



# Uptake and accumulation of heavy metals by water body and *Azolla filiculoides* in the Anzali wetland

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## Abstract

The Anzali international wetland is a valuable habitat to a wide variety of aquatic flora and fauna. This wetland ecosystem is being threatened due to various pollutants particularly the heavy metals discharging from different point and non-point sources. Concentration of various heavy metals (Cr, Pb, Hg, Cu, Zn, Cd, Ag and Ti) was examined in the water body and *Azolla filiculoides* (an exotic fern in the Anzali wetland). The samples regarding water and *A. filiculoides* were randomly carried out in three parts of the wetland (eastern, central and western parts) in May 2015. After preparing and digesting the samples, the concentration of the heavy metals was measured using ICP OES method. The obtained results showed that the concentration of Zn in water and *A. filiculoides* samples was higher than other heavy metals in all parts of the wetland ( $p < 0.05$ ). Overall, concentration of the heavy metals was significantly higher in the eastern part of the wetland than other two sampling locations ( $p < 0.05$ ). Also with comparing the concentration of the heavy metals in water and *A. filiculoides*, it was found that all examined metals (except Cd and Ag) in *A. filiculoides* had significantly higher concentration than water sample, while Cd and Ag in water and *A. filiculoides* did not have a significant difference with each other. Based on the results, it can be concluded that *A. filiculoides* can effectively absorb Cr, Pb, Zn, Hg, Cu, Cd, Ag and Ti so that it might be used as a valuable species for the bioremediation and the removal of heavy metals from the wetland.

**Keywords** Anzali wetland · *Azolla filiculoides* · Heavy metals

## Introduction

Wetlands are considered important ecosystems to improve water quality (Zarkami et al. 2020). Since the last decades, these valuable ecosystems are facing with various sources of pollutants particularly with the heavy metals (Bonanno and Giudice 2009). Heavy elements are a group of metals and quasi-metals that are toxic and dangerous even in the low level of concentration. Based on this, they are classified as primarily toxic pollutants in terms of pollution level (Modrzewska and Wyszowski 2014). The main concern for heavy metals is the expansion of the source of emission,

toxicity and environmental stability (Folkesson et al. 2009). These metal pollutants directly affect water characteristics, decrease biological activity and reduce the bioavailability of food in water and pose a serious threat to the health of organisms when entering the food chains (Tokhi et al. 2008) because they can be accumulated in aquatic ecosystems as well as in the tissue of organisms such as bivalve molluscs and a variety of fish species. Then, the metals are in turn transmitted through the food chains to birds, fish-eating animals, and eventually to humans (El Nemr et al. 2007). Another problem is that such metals are not metabolized in the body of aquatic organisms or human. In fact, they are not excreted from the body of organisms so that they are deposited and accumulated in the tissue of organisms such as fat, muscles, bones and joints causing many complications and diseases in the body (Essien et al. 2009). Heavy metals can cause a variety of effects on aquatic organisms such as retarded growth, behavioral and genetic changes, and occasionally biological decline in aquatic life, and also genetic changes and cancer incidences in people (Guveni and Akinci 2011).

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Despite, some metals consisting of copper, zinc and nickel are essential elements for organisms. In contrast, some of metals such as cadmium, lead, mercury and arsenic pose a serious threat to the health of living things (El Nemr et al. 2007). Though some metals in the environment are of natural origin, the metals particularly those resulting from industrial pollution can be poisonous, even destruct living things and upset the balance of aquatic organisms (Morillo et al. 2004). The main source of heavy metals discharging in aquatic environments are various factories and industries whose effluents contain high amounts of the heavy metals (Mishra et al. 2010).

The Anzali wetland is one of the most important wetlands in the north of Iran with an area of about 20,000 hectares. This wetland is located in the south of the Caspian Sea and in the province of Guilan (Pourang et al. 2010). Due to the high diversity of fauna and flora communities in this valuable wetland and relatively long retention time of inflowing water, the Anzali wetland effectively modulates the physical and chemical properties of water entering the Caspian Sea (Mansuri et al. 2013). With the arrival of heavy metals into the Anzali wetland, a portion of the metals enters the Caspian Sea causing biological magnification in the marine food chain through trophic levels. This has led to many concerns about the accumulation of metals in sediments, plants, animals and ultimately humans (Shokrzade et al. 2009).

