant species documented in the literature to accumulate high levels of metals in leaves. In the last decade, extensive research has been conducted to investigate the importance of metal in biology. Some macrophytes are found to remove different concentrations of arsenic ions, which make them suitable to act as bio-monitors for metals, and have ability to act as biological filters of the aquatic environment [48, 49, 50, 51].

The plants using in phytoremediation should have the following criteria will be suited as good remediates plants

a) Extract large concentrations of heavy metals,

b) Rapid growth, easy spreading and easy harvesting,

c) High translocation factors (TF) and high bio-concentration factor (BCF),

d) Produce a large quantity of plant biomass, and

e) Must have mechanisms to detoxify or tolerate high metal concentrations accumulated in their shoots.

Based on these above criteria plants for the present study were selected.

For aquatic macrophytes that possess roots but do not have a close physical association with sediments, the water is undoubtedly the principal source of elements. The uptake of trace metals through the root systems and subsequent release of metals during decomposition of plant material and transmission of these metals to organisms of higher trophic levels represent a pathway of cycling of trace metals in aquatic ecosystems.


1.9 Types of Phytoremediation

Plants utilize several methods to remediate the polluted sites. Phytoremediation technology can be subdivided, on the basis of its underlying process and applicability, as follows:

1. Phytoextraction,
2. Phytostabilization,
3. Phytovolatization,
4. Phytodegradation (Phytotransformation) and
5. Rhizofiltration.

Table 1.2 Different phytoremediation processes [52]

<table>
<thead>
<tr>
<th>Process</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phytoextraction</td>
<td>In this process, plants uptake pollutants from soil and water, and translocate to and store in the harvestable biomass of the plants. Phytoextraction aims to remove pollutants from the contaminated sites. This process is usually observed in hyperaccumulating plants, resistant to the pollutants.</td>
</tr>
<tr>
<td>Phytostabilization</td>
<td>Plants reduce mobility and phytoavailability of contaminants in the environment. This process does not remove pollutants from contaminated sites but reduces mobility and excludes metals from plant uptake.</td>
</tr>
<tr>
<td>Phytovolatilization</td>
<td>Hyperaccumulating plants uptake pollutants from soil and water, and translocate to the aerial parts of the plants, and volatilize the pollutants in the air.</td>
</tr>
<tr>
<td>Phytotransformation</td>
<td>This process is one kind of plant’s defence mechanism to the environmental pollutants. The hyperaccumulating plants modify, inactivate, degrade (phytodegradation), or immobilize (phytostabilization) the pollutants through their metabolism.</td>
</tr>
<tr>
<td>Rhizofiltration</td>
<td>Usually aquatic plants perform this process. The hyperaccumulating aquatic plants adsorb and absorb pollutants from aquatic environments (water and wastewater).</td>
</tr>
</tbody>
</table>

1.10 Quantification of phytoremediation efficiency

The efficiency of phytoremediation can be quantified by calculating bioconcentration factor (BCF) and translocation factor (TF). Both BCF and TF are important in screening hyperaccumulators for phytoextraction of heavy metals. The evaluation and selection of plants for phytoremediation purposes entirely depend on
Chapter 1

BCF and TF values [53]. BCF is a more important measure than shoot metal concentration when considering the potential of a given candidate species for phytoremediation [11]. Translocation factor value greater than 1 indicates the translocation of the metal from root to above-ground part [12].

1.10.1 Bio-concentration factor (BCF)

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. [54] reported that BCF can be calculated as follows

\[
\text{BCF} = \frac{\text{Concentration of metal in plant tissue}}{\text{Initial Concentration of metal in external solution}}.
\]

Bioconcentration factor indicates the efficiency of a plant species in accumulating a metal into its tissues from the surrounding environment [55]. Bioconcentration factor (BCF) is a useful parameter to evaluate the potential of the plants in accumulating metals/metalloids and this value was calculated on dry weight basis. The ambient metal/metalloid concentration in water was the major factor influencing the metal/metalloid uptake efficiency. In general, when the metal/metalloid concentration in water increases, the amount of metal/metalloid accumulation in plants increases, accordingly the BCF values also increases [56, 57].

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; >1,000mg kg\(^{-1}\) for Co, Cu, Ni, As, and Se; and >100 mg kg\(^{-1}\) for Cd [58, 59]; 2) the bioconcentration factor (BCF) is >1.0, and sometimes reaches 50 to 100 [60] and 3) the translocation factor is>1.0 [61]. BCF values can be indexed to estimate the capacity for plants to accumulate heavy metals. Based on many studies, BCF values differ with the type and concentration of heavy metals, plant species, and environmental conditions [62, 63].

