

Examination of Toxic Metals (mercury) in Coot (*Fulica atra*) and Cormorant (*Phalacrocorax carbo*) Collected in Anzali and Gomishan Wetlands, Iran

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ABSTRACT

Background: Present study was carried out in north of Iran to investigate the concentration of mercury (Hg) as one of the most toxic metals in tissues of two species of birds.

Methods: In this study, 15 birds from coot (*Fulica atra*) and 18 birds from cormorant (*Phalacrocorax carbo*) were hunted in Anzali and Gomishan wetlands. Data-analyses carried out using SPSS software version 17 licenced by Tarbiat Modares University. Data-test normality performed based on Sharpiro-Wilk's test and P-value was set on $P < 0.05$.

Results: Based on the study, for two species, mercury concentration in feather was in maximal and in muscles it was in minimal concentration. For coot muscles, kidney, liver tissues and feathers, it was 0.03, 0.07, 0.09, and 0.23 mg/kg, and for cormorant it was 2.26, 3.59, 5.67, and 8.68 mg/kg. In general, mercury (Hg) concentrations in coot were lower than cormorant ($P < 0.05$). No significant differences were found between them in terms of sex ($P < 0.05$).

Conclusion: Concentration of mercury was not the same in two species. Statistical analysis didnot show a significant differences between male and female tissues of them. Our data can contribute to develop management programs for understanding the ecotoxicological status of the Caspian basin and its coastal environment.

Keywords: Bioindicator, Caspian Sea, Coot, Cormorant, Mercury

INTRODUCTION

Mercury (Hg) is released and mobilized through natural processes and by anthropogenic activities (1). Mercury anthropogenic sources, such as fossil fuels combustion, nonferrous metal production, and waste incineration, have been suggested to contribute significantly to the Hg contamination (2). Caspian Sea area is the largest landlocked body of water on earth and home to numerous ecosystems. The coastal wetlands of the Caspian basin include many shallow and saline water pools, which attract a variety of birds and support great biodiversity; more than 400 species of birds are unique to the Caspian Sea area (3). It also serves as a sink for many contaminants and high concentrations of Hg which are found in coastal sediment and Caspian sturgeon (4). Sources of Caspian pollution includes oil discharges and spills from Republic of

Azerbaijan and untreated waste from the Volga (including drainage from heavy industrial and municipal sewage) which seriