



A COMPARATIVE STUDY AMONG THREE SPECIES OF AQUATIC PLANTS IN THE ABILITY OF ABSORPTION AND BIO-ACCUMULATION OF SOME HEAVY METALS ON EUPHRATES RIVER IN AL-RAMADI CITY

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Abstract

As a result of the recent events that happen in AL-Anbar Governorate Since 2014, and because of the wars occurred in these regions, the water bodies especially, Euphrates River exposed to pollution with heavy metals that come from explosives and poisonous gases that leading to new problem for Iraqi environment which suffer from lots of them before. The objective of this work was to determine the bio-accumulation ability of aquatic plants for some heavy metals as bio-indicator. The present study was conducted along the bank of Euphrates River in Al-Ramadi City, in order to determine the concentrations of six heavy metals; Lead (Pb), Zinc (Zn), Nickel (Ni), Copper (Cu), Cadmium (Cd) and chromium (Cr) in the roots and shoot of three types of aquatic plants along the river; (*Phragmites australis*, *Typha domingensis* and *Ceratophyllum demersum*). Three sites along the river were selected for sampling; the samples were collected monthly from October 2017 until March 2018. plants samples were prepared and digested with appropriate acids in order to reveal the concentrations of these metals by using Flame Atomic Absorption Spectrophotometer (FAAS). The obtained results from this study showed the accumulation in roots of plants were higher slightly than shoot. The highest mean of absorbent Zn was 48.75 mg/l in *Ceratophyllum demersum* root and the lowest mean of absorbent Cd was 1.09 mg/l in *Phragmites australis* root. While, the highest mean of Zn was 37.44 mg/l in *Ceratophyllum demersum* shoot and the lowest mean of Cd was 0.89 mg/l. However, statistical analysis showed significant differences between roots and shoot of studied plants. The results clearly conclude that the Euphrates River is contaminated with toxic metals and the plants are considered as a good potential bio-indicators for pollution with metals by reflecting the concentrations of such metals in their environment.

The issue of water pollution in hazardous materials is one of the most important topics in the life of the population. Therefore, the research was the first to be completed after the terrorist attacks and military operations in Anbar province since 2014, especially if we know that there is a current international water crisis in Iraq that threatens the lives of 4 million citizens.

Key words: Aquatic plants, Euphrates River, Bio-accumulation

Introduction

Essential heavy metals are important constituents of several key enzymes in plants and animals, they extend biochemical and physiological functions and play significant roles in different oxidation-reduction reactions (Who, 1996). Although plants require certain heavy metals for their growth and upkeep, too much quantity of these metals can become toxic to plants (Djingova and Kuleff, 2000). Also, the toxic heavy metals accumulation in living plant cells causes different deficiencies including reduction of cell activities and inhibition of plant growth (Farooqi *et al.*, 2009), as well as accumulation of heavy metals in plant tissues in concentrations above the permitted levels which is representing a threat to the life of humans, and animals feeding on these crops and leading to contamination of food chain (Amin *et al.*, 2010) and their absorption and accumulation depend in the first

place, on the movement of the metals from the solution in the soil to the root, stems and leaves. In plants, the bioaccumulation point to the assemblage of contaminants, some of them are more suitable to be phyto-adapt than others (Kabata-Pendias, 2011). The plants take up metals and metalloids by the process, phyto-extraction, phyto-filtration and rhizo-remediation. However, the heavy metals and metalloids cannot be metabolized but accumulate in the plant biomass (Núñez-López *et al.*, 2008). Yet, the first organs to begin into connect with toxic elements are roots which usually accumulates metals more than shoots (Rout *et al.*, 2001). Many researchers have reported that aquatic macrophytes such as a genus of *Typha*, *Phragmites*, *Eichhornia*, *Azolla* and *Lemna* are wetland plants possible for removal of heavy metal and metalloids due to their morphological change (Rai, 2008; Dipu *et al.*, 2012). Also Aquatic macrophytes have great

potential to accumulate heavy metals inside their tissues hundred thousand times greater than in the associated water (Mishra *et al.*, 2008). Heavy metal indicator plants render biological and ecological functions in that they are possible indicators of pollution and useful in absorption of pollutants (Kvesitadze *et al.*, 2006). Therefore, plants have been used intensively as bio-monitors and bio-indicators of environmental pollutants in urban, rural and in remote areas (Ataabadi *et al.*, 2010; Radulescu *et al.*, 2010).

