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EXAMINATION OF THE ACCUMULATION OF THE HEAVY METALS IN SOME ORGANS OF *FULICA ATRA* IN GOMISHAN INTERNATIONAL WETLAND

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ABSTRACT

This research is done to measure the concentration of heavy metal Pb and Cd in muscle, liver, bone and feather of *Fulica atra* in international gomishan wetland. The purposes of this research is to compare the rate of concentration of those metals in the tissues and compare them with rate concentration of metals in standard metods and other researches. Samples of coots have been taken from five stations of international gomishan wetland on February 2014 and after that put samples in icebox below 4˚C and send them to laboratory and put all samples in refrigerator with -18˚C. MOOPAM method use to extract heavy metals from these tissues and measure the concentration with atomic absorption machine. Totally the average of concentration result for lead was 1.81 µg/g in muscle, 6.47 µg/g in liver, 19.93 µg/g in bone and 4.15 µg/g in feather and for Cadmium was 0.35 µg/g in muscle, 1.63 µg/g in liver, 2.64 µg/g in bone and 2.07 µg/g in feather (dry weight). The results indicated that the average Lead and cadmium levels were highest in bone and lowest in muscle.

One-way analysis of variance (ANOVA) showed no significant difference in the average in lead and cadium concentration in terms of the type of stations ($P>0.05$) while this difference was significant for lead and cadmium between tissues of the coot($P<0.05$). The results do not show the severe exposure of the coots to the metals lead and cadium, but due to the high concentration of lead and cadium in the bone, it may be exposed to the pollutions.

Keywords: Lead, Cadmium, *Fulica atra*, gomishan, atomic absorption

INTRODUCTION

Today, population growth, development of various industries, and the expansion of agricultural areas have caused to enter the high volume of the pollution to the aquatic environment (Lamanso et al., 1991).Among pollutants of aquatic ecosystems, heavy metals due to toxic effects and the high potential of bio-accumulate in many aquatic species are remarkable. Pollution of water ecosystems by different pollutants can be confirmed by examining the water, sediment and aquatic organisms. Accumulation of heavy metals in each of these components can lead to serious ecological changes (Altindag and Yigit, 2005). Although the nature has high self-purification capacity in different pollutants, but the high pollutants in most of the modes remove the optimization property and causes the fundamental changes in ecosystem and extinction of many plant and animal species, and being unusable of water, soil and air (Mohammadi and Samaei, 2006). Wetland areas as one of the most fragile ecosystems in the dynamics of the population and changes in habitat, havethe major role in variety of the waterfowl (Holloway, 1993) and due to human and natural interference, it is considered as one of the major dangers for the ecosystems (Graveland, 1999). Pollution is one of the main factors in destroying the wetlands and reducing their ecologic functions. As the wetlands, especially the stagnant wetlands have long rotation time, adjacent industries with them cause to accumulation of the wetlands, most of the pollutants interacted chemically, and the pollutants sedimentin bed of wetlands (Behrouzi Rad, 2007). To evaluate the changes in an ecosystem, it is impossible to evaluate all components and relations, so selecting the ecological components such as birds, can be used as a biological indicator. birds may be exposed to differentchemicals such as heavy metals through direct contact with contaminated food and water (Savinov et al., 2003). The best organisms to determine the quality of the environment, ecosystems, the extent of contamination and human survival are birds. Also, in Ecotoxicology, the birds are used as the ecological indicator for the heavy metals due to wide geographical distribution, long lifespan and their proper place in the food chain (Spalding et al., 1994). birds due to exposure to high levels of the food chain and their oral administration have of particular importance for monitoring environmental pollution (Khosravi et al., 2011). birds can keep high levels of metals in their tissues, including the liver and kidneys. So many physiological and biological processes in birds may be affected by pollution from heavy metals (Scheuhamme, 1987). For many years, many of the birds have been used as the primary providers for of environmental pollutants such as DDT, pesticides and heavy metals. (Burger, 1993). Stability of heavy metals due to their distribution in the food chain of animals so that its value in the food chain can be several times higher than that in water (Khodabandeh, 2001).

