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Study on bioaccumulation of heavy metals (cadmium, nickel, zinc and lead) in the muscle of wels catfish (*Silurus glanis)* in the Anzali Wetland

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Introduction

After entering aquatic ecosystems, heavy metals accumulate in the organs and tissues of aquatics including fish and finally enter the food chain. These heavy metals can enter the human body through eating contaminated fish. Absorbance values and accumulation of heavy metals in aquatics especially the fish depend on ecological conditions, physical, chemical and biological factors of water, the element type, and the physiological state of the aquatic (Oryan *et al.*, 2010). Among aquatic ecosystems, wetlands and rivers are considerably important from the point of ecology. Rivers act as carriers of nutrients and non–nutrients and wetlands act as sinks for these substances. Now this effect will be more apparent if the wetland is an intermediary between a river and a static ecosystem. The Anzali Wetland is the type of ecosystem that embraces water from 11 rivers from one side and passes water into the Caspian Sea through a second outlet on the other side. The rivers passing through urban and rural areas and forests carry different types of organic matter, minerals, sediment, and industrial sewage (Taheri, 2013). The Anzali Wetland is one of the valuable wetlands registered in the Ramsar International Convention the contamination of which has become the main challenge for the people and officials during the past two decades (Khosravi, 2011). Wels catfish with the scientific name *Silurus glanis* belongs to the family wels catfish (*Siluridae*) and is distributed in Eastern Europe, Asia Minor and Central Asia. Wels catfish is found in the Anzali Wetland, but in spite of the high food variety it mostly feeds on low-value creatures such as insects, crustacean

(*Macrobrachium rosenbergii*), amphibian (frog) and low-value fish (*Carassius carassius*, *Rhinogobius similes*, Gambusia). Wels catfish is one of the fishes of economic importance of the Anzali Wetland, and can be used for meat production in terms of its commercial fishing and satisfactory growth (Abbasi, 2005). Many studies have been done to measure heavy metal concentrations in wels catfish in various environments in the world and Iran. Some of these studies include the accumulation of copper, zinc, chromium, zinc and lead was studied in the muscle tissue of the species *Pseudoplatystoma corruscans* caught from two Paraopeba River stations in Brazil (Fábio *et al.,* 2015), Determination of heavy metal concentrations in muscle, liver, intestine and gills of wels catfish (*Clarias gariepinus*) sampled from Nigerian waters (Adegunloye *et al.,* 2015), Investigation of the amount of heavy metal contaminants in edible tissues of *(S. glanis)* collected from the Danube river of Europe (Peycheva *et al*., 2014).

Materials and methods

The Anzali International Wetland is located on the southern coast of the Caspian Sea at 37° 28′ northern latitude and 49° 25′ eastern longitude in the Guilan Province (Sadeghi Rad, 1997). In this research 30 wels catfish were caught using boats and gillnets from 3 stations (Abkenar, Hendekhaleh and Shaykhan) of the Anzali Wetland in 2012 (Fig. 1). The samples were gutted, peeled and filleted. Then the fillets of muscle tissue from each station were homogenized by a bone-consuming device. For the assessment of cadmium, nickel, lead and zinc approximately 20-30 g of homogenized samples of fish were freeze dried (CHRIST-LCG Model, Germany) at -55°C for 10 hours. The samples were made soft and smooth using an electric mill.

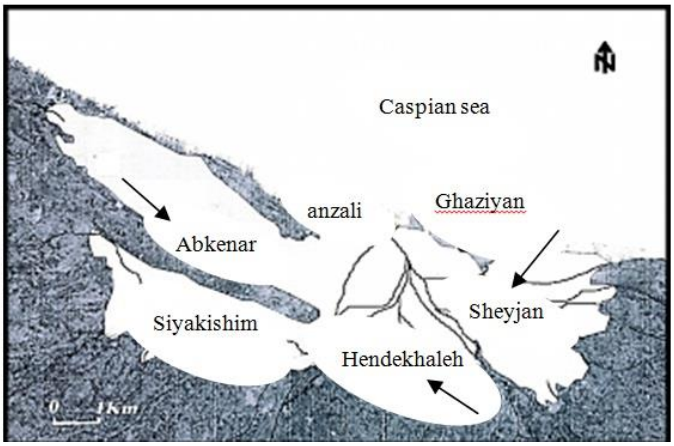

Figure 1: Location of research stations in the Anzali Wetland.

