

# Heavy metal biomonitoring and phytoremediation potentialities of aquatic macrophytes in River Nile

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**Abstract** The concentrations of Cd, Cu, Pb, and Zn in sediments, water, and different plant organs of six aquatic vascular plant species, *Ceratophyllum demersum* L. *Echinochloa pyramidalis* (Lam.) Hitchc. & Chase; *Eichhornia crassipes* (Mart.) Solms-Laub; *Myriophyllum spicatum* L.; *Phragmites australis* (Cav.) Trin. ex Steud; and *Typha domingensis* (Pers.) Poir. ex Steud, growing naturally in the Nile system (Sohag Governorate), were investigated. The aim was to define which species and which plant organs exhibit the greatest accumulation and evaluate whether these species could be usefully employed in biomonitoring and phytoremediation programs. The recorded metals in water samples were above the standard levels of both US Environmental Protection Agency and Egyptian Environmental Affairs Agency except for Pb. The concentrations of heavy metals in water, sediments, and plants possess the same trend: Zn > Cu > Pb > Cd which reflects the biomonitoring potentialities of the investigated plant species. Generally, the variation of heavy

element concentrations in water and sediments in relation to site and season, as assessed by two-way repeated measured ANOVA, was significant ( $p < 0.05$ ). However, insignificant variations were observed in the concentrations of Pb and Cd in sediments in relation to season and of Cu and Zn in relation to site. Results also showed that the selectivity of the heavy elements for the investigated plants varied significantly ( $p < 0.05$ ) with species variation. The accumulation capability of the investigated species could be arranged according to this pattern: *C. demersum* > *E. crassipes* > *M. spicatum* > *E. pyramidalis* > *T. domingensis* > *P. australis*. On the basis of the element concentrations, roots of all the studied species contain higher concentrations of Cu and Zn than shoots while leaves usually acquire the highest concentrations of Pb. Cd concentrations among different plant organs are comparable except in *M. spicatum* where the highest Cd concentrations were recorded in the leaves. Our results also demonstrated that all the studied species can accumulate more than 1,450-fold the concentration of the investigated heavy elements in water rendering them of interest for use in phytoremediation studies of polluted waters. Given the absence of systematic water quality monitoring, heavy elements in plants, rather than sediments, provide a cost-effective means for assessing heavy element accumulation in aquatic systems during plant organ lifespan.

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## Introduction

Water pollution is considered to be one of the most dangerous hazards affecting both developing and developed countries. The large-scale industrialization and production of variety of chemical compounds has led to global deterioration of the environmental quality (Chakravarty et al. 2010). In Egypt, the pollution of River Nile system (main stem Nile, drains and canals) has increased in the past few decades because of increases in population; several new irrigated agriculture projects, and other activities along the Nile. As the program to expand irrigated agriculture moves forward, the dilution capacity of River Nile system will diminish at the same time that the growth in industrial capacity is likely to increase the volume of pollutants discharged to the Nile (APRP 2002).

It has been known for long time that aquatic plants, both living and dead, are heavy metal accumulators and, therefore, the use of aquatic plants for the removal of heavy metals from wastewater has gained high interest (Kuyucak and Volesky 1989). Moreover, aquatic plant-based treatment systems are low-cost technologies which can be adopted by developing countries for recycling/treatment of waste water, especially contaminated by heavy/toxic metals (Figueira and Ribeiro 2005). This technology is considered as an alternative solution for conventional methods to clean up heavy metals from contaminated waters (Khambhaty et al. 2009) that leads to its selection for phytoremediation of various industrial events (Verma et al. 2005). This technology can be applied to both organic and inorganic pollutants present in soil (solid substrate), water (liquid substrate), or the air (Salt et al. 1998).

In the present study, the concentrations of Cd, Cu, Pb, and Zn in sediments, water and different plant organs of six aquatic vascular plant species, *Ceratophyllum demersum* L. *Echinochloa pyrami-*

*dalis* (Lam.) Hitchc. & Chase.; *Eichhornia crassipes* (Mart.) Solms-Laub; *Myriophyllum spicatum* L.; *Phragmites australis* (Cav.) Trin. ex Steud; and *Typha domingensis* (Pers.) Poir. ex Steud, growing naturally in the Nile system (Sohag Governorate) were investigated. The aim was to: (1) evaluate the relationship among heavy metals concentrations in the water, sediments and aquatic macrophytes; (2) determine the heavy metal selectivity of each plant species and of different organs; and (3) define which species and which plant organs exhibit the greatest accumulation. The use of these species for biomonitoring and phytoremediation programs has also been considered.

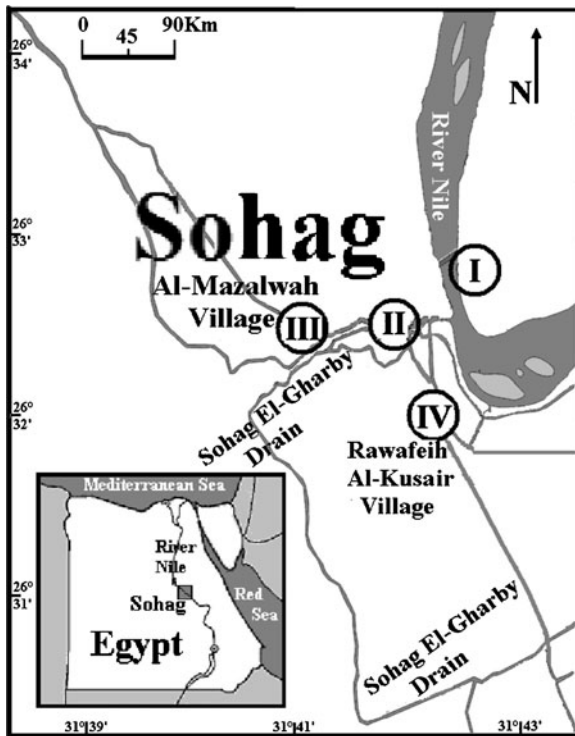
## Study area

Sohag is one of the rural governorates in Upper Egypt. Its capital, Sohag City, is located 467 km south of Cairo. Sohag Governorate is one of the most populated areas in Egypt, which include about 4 million persons. Geographically, the Governorate is a narrow strip of land along both banks of River Nile, with a length of 110 km (Fig. 1). Four sampling sites were selected to represent different pollution sources (Fig. 1). Site I is at the main stream of River Nile east bank and affected by industrial effluents of a nearby outlet of a beverage factory (N: 26° 33' 29"; E: 31° 42' 33"); site II is located at Sohag Al-Gharby drainage canal (N: 26° 32' 18"; E: 31° 41' 79"), affected mainly by agricultural waste water; site III is opposite to the major outfall of domestic waste water station of Al-Mazalwah village (N: 26° 31' 65"; E: 31° 39' 41") and site IV is located below Rawafeih Al-Kusair village (N: 26° 31' 16"; E: 31° 42' 39"), affected by both domestic and agricultural waste water drainage.

## Materials and methods

### Sampling

Surface water, sediments as well as six native aquatic plant species (*C. demersum*; *E. pyramidalis*; *E. crassipes*; *M. spicatum*; *P. australis*; and *T. domingensis*) were sampled from four



**Fig. 1** Location map of Sohag City showing the selected sites (I, II, III, IV)

sites representing three different pollution sources according to the range of distribution of the study species (Fig. 1). All samples (water, sediments, and plants) were collected in triplicates, monthly, throughout the year (from January 2006 to December 2006) and preserved in cool ice boxes throughout the field trip and during transportation to the laboratory. However, pH, water temperature, conductivity, and dissolved oxygen (DO) were measured immediately after collection using a pH meter; a dry mercury thermometer; a handheld conductivity meter PC 300 (Eutech instruments); and a Schott handy lab OX 1/SET portable meter, respectively. Each water sample was divided into two aliquots: one was acidified immediately in the field with nitric acid (1 ml  $\text{HNO}_3 \text{ L}^{-1}$ ) for heavy metals determination, while the other stored in a separate bottle and immediately refrigerated upon reaching the lab until nutrient analysis. Analysis for biochemical oxygen demand (BOD) and nutrients were completed in the lab. Aquatic macrophytes were identified according to Boulos (2005).

