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Original Article

# Phytoremediation efficiency of pondweed (*Potamogeton crispus*) in removing heavy metals (Cu, Cr, Pb, As and Cd) from water of Anzali wetland

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**Abstract:** Plant-based remediation (i.e. phytoremediation) is one of the most significant eco-sustainable techniques to cope with devastating consequences of pollutants. In the present study, the potential of a wetland macrophyt (i.e. *Potamogeton crispus*) for the phytoremediation of heavy metals (i.e. Cu, Cr, Pb, As and Cd) in the Anzali wetland was evaluated. The results showed that *P. crispus* tends to accumulate notable amounts of Cu, Cr, Pb, As and Cd according to their assayed concentrations as follows: 8.2 µg $g^{-1}$ dw, 0.97 µg $g^{-1}$ dw, 6.04 µg $g^{-1}$ dw, 2.52 µg $g^{-1}$ dw and 0.34 µg $g^{-1}$ dw, respectively. Further accurate perception of the phytoremediation efficiency were conducted using both bioconcentration factor and translocation factor. The average of the highest bioconcentration factors was presented in a descending order as: $2.9\times10^3$, $1.9\times10^3$, $1.17\times10^3$, $0.68\times10^3$ and $0.46\times10^3$ for the Cu, Cr, Pb, Cd and As, respectively. Based on the results, *P. crispus* presents high potential to absorb all the alluded metals except for As and partly Cd. Correspondingly, the mean values of translocation factor were reported in the range of 0.41 to 2.24. Eventually, relying on the observed findings, the results support the idea that *P. crispus* species would be employed as the prospective candidate for the phytoremediation processes in Anzali wetland.

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## Introduction

Heavy metals pollution has been posed as a growing predicament worldwide. Hence, the natural environment quality levels tends to be worsened due to poor problem-solving skills and inadequate eco-suited techniques to diminish the detrimental demeanor of pollutants. Unlike organic pollutants, non-biodegradability property of heavy metals displays the immediate cause of bioaccumulation and its harmful subsequences on food chains (Khan et al., 2010; Hamidian et al., 2014). Therefore an acute health risk occurs to the environment and its living organisms. Thus, removing heavy metals from natural medium requires necessary action (Ali et al., 2013).

The heavy metals are divided broadly into essential and non-essential. Essential heavy metals such as Zn, Cu, Mn, Fe, Cr and Ni are necessary for functions in trace quantities in biological operations (Cempel and Nikel, 2006). In contrast, Hg, Cd, As and Pb known as non-essential heavy metals which are not only unnecessary for organism, but also have toxic and deleterious effects (Dabonne et al., 2010). Major origin of contaminants such as heavy metals are anthropological activities including industrial and municipal effluents, mining, untreated wastewater and agrochemicals and also, natural-driven origins such as volcanic eruption, ore weathering and mineral deposition (Kabata-Pendias and Pendias, 1989; Rai et al., 2008).

Concern about marked release of heavy metal pollutants and cleaning up of contaminated areas has been increasing as the major controversial and disputed issues in the contemporary time. Therefore,