Some plant species such as *Phragmites australis* and *Ceratophyllum demersum* used as accumulators of such metals inside tissues due to the ability of these plants on the bioaccumulation of heavy metals, and for decreasing the negative effects on the ecosystem in a process called Phyto-remediation which reduce the concentrations of contaminants and remove them from the aquatic environment (Wafa'a *et al.*, 2009). In Iraq, many researchers used aquatic plants as bio-indicators for heavy metals such as studies of (Saleeh *et al.*, 2010; Khair Allahand Al Khfaji, 2017; Farhood, 2017; Al Khafaji and Hussain, 2014; Al-Ghanemi, 2010; Hanaf, 2009).

This study aims to determine the concentrations, variations for the accumulation of some heavy metals in three plants (*Phragmites australis*, *Typha domingensis* and *Ceratophyllum demersum*), of Euphrates River in Al-Ramadi City and from which can estimate the levels of these metals in the area of study.

Materials and Methods

Description of study area

Euphrates is considered one of the longest rivers in western Asia. Al-Ramadi city occupies a highly strategic position on the Euphrates. The city extends along Euphrates and is the largest city in Al-Anbar governorate. Making it a highly populated city with diverse professional, commercial and industrial activities in various fields, in which such activities may drain their wastes directly to the river water body. However three sites were selected along the course of the Euphrates River through the Ramadi city. These sites were determined by using Global Position System (GPS) as it seen (in Fig.1; Table1)

Table 1 : Coordinates of Study Sites

Longitude (E°)	Latitude (N°)	Sites
43°15'22.08"	33°27'21.83"	S.1
43°19'19.32"	33°26'37.07"	S.2
43°20'49.56"	33°28'19.50"	S.3

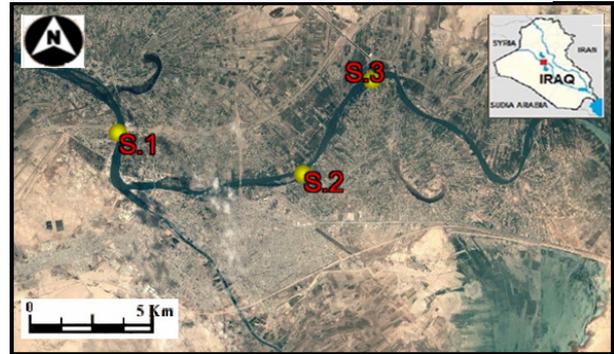


Figure 1 : Study Sites at Euphrates River, Ramadi City. (Google Earth)

Site number 1 is located near Palestine Bridge and it considers as upstream of the Euphrates River in Ramadi City. This site is characterized with many villages nearby. The plants occur rarely and the common is *Phragmites australis*, *Typha domingensis* and *Ceratophyllum demersum*. Regarding site number 2, it is characterized with high human population, there for it is affected by their activities and likely contaminated with different pollutants. Various plant species are common with considerable densities. However, Site (3) is surrounded by agricultural areas; citizens occupy many occupations in addition to agriculture. There are many plant species such as *Phragmites australis*, *Typha domingensis* and *Ceratophyllum demersum*. Forms of the three aquatic plants are show in figures below (Fig.2, 3 and 4).

Plant Specimens

Aquatic plant specimens including root and shoot were collected from three sites (Site 1, Site 2 and Site 3). The specimens were washed firstly with river water, then with distilled water, and transferred to the laboratory in sterilized poly-ethylene containers. Plant samples were air dried for about 72 hrs. Then grounded using the blender and kept in dark at 4°C until the analysis process started.

