



***In situ* phytoaccumulation of elements by native aquatic and wetland plants- Ecofriendly approach**

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Abstract

Phytoaccumulation is the process that introduces plant into environment and allow them to assimilate the contaminant into their roots and leaves. Such process has been used to clean-up heavy metals, pesticides, xenobiotics, organic compounds and acid mine drainage. Aquatic macrophytes are widely distributed in various wet environment from fresh to salt water and they play an important role in heavy metal cycling in the wet land due to uptake, storage and release processes in the form of phytoaccumulation. Phytoaccumulation is cost effective, environment friendly, aesthetically pleasing approach and most suitable for developing countries. *In situ* phytoaccumulation of metals from the water source by native aquatic and wetland plants selected for this research work are *Aponogeton natans*, *Eichhornia crassipes*, *Lemna polyrrhiza*, *Pistia stratiotes*, *Scripus maritimus* and *Typha angustata*. The SEM-EDX elemental analysis of *in situ* phytoaccumulation resulted in the maximum accumulation of chlorine 2.94%, sodium 2.29% and silica 1.52% by *A. natans*; *E. crassipes* accumulated 1.20% of potassium; *L. polyrrhiza* 4.93% of potassium, 4.59% of calcium, 2.25% of phosphorus, 2.17% of sulphur, 1.98% of chlorine, 1.75% of iron and 1.38% of magnesium; *P. stratiotes* accumulated 9.07% of silica, 2.04% of potassium, 1.30% of sodium; *S. maritimus* accumulated chlorine 1.82%, potassium 1.13%, calcium 1.04%, sodium 1.00% and 0.77% of fluorine; *T. angustata* accumulated 2.84% of chlorine, 1.56% of potassium and 0.92% of calcium.

Keywords: phytoaccumulation, SEM-EDX elemental analysis, Native aquatic, wetland plants

Introduction

Contamination of water and wastewater with heavy metals is emerging as a global environmental challenge. Increasing urbanization, industrialization and over population is leading to the degradation of the environment, affecting crop yield and contributing to bioaccumulation in the food chain. The main hazardous contents of water pollution are heavy metals and elements. Water bodies are the main target for disposing of such pollutants directly or indirectly. The prevailing purification technologies used to remove the contaminants are too costly and sometimes non-eco-friendly also. Therefore, the research is oriented towards low cost and eco-friendly technology for water purification, which will be beneficial to the community (Dhote and Dixit, 2009) [19]. Heavy metal and elemental pollution are mainly due to the results of human activities such as agriculture, mining, construction and industrial process including E-waste (Vahdatiraad and Khara, 2012) [57]. The widespread accumulation of heavy metals and elements in water and soil has become a serious problem. The concentrations of toxic metals in polluted water and soils are often many times greater than the acceptable values, thus exerting a toxic effect on the majority of higher plants. Toxic metals and elements can affect the biosphere for long period of time and leached through the soil layers leading to the contamination of soil and water (Wang *et al.*, 2003). Heavy metals are non-degradable by any biological or typical process and are persistent in the soil for a long period which pose a long term threat for the environment. By either biological or typical process, heavy metals are non-degradable and remain in the soil for a long time, which poses a long-term environmental hazard (Suman *et al.*, 2018). At least 20 metals are classified as toxic with half of them emitted into the environment that poses great risks to human health (Akpore and Muchie, 2010) [7, 8]. The common heavy metals like Cd, Pb, Co, Zn and Cr are phytotoxic at both low concentration as well as high concentrations and are detected in wastewater especially, the urban water bodies (Divya *et al.*, 2012). Recently, there has been an increasing interest in using biological indicators and remediators such as plants for soil, and water pollution (Ruiz and Velasco, 2010). Phytoaccumulation is the process that introduces plants into environment and allows them to assimilate the contaminants into their roots and leaves. Such process has been used to clean up heavy metals, pesticides and xenobiotics (Suresh and Ravishankar, 2004), organic compounds (Newman and Reynolds, 2004) and acid mine drainage (Archer and Caldwell, 2004), basically involves the extraction or inactivation of these metals in soils and water (Lombi *et al.*, 2001) [37]. The bioaccumulation of Cu, Fe, Mn, Na, Pb, and Zn in *Lemna minor* was examined, as well as the effects of Cd and Ni on the bioaccumulation of B, Ca, Cu, Fe, Mg, K, Mn, Na, Pb, and Zn in *L. minor* (2021) United Nations Environment Program defined phytoremediation as “the

