Phytofiltration of arsenic and cadmium from the water environment using *Micranthemum umbrosum*

（*Micranthemum umbrosum* を用いた水圏環境よりのヒ素及びカドミウムの浄化）

Dissertation in fulfillment of the requirement for the degree of

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Abstract

Heavy metal pollution in aquatic environment due to natural sources and anthropogenic activities, is posing a dreadful threat to the human health. Among different heavy metals, arsenic (As) and cadmium (Cd) are the two most toxic and carcinogenic agent that extensively contaminates the water bodies. There are some physical and chemical remediation methods that have some limitations like high production technology, costly, destruction of native micro flora and fauna, and creation of secondary pollutions. In contrast, phytofiltration is a novel, cost effective, environmental friendly, aesthetic and solar-driven technology, using aquatic plants to remove As and Cd from contaminated water without causing any or little secondary pollution. A small number of aquatic plants were identified to uptake contaminants from aquatic environment. Among them very few could accumulate more than one pollutant in their bodies. *Micranthemum umbrosum* (J.F. Gmel) S.F. Blake, commonly known as Water fern, Baby's tears, or Pearl grass, belongs to the family Linderniaceae, is one of them, that significantly absorbs both As and Cd from contaminated water.

After culturing *M. umbrosum* for 7 days in a hydroponic experiment, the accumulation of about 1220 µg As g^{-1} and 800 µg Cd g^{-1} were observed in the leaves, from 1000 µg As L^{-1} and 1000 µg Cd L^{-1} of water, respectively and it can removed 79.3–89.5% As and 60–73.1% Cd from 200 to 1000 µg As L^{-1} and 300 to 3000 µg Cd L^{-1} solutions, respectively. Plant and water samples were analyzed for assessing the As and Cd accumulations, translocations, phytotoxic effects, uptake mechanisms and kinetics, and for evaluating the potential of *M. umbrosum* as As and Cd phytofiltrator.

For As treatment, root to stem and stem to leaf translocation factors greater than 1.0 indicated that accumulation of As in leaves was large compared to that in stem and roots but there are little differences in accumulation of Cd in roots, leaves and stem. It is easy to clean up aquatic environment rather than soil due to most of the soil phytoremediators accumulated contaminants in their root parts which is sometimes very difficult to harvest and removal from the contaminated soil environment. However, the absorption pattern of As and Cd within *M. umbrosum* was leaf > stem > root. Bio-concentration factors (2350 for As and 3027 for Cd) for *M. umbrosum* were higher than for other As and Cd phytoremediators, indicates its hyperaccumulation of As and Cd from contaminated water environment. The analysis of

different photosynthetic pigments and macro micro-nutrient concentration within plant body indicated that the plant showed more resistance to internal and external As concentrations than to that of Cd.

Absorption uptake kinetics within *M. umbrosum* was studied by using Michaelis Menten equation from different As species like arsenite, monomethylarsinic acid (MMAA), dimethylarsinic acid (DMAA) and Cd. The uptake of inorganic As species was much greater than that of organic As and was found at above the substrate concentration. Concentration dependent arsenite and Cd uptake influx were linear up to 500 µg L^{-1} and after that decreased, probably due to the toxicological inhibition. However, Cd showed similar uptake pattern to that of inorganic As species, and the data was better fitted to a non-linear than a linear model. Higher V_{max} and lower K_m value indicated that this plant has high affinity to uptake inorganic arsenite than Cd, organic MMAA and DMAA; and the uptake order was inorganic arsenite > Cd > MMAA > DMAA.

As and Cd uptake mechanism within *M. umbrosum* was investigated by using Gel chromatography column made from *Sephadex* G-50 (fractionation range is about 1500-30000 MW) and *Sephadex* G-15 (fractionation range is about 700-1500 MW) beads. After analysis of As, Cd, protein and thiol contents in each 2 mL fractionation collected from gel filtration column, we concluded that As within plants appeared to involve an induction of thiol synthesis or binding with low molecular weight substances that have thiol group(s) whereas Cd showed a different mechanism to that of As. Amino acid profile studied also showed that Cd uptaking mechanism and binding substances in *M. umbrosum* is different from algae and other plants which is not phytochelatin or thiol complex formation.

M. umbrosum showed good As phytofiltration capabilities without any phytotoxic effects, but it was found to be a moderate accumulator of Cd with some phytotoxic effect and it can lower the As toxicity to a level (about 25 µg As L^{-1}) close to the limit recommended by the World Health Organization (10 µg As L^{-1}) but below the limit recommended by Bangladesh and China Government (50 µg As L^{-1}). So *M. umbrosum* has the high As and Cd phytofiltration potency at low level (500 µg L^{-1}) As and Cd contaminated water and it can be used as ornamentation for room in addition to As and Cd accumulation from water, as it is popular as a green aquarium plant, from the aesthetic point of view of phytoremediation.

Chapter I: General Introduction

1.1 Introduction

1.1.1 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 (Greipsson, 2011). It can be used for removal of heavy metals and radionuclides as well as for organic pollutants such as polynuclear aromatic hydrocarbons-PAHs, polychlorinated biphenyls, and pesticides. It is a novel, cost-effective, efficient, environmental friendly, in *situ* applicable and solar-driven remediation strategy with good public acceptance in the aesthetic point of view (Ali *et al*., 2013) compared to other traditional physical and chemical remediation technologies (Fig. 1.1). Phytoremediation is also a low cost and conventional clean-up technology. For example, the cost of cleaning up one acre of sandy loam soil with a contamination depth of 50 cm with plants was estimated at $60,000-$100,000 compared to $400,000 for the conventional excavation and disposal method (Henry, 2000). On the other hand, plant generally handles the contaminants without affecting soil or water environment, thus conserving its utility and quality. Green plants have an enormous ability to uptake pollutants from the environment, and accomplished their detoxification by various mechanisms. It is suitable for application at very large field sites where other remediation methods are not cost effective or practicable (Garbisu and Alkorta, 2003). Phytoremediation has low installation and maintenance costs compared to other remediation options (Van Aken, 2009). Cost for phytoremediation can reduce as 5% less than cost for alternative clean-up methods (Prasad, 2003). From an economic point of view, the purpose of phytoremediation in polluted land can be threefolds: (1) risk containment

(phytostabilization); (2) phytoextraction of metals with market value such as Ni, Tl and Au (Sheoran *et al.*, 2013); (3) durable land management where phytoextraction gradually improves soil quality for subsequent cultivation of crops with higher market value (Vangronsveld *et al*., 2009). Furthermore, fast-growing and high-biomass producing plants such as willow, poplar and jatropha could be used for both phytoremediation and energy production (Abhilash *et al.*, 2012). Phytoremediation also enjoys popularity with the general public as a ‘‘green clean’’ alternative to chemical plants and bulldozers (Pilon-Smits, 2005). Then phytoremediation is a sustainable technology which has good future perspective to environmental pollution remediation without disturbing natural condition.

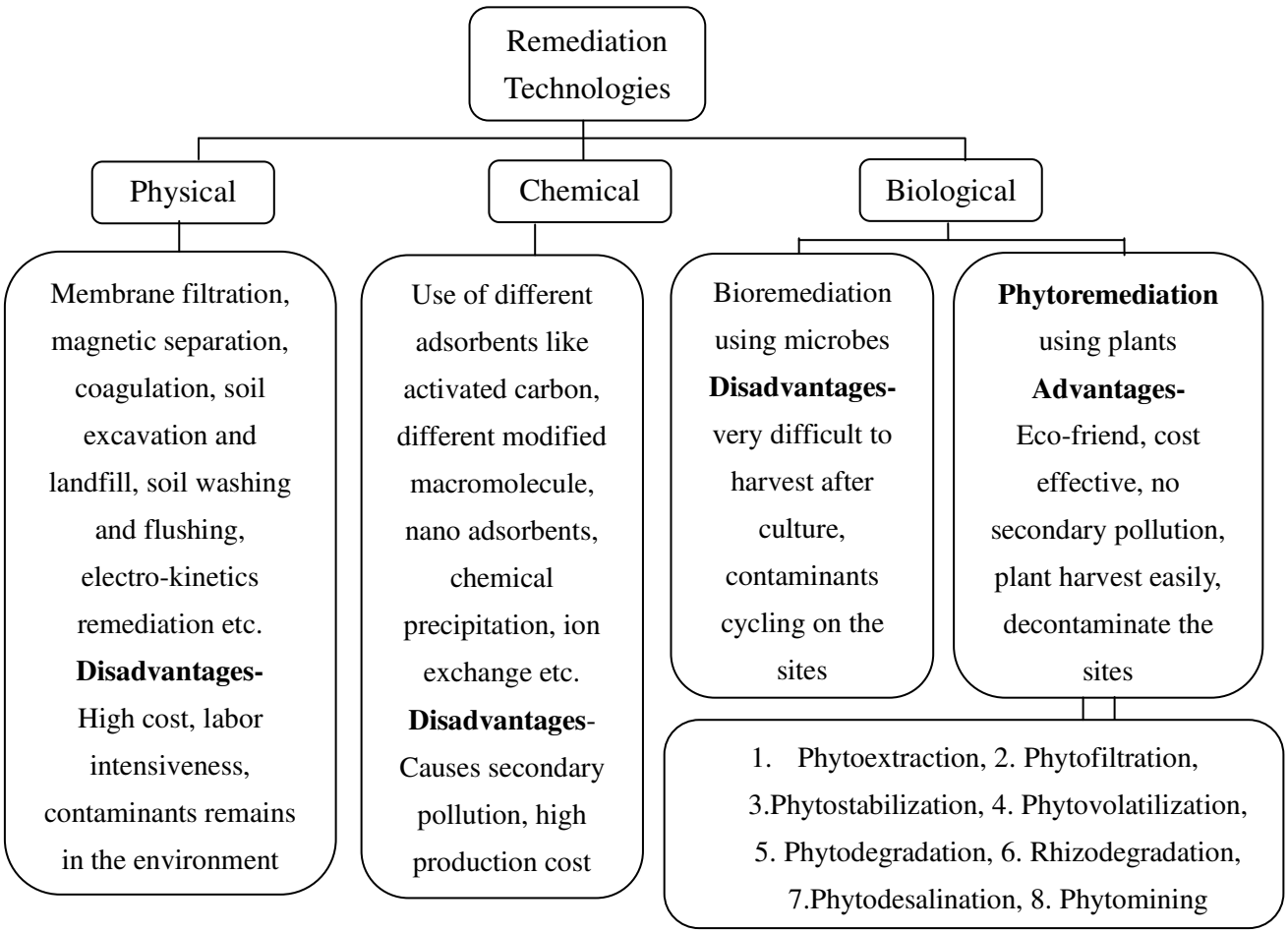


Fig. 1.1 Flow chart of different environmental remediation technologies

1.1.2 Phytoremediation of organic pollutants

Phytoremediation for organic pollutants is often inefficient because plants do not completely degrade these compounds through their rhizosphere or within plant system. Another reason is that organic pollutants are frequently found in heterogeneous streams mixed with other chemicals and distributed in a highly non-uniform manner. For example, ammoniacal liquor and coal tar are highly polluting products of former gas industry. Ammoniacal liquor contains phenol, ammonia, cyanides and sulphates; and coal tars contain high concentration of PAHs, volatile aliphatic and aromatic components as well as phenolic tar acids (Harvey *et al.,* 2002). Naturally, microorganisms can rapidly degrade the lower concentration of organic pollutants in the environment. Bellin and O`Conner (1990) reported that plant can uptake pentachlorophenol at concentration above 10 mg kg^{-1} due to soil microorganism degraded them at lower concentration. The fate of the organic contaminants in the rhizosphere-root system largely depends on its physicochemical properties. Organic xenobiotics with a log K_{ow} (octanol/water partition coefficient) < 1 are considered to be very water-soluble, and plant roots do not generally accumulate them at a rate surpassing passive influx into the transpiration stream (Cunningham and Berti, 1993). Contaminants with a log K_{ow} > 3.5 show high sorption to the roots, but slow or no translocation to the stems and leaves (Trapp *et al.*, 2001). However, plants readily take up organic xenobiotics with a log K_{ow} between 0.5 and 3.5, as well as weak electrolytes (weak acids and bases or amphoteres as herbicides). These compounds seem to enter the xylem faster than the soil and rhizosphere micro flora can degrade them, even if the later is enriched with degrading bacteria (Trapp *et al.*, 2000). Once taken up, plants metabolize these contaminants, although some of them, or their metabolites, such as trichloroethene (TCE), which is transformed into trichloro acetic acid, can be toxic (Doucete *et al.*, 1998). Alternatively, plants preferentially release volatile pollutants, such as benzene, toluene,

ethylbenzene and xylene compounds and TCE and their metabolites, into the environment by evaporation via the leaves, which calls into question the merits of phytoremediation (Van der Lelie *et al.*, 2001; Schwitzguebel *et al*., 2002; Ma and Burken, 2003; Burken and Schnoor, 1999). Some enhanced engineered phytoremediation technology used by some researchers to mitigate this toxic evaporation of organic pollutants. Barac *et al.* (2004) examined that endophytic bacteria equipped with the appropriate degradation pathway improve *in planta* degradation of toluene. After surface-sterilized lupine seeds were successfully inoculated with the recombinant strain, the engineered endophytic bacteria strongly degraded toluene, resulting in a marked decrease in its phytotoxicity, and a 50–70% reduction of its evapotranspiration through the leaves. Thus it is difficult to remediate organic pollutants using plant itself only, without association of microorganisms.

1.1.3 Phytoremediation of heavy metals/inorganic pollutants

Heavy metal pollutions or inorganic pollutants have become one of the most serious environmental problems in the world today. This problem is more and more severe with increasing industrialization and disturbance of natural biogeochemical cycles. The mobilization of heavy metals through extraction from ores and subsequent processing for different applications has led to the release of different elements such as cadmium (Cd), arsenic (As), lead (Pb), mercury (Hg), chromium (Cr), nickel (Ni), copper (Cu), zinc (Zn) and so on, into the environment (Ali *et al*., 2013). Unlike organic substances, heavy metals are essentially non-biodegradable, and tend to accumulate in the environment and living organisms. Many heavy metal ions are known to be toxic or carcinogenic to human being. Thus, the treatment of heavy metals is of special concern now-a-days due to their recalcitrance and persistence in the environment (Fu and Wang, 2011). Phytoremediation technology can successfully be used to remedy of these 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; and up to 22% of soil exchangeable Cd from contaminated site (Brown *et al.*, 1995; Gerard *et al*., 2000). *Brassica juncea*, commonly called as Indian mustard, has been found to be a good ability to transport lead from the roots to the shoots. Some calculations indicate that *Brassica juncea* is capable of removing 1,550 kg of lead per acre (Henry, 2000). On a worldwide basis, plant species having more than 1,000 mg kg^{-1} metal removing ability are known more than 320 for Ni, 30 for Co, 34 for Cu, 20 for Se, 14 for Pb and one plant species for Cd. The species involved in hyperaccumulation have been tabulated by Reeves and Baker (2000). Metal removing ability exceeding 10,000 mg kg^{-1} has been recorded 11 plant species for Zn and 10 plant species for Mn (Reeves and Baker, 2000). *Pteris vittata* has been shown to accumulate as much as 14,500 mg As kg^{-1} fronds from soil without showing toxicity symptoms (Ma *et al*., 2001). Now-a-days, scientists are trying to find more suitable plants or develop genetically engineered plants for effective phytoremediation of inorganic pollutants from the environment.

1.1.4 Why As and Cd Chosen here?

As is one of the 20^{th} most abundant elements in the Earth`s crust (Woolson, 1975), 22^{nd} in seawater (Brown *et al*., 1991) and 12^{th} in the human body (Mandal and Suzuki, 2002). It is ubiquitous and thus found in many environments (Fig. 1.2). As is known to be highly toxic to living species especially human beings. Groundwater As contamination poses a dreadful threat to millions of people across the world. More than 80 million residents in Southeast Asia (Smith *et al*., 2000; Nordstrom 2002) are estimated to face the risk from consuming As-contaminated groundwater (Fig. 1.2). As has been detected from several parts of Bangladesh, India, China, Nepal, Pakistan, and most Southeast Asian countries including

Cambodia, Vietnam, Myanmar, Laos, Thailand, Taiwan and Indonesia (Kim *et al*., 2011). Many states within the United States have been reported with significant concentrations (up to 50 ppm) of As in the groundwater (Tchounwou *et al*., 2003; Knobeloch *et al*., 2006). In Latin America, the problem of As contamination in water is known in 14 out of 20 countries: Argentina, Bolivia, Brazil, Chile, Colombia, Cuba, Ecuador, El Salvador, Guatemala, Honduras, Mexico, Nicaragua, Peru, and Uruguay (Bundschuh *et al*., 2012). In countries such as Romania, Hungary, Italy and Spain, As concentrations have been elevated, and special treatment steps have been recommended to reduce As to acceptable levels (Van Halem *et al.*, 2009).

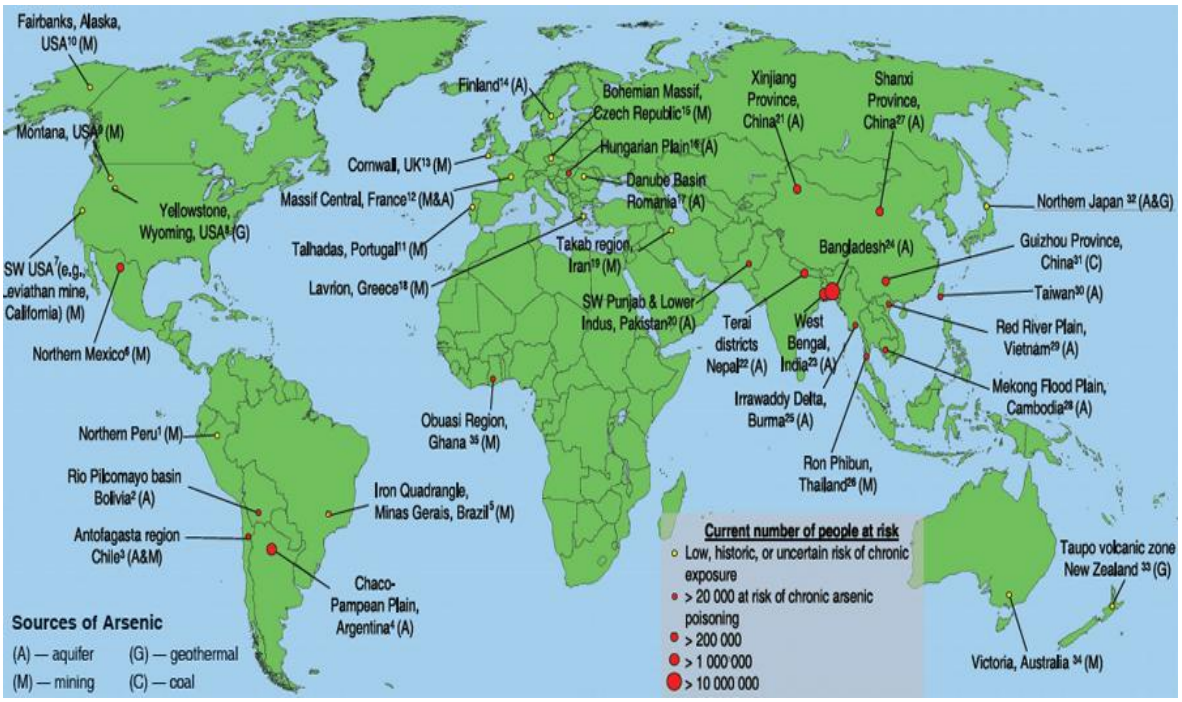


Fig. 1.2 Worldwide As distribution in different environment and risk to people (Sources: http://www.iupac.org/publications/ci/2008/3004/map_popup.html)

However, a number of large aquifers with naturally occurring As at concentrations greater than World Health Organization (WHO) permissible limit of 10 µg L^{-1} (WHO, 2011) or even significantly higher have been identified in several parts of the world (Kim *et al*., 2011). Some of the worst reports have been evidenced from Bangladesh and West Bengal

in India. Water analyses of all 64 districts of Bangladesh (Fig. 1.3) reported 27.2 and 42.1 % of the tube wells with As above 50 and 10 µg L^{-1}, respectively, and 7.5 % contained As above 300 µg L^{-1} (Chakraborti *et al*., 2010). In all the 19 districts of west Bengal, India, 48.1 % have As above 10 µg L^{-1} (WHO guideline), 23.8 % above 50 µg L^{-1} (Indian standard), and 3.3 % above 300 µg L^{-1} (concentration expected to produce overt arsenical skin lesions) (Chakrabarti *et al*., 2009). As toxicity depends on As species; and generally inorganic As species (arsenite and arsenate) are more toxic as compared with organic As species (Meharg and Hartley-Whiteker, 2002; Ng, 2005). Table 1.1 represents the different forms of As that exist in the environment (Rahman and Hasegawa, 2011).

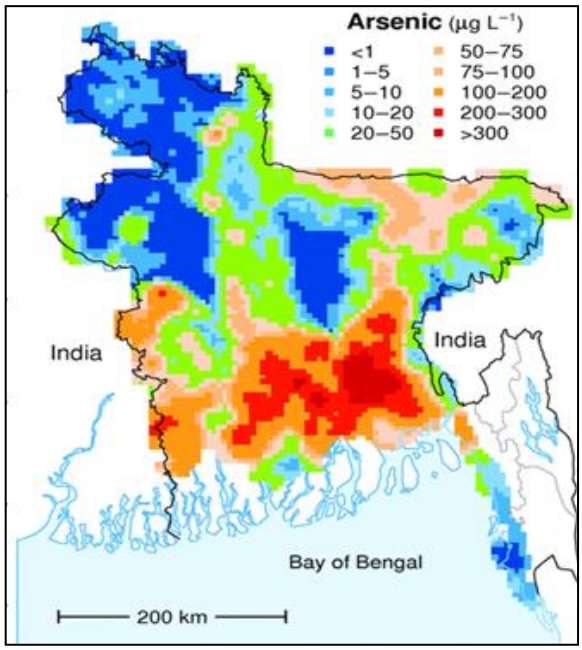


Fig. 1.3 As contamination in different parts of Bangladesh

The toxicity level of the various As species is As (III)>As(V)>DMAA>MMAA (Petrick *et al*., 2000). Groundwater (the main drinking water source in many countries), soils, sediments and food chains contaminated with As are due to natural geochemical or anthropogenic influence, causes skin lesions, cancers, and many other diseases in human beings (Figs. 1.4 and 1.8) (Dhankher, 2005; Ducker *et al*., 2005; Mondal *et al*., 2006; William *et al.*, 2006). It has been reported as the possible cause of the death of such

notables as Napolean and the American president Zachary Taylor due to As poisoning (Feldmann, 2001).

Table 1.1 Different forms of As in the environments

Name	Abbreviation	Formula/Structure	References
Inorganic arsenicals			
Arsine	As(-III)	AsH_3	*
Arsenious acid or arsenite	As(III)	$As^{3+}(OH)_3$	*, **
Arsenic acid or arsenate	As(V)	$H_3As^{5+}O_4$	*, **
Methylarsenicals			
Methylarsine	-	AsH_2CH_3	*, **
Dimethylarsine	-	$AsH(CH_3)_2$	*, **
Trimethylarsine	-	$As(CH_3)_3$	*, **
Monomethylarsonous acid	MMAA(III)	$As(OH)_2CH_3$	*, **
Dimethylarsinous acid	DMAA(III)	$As(OH)(CH_3)_2$	*, **
Monomethylarsonic acid	MMAA(V)	$AsO(OH)_2CH_3$	*, **
Dimethylarsinic acid	DMAA(V)	$AsO(OH)(CH_3)_2$	*, **
Trimethylarsine oxide	TMAO	$AsO(CH_3)_3$	*, **
Trimethylarsonium ion	TMA^+	$As^+(CH_3)_4$	***
Organoarsenicals			
Arsenocholine	AsC	$(CH_3)_3As^+CH_2CH_2O$	**
Arsenobetaine	AsB	$(CH_3)_3As^+CH_2COO^-$	**
Rosarsone	-	$C_6H_6AsNO_6$	**
Arsenosugars			
dimethylarsinoylribosides	AsS		****
trialkylarsonioribosides	AsS		
Others			
Dimethylarsinoylethanol	DMAE		*****
Glycerophospho(arsenocholine)	GPAC		
glycerophosphatidylarseoncholine	-		***

*Maher (1984), Kaise *et al*., (1988), Francesconi and Edmonds (1996), Craig (2003), Sharma and Sohn (2009); **Craig (2003) and O'Day (2006); ***O'Day (2006), Sharma and Sohn (2009); ****Francesconi and Edmonds (1996), Sharma and Sohn (2009); *****Francesconi and Edmonds (1996).

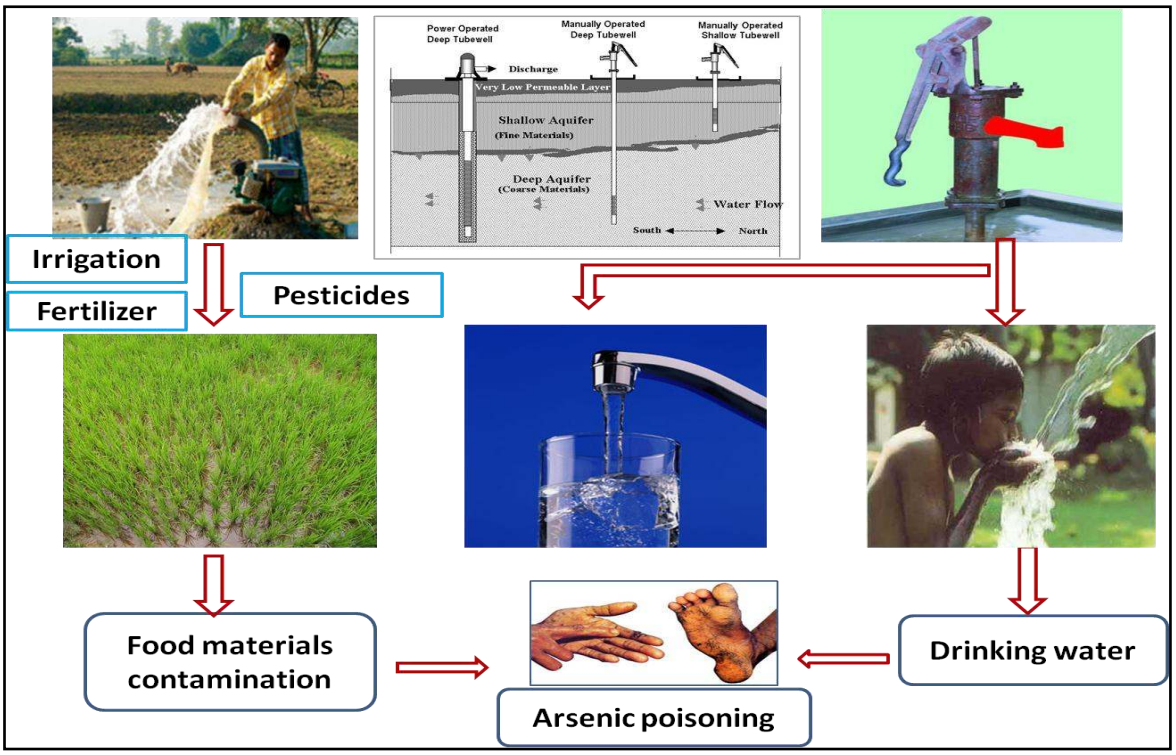
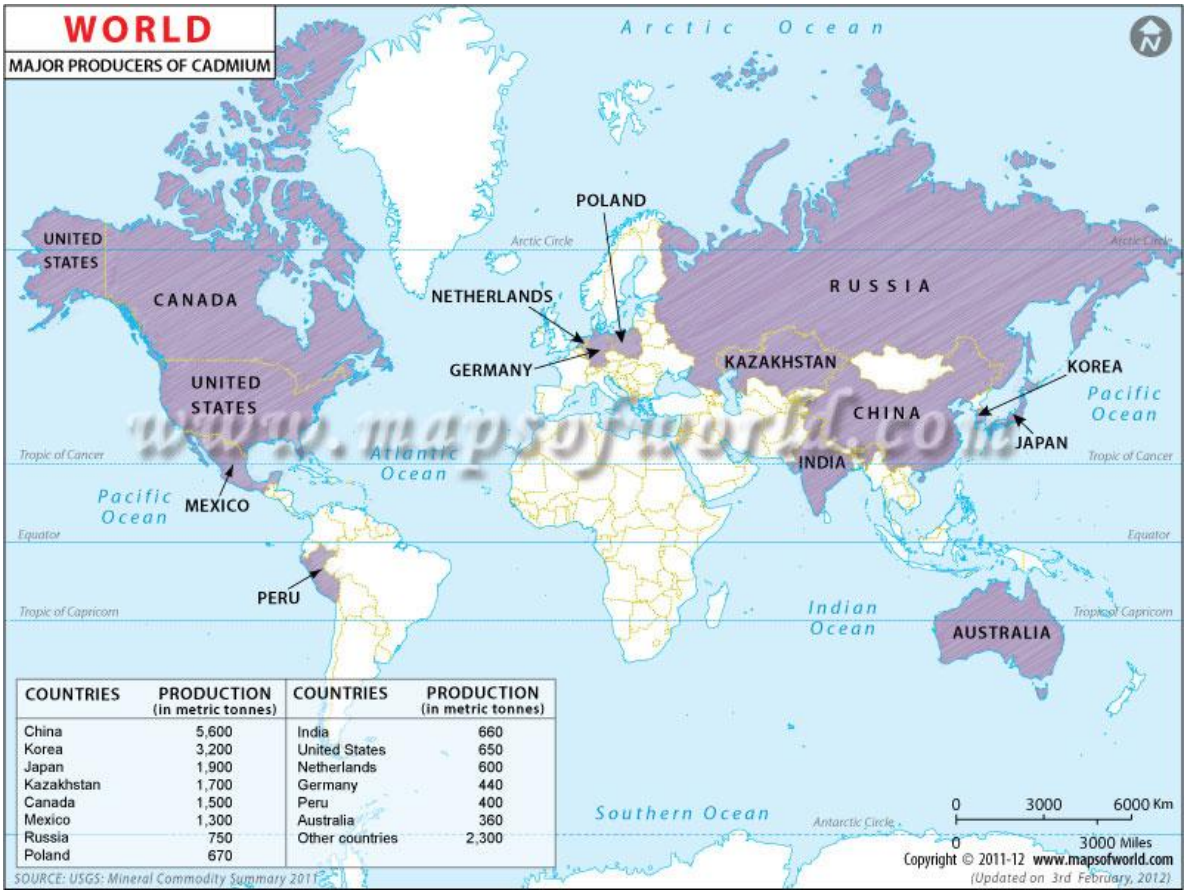


Fig. 1.4 As contamination via anthropogenic and natural sources

Cd listed as number 7 (of 275) in the priority list of hazardous materials (ASTDR, 2011) Cd is a ubiquitous non essential element that possesses high toxicity, and is easily accumulated from the environment by organisms (Rahimi and Nejatkhan, 2010). Anthropogenic pathways by which Cd released in to environment are through industrial waste from processes such as electroplating, manufacturing of plastics, paint pigments, alloy preparation and batteries that contain Cd (Fig. 1.7). Cd is also used for luminescent dials, in photography, rubber curing, and as fungicides (Kirkham, 2006). China, Korea and Japan are the most Cd producing country (Fig. 1.5). In the decades leading up to World War II, mining operations contaminated the Jintsu River flow from Gifu prefecture to Toyama prefecture in Japan (Fig. 1.6) with Cd and traces of other toxic metals. As a consequence, Cd accumulated in the rice crops growing along the riverbanks downstream of the mines. Some members of the local agricultural communities consuming the contaminated rice developed *itai-itai* disease (Fig. 1.8) (Nogawa *et al*., 2004). It can cause

a variety of human diseases, such as renal tubular dysfunction, pulmonary emphysema and osteoporosis/osteomalacia (Fig. 1.8) without an efficient chelation treatment for reducing Cd body burden (Wagner, 1993; Satarug *et al.*, 2010). The high toxicity and great solubility of Cd in water make a significant pollutant (Lockwood, 1976). So far there is no evidence of its essentiality in plant growth. Moreover, Cd can be taken up and accumulated by many plants using pathways for essential elements (Shah, 2011), through which it enters into the food chain. More than 30 µg L^{-1} Cd was recorded in the drinking water, though the recommended Cd level in drinking water is only 3 µg L^{-1} (WHO, 2011). Therefore, the removal of As and Cd from contaminated water has been of the utmost importance in order to minimize their impacts on ecosystems.