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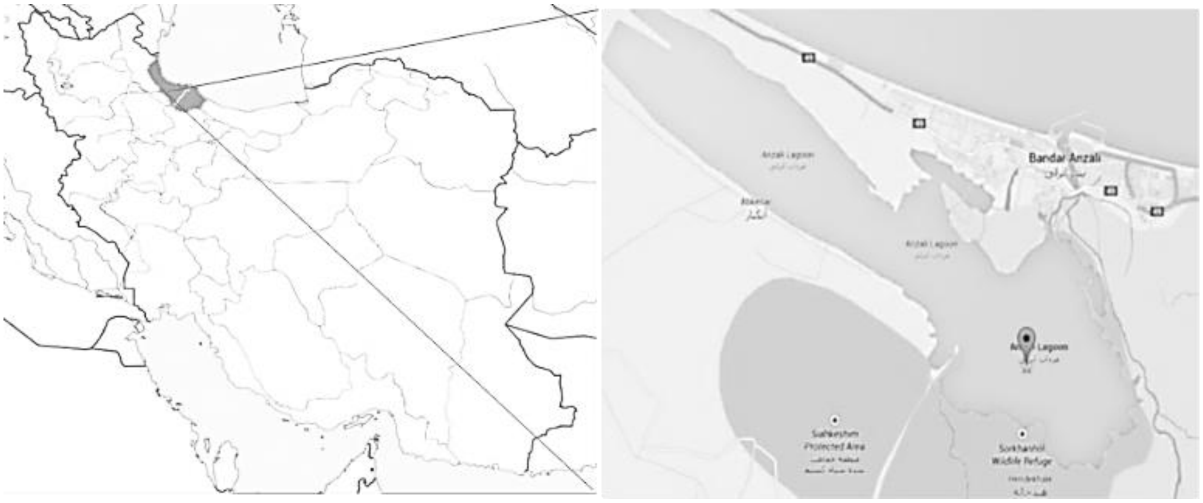

Figure 1. Location of Anzali wetland in Iran

many physical, chemical and biological approaches have been applied to reduce the impacts of pollutants especially heavy metals (Sheoran et al., 2011). Most of these methods appear to cost high and disruptive to the natural properties of environment and ended to soil erosion rapidly, and may also cause multiplied environmental problems (Ali et al., 2013). The most innovative and effective alternative method to omit the heavy metals from environment is phytoremediation that is a green, safe, solar-oriented and cost effective method using the capability of plant species to absorb high levels of metals in specific tissues like roots and leaves especially in the aquatic substratum (Carranza-Ắlvarez., 2008; Sigh and Prasade, 2011). The inception of phytoremediation idea was owing to Chaney (1983) and came to apply eagerly during the recent two decades as a better way to solve contamination problems particularly by aquatic plants for removal of pollutants from contaminated surface water (i.e. phytofilteration) (Mukhopadhyay and Maiti, 2010). Based on Ali et al. (2013), proper plant species for phytoremediation needs some distinctive features such as to spread widely, easy to grow and mainly high potential to accumulate heavy metals. The aquatic macrophyts have innate ability to uptake heavy metals from polluted water medium and wastewater (Hamidian et al., 2014). The aquatic plants can absorb more Cu, As, Br, Cr, Cd, Sr, V and Pb than terrestrial plants (Sood et al., 2012). Phytoremediation and metal removal using aquatic macrophyts can be enormously reinforced by preferring appropriate plants species (Fritioff and Greger, 2003; Hamidian et al., 2014).

Identification of proper plants for phytoremediation in a contaminated area is a full-scale model to survey its mechanisms of heavy metals purification and accumulation (Carranza-Alvarez et al., 2008; Hamidian et al., 2014). Therefore, this study aimed to assess the phytoremediation potential of a native and dominant floating plant in Anzali wetland, *Potamogeton crispus* (Pondweed), and evaluate its innate capability to accumulate heavy metals as hyper-accumulators to meet the phytoremediation purposes. *Potamogeton crispus* is a widespread floating species in wetland ecosystems especially in Anzali wetland (Pajevic et al., 2008). This species is a floating plant and its metal-accumulation aspect is related to its surrounding water metal concentrations (Favas and Pratas, 2013). Hence, this study intends to determine the extent to which the above-mentioned species is well-suited for phytoremediation and whether could be used as a proper accumulator species to uptake specific heavy metals including Cu, Cr, Pb, As and Cd based on (1) to investigate the concentration of metals in water and plant body, (2) to determine the removal potential of Cu, Cr, Pb, As and Cd using *P. crispus*, (3) to estimate the metal transportability according to bioconcentration factor (BCF) for water and plant tissues; Moreover, transfer factor (TF) for shoot and root and (4) to confirm the positive correlation

Table 1. Heavy metals in *Potamogeton crispus* species tissues (μg$^{-1}$, dry weight) and in Fresh water (μg lit$^{-1}$).