efficient use of plants to remove, detoxify or immobilize environmental contaminants” (UNEP, 2019). Phytoremediation is an eco-friendly and advantageous technique for the cleaning of contaminated media. The mechanism involves is absorption of pollutants through roots, accumulation in body tissues, decompose and transforming pollutants to a less harmful forms. Various phytoremediation techniques used in different media have been extensively discussed by several workers worldwide (Favas et al., 2018; UNEP, 2019; Vidal et al., 2019; Yadav et al., 2018)

Aquatic macrophytes are widely distributed in various wet environments, from fresh to salt water (Bonanno and Giudice, 2010) and they play an important role in heavy metals cycling in the wet lands due to uptake, storage and release processes. Phytoaccumulation is a cost effective, environmental friendly, aesthetically pleasing approach most suitable for developing countries (Ghosh and Singh, 2005). The technologies based on the phytoaccumulation process is applied to both organic and inorganic pollutants present in soil (solid substrate) and water (liquid substrate). Aquatic plants can be used as the natural catalysis to absorb and accumulate heavy metals in their tissues from waste water (Vymazal and Kropfelova, 2008). In phytoaccumulation the biosorbed toxic metals and elements in plant tissues are harvested for metal recovery and reuse. Normally, the plants termed hyperaccumulators are preferably used, since they have the ability to withstand and build up high concentrations of metals, when compared to other plants. These plants can be processed to recover the metals biosorbed during the phytoaccumulation process. According to Eid (2011), the minimum threshold tissue concentrations for Co, Cu, Cr, Pb or Ni hyperaccumulators should be 0.1% dry weight, while for Zn or Mn the threshold is 1%. With the heavy metal and elements accumulation in water bodies as ecological problems in view, the motivation of this work is to determine the efficient species of aquatic plant for water quality remediation of heavy metals and elements. Phytoaccumulation using aquatic plants is evolving as a cost-effective and alternative to high energy, high cost conventional method, thus considered to be a ‘Green Technology’ in the clean-up process (Kavitha et al., 2013) [34]. The work is focuses on the aquatic ecosystem of river Cauvery water bodies of study area in Tiruchirappalli, Tamil Nadu, India.

Materials and methods

Collection of Samples

The area under study for this work was identified based on the need, diversity and extend of pollution produced by the anthropogenic activities. Cauvery is a major river in Tamil Nadu catering to the needs of agriculture and human consumption. It branches as Uyyakondan Canal (irrigation canal) at Tiruchirappalli District and runs through regions where major anthropogenic activities are carried out as day to day life and the water get contaminated with pollutants. The water samples were collected at the sampling sites of this canal where the native aquatic and wetland plants are growing. The plants growing in site-I is *Aponogeton natans* (Aponogetonaceae) and *Eichhornia crassipes* (Pontederiaceae), at site-II *Lemna polyrrhiza* (Lemnaceae) and *Pistia stratiotes* (Araceae) and at site-III *Scirpus maritimus* (Cyperaceae) and *Typha angustata* (Typhaceae) (Fig. 1: Sites I, II and III). These plants were tested for the phytoaccumulation of metals and elements *in situ* conditions. The water samples were collected from three sites filled in properly cleaned, sterilized, wide mouth plastic bottles and stored in dark at a temperature of 4 °C. The water samples were later utilised for physico-chemical characterization studies.