COUNTRIES	PRODUCTION (in metric tonnes)	COUNTRIES	PRODUCTION (in metric tonnes)
China	5,600	India	660
Korea	3,200	United States	650
Japan	1,900	Netherlands	600
Kazakhstan	1,700	Germany	440
Canada	1,500	Peru	400
Mexico	1,300	Australia	360
Russia	750	Other countries	2,300
Poland	670		

Fig. 1.5 Worldwide Cd productions

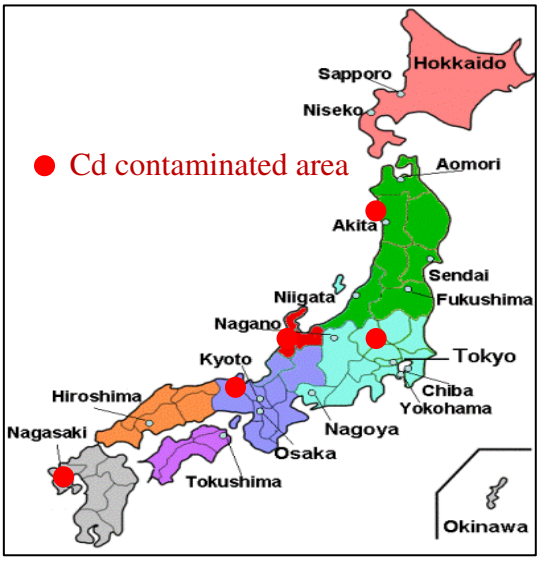


Fig. 1.6 Cd contamination in Japan

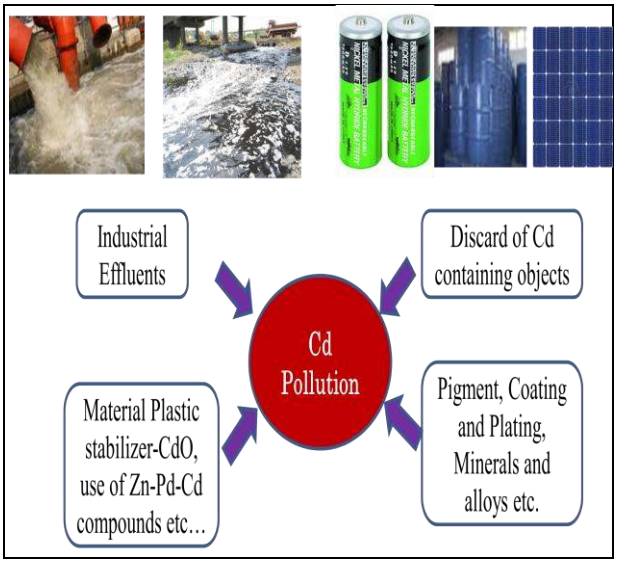


Fig. 1.7 Cd contamination by anthropogenic ways

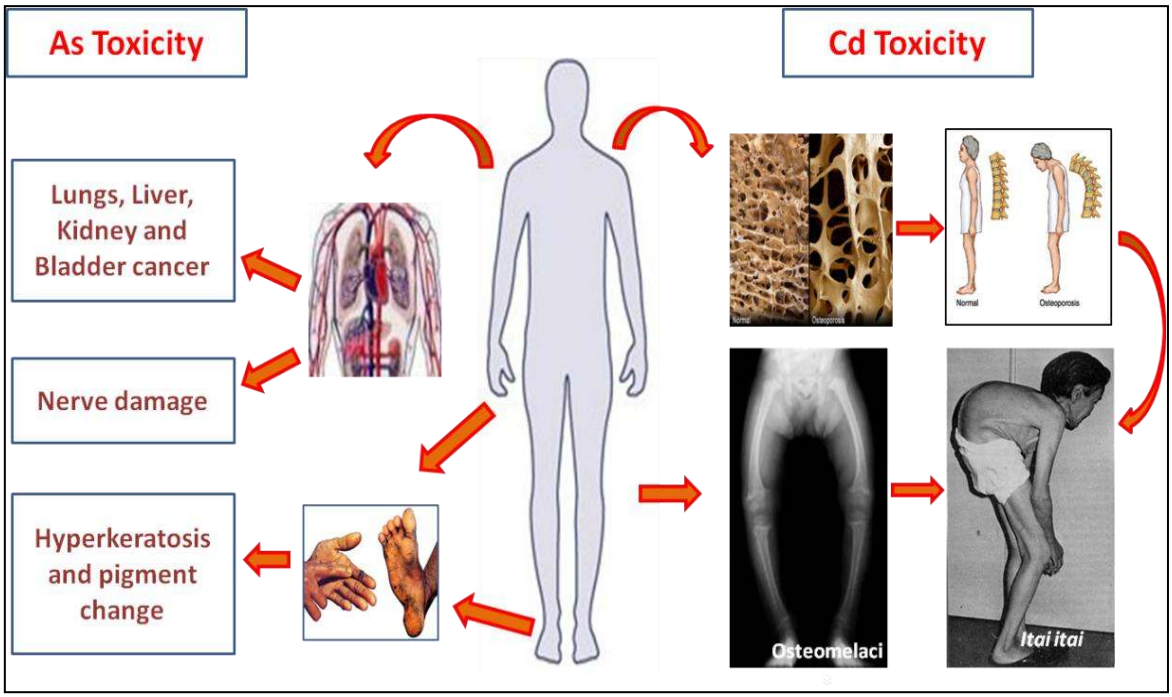


Fig. 1.8 Toxicity induced in human body due to As and Cd exposure

1.1.5 Heavy metals contamination in soil and water environment and phytoremediation

Heavy metal enters into the environment through natural and anthropogenic sources, and

causes food chain contamination via soil and water pollution (Fig. 1.9). The adverse effect of water pollution is much greater than the soil pollution. If the drinking water is polluted, it causes very severe effects on living beings including human even at micro level concentration. For example a recommendation limit of As in drinking water is only 0.1 mg L^{-1} (WHO, 2011) where in cultivated soil is 20 mg kg^{-1}. Therefore it deserves special attention to cleanup of contaminated water bodies. Phytoremediation technology can be used for removal of these heavy metals both form contaminated soil and water sites; however, it is easy to use this technology for decontamination of water bodies rather than soil. Because soils is a highly heterogeneous body rather than the water. Most of the plant species (phytoremediators) have lower accumulation rate of toxic elements from soils and lower translocation rate from root to shoot. Most of the elements deposits in the roots

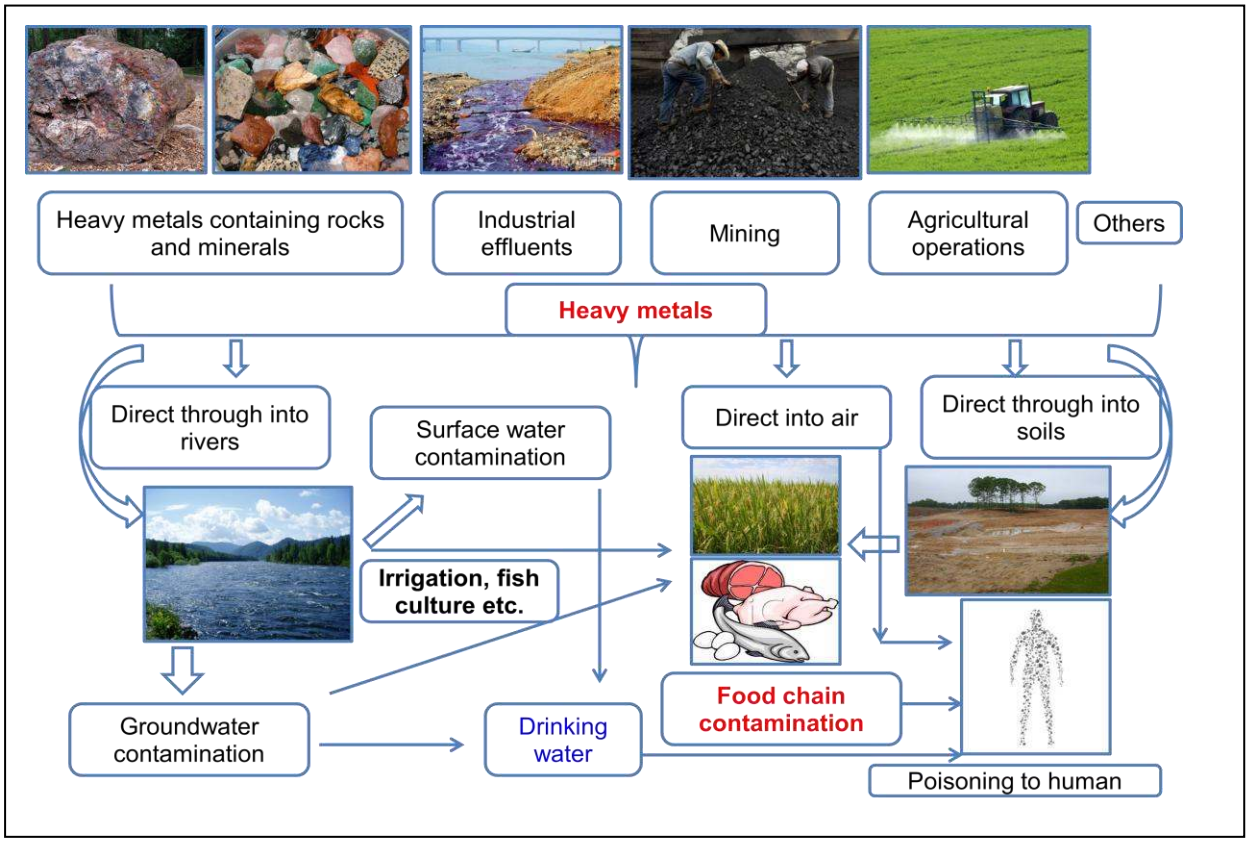


Fig. 1.9 Heavy metals exposure to soil and water environment causing food chain contamination

which are difficult to harvest and collect all roots to remove pollutants. For another reason, most of the phytoremediators can uptake only one toxic elements from soils, and it will take long time for complete removal of contaminated sites. Moreover there is a chance to recontamination of the pollutant or food chain contamination due to lack of knowledge about proposal disposal or through animal feeding. On the other hand, phytoremediation can successfully be used for water pollution remediation due to easy to culture and harvest of aquatic hyperaccumulating plants. In addition, plants can absorb high amounts of pollutant(s) in their body as most of the heavy metals are ionic form in the water bodies. However, different techniques of phytoremediation, such as phytoextraction, phytofiltration, phytostabilization, phytovolatilization, phytodegradation, rhizodegradation, phytodesalination and phytomining which are applying for soil and water decontamination as described below.

1.1.6 Types of phytoremediation

1.1.6.1 Phytoextraction

Phytoextraction (also known as phytoaccumulation, phytoabsorption or phytosequestration) is the uptake of contaminants from soil or water by plant roots and finally accumulation in above ground biomass i.e., shoots (Rafati *et al*., 2011). Metal translocation to shoots is a crucial biochemical process, and is desirable in an effective phytoextraction because the harvest of root biomass is generally not feasible (Tangahu *et al.*, 2011).

1.1.6.2 Phytofiltration

Phytofiltration is the removal of pollutants from contaminated surface waters or waste waters by plants (Mukhopadhyay and Maiti, 2010). Phytofiltration may be rhizofiltration

(use of plant roots) or blastofiltration (use of seedlings) or caulofiltration (use of excised plant shoots; Latin caulis = shoot) (Mesjasz-Przybylowicz *et al.*, 2004). In phytofiltration, the contaminants are absorbed or adsorbed and thus their movement to water environment is minimized.

1.1.6.3 Phytostabilization

Phytostabilization or phytoimmobilization is the use of certain plants for stabilization of contaminants in contaminated soils (Singh, 2012). This technique is used to reduce the mobility and bioavailability of pollutants in the environment, thus preventing their migration to groundwater or their entry into the food chain. Plants can immobilize heavy metals in soils through sorption by roots, precipitation, complexation or metal valence reduction in rhizosphere (Barcelo and Poschenrieder, 2003; Wuana and Okieimen, 2011). By excreting special redox enzymes, plants skillfully convert hazardous metals to a relatively less toxic state and decrease possible metal stress and damage. For example, reduction of Cr(VI) to Cr(III) is widely studied, the latter being less mobile and less toxic (Wu *et al*., 2010). Phytostabilization limits the accumulation of heavy metals in biota, and minimizes their leaching into underground waters. However, phytostabilization is not a permanent solution because heavy metals remain in the environment; only their movement is limited. Actually, it is a management strategy for stabilizing (inactivating) potentially toxic contaminants (Vangronsveld *et al*., 2009).

1.1.6.4 Phytovolatilization

Phytovolatilization is the uptake of pollutants from soil by plants, their conversion to volatile form and subsequent release into the atmosphere. This technique can be used for organic pollutants and some heavy metals like Hg and Se. However, its use is limited by

the fact that it does not remove the pollutant completely; only it is transferred from one segment (soil) to another (atmosphere) from where it can be redeposit. Phytovolatilization is the most controversial of phytoremediation technologies (Padmavathiamma and Li, 2007).

1.1.6.5 Phytodegradation

Phytodegradation is the degradation of organic pollutants by plants with the help of enzymes such as dehalogenase and oxygenase; it is not dependent on rhizospheric microorganisms (Vishnoi and Srivastava, 2008). Plants can accumulate organic xenobiotics from polluted environments, and detoxify them through their metabolic activities. From this point of view, green plants can be regarded as ‘‘Green Liver’’ for the biosphere. Phytodegradation is limited to the removal of organic pollutants only because heavy metals are non biodegradable. Recently, scientists have shown their interest in studying phytodegradation of various organic pollutants including synthetic herbicides and insecticides. Some studies have reported the use of genetically modified plants (e.g., transgenic poplars) for this purpose (Doty *et al*., 2000).

1.1.6.6 Rhizodegradation

Rhizodegradation refers to the breakdown of organic pollutants in the soil by microorganisms in the rhizosphere (Mukhopadhyay and Maiti, 2010). Rhizosphere extends about 1 mm around the root, and is under the influence of the plant (Pilon-Smits, 2005). The main reason for the enhanced degradation of pollutants in the rhizosphere is likely the increase in the numbers and metabolic activities of the microbes. Plants can stimulate microbial activity about 10–100 times higher in the rhizosphere by the secretion of exudates containing carbohydrates, amino acids and flavonoids. The release of nutrients-containing exudates by plant roots provides carbon and nitrogen sources to the

soil microbes, and creates a nutrient-rich environment in which microbial activity is stimulated. In addition, to secreting organic substrates for facilitating the growth and activities of rhizospheric microorganisms, plants also release certain enzymes for degrading organic contaminants in soils (Kuiper *et al*., 2004; Yadav *et al*., 2010).

1.1.6.7 Phytodesalination

Phytodesalination refers to the use of halophytic plants for removal of salts from salt-affected soils in order to enable them for supporting normal plant growth (Manousaki and Kalogerakis, 2011; Sakai *et al*., 2012). Halophytic plants have been suggested to be naturally better adapted to cope with heavy metals compared to glycophytic plants (Manousaki and Kalogerakis, 2011). According to an estimation, two halophytes, *Suaeda maritima* and *Sesuvium portulacastrum* could remove 504 and 474 kg of sodium chloride, respectively, from 1 ha of saline soil in a period of 4 months. Therefore, *S. maritima* and *S. portulacastrum* could be successfully used to accumulate NaCl from highly saline soils (Ravindran *et al.*, 2007). Another study has reported accumulation of about 1 t ha^{-1} of Na^{+} ions in the above ground biomass of the obligate halophyte *S. portulacastrum* cultivated on a salinized soil. The resultant decrease in salinity of the phytodesalinized soil significantly reduced the negative effects on the growth of the test culture of the glycophytic crop, *Hordeum vulgare* (Rabhi *et al*., 2010).

1.1.6.8 Phytomining

It is an emerging technique to detect the reserve of valuable elements (like gold) in a particular underground place (Lintern *et al*., 2013). 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 (Sheoran

et al., 2013). Table 1.2 summarizes the different techniques (Ali *et al.*, 2013; Sheoran *et al*., 2013) of phytoremediation. Fig. 1.10 illustrates this technique for easy understanding. Among different phytoremediation techniques, we used phytofiltration methods in this study for removal of As and Cd from contaminated water environment.

Plants suitable for phytoremediation should have the following characteristics-

i) Hyperaccumulation and hypertolerance

ii) High growth rate

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

iv) Production of above ground biomass

v) Widely distributed and highly branched root system

vi) Translocation of the accumulated heavy metals from root to shoots

vii) Good adaptation to prevailing environmental and climatic conditions

viii) Resistance to pathogen and pest

ix) Easy to cultivation and harvest

x) Repulsion to herbivores to avoid food chain contamination

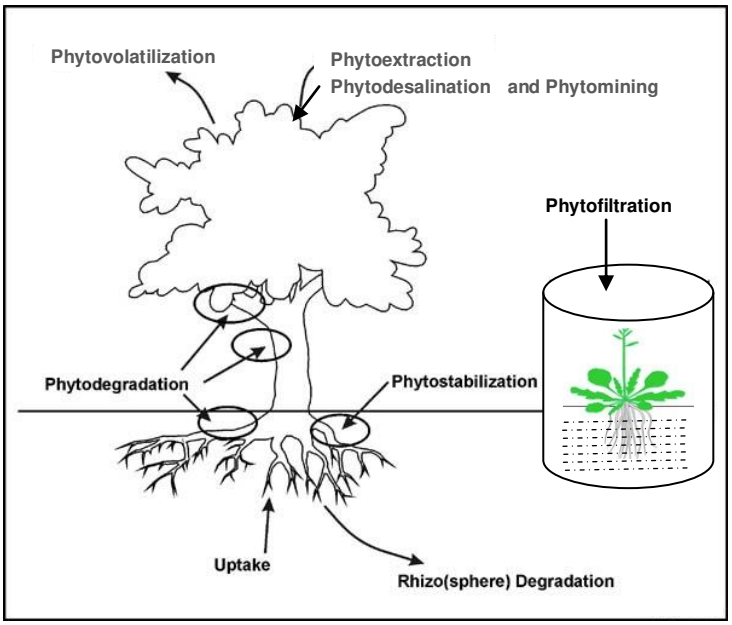


Fig. 1.10 Different techniques of phytoremediation
(Modified from www.phytoremediation.com/jpg)

Table 1.2 Quick views of different techniques for phytoremediation

Technique	Description	Substrate
Phytoextraction	Uptake of contaminants and stored in harvestable biomass	Soil/water
Phytofiltration	Accumulation of pollutants from contaminated water	Water
Phytostabilization	Limiting the mobility and bioavailability of pollutants in environment by plants	Soil
Phytovolatilization	Plants used to convert pollutants to volatile form and subsequent release into the atmosphere	Soil/Water
Phytodegradation	Degradation of organic xenobiotics by enzymatic reactions within plant tissue	Soil/water
Rhizodegradation	Degradation of organic xenobiotics in the rhizosphere by microorganisms	Soil
Phytodesalination	Accumulation of excess salts from saline soils by halophytes	Soil
Phytomining	Accumulation and deposition of mining elements within the harvestable parts of plants and then extract as a bio-ore	Soil

1.1.7 Hyperaccumulator of Heavy metals

The term ‘‘hyperaccumulator’’ was first coined by Brooks *et al*. (1977) to define plants with Ni concentrations higher than 1,000 mg kg^{-1} dry weigh (0.1%). The standard for hyperaccumulators has not been defined scientifically (Nazir *et al.*, 2011). However, individual authors or research groups have defined hyperaccumulators. To the authors' notice, the most cited criteria for hyperaccumulation of metals is that of Baker and Brooks (1989) (with 1735 citations, 23th April, 2014) according to which 'hyperaccumulators are plant species, having greater than 100 mg kg^{-1} dry weight Cd, or greater than 1,000 mg kg^{-1} dry weight Ni, Cu, As and Pb or greater than 10,000 mg kg^{-1} dry weight Zn and Mn in their shoots when grown on metal rich soils'. van der Ent *et al*. (2013) admit that criteria

commonly used for hyperaccumulation of some metals are unnecessarily conservative, and propose that criteria for hyperaccumulation of such metals be lowered. They recommend the following concentration criteria for different metals and metalloids in dried foliage with plants growing in their natural habitats: 100 mg kg^{-1} for Cd, Se and Tl; 300 mg kg^{-1} for Co, Cu and Cr; 1,000 mg kg^{-1} for Ni, Pb and As; 3,000 mg kg^{-1} for Zn; 10,000 mg kg^{-1} for Mn. Generally, hyperaccumulators achieve 100-fold higher shoot metal concentration (without yield reduction) compared to crop plants or common nonaccumulator plants (Lasat, 2002; Chaney *et al.*, 2007). Hyperaccumulators achieve a shoot-to-root metal concentration ratio (called translocation factor, TF) of greater than one (Tangahu *et al*., 2011; Badr *et al.*, 2012). However, TF cannot be used alone to define hyperaccumulation although it is a useful measure in supporting other evidence of hyperaccumulation (van der Ent *et al*., 2013). Zayed *et al*. (1998) defined hyperaccumulator on the basis of BCF value (greater than 1,000). Hyperaccumulators can be used for phytoremediation of toxic and hazardous heavy metals as well as for phytomining of precious heavy metals (such as Au, Pd and Pt). So exploring more effective hyperaccumulators for heavy metals is a key step for successful phytoremediation for environmental pollutants.

1.1.8 Phytofiltration of heavy metals using aquatic plant

Water environment are being contaminated by various toxic heavy metals through anthropogenic activities and from natural sources. This contamination has a great adverse effect on human health via food chain contamination as well as total ecosystem. Therefore, remediation of contaminated aquatic environment is important as it is for terrestrial environment. Phytoremediation of the toxic contaminants can be readily achieved by aquatic plants since the process involves biosorption and bioaccumulation of the soluble and bioavailable contaminants from water (Brooks and Robinson, 1998), called as

phytofiltration. 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. Several aquatic plants have been investigated for the remediation of natural and wastewater contaminated with Cu(II), Cd(II) and Hg(II) (Sen and Mondal, 1987; Selvapathy and Sreedhar, 1991; Alam *et al*., 1995). *Microspora* and *Lemna minor* were studied for Pb and Ni remediation (Axtell *et al*., 2003). Five common aquatic plant species (*Typha latifolia, Myriophyllum exalbescens, Potamogeton epihydrus, Sparganium angustifolium* and *Sparganium multipedunculatum*) were tested for Al phytoremediation (Gallon *et al*., 2004). Parrot feather (*Myriophyllum aquaticum*), creeping primrose (*Ludwigina palustris*) and water mint (*Mentha aquatic*) have been reported to remove Fe, Zn, Cu, and Hg from contaminated water effectively (Kamal *et al*., 2004). The *L. minor* was reported to accumulate Cu and Cd from contaminated wastewater (Kara, 2004; Hou *et al*., 2007). The submerged aquatic plant *Myriophyllum spicatum* L. has been reported as an efficient plant species for the metal-contaminated industrial wastewater treatment (Lesage *et al*., 2007).

1.1.9 Phytofiltration of As and Cd using aquatic plant

As and Cd contaminations in the environment are mainly due to the ground water and industrial waste water contamination, respectively. Thus aquatic plants will be a good tool for the remediation of As and Cd in aquatic environment, and some species have already been reported to accumulate As and Cd from water. A number of aquatic plants that have been reported for As and Cd accumulation is given in the Table 1.3 and Fig. 1.11.

Table 1.3 Aquatic plant that accumulate As and Cd from water environment

Scientific name	Element uptake	Amount uptake mg kg^{-1} (DW)	References
Eleocharis acicularis (L.) Roem. & schult.	As	1470	Sakakibara *et al*., 2011
Pteris vittata L.	As	1400	Baldwin and Butcher, 2007
Pteris cretica (L.) cv. *Mayii*	As	1200	
Wolffia globosa (Roxb.) Hartog & Plas	As	>1000	Zhang *et al*., 2009
Eichhornia crassipes (Mart.) Solms	As	345	Chigbo *et al*., 1982
Hydrilla verticillata (L.f.) Royle	As	325	Lee *et al*., 1991
Azolla caroliniana Willd.	As	280	Zhang *et al*., 2008
Spirodela polyrhiza (L.) Schleid.	As	0.353 µmol g^{-1}	Rahman *et al*., 2007
Micranthemum umbrosum (J.F.Gmel.) Blake	**As**	**1220**	**Current study**
Limnocharis flava (L.) Buchenau	Cd	>1000	Abhilash *et al*., 2009
Azolla pinnata R. Br.	Cd	740	Rai, 2008
Eichhornia crassipes (Mart.) Solms	Cd	575	Chigbo *et al*., 1982
Wolffia globosa (Roxb.) Hartog & Plas	Cd	500	Xie *et al*., 2013
Echinochloa polystachya (Kunth) Hitchc	Cd	230-300	Solis-Dominguez *et al*., 2007
Helianthus annuus L.	Cd	40-330	Zhi-xin *et al*., 2007
Rorippa globosa (Turcz. Ex Fisch. & C.A. Mey.) Hayek	Cd	>100	Wei *et al*., 2008
Micranthemum umbrosum (J.F.Gmel.) Blake	**Cd**	**800**	**Current study**

Eleocharis acicularis *Pteris vittata* *Pteris cretica* cv *Mayii* *Wolffia globosa* *Eichhornia crassipes*

Hydrilla verticillata *Azolla caroliniana* *Spirodela polyrhiza* *Limnocharis flava* *Azolla pinnata*

Echinochloa polystachya *Helianthus annuus* *Rorippa globosa* ***Micranthemum umbrosum***

Fig. 1.11 Representative As and Cd accumulating aquatic plants

1.2 Features of *Micranhemum umbrosum*

M. umbrosum, is an aquatic fern commonly named as water fern or Baby`s tears or pearl grass, which is a beautiful green aquarium plant mainly originated from USA under the family of *Linderniaceae*. Its height about 10-20 cm. This is a herb type plant and annual or perennial life cycle (USDA NRCS PLANTS Database). Its Geographical distribution from North America to South America.

The taxonomic nomenclature (Integrated Taxonomic Infromation System, ITIS, via Catalogue of Life, 2006 version) of this plant is as follows-

Kingdom- Plantae

Phylum- Magnoliophyta

Class- Magnoliopsida

Order- Lamiales

Family- Linderniaceae

Genus- *Micranthemum*

Species- *Micranthemum umbrosum* (J.F.Gmel.) Blake

The main features of *M. umbrosum* are-

i) whole plant can be easily removed from water environment

ii) growth rate is high and relatively vigorous

iii) easy to propagate by stem cutting

iv) it grows under emerged and submerged conditions

v) its light requirement for growth is moderate, and

vi) it can be used as ornamentation for house in addition to clean up contaminants from water.

This plant has been used here for the remediation of As and Cd from the contaminated water environment as there was no data regarding phyoremediation of As and Cd using *M. umbrosum*.

1.3 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? 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. There may be some processes for the disposal of these aquatic plants, but it is difficult to elucidate

whether this would be economically and environmentally feasible or not. However, there was little research about that. Here some possible ways discuss to handle the post harvest plant used for phytoremediation of heavy metals (Rahman and Hasegawa, 2011; Ali *et al*., 2013).

1.3.1 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. Previously water hyacinth has been used in this purpose (Thomas and Eden, 1990). However, fresh aquatic plants have high moisture content. Therefore, it may take longer time for drying. In addition, there is no evidence whether contaminant is completely vanished after burning the plants. Incineration of the plants with high heavy metal contents may also be a source of toxic emission in the air. It has been reported that burning high As-containing coal is one of the major sources of As exposure (10–20% of total As exposure) for the population of Guizhou, China (Liu *et al*., 2002). Another study also revealed that burning coal with high arsenic content increased arsenic content in hair, urine, and blood in children residing in polluted area (Bencko and Symon, 1977). So, burning hyperaccumulating aquatic plants would not be environmentally safe, and would be hazardous for human health.

1.3.2 Hydrolysis and fermentation

Liquid fuel, such as ethanol, may be produced in aquatic plants during phytoremediation by hydrolysis together with fermentation. Hydrolysis and fermentation also require yeast fermentable sugars that may available only to a low extent in aquatic phytoremediating plants. Some kinds of pre-treatment are, therefore, needed to make the sugar more easily available for chemical hydrolysis (Gunnarsson and Petersen, 2007). The pre-treatment

requires a relatively high temperature, strong acids and pressurized reactors. Thomas and Eden (1990) 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. Even it is economically feasible to produce fuel from phytoremediating aquatic plants, heavy metal contents in by-product sludge and its recontamination possibility should be tested.

1.3.3 Briquetting

Briquettes have been widely sold commercially for cooking food. Briquetting would be a good option for the treatment of the phytoremediating aquatic plants. Thomas and Eden (1990) reported briquetting as a possible treatment of water hyacinth. The briquettes are made by sun-drying the water hyacinth for few days, disintegrating, screening and chopping the dried water hyacinths to pieces about 6 mm long. The shredded water hyacinth can then be compressed into briquettes or pellets. The material resulting after briquetting water hyacinth has an energy density of 8.3 GJ m^{-3}, which is comparable to charcoal which has 9.6 GJ m^{-3} (Thomas and Eden, 1990; Gunnarsson and Petersen, 2007).

1.3.4 Bio-recovery or disposed as hazardous waste

Phytoremediation plants can be either disposed as hazardous waste safely in specialized dumps (Fig. 1.12) like other hazardous materials or if economically feasible, bio-recovery of precious and semiprecious metals (called phytomining) (Fig. 1.13). Plant biomass containing accumulated heavy metals can be combusted to get energy. The remaining ash is considered as ‘‘bio-ore’’. This bio-ore can be processed for the recovery or extraction of the heavy metals. The commercial viability of phytomining depends on many factors like the efficiency of phytoextraction and current market value of the processed metals.

Phytomining has been commercially used for Ni and it is believed that it is less expensive than the conventional extraction methods. Using *Alyssum murale* and *Alyssum corsicum*, one can grow biomass containing 400 kg Ni ha^{-1} with production costs of \$250–500 ha^{-1}.

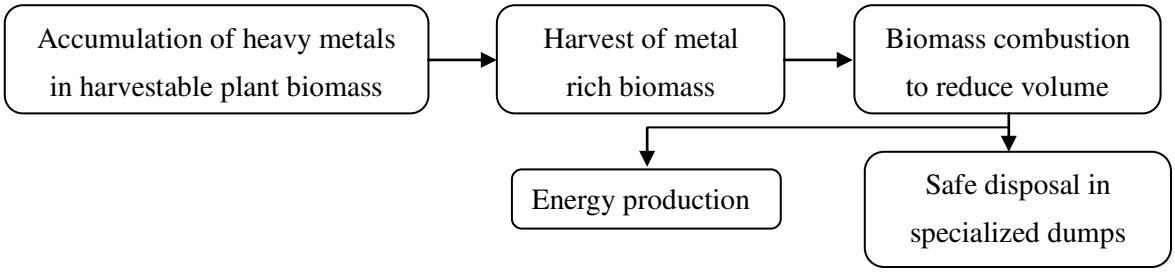


Fig.1.12 Post-harvest treatment of Phytoremediator plants (modified from Ali *et al*., 2013)

Considering Ni price of \$40 kg^{-1} (in 2006, Ni metal was trading on the London Metal Exchange at more than \$40 kg^{-1}), Ni phytomining has become a highly profitable agricultural technology (crop value = \$16,000 ha^{-1}) for Ni-contaminated or mineralized soils (Chaney *et al.*, 2007). The enforcement of more strict legislation for limiting environmental pollution would make bio-based mining more attractive (Siddiqui *et al*., 2009).

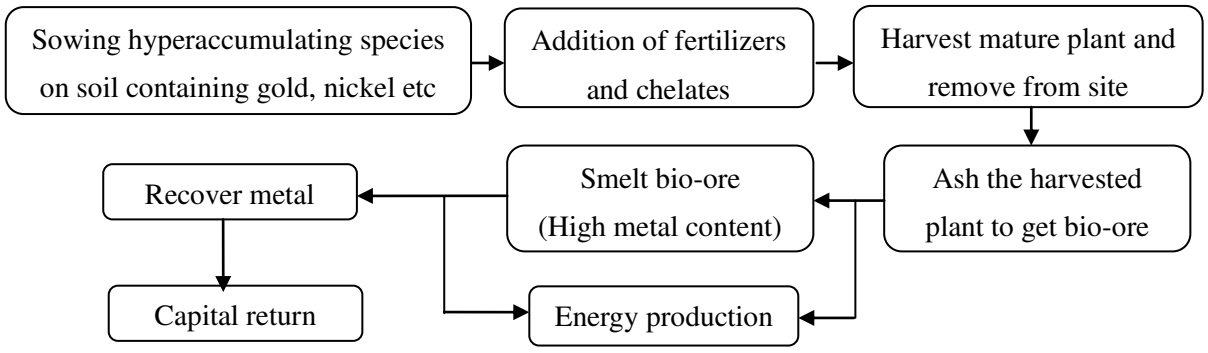


Fig.1.13 Integrated process of metal recovery or phytomining (modified from Sheoran *et al*., 2009)

1.4 Limitation of phytoremediation

Although phytoremediation is a cost-effective, efficient, eco-friendly and solar driven technology for remediation of contaminated environment compares to others physical and

chemical approaches, but it also suffers from some limitations, such as-

a) Hyperaccumulators are limited in number in nature with low biomass and slow growth rate.

b) Long time required to clean up contaminated sites.

c) Limited bioavailability of the contaminants in the environment to plant uptake.

d) It is only applicable to low to moderate level of contamination sites because plant growth affected or not sustained in heavily polluted sites.

e) Risk of food chain contamination due lacking of proper post harvest care.

f) Disposal cost of harvested biomass at waste management facilities.

1.5 Phytoremediation research-future perspective

Phytoremediation of environmental contaminants is a relatively promising field of research which is currently limited to laboratory and green house scale studies due to above limitations, and only a few studies have been conducted to test the phytoremediation potency in the field level. There are many factors which may affect phytoremediation in the field including variations in temperature, nutrients, precipitation and moisture, plants pathogens, uneven distribution of contaminants, soil type, soil structure, soil/water pH, and redox potential (Vangronsveld *et al.*, 2009) and other environmental conditions.

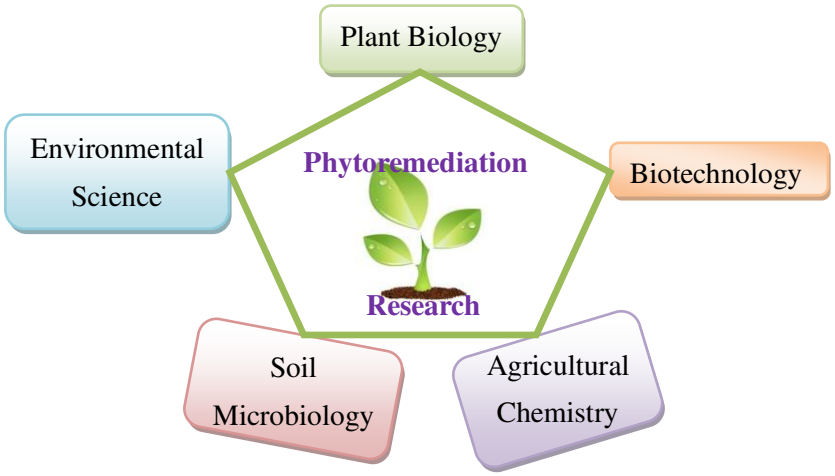


Fig. 1.14 Interdisciplinary phytoremediation research

However, phytoremediation efficiency of different plants for specific target heavy metals has to be tested in field conditions in order to realize the feasibility of this technology for concrete commercialization. To overcome the above limitations, we should conduct more interdisciplinary research (Fig. 1.14) to find out more effective natural hyperaccumulators, to discover uptake mechanism, binding substances and clear understanding of coordination chemistry of metals within plant tissue (Saraswat and Rai, 2011); and to identify the gene responsible for hyperaccumulation for the purpose to develop transgenic variety (Thakur, 2006). In spite of many challenges, phytoremediation is an aesthetic, green and solar driven technology with good public acceptance, and has a great potential in future.

1.6 Aims and objectives

The main purpose of the current research is to develop an effective As and Cd phytofiltration technique for the removal of As and Cd from the aquatic environment using *M. umbrosum* plant. To attain this purpose, the specific objectives of this research are shown as follows-

1. To review the heavy metal contamination in the environment, effects and proposed remediation techniques with their advantages and disadvantages.
2. To examine the potentiality of *M. umbrosum* for phytofiltration of As and Cd from contaminated water.
3. To analysis the As and Cd uptake and translocation pattern within *M. umbrosum*.
4. To assess the As and Cd hyperaccumulation characteristics of *M. umbrosum*, such as total metal absorption, and BCF.
5. To examine the phytotoxicity of As and Cd on *M. umbrosum*.
6. To study the uptake kinetics of different As species and Cd within *M. umbrosum*.
7. To find out the possible uptake mechanism of As and Cd within *M. umbrosum*.

1.7 Outline of thesis

The total research work presented in this dissertation is organized into five chapters. The introductory chapter I comprehensively introduction of the overall background, concept and purpose of this study along with the review of relevant literatures to understand the heavy metal contamination in the environment, adverse effect and proposed remediation techniques emphasized on phytoremediation techniques. Different phytoremediation techniques have also been discussed with different plants and heavy metals to obtain relevant knowledge about their prospects, drawbacks and mechanism of heavy metals remediation from aquatic environment. Chapter II presents the preparation of phytoremediation techniques using different naturally grown weeds from naturally and artificially As contaminated soils. Chapter III indicates the As and Cd phytofiltration potential, and the hyperaccumulation characteristics of *M. umbrosum* was evaluated by several determining factors. In Chapter IV, the phytotoxicity of As and Cd on *M. umbrosum* by analyzing the effects of these heavy metals on growth, photosynthetic pigments, macro- and micro-nutrient status within plant was discussed. In addition, the chapter IV describes the As and Cd uptake kinetics and mechanism of *M. umbrosum* plant. Finally chapter V summarizes the all chapters as total conclusions with some recommendations.

References

Abhilash, P.C., Powell, J.R., Singh, H.B., Singh, B.K., 2012. Plant-microbe interactions: novel applications for exploitation in multipurpose remediation technologies. Trends Biotechnol. 30, 416–420.

Abhilash, P.C., Vimal, C.P., Srivastava, P., Rakesh, P.S., Chandran, S., Singh, N., Thomas, A.P., 2009. Phytofiltration of cadmium from water by *Limnocharis flava* (L.) Buchenau grown in free-floating culture system. J Hazard Mater. 170 (2-3), 791-797.

Agency for Toxic Substances and Disease Registry (ATSDR) [Internet], 2011. CERCLA Priority List of Hazardous Substances. Atlanta, GA: Agency for Toxic Substances and Disease Registry; [updated 2011 Oct; cited 2012 Mar 05]. Available from: http://www.atsdr.cdc.gov/SPL/index.html.

Alam, B., Chatterjee, A.K., Duttagupta, S., 1995. Bioaccumulation of Cd(II) by water lettuce. Pollut Res. 14, 59–64.

Ali, H., Khan, E., Sajad, M.A., 2013. Review-Phytoremediation of heavy metals-concepts and applications. Chemosphere, 91, 869-881.

Axtell, N.R., Sternberg, S.P.K., Claussen, K., 2003. Lead and nickel removal using *Microspora* and *Lemna minor*. Bioresour Technol. 89, 41–48.

Badr, N., Fawzy, M., Al-Qahtani, K.M., 2012. Phytoremediation: an economical solution to heavy-metal-polluted soil and evaluation of plant removal ability. World Appl Sci J. 16, 1292–1301.

Baker, A.J.M., Brooks, R.R., 1989. Terrestrial higher plants which hyperaccumulate metallic elements. A review of their distribution, ecology and phytochemistry. Biorecovery 1, 81–126.

Baldwin, P.R., Butcher, D.J., 2007. Phytoremediation of arsenic by two hyperaccumulators in a hydroponic environment. Microchem J. 85, 297–300.

Barac, T., Safiyh, T., Brigitte, B., Ann, P., Licy, O., Jan, V.C., Vangronsveld, J., Daniel van der, L., 2004. Engineered endophytic bacteria improve phytoremediation of water-soluble, volatile, organic pollutants. Nature Biotech. 22 (5), 583-588.

Barcelo, J., Poschenrieder, C., 2003. Phytoremediation: principles and perspectives. Contrib Sci. 2, 333–344.

Bellin, C.A., O`Conner, G.A., 1990. Plant uptake of pentachlorophenol from sludge-amended soils. J Environ Qual. 19, 603-608

Bencko, V., Symon, K., 1977. Health aspects of burning coal with a high arsenic content: I. Arsenic in hair, urine, and blood in children residing in a polluted area. Environ Res. 13, 378–385.

