

## CHAPTER 4

### 4 Phytoremediation of Arsenic (As) by *Trapa natans* in Hydroponic System

#### 4.1 Introduction

Water contamination caused by heavy metals is a major problem worldwide. Both wastewater and insufficiently treated industrial water contribute continuously to disseminate either organic or inorganic contaminants (a danger to both the ecosystem and human health). In contrast to organic contaminants, heavy metals persist and are likely to accumulate in the environment.

Arsenic (As) is a naturally occurring element that is chemically classified as a metalloid, and it is widely distributed in natural environments. Inorganic As is a serious toxicant and can cause a variety of adverse health effects to humans after chronic exposures. The problem of naturally occurring As pollution in groundwater is a burning issue which has now been recognised as one of the greatest environmental hazards, threatening the lives of the million across the globe [1, 2, 3, 4]. Paths of entry include volcanic ash and weathering of the arsenic-containing minerals. Usually, As is found in the environment combined with other elements and inorganic As exists in combinations with elements such as oxygen, sulfur, and chlorine, whereas As combined with carbon and hydrogen is referred to as organic arsenic. Inorganic arsenic occurs naturally in soil and rocks, particularly in copper and lead containing minerals and ores.

During the past two decennia, occurrence of high concentrations of arsenic in drinking-water has been recognized as a major public-health concern in several parts of the world [1, 3]. The occurrence of arsenic (As) in groundwater pose a serious threat around the world. The problem is particularly severe in the alluvial and deltaic aquifer of India and Bangladesh where a large part of the population depends on groundwater as the source of drinking water and do not have basic purification facilities. Long-term ingestion of drinking water having As concentration beyond the permissible limit of 10 µg/L leads to detrimental effects on human health. Epidemiological studies have shown that inorganic As is a serious toxicant and can cause a variety of adverse health effects, such as cardiovascular, dermal changes, respiratory, neurological, pulmonary,

gastrointestinal, hematological, hepatic, renal, developmental, reproductive, immunologic lead to cancer and other degenerative effects of the circulatory and nervous system [3, 5]. In view of the above perspective WHO in 1993 has lowered its earlier permissible limit of 50 µg/L in drinking water to 10 µg/L.

In the state of Assam the conditions are very similar to those found in the Middle Gangetic Plain, with extensive Holocene Alluvial deposits along the flood plains of the river Brahmaputra. The immediate source material for As in groundwater is likely to be ferric arsenate, derived from an alteration product of the mineral arsenopyrite that was geologically transported to the Bengal delta and Assam Valley [5]. Contamination is believed to occur by chemical and biological processes that lead to the formation of arseneous acids from buried arsenate, chemically the plausible reaction would be hydrolysis of arsenate. Supply of excess oxygen during withdrawal of groundwater from tube wells appears to be responsible for hydrolysis [5].

Arsenic is not only toxic but also known to be carcinogenic and in the environment has both natural and anthropogenic origin. As is classified as Group-1 carcinogen to humans based on strong epidemiological evidence [6] and when absorbed into the body, arsenic undergoes some accumulation in soft tissue organs such as the liver, spleen, kidneys, and lungs, but the major long-term storage site for arsenic is keratin-rich tissues, such as skin, hair, and nails, making the measurement of arsenic in these biological specimens useful for estimating total arsenic burden and long-term exposure under certain circumstances [7]. 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 [8].

Arsenic uptake by plants is associated with the phosphate ion ( $\text{H}_2\text{PO}_4^-$ ) uptake mechanism, where presumably  $\text{As}^{\text{V}}$  is taken up as a  $\text{H}_2\text{PO}_4^-$  analog [9, 10, 11]. Ma et al. [12] reported As concentrations in the Brake fern, *Pteris vittata*, of up to 22 000 mg/kg<sup>-1</sup> (2.2%) on a dry-matter basis.

Human development has led to an increase in energy consumption and more waste production. Moreover, these developments are often linked to environmental pollution and raise new challenges in the field of environmental protection and conservation [13]. Toxic substances from industrial wastes or residential wastes are released into the environment and contribute a variety of toxic effects on living organisms in the food chain [14] by bioaccumulation and

biomagnification [15]. Most of the industries discharge untreated metal containing effluents into water bodies that results in the contamination of water and finally lead to health hazards for living beings. Arsenic (As) contamination of ground water is a global difficulty due to the risk arsenic poses to plants, animals and human health [16]. Many countries around the world (including Taiwan, Argentina, India, Bangladesh, Mexico, Hungary, and Chile) have reported extensive arsenic contamination of their groundwater supplies [17, 18]. Use of this contaminated water for irrigation of crops has led to elevated concentrations of arsenic in agricultural soils. The detrimental effects of arsenic contamination range from skin diseases to serious diseases such as cancer.

Arsenic can exist in four oxidation states (-3, 0, +3, +5) and is unique among oxyanion-forming elements. The distribution and speciation of arsenic in the environment is a function of pH, redox potential (Eh) and microbial activity [18, 19]. The As(III) is generally predominant in anoxic ground water whereas As(V) is found in aerobic surface water. Toxicity of arsenic depends on its oxidation states, As(III) is more toxic than As(V) due to its greater cellular uptake [20, 21]. The provisional limit of arsenic in drinking water as recommended by the World Health Organization (WHO) is 10 µg/L. People have become more concerned because of its chronic and epidemic toxic effects to humans through widespread contamination of water and food crops through natural release of the element from aquifer rocks in Bangladesh [22, 23] and West Bengal, India [24].

Most of the areas within the Northeastern states of India with high As concentration implying that millions of people are at serious risk of As poisoning [25]. A study conducted by The North Eastern Regional Institute of Water and Land Management (NERIWALM) in 2007 revealed that arsenic levels in some aquifers of Assam, Manipur, Tripura and Arunachal Pradesh were above 300 ppb. According to the WHO, consumption of water contaminated with arsenic levels of over 50 ppb can cause skin lesions and even cancer. Thousands of underground water sources in India's northeast are unfit for consumption due to highly toxic contamination of arsenic [4]. In a recent study, Assam deep well arsenic concentrations were found to be very high, which may result in severe problems of arsenicosis in the near future [26, 27]. So, a long term environmental planning is required to get rid of the danger of this pollution of groundwater. In view of this, various conventional methods such as electro dialysis, reverse-osmosis, and

adsorption, are used for purification of wastewater and to remove these toxic contaminants [28, 29 30]. But most of these methods are expensive and not eco-friendly. Phytoremediation is an emerging technology for cleaning up contaminated sites that has aesthetic advantages and long-term applicability [31]. It can also be defined as the use of green plants to remove pollutants from the environment or to render them harmless [32, 33] and can be applied to both organic and inorganic pollutants present in soil, water, or the air by efficient use of plants to remove, detoxify or immobilize contaminants through the natural, biological, chemical or physical activities or processes of the plant [34, 35]. There are various aspects of phytoremediation: phytoextraction, phytodegradation, rhizofiltration, phytostabilization and phytovolatilization. Phytoextraction involves using hyperaccumulating plants to remove the contaminant from the contaminated media and concentrate it in their above ground plant tissue, which is periodically harvested. The metal-enriched plant residue can be disposed of as hazardous material and, if economically feasible, used for metal recovery [35]. The key to using hyperaccumulators in phytoremediation lies in the rate of biomass production, coupled with the concentration of the element transferred to the plant matter [36]. Many hyperaccumulating plants species have been reported but the fern *Pteris vittata* was the first arsenic hyperaccumulator reported [37] and was discovered in an abandoned wood treatment site in Central Florida. Many plant species like *Pityrogramma calemelanos* [38] and several species of *Pteris* [12, 37, 39, 40, 41] and Brassica family [42] have been reported to be arsenic hyperaccumulators. In recent studies, some species of submerged macrophytes have shown considerable potential to accumulate As and to act as phytofiltrators of As-contaminated water, such as *Hydrilla verticillata* (L.f.) Royle [43] and *Vallisneria neotropicalis* [44]. After heavy metal accumulation by plants, there is some evidence that subcellular distribution and different chemical forms of heavy metals may be associated with metal tolerance and detoxification in plants [45, 46].

*Trapa natans*, commonly known as water chestnut, is an aquatic macrophyte typical of natural wetlands. It possesses a submerged flexuous stem that anchors into the mud and extends upwards to the water surface and features a rosette of floating, fan-shaped leaves having a slightly inflated petiole. Recently it was also found to have phytoremediative potential and research work is being done in the respective field. Manganese tolerance and the ability of *Trapa natans* L. to

hyperaccumulate the metal inside specialized cells (20000 g/g [dry weight]) were studied [47]. In previous studies, it was shown that the manganese resistance of this plant is linked to induction of chelating phenolic in the floating leaves [48]. Leaves are, in fact, rich in phenolic compounds and anthocyanin [48]. Anthocyanin accumulation in leaves might be suggested that it may play a role in the mechanisms reducing the toxic effects of the metal. The arsenic accumulation properties of *T. natans* have not been investigated yet. Further, the alarming pictures of arsenic contamination of ground water and continuous consumption of this water by the local population have the potential of posing serious health hazards. Keeping the above facts in mind, the present investigation was carried out to test the efficacy of *T. natans* as an As hyperaccumulator. The phytoaccumulation potential of *T. natans* and translocation in treated plants was also studied. Further *T. natans* biomass is characterized by SEM-EDX and FTIR techniques to know the absorption of arsenic ions.

## **4.2 Phytoremediation of As**

The first report of a fern, which accumulate arsenic, was published by Ma et al. [12]. They reported that *Pteris vittata* was a highly efficient accumulator of arsenic. *Pteridophytes* (or ferns or horsetails) are little known phyla of plant kingdom. As mentioned above, they are ancient plants with a long fossil record and they are diverse both in habitat and morphology. *Pteris*, one of the two genera have been found, so far, to accumulate arsenic has been detected in rocks from the Miocene period.

Arsenic tolerant flora has been found in the South West of England on highly acidic mine containing 10,000-30,000 mg/kg arsenic in the surface horizons [49]. Arsenate, the dominant form of arsenic in well drained soils, competes for uptake with phosphate in plants and alteration of the phosphate transport system is necessary if plants are to suppress arsenic uptake [50]. A comprehensive review by Meharg and Hartley-Whitaker [51] of arsenic uptake by plants suggests very complex interrelationships between mycorrhizal associations and arsenate transport within the plants. At present, *Pityrogramma calemelanos* [38] and several species of *Pteris* family [12, 51 52] have been reported to be arsenic hyper-accumulators. *Pteris cretica*, a fern that has naturalized in Cornwall, does hyperaccumulate arsenic. However, although two native ferns of Cornwall, *Athyrium filix-femina*

(Lady Fern) and *Phyllitis scolopendrium* (Harts Tongue fern), appear to be primary colonizer of arsenic rich coal mine waste in Cornwall [53]. Francesconi et al. [38] approximately calculated the reduction in arsenic concentration from soil containing 500mg/kg arsenic by using the plant *Pityrogramma calemelanos*.