Various wetland plants can accumulate heavy metals such as lead and cadmium serving as a bioremediation agent (Khosravi 2005). The chemical composition of heavy metals, plant age, physiology of absorption and excretion of elements from the body of organisms, physical and chemical factors and the function of heavy elements in various organs of aquatic organisms can have a significant impact on the absorption of heavy metals by aquatic plants (Almeida et al. 2011). Many studies have been conducted to determine the accumulation of heavy metals in aquatic plants (Axtell et al. 2003; Bonanno and Giudice 2009; Engi et al. 2015). For instance, earlier report (Favas and Pratas 2013) showed the importance of absorption of heavy metals by some aquatic plants such as *Ranunculus trichophyllus*, *Azolla caroliniana* and *Juncus*. In another study (Hamidian et al. 2014), the sequence of absorption of some heavy metals by *Nelumbo nucifera* was obtained as  $Cu > As > Cr > Pb > Cd$ , respectively.

*A. filiculoides* is an invasive free-floating plant in the Anzali wetland belonging to salvinaceae family. This fern fixes nitrogen with the symbiosis of *Anabaena* cyanobacteria and is used for agricultural purposes (Zazouli et al. 2013). *A. filiculoides* is found in several parts of the world including south Asia and particularly in tropical and subtropical regions. It has high reproduction rate and can grow quickly under favorable environmental conditions (Larsson 2011). The plant was imported from Philippines to Guilan province

in 1983 with the aim of nitrogen fixation in the rice fields (Khosravi 2005). Then, *A. filiculoides* occupied a large area of natural catchments and northern paddy fields in a short time (Hashemloian and Azimi 2009) due to inappropriate management by the government as well as lack of natural predators. Based on this, *A. filiculoides* has grown very quickly in the northern of Iran and occupied a large area of the Anzali wetland. It has adversely affected the water quality of the wetland and has caused many ecological problems for native animal and also plant species such as *Nelumbo nucifera* (Indian lotus) and *Lemna minor* (duckweed) (Najafi and Baghestani 2009; Sadeghi et al. 2014). Despite many problems caused by *A. filiculoides* in Iranian wetlands, this water fern is considered as a valuable species to absorb various pollutants particularly the heavy metals ones. Thus, determination of concentration of the heavy metals in the Anzali wetland is necessary due to the disturbing effects of such metals on the native aquatic organisms. The present study aims to determine the amount of heavy metals in water sample and also the accumulation capacity and potential bio-monitoring capability of the heavy metals by *A. filiculoides*.