Table 1: Overview of physio-chemical characterization of water samples of three different sites of study area were compared with the WHO standards.

Parameters	Units	Sample Site I	Sample Site II	Sample Site III	WHO Standards
Colour	-	Light Tann	Light Tann	Light Tann	-
Odour	-	Faint Fishy	Faint Fishy	Faint Fishy	-
pH	-	7.26 ± 0.11	7.43 ± 0.05	7.26 ± 0.11	7.0 - 8.5
Electrical Conductivity (EC)	µmos/cm	733.0 ± 15.27	743.0 ± 15.27	746.0 ± 11.54	500 - 1500
Total Solids (TS)	mg/l	843.0 ± 20.81	830.0 ± 10.00	850.0 ± 10.00	500 - 1500
Total Suspended Solids (TSS)	mg/l	650.0 ± 10.00	650.0 ± 15.27	623.0 ± 15.27	100 - 600
Total Dissolved Solids (TDS)	mg/l	1063.0 ± 15.27	1116.0 ± 15.27	1136.0 ± 15.27	850 - 1500
Biochemical Oxygen Demand (BOD)	mg/l	12.66 ± 1.15	15.33 ± 1.15	13.66 ± 1.52	≥ 5.0
Chemical Oxygen Demand (COD)	mg/l	166.0 ± 11.54	173.0 ± 11.54	173.0 ± 5.77	250
Dissolved Oxygen (DO)	mg/l	8.4 ± 0.20	6.9 ± 0.36	8.23 ± 0.25	≤ 6.0
Total Hardness as CaCO ₃ (TH)	mg/l	136.0 ± 4.10	133.0 ± 4.16	134.0 ± 5.03	500
Carbonate Alkalinity level	mg/l	142.0 ± 3.65	140.0 ± 2.00	132.0 ± 3.00	200
Bicarbonate Alkalinity	mg/l	79.0 ± 3.60	86.3 ± 1.52	84.3 ± 1.76	50
Calcium (Ca)	mg/l	210.0 ± 0.61	220.0 ± 0.42	220.0 ± 0.42	75 - 200
Magnesium (Mg)	mg/l	1.24 ± 0.05	1.12 ± 0.21	1.10 ± 0.42	2.0
Chloride (Cl ₂)	mg/l	230.0 ± 0.21	220.0 ± 0.42	210.0 ± 3.20	200 - 600
Chromium (Cr)	mg/l	2.50 ± 0.20	2.26 ± 0.15	2.36 ± 0.15	0.05
Copper (Cu)	mg/l	3.46 ± 0.30	34.6 ± 0.30	3.26 ± 0.15	1.0

Iron (Fe)	mg/l	2.60 ± 0.15	1.90 ± 0.30	2.20 ± 1.21	4.5
Lead (Pb)	mg/l	1.40 ± 0.26	1.0 ± 0.20	1.20 ± 0.20	0.10
Zinc (Zn)	mg/l	4.16 ± 0.15	3.70 ± 0.15	3.70 ± 0.10	5.0
* The mean difference is significant at 0.01 level					

Table 2: *In situ* phytoaccumulation of elements by native aquatic and wetland plants taken up for this study by SEM-EDX elemental analysis (%)

Elements/Metals	<i>Aponogeton natans</i>	<i>Eichhornia crassipes</i>	<i>Lemna polyrrhiza</i>	<i>Pistia stratiotes</i>	<i>Scirpus maritimus</i>	<i>Typha angustata</i>
Carbon (C)	55.73	64.80	45.28	56.54	60.37	50.00
Nitrogen (N)			5.26			
Oxygen (O)	33.94	32.81	28.65	25.74	33.29	41.79
Fluorine (F)				0.77		
Sodium (Na)	2.29		0.80	1.30	1.00	0.82
Magnesium (Mg)	0.86	0.16	1.38	0.37	0.36	0.15
Aluminium (Al)	0.30			0.41	0.22	0.12
Silica (Si)	1.52	0.12	0.67	9.07	0.77	1.24
Phosphorus (P)		0.19	2.25	0.29		
Sulphur (S)	0.15	0.07	2.17	0.22		0.40
Chlorine (Cl)	2.94	0.44	1.98	0.13	1.82	2.84
Potassium (K)	0.47	1.20	4.93	2.04	1.13	1.56
Calcium (Ca)		0.22	4.59	1.14	1.04	0.92
Manganese (Mn)			0.29			
Iron (Fe)	0.19		1.75			
Copper (Cu)	0.15					0.15
Titanium (Ti)				0.52		