Brooks, R.R., Robinson, B.H., 1998. Aquatic phytoremediation by accumulator plants. In: Brooks, R.R. (Ed.), Plants that Hyperaccumulate Heavy Metals: Their Role in Archaeology, Microbiology, Mineral Exploration, Phytomining and Phytoremediation. CAB International, Wallingford, pp. 203–226.

Brooks, R.R., Lee, J., Reeves, R.D., Jaffrre, T., 1977. Detection of nickeliferous rocks by analysis of herbarium specimens of indicator plants. J Geochem Explor. 7, 49–57.

Brown, J., Colling, A., Park, D., Phillips, J., Rothery, D., Wright, J., 1991. Chemical cycles in the oceans. In: Bearman, G. (Ed.), Ocean Chemistry and Deep-Sea Sediments. The Open University & Pergamon Press, Buckinghamshire, pp. 18–60.

Brown, S.L., Chaney, R.L., Angle J.S., Baker A.J.M., 1995. Zinc and cadmium uptake by hyperaccumulator Thlaspi caerulescens grown in nutrient solution. – Soil Sci Soc Am J. 59,125–133.

Bundschuh, J., Litter, M.I., Parvez, F., Roma´n-Ross, G., Nicolli, H.B., Jean, J.S., Liu, C.W., Lo´pez, D., Armienta, M.A., Guilherme, L.R., Cuevas, A.G., Cornejo, L., Cumbal, L.,Toujaguez, R., 2012. One century of arsenic exposure in Latin America: a

review of history and occurrence from 14 countries. Sci Total Environ. 429, 2–35.

Burken, J.G., Schnoor, J.L., 1999. Distribution and volatilization of organic compounds following uptake by hybrid poplar trees. Int J Phytorem. 1, 139–151.

Chakrabarti, D., Das, B., Rahman, M.M., Chowdhury, U.K., Biswas, B., Goswami, A.B., Nayak, B., Pal, A., Sengupta, M.K., Ahmed, S., Hossain, A., Basu, G., Roychowdhury, T., Das, D., 2009. Status of groundwater arsenic contamination in the state of West Bengal, India: a 20-year study report. Mol Nutr Food Res. 53, 542–551.

Chakraborti, D., Rahman, M.M., Das, B., Murrill, M., Dey, S., Chandra Mukherjee, S., Dhar, R.K., Biswas, B.K., Chowdhury, U.K., Roy, S., Sorif, S., Selim, M., Rahman, M., Quamruzzaman, Q., 2010. Status of groundwater arsenic contamination in Bangladesh: a 14-year study report. Water Res. 44, 5789–5802.

Chaney, R.L., Angle, J.S., Broadhurst, C.L., Peters, C.A., Tappero, R.V., Sparks, D.L., 2007. Improved understanding of hyperaccumulation yields commercial phytoextraction and phytomining technologies. J Environ Qual. 36, 1429–1443.

Chanakya, H.N., Borgaonkar, S., Meena, G., Jagadish, K.S., 1993. Solid-phase biogas production with garbage or water hyacinth. Bioresour Technol. 46, 227–231.

Chigbo, F., Smith, R. W., Shore, F. L., 1982. Uptake of arsenic, cadmium, lead and mercury from polluted waters by the water hyacinth (*Eichhornia crassipes*). Water Sci Tech. 19, 123-128

Craig, P.J. (Ed.), 2003. Organometallic Compounds in the Environment. Wiley, West Sussex, England, p. 415.

Cunningham, S.D., Berti, W.B., 1993 Remediation of contaminated soils with green plants: an overview. In Vitro Cell Dev Biol. 29, 207–212.

Dhankher, O.P., 2005. Arsenic metabolism in plants: an inside story. New Phytol. 168, 503–505.

Doty, S.L., Shang, Q.T., Wilson, A.M., Moore, A.L., Newman, L.A., Strand, S.E., Gordon, M.P., 2000. Enhanced metabolism of halogenated hydrocarbons in transgenic plants containing mammalian P450 2E1. Proc Natl Acad Sci. USA 97, 6287–6291.

Doucette, W.J., Bugbee, B., Hayhurst, S., Plaehn, W.A., Downey, D.C., Taffinder, S.A., Edwards. R., 1998. Phytoremediation of dissolved-phase trichloroethylene using mature vegetation. pp. 251-256. In G.B. Wickramanayake and R.E. Hinchee (eds.) Bioremediation and Phytoremediation, Chlorinated and Recalcitrant Compounds. Battelle Press, Columbus, Ohio, USA.

Ducker, A.A., Carranza, E.J.M., Hale, M., 2005. Arsenic geochemistry and health. Environ Int 31, 631–641.

Feldmann, J., 2001. An appetite for arsenic. Chemistry in Britain 37, 31-32.

Francesconi, K.A., Edmonds, J.S., 1996. Arsenic and marine organisms. Adv Inorg Chem. 44, 147–189.

Fu, F., Wang, Q., 2011. Removal of heavy metal ions from wastewaters: A review. J Environ Manage. 9, 407-418.

Gallon, C., Munger, C., Premont, S., Campbell, P.G.C., 2004. Hydroponic study of aluminum accumulation by aquatic plants: effects of fluoride and pH. Water Air Soil Pollut. 153, 135–155.

Garbisu, C., Alkorta, I., 2003. Basic concepts on heavy metal soil bioremediation. Eur J Miner Process Environ Prot. 3, 58–66.

Gerard, E., Echevarria, G., Sterckeman, T., and Morel, J.L. P., 2000. Availability of Cd to three plant species varying in accumulation pattern. J Environ Qual. 29, 1117-1123.

Greipsson, S., 2011. Phytoremediation. Nat Educ Knowl. 2, 7.

Gunnarsson, C.C., Petersen, C.M., 2007. Water hyacinths as a resource in agriculture and energy production: a literature review. Waste Manage. 27, 117–129.

Harvey, P.J.1., Campanella, B.F., Castro, P.M., Harms, H., Lichtfouse, E., Schäffner, A.R., Smrcek, S., Werck-Reichhart, D., 2002. Phytoremediation of polyaromatic hydrocarbons, anilines and phenols. Environ Sci Pollut Res Int. 9, 29–47.

Henry, J.R., 2000. In An Overview of Phytoremediation of Lead and Mercury. –NNEMS Report. Washington, D.C.

Hou, W.H., Chen, X., Song, G.L., Wang, Q.H., Chang, C.C., 2007. Effects of copper and cadmium on heavy metal polluted waterbody restoration by duckweed (*Lemna minor*). Plant Physiol Biochem. 45, 62–69.

Iqbal, M.P., 2012. Lead pollution—a risk factor for cardiovascular disease in Asian developing countries. Pak J Pharm Sci. 25, 289–294.

Islam, M.S., Ueno, Y., Sikder, M.T., Kurasaki, M., 2013. Phytofiltration of Arsenic and Cadmium from the Water Environment Using *Micranthemum umbrosum* (J.F. Gmel) S.F. Blake as a Hyperaccumulator. Int J Phytoremediation, 15, 1010-1021.

Kaise, T., Hanaoka, K.I., Tagawa, S., Hirayama, T., Fukui, S., 1988. Distribution of norganic arsenic and methylated arsenic in marine organisms. Appl rganomet Chem. 2, 539–546.

Kamal, M., Ghaly, A.E., Mahmoud, N., Cote, R., 2004. Phytoaccumulation of heavy metals by aquatic plants. Environ Int. 29, 1029–1039.

Kara, Y., 2004. Bioaccumulation of copper from contaminated wastewater by using *Lemna minor*. Bull Environ Contam.Toxicol. 72, 467–471.

Kim, K.W., Chanpiwat, P., Hanh, H.T., Phan, K., Sthiannopkao, S., 2011. Arsenic geochemistry of groundwater in Southeast Asia. Front Med. 5, 420–433.

Kirkham, M.B., 2006. Cadmium in plants on polluted soils: effects of soil factors, hyperaccumulation, and amendments. Geoderma. 137, 19–32.

Knobeloch, L.M., Zierold, K.M., Anderson, H.A., 2006. Association of

arsenic-contaminated drinking-water with prevalence of skin cancer in Wisconsin's Fox River Valley. J Health Popul Nutr. 24, 206–213.

Kuiper, I., Lagendijk, E.L., Bloemberg, G.V., Lugtenberg, B.J.J., 2004. Rhizoremediation: a beneficial plant-microbe interaction. Mol Plant-Microbe Interact. 17, 6–15.

Lasat, M.M., 2002. Phytoextraction of toxic metals: a review of biological mechanisms. J Environ Qual. 31, 109–120.

Lee, C.K., Low, K.S., Hew, N.S., 1991. Accumulation of arsenic by aquatic plants. Sci Total Environ. 103, 215–227.

Lesage, E., Mundia, C., Rousseau, D.P.L., Van de Moortel, A.M.K., Du Laing, G., Meers, E., Tack, F.M.G., De Pauw, N., Verloo, M.G., 2007. Sorption of Co, Cu, Ni and Zn from industrial effluents by the submerged aquatic macrophyte *Myriophyllum spicatum* L. Ecol Eng. 30, 320–325.

Lintern, M., Anand, R., Ryan, C., Paterson, D., 2013. Natural gold particles in Eucalyptus leaves and their relevance to exploration for buried gold deposits. Nature Communication. 4, 2614.

Liu, J., Zheng, B.S., Aposhian, H.V., Zhou, Y.S., Chen, M.L., Zhang, A.H., Waalkes, M.P., 2002. Chronic arsenic poisoning from burning high-arsenic-containing coal in Guizhou, China. J Peripher Nerv Syst. 7, 205–212.

Lockwood, M.P., 1976. Effects of pollutants on aquatic organisms. New York (NY): Cambridge University Press.

Ma, L.Q., Komar, K.M., Tu, C., Zhang, W., Cai, Y., and Kenelley, E.D., 2001. A fern that hyperaccumulates arsenic. Nature, 409; 579.

Ma, X., Burken, J.G., 2003. TCE diffusion to the atmosphere in phytoremediation applications. Environ Sci Technol. 37, 2534–2539.

Maher, W., 1984. Arsenic in marine organisms. Biol Trace Elements Res. 6, 159–164.

Manousaki, E., Kalogerakis, N., 2011. Halophytes present new opportunities in phytoremediation of heavy metals and saline soils. Ind Eng Chem Res. 50, 656–660.

Mandal, B.K., Suzuki, K.T., 2002. Arsenic round the world: a review. Talanta 58, 201–235.

Meharg, A.A., Hartley-Whitaker, J., 2002. Arsenic uptake and metabolism in arsenic resistant and nonresistant plant species. New Phytol. 154, 29-43.

Mesjasz-Przybylowicz, J., Nakonieczny, M., Migula, P., Augustyniak, M., Tarnawska, M., Reimold, W.U., Koeberl, C., Przybylowicz, W., Glowacka, E., 2004. Uptake of cadmium, lead, nickel and zinc from soil and water solutions by the nickel hyperaccumulator Berkheya coddii. Acta Biol Cracov Bot. 46, 75–85.

Mondal, P., Majumder, C.B., Mohanty, B., 2006. Laboratory based approaches for arsenic remediation from contaminated water: Recent developments. J Hazard Mater. 137, 464–479.

Mukhopadhyay, S., Maiti, S.K., 2010. Phytoremediation of metal enriched mine waste: a review. Global J Environ Res. 4, 135–150.

Nazir, A., Malik, R.N., Ajaib, M., Khan, N., Siddiqui, M.F., 2011. Hyperaccumulators of heavy metals of industrial areas of Islamabad and Rawalpindi. Pak J Bot. 43, 1925–1933.

Ng, J.C., 2005. Environmental Contamination of Arsenic and its Toxicological Impact on Humans. Environ Chemi. 2, 146-160.

Nogawa, K., Kobayashi, E., Okubo, Y., Suwazono, Y., 2004. Environmental cadmium exposure, adverse effects, and preventative measures in Japan. Biometals, 17 (5): 581–587.

Nordstrom, D.K., 2002. Public health–worldwide occurrences of arsenic in ground water. Science. 296(5576): 2143–2145.

O'Day, P.A., 2006. Chemistry and mineralogy of arsenic. Elements 2, 77–83.

Padmavathiamma, P.K., Li, L.Y., 2007. Phytoremediation technology: hyperaccumulation metals in plants. Water Air Soil Pollut. 184, 105–126.

Petrick, J.S., Ayala-Fierro, F., Cullen, W.R., Carter, D.E., Aposhian, H.V., 2000. Monomethylarsonous acid (MMAIII) is more toxic than arsenite in change human hepatocytes. Toxicol App Pharmacol. 163, 203–207.

Pilon-Smits, E., 2005. Phytoremediation. Annu Rev Plant Biol. 56, 15–39.

Prasad, M.N.V., 2003. Phytoremediation of metal-polluted ecosystems: hype for commercialization. Russ J Plant Physiol. 50, 686–700.

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

Rabhi, M., Ferchichi, S., Jouini, J., Hamrouni, M.H., Koyro, H.-W., Ranieri, A., Abdelly, C., Smaoui, A., 2010. Phytodesalination of a salt-affected soil with the halophyte *Sesuvium portulacastrum* L. to arrange in advance the requirements for the successful growth of a glycophytic crop. Bioresour Technol. 101, 6822–6828.

Rafati, M., Khorasani, N., Moattar, F., Shirvany, A., Moraghebi, F., Hosseinzadeh, S., 2011. Phytoremediation potential of *Populus alba* and *Morus alba* for cadmium, chromuim and nickel absorption from polluted soil. Int J Environ Res. 5, 961–970.

Rahimi, B., Nejatkhan, M.P., 2010. Availability, accumulation and elimination of cadmium by *Artemia urmiana* in different salinities. J Biol Environ Sci. 4, 149-157.

Rahman, M.A., Hasegawa, H., Ueda, K., Maki, T., Okumura, C., Rahman, M.M., 2007. Arsenic accumulation in duckweed (*Spirodela polyrhiza* L.): a good option for phytoremediation. Chemosphere, 69, 493–499.

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

Rahman, M.A., Hasegawa, H., Ueda, K., Maki, T., Rahman, M.M., 2008b. Arsenic uptake by aquatic macrophyte *Spirodela polyrhiza* L.: interactions with phosphate and iron. J Hazard Mater. 160, 356–361.

Rahman, M.A., Kadohashi, K., Maki, T., Hasegawa, H., 2011. Transport of DMAA and MMAA into rice (*Oryza sativa* L.) roots. Environ Exp Bot.72, 41-46.

Rai, P.K., 2008. Phytoremediation of Hg and Cd from industrial effluents using an aquatic free floating macrophyte *Azolla pinnata*. Int J Phytoremediation, 10, 430–439.

Ravindran, K.C., Venkatesan, K., Balakrishnan, V., Chellappan, K.P., Balasubramanian, T., 2007. Restoration of saline land by halophytes for Indian soils. Soil Biol Biochem. 39, 2661–2664.

Reeves, R.D., Baker, A.J.M., 2000. Metal accumulating plants. In Phytoremediation of Toxic Metals: Using plants to clean up the environment. (ed. Raskin, I. and Ensley, B.) – Wiley, New York. pp 193-229

Sakai, Y., Ma, Y., Xu, C., Wu, H., Zhu, W., Yang, J., 2012. Phytodesalination of a salt affected soil with four halophytes in China. J Arid Land Stud. 22, 1–20.

Sakakibara, M., Ohmori, Y., Ha, N.T.H., Sano, S., Sera, K., 2011. Phytoremediation of heavy metal contaminated water and sediment by *Eleocharis acicularis.* Clean: Soil, Air, Water 39, 735–741.

Saraswat, S., Rai, J.P.N., 2011. Complexation and detoxification of Zn and Cd in metal accumulating plants. Rev. Environ Sci Biotechnol. 10, 327–339.

Satarug, S., Garrett, S.H., Sens, M.A., Sens, D.A., 2010. Cadmium, environmental exposure, and health outcomes. Environ Health Perspect. 118, 182–190.

Schwitzguebel, J.-P., van der Lelie, D., Glass, D.J., Vangronsveld, J., Baker A., 2002. Phytoremediation: European and American trends, successes, obstacles and needs. J Soil Sed. 2, 91–99.

Selvapathy, P., Sreedhar, P., 1991. Heavy metals removal by water hyacinth. J Ind Public Health Eng. 3, 11–17.

Sen, A.K., Mondal, N.G., 1987. Salvinia natans as the scavenger of Hg (II). Water Air Soil Pollut. 34, 439–446.

Shah, K., 2011. Cadmium metal detoxification and hyperaccumulators. In: Sherameti I, Varma A, editors. Detoxification of heavy metal. Berlin (Germany): Heidelberg. pp. 181–203.

Sharma, V.K., Sohn, M., 2009. Aquatic arsenic: toxicity, speciation, transformations, and remediation. Environ Int. 35, 743–759.

Sheoran, V., Sheoran, A. S., Poonia, P., 2013. Phytomining of gold: A review. J Geochem Explor. 128:42-50

Sheoran, V., Sheoran, A., Poonia, P., 2011. Role of hyperaccumulators in phytoextraction of metals from contaminated mining sites: a review. Crit. Rev. Environ Sci Technol. 41, 168–214.

Sheoran, V., Sheoran, A.S., Poonia, P., 2009. Phytomining: a review. Min Eng. 22 (12), 1007–1019.

Siddiqui, M.H., Kumar, A., Kesari, K.K., Arif, J.M., 2009. Biomining—a useful approach toward metal extraction. American-Eurasian J. Agron. 2, 84–88.

Singh, S., 2012. Phytoremediation: a sustainable alternative for environmental challenges. Int J Gr Herb Chem. 1, 133–139.

Smith, A.H., Lingas, E.O., Rahman, M., 2000. Contamination of drinking water by arsenic in Bangladesh: a public health emergency. Bull World Health Organ. 78, 1093-1103.

Solís-Domínguez, F.A., González-Chávez,M.C.,Carrillo-González, R., Rodríguez-Vázquez, R., 2007. Accumulation and localization of cadmium in *Echinochloa polystachya* grown within a hydroponic system. J Hazard Mater. 141, 630-636.

Tangahu, B.V., Abdullah, S.R.S., Basri, H., Idris, M., Anuar, N., Mukhlisin, M., 2011. A review on heavy metals (As, Pb, and Hg) uptake by plants through phytoremediation. Int J Chem Eng. Article ID 939161, pp: 1-31. http://dx.doi.org/10.1155/2011/939161

Tchounwou, P.B., Patlolla, A.K., Centeno, J.A., 2003. Carcinogenic and systemic health effects associated with arsenic exposure—a critical review. Toxicol Pathol. 31, 575–588.

Thakur, I.S., 2006. Environmental Biotechnology. I. K. International, New Delhi.

Thangavel, P., Subbhuraam, C., 2004. Phytoextraction: role of hyperaccumulators in metal contaminated soils. Proc Indian Natl Sci Acad. Part B 70, 109–130.

Thomas, T.H., Eden, R.D., 1990. Water hyacinth – a major neglected resource. In: Sayigh, A.A.M. (Ed.). Energy and the Environment. Proceedings of the 1st World Renewable Energy Congress, Reading, UK, pp. 2092–2096.

Trapp, S., Köhler, A., Larsen, L.C., Zambrano, K.C., Karlson, U., 2001. Phytotoxicity of fresh and weathered diesel and gasoline to willow and poplar trees. J Soils Sed. 1, 71–76.

Trapp, S., Zambrano, K.C., Kusk, K.O., Karlson, U., 2000. A phytotoxicity test using transpiration of willows. Arch Environ Contam Toxicol. 39, 154–160.

Van Aken, B., 2009. Transgenic plants for enhanced phytoremediation of toxic explosives. Curr Opin Biotechnol. 20, 231–236.

van der Ent, A., Baker, A.J.M., Reeves, R.D., Pollard, A.J., Schat, H., 2013. Hyperaccumulators of metal and metalloid trace elements: facts and fiction. Plant Soil. 362, 319–334.

Van der Lelie, D., Schwitzguebel, J.P., Glass, D.J., Vangronsveld, J., Baker A., 2001. Assessing phytoremediation's progress in the United States and Europe. Environ Sci Technol. 35, 446A–452A.

van Halem, D., Bakker, S.A., Amy, G.L., van Dijk, J.C., 2009. Arsenic in drinking water: not just a problem for Bangladesh. Drink. Water Eng Sci Discuss. 2, 51–64.

Vangronsveld, J., Herzig, R., Weyens, N., Boulet, J., Adriaensen, K., Ruttens, A., Thewys, T., Vassilev, A., Meers, E., Nehnevajova, E., Van der Lelie, D., Mench, M., 2009. Phytoremediation of contaminated soils and groundwater: lessons from the field. Environ Sci Pollut Res. 16, 765–794.

Vishnoi, S.R., Srivastava, P.N., 2008. Phytoremediation-green for environmental clean. In: The 12th World Lake Conference, pp. 1016–1021.

Wagner, G.J., 1993. Accumulation of cadmium in crop plants and its consequences to human health. Adv Agron. 51, 173–212.

Wei, S., Zhou, Q., Saha, U.K., 2008. Hyperaccumulative characteristics of weed species to heavy metals. Water Air Soil Pollut. 192, 173–181.

WHO, 2011. Guidelines for drinking-water quality, 4th ed. Chemical fact sheets-chemical contaminants in drinking water. Geneva: World Health Organization.

Williams, P.N., Islam, M.R., Adomako, E.E., Raab, A., Hossain, S.A., Zhu, Y.G., Feldmann, J., Meharg, A.A., 2006. Increase in rice grain arsenic for regions of Bangladesh irrigating paddies with elevated arsenic in groundwaters. Environ Sci Technol. 40, 4903–4908.

Woolson, E.A. (Ed.), 1975. *Arsenical Pesticides.* American Chemical Society, Washington DC, 176.

Wu, G., Kang, H., Zhang, X., Shao, H., Chu, L., Ruan, C., 2010. A critical review on the bio-removal of hazardous heavy metals from contaminated soils: issues, progress, eco-environmental concerns and opportunities. J Hazard Mater. 174, 1–8.

Wuana, R.A., Okieimen, F.E., 2011. Heavy metals in contaminated soils: a review of sources, chemistry, risks and best available strategies for remediation. ISRN Ecology

2011, 1–20.

Xie, W. Y., Huang, Q., Li, G., Rensing, C., Zhu, Y. G., 2013. Cadmium accumulation in the rootless macrophyte *Wolffia globosa* and its potential for phytoremediation. Int J Phytoremediation,15, 385-397,

Yadav, R., Arora, P., Kumar, S., Chaudhury, A., 2010. Perspectives for genetic engineering of poplars for enhanced phytoremediation abilities. Ecotoxicology 19, 1574–1588.

Zayed, A., Gowthaman, S., Terry, N., 1998. Phytoaccumulation of trace elements by wetland plants: 1. Duckweed. J Environ Qual. 27, 715–721.

Zhang, X., Lin, A.-J., Zhao, F.-J., Xu, G.-Z., Duan, G.-L., Zhu, Y.-G., 2008. Arsenic accumulation by the aquatic fern Azolla: Comparison of arsenate uptake, speciation and efflux by Azolla caroliniana and Azolla filiculoides. Environ Pollut. 156, 1149–1155.

Zhang, X., Zhao, F.J., Huang, Q., Williams, P.N., Sun, G.X., Zhu, Y.G., 2009. Arsenic uptake and speciation in the rootless duckweed *Wolffia globosa*. New Phytol. 182, 421–428.

Zhi-xin, N.I.U., Li-na, S., Tie-heng, S., Yu-shuang L.I., Wang, H., 2007. Evaluation of phytoextracting cadmium and lead by sunflower, ricinus, alfalfa and mustard in hydroponic culture. J Environ Sci. 19, 961-967.

Chapter II: Preparation of experiment - Phytoaccumulation of arsenic from arsenic contaminated soils by *Eichhornia crassipes* L., *Echinochloa crusgalli* L. and *Monochoria hastata* L. in Bangladesh

Preface

This study was mainly done for the purpose of preparation of phytofiltration experiment and to get initial laboratory and field basis information and also compared water phytoremediation technique with soil phytoremediation. Current phytofiltration research is an output of series of experiment like field level As contaminated soil sampling, to find out As accumulating plant and to examine how to culture them in naturally and artificially As contaminated soil. After analysis of data for As accumulation, uptake pattern and their phytoremediation ability to remove As from soil were compared among the tested plants.

Abstract

As phytoaccumulation study was conducted with three plant species namely *Eichhornia crassipes* L. (water hyacinth), *Echinochloa crusgalli* L. (barnyard grass) and *Monochoria hastata* L. (water taro) in crop land soils contaminated by naturally and artificially from sodium arsenite ($NaAsO_2$). Phytoaccumulation of As increased significantly with increasing soil As levels. In artificially As contaminated soils, highest As concentration was recorded in water hyacinth (67.9 and 46.8 mg kg^{-1} root and shoot, respectively) followed by water taro and barnyard grass at 100 mg As kg^{-1} treated soil. For naturally As contaminated soils, the highest accumulation of As in barnyard grass (56.9 and 26.5 mg kg^{-1} root and shoot, respectively) followed by water taro and water hyacinth in *Paranpur* soils (116 mg As kg^{-1} soil). The enrichment factor of As in both artificially and naturally arsenic contaminated soils, root and shoot parts of these plant species were found to be in the sequence of soil>root>shoot. In most cases, As translocation factor of soil to root and root to shoot was 0.5 to 1.0. Highest bio-concentration factor value (2300) was found in barnyard grass root. The value was higher than water taro (2184) and water hyacinth (1336). In addition, these values from the plant parts grown in the contaminated site were always more than 10 times higher (293-2300) compared with those in uncontaminated site. Current study revealed that these plant species can be used as As accumulator in As contaminated soils.

2.1 Introduction

As pollution in ground water has become a major public concern in many countries especially in Bangladesh. Approximately 35–77 million people out of 125 million populations in Bangladesh have faced the risk of As in their drinking water (Smith *et al*., 2000). The As contaminated areas in Bangladesh have shown as more than 20 mg As kg^{-1} soil (Zaman *et al*., 2008). High concentration As in surface soil was detected to depend on As contaminated ground water irrigation (Mandal *et al*., 1996), application of As-based herbicides and pesticides, fertilizers such as chicken manure from Roxarsone fed chicken and mining activities (Onken and Hossner, 1996; Kris, 2001). These pollutions could pose a serious threat to plants, human health and other organisms through the food chain pathways (Arif, 2001; Bruce *et al*., 2003; Duxbury *et al*., 2003; Williams *et al*., 2006; Zhu *et al*., 2008). As is classified as Group-1 carcinogen to humans based on strong epidemiological evidence (Tchounwou *et. al*., 2003). There were about more than fifty arsenicosis patients identified in *Nalitabari* upazila (total population 0.27 million) under *Sherpur* District of Bangladesh due to drinking of As-contaminated water (The Daily Amar Desh, December, 2011). According to WHO, the mean daily intake of As through food by adults is in the range of 17-129 µg. Average As concentration in rice grain produced in different parts of Bangladesh is around 480 µg kg^{-1}. Considering average consumption of rice grain 454 gm/capita/day average As intake by a Bangladeshi people through only rice grain is 218 µg day^{-1} (SOS-arsenic.net, 2005). As toxicity depends on its speciation, and generally inorganic As species are more toxic compared with that of organic species (Meharg and Hartley-Whitaker, 2002; Jack, 2005). As(III) is more toxic as compared with As(V), and dimethylarsinic acid (DMAA) and monomethylarsonic acid (MMAA) are more toxic than their parent compounds (Petrick *et al*., 2000). As remediation technologies from soils include excavation, immobilization, vitrification, soil washing/flushing and phytoremediation (Rahman and Hasegawa, 2011). Phytoremediation is a low cost and eco-friendly technology for cleaning up the metal-contaminated sites (Vamerail *et al*., 2010). Phytoaccumulation is one of the phytoremediation processes that plants uptake contaminants from the environment and store them in their body. Some terrestrial plant species such as *Agrostis castellana*; *Agrostis delicatula* (Koe, 1994), *Bidens cynapiifolia* (Bech *et al*., 1997), *Pteris vittata* L. (Ma *et al*., 2001) and *Pityrogramma calomelanos* L. (Gulz *et al*., 2005) have been reported to accumulate As from soils. Among them *Pteris vittata* L. accumulates a formidable amount of As from soil (Ma *et al*., 2001) and stores it

in the fronds (Tu *et al*., 2002).

Aquatic macrophytes have ability to concentrate heavy metals in their roots and shoots as well as leaves. However, the accumulation of heavy metals is much higher in roots of these plants (Mishra *et al*., 2009; Paiva *et al*., 2009; Mufarrege *et al*., 2010). Mishra and Tripathi (2008) compared the phytoremediation potential of three aquatic macrophytes, and concluded that *Eichhornia crassipes* was more efficient candidate for removal of heavy metals (Fe, Zn, Cu, Cr, and Cd) followed by *Pistia stratiotes* and *Spirodela polyrrhiza*. Rahman *et al*. (2007) performed a hydroponic experiment with *Spirodela polyrrhiza* L., and found that it uptake about 0.353 µM As g^{-1} from 4.0 µM arsenate solution. Rahman *et al*. (2008) also reported that external supplementation of ethylene diamine tetra acetic acid (EDTA) in the growth medium of *Spirodela polyrhiza* increased the uptake of As(V) and As(III).

Many researches were conducted on phytoremediation of As using hyper accumulator. There are some problems of the application of hyper accumulators to contaminated soils such as a small biomass, and a limited adaptation capacity to the growth condition and cultivation. 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 (Murakami and Ae, 2009). Then the current research focused on phytoremediation of crop land surface soils using adaptable and high biomass content plants, where As built up by using of As contaminated irrigation water, fertilizers, manures and pesticides; and artificially from $NaAsO_2$. Plant species used in this study were common in Bangladesh and can easily grow on the crop land in moist or submerged condition especially in rice field. To study remediation of As contaminated crop land surface soils, these plant species were examined for the phytoaccumulation of As and clean up the soil environment in a eco-friend way.

2.2 Materials and Methods

2.2.1 Study area, sample collection and preparation

Soils were collected from Bangladesh Agricultural University, Mymensingh (Latitude: 24.75° N, Longitude: 90.4° E, Altitude: 17 m), campus at 0-15 cm depth for artificial As contamination. Naturally As contaminated soil was collected from the three As contaminated sites (*Paranpur, Kamorpur and Dholdi*) of *Faridpur* Sadar Upazilla under *Faridpur* district (Latitude: 23.6° N, Longitude: 89.83° E, Altitude: 11 m), Bangladesh,

which was known as severely As contaminated area (Hossain *et al*., 2001). Soil characteristics of control, artificial and naturally As contaminated soil were given in the Table 2.1. Exactly 5.0 kg soil was taken in a series of plastic pots. The pots were maintained in natural condition listed in Table 2.1. The experiments were laid out in a Completely Randomized Design (CRD) with three replications. For artificial As contamination of soil, there were four treatments of As *viz*., 30, 50, 70 and 100 mg As kg^{-1} (ppm) soil from sodium arsenite ($NaAsO_2$) with control soil (Table 2.1) and three replications in both for artificially and naturally As contaminated soils were done. Initially required amounts of As dissolved in de-ionized water and mixed properly with soil then 20 mg N from urea and P from triple super phosphate were also added per kg soil before planting. Plant seedlings were collected from Agronomy field of Bangladesh Agricultural University, Mymensingh. Each plant was grown on each pot. The plants (water hyacinth, barnyard grass and water taro) were irrigated daily with As free tap water. Plants were uprooted at 45 days after transplanting. Plant height was measured from the ground level to the top of the plants and number of leaves for each plant was recorded at full maturity. Then about 2-3 g air dried plant samples were dried at 65ºC for 48 h according to Rahman *et al*. (2007). The oven dried samples were cooled and weighed (by digital balance) separately for root and shoot. This procedure was repeated until constant weight was obtained.

Table 2.1 Agro-ecological zone (AEZ), soil series, name of soil, arsenic content and pH value of the *Faridpur* soils and Bangladesh Agricultural University (BAU) farm soils

Experiment	AEZ	Soil Series	Name of soils	As (ppm)	pH
Control	Brahmaputra-Jamuna Floodplain	Sonatala	BAU Farm soil	4.3	6.7
Faridpur soil (Naturally As contaminated soils)	Low Ganges River Floodplain	Ishurdi	*Paranpur* Soil	116.0	7.5
			Kamorpur Soil	47.3	7.4
			Dholdi Soil	22.0	7.5
Artificially As contaminated soil	Brahmaputra-Jamuna Floodplain	Sonatala	Soil 1	34.3	6.7
			Soil 2	54.3	6.7
			Soil 3	74.3	6.5
			Soil 4	104.3	6.8

2.2.2 As analysis

Exactly 0.5 g (oven dry basis) for plant and soil samples was taken into a digestion tube. Five mL of 65% HNO_3 (analytical reagent grade) were added, and samples were kept under fume hood for 12 h. Then the samples heated on a digestion chamber at 95ºC for 2 h. After cooling to room temperature, 3 mL of 30% hydrogen peroxide were added to the digests, and the samples were heated again at 120ºC for 20 min. The digested samples were diluted to 10 mL using de-ionized water, and filtered with Whatman No. 42 filter paper. They are stored in 15 mL plastic bottles until measurement. Total As contents in the plant and soil were determined with a hydride generator Atomic Absorption Spectrophotometer (Varian, UK) as described by Welsch *et al.* (1990).

2.2.3 Enrichment factor (EF)

The EF has been calculated to derive the degree of soil contamination and heavy metal accumulation in soil and in plants growing on contaminated site with respect to soil and plants growing on uncontaminated soil (Kisku *et al*., 2000).

EF = Concentration of As in soil or plant parts at contaminated site/ Concentration of As in soil or plant parts at uncontaminated site.

The enrichment factor in the plant parts is an important criterion for the selection of suitable crop species which can be selected for cultivation in a field having higher level of metal contamination or receiving industrial effluent (Barman and Bhargava, 1997).

2.2.4 Translocation factor (TF)

TF or mobilization ratio was calculated to determine relative translocation of metals from soil to other parts (root and shoot) in the plant species (Barman *et al*., 2000; Gupta *et al*., 2008).

TF =Concentration of As in plant tissue (parts)/Concentration of As in corresponding soil or root

2.2.5 Bio-Concentration factor (BCF)

The BCF provides an index of the ability of the plant to accumulate the metal with respect to the metal concentration in the substrate. The result of BCF was calculated (L kg^{-1}) as follows (Snyder, 2006).

BCF=Concentration o As in plant tissue (mg kg^{-1})/Initial concentration of As in external solution (mg L^{-1})

2.2.6 Statistical Analysis

The data were carried out for statistical analyses. For each pot, the mean values, standard deviations (SD) and confidence ranges were calculated at the 0.05 probability level as per Duncan's Multiple Range Test (DMRT). Significance of differences between the means was checked by least significant difference (LSD) test. Statistical analysis was performed by MSTATC program and as outlined by Gomez and Gomez (1984).

2.3 Results and discussion

2.3.1 Effects of As on leaves production

Increasing dose of As decreased significantly the number of leaves of three plants in artificially As contaminated soil. Water hyacinth showed the maximum number of leaves in the artificially soil contained 34.3 ppm As (Fig. 2.1a). In cases of barnyard grass and water taro, the highest number of leaves per plant was observed in the control group. The number of leaves varied from 16 to 5, 11 to 5 and 10 to 6 in water hyacinth, barnyard grass and water taro, respectively (Fig. 2.1a) due to different As treatments. In naturally As contaminated soil, the highest number of leaves (32) was found in the water hyacinth at 22 mg As kg^{-1} soil and the lowest number of leaves (9) was found in the barnyard grass at 116 mg As kg^{-1} soil as shown in Fig. 2.1d. Similar results were also shown by Mitra (2004) and Sultana (2006). They reported that the number of leaves in some weed species decreased with the increase of soil As concentration.

2.3.2 Effects of As on plant height

Increasing levels of As decrease the plant height from 34.3 ppm to onwards (Figs. 2.1b, and 2.1e). The maximum height was obtained by 34.3 ppm As treatment for barnyard grass (85 cm) and minimum height was obtained by 104.3 ppm As for water hyacinth as presented in Fig. 2.1b. In naturally As contaminated soil, the highest plant height was obtained in barnyard grass (85 cm) at 22 ppm and lowest was at 116 ppm As for water hyacinth (17 cm) as shown in Fig. 2.1e. As can help the growth of these plants at certain lower level but in excess amount of As, plant growth was decreased. It suggests that calcium content in leaf and stem was reduced by As treatment (Petrick *et al.*, 2000). Bindu *et al*. (2010) reported that significant decrease in the relative growth, biomass productivity and total chlorophyll content was noticed in the taro plant (*Colocasia esculenta*) with an increase in Pb and Cd concentrations in the solution and exposure time.