Samples Preparation

Extraction heavy metals From Plant's Tissues

The Heavy metals extracted from a plant according to (APHA, 1998), where 1.0g of dried and grounded plant was taken into a conical flask. 5ml of concentrated nitric acid were added and swirl carefully, then left on the rack from 6 to 8 hour. After pre-digestion, 10 ml of di-acid ($\text{HNO}_3 + \text{HClO}_4$) were added. Then placed in the rack which placed in the block digester at a temperature 180-200 °C until the dense white fumes release and transparent white contents are left. Then the sample left to cool at the room temperature and filtered through what man NO.1 filter paper and brought to 50 ml volume to be prepared for analysis

Heavy Metals Determination

The concentrations of heavy metals (Pb, Cd, Cr, Zn, Ni, and Cu) in the extracts were determined by using the atomic absorption spectrophotometer (Perkin Elmer 5000) with hollow cathode lamps for each metal. As well as the standard solutions of each metal were prepared from stock solution as needed.



Figure 2: *Typha domingensis*



Figure 3: *Phragmites australias*



Figure 4: *Ceratophyllum demersum*

Statistical analysis

The statistical analyses were performed by using Statistical Package for the Social Science (SPSS)

software (version 23). One –way ANOVA were used to determine the least significant difference (LSD) between roots also between shoot of plants as well as at sites between sites. T-test was used to determine the significant differences between root and shoot of each plant also among plants. The level of significance was set at $P < 0.05$

Result and Discussion

Six heavy metals (Pb, Zn, Ni, Cu, Cd and Cr) have been measured in these aquatic plants to determine the ability of these plants to accumulate the metals in their tissues (root and shoot). The mean concentrations of Pb in root and shoot of those aquatic plants show in (Fig.5) The mean concentrations of Pb in *Phragmites australis* root was 27.64 ppm. The lowest value was observed at Site 1 in November, while the highest was at Site 2 in March, which are 12 ppm and 55 ppm respectively. While the highest concentration of Pb in *P. australis* shoot 40 ppm was found at Site 2 in March and the lowest concentration 10 ppm was found at Site 1 in November with mean 23.31 ppm.

For *Ceratophyllum demersum* root, the mean Pb concentration was 41.62 ppm during the study period. The highest Pb concentration was 60.2 ppm found at Site 3 in March, while the lowest was 30 ppm at Site 1 in October. In *C. demersum* shoot, the highest value 51 ppm was recorded at Site 3 in March and the lowest value 28 ppm at Site 1 in October and November with mean of 35.94 ppm.

Also the study showed Pb concentration in *Typha domingensis* root, the highest value 50.8 ppm was found at Site 2 in March, and the lowest value 20 was at Site 1 in October. The mean was 38.16 ppm, While the highest values of pb in *T. domingensis* shoot was 40.1 ppm at Site 2 during March and the lower value 22 ppm at Site 1 during November. with mean 32.91 ppm.

The mean concentrations of Zn in root and shoot of those aquatic plants show in (Fig.6). For *P. australis* root, the highest value 50 ppm was recorded at Site 3 in March and the lowest value was 12.5 ppm at Site 1 in November with mean of 33.31 ppm. While the mean concentrations of Zn in *P. australis* shoot was 24.44 ppm. The highest value was observed at Site 2 in March and the highest was at Site 2 and Site 3 in November, are 51 ppm and 7.5 ppm respectively.

For *C. demersum* root the mean value of Zn during the study period was 48.75 ppm. The highest value 67 ppm was recorded at Site 2 in March, whilst the lowest value 33 ppm was recorded at Site 1 in October. Regarding *C. demersum* shoot, the mean concentration of Zn was 37.44 ppm. The highest value was found at

Site 2 in March, while the lowest was at Site 3 in November being 61 ppm and 28 ppm respectively.

Also the study showed that the mean concentration of Zn in *T. domingensis* root was 36.27 ppm, the highest value 46 ppm in March at Site 2, and the lowest value 13 ppm in November at Site 3. In *T. domingensis* shoot, the highest value 45 ppm was found at Site 3 in October, whilst the lower value 10 ppm at Site 3 in November. The mean value was 29.51 ppm.

Regarding the mean value of Ni in the *P. australis* root. The mean value of Ni was 4.38 ppm during the study period. The highest value 13 ppm was at Site 2 in March 2018, while the lowest value was 1.1 ppm at Site 1 in November. For *P. australis* shoot, the highest Ni value was 10.2 ppm recorded in March at Site 2, whilst the lowest value was 0.9 in November 2017 at Site 1 with the mean of 3.54 ppm.

