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Heavy metals in sediments and their bioaccumulation in *Phragmites australis* in the Anzali wetland of Iran

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Abstract Accumulation of metals in both sediments and *Phragmites australis* organs was studied. Samples were collected from seven stations located in Anzali wetland, Iran. The samples were analyzed by inductively coupled plasma mass spectrometry (ICP-MS). The results showed that concentration of the studied metals (except As and Cd) were higher in sediments than in *P. australis* organs. Metal accumulation was found to be significantly ($P<0.05$) higher in roots than in above-ground organs of *P. australis*. The bioaccumulation factor (BAF) and the transfer factor (TF) also verified the highest rate of metal accumulation in roots and their reduced mobility from roots to the above-ground organs. Pearson correlation coefficient showed significant relationships between metal concentrations in sediments and those in plant organs. It should be pointed out that sediment and plant samples exhibited higher metal concentrations in eastern and central parts than in western and southern parts of the wetland. The mean concentrations of all studied elements (except for Fe, V and Al) were higher in these sediment samples than in the Earth's crust and shale. High accumulation of metals in *P. australis* organs (roots and shoots) is indicative of their high bioavailability in sediments of the wetland. The correlation between metal concentrations in sediments and in *P. australis* indicates that plant organs are good bioindicators of metal pollution in sediments of Anzali wetland.

Keyword: transfer factor; bioaccumulation factor; aquatic plant; phytotoxic level; trace elements

1 INTRODUCTION

Metals enter into the aquatic environments from natural and anthropogenic sources (Suárez-Serrano et al., 2010; Abdallah and Mohamed, 2015). Industrial wastewaters, agricultural runoffs and weathering of rocks are processes that play important roles in release of heavy metals into the water sources (Nasehi et al., 2013; Xiao et al., 2013). Heavy metals in aquatic ecosystems are long-term contaminants because of their environmental stability, high toxicity and ability to transfer to food chain (Eid et al., 2012; Lü et al., 2015). Therefore, investigation of the accumulation and availability of metals to living organisms in aquatic ecosystems is of great importance. Wetland sediments act as "sources" of and "sinks" for these pollutants (Bai et al., 2014). In aquatic environments, most of the contaminants, particularly trace elements, are readily adsorbed onto the suspended solids and deposited in bed sediments. Metals stabilized in sediments can also enter water columns through chemical and biological processes (Devesa-Rey et al., 2010; Abdallah and Mohamed, 2015). Sediments, smooth out environmental variations in the overlying water, and thus are an appropriate tool for the monitoring pollution in aquatic environments (Karbassi et al., 2008; Xiao et al., 2013). However, the chemical properties of sediments alone fail to provide enough biological information on potential risks to the organisms (Piva et al., 2011). Bioavailability of contaminants, particularly heavy metals, depends on different physical (such as grain size and suspended solids) and chemical (such as

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solubility and pH) factors. However, measurement of bioaccumulation of metals in organisms is required to determine the bioavailability of metals (Calace et al., 2005; Champan, 2007). Biomonitoring has also proved to provide a suitable framework within which to measure heavy–metal accumulation and their bioavailability. Aquatic plants, as the first trophic level of the food chain, take up the heavy metals, and thus can be indicative of the relative increase in concentration of elements in water or sediments of an ecosystem (Bonanno, 2011; Ganjali et al., 2014). Therefore, sediments and aquatic plants, as two complementary factors, can show metal pollution in an aquatic environment. Various research has been conducted on metal pollution in sediment and aquatic plants in wetlands (Ganjali et al., 2014; Wang et al., 2014, 2015) Compared to other types of flora and fauna, wetland macrophytes show a higher capacity for accumulation of metals (Bonanno and Lo Giudice, 2010; Ganjali et al., 2014). Selection of the species to monitor depends on local conditions and availability of aquatic macrophytes (Bonanno and LoGiudice, 2010). *Phragmites australis*, an emergent macrophyte, adsorbs heavy metals from sediments and is grown widely in constructed wetlands for the treatment of metal-containing wastewaters (Grisey et al., 2012; Eid and Shaltout, 2014). Furthermore, some characteristics, including immobility and being in continuous contact with contaminants, wide distribution across aquatic environments, and being a perennial plant (allowing integration of long-term contamination of the environment), make it a good candidate for biomonitoring contaminants in aquatic environments (Bonanno, 2011; Srivastava et al., 2014).

In the Anzali wetland, *P. australis* has been examined as a bioindicator of metals. The main aim of the present work has been to investigate the quality of aquatic environment in this wetland, that has been subject to serious sources of pollution. We also try to find out the ability of *P. australis*' different organs in metal absorption. For this purpose the concentrations of As, Cd, Co, Cu, Cr, Zn, V, Pb, Ni, Mn, Fe and Al in sediments, as well as in roots and shoots of *P. australis* in the Anzali wetland (Ramsar site) were investigated. It was also attempted to investigate the relationship between the concentrations of heavy metals in sediments and those in *P. australis* organs. Furthermore, the differences in accumulation of heavy metals in different parts of *P. australis* were investigated, and the most appropriate organ found has been is assessed for its suitability in monitoring heavy metals in sediments in the study area.