**Table 4.1 Summary of studies investigating As uptake by plants**

Origin of As	Plants species	Field exp. or Lab exp.	Conc. of As (ppm)	References
Weathering of rocks and soils	<i>Pteris vittata</i>	Lab exp.	0.046	[54]
Wood preservatives	<i>Pteris vittata</i>	Lab exp.	50 & 200	[55]
Mining area	<i>Artemisia aucheri, Astragalus myriacanthus, Ferula oopoda, Gundelia tournefortii and Rumex ribes</i>	Lab exp.	102.5 to 300	[56]
Mining area	<i>Pteris vittata and Brassica juncea</i>	Field exp.	80	[57]
Industrial wastes	<i>Pteris vittata</i>	Field exp.	800	[58]
Soluble salt in water	<i>Brassica rapa</i>	Field exp.	10,100 and 1000	[59]

### 4.3 Arsenic bioaccumulation coefficient

The concentration of As in the plant alone may not necessarily explain its efficiency in extracting the element from soil or water, because the substrate As concentration partially determines plant uptake. It is therefore necessary to compare the concentration of As in the plant in relation to the concentration found in the environment in which the plant is growing. Logically, a plant growing in a medium rich in (bioavailable or total) As would be expected to contain a higher amount of that element in its tissues. However, in phytoremediation, an important trait of the plants is their ability to extract As even if it is present in low concentrations in the substrate.

## 4.4 Materials and Methods

### 4.4.1 Experimental set-up and design

Healthy and young plants of *T. natans* were collected in clean polythene bags from Joysagar pond (not contaminated with arsenic) of Sibsagar District of Assam, India. The collected plants were washed and rinsed properly with tap water and

placed in plastic tubs under natural sunlight for seven days to let them adapt to the new environment (acclimatization). After acclimatization, the plants were taken out and washed again properly. Plants of similar size and weight (35 g) were selected for the experiment. The treatment of arsenic to *T. natans* was given in the concentration of 1.28 ppm and 10.80 ppm. Hoagland's nutrient solution was prepared and added to each of the tubs including the control to supplement nutritional requirements. The nutrient solution consisted of 4.0 mM CaNO<sub>3</sub>, 2.0 mM MgSO<sub>4</sub>, 4.0 mM KNO<sub>3</sub>, 0.4 mM(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 2 μM MnSO<sub>4</sub>, 0.3 μM CuSO<sub>4</sub>, 0.8 μM ZnSO<sub>4</sub>, 30 μM NaCl, 0.1 μM Na<sub>2</sub>Mo<sub>4</sub>, 1.43 μM KH<sub>2</sub>PO<sub>4</sub>, 10 μM H<sub>3</sub>BO<sub>3</sub> and 20 μM Fe-Na-EDTA (All obtained from Merck Specialties) [60].

The Hoagland solution was modified by omitting ferrous sulfate in order to prevent the As precipitation by iron. The entire set up ran for seven days. Chlorophyll (in 3<sup>rd</sup>, 5<sup>th</sup> and 7<sup>th</sup> day intervals) and proline (on 7<sup>th</sup> day) content in both control and As treated plants was measured using UV-Visible spectrometer [UV-1700 Pharma Spec.]. Morphological symptoms were recorded after 2<sup>nd</sup>, 5<sup>th</sup> and 7<sup>th</sup> day intervals. Milli-Q water was added daily to compensate for water loss through plant transpiration, sampling and evaporation. The As (III) stock solution (1000 mg/L) was prepared in ultra-pure water with analytical grade NaAsO<sub>2</sub> and other solutions were prepared from this stock solution. Arsenic accumulation in different parts of plants was determined after harvest.

#### **4.4.2 Chlorophyll Extraction and Estimation**

The method used for estimation of chlorophyll was given by Anderson and Boardman [61]. Total chlorophyll content was determined by using the following formula:

Total chlorophyll= {(12.7XA<sub>645</sub>) + (8.02XA<sub>663</sub>)} X V/1000XW mg chlorophyll/g fresh leaf weight.

Where,

A<sub>645</sub>= Absorbance at 645 nm wavelength

A<sub>663</sub>= Absorbance at 663 nm wavelength

V= Final volume of the extract (mL)

W= Fresh weight of the leaf (g)

#### **4.4.3 Proline Estimation**

Proline is a α-amino acid, one of the twenty DNA-encoded amino acids. Its codons are CCU, CCC, CCA and CCG. It is not an essential amino acid, which

means that the human body can synthesize it. Proline is the precursor to hydroxyproline, which is a major amino acid found in the connective tissue in the body i.e. collagen. Proline is different from other amino acids in that it has a secondary amino group and contains a pyrrole ring, such as found in hemoglobin and the cytochromes.

In plants, proline is a non-essential amino acid. It acts as osmoprotectant. Under stress condition, concentration of proline increases. It protects the structure of protein and acts as dehydration inhibitor.

The method used for estimation of proline was given by Bates et al. [62]. Proline content was determined by the following formula: mM of proline/gram tissue = mg proline /ml x ml of toluene/115.5 x 5/g sample

**Procedure:** 0.5gm of sample is taken in a mortar with 10ml of 3% aqueous sulphosalicylic acid and filter with Whatmann No. II filter paper. The extraction is repeated and the filtrate is cooled. To 2ml of filtrate, 2ml of each of glacial acetic acid and Ninhydrin are added and mixed. Then it is kept in a boiling water bath for 1hr and then the reaction is terminated by placing in ice-bar. 4ml of toluene is added and mixed vigorously for 20-30 sec. Aspirate the chromophore (toluene layer) and warm to room temperature. Absorbance of the red color is measured at 520 nm against a reagent blank. The amount of proline is calculated using a standard curve prepared from pure proline and expressed on fresh weight basis of the sample. Standard curve must be prepared in the range of 0.1-36  $\mu$ mol.

#### 4.4.4 Sample Preparation for As Accumulation Analysis

On the seventh day of the treatment, the plants were harvested from each tub. They were washed properly with tap water and then deionized water. Plants were then separated into leaves, shoot and root. All plant parts were then oven dried using digestion bomb at 65<sup>0</sup>C to a constant weight. After measuring dry weight, the plants were ground. Approximately 0.5g of each plant sample was taken and mixed with 15 ml high purity HNO<sub>3</sub>-HClO<sub>4</sub> (3:1) acid, and allowed to stand at room temperature overnight and then heated to 140-180<sup>0</sup>C for complete digestion (Reagent blanks were processed to ensure that As was not added during sample preparation). The digested solution was analyzed for As using ICP-OES [63].

##### 4.4.4.1 Bioconcentration Factor (BCF)

Bioconcentration of heavy metal by aquatic organisms is described as the bioconcentration factor (BCF), which is the ratio of heavy metal accumulated by



plants to that dissolved in the surrounding medium. For this, two bioconcentration factors were computed from the plant compartment concentrations as:

$$BCF^a = C_{\text{roots}}/C_{\text{water}} \quad (1)$$

$$BCF^b = C_{\text{aerial (shoot+leaf)}}/C_{\text{water}} \quad (2)$$

#### **4.4.4.2 Translocation Factor (TF)**

The translocation of heavy metal from the roots to harvestable aerial part is generally expressed as the translocation factor (TF). It was calculated on a dry weight basis by dividing the heavy metal concentration in aerial parts (peduncles + leaf) by the heavy metal concentration in root. Based on the above two equations (1) and (2), the translocation factor can be expressed as

$$TF = BCF^b/BCF^a \quad (3)$$

To evaluate the potential of plants for phytoextraction, the translocation factor (TF) was used. This ratio is an indication of the ability of the plants to translocate metal from the roots to the aerial parts of the plants [64]. It is represented by the ratio

$$TF = \text{Metal concentration (shoot + leaves)}/\text{Metal concentration (roots)}.$$

Metals that are accumulated by plants and largely stored in roots of the plants are indicated by TF values <1, with value >1 indicating that metals are stored in the stem and the leaves.

One reason for slow translocation of As from root to shoot could be due to that trivalent arsenite are easily trapped in the root, but under anaerobic conditions, much of the As in the cells was a pentavalent arsenate and this arsenate again is partly reduced to arsenite due to the activity of endogenous arsenate reductase enzyme, conjugated with thiols, and sequestered in the root vacuole [65].

#### **4.4.5 SEM-EDX Studies**

Roots, shoots and leaves were subjected to primary fixation using 3% glutaraldehyde in 0.05M phosphate buffer for 90 minutes, followed by secondary fixation in 2% osmium tetroxide in 0.01M Sodium cacodylate buffer for 30 min. [66]. The samples were dehydrated in an acetone series. SEM images were recorded using SEM model JEOL JSM-6390 LV, attached with energy dispersive X-ray unit, with an accelerating voltage of 20 kV.

#### 4.4.6 Sample Preparation for the Fourier Transform Infrared Spectrometer (FTIR)

An infrared spectrum of *T. natans* root, shoot and leaf biomass with or without arsenic loaded were obtained to determine which functional groups may have contributed to the arsenic absorption. Plants were washed with tap water followed by double distilled water. All the plant parts were dried at 65 °C for 48 hours. All the plant parts were weighted separately (as 1% w/w) with potassium bromide (KBr) to be ready for FTIR [(Spectrum -100) Perkin-Elmer] measurements.

### 4.5 Results

#### 4.5.1 Morphological Changes due to As treatment in *T. natans*

The plants were brought for the experiment was healthy and green.

**Table 4.2** Some of the morphological changes on 3<sup>rd</sup>, 5<sup>th</sup> and 7<sup>th</sup> day intervals

Species	Concentrations	Morphological change on		
		3 <sup>rd</sup> day	5 <sup>th</sup> day	7 <sup>th</sup> day
<i>T. natans</i>	Control	No change	No change	No change
	1.28 ppm	Slight yellow on leaf tip.	Brown spots along with chlorosis.	Leaf fall
	10.80 ppm	Yellow color spreads to middle of leaf.	Leaf fall	Severe chlorosis with brown spots on whole leaf.