1.10.2 Translocation factor (TF)

Translocation factor indicates the efficiency of the plant in translocating the accumulated metal from its roots to shoots. It is calculated as follows [64]

\[
\text{Translocation Factor (TF)} = \frac{C_{\text{shoot}}}{C_{\text{root}}}
\]

Where \(C_{\text{shoot}}\) is concentration of the metal in plant shoots and \(C_{\text{root}}\) is concentration of the metal in plant roots.
The efficiency of phytoextraction can be quantified by calculating bioconcentration factor and translocation factor.

Plant species were categorized according to their TF values into four groups. First group contain hyperaccumulator plants with TF values above ten. Second group contains hypertolerant plants with TF values above one but below ten. Third group contains tolerant plants with TF values less than one. This groups of plants have adopted an exclusion strategy. This strategy allows them to form metal stable complexes in their root cells, which results in a limited metal translocation to above-ground parts. The fourth group, categorized as excluders, as these plants can grow in heavy-metal polluted soils without accumulating significant quantities [65]. These plant species have TF values < 0.1. Possibly these plants use mechanism that avoids excessive uptake of metals and metal is absorbed and translocated only in nontoxic quantities. Since plants employed in phytoextraction treatments must accumulate more than 1000 mg/kg DW, should exhibit values of TF and BCF larger than unity, and should produce high quantities of biomass [66, 67, 68].

1.10.3 Biological accumulation coefficient (BAC)
The BAC is the concentration of metals in the plant shoots divided by the metal concentration in the soil/water.

\[
BAC = \frac{\text{Metal concentration in shoots}}{\text{Metal concentration in soil/water}}
\]

BAC factors >1 indicates that the plant species has the ability to store metals from the soil/water into the shoots (Baker & Brooks; Baker, Reeves & Hajar; Brown, Chaney & Baker; Wei, Chen & Huang as cited in Khan and Uzair [69].

Plants with high BAC (greater than 1) are suitable for phytoextraction; those with high BCF (higher than 1) and low TF (lower than 1) have potential for phytostabilisation [13].

Both BCF and TF are important in screening hyperaccumulators for phytoextraction of heavy metals. The evaluation and selection of plants for phytoremediation purposes entirely depend on BCF and TF values [53]. BCF is a more important measure than shoot metal concentration when considering the potential of a given candidate species for phytoextraction [11]. Translocation factor value greater than 1 indicates the translocation of the metal from root to above-ground part [12]. According to Yoon et al. [13], only plant species with both BCF and TF greater than 1 have the potential to be used for phytoextraction. Hyperaccumulators have BCF greater than 1, sometimes reaching 50–100 [48].
Bio-concentration factor (BCF) is a useful parameter to evaluate the potential of the plants in accumulating metals/metalloids and this value was calculated on dry weight basis. The ambient metal/metalloid concentration in water was the major factor influencing the metal/metalloid uptake efficiency. In general, when the metal/metalloid concentration in water increases, the amount of metal/metalloid accumulation in plants increases, accordingly the BCF values also increases [70, 71]. In the present thesis the effects of bio-concentration factor values of Pb$^{2+}$, Cd$^{2+}$, As and Sb$^{2+}$ at different concentration and exposure times were presented.

1.11 Heavy metals uptake, translocation, and tolerance mechanisms
There are several ways by which plants clean up contaminated sites. Plants initiate a range of potential cellular mechanisms to nullify and attenuate the adverse effects of heavy metals. The root system provides an enormous surface area that absorbs and accumulates the water and nutrients essential for growth along with other non-essential contaminants. Plant roots change the soil/water-root interface as they release the organic and inorganic exudates in the rhizosphere, which affect the aggregation, stability of the contaminants. After entry of heavy metals/metalloids from soil/water solution into roots, they are either stored in the roots or translocated to the shoots primarily through xylem vessels [72, 73] where they are mostly deposited in vacuoles. Vacuoles are the cellular organelles with low metabolic activities [74]. The entire mechanism of phytoextraction of heavy metals has five basic aspects: mobilization of the heavy metals in substrate, 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. Metal tolerance is a key prerequisite for metal accumulation and hence phytoremediation [75, 76].