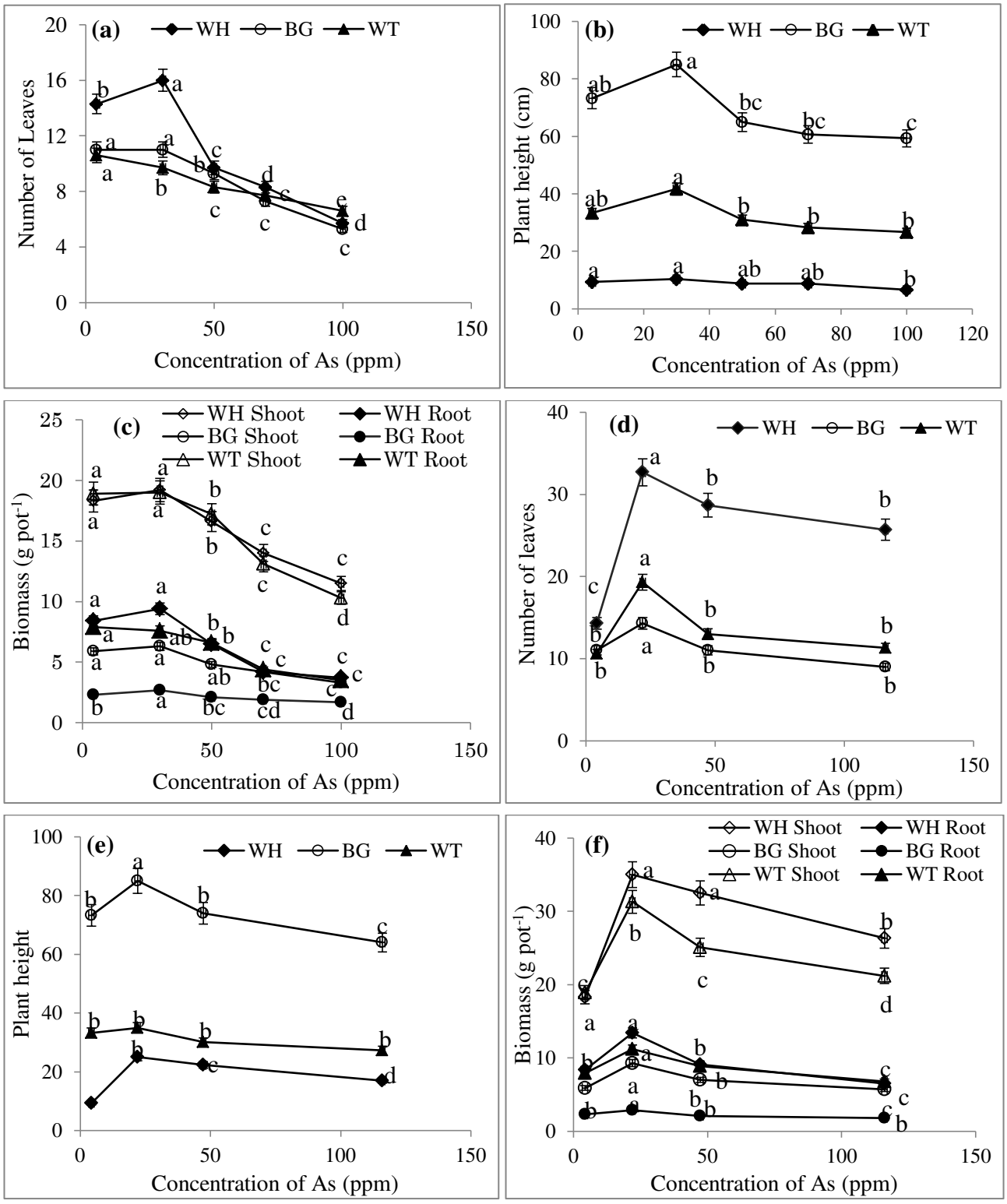


Fig. 2.1 Effects of different concentration of As (ppm or mg As kg^{-1} soil) on the production of leaves [(a), (d)], plant height [(b), (e)] and biomass production [(c), (f)] of water hyacinth (WH), barnyard grass (BG) and water taro (WT) in artificially [(a),(b),(c)] and naturally [(d),(e),(f)] As contaminated soils. Common letter did not differ at 5% level of probability as per DMRT (n=3).

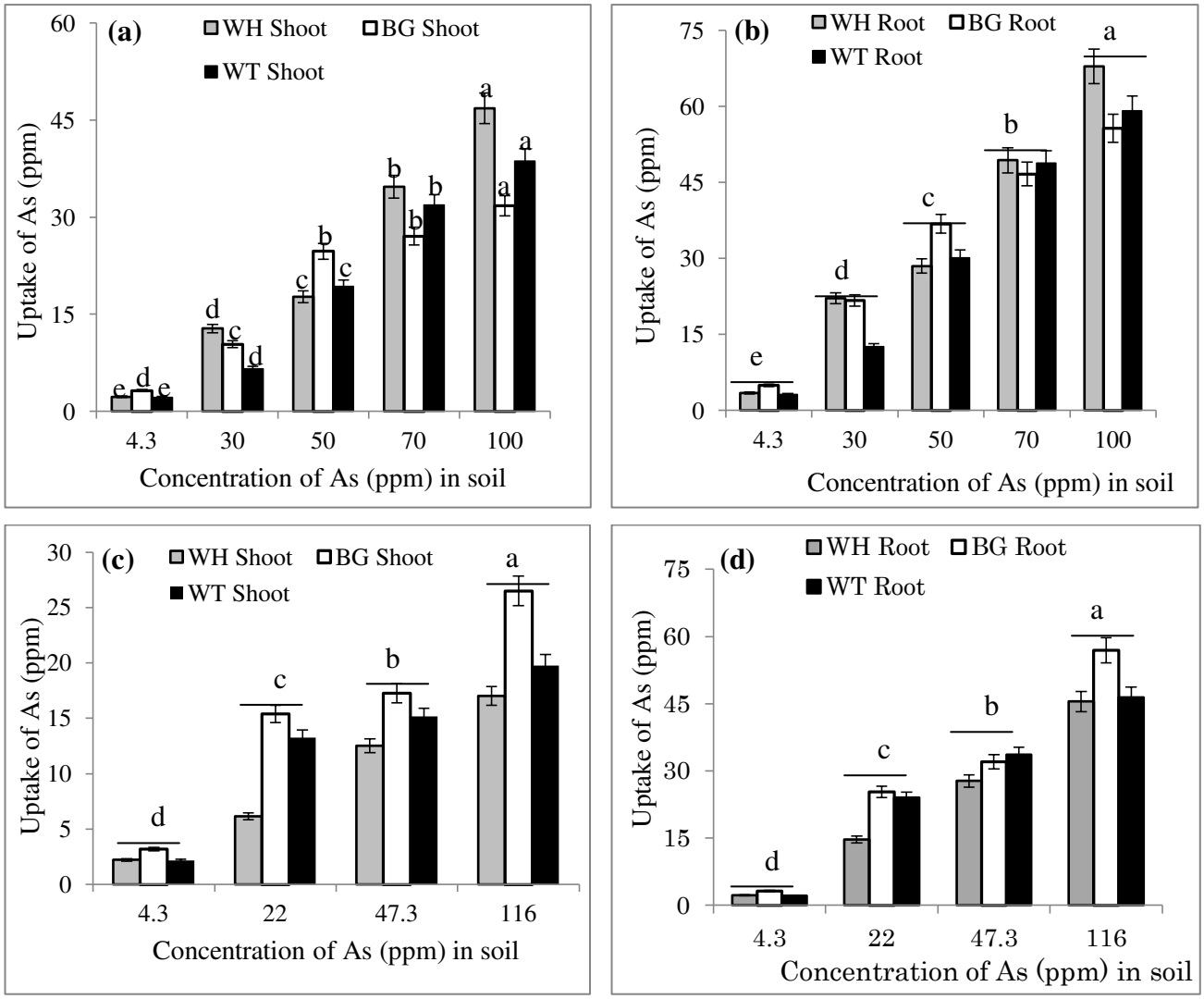


Fig. 2.2 As uptaken (ppm or mg As kg^{-1} biomass, oven dry basis) by water hyacinth (WH), barnyard grass (BG) and water taro (WT) shoot (a, d) and root (b, c) from artificially (a, b) and naturally (c, d) As contaminated soil. Common letter did not differ at 5% level of probability as per DMRT (n=3)

2.3.3. Effects of As on biomass production

Results shown in Figs. 2.1c and 2.1f indicated that production of shoot and root biomass was affected by increasing As levels. It was significantly ($p<0.05$) reduced the water hyacinth root, barnyard grass and water taro shoot, and root biomass content (Fig. 2.1c). In most cases, the biomass content was increased at 34.3 ppm As, and then decreased with increasing As levels (Figs. 2.1c and 2.1f). The highest biomass (root and shoot combined) was found in water hyacinth (48.4 g) at 22 ppm As (Fig. 2.1f), and lowest biomass (root and shoot combined) was found in barnyard grass (5 g) at 104.3 ppm As (Fig. 2.1c). Sultana and Kobayashi (2011) described that growth inhibition of biomass production in *barnyard grass* occurred with increasing As concentration. Ebel *et al*. (2007) have been reported that water hyacinth grow very fast, and enormous biomass production rate and high tolerance to heavy metals polluted

wastewater. Giraldo and Garzon (2002) also reported that water hyacinth represents a reliable alternative for As bioremediation in aquatic system even though the plant may cause severe water management problems because of its huge vegetative reproduction and high growth rate.

2.3.4 As accumulation in plant parts

Phytoaccumulation of As was significantly increased in these plant species with increasing As levels in soil (Fig. 2.2). As accumulation both in root and shoot was determined (Tables 2.2 and 2.3). In artificially As contaminated soil, highest amount of As accumulation was found in 104.3 ppm As treatment. The relative distribution of As in the shoot and root has been presented in Figs. 2.2a and b. The highest As concentration in shoot was found in water hyacinth (46.8 mg As kg^{-1} shoot) followed by barnyard grass (31.8 mg As kg^{-1} shoot) and water taro (38.6 mg As kg^{-1} shoot) as shown in Fig. 2.2a. In the cases of roots, the highest As concentration was found in water hyacinth (67.9 mg As kg^{-1} root) followed by water taro (59.1 mg As kg^{-1} root) and barnyard grass (55.6 mg As kg^{-1} root) as shown in Fig. 2.2b. Alvarado *et al*. (2008) showed that water hyacinth has high As removal efficiency (removal rate of 600 mg As ha^{-1} d^{-1}) from water due to its high biomass production, favourable climatic conditions under field environment and a removal recovery of 18% under laboratory conditions. In naturally As contaminated soil, highest amount of As accumulation was found in the root of barnyard grass as 56.9 mg As kg^{-1} root at 116 ppm As containing soil, and minimum amount of As was found in the shoot of water hyacinth as 6.17 mg As kg^{-1} shoot at 22 ppm As treatment (Table 2.2). In both shoot and root, the As concentration increased progressively with increased levels of As (Figs. 2.2c and 2.2d). The highest concentration of As in shoot was found in case of barnyard grass (26.5 mg As kg^{-1} shoot) at 116 ppm As treatment (Fig. 2.2c) followed by water taro (19.77

mg As kg^{-1} shoot) and water hyacinth (17.03 mg As kg^{-1} shoot) shown in Table 2.2. The highest As removal efficiency was found in water hyacinth compared with other plants due to high biomass production (Fig. 2.1c). Mishra *et al*. (2008) also compared As removal efficiency of *Eichhornia crassipes*, *Lemna minor* and *Spirodela polyrhiza* from tropical opencast coalmine effluents, and observed that *E. crassipes* had the highest removal efficiency (80%). This study indicated that As accumulated in the root was higher than that in shoot (Fig. 2.2). Tlustos *et al.* (1998), Mitra (2004) and Sultana (2006) also reported similar results that some weeds like *joina, water cress* accumulate higher amount of As in root than shoot. Sultana and Kobayashi (2011) found that barnyard grass accumulated higher As in root compared with that in shoot. According to Hoffmann *et al.* (2004), As uptake by *Salvinia minima* was increased with increasing As exposure time and concentration in the growth solution. As accumulation in brake fern (*Pteris vittata*) also increased by increasing As levels in soils (Ma *et al*., 2001). High concentration of As (138 mg kg^{-1} fresh wt) has also been found in naturally grown watercress (*Nasturtium microphyllum*) in Taupo Volcanic zone, New Zealand (Robinson *et al*., 2006). These results also supported the results obtained in this study.

2.3.5 Enrichment factor (EF)

EF in the root and shoot parts of three plants under the naturally and artificially As contaminated soils was shown in Tables 2.2 and 2.3. High EF indicates higher availability and distribution of As in soil contaminated by irrigation water or As containing pesticides, fertilizers etc., and increasing the metal accumulation in plants species grown on the contaminated soil (Kisku *et al.*, 2000; Gupta *et al*., 2008). In most cases, the sequence of EF is soil>root>shoot. The EF increases in different plant parts with increasing As concentration in soil. The EF values in this study indicated higher accumulation of As by

roots than shoot for all plant species from naturally (Table 2.2) and artificially (Table 2.3) As contaminated soil hence they are suitable for As phytoaccumulation, and both root and shoot can be harvested to clean up As. Ramesh *et al*. (2010) investigated the accumulation of Cd, Zn, Cr, Pb, Cu, Ni, Mn and Fe in fields contaminated with fly ash from a thermal power plant and subsequent uptake in different parts of naturally grown 11 plants species. As the results, among the eight metals, the maximum EF was found in case of Cd followed by Fe for soil, root and shoot part but in overall, the sequence did not follow any specific pattern – some are more than one and in some cases less than one.

2.3.6 Translocation factor (TF)

TF or mobilization ratio of metals from soil to root and root to shoot has been estimated (Tables 2.2 and 2.3). In naturally As contaminated soil, TFs for soil to root and root to shoot increase with decrease of As concentration in the soil (Table 2.2). In case of 22 ppm As treatment, the translocation of As from soil to root was found to be in the order of barnyard grass (1.15) >water taro (1.09) >water hyacinth (0.67), and when these values were compared with control value it was observed to be higher in the contaminated site in the case of barnyard grass and water taro (Table 2.2). The same order was also found in TF for root to shoot; however, all TF values were less than 1.0 indicating that shoot accumulated lower As compared with root. Marin *et al*. (1992) found that TF of root to shoot for inorganic As was less than 0.2 for rice cultivars; however, in this study TFs of root to shoot for water hyacinth, barnyard grass and water taro ranged 0.37-0.45, 0.47-0.61 and 0.43-0.55, respectively (Table 2.2).

In artificially As contaminated soils, TFs of soil to root and root to shoot for each plant species were lower than the uncontaminated soil (TF was less than 1.0) as listed in Table 2.3. Rabb *et al.* (2007) studied 46 plant species to determine uptake and translocation into

shoot of As, methyl arsonate and dimethylarsinate, and found that none of the plant species had exceeded 0.9 of TFs of shoot to root for arsenate (V). TF values range from 0.01 to 0.84. In this study, TFs of root to shoot for water hyacinth, barnyard grass and water taro range 0.58-0.70, 0.48-0.67 and 0.53-0.65, respectively under the artificial As contaminated soil (Table 2.3). Ramesh *et al.* (2010) reported that order for translocation of metals from soil to root was Cu (1.03)> Ni (0.96) > Mn (0.85) > Zn (0.67) >Pb (0.58) > Cd (0.50) > Fe (0.48) in 11 plant species. TFs for Cd, Zn, Cu, Ni, Mn and Fe in the contaminated site were higher than TF in control site. They also reported that order of TF of root to shoot was Mn (1.38) > Fe (1.27) > Pb (1.03) > Ni (0.94) > Zn (0.85) > Cd (0.82) > Cr (0.73). Totally, the TF values from soil to root and root to shoot were lower than EF values from those. One reason for slow translocation of As from root to shoot could be due to that trivalent arsenite was easily trapped in the root; however, under anaerobic conditions, much of the As in the cells was As(V), and As(V) might be partly reduced to arsenite due to the activity of endogenous arsenate reductase enzyme with conjugation to thiols, resulting that As is sequestered in the root vacuole (Zhu and Rosen, 2009). To express the gene for arsenate reductases, Dhankher *et al*. (2002) reported that over expressing of the gene for *Escherichia coli* arsenate reductase, *arsC*, in A*rabidopsis thaliana* under the control of a light-responsive transcription factor led to hypersensitivity to arsenic, and arsenite formed $As(GS)_3$ conjugates. Other factor that influences the TF for the different As species is the ability of plants to complex inorganic arsenic as As–phytochelatin (PC) complexes. In experiments with *Helianthus annuus,* it was reported that the formation of these complexes was predominantly in the root system (Raab *et al*., 2005). Since As–PC complexes seem not to be transport forms of As, their formation might reduce the translocation of inorganic arsenic (Pickering *et al*., 2000; Raab *et al*., 2005).

Table 2.2 EF and TF of as from soil to root (S→R), root to shoot (R→St) and As uptake (ppm) by water hyacinth (WH), barnyard grass (BG) and water taro (WT) in naturally soils contaminated and uncontaminated with As

Plant	Treatment of As ppm	Location	Naturally As contaminated soil			Uncontaminated soil			EF
			As uptake (ppm)	TF		As uptake (ppm)	TF		
				S→R	R→St		S→R	R→St	
WH	22	Shoot	6.17	0.67	0.42	2.2	0.80	0.65	2.77
		Root	14.7			3.4			4.27
		Soil	22			4.3			5.12
	47.3	Shoot	12.5	0.59	0.45	2.2	0.80	0.65	5.62
		Root	27.8			3.4			8.07
		Soil	47.3			4.3			11.00
	116	Shoot	17.0	0.39	0.37	2.2	0.80	0.65	7.64
		Root	45.5			3.4			13.23
		Soil	116			4.3			26.98
BG	22	Shoot	15.4	1.15	0.61	3.2	1.14	0.65	4.81
		Root	25.3			4.9			5.17
		Soil	22			4.3			5.12
	47.3	Shoot	17.3	0.68	0.54	3.2	1.14	0.65	5.40
		Root	32.1			4.9			6.56
		Soil	47.3			4.3			11.00
	116	Shoot	26.5	0.49	0.47	3.2	1.14	0.65	8.28
		Root	56.9			4.9			11.64
		Soil	116			4.3			26.98
WT	22	Shoot	13.3	1.09	0.55	2.2	0.74	0.68	6.12
		Root	24.0			3.2			7.56
		Soil	22.0			4.3			5.12
	47.3	Shoot	15.2	0.71	0.45	2.2	0.74	0.68	6.99
		Root	33.6			3.2			10.58
		Soil	47.3			4.3			11.00
	116	Shoot	19.8	0.40	0.43	2.2	0.74	0.68	9.11
		Root	46.4			3.2			14.59
		Soil	116			4.3			26.98

Table 2.3 EF and TF of as from soil to root (S→R), root to shoot (R→St) and As uptake (ppm) by water hyacinth (WH), barnyard grass (BG) and water taro (WT) in artificially soils contaminated and uncontaminated with As

Plant	Treatment of As	Location	Artificially As contaminated soil			Uncontaminated soil			EF
			As uptake (ppm)	TF		As uptake (ppm)	TF		
				S→R	R→St		S→R	R→St	
WH	34.3 ppm	Shoot	12.8	0.64	0.58	2.2	0.80	0.65	5.74
		Root	22.1			3.4			6.43
		Soil	34.3			4.3			7.98
	54.3 ppm	Shoot	17.7	0.52	0.62	2.2	0.80	0.65	7.94
		Root	28.5			3.4			8.28
		Soil	54.3			4.3			12.63
	74.3 ppm	Shoot	34.7	0.66	0.70	2.2	0.80	0.65	15.56
		Root	49.4			3.4			14.35
		Soil	74.3			4.3			17.28
	104.3 ppm	Shoot	46.8	0.65	0.69	2.2	0.80	0.65	21.00
		Root	67.9			3.4			19.74
		Soil	104.3			4.3			24.26
BG	30 ppm	Shoot	10.4	0.63	0.48	3.2	1.14	0.65	3.24
		Root	21.7			4.9			4.43
		Soil	34.3			4.3			7.98
	50 ppm	Shoot	24.7	0.68	0.67	3.2	1.14	0.65	7.73
		Root	36.8			4.9			7.53
		Soil	54.3			4.3			12.63
	70 ppm	Shoot	27.1	0.63	0.58	3.2	1.14	0.65	8.46
		Root	46.6			4.9			9.54
		Soil	74.3			4.3			17.28
	100 ppm	Shoot	31.8	0.53	0.57	3.2	1.14	0.65	9.93
		Root	55.6			4.9			11.38
		Soil	104.3			4.3			24.26
WT	30 ppm	Shoot	6.6	0.37	0.53	2.2	0.74	0.68	3.04
		Root	12.5			3.2			3.94
		Soil	34.3			4.3			7.98
	50 ppm	Shoot	19.3	0.56	0.64	2.2	0.74	0.68	8.91
		Root	30.2			3.2			9.48

		Soil	54.3			4.3			12.63
	70 ppm	Shoot	31.9	0.66	0.65	2.2	0.74	0.68	14.70
		Root	48.8			3.2			15.36
		Soil	74.3			4.3			17.28
	100 ppm	Shoot	38.6	0.57	0.65	2.2	0.74	0.68	17.80
		Root	59.1			3.2			18.59
		Soil	104.3			4.3			24.26

2.3.7 Bio-Concentration factor (BCF)

BCF is a useful parameter to evaluate the potential of the plants in accumulating metals, and this value was calculated on a dry weight basis. The BCF values of root and shoot of each plant were calculated separately for naturally and artificially As contaminated soil (Table 2.4). BCF was always higher in root than in shoot. The highest BCF value (2300) was recorded in barnyard grass root at 22 ppm As treatment, and lowest in water hyacinth shoot at 116 ppm As treatment (293.6) for naturally As contaminated soil (Table 2.4). In case of artificially As contaminated soil, highest BCF found in water hyacinth root (1475) at 34.3 ppm As whereas lowest in water taro shoot (440) at 34.3 ppm As concentration in the soil (Table 2.4). In all cases, BCF was 10-40 times higher than in control or uncontaminated site for shoot and root in all plants. Anwar *et al.* (2006) conducted an experiment about exposure and bioavailability of As in contaminated soils from the La Parrilla mine, Spain using *Pteridium aquilinum*, *Erica australis*, *Juncus effuses*, *Phalaris caerulescens* and *Spergula arvensis*, and measured BCF as 3.2 to 593.9 for one site and 2.1 to 20.7 for other As contaminated site. Giri and Patel (2011) also found the maximum values of BCF for Cr (VI) and Hg (II) were found to be 413.33 and 502.40 L kg^{-1}, respectively in water hyacinth where the initial concentration was 0-4 ppm Cr and 0-20 ppm Hg in hydroponic culture. These results were supported our current research. In this study, highest BCF value indicated that these plants might have the great potentiality for As phytoaccumulation in As contaminated crop land soils for future applications.

Table 2.4 BCF of shoot and root of water hyacinth (WH), barnyard grass (BG) and water taro (WT) for As accumulation in naturally and artificially As contaminated soils

Plant	Naturally As contaminated soil			Artificially As contaminated soil		
	As treatment (ppm)	Plant parts	BCF	As treatment (ppm)	Plant parts	BCF
WH	4.3 (Control)*	Shoot	38.5	34.3	Shoot	853.3
		Root	59.3		Root	1475
	22	Shoot	560.9	54.3	Shoot	708.0
		Root	1336		Root	1140
	47.3	Shoot	529.8	74.3	Shoot	991.4
		Root	1174		Root	1410
	116	Shoot	293.6	104.3	Shoot	936.6
		Root	784.5		Root	1358
BG	4.3 (Control)*	Shoot	55.2	34.3	Shoot	691.3
		Root	84.3		Root	1445
	22	Shoot	1400	54.3	Shoot	989.2
		Root	2300		Root	1473
	47.3	Shoot	730.2	74.3	Shoot	773.4
		Root	1356		Root	1332
	116	Shoot	456.9	104.3	Shoot	635.4
		Root	981.6		Root	1113
WT	4.3 (Control)*	Shoot	37.4	34.3	Shoot	440.0
		Root	54.8		Root	836.0
	22	Shoot	1206	54.3	Shoot	773.2
		Root	2185		Root	1206
	47.3	Shoot	641.4	74.3	Shoot	911.4
		Root	1422		Root	1395
	116	Shoot	340.9	104.3	Shoot	772.6
		Root	800.0		Root	1182

*Control is both for naturally and artificially contaminated soils.

2.4 Conclusions

Water hyacinth, barnyard grass and water taro were efficient for phytoaccumulation of As in contaminated soils. These plants showed good growth parameter like height, leaves and biomass production up to 25-30 ppm As concentration in the soil, and then they were gradually decreased. The highest recovery was recorded in water hyacinth due to higher biomass production. The weather of Bangladesh is very suitable to grow these plants spontaneously in moist and submersed soil condition, so this plant might be considered for cleaning up As contaminated surface soils in Bangladesh. From values of EF, TF and BCF, it can be concluded that accumulation of As in roots was always higher than that in shoots; however, the plant was easily uprooted during moist or submersed soil condition. So all of these are suitable for As phytoaccumulation in crop land soil, and have the great potentiality for future applications as an As accumulator in the As contaminated area.

References

Alvarado, S., Guedez, M., Lue-Meru, M.P., Nelson, G., Alvaro, A., Jesus, A.C., Gyula, Z., 2008. Arsenic removal from waters by bioremediation with the aquatic plants water hyacinth (*Eichhornia crassipes*) and lesser duckweed (*Lemna minor*). Bioresour Technol. 99,8436–8440.

Anawar, H.M., Sanchez, A.C., Murciego, A., Buyolo, T., 2006. Exposure and bioavailability of arsenic in contaminated soils from the La Parrilla mine, Spain. Environ Geol. 50,170-179.

Arif, M.I., 2001. Arsenic in food chain due to arsenic contaminated groundwater, MS Thesis, Department of Agricultural Chemistry, BAU, Mymensingh, Bangladesh.

Baldwin, P.R., Butcher, D.J., 2007. Phytoremediation of arsenic by two hyperaccumulators in a hydroponic environment. Microchemical J. 85, 297-300.

Barman, S.C., Bhargava, S.K., 1997. Accumulation of heavy metals in soil and plants in industrially polluted fields, (In Cheremissinoff, P.N. (Ed.), Ecological issues and Environmental Impact Assessment, Gulf Publishing Company, Houston, Texas, USA. pp. 289-314.

Barman, S.C., Sahu, R.K., Bhargava, S.K., Chatterjee, C., 2000. Distribution of heavy metals in wheat, mustard and weed grains irrigated with industrial effluents. Bull Environ Contam Toxicol. 64, 489-496.

Bindu, T., Sumi, A.M.M., Ramasamy, A.E.V., 2010. Decontamination of water polluted by heavy metals with Taro (*Colocasia esculenta*) cultured in a hydroponic NFT system. Environmnetalist, 30, 35-44.

Bruce, S.L., Noller, B.N., Grigg, A.H., Mullen, B.F., Mulligan, D.R., Ritchie, P.J., Currey, N., Ng, J.C., 2003. A field study conducted at Kidston gold mine, to evaluate the

impact of arsenic and zinc from mine tailing to grazing cattle. Toxicol Lett. 137, 23–34.

De Koe, T., 1994. *Agrostic castellana* and *Agrostis delicatula* on heavy metal and arsenic enriched sites in NE Portugal. Sci Total Environ. 145, 103–109.

Dhankher, O.P., Li, Y., Rosen, B.P., Shi, J., Salt, D., Senecoff, J.F., Sashti, N.A., Meagher, R.B., 2002. Engineering tolerance and hyperaccumulation of arsenic in plants by combining arsenate reductase andγ-glutamylcysteine synthetase expression. Nature Biotechnol. 20, 1140 – 1145.

Duxbury, J., Mayer, A., Lauren, J., Hassan, N., 2002. Food chain aspects of arsenic contamination in Bangladesh: effects on quality and productivity of rice. J Environ Sci Health A Toxic/Hazard Subs Environ Eng. 38, 61–69.

Ebel, M., Evangelou, M.W.H., Schaeffer, A., 2007. Cyanide phytoremediation by water hyacinths (*Eichhornia crassipes*). Chemosphere 66, 816–823.

Giraldo, E., Garzon, A., 2002. The potential for water hyacinth to improve the quality of Bogota River water in the Muna reservoir: comparison with the performance of waste stabilization ponds. Water Sci Technol. 45, 103–110.

Giri, A.K., Patel, R.K., 2011. Toxicity and bioaccumulation potential of Cr (VI) and Hg (II) on differential concentration by *Eichhornia crassipes* in hydroponic culture. Water Sci Technol. 63, 899-907.

Gomez, K.A., Gomez, A.A., 1984. Statistical Procedures for Agricultural Research. 2nd edn. John Wiley and Sons. New York, pp. 680.

Gulz, P.A., Gupta, S.K., Schulin, R., 2005. Arsenic accumulation of common plants from contaminated soils. Plant soil, 272, 337-347.

Gupta, S., Nayek, S., Saha, R.N., Satpati, S., 2008. Assessment of heavy metal accumulation in macrophyte, agricultural soil and crop plants adjacent to discharge zone of sponge iron factory. Environ Geol. 55, 731-739.

Hoffmann, T., Kutter, C., Santamaria, J., 2004. Capacity of Salvinia minima Baker to tolerate and accumulate As and Pb. Eng Life Sci. 4, 61–65.

Hossain, M.M., Sattar, M.A., Hashem, M.A., Islam, M.R., 2001. Arsenic contamination in some selected soils of Bangladesh. Online J Biol Sci. 1, 989-992.

Jack, C.N., 2005. Environmental contamination of arsenic and its toxicological impact on humans. Environ Chem. 2, 146–160.

Kisku, G.C., Barman, S.C., Bhargava, S.K., 2000. Contamination of soil and plants with potentially toxic elements irrigated with mixed industrial effluent and its impact on the environment. Water Air Soil Pollut. 120, 121-137.

Kris, C., 2001. Chickens, manure, and arsenic *Environ Sci Technol 35*(9): 184A–185A.

Ma, L.Q., Komar, K.M., Tu, C., Zhang, W., Cai, Y., Kennelley, E.D., 2001. A fern that hyperaccumulates arsenic. Nature, 409, 579.

Mandal, B.K., Chowdhury, T.R., Samanta, G., Basu, G., Chowdhury, P.P., Chanda, R., et al., 1996. Arsenic in groundwater in seven districts of West Bengal, India – The biggest arsenic calamity in the world. Curr Sci.70, 976–986.

Marin, A.R., Masscheleyn, P.H., Patrick, W.H., 1992. The influence of chemical form and concentration of arsenic on rice growth and tissue arsenic concentration. Plant Soil, 139, 175-183.

Mishra, V., Upadhyay, A., Pathak, V., Tripathi, B., 2008. Phytoremediation of mercury and arsenic from tropical opencast coalmine effluent through naturally occurring aquatic macrophytes. Water Air Soil Pollut. 192, 303–314.

Mitra, N., 2004. Phytoremediation of arsenic contaminated soils by naturally grown weeds, M.S. Thesis, Department of Agricultural Chemistry, Bangladesh Agricultural University, Mymensingh, Bangladesh.

Meharg, A.A., Hartley-Whitaker, J., 2002. Arsenic uptake and metabolism in arsenic resistant and nonresistant plant species. New Phytol. 154, 29–43.

Mishra, V.K., Tripathi, B.D., 2008. Concurrent removal and accumulation of heavy metals by the three aquatic macrophytes. Bioresource Technol. 99, 7091–7097.

Mishra, V.K., Tripathi, B.D., Kim, K.H., 2009. Removal and accumulation of mercury by aquatic macrophytes from an open cast coal mine effluent. J Hazard Mater. 172, 749–754.

Mufarrege, M.M., Hadad, H.A., Maine, M.A., 2010. Response of *Pistia stratiotes* to heavy metals (Cr, Ni, and Zn) and phosphorous. Arch Environ Contam Toxicol. 58, 53–61.

Murakami, M., Ae, N., 2009. 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.

Onken, B.M., Hossner, L.R., 1996. Determination of arsenic species in soil solution under flooded conditions. Soil Sci Soc Am J. 60, 1385–1392.

Paiva, B.L., de Oliveira, J.G., Azevedo, R.A., Ribeiro, D.R., da Silva, M.G., Vito´ria, A.P., 2009. Ecophysiological responses of water hyacinth exposed to Cr^{3+} and Cr^{6+}. Environ Exp Botany, 65, 403–409.

Petrick, J.S., Ayala-Fierro, F., Cullen, W.R., Carter, D.E., Vasken Aposhian H., 2000. Monomethylarsonous acid (MMA-III) is more toxic than arsenite in change human hepatocytes. Toxicol Appl Pharmacol. 163, 203–207.

Pickering, I.J., Prince, R.C., George, M.J., Smith, R.D., George, G.N., Salt, D.E., 2000. Reduction and coordination of arsenic in Indian Mustard. Plant Physiol. 122, 1171-1178.

Poschenrieder, B.J.C., Llugany, M.J., Tume, B.P., Toloias, F.J., 1997. As and heavy metal contamination of soil and vegetation around a copper mine in Northern Peru. Sci Total Environ. 203, 83–91.

Raab, A., Schat, H., Meharg, A.A., Feldmann, J., 2005. Uptake, translocation and transformation of arsenate and arsenite in sunflower (*Helianthus annuus*)–Part 1: formation of arsenic-phytochelatin complexes during exposure to high arsenic concentrations. New Phytol. 168, 551-558.

Raab, A., Willium, N.P., Meharg, A.A., Feldmann, J., 2007. Uptake and translocation of inorganic and methylated arsenic species by plants. Environ Chem. 4, 197-203.

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

Rahman, M.A., Hasegawa, H., Ueda, K., Maki, T., Okumura, C., Rahman, M.M., 2007. Arsenic accumulation in duckweed (*Spirodela polyrhiza* L.): A good option for phytoremediation. Chemosphere, 69, 493–499.

Rahman, M.A., Hasegawa, H., Ueda, K., Maki, T., Rahman, M.M., 2008. Influence of EDTA and chemical species on arsenic accumulation in *Spirodela polyrhiza* L. (duckweed). Ecotoxicol Environ Safety. 70, 311–318.

Robinson, B., Kim, N., Marchetti, M., Moni, C., Schroeter, L., van den Dijssel, C., Milne, G., Clothier, B., 2006. Arsenic hyperaccumulation by aquatic macrophytes in the Taupo Volcanic Zone, New Zealand. Environ Exp Botany, 58, 206–215.

Singh, R., Singh, D.P., Kumar, N., Bhargava, S.K., Barman, S.C., 2010. Accumulation and translocation of heavy metals in soil and plants from fly ash contaminated area. J Environ Biol. 31, 421-430.

Smith, A.H., Lingas, E.O., Rahman, M., 2000. Bull. World Health Organization 78, 1093.

Snyder, K.V.W., 2006. Removal of Arsenic from Drinking Water by Water Hyacinths (*Eichhornia crassipes*). *J. U.S. S J W P, Volume 1*.

SOS-arsenic.net. 2005. 35 million (3.5 crore) people facing arsenic contamination-Poor Mitigation Activities**.** http://www.sos-arsenic.net/english/latest.html.

Sultana, R., Kobayashi, K., 2011. Potential of barnyard grass to remediate arsenic-contaminated soil. Weed Biol Manage. 11, 12–17.

Sultana, R., Zaman, M.W., Islam, S.M.N., 2006. Interaction of arsenic with other elements in arsenic hyperaccumulating weeds. J Bangladesh Agricul Univ. 4, 211-217.

The Daily Amar Desh, 2011. *Nirapod panir obave Arsenic atonke Nalitabarir ordhek jonogosti*-(Crisis of safe drinking water among the half population of Nalitabari upazila due to Arsenic contamination), The daily Bengali newspaper of Bangladesh. Amar Bangla Page (2nd December 2011).

Tchounwou, P.B., Patlolla, A.K., Centeno, J.A., 2003. Carcinogenic and systemic health effects associated with arsenic exposure-a critical review. Toxicol Pathol. 31, 575-588.

Tlustos, P., Balik, J., Szakova, J., Pavlikova, D., 1998. The accumulation of arsenic in radish biomass when different forms of arsenic were applied to the soil. Rostlinna Vyroba Czech Univ of Agril. 44(1), 7-13.

Tu, C., Ma, L.Q., Bondada, B., 2002. Arsenic accumulation in the hyperaccumulator Chinese brake and its utilization potential for phytoremediation. Environ Qual. 31, 1671–1675.

