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Heavy metals phytoremediation by aquatic plants (*Hyrocotyle ranocloides, Ceratophyllum demersum*) of Anzali lagoon

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ABSTRACT: Wetlands are often considered sinks for contaminants, and Aquatic plants have been shown to play important roles in wetland biogeochemistry through their active and passive circulation of elements. In this research, in order to evaluate the concentration level of heavy metals released by the urban, industrial and agricultural activities at the margin of the rivers flowing into Anzali lagoon, as well as finding lead and cadmium accumulated in two plant species quoted below, a sampling has been made on two kinds of aquatic plants in the lagoon at three randomized stations based on the density of the plant coverage. In this project two regional aquatic plant species as*Hyrocotyleranocloides, Ceratophyllumdemersum*have been investigated for absorption of lead and cadmium. Results show an average concentration of lead in *Ceratophyllum* being 53.11 ppm, for *Hydrocotyle* 77.8 ppm, and of cadmium in *Ceratophyllum* being 4.46 ppm and for *Hydrocotyle* 6.28 ppm. According to results, lead has been the most abundant between these two metals inside plant organs regardless the specie. The statistic test gives the results under the confidence level 95% proving the amount of the absorption being significant in the stem of *Hydrocotyle* plant rather than in the root or the leaf compared with other species.

Keywords: *Heavy metals; Aquatic plants; Anzali lagoon; phytoremediation*

INTRODUCTION

The Anzali lagoon is located in the province of Gilan in Northern Iran. It is situated at the southwestern coast of the Caspian Sea, near to the city of Bandar-e-Anzali, and made of a pretty diverse wetland flora and fauna. It's located between 37°28′ N and 49°25′ E. It is 26 km long and 2.0 - 3.5 km wide, surrounded by reed-beds extended to its eastern limits by a margin up to 7 km wide. Over 11 rivers are entered the Anzali lagoon in their way towards the Caspian Sea, the water system consists of large, eutrophic freshwater basins, shallow impoundments, marshes and seasonally floodegrasslands. The wetland is bordered to the north by sand dunes and to the south by cultivated land (mainly rice fields) and patches of woodland. (Ramezanpoor, 2004). The Anzali, like other lagoons, is affected by human activities resulting in contamination of the water. The pollution with petroleum, heavy metals, xenobiotics, organic compounds and other contaminants is an emergent environmental factor that harms both terrestrial and aquatic ecosystems. Heavy metal pollution is mainly due to the result of human activities such as agriculture, mining, construction and industrial processes (Hoseinizadeh *et al.*, 2011).

The commercial use of phytoremediation is acceptable way to refine contaminated regions, so the role of macrophytes in the heavy metal cycling, in aquatic ecosystems must first be noticed. Macrophytes are important in the biological monitoring of aquatic ecosystems, as changes in the composition of the aquatic vegetation are considered a greate biological indicator of the quality of water (Schneider and Melzer, 2003). Most macrophytes which are mainly submerged and floating, have the capacity to tolerate high levels of heavy metal by forming chelates and by subcellular compartmentation. In these aquatic plants, phytochelatines and metalothionines are the main cytoplasmic chelators of heavy metals. Furthermore, macrophytes are involved in bioremediation due to their high tolerance to metals and the affect on ion solubility through the release of O2 from their roots (Stoltz, 2004).

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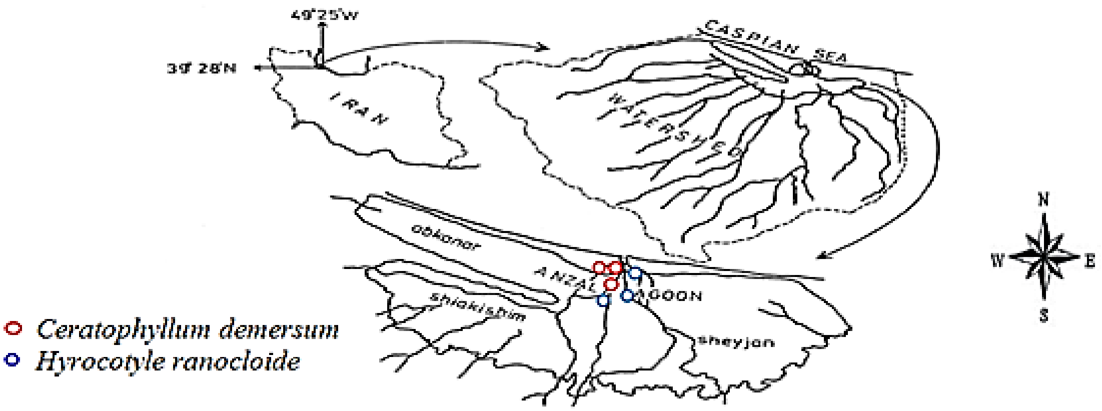