The mechanisms of metals or metalloids detoxification, accumulation, and tolerance have become the basis for using plants for the remediation of metal-contaminated site. Plants are very well equipped with a wide array of defense mechanisms to cope with metal accumulation and toxicity. Plants have a range of intracellular ligands potentially involved in metal accumulation and detoxification. 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: phytochelatins (PCs) and metallothioneins. The interaction of plant roots and substrate microbes can improve metal bioavailability in rhizosphere through
secretion of proton, organic acids, phytochelatins (PCs), amino acids, and enzymes. Chelation through the induction of metal-binding peptides and the formation of metal complexes is one of the existing mechanisms. The members of the third class of MTs, the PCs, are responsible for the formation of complexes with heavy metals in plants [77, 78]. These peptides are enzymatically derived and synthesized on exposure of the cell to toxic metals. The structure of PCs is (γ-Glu-Cys)nX, in which X is Gly, γ-Ala, Ser or Glu and n = 2-11 depending on the organism, although the most common forms have 2-4 peptides [77]. The biosynthesis of PCs is induced by many metals including Cd, Hg, As, Ag, Cu, Ni, Au, Pb and Zn; however, Cd is by far the strongest inducer [79]. It is now clear that a large number of low molecular organic acids also involve in plant metal detoxification besides sulphur-containing proteins and peptides.

Heavy metal sequestration in the vacuole is one of the ways to remove excess metal ions from the cytosol and may reduce their interactions or toxicity with cellular metabolic processes [80, 81]. Compartmentalization of complexed metals in vacuoles is part of the tolerance mechanism in metal hyperaccumulators [60, 76]. 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 within the cell [82, 83].

The overall journey of heavy metals from soil/water solution to the vacuoles is controlled and regulated by a variety of molecules. Some molecules are involved in the cross-membrane transport of the heavy metals and others are involved in their complexation and subsequent sequestration. Uptake of heavy metal ions from soil solution is mediated by specialized transporters (channel proteins) or H+ coupled carrier proteins present in the plasma membrane of the root [84]. For example, transporters of the ZIP (zinc–iron permease) family contribute to the uptake of Zn²⁺ and Fe²⁺ [85]. Nramp (natural resistance-associated macrophage) is another family of proteins, which plays an important role in transport of divalent metal ions [86]. Non-essential heavy metals may effectively compete for and enter roots through the same transmembrane transporters used by essential heavy metals having similar oxidation states and ionic radii [87, 88].
1.12 Metals/metalloids under Present Study

1.12.1 Lead (Pb)

Lead (Pb) is an important environmental pollutant extremely toxic to plants and other living organisms. Out of several stable isotopes, it is the most abundant. Lead occurs as Galena (PbS). Early, lead was used in water pipes and pipe joints. Pb is a very heavy, soft, highly malleable, bluish – grey colour metal and 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 [89].

There are various industries and Pb smelting units in India which causes a marked increase in Pb concentration in air. In the eastern part of Kolkata, there are a large number of small scale lead smelting units run by local people who remelt lead from discarded automotive batteries and other such items in poorly designed furnaces without adequate preventive measures. This results in spreading out of lead fumes in a thickly congested areas which ultimately enters into the environment.

1.12.1.1 Uptake of Pb by plant

Although lead is not an essential element for plants, it gets easily absorbed and accumulated in different plant parts [90].

1.12.1.2 Pb toxicity

It is easily taken up by plants from the soil and the surrounding environment and is accumulated in different organs. Although plants has the ability to accumulate large amounts of Pb without visible changes in their appearance or yield but Pb is toxic and harmful to plants [91]. Naresh Kumar et al. [92] studied the Pb-Stress Induced Oxidative Stress caused alterations in antioxidant efficacy in two groundnut (Arachis hypogaea L.) cultivars and showed that Pb-stress results in an increase in free radicals (O$_2^-$ and H$_2$O$_2$) generation in both groundnut cultivars. Pb is considered as a general protoplasmic poison, which is cumulative, slow acting and subtle. Soils contaminated with Pb causes sharp decreases in crop productivity thereby posing a serious problem for agriculture [93]. Studies have demonstrated that the bulk of the Pb taken up by plants remains in the roots restricting its translocation to above ground parts [94, 95]. It has been demonstrated that at the root surface Pb binds to carboxyl groups of mucilage uronic acids which restricts metal uptake into the root.
and establishes an important barrier protecting the root system. However, some of the bound Pb gets released when mucilage is biodegraded [96]. The pattern of distribution of Pb in the roots considerably differs depending on whether the concentrations of Pb are lethal or non-lethal [97]. At lower concentrations, Pb ions predominantly flow in the apoplast, whereas at higher concentrations, the barrier function of plasmalemma is damaged and a greater amount of Pb enters into the cells.