2 MATERIAL AND METHOD

2.1 Study area

Anzali wetland, a freshwater, eutrophic and shallow wetland, lies between latitudes 37°22′ and 37°32′N and longitudes 49°15′ and 49°36′E. It is located in the south-west of the Caspian Sea with an area of about 193 km^2. Anzali has been registered as an international wetland since 1975 under the Ramsar Convention (Vesali Naseh et al., 2012; Zamani Hargalani et al., 2013). The catchment of the wetland covers about 3 610 km^2 and is limited by the Caspian Sea to the north, the Alborz mountain range to the south, the Talish Mountains to the west and the Sefid Rud delta to the east. Approximately 93 525 and 196 020 ha of the catchment are covered with farm lands (particularly rice farms) and forest lands, respectively. The wetland, with mean annual precipitation and evaporation rates of about 1 280 mm and 980 mm respectively, does not have a dry season (JICA, 2004). It is covered with reed beds and plays a key role in spawning and development of fish and also provides many water birds with a place for breeding as well for staging and wintering (Vesali Naseh et al., 2012; Zamani Hargalani et al., 2013). Anzali wetland's environment has been put in jeopardy due by contaminants produced as a result of urbanization, population growth, and agriculture, industry and tourism (Jamshidi-Zhanjani and Saeedi, 2013).

2.2 Sampling and chemical analysis

In April 2013, the first set of samples was collected from surface sediments using a Peterson grab sampler, while the second set was collected from *P. australis* aquatic plants based on vegetation density at 7 stations in Anzali wetland. The sediment and plant samples were taken to the laboratory. To analyze metals in plant samples, roots, stems and leaves were segregated and washed with distilled water. The samples were first air dried for about 15 days and then placed in an oven for 24 h to be thoroughly dried at 65°C. The dried roots, stems and leaves were then powdered using an agate mortar and pestle (Bonanno and Lo Giudice, 2010; Hosseini Alhashemi et al., 2011). Since the tissues of *P. australis* contain large amounts of lignin and cellulose, they are very difficult to digest

Table 1 Comparison amongst metal concentration in Anzali wetland sediment and other locations

Zn (mg/kg)	V (mg/kg)	Pb (mg/kg)	Ni (mg/kg)	Mn (mg/kg)	Fe (%)	Co (mg/kg)	Cu (mg/kg)	Cr (mg/kg)	Cd (mg/kg)	As (mg/kg)	Al (%)	Location
120	122	24	89	1270	4.32	22	55	118	0.38	20	2.9	Anzali wetland, Iran; this study
62	35	17	84	616	2.97	15	25	54	-	-	-	Gowatr Bay, Iran; Moore et al. (2015)
129	-	31	44	-	-	-	43	81	0.67	38	-	Baiyangdian Lake, China; Gao et al. (2013)
83	-	21	36	-	-	-	22	79	0.68	31	-	Yellow River Delta, China; Lu et al. (2014)
49	-	11	30	478	1.57	-	20	28	0.22	3.5	-	Lake Çıldır, Ardahan, Turkey; Kükrer et al. (2014)
251	-	-	157	351	0.78	-	103	-	71	-	11.18	Fusaro Lagoon, Southern Italy; Arienzo et al. (2014)
75	130	14	80	950	4.10	20	50	100	0.2	5	8.20	Mean Earth's crust
95	130	20	68	850	4.70	19	45	90	0.3	13	8.10	Shale values

(Du Laing et al., 2009). Among the studied methods, microwave digestion has proved to be the best digestion method for the analysis of heavy metals in *P. australis* (Du Laing et al., 2009). Therefore, about 0.5 g of each plant was put into microwave vessels and digested with 7 mL of HNO_3 (65% v/v) and 1 mL of H_2O_2 (30% w/v) at high temperature and pressure. Subsequently, the samples were cooled and filtered at room temperature and then made up to volume with HNO_3 (4% v/v) in a 50 mL volumetric flask (Bonanno, 2011). For determination of the total metal content in sediment samples, air-dried samples were passed through a mesh size less than 63 µm and subsequently powdered by an agate mortar and pestle. About 0.5 g of powdered sample was treated with 5 mL aqua regia in a TFM beaker at about 125°C. This was followed by 3 mL $HClO_4$ for digestion of organics. The samples were then cooled at room temperature, filtered and made up to volume by 1N HCl in a 50 mL volumetric flask (U. S. EPA3050, 1996; Chester and Hughes, 1967; Gibbs, 1973; Tessier et al., 1979). Finally, all the digested samples were analyzed for metals through inductively coupled plasma mass spectrometry (ICP-MS; Agilent 4500-MS).

2.3 Statistical analyses

The correlation between the concentration of trace elements in sediments and plant organs was tested using the Pearson coefficient. One-way ANOVA, followed by Dunkan test using SPSS statistical software. Translocation factor (TF) or bioaccumulation factor (BAF) for the concentration of heavy metals in sediments and plant was shown using the ratios: [Trace element] Root / [Trace element] sediment and [Trace element] Leaves / [Trace element] Root (Wang et al., 2015; Xiao et al., 2015).