Fig. 1: Map of the sampling site in Anzali international lagoon

The ability to effectively up taking metal ions out of solution and to concentrate high levels of these contaminants in plant tissue are dependent on plant species and metal ions, Some factors involved in the determining such differences is the rate of chelation, ionic exchange, chemical precipitation, translocation of metal ions and precipitation made by root exudates or by microorganisms (Suñe *et al.*, 2007).

The uptaking of heavy metal ions by the biomass of aquatic macrophytes was studied by (Bunluesin *et al.*, 2007). They found that *Hydrilla verticillata* is a reliable biosorbent for treatment of wastewater with heavy metal contaminants. Boyd observed that aquatic plants are suitable for wastewater treatment because they have tremendous capacity of absorbing nutrient and metals from the water so bring the pollution load down. Since plant species can differ in rates of metal uptake, allocation and excretion, metal dynamics in wetlands may be influenced by the composition of plant communities, use of plants in wastewater treatment or phytoremediation, could not be exceeded without environmental damage (Pajević *et al.*, 2008). Our purpose was to determine the pollutant situations of the Anzali lagoon in the Gilan in Northern Iran, by measuring the heavy metal content of macrophytes.

MATERIALS AND METHODS

In order to evaluate the concentration level of heavy metals released by the urban, industrial and agricultural activities at the margin of the rivers flowing into Anzali lagoon, two plant species from three stations were collected to study heavy metals accumulation(Fig. 2: the location of sampling stations). Samples were collected by using a random block system on June 2011. This regional aquatic plant species as *Hyrocotyleranoncloides*, *Ceratophyllumdemersum* have been investigated in this project. Experimental factors were heavy metals (Pb, Cd) and organs of plants were (leave, stem and root). Plant tissues were carefully separated, the samples was kept in freezer (<-18°c) until experiment, Plant samples were dried at 105°C for 24 h, then1g of powdered samples were taken and weighted. Then, samples were digested by a10ml HNO^3digestion method based on ASTAM standards. Then, the mixture was swirled and allowed to stand for 30 min. Then the beaker was covered with acid, placed on Heater and piecemeal the temperature increased until the mixture was boiling. The boiling was continued until evaporation occurred and acid fumes were emitted. Heating was terminated when a clear liquid was obtained. Thereafter, deionized water was added to bring the digest to 50 ml. the digested solution was analyzed for Cd and Pb, content by flame atomic absorption using AAS spectrometer (Varian Spectra AA200). (A.S.T.M., 2000).

Concentration of Cd and Pb in plant samples, were expressed in terms of mg kg^{-1} on a dry weight basis. Data analysis was done by SPSS program. Analysis of variance (ANOVA) was employed to detect significant difference between or among samples.

RESULTS AND DISCUSSION

The Results of data variance analysis were demonstrated that the kind of organ of two aquatic plants and the kind of heavy metal with 95% had significant effect on pollutant accumulation. Between the organs of aquatic plant lowest heavy metal accumulation were observed in leave and root tissues and highest heavy metals accumulation were observed in stem (with only exception in *Ceratophyllum* concentration of cd in the leave were found to be higher than stem) (Table 2). Among of heavy metals of two aquatic plants, concentration of Pb was found to be higher than Cd (Table 2).

The mean compare, show that, the highest pollutant accumulation was observed in *Hydrocotyleranoncloides*. In the field pollutant, aquatic plants have been used for the elimination of heavy metals from aquatic environments.