In general, the apparent concentration of Pb in aerial parts of the plant decreases as the distance from the root increases. This occurs due to greater localization of Pb in cell walls of the root than in other parts of the plant. Further, binding of Pb occurs more in lignified rather than non-lignified tissues. Suchodoller [98] found that in barley much of the applied Pb was retained in the root epidermis while a small amount could be detected in the vascular tissues. This suggests that the extent of localization of Pb in different tissues of the plant is also dependent on the plant species. In seeds, the testa prevents entry of Pb into the internal tissues until it is ruptured by the developing radicle. Once the testa is ruptured, Pb is taken up very rapidly, with notable exceptions occurring in the meristematic regions of the radicle and hypocotyls [94]. In the cotyledons, Pb moves through the vascular tissues and tends to accumulate in discrete areas in the distal parts [94]. The content of Pb in various plant organs tends to decrease in the following order: roots>leaves>stem>inflorescence>seeds. However this order can vary with plant species [99]. In onion (Allium cepa) plants, absorbed Pb is localized in highest concentration in the root tips followed by proximal parts of the root, while its lowest concentration is found in the root base [100]. Leaves differ in their abilities to accumulate Pb depending on age. Maximum Pb content is found in senescing leaves and least in young leaves [101].

1.12.1.3 Physiological, biochemical and ultrastructural effects of Pb

Ultrastructural studies have revealed that variable amounts of Pb deposits are present mainly in the intercellular space, cell wall and vacuoles, whereas small deposits of this metal are seen in the ER, dictyosomes and dictyosome derived vesicles. The cell wall and vacuole together account for about 96 % of absorbed Pb [102]. The fact that Pb is found in the ER and dictyosome is apparently related to metal secretion of the cell surface into the vacuole. A small quantity of Pb reaches nuclei, chloroplasts and mitochondria and exerts its toxic effects on these organelles. In leaf
cells of *Potamogeton* sp. it was shown that the electrochemical potential gradients between cell vacuoles and the outside bathing solution ranged from -150 to -240 mV [103] which could favor a passive influx of Pb into vacuoles during Pb treatment. Of particular interest is the invagination of the plasmalemma to form pinocytotic vacuoles in many plant species. In *Stigeoclonium* the formation of such vacuoles is important for the sequestration of excess metal ions, as these vacuoles could protect the cell contents from toxic effects of Pb [104]. Sometimes, particularly in close proximity to the plasmodesmata, the larger Pb particles appear to occupy much of the volume of the cell wall. In the other regions where the cell wall is much thicker and more substantial, smaller Pb particles accumulate within the cell wall towards its periphery. The deposition of these smaller Pb particles occurs possibly through the action of pinocytotic vesicles [105]. While considerable information is available on Pb distribution the cells of terrestrial plants, little is known about Pb localization in the cells of aquatic plants. *Lemna minor* L. (duck weed) plants when treated with Pb for one hour showed maximum concentration of Pb in small vacuoles [106]. After 6 and 12 h of Pb treatment, the content of in cell walls gradually increased. The localization of Pb between vacuoles and cell walls possibly results due to redistribution of Pb and it reflects increased apoplastic transport [106]. The presence of Pb in small vesicles in *Lemna minor* suggests that endocytosis plays an important role in Pb uptake in these plants.

1.12.1.4 Tolerance and Detoxification Mechanism of Pb by plant

Mechanisms of Pb-detoxification include sequestration of Pb in the vacuole, phytochelatin synthesis and binding to glutathione and amino acids etc. Pb tolerance is associated with the capacity of plants to restrict Pb to the cell walls, synthesis of osmolytes and activation of antioxidant defense system. Remediation of soils/water contaminated with Pb using phytoremediation and rhizofiltration technologies appear to have great potential for cleaning of Pb-contaminated soils/water.

1.12.2 Arsenic (As)

Arsenic is considered as one of the most significant pollutant in many countries of world. It can be easily solubilized in groundwater depending on pH, redox conditions, temperature, and solution composition [107, 108]. There are many possible routes of human exposure to arsenic from both natural and anthropogenic sources. Arsenic is mobilized by natural weathering reactions, biological activity, mining activity, geochemical reactions, and volcanic eruptions [109]. Most
environmental arsenic problems are the result of mobilization under natural conditions. Arsenic from weathered rocks and soils dissolves in groundwater. Arsenic concentrations in groundwater are particularly high in areas with geothermal activity. Inorganic arsenic derived from rocks such as arsenic trioxide (\(\text{As}_2\text{O}_3\)), orpiment (\(\text{As}_2\text{S}_3\)), arsenopyrite (\(\text{AsFeS}\)) and realgar (\(\text{As}_4\text{S}_4\)) is most prevalent [110].