The results during study period showed that the highest concentration of Ni in *C. demersum* root was 15 ppm, observed at Site 2 during March, while the lowest concentration 2 ppm was at Site 1 during October, with a mean of 7.16 ppm. For *C. demersum* shoot, the mean concentration of Ni was 6.22 ppm, while highest concentration was 12 ppm found in March at Site 2, the lowest concentration was 1.8 ppm measured in November at Site 1.

Pertains *T. domingensis* root, the mean concentration was 6.44 ppm, the high value 12.5 ppm was recorded at Site 2 in March, whereas the low value of 1.4 ppm was recorded at Site 1 during October. For *T. domingensis* shoot the study showed high value of 12 ppm at Site 2 during March and the low value 1.5 ppm was recorded at Site 1 during November with the mean of 5.83 ppm. The mean concentrations of Ni in root and shoot of those aquatic plants shown in (Fig.7).

Pertains the mean concentration of Cu. The mean concentration of Cu in *P. australis* root was 3.99 ppm. The highest value was 6.5 ppm recorded in March 2018 at Site 2, while the lowest value 2.2 ppm showed in November 2017 at Site 1, in *P. australis* shoot, concentration ranged from 6.3 ppm and 1.8 ppm at Site 3 in October and Site 1 in November respectively with mean of 3.23 ppm.

Regarding *C. demersum* root, the highest concentration of 12 ppm was found at Site 2 in March, whilst the lowest 2.8 ppm was at Site 1 in October. The mean was 6.65 ppm. For *C. demersum* shoot the mean concentration was 5.21 ppm, the values ranged from 9.5 ppm at Site 2 in March and 2.4 ppm at Site 1 in October.

Concerning *T. domingensis* root, the high value was 12.5 ppm at Site 2 in February, whereas the lower value was 2 at Site 1 in October with mean of 6.89 ppm.

As for *T. domingensis* shoot, the values ranged from 10.5 to 2.3 ppm. The maximum concentration of Cu was recorded in March at Site 2, whereas the minimum concentration was measured in November at Site 1 with mean value 6.24 ppm. The mean concentrations of Cu in root and shoot of those aquatic plants shown in (Fig.8).

The mean concentration of Cd in root and shoot of those aquatic plants are shown in (Fig.9). For *P. australis* root, the highest Cd value 1.6 ppm recorded in October at Site 3, whilst the lowest value was 0.7 in October at Site 1 with mean of 1.09 ppm. For *P. australis* shoot the maximum value 1.7 ppm was recorded at Site 3 in October, while the minimum value 0.5 ppm was measured at Site 1 in November with mean of 0.89 ppm.

As for *C. demersum* root, the mean concentration of Cd was 1.96 ppm. The highest concentration was 3.3 ppm found in March at Site 2, the lowest concentration was 1 ppm measured in October at Site 1, while in *C. demersum* shoot, the maximum value was 3 ppm and the minimum value 0.9 ppm was recorded in March at Site 2 and in October at Site 1 respectively with mean value of 1.68 ppm.

Regarding *T. domingensis* root, the results shows that the highest value of 3.5 ppm at Site 2 during March and lowest value 0.9 ppm was recorded at Site 1 during October with mean value of 1.98 ppm, while in *T. domingensis* shoot the value was ranged from 3 ppm in March at Site 2 to 0.9 ppm in November at Site 1, with mean value of 1.82 ppm.

As regards for the mean concentration of Cr, the mean concentrations of Cr in *P. australis* root was 2.59 ppm. The highest value was 6.1 ppm recorded in October at Site 2 and Site 3, whereas the lowest value 1 ppm was found in November at Site 1. For *P. australis* shoot, concentrations values of Cr was ranged from 6.5 to 0.9 ppm. The maximum concentration was found at Site 3 in October and minimum concentration was at Site 1 in November having a mean value of 2.21 ppm.

For *C. demersum* root, the highest concentrations of Cr 9.2 ppm was recorded in March at Site 2, while the lowest 2 ppm was found in October at Site 1 with mean of 5.82 ppm, as for *C. demersum* shoot, the values of Cr ranged from 7.7 to 1.6 ppm. The maximum value was recorded at Site 2 in March and the minimum value was observed at Site 1 in October having a mean value of 5.17 ppm.