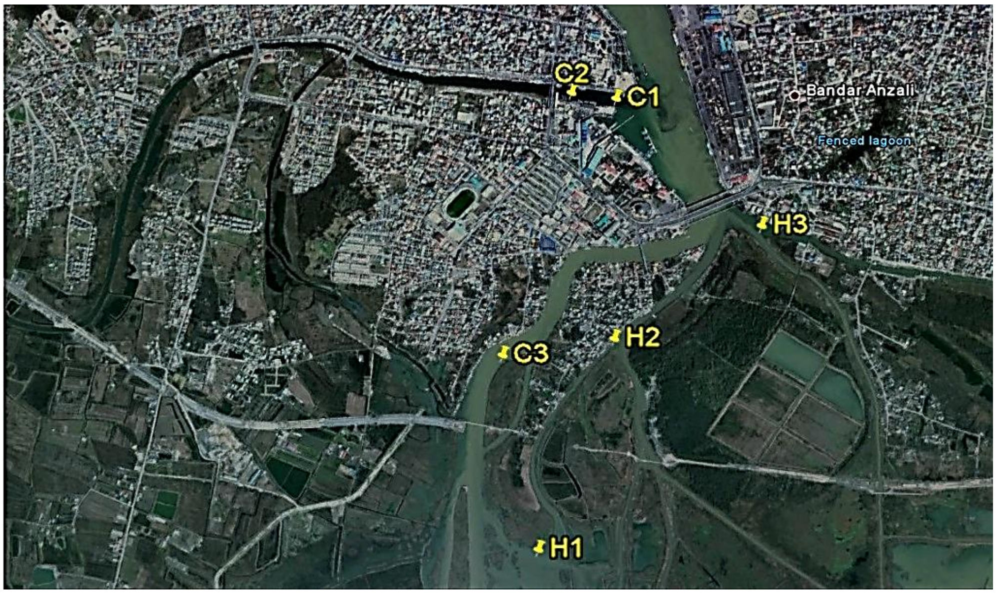

Fig. 2: Location of sampling stations in Anzali lagoon (2012 Google earth)

Table1: sampling stations and their geographical location

Station	plant	Longitude and Latitud
1	*Ceratophyllumdemersum*	37°28′ 11.24" N–49 ° 27′ 47.36"E
2	*Ceratophyllumdemersum*	37°28′ 12.12" N–49 ° 27′ 39.29"E
3	*Ceratophyllumdemersum*	37° 27′ 32.00" N– 49 ° 27′ 26.85"E
1	*Hydrocotyleranoncloides*	37° 27′ 51.72" N– 49 ° 28′ × 13.56"E
2	*Hydrocotyleranoncloides*	37° 27′ 34.51" N– 49 ° 27′ 46.93"E
3	*Hydrocotyleranoncloides*	37° 27′ 2.58" N– 49 ° 27′ 33.29"E

Table 2 :Comparison of Mean on Accumulation Heavy Metals by organs of aquatic plants(mg/kg)

Treatment	*Hydrocotyle ranoncloides*	*Ceratophyllum demersum*
Heavy Metal		
Pb	77.80	53.11
Cd	6.28	4.46
Organs of plant		
Leaf (pb)	71.05	50.64
Stem (pb)	85.89	55.58
Root (pb)	76.48	-
Leaf (cd)	6.27	5.28
Stem (cd)	6.62	3.65
Root (cd)	5.97	-