3 RESULT AND DISCUSSION

3.1 Metal concentration in sediment

Table 1 shows the concentration of elements that were found in the Anzali wetland sediments along with mean Earth's crust and shale values. The mean concentrations of these elements in sediments followed the order: Fe>Al>Mn>V>Zn>Cr>Ni>Cu>Pb>Co>As>Cd.

Generally, concentration of all the studied metals (except for Fe, V and Al) in the sediments was higher than the mean concentration in the Earth's crust and shale. Concentrations of metals in the sediments were also compared by Ontario Ministry of the Environment and Energy Guideline and Guideline for Use at Contaminated Sites in Ontario (1998). The amounts found of Ni (88 mg/kg), Mn (1 270 mg/kg), Cu (54 mg/kg), Cr (117 mg/kg) and As (19.8 mg/kg) were higher than the standard limits, while the amounts of other metals were lower than the limits suggested in the Ontario Guideline. Standard limits of 6, 26, 16, 460, and 16 mg/kg have been specified for As, Cr, Cu, Mn, and Ni, respectively, in the Ontario Guideline.

The concentration of metals in the samples reveals that accumulation of metals in the sediments is higher in the eastern and central parts than in the western and southern parts of the wetland. The populated cities and industrial centers are mainly located at the eastern and central parts of the wetland and discharge domestic, industrial and agricultural wastewaters, which may be the main contributors of pollution in these area.

Metal concentrations in the sediments of Anzali wetland and in other aquatic ecosystems are compared in Table 1. In the sediments of Anzali wetland, concentrations of Cr, Mn and Fe were higher than, and concentrations of As, Cd, Pb and Zn similar to the

Table2 Trace element concentration in plant organs (mg/kg) and phytotoxic levels (mg/kg DW)

Phytotoxic levels	Leaf	Stem	Root	Element
1 000–3 000 [1]	108.2 [a]	53.6 [a]	1 839.5 [a]	Al
-	16.5 [b]	11.8 [a]	28.3 [c]	As
5–700 [3]	0.17 [a]	0.09 [a]	0.35 [b]	Cd
15–50 [1]	0.31 [a]	0.22 [a]	1.1 [a]	Co
0.5 [2]	1.88 [a]	0.99 [a]	6.96 [a]	Cr
25–40 [3]	6.15 [a]	3.94 [a]	23.2 [b]	Cu
1 000–3 000 [1]	222.7 [a]	165.3 [a]	3 463.8 [b]	Fe
5–500 [2]	131.2 [a]	104.7 [a]	237.2 [b]	Mn
-	3.11 [a]	2.61 [a]	4.69 [a]	Ni
30–300 [4]	5.08 [ab]	2.9 [a]	6.65 [b]	Pb
5–10 [1]	0.61 [a]	0.26 [a]	0.81	V
500–1 500 [3]	19.24 [a]	15.46 [a]	39.6 [b]	Zn

Different letters (a, b, c) indicate significant differences among organs per element; (P<0.05, Post hoc Duncan test); Kabata-Pendias and Pendias (2001)[1], Allen (1989)[2], Chaney (1989)[3], Roos (1994)[4].

sediments of other areas. Generally, it seems that metal pollution in the sediments of Anzali wetland is not much more serious than in other areas. (Areas shown in Table 1). Total organic matter (TOM) and grain size are among the factors affecting the spatial distributions of metals in sediments. Fine-grained sediments have higher ionic absorption power. Therefore, the fine-grained sediments tend to carry more organic materials and pollutants than coarse-grained sediments (Darvish Bastami et al., 2012). This may be one of the reasons for the variation in metal contents in sediments of the different areas.

3.2 Metal concentration in plants

3.2.1 Accumulation of different metals in *Phragmites australis*

The mean concentrations of metals measured in roots, stems and leaves of *P. australis* in the Anzali wetland are compared with phytotoxic levels (Table 2). The mean concentration of trace elements in *P. australis* organs follows the order:

In roots:
Fe>Al>Mn>Zn>As>Cu>Cr>Pb>Ni>Co>V>Cd;

In stems:
Fe>Mn>Al>Zn>As>Cu>Pb>Ni>Cr>V>Co>Cd;

In leaves:
Fe>Mn>Al>Zn>As>Cu>Pb>Ni>Cr>V>Co>Cd.

Fe and Mn are known to be essential micronutrients for plants and they need to be present for enzyme activities and photosynthesis (Sasmaz et al., 2008). Iron toxicity in plants is related to the amount of Fe^{2+} adsorbed by the roots. Availability of Fe to plants depends on different factors including pH and dissolved O_2 in the soil (Goulet and Pick, 2001). High concentration of Fe in plants can induce formation of free radicals and consequently damage the cellular structure, membrane, protein molecules and DNA (Gill, 2014). At the studied stations, Table 1 shows that Fe concentrations in roots, stems and leaves were in the ranges 2 946–4 109 mg/kg, 105–196 mg/kg and 138–292 mg/kg, respectively.

Due to the abundance of Mn in lithosphere, it is usually found in most plant organs in high concentrations (Bonanno, 2012). Excessive increase of Mn in plants can disrupt the photosynthesis process by blocking the needed Fe (Gill, 2014). Manganese concentration in roots, stems and leaves was in the ranges 230–303 mg/kg, 81–133 mg/kg and 103–195 mg/kg respectively.

Previous studies have shown that low Al concentrations can stimulate growth in plants (Kabata-Pendias and Mukherjee, 2007). However, high concentration of Al is toxic to plants and may cause damage to the structure of cytoskeleton, calcium homeostasis and phosphorus metabolism and can lead to oxidative stress in plants (Miyasaka et al., 2004). Al accumulations in roots, stems and leaves were respectively in the ranges of 1 400–2 489 mg/kg, 33.5–79 mg/kg and 54.5–142 mg/kg at different stations. Results obtained from the plant samples revealed that Mn concentration was at the phytotoxic level in roots, stems and leaves whereas Al and Fe were at the phytotoxic level only in roots.

Zn concentration in samples ranged from 22.1–59 mg/kg in roots, 12.3–17 mg/kg in stems, and 19.7–30.2 mg/kg in leaves. Zinc is an essential element for the growth of plants and plays a vital role in many metabolic and physiological processes within plants (Gill, 2014).

Zinc toxicity in plants can lead to poor or reduced root and shoot growth as well as chlorosis of leaves (Malik et al., 2011).

Copper is classified as a micronutrient element and plays a significant role in the vital activities of plants such as CO_2 absorption and ATP synthesis. Higher concentrations of Cu can lead to oxidative stress and growth inhibition in plants (Gill, 2014). Copper was found to be in the ranges of 19.9–25.6 mg/kg in roots, 3.1–4.7 mg/kg in stems, and 5.4–7 mg/kg in leaves of *P. australis* at different stations in the Anzali wetland.

The results also revealed that the mean concentrations of Zn and Cu in the plant samples were below the phytotoxic level.

Chromium is toxic to plants and its higher concentrations can affect the physiological processes of plants. Reduction of chlorophyll and photosynthesis as well as reduced plant growth are among the adverse effects of Cr (Chatterjee et al., 2015). Concentration of Cr in the roots, stems and leaves collected from the Anzali wetland ranged from 5–11.2 mg/kg, 0.52–1.9 mg/kg and 0.6–3.32 mg/kg respectively. The mean concentrations of Cr were obtained to be far above the phytotoxic level of Cr (0.5 mg/kg) specified for plants.

Excess Pb in plants can reduce germination percentage and have adverse effects on metabolism. Moreover, lead toxicity inhibits root elongation (Kopyra and Gwóźdź, 2003). The contents of Pb in roots, stems and leaves of *P. australis* were respectively in the ranges 2.64–10.7 mg/kg, 1.19–4.8 mg/kg and 0.6–3.32 mg/kg. These values are far below the phytotoxic level specified for Pb.

The most evident symptoms of nickel toxicity are chlorosis and necrosis. At high concentrations, Ni can form reactive oxygen species (ROS) in plants and induce membrane lipid peroxidase (Pandey and Sharma, 2002). Although Co has a positive effect on some plants, it is not regarded as an essential element for their growth (Sasmaz et al., 2008). Little is known about the toxicity of cobalt to plants; however, a few related studies have suggested that increase of cobalt in plants may influence their growth and biomass and cause problems in transfer of essential elements, including Zn, Cu, P, S and Mn, from roots to above-ground organs (Gill, 2014). Cobalt content in roots, stems and leaves of *P. australis* was in the ranges 0.77–1.67 mg/kg, 0.14–0.31 mg/kg and 0.14–0.56 mg/kg respectively, in the plant samples of Anzali wetland, far below phytotoxic levels.

Cadmium is not an essential element for plants and its accumulation in plants would be toxic. It is easily absorbed and transferred to the different parts of plants through metabolism (Bonanno and Lo Giudice, 2010). It can also result in many morphological, physiological, biochemical, and structural changes in plants (Benavides et al., 2005). Compared to the studied metals,Cd had the lowest concentrations in the plant samples with the ranges 0.19–0.52 mg/kg in roots, 0.06–0.16 mg/kg in stems and 0.1–0.25 mg/kg in leaves. These values are considerably below the phytotoxic level.

The highest amount of V, ranging from 0.45 to 1.28 mg/kg, was found in the roots of *P. australis*. In the Anzali wetland, V content was about 0.001–0.49 mg/kg and 0.34–1.05 mg/kg in the stems and leaves respectively, below the phytotoxic level. Since the roots of *P. australis* can set up a protective mechanism to prevent uptake of vanadium (Bonanno, 2011), a lower amount of V can enter *P. australis* as compared to the sediments.