Pertains *T. domingensis* root, the mean concentrations of Cr 5.87 ppm, the highest value of concentrations 9.3 ppm was observed in March at Site 2, whilst the lowest value 1.3 ppm was observed in October at Site 1. In *T. domingensis* the concentration

values ranged from 8.2 ppm at Site 2 in March to 1.4 ppm at Site 1 in November with mean value of 5.27 ppm. The mean concentrations of Cr in root and shoot of those aquatic plants present in (Fig.10).

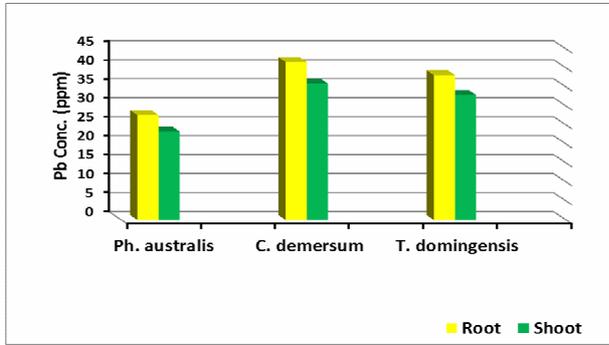


Figure 5 : Mean concentration of Pb in roots and shoot of studied plants

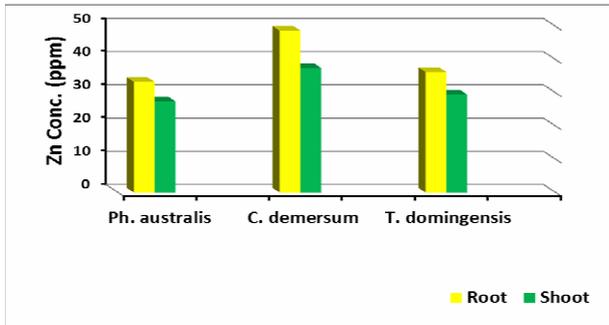


Figure 6: Mean concentration of Zn in roots and shoot of studied plants

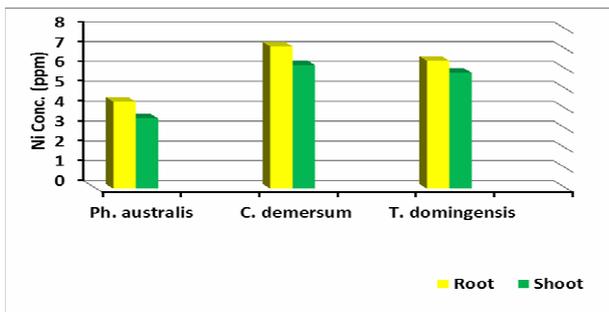


Figure 7: Mean conc. of Ni in root and shoot of studied plants

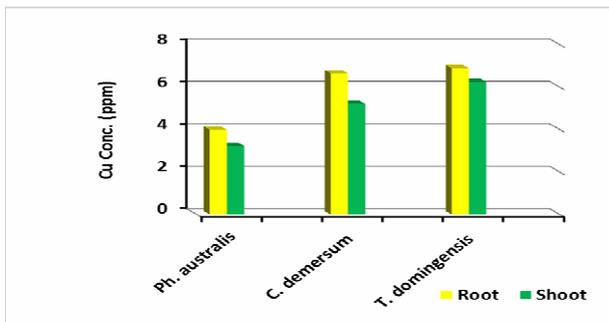


Figure 8: Mean conc. of Cu in root and shoot of studied plants

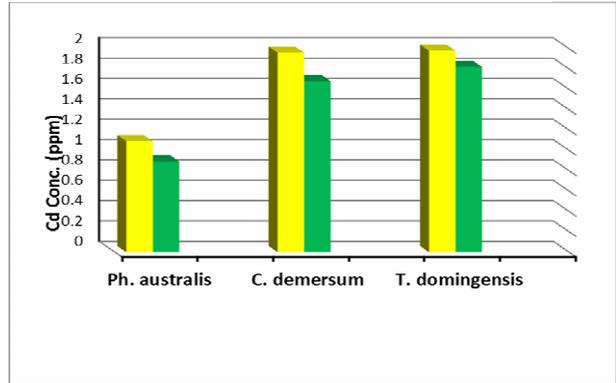


Figure 9: Mean conc. of Cd in root and shoot of studied plants

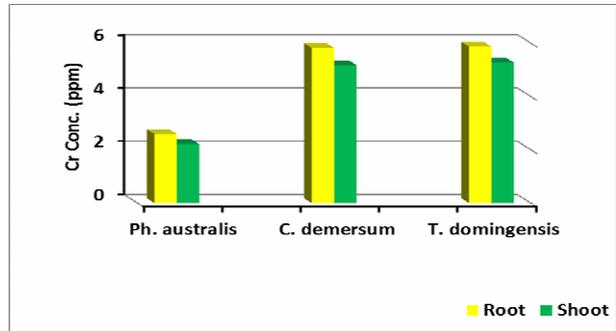


Figure 10: Mean conc. of Cr in roots and shoot of studied plants

According to the aforementioned results, it is clear that there were differences in the concentrations of heavy metals between sites. The higher levels of these metals in roots and shoot of those plants (*P. australis*, *C. demersum* and *T. domingensis*) were found at Site 2 than at Site 1 (the control site), this may be due to exposure of Site 2 to different types of pollutants such as sewage, chemicals used for fishing, oil spilt from boats, while Site 1 was less polluted. Also the concentrations of these metals in root and shoot of plants during March was more than their concentrations in October and November, this may be due to rainfall which decreasing the pH and leads to elevating the concentrations of heavy metals. However, the accumulation of heavy metals different between the plants and also between the plant parts (root, stem, leaves), (Bareen and Khilji 2008). The statistical analysis shows significant different ($p < 0.05$) between plants roots as well as between shoots in sites and also between roots as well as between shoot of plants as present in Table (2). The concentration of heavy metals in *P. australis* root and shoot showed a significant difference ($p < 0.05$) with root and shoot of *C. demersum* and *T. domingensis*. Yet, the mean concentrations of Pb, Zn, and Ni in the roots and shoot of the plant were higher in *C. demersum*, but mean concentrations of Cu, Cd and Cr were higher in

