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The study of Forest Hara Biosphere Reserve in coast of Persian Gulf and the importance of heavy metal accumulation; Case study: feathers of great cormorant

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Abstract. *Mirsanjari MM, Sheybanifar F, Arjmand F. 2014. The study of forest Hara Biosphere Reserve in coast of Persian Gulf and the importance of heavy metal accumulation; Case study: feathers of great cormorant. Nusantara Bioscience 6: 159-164.* In recent years, concerns about the long term effects of heavy metals as environmental polluters have arisen, since considerable quantities of heavy metals have been released into the environment as a result of extensive human activities. Heavy metal has been determined as a serious threat to the stability of ecosystems. In this study, we examined the levels of zinc, copper, lead, and cadmium in the feathers of twenty great cormorants (*Phalacrocorax carbo*), collected from Hara Biosphere Reserve during November and December in 2012. The results revealed that the mean concentration of heavy metals in the feathers of males is significantly higher than females ($P < 0.05$). In addition, no significant difference was observed in heavy metal concentration between juvenile and adult birds. Moreover, according to the results, the high concentration of heavy metals in some samples indicated this fact that birds are potentially exposed to the risk of heavy metals in their habitat.

Keywords: Feather, great cormorant, Hara Biosphere Reserve, heavy metals, *Phalacrocorax carbo.*

INTRODUCTION

Environmental pollution, especially marine pollution, is one of the complex and minatory problems that human is facing at the present time. Many of human activities have irrecoverable outcomes and affect the marine environment in such a way that destruction of marine zones has become a significant warning. Sea has been one of the important sources with special advantages for mankind in all the times and still is, and therefore, protection of its environment and the animals living in the sea is of a considerable influence on the life process of mankind (Abdolvan and Safahieh 2009). Heavy metals are the most important contaminants that enter into the sea through rivers and shore zones and aggregate in the aquatic body through the food chain (Pourkhabaz and Norouzi 2012).

Most of these elements are not necessary for animals but have high toxic properties. One of the most imperative problems about heavy metals refers to their insoluble characteristic in the body. Indeed, heavy metals never excrete from the body, but they sediment in fat, muscles, bones, and joint tissues and may cause illnesses and various other phenomena (Harikumar et al. 2009).

One of the most important ecosystems in Iran is Hara Biosphere Reserve, located in the southern part of the country, and in the northwest of Qeshm Island in the protected zone of Hara. Since it is situated near the city of Bandar Abbas (the largest southern port of Iran in the Persian Gulf) and because ofits location at the margins of the northern coast of Qeshm Island (the largest island and commercial-industrial free zone of the country in the Persian Gulf), this region has been subjectedto urban and industrial pollutions. One of the most important pollutant sources, which has jeopardized the life of this ecosystem with serious threats in the recent decade, is the entry of heavy metals from various pollutant sources (Pourkhabaz and Norouzi 2012).

Heavy metals can originate serious effects on the ecosystem stability. In assessment of an ecosystem changes, studying all the elements and the relations between them appears to be impossible. However, application of monitoring species can provide trustworthy data for evaluating the environmental quality. For instance, biological monitoring of seashore zones with the aid of sea animals is a useful method to study the pollutions, which have been caused by the wastewater discharges and wastes of human activities (Furness 1993).

Birds, in comparison with other animals, are much more sensitive to environmental pollution (Furness 1993). They may distribute chemicals, such as heavy metals, through direct connection or using polluted water or food (Savinov et al. 2003). In other way, because of their indirect connection to polluted sources, they can determine the level of toxic element concentration in the marine ecosystem food chain. Therefore, the birds' pollution of heavy metals can be used to anticipate the pollution level in the food chains (Burger 1993). The high concentrations of heavy metals in birds' organs can cause the thinning of eggshell, the reduced reproduction, decreasing of the body's immune system, undesirable effects on thebirds' growth, and different abnormalities in the fetus. As a consequence, the population of birds and therefore,the biodiversity of an ecosystem will beaffected (Burger and Gochfeld 2000).

Another reason for studying heavy metals as environmental pollutants is because of the concerns about the public health. Since humans consume huntable aquatic birds, if these birds are polluted with heavy elements, they can bring many damages and complications to human lives. On the other hand, the frequency of food chain in the world of living beings, and the stability and consistency of heavy metals in theirbodies,have made the investigationsabout the effect of heavy metals on the life of aquatic organismsvery critical and indispensable (Mansouri et al. 2012).