| Element | Sample (Mean ± SD, n=20) | | | BCF | TF |
|---|---|---|---|---|---|
| | Water (μg L$^{-1}$) | Shoot (μg g$^{-1}$) | Root (μg g$^{-1}$) | | |
| Cu | 2.82 ± 0.14 | 4.35 ± 0.12 | 8.2 ± 0.17 | 2900 | 0.530 |
| Cr | 0.51 ± 0.03 | 0.471 ± 0.024 | 0.97 ± 0.05 | 1900 | 0.485 |
| Pb | 5.14 ± 1.43 | 6.04 ± 0.2 | 2.686 ± 0.134 | 1170 | 2.25 |
| As | 5.42 ± 0.65 | 1.037 ± 0.052 | 2.525 ± 0.127 | 460 | 0.411 |
| Cd | 0.50 ± 0.01 | 0.235 ± 0.012 | 0.344 ± 0.02 | 688 | 0.683 |

Bioconcentration factor (BCF) = Concentration in plant/Concentration in water
Translocation factor (TF) = Concentration in shoot / Concentration in root

between metal concentration in water and plant in the eastern part of Anzali wetland in Iran.

## Material and methods

***Description of study area:*** Anzali wetland is an outstanding coastal lagoon that is located in the southwest of Caspian Sea including an area of about 200 km$^2$ located between 37°28´N and 49°25´E. It is an excellent natural aquatic ecosystems that enhances a widely heterogeneous floristic composition and wildlife refuges. The wetland is covering 1% of bird wintering immigrant community of middle-east region (Jafari, 2009). Anzali wetland similar to other coastal ecosystems is likely to be affected by anthropocentric activities. Hence, the contaminants deriving from industrial and urban effluents, agrochemicals and untreated wastewater can cause irretrievable adverse effects on this natural ecosystem (Fig. 1).

***Preparation of plant samples:*** Sampling was performed in October 2013. The water samples were removed from five stations and prepared by filtration and addition of 2% $HNO_3$ subsequently. Also, twenty plant samples were collected from the same site. The body samples were washed with tap and distilled water to wipe out any adhering substances. The root and body of plants were homogenized and then dried in oven (60°C for 24 hrs). Then, the dried samples were pulverized and obtained powder was sieved (0.15 mm) (Kalra, 1998). According to the digestion protocol, 0.5 gram samples of plant tissues put into the digestion tube and then 10 ml of nitric acid was added and stored overnight. Furthermore, samples were heated (120°C) for 4 hrs. Finally, the samples were transferred into 25 mL volumetric flasks and after addition of 3 mL diluted hydrochloric acid, filled with distilled water. Inductivity coupled Plasma-Optical Emission Spectrometry (ICP-OES) (PerkinElmer, USA) was used to measure the concentration of Cu, Cr, Pb and Cd. The detection limits for the analytical instrument were 2.5, 0.5, 0.5, 5 ppb for Cu, Cd, Cr and Pb, respectively. As concentrations were assayed by HG-FAAS (hydrogen generation flame atomic absorption spectroscopy) with detection limit of 1 ppb. The analysis procedure was confirmed by analyzing 2 blanks and 2 spiked specimens for each twenty plant samples considering all laboratory conditions being equal.

***Statistical Analysis:*** All statistical analysis were performed using Origin 8.0 software. Each metal was analyzed individually. Data was analyzed for normalization using Kolmogorov-smirnov test. ANOVA and Duncan tests were applied to parallel the mean values of metals in different sampling sites. Pearson correlation coefficients were used for estimating the correlation among metal concentrations in the water and plant tissues. Also, comparisons between metal concentrations in water and plant tissues were performed using t-test. When concentrations were not definite (e.g. below the detection limit), they were considered as half of the

