Journal of Novel Applied Sciences
Available online at www.jnasci.org
©2013 JNAS Journal-2013-2-11/644-649
ISSN 2322-5149 ©2013 JNAS

A Study On Concentration Of Heavy Metals (Pb, Ni, Cu, Fe, and Zn) In Liver And Muscle Tissues Of Loach Fish (Paracobitis Rhadinaea) In Sistan's Chahnimeh reservoirs,Iran

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ABSTRACT: In this research, in order to study the concentrations of heavy metals Cu, Zn, Pb, Ni, and Fe in Loach fish (*Paracobitis rhadinaea*), sampling was done in Chahnime water reservoirs. Afterwards, the fish were transported to the laboratories at Hamun International Wetland Research institute for preparation and digestion in order to measure the concentration of heavy metals. The concentration of heavy metals was measured using the Konic NOVAA 300 atomic absorption spectrophotometer on the basis of micrograms per gram dry weight. In the muscles, the concentrations of Cu, Fe, Ni, Pb, and Zn were measured to be 10.40±075, 196.04±10.88, 3.16±0.10, 4.35±0.68, and 118.04±50.08 µg per gram dry weight respectively. In the liver, the concentrations of Cu, Fe, Ni, Pb, and Zn were measured to be 35.27±16.13, 467.11±117.47, 15.15±3.69, 13.15±6.93, and 198.20±50.08 µg per gram dry weight respectively. Analysis of the acquired data showed that metal concentration pattern for liver and muscle tissues is similar and the highest concentrations were those of Fe and Zn. Concentrations of Cu, Pb, and Ni too did not show any significant difference between the two tissues. Pb, Fe, and Ni showed higher concentrations than the critical limits set by some standards (WHO and FDA) which could be stimulated by the environmental factors e.g. food, water and surrounding environment. The significant relationship between Zn concentration in liver and muscles shows its similar concentration pattern between both tissues and that changes which affect liver concentration would stimulate concentration changes in muscles too.

***Keywords*:** Heavy metals, Loach fish (*Paracobitis rhadinaea*), and Sistan's Chahnimeh reservoirs.

INTRODUCTION

In recent years, an increasing different types of pollutants have contaminated the waters and are thought of as being a serious threat. Because of the adjacency of human activity to these ecosystems, they are more vulnerable than other natural ecosystems (Botte et al., 2007). Heavy metals are of especial importance because of, toxic effects, bioaccumulation in different aquatics, and that they can enter the food chain (Demirak et al., 2006). Biological processes are unable to decompose the heavy metals, thus, if they find a way to enter the natural environments, they could remain in living creatures' tissues (UNEP, 2001; Gbaruko and Friday, 2007). The fish are studied as pollution indicators of the waters and concentration of the heavy metals in their tissues is thought to be a result of being exposed to pollutants (Evans et al., 1993). Concentration of heavy metals in sedentary fish (nonimmigrant) is a suitable indicator of studying water ecosystems which are affected by human activities (Jorgensen and Pedersen, 1994). Muscle tissues are one of the most important body parts of the fish that is used for human consumption as food (Evans et al., 1993). The absorption rate is different for different body parts of the fish (muscles, liver, ovary, and gills) and is dependent on bioaccumulation while also being relevant to physiology and food habits of the fish (Canli and Atli, 2003).

For the purposes of assessing the health and quality of water ecosystems, it is a suitable model and the physiological changes of fish's body are considered as biomarkers of environmental pollutants (Kock et al, 1996). Chahnimeh water reservoirs are Sistan's only water resources at present. On the other hand, these resources, because of drought and also Hamun international wetland going dry, are the last biological resort in the region. Also, the main water supply for Chahnimeh reservoirs is Helmand River which flows in Afghanistan and there is no reliable information on its health and the route in which it flows regarding the issue of contamination with heavy metals. Thus, studying the concentration of heavy metals in these water resources is important. Therefore, since the Sistan's Sweeper Fish lives in Chahnimeh water reservoirs and is a benthic animal (Annandale and Hora, 1920), the research studied this fish to monitor Chahnimeh water reservoirs for contamination. This research aims to measure the concentration of heavy metals (Cu, Zn, Pb, Ni, and Fe) in liver and muscle tissues of the Sweeper fish in Chahnimeh water reservoirs and study the significant relationship, if any, between the concentrations of heavy metals in each of the tissues and also to compare the concentrations with the standards.