It has been proven that metals, such as mercury, selenium, and cadmium, can cause the decreaseinthe birds' growth and weight and can have adverseimpacts on the birds' survival and reproduction success. The morphological parameters, such as the birds' body weight, tarsus length, and wing length, have many relationships with the concentration of heavy metals in the birds' body. Therefore, they are used as the indicators of survival and reproduction success in various species of birds (Dauwe et al. 2005). Hence, birds can be usedin environmental studies to assess the health of an ecosystem (Malik and Zeb 2009). Different species of birds including herons and egrets are used as biomonitors or bioindicators to measure the environmental pollution (Horari et al. 2007).

Among different tissues, mostly the soft tissues of birdsareused for the monitoring operation. In many studies, the tissues of muscle, liver, kidney, spleen, heart, lungs, fat, blood, brain, and bone, and in some others, the feather and egg have been used toinvestigate the concentration of heavy metals in the environment. It should be noted that in various tissues, different concentrations of aspecific type of metalcan beaccumulated. Therefore, in order to evaluate the condition of the environment, it is necessary to use different organs of the bird (Zhang and Ma 2011). Different studies have been conducted in this field, each of which has made use of different tissues (Malik and Zeb 2009).

Heavy metals can easily adjoin the protein molecules existing in the feather, during the short growth period of feather. In this short period, the feather connects to the main artery of the body, through a small blood vessel (Burger 1993). After completion of the feather, the blood vessels are reduced and therefore, the physiological connection of feather to the body is blocked (Denneman and Douben 1993). But the rates of heavy metals remain in the feathers forever (Braune and Gaskin 1987). Therefore, the feather tissue can indicate the rate of heavy metals in blood and other internal tissues in the first week of its formation (Lewis and Furness 1991).

The selection criteria for choosing the suitable bird species (generally the suitable environmental monitors) include: sedentary species, proper distribution, easy identification, long life, availability during the whole year, carnivorous species, stability against fluctuations, and physiochemical features (Lafabrie et al. 2013).

The birds that have most of these characteristics are not numerous. In fact, the maximum reflection of the heavy metals ratio is the purpose of these limitations and therefore, according to the above conditions, great cormorant (*Phalacrocorax carbo*) was selected as a suitable species to evaluate the heavy metals concentration. great cormorant is a species dependent on the aquatic ecosystem with a plentiful population and a wide distribution. In addition, its special piscivorous behavior has been always attractive for researchers. This species also spawns in Hara Biosphere Reserve and is known as a resident species, even though it is very hard to distinguish whether if the collected samples have been born at the same place or they have migrated from other locations, since it needs more attention and study. In addition, the position of egrets, at the top of the aquatic ecosystem cycle, has made them very sensitive to environmental changes. Thus, this species can be employed as a useful tool to study aquatic ecosystem' changing quality. Therefore, the piscivorous and residential features of these species (breeding and overwintering) in Hara Biosphere Reserve led to conclude that this species is a suitable tool to study heavy metals status (zinc, copper, lead, and cadmium).

The purpose of the present study is to compare the concentration of heavy metals (i.e. cadmium, copper, zinc, and lead) in the feather tissue of the great egret between the male and female egrets, and also to compare the concentration of heavy metals between the mature and immature egrets.

MATERIALS AND METHODS

The range of study

Hara Biosphere Reserve is located in the south of Iran in the Strait of Khuran, between Qeshm Island and the mainland of Iran, in the Persian Gulf, and with 86,581 hectares area. Its latitude and longitude coordinates are 36°40' to 37° and 55°21' to 55°52' E, respectively (Figure 1). It is situated in the Mehran River delta and hosts the largest seabirds along the Persian Gulf shoreline and, therefore, it would represent a center of biodiversity in Iran. The variety in this biosphere reserve and its unique mangrove trees provides a diverse habitat for birds like egrets, herons, pelicans, and plovers. Based on the available statistics, in 2010 (Neinavaz et al. 2010), the biosphere reserve had been the host of 36 species and 13,000 water birds and wader birds. Hara also serves as a breeding and spawning habitat for fishes, shrimps, and other crustaceans.

This area joined the "Man and the Biosphere Program" (MAB) of UNESCO (1997). On the other hand, this region is one of the protected areas in Iran introduced by the Department of Environment. The entire region was selected under the category of "Wetlands of International Importance as the Habitat of Aquatic Birds". In addition, this region was introduced as one of the important bird areas by the BirdLife International Organization (Neinavaz et al. 2010).