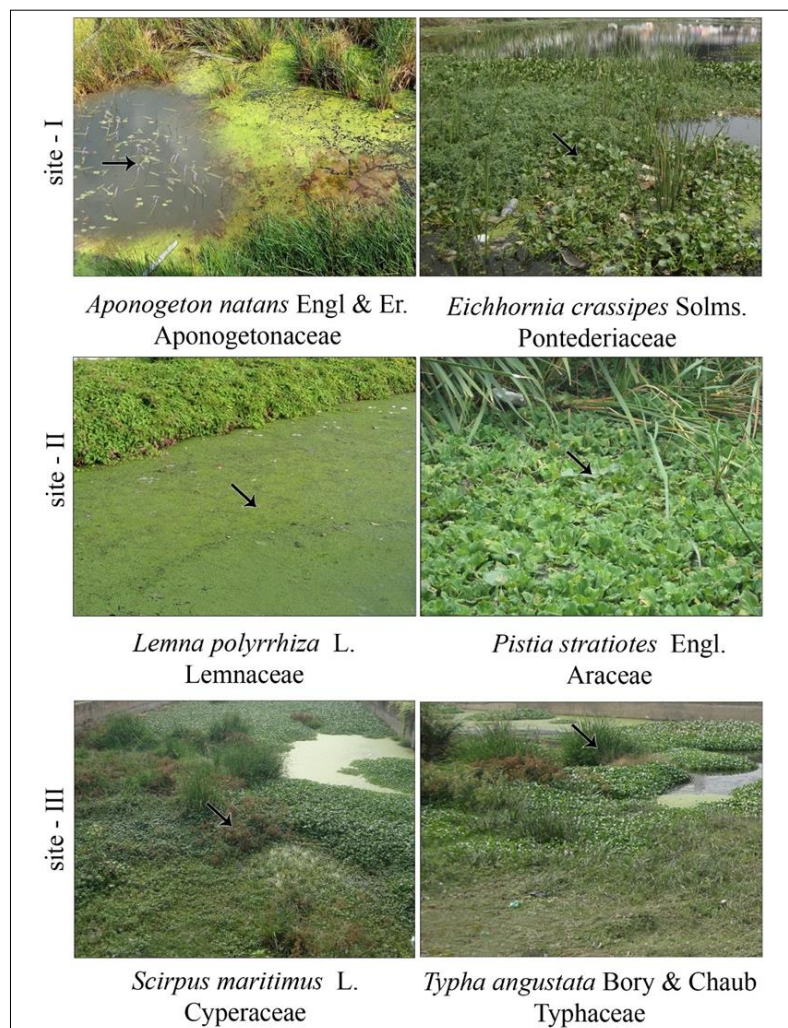


Fig 1: Sampling sites of polluted water and habitat of native aquatic and wetland plants