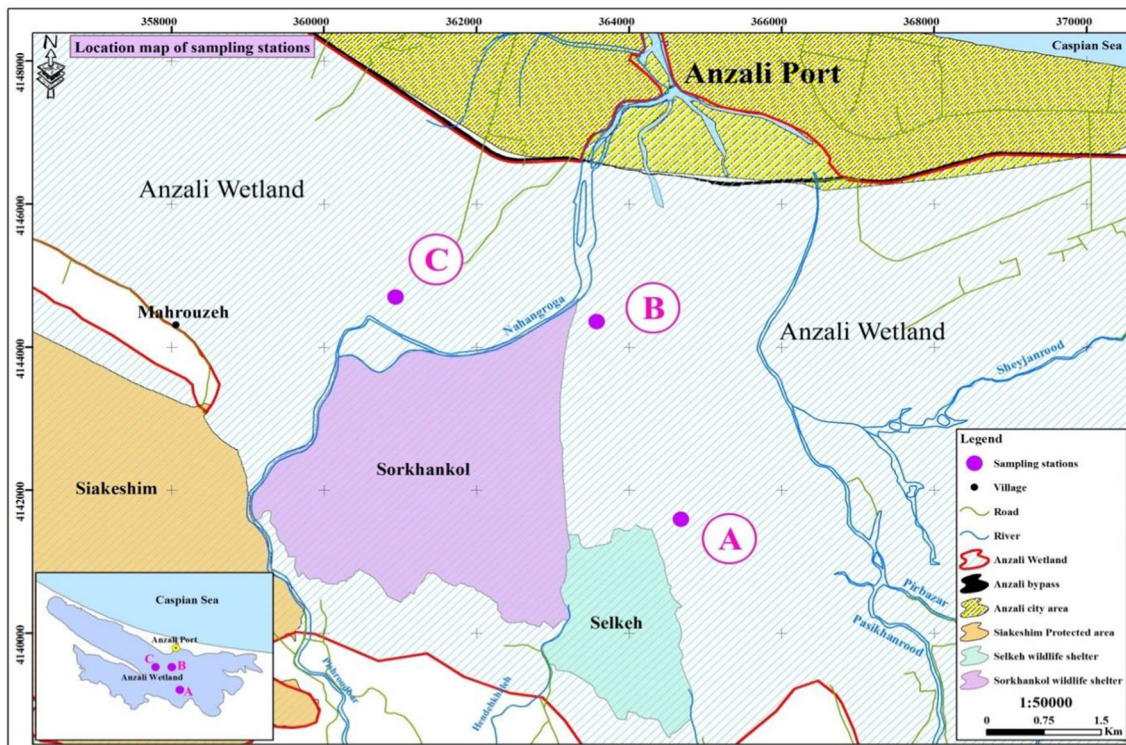
## Materials and methods

### The study area

The Anzali international wetland lies at  $37^{\circ} 22' - 37^{\circ} 30' N$  and  $49^{\circ} 15' - 49^{\circ} 33' E$  coordinates. This wetland consists of four distinct parts: central, eastern, western and Siahkeshim (south-east section of the central part) (Pourang et al. 2010). This ecosystem is an important spawning and nursery habitat for various fish species, and a breeding and wintering area for many waterfowls migrating from Siberia and other parts of the world. Many types of pollution sources enter the wetland resulting from the direct discharge of agricultural, urban and industrial wastewaters. In the present study, three sampling locations were randomly selected. The samples were taken from water body and *A. filiculoides* at each three parts of the wetland including central, eastern and western parts in June 2014 (Fig. 1).

### Water samples

Water samples were taken in the middle depth of water column using Ruttner sampler with three replicates at each station. The samplers were already washed with distilled water and rinsed with 2% nitric acid to avoid contaminating the samples. The three samples (taking from three stations) were put in a container. Then, the samples passed through a Whatman filter paper 42 microns into a plastic bottle and immediately transferred to the laboratory (APHA/AWWA/WEF 1998). To digest the water sample, 500 ml of water



**Fig. 1** Map of study areas with indication of the sampling sites in three parts of the Anzali wetland (**a** eastern part, **b** central part, **c** western part)

was taken from each sampling site and placed in a hot water bath at a temperature below 100°C until the sample volume reached 50 ml. After adjusting the acidity of the samples to pH < 2, they were stored in a refrigerator at 4°C (Ahmad et al. 2010).

### **A. *filiculoides* samples**

*A. filiculoides* specimens were collected at the same locations which were taken for the water samples (eastern, central and western wetland). Then, the samples were placed in a polyethylene container. The samples were transferred to the laboratory and they were initially rinsed with tap water and followed by the distilled water. The samples were then spread on a lace fabric for 72 h to dry. The dried *A. filiculoides* samples were placed in an oven at 180 °C for 3 h. Then, the specimens were smashed and sieved. 1 g of sieved samples (from each sampling station) was added into digestion tubes (Khosravi 2005). The tubes were thus placed in a 90 °C water bath. To digest the samples, 1 ml of 65% nitric acid and after 20 min, 1 ml of hydrochloric acid and 2 ml of 65% nitric acid were added to the tubes. The pipes were then placed in warm water bath at a temperature below 100 °C. The tubes were then removed from the bath and placed in a reagent machine at 120 °C for 2 h. The samples were once again transferred to the bathroom until the volume of

samples reached 50 cc. The samples were passed through a 42-micron Whatman filter paper and stored in a cylindrical polyethylene vessel (Shafi et al. 2015). After digestion process, the samples (water and *A. filiculoides*) were transferred to the laboratory (Faculty of Environmental Health, University of Guilan). Then standard solutions in specific concentration ranges (0.01, 0.1, 1.0, 5.0 mg/l) was prepared for the given metals in the laboratory. In the next step, the calibration operation of the device was performed to draw the calibration curve.

Finally, the samples were injected into the ICP OES (SPECTRO ARCOS, AMETEK), and the concentrations of heavy metals were read (all laboratory materials used belonged to the Merck Germany).