## Analytical techniques

The water samples for nutrient determination were filtered within 1 h of collection using Millipore filter paper type HA 0.45  $\mu\text{m}$  pore size (APHA 1995). Nitrite was determined after filtration of the water samples using methods described by Dewis and Freitas (1970) while nitrate was determined using the sodium salicylate method (DEWAS 1980). Reactive and total phosphorus were determined according to the molybdenum blue method (APHA 1995). Sediment samples were wet-sieved through a 63-mm sieve, washed with de-ionized water, dried at  $105^\circ\text{C}$  and homogenized. One gram of homogenized samples was digested using  $\text{HNO}_3\text{-HF-H}_3\text{BO}_4$  acids according to Wade et al. (1993). Plants were harvested and sorted by species in the laboratory; carefully washed using tap water then distilled water to remove all the debris and other foreign particles (O'Halloran et al. 1997). Plants were then separated into aboveground parts (stems and leaves) and belowground parts (roots of floating species and roots plus rhizomes of emergent macrophytes). After separation, each part was oven-dried at  $75^\circ\text{C}$  and grounded to a fine powder. Approximately 0.2 g of homogeneous samples of leaves, rhizomes and roots powder were weighed and digested according to method described by Allen (1989). Concentrations of selected metals in water, sediment, and plant samples were determined by a flame atomic absorption spectrophotometer (Varian, spectra AA220). All the analyses were carried out on three subsamples.

## Data analysis

In the present investigation, the bioaccumulation factor (BF) was calculated using the formula outlined by Sadiq (1992):

$$\text{BF} = \frac{\text{element concentration in plant}}{\text{element concentration in water}}$$

Statistical tests including paired Student's *t* test, two-way repeated measured and three-way analysis of variance (ANOVA) together with simple linear correlation coefficient were performed with

SPSS, version 13 computer package (Levesque 2007).

## Results and discussion

### Physicochemical characteristics of water

Each water body has an individual pattern of physical and chemical characteristics which are determined largely by the climatic, geomorphologic, and geochemical conditions prevailing in the drainage basin and the underlying aquifer. Several studies on the physicochemical characteristics of the River Nile water were reported by Abou El-Atta (1978), Saad (1980), Soltan (1988), Abdel-Satar (1994), Ghallab (2000) and Elewa et al. (2001).

In the present study, the mean water temperature of the selected sites (Table 1) varied between 18°C in winter and 29°C in summer. The variation in the temperature of water samples was highly significant ( $p < 0.001$ ) among different seasons. Conversely, the variation of temperature among studied sites were not significant ( $p > 0.05$ ). However, site IV (Rawafeih Al-Kusair drain) acquired the highest temperature at all seasons. The wa-

ter temperature of the irrigation, drainage, and sewage canals are relatively higher compared to those of the open River Nile (site I). This could be attributed to the fact that these canals are narrower and shallower than River Nile and they are characterized by dense vegetation of hydrophytes.

The average values of pH of the selected sites (Table 1) were generally in the neutral and slightly alkaline side; being ranged between 7.1 and 8.1. However, pH values of water samples varied significantly ( $p < 0.001$ ) among the different seasons. According to Samaan (1974) and Kwaitkowski and Roff (1976), the changes in pH are mainly due to photosynthesis activities of phytoplankton and aquatic plants, and respiration of animals and plants as well as variations in temperature. In accordance with (Mohammed et al. 1984), our results indicated that the water of River Nile at Sohag City is slightly alkaline and lies within the permissible range (EPA 2006).

The conductivity of most fresh water ecosystems ranges from 10 to 1,000  $\mu\text{S}/\text{cm}$  but may exceed 1,000  $\mu\text{S}/\text{cm}$ , especially in polluted waters. Conductivity measurements are useful in rivers for the management of temporal variations in total dissolved solids and major ions (Chapman

**Table 1** Physicochemical characteristics of the water samples (average  $\pm$  sd) collected from the selected sites during different seasons

Seasons	Site	Physicochemical characteristics of water				
		T (°C)	pH	E.C ( $\mu\text{S}/\text{cm}$ )	DO (mg/L)	BOD (mg/L)
Winter	I	18.2 $\pm$ 0.5	8.0 $\pm$ 0.2	552.5 $\pm$ 217.1	9.8 $\pm$ 0.2	1.6 $\pm$ 0.3
	II	19.1 $\pm$ 0.6	8.0 $\pm$ 0.6	511.8 $\pm$ 221.9	8.7 $\pm$ 0.6	3.9 $\pm$ 0.7
	III	18.1 $\pm$ 0.7	7.8 $\pm$ 0.3	1225.5 $\pm$ 639.0	8.3 $\pm$ 1.0	3.9 $\pm$ 0.4
	IV	21.9 $\pm$ 2.6	7.6 $\pm$ 0.6	1029.3 $\pm$ 554.5	7.7 $\pm$ 0.7	4.4 $\pm$ 0.5
Spring	I	23.3 $\pm$ 2.1	7.1 $\pm$ 0.3	382.5 $\pm$ 78.0	8.2 $\pm$ 0.3	1.4 $\pm$ 0.3
	II	23.8 $\pm$ 2.2	7.6 $\pm$ 0.2	331.3 $\pm$ 19.3	7.6 $\pm$ 0.3	2.6 $\pm$ 0.5
	III	23.0 $\pm$ 3.1	7.7 $\pm$ 0.3	971.8 $\pm$ 83.3	7.1 $\pm$ 0.6	2.9 $\pm$ 0.4
	IV	24.4 $\pm$ 2.0	7.2 $\pm$ 0.2	688.5 $\pm$ 72.0	6.5 $\pm$ 0.4	3.1 $\pm$ 0.7
Summer	I	26.6 $\pm$ 1.4	7.8 $\pm$ 0.4	342.2 $\pm$ 40.7	7.4 $\pm$ 0.2	1.4 $\pm$ 0.7
	II	26.0 $\pm$ 0.9	7.8 $\pm$ 0.4	329.3 $\pm$ 20.8	6.3 $\pm$ 0.3	1.6 $\pm$ 1.2
	III	29.2 $\pm$ 1.3	7.7 $\pm$ 0.3	890.5 $\pm$ 180.8	6.0 $\pm$ 0.4	2.0 $\pm$ 0.9
	IV	28.8 $\pm$ 1.0	7.5 $\pm$ 0.3	826.2 $\pm$ 124.2	5.8 $\pm$ 0.2	2.0 $\pm$ 0.4
Autumn	I	22.7 $\pm$ 3.3	8.1 $\pm$ 0.6	405.8 $\pm$ 68.3	8.4 $\pm$ 0.8	1.0 $\pm$ 0.1
	II	24.8 $\pm$ 4.7	7.9 $\pm$ 0.3	357.7 $\pm$ 28.2	7.3 $\pm$ 0.5	3.0 $\pm$ 0.4
	III	24.2 $\pm$ 4.2	8.0 $\pm$ 0.2	821.8 $\pm$ 146.1	7.1 $\pm$ 0.3	5.0 $\pm$ 0.3
	IV	25.2 $\pm$ 2.5	7.8 $\pm$ 0.1	721.0 $\pm$ 46.8	6.9 $\pm$ 0.3	5.8 $\pm$ 0.4

T temperature; pH hydrogen ion concentration, E.C electrical conductivity, DO dissolved oxygen, BOD biochemical oxygen demand

1996). In this study, the seasonal variation in the electric conductivity (Table 1) of water samples of different studied sites was highly significant ( $p < 0.001$ ) while spatial variation was insignificant. Results also showed that water conductivity was higher at site III (Al-Mazalwah drain) and site IV (Rawafeih Al-Kusair drain) than the other sites. This could be attributed to the huge amounts of domestic waste water discharged into these sites from the nearby villages. According to the EPA (1997), discharges to streams can change the conductivity depending on their make-up. A failing sewage system would raise the conductivity because of the presence of chlorides, phosphates, and nitrates. Conductivity is also affected by temperature, (the warmer the water, the higher the conductivity). This has been proved by the results of the temperature which show that the water temperature was higher at sites III and IV than that at the other two sites.

In this study, both seasonal and spatial variations of the DO in all sites were highly significant ( $p < 0.001$ ). In all sites, the dissolved oxygen acquired its maxima in winter (Table 1). This is attributed to the activities of air movement which allow more transfer of oxygen across the air–water interface and also due to turbulence in the flowing water (Maria Adelaid et al. 2000). Relatively lower temperature in winter may increase the ability of water to hold dissolved oxygen (Radwan et al. 2003). Sites I (River Nile) and II (Sohag Al-Gharby drainage canal) acquired relatively higher values (9.83 and 8.71 mg L<sup>-1</sup>, respectively) of DO than the others. In fresh waters, DO ranges from 15 mg L<sup>-1</sup> at 0°C to 8 mg L<sup>-1</sup> at 25°C. Concentrations in unpolluted waters are usually close to, but less than, 10 mg L<sup>-1</sup> (Environment Canada 1987; Committee for Fisheries 1993; WHO 1993; Gray 1994). Waters of Sites III (Al-Mazalwah drain) and IV (Rawafeih Al-Kusair drain) are heavily polluted and subjected to the discharge of both domestic and agricultural waste waters. The introduction of excess of organic matter may result in a depletion of oxygen from an aquatic system mainly during warm stagnant condition (Maria Adelaid et al. 2000), as a result of the increased microbial activity (respiration) occurring during the degradation of the organic matter (WHO 1996).

Oxygen concentrations below 5 mg L<sup>-1</sup> may adversely affect the functioning and survival of biological communities and below 2 mg L<sup>-1</sup> may lead to the death of most fishes (Gower 1980; Chapman 1996). It is clear from the results (Table 1) that the oxygen content recorded at Sites III and IV, in summer, were slightly higher (5.95 and 5.8 mg L<sup>-1</sup>, respectively) than the minimum value required for maintaining the fish population.

In this study, the BOD values (Table 1) of the selected sites varied significantly ( $p < 0.05$ ) among the investigated sites. Conversely, the seasonal variation was not significant. BOD values per site were arranged according to the pattern: Sites IV > III > II > I. This implies that Site IV was the most polluted one which adds more support to the negative impact of domestic and agricultural waste water discharge in this site on the water quality. It is reported that a clean stream would normally have a BOD of 2 mg L<sup>-1</sup> and if the BOD exceeded 4 mg/L, the stream was in the average of becoming a nuisance. Grossly polluted water is characterized by having BOD of 12 mg L<sup>-1</sup> or more on average (Tebbut 1979; WHO 1996). As such, it seems that the water quality of all the selected sites except Site I lie in the nuisance range. Moreover, the presence of toxic substances in water may affect microbial activity leading to a reduction in the measured BOD (Velz 1984).

Seasonal and spatial variation of the water nitrite (Table 2) was highly significant ( $p < 0.01$ ). The maximum value of nitrite (0.13 mg L<sup>-1</sup>) was recorded at Site II (Sohag Al-Gharby drainage canal), while, the minimum value (0.03 mg L<sup>-1</sup>) was recorded at Site III (Al-Mazalwah drain). Because of the possibility of the simultaneous occurrence of nitrite and nitrate in drinking water, the sum of the ratios of the concentrations of each to WHO guideline value should not exceed 1 (WHO 1985). Nitrite concentrations in freshwaters are usually very low, 0.001 mg L<sup>-1</sup> NO<sub>2</sub>-N, and rarely higher than 1.0 mg L<sup>-1</sup> NO<sub>2</sub>-N (Environment Canada 1987; Committee for Fisheries 1993; WHO 1993; Gray 1994). High nitrite concentrations are generally indicative of industrial effluents and are often associated with unsatisfactory microbiological quality of water.

As such, the recorded concentration of nitrite in all the selected sites lies within the permissible limits indicated by WHO. This is because none of the examined sites is subjected to industrial effluents except Site I which is located in the Nile stream.

Data collected from the present study indicated that water nitrate content (Table 2) varied significantly at both the temporal and spatial levels ( $p < 0.05$ ). Sites II (23.34 mg L<sup>-1</sup>) and III (20.29 mg L<sup>-1</sup>) were characterized by having relatively high concentrations of nitrate than other sites as they receive agricultural waste waters from the surrounding cultivated lands, and domestic waste water, respectively. Surface waters can have nitrate concentrations up to 5 mg L<sup>-1</sup> NO<sub>3</sub>-N, but often less than 1 mg L<sup>-1</sup> NO<sub>3</sub>-N. Concentrations in excess of 5 mg L<sup>-1</sup> NO<sub>3</sub>-N usually indicate pollution by human or animal waste or fertilizer run-off. WHO recommended maximum limit for NO<sub>3</sub><sup>-</sup> in drinking water is 11.3 mg L<sup>-1</sup> as NO<sub>3</sub>-N, and waters with higher concentrations can represent a significant health risk. The nitrate content of all the examined sites exceeded 5 mg L<sup>-1</sup> (7–23 mg L<sup>-1</sup>) indicating the polluted status of water as a result of heavy applications of fertilizers in the agricultural lands in the vicinity of the study area.

Natural sources of total phosphorus are mainly the weathering of phosphorus-bearing rocks and

the decomposition of organic matter. Domestic waste waters (containing detergents), industrial effluents, and fertilizer run-off contribute to elevated levels in surface waters. In most natural surface waters, phosphorus ranges from 0.005 to 0.020 mg L<sup>-1</sup> PO<sub>4</sub>-P (Environment Canada 1987; Committee for Fisheries 1993; WHO 1993; Gray 1994). According to the European Union, the maximum allowable concentration of phosphorus is 5 mg L<sup>-1</sup>. The recorded concentration of the total phosphorus in the selected sites (Table 2) clearly demonstrates its significant seasonal and spatial fluctuations ( $p < 0.05$ ) with the highest values of 1.56 mg L<sup>-1</sup> recorded at Site III. This is mainly related to the agricultural cycle and source of the drainage water of the study sites. On the other hand, the reactive phosphorous showed only seasonal variations ( $p < 0.001$ ).

#### Heavy metals in water, sediments and plants

In the present study, heavy metal concentrations in water and sediments (Fig. 2) were decreased in sequence of Zn > Cu > Pb > Cd. The results are in agreement with Rabie et al. (1996). They indicated that the content of heavy metals (in micrograms per milliliter) in the Nile water at El-Saff were 0.09, 0.02, 0.05, and 0.00 for Zn, Cu, Pb, and Cd, respectively. Abdel-Shafy and

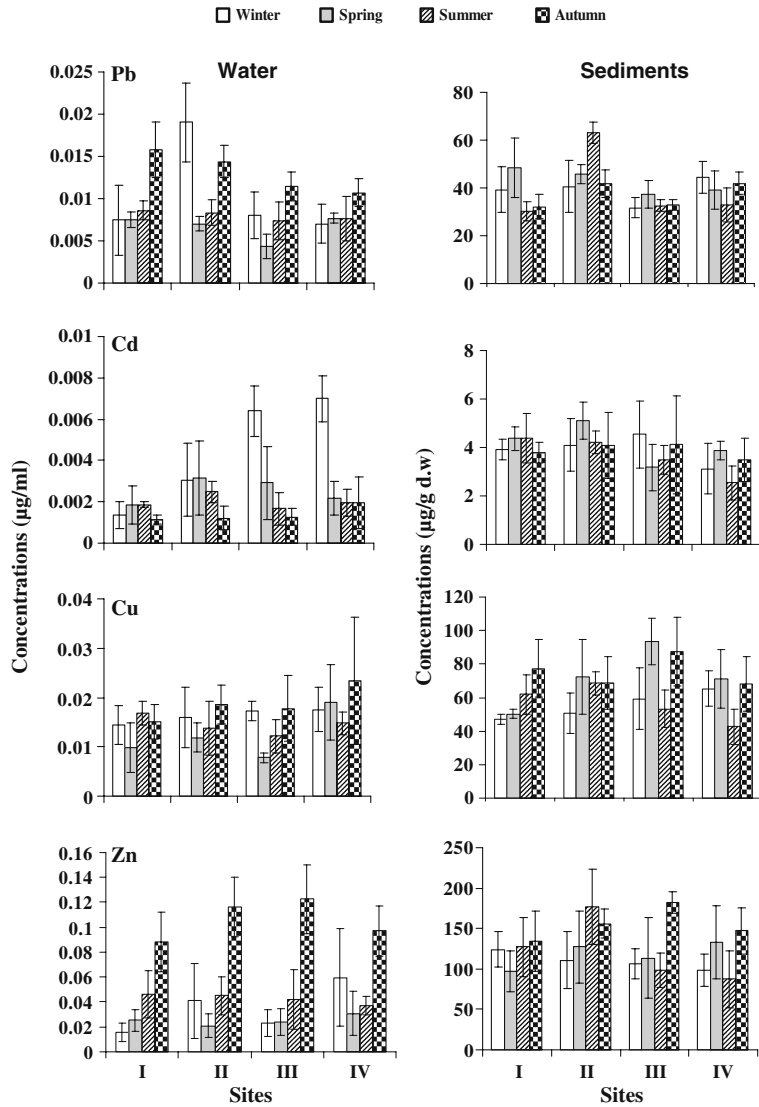
**Table 2** Nutrient salts content of the water samples (average ± sd) collected from the selected sites during different seasons