Lead and cadmium are common pollutants as heavy metals that are scattered throughout ecosystems. Although these two elements naturally present in marine environments, they are non-essential elements that their appearance in the diet can be toxic for consumers such as waterfowl(Scheuhamme, 1987). Toxic metals lead and cadmium can have the harmful effects on various physiological systems along with the endocrine systems in birds in the natural and laboratory environment(Martin et al., 2003). All birds' species are sensitive to lead poisoning, but generally wild birds, especially waterfowl, mostly affected by lead poisoning. This is probably due to their eating habits and lifestyle (Rahimi, 1387).

Lead causes weight loss and reproduction loss in birds (Kim et al., 2007), while the toxic effects of cadmium include reducing egg production in birds, kidney damage, damage to the testicles and change of reaction behavior (Furness, 1993). Accumulation of heavy metals lead and cadmium in different organs of the birds can cause the exposure of metalsat early and late times for the birds. Gomishan wetland is one of the most important habitats for thewaterfowl in the Caspian Sea, and there are many birds that can bring chick in winter. This study studies the heavy metals lead and cadmium in different organs of coot in the international Gomishan wetland and analyzes the pollution conditions in the region.

Selecting appropriate species

The important point in this research, the choice of appropriate species. The points that cause the selection of *Fulica atra* as the appropriate species among the birds of region, including:

-Aborigines

-appropriate number compared to other birds

-Identify and collect the birds

-The desirability of the food in the region

-lack of restrictions for hunting

- Diet bird (Omnivore)

By selecting this species, this birds can show the maximum reflect amount of heavy metals in wetland habitats.

This bird is like the birds that depend on water, and it is in the marshlands areas with open water, marshes, and lakes and even large rivers. In winter, this bird is native and abundant, a large number of these birds can be seen in the wetlands of Iran (Mansoori, 2008). *Fulica atra* is omnivorous but prefer to eat plant material and algae, aquatic plants and plankton. Animal feedincludes mollusks, invertebrates, insects and worms. *Fulica atra* is the most waterfowl that it is hunted in Iran and its consumption is very good (Yazdandad, 2007).

Figure 1: *Fulica atra*

The studied area

Gomishan international wetland is located in north of Iran and southeastern of the Caspian Sea and its geographic position is 53 degrees 58 minutes east longitude and 37 degrees 20 minutes north latitude. This wetland is 23 meters below the surface of the open sea and it is a shallow brackish wetland that its level changes seasonally–flood. Its average depth is one meter, but in the North parts of West there are more than 2.5 meters. The area of this wetland has changed significantly in the last fifty years due to climate change and sea level rise of the Caspian Sea, as well as drying areas for wetland margins. The current area is about 20,000 hectares. Conversion of grasslands that surround the wetlands to agricultural land, grazing livestock, aquaculture activities, hunting and overfishing are the most important factors that threaten this valuable wetland (Mansoori, 2009).

MATRIALS AND METHODS

Sampling was accomplished in five stations along South to North of wetland by hunting in winter 2013. Stations were determined based on the distribution and abundance of coots. From each station, the three coots and a total of 15 coots were collected. After collecting the samples were coded, weighed and biometered. Digital scale with the accurance 0.01 g is used for weighing. Biometry includes factors like throughout the entire body, the ends of the wings, and tail and beak length in cm. (Table 1)

After removing muscle, liver, bone andfeather , tissues inside the plastic bag got pollution-free and were stored at -18°C during the analysis. The samples after removing the frozen mode, it is placed at 105˚C for 24 hours to lose its moisture and to be completely dried (Moopam, 1999). Moisture percentage was determined separately for each of the tissues to be used for calculating the concentrations of metals in wet weight. The dried samples were ground by mortar and 1 g of each tissue was placed in 100 ml Beaker, and 10 ml of nitric acid(65%) for digestion of the samples and 2 ml of hydrogen peroxide to dissolve fat in the tissues were added; after a while that the achieved blubber decreased, the beaker that contains the sample was heated on the heater at 90˚ C for 3 hours to be solved in acid completely till it becomes yellow and clear(Moopam, 1999). Since after removing the samples from the heater, by adding distilled water may form sediment, all solution samples filtered by funnel and filter paper 0.45 micron (Whatman No. 42), and transferred to volumetric flask and then the flasks dilute to 50 ml with distilled water(ASTM, 1994). Atomic absorption was used to measure the concentration of metals. First, to provide standards and limits the concentration of each of the discussed elements, preliminary analysis and standards for each element were prepared.