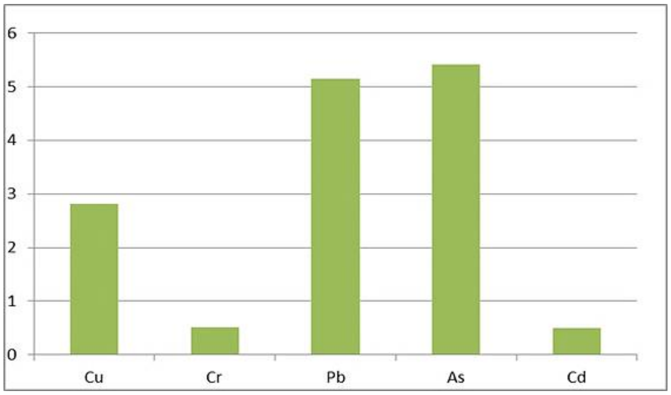

Figure 2. Concentration of heavy metals in water (μg.lit$^{-1}$).

detection limit. Data were reported based on mean values of at least triplicates with standard deviation (SD).

## Result and Discussion

***Variation of heavy metals in water and plant tissues:*** No significant difference was observed in heavy metal concentrations of different sampling sites, in the same time (Table 1). Thus, it can be inferred that the metal ions were equally distributed throughout the wetland. However, the concentration of heavy metals in station 1 (Pirbazar river outfall to the wetland) and station 3 (Hendekhaleh river outfall to the wetland), which are located in the east and southeast of Anzali wetland, respectively, were little higher than those of other stations. Meanwhile, based on geographical interpretation of the sampling sites, it was obvious that heavy metal effluents outfall were participated in the contaminating of the water substratum (Pajevic et al., 2008; Pratas et al., 2012). The results showed a significant differences between the heavy metals concentration in plant tissues and water ($P<0.05$). The concentrations of heavy metals in water are shown in Figure 2. The heavy metals concentration in plant tissues are depicted in Figure 3. Based on the results, a range between 0.12 μg g$^{-1}$ dw and 14.05 μg g$^{-1}$ dw in plant and a range between 0.5 μg lit$^{-1}$ and 6.91 μg lit$^{-1}$ in water were recorded. This results show a significant differences in the different tissues of plant and water.

***Copper:*** Cu not only is a necessary nutrient for plants, but also a toxic element at extra concentration. It seems having high level of potency to accumulate in the lower tissues of plants (Kabata-Pendias and Pendias, 2001). The results revealed that the concentration of Cu in the water, *P. crispus* roots and shoots tissues were 2.82 μg lit$^{-1}$, 8.20 μg g$^{-1}$ dw and 4.01 μg g$^{-1}$ dw, respectively. The highest registered BCF was calculated in *P. crispus* with the average of $2.9\times10^3$. Similarly, the TF were calculated in the range of 0.17 and 0.56. Moreover a significant ($P<0.001$) positive correlation between the Cu concentration in the plant and water was found. This result shows the positive aspects of *P. crispus* as a reliable accumulator species to omit Cu-oriented contaminants loaded to Anzali wetland.

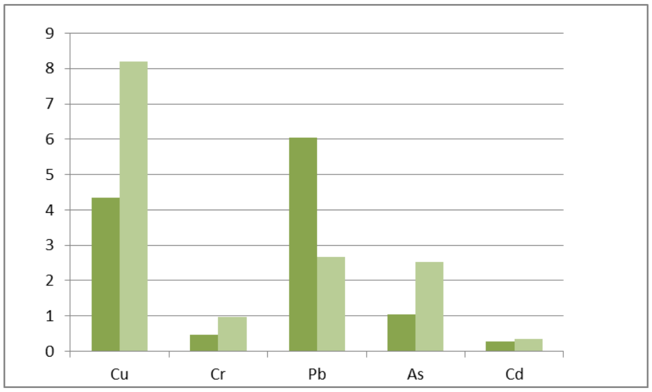

Figure 3. Concentration of heavy metals in *Potamogeton Crispus* tissues (root (first column) and shoot (second column)) (μg g$^{-1}$ dw).

***Chromium:*** Contents of Cr in plants have appealed warning notice not only due to its main function as an essential metal, but also because of its carcinogenic impacts. Chromium is slightly available to plants, thus it is accumulated eminently in roots. Chromium concentration vary between 0.4 and 3.2 mg kg$^{-1}$ in rooted emergent plants species (Kabata-Pendias, 2011). Nonetheless, chromium concentrations more than 10 mg kg$^{-1}$ have phytotoxic (Pais and Jones, 2000). In the current investigation, the mean values of Cr in the water and *P. crispus* roots and shoots were calculated to be 0.51 μg lit$^{-1}$, 0.97 μg g$^{-1}$ dw and 0.47 μg g$^{-1}$ dw, respectively. The Cr values in the *P. crispus* tissues were measured in a descending order from root to shoot. Although, Cr concentration of *P. crispus* was shown positive correlation with its concentration in water.

Low transport of Cr from root to aerial parts in

*Potamogeton* sp. can be described due to being as a non-essential element for plant growth (Shewry and Peterson, 1974). On the other hand, the high accumulation of Cr in some wetland plants depends on the plant ability to decrease toxic Cr(VI) to the non-toxic Cr(III) form in roots and then trasmittancy of Cr(III) to the shoots (Lytle et al., 1998). The highest BCF was calculated with the average of $1.9\times10^3$. Also, the translocation factor values ranged between 0.47 and 0.6 in *P. crispus*. Based on TFs, Cr transfer ability of *P. crispus* from root to shoot, implying low transfer rate of Chromium due to incompetence in plant transfer system.

According to the results a significant ($<0.05$) positive correlation among Cr concentrations in tissues and water was detected. Owing to the results, *P. crispus* seems to act as a proper alternative to reduce negative effects of pollution especially in the Cr-based contaminated aquatic environments.

***Arsenic:*** As is a prevalent metalloid that found in water, atmosphere, minerals and living organisms (Adriano, 2001). Concentration of As in unpolluted surface water differs from 1 to 10 μg $lit^{-1}$. In freshwater, the As concentrations were reported in the range of 0.15–0.45 and 2 μg $lit^{-1}$ (Sharma and Sohn, 2009; Smedley and Kinniburgh, 2002). Although, aquatic macrophyts have a significant effect on the As uptake, but the presence of As in plants is calculated more than 1 mg $kg^{-1}$ (Sasmaz and Obek, 2009). García et al. (2010) noticed that As can hardly is removed via direct absorption by plants. In addition, Heung Lee (2013) mentioned that 0.5–1% of the total As input was accumulated in plant tissues. In the current investigation, the mean concentration of As in water, *P. crispus* roots and shoots tissues were measured as 5.4 μg $lit^{-1}$, 2.52 μg $g^{-1}$ dw and 1.03 μg $g^{-1}$ dw, respectively. Besides, the assayed metal concentrations in plant were more considerable than those of water. The highest BCF was $0.46\times10^3$ and TFs values were varied between 0.28 and 0.42. TFs quantities pronounced that As were not transferred from roots to shoots efficiently. Moreover, a significant ($P<0.001$) positive correlation between As concentrations in the *P. crispus* and water was recognized. According to results, *P. crispus* does not appear to be a proper aquatic species to omit As.

***Lead:*** Pb is a major pollutant of the environment and absorbed by aerial tissues of plant passively and tightly is bound in root (Kabata- Pendias, 2011). The Pb transfer from roots to aerial parts is limited, whereas Raskin (1996) described, only 3% of the Pb in roots was transferred to the upper tissues. Pb is a gradually phytoavailable metal thus, hard to be phytofilter (Kabata-Pendias, 2011). In spite of above mentioned reports, in present study, the mean concentration of lead in water, *P. crispus* roots and shoots tissues were detected as 5.13 μg $lit^{-1}$, 2.68 μg $g^{-1}$ dw and 6.04 μg $g^{-1}$ dw, respectively. The most concentration of Pb was found in the shoots of *P. crispus* (6.04 μg $g^{-1}$ dw). This value were correlated with the Pb value of water. As a result, *P. crispus* was able to meet strong Pb concentrations overstepped that of the surrounding substratum. The highest BCFs of Pb in the *P. crispus* was $1.17\times10^3$. Also, the TFs were in the range of 1.77-2.24. Based on the results, the concentration of Pb in aerial parts is higher than that of roots, therefore Pb transferring from root to aerial part is feasible and furthermore, a well-developed root-rhizome form was not detected in plant species (Demirzen and Aksoy, 2004; Aksoy et al., 2005).