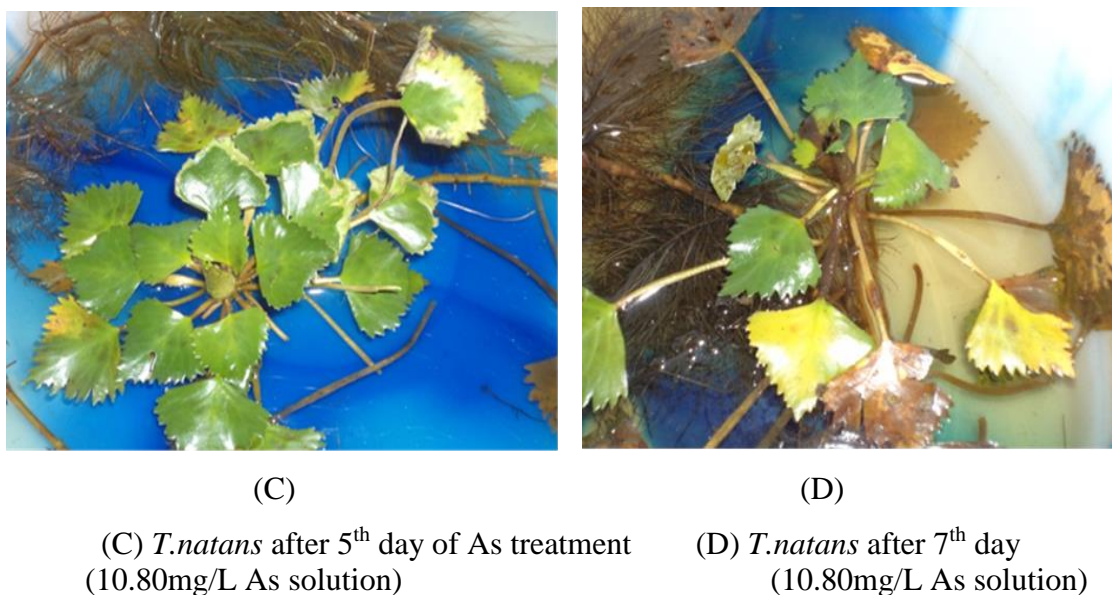
(A)

(A) *T. natans* in control condition



(B)

(B) *T. natans* after 3<sup>rd</sup> day of As treatment (10.80 mg/L As solution)



**Figure 4.1** Some of the morphological changes in *T. natans* on 3<sup>rd</sup>, 5<sup>th</sup> and 7<sup>th</sup> day intervals

#### 4.5.2 Effect of Arsenic on Total Chlorophyll and Proline Content

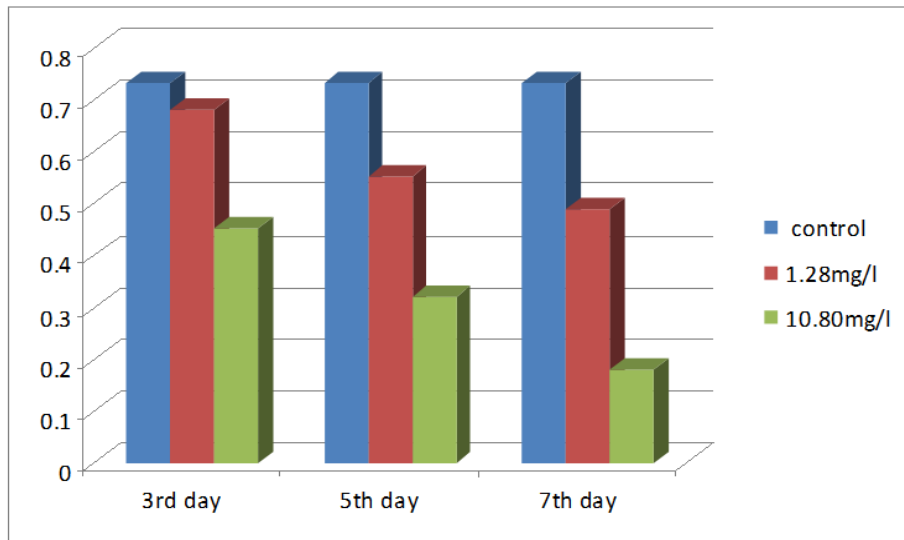
Arsenic accumulation results in a decrease in chlorophyll content in *Trapa natans*. Total chlorophyll content was found to be reduced as the concentration of arsenic increases (**Figure 4.2**). However, chlorophyll concentration was remained unchanged i.e. 0.73 mg chlorophyll/g fresh weight in control plants. At concentrations, 1.28 mg/L and 10.80 mg/L, chlorophyll content was reduced gradually from 0.68 mg chlorophyll/g fresh leaf weight, 0.55 mg chlorophyll/g fresh leaf weight, 0.49 mg chlorophyll/g fresh leaf weight and 0.45 mg chlorophyll/g fresh leaf weight, 0.32 mg chlorophyll/g fresh leaf weight, 0.18 mg chlorophyll/g fresh leaf weight for both arsenic treatments respectively on 3<sup>rd</sup>, 5<sup>th</sup> and 7<sup>th</sup> days of intervals. Proline concentration increases along with the increase in the concentration of arsenic (**Figure 4.2A**). The concentration of proline increased from 8.31  $\mu$ M /gm leaf tissue to 11.02  $\mu$ M proline/ gm tissue in 1.28 mg/L and 10.80 mg/L of arsenic concentration respectively.

**Table 4.3** Total chlorophyll content in the *T. natans* species

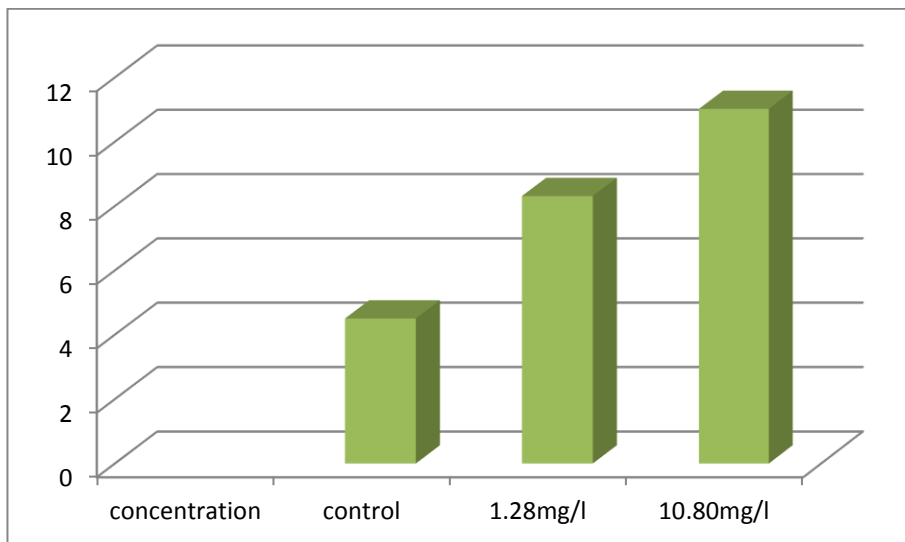
SPECIES	Concentration of stock solution (mg/L)	Chlorophyll content in mg/g fresh wt. on		
		3 <sup>rd</sup> day	5 <sup>th</sup> day	7 <sup>th</sup> day
<i>T. natans</i>	Control	0.73	0.73	0.73
	1.28	0.68	0.55	0.49
	10.80	0.45	0.32	0.18

**Table 4.3 (A) Amount of proline synthesis in the *T. natans* is given below**

SPECIES	Concentration of stock solution (mg/L)	Proline content in u mol/g of tissue on 5 <sup>th</sup> day
<i>T. natans</i>	Control	4.5
	1.28	8.31
	10.80	11.08



**Figure 4.2 Graphical representation of effect of arsenic on total chlorophyll content**



(A)

**Figure 4.2(A) Graphical representation of amount of proline synthesis under stress condition**

Proline accumulation in plants is a response to heavy metals to maintain the osmotic balance in cells of plants, which can be used as a physiological parameter for the tolerance response of plants. Singh et al. [67] suggested that the accumulation of proline in plants protect the cell membrane and proton pump against damage. Qureshi et al. [68] found that in Pb -induced oxidative stress and metabolic alterations in *Cassia angustifolia*, the proline concentrations increased at 60 days after treatment. The results of our study showed that the concentration of proline in the leaves increased from 8.31 micro mol proline/gm tissue in 1.28 mg/L As to 11.02 micro mol proline/ gm tissue in 10.80 mg/L of As concentration, indicating that *T. natans* was able to respond against As stress by Proline osmotic regulation.

The involvement of proline in response of plants to saline, water and low temperature stresses has been known since a relatively long time. Increase of this amino acid also has been reported as a general response to stress in plants [69]. Accumulation of proline in response to excess metal such as Cu, Cd, Zn and Ni was described by several authors in various plants [70].

#### **4.5.3 ICP Analysis**

This study was undertaken to evaluate the potential of *T.natans* for the remediation of As from hydroponic solution of different concentration namely from 1.28 mg/L and 10.80mg/L. The ability of the plant to take up As from the aqueous solution is evident from the analysis of the solution obtain by digesting different parts of the *T.natans* withdrawn at different interval of time using ICP-OES (Opima-2100 Perkin Elmer).

**Table 4.4 Accumulation of As by the *T. natans***

Initial Concentration of Solution (mg/L)	Concentration in Root (mg/kg) dry wt.	Concentration in Fruit (mg/kg) dry wt.	Concentration in Shoot (mg/kg) dry wt.	Concentration in Leaf (mg/kg) dry wt.
1.28	310.0	190.0	170.0	150.0
10.80	2890.0	1734.0	1570.0	570.0

Accumulation of As in the plant organs was highest in roots in both the sets of initial concentrations. The fruit accumulated more As than shoot and leaf. The order of As accumulation is root>fruit>shoot>leaf. Direct sorption of As in the

solution happens through the leaves; according to the morphology of this plant, leaves are in contact with the solution having large surface area [71].

#### 4.5.4 Translocation of As

##### 4.5.4.1 BCF and TF

The bioconcentration factor (BCF) is a useful parameter to evaluate plant's potentiality to accumulate metal, it provides the ability index of a plant to accumulate metals with respect to metal concentration in the substrate and it was calculated on a dry weight basis [72]. 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.

The bio-concentration factor of 640.62 was obtained in plants treated with 1.28 mg/L of As solution and 626.29 in 10.80 mg/L of As concentration respectively. The maximum bioconcentration factor (BCF) values were found at the 7<sup>th</sup> day in 1.28 mg/L As concentration and translocation factor (TF) was also high (1.64) in the same concentration. The experimental results demonstrated that this aquatic macrophyte has a phytoremediation potential for removing As from As contaminated water.