The results of atomic absorption was calculated by the following formula:

$$M = \frac{C \times V}{W}$$

The components of formula are:

M: The final results of the sample in µg/g(dry weight)

C: The obtained concentration from the system (µg/ml)

V: The final volume of the sample (ml)

W: Weight of the dried sample (g)

Statistical Analysis of the Samples

For data analysis software (version 19) Spss and Excel were used. In order to objective compare of the average of the obtained data from the distribution histograms were used. The normality of all data were examined by kolmogorov-smirnov normality test.

In order to compare the average of Lead and Cadmium in the tissues, One way analysis ANOVA and Tukey test in the significance a=0.05 were used to determine the significant difference between the averages of Lead and Cadmium in the tissues of muscle, bone, liver, and feather. The statistical results that P is lower than 0.05 were considered as the criteria of difference, and also these resultd were used to analyze the correlation of the Perison test.

RESULTS

The average of Lead and Cadmium among the tissues (except between feather and liver in Cadmium) was significantl different. It seems that the most important of difference in average concentration of Lead and Cadmium in different tissues is the different structure of *Fulica atra*, the special metabolismic activity and the ability to attract and repel a variety of different tissues in birds(Ferns and Anderson, 1994). There was no significant difference between the stations in comparison of the average Lead and Cadmium in different tissues of *Fulica atra* (P>0.05).

Similar types of food for coots in a certain geographic area of wetland, and they are not living in the wetlands and the continuos flight to get food are the reasons of no significant difference between their stations. The amount of lead and cadmium in muscle had a significant difference with other tissues and it was lower than the average of other tissues and international standards, which in this case did not threaten the health of consumers. One reason for the lower levels of heavy metals in muscle tissue compared with other tissues, dilution concentrations of heavy metals in the muscle is growing (Lewis and Furness, 1991). There is no defined standard for the metal in other tissues compared to muscle tissue, however, there are higher levels of heavy metals in the liver.In other word, the liver is the most important center for accumulation of heavy metals. That is why this tissue is used as a biomarker for contamination of aquatic ecosystems and the food chain (Endo et al., 2008). Average lead and cadmium in bone was very high. Increase the amount of these metals in the bones is the evidence of storage and accumulation of lead and cadmium in a long time in the body as the main fabric store of these metals. The amounts of lead and cadmium in the feather was significant and it was the evidence of the disposal and storage of these metals by the tissue in the birds, thus, the feather can have the role of storage and removal of heavy metals. The amount of heavy metal in feather indicate the amount of metal in the blood of the bird during the formation and growth(Burger, 1993). Feather is non-destructive tissue In addition, it can be obtained from the live bird that it is the special feature for the rare species (Burger, 1994).

Table 1: The results of the biometric samples

	Weight (g)	The length of two wings (cm		The length of beak(cm)	
Average±SD	**569.4±93.5**	**43.76±1.57**	**71.37±2.2**	**6.47±0.54**	**2.36±0.19**
Min-Max	**448.7-755**	**41.7-46.3**	**67.5-75.2**	**5.5-7.5**	**2.1-2.7**

Table 2: Lead and cadmium concentration (µg/g D.W) in the tissues of *Fulica atra*

Tissue	Muscle		Bone		Liver		Feather	
Metal	**Pb**	**Cd**	**Pb**	**Cd**	**Pb**	**Cd**	**Pb**	**Cd**
Average	**1.81**	**0.35**	**19.93**	**2.64**	**6.47**	**1.63**	**4.15**	**2.07**
Standard deviation	**±0.59**	**±0.12**	**±1.68**	**±0.62**	**±1.37**	**±0.5**	**±0.96**	**±0.51**
Minimum	**0.92**	**0.18**	**17.23**	**1.39**	**4.36**	**0.88**	**2.39**	**1.36**
Maximum	**2.64**	**0.57**	**22.38**	**3.45**	**9.15**	**2.49**	**6.12**	**3.35**