Vamerali, T., Bandiera, M., Mosca, G., 2010. Field crops for phytoremediation of metal-contaminated land: A review. Environ Chem Lett. 8, 1–17.

Welsch, F.P., Crock, J.G., Sanzolone, R., 1990. Trace level determination of arsenic and selenium using continuous-flow hydride generator atomic absorption spectrophotometry (HG-AAS). *In*. Arbogast BF (ed.). Quality assurance manual for the Branch of Geochemistry, US Geological Survey. USA, pp. 38-45.

WHO (World Health Organization). 1999. Arsenic in drinking water URL: http/www. who. Int/inf-fs/en/fact. 210. Hotmai1.

Williams, P.N., Islam, M.R., Adomako, E.E., Raab, A., Hossain, S.A., Zhu, Y.G., Feldmann, J., Meharg, A.A., 2006. Increase in rice grain arsenic for regions of Bangladesh irrigating paddies with elevated arsenic in groundwaters. Environ Sci Technol. 40, 4903–4908.

Zaman, M.W., Shariful, I.M., Mokhlesur, R.M., 2008. Remediation of arsenic contaminated soils by naturally grown weeds. J Agrofor Environ. 2(1), 123-126.

Zhu, Y.G., Sun, G.X., Lei, M., Teng, M., Liu, Y.X., Chen, N.C., Wang, L.H., Carey, A.M., Deacon, C., Raab, A. *et al*., 2008. High percentage inorganic arsenic content of mining impacted and nonimpacted Chinese rice. Environ Sci Technol. 42, 5008–5013.

Zhu, Y.G., Rosen, B., 2009. Perspective for genetic engineering for the Phytoremediation of arsenic-contaminated environments: from imagination to reality? Current Opin Biotechnol. 20, 220-224.

Chapter III: Phytofiltration of arsenic and cadmium from the water environment using *Micranthemum umbrosum* (J.F. Gmel) S.F. Blake as a hyperaccumulator

Abstract

Arsenic (As) and cadmium (Cd) pollution in water is an important global issue. Phytofiltration is an eco-friendly technology that helps clean up pollutants using ornamental plants, such as *Micranthemum umbrosum* (J.F. Gmel) S.F. Blake. After a seven-day hydroponic experiment, *M. umbrosum* removed 79.3–89.5% As and 60–73.1% Cd from 0 to 1.0 µg As mL^{-1} and 0.3 to 30.0 µg Cd mL^{-1} solutions, respectively. For As treatment, root to stem and stem to leaf translocation factors greater than 1.0 indicated that accumulation of As in leaves was large compared to that in stem and roots. However, the accumulation of Cd in roots was higher than that in the leaves and stem. In addition, *M. umbrosum* completely removed Cd within three days from 0.38 to around 0 µg mL^{-1}Cd in the solution when the plant was exchanged daily. Bio-concentration factors (2350 for As and 3027 for Cd) for *M. umbrosum* were higher than for other As and Cd phytoremediators. The results show that *M. umbrosum* can be an effective accumulator of Cd and a hyper-accumulator of As, as it can lower As toxicity to a level close to the limit recommended by the World Health Organization (0.01 µg As mL^{-1}).

3.1 Introduction

Arsenic (As) and cadmium (Cd) are the most toxic and carcinogenic substances among all of the possible xenobiotics (USEPA-IRIS, 2010), occurring naturally or as a result of anthropogenic influences, and pose a serious threat to environmental and human health worldwide. Contamination in drinking water has been recognized as a serious global problem. For example, As threatened the health of more than 80 million people in Bangladesh (Smith *et al.*, 2000) and West Bengal, India (Nordstrom, 2002). Studies have shown that As also enters the food chain via crop uptake from soils contaminated by

As-contaminated irrigation water or mining activities (Williams *et al.*, 2006; Zhu *et al*., 2008). In Bangladesh, concentrations of As in well water were found to be high, ranging from less than 1 µg L^{-1} to more than 300 µg L^{-1} (Smith *et al*., 2000), whereas the As standard for drinking water is 10 µg L^{-1} (WHO, 2011). The International Program on Chemical Safety (IPCS, 2001) reported that long-term exposure to As in drinking water increased the risk of cancer in the skin, lung, bladder and kidney, as well as other skin changes such as hyperkeratosis and changes of dermal pigmentation.

Cd and its compounds are used in the steel industry, in plastic and batteries, and are released to the environment through disposal of mining or industrial effluents, wastewater and often from fertilizers. It causes kidney damage, osteomelacia, osteoporosis, and itai itai disease, and has carcinogenic effects (WHO, 2011). Langner *et al*., (2012) reported that Cd concentration of sediments in the Upper Clark Fork River, Montana, USA showed 4.4 (range 0.6–6.9) mg kg^{-1}. In addition, Marine black shale's and slates have frequently been found to contain anomalously high concentrations of Cd (<240 mg/kg) (OECD, 1994). In potable water in Saudi Arabia, 1–26 µg L^{-1} Cd was reported (Mustafa *et al.*, 1988) and the maximum value recorded was 100 µg L^{-1} in the Rio Rimao in Peru (WHO/UNEP, 1989), whereas the maximum Cd tolerance level in water is only 3 µg L^{-1} (WHO, 2011). As and Cd are classified as Group 1 and Group 2A carcinogenic compounds to humans, respectively (IARC, 1987). Therefore, remediation of As and Cd from water and soil is an important global issue. Among various technologies such as precipitation, membrane filtration, adsorption, ion exchange, permeable reactive barriers, biological treatment and phytoremediation (Rahman *et al*., 2011), phytofiltration is a type of phytoremediation and is an emerging, eco-friendly technology in which green plants are used to remediate or remove metals from contaminated water (Dushenkov and Kapulnik, 2000). Several studies have focused on phytoremediation of heavy metals from water and soil; however, few

plants showed the ability to translocate high amounts of As from root to shoot (Rahman *et al*., 2011). Raab *et al*. (2007) studied 46 different plant species in terms of As accumulation, and found that translocation factors of these plants never exceeded 0.9 for As(V). The fact that their translocation factors were less than 1 indicated partial immobilization of As in their roots and a low conveyance of As to the shoots. This immobilization reduces the phytoavailability of contaminants from the environment (Vamerali *et al*., 2010). *Pteris vittata* L. has shown the highest ability to accumulate and translocate As from root to shoot (Ma *et al*., 2001). *Spirodela polyrhiza* L. (Rahman *et al*., 2007), *Lemna gibba* L. (Mkandawire and Dudel, 2005), *Polygonum hydropiper* L. (Robinson *et al*., 2005), and *Azolla caroliniana* L. (Zhang *et al*., 2008) were also identified as As accumulators. In addition, *Nymphaea aurora* L. (Schor-Fumbarov *et al*., 2003), *Solanum nigrum* L. (Sun *et al*., 2007), *Thlaspi caerulescens* J. & C. Presl. (Zhao *et al*., 2003), and *Arabidopsis halleri* L. (Küpper *et al*., 2000) were recognized as Cd accumulators. However, these plants have low bio-concentration factors and low root to shoot translocation factors. This indicates the difficulty in employing these plants for As and Cd phytoremediation at a field scale. Therefore, it is necessary to identify plants having high bio-concentration factors and translocation factors (i.e., greater than 1) that can remove As and Cd from contaminated drinking water to levels below the tolerable limit.

Micranthemum umbrosum (J.F. Gmel) S.F. Blake, commonly known as Water fern, Baby's tears, or Pearl grass, belongs to the family Linderniaceae, and it is widely used as an aquarium ornamental plant. In this study, this plant was employed to remediate As and Cd contaminated water for the following reasons: i) the whole plant can be easily removed from a water environment; ii) its growth rate is high and relatively vigorous; iii) it grows under submerged conditions; iv) its light requirement for growth is moderate; and v) It can

be used as ornamentation for room in addition to accumulation of As and Cd from water as it is popular as an aquarium plant. There is currently no data regarding phyoremediation of As and Cd using *M. umbrosum*. In this study, to understand whether *M. umbrosum* would be a good candidate for phytoremediation, metal accumulation pattern in *M. umbrosum* grown in water including As or Cd was investigated. In addition, As and Cd bio-concentration factors and translocation factors of *M. umbrosum* were determined.

3.2 Materials and Methods

3.2.1 Plant and Culture Conditions

M. umbrosum (J.F. Gmel) S.F. Blake, was obtained from Aqua Friend Hokusui (Hokkaido, Japan). Initially, the plants were acclimated for 1 week in water containing plant nutrients (contains some essential trace elements and potassium, Aqua Design Amano, Nigata, Japan) under laboratory conditions, to allow for adaptation prior to the experiment. Then, *M. umbrosum* was grown in glass pots (volume: 765.45 cm^3) in Milli-Q water (Millipore-Gradient A10, Milli-Q Gradient ZMQG) containing 0.2, 0.45, and 1.0 μg mL^{-1} As (as $NaAsO_2$), or 0.3, 3.0, and 30.0 μg mL^{-1} Cd solutions (as $CdCl_2.2.5H_2O$). The exposure experiments were carried out for 7 days under the following conditions: 14:10 h light/dark cycle, 100–125 μmol m^{-2} s^{-1} light intensity, and 75% humidity at 21±1°C. The pH value of the solutions was maintained at 6.8. After every 24 h, water samples were collected from each pot to measure the As and Cd concentrations in the water. Each experiment was performed thrice. Milli-Q water containing adequate concentrations of As and Cd was added daily to compensate for the water loss due to plant transpiration and evaporation. After 7 days, the plants were harvested, rinsed four times with Milli-Q water, and then placed on clean absorbent paper for water removal from the plant surface. The plants were then separated into root, stem, and leaf for measurement of the metal accumulation, bio-concentration factors, and translocation factor in each component.

3.2.2 Metal Analysis

After harvesting the plants, the whole plants were washed by Milli-Q water for three times then the roots, stems and leaves were separated and placed on paper and air dried on the plastic table under room temperature for 24 h. The As treated samples were dried for 48 h in an oven at 65°C (Constant Temperature Oven, DKN602, Yamato Scientific Co. Ltd., Japan) until they reached a constant weight. Dried samples were weighed on a digital balance (A&D Co. Ltd, Japan, HF-200, Max 210 g, d = 0.001 g). After cutting the samples, 20–40 mg samples of root, stem, or leaf were separately placed into 15-mL polyethylene tubes (Thermo Fisher Scientific, NY). Two mL of 65% HNO_3 (Wako Pure Chemical Ind. Ltd., Japan) was added, and the samples were kept under the fume hood for 12 h. Then, the samples were heated on a heating block (TAH-2G, Dry Thermo Unit, Japan) using lids at 95°C for 2 h to digest. After cooling, 1 mL of 30% H_2O_2 (Wako Pure Chemical Ind. Ltd., Japan) was added, and the samples were heated again at 105°C for 20 min (Rahman *et al*., 2007). Digested samples were diluted up to 10 mL with Milli-Q water using 10-mL volumetric flasks (Pyrex, IWAKI Glass), as described by Cai *et al*. (2000) and Rahman *et al*. (2007). To digest the wet-weighed Cd-treated samples, they were treated with 2 mL of 68% HNO_3 (Walko Pure Chemical Ind. Ltd., Japan), and subsequently heated at 110°C for 2 h. The digested samples were diluted up to 10 mL with Milli-Q water using 10-mL volumetric flasks. Both sets of diluted samples were then filtered using a 0.45-µm syringe-driven filter unit (Millipore, Billerica, USA) and stored in 15-mL polyethylene bottles. The As and Cd contents were measured using an inductively coupled plasma-mass spectrophotometer (ICP-MS; SPQ 6500, Plasma Quadrupole Mass Analyser, SII-Seiko Instrument, Japan) and a flame-type atomic absorption spectrophotometer (AAS; model 180-80, Hitachi, Japan), respectively. The accuracy of the analysis was checked by the use of certified standard reference material for As (013-15481, Lot ALK 9912, 1000 ppm) and

Cd (036-16171, Lot TSP9842, 1000 ppm) obtained from Wako Pure Chemical Ind. Ltd., Japan. The results were expressed as µg g^{-1} dry weight for As and µg g^{-1} wet weight for Cd in root, stem, and leaf.

3.2.3 Bio-concentration Factor (BCF)

The BCF was determined as an index of the plant's ability to accumulate a metal with respect to the metal concentration in the substrate. The BCF was calculated (L kg^{-1}) as follows (Snyder, 2006):

BCF = As in the plant component (root, stem, or leaf) (mg kg^{-1})/As in the substrate water (mg L^{-1})

3.2.4 Translocation Factor (TF)

The TF was calculated to determine the relative translocation of metals from the water to the various plant components (root, stem, and leaf) (Barman *et al*., 2000; Gupta *et al.*, 2008).

TF = Concentration of As in plant tissue (root, stem, or leaf)/Concentration of As in corresponding water or root

3.2.5 Statistical Analysis

The mean, standard deviation (SD) and standard error of mean (SEM) were calculated, and *t*-test was performed to determine any significant differences among treatments at the 0.1%, 1%, and 5% levels using the Microsoft Excel-2007 program.

3.3 Results and Discussions

3.3.1 Phytofiltration of As from Water

As concentration in the solution decreased with increasing time, and *M. umbrosum* significantly removed (when compared to the previous day) As up to the third, sixth, and fourth day from the 0.2, 0.45, and 1 µg As mL^{-1} solutions, respectively (Fig. 3.1). For the 0.2 and 0.45 µg mL^{-1} As solutions, the water contained only 0.041 (Fig. 3.1a) and

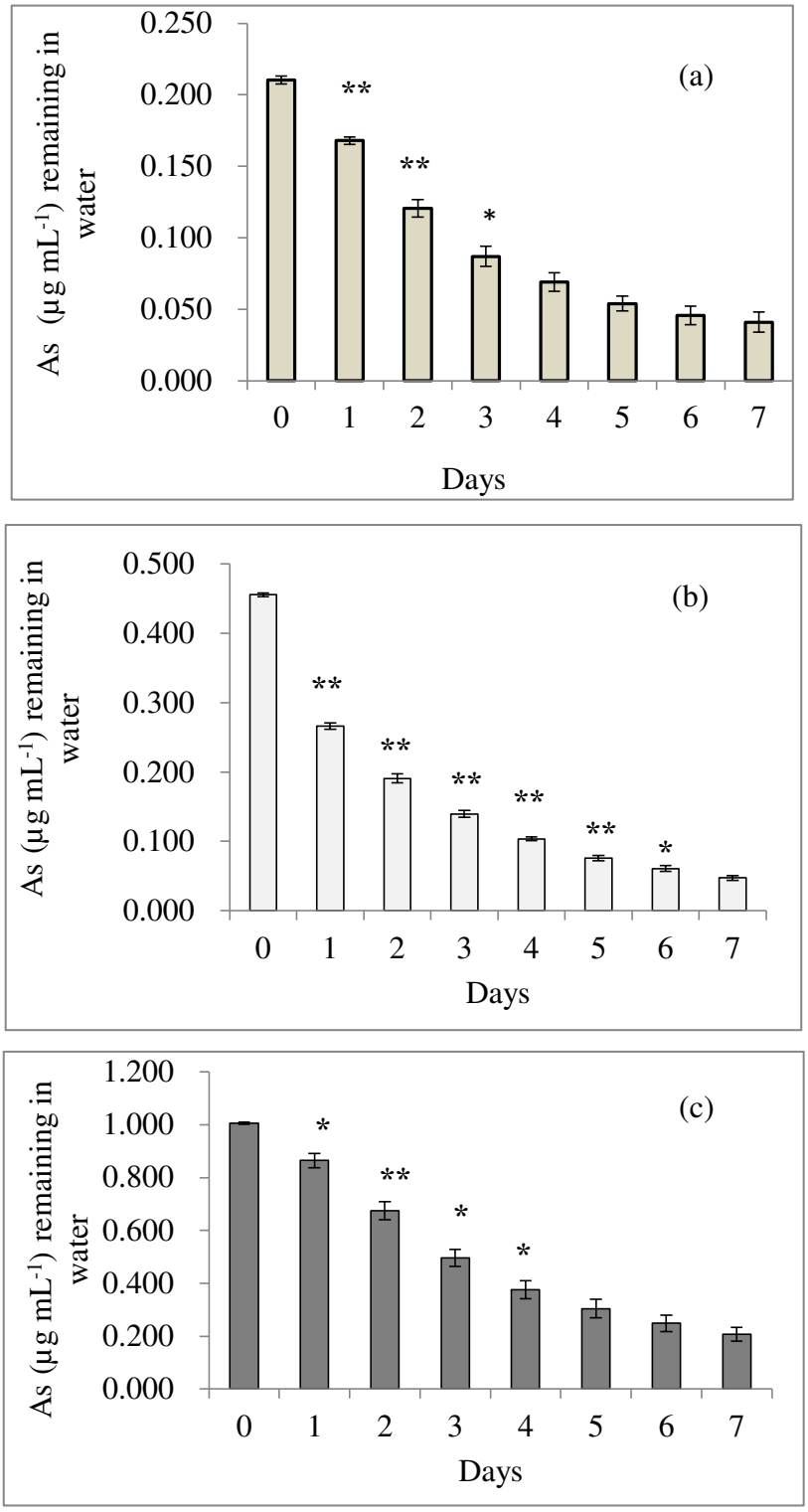


Fig. 3.1 Remaining As (µg mL^{-1}) in water in which *M. umbrosum* was grown with 0.2 (a), 0.45 (b), and 1.0 (c) µg As mL^{-1}. Error bar indicates mean ± S.E.M. (n = 3). ** and *denote significant differences at $P < 0.01$ and 0.05, respectively, compared to previous days.

0.047 µg mL^{-1} As (Fig. 3.1b), respectively, after seven days of growing *M. umbrosum*. However, an As concentration of 0.207 µg mL^{-1} was observed in the water when the initial As concentration was 1.006 µg mL^{-1} (Fig. 3.1c). In addition, As concentration remained constant in the control treatment without plants (data not shown). Therefore, at lower initial concentrations (0.2 and 0.45 µg As mL^{-1}), *M. umbrosum* removed As from the water to achieve a final concentration below the maximum level (0.05 µg mL^{-1}) prescribed by the Bangladesh Government (World Bank, 2005). As listed in Table 3.1, the plant removed As from the water solution to differing extents as the As concentration was increased (80.5, 89.6, and 79.3% As were removed from water containing 0.2, 0.45, and 1.0 µg As mL^{-1}, respectively). This tendency might be due to As inhibiting growth of the plant at a concentration of 1 µg As mL^{-1} since at 1.8 µg mL^{-1} As, the plant died (data not shown). Growth of *Wolffia globosa* was also significantly inhibited ($P < 0.001$) by arsenate at more than 30 µM concentration and by arsenite at more than 10 µM concentration, and it decreased total As concentration in the solution from 200 to 116 µg L^{-1} within 48 h (Zhang *et al*., 2009).

3.3.2 As Accumulation in Plant Material

As accumulation patterns in the root, stem, and leaf of *M. umbrosum* 7 days after incubation are shown in Fig. 3.2. The leaf component took up significantly ($P < 0.001$ and 0.005) higher amounts of As than the corresponding stem and root components. The As accretion patterns from contaminated water to root, root to stem, and stem to leaf showed high accumulation for each treatment (Fig. 3.2). Leaf and stem contained 1179.3±11.6 and 1001±16.5 µg As g^{-1} (dry wt. basis) at the 1 µg As mL^{-1} dose, whereas 802±18.7 and 470±14.5 µg As g^{-1} was accumulated in leaves at the 0.45 and 0.2 µg As mL^{-1} doses, respectively (Fig. 3.2). These results are consistent with studies of Zhang *et al*., (2009) who reported that *Wolffia globosa* accumulated 1057±61 mg As kg^{-1} dry weight after 7

days growth in 15 µM As solution. Rahman *et al*. (2007) also showed that *Spirodela polyrhiza* took up 0.353±0.003 µmol As g^{-1} dry weight 6 days after exposure to 4 µM As. However, compared to these previous studies, the plant used in this study took up much more As from the As-contaminated water. Therefore, *M. umbrosum* has a high potential for As remediation from contaminated drinking water.

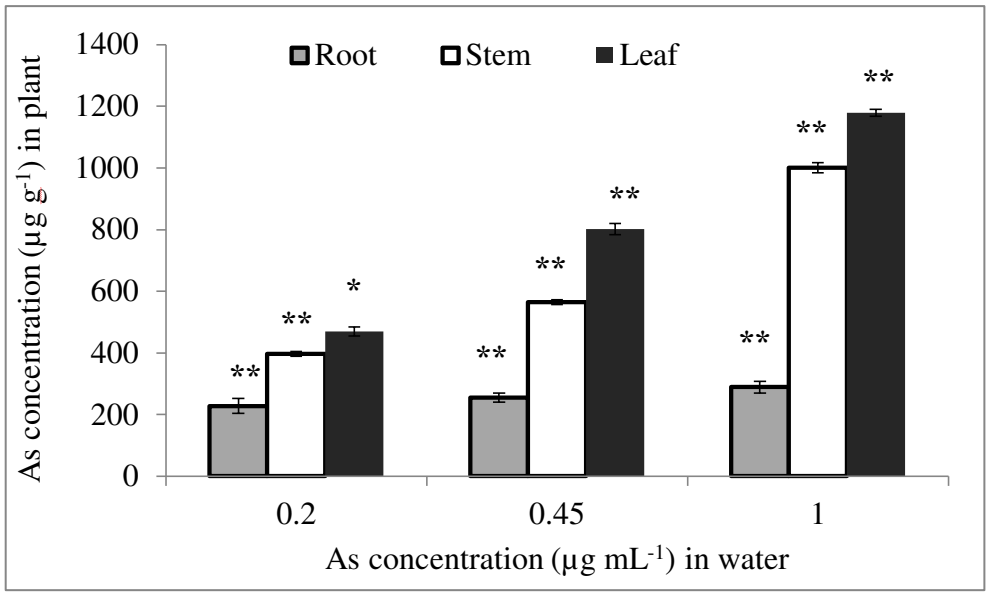


Fig. 3.2 As accumulation in root, stem and leaf of *M. umbrosum* seven days after exposure to 0.2, 0.45, and 1.0 µg As mL^{-1} water. Error bars indicate mean ± S.E.M. (n = 3). ** and * denote significant differences at $P < 0.001$ and 0.005, respectively, compared to As from water to root, root to stem, and stem to leaf.

3.3.3 Phytofiltration of Cd from Water

Cd concentrations in the water were detected according to the time-dependent manner in which plants were grown (Fig. 3.3). The Cd concentrations in the water gradually decreased day by day. The pattern of Cd decrease was similar across the 0.3, 3, and 30 µg Cd mL^{-1} treatments (Fig. 3.3). The rate of Cd concentration decrease was observed to be largest on the first day with a strongly significant difference observed in the 0.3 µg Cd mL^{-1} treatment ($P < 0.05$) (Fig. 3.3a). The rate of decrease exponentially declined day by day. As shown in Fig. 3.3, at 0.3, 3, and 30 µg Cd mL^{-1} concentrations, *M. umbrosum*

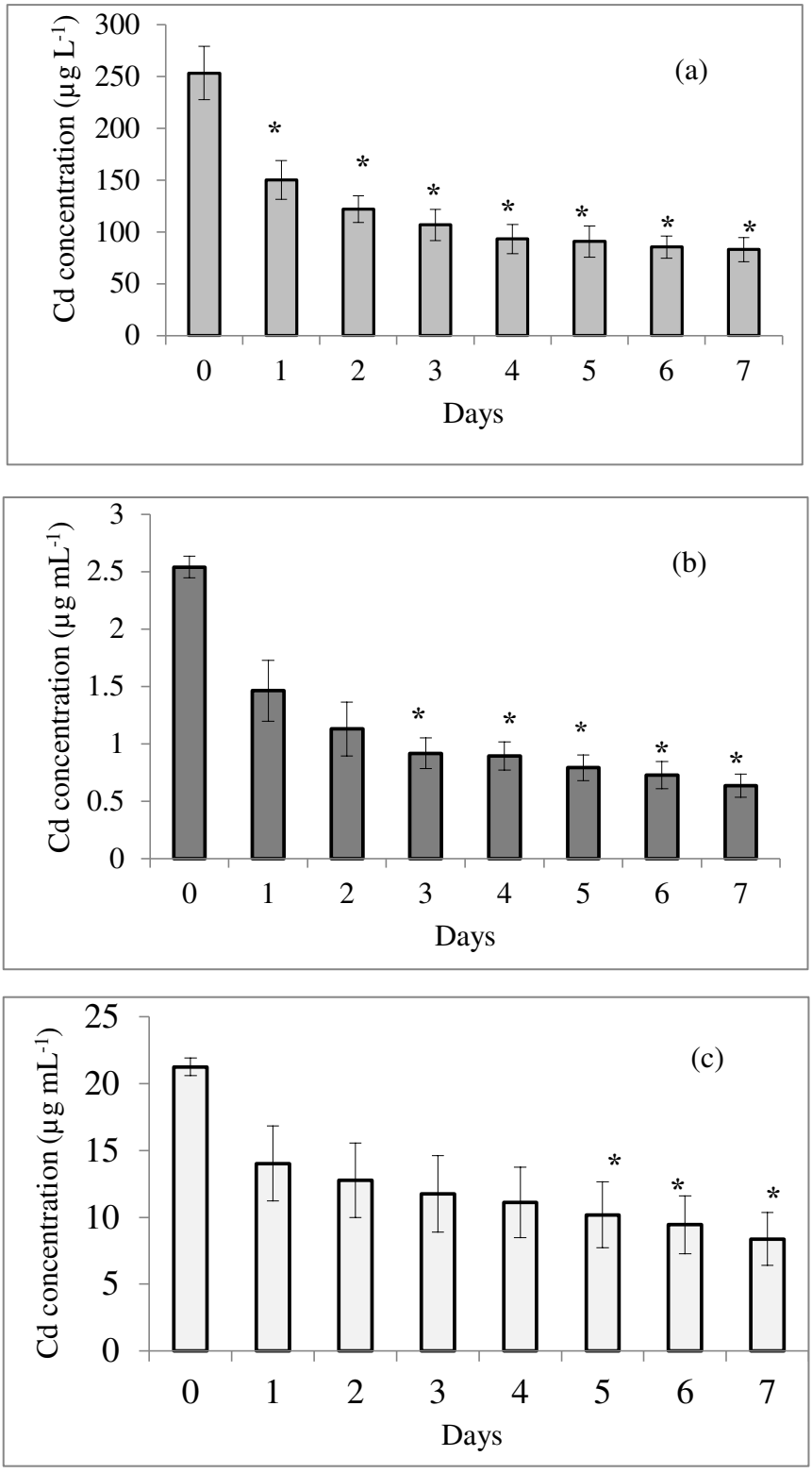


Fig. 3.3 Remaining Cd in water in which *M. umbrosum* was grown with 0.3 (a), 3.0 (b), and 30 (c) µg Cd mL^{-1}. Error bar indicates mean ± S.E.M. (n = 3). * denotes significant differences at $P < 0.05$, compared to day 0.

could not completely remove Cd from the solution. Therefore, the plants were replaced with new ones each day and Cd concentration in the water was measured. Under these conditions, it was observed that when initial Cd concentration in the water was 0.38 µg mL^{-1}, Cd in the water was completely remediated after three days (data not shown). Abhilash *et al*. (2009) conducted an experiment using *Limnocharis flava L.* grown in 0.5, 1, 2, and 4 mg Cd L^{-1} solutions, and found that after 30 days, more than 93% of the Cd was removed. However, here, *M. umbrosum* can remove around 100% of the Cd within 3 days by replacing the plants with new ones each day. When the plants were not replaced, 70.4, 73.1, and 60% Cd were removed after 7 days from the 0.3, 3.0, and 30 µg Cd mL^{-1} solutions, respectively (Fig. 3.3).

3.3.4 Cd Accumulation in the Plant

Cd accumulation in the leaves, stem, and roots of *M. umbrosum* is shown in Fig. 3.4. The amount of Cd accumulation in the plant components was in the following order: roots>leaves>stems. Cd accumulation in each component was significantly increased by the increase in Cd levels in the hydroponic solution (Fig. 3.4). In the case of 30 µg Cd mL^{-1} exposure, the Cd contents in the roots (13296.2±1962.6 µg g^{-1} wet weight) were higher than those in the corresponding stems (3377.7±208.0 µg g^{-1} wet weight) and leaves (4491.4±300.3 µg g^{-1} wet weight). The accumulation of Cd in the various parts of aquatic macrophytes under laboratory conditions has been reported in several species of aquatic plants such as *Limnocharis flava* (Abhilash *et al*., 2009), *Ipomea auqatica* (Wang *et al.*, 2008), *Potamogeton natans* (Fritioff and Greger, 2006), *Lemna minor* (Hou *et al*., 2007), and *Elodea canadensis* (Fritioff and Greger, 2007). Cd concentrations were reported to be higher in the roots in most of these studies. The high Cd concentrations in the roots of *M. umbrosum* were because of the numerous fibrous roots of this plant, as mentioned by Abhilash *et al.* (2009) for *Lamna flava*. Similarly, with 3 and 0.3 µg Cd mL^{-1} exposure, Cd

contents in the root were also slightly higher than those in the stem and leaf (Fig. 3.4). However, in the case of treatment with different As concentrations, roots contained lower amounts of As as compared with stems and leaves. The reason for differing accumulation of Cd and As in the plant components is still unclear; a possible reason could be the usage of different uptake and translocation mechanisms for As and Cd (Schiorup and Larsen, 1981).

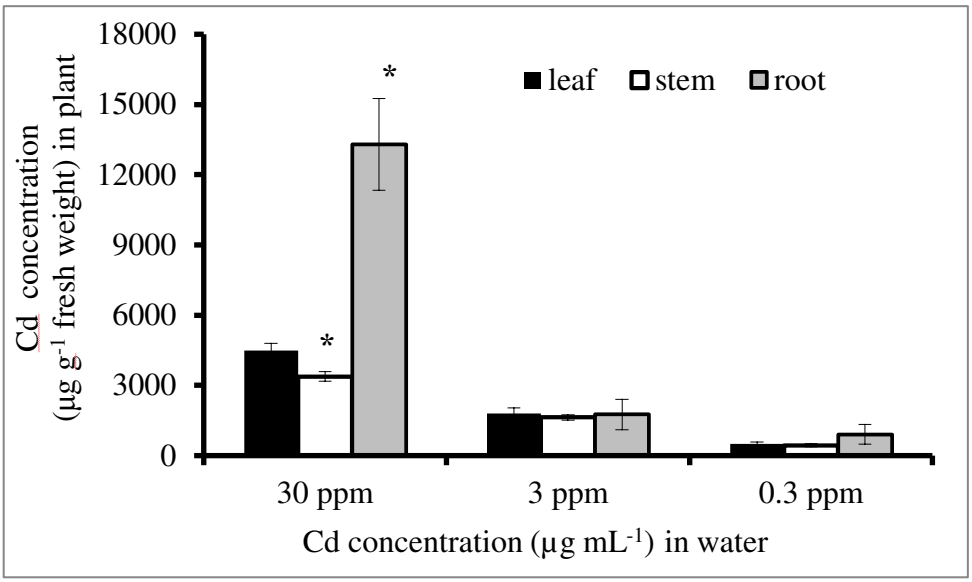


Fig. 3.4 Cd accumulation in leaf, stem and root of *M. umbrosum* seven days after exposure to 30, 3.0, and 0.3 µg Cd mL^{-1} water. Error bars indicate mean ± S.E.M. (n = 3). * denotes significant differences at $P < 0.05$, compared to Cd from root to stem.

3.3.5 BCF of As and Cd in *M. umbrosuum*

BCF is defined as the ratio of metal concentration in the plant to the initial concentration of metal in the feed solutions. Higher values of BCF indicate the ability of plants to concentrate metals in their tissues (Abhilash *et al*., 2009). The BCF values for different components (root, stem, and leaf) of *M. umbrosum* for As and Cd at different exposure levels were calculated (Tables 3.1 and 3.2). The highest BCF value was obtained after exposure to 0.2 µg As mL^{-1} (2350±72.3 for leaf) and 0.3 µg Cd mL^{-1} (3026.91±1389.12 for root), and the lowest BCF value was found for both As and Cd at the highest concentration

treatment in the experiments (Tables 3.1 and 3.2). It was observed that the plant was a good accumulator of As and Cd if the water contained concentrations 50 times (up to 500 µg L^{-1}) and 100 times (up to 300 µg L^{-1}) the maximum levels of As (10 µg L^{-1}) and Cd (3 µg L^{-1}) recommended by the World Health Organization, respectively (WHO, 2011). From the point of view of phytoremediation, a good accumulator has been defined as having the ability to concentrate the heavy metal in its tissues. In general, a plant with a BCF of more than 1000 is considered a hyperaccumulator. A plant with a BCF of 1 to less than 1000 is

Table 3.1 BCF values (dry weight basis), root to stem and stem to leaf TF values, and As removal efficiency (%) of *M. umbrosum*. (n = 3)

Conc. of As (µg mL^{-1})	Plant parts	BCF [Mean±SE]	TF	% Removed
0.2	Root	1140±121.4		
	Stem	1983±38.4	1.74	80.48
	Leaf	2350±72.3	1.18	
0.45	Root	567.4±32.9		
	Stem	1253.3±17.3	2.21	89.56
	Leaf	1782.2±41.5	1.42	
1	Root	289.3±19.2		
	Stem	1001±16.5	3.46	79.3
	Leaf	1179.3±11.6	1.27	

considered an accumulator, and with a BCF of less than 1 as an excluder (Zayed *et al*. 1998). In addition, a plant is defined to be a hyper-accumulator if it can concentrate the pollutants in any above ground tissue of dry weight; which varies according to the pollutant involved: >1000 mg/kg for Ni, Cu, Co, Cr or Pb; >10,000 mg/kg for Zn or Mn (Morel *et al*., 2006). In this study, as the BCF value of *M. umbrosum* was shown to be higher than 1000 in the leaf, stem, and root in the 0.2 µg As mL^{-1} and 0.3 µg Cd mL^{-1} treatments, and leaf and stem in the 0.45 and 1.0 µg As mL^{-1} treatments (Tables 3.1 and

3.2), the plant can be recognized as a hyperaccumulator for As and Cd. Some plant species have shown similar or higher accumulation of As and Cd. For example, BCF values of *W. globosa* were 940 and 476 for 15 µM arsenite and 30 µM arsenate, respectively (Zhang *et al*., 2009). Abhilash *et al*. (2009) reported Cd BCF values of more than 934 in *L. flava*. In addition, Sela *et al*. (1989) reported markedly high BCF values (24000) for Cd in the roots of *Azolla filiculoids*. However, some other plant species were shown to have lower accumulation of As and Cd, and low BCF values. Anwar *et al*. (2006) assessed the exposure and bioavailability of As using *Pteridium aquilinum*, *Erica australis*, *Juncus effuses*, *Phalaris caerulescens*, and *Spergula arvensis* plant species in contaminated soils from the *La Parrilla* mine, Spain. They reported BCF values of 2.1 to 593.9 for the As contaminated site. Brix *et al*. (1983) found a BCF value of 6 for *Zosterna marina* grown in a Cd-contaminated site.

3.3.6 TF of As and Cd in *M. umbrosum*

TF values of the various As and Cd treatments for root to stem and stem to leaf transfers are given in Tables 3.1 and 3.2. All TF values for the As treatments, and TF values of stem to leaf for the Cd treatments, were greater than 1.0. It was indicated that As was readily translocated from root to stem and stem to leaf. Abhilash *et al.* (2009) reported that TF values for *L. flava* were from 0.90 to 4.41 for 0.5, 1, 2, and 4 mg Cd L^{-1} treatments after 3, 7, 21, and 30 days. Rabb *et al*. (2007) studied 46 plant species to determine translocation into the shoots for arsenate, methyl arsonate, and dimethylarsinate. They found, for arsenate (V), that none of the plant species had a TF of more than 0.9 for shoot to root transfer. In this study, high TF values (>1) of root to stem and stem to leaf for As, and stem to leaf for Cd, revealed that *M. umbrosum* is a good phytofiltrator as compared with other species.