MATERIALS AND METHODS

Sampling

In order to measure the concentration of heavy metals in the fishes, a number of 20 fishes were captured from the Chahnimeh water reservoirs (figure 1) by the help of local fishers using gill and fin nets and were taken to the laboratory of Hamun Wetland Research Center in containers full of ice.

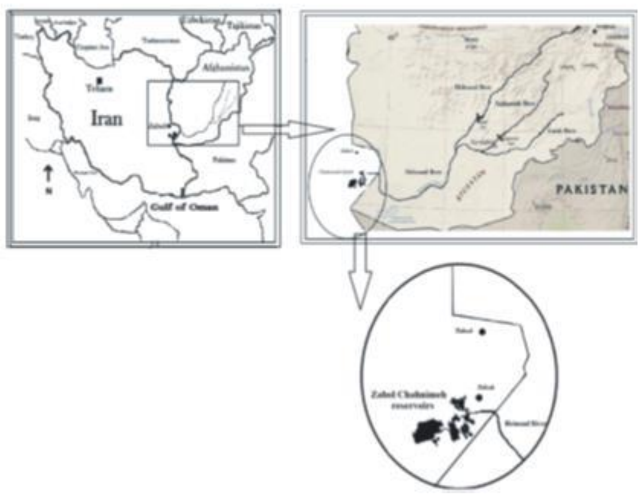

Figure 1. The arrows indicate the location of the study area (Dahmardeh Behrooz et al., 2012)

Preparation

To prepare the fish, biometry was done first and then samples were taken from the liver and muscles. The samples, then, were placed in the oven in 80˚c until there was no more weight loss and the samples were totally dry. The samples were again weighed to measure the dry weight. Next, the dried tissues were crushed in porcelain mortar and kept in polyethylene containers the next step, digestion (Yap et al., 2004).

Sample Digestion

To digest the fish tissues, 1 gram of the dried samples, using nitric acid 65%, were kept in the hot plate digester for 1 hour at 40˚c and then at 140˚c for 3 hours to complete the digestion. Afterwards, the solutions were kept in the room temperature to get cooled down. Next, each of the solutions were diluted to the volume of 40 ml using double distilled water. These solutions, then, were filtered using the whatman 42 microns filter paper (Kavun and Podyuiskay, 2009; Yap et al., 2004) and were kept in refrigerator until the time of measurement. The concentration of heavy metals were measured using the atomic absorption device Konic NOVAA300 and the final concentration was calculated using the following equation (in micrograms per gram dry weight).

Statistical Analysis of the Data

To determine the type of the statistical test, first the data were checked to be normal and after that, the t-test was used to compare the heavy metal concentration between tissues. ANOVA test was used for compare the pattern of heavy metals accumulation in tissues. Pearson correlation test was used to study the relationship between the heavy metal concentration in liver and muscle tissues. For statistical analysis, SPSS v13 was used and charts were drawn using Microsoft Excel.