Season	Site	Nutrient salts			
		NO <sub>2</sub> -N (mg/L)	NO <sub>3</sub> -N (mg/L)	PO <sub>4</sub> -P (mg/L)	TP (mg/L)
Winter	I	0.08 ± 0.01	7.31 ± 4.53	0.26 ± 0.24	0.32 ± 0.17
	II	0.09 ± 0.04	12.86 ± 2.68	0.15 ± 0.14	0.50 ± 0.40
	III	0.05 ± 0.02	19.44 ± 6.15	0.16 ± 0.14	1.56 ± 0.81
	IV	0.09 ± 0.03	16.39 ± 6.81	0.11 ± 0.07	0.40 ± 0.18
Spring	I	0.12 ± 0.04	11.59 ± 1.79	0.09 ± 0.08	0.20 ± 0.14
	II	0.13 ± 0.06	12.06 ± 8.03	0.08 ± 0.07	0.25 ± 0.15
	III	0.06 ± 0.02	14.09 ± 5.17	0.18 ± 0.15	0.28 ± 0.14
	IV	0.11 ± 0.07	13.11 ± 8.69	0.16 ± 0.15	0.32 ± 0.26
Summer	I	0.08 ± 0.06	13.58 ± 4.77	0.38 ± 0.36	0.79 ± 0.20
	II	0.06 ± 0.05	23.34 ± 3.35	0.24 ± 0.23	0.91 ± 0.28
	III	0.03 ± 0.01	20.29 ± 6.68	0.24 ± 0.22	0.89 ± 0.21
	IV	0.09 ± 0.05	12.92 ± 1.71	0.43 ± 0.33	1.24 ± 0.16
Autumn	I	0.04 ± 0.01	13.40 ± 2.38	0.29 ± 0.10	0.80 ± 0.38
	II	0.07 ± 0.05	21.08 ± 4.76	0.59 ± 0.35	0.87 ± 0.46
	III	0.04 ± 0.01	11.37 ± 4.16	0.39 ± 0.35	0.98 ± 0.45
	IV	0.06 ± 0.01	10.76 ± 6.57	0.30 ± 0.24	1.29 ± 0.44

NO<sub>2</sub>-N nitrite; NO<sub>3</sub>-N nitrate; PO<sub>4</sub>-P orthophosphate; TP total phosphorus



**Fig. 2** Average concentrations ( $\pm$ S.D) of the studied heavy metals in water ( $\mu\text{g/ml}$ ) and sediments ( $\mu\text{g g}^{-1}$  d.w) collected from the selected sites during different seasons



Aly (2002) reported that the water quality gradually deteriorates in the downstream direction due to the poorly treated wastewater discharges from both domestic and industrial activities and uncontrolled mixing with water from drains. Therefore, they contain high levels of various pollutants. In general, the obtained results in this study agree with findings obtained by the former investigators. Generally, the variation of heavy element concentrations in water and sediments in relation to site and season, as assessed by the two-way repeated measures ANOVA, was significant ( $p < 0.05$ , Table 3). However, insignificant vari-

ations were observed for the concentration of Pb and Cd in sediments in relation to season and of Cu and Zn in relation to site. On the other hand, the interaction of season and site exerts significant variations in the concentrations of Pb, Cd, and Zn in water and on Pb only in sediments.

Owing to its high persistence and several past and present uses (Chen et al. 2006), Pb is defined by the United State Environmental Protection Agency (USEPA) as potentially hazardous to most forms of life. More than two-thirds of the nations in Africa have maximum lead levels above the world median value. In Egypt, progressive

**Table 3** *F* values of variation of heavy element concentrations in water and sediments in relation to variation in site and season

Variables	Pb		Cd		Cu		Zn	
	W	Sed	W	Sed	W	Sed	W	Sed
Season	32.853*	1.379	37.966*	0.487	6.981*	6.183*	78.036*	4.325*
Site	13.433*	9.549*	12.699*	2.910*	4.422*	2.225	2.120*	1.452
Season × site	7.641*	4.142*	10.456*	0.922	1.488	2.137	2.722*	1.675

\**p* < 0.05

industrial activity has resulted in increased environmental pollution and attendant health problems (Sridhar et al. 2000). Generally, Pb concentrations of the selected sites were much higher in sediments than in water (Fig. 2). The concentration of Pb in water acquired its maxima in autumn in all sites except Site II (Sohag Al-Gharby drainage canal). The highest Pb concentrations of site II is recorded in winter that may be related to the decay of plankton and precipitation of organic matter associated with Pb to the sediment. This finding is in agreement with the report of Gohar (1998). Water and sediments of this site is characterized by having the highest Pb concentrations ( $0.019 \mu\text{g ml}^{-1}$  and  $63.16 \mu\text{g g}^{-1}$  d.w, respectively) compared to other sites. This site, besides being subjected to agricultural run-off, is also affected by aerial Pb deposition from a nearby road with heavy traffic. Pb continues to enter the environment primarily by anthropogenic means, retaining its status as a priority pollutant (EPA 2006).

Pb is a non-essential and toxic element to plants. Results obtained from three-way ANOVA (Table 4) showed that Pb concentrations in plant tissues varied significantly (*p* < 0.05) with the variation in seasons, sites, and plant species.

**Table 4** *F* values of variation of heavy elements concentrations in the studied plants in relation to variation in site, season, and species

Variables	Pb	Cd	Cu	Zn
Season	31.382*	3.351*	3.026*	0.729
Site	3.259*	0.046	5.053*	5.623*
Species	9.388*	6.683*	8.748*	6.497*
Season × site	0.629	0.230	0.203	0.578
Season × species	1.154	1.006	0.851	3.608*
Site × species	1.372	0.518	0.342	1.052
Season × site × species	0.908	0.337	0.583	0.422