**1.12.2.1 As in water**

As is one of the most deleterious and widespread pollutants in aquatic system. Arsenic concentrations in ground water are of increasing environmental concern because of the risk arsenic poses to plants, animals and human health [111]. The EPA is in the process of setting the new arsenic standard for drinking water at 10ppb (\(\mu\text{g/L}\)) to protect humans against the effect of long term, chronic exposure to arsenic in drinking water. Roughly 5% (or 3000) of community water systems serving 11 million people will have to take corrective action to lower the current levels of arsenic in their drinking water [112]. Higher levels of arsenic are found in ground water sources than in surface-water sources. Arsenic in ground water is mainly inorganic with arsenate comprising about 50% of the total [113].

**1.12.2.2 The Toxicity of As**

The results from a number of hydroponic experiments agree that As phytotoxicity depends on the chemical species supplied to the plant, but disagree on the identity of the most phytotoxic form of As [114, 115, 116]. These hydroponic experiments provide the clearest insights into the potency of externally supplied As on whole plant growth because they eliminate the complex and confounding phytoavailability issues that arise from differences in the mobility of various As species through the diverse growth substrates. The studies generally agree with the hydroponic survey of 46 different plant species [117] that the uptake of As by plants has the order \(\text{As}^{\text{III}} > \text{As}^{\text{V}} > \text{MMA}^{\text{V}} > \text{DMA}^{\text{V}}\), while translocation from the roots to the rest of the plant has the order \(\text{DMA}^{\text{V}} > \text{MMA}^{\text{V}} > \text{As}^{\text{V}} \geq \text{As}^{\text{III}}\).

An important mode of action of AsV toxicity may be the replacement of phosphate (P) in critical biochemical reactions. Substitution of P by AsV has been demonstrated by many researchers to occur in numerous biochemical reactions, and any reaction with (inorganic phosphate) \(\text{Pi}\) or a Pi-ester as a substrate is a potential target for AsV disruption [118, 119].

Arsenate (As) is an analog of phosphate (P) and competes for the same uptake carriers in the root plasmalemma of plants [120]. The As tolerance has been
identified in a number of plant species [121]. The As tolerance in grasses results from suppression of a high-affinity P/As uptake system [120]. This suppression reduces As influx to a level at which plant can easily detoxify it, presumably by constitutive mechanisms [121].

1.12.3 Cadmium (Cd)
Cadmium (Cd) is a heavy metal of particular concern with respect to environmental quality and health [122]. As a non-essential trace metal for biological functions, it is highly toxic to plants and animals [123, 124]. Anthropogenic pathways by which Cd release into the environment are through industrial waste from processes such as electroplating, manufacturing of plastics, paint pigments, alloy preparation and batteries that contain cadmium. Household appliances, automobiles and trucks, agricultural implements, airplane parts, industrial tools, hand tools and fasteners of all kinds (e.g. nuts, bolts, screws, and nails) are commonly Cd coated. Cadmium is also used for luminescent dials, in photography, rubber curing, and as fungicides [125]. Sequestration of Cd at elevated concentrations not only results in toxicity symptoms but also cause structural and ultrastructural changes [126].

1.12.3.1 Physiological, biochemical and ultrastructural effects of Cd
The effect of excessive concentrations of cadmium (Cd$^{2+}$), on plant species has been extensively studied with biological and ecological consequences that depend on its availability in the soil or water. Plants grown in soil containing high levels of Cd show visible symptoms of injury reflected in terms of chlorosis, growth inhibition, browning of root tips and finally death [127, 128, 129]. The inhibition of root Fe(III) reductase induced by Cd led to Fe(II) deficiency, and it seriously affected photosynthesis [130]. The presence of heavy metals in the rhizosphere causes a decrease in plant growth [131, 132] and disturbs cellular metabolism [133] thus, negatively affecting important processes, such as the transport of water [134] oxidative phosphorylation in the mitochondria [135] photosynthesis [136] and alteration in chlorophyll contents [137]. The damage to photosynthesis occurs mainly from an impaired efficiency of the Rubisco activity, a decrease in chlorophyll and an increase in lipid peroxidation within these organelles [138]. Fodor et al. [139] found that cadmium treatments have been shown to reduce ATPase activity of the plasma membrane of wheat and sunflower roots.
Cd$^{2+}$ does not have a biological function and is extremely toxic to plants and animals [140]. Anatomic and structural changes are known to be some of the effects of Cd in plants [141, 142]. Studies in the literature have shown an increase in the vacuoles, condensation of cytoplasm due to an increase of the matrix density, reduction of the mitochondrial cristae, severe plasmolysis and high condensation of chromatin [143], disorganization of the chloroplast structure [144] and disruption of the nuclear envelope, plasmalemma and mitochondrial membranes [145].

1.12.4 Antimony (Sb)

Antimony (Sb) is a toxic element with adverse effects to humans and the environment and without any known physiological function [146]. It can exist in a variety of oxidation states (+5, 0, +3, -3), but it is mainly found in the environment as trivalent and pentavalent. Water-soluble Sb is comparable in its toxicological behavior to arsenic (As), hence trivalent species are more toxic than pentavalent ones [147]. Anthropogenic sources of atmospheric Sb include tire, brake, engine and vehicle components deterioration [148] and to a lesser extent fuel combustion [149]. In recent years Sb has been linked to traffic emissions [150] and recognized as a traffic related element (TRE) associated with particulate matter [151] and as a consequence Sb has become an element of increasing environmental concern.

1.12.4.1 Sb Uptake

Different species of plants have been shown to differ in their Sb uptake capacities and tissue storage targets. Many plants accumulate Sb in the roots [152, 153, 154] while maize and radish accumulate more Sb in the shoots [155, 156]. Similar to other plants, wheat and rice take up more SbIII than SbV [157, 158] but rye takes up more SbV than SbIII [158].

Tschan et al. [159] investigated the extent of Sb uptake by maize (Zea mays) and sunflower (Helianthus annuus) from nutrient solutions containing concentrations from 3 to 24 mg/L of potassium antimonate, with the aim of determining the potential of Sb to enter the food chain. Tisarum et al. [160] investigated the (Sb) up take and translocated in plants. Sb accumulated both in roots and shoots of clover without any negative effect on root growth, cellular viability and lipid peroxidation. This absence of toxicity symptoms in clover plants could be very dangerous because Sb can be in avertedly incorporated into the trophic chain causing toxic effects both in animals and humans. The absence of toxic effects on plants does not seem to be due to detoxification by phytochelatins because the use
Chapter 1

of the gamma-glutamylcysteine synthetase inhibitor, L-buthionine-[S, R] sulfoximine (BSO) did not enhance Sb toxicity to plants.

1.13 The fate of plant after phytoremediation

An important question of phytoremediation study is always, what will be the fate of plants after being used for phytoremediation of heavy metals/metalloids? Because the accumulation and removal of the metals from water by aquatic plants would not be enough for the successful implementation of this emerging technology without proper management. Here some possible ways discuss to handle the post-harvest plant used for phytoremediation of heavy metals [161, 162].

**Figure 1.1 Post-harvest treatment of Phytoremediator plants, modified from [162]**

1.13.1 Phytomining

It is an emerging technique to detect the reserve of valuable elements (like gold) in a particular underground place [163]. There are some hyperaccumulating plants that can accumulate rare metals such as gold and nickel from soil in there harvestable part, and after that these valuable elements can be extract from plants [164].

It is a recent more advanced technology of phytoremediation to produce low volume, sulphide-free ‘bio-ore’, which can either be safely disposed of or, if the target metal is of sufficient economic value, smelted, and recovered. This technology has potential application in the mineral industry to return an economic profit by commercial production of metals via cropping. There are also enormous potential environmental benefits from these phenomena. Certain plants use to extract metals from soil and water is commercially feasible. These specially selected plants-called hyperaccumulators- are known for their ability to take up and store particular metals. Recovery of metals from these plants can be defined as phytoextraction. There are two basic applications: Phytomining, where valuable naturally occurring elements are harvested and Phytoremediation, where non-naturally occurring
contaminants are recovered for secure disposal or reuse. Both are relatively new technologies and require fundamental research before becoming widely adopted by either the mining industry or environmental practitioners. These plants would be burned after harvest to create a biomass energy byproduct, with the respective metal recovered from the ash. In this way the land can then be converted to productive farming activities providing sustainability for the region and a natural method for these local people to transition their economy from mining to agriculture. Hyperaccumulators are good candidates in phytoremediation, particularly for the removal of heavy metals. Phytomining efficiency of plants can be substantially improved using genetic engineering technologies. Metal and metalloid contaminations seriously threaten the health of a large number of people worldwide and require novel, low-cost, flexible and effective phytoremediation technologies. The identification of unique genes from natural hyperaccumulators and their subsequent transfer to fast growing species is a promising approach to enhance the phytoremediation abilities of plant species. Recent research results, including over expression of genes whose protein products are involved in metal uptake, transport, and sequestration, or act as enzymes involved in the degradation of hazardous organics, have opened up new possibilities in phytoremediation.