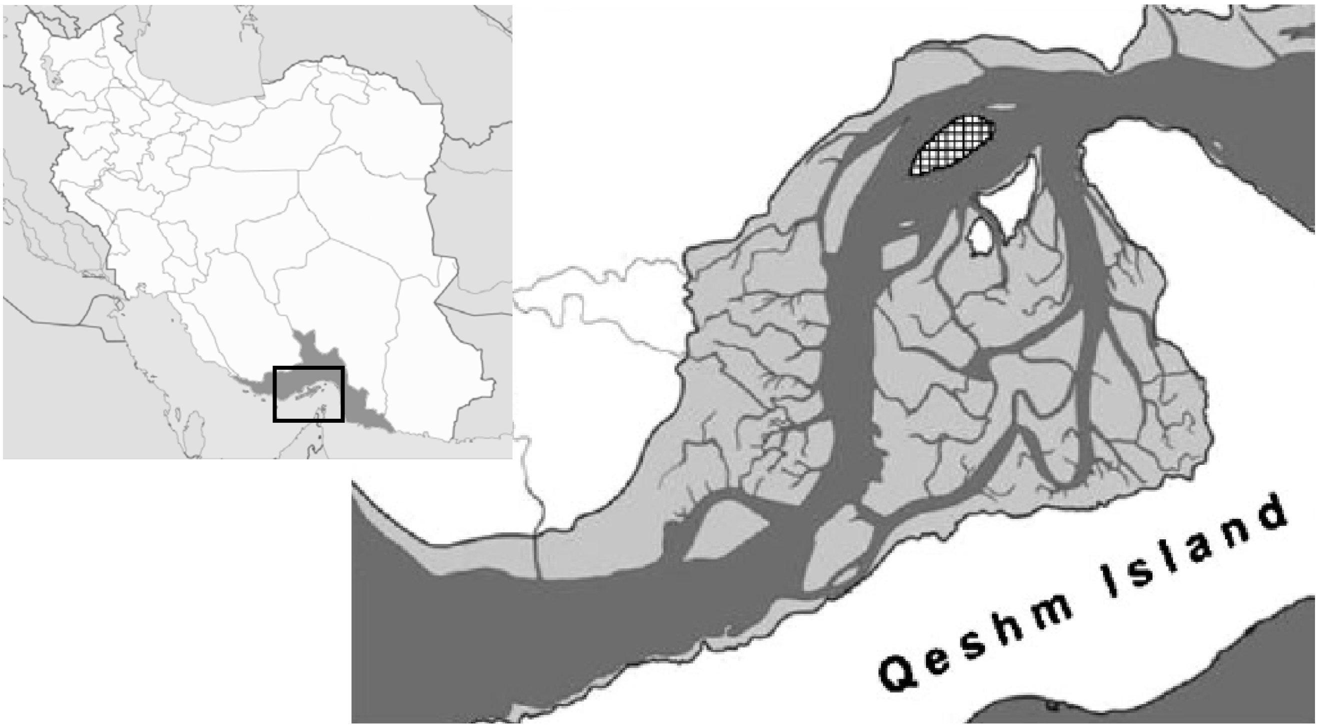

Figure 1. The location of the Hara Biosphere Reserve in coast of Persian Gulf, Iran.

Samples preparation

After primary studies about the studied areas, feathers of 20 great cormorant samples were collected in November and December 2012 from Hara biosphere reverse. After collection, they were transferred to the laboratory and washed with distilled water and acetone in order to remove external pollutions and then, placed in the oven at 65 °C for 24 hours (Burger and Gochfeld 2007). Samples were subsequently cut to smaller pieces by scissors and weighed by the scale of 1 gram. In the digestion phase, 8 ml of nitric acid (65%) and 2 ml of perchloric acid (60%) (i.e. at the ratio of 4: 1) were added to each sample (1 gram of the weighted sample). In a hot-block digester, they were digested firstly at 40 °C for 1 hour and then, at 140 °C for 3 hours. Afterwards, samples were screened by Whatman paper No. 1 and the solution's volume reached 25 ml by using distilled water. At the end, all samples were kept in the refrigerator in polyethylene jars in order to later evaluate heavy metals concentration by atomic absorption machine (Yap et al. 2002).