- *Ceratophyllumdemersum* isa rootless plant

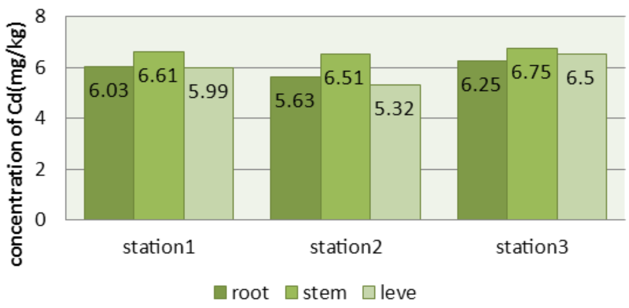

Fig. 3: Mean concentrations of Cd in *Hyrocotyleranoncloides*

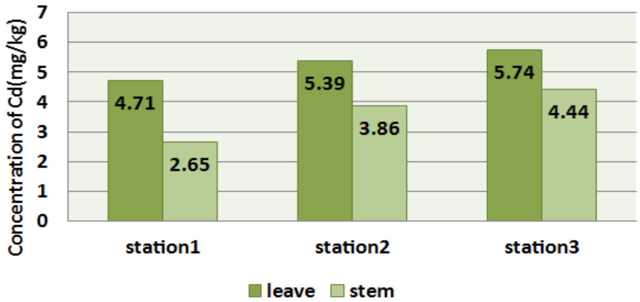

Fig. 4: Mean concentrations of Cd in *Ceratophyllumdemersum*

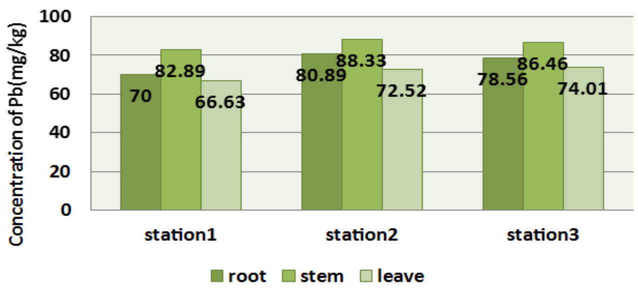

Fig. 5: Mean concentrations of Pb in *Hyrocotyleranoncloides*

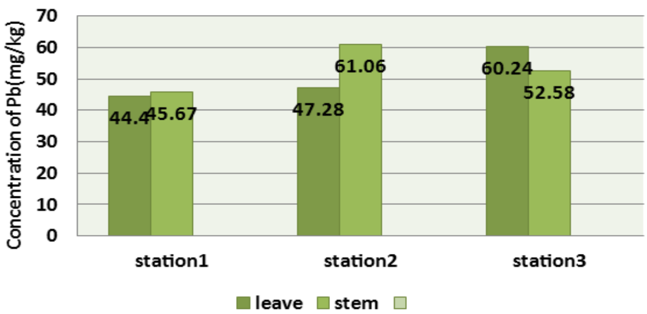

Fig. 6: Mean concentrations of Pb in *Ceratophyllumdemersum*

The mean compare of heavy metals content between two plants show that, the highest pollutant accumulation was observed in *Hydrocotyleranoncloides* (Table 2). Different plant species have different uptake patterns of metals and can have different impacts on wetland ecosystems. Since *Hyrocotyleranoncloides* than *Ceratophyllumdemersum* more amounts heavy metals, Seems that Potential of different macrophytes in amending water quality. Denny (1980, 1987) recorded that in aquatic plants main route of heavy metal uptake was through the roots. In the emergent macrophyte like *Hydrocotyleranoncloides,* roots are attached in the soil and play important role in removal heavy metals and nutrient from the environment. A supportive tissue in the emergent macrophyte is more than floating and submerges macrophytes and they could have more potential for storing over a longer period. Cowgill (1974) noted that submerged rooted plants have potential from water as well as sediments, but in the rootless plants extracted metals is only from water. Guimaraes *et al.* (2000) suggested that roots of the freshwater macrophytes *Eichhorniaazurea, E. crassipes, Paspalum sp., Eleocharissellowiana, Salvinia sp., S. rotundifolia and Scirpuscubensis* had order of magnitude higher level metals in their roots than the underlying lake sediments.

Among the organs of aquatic plant lowest heavy metal pollutant accumulation were observed in leave and root tissues and highest heavy metals pollutant accumulation were observed in stem (with only exception in *Ceratophyllum* concentration of cd in the leave were found to be higher than stem) (Table 2). These data, similarly as the results of the study was done by the hoseinizade (2007) at the Anzali lagoon.