Chemical digestion of samples was done by wet digestion method using acid mixture (HNO_3/ $HClO_4$). After cooling the digested samples were passed through Whatman filter paper (number 42) and made up to a volume of 25 mL in a volumetric flask using double-distilled water. The samples were poured into polyethylene bottles with labels and sample codes. The obtained sample solution was injected into the device and concentration of heavy metals including cadmium, nickel, lead and zinc was determined by flame atomic absorption spectrometry (AA/680, Model Shimadzu Company, Japan) (Roger, 1994, MOOPAM, 1999). To draw a calibration line, standard solutions with different concentrations that was prepared from a stock standard solution of 1000 ppm concentration. All laboratory reagents used were of analytical grade and were obtained from the Merck Company (Germany). The intensity of the signal of the analyte was confirmed using the Standard Addition method and the method of sample preparation was ensured by calculating percent recovery. In this study, 10 mL of standard solution of the metals to be determined were prepared in two different concentrations (in ppm) and added to the test sample. It is worth noting that two samples were prepared in the same condition and the standard solution was added to just one of them. Then the concentration of the metal was determined separately and the percent recovery percentage of each metal was calculated. The results of standard addition to fish samples and percent recovery of heavy metals (98.3-92.8) showed an appropriate confidence level of the method used for the determination of heavy metals. The standard used in this method for calculating the percent recovery and calibration curve was "Standard Reference Materials" (SRM). Normal distribution of data was shown using Kolmogorov - Smirnov, and the statistical analyses of the obtained data were computed using SPPS-17 software. One way analysis of variance (ANOVA) was used to determine whether there were any statistically significant differences between the means of two groups and Tukey's test was used to determine differences between means at significance level ($p<0.05$). Excel software was used to plot the graphs.

Results and discussion

In this study, levels of accumulating heavy metals in edible muscle tissue of wels catfish in three stations (west, east and center) were Zn>Pb>Ni. This increase was significant at a statistical confidence level of 95% ($p<0.05$). The concentrations of heavy metals cadmium, nickel, zinc and lead in muscle tissue of wels catfish showed no significant differences between the studied stations ($p>0.05$). Also average concentrations of heavy metals were compared to standard guidelines on the World Health Organization (WHO). The mean cadmium concentration in

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wels catfish in the west and central wetland were 0.05 and 0.02 µg g^{-1} dry weight, respectively and its accumulation in east wetland wels catfish was below the detection limit set. The mean nickel concentrations in wels catfish samples from the west, central and east wetland were equivalent to 0.44, 0.37 and 0.22 µg g^{-1} d.w, respectively. Also mean zinc concentrations in wels catfish collected from west, central and east wetland were 22.35, 20.25, 22.35 µg g^{-1} d.w, respectively. Toxicity levels of cadmium, nickel, and zinc in all three regions were well below the permissible levels set by WHO. Mean concentrations of lead wels catfish samples from the west, central and east wetland were 0.49, 0.5, 1.11 µg g^{-1} d.w, respectively. Compared to WHO guidelines (0.5), toxicity level of Pb observed in the west and central wetland samples were close to allowable levels for this metal, while Pb levels in the East wetland samples exceeded the recommended limit (Table 1).

Table 1: Accumulation (Mean±standard error) of heavy metal (mg g^{-1} dry weight) in edible muscle of wels catfish caught from three stations in the Anzali Wetland (*p*>0.05).