**Table 4.5 BCF and TF of *T.natans* on 7<sup>th</sup> day**

Metal concentration (mg/L)	BCF	TF
1.28	640.62	1.64
10.80	626.29	1.34

Metals that are accumulated by plants and largely stored in the roots of plants are indicated by TF values < 1, TF values greater than 1 is indicated translocation to the aerial parts of the plants. Zhu et al. [73] reported that the BCF factor values of *Eichhornia crassipes* are very high for Cd, Cu, Cr and Se at low external concentration, and they are found to decrease with the increase in external concentration [74, 75]. The As accumulation in *T. natans*, however, was considerably low compared to the results of Robinson et al. who observed more than 1000mg kg<sup>-1</sup> of As accumulation in samples of *E. densa* (dry weight) collected in the Waikato river system [18]. Plants which have the ability to accumulate heavy metal in the tissues are generally classified as a good accumulator. Generally it is considered that a plant useful for phytoremediation should have a BCF value greater than 1000 [72]. In the present study, the BCF

value of *T. natans* was 640.62 in 1.28 mg/L and 626.29 in 10.80 mg/L As concentration respectively. Based on BCF values *T. natans* may be considered as a moderate accumulator. Giri and Patel [76] also found the maximum values of BCF for Cr (VI) and Hg (II) were found to be 413.33 and 502.40 L/kg respectively in water hyacinth where the initial concentration was 0-4 ppm Cr and 0-20 ppm Hg in hydroponic culture and this result was supported our current research. This study showed highest BCF value which indicated that this plant might have the great potentiality for using As phytoaccumulation. Yanqun et al. [77] reported that a TF value greater than 1, the plants are considered as an accumulator species, whereas TF lesser than 1 is an excluder species in As contaminated environment. The  $TF > 1$  indicated that there is a transport of metal from root to leaf probably through an efficient metal transporter system [40], metals sequestration in the leaf vacuoles and apoplast [78].

Low acropetal translocation efficiency of As was observed in *T. natans*, which is in accordance with previous findings of [52]. The TF value in 1.28 mg/L and 10.80 mg/L As concentration was greater than 1 (TF= 1.64 and 1.34), indicating that translocation of As from roots to shoots parts of the plant occurred.

In *T. natans* for both concentrations TF values was  $>1$ , so we can concluded that As was stored in aerial parts of the plant. TF of metals from root to shoot exceeded the critical value (1.0), which indicates that *T. natans* has the ability to transfer As from roots to shoots effectively. These results show that *T. natans* may potentially be useful for removal of As from the As contaminated wastewater. The translocation factors must be invariably higher than one ( $TF > 1$ ). This indicates an efficient ability to transport metals from roots to shoots and most likely, the existence of tolerance mechanisms to cope with high concentrations of metals [79]. Zhao et al. [40] reported that the formation of As-phytochelation (PC) complexes in roots and possible subsequent sequestration in root vacuoles limits the translocation of As from roots to shoots which maybe the probable reasons for low translocation of As in *T. natans*.

#### **4.5.5 SEM-EDX Studies**

##### **4.5.5.1 Distribution of Arsenic in Leaves**

Arsenic uptake, localization and physiological traits are studied in hydroponically grown *T. natans* plants at two different concentration (1.28mg/L and 10.80mg/L). No metal toxicity was found in control *T. natans* during the entire period of study.

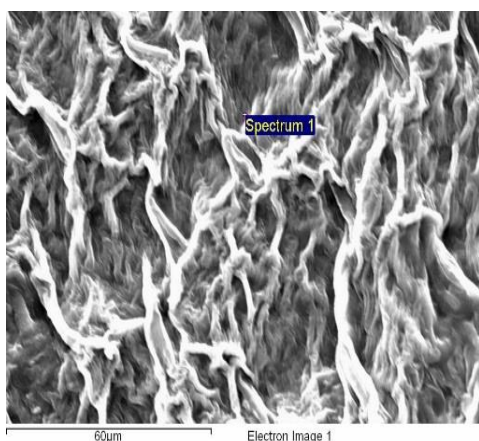


However a substantial toxicity in the form chlorosis of As was found in *T. natans* on 3<sup>rd</sup>, 5<sup>th</sup> and 7<sup>th</sup> days of intervals with respect to control.

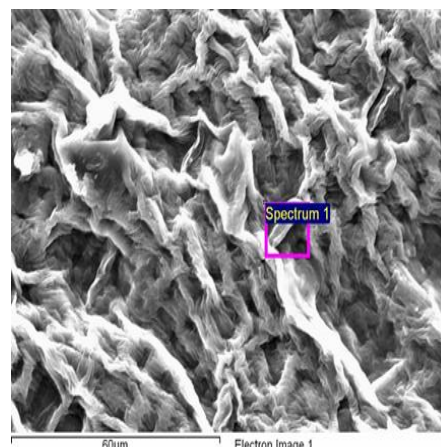
Scanning electron microscope (SEM) studies showed the inclusion of As within the root hairs, vascular bundle of the stem and leaf. This result was confirmed by EDX spectroscopy. The internalization & accumulation of As in different parts of *T. natans* biomass have been investigated with the help of ICP-OES.

It is also important to study the structural changes in different plant parts to understand the overall process of phytoremediation. Hence in this study the physiological, morphological and anatomical characters of *T. natans* plants were evaluated with reference to their As phytoextraction potential.

SEM equipped with EDX revealed the accumulation of arsenic in the tissues and the cell walls. SEM micrograph of the upper surface section of leaves of control and treated plants showed both morphological as well as anatomical differences (**Figure 4.3A, B**). Treated plants showed gradual changes in leaf structure and significant foliar structural changes as compared to control plants. The leaves of treated plants showed a breakdown of epidermal cells, palisade parenchyma cells followed by further loss of cell shape and decrease in intercellular spaces compared to the control plants (**Figure 4.3C, D**). There is no arsenic peak seen in control leaves of *T. natans* (**Figure 4.3E**). Other elements viz. calcium, potassium, carbon, oxygen, sodium, zinc, magnesium and sulfur were also found to be present. Treated leaves showed arsenic accumulation of 1.77 wt %. However, it is less than in the root and shoot. EDX analysis further confirmed that leaf structural changes are due to accumulation of arsenic in treated leaves of *T. natans* (**Figure 4.3F**).

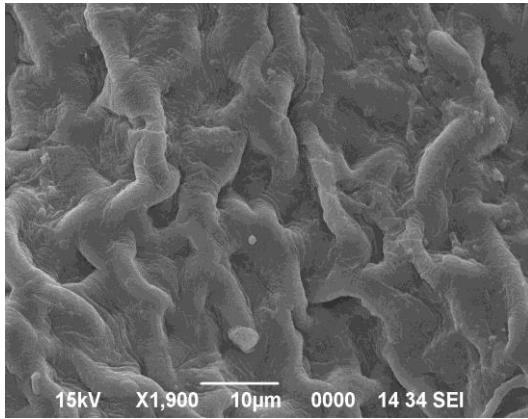


(A)

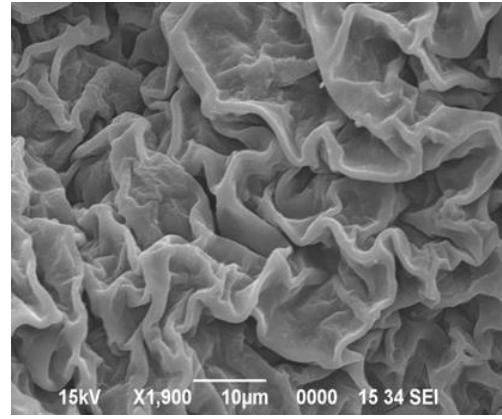


(B)

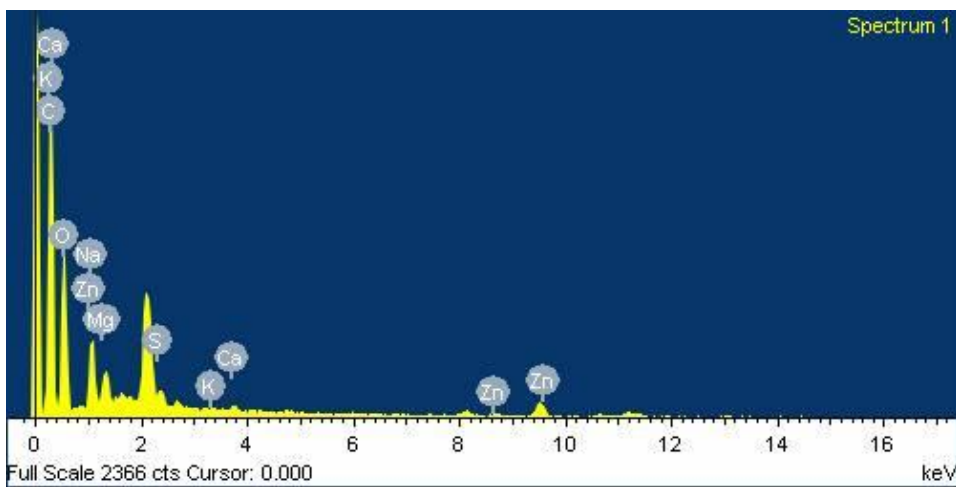




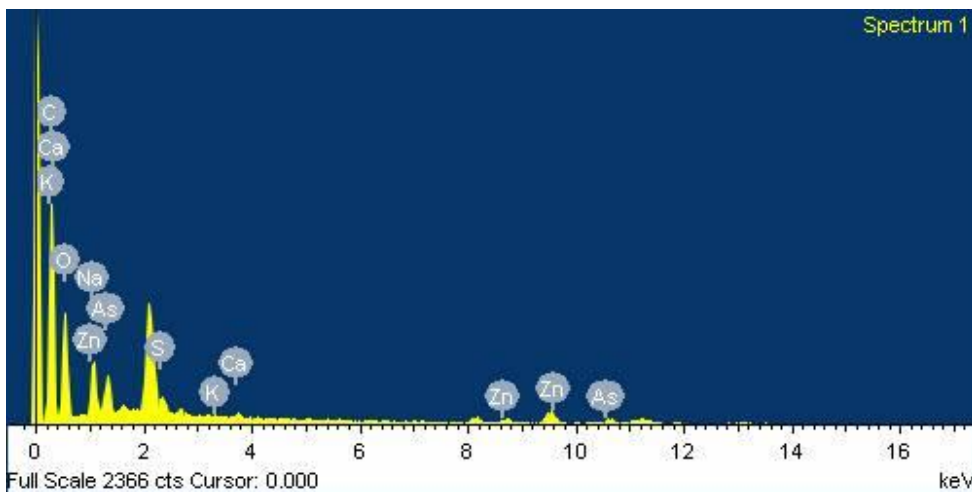
(C)



(D)



(E)

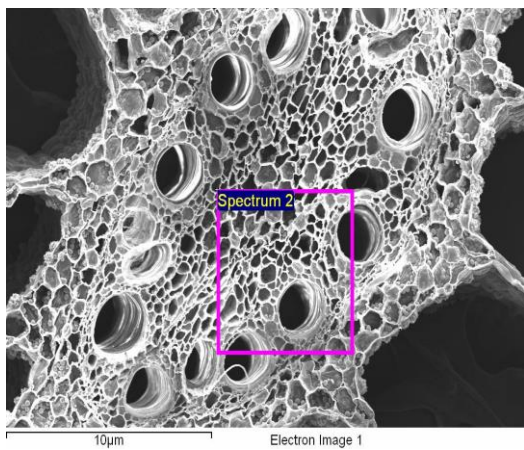


(F)

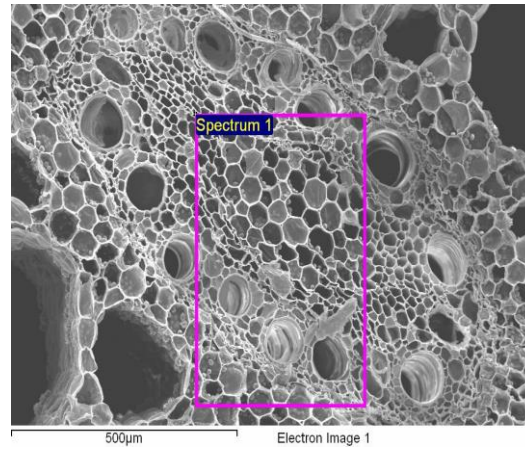
Figure 4.3 (A) *T. natans* in control condition; (B) *T. natans* after arsenic treatment; (C) SEM micrograph of leaves of control *T. natans*; (D) Leaves of arsenic treated *T. natans*; (E) EDX of leaves of control *T. natans*; (F) EDX of leaves of arsenic treated *T. natans*

#### 4.5.5.2 Distribution of Arsenic in Shoots

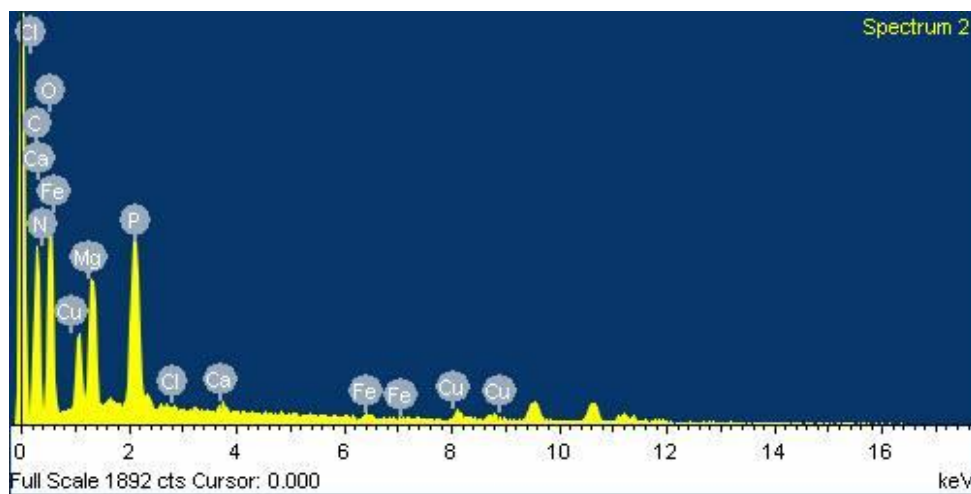
The SEM micrograph of arsenic treated shoots (**Figure 4.3G**) showed precipitates binding all along the cell walls of vascular bundles compared to the control plants (**Figure 4.3H**). In control plants, the clotted deposition was not observed along the cell walls of the vascular bundles. The shoots of treated plants also showed thickened cell wall in both xylem and phloem vessels, which are not seen in shoots of control plants. EDX of control shoots of *T. natans* (**Figure 4.3I**) shows the absence of arsenic, but other elements like oxygen, nitrogen, carbon, calcium, manganese, sodium, and potassium were found to be present. The SEM-EDX micrograph of arsenic treated shoot of *T. natans* (**Figure 4.3J**) showed arsenic (7.00 wt %), which confirms the uptake of arsenic by the plant shoot.



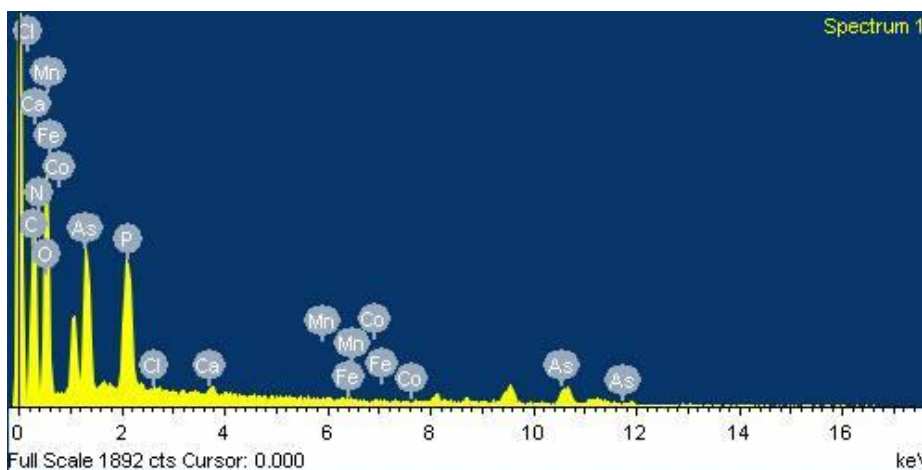
(G)



(H)

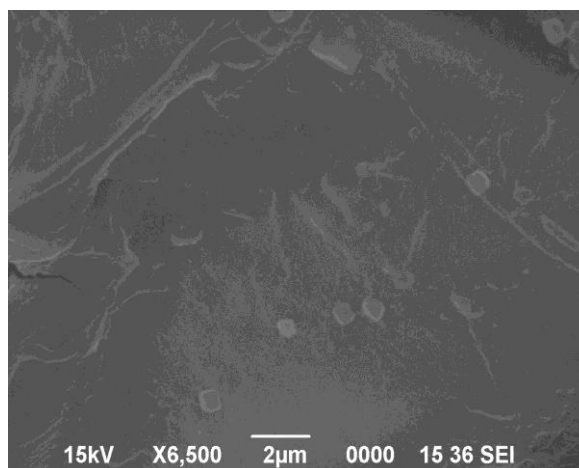


(I)



(J)

Figure 4.3 (G) Arsenic treated shoot of *T. natans*; (H) Control shoot of *T. natans*; (I) EDX of shoot of control *T. natans*; (J) EDX of shoot of arsenic treated *T. natans*



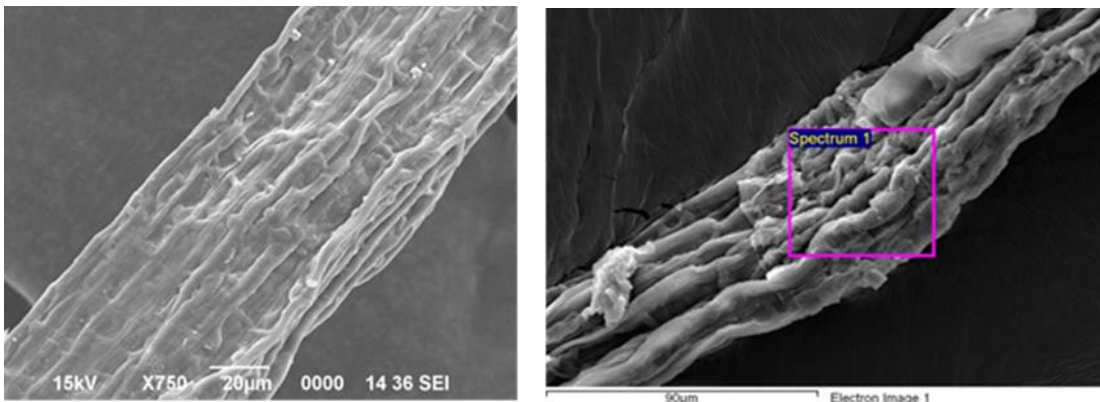
(K)

Figure 4.3 (K) Internal distributions of electron dense deposition of elements in the vascular bundles of *T. natans* shoot (As treated)

#### 4.5.5.3 Distribution of Arsenic in Roots

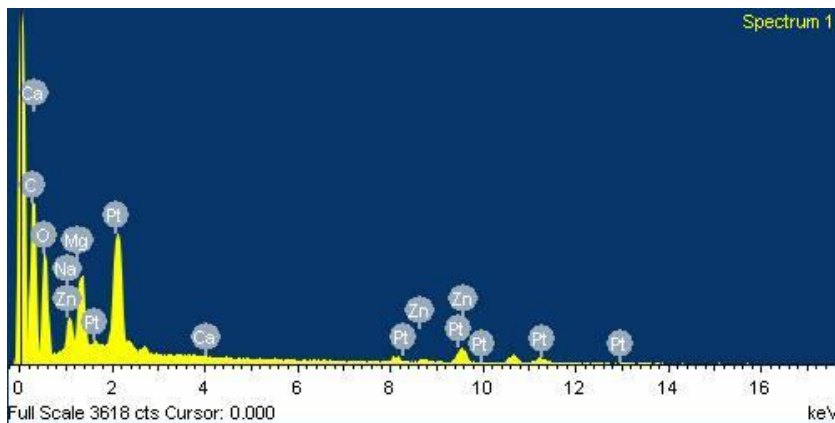
The SEM micrograph of *T. natans* root exposed to arsenic showed some anomalous growth in thickness with large apertures in the central stele region. Observations of treated root (Figure 4.3L) revealed small inclusions on the root surface that were not observed in the root surface of control plants (Figure 4.3M). EDX revealed that the inclusions are rich in arsenic. Arsenic peak was found to be absent in control plants (Figure 4.3N). EDX of treated root (Figure 4.3O) shows the arsenic peak along with other elements such as carbon, sodium, phosphorous and chloride. As the EDX of control roots showed no arsenic, it can be concluded that treated plant root has accumulated the arsenic from the solution. So, it

becomes clear from our results that *T. natans* was found to store the highest concentration of arsenic in roots (7.73 wt %), followed by shoots (7.00 wt %), and leaves (1.77 wt %). It decreases in the following order: root>shoot>leaf.