A highly significant ($P<0.001$) positive correlation between Pb in *P. crispus* and water was found. Based on the findings, *P. crispus* seems to be an appropriate species to omit Pb from wastewater (Favas et al., 2012).

***Cadmium:*** Cadmium is noted as one of the main eco-toxic metals that reveals catastrophic effects on the plants and entire physiological processes of living organisms (Kabata-Pendias, 2011). Although Cd is a non-essential element for metabolic processes, it is easily absorbed by both root and leaf. There are proofs that acceptable values of Cd is absorbed passively by root and leaf (Kabata-Pendias, 2011). Hence, the linear relationship between Cd in plant and water medium was reported (Kabata-Pendias, 2011). Cd values in natural water are normally lower than 0.001 μg $mL^{-1}$ and can be reached up to 1.9 μg $g^{-1}$ in stems and leaves of aquatic plants (Friberg et al., 1986; Kabata-Pendias, 2004). The growth of plants in higher concentrations of Cd usually is stopped (Wang and Zhou, 2005). According to the results, the mean values of Cd in the water and *P. crispus* roots and shoots were recorded as 0.50 μg $lit^{-1}$, 0.34 μg $g^{-1}$ dw and 0.23 μg $g^{-1}$ dw, respectively. The highest Cd concentrations in the roots of *P. crispus* (0.34 μg $g^{-1}$ dw) indicates that this species is capable to accumulate a high Cd concentration. Therefore, the highest BCFs were estimated $0.68\times10^3$ for the root of *P. crispus*, indicating its moderate ability to accumulate Cd. Similarly, the TF recorded between 0.56 and 1.52. TFs reveal that Cd was not transferred from roots to leaves efficiently but was absorbed greater than Cu, Cr and As. A significant ($P<0.001$) positive correlation between Cd concentration and plant and water was observed, therefore *P. crispus* seems to be an appropriate species to remove Cd-derived pollutants from surrounding water effectively.

***Quantification of phytoremediation possibility:*** Evaluation of phytoremediation efficiency is determined using Bioconcentration Factor (BCFs) and Translocation Factor (TFs) (Pratas et al., 2012; Ali et al., 2013). Both factors (BCFs and TFs) would help trial to establish a greater degree of accuracy on phytoremediation efficiency. The both factor values are presented in Table 1.

***Bioconcentration factor:*** BCFs demonstrate the capability of plant to uptake heavy metals from surrounding medium, indicating that plants are able to accumulate heavy metals and therefore more appropriate for phytoremediation (Pratas et al., 2012). The bio-concentration factor is defined as metal concentration in dry mass in relation to its concentration in external substratum (Favas and Pratas, 2012). It is calculated as follows (Zhuang et al., 2007).

$$\text{Bioconcentration Factor (BCF)} = \frac{C\ harvested\ tissues}{C\ water(substratum)}$$

Where *C harvested tissues* is the concentration of the metal in the plant harvested tissue and *C water(substratum)* is the concentration of the same metal in water (substratum). A BCF value of > 1000 indicates a considerable hyper-accumulation potency of plant (Boonyapookana et al., 2002). In present study, the highest BCF value was calculated in *P. crispus* for Copper ($2.9\times10^3$), showing that *P. crispus* exhibits acceptable efficiency to decline Cu in water. Similarly, the highest Chromium BCFs with the average of $1.9\times10^3$ was reported in this plant species. Also, the highest BCFs for Pb, Cd and As were as $1.17\times10^3$, $0.68\times10^3$ and $0.46\times10^3$, respectively. Consequently, *P. crispus* appears to be a noticeable accumulator in Anzali wetland for all mentioned metals except for As.