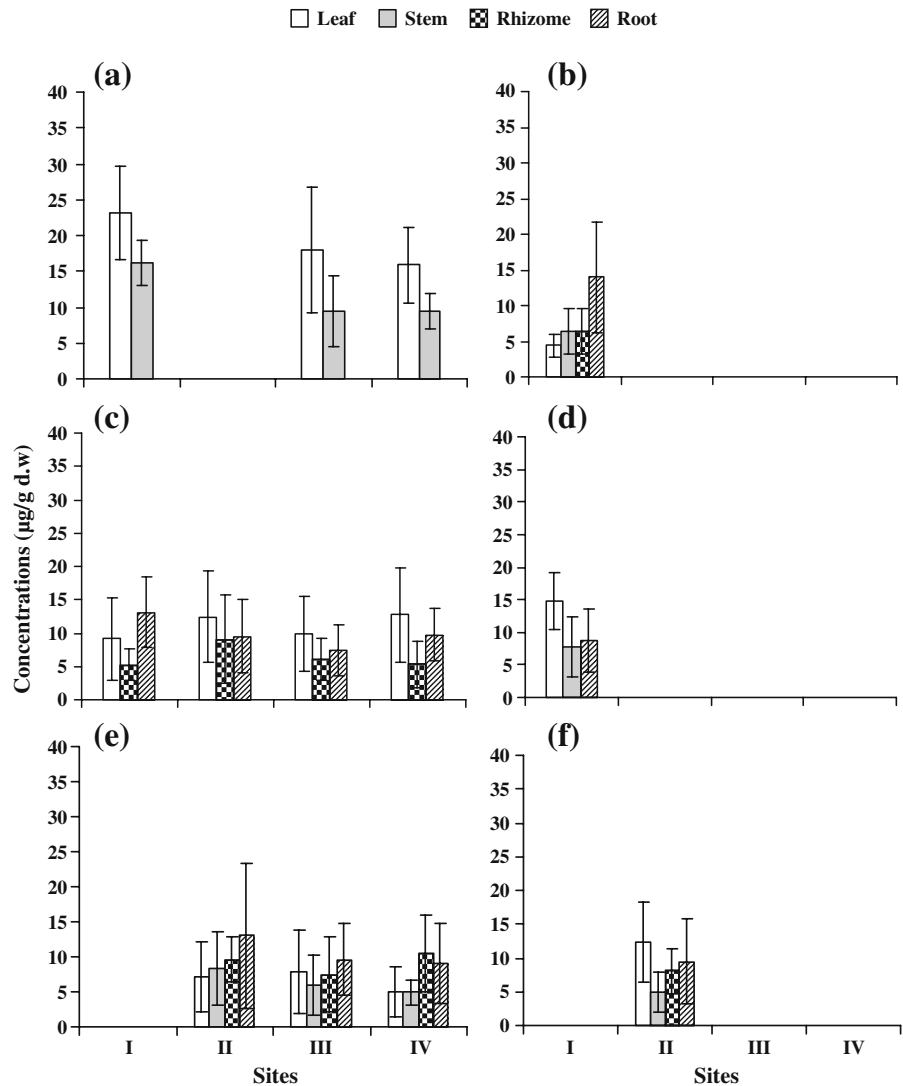
\**p* < 0.05

However, the interaction of these factors exerts insignificant variation on the element concentrations in the studied plants. Seasonally, the highest value of Pb concentration, in all plants, was recorded in winter. Generally, Pb concentrations in all six plants (Fig. 3) were notably higher at Sites I–IV, subjected to airborne Pb deposition emitted from a highway with heavy traffic crossing these sites. However, plants collected from Site II are characterized by having the highest Pb content (Fig. 3) except *C. demersum* (not recorded in Site II). It is worth noting that water and sediments of Site II are also characterized by having the highest Pb concentrations (Fig. 2) which reflect the biomonitoring potentialities of the examined plant species. Pb content of the studied species could be arranged according to the pattern: *C. demersum* > *M. spicatum* > *E. pyramidalis* > *E. crassipes* > *P. australis* > *T. domingensis*. The highest Pb concentration,  $31.55 \mu\text{g g}^{-1}$  d.w (dry basis), was detected in *C. demersum* leaves in winter. Moreover, *C. demersum* attained the highest BF for Pb (4986.2) at Site I (Table 5). This agrees with the earlier finding of Baudo et al. (1981); they reported that submerged plants had higher metal uptake ability due to more surface/biomass ratio. Pb accumulation by aquatic plants has been reported earlier in *C. demersum* (Gupta and Chandra 1994; Rai et al. 1995). Mishra et al. (2006) and Shaltout et al. (2010) concluded that *Ceratophyllum* species accumulate high amount of Pb and showed potential to be used as a phytoremediator species in aquatic bodies having moderate pollution of Pb.

Pb is believed to be the metal of least bioavailability and the most highly accumulated metal in root tissue while Pb shoot accumulation is much lower in most plant species (Kabata-Pendias and



**Fig. 3** Average annual concentrations ( $\pm$ S.D) of Pb in the different organs of the studied species: **a** *C. demersum*, **b** *E. pyramidalis*, **c** *E. crassipes*, **d** *M. spicatum*, **e** *P. australis*, and **f** *T. domingensis* collected from the selected sites



Pendias 2001). Similarly, the results obtained from plant analysis revealed that Pb contents in roots of most of the studied species were always higher than those in shoots.

Cadmium (Cd) is one of the most toxic heavy metals and is considered non-essential for living organisms (Woodbury 1998). Plants treated with higher concentrations of Cd usually become stunted in growth (Wang and Zhou 2005). The Cd concentration in unpolluted waters is usually below  $0.001 \mu\text{g ml}^{-1}$  (Friberg et al. 1986). Data collected from the present study showed that water Cd level exceeds the upper limit,  $0.002 \mu\text{g ml}^{-1}$ , of The US Environmental Protection Agency (EPA

2006) and the Egyptian standards according to Egyptian Environmental Affairs Agency (EEAA 2008) of  $0.002 \mu\text{g ml}^{-1}$  at all sites. This is may be due to: (1) the great tendency of Cd to be adsorbed on the suspended matter (Laxen 1985); (2) the mobilization of Cd from sediment to the above water layer (Gohar 1998); (3) the high Cd uptake by phytoplankton and zooplankton and other organisms in water (Harrison and De Mora 1996). The maximum Cd water concentration (Fig. 2) was attained at Sites III and IV ( $0.0063$  and  $0.0069 \mu\text{g ml}^{-1}$ , respectively). This is may be attributed to human activities, the marked depletion of dissolved oxygen, relatively low pH value,

**Table 5** Bioaccumulation factor (BF) of Pb, Cd, Cu, and Zn in the studied species collected from the selected sites

Heavy metals	Site	Species					
		<i>C. demersum</i>	<i>E. pyramidalis</i>	<i>E. crassipes</i>	<i>M. spicatum</i>	<i>P. australis</i>	<i>T. domingensis</i>
Pb	I	<b>4986.2</b>	4500.2	3928.0	4439.5	NR	NR
	II	NR	NR	2638.4	NR	3418.7	2573.3
	III	3757.0	NR	3534.8	NR	4772.6	NR
	IV	3398.3	NR	3525.6	NR	3694.3	NR
Cd	I	4017.4	6306.5	4234.3	<b>12820.4</b>	NR	NR
	II	NR	NR	3956.2	NR	5112.6	4979.5
	III	2504.5	NR	3776.4	NR	3673.5	NR
	IV	2471.7	NR	3415.0	NR	3183.9	NR
Cu	I	1457.9	3749.0	2881.3	2099.6	NR	NR
	II	NR	NR	<b>4739.7</b>	NR	3500.3	4193.2
	III	2550.2	NR	4426.8	NR	2867.2	NR
	IV	2209.3	NR	3799.3	NR	2493.5	NR
Zn	I	3350.3	<b>6738.4</b>	4414.2	4120.5	NR	NR
	II	NR	NR	3799.2	NR	3754.3	4303.4
	III	3086.9	NR	3272.9	NR	4362.6	NR
	IV	3389.3	NR	3691.7	NR	3420.5	NR

NR not recorded; bold value = highest value

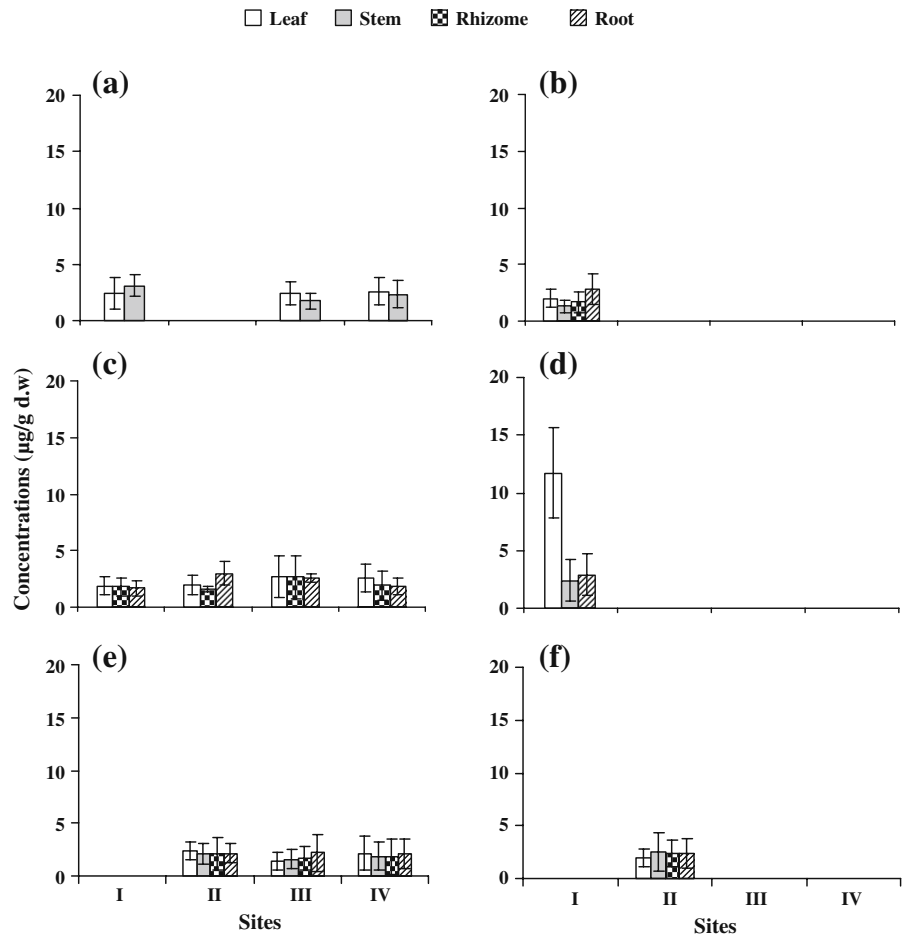
and low salinity which enhance Cd precipitation at these sites (El-Rayis and El-Sabrouti 1997).