Arsenic toxicity depends on its chemical forms in the environment and the types of plant species. Because of having an affinity with phosphorous, arsenic can easily be absorbed by the plant system. It can lead to poor or reduced root and shoot growth, reduced germination, impaired photosynthesis and eventually plant death (Gupta and Khan, 2015). The amount of As in roots, stems and leaves was found to be in the ranges of 14.8–48.7 mg/kg, 8.8–16 mg/kg and 7.6–24.1 mg/kg, respectively. Accumulation of As is higher in roots than in above ground shoots because *P. australis* absorbs As from the environment through rhizofiltration process (Ghasemzadeh et al., 2008). The presence of elements in plants can be ascribed to the pollution sources in the wetland. Urban development, population growth, and industrial and agricultural activities are known to be the most important pollution sources which release the pollutants (such as metals) directly or through rivers into the wetland.

3.2.2 Distribution heavy metals in organs of *Phragmites australis*

There was a significant statistical difference between the rates of metals accumulation in the *P. australis* organs (Table 2). Bioaccumulation of the metals in organs of *P. australis* in various stations in the Anzali wetland, follows the order: root>leaf>stem. The highest bioaccumulation of metals was found in the roots of *P. australis*, and the lowest was observed in the stems. Roots of aquatic plants absorb heavy metals from sediments and store them in high concentrations (Baldantoni et al., 2004). This can be attributed to the fact that roots are the first organs which come into contact with metals. Therefore, metal ions are deposited in roots and prevented from being transferred to the above-ground organs. Therefore, roots play a significant role for immobilization of metals (Benavides et al., 2005). Since an important role of the stem is transport of nutrients and it has a little contact with pollutants, detoxification enzyme activity is very low in it

(Pbugmacher et al., 1999). This can be the main reason for low accumulation of metals in stems. However, leaves are a place for photosynthesis and therefore, have a high rate of metabolic activity and a stronger defense system and detoxifying enzymes compared to the stems (Pbugmacher et al., 1999). Heavy metals become hazardous to the plant when they reach the cell cytosol. Cells store metals in leaf-cell vacuoles after changing them into a form tolerable for plants (Michalak, 2006; Bonanno, 2011). Thus, metal accumulation is higher in leaves than in stems.

The TF calculated for each element in the *P. australis* organs has been presented in Table 3. The mean TF indicates that metals mobility in *P. australis* is higher than metals translocation from the sediments to the plant. Translocation of metals within the plant tissues depends on the type of metal and the related organ (Bonanno, 2011).

Arsenic and Cd exhibited the highest rates of translocation from the sediments to the roots of *P. australis*. Phosphorous is one of the essential elements in plants; Arsenic is very similar to phosphorus and enters the plants through the same path for phosphorous transportation (Gupta and Khan, 2015). Such a similarity seems to be the main reason for the high uptake of As onto the plants. Cadmium has a high mobility in soil and thereby has a high availability and uptake rate in plants (Bonanno and Lo Giudice, 2010). Translocation Factor (TF) indicated that the elements accumulated in *P. australis* were stored in large amounts in roots. It should be pointed out that its value for all studied metals in stem/root was less than 1.

Table 3 Bioaccumulation and translocation factors in *Phragmites australis*

Leaf/Root	Stem/Root	Root/Sediment	Element
0.05	0.002	0.62	Al
0.58	0.41	1.42	As
0.48	0.25	0.92	Cd
0.28	0.2	0.04	Co
0.27	0.14	0.05	Cr
0.26	0.16	0.42	Cu
0.06	0.04	0.08	Fe
0.10	0.45	0.18	Mn
0.66	0.55	0.05	Ni
0.76	0.43	0.28	Pb
0.75	0.32	0.006	V
0.48	0.39	0.33	Zn
0.39	0.27	0.36	Mean

Metal mobility was also reduced from roots to the above-ground organs. The highest rate of translocation from root to stem was found in Ni and Mn. Vanadium and Pb possess the highest rate of translocation from root to leaf. In general, the rate of metal translocation was higher from root to leaf than from root to stem.

3.3 Relationship between heavy metal concentration in sediment and plant

Accumulation of trace elements in sediments and *P. australis* organs at different stations in Anzali wetland has been presented in Fig.2.

A significant difference was observed between the accumulation of heavy metals in sediments and that in plant organs. Plants collected from various stations in the Anzali wetland were highly contaminated with metals. The highest concentration of Fe, Al and Mn, and the lowest concentration of Cd were observed in the sediment and in *P. australis* samples. Totally 12 metals were identified in the sediments and plant organs, and a significant positive, correlation ($P<0.05$) was observed between metal concentration (except for Al) in sediments and their accumulation in *P. australis* organs. (Table 4). As can be seen, metals in the Anzali wetland sediments bear the highest correlation with the metals in the *P. australis* roots. Moreover, among the metals in the sediments, As, Co, Cd and Zn had the highest correlation with the metals in roots. Most of the contaminants, particularly metals, can be absorbed by suspended particles in aquatic environments. Some of these particles are deposited and stabilized in sediments and, due to low solubility of heavy metals, they subsequently develop bonds with different organic and inorganic matters

Table 4 Correlation between metal concentration in sediment and plant organs

	Zn	V	Pb	Ni	Mn	Fe	Cu	Cr	Co	Cd	As	Al
Sediment-root	0.836**	0.663*	0.769**	0.570	0.702*	0820**	0.770**	0.614	0.953**	0.873**	0.901**	0.261
Sediment-stem	0.787**	0.701*	0.754*	0.232	0.602	0.301	0.299	0.584	0.105	0.679*	0.747*	0.317
Sediment-leaf	0.616	0.797**	0.786	0.324	0.740	0.527	0.383	0.502	0.175	0.878	0.800	-0.14

* Correlation is significant at level 0.05 (2-tailed); ** Correlation is significant at level 0.01 (2-tailed).