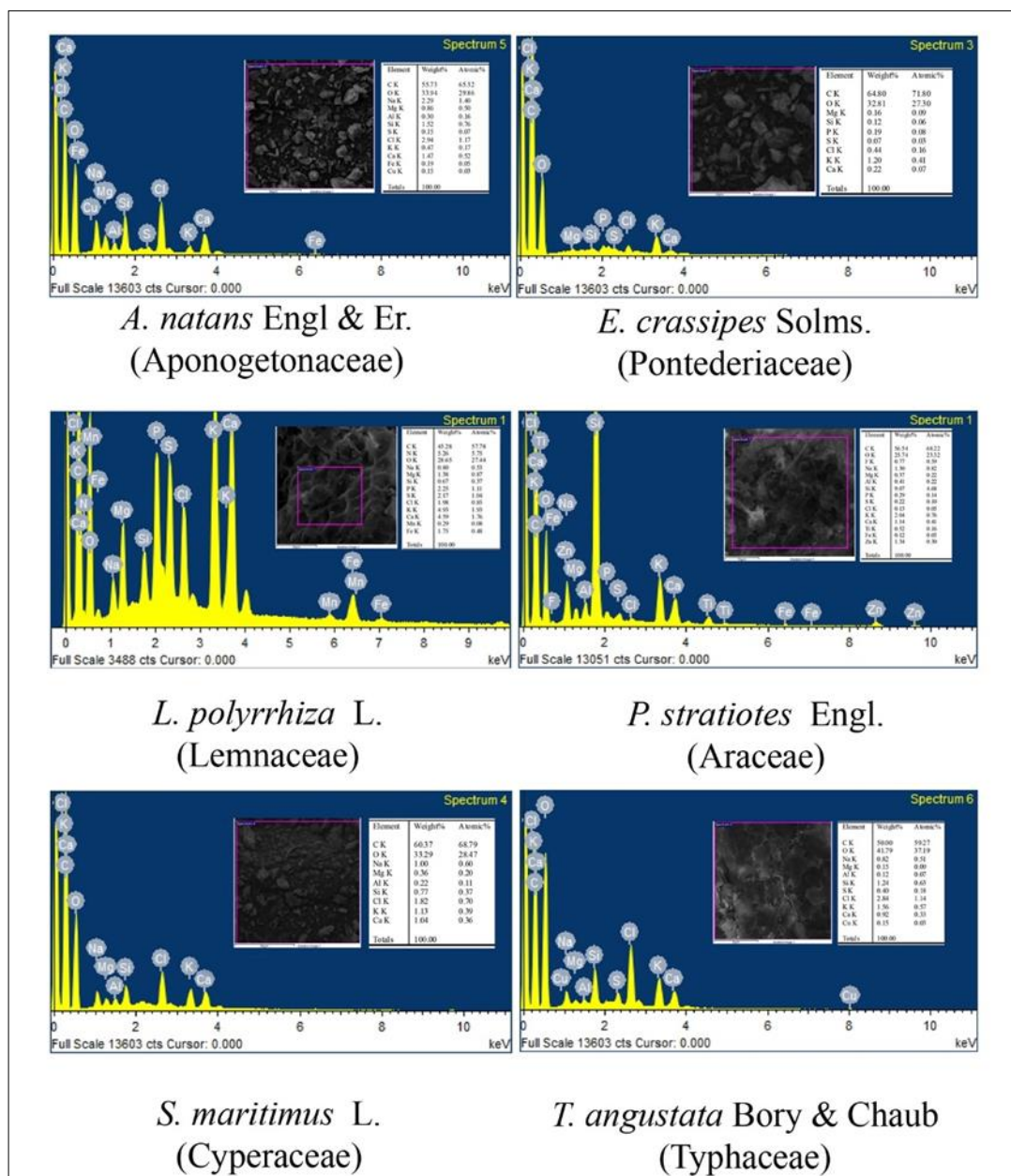


Fig 2: EDX Elemental analysis: *In situ* phytoaccumulation of elements in native aquatic and wetland plants

Physico-chemical Analysis of Water Samples

Standard methods (APHA, 2005) [11]. Were used for analyses of various physico-chemical parameters of the water samples. The physico-chemical parameters like colour, odour and pH (pH meter Model EC10), BOD, COD and DO were studied immediately at the laboratory.