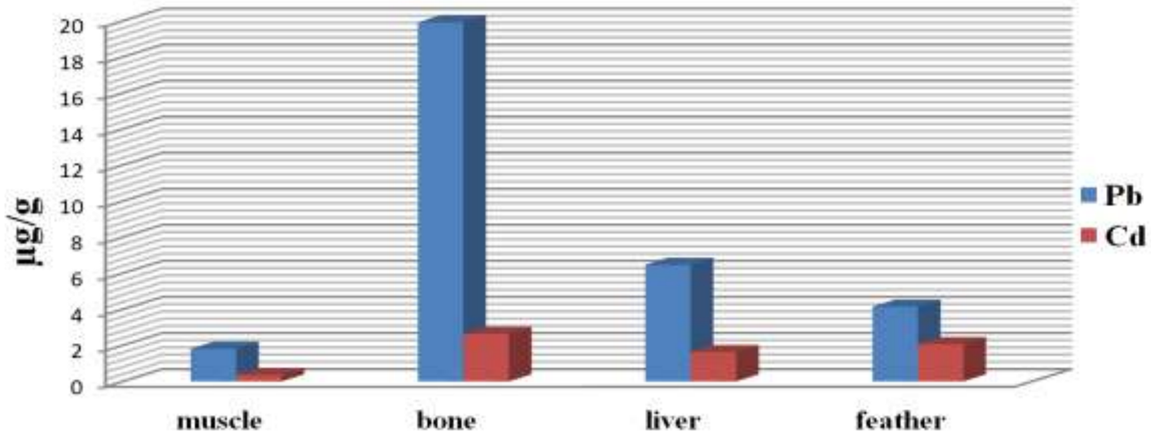

Figure 2- The comparison of the tissues in the average of lead and cadmium (µg/gD.W)

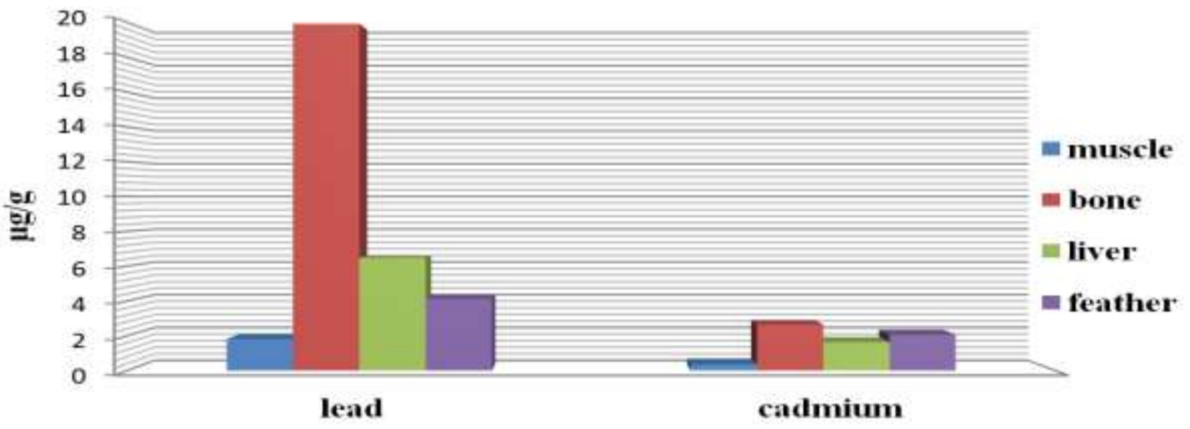

Figure 3- The comparison of the average in lead and cadmium in tissues(µg/g D.W)

High correlation was seen between heavy metals and weight in the bone (r=0.82, r=0.92). Low metabolic rate, long life and be at the top of the food pyramid, and also tend to bioaccumulate, increasing half-life and durability of heavy metals in the body are the main reasons for the increase in their values with an increase in weight, length and age of the bird (Szefer et al., 2003). High correlation was seen between lead and weighted in the Liver and feather (r=0.83, r= 0.77). Food needs look to increase the weight and continuous contact with the pollution, increase the lead in organs such as the liver and feather. After one to two months, lead moves from the soft tissue to bone and leadcompounds with hydroxyapatite lattice(Haddad et al., 1998). The correlation between liver and bone lead level was high (r=0.79) it means that the exposure of the coots with lead happenedearly and late. Both liver and bone are considered as the main center of lead storage with the difference that the time is different for the two tissues. There was a high correlation between Lead and cadmium in the bone (r=0.93). It seems that the bird simultaneously exposed to both lead and cadmium for the long time. No correlation between the metals andweight was found in muscle tissue, because of the failure to comply with the particular pattern of muscle, for the accumulation of heavy metals. Also,the excretory and absorbtion organs of the body such as the liver and bone by their activitesprevent theaccumulation of metals in tissues like muscle.