Table 3.2 BCF values (fresh weight basis), root to stem and stem to leaf TF values, and Cd removal efficiency (%) of *M. umbrosum*. (n = 3)

Conc. of Cd (μgmL^{-1})	Plant parts	BCF [Mean±SE]	TF	% Removed
0.3	Root	3026.91±1389.12		
	Stem	1473.91±219.02	0.49	70.4
	Leaf	1686.56±277.22	1.14	
3.0	Root	585.14±215.21		
	Stem	542.97±39.18	0.93	73.1
	Leaf	596.49±86.06	1.10	
30	Root	443.21±65.42		
	Stem	112.59±6.93	0.25	60
	Leaf	149.71±10.01	1.33	

3.4 Conclusion

Water pollution by heavy metals such as As and Cd is a serious problem for humans and aquatic organisms. One approach to remedy this pollution is to develop cost effective, practically applicable, novel, and eco-friendly phytoremediation technologies. Although many studies have already been conducted using plants to remediate contaminants from water bodies, the lack of suitable plants is still limiting the effectiveness of phytoremediation. In the present study, we used *M. umbrosum* in a hydroponic environment to evaluate its phytofiltration potential for two noxious metals, As and Cd. It was revealed that *M. umbrosum* was a suitable plant for the phytofiltration of low-level As and Cd contamination in water because of i) a high removal rate (79.3–89.5% As and 60–73.1% Cd), ii) an enough BCF (2350 for As and 3026.91 for Cd), iii) a TF value of more than 1, and iv) ease of culturing and harvesting. Therefore, the proposed plant is a candidate as a good phytoremediator for As- and/or Cd-contamination. Further investigation will be needed to clarify the mechanism of metal accumulation in *M. umbrosum* in order to use it as an effective phytofilter for As and Cd removal from drinking water.

References

Abhilash, P.C., Vimal, C.P., Srivastava, P., Rakesh, P.S., Chandran, S., Singh, N., Thomas, A.P., 2009. Phytofiltration of cadmium from water by *Limnocharis flava* (L.) Buchenau grown in free-floating culture system. J Hazard Mater. 170(2-3), 791-797.

Anawar, H.M., Sanchez, A.C., Murciego, A., Buyolo, T., 2006. Exposure and bioavailability of arsenic in contaminated soils from the La Parrilla mine, Spain. Environ Geol. 50(2), 170-179.

Barman, S.C., Sahu, R.K., Bhargava, S.K., Chatterjee, C., 2000. Distribution of heavy metals in wheat, mustard and weed grains irrigated with industrial effluents. Bull Environ Contamin Toxicol. 64(4), 489-496.

Brix, H., Lyngby, J.E., Schierup, H.H., 1983. Eelgrass (*Zostera marina* L.) as an indicator of trace metals in the Limfjord, Denmark. Mar Environ Res. 8(3), 165-181.

Cai, Y., Georgiadis, M., Fourqurean, J.W., 2000. Determination of arsenic in seagrass using inductively coupled plasma mass spectrometry. Spectrochimica Acta Part B. 55(9), 1411-1422.

Dushenkov, S., Kapulnik, Y., 2000. Phytoremediation of Toxic Metals. In: Raskin I, Ensley BD. eds. Using Plants to Clean Up the Environment. New York: John Wiley & Sons. p. 89-106.

Fritioff, A., Greger, M., 2007. Fate of cadmium in *Elodea canadensis*. Chemosphere, 67(2), 365–375.

Fritioff, A., Greger, M., 2006. Uptake and distribution of Zn, Cu, Cd, and Pb in an aquatic plant *Potamogeton natans*. Chemosphere, 63(2), 220–227.

Gupta, S., Nayek, S., Saha, R.N., Satpati, S., 2008. Assessment of heavy metal accumulation in macrophyte, agricultural soil and crop plants adjacent to discharge zone of sponge iron factory. Environ Earth Sci. 55(4), 731-739.

Hou, W., Chen, X., Song, G., Wang, Q., Chang, C.C., 2007. Effects of copper and cadmium on heavy metal polluted waterbody restoration by duckweed (*Lemna minor*) Plant Physiol Biochem. 45(1), 62-69.

IARC, 1987. Overall evaluations of carcinogenicity: An updating of IARC Monographs Volumes1–42. Lyon, International Agency for Research on Cancer. p 100–106 (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Supplement 7).

IPCS, 2001. Arsenic and arsenic compounds. Geneva: World Health Organization, International Programme on Chemical Safety (Environmental Health Criteria 224; http://www.inchem.org/documents/ehc/ehc/ehc224.htm).

Küpper, H., Lombi, E., Zhao, F.J., McGrath, S.P., 2000. Cellular compartmentation of cadmium and zinc in relation to other elements in the hyperaccumulator *Arabidopsis halleri.* Planta, 212(1), 75–84.

Langner, H.W., Greene, E., Domenech, R., Staats, M.F., 2012. Mercury and other mining-related contaminants in ospreys along the Upper Clark Fork River, Montana, USA. Arch Environ Contam Toxicol. 62(4), 681-695.

Ma, L.Q., Komar, K.M., Tu, C., Zhang, W., Cai, Y., Kennelley, E.D., 2001. A fern that hyperaccumulates arsenic. Nature, 409, 579.

Mkandawire, M., Dudel, E.G., 2005. Accumulation of arsenic in *Lemna gibba* L. (ducweed) in tailing waters of two abandoned uranium mining sites in Saxony, Germany. Sci Total Environ. 336(1-3), 81–89.

Morel, J., Echevarria, G., Goncharova, N., 2006. Phytoremediation of Metal Contaminated Soils. Springer, Netherlands.

Mustafa, H.T., Hassan, H.M., Abo-Melha, A., Rihan, T.I., 1988. Cadmium and zinc concentrations in the potable water of the eastern provinces of Saudi Arabia. Bull Environ Contam Toxicol. 40(3), 462–467.

Nordstrom, D.K., 2002. Public health–worldwide occurrences of arsenic in ground water. Science, 296(5576), 2143–2145.

OECD, 1994. OECD environment monograph series No. 104: Risk Reduction Monograph No. 5: Cadmium. Background and National Experience with Reducing Risk. Environmental Directorate. Organisation for Economic Co-operation and Development, Paris 1995 (cancels and replaces the same document distributed on 15 Nov. 1994). p. 33.

Raab, A., Williams, N.P., Meharg, A., Feldmann, J., 2007. Uptake and translocation of inorganic and methylated arsenic species by plants. Environ Chem. 4(3), 197-203.

Rahman, M.A., Hasegawa, H., 2011. Review on Aquatic arsenic: Phytoremediation using floating macrophytes. Chemosphere, 83(5), 633-646.

Rahman, M.A., Hasegawa, H., Ueda, K., Maki, T., Okumura, C., Rahman, M.M., 2007. Arsenic accumulation in duckweed (*Spirodela polyrhiza* L.): A good option for phytoremediation. Chemosphere, 69(3), 493–499.

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. Collingwood, Victoria: CSIRO. pp. 235–247.

Schiorup, H.H., Larsen, V.J., 1981. Macrophyte cycling of zinc, copper, lead and cadmium in the littoral zone of a polluted and a non-polluted lake. 1. Availability, uptake and translocation of heavy metals in *Phragmites australis* (Cav.) Trin Aquat Bot. 11, 197-210.

Schor-Fumbarov, T., Keilin, Z., Tel-Or, E., 2003. Characterization of cadmium uptake by the water lily *Nymphaea aurora*. Int J Phytorem. 5(2), 169–179.

Sela, M., Garty, J., Tel-Or, E., 1989. The accumulation and the effect of heavy metals on the water fern *Azolla filiculoides*. New Phytol. 112(1), 7-12.

Smith, A.H., Lingas, E.O., Rahman, M., 2000. Contamination of drinking water by arsenic in Bangladesh: a public health emergency. Bull World Health Organ. 78, 1093-1103.

Snyder, K.V.W., 2006. Removal of Arsenic from Drinking Water by Water Hyacinths (*Eichhornia crassipes*). J U S S J W P. 1, 41-58.

Sun, R.L., Zhoua, Q.X., Sun, F.H., Jin, C.X., 2007. Antioxidative defense and proline/phytochelatin accumulation in a newly discovered Cd-hyperaccumulator, *Solanum nigrum* L. Environ Exp Botany. 60(3), 468–476.

USEPA-IRIS, 2010. Toxicological Review of Inorganic Arsenic (Cancer)-External Review Draft. U.S. Environmental Protection Agency, Washington, DC, EPA/635/R-10/001, 2010.

Vamerali, T., Bandiera, M., Mosca, G., 2010. Field crops for phytoremediation of metal-contaminated land: A review. Environ Chem Lett. 8(1), 1–17.

Wang, K.S., Huang, L.C., Lee, H.S., Chen, P.Y., Chang, S.H., 2008. Phytoextraction of cadmium by *Ipomoea aquatica* (water spinach) in hydroponic solution: Effects of cadmium speciation. Chemosphere, 72(4), 666-672.

WHO, 2011. Guidelines for drinking-water quality, 4th ed. Chemical fact sheets-chemical contaminants in drinking water. Geneva: World Health Organization.

WHO/UNEP, 1989. GEM-Global fresh water quality. Published on behalf of the World Health Organization/United Nations Environment Programme. Oxford: Blackwell Reference.

Williams, P.N., Islam, M.R., Adomako, E.E., Raab, A., Hossain, S.A., Zhu, Y.G., Feldmann, J., Meharg, A.A., 2006. Increase in rice grain arsenic for regions of Bangladesh irrigating paddies with elevated arsenic in groundwaters. Environ Sci Technol. 40(16),

4903–4908.

World Bank, 2005. Environment and Social Unit-South Asia Region. Towards a More Effective Operational Response. Arsenic Contamination of Groundwater in South and East Asian Countries Volume II Technical Report. Environmental Study.

Zayed, A., Gowthaman, S., Terry, N., 1998. Phytoaccumulation of trace elements by wetland plants: 1. Duckweed. Environ Qual. 27(3), 715–721.

Zhang, X., Zhao, F.J., Huang, Q., Williams, P.N., Sun, G.X., Zhu, Y.G., 2009. Arsenic uptake and speciation in the rootless duckweed *Wolffia globosa*. New Phytologist 182(2), 421-428.

Zhang, X., Lin, A.J., Zhao, F.J., Xu, G.Z., Duan, G.L., Zhu, Y.G., 2008. Arsenic accumulation by the aquatic fern *Azolla*: Comparison of arsenate uptake, speciation and efflux by *Azolla caroliniana* and *Azolla filiculoides*. Environ Pollut. 156(3), 1149–1155.

Zhao, F.J., Lombi, E., McGrath, S.P., 2003. Assessing the potential for zinc and cadmium phytoremediation with the hyperaccumulator *Thlaspi caerulescens*. Plant Soil. 249(1), 37-43.

Zhu, Y.G., Sun, G.X., Lei, M., Teng, M., Liu, Y.X., Chen, N.C., Wang, L.H., Carey, A.M., Deacon, C., Raab, A., Meharg, A.A., Williams, P.N., 2008. High percentage inorganic arsenic content of mining impacted and non-impacted Chinese rice. Environ Sci Technol. 42(13), 5008–5013.

Chapter IV: Phytofiltration of arsenic and cadmium by using *Micranthemum umbrosum*: Phytotoxicity, uptake kinetics, and mechanism

Abstract

As and Cd are noxious and carcinogenic pollutants that can be removed from water by using emerging, ecofriendly, phytofiltration technology that employs *Micranthemum umbrosum*. After culturing *M. umbrosum* for 7 days in a hydroponic experiment, accumulation of 1219±44 µg As g^{-1} and 799±31 µg Cd g^{-1} were observed in the leaves, from 1000 µg As L^{-1} and 1000 µg Cd L^{-1} of water, respectively. Plant and water samples were analyzed for assessing the As and Cd accumulations, phytotoxic effects, uptake mechanisms and kinetics, and for evaluating the potential of *M. umbrosum* in As and Cd phytofiltration. The uptake pattern was leaf > stem > root for both pollutants. The plant showed more resistance to internal and external As concentrations than to that of Cd. Uptake of inorganic As species was much greater than that of organic As, and was found at above the substrate concentration. However, Cd showed similar uptake pattern to that of inorganic As species, and the data was better fit to a non-linear than a linear model. Low molecular weight substances having thiol group(s) may be responsible for the binding of As in plants whereas Cd showed a different mechanism to that of As. *M. umbrosum* showed good As phytofiltration capabilities without any phytotoxic effects, but it was found to be a moderate accumulator of Cd with some phytotoxic effect.

4.1 Introduction

As and Cd are classified as group 1 carcinogenic compounds to human (IARC, 2012). As is one of the 20th most abundant elements in the Earth`s crust (Woolson, 1975), thus found in many environments, and is highly toxic to living species especially human beings. Groundwater (the main drinking water source in many countries), soils, sediments and

food chains contaminated with As, irrespective of whether the contamination is due to natural geochemical or anthropogenic influence, causes skin lesions, cancers, and many other diseases in human beings (Dhankher, 2005; Ducker *et al*., 2005; Mondal *et al*., 2006; William *et al*., 2006).

In some areas of Bangladesh and India, As concentration in groundwater has exceeded 2000 µg L^{-1} (British Geological Survey, 2000; Hossain, 2006). According to the British Geological survey (2000), 51% of tube well water samples collected from 41 out of 64 districts contained more than 10 µg L^{-1} of As, 35% were above 50 µg L^{-1}, 25% were above 100 µg L^{-1}, 8.4% were above 300 µg L^{-1} and 0.1% were above 1000 µg L^{-1}, whereas the WHO`s permissible limit for As in drinking water is 10 µg L^{-1} (WHO, 2011), and the national standard for drinking water in Bangladesh is 50 µg L^{-1} (World Bank, 2005). There are different forms of As that exist in the environment e.g., inorganic (arsine, arsenious acid, arsenite, arsenic acids or arsenate), organic (monomethyl arsonic acid [MMAA] and dimethylarsinic acid [DMAA]), biological, and other forms (Rahman and Hasegawa, 2011). As toxicity depends on the As species; and generally inorganic As species (arsenite and arsenate) are more toxic as compared with organic As species (Meharg and Hartley-Whiteker, 2002; Ng, 2005). The toxicity level of the various As species is As (III)>As(V)>DMAA>MMAA (Petrick *et al.*, 2000).

Cd is a widespread toxic heavy metal that is mainly released into the environment by paints and pigments, plastic stabilizers, electroplating, incineration of cadmium-coated plastics, by-products of cement, and phosphate fertilizer factories (Sanita di Toppi and Gabbrielli, 1999; Salem *et al*., 2000; Pulford and Watson, 2003). It causes carcinogenic, mutagenesis, interferes with calcium regulation in biological systems, renal failure, and chronic anemia (Degraeve, 1981; Salem *et al*., 2000). More than 30 µg L^{-1} Cd was recorded in the drinking water, though the recommended Cd level in drinking water is only

3 µg L^{-1} (WHO, 2011).

Therefore, the removal of As and Cd from contaminated water has been of the utmost importance in order to minimize their impacts on ecosystems. This has proven good challenging with respect to cost and technical complexity (Barcelo and Poschenrieder, 2003). Different physical, chemical, and biological approaches have been employed for this purpose. Generally, the physical and chemical methods have incurred limitations such as high cost, labor intensiveness, irreversibility of the changes to environmental properties, and secondary pollution (Ali *et al*., 2013). One novel approach is phytofiltration, which has been proposed as a promising, environment friendly, esthetically pleasant technology is by using live plants to remove As and Cd from contaminated water. There are some plants that can accumulate As and Cd in their harvestable parts. *Pteris vittata* L. (Ma *et al*., 2001), *Wolffia globosa* (Zhang *et al*., 2009), *Spirodela polyrhiza* L. (Rahman *et al*., 2007a), *Lemna gibba* L. (Mkandawire and Dudel, 2005), *Polygonum hydropiper* (Robinson *et al*., 2005), and *Azolla caroliniana* (Zhang *et al*., 2008) were identified for As accumulators. *Limnocharis flava* L. (Abhilash *et al*., 2009), *Nymphae aurora* (Schor-Fumbarov *et al*., 2003), *Solanum nigrum* L. (Sun *et al*., 2007), *Thlaspi caerulescens* (Zhao *et al*., 2003), and *Arabidopsis halleri* (Küpper *et al*., 2000) were found to be Cd accumulators. Few aquatic plants could accumulate more than one pollutant in their bodies. *M. umbrosum*, commonly called the Water fern or Baby`s tears, is one of them that has been identified as an As hyperaccumulator because of its high bio-concentration factors (>1000) and translocation factor (>1.0) and also a moderate Cd accumulator at low concentrations, and it can remove 79.3–89.5% As and 60–73.1% Cd from 0 to 1.0 µg As mL^{-1} and 0.3 to 30.0 µg Cd mL^{-1} solutions, respectively (see Chapter III). Plants exposed to heavy metals showed tolerance and hyper accumulation by adjusting and/or altering some physiological mechanism depending on the type of pollutant, dose intensity and plant species; (Fig. 4.1 and 4.2).

Plant uptake heavy metals from solution through their roots and in submerged condition, whole plant body acted as a active site for absorption (Rahman and Hasegawa, 2011). After entry into roots, heavy metal ions can either be stored in the roots or translocated to the shoots primarily through xylem vessels (Prasad, 2004; Jabeen *et al*., 2009) where they are mostly deposited in vacuoles. Heavy metal sequestration in the vacuole is one of the ways to remove excess metal ions from the cytosol, and may reduce their interactions with cellular metabolic processes (Assuncao *et al*., 2003; Sheoran *et al*., 2011). Compartmentalization of complex metals in vacuoles is part of the tolerance mechanism in metal hyperaccumulators (Cluis, 2004; Tong *et al*., 2004). 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 (Mejare and Bulow, 2001; Memon and Schroder, 2009). The most important peptides/proteins involved in metal 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 (Karenlampi *et al*., 2000). PCs are small glutathione (GSH)-derived, enzymatically synthesized peptides, which bind metals and are principal part of the metal detoxification system in plants (Clemens, 2001; Cobbett and Goldsbrough, 2002; Yurekli and Kucukbay, 2003; Fulekar *et al*., 2009). They have the general structure of $(\gamma\text{-glutamyl-cysteinyl})_n$-glycine where n = 2–11 (Inouhe, 2005). Synthesis and chemical structures of GSH and PCs is shown in Fig. 4.1 and Fig. 4.3, respectively. Modification or over expression of GSH (glutathione) and PCS gene has significant potential for increasing

heavy metal accumulation and tolerance in plants (Seth, 2012). Cd also increased synthesis of PCs, but reduced the synthesis of ascorbate and antioxidant enzymes in *Pistia stratiotes* L. compared to *Eichhornia crassipes* (Mart.) Solms (Sanita di Toppi *et al*., 2007), whereas Cd compartmentalization occurred in the epidermal vacuoles of *Thlaspi caerulescens* leaves (Küpper *et al.*, 2004).

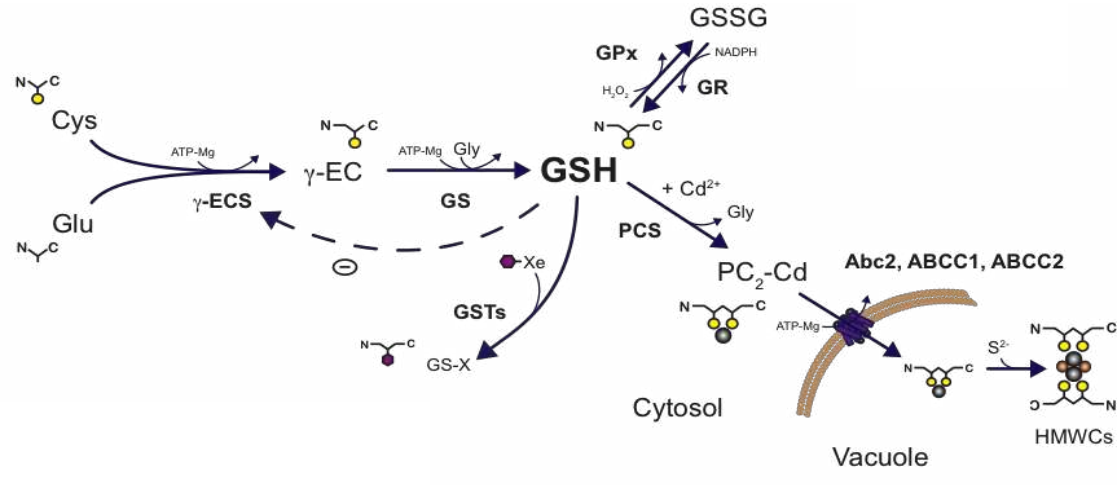


Fig. 4.1 Glutathion (GSH) and phytochelatin synthesis in plant (Modified from Mendoza-Cozatl *et al*., 2010).

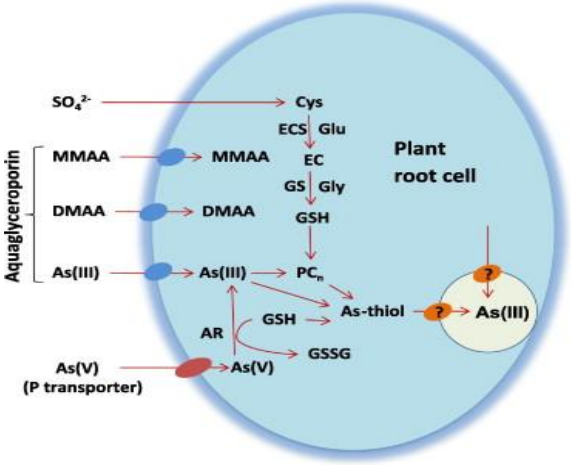


Fig. 4.2 Mechanisms of arsenic uptake into plant cells (Tripathi *et al*., 2007). As(V) is transported through phosphate transporters, and As(III) and organoarsenic species (MMAA and DMAA) might be through aquaglyceroporins (Tripathi *et al*., 2007; Rahman *et al*., 2008; Zhao *et al*., 2009; Rahman *et al.*, 2011).

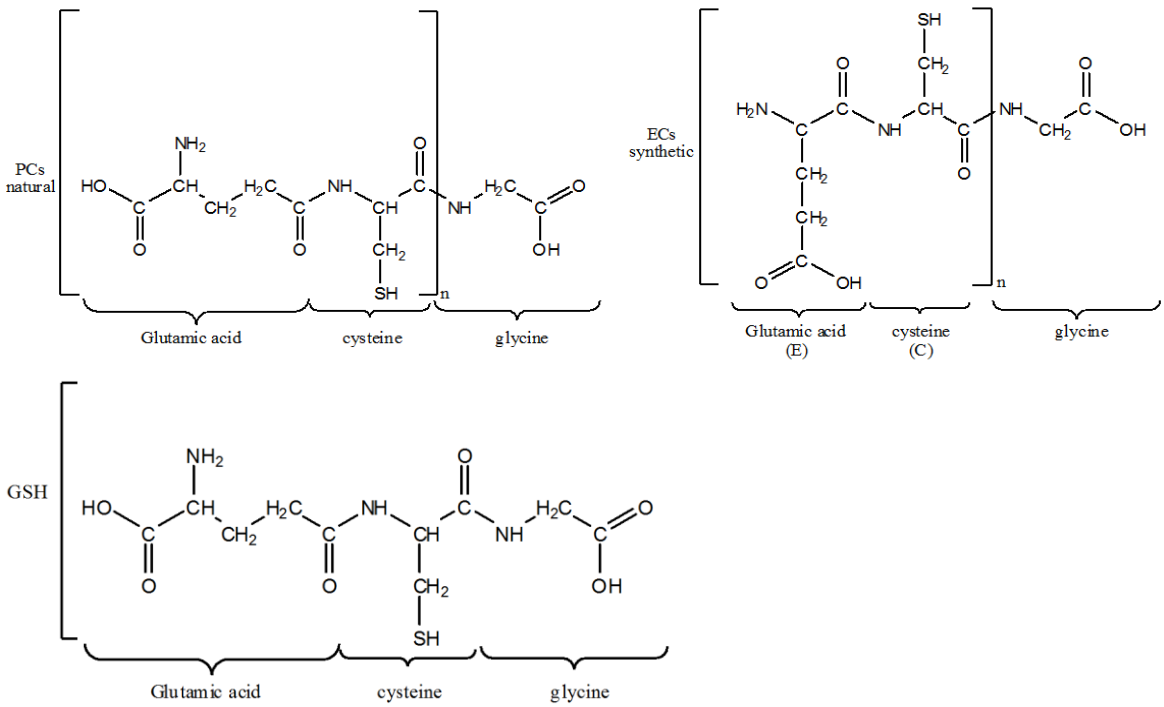


Fig. 4.3 Chemical structures of molecules binding heavy metals: natural phytochelatin (PC), synthetic phytochelatin (EC) - glutamic acid (glu-E) and cysteine (Cys-C) (Bae *et al*., 2000) and glutathione (GSH) (Bae and Mehra, 1997).

In the case of As, main route of As(V) uptake within plant is through phosphate transporter (Asher and Reay, 1979; Meharg *et al*., 1994) and As(III) is through aquaglyceroporins (Meharg and Jardine, 2003; Isayenkov and Maathuis, 2008; Ma *et al*., 2008). As substantially increases the synthesis of glutathione (GSH) and PCs (Schat *et al*., 2002; Grill *et al*., 2006). Raab *et al*. (2005) identified 14 different As complexes, including PCs, in *Helianthus annuus*, but As appears to be present in its unbound inorganic form in *Pteris vittata* hyperaccumulator (Rabb *et al*., 2004; Pickering *et al*., 2006). These phenomena suggested that As and Cd hyper accumulation occurred through different mechanisms in various plant species. *M. umbrosum* plant have the tendency to absorb As and Cd (Islam *et al*., 2013). However, interestingly, the current study showed that this plant has different uptake mechanisms with different As and Cd doses exposures and different response to growth with respect to photopigments production and macro and micronutrient uptake.

Thus, in the present study, we evaluated the potential of *M. umbrosum* for the

phytofiltration of As and Cd from contaminated water by investigating the phytotoxic effects, effects on macro and micro nutrient uptake, uptake kinetics, and possible uptake mechanisms that these two carcinogenic elements showed in this plant.

4.2 Materials and Methods

4.2.1 Plant culture

M. umbrosum was obtained from Aqua Friend Hokusui (Hokkaido, Japan). Initially, the plants were grown in hydroponic cultures in laboratory conditions for seven days to allow for adaptation. Then about 3.5 g (fresh weight) of *M. umbrosum* were grown in glass pots in Milli-Q water (Millipore-Gradient A10, Milli-Q Gradient ZMQG) containing 0, 200, 500, 1000 µg As L^{-1} [from sodium (meta) arsenite, $NaAsO_2$], and 0, 300, 1000 µg Cd L^{-1} from $CdCl_2 \cdot 2.5H_2O$ with Hoagland nutrient solution (Hoagland and Arnon, 1950) as a nutrient source. Another experiment was conducted in dark condition and excised shoot and root part separately treated with 500 µg As L^{-1} and 400 µg Cd L^{-1} treatments. The pH of the solution was adjusted to 6.0 by adding KOH or HCl. The experiments were carried out for 7 days in a growth chamber under a controlled environment at the following conditions: 14L:10D light/dark cycle, 100-125 $\mu mol.m^{-2}.s^{-1}$ light intensity, 75% humidity and 21 ± 1ºC. Three replications were done in all cases and a control was maintained both for the metal and the plant.

4.2.2 Sampling and photopigments analysis

Leaf sampling were done at 0, 4, and 7 day intervals and immediately used for the estimation of chlorophyll a, chlorophyll b, total chlorophyll, carotenoids and anthocyanine contents. Chlorophyll and carotenoid concentrations in leaves were extracted using 80% chilled acetone; contents of these were estimated using the equation given by Lichtenthaler

and Wellburn (1983). Anthocyanine content was also estimated in leaves as described by Sims and Gamon (2002). Two milliliters water samples were collected from each pot at 24 h intervals to measure the As and Cd status in the water. After 7 days, whole plants were harvested and rinsed with Milli-Q water three times to remove any apoplastic As and Cd, then kept in clean absorbent paper to remove the remaining water from the surface. Final fresh weight was taken on a digital balance (A&D Co. Ltd, Japan, HF-200, Max 210 g, d = 0.001 g). Then, the whole plants were separated into leaves, stems and roots for analysis of As, Cd, potassium (K), calcium (Ca), magnesium (Mg), manganese (Mn), and zinc (Zn) contents.

4.2.3 Sample preparation and chemical analysis

Separated leaf, stem, and root samples were kept for 24 h for air drying at room temperature on absorbent paper. Then, most of the samples (some of 0 and 1000 μg L^{-1} As and Cd treated leaf were kept fresh for amino acid and SH content analyses) were oven dried at 65ºC (Constant Temperature Oven, DKN602, Yamato Scientific Co. Ltd., Japan) for at least 48 h until they reached a constant weight. After grinding the samples, 25-40 mg samples of roots, stems, or leaves were separately placed into 15 mL polyethylene tubes (Thermo Fisher Scientific, NY, USA). Two milliliters of 65% HNO_3 (Wako Pure Chemical Ind. Ltd., Japan) were added, and the samples with HNO_3 were kept under the fume hood for 12 h. Then, the samples were covered and heated on a heating block (TAH-2G, Dry Thermo Unit, Japan) at 95ºC for 2 h to digest. After cooling, 1 mL of 30% H_2O_2 (Wako Pure Chemical Ind. Ltd., Japan) was added, and the samples were covered and heated again at 105ºC for 20 min (Rahman *et al*., 2007a). The digested samples were diluted up to 10 mL with Milli-Q water using 10 mL volumetric flasks (Pyrex, IWAKI Glass), as described by Cai *et al*. (2000). The diluted samples were then filtered using a

0.45-µm syringe-driven filter unit (Millipore, Billerica, USA) and stored in 15 mL polyethylene bottles. As, Mg, Mn and Zn contents were measured using an inductively coupled plasma-mass spectrophotometer (ICP-MS; Agilent G1820 Model), whereas Cd, K and Ca contents were measured by a flame-type atomic absorption spectrophotometer (AAS; Model 180–80, Hitachi, Japan). The accuracy of the analysis was checked using certified standard reference materials for As (013–15481, Lot ALK 9912, 1000 mg L^{-1}) and Cd (036–16171, Lot TSP9842, 1000 mg L^{-1}) obtained from Wako Pure Chemical Ind. Ltd., Japan.

4.2.4 Uptake kinetics of inorganic and organic As species; and Cd

Approximately 3.5 g of fresh plant (whole) were cultured in 0, 200, 500, 1000 µg As L^{-1} [each from sodium (meta) arsenite ($NaAsO_2$, Sigma-Aldrich, India), DMAA ($C_2H_7AsO_2$, TCI, Tokyo, Japan), and MMAA (CH_5AsO_3, Wako Pure Chemical Ind. Ltd., Japan)], and 0, 300, 1000 µg Cd L^{-1} (from $CdCl_2.2.5H_2O$, Wako Pure Chemical Ind. Ltd., Japan) contaminated water with Hoagland nutrient solution (Hoagland and Arnon, 1950) for 24 h. Then plant samples were collected and washed with ice-cold deionized water to remove As and Cd from plant`s surface (Irtelli and Navari-Izzo, 2008). Water samples were also collected for each pot to determine the As and Cd absorption by the plants after the first 24 h. Kinetics parameters were measured by fitting data to the Michaelis-Menten function. MMAA and DMAA were only used for the uptake kinetics study, so no data for MMAA and DMAA, other than uptake kinetics, are shown in this manuscript.

4.2.5 Separation and quantification of thiol containing peptides

As- and Cd-treated (1000 µg L^{-1}) leaf samples (500 mg fresh wt) were homogenized in a stirrer (Yamato Labo-Stirrer, L-35, Japan) in 5 mL of ice cold 50 mM Tris-Cl buffer, pH

7.4, containing 0.1% sodium dodecyl sulfate (SDS). Homogenized samples were then centrifuged (Kubota 6200 Centrifuger, Japan) at 10,000 × g for 30 min at 4ºC. The obtained supernatants were filtered through a 0.45-µm syringe-driven filter unit (Millipore, Billerica, USA) and immediately 3 mL of supernatant was applied on a gel filtration column chromatography equipped with a column (1.1 × 110 cm^2) containing Sephadex G-50 (Pharmacia, Sweden). The chromatography was carried out in the presence of 50 mM Tris-Cl buffer, pH 7.4 (Schmoger *et al.*, 2000) and eluted at a flow rate of 2.5 min/mL. Sixty fractions of the eluent (2 mL fraction size) were measured for As (using ICP-MS) or Cd (by AAS) and absorbance at 280 nm using a UV/VIS spectrophotometer (Beckman, DU-65 Spectrophotometer, USA) for protein quantification (Walker, 1996). Thiol contents in each fraction were detected at 412 nm with Ellman`s reagent (5-5´dithio(2-nitrobenzoic acid) (Sanita di Toppi *et al*., 2007). For Cd-treated leaves, we again concentrated the Cd containing fractions and applied them to a gel filtration column chromatography equipped with a column (1.1 × 110 cm^2) containing Sephadex G-15 (Pharmacia, Sweden). The chromatography was carried out with 50 mM Tris-Cl buffer, pH 7.4, at a flow rate of 0.8 min/mL (Schmoger *et al*., 2000). Sixty fractions of the eluent (2 mL fraction size) were tested for Cd (by AAS). After drying Cd containing fractions using a SpeedVac Concentrator SVC100H (Savant, USA), performic acid oxidation (Walker, 1996) was performed using 1.5 mL performic acid ($HCOOH:H_2O_2$ = 9:1) at 6ºC for 24 h. Again, samples were dried and analyzed for cysteine or cysteic acid and other amino acids using a High Performance Amino Acid Analyzer (Hitachi High-Technologies Corporation, Tokyo, Japan).

4.2.6 Statistical analysis

Results were expressed as the means ± standard error (SE) of three replicates. Significance

degree was calculated using a *t*-test and curve fitting was done using the computer package Microsoft Excel program (Microsoft Office 2007 Professional).

4.3 Results

4.3.1 As and Cd contents in each parts of the plant and growth medium

The concentrations of the metals studied in the different parts of *M. umbrosum* and the water are depicted in Figs. 4.4. As and Cd concentrations in the plant increased significantly with increasing added As and Cd levels in hydroponic solution (Figs. 4.4a and b). The maximum accumulation of As (about 1220 µg g^{-1}) and Cd (800 µg g^{-1}) were found in leaves at 1000 µg L^{-1} treatment of As or Cd (Figs. 4.4a and b). As accumulation order was root < stem < leaf. Cd uptake occurred significantly from Cd-tainted water but translocation was not significant from root to stem to leaf (Fig. 4.4b).

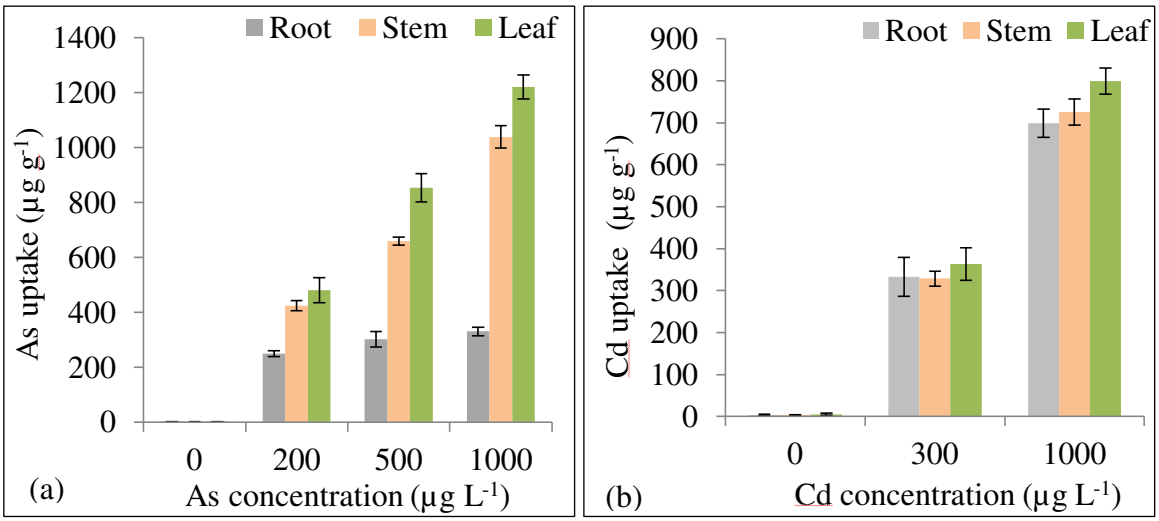


Fig. 4.4 As (a) and Cd (b) uptake pattern in root, stem and leaf of *M. umbrosum* seven days after exposure to 0, 200, 500 and 1000 µg As L^{-1} and 0, 300 and 1000 µg Cd L^{-1}. Error bars indicate mean ± standard error (n =3).

Plant cultured in the excised root and shoot condition found that shoot absorbed higher amount of As (160 µg g^{-1}) than root (Fig. 4.5a) whereas Cd absorption showed vice versa result (Fig. 4.5b). The amounts of absorption were much lower than the normal culture

without separating root and shoot part (Fig. 4.4). But in the dark condition, both As and Cd treated plant become died within 7 days, so absorption also very lower (Fig. 4.5) than plant cultured in light conditions (Fig. 4.4).