Cd is considered as a heavy metal of most concern because it shows the greatest mobility in the soil environment (Wilson and Bell 1996). Generally, Cd concentrations in the sediments (Fig. 2) of the studied sites under investigation relatively low ( $<6 \mu\text{g g}^{-1}$  d.w). The highest Cd concentration of  $5.1 \mu\text{g g}^{-1}$  d.w was recorded at Site II that marked by agricultural discharge from farmland throughout the year. The main anthropogenic sources of Cd are the amendment of agricultural soils with Cd-contaminated bio-solids, phosphate fertilizers, and industrial by-products (Westfall et al. 2005). The values of extractable Cd from sediments samples ranged between 2.53 and  $5.1 \mu\text{g g}^{-1}$  d.w. This range was higher than the levels that recorded by Abd El-Hady (2007) for River Nile at El-Giza area. He reported that the Cd concentration of the sediment ranged between 0.03 and  $0.26 \mu\text{g g}^{-1}$  d.w.

With regards to plant tissue, Cd contents showed significant variation ( $p < 0.05$ ) in relation to variation in season and plant species (Table 4). On the contrary, variation in sites and the interaction of all variables exerts insignificant variation on plant Cd content. The results indicated that Cd could be accumulated in all plant organs (leaves, rhizomes, and roots). The distribution of Cd within plant organs is quite variable and clearly

illustrates its rapid translocation from roots to shoots (Kabata-Pendias and Pendias 2001). Cd content of the studied species (Fig. 4) could be arranged according to the pattern: *M. spicatum* > *C. demersum* > *E. crassipes* > *E. pyramidalis* > *T. domingensis* > *P. australis*. The highest uptake of Cd ( $18.71 \mu\text{g g}^{-1}$  d.w) was attained by *M. spicatum* leaf at Site I (Fig. 4). Moreover, *M. spicatum* attained the highest BF for Cd at Site I, accumulating up to 12,820.4-fold of its concentration in water (Table 5). Cd accumulation by *M. spicatum* indicates a fact that this species may be useful vehicle for absorbing cadmium from nutrient-rich waters, so long as the Cd concentration falls within the range between 0.04 and  $7.63 \text{ mg L}^{-1}$  (Sajwan and Ornes 1996). Ngayila et al. (2007) concluded that *M. spicatum* is able to absorb Cd from synthetic freshwater. Among the aquatic macrophytes, *Myriophyllum* species could be used in ecological surveys as in situ biomonitors of water quality due to its ability to concentrate pollutants in their tissues and reflect the environmental pollution (Kamal et al. 2004; Nimptsch et al. 2005). In *Myriophyllum* species, there was heavy metal uptake by the roots which were transported to stems and leaves (Cardwell et al. 2002). Generally, the Cd content of most plants does not exceed  $1.9 \mu\text{g g}^{-1}$  d.w (Outridge and Noller 1991). However, the Cd

**Fig. 4** Average annual concentrations ( $\pm$ S.D) of Cd in the different organs of the studied species: **a** *C. demersum*, **b** *E. pyramidalis*, **c** *E. crassipes*, **d** *M. spicatum*, **e** *P. australis*, and **f** *T. domingensis* collected from the selected sites



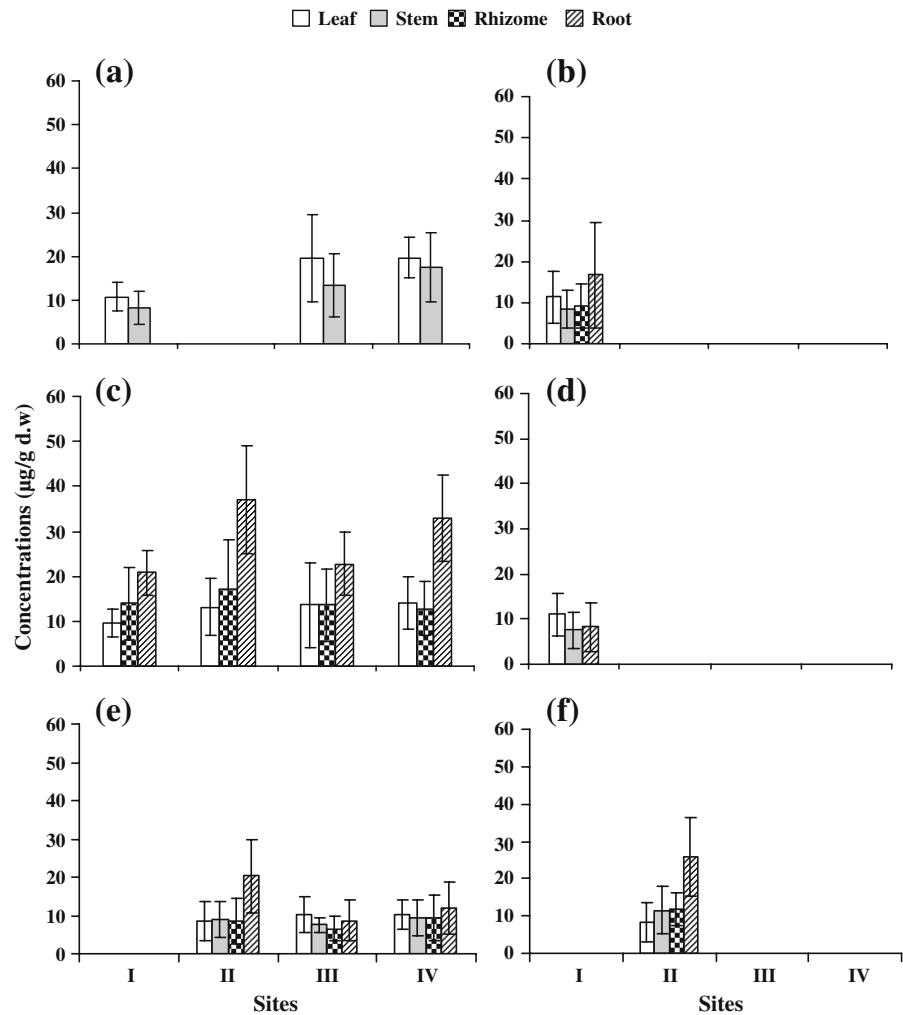
content recorded for the studied species reached up to 18.71  $\mu\text{g g}^{-1}$  d.w.

Copper (Cu) is a micronutrient essential for plants at very low concentrations. However, excessive concentrations of this metal are considered to be highly toxic. Mining, smelting and applications of fertilizers and sewage sludge, together with the use of fungicides containing Cu, and other human activities, has lead to widespread soil contamination with Cu (Hong-yun et al. 2005). The dissolved fractions of Cu (Fig. 5) were notably higher in all sites than the upper limit, 0.013  $\mu\text{g ml}^{-1}$ , of EPA (2006) and the Egyptian standards (EEAA 2008). The concentration of Cu in water acquired its maxima (0.0233  $\mu\text{g ml}^{-1}$ ) at site IV in autumn (Fig. 2). The distribution pattern of Cu in the sediments of the studied sites indicated that all sites were heavily enriched with this element ( $>45 \mu\text{g g}^{-1}$  d.w.), with the maximum

value being attained at Site III (93.41  $\mu\text{g g}^{-1}$  d.w.). Agriculture on soils around the studied sites may be the main reason for its higher concentrations as Cu is used in the manufacturing of fertilizers and algicide. In general, the obtained results are higher than those obtained by Rashed et al. (1995) who reported that Cu concentration in the sediment of Nile Delta ranged between 10 to 81  $\mu\text{g g}^{-1}$  d.w.