SEM-EDX Elemental Analysis (Khambhaty *et al.*, 2009; Giri and Patel, 2012)

The Scanning Electronic Microscopy equipped with Energy Discharge X-ray (SEM-EDX) analysis was performed to determine the accumulation of metals and elements by the aquatic plants by phytoaccumulation process. The phyto-accumulated plant samples were dried in shade and were ground into fine powder and then placed in the steel- stubs with carbon tab stuck on the stub and sputter coated with gold particles. Elemental analysis and SEM images completed with 3.5 nm and 2.5 nm resolution for tungsten filament and LaB6 and EDX detector resolution 133 eV of the sample were assessed (SEM-TECON, Czechoslovakia, EDX – Oxford Instrument, INCA – software).

Results and Discussions

Rapid industrialization and urbanization have caused elevated release of toxic heavy metals which ultimately increase the load of pollutant in the biosphere (Lien and Wilkin, 2005) [36]. Regarding the role of heavy metals in living systems, they are divided into two classes: essential and non-essential. Essential heavy metals are those, which are needed by living organisms for their growth, development and physiological functions like Mn, Fe, Ni, Cu and Zn (Gohre and Paszkowski, 2006; Gaikwad Rupali and Khan Shahana, 2014) [26, 23], while non-

essential heavy metals are those, which are not needed by living organisms for any physiological functions like Cr, Cd, Pb, Hg and AS (Peng *et al.*, 2009).

Higher levels of heavy metals disturb the normal physiology and biochemistry of living systems (Hazrat Ali *et al.*, 2012; Akpor *et al.*, 2014) [7, 29]. The most hazardous heavy metal are As, Cd, Cr, Cu, Hg, Pb, Sn and Zn (Reeves, 2003; Gosh, 2010) [50, 27]. The concentrations of heavy metals increase in the environment from year to year. Therefore, decontamination of heavy metal contaminated water and soils is very important for maintenance of environmental health and ecological restoration (Govindasamy *et al.*, 2011) [28]. Different physical and chemical methods are used for this purpose with each method having its merits and demerits and generally considered as destructive, expensive, labour-intensive and causing secondary problems (Wu *et al.*, 2010). In comparison, phytoaccumulation is a novel, less-expensive, efficient, environmental and eco-friendly remediation strategy with good public acceptance (Revathi *et al.*, 2011) [51]. Phytoaccumulation technology was conducted with special attention to native aquatic and wetland plants growing in three different sites of Uyyakondan canal of river Cauvery. The research work pertaining to phytoaccumulation and remediation of heavy metals and its utilization as green eco-friendly approach.