(Devesa-Rey et al., 2010). Sediment-rooted macrophytes, including *P. australis*, are mostly influenced by the metals existing in sediments rather than those existing in water. Therefore, a higher bioaccumulation will be found in *P. australis* if sediments are contaminated with heavy metals (Ganjali et al., 2014). Metal accumulation in aquatic plants depends on various factors including metal concentration in the environment, physical and chemical properties of water and sediments, contact time, condition of plant growth, type of absorption mechanism and time of sampling (Du Laing et al., 2009; Bonanno, 2011). However, high accumulation of metals in *P. australis* is indicative of their high bioavailability in the Anzali wetland sediments and their high absorption by *P. australis* in the study area.

Table 5 shows metal concentrations in roots, stems and leaves of *P. australis* reported in previous studies. Ganjali et al. (2014) investigated the accumulation of Fe, Cu, Cd and Ni in sediments and *P. australis* in Anzali wetland. Comparison of the results obtained in the present study with the ones obtained by Ganjali et al. (2014) demonstrated the differences in amount of the studied metals in sediments and plant. Hosseini Alhashemi et al. (2011) studied heavy metal distribution in both sediments and *P. australis* in Shadegan wetland. Comparison of the results obtained in the present study with those obtained by Hosseini

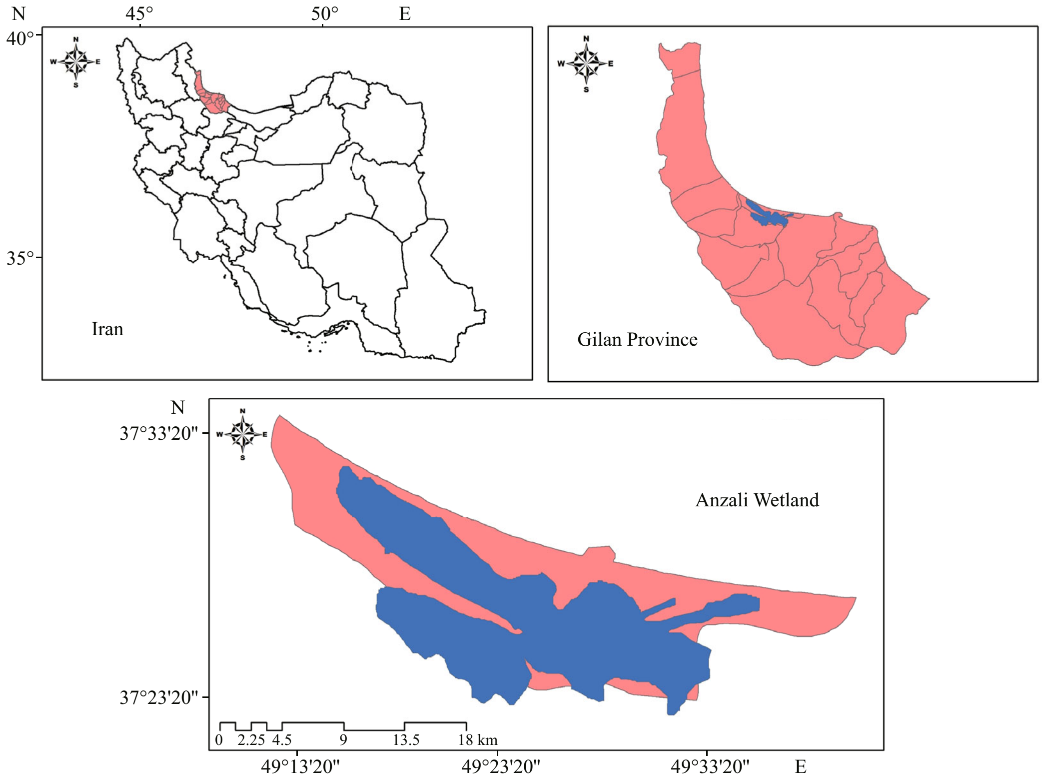

Fig.1 Location of the study area

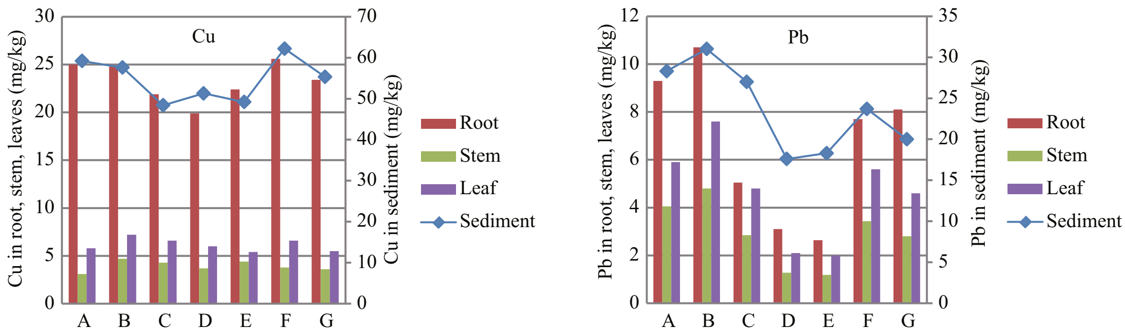

Fig.2 Mean Concentration of heavy metals from sediment and organs of phragmites australis in Anzali Wetland