T. dominensis. This results are in agreement with previous studies of (Mashkool, 2012; Farhood, 2017; Al-Khafaji *et al.*, 2012).

Therefore the plants used as a good indicator of pollution with heavy metals due to having the ability to absorb heavy metals from sediments and soils and accumulate them in their tissues (Cheng, 2003). Thus, the accumulation of HMs in aquatic plants came from their environment and they are considered as a good indicator for study the change of HMs in environment (Radeef *et al.*, 2013). Many previous studies used many plants as an indicator of HMs pollution such as studies of (Farhood, 2017, Khair Allah and Al-Khafaji, 2017; Al-Helaly, 2010; Mashkool, 2012). This study showed that the three aquatic plants were varied from one to another in the accumulation of HMs in their tissue. also the result showed that mean concentrations of heavy metals in plants roots (*P. australis*, *C. demersum* and *T. domingensis*) was higher than the mean concentration of heavy metals in plants shoots (*P. australis*, *C. demersum* and *T. domingensis*). However, the mean concentration of Pb, Zn and Ni in root and shoots of plants were higher in *C. demersum* while the mean concentration of Cu, Cd and Cr were higher in *T. domingensis*.

Statistical analysis shows there was no significant differences ($p>0.05$) in the concentrations of metals (except Cd) between root and shoot of *P. australis* also it found no significant difference in concentrations of these metals (except Pb and Zn) between root and shoot of the *C. demersum* as well as *T. domingensis*. Additionally, the concentrations of metals (except Ni, Cd and Cr) between root and shoot of all plants showed significant differences ($P<0.05$) as present in Table (2). The highest mean of concentrations of all HMs were found in the *C. demersum*, this may be due to the exposure of all parts of plant to HMs that occur in water and sediment because it is the first submersible plant (Fritioff, 2005). While the lowest mean were found in the *P. australis*, this may be due to the degree of development and complexity of plant that makes it no accumulated for high levels of elements especially toxic compounds (Peralta-Videa *et al.*, 2009). Skorbilowicz (2009) reported to the existence mechanisms of physiology in plants high quality to get rid of elements, especially toxic materials. The results are in agreement with previous studies of (Hussain, 2014; Al-Khafaji and Hussein, 2015; Farhood, 2017).