RESULTS AND DISCUSSION

Results

Results of the statistical analysis are shown in figures 1 to 3. For the muscles, concentrations of the metals Cu, Fe, Ni, Pb, and Zn were found to be 10.40±0.75, 196.04±10.88, 3.16±0.10, 4.35±0.68, and 118.04±50.08 µg/g dry weight respectively. For the liver, concentrations of the metals Cu, Fe, Ni, Pb, and Zn were found to be 35.27±16.13, 467.11±117.47, 15.15±3.69, 13.15±6.93, and 198.20±50.08 µg/g dry weight respectively. Statistical analysis showed that the metal concentration pattern in both tissues is similar. The highest concentrations in liver and muscle tissues was that of the Fe and then Zn. Other metals did not show any significant concentration differences (ANOVA, $P<0.05$) (figures 2 and 3). T-test was used to compare the concentrations of every metal between liver and muscle tissues which resulted in significant differences between the two for every one of the metals. The highest concentrations were those of the liver (t-test, $P<0.05$) (fig 4).

Studying the relationship between metal concentrations in liver and muscle tissues, only Zn showed a positive significant relationship ($P<0.05$) (fig 5).

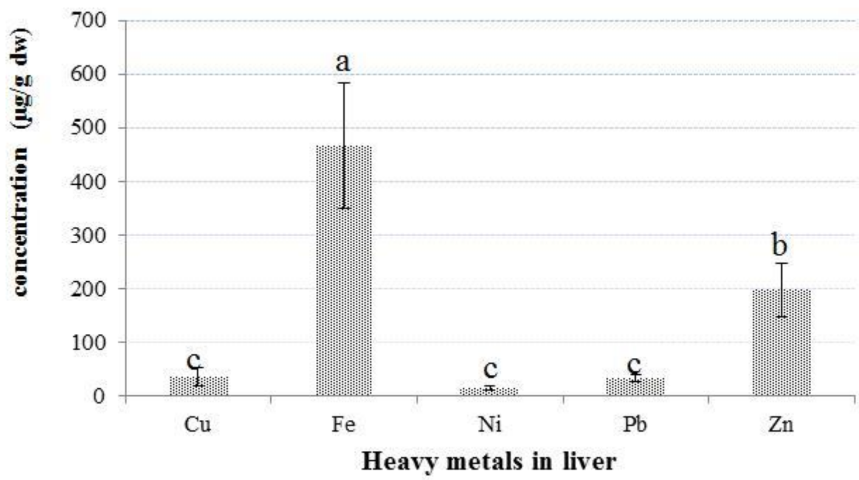

Figure 2. heavy metals in tissue of liver

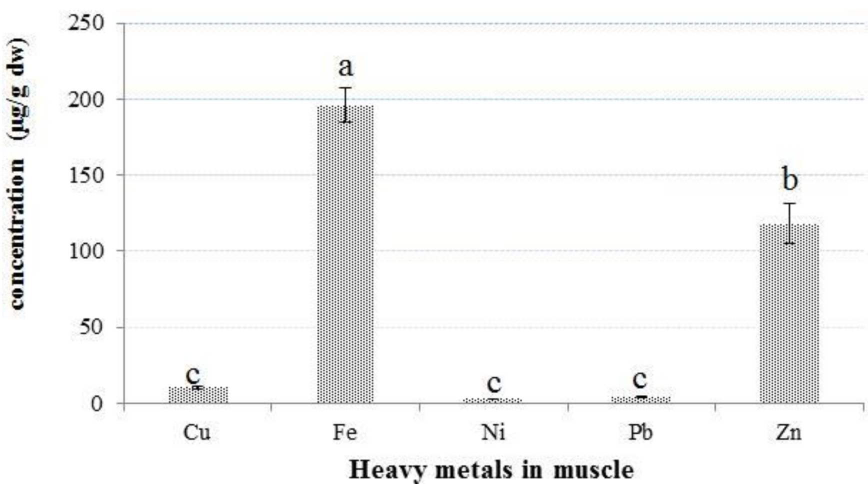

Figure 3. heavy metals in tissue of muscle

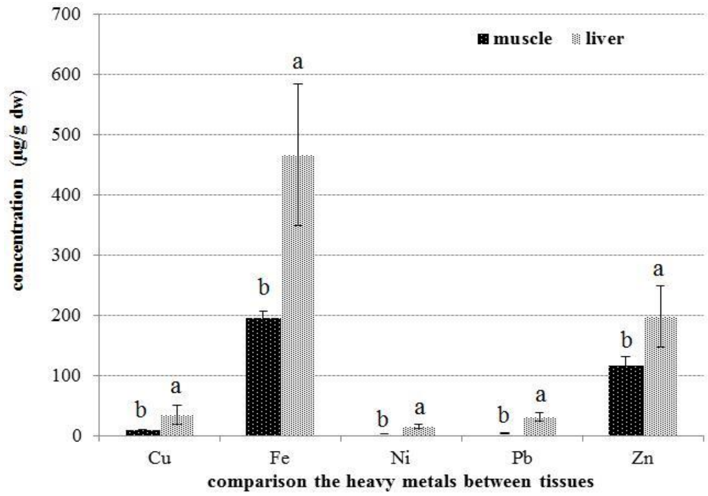

Figure 4. heavy metals between tissues tissues of liver and muscle

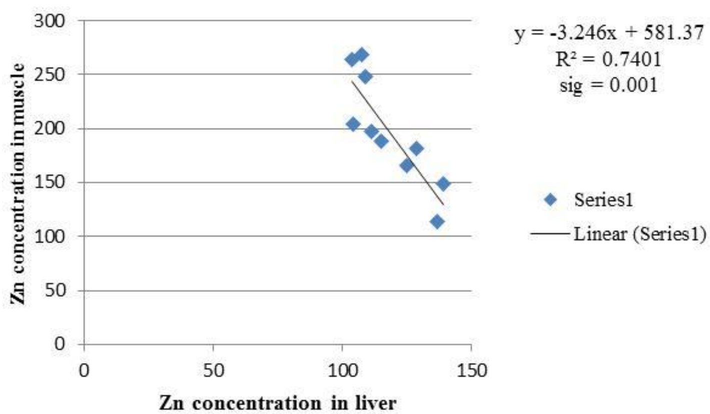

Figure 5. significant correlation of Zn concentration between liver and muscle

Discussion

Heavy metals pick their target body part based on the amount of its metabolic activity. This point explains why heavy metals concentrate more in tissues like liver and muscles (tissues that have low metabolic activity) (Filazi et al., 2003).

There has been many studies on concentration of heavy metals in fish tissues which show that metals may concentrate in different fish body parts with different concentrations. The results of this study indicate that Cu, Pb, Fe, Zn, and Ni concentrate more in liver than muscles. The followings may be the reasons that help explain this: liver is the tissue in charge of metal compounds, it has a higher potential for bioaccumulation of heavy metals, and it is also a target tissue for detoxification (Staniskiene et al., 2006).