In order to ensure about accuracy of the digestion process, in each digestion round, there was a control sample to subtract the sizable results from concentration of the metal in different samples. In this study, measurement of heavy metals concentration was performed by atomic absorption machine (model contrAA 700).

Final metal concentration was calculated by the following relation in terms of microgram per gram dry weight:

$$M = CV / W$$

C : concentration taken from machine
V : final volume of sample (25ml in this study)
W: amount of dry material in terms of gram for digestion
M : final concentration of sample in terms of ppm per 1 gram dry material.

Statistical analysis

In this study, in order to perform statistical analysis, SPSS software (version 19), and to draw diagrams, Excel software (version 2007) have been employed. Firstly, the data adherence from the normal distribution was studied by the Shapiro-Wilk W-test. Because of the non-normality of data, normal shapes were not obtained and hence, a nonparametric test (the Mann-Whitney U-test) was utilized in order to compare the concentration of heavy metals in male/female and mature/immature samples. Furthermore, in order to investigate the relation of heavy metals in feather tissues, Spearman's correlation test was applied.

RESULTS AND DISCUSSIONS

The average, minimum and maximum rate of zinc, copper, cadmium and lead in the great cormorant tissue showed in the Table 1. Maximum average concentration belongs to zinc and the minimum to cadmium. According to Table 1, the maximum and average rates of heavy metal concentrations in adult and juvenile cormorants belong to zinc and the minimum to cadmium. According to the results, the average zinc concentration in cormorants' feather is 157.5 $\mu g.g^{-1}$. In comparison with the average zinc concentration in Lucia and Andre's (2010) studies on the greylag goose in France, it was realized that the rate of zinc in great cormorant in Hara Biosphere Reserve is much higher.

The average copper concentration in feather of cormorant in this study was obtained 9.11 µg.g^{-1} (Table 1). In this study, the measured copper concentration was equal to the reported copper concentration in Barbieri and Elisangela's (2009) studies on feathers of gull in Brazil and even studies of Mansouri et al. (2012) on egrets in Hara Biosphere Reserve. Copper and zinc are known as essential metals for the body. However, when they are increased in the animal body, they will impose bad effects on the animal health. Essential metals are necessary in all tissues and play a key role in chicken development and bone growth (Honda et al. 1986). The resultant wastes from ship painting and scrubbing in shipbuilding plants in Bandar Abbas, which is a coastal city located in the south of Iran and near Hara Biosphere Reserve, are full of zinc. This problem is the most important factor in increasing the concentration of zinc and copper in birds' tissues.

In the recent study, the average lead concentration in egrets' feather is 0.67 µg.g^{-1}, that in comparison with Malik and Zeb (2009) studies on the cattle egret in Pakistan and Mansouri et al. (2012) studies on siberian gull in Hara Biosphere Reserve is very low and approximately equal to the measured lead level in Tsipoura and Burger (2011) study on canadian goose in New Jersey, USA. Because of the structural similarities of lead and calcium, it can be easily placed instead of that in bones, feathers, hairs, and nails (Metcheva et al. 2006). Thus, according to many researches, the amount of lead stored in the feathers of birds is higher than other tissues (Burger 1993).

In this study, the high lead concentration in some of the samples from Hara Biosphere Reserve demonstrated this fact that the birds' environment is under extreme pressure due to existence of lead. Lead is a contaminant, which can be found everywhere and enters into the sea through various industrial wastes from the printing industry, oil refinery, etc. (Lakshmanan et al. 2009). Human activities, along with the developments of the oil refinery and petrochemical plants, added to zinc and lead factories in Qeshm, and marine transportation in the south of Iran, may play the most important roles in absorption of this metal in the feather tissue of this bird. The existence of lead in the feathers of the great egret in Hara Biosphere Reserve can be due to the presence of oil refineries and usage of petrol nearby the studied area. Thus, in the present study, it seems that in Hara Biosphere Reserve, the external pollution would have more effects than diets on lead accumulation in the great egret.

Burger and Gochfeld (2000) indicated that the undesirable effects of lead in birds occurs atthe concentration of 4000 ppb, althoughaquatic birds are able to tolerate even the concentrations higher than this level. If the lead concentration in birds' feather reaches over 4000 ppb, it would bring some problems, such as the decrease in the bird's perception, decrease of chickens' survival, inability in recognizing the sibling species, and the behavioral and nutritional problems (Burger and Gochfeld 2009). Moreover, due to its structural similarity, lead may interfere in calcium metabolism (Hutton and Goodman 1980). However, in the present study, the lead concentration is much lower than the effective threshold.