Plant Cu content varied significantly ( $p < 0.05$ ) with the variation in seasons, sites, and plant species. However, the interaction of these variables exerts insignificant variation on plant Cu content (Table 4). The highest value of Cu concentration, in most plants, was recorded in winter. Generally, roots of all plants attained higher Cu concentrations than other organs (Fig. 5). This is in line with the findings of Fawzy and Badr (2006) who reported that roots of *E. crassipes*,

**Fig. 5** Average annual concentrations ( $\pm$ S.D) of Cu in the different organs of the studied species: **a** *C. demersum*, **b** *E. pyramidalis*, **c** *E. crassipes*, **d** *M. spicatum*, **e** *P. australis*, and **f** *T. domingensis* collected from the selected sites



*P. australis* and *T. domingensis* can accumulate higher amounts of Cu than shoots. The maximum Cu concentration ( $43.48 \mu\text{g g}^{-1} \text{d.w}$ ) was attained by *E. crassipes* root (Fig. 5) at site II with the highest bioaccumulation factor of 4,739.8 (Table 5). The main source of pollution in this site is agriculture drainage that transports huge amounts of fertilizers by-products. Cu content of the studied species could be arranged according to the pattern: *E. crassipes* > *T. domingensis* > *P. australis* > *C. demersum* > *E. pyramidalis* > *M. spicatum*.

Cu concentration in plant tissues ranged from 5 to  $20 \mu\text{g g}^{-1} \text{d.w}$ . Above the upper limit, toxicity effects are likely to occur (Kabata-Pendias and Pendias 1992; Borkert et al. 1998). Higher concen-

trations of Cu in the roots of *T. domingensis* and *P. australis* collected from sites with relatively higher pH values may be attributed to the presence of plaque, a metal-rich rhizoconcretions composed of iron hydroxides and other metals that are mobilized and precipitated on the root surface (Sundby et al. 1998). This is in agreement with the finding of Weis and Weis (2004) who reported that at higher pH conditions ( $\geq 8.0$ ), the presence of plaque enhanced Cu uptake into roots.

In the past few years, emphasis has been given to use aquo-vascular plants such as *E. crassipes* for the removal of toxic metals from wastewater (Deng et al. 2004; Gupta and Chandra 1998). Accumulation of Cu by the plants in our study showed greater efficiency because of being rooted

plants. The accumulation of metals by plants is sufficiently high such that its use was recommended for metal recovery from the nuclear wastes (Rai et al. 1994; Cosstley and Wallis 2001). *E. crassipes* has already been shown to possess the ability for the sorption of Cu (Muramoto and Oki 1983; Vaidyanathan et al. 1985). Moreover, results obtained by Nor (1994) showed that *E. crassipes* has a tremendous capacity to absorb phenolic compounds as well as Cu and Zn. In general, the metal removal efficiency of *E. crassipes* is high at higher metal concentration but at the same time toxicity symptoms also appear. Zhu et al. (1999) used *E. crassipes* grown hydroponically for treating Cu-contaminated water with initial concentration of 10  $\mu\text{g/ml}$  for 14 days. The Cu concentrations were 130  $\mu\text{g g}^{-1}$  d.w. (dry basis) in the examined plants' shoots and 2800  $\mu\text{g g}^{-1}$  d.w. (dry basis) in the roots.

Although Zn is essential trace element, high levels can cause harmful health effects. Toxicity of high level Zn concentrations in man is well known (Clark et al. 1981). Water concentrations of Zn were significantly higher than the other metals in all the studied sites and attained its maximum value of 0.122  $\mu\text{g ml}^{-1}$  at site III (Fig. 2). This is mainly due to the sewage discharges from sanitary domestic and agricultural effluents. The recorded value of zinc slightly exceeds the recommended upper limit, 0.12  $\mu\text{g ml}^{-1}$ , for discharge in fresh water requires by the USEPA and the Egyptian standards (EEAA). However, the results obtained by El-Gendi (2003) showed that the level of Zn in Nile water is 0.012  $\mu\text{g ml}^{-1}$  which is below the critical levels reported for this element in irrigation water. This reflects the remarkable increase of Zn concentration in Nile water within the last few years. Rashed et al. (1995) reported that Zn concentration in the sediment of Nile Delta ranged between 18 to 104  $\mu\text{g g}^{-1}$  d.w. According to Kloke (1979), toxicity level of this element is around 300  $\mu\text{g g}^{-1}$  d.w. Abdel-Sabour et al. (1996) indicated that the use of Cairo sewage effluent, on El-Gabal El-Asfer farm, for 12 and 50 years resulted in an increase in soil Zn content by about 10- to 14-fold, respectively.

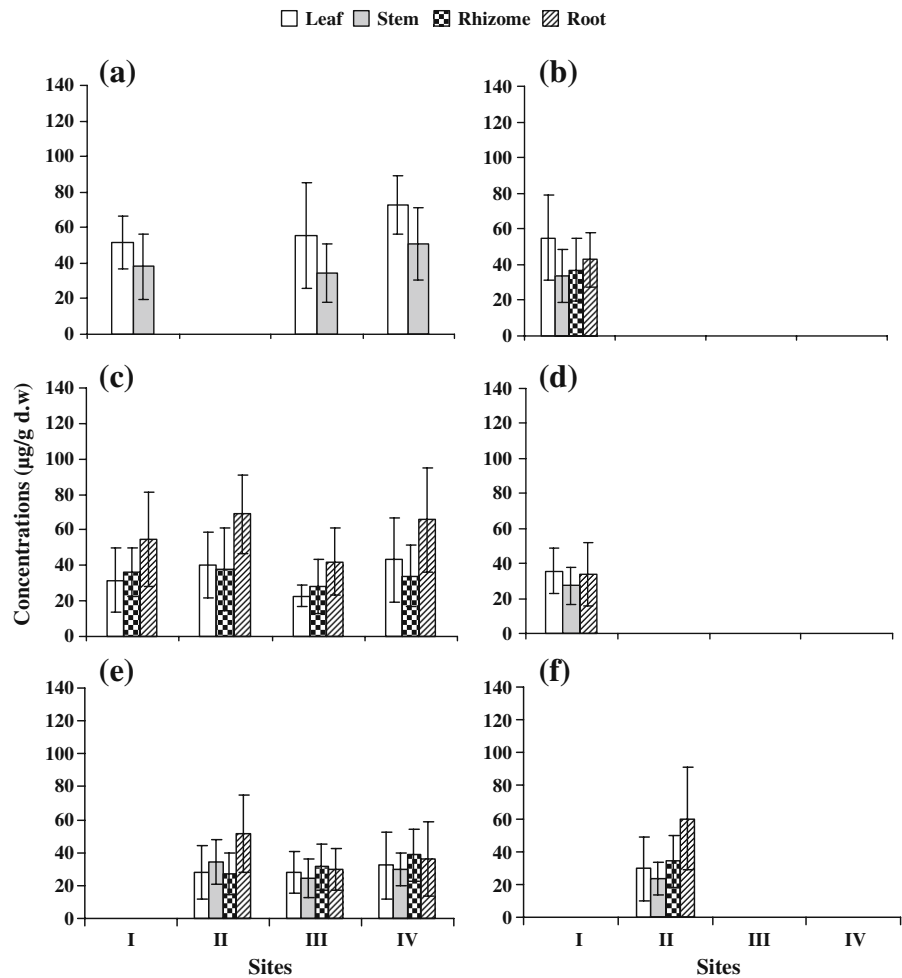
The Zn contents of the studied plants showed significant variation ( $p < 0.05$ ) in relation to variation in season and plant species (Table 4).

In contrary, data analysis showed that variation in sites and the interaction of all variables exerts insignificant variation on plant Zn content except for the interaction of season and species. Zn content of the studied species could be arranged according to the pattern: *E. crassipes* > *C. demersum* > *E. pyramidalis* > *T. domingensis* > *P. australis* > *M. spicatum*.