Physico-chemical Characterization of Water Samples

The parameters such as colour, odour, *pH*, EC, TS, TSS, TDS, COD, total hardness as CaCO₃, carbonate alkalinity, iron, and zinc are well within the standards (WHO, 2005), whereas, Biochemical Oxygen Demand, Dissolved Oxygen, Bicarbonate alkalinity, Chromium, Copper and Lead have shown higher value than the standard (WHO, 2005). The BOD reflects the high concentration of substances that can be biologically degradable and consumption of oxygen by microbes, potentially results in dissolved oxygen. DO is an index of physical and biological process in water and the most important parameter to access the water quality. It measures the molecular oxygen utilized during specified incubation period for degradation of organic material and the oxygen used to oxidize inorganic material. The results of the present study indicates the maximum of 15.33 ± 1.15 mg/l as against WHO standard of 5.0 mg/l. DO range between 6.9 ± 0.36 to 8.4 ± 0.20 mg/l as against 6.0 mg/l, WHO standard. The variations occur due to the contamination of pollutant and in source, play a vital role (Okunade and Adekalu, 2013; Ameer Basha and Rajaganesh, 2014). Bicarbonate alkalinity concentrations fluctuated from 79.0 ± 3.60 to 86.3 ± 1.52 mg/l in the three sites. The heavy metals such as Chromium, Copper, Magnesium and Zinc concentrations are found to be in higher range than standards (WHO, 2005). Chromium concentration was 2.26 ± 0.15 to 2.50 ± 0.20 mg/l as against 0.05 mg/l (WHO standards), Copper concentration varied from 3.26 ± 0.15 to 3.46 ± 0.30 mg/l (1.0 mg/l) (WHO, 2005), lead concentrations were much higher than the 0.10 standards (WHO, 2005), i.e., it ranges from 1.0 ± 0.20 to 1.40 ± 0.26 mg/l. Due to the contamination of pollutant by various activities including E-waste leads to dispose off heavy metals into rivers (Nyamangara *et al.*, 2008) [42] (Table-1). There is a strong correlations between the *pH*, extent of degradation and concentration of heavy metal like Cr, Pb, Fe, Cd, Cu, Ni, Mn and Zn when accumulation in the water system known to cause many undesirable effects in the environment and cause health hazard (Tendai Nyakungu and Sharron Mbera, 2013) [55]. The variations in the concentrations of these heavy metals are mostly derived from industrial, agricultural and domestic activities. As a result, water bodies which are major receptacles of treated and untreated or partially treated industrial wastes have become highly polluted. The resultant effects of this on public health and the environment are usually great in magnitude as reported by Osibanjo *et al.* (2011) [44].

In situ Phytoaccumulation of Metals by Native Aquatic and Wetland Plants: SEM-EDX Elemental Analysis

The phytoaccumulation of 2.94% of chlorine, 2.29% of sodium, 1.52% of silica by *A. natans*, 1.20% of potassium by *E. crassipes*. In *L. polyrrhiza*, the accumulation of potassium was 4.93%, calcium 4.59%, phosphorous 2.25%, sulphur 2.17%, chlorine 1.98%, iron 1.75%, and magnesium 1.38%. *Pistia stratiotes* accumulated maximum percentage of silica 9.07% followed by potassium 2.04% and sodium 1.30%. *S. maritimus* plant accumulated chlorine 1.82%, followed by potassium 1.13%, calcium 1.04%, and sodium 1.00%. The plant *T. angustata* accumulated chlorine 2.84%, potassium 1.56% and calcium 0.92%. The phytoaccumulation of magnesium, silica, chlorine and potassium was shown by all the plants tested for this research work, whereas certain metals phytoaccumulation like manganese is unique for *L. polyrrhiza*, iron for *A. natans* and *L. polyrrhiza*, copper for *A. natans* and *T. angustata*, titanium and fluorine for *P. stratiotes*. The accumulation of sodium was found in all the plants except *E. crassipes*, aluminium was accumulated by *A. natans*, *P. stratiotes*, *S. maritimus* and *T. angustata*. Phosphorous was accumulated by *E. crassipes*, *L. polyrrhiza* and *P. stratiotes*. Sulphur was accumulated by all the plants except *S. maritimus*, calcium except *A. natans* (Table-2).

Aquatic plants as well as wetland plants have tremendous capacity of absorbing trace elements and heavy metals from the water and wetland (Alkorta and Garbisa, 2001; Dipu Sukumaran, 2013) [9]. However, excessive accumulation of these metals and elements can be toxic to plants. The ability to both tolerate elevated levels of metals and accumulate them in very high concentrations has been evolved both independently and together in number of different plant species (Abida Begum *et al.*, 2008; Abida Begum *et al.*, 2009). Such conditions have been found in the present study, that each plant species has accumulated tolerance level for the metals and much variations are found as reported by Abida Begum and Harikrishna (2010) [1].