The average cadmium concentration in great cormorant in this study is 0.022 µg.g^{-1}, which in comparison to Kim and Koo's (2007, 2008) studies on black crowned night heron and Mansouri et al. (2012) studies on western reef egret in Hara Biosphere Reserve, is very low and approximately equal to the measured cadmium level in Burger's studies on a kind of gull in America (Burger et al. 2007). Cadmium is known as one of the poisonous and non-essential metals. Undesirable effects of cadmium in birds include the damage to kidneys, behavioral changes, prevention of egg production, thinning of the eggshell, and the damage to testicles (Furness 1993); and at the population level, it leads to the decrease of bones' growth rate, and the decrease of the reproduction success (Spahn and Sherry 1999). The behavioral problems of birds resulting from cadmium occur at the concentrations much lower than the lead and mercury concentrations (Eisler 1985). Berger (1993) stated that the detrimental effects of cadmium in the birds usually occur at the concentrations between 100 and 2000 ppb in birds' feathers. In this study, the cadmium concentration is much lower than the effective threshold.

The most important factor in increasing the cadmium level in Hara Biosphere Reserve is due to oil pollutions, since cadmium is an oil element. Either if the oil pollution has been accumulated by accident or sudden events, it has been caused by the discharges of ships' ballast water and this problem happens mostly in water (Malekpouri 2001). External pollution resulting from artificial sources has been recognized as one of the most important factors in increasing the concentration of heavy metals in birds during the feather-garnishing stage. It is considered as one of the ways that heavy metals can enter the birds' feathers (Dauwe et al. 2004, 2005). De Luca-Abbott et al. (2001) showed that diet in the family of herons hasa great influence on increasing the concentration of heavy metals in their body. In other way, Burger and Gochfeld (2007, 2008) claimed that the concentration of heavy metals in birds is under impact of their living habitat, atmospheric pollution, and their diet. Almost 87% of heavy metals in birds' feathers have external sources, such as environmental pollutions and sedimentation of lead through the preening process by the beak, which can be even stored in there. Only 13% of heavy metals enter through feeding and store in feathers.

Although the average concentrations of zinc, copper, lead, and cadmium are higher in adult birds, but the results of the Mann-Whitney U-test showed that there is no significant difference between zinc, copper, lead, and cadmium concentrations in adult and juvenile cormorants ($P > 0.05$) (Table 3).

The increasing amounts of heavy metals concentrations have been reported in many marine birds, especially in their feathers (Barbieri and Elisangela 2009; Burger and Gochfeld 2000). It is because adult birds have longer times for biological aggregation of heavy metals in their tissues (Burger 1994). On the other hand, Burger's studies (1996) on feather of a kind of gull at Captree, Long Island showed that the cadmium concentration in juvenile birds is higher than adults. They concluded that it could be due to different feeding style in adults and juveniles.

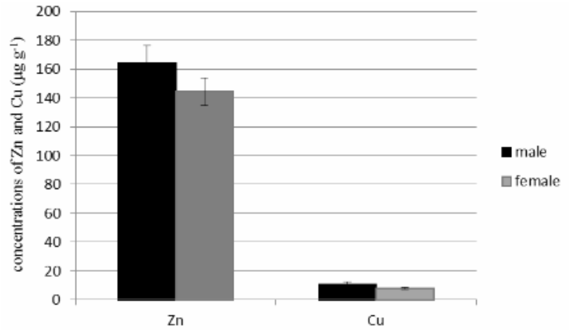

Figure 1. Comparison of zinc and copper concentrations between males and females of *P. carbo* in Hara Biosphere Reserve, Iran.

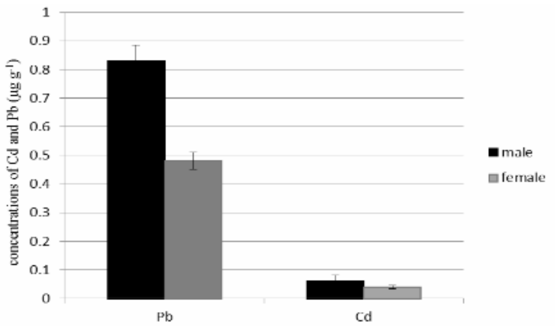

Figure 2. Comparison of lead and cadmium concentrations between males and females of *P. carbo* in Hara Biosphere Reserve, Iran.