Liver is considered to be a suitable indicator for studying long term exposure to heavy metals. And it is because this tissue is the metabolism place of metals which makes it a good indicator of being contaminated with heavy metals (Filazi et al., 2003). So, the higher concentrations of metals in liver, in comparison to the muscles, may be a result of what roles that liver plays. Bandani et al, (2010) have found similar results studying different Carp fish tissues in Golestan Province. Jent et al, (1998) found the highest concentrations of Cu and Zn to be in liver. Rashed et al, (2001) found similar results studying Cu concentrations in different tissues of Tilapia captured from Nasser Lake. They concluded that the high Cu concentrations in liver is a result of its need for Cu. Jalali, Ja'fari and Aqazade, (2007) stated that red blood cells would disperse the Pb in all of the tissues and this metals tends to concentrate more in liver and kidney. Fazeli et al, (2005) in their study on Mullet fish, Askari Saari et al (2009) studying two types of Barbus grypus and Liza subviridis from Bahmanshir River, and Rezaee et al, (2005) in their study on Mullet (Liza dussumieri) fish contended that the metal concentration in liver was higher than muscles. In this study, Fe and Zn showed the highest concentrations in liver and muscle tissues of Loach fish from Sistan's Chahnime water resources. These elements have a role in cellular metabolisms and their needed low concentrations is controlled by homeostasis mechanisms (Ashraf and Jaffar, 1988). Zn and Fe are required in liver tissues for bile secretion and making hemoglobin (Chen et al., 2012). Thus, it may explain the higher concentrations of these two in this tissue for it may need these metals more (Foruqi, 2006; Sankar et al., 2006, Bols et al., 2001). Beheshti et al, (2010) found similar results in their study on different tissues of Liza subviridis captured from Dez River in Khuzestan Province. They explained the high concentrations of Zn and Fe in liver as a result of their role in this tissue.