1.13.2 Carbonization and incineration
The high heavy metal content aquatic plants may be used for the making charcoal and the by-product gas can be used as fuel. Water hyacinth has been used in this purpose [165].

1.13.3 Hydrolysis and fermentation
Hydrolysis and fermentation also require yeast fermentable sugars that may available only to a low extent in aquatic phytoremediating plants. Thomas and Eden [165] conclude that hydrolysis of water hyacinths to produce fuel is only feasible in situations where there is a high need for ethanol as a liquid fuel because of the negative energy balance.

1.14 Diatoms
1.14.1 Definition and classes
Diatoms are one of the most beautiful organisms to look at under a microscope. They are unicellular and eukaryotic microorganisms that form an important component of the aquatic ecosystem. Diatoms are protists belonging to phylum
Bacillariophyta and class Bacillariophyceae. Their cell walls are shaped like tiny glass pillboxes, with an amazing array of sizes, shapes and ornamentation. Diatoms show enormous species diversity and are inhibit both in fresh and marine water environments. There are more than 100000 diatom species, and each of them forms unique frustules (cell wall) made of amorphous silica composites. Each species form different frustules forms and a variety of pore types. Figure 1.2 shows a secondary electron micrograph, using a field emission scanning electron microscope of diatoms.

![Figure 1.2 General Morphology of the Diatom frustule (Source: Author)](image)

Diatoms are unicellular, photosynthesizing algae, also called phytoplankton, that belong to the group of Phaeophyceae (brown algae) [166]. They are ubiquitous and are distributed in both fresh as well as marine ecosystems. They are found throughout the world’s oceans and freshwater systems mainly in the surface photic layer where they receive sufficient light to support growth and primary production. With over 200,000 species under 250 genera, the diatoms constitute one of the largest groups of photosynthetic algae. Because of their wide spread distribution, the diatoms play critical ecological functions in terms of generation of an estimated 40% of the 50 billion tons of organic carbon in the global carbon cycling. The diatoms have interesting structural features. Apart from the importance of these organisms as major contributors to the overall carbon-life cycle, they also represent a primary food source to other aquatic organisms.

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. To overcome the limitation of their silica based frustule composition, diatom frustules have been converted into a variety of materials including silicon, silicon carbide, silver, gold, palladium and carbon in the
present day. The compositions and the extent of shape preservation of the replicas are examined and evaluated with different characterization methods such as X-ray diffraction, SEM and FTIR analyses.

Depending on the diatom species and the culturing conditions, these frustules can display a wide range of different morphologies. A collection of SEM images of diatom frustules, which demonstrates the diversity of diatom frustule morphologies, is shown in Figure 1.3. Diatoms are microscopic (~1–500 µm in length) single-celled algae with characteristic rigid cell walls (frustules) composed of amorphous silica. They are ubiquitous organisms found in a wide variety of habitats and are thought to be responsible for up to 25% of the world’s net primary production of organic carbon [167]. There are currently estimated to be over 100,000 different species, classified by their unique frustule morphologies [168].

![Figure 1.3 Diatom, Navicula sp. (Source: Author)](image)

Diatoms are single celled organisms which secrete intricate skeletons. These may be elongate with a bilateral plane of symmetry or they may be round and radially symmetrical. Many diatoms are slightly asymmetrical though they generally fall into one of these two categories.