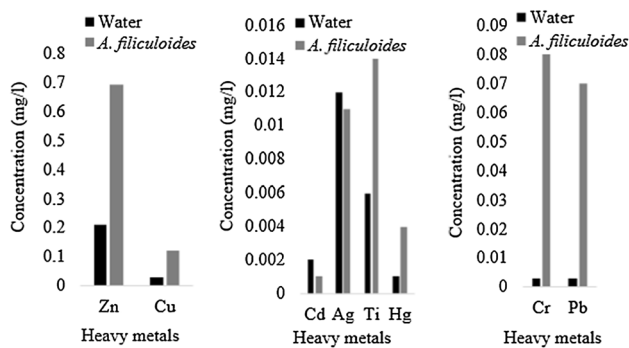
### **Data processing and analysis methods**

The normality of data for the recorded heavy metals parameters was examined by Kolmogorov–Smirnov test (SPSS 23.0 software for windows). After normalizing the data, a one-way ANOVA was conducted to determine the differences between the observations. The Tukey post hoc test (at the 95% confidence level) was used to compare the mean differences of samples at each part of wetland.

**Table 1** Concentration of heavy metals (Cr, Pb, Zn, Hg, Cu, Cd, Ag, Ti) (mean  $\pm$  standard deviation) in water and *A. filiculoides* samples (mg/l) in the eastern, central and western parts of the Anzali wetland.

Metals	Samples	Eastern	Central	Western
Cr (mg/l)	Water	0.020 $\pm$ 0.010 <sup>Ab</sup>	0.003 $\pm$ 0.001 <sup>Bb</sup>	0.003 $\pm$ 0.001 <sup>Bb</sup>
	<i>A. filiculoides</i>	0.130 $\pm$ 0.020 <sup>Aa</sup>	0.080 $\pm$ 0.010 <sup>Ba</sup>	0.040 $\pm$ 0.010 <sup>Ca</sup>
Pb (mg/l)	Water	0.020 $\pm$ 0.002 <sup>Ab</sup>	0.003 $\pm$ 0.001 <sup>Bb</sup>	0.004 $\pm$ 0.001 <sup>Bb</sup>
	<i>A. filiculoides</i>	0.150 $\pm$ 0.010 <sup>Aa</sup>	0.070 $\pm$ 0.030 <sup>Ba</sup>	0.065 $\pm$ 0.030 <sup>Ba</sup>
Zn (mg/l)	Water	0.290 $\pm$ 0.010 <sup>Ab</sup>	0.209 $\pm$ 0.020 <sup>Bb</sup>	0.169 $\pm$ 0.010 <sup>Cb</sup>
	<i>A. filiculoides</i>	0.736 $\pm$ 0.220 <sup>Aa</sup>	0.695 $\pm$ 0.170 <sup>Ba</sup>	0.554 $\pm$ 0.230 <sup>Ca</sup>
Hg (mg/l)	Water	0.004 $\pm$ 0.001 <sup>Ab</sup>	0.001 $\pm$ 0.001 <sup>Bb</sup>	0.001 $\pm$ 0.001 <sup>Bb</sup>
	<i>A. filiculoides</i>	0.011 $\pm$ 0.002 <sup>Aa</sup>	0.004 $\pm$ 0.001 <sup>Ba</sup>	0.005 $\pm$ 0.001 <sup>Ba</sup>
Cu (mg/l)	Water	0.034 $\pm$ 0.001 <sup>Ab</sup>	0.031 $\pm$ 0.001 <sup>Bb</sup>	0.027 $\pm$ 0.001 <sup>Cb</sup>
	<i>A. filiculoides</i>	0.185 $\pm$ 0.020 <sup>Aa</sup>	0.125 $\pm$ 0.010 <sup>Ba</sup>	0.120 $\pm$ 0.010 <sup>Ba</sup>
Cd (mg/l)	Water	0.001 $\pm$ 0.000 <sup>Aa</sup>	0.001 $\pm$ 0.001 <sup>Aa</sup>	0.002 $\pm$ 0.001 <sup>Aa</sup>
	<i>A. filiculoides</i>	0.001 $\pm$ 0.000 <sup>Aa</sup>	0.001 $\pm$ 0.001 <sup>Aa</sup>	0.001 $\pm$ 0.001 <sup>Aa</sup>
Ag (mg/l)	Water	0.012 $\pm$ 0.001 <sup>Ab</sup>	0.012 $\pm$ 0.002 <sup>Aa</sup>	0.012 $\pm$ 0.001 <sup>Aa</sup>
	<i>A. filiculoides</i>	0.012 $\pm$ 0.001 <sup>Aa</sup>	0.011 $\pm$ 0.001 <sup>Aa</sup>	0.011 $\pm$ 0.001 <sup>Aa</sup>
Ti (mg/l)	Water	0.014 $\pm$ 0.001 <sup>Ab</sup>	0.006 $\pm$ 0.002 <sup>Bb</sup>	0.003 $\pm$ 0.001 <sup>Cb</sup>
	<i>A. filiculoides</i>	0.020 $\pm$ 0.050 <sup>Aa</sup>	0.014 $\pm$ 0.030 <sup>ABa</sup>	0.011 $\pm$ 0.001 <sup>Ba</sup>

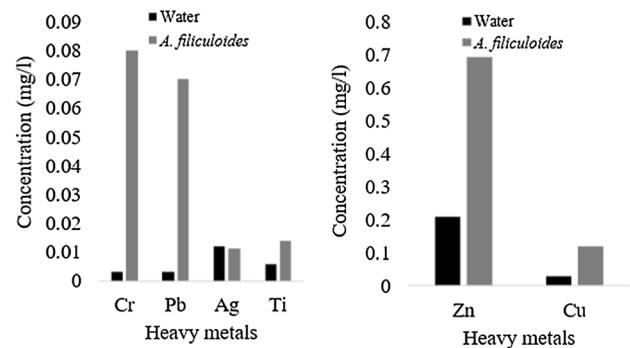
The values specified with different letters indicate a significant difference between the sampling stations (Tukey post hoc test at the 95% confidence level)



**Fig. 2** Heavy metals in the samples of water and *A. filiculoides* in the eastern part of the wetland

## Results

The obtained results concerning the concentration of heavy metals in water and *A. filiculoides* samples are presented in Table 1. The comparison of the accumulation of the heavy metals in the samples related to water body and *A. filiculoides* is also shown in Figs. 1, 2 and 3. The results of Tukey post hoc test ( $p < 0.05$ ) showed that compared to other heavy metals, Zn had the highest concentration in water and *A. filiculoides* in eastern, central and western parts of the wetland (Table 1). As presented in the table, there was a significant difference between the concentration of all heavy metals in all three parts of the wetland. In total, the amount of heavy metals in the eastern part was higher than other two parts of wetland ( $p < 0.05$ ).



**Fig. 3** Concentration of heavy elements in the samples of water and *A. filiculoides* in the central part of the wetland

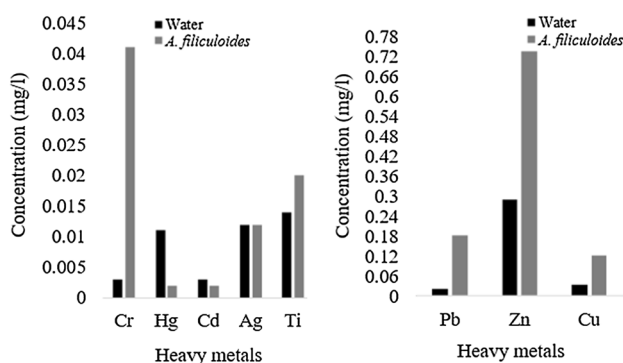
Also, with comparing the concentrations of heavy metals in water and *A. filiculoides* samples, it was found that the accumulation of the heavy metals (except Cd and Ag) in *A. filiculoides* was significantly higher than water samples ( $p < 0.05$ ). As mentioned above, the accumulation of Cd and Ag in water and *A. filiculoides* did not differ significantly from each other.

As shown in Fig. 2, the concentration of heavy metals in the eastern part of the wetland (except Cd and Ag) were significantly different in the water and *A. filiculoides* samples so that the values of metals in *A. filiculoides* were significantly higher than water sample. Zn had the highest concentration in both water and *A. filiculoides* so that the concentration of Zn was  $0.290 \pm 0.010$  in water and  $0.736 \pm 0.220$  mg/l in *A. filiculoides*. Cd had the lowest amount of concentration in water and *A. filiculoides* samples ( $0.001 \pm 0.000$ ).

The average concentration of Cr, Pb, Zn, Hg, Cu, Cd, Ag and Ti in the water sample were 0.020, 0.020, 0.290, 0.004, 0.034, 0.001, 0.012 and 0.014 mg/l in the eastern part of wetland, respectively. This indicates that the sequence of concentrations of heavy metals in water was obtained as  $Zn > Cu > Cr > Pb > Ti > Ag > Hg > Cd$ . The average concentration of Pb, Cu, Zn, Hg, Ti, Cd, Ag and Cr for *A. filiculoides* in the eastern part of wetland were obtained as 0.130, 0.180, 0.730, 0.011, 0.156, 0.001, 0.012 and 0.020 mg/l, respectively. As a result, the concentration sequence of the metals in the eastern part for *A. filiculoides* was reported as  $Zn > Cu > Pb > Cr > Ti > Ag > Hg > Cd$ .

In the central part of the wetland, the values of heavy metals except Cd and Ag were significantly different in the samples of water and *A. filiculoides* indicating that the values of metals in *A. filiculoides* were significantly higher than water (Fig. 3). Concentration of Zn in water ( $0.209 \pm 0.020$  mg/l) and *A. filiculoides* ( $0.695 \pm 0.170$  mg/l) had the highest amount compared to other metals. Cd had the lowest amount both in water and *A. filiculoides* samples ( $0.001 \pm 0.001$ ). The average concentration of Cr, Pb, Zn, Hg, Cu, Cd, Ag and Ti in water sample were 0.003, 0.003, 0.209, 0.001, 0.031, 0.001, 0.012 and 0.006 mg/l in the central part of the wetland, respectively. So, the sequence of metals concentrations in water was observed as  $Zn > Cu > Ag > Ti > Pb > Cr > Hg > Cd$ , respectively. Also, the mean concentrations of these heavy metals in *A. filiculoides* in the central part were reported as 0.080, 0.070, 0.695, 0.004, 0.125, 0.001, 0.011 and 0.014 mg/l, respectively. The sequence of concentration of metals in *A. filiculoides* in the central part of the wetland was  $Zn > Cu > Cr > Pb > Ti > Ag > Hg > Cd$ .

In the western part of the wetland (Fig. 4), the values of all heavy metals except Cd and Ag were significantly different in water and *A. filiculoides* samples so that the values of metals in *A. filiculoides* were significantly higher than water sample. In the western part of the wetland, Zn had the highest concentration in water ( $0.169 \pm 0.010$ ) and *A. filiculoides* ( $0.554 \pm 0.230$  mg/l), respectively. On the other



**Fig. 4** Concentration of heavy elements in the samples of water and *A. filiculoides* in the western part of the wetland

hand, Cd had the lowest values in water ( $0.001 \pm 0.001$ ) and *A. filiculoides* ( $0.002 \pm 0.001$ ), respectively. In the western part of the wetland, the average concentration of metals (in water samples) consisting of Cr, Pb, Zn, Hg, Cu, Cd, Ag and Ti were obtained at 0.003, 0.004, 0.169, 0.001, 0.027, 0.002, 0.012 and 0.003 mg/l, respectively. The sequence of metal concentration in water sample was  $Zn > Cu > Pb > Cr > Ti > Cd > Hg$ , respectively. In addition, in the western part of the wetland, the average concentration of heavy metals in *A. filiculoides* was 0.040, 0.065, 0.550, 0.005, 0.156, 0.001, 0.011 and 0.011 mg/l, respectively. Also, in the western part of the wetland, the sequence of metal concentrations in *A. filiculoides* was obtained as  $Zn > Cu > Pb > Cr > Ti > Ag > Hg > Cd$ .

## Discussion

The potential and capabilities of some of the aquatic plants like *A. filiculoides* should be identified for the treatment of wetlands because such plants are considered as irreplaceable filters that play an important role for the health of wetland. Based on this, in recent years, much attention has been paid to the accumulation of heavy metals by aquatic plants through phytoremediation process (Foroughi and Toghiani 2012). For instance, many authors (Robinson et al. 2003; Pratas et al. 2012; Hamidian et al. 2014; Torbati and Keshipour 2020) proved the importance of phytoremediation using hydrophytes like *Lemna gibba* L, *Lemna minor*, *Callitriche stagnalis* and *Fontinalis antipyretica*. Other studies (Sasmaz et al. 2008; Pratas et al. 2012) also confirmed the importance of *Typha latifolia* for the phytoremediation process in wetland and calculated the biological condensation and transmission factors to remove Zn. Based on this, in the present study, the concentration of heavy metals in water and *A. filiculoides* samples was evaluated in three parts of the Anzali wetland. Then, the capability of *A. filiculoides* (as a phytoremediation agent) was compared with the samples taken from water body concerning metal accumulation in the sampling locations.