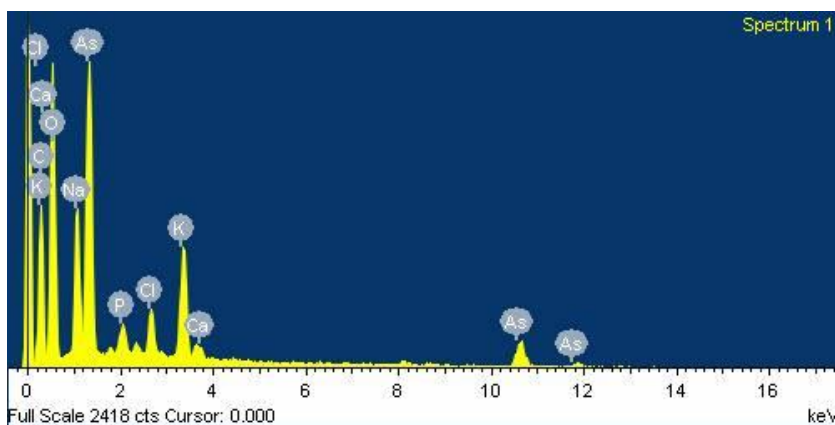


(L)

(M)



(N)



(O)

**Figure 4.3 (L) Arsenic treated root of *T. natans*; (M) Control root of *T. natans*; (N) EDX of root of control *T. natans*; (O) EDX of root of arsenic treated *T. natans***

These results are in accordance with Prajapati et al. [80] who investigated aquatic plants for Cr remediation. Retention of Cr was more in roots as compared with shoots, confirming the findings of Rehman and Haesgawa [81] who found that arsenic translocation in *P. stratiotes* was slow and most of the arsenic remained adsorbed onto root surfaces from solution. Earlier findings have also revealed that arsenic compounds are less readily translocated through the root system of aquatic plants.

#### **4.5.6 Fourier Transform Infrared Spectroscopy (FT-IRS)**

To investigate the different functional groups of control and metal loaded root, shoot and leaf, FT-IR (Spectra-100) was carried out (**Figure 4.4.1** to **Figure 4.4.6**). The shifts in the absorption peaks generally observed indicate the existence of a metal binding process taking place on the plant biomass. FT-IRS indicates that carboxylic, alcoholic; amide and amino groups are responsible for the binding of the metal ions. The spectra of leaf exhibits a broad absorption band of  $3322.27\text{ cm}^{-1}$  (control) due to bonded  $\text{-OH}$  stretching vibration which is shifted to  $3282.66\text{ cm}^{-1}$  (arsenic loaded) due to complexation of  $\text{-OH}$  groups. A change in peak position in the spectrum of the arsenic loaded biomass indicates the involvement of these groups in the biosorption of arsenic ions. The adsorption band at  $1734.91\text{ cm}^{-1}$  (control) is assigned to  $\text{C=O}$  stretching and the band at  $1669.51\text{ cm}^{-1}$  (control) is assigned to carboxylation of amide ( $\text{NH}_2$ ) group. Shifting of these peaks to a different region in arsenic loaded spectra indicated association of these groups in arsenic bindings. The absorption peaks at  $1454.70\text{ cm}^{-1}$  (control) could be attributed to the presence of  $\text{C-H}$  asymmetry, which shifted to  $1453.52\text{ cm}^{-1}$  (arsenic loaded). The band at  $1336.31\text{ cm}^{-1}$  (control) has been shifted insignificantly to  $1336.62\text{ cm}^{-1}$ , due to  $\text{CH}_3$  asymmetric stretching. Another shift was observed from  $1193.12\text{ cm}^{-1}$  (control) to  $1180.80\text{ cm}^{-1}$  (treated) and  $1136.77\text{ cm}^{-1}$  (control) to  $1129.50\text{ cm}^{-1}$  (treated) may be due to interaction of nitrogen from the amino group with arsenic ions. The minor shift of the peak at  $896.63\text{ cm}^{-1}$  (control) also suggests the involvement of  $\text{CH}_2$  group in binding arsenic ions. The other weak absorption peak shifted from  $779.28\text{ cm}^{-1}$  to  $780.14\text{ cm}^{-1}$ ,  $756.23\text{ cm}^{-1}$  to  $754.69\text{ cm}^{-1}$ ,  $656.78\text{ cm}^{-1}$  to  $649.41\text{ cm}^{-1}$ ,  $615.96\text{ cm}^{-1}$  to  $611.37\text{ cm}^{-1}$ ,  $518.29\text{ cm}^{-1}$  to  $524.79\text{ cm}^{-1}$ ,  $464.21\text{ cm}^{-1}$  to  $463.90\text{ cm}^{-1}$  and  $411.62\text{ cm}^{-1}$  to  $406.66\text{ cm}^{-1}$  corresponding to the thiol and sulfhydryl groups with arsenic ions.



**Table 4.6. Assignment of important bands for FTIR Spectra of control and arsenic treated leaves**

Control Leaves (cm <sup>-1</sup> )	Arsenic Treated Leaves (cm <sup>-1</sup> )	Functional Groups and Assignment
3322.27	3282.66	Bonded O-H stretching or N-H stretching vibration
1734.91	1671.77	C=O stretching
1669.51	1650.03	Carboxylation of (NH <sub>2</sub> ) amide group
1454.70	1453.52	C-H asymmetry
1336.31	1336.62	CH <sub>3</sub> symmetric stretching
1193.12	1180.80	Interaction of nitrogen from amino group
1136.77	1129.50	Interaction of nitrogen from amino group
896.63	886.02	CH <sub>2</sub> stretching
779.28	780.14	Thiol and Sulphydral groups
756.23	754.69	Thiol and Sulphydral groups
656.78	649.41	Thiol and Sulphydral groups
615.96	611.37	Thiol and Sulphydral groups
518.29	524.79	Thiol and Sulphydral groups
464.21	463.90	Thiol and Sulphydral groups
411.62	406.66	Thiol and Sulphydral groups

**Table 4.7 Assignment of important bands for FTIR Spectra of control shoot and arsenic treated shoot**

Control Shoot (cm <sup>-1</sup> )	Arsenic Treated Shoot (cm <sup>-1</sup> )	Functional Groups and Assignment
3206.15	3203.02	-NH stretching
2881.06	2880.65	CH <sub>3</sub> -CH asymmetry/symmetry stretching
1669.71	1670.78	C=O stretch
1194.12	1193.63	Interaction of N from amino group with As ions
1121.67	1122.67	C-O stretch
835.62	836.08	CH <sub>2</sub> stretch
421.54	420.87	Thiol and Sulphydral groups

**Table 4.8 Assignment of important bands for FTIR Spectra of control root and arsenic treated root of *T. natans***

Control Root (cm <sup>-1</sup> )	Arsenic Treated Root (cm <sup>-1</sup> )	Functional Groups and Assignment
3316.53	3315.42	-OH stretching
3202.97	3199.86	-NH stretching
2967.82	2968.17	Methyl C-H asymmetry/symmetry stretching
1193.09	1193.56	C-O stretching
886.30	885.26	CH <sub>2</sub> stretching
800.60	807.93	Thiol and Sulphydral group
753.16	754.83	Thiol and Sulphydral group
535.93	539.87	Thiol and Sulphydral group
464.17	463.56	Thiol and Sulphydral group
414.90	416.82	Thiol and Sulphydral group