But in a research study carried out by Ebadati (2005) at the Miankaleh lagoon, in *phragmites australis*, *Typha latifolia* highest concentration was observed in roots and in *Potamogaton crispus* there was identical concentration in various organs. When wetland plants translocate metals from root tissue to aerial tissue, they are accumulated in leaves and stems. The degree of upward translocation is dependent on the species of plant, the characteristic metal and a number of environmental conditions (Suñe *et al.,* 2007). Fitzgerald *et al.* (2003) noted that copper accumulated mainly in the roots of monocots and dicots, while lead accumulated in the roots of monocots but in the shoots of dicots, For *A. tripolium*, lead accumulation was observed in the roots at low salinity, while at higher salinity a greater ratio of lead was translocated to the shoots.

The grey mangrove, *A. marina*, accumulated metals in roots compared to the concentrations in sediments. The degree of accumulation in tissues varied according to the metal. In studies comparing the two species, *P. australis* and *S. alterniflora*, *P. australis* is known to take up metals from sediments, but *S. alterniflora* concentrates a greater parts of its body-burden of metals in root and rhizome tissue, translocating smaller amounts to aerial tissues. Kraus (1987) noted that, in plants collected from the same contaminated site in the Hackensack Meadowlands, NJ, the concentrations of Ni, Cd and Pb in the roots of *P. australis* is higher than *S. alterniflora*. The levels of metals in stem were higher in *S. alterniflora*, and leaf levels were comparable in the two plants. In *Ph. Australis* the cadmium accumulation in roots was more than shoots, which would suggest limited mobility of Cd in this species (Kozlowska *et al.,* 2009). Various environmental factors, such as temperature or solar exposure, etc., had a considerable effect on the activity of heavy metals. Thus, in the compared study between two species, *P. australis* have a tighter limitation on upward movement of metals, while *S. alterniflora* transports greater amounts to aerial tissues, especially leaves (Kozlowska *et al.*, 2009). In the other species, under identical experimental conditions *Typhalatifolia,* accumulated three times more cadmium than *Ph. australis* and higher concentrations were observed in shoots. But in submerge macrophyte, the whole plant plays important role for removal of contaminant. Denny recorded that the dependence upon roots for heavy metal uptake was in rooted floating leaved species with lesser dependence in submerged species. He also observed that tendency to use shoots as sites of heavy metal uptake instead of roots enhances with progression towards submergence and simplicity of shoots structure.

The translocation of metals from belowground to aerial tissues and their release from leaf tissue may be important steps in metal flux in ecosystems. Although the metals that remain in the roots are generally considered ‘‘out of trouble’’ as far as release to the environment is concerned, studies are needed regarding the turnover of nutritive roots and the potential release of metals from decomposing roots.

The results obtained in this study indicated that, the highest water pollution in *Hydrocotyleranoncloides* was observed in station3 and a lower pollution was observed in station1and 2, it could be because of the station 3 is situated near by the protected area. And for *Ceratophyllumdemersum* the lowest concentration was observed in station1 and station 2, and the highest in station 3 because it’s located on the pathway of boats circulation at the big river (Nahang rouga).

In the analyzed, Pb content was at least several-fold higher than of Cd content. Cadmium is an element of a much higher mobility than lead, but its content in the environment is much lower, which was also certified in our study. It’s probably because of the widespread use of fossil fuels; lead is the most common contaminant of environment. There are various industries in the region; most common source of lead is dumping of industrial effluent into the river, that containing various heavy metals. Based on the heavy metals concentrations in plants, the chemical composition of macrophytes is an evidence of the heavy metal pollution of the aquatic ecosystems. Also, these macrophytes may be useful in purifying natural aquatic environment as part of program for supportable development of aquatic resources.

CONCLUSION

This study shows that Macrophytes are biological filters that refine water bodies by accumulating dissolved metals. These aquatic plants are useful indicators for monitoring of ecological condition of ecosystems. So the purpose of this study is to realize the importance of macrophytes in bioremediation and controlling the heavy metal pollution, therewith suggesting the remedial measures for the protection and refund of anzali lagoon ecosystem. Our results show that purification efficacy, uptake and accumulation rates of studied heavy metals depended both on plant species and kind of heavy metals.

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