Many reports have been put towards in phytoaccumulation of elements by plants. Nur Zaida and Piakong (2011)^[41], Aurangzeb *et al.* (2014)^[45], Ijaola Taiwo *et al.* (2015)^[32], Rezanian *et al.* (2015)^[52] and Priyanka Saha *et al.* (2017)^[48] have studied phytoaccumulation of metals of *E. crassipes* and proved to be the best phytoremediant for polluted water as its efficiency was 82.8% for Cadmium, 78.6% for copper, 74% for Arsenic, 73% for Aluminium and Lead. According to the present study the efficiency of *E. crassipes* is 1.20% for Potassium. Yesim Kara (2003), Brahaite *et al.* (2015), Rashidi Othman *et al.* (2015) and Chunguang Liu *et al.* (2017) were studied *Lemna minor* for phytoaccumulation work and Ahmet Sasmaz and Erdal Obek (2009) and Nabila Khellaf and Mostep Zerdaoni (2012) studied *L. gibba* for phytoaccumulation and found these plants are able to accumulate Cd, Cu, Ni, Zn, Pb and Fe, the present investigation with *L. polyrrhiza* have shown accumulation of 4.93% of Potassium, 2.17% of Sulphur, 1.98% of Chlorine, 1.75% of Iron and 1.38% of Magnesium were new research reports (Abubacker and Sathya, 2017a). Nur Zaida and Piakong (2011)^[41], Aurangzeb *et al.* (2014)^[45] and Brahaite *et al.* (2015) reported that *P. stratiotes* was found to be effective in the bioaccumulation of Pb 70.7% and Cu 66.5%, the present result with *P. stratiotes* have shown the phytoaccumulation of 9.07% Silica, followed by 2.04% of Potassium and 1.30% of Sodium. Hehe Sun *et al.* (2013), Brankovic *et al.* (2015), Phillips *et al.* (2015)^[47], and many other workers have studied the phytoaccumulation potentials of *Typha angustifolia* and found that this plant have accumulated Mn, Cu, Cd, Pb and Zn more effectively, the present study with this plant have shown the maximum accumulation of chlorine 2.84%, Potassium 1.56% and Calcium 0.92%. These results are deviated from the earlier reports and the accumulation of chlorine is the report first of its kind for *T. angustifolia* plant Ahmet Sasmaz and Erdal Obek (2009)^[46].

As far as the phytoaccumulation reports of *Aponogeton natans* and *Scripus maritimus* not much work has been reported, instead Peng *et al.* (2007)^[45] used *Potamogeton pectinatus* plant a close taxonomic relative to *Aponogeton natans* plant. Peng *et al.* (2007)^[45] revealed that *P. pectinatus* plant was able to accumulate highest concentration of Cd, Pb, Cu, Zn and Mn, the present study with *A. natans* revealed phytoaccumulation of Chlorine 2.94%, Sodium 2.29%, and Silica 1.52% and the present work *S. maritimus* has accumulated Chlorine 1.82%, Potassium 1.13%, Calcium 1.04% and Sodium 1.00%. Many workers have studied the phytoaccumulation of taxonomically related wetland genera like *Cynodon dactylon*, *Cyperus rotundus*, *Eleusine indica* (Bui Thi Kim Anh *et al.*, 2011), *Phragmites australis* (Judith Weis and Peddrick Weis, 2004) and according to their report these plants are capable of accumulating Arsenic, Lead, Cadmium and Zinc very effectively.

The present report reveals that the *in situ* phytoaccumulation of metals by native aquatic and wetland plants technologies entirely based on the phyto-remediation technique applied to both organic and inorganic pollutants present in soil (solid substrate), water (liquid substrate) or air (Aurangzeb, 2014)^[45]. The uptake of metals by plants, tolerance by plants, has been extensively used for phytoaccumulation research (Miretzky *et al.*, 2010). The variations noticed in the accumulation of metals in the present *in situ* research work with the aquatic and wet land plants are entirely due to the types of metal pollutant reach the aquatic and the adjacent wet land during the time of study.

Conclusions

These aquatic plants are capable of multiplying at a faster rate, as well as they have the ability to accumulate various elements in their biomass.

It is very easy to harvest, possible to convert them into manure.

It is an eco-friendly approach.

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