To be continued

Fig.2 Continued

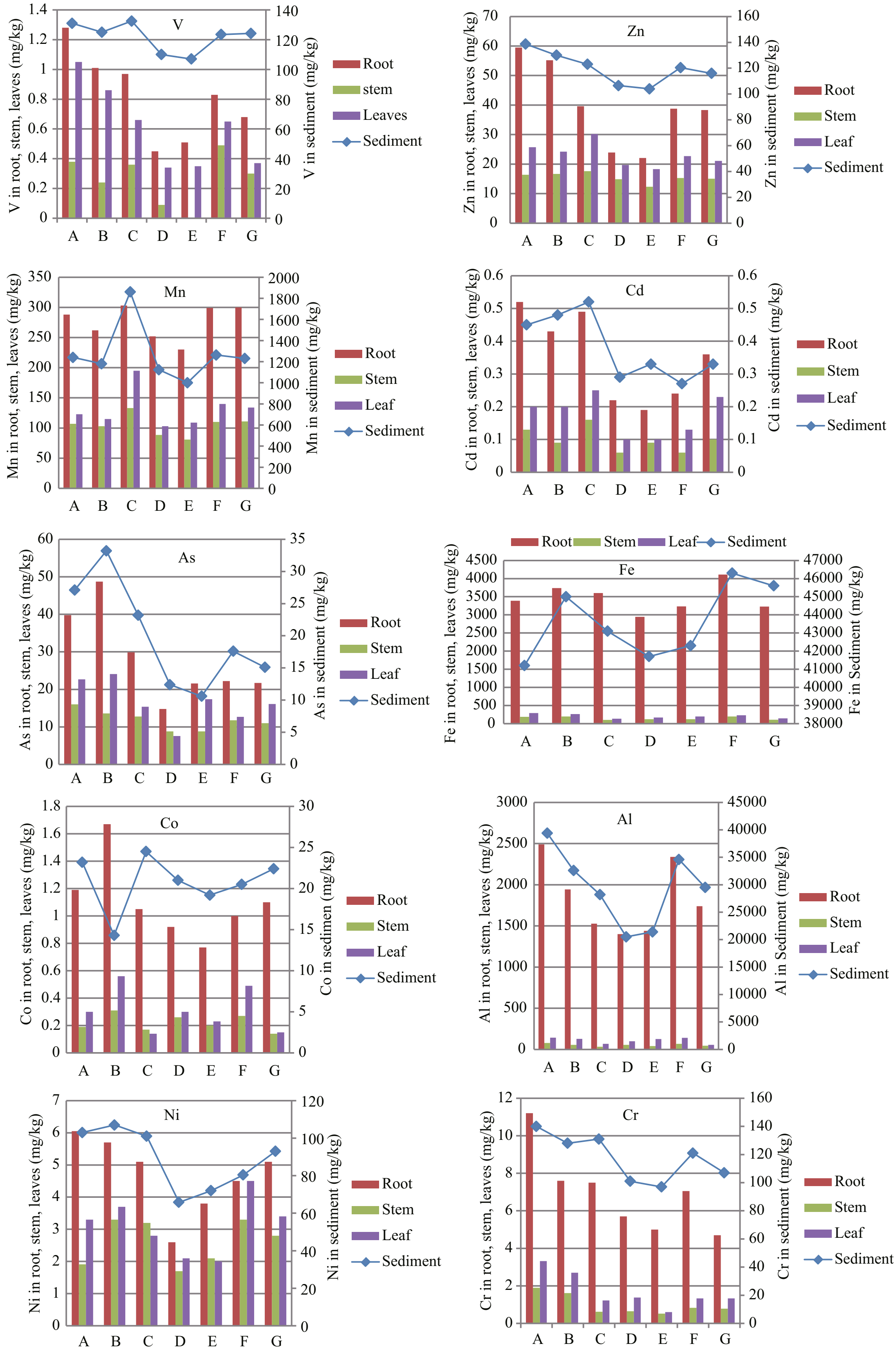

Table 5 Reported metal concentrations in organs of *Phragmites australis* (mg/kg DW)