The skeleton of a diatom or frustule is made of very pure silica coated with a layer of organic material. This skeleton is divided into parts, one of which (epitheca) overlaps the other (the hypotheca) like lid of a box joined with the connecting bands. The connecting bands together form a griddle, which are also made of silica. The griddle bands form the rims of the two valves and allow unidirectional growth of the diatom during vegetative division.
There are two basic body shapes of diatoms based on symmetry, centric (round with radial symmetry, Order Centrales) and pinnate (thin ellipse with bilateral symmetry, Order Pennales) and either may be found in the plankton or on the benthos. While the pinnate diatoms are solitary cells (although often living in dense assemblages or even forming tubes together), the centric diatoms may be solitary or chain forming, linked by projections from their cell wall or membrane. These two major taxonomic divisions also reflect a major ecological difference [169]. Centric diatoms are mainly holoplanktonic or meroplanktonic, with only a few genera that are associated with substrates throughout their life cycles. Araphidineae (pinnate diatoms without a raphe system on one valve) are attached to sand grains, rocks and biological substrates, whereas genera of the Biraphidineae (pinnate diatoms with two raphe systems on both valves) are almost completely attached to mud with only a few planktonic species. They are the major primary producers (both pelagic and benthic environment) and are thought to be responsible for up to 25% of the world’s net primary productivity [170] whereas total phytoplankton account for up to 40% of the global primary production [171]. At least some can live heterotrophic ally in the dark, if supplied with a suitable source of carbon [168]. Dinoflagellates form the other important component of the marine and freshwater phytoplankton.

1.14.2 Diatom structure and function

The different shapes and sizes of diatoms are numerous. They can vary from circular to triangular with sizes between five to a few hundred micrometers. These organisms share typical characteristics with the standard plant cell: they have a nucleus containing DNA, mitochondria, chloroplasts for photosynthesis, and a cell membrane [172]. Each cell contains one or more chloroplasts, often brown in colour, although the colour may vary with taxon and can even be greenish in some species [166]. However, the by far most distinguishing feature of the diatom structure is the elaborate and self-assembled micro and nanoporous outer cell wall, the frustule [173]. This highly elaborate structure is composed of two parts, or valves; called the epitheca and the hypotheca. Each valve is associated with a series of bands called girdle bands or cingulum that holds the two valves together. The cingula are in most diatoms perforated strips of silicate, but in other species they can also be folded to form channel-like structures [166]. The epitheca is slightly larger than the hypotheca and encloses the former as a lid on a petri dish. Through cell
division the epitheca of each new diatom is the valve inherited from the mother diatom while the hypotheca is synthesized during the cell division.

![Figure 1.4 Scanning electron microscopy of four different diatom species representing centric and pennate symmetry [174]](image)

The pore pattern on the frustule-valves is highly species-specific and makes the basis for diatom categorization. According to the pore-symmetry on the frustule, diatoms are thus divided into two main groups, namely the centric diatoms, having a radial symmetry, and pennate diatoms, having symmetry along a bilateral line. These main groups can further be divided into two sub-groups, the raphid and araphid pennate diatoms, and the radial and polar centric diatoms [174].

Diatom frustules are covered with a 3D-network of organic material mainly composed of polysaccharides and proteins. This layer is connected to a variety of surface moieties like amino, carboxyl and silanol groups that control both surface charge, and affinity to metal ions [175, 176].

1.14.3 Metal toxicity and interaction with cellular surfaces in diatoms

Diatoms are known to have a high affinity towards a variety of dissolved trace elements/metals and they will therefore exert a strong control on the transfer of heavy metals along the water column to the sediments. Along with the fact that they constitute one of the fundaments in the trophic food chains they are also responsible for much of the bioaccumulation of certain heavy metals, in particular Cd and Zn, throughout the web of food chains [176]. The study of the toxicity and bioaccumulation of metals in diatoms will therefore help understanding to which degree these metals will proceed to other organisms, and also how the diatoms control the metal concentration in freshwater and marine environments.
1.14.3.1 Metal accumulation in diatoms

The capacity of diatoms to accumulate metals from water producing an internal concentration greater than in their surroundings has been reported and discussed in various literature [177, 178]. The ratio of CFs (Concentration Factors) quantifies this ability to concentrate metals in plankton and epilithic diatoms have been proved to show high CFs [179]. Uptake of pollutants from surrounding water by algal cells could be the results of many processes. The chemical compound may be metabolically active, which can act as an essential nutrient or mineral and be transported across the cell membrane and thus enter into biochemical process [180]. In case of metal, Cambell et al. [181] recognised the interaction of metal with an algal cell with thus normally involve the following steps: i) diffusion of the metal from the bulk solution to the biological surface; ii) sorption/ surface complexation of metal at passive binding sites within protection layer, or at sites on the outer of surface of the plasma membrane; iii) uptake or “internalization” of the metal (transport across plasma membrane).

1.14.3.2 Economic importance

Diatoms constitute major plankton of sea/freshwater system. They are the major source of food for aquatic animals.

- Due to the deposition of diatom shells in sea bed over millions of years caused the formation of thousands of meter thick diatomaceous earth. Diatomaceous earth is highly absorbent

References


Chapter 1

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