Table 3: The amount of correlation between the weight of *Fulica atra*, lead and cadmium

Tissue		Muscle		Bone		Liver		Feather	
	Metal	pb	cd	pb	cd	pb	cd	pb	cd
Muscle	pb	1	0.25-	0.11	0.22	0.05	0.13	0.53	0.08
	cd	0.25-	1	0.04	0.19	0.03	0.02	0.28	0.04
Bone	pb	0.11	0.04	1	0.93	0.79	0.28	0.66	0.23
	cd	0.22	0.19	0.93	1	0.26	0.12	0.12	0.44
Liver	pb	0.05	0.03	0.79	0.26	1	0.55	0.53	0.12
	cd	0.13	0.02	0.28	0.12	0.55	1	0.33	0.57
Feather	pb	0.53	0.28	0.66	0.12	0.53	0.33	1	0.52
	cd	0.08	0.04	0.23	0.44	0.12	0.57	0.52	1
Weight (g)		0.1	0.07-	0.92	0.82	0.82	0.35	0.77	0.48

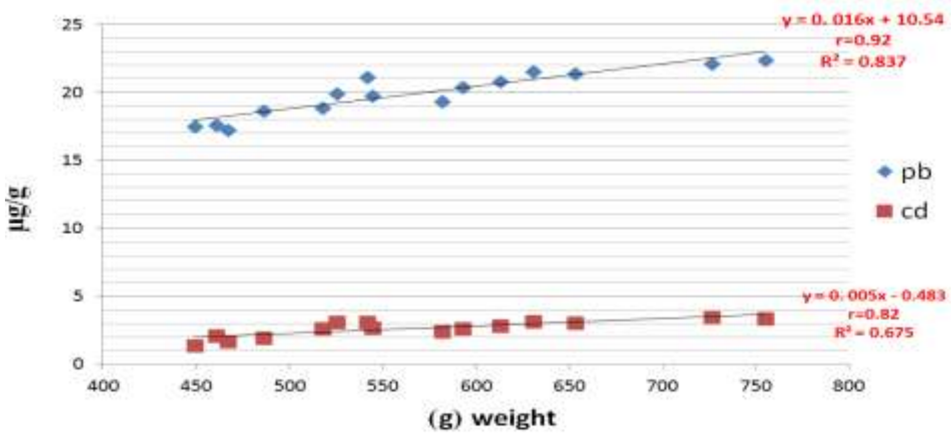

Figure 4: Comparisonofthe weight of *Fulica atra* with the concentration of lead and cadmium in bone

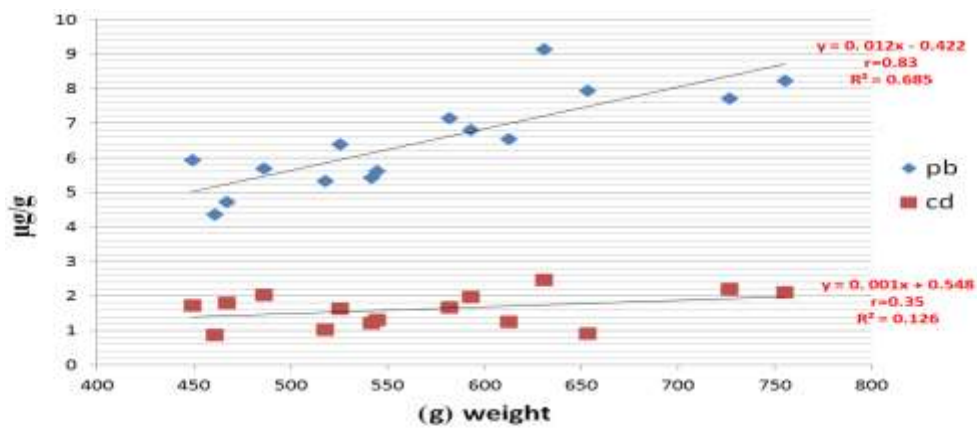

Figure 5: The comparison of the weight of *Fulica atra* with the lead and cadmium concentration in liver

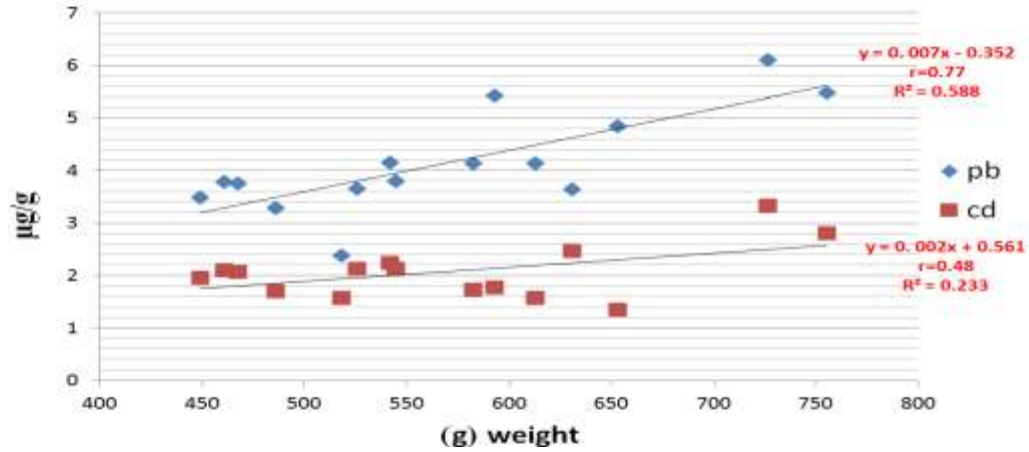

Figure 6: The comparison of the weight of *Fulica atra* with the lead and cadmium concentration in feather