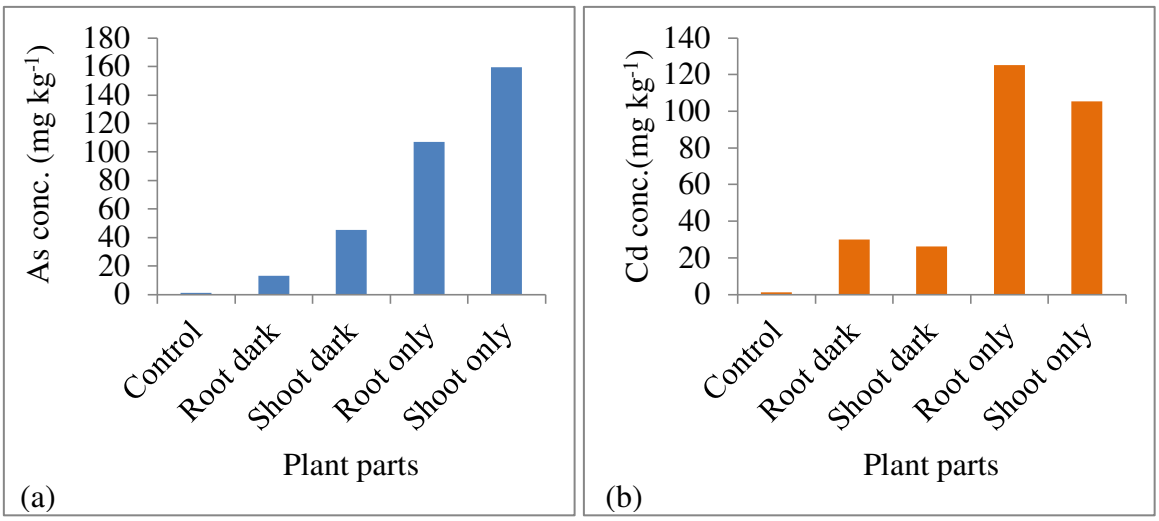


Fig. 4.5 As (a) and Cd (b) uptake by root and shoot (stem and leaf) of *M. umbrosum* seven days after exposure to 500 µg As L^{-1} and 400 µg Cd L^{-1} water in dark condition and excised root and shoot part.

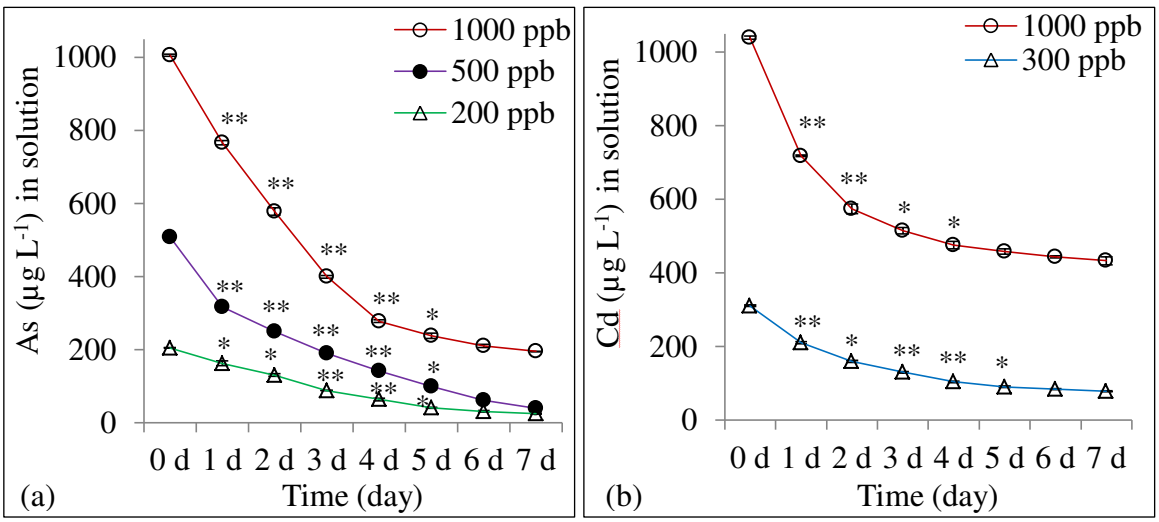


Fig. 4.6 As (a) and Cd (b) remaining (µg L^{-1}) in water in which *M. umbrosum* was grown with 0, 200, 500 and 1000 µg As L^{-1} and 0, 300 and 1000 µg Cd L^{-1}. Error bars indicate mean ± standard error (n =3). ** and * denote significant differences at $P < 0.01$ and 0.05, respectively, compared to previous days.

Remaining As and Cd concentrations in the solution at each day after culturing *M.*

umbrosum for 7 days are shown in Fig. 4.6. It was indicated that As and Cd concentrations decreased significantly up to the 5th day for As (Fig. 4.6a) and 4th day for Cd (Fig. 4.6b), respectively. After 7 days, total remaining As and Cd concentrations in the solution were below 50 µg L^{-1} for As (Fig. 4.6a) and 100 µg L^{-1} for Cd (Fig. 4.6b) in the treatment of 500 µg L^{-1} As and 300 µg L^{-1} Cd, respectively. The accumulation order was leaf > stem > root for both pollutants.

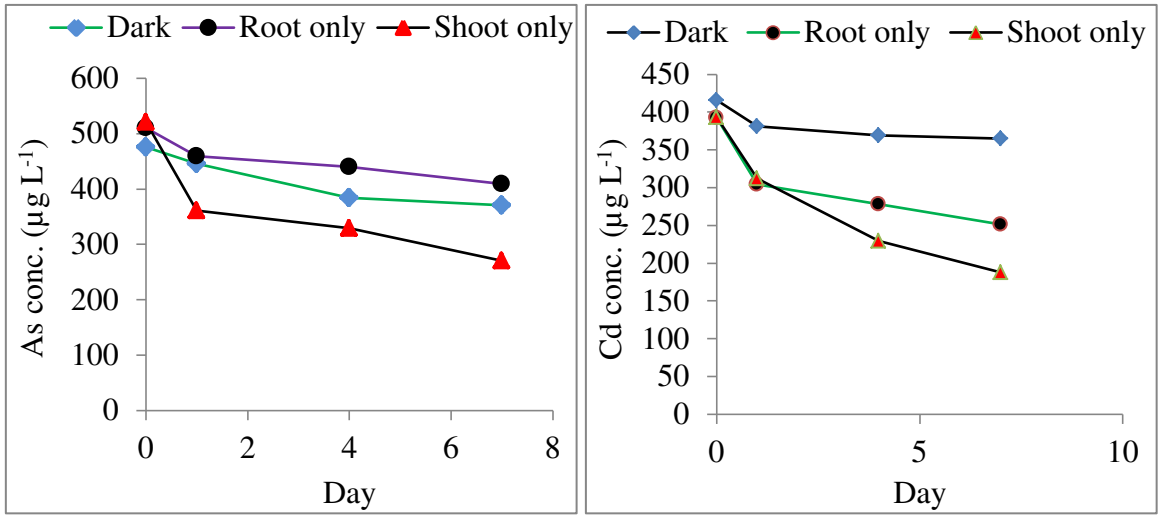


Fig. 4.7 As (a) and Cd (b) remaining (µg L^{-1}) in water in which *M. umbrosum* was grown with 500 µg As L^{-1} and 400 µg Cd L^{-1} water in dark condition and excised root and shoot part.

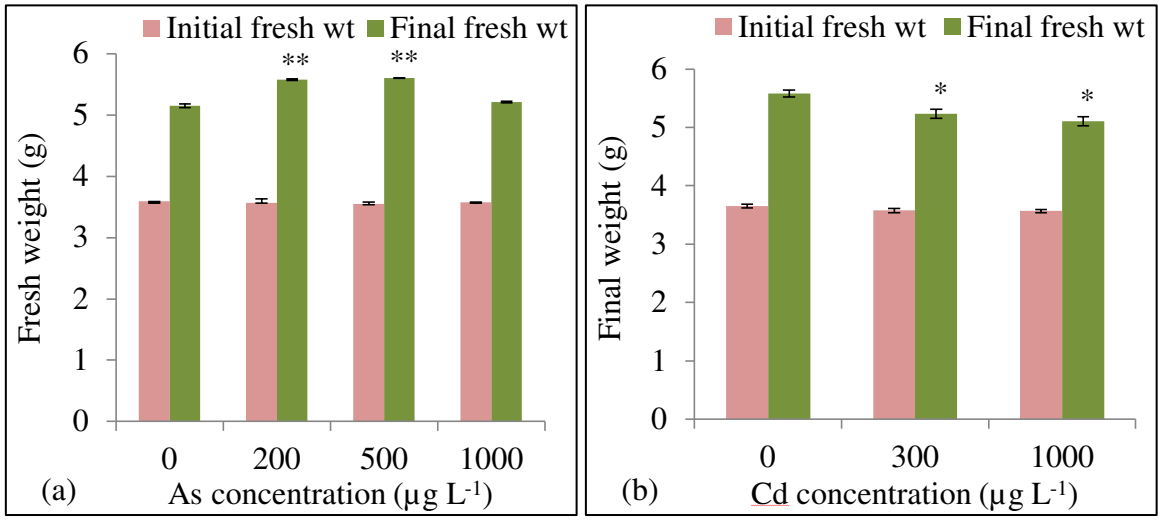


Fig. 4.8 Initial and final fresh weights of plant grown in 0, 200, 500 and 1000 µg As L^{-1} (a) and 0, 300 and 1000 µg Cd L^{-1} (b). Error bars indicate mean ± standard error (n =3). ** and * denote significant differences at P < 0.01 and 0.05, respectively, compared with their respective control.

In dark condition, the amount of As and Cd level were not so changed after 7 days but small decreasing trends were found in excised root and shoot treatment (Fig. 4.7).

4.3.2 Phytotoxicity of As and Cd on *M. umbrosum*

Phytotoxicity of As and Cd on *M. umbrosum* was evaluated in response to measuring the final fresh weight and contents of the photosynthetic pigments such as chlorophyll a, chlorophyll b, total chlorophyll, carotenoids and anthocyanin. As shown in Fig. 4.8a, the plant growth increased significantly ($p < 0.01$) with increasing As concentration in the growth medium (up to 500 µg As L^{-1}). Similarly total chlorophyll (Fig. 4.9c) and anthocyanins (Fig. 4.9e) contents in leaves increased significantly up to 500 µg As L^{-1}. Contents of chlorophyll a (Fig. 4.9a) and carotenoids (Fig. 4.9d) were observed to increase for the entire sampling period as compared with their controls. However, in the case of chlorophyll b, it increased significantly up to 4 days but later increases were not significant as compared with the controls (Fig. 4.9b). On the other hand, growth was significantly inhibited ($p < 0.05$) with elevated levels of Cd concentration in the hydroponic medium (Fig. 4.8b) because of decreases in the photosynthetic pigments (Figs. 4.10a-e). The plants become died during culturing in the dark condition for 7 days (data not shown).

4.3.3 Macro and micro elemental compositions of *M. umbrosum*

The concentrations of essential macro-(K, Ca and Mg) and micro-(Mn and Zn) nutrient elements in the plant parts were examined after 7 days to find out the effects of As and Cd on uptake of these mineral nutrient elements, and to determine the implications for water management (fertilization in particular) of *M. umbrosum* in As and Cd phytofiltration practices (Tables 4.1 and 4.2).

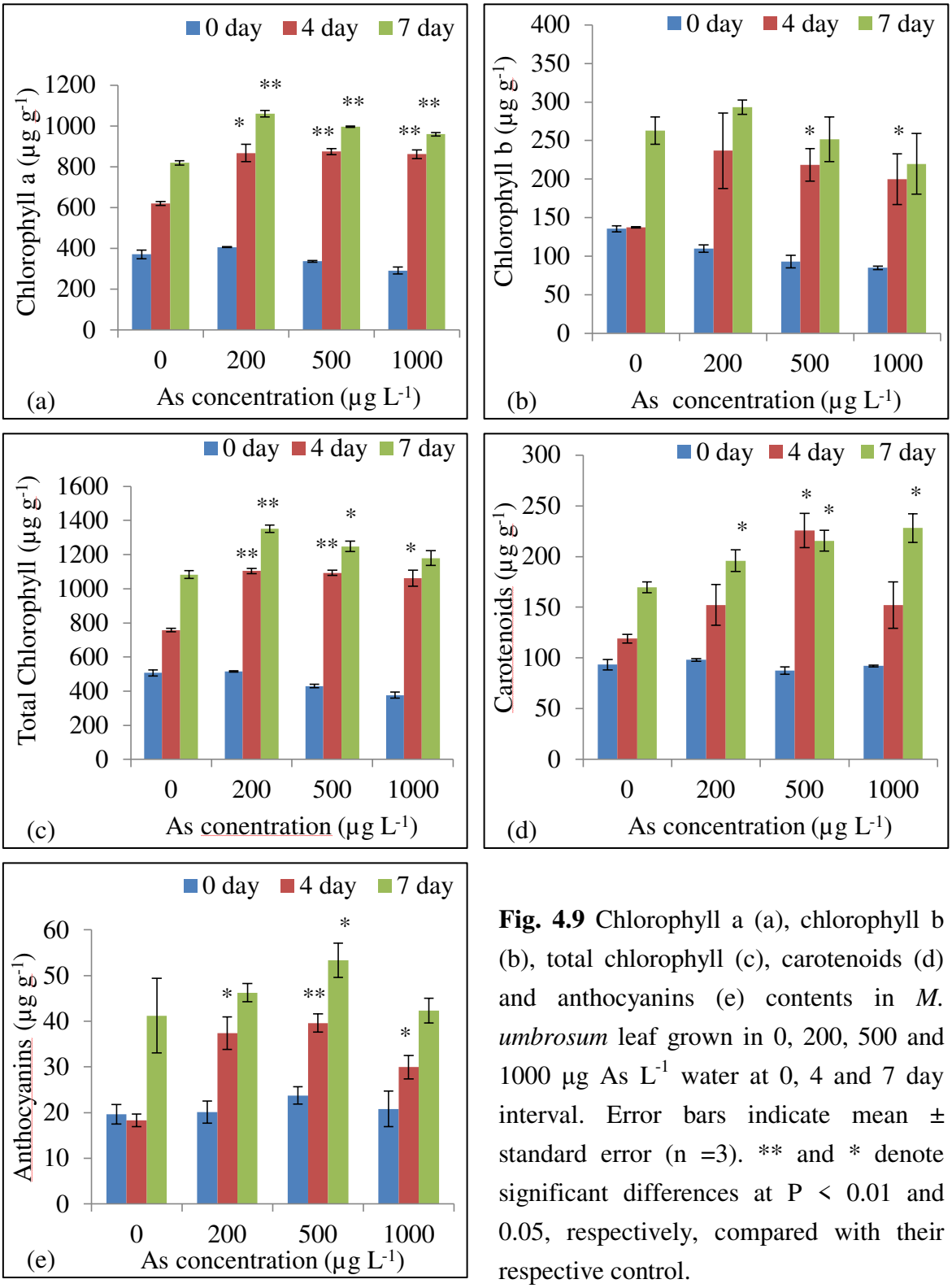


Fig. 4.9 Chlorophyll a (a), chlorophyll b (b), total chlorophyll (c), carotenoids (d) and anthocyanins (e) contents in *M. umbrosum* leaf grown in 0, 200, 500 and 1000 µg As L^{-1} water at 0, 4 and 7 day interval. Error bars indicate mean ± standard error (n =3). ** and * denote significant differences at $P < 0.01$ and 0.05, respectively, compared with their respective control.

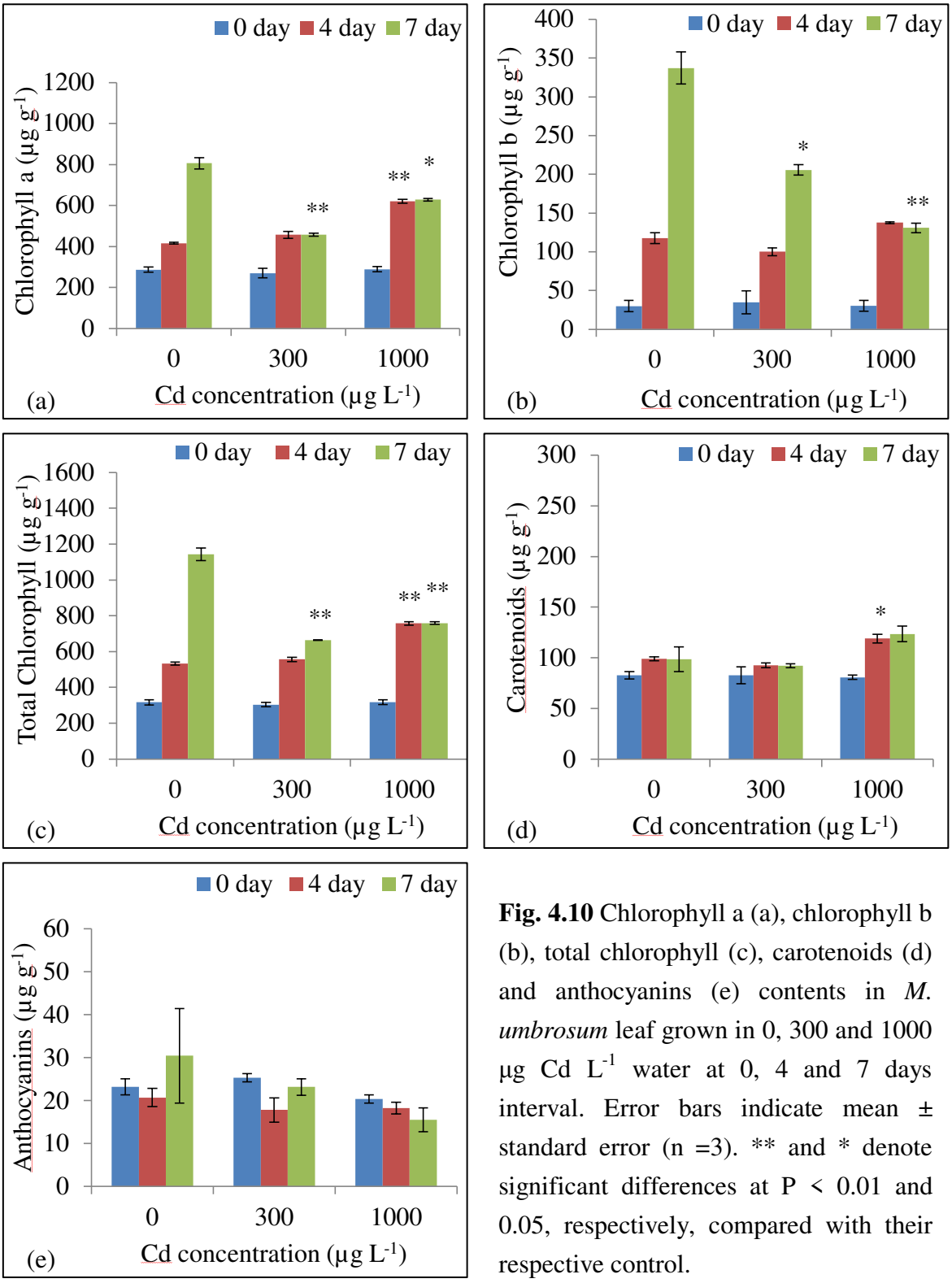


Fig. 4.10 Chlorophyll a (a), chlorophyll b (b), total chlorophyll (c), carotenoids (d) and anthocyanins (e) contents in *M. umbrosum* leaf grown in 0, 300 and 1000 µg Cd L^{-1} water at 0, 4 and 7 days interval. Error bars indicate mean ± standard error (n =3). ** and * denote significant differences at P < 0.01 and 0.05, respectively, compared with their respective control.

The present study showed that treatment of *M. umbrosum* with As supply increased K and Mg concentrations in the stems and leaves, respectively, up to 500 µg As L^{-1} treatment,

later on, decreases were seen compared to the control; however, As accumulation negatively influenced Ca accumulation in the leaves (Table 4.1). K and Mg have some positive effects on As uptake and growth of *M. umbrosum* at certain level (Fig. 4.8a).

Table 4.1 Composition of nutrient elements (oven dry basis) of *M. umbrosum* plant parts grown in As tainted water

Dose (µg L^{-1})	Plant parts	K (mg g^{-1})	Ca (mg g^{-1})	Mg (µg g^{-1})	Mn (µg g^{-1})	Zn (µg g^{-1})
0	Leaf	23.3±0.59	3.5±0.10	22.7±0.37	225.1±8.32	82.6±1.46
	Stem	17.5±0.73	2.9±0.15	18.0±1.11	120.1±3.94	39.3±1.88
	Root	8.4±0.63	3.9±0.08	4.9±1.62	94.0±5.65	37.9±1.19
200	Leaf	24.6±0.33	3.6±0.10	**40.9±0.72	**135.1±2.00	**70.0±1.84
	Stem	**25.7±0.69	4.7±0.26	**32.6±1.89	144.0±4.18	79.9±1.95
	Root	10.8±0.81	3.0±0.12	9.0±0.81	170.6±2.05	99.7±4.58
500	Leaf	21.9±0.30	*3.4±0.06	**32.5±3.10	**83.6±1.39	**63.3±1.37
	Stem	**26.0±0.31	4.2±0.14	**28.2±1.19	**92.8±2.85	78.6±4.30
	Root	12.1±1.32	4.9±0.20	6.3±0.75	148.6±7.73	93.7±2.26
1000	Leaf	17.2±0.65	**2.8±0.05	*24.0±1.51	**68.5±3.49	**52.8±2.65
	Stem	**22.5±0.48	4.1±0.03	15.9±1.31	*89.8±0.98	74.8±2.74
	Root	9.5±1.02	4.9±0.24	3.8±1.46	108.5±6.90	89.2±4.27

Each value indicated as Mean ± standard error (n=3); ** and * showed significantly difference against control or 0 (µg L^{-1}) at $p < 0.01$ and 0.05, respectively.

Enhanced Cd treatment decreased the Ca and Mg contents in the roots and leaves whereas K content increased in roots (Table 4.2). Cd might be competing with Ca and Mg during *M. umbrosum* uptake as all are divalent cations. Thus, potassic fertilizers could enhance the storage of Cd in *M. umbrosum* roots. Mn showed negative correlation with As accumulation (Table 4.1), however, significant positive correlation (Table 4.2) on Cd uptake within *M. umbrosum* as compared with the control. Zn concentration in leaves, ranging from around 50 to 70 µg g^{-1} for As (Table 4.1) and 65 to 70 µg g^{-1} for Cd treatment (Table 4.2), was significantly decreased by As and Cd treatment.

Table 4.2 Composition of nutrient elements (oven dry basis) of *M. umbrosum* plant parts grown in Cd contaminated water

Dose (µg L^{-1})	Plant parts	K (mg g^{-1})	Ca (mg g^{-1})	Mg (µg g^{-1})	Mn (µg g^{-1})	Zn (µg g^{-1})
0	Leaf	23.3±0.59	3.5±0.10	22.7±0.37	225.1±8.32	82.6±1.46
	Stem	17.5±0.73	2.9±0.15	18.0±1.11	120.1±3.94	39.3±1.88
	Root	8.4±0.63	3.9±0.08	4.9±1.62	94.0±5.65	37.9±1.19
300	Leaf	23.6±0.93	3.7±0.08	**19.0±0.11	**407.9±21.71	*71.7±2.43
	Stem	21.3±1.35	4.6±0.38	14.4±1.61	**361.4±22.37	67.2±4.73
	Root	*12.5±0.83	*3.6±0.17	5.6±0.65	*301.1±30.53	48.0±2.35
1000	Leaf	23.3±1.09	3.3±0.11	*20.2±0.41	*301.7±0.89	**67.4±2.03
	Stem	18.7±0.52	3.8±0.06	20.3±0.53	**168.9±5.36	54.6±2.62
	Root	**14.9±0.73	**1.1±0.01	7.0±0.79	*155.8±11.12	42.4±1.04

Each value indicated as Mean ± standard error (n=3); ** and * showed significantly difference against control or 0 (µg L^{-1}) at $p < 0.01$ and 0.05, respectively.

4.3.4 As and Cd uptake kinetics

The long term (24 h) concentration-dependent As (inorganic arsenite, organic MMAA and DMAA) and Cd influx isotherm exhibited a hyperbolic pattern in relation to the external concentration (Figs. 4.11b and d), and the data fit moderately well for arsenite and Cd, but not for MMAA and DMAA using *Michaelis-Menten* equation for linear regression model (Table 4.3). However, uptake kinetics data were described satisfactorily by the *Michaelis-Menten* equation using non-linear curve fitting by applying the natural logarithm of As and Cd influx within *M. umbrosum* (Figs. 4.11a and c) rather than the linear one (Tables 4.3 and 4.4). The V_{max} for inorganic arsenite (403.4 µg g^{-1} DW 24 h^{-1}) was about 5 to 9 times greater than that of MMAA and DMAA (Table 4), and marginally more than Cd (365.0 µg g^{-1} DW 24 h^{-1}). However V_{max} values were almost similar using non-linear and linear regression models but the K_m values were quite different from each other (Tables 4.3 and 4.4).

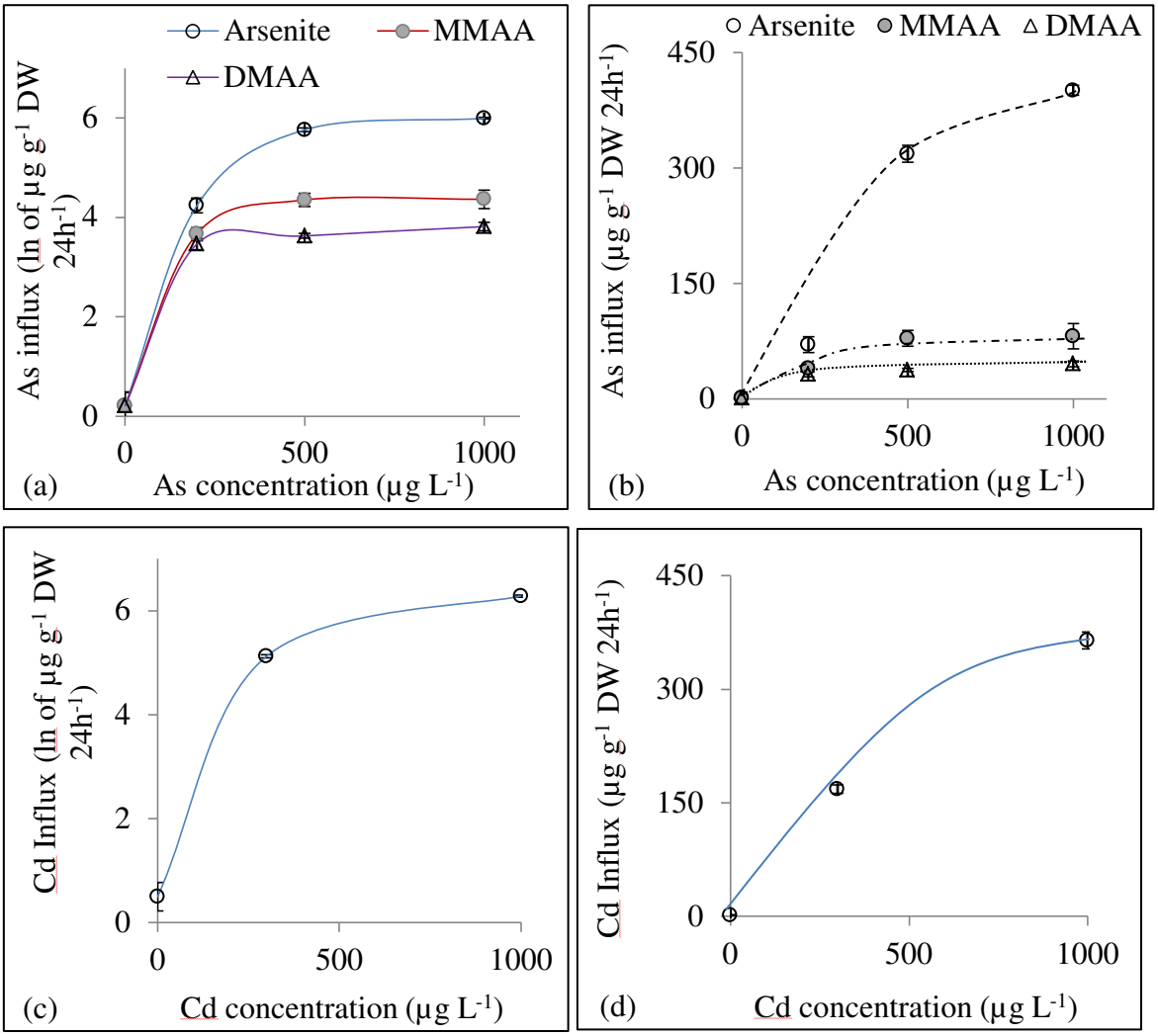


Fig. 4.11 Concentration dependent kinetics for arsenite, MMAA, DMAA (a, b) and Cd (c, d) within *M. umbrosum,* curve fitting with *Michaelis-Menten* non-linear (a, c) and linear (b, d) model.

Table 4.3 Linear regression model for uptake kinetic parameters of inorganic and organic As species; and Cd influx into *M. umbrosum*

	Linear regression model				
Species	V_{max} (µg g^{-1} DW $24h^{-1}$)	K_m (µg L^{-1})	R^2	*a* (slope)	*b* (Intercept)
Arsenite	420	454.3	0.9027	0.419	19.6
MMAA	90	354.4	0.7679	0.076	18.0
DMAA	55	378.6	0.7047	0.038	13.3
Cadmium	390	481.6	0.9684	0.348	27.2

Table 4.4 Non-linear model for uptake kinetic parameters of inorganic and organic As species; and Cd influx into *M. umbrosum*

Species	Non-linear model		
	V_{max} (µg g^{-1} DW $24h^{-1}$)	K_m (µg L^{-1})	R^2
Arsenite	403.4	141.7	0.9616
MMAA	81.45	120.2	0.9129
DMAA	44.70	94.21	0.8468
Cadmium	365.0	120.1	1.0000

4.3.5 As and Cd uptake mechanism

After Sephadex G-50 gel filtration of leaf extract from the plant treated with 1000 µg L^{-1} As and Cd, the maximum amounts of As (Fig. 4.12a) and Cd (Fig. 4.13a) were found at 90-108 and 80-104 mL of eluents, respectively. Absorbances at 280 nm were also counted for each fraction because proteins give the maximum absorbance at this wavelength. According to Fig. 4.12a, small amounts of As were scarcely observed in the high molecular weight fraction, whereas Cd-binding substance(s) might be those other than proteins, as they gave different peaks (Fig. 4.13a) in the G-50 column. SH (thiol) contents were measured by recording the absorbance at 480 nm. Data represented in Fig. 4.12b show that the maximum amounts of thiol (9 µM) were found in the fractions having the maximum amounts of As. On the contrary, control leaf eluent samples have lower thiol content (2.8 µM) when treated with the 0 mg As L^{-1} solution (Fig. 4.12c). So current study shows that As induced low molecular thiol compound(s) in *M. umbrosum* for detoxification or enhanced the accumulation of As from the water environment. In the case of Cd, no thiol formation occurred in high Cd-containing eluents (Fig. 4.13b). For confirmation of whether Cd formed any substances having thiol compounds or not, we again purified the eluents containing high Cd contents through a Sephadex G-15 gel filtration column and after performic acid treatment we measured the cysteic acid/cysteine

(thiol containing peptides), and other amino acids. The data listed in Table 4.5 indicate that only 0.95 nmol of cysteic acid which is 2.13% of the total amino acids were present in the eluents containing high levels of Cd. This means that Cd might follow different uptake mechanism than that for As.

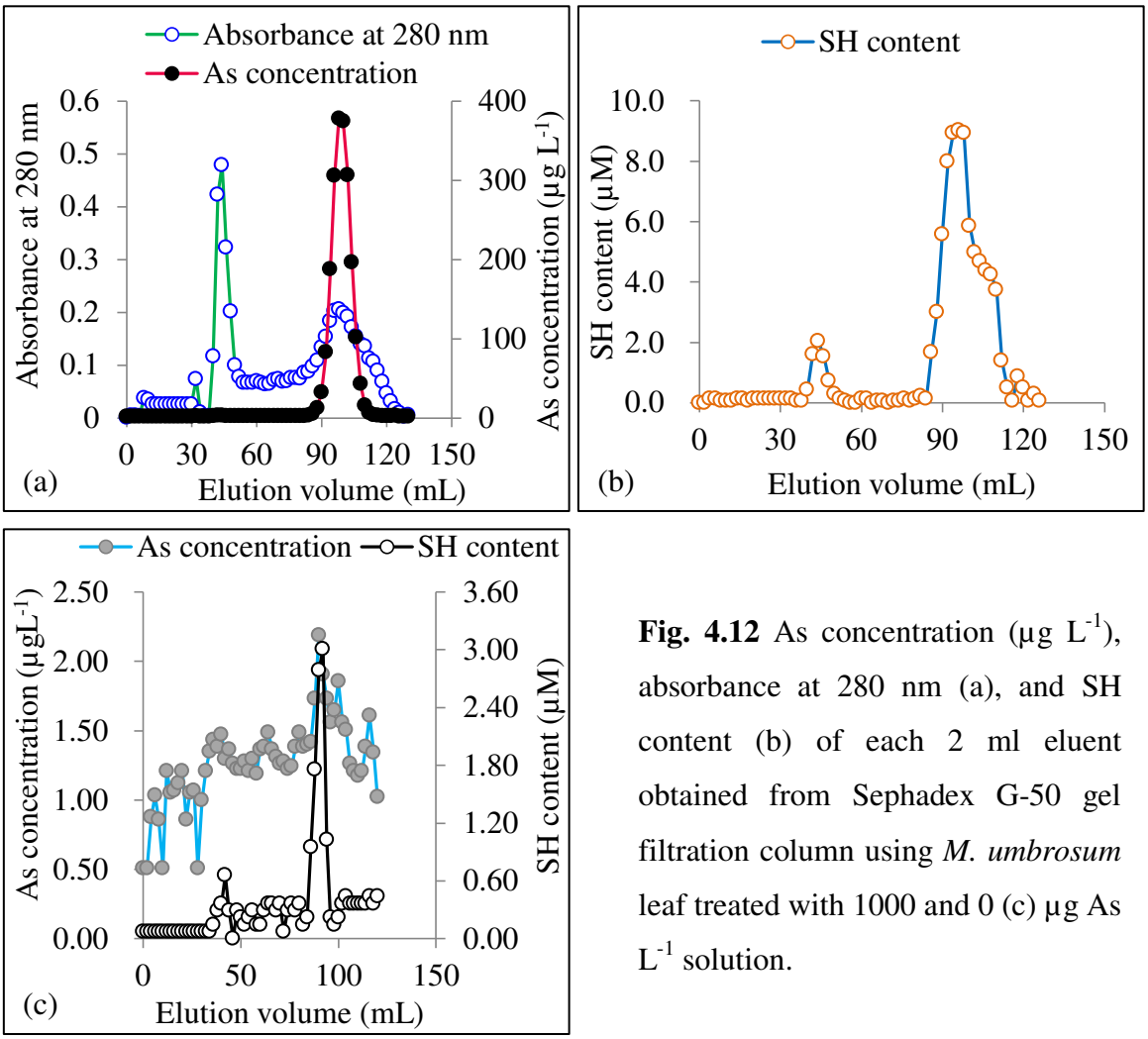


Fig. 4.12 As concentration (µg L^{-1}), absorbance at 280 nm (a), and SH content (b) of each 2 ml eluent obtained from Sephadex G-50 gel filtration column using *M. umbrosum* leaf treated with 1000 and 0 (c) µg As L^{-1} solution.

4.3.6 Phytofiltration potential

M. umbrosum shows effective phytofiltration of As and moderate accumulation for Cd, because it can decrease the total As concentration from about 500 and 200 to 40 and 25 µg L^{-1} (Fig 4.6a), respectively, without showing any phytotoxic effects. This are ranges below the national standard for drinking water in Bangladesh and China, which is 50 µg L^{-1} (World Bank, 2005). However, while Cd concentration in the solution could be lowered

from around 1040 and 310 to 415 and 80 µg L^{-1} (Fig 4.6b), respectively, with distinguished phytotoxic effects were seen. As and Cd concentration remained stable (205-220 and 1000-1017 µg L^{-1} for As and Cd, respectively) in the control treatment without plants for the entire experimental period. As and Cd uptake kinetic data also showed that *M. umbrosum* is a better accumulator of inorganic As than Cd and organic As at lower substrate concentration (Tables 4.3 and 4.4). As uptake was involved the thiol formation mechanism, which was different from the Cd uptake mechanism in *M. umbrosum.*

Table 4.5 Amino acid contents in 1000 µg L^{-1} Cd treated leaf (n=3)

Amino acids	Mean (nmol ± SEM)	%	Amino acids	Mean (nmol ± SEM)	%
CysCOOH	**0.95**±0.06	**2.13**	Met	0.04±0.01	0.08
Asp	5.87±2.73	13.19	Ile	2.41±1.00	5.43
Thr	2.98±1.34	6.69	Leu	3.66±1.52	8.23
Ser	3.09±1.30	6.94	Tyr	0.05±0.03	0.11
Glu	5.20±2.20	11.69	Phe	1.53±0.71	3.43
Gly	5.49±1.96	12.34	Lys	3.09±1.46	6.95
Ala	4.43±1.90	9.96	His	0.75±0.30	1.68
Cystine	0.11±0.03	0.25	Arg	1.22±0.51	2.74
Val	3.64±1.57	8.17	**Total**	44.48±17.89	100

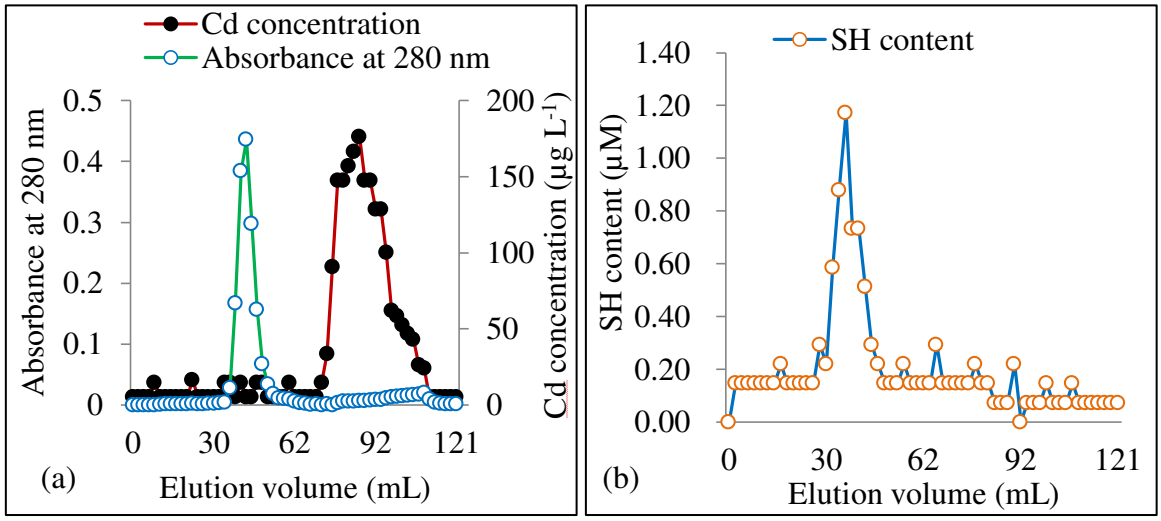


Fig. 4.13 Cd concentration (µg L^{-1}), absorbance at 280 nm (a), and SH content (b) of each 2 ml eluent obtained from Sephadex G-50 gel filtration column using *M. umbrosum* leaf treated with 1000 µg Cd L^{-1} solution.

4.4 Discussion

In this study, *M. umbrosum* was shown to be a strong accumulator of As, because it accumulated more than 1,000 µg As g^{-1} of stem and leaf biomasses (Fig. 4.4a) and also reduced the As concentration in the solution from 200 to 25 µg L^{-1} (Fig. 4.6a), whereas other plants used for phytofiltration, such as *Wolffia globosa*, decreased the total As concentration in the solution from 200 to 116 µg L^{-1} within the first 48 h, but no further decrease in As concentration was noted (Zhang *et al*., 2009). However, *M. umbrosum* was shown to be a moderate accumulator for Cd based on the <1000 µg Cd g^{-1} root and shoot biomass uptake that was observed (Fig. 4.4b). During excised root and shoot treatment, the plant parts shows high activity at first day but later on not so significant change of As (Fig. 4.7a) and Cd (Fig. 4.7b) in the treated water might be due to separating root and shoot part causes plant injury and developing of necrosis and chlorosis. This plant showed more resistant internally to high As and Cd accumulation than to external treatment. The As accumulation capacity in *M. umbrosum* is much higher than any non-hyper accumulator species, which suffer from As phytotoxicity when tissue As concentration exceeds 10-100 µg g^{-1} (Kabata-Pendias and Pendias, 1992). Cd-treated plant roots contained relatively higher concentrations of Cd than the As concentrations in the roots of As-treated plants. These results were consistent with the studies of Abhilash *et al.* (2009), who reported that *Lemna flava* roots contained higher amount of Cd than the peduncle and leaf separately, but that the total Cd concentration in the aerial parts was higher than in the roots.

Accumulations of As and Cd within *M. umbrosum* were significant up to 5^{th} (Fig. 4.6a) and 4^{th} day (Fig. 4.6b), respectively, since *M. umbrosum* can grown under-water in merged conditions and the whole plant can act as an active site for As and Cd uptake as there is no evidence of Fe-oxide deposition or physiochemical adsorption of these pollutants in the findings described by Robinson *et al*. (2006).

There is a co-relation between plant growth and photosynthetic pigments. The final fresh weight (Fig. 4.8a) of *M. umbrosum* showed that As initially enhanced the growth significantly ($p < 0.01$) up to the concentration of 500 µg L^{-1} in hydroponic culture due to the increase in photosynthetic pigments (Figs. 4.9a-e), but at higher concentration, the growth of the plant was reduced. Therefore it appeared that low-level As concentration stimulated plant growth. Mirza *et al*. (2010) also reported that growth and total chlorophyll contents of *Arundo donax* L increased in solutions up to the 600 µg As L^{-1}, with subsequent decreases at 1000 µg L^{-1}. Rahman *et al.* (2007b) found that chlorophyll contents and rice plant growth decreased with increasing As concentration in the soil. On the other hand, the final fresh weight was inversely characterized by Cd treatment (Fig. 4.8b) due to the significant reduction in chlorophylls a and b, and other pigments (Figs. 4.10a-e), which resulted in the development of some toxic symptoms, such as the yellowing of leaves and necrotic leaf margin. Stobart *et al*. (1985) also concluded that Cd inhibited the formation of chlorophylls by interfering with protochlorophyllide reduction and the synthesis of aminoevulinic acid in barley. Heavy metals generate reactive oxygen species, which damage photosynthetic pigments, in the plants grown under stress conditions (Romero-Puertas *et al*., 2002). Thus, the increased chlorophyll a and carotenoid levels in the As-treated leaves (Figs. 4.9a and d) is probably a part of a strategy adopted by the plant to counteract the toxic effects of the free radicals generated under As stress; a finding that agrees with other reports on other aquatic plants (Aslan *et al*., 2003).

As supply significantly increased K and Mg contents in shoot parts (Table 4.1). K in plants is preferentially transported to young meristematic tissues and has close relationships with protein synthesis, cytokine supply and plant growth (Mengel and Kirkby, 1987) whereas Mg serves as a core element of the chlorophyll molecule (Jones, 1998). Therefore K and Mg concentrations in the shoots of *M. umbrosum* had a significant relationship with plant

biomass production up to 500 µg As L^{-1} treatment. Carbonell *et al*. (1998) also reported that inorganic As increases K concentrations in *Spartina alterniflora* Loisel shoots, and a reduction in total dry biomass at a high level of As. On the other hand, K also serves as a dominant cation for counter balancing anions in plants (Marschner, 1995). Therefore, enhanced As uptake in *M*. *umbrosum* results in an increase in K concentration to balance the excessive anion presence caused by As hyper-accumulation. Tu and Ma (2005) also found that K might function as a counter cation for As hyper-accumulation in *Pteris vittata*. Increasing Mg concentration in *M*. *umbrosum* might indicate the increasing chlorophyll contents (Figs. 4.9a-c) and enhanced plant growth (Fig. 4.8a) in As treatment and vice versa for Cd (Figs. 4.8b and 4.10a-c). Ca is an essential macronutrient elements for plants (Jones, 1998), and its concentration decreases with increased As or Cd concentration in the growth medium of *M*. *umbrosum* (Tables 4.1 and 4.2). This may suggest that Ca has a limited role in the defense mechanism of the plant against As and Cd toxicity. Tu and Ma (2005) also reported that Ca concentration decreased with increasing As in the fronds of *Pteris vittata*, an As hyperaccumulator. Micronutrients (Mn and Zn) concentrations were higher in Cd-treated leaves (Table 4.2) than As treated leaves (Table 4.1). Mn and Zn concentrations significantly decrease with increased As level compare to respective measurement in the control (Table 4.1). Carbonell-Barrachina *et al*. (1997) showed that As caused a reduction in micronutrient contents (B, Cu, Mn, and Zn) in tomato plants (*Lycopersicum esculentum* Mill). Increasing micronutrients concentration in the Cd-stressed plants may be related to a "concentration effect", since biomass decreased with elevated doses of Cd in the hydroponic solution.

The long term (24 h) concentration-dependent arsenite and Cd uptake influxes were linear up to the 500 µg L^{-1} treatment (Figs. 4.11c and d). Decreased As and Cd influx after this level (500 µg L^{-1}) is probably due to toxicological inhibition. Meharg and Jardine (2003)

reported that the time dependent uptake of 0.1 mM arsenite in excised rice roots showed linear influx up to 30 min, and no further influx thereafter due to toxicological inhibition. V_{max} and K_m (*Michaelis-Menten* parameter) were calculated from these concentration-dependent experiments. The V_{max} value of arsenite, MMAA, DMAA and Cd were almost similar in order of magnitude by using linear and non-linear models (Tables 4.3 and 4.4), but the data were more well-fitted to the non-linear model as R^2 values: 0.9616, 0.9129, 0.8468 and 1.0, respectively than linear model (R^2 value 0.9027, 0.7679, 0.7047 and 0.9684, respectively). Different K_m values resulted from the different calculation methods, for example using natural logarithm (*ln*) to fit the data in the case of the non-linear model. However, the higher maximum K_m value for Cd (around 480 µg L^{-1}) than As (around 455 µg L^{-1}) indicated that *M. umbrosum* has higher affinity to uptake As (V_{max} = 420 µg g^{-1} DW 24 h^{-1}) than Cd (V_{max} = 390 µg g^{-1} DW 24 h^{-1}). At a slow rate, MMAA uptake showed hyperbolic curve and the limited uptake of DMAA occurred because to aerial tissues contain a smaller concentration of this species (data not shown), and its translocation from root to shoot is restricted (Odanaka *et al*., 1987; Carbonell-Barrachina *et al*., 1998). Abedin *et al*. (2002) also found the similar uptake kinetics of MMAA and DMAA in rice plants.

Arsenite within *M. umbrosum* appeared to involve an induction of thiol synthesis or binding with protein –SH groups (Figs. 4.12a and b), and the importance of these thiol groups to combat the biotic and abiotic stresses, including the stress imposed by As (Hartley-Whitaker *et al*., 2001; Zhao *et al*., 2003; Mishra *et al*., 2008; Srivastava and D'Souza 2009; Srivastava *et al*., 2010) at a specific level (500 µg As L^{-1}) compared to the respective control (Fig. 4.12c). Recent studies also support these current findings, and showed that eight species were identified as thiol-bound As species (PCs, GSH and cysteine), including three newly identified complexes: Cys-As(III)-PC_2, Cys-As-$(GS)_2$, and

GS-As(III)-desgly-PC_2 in C*eratophyllum demersum* macrophytes (Mishra *et al*., 2013). By contrast, Cd (Figs. 4.13a and b) is likely to be taken up by a mechanism other than thiol formation. Further confirmation by measuring the content of amino acids (Table 4.5) concluded that only 2.13% of the amino acids present in the Cd treated leaves samples were thiol containing cysteic acid. Cosio *et al*. (2004) found that Cd accumulation increases in *Thlaspi caerulescens* 'Ganges' and decreases in *Arabidopsis halleri* protoplasts indicating that Cd-permeable transport proteins are differentially regulated. They also concluded that Cd could be transported by a Zn and Ca pathway in *Thlaspi caerulescens* 'Prayon', whereas in 'Ganges', Cd was transported by other pathways (Cosio *et al*., 2004). Thus, Cd uptake mechanisms might vary from one species of plant to another. More intensive research, such as vacuole sequestration and other mechanism will be conducted in the future to determine the Cd uptake mechanism in *M. umbrosum.*

4.5 Conclusions

M. umbrosum has the potential to be an As and Cd phytofiltration species in drinking water contaminated with low levels of As and Cd without any phytotoxic effect, in addition to its beautification potential (from the aesthetic view point of phytoremediation) by culturing in an aquarium. This plant has high affinity to uptake inorganic As rather than Cd and organic As species, and the intensity of uptake order is Arsenite > Cd > MMAA > DMAA. As induced low molecular thiol containing compound(s) within *M. umbrosum* for detoxification or enhanced the accumulation of As from the water environment but Cd follow different uptake mechanism than that for As. Furthermore, it may play a significant role in the understanding of the As and Cd mobility and detoxification mechanisms within other aquatic plant systems. More intensive biochemical and physiological parameters should be analyzed before applying these findings at field levels.

References

Abedin, M.J., Feldmann, J., Meharg, A.A., 2002. Uptake kinetics of arsenic species in Rice plants. Plant Physiol. 128, 1120-1128.

Abhilash, P.C., Vimal, C.P., Srivastava, P., Rakesh, P.S., Chandran, S., Singh, N., Thomas, A.P., 2009. Phytofiltration of cadmium from water by *Limnocharis flava* (L.) Buchenau grown in free-floating culture system. J Hazard Mater. 170, 791-797.

Ali, H., Khan, E., Sajad, M.A., 2013. Review-Phytoremediation of heavy metals-concepts and applications. Chemosphere, 91, 869-881.

Asher, C.J., Reay, P.F., 1979. Arsenic uptake by barley seedlings. Funct. Plant Biol. 6, 459–466.

Aslan, M., Unlu, M.Y., Turkmen, N., Yilmaz, Y.Z., 2003. Sorption of Cadmium and Effects on Growth, Protein Content, and Photosynthetic Pigment Composition of *Nasturtium officinale* R. Br. and *Mentha aquatica* L. Bull Environ Contam Toxicol. 71, 0323-0329.

Assuncao, A.G.L., Schat, H., Aarts, M.G.M., 2003. *Thlaspi caerulescens*, an attractive model species to study heavy metal hyperaccumulation in plants. New Phytol. 159, 351–360.

Bae, W., Mehra, R.K., 1997. Metal-Binding characteristics of a phytochelatin analog $(Glu\text{-}Cys)_2Gly$. Elsevier Sci., 201-210.

Bae, W., Chen, W., Mulchandani, A., Mehra, R.K., 2000. Enhanced bioaccumulation of heavy metals by bacterial cells displaying synthetic phytochelatins. Biotechnol Bioeng. 70: 518-524 .

Barcelo, J., Poschenrieder, C., 2003. Phytoremediation: principles and perspectives. Contrib Sci. 2, 333–344.

British Geological Survey, 2000. *Executive summary of the main report of Phase I, Groundwater Studies of Arsenic Contamination in Bangladesh*, by British Geological

Survey and Mott MacDonald (UK) for the Government of Bangladesh, Ministry of Local Government, Rural Development and Cooperatives, Department of Public Health Engineering, and Department for International Development (UK). http://www.dainichi-consul.co.jp/english/article/DFID-sum.html [accessed- 11Nov. 2000].

Cai, Y., Georgiadis, M., Fourqurean, J.W., 2000. Determination of arsenic in sea grass using inductively coupled plasma mass spectrometry. Spect Acta Part B. 55, 1411-1422.

Carbonell, A.A., Aarabi, M.A., Delaune, R.D., Gambrell, R.P., Patrick, J.W.H., 1998. Arsenic in wetland vegetation: availability, phytotoxicity, uptake and effects on plant growth and nutrition. Sci Total Environ. 217, 189e199.

Carbonell-Barrachina, A.A., Burlo´-Carbonell, F., Mataix-Beneyto, J., 1997. Effect of sodium arsenite and sodium chloride on bean plant nutrition (macronutrients). J Plant Nutr. 20, 1617-1633.

Carbonell-Barrachina, A.A., Aarabi, M.A., DeLaune, R.D., Gambrell, R.P., Patrick, W.H., 1998. The influence of arsenic chemical form and concentration on *Spartina patens* and *Spartina alterniflora* growth and tissue arsenic concentration. Plant Soil, 198, 33–43.

Clemens, S., 2001. Developing tools for phytoremediation: towards a molecular understanding of plant metal tolerance and accumulation. Int J Occup Med Environ Health ,14, 235–239.

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

Cobbett, C., Goldsbrough, P., 2002. Phytochelatins and metallothioneins: roles in heavy metal detoxification and homeostasis. Annu Rev Plant Biol. 53, 159–182.

Cosio, C., Martinoia, E., Keller, C., 2004. Hyperaccumulation of Cadmium and Zinc in

Thlaspi caerulescens and *Arabidopsis halleri* at the leaf cellular level. Plant Physiol. 134, 716-725.

Degraeve, N., 1981. Carcinogenic, teratogenic and mutagenic effects of cadmium. Mutation Res. 86, 115–135.

Dhankher, O.P., 2005. Arsenic metabolism in plants: an inside story. New Phytol. 168, 503–505.

Duker, A.A., Carranza EJM, Hale M., 2005. Arsenic geochemistry and health. Environ Int. 31, 631–641.

Ellman, L.G., 1959. Tissue sulfhydryl groups. Arch Biochem Biophys. 82, 70-77.

Fulekar, M., Singh, A., Bhaduri, A.M., 2009. Genetic engineering strategies for enhancing phytoremediation of heavy metals. Afr J Biotechnol. 8, 529–535.

Grill, E., Mishra, S., Srivastava, S., Tripathi, R.D., 2006. Role of phytochelatins in phytoremediation of heavy metals. In: Environ. Bioremed. Tech. (Eds.: S.N. Singh and R.D. Tripathi). Springer, Heidelberg. pp. 101-145.

Hartley-Whitaker, J., Ainsworth, G., Meharg, A.A., 2001. Copper- and arsenate-induced oxidative stress in *Holcus lanatus* L. clones with differential sensitivity. Plant Cell Environ. 24, 713–722.

Hoagland, D.R., Arnon, D.I., 1950. The Water-culture method for growing plants without soil. *Circular.* California Agric Exp Sta. 347, 32 (2^{nd} Ed)

Hossain, M.F., 2006. Arsenic contamination in Bangladesh – a review. Agric Ecosyst Environ. 113, 1–16.

IARC, 2012. International Agency for Research on Cancer (IARC) Monographs on the Evaluation of Carcinogenic Risks to Humans, Agents classified by the IARC Monographs, Volume 23, Sup 7, 100C and Volume 58, 100C (last update, Jan 2014).

Inouhe, M., 2005. Phytochelatins. Braz J Plant Physiol. 17, 65–78.

Irtelli, B., Navari-Izzo, F., 2008. Uptake kinetics of different arsenic species by Brassica carinata. Plant Soil, 303, 105-113.

Isayenkov, S.V., Maathuis, F.J.M., 2008. The *Arabidopsis thaliana* aquaglyceroporin AtNIP7; 1 is a pathway for arsenite uptake. FEBS Lett. 582, 1625–1628.

Islam, M.S., Ueno, Y., Sikder, M.T., Kurasaki, M., 2013. Phytofiltration of Arsenic and Cadmium from the Water Environment Using *Micranthemum umbrosum* (J.F. Gmel) S.F. Blake as a Hyperaccumulator. Int J Phytoremediation, 15, 1010-1021.

Jabeen, R., Ahmad, A., Iqbal, M., 2009. Phytoremediation of heavy metals: physiological and molecular mechanisms. Bot Rev. 75, 339–364.

Jones, J.J.B., 1998. Plant Nutr. Manual. CRC Press, Boca Raton, FL, USA.

Kabata-Pendias, A., Pendias, H., 1992. *Trace elements in soils and plants*. Boca Raton, FL, USA: CRC Press.

Karenlampi, S., Schat, H., Vangronsveld, J., Verkleij, J., van der Lelie, D., Mergeay, M., Tervahauta, A., 2000. Genetic engineering in the improvement of plants for phytoremediation of metal polluted soils. Environ Pollut. 107, 225–231.

Küpper, H., Lombi, E., Zhao, F.J., McGrath, S.P., 2000. Cellular compartmentation of cadmium and zinc in relation to other elements in the hyperaccumulator *Arabidopsis halleri.* Planta 212, 75–84.

Ku¨pper, H., Mijovilovich, A., Meyer-Klaucke, W., Kroneck, P.M.H., 2004. Tissue- and age-dependent differences in the complexation of cadmium and zinc in the Cd/Zn hyperaccumulator *Thlaspi caerulescens* (Ganges ecotype) revealed by X-ray absorption spectroscopy. Plant Physiol. 134, 748–757.

Lichtenthaler, H.K., Wellburn, A.R., 1983. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. Biochem Soc Trans. 11, 1-592.

Ma, L.Q., Komar, K.M., Tu, C., Zhang, W., Cai, Y., Kennelley, E.D., 2001. A fern that hyperaccumulates arsenic. Nature, 409,579.

Ma, J.F., Yamaji, N., Mitani, N., Xu, X.Y., Su, Y.H., McGrath, S.P., Zhao, F.J., 2008. Transporters of arsenite in rice and their role in arsenic accumulation in rice grain. Proc Natl Acad Sci. 105, 9931-9935.

Marschner, H., 1995. *Mineral Nutrition of Higher Plants,* second ed. Academic Press Limited, London, UK.

Meharg, A.A., Naylor, J., Macnair, M.R., 1994. Phosphorus nutrition of arsenate-tolerant and nontolerant phenotypes of *velvetgrass*. J Environ Qual. 2, 234–238

Meharg, A.A., Hartley-Whitaker, J., 2002. Arsenic uptake and metabolism in arsenic resistant and nonresistant plant species. New Phytol. 154, 29-43.

Meharg, A.A., Jardine, L., 2003. Arsenite transport into paddy rice (*Oryza sativa*) roots. New Phytol. 157, 39–44.

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

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

Mendoza-Cózatl, D.G., Zhai, Z., Jobe, T.O., Akmakjian, G.Z., Song, W.Y., Limbo, O., Russell, M.R., Kozlovskyy, V.I., Martinoia, E., Vatamaniuk, O.K., Russell, P., Schroeder, J.I., 2010. Tonoplast-localized Abc2 transporter mediates phytochelatin accumulation in vacuoles and confers cadmium tolerance. J Biolog Chem. 285, 40416-26.

Mengel, K., Kirkby, E.A., 1987. *Principles of Plant Nutrition,* fourth ed. International Potash Institute, Worblaufen-Bern, Switzerland.

Mirza, N., Mahmooda, Q., Pervez, A., Ahmad, R., Farooq, R., Shah, M.M., Azim, M.R.,

2010. Phytoremediation potential of *Arundo donax* in arsenic-contaminated synthetic wastewater. Bioresour Technol. 101, 5815-5819.

Mishra, S., Srivastava, S., Tripathi, R.D., Trivedi, P.K., 2008. Thiol metabolism and antioxidant system complement each other during arsenate detoxification in *Ceratophyllum demersum* L. Aquat Toxicol. 86, 205–215.

Mishra, S., Wellenreuther, G., Mattusch, J., Stärk, H.J., Küpper, H., 2013. Speciation and Distribution of Arsenic in the Nonhyperaccumulator Macrophyte C*eratophyllum demersum.* Plant Physiol. 163, 1396-1408.

Mkandawire, M., Dudel, E.G., 2005. Accumulation of arsenic in *Lemna gibba* L. (ducweed) in tailing waters of two abandoned uranium mining sites in Saxony, Germany. Sci Total Environ. 336, 81–89.

Mondal, P., Majumder, C.B., Mohanty, B., 2006. Laboratory based approaches for arsenic remediation from contaminated water: Recent developments. J Hazard Mater. 137, 464–479.

Ng, J.C., 2005. Environmental Contamination of Arsenic and its Toxicological Impact on Humans. Environ Chem. 2, 146-160.

Odanaka, Y., Tsuchiya, N., Matano, O., Goto, S., 1987. Absorption, translocation and metabolism of the arsenical fungicides, iron methanearsonate and ammonium iron methanearsonate, in rice plants. J Pest Sci. 12, 199–208.

Petrick, J.S., Ayala-Fierro, F., Cullen, W.R., Carter, D.E., Aposhian, H.V., 2000. Monomethylarsonous acid (MMAIII) is more toxic than arsenite in change human hepatocytes. Toxicol. App. Pharmacol. 163, 203–207.

Pickering, I.J., Gumaelius, L., Harris, H.H., Prince, R.C., Hirsch, G., Banks, J.A., Salt, D.E., George, G.N., 2006. Localizing the biochemical transformations of arsenate in a hyperaccumulating fern. Environ Sci Technol. 40, 5010–5014.

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

Pulford, I., Watson, C., 2003. Phytoremediation of heavy metal-contaminated land by trees-a review. Environ Int. 29, 529–540.

Raab, A., Feldmann, J., Meharg, A.A., 2004. The nature of arsenic–phytochelatin complexes in *Holcus lanatus* and *Pteris cretica.* Plant Physiol. 134, 1113–1122.

Raab, A., Schat, H., Meharg, A.A., Feldmann, J., 2005. Uptake, translocation and transformation of arsenate and arsenite in sunflower (*Helianthus annuus*): formation of arsenic–phytochelatin complexes during exposure to high arsenic concentrations. New Phytol. 168, 551–558.

Rahman, M.A., Hasegawa, H., Ueda, K., Maki, T., Okumura, C., Rahman, M.M., 2007a. Arsenic accumulation in duckweed (*Spirodela polyrhiza* L.): A good option for phytoremediation. Chemosphere, 69, 493–499.

Rahman, M.A., Hasegawa, H., Rahman, M.M., Islam, M.N., Miah, M.A.M., Tasmen, A., 2007b. Effect of arsenic on photosynthesis, growth and yield of five widely cultivated rice (Oryza sativa L.) varieties in Bangladesh. Chemosphere, 67, 1072-1079.

Rahman, M.A., Hasegawa, H., Ueda, K., Maki, T., Rahman, M.M., 2008. Arsenic uptake by aquatic macrophyte Spirodela polyrhiza L.: interactions with phosphate and iron. J Hazard Mater. 160, 356–361.

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

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.* Collingwood, Victoria:

CSIRO. pp. 235–247.

Robinson, B., Kim, N., Marchetti, M., Moni, C., Schroeter, L., Dijssel, C., Milne, G., lothier, B., 2006. Arsenic hyperaccumulation by aquatic macrophytes in the Taupo Volcanic Zone, New Zealand. Environ Exp Bot. 58, 206–215.

Romero-Puertas, M.C., Palma, J.M., Gómez, M., Del Río, L.A., Sandalio, L.M., 2002. Cadmium causes the oxidative modification of proteins in pea plants. Plant Cell Environ. 25, 677-686.

Salem, H.M., Eweida, E.A., Farag, A., 2000. Heavy Metals in Drinking Water and their Environmental Impact on Human Health. ICEHM2000, Cairo University, Egypt, pp. 542–556.

Sanita' di Toppi, L., Gabbrielli, R., 1999. Response to cadmium in higher plants. Environ Exp Bot. 41, 105–130.

Sanita` di Toppi, L., Vurro, E., Rossi, L., Marabottini, R., Musetti, R., Careri, M., Maffini, M., Mucchino, C., Corradini, C., Badiani, M., 2007. Different compensatory mechanisms in two metal-accumulating aquatic macrophytes exposed to acute cadmium stress in outdoor artificial lakes. Chemosphere, 68, 769-780.

Schat, H., Llugany, M., Vooijs, R., Hartley-Whitaker, J., Bleeker, P.M., 2002. The role of phytochelatins in constitutive and adaptive heavy metal tolerances in hyperaccumulator and nonhyperaccumulator metallophytes. J Exp Bot. 53, 2381-2392

Schor-Fumbarov, T., Keilin, Z., Tel-Or, E., 2003. Characterization of cadmium uptake by the water lily *Nymphaea aurora.* Int J Phytoremediation, 5, 169–179.

Schmoger, M.E.V., Oven, M., Grill, E., 2000. Detoxification of arsenic by phytochelatins in plants. Plant Physiol. 122, 793-801.

Seth, C.S., 2012. A review on mechanisms of plant tolerance and role of transgenic plants in environmental clean-up. Bot Rev. 78, 32–62.

Sheoran, V., Sheoran, A., Poonia, P., 2011. Role of hyperaccumulators in phytoextraction of metals from contaminated mining sites: a review. Crit. Rev. Environ. Sci. Technol. 41, 168–214. Siddiqui, M.H., Kumar, A., Kesari, K.K., Arif, J.M., 2009. Biomining—a useful approach toward metal extraction. American-Eurasian J Agron. 2, 84–88.

Sims, D.A., Gamon, J.A., 2002. Relationships between leaf pigment content and spectral reflectance across a wide range o species, leaf structures and developmental stages. Remote Sens Environ. 81, 337-354.

Srivastava, S., D'Souza, S.F., 2009. Increasing sulfur supply enhances tolerance to arsenic and its accumulation in *Hydrilla verticillata* (L.f.) Royle. Environ Sci Technol. 43, 6308–6313.

Srivastava, S., Mishra, S., Dwivedi, S., Tripathi, R.D., 2010. Role of thiol metabolism in arsenic detoxification in *Hydrilla verticillata* (L.f.) Royle. Water Air Soil Poll. 212, 155-165

Stobart, A.K., Griffiths, W.T., Bukhari, J.A., Sherwood, R.P., 1985. The effect of Cd^{2+} on the biosynthesis of chlorophyll in leaves of barley. Plant Physiol. 63, 293-298.

Sun, R.L., Zhoua, Q.X., Sun, F.H., Jin, C.X., 2007. Antioxidative defense and proline/phytochelatin accumulation in a newly discovered Cd-hyperaccumulator, *Solanum nigrum* L. Environ Exp Bot. 60, 468–476.

Tong, Y.P., Kneer, R., Zhu, Y.G., 2004. Vacuolar compartmentalization: a second generation approach to engineering plants for phytoremediation. Trends Plant Sci. 9, 7–9.

Tripathi, R.D., Srivastava, S., Mishra, S., Singh, N., Tuli, R., Gupta, D.K., Maathuis, F.J.M., 2007. Arsenic hazards: strategies for tolerance and remediation by plants. Trends Biotechnol. 25, 158–165.

Tu, C., Ma, L.Q., 2005. Effects of arsenic on concentration and distribution of nutrients in

the fronds of the arsenic hyperaccumulator *Pteris vittata* L. Environ. Poll. 135, 333-340

Walker, J.M. (Ed.), 1996. *The Protein Protocols Handbook*, Humana press Inc.,Totowa, New Jersey, USA..

WHO., 2011. *Guidelines for drinking-water quality*, 4th ed. Chemical fact sheets-chemical contaminants in drinking water. Geneva:World Health Organization.

Williams, P.N., Islam, M.R., Adomako, E.E., Raab, A., Hossain, S.A., Zhu, Y.G., Feldmann, J., Meharg, A.A., 2006. Increase in rice grain arsenic for regions of Bangladesh irrigating paddies with elevated arsenic in groundwaters. Environ Sci Technol. 40, 4903–4908.

Woolson, E.A. (Ed.), 1975. *Arsenical Pesticides.* American Chemical Society, Washington DC, 176.

World Bank., 2005. Environment and Social Unit-South Asia Region. *Towards a More Effective Operational Response. Arsenic Contamination of Groundwater in South and East Asian Countries Volume II* Technical Report.

Yurekli, F., Kucukbay, Z., 2003. Synthesis of phytochelatins in Helianthus annuus is enhanced by cadmium nitrate. Acta Bot Croat. 62, 21–25.

Zhang, X., Lin, A.J., Zhao, F.J., Xu, G.Z., Duan, G.L., Zhu, Y.G., 2008. Arsenic accumulation by the aquatic fern *Azolla*: Comparison of arsenate uptake, speciation and efflux by *Azolla caroliniana* and *Azolla filiculoides*. Environ Poll. 156, 1149–1155.

Zhang, X., Zhao, F.J., Huang, Q., Williams, P.N., Sun, G.X., Zhu, Y.G., 2009. Arsenic uptake and speciation in the rootless duckweed *Wolffia globosa*. New Phytol. 182, 421-428.

Zhao, F.J., Ma, J.F., Meharg, A.A., McGrath, S.P., 2009. Arsenic uptake and metabolism in plants. New Phytol. 181, 777–794.

Zhao, F.J., Lombi, E., McGrath, S.P., 2003. Assessing the potential for zinc and cadmium

phytoremediation with the hyperaccumulator *Thlaspi caerulescens*. Plant Soil, 249, 37-43.

Zhao, F.J., Wang, J.R., Barker, J.H.A., Schat, H., Bleeker, P.M., McGrath, S.P., 2003. The role of phytochelatins in arsenic tolerance in the hyperaccumulator *Pteris vittata*. New Phytol. 159, 403–410.

Chapter V
Total Conclusions

Remediation in an eco-friend and sustainable way for contaminants from environments is now a burning issue in the world. Current research addressed to find out the eco-friend and sustainable solution of two carcinogenic and ubiquitous As and Cd pollution in the aquatic environment. At first, the following hypothesis or questions were considered before starting this study; - 1) Is phytoremediation sustainable, low cost and environmental friendly technology other than traditional physical or chemical methods? If so, 2) Is there any plants that has the capability to remove As and Cd from contaminated soil and water? If any plants is suitable for this phytoremediation, then 3) How many amounts of As and Cd can be uptake, and translocate to their harvestable parts? 4) Is there any phytotoxic effects of As and Cd on this plant? 5) Does absorption data fit any model? 6) What possible uptake mechanisms/binding substances for As and Cd within this plant? Finally 7) Is it potential for As and Cd phytofiltration from contaminated water environment?

To consider above these questions, different chapters were organized in this dissertation. Chapter I described the answer of question 1, and summarizes that phytoremediation is an eco-friend, sustainable, low cost, ecologically and aesthetically accepted solar driven technology that can be used for clean up As and Cd without causing any secondary pollution. There are some plants that can apply for As or Cd removal from soil and water. These statements were described in chapter I and II. Chapter II also presented the preparation of phytoremediation research in both naturally and artificially As contaminated soils using laboratory and field experiments. Among different plant species, an aquarium green plant named *Micranthemum umbrosum* has the potency to uptake both As and Cd from contaminated water environment. Chapter III indicates its potentiality of *M. umbrosum* as an As hyperaccumulator due to its high As accumulation (greater than 1000 $\mu g\ L^{-1}$) and high BCF value (>1000), and moderate accumulator of Cd. The accumulation

pattern is leaf > stem > root. This uptake pattern is clear for As rather than Cd. Chapter IV comprehensively described the answer of questions 4-7. From the analyses of macro- and micronutrients and different kinds of photpigments such as chlorophyll a, chlorophyll b, total chlorophyll, carotenoid and anthocyanin contents in the plant, As increases the growth of plant up to 500 μg L^{-1} without showing any phytotoxic effects. On the other hand, Cd shows little phytotoxic effects as decreases the growth of plant with increasing Cd concentration in water. Thus *M. umbrosum* is markedly excellent As phytofiltrator without phytotoxic effects but is moderate Cd accumulator with little phytotoxic effects. The plant showed more resistant at internal and external As concentration than Cd. Uptake of inorganic As much more higher than organic As, and absorption data were fitted well with *Michaelis-Menten* non linear model rather than linear model. Uptake kinetics data (V_{max} and K_m) indicated that the plant has high affinity to uptake inorganic As rather than Cd and organic As species, and the intensity of uptake order is Arsenite > Cd > MMAA > DMAA. Results of gel filtration chromatography and amino acid analyses showed that As induced low molecular thiol containing compound(s) in *M. umbrosum* for detoxification or enhancement of As accumulation from the water environment but Cd follow different uptake mechanism than that for As.

M. umbrosum can be an effective accumulator of Cd and a hyperaccumulator of As, as it can lower As toxicity to a level close to the limit recommended by WHO (10 μg As L^{-1}) and below the limit recommended by Bangladesh and China Government (<50 μg L^{-1}). Furthermore, it may play a significant role in the understanding of the As and Cd mobility and detoxification mechanisms within other aquatic plant systems. In addition, further investigation of precise mechanism for accumulation of As and Cd in *M. umbrosum* will be needed to understand metal sequestering system in organisms.