Table 1 demonstrates heavy metal concentration pattern of Zn, Cu, Pb, Ni, and Zn in Loach fish compared to several other fishes which shows that Fe and Zn have higher concentrations in every tissue compared to other metals. Results of the present study regarding concentration patterns of heavy metals are in agreement with several other such studies.

Table 1. comparison the heavy metals (µg/g dry weight) in tissues of fish with other studies

Species	tissue	Cu	Fe	Ni	Pb	Zn	Reference
Mugil cephalus	Liver	4.41	----	---	7.60	---	Canli and Atli, 2003
	muscle	---	----	4.41	5.32	37.39	
Clarias Gariepinus	muscle	1.65	8.01	---	1.18	5.24	Eneji et al., 2011
Chrysichthys nigrodigitatus	muscle	0.59	197.60	---	0.50	7.75	Nwani et al., 2010
Barbus capitu	Liver	4.72	---	3.26	4.28	---	Canli and Kalay, 1998
	muscle	1.23	---	0.52	1.32	---	
Cyprinus carpio	Liver	2.63	---	1.10	1.26	---	Vindohini and Narayanan, 2008
	muscle	0.85	---	0.67	1.38	---	
Paracobitis rhadinaea	Liver	35.27	467.11	15.15	32.15	198.19	Present study
	muscle	10.40	196.04	3.16	4.35	118.04	

To compare the concentration of heavy metals in Chahnimeh Loach fish muscle tissues, the concentrations were calculated on the basis of micrograms per gram wet weight. The tissues were weighed before drying them and they were also weighed after it too. Then, considering the difference, it was concluded that 1 gram dry weight equals 4.17 grams wet weight. And the heavy metal concentration in wet weight was calculated on this basis. Ni and Pb showed higher concentrations than that of the WHO standard. Also, Fe shoed a higher concentration than that of the FDA standard. The study showed a significant relationship between the heavy metal concentrations in both tissues and Zn showed a positive significant correlation which shows that Zn concentration pattern in muscle tissues can be affected by its concentration changes in liver (table 2).

Table 2. comparisons the heavy metals (µg/g wet weight) in muscle of fish with available standards

standards	Cu	Fe	Ni	Pb	Zn	Reference
WHO[1]	10	-	0.38	0.40	30	European Commission, 2000; Pourang et al., 2004
FDA[2]	-	0.5	1	5	35	Pourang et al., 2004; Amini Ranjbar and Sotoudeh, 2005
NHMRC[3]	10	-	-	1.5	150	Pourang et al., 2004; Amini Ranjbar and Sotoudeh, 2005
UK MAFF[4]	20	-	-	2	50	Pourang et al., 2004; Amini Ranjbar and Sotoudeh, 2005
Muscle of *Paracobitis rhadinaea*	2.49	47.01	0.75	1.04	28.30	Present study

CONCULSION

According to the results of this research, highest heavy metal concentrations are in liver which can be a result of its role and function in processes like detoxification. Pb, Ni, and Fe showed higher concentrations than some of the standards which may be caused by getting more of them from the habitat, water, and food. Thus, the environment should be monitored constantly in order to determine its health condition.

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