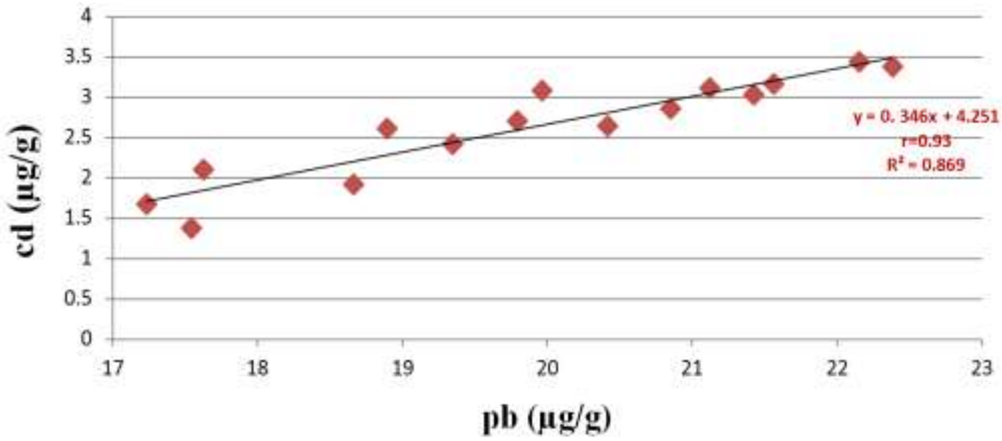

Figure 7: The comparison ofconcentration of lead and cadmium in bone

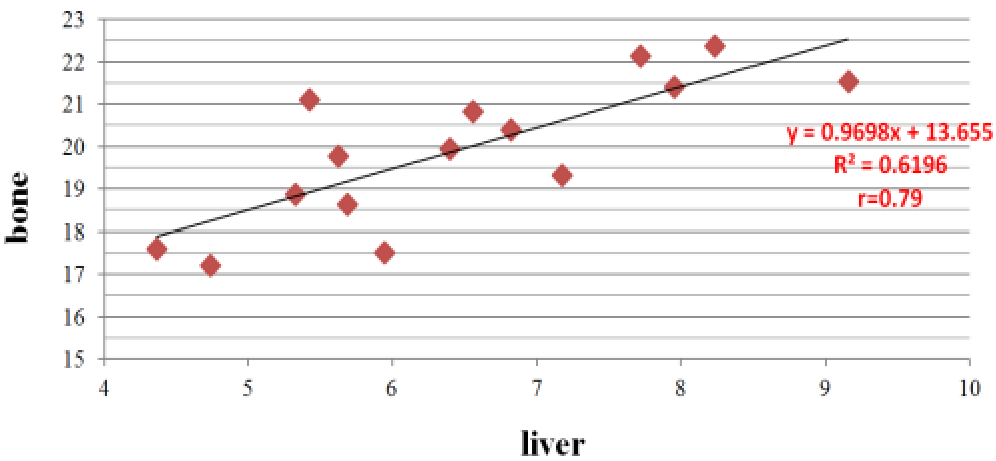

Figure 8: The comparison of lead between the liver and bone

ARGUMENTS

Acorrding to Rudd and Craig, Continuous monitoring of the amount of pollutants in the number of species and recognition effects need to understand a wide range of physical and chemical factors to the ecological, such as recognition of the effects of interactions with other components of the ecosystem, determination of pollutant transfer rate at various levels and the measurement of the digestion which describes the resistance percentage of species at different levels (Moriarty, 1983).

Concentrations of the metals in soft tissues such as liver ofbirds can be an indicator of recent exposure to sources of contamination, while concentration of the metals in bone is the reflection of chronic exposure to the contaminated sources. Probably, dietis as the important resource of eating metals for the waterfowl. Meanwhile, the food effectsare greater than the effects of the geographical area (Van Eeden, 2003).

In this study, the concentration of lead and cadmium in the liver of *Fulica atra*estimates 1.84 and 0.46 (µg/g wet weight) that was approximately 2 times more than Kreuzer & Wissmath and Bagley & Locke studies and it was lower than Bagley & Locke studies(1967).Comparing to Van Eeden studies during 1991 to 1993 lead in liver was lower, but Cadium was somewhat higher, and in comparingto Lukasz, cadmium in muscle and liver ofcoot was half of the above amount in Gomishan wetland.

Szarek et al in 1993 and 1995 measured cadmium in liver of 48 *Fulica atra* 5 to 6 months and cadmium in industrial areas was reduced from 0.04 to 0.03 and it is increased in agricultural areas from 0.03 to 0.04 (µg/g wet weight). It seems that the development of agricultural areas and the increasing use of phosphate fertilizers is responsible for the increase in agricultural areas. Cadium in liver in research of szarek was lower than the cadium of liver in cootsof gomishan wetland that it is justified due to the age of the cootsin Poland (5 to 6 month). Lead in the feather of *Fulica atra* in the current research was in board research of Guo and Miao in China, but its cadium was more. Due to the board research of Guo and Miao that was a salina, low cadium of feather is justified in comparison to Gomishan wetland, as the extensive agricultural areas around Gomishan wetland are polluted with cadium due to the high amount of phosphate fertilizers.

The amount of lead and cadmium in bone of coots in Gomishan wetland was lower in comparison with Van Eeden study, but it was higher in comparison with Taggart and Binkowski studies. Since bone tissue is the main reserve of the metal it is the beginning of feed ofbird, location and route of migration according to the type of feeding in the long time determine the amounts of these metals in the bone, that is why with regard to the amount of metal in the bone, it certainly cannot be argued that the sample is infected and due to the age of bird, pollution can come from different places the country. Change in the mean concentrations of lead and cadmium in different tissues of the bird indicated that bird has the ability to adjust the level of concentration in organs or specific tissues that it may be the result of the coot ability to regulate the body of metal through metabolic transactions, or it is not far-reaching changes in the nature of metals (Van Eden, 2003). Normal concentration of lead in liver was reported about 2 (µg/g wet weight) by Scheuhammer & Clark, and a concentration of 2 to 10 contact of lead contamination (in the contact area or toxic levels) the concentration of more than 10 (µg/g wet weight) were reported as the severe toxicity (Odiete, 1999). Therefore, lead in liver of coot in Gomishan wetland was normal (1.84 µg/g w.w). But factors such as diet, age, chemical composition of heavy metals, adsorption and desorption of heavy metals in body, the function of metals in organs can affect the concentration of metals in different tissues of bird.