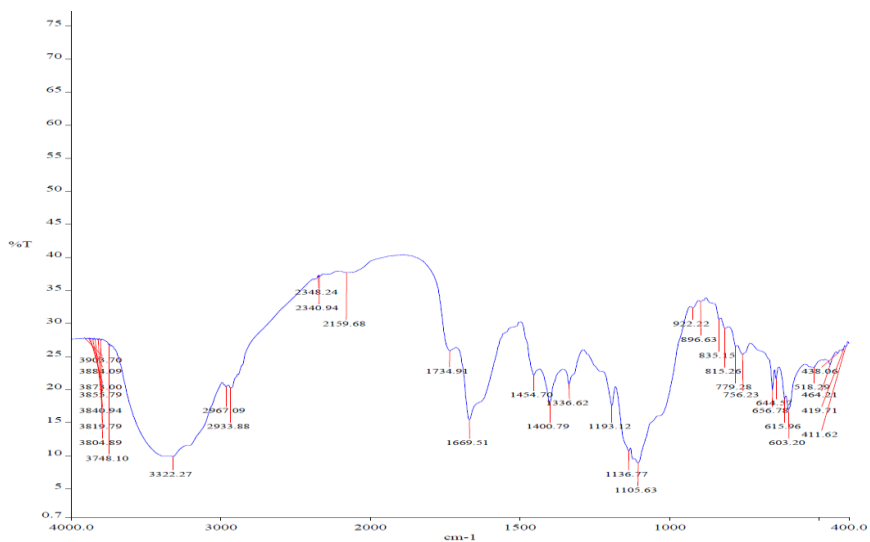


Figure 4.4.1 FTIR spectra of control leaf of *T. natans*

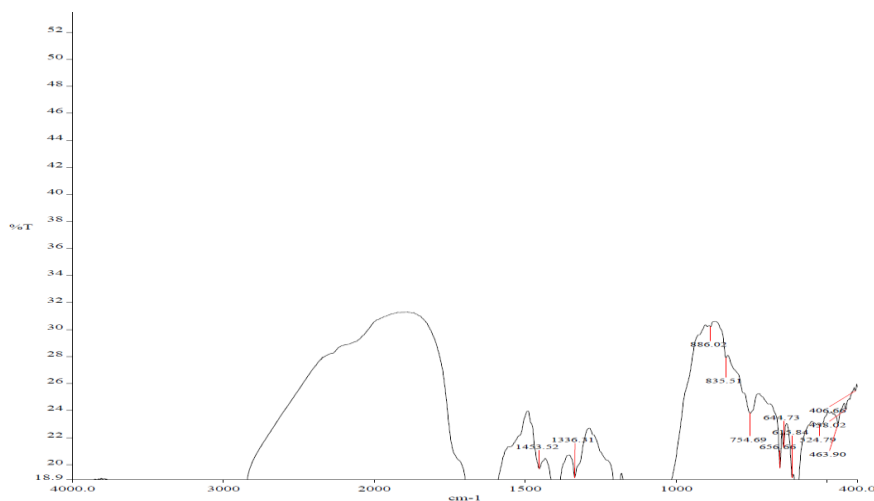


Figure 4.4.2 FTIR spectra of arsenic treated leaf

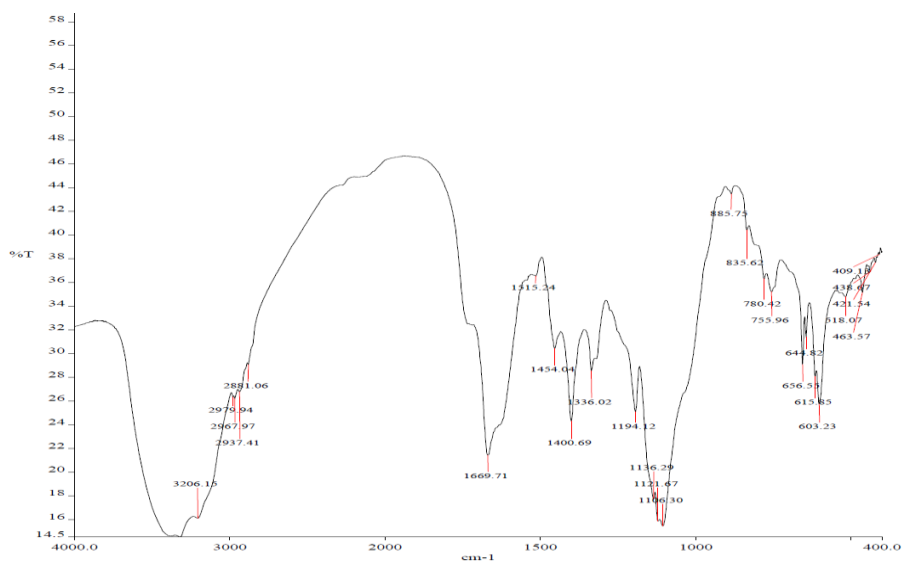


Figure 4.4.3 FTIR spectra of control shoot of *T. natans*

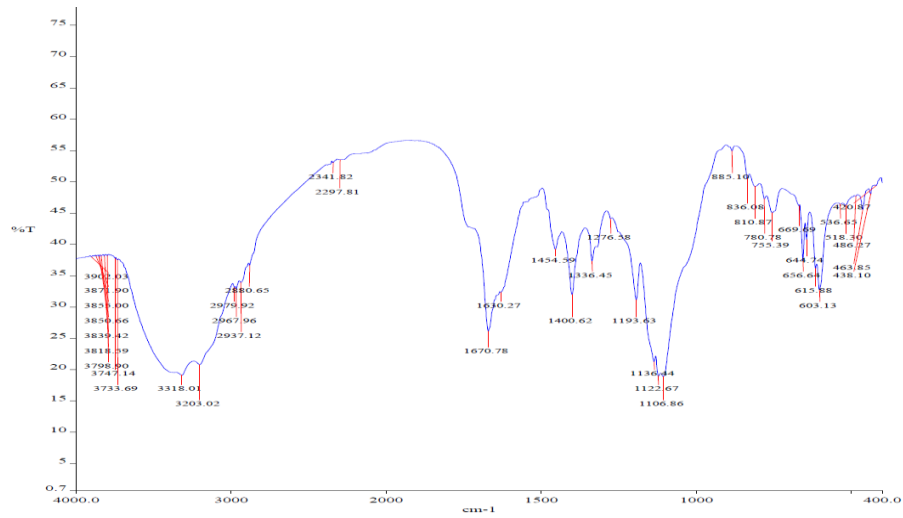


Figure 4.4.4 FTIR spectra of arsenic treated shoot

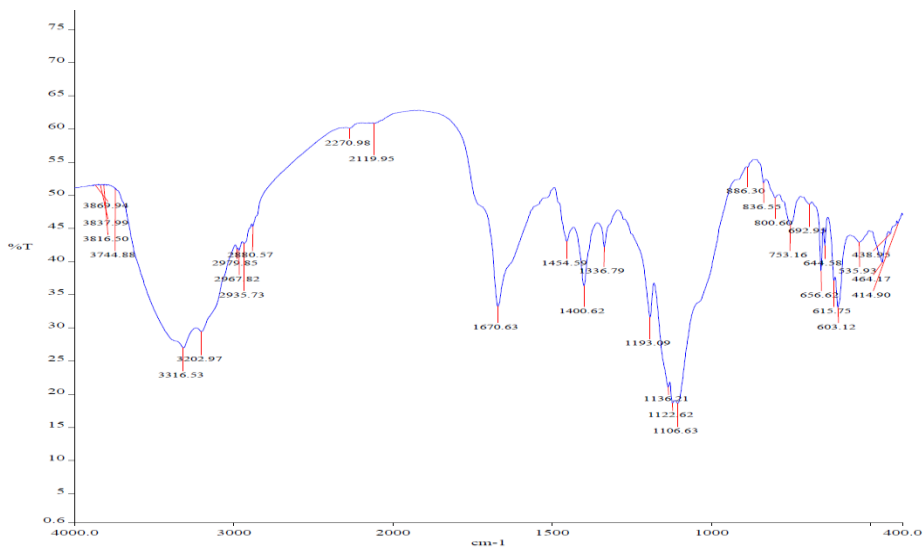


Figure 4.4.5 FTIR spectra of control root of *T. natans*

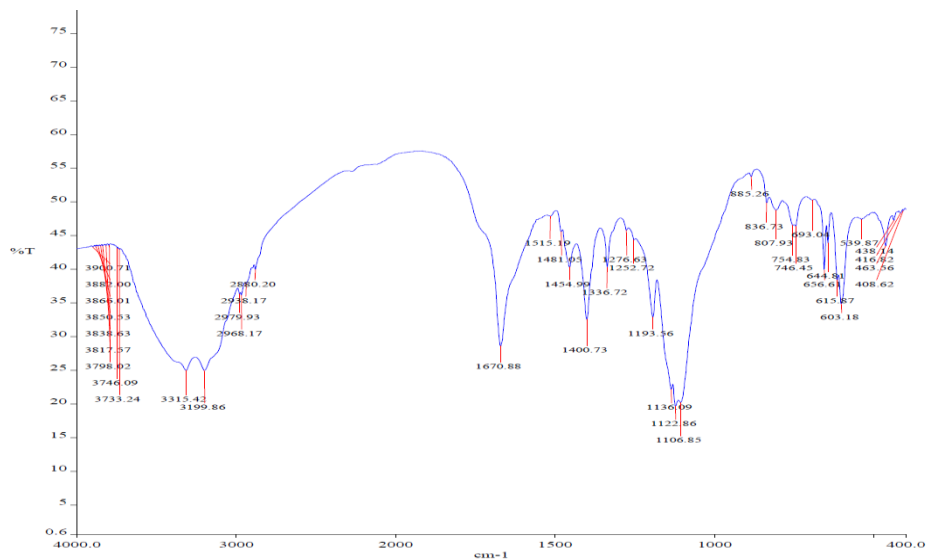


Figure 4.4.6 FTIR spectra of arsenic treated root



The shoot spectra exhibit a broad absorption band at  $3206.15\text{ cm}^{-1}$  (control) due to  $\text{-NH}$  stretching vibration which shifted to  $3203.02\text{ cm}^{-1}$  (with arsenic loaded) may be due to complexation of  $\text{-OH}$  groups. The next absorption peak at  $2881.06\text{ cm}^{-1}$  (control) may be due to the presence of  $\text{CH}_3\text{-CH}_2$  asymmetric/symmetric group which is shifted to lower frequency and appeared at  $2880.65\text{ cm}^{-1}$  (with As loaded). The shift of absorption peak may be due to complexation of different binding groups present in the biomass of *T. natans* with arsenic ions.

Another shift observed from  $1669.71\text{ cm}^{-1}$  (control) to  $1670.78\text{ cm}^{-1}$  (with arsenic loaded) may be due to the interaction of  $\text{C=O}$  group with arsenic ions. The band at  $1194.12\text{ cm}^{-1}$  (control) has been shifted to  $1193.63\text{ cm}^{-1}$  (with arsenic loaded). This may be due to the interaction of nitrogen from amino group with arsenic ions. Another band at  $1121.67\text{ cm}^{-1}$  (control) has been shifted to  $1122.67\text{ cm}^{-1}$  (with arsenic loaded) due to the complexation of carboxyl group with arsenic ions. The other weak absorption peak shifted from  $835\text{ cm}^{-1}$  (control) to  $836.08\text{ cm}^{-1}$  (with arsenic loaded) corresponding to the carboxyl group with arsenic ions and a shift from  $421.54\text{ cm}^{-1}$  (control) to  $420.87\text{ cm}^{-1}$  (with arsenic loaded) is corresponding to the thiol and sulfhydryl groups with arsenic ions. The other bands in this spectrum have not been significantly shifted. The above changes in the spectra may be attributed to the interaction of arsenic ions with the hydroxyl, amide, thiol, and amino groups present in the shoot biomass.

The root spectra exhibited broad absorption at  $3316.53\text{ cm}^{-1}$  (control) shifted to  $3315.42\text{ cm}^{-1}$  (loaded with As) due to bonding with  $\text{O-H}$  stressing variation. Again the band at  $3202.97\text{ cm}^{-1}$  (control) has been assigned to  $\text{N-H}$  stressing, shifted to  $3199.86\text{ cm}^{-1}$  (with arsenic loaded)-may be due to complexation of  $\text{N-H}$  group with arsenic. A shift in band position at  $2967.82\text{ cm}^{-1}$  (control) to  $2968.17\text{ cm}^{-1}$  (with arsenic loaded) possibly for  $\text{CH}_3$  asymmetric stress and  $\text{CH}_3$  asymmetric deformation. The comparison between control and arsenic loaded *T. natans* shows a slight shift in band for  $\text{C-O}$  stretching (from  $1193.09\text{ cm}^{-1}$  to  $1193.56\text{ cm}^{-1}$ ).

Another shift at  $886.30\text{ cm}^{-1}$  (control) to  $885.26\text{ cm}^{-1}$  (arsenic loaded) was observed may correspond to the  $\text{CH}_2$  groups of *T. natans* bonded with arsenic ion. The other weak absorption peak shifted from  $800.60\text{ cm}^{-1}$  to  $807.93\text{ cm}^{-1}$ ;  $753.16\text{ cm}^{-1}$  to  $754.83\text{ cm}^{-1}$ ;  $535.93\text{ cm}^{-1}$  to  $539.87\text{ cm}^{-1}$ ;  $464.17\text{ cm}^{-1}$  to  $463.56\text{ cm}^{-1}$  and  $414.90\text{ cm}^{-1}$  to  $416.82\text{ cm}^{-1}$  corresponding to the thiol or sulfhydryl group with

arsenic ions. Assignment of the different chemical groups is shown in **Table 4.6** to **Table 4.8**.