Heavy metal	West wetland	Central wetland	East wetland	WHO
Cadmium	0.05±0.07	0.02 ±0.07	ND	0.2
Nickel	0.44±0.38	0.37± 0.07	0.22±0.03	0.38
Zinc	22.35±1.90	20.25±1.23	22.35±1.27	100
Lead	0.49±0.47	0.5± 0.47	1.11±1.11	0.5

The results this study showed that the accumulation pattern of Cd, Ni, Zn and Pb in edible muscle tissue of wels catfish in the three stations (west, central and east) of the Anzali Wetland was: Zn>Pb>Ni>Cd. These results are similar to the study conducted by Peycheva *et al*. (2014) on the accumulation pattern (Pb<Ni<Cd) of metals (lead, nickel, cadmium) in the muscle tissue of the wels catfish (*S. glanis*) collected from the Danube River in Europe. Also, similar results were reported in studies by Olatunji *et al*. (2011) who reviewed accumulation of heavy metals (Cd, Zn, Pb) in the edible muscle tissue of wels catfish Silver (*Chryshchythys nigrogitatus*) caught from the River Cross Nigeria. The accumulation of metals based on their concentrations was reported as Zn<Cd<Pb. In another study by Indrajit *et al.* (2011) on the heavy metals level of *C. nigrogitatus*, sampled from the Yamuna river of Delhi metal accumulation was reported as Cd<Ni<Pb. Among heavy metals, metals such as nickel are non-essential metals. This means that they are not required by the body for metabolism and even small amounts of this metal are harmful. Considering oil extraction from the Caspian Sea and transport of ships carrying oil from sea, sewage to sea, the subsequent mixing of sea water and wetland and the presence of heavy

metals such as nickel in oil, aquatic animals are always exposed to these heavy metals. In addition, nickel has been found in car smoke, industrial wastes, burning wastes, crust and fertilizers. Due to the proximity of the wetland to gas stations, and agricultural fields, untreated industrial and municipal wastewater enters aquatic environments and the wetland (Khanipour and Seifzadeh, 2016). In this study, nickel toxicity level was below permissible limits in all three regions. Values higher than standard levels of the other heavy metals that are required in small amounts can lead to bad effects. Zinc belongs to this category (Ebrahimi *et al.*, 2012). High accumulation of zinc can be connected to its critical role in enzymatic processes, and metabolic processes of aquatic animals, as well as to its geological origin and natural presence. In addition, Zn excretion rate is slower compared to its bioaccumulation (Selda Tekin *et al*., 2005). Accumulation rates of lead in the west and central wetland and near the eastern wetland were higher than the standard levels set by WHO. The most polluted river entrance to the East wetland is Pir Bazar River that receives municipal and industrial wastewater. Different industries, interface trunks, steel making, ceramics, plastics, and milk industries in the cities of Rasht, Bandare Anzali, Foman and Somesara are the main sources of Pb. The waste from all these industries is emptied in this river which eventually enters the (east) wetland. Therefore further research should be done to determine the sources of this metal in the east basin of the wetland. In this study, no significant differences were reported in metal accumulation levels in wels catfish in the three stations in the Anzali Wetland. Ashja Ardalan and Sohrabi (2009) measured Zn and Pb levels in muscle tissue of *Perca fluviatilis* in two regions Abkenar and Sheyjan of the Anzali Wetland. The results showed no significant differences (p>0.05) in lead amounts between samples of Abkenar area and Sheyjan. In another study (Askari Sari *et al*, 2010) the accumulation levels of heavy metals was investigated in tissues of *Liza abu* of Karoon and Bahmanshir Rivers. No differences were found in metal concentrations in the studied fish tissues between the two rivers. Also Khalifi (2013) studied contamination levels in liver tissue in silver carp (*Hypophthalmichtys moltrix*) to Pb in 6 stations in the Anzali Wetland. The Pb levels did not show significant differences between all stations. These findings agree with the results of the present research. It is suggested that a comprehensive research be carried out on rivers in the wetland catchment area, and human activities on the margin of the wetland. We also need to find ways to remove non-biological resources in the wetland, and continuously monitor industrial pollutants and wastewater.

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