***Translocation Factor:*** Translocation factor explains the capacity of plant in transporting the concentrated metals from root to aerial parts (Ali et al., 2013). This factor is defined as the metal concentration in plant shoot in relation to its concentration in plant root (Pratas et al., 2012). It is calculated as follows (Padmavathiamma and Li, 2007):

$$\text{Translocation Factor (TF)} = \frac{C\ shoot}{C\ root}$$

Where *C shoot* is the concentration of metal in plant shoots and *C root* is its concentration in plant roots. The TF with higher valuse of 1, imply the high potency of plant metal transport systems (Zhao, 2002). Also, Kabata-Pendias and Pendias (2000) reported that the translocation factor in the range of 0.01-1 indicates the moderate bioavailiblity and accumulation of metals in aerial tissues of plants. In the present study, the mean values of TF in the *P. crispus* were calculated lower than 1 for Cu, As, Cr due to dysfunction in metal transmittancy operations (Sasmaz et al., 2008). The mean TFs in the plant evaluated higher than1 for Pb and relatively Cd. The highest TFs refer to *P. crispus* (2.24) for Pb, due to high transmittancy of Pb from roots to leaves actively. The mean TFs were presented in a descending order as Pb, Cd, Cu, Cr and As. This results confirmed that above-mentioned heavy metals were not transferred from roots to aerial parts efficaciously except for Pb and partly Cd.

***General conception:*** Many works have been conducted to assess the bioaccumulation of heavy metals using aquatic macrophyts (Ye et al., 1997; Robinson et al., 2003; Pajevic et al., 2008; Carranza-Alvarez et al., 2008; Sasmaz et al., 2008; Alonso-Castro et al., 2009; Bonanno and Giudice, 2010; Pratas et al., 2012; Favas et al., 2012; Hamidian et al., 2014). Also, Rai (2008) has been emphasized to introduce *P. crispus* as proper metal accumulator. Ali et al. (1999) noticed the prominence of *P. crispus* to phytoremediate Cu, Cr, Pb and Zn. Demirzen and Aksoy (2004) investigated remediation potential of *Potamogeton* sp. to accumulate Cd, Pb, Cr, Ni, Zn and Cu in wetlands. Furthermore, Fritioff and Greger (2006) recognized *P. crispus* ability to participate on the heavy metals phytoremediation process. Moreover, Pajevic et al. (2008) pronounced that *Potamogeton* sp. can be used as reliable accumulators for heavy metals (Fe, Mn and Cd) pollution.

Numerous factors may influence on the concentration-dependent variation of heavy metals in the plant species such as total concentration of metals in aquatic environment, the wide scope of metal species, various metal mechanisms and movability, and also plant–water interface. In addition, several physiochemical factors and physiological features such as water depth, water overflow, natural attributes of heavy metals, water pH, organic compound volume and biological characteristic of each plant species are used to determine whether a particular heavy metal is likely to be accumulated (Caranz-Alvarez et al., 2008).

## Conclusion

Macrophyts are the key elements of wetland ecosystems. They not only uptake heavy metals, but also exhibit inherent strength to clean up surrounding water by absorbing of increasing pollutants particularly heavy metals (Jenssen et al., 1993). In addition, the highlighted functions of wetland macrophyts on phytoremediation and bioaccumulation assist to achieve a profound binary role (Rai et al., 2008).

Eventually, this study showed a concentration-bound accumulation of Cu, Cr, Pb, As and Cd in *P. crispus* tissues. Namely, the metals concentrations in plant species was increased in a linear model along with increase in the water. The inspected positive correlation between metal concentrations in *P. crispus* and water, revealed its valuable role to remove metallic pollutants. The root of *P. crispus* is more adapted to concentrate metals than aerial parts, due to confined metal transporting system of plant. By returning to the objectives, it is now possible to state that *P. crispus* is an important qualified representative to meet the phytoremediation needs in the polluted substratum of Anzali wetland by Cu, Cr, Cd, Pb and As, respectively.

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