## 4.6 Discussion

The aim of the present study is to assess the ability of hydroponically grown *T. natans* to accumulate arsenic from the water. The ultimate goal is to assess the application of these plants in the phytoremediation of metal contaminated water. *T. natans* stands out as a good candidate for phytoremediation hydroponically.

The movement of metal/metalloids containing solution from the root to the shoot is termed as translocation is primarily controlled by two processes root pressure and leaf transpiration. Some metals are accumulated in roots, probably due to the some physiological barriers against metal/metalloid transport to the aerial parts [82, 83].

The metals accumulation increased linearly with the solution concentration in the order of leaves > stems > roots in *Eichhornia crassipes* [84, 85, 86, 87, 88]. Metalloid ions penetrated plants by passive process, mostly by exchange of cations which occurred in the cell wall. All heavy metals were taken up by plants through absorption, translocation and released by excretion. It can be proposed that the roots reached saturation during the period and there exists some mechanism in roots that could detoxify heavy metals or transfer them to aerial parts.

The plant shows TF values greater than 1, indicating the ability of the plant to translocate As from root to shoot. Arsenic accumulation in plant biomass was found to increase with increasing arsenic concentration and with increasing days of exposure. Accumulation of As in *T. natans* followed a pattern observed in *P. griffithii* for cadmium accumulation [89]. A similar trend was observed in *E. crassipes* for zinc accumulation [90]. In contrast to our results, accumulation of arsenic was found to increase linearly with the solution concentration in the order of leaves > stems > roots in *E. crassipes* [84, 85, 86]. However, to the best of our knowledge the reason is still unclear but it might be due to the presence of a different transporter system in different macrophytes. The root sorption in *T. natans* is more because of sorption of arsenic ions by root surface area and also might be due to the direct contact of roots with the metal solution. Hydroponic culture is an efficient method for removal of arsenic ions as the free floating plant *T. natans* is arsenic tolerant. The surface morphology of *T. natans* shoots biomass without and with removal of As ions during absorption process was observed with

the help of SEM-EDX. (**Figure 4.3C, D**) shows the morphological changes with respect to shape and size of the leaves after absorption of As ions. It can be clearly observed that the surface of materials shape has been changed into a new shiny bulky particle and whitish patches structure the adsorption of after As ions. So, it was concluded that, As ions were adsorbed on the surface of the extract materials. These results are further confirmed with the results of FTIR spectra analysis.

In the shoot, arsenic binding along the cell walls of vascular bundles and thickening of the cell wall in both xylem and phloem vessels was observed. Small inclusions on the root surface along with anomalous growth in thickness with large apertures in the central stele region of root was also observed. The EDX spectra showed the characteristic peak in treated plant biomass, confirming that *T. natans* can absorb and accumulate arsenic. The SEM technique has been used in several studies to investigate the internal distribution of metals in plant tissues [91]. In the previous studies it has been used for the detection of copper, zinc, cadmium, lead and arsenic in different hyperaccumulators [89]. Numerous chemical groups responsible for metal bindings in plants include carboxyl, amino, sulphonate and hydroxyl and their importance in metal binding depends on factors such as the quantity of sites, their accessibility and the affinity between the sites and the metal. The hydroxyl, carbonyl, carboxyl, sulfonate, amine, amide, imidazole, and phosphonate groups are the main functional groups responsible for a biosorption process [92, 93].

Pickering et al. [11] reported that As(III) in *Brassica juncea* is coordinated with three sulfur groups. Our FT-IR analysis confirms the presence of hydroxyl, amide, thiol, sulfahydral and amino groups present in *T. natans* biomass that might interact with arsenic during the absorption process. The shift from  $1669.71\text{cm}^{-1}$  (control) to  $1670.78\text{cm}^{-1}$  (with arsenic loaded) may be due to the interaction of C=O group with arsenic ions [94, 95]. The weak absorption peak shifted from  $800.60\text{cm}^{-1}$  to  $807.93\text{cm}^{-1}$ ;  $753.16\text{cm}^{-1}$  to  $754.83\text{cm}^{-1}$ ;  $535.93\text{cm}^{-1}$  to  $539.87\text{cm}^{-1}$ ;  $464.17\text{cm}^{-1}$  to  $463.56\text{cm}^{-1}$  and  $414.90\text{cm}^{-1}$  to  $416.82\text{cm}^{-1}$  corresponding to the thiol or sulfhydral group with arsenic ions [96]. The high removal efficiency and greater accumulation capacity of arsenic ions make *T. natans* an excellent choice for phytoremediation processes. Uptake and accumulation of metals at higher concentrations can be cytotoxic in some plant species, causing structural and ultra

structural changes affecting the growth and physiological well being of the plants [97]. Morphological changes like leaf chlorosis and necrosis with premature leaf fall was observed in treated plants with days of exposure. Decline in chlorophyll content in *T. natans* might be caused by an inhibition of an important enzyme of chlorophyll biosynthesis, d--aminolevulinic acid dehydrogenase (d-ALAD) and protochlorophyllide reductase [98]. Proline is a non-essential amino acid. It acts as an osmoprotectant. Under stress condition, concentration of proline increases. It protects the structure of protein and acts as a dehydration inhibitor. Proline accumulation in plants is a response to heavy metals accumulation in order to maintain the osmotic balance in the cells of plants [99]. These results suggested that the accumulation of proline in plants protects the cell membrane and proton pump against damage indicating that *T. natans* is able to respond against arsenic stress by proline osmotic regulation.

Infrared spectra of the *T. natans* shoot biomass without arsenic ions loaded are obtained to determine which functional groups may have contributed to the arsenic ions adsorption are presented in **Figure 4.4.3**. The FTIR spectra of the shoot biomass loaded with arsenic ions are presented in **Figure 4.4.4** which displays a number of absorption peaks, indicating the complex nature of the biomass. The spectra of loaded with arsenic and without are compared and found the following shift is observed in spectra. The spectra of extract materials exhibits a broad absorption band at  $3225.78\text{ cm}^{-1}$  due to bonded  $\text{-OH}$  stretching vibration which is shifted to  $3195.91\text{ cm}^{-1}$  may be due to complexation of  $\text{-OH}$  groups with metal. The band at  $2,918.50\text{ cm}^{-1}$  has been shifted insignificantly. The new peak at  $2,351.75\text{ cm}^{-1}$  may be due to the complexation of  $\text{-SH}$  group with arsenic ions [96]. The next absorption peak at  $1,638.96\text{ cm}^{-1}$  may be due to the presence of amide group (N-H stretching and C=O stretching vibration) is shifted to higher frequency and appeared at  $1,645.03\text{ cm}^{-1}$  may be due to the complexation of amide group with arsenic ions [100]. Another peak at  $1,319.75\text{ cm}^{-1}$  has been shifted insignificantly. Another shift was observed from  $1,163.54\text{ cm}^{-1}$  to  $1,169.27\text{ cm}^{-1}$  and  $1,022.47\text{ cm}^{-1}$  to  $1,023.65\text{ cm}^{-1}$  may be due the interaction of nitrogen from amino group with arsenic ions [101, 102]. The other weak absorption peak shifted from  $780.91\text{ cm}^{-1}$  to  $780.64\text{ cm}^{-1}$  and  $670.09\text{ cm}^{-1}$  to  $668.44\text{ cm}^{-1}$  corresponding to the thiol or sulfhydryl group with arsenic ions [96]. The above changes in the

spectra may be attributed to the interaction of arsenic ions with the hydroxyl, amide, thiol and amino groups present on the shoot biomass.

#### **4.7 Conclusion**

The present study proved *T. natans* is a good hyperaccumulator of arsenic in the roots and aboveground plant parts. Regardless of the concentration, the roots were found to be most efficient in the accumulation of arsenic. Although some morphological symptoms of toxicity were observed at higher arsenic concentration, the plants were able to resist arsenic toxicity due to proline synthesis and accumulation. On the basis of our results, *T. natans* can be recommended for the removal of arsenic from contaminated water.

It is necessary to elucidate what internal/external factors play important roles in metal and metalloid (e.g. As) uptake and tolerance in order to select suitable wetland plants with high levels of tolerance.

In this study, the characteristics of accumulation and transportation of As in wetland plants are summarized as follows-

1) The distribution of As in the root is greater than in shoot; after uptake of As by roots, most of the As will be stored in root tissues, and less transported in to the shoots, 2) Plaque can act as a buffer area for the uptake of toxic metals (As) into root tissues, but it does not block the As transport into root tissues.

In the present study, the aquatic macrophyte *T. natans* which is easily cultivated and controlled, and well adapted to contaminated environment was tested for its ability to accumulate As from metal solution in laboratory experiments. The results indicate that the plants stand out as a good candidate for phytoremediation hydroponically. The following conclusion may be drawn from the present investigation:

FT-IR analysis reveals that the arsenic ions may be coordinate with the hydroxyl, amide, thiol, and amino groups present in the biomass. *T. natans* was found to store As in roots in highest concentration (7.73wt %), followed by shoot (7.00wt %), root hair (4.88wt %), and leaf (1.77wt %) It decreases in the following order: root>shoot>root hair>leaf. It is revealed by EDX analysis. The

root accumulation is more than shoot and leaf because of sorption of As ions by root surface area and also direct contact with the metal solution. Scanning-electron microscopy analysis showed that As deposition was in vascular tissue in root as well in shoot in the plant. Plants showed As toxicity like chlorosis, and curled leaf was observed during the experimentation period. As concentration in plant biomass was found to increase with increasing initial metal concentration and with increasing days of exposure. The accumulation of As in different parts of *T. natans* are in the following order: root>fruit>shoot>leaf. The results indicate that *T. natans* has the capability to transport As from roots to shoots region. Arsenic concentration reduced the total chlorophyll content with increasing concentration and days of exposure. However proline concentration increases with increasing concentration of As. Morphological changes showed leaf chlorosis and necrosis with leaf fall. Appearance of new leaves and roots, though unhealthy indicates that the plant has the potential to survive under stress condition to a great extent. From the above discussion it is revealed that hydroponic culture is an efficient method for screening of arsenic ions tolerant for free floating plants of *T. natans*.

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