As stated in the results, the highest and lowest amount of heavy metals in water and *A. filiculoides* samples were observed in Zn and Cd in all sampling sites, respectively. The importance of Zn for *A. filiculoides* has been already confirmed by several studies (MacFarlane et al. 2003; Kara 2005; Sadeghi et al. 2014) so Zn is an essential element for the growth of *A. filiculoides* playing an important role in many metabolic and physiological processes of this water velvet (MacFarlane et al. 2003; Kara 2005; Sadeghi et al. 2014). Zn is also an important metal as an activator of some vital enzymes including carbonic anhydrase and phospholipases for *A. filiculoides*. Based on this, the effect of increasing Zn concentration on the tissue of *A. filiculoides* in the

sampling sites can be due to the necessity of the given metal for the growth of the water fern (MacFarlane et al. 2003; Kara 2005).

Unlike essential elements such as Cu and Zn, many heavy metals might have devastating effect on the ecological balance of aquatic life and biodiversity in the study wetland. The results showed that the amounts of metals except Cd and Ag in the eastern part of wetland were significantly higher than the central and western parts of wetland. This indicates that the eastern part is the most polluted areas concerning Cr, Pb, Zn, Hg, Cu and Ti than the central and western parts of the wetland. One of the main reasons for the high concentration of heavy metals in the eastern part of the wetland is due to the presence of various industries activities and also intensive farming practices in the vicinity of this part of wetland which discharge too much wastewaters into the wetland (Vesali Naseh et al. 2012).

The statistical test showed that the concentration of Cr, Pb, Zn, Hg, Cu and Ti in *A. filiculoides* sample was significantly higher than water sample in all three parts of the wetland. The discharge of urban wastewater, the wastes of petroleum products, the pollution resulting from various industrial, agricultural and landfill are the main sources of heavy metals pollution in the Anzali wetland (Ganjali and Ghasemi 2016). One of the effective ways in reducing the concentration of these heavy metals in the Anzali wetland might be phytoremediation process by hydrophytes like *A. filiculoides*. Due to intensive distribution of *A. filiculoides* in the most parts of the Anzali wetland, this fern is largely able to prevent contamination and treat the wetland (Mansuri et al. 2013).

Light intensity, oxygen content and temperature are important factors for the growth of *A. filiculoides* (Sadeghi et al. 2014). The given parameters play important role in the absorption of heavy metals by this water fern. Also, the energy from photosynthesis and released oxygen provides the necessary conditions to the active absorption of the heavy metals by this water fern (Arts et al. 2008).

The earlier report showed that the biological transmission factor for Zn was higher than one which indicates the efficiency of *A. filiculoides* in the transfer of the metal from root to upper organs (Sasmaz et al. 2008). The results of the present study are in line with earlier reports (Shafi et al. 2015) demonstrating that *A. filiculoides* had the highest levels of Zn in its tissues compared to Cu, Pb, Cd and Cr. The higher amount of metal accumulation in the roots compared to the leaf tissue of *A. filiculoides* indicates poor transfer of ions in the leaf of the plant (Shafi et al. 2015). This has been confirmed by previous studies for *A. filiculoides* as well as other plants (Sela et al. 1989) who reported that the content of heavy metals in *A. filiculoides* root was two to five times higher than leaf tissue (Sela et al. 1989). According to a research study (Yabanli et al.

2014), accumulation of Cr in the roots of some macrophytes such as *Myriophyllum spicatum* was higher than the stem and lower than the leaves. In addition, *A. filiculoides* has symbiotic relationship with some cyanobacteria such as *Anabaena* in its roots (Larsson 2011) which can help the plant.

Moreover, another study (Shafi et al. 2015) showed that *A. filiculoides* can accumulate the highest amount of Zn in its tissues compared to Cu, Pb and Cd which could be due to the necessity of Zn for the vital activities of the fern. The higher accumulation of metals in the roots compared to the leaf tissue of *A. filiculoides* indicates poor transport of ions in this water fern so that the outcomes are consistent with the results of the present research since adsorption of Zn after Ti by *A. filiculoides* in all three parts of the wetland showed relatively higher values than other metals.