Zn	V	Pb	Ni	Mn	Fe	Co	Cu	Cr	Cd	As	Al	Organs	Location
104± 9.28	9.2±1.32	16.54±97	9.12±0.2	475±11.91	5561±673	8±0.59	14.98±0.93	6.97±0.19	1.13±0.08	<0.05	3153±264	Root	Imera meridionale River, Italy Bonanno (2011)
10.04±0.8	<0.05	9.87±0.8	0.48±0.08	27.92±2.3	120±10.6	0.12±0.01	2.31±0.2	0.4±0.04	0.68±0.06	<0.05	67.3±8.5	Stem	
28.4±1.72	<0.05	13.2±0.74	1.69±0.15	308.3±11.4	453±38.4	0.22±0.01	4.13±0.19	0.69±0.04	1.05±0.1	<0.05	389±27.3	Leaf	
14.1±8.2	7.5± 3.7	5.4±1.5	12.31±7.1	1236.6±2430	-	3.02±1.6	8.64±2.5	6.32± 2.5	0.2±11	-	-	Root	Shadegan wetland, Iran Hosseini Alhashemi et al. (2011)
10.54±6.6	9.27±7.6	6.19±4.9	2.77±2.1	23.4±10.7	-	1.33±0.84	8.25±7.5	1.27±1.7	0.19±0.27	-	-	Stem	
12.32±1.7	6.7±5.4	2.99±1.31	1.82±0.95	66.5±17.9	-	0.6±0.2	5.99±1.38	1.24±0.13	0.17±0.02	-	-	Leaf	
-	-	-	12.04±1.2	-	459.7±23.02	-	12.16±0.16	-	1.93±0.3	-	-	Root	Anzali wetland of Iran Ganjali et al.(2014)
-	-	-	3.59±0.3	-	31.3±4.3	-	3.33±0.85	-	1.43±0.18	-	-	Stem	
			2.3±0.28		122.9±9.2		5.09±0.77		1.75±0.44			Leaf	
71.8±362	-	1.63±1.7	5.7±1.69	961±551	2081±1203	0.65±0.28	11.86±4.89	9.73±5.02	0.08±0.03	-	-	Root	Courseof River Cybina, Maltański Reservoir (Western Poland) Rzymski et al. (2014)
-	-	-	-	-	-	-	-	-	-	-	-	Stem	
62.32±20.4	-	1.67±0.44	2.54±0.84	580.6±429	325.2±176	0.2±0.2	5.23±1.37	4.5±0.9	0.11±0.01	-	-	Leaf	
-	9.09±0.98	17.63±9.6	21.53±2.8	-	-	3.62±0.53	-	30.62±4.9	0.739±0.2	3.64±1.4	-	Root	Constructed Treatment Wetland, Municipal Wastewater Morari et al. (2015)
-	<0.14	<0.34	0.45±0.24	-	-	<0.14	-	0.5±0.37	0.03>	0.43±0.13	-	Stem	
-	0.46±0.1	0.98±0.31	0.84±0.15	-	-	<0.14	-	0.92±0.06	0.039	0.38±0.05		Leaf	

Alhashemi et al. (2011) shows identical TF values for Zn, Cu and Pb and totally different TF values for other metals. The differences between the results obtained in the present study and those reported in the previous ones can be attributed to the differences in contamination rates and physical and chemical characteristics of the sediments and water. Also, analytical methods applied to digest the *P. australis* material prior to analysis may have significantly affected the concentration of the observed metals (Du Laing et al., 2009)

The studies so far conducted support the results obtained in this study and imply that, in *P. australis*, the concentration of accumulated metals is higher in under-ground organs than in above-ground ones. In the other studies, metal accumulation follows the order: roots> leaves>stems (Bonanno, 2011; Hosseini Alhashemi et al., 2011; Ganjali et al., 2014; Rzymski et al., 2014; Morari et al., 2015).

In the studies conducted by Bonanno (2011), Ganjali et al. (2014) and Hosseini Alhashemi et al. (2011), a significant, linear correlation was observed between metal concentrations in sediments and their accumulation in *P. australis* organs. In full compliance with the results of the present study, they also suggested *P. australis* is useful for biomonitoring.

4 CONCLUSION

Concentrations of metals in the sediment and *P. australis* samples collected from Anzali wetland were measured. Results indicated that the concentration of most of metals in the sediment samples was higher than the mean concentrations of the Earth's crust and shale. Moreover, the metals As, Cr, Cu, Ni and Mn had higher concentrations than the limits specified in the Ontario Guidelines, indicating that area of study is polluted. Investigation on the plant samples revealed that they are contaminated with metals. This also confirmed the high bioavailability of these metals in the wetland sediments. Statistical analysis demonstrated a high correlation between metal concentration in the sediments and in the *P. australis* organs, with the highest correlation observed between sediment and roots. According to the present study, *Phragmites australis* has many advantages, such as worldwide distribution, good growth and high toxic tolerance particularly to heavy metals and herbicides. Since it has good accumulation rate, therefore, it can be used as a biomonitor to reflect metal pollution in sediments. Anzali international wetland is a unique and worthwhile ecosystem and, therefore, it seems necessary to develop an appropriate strategy to reduce the discharge of pollutants into it. Such strategies may include management of pesticide application and chemical fertilizers used over the wetland catchment area and finally treatment of the municipal and industrial wastewaters. The continuous monitoring of metal pollution would be essential. Selection of appropriate bioindicators can substantially contribute to monitoring program.

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