Table 1. Values of heavy metal concentration (mean±SD, µg g^{-1}) in feathers of *P. carbo* in Hara Biosphere Reserve, Iran.

Sample	Zinc	Copper	Lead	Cadmium
Male	164.5±38.33	10.55±1.69	0.83±0.28	0.06±0.01
Female	144.5±12.61	7.74±1.33	0.48±0.15	0.04±0.0006
Adult	159.7±28.9	9.51±2.1	0.71±0.26	0.027±0.011
Juvenile	133.8±16.86	8.5±2.4	0.65±0.44	0.016±0.013
Total	157.5±30.23	9.11±2.15	0.67±0.24	0.022±0.02

Table 2. Results of paired samples Mann-Whitney U test for comparing concentration (µg g^{-1}) between males and females.

Element	Sex	No.	P Value
Zinc	Male	9	0.031*
	Female	11	
Copper	Male	9	0.01*
	Female	11	
Lead	Male	9	0.002*
	Female	11	
Cadmium	Male	9	0.000*
	Female	11	

Note: p< 0.05, indicates significant differences (Confidence level 95%)*.

Table 3. Results of paired samples Mann-Whitney U test for comparing concentration (µg g^{-1}) between adults and juveniles.

Element	Age	No.	P Value
Zinc	Adult	8	0.51
	Juvenile	12	
Copper	Adult	8	0.33
	Juvenile	12	
Lead	Adult	8	0.14
	Juvenile	12	
Cadmium	Adult	8	0.23
	Juvenile	12	

Note: p< 0.05, indicates significant differences (Confidence level 95%)*.

Figures 1 and 2 displayed that the average of zinc, copper, lead, and cadmium concentration in feather tissue is much higher in males than females. In this research, the Mann-Whitney U-test results were used for comparing the concentration differences between two genders (Table 2) and it was shown that there is a significant difference among zinc, copper, lead, and cadmium concentrations in feather tissue of males and females ($P < 0.05$). There are only few researches that investigated the role of gender in aggregation of heavy metals in feather tissue (Zamani et al. 2010). In studies of Burger (2007), it was obtained that in male gull, the chromium and manganese concentrations are significantly higher than females (Burger and Gochfeld 2007). The females can expel heavy metals through the spawning process, and it is the only excretion way, in which males are unable (Burger 1993).

Hara Biosphere Reserve, which is considered as the largest mangrove population of Iran, is of significant importancefrom anecological perspective,since it acts as a nutritional filter and natural absorber. These forests protect the coastal areas from the collision of heavy sea currents. They also have commercial advantages since they provide the grounds for reproduction of many aquatic species. However, the increase of heavy metals in the natural waterways, leading to mangrove forests,has remarkably threatened this ecosystem (Hoff et al. 2002).

The most important factors affecting the destruction of mangrove forests are, the increasing trend of large industries in Hormozgan, holding military maneuvers in the region, establishing oil platforms and rigs, not observing the recognized standards forthe marine environment, entry of industrial and urban sewage and sullage to the sea, the growing population in the coastal areas, using the branches of Hara trees for feeding livestock, building dams onthe upstream and hence, blocking the sweet water rivers thatplay the critical role of vital artery for mangroves, and building pools for shrimp cultivation in the vicinity of mangroves and entering their sewage into the sea.All the aforementioned are amongst the factors threatening the natural ecosystem of the Persian Gulf and also the Hara forests located in this region. All these activities have deleterious effects on the environment in such a way that the natural self-purification function would beunable to tolerate the high volume of pollutants entering the environment (Norouzi and Pourkhabaz 2012).

Therefore, studying the accumulation of heavy metals in the constituent elements of this reserve,i.e. water, sediment, and the beings living in them, is required for monitoring the pollution of heavy metals in this ecosystem andfor presenting different solutions with the purpose toreduce the pollutions and revitalizethe lost functions of this ecosystem.

In the end, it can be concluded that the pollution conditions for cadmium, copper, zinc, and lead is not at the toxic level. In addition, since this habitat is of biological significance, the feather tissue of this bird can be employed as a biomonitor to study the region pollution. The results presented that there is a significant difference in heavy metal concentration between males and females. In addition, no significant difference was witnessed in heavy metal concentrations between adult and juvenile birds. Moreover, it was perceived that the concentration of heavy metals in birds is affected by their living habitat, atmospheric pollution, and their diet.

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