Previous report (AitAli et al. 2002) confirmed that the uptake of Cu might show an increase in *A. filiculoides* by increasing the concentration of Cu in the growth medium. In the present study, the highest absorption of Cu in *A. filiculoides* was obtained as 0.156 mg/l in the eastern part of the wetland which indicates the high potential of this plant in removing Cu in the given part of the wetland. According to earlier research (Hoseinizadeh et al. 2011), the sequence of the concentration of heavy metals in the roots of some macrophytes such as *Trapa natans* in the Anzali wetland was similar to the results of the present study like  $Cd < Cr < Cu < Zn$  (Hoseinizadeh et al. 2011).

The outcomes of the current research indicated that the most contaminated areas for the heavy metals for *A. filiculoides* sample were the eastern followed by the central and western parts of Anzali wetland, respectively. This can be justified by the fact the western part is the deepest part of the wetland and the Siahkeshim (a protected area) is located in this part. Due to its special characteristics, this protected area is one of the least polluted areas in the wetland, while the eastern part of the Anzali wetland has the lowest depth. Moreover, Pirbazar River (one of the highly polluted rivers in the Anzali wetland watershed) enters the eastern part so that the discharged waters from Pirbazar River into the region have high concentrations of pollutants such as Ti, Zn and Cu (Vesali Naseh et al. 2012). A large volume of sewage from the Pirbazar River enters the eastern part of the wetland. This river originates from two highly polluted rivers including Goharrood and Zarjoub. About 15% of the pollution and 25% of the sedimentary loads are transported to the central part of the wetland through the Pirbazar River, which has recently increased the growth of aquatic plants including *A. filiculoides* in this part of the wetland. As a result, the self-purification capacity of the central has increased than the eastern part of the wetland which might reduce pollution loads in this area (Hoseinizadeh et al. 2011; Esmacilzadeh et al. 2016).

In another study (Sartaj et al. 2005), the highest levels of Cr and Zn contamination were found in the sediments of Pirbazar (the eastern part of the wetland). The research showed that the amount of Zn and Cr decreased with the distance from the estuary (Sartaj et al. 2005). In another study (Vesali Naseh et al. 2012), the sequence of metals in the water samples of the Anzali wetland was obtained as  $Zn > Ni > As > Pb > Cd$ . The highest concentration of Zn and Pb was similar to the present research in the eastern part of the wetland (Vesali Naseh et al. 2012).

There are various metals and smelting industries, factories and chemical and petrochemical industries in the vicinity of the Anzali wetland watershed. The effluents have high amounts of heavy metals such as Zn, Hg, Cr and Pb (Zare Khosheghbal et al. 2013). Hg, Cr, Cu, Ni and Zn are the most widely used industrial metals found in the effluents of various industries, especially metal plating industries, as well as agricultural and municipal wastewater in the Anzali wetland watershed (Karbassi, et al. 2008). In addition, one of the sources of metal input to the Anzali wetland is the Anzali port which is in the vicinity of the Anzali wetland. The reason is that there are a lot of shipping activities and oil tanker traffic in Anzali port.

Cyanobacteria have high absorption and accumulation capacity for the heavy metals which are used for the purification of industrial wastewater (Afdal 2008; Laloknam et al. 2009). Therefore, the absorption and accumulation potential of the *A. filiculoides* roots are very important (Sood et al. 2012). The high ability of *A. filiculoides* in absorbing and accumulating heavy metals has made it possible to introduce this plant as an effective macrophyte in biomonitoring of water quality in the Anzali wetland for the heavy metals and in refineries. The previous research showed that different *Azolla* species can be used to remove colored contaminants from water and sewage (Zazouli et al. 2013).

According to the results of the present study, it can be concluded that *A. filiculoides* is a very suitable plant for the purposes of phytoremediation in the Anzali wetland. In general, a suitable plant species (as a bioremediation) should have specific characteristics such as rapid and abundant growth, high dispersion and potential for absorption and accumulation of pollutants (Hamidian et al. 2014). As a conclusion derived from the study, *A. filiculoides* has all these conditions in the study areas.

## Conclusion

Heavy metal pollution in aquatic ecosystems is a serious concern for the inhabitants of the ecosystem and the health of humans. Therefore, it is essential to evaluate the extent and sources of the heavy metal pollution in the aquatic ecosystems. Based on the conclusion derived from the present

study, due to the expansion of industrial, tourist and petroleum activities around the Anzali wetland, the concentration of heavy metals may increase to hazardous levels. Therefore, continuous monitoring of the area using aquatic plants such as *A. filiculoides* is essential for the protection of plant and animal life the Anzali wetland.

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## Declarations

**Conflict of interest** The authors declare that they have no conflict of interest.

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