The upper toxic levels of Zn in various plants range from 100 to 500  $\mu\text{g g}^{-1}$  d.w. (Waganov and Nizharadze 1981). The results demonstrated that roots often contain more Zn than shoots. The highest Zn root concentration, 102.9  $\mu\text{g g}^{-1}$  d.w., was attained by *E. crassipes* at Site II (Fig. 6). However, *E. pyramidalis* attained the highest BF for Zn (6,738.4) at Site I (Table 5). The roots are thought to be important for element uptake in free-floating plants as well (Sharma and Gaur 1995). Although the highest zinc concentrations in sediments were recorded at site III, the maximum plant zinc content was recorded at site II. This is mainly attributed to the antagonistic effect of Cu on the uptake of zinc (Figs. 2, 5 and 6). Higher Cu concentrations in the sediments may reduce zinc availability and uptake by plants (Kabata-Pendias and Pendias 2001). Previous studies on the accumulation of various metal ions by aquatic plants have shown that the deposition of most metals was higher in roots than the other parts of plants (Zaranyika and Ndapwadza 1995; Chandra and Kulshreshtha 2004; Fawzy and Badr 2006). This is in line with the findings of the present study (Fig. 6). *E. crassipes* was tested for concurrent removal of Zn. These plants have removed the metal successfully without production of toxicity (Chandra and Kulshreshtha 2004).

The mean concentration of Zn in normal plants (aboveground tissues) is 66  $\mu\text{g g}^{-1}$  (Outridge and Noller 1991), and the toxic level is up to 230  $\mu\text{g g}^{-1}$  (Borkert et al. 1998; Long et al. 2003). The ranges of Zn in plants presented here were generally higher than the levels reported for other plants (Cardwell et al. 2002), but still within the range of contaminated plants (100–400  $\mu\text{g g}^{-1}$  d.w.) reported by Outridge and Noller (1991). Abouloos et al. (1996) mentioned that Zn content of plant increased with increasing levels of Zn in the sediments. Abd-El-Fattah et al. (2002) found that

**Fig. 6** Average annual concentrations ( $\pm$ S.D) of Zn in the different organs of the studied species: **a** *C. demersum*, **b** *E. pyramidalis*, **c** *E. crassipes*, **d** *M. spicatum*, **e** *P. australis*, and **f** *T. domingensis* collected from the selected sites



the concentration and uptake of elements were higher for plant irrigated with municipal sewage water compared to river water. El-Naim and El-Houseini (2002); Abdel-Sabour and Rabie (2003) reported that sewage sludge and irrigation with different wastewater caused limited increase in the contents of heavy metals in the aquatic plants. Kandil et al. (2003) found highly significant correlations between the soil content of macro, micro-nutrients and heavy metals and its accumulation in shoots of plants.

The correlation coefficients among heavy elements concentrations in water, sediments, and different plant organs were presented in Table 6. The correlation between heavy metals concentrations in water to those in sediments are highly significant,  $p < 0.001$ . Similarly, the element concentrations in water were correlated to element

contents in different organs of *C. demersum* ( $p < 0.001$ ), *M. spicatum* and *E. crassipes* ( $p < 0.01$ ). On the contrary, element concentrations in sediments are not significant with those detected in the stem of *C. demersum*, leaf of *M. spicatum* and rhizome of *E. crassipes*. The element concentrations in sediments were significantly correlated ( $p < 0.001$ ) to those found in the stem, rhizome, and root of *P. australis*, and leaf and root of *E. pyramidalis* and *T. domingensis*

The correlation found between element concentrations in the tissue of submerged and floating species analyzed with those in water suggests direct absorption of these elements by shoots from the water column with consequent accumulation. Guilizzoni (1991) states that some rooted submerged plants may absorb metals directly from water when they are not readily available in



**Table 6** Simple linear correlation coefficient (r) between heavy elements concentrations in water (W), sediments (Sed) and plant organs (L = leaf, S = stem, Rh = rhizome, R = root)

Species	Factor	Pb		Cd		Cu		Zn	
		W	Sed	W	Sed	W	Sed	W	Sed
(n = 48)	W	1.00		1.00		1.00		1.00	
	Sed	-0.53***	1.00	-0.42**	1.00	-0.54***	1.00	0.63***	1.00
<i>C. demersum</i> (n = 36)	L	0.65***	-0.16	-0.59***	-0.60***	0.59***	-0.14	0.65***	-0.68***
	S	-0.59***	0.43**	-0.66***	0.03	0.59***	-0.18	0.63***	0.11
<i>E. pyramidalis</i> (n = 12)	L	0.83***	-0.55*	-0.95***	-0.97***	-0.01	-0.81***	-0.35	-0.95***
	S	-0.50	0.46	-0.65**	-0.64**	-0.15	-0.88***	-0.64**	-0.99***
	Rh	0.10	0.20	-0.87***	-0.84***	-0.16	-0.56*	-0.61*	-0.99***
	R	0.06	0.62*	-0.81***	-0.83***	-0.38	-0.86***	-0.29	-0.91***
<i>E. crassipes</i> (n = 48)	L	0.57***	-0.23	-0.44**	-0.25	-0.47**	0.35*	0.36*	0.00
	Rh	0.35*	0.10	-0.46**	-0.24	0.42**	0.16	0.39**	0.12
	R	-0.07	0.39**	-0.52**	0.05	0.35*	0.52***	0.66***	0.56***
<i>M. spicatum</i> (n = 12)	L	0.59*	0.05	-0.71**	-0.24	0.58*	0.16	0.70**	0.31
	S	-0.29	-0.04	-0.57**	-0.56	-0.13	-0.60*	0.75**	0.12
	R	0.62*	0.06	-0.78**	-0.02	0.62*	-0.68*	-0.58*	0.57*
<i>P. australis</i> (n = 36)	L	0.52***	-0.16	0.06	-0.53***	-0.23	-0.26	0.04	-0.19
	S	0.74***	-0.64***	0.01	-0.55***	-0.04	0.12	0.09	0.02
	Rh	0.22	0.55***	0.15	-0.53***	-0.15	0.54***	-0.08	-0.66***
	R	0.52***	0.51***	0.20	0.59***	-0.15	-0.53***	-0.14	0.54***
<i>T. domingensis</i> (n = 12)	L	1.00***	-0.69***	-0.95***	-0.26	0.36	-0.92***	0.08	0.83***
	S	0.85***	-0.29	-0.71**	-0.58*	-0.27	0.03	0.61*	0.96***
	Rh	0.89***	-0.46	0.50	-0.24	0.56*	-0.13	0.03	0.25
	R	0.84***	-0.60*	-0.48	-0.71**	-0.02	-0.72**	-0.21	-0.70**

\*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001

sediments and/or in high concentration in the surroundings. On the other hand, the correlation found between element contents in the studied emergent species highlighted their ability to take up and accumulate elements which are abundant in water and sediments.

**Conclusions**

In the course of this study, it is concluded that metal concentrations in water samples, except for Pb, were above the standard levels of EPA and EEAA (0.065, 0.002, 0.013, and 0.120 µg ml<sup>-1</sup> for Pb, Cd, Cu, and Zn, respectively). The concentration of heavy metals in water, sediments, and plants possess the same trend: Zn > Cu > Pb > Cd, which reflects the biomonitoring potentialities of the examined plant species. Moreover, heavy metal content of the studied plants varies significantly in relation to variation in species, seasons (except Zn) and sites (except Cd). Accordingly, the studied species can be used in monitor-

ing both temporal and spatial variations in heavy metal concentrations of polluted water.

Sohag drains receive high concentrations of organic and inorganic pollutants from industrial, domestic, as well as diffuse agricultural wastewater. Priority should be given to those drains receiving high loads of pollution such as: Rawafeih Alku-sair drain, Almazalwa drain, and Sohag Algharbi drainage canal, and measures should be taken for the treatment of their water loads before being delivered into River Nile.

In approaching biomonitoring studies, it may be useful to investigate several species together in order to identify the best biomonitor. The accumulation potentiality of the investigated plants could be arranged according to this pattern: *C. demersum* > *E. crassipes* > *M. spicatum* > *E. pyramidalis* > *T. domingensis* > *P. australis*. The investigated plants can survive in extreme conditions and can tolerate very high concentrations of heavy metals which make them an excellent choice for phytoremediation and biomonitoring programs.

In this investigation, substantial information on the temporal and spatial distribution of heavy elements in the study area was provided by analyzing different organs of the investigated species. The allocation of the highest concentration of Pb to *C. demersum* leaf and the highest Cd concentrations in *M. spicatum* leaf suggested these plants as ideal candidates for phytoremediation programs of these elements.

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