Table 2 : Mean concentrations± standard deviation of heavy metals in the root and shoot of aquatic plants.

Heavy metals	<i>P. australis</i>						<i>C. demersum</i>						<i>T. domingensis</i>						LSD
	Site 1		Site 5		Site 6		Site 1		Site 5		Site 6		Site 1		Site 5		Site 6		
	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	
Pb	19.50 ±4.14	17.67 ±6.12	35.92 ±12.21	28.75 ±11.29	27.50 ±7.71	23.5 ±9.99	36.83 ±4.88	30.5 ±2.74	46.00 ±8.05	40.5 ±7.18	42.03 ±11.02	36.83 ±7.99	31.00 ±7.32	25.7 ±2.82	43.13 ±6.51	37.35 ±3.09	40.33 ±6.19	35.67 ±1.63	13.289
Zn	27.30 ±10.90	22.62 ±6.66	38.42 ±3.77	32.95 ±18.02	34.20 ±12.21	26.75 ±13.67	41.17 ±6.55	34 ±5.51	57.08 ±7.02	41.5 ±12.14	48.00 ±10.02	36.83 ±9	36.12 ±6.56	29.37 ±3.91	38.20 ±7.92	29.83 ±3.87	34.50 ±11.95	29.33 ±11.6	24.691
Ni	2.85 ±2.52	2.33 ±1.68	5.55 ±4.74	4.75 ±3.83	4.73 ±3.76	3.53 ±2.83	3.78 ±2.80	3.6 ±3.25	9.15 ±3.25	7.83 ±7.4	8.55 ±2.96	7.23 ±1.92	3.07 ±2.45	2.54 ±1.36	8.68 ±2.18	8.22 ±2.06	7.58 ±2.39	6.83 ±1.66	7.176
Cu	3.03 ±0.79	3.38 ±0.56	4.68 ±1.45	3.3 ±1.22	4.27 ±1.49	3.42 ±1.58	3.58 ±0.66	3.05 ±0.6	9.42 ±2.06	6.97 ±2.25	6.95 ±2.30	5.6 ±1.84	3.18 ±0.86	2.82 ±0.46	9.15 ±2.67	8.33 ±1.72	8.35 ±1.51	7.58 ±0.92	9.256
Cd	0.93 ±0.18	0.75 ±0.19	1.20 ±0.19	0.93 ±0.18	1.15 ±0.26	0.98 ±0.41	1.32 ±0.29	1.13 ±0.23	2.68 ±0.44	2.15 ±0.56	1.88 ±0.29	1.77 ±0.24	1.17 ±0.24	1.12 ±0.21	2.57 ±0.59	2.27 ±0.45	2.22 ±0.20	2.08 ±0.27	2.384
Cr	1.83 ±0.83	1.62 ±0.64	3.12 ±1.79	2.6 ±1.84	2.82 ±1.88	2.4 ±2.09	2.68 ±0.86	2.23 ±0.73	7.83 ±1.07	6.77 ±0.73	6.93 ±0.61	6.52 ±0.48	2.47 ±1.16	1.98 ±0.65	8.00 ±1.06	7.32 ±0.75	7.13 ±0.98	6.5 ±0.51	2.0760

Conclusion

In conclusion, The aquatic plants accumulate high levels of these metals in their tissues (roots and shoot) and the accumulation are different between plants and also between root and shoot of plants; as wells as in sites and months, how ever the accumulation in roots was higher slightly than shoot. And the accumulation of (pb, Ni, Zn) in root and shoot was higher in *C. demersum* while the accumulation of (Cu, Cd, Cr) was higher in root and shoot of *T. domingensis* Therefore, those plants can use as indicator for pollution with heavy metals. As well as using in the biological treatment of water instead of chemicals.

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