Levels of metals are different among the variety of birds and depend on the ecology of food, biochemical and physiological contact with the environment and the characteristics bird (Savinov et al., 2003).

Many researches showed that the food chain related to the bird habitat and diet has a major role in the accumulation of heavy metals (Van Eeden, 2003).

Gomishan International wetland affected by human pollution, such as sewage overflows, agricultural and industrial shell casing and lead from hunters and other activities (Tabari et al., 2010).

Also, coot fed by plants, invertebrates, worms, benthos and bed things that have a lot of metal. All of these factors are the evidence of of accumulation of heavy metals in different parts of the bird due to feeding from the contaminated areas and the similarity in food vaiety in wide areas of the wetland. Human activities in the area around the wetland and urban wastewater, agricultural and industrial wastes can be a major source of pollution as a factor affecting the amount of these metals in wetland creatures. So, finding the path of pollution sources in the area around the pond is important. Population growth and increase of industrial activities lead to organic and inorganic pollutants in the southeastern coast of the Caspian Sea and physical purification is applied on these pollutants. The large amounts of pesticides and fertilizers containing metal compounds in agricultural lands of surrounding wetlands areas are used Gomishan international wetland and these materials flew into rivers and the Caspian Sea through surface runoff (Tabari et al., 2010). Rural and urban sewage, and agricultural waste flew directly to Gorgan river and surrounding channels such as Channel Alagol and Gomishan wetland is the final acceptor. Since the density of the population and industries in the area around wetlands, Gomishan agricultural wastewater surrounding wetlands have the major role in polluting the area, especially, it includes the large areas of the East Wetlands and it includes the large numbers of agricultural areas and the major share of the fertilizer and agricultural chemical containing heavy metals, especially lead and cadmium.

Finally, it can be concluded that regarding the concentration of lead and cadmium in the tissues of coot in Gomishan international wetland, coot was exposed to low-level contamination, but the amount of these elements is not in the extent to make toxicity in coots. But according to timing migration of the bird to other areas of the Caspian Sea, including Anzali andFereidounkenar wetlands, time sampling of them is important because different tissues of bird have a certain time limit in storing other heavy metals. Therefore, the period between the bird migration to the studied area and sampling of them determine the pollution in this area. The type of tissue in this bird can be the most factor in determining the pollution. So, the liver shows the pollution of the area in recent months, while the bone indicates the contamination in the food during the life of the bird. The species and the type of the birds affect the physiological ability, especially the liver and feather. That is why liver some species have higher filtration efficiency and holds pollution in few time. Age, sex, temperature of the environment, diet, chemical compound of the pollutant and absorbtion physiology, and releasing the elements in body are important factors. This fact is the same as the feather, regarding that the time of feather loss changes from months to years according to species, it is a good criteria for the enterance of pollution to the tissue, as it shows the amount of heavy metal in the feather, the amount of metal in blood during the growth of feather.

Table 4: The comparison of the average of lead and cadmium in the muscle of coot with the presented standards (mg/kg wet weight)

Resources	Lead	Cadmium	Standards
WHO, 1996)(	0.5	0.2	(WHO)
(Chen & Chen, 2001)	5	1	FDA) (
Pourang *et al*., 2004)(	1.5	0.05	(NHMRC)
(Pourang *et al*., 2004)	2	0.2	(UKMAFF)
	0.74-0.24	0.05-0.16	The concentration of metal in muscle in the current research (Min- Max)
	0.5	0.1	The concentration of metal in muscle in the current research (average)

RECOMMENDATIONS

1. Evaluation ofcontamination of other heavy metals in other species of birds living in the Gomishan wetland.

2. Evaluation of heavy metals in the diet Gomishan wetlandsbirds such as *Fulica atra*.

3. Identification of genetic and physiological effects of heavy metals in Gomishan wetlands*Fulica atra* population.

4. The use of other organs such as kidney, air sacs, egg and shells, the beak and nails to determine the amounts of heavy metals.

5. Study and identify pathways of pollutants from sources to the Gomishan wetland.

6. Evaluation of organic pollutants in different organs of birds in the area.

7. According to the importance of preserving the lives of some rare species, the use of organs such as feathers, nails and their relation of concentrations in organs with the main disposal and storage organs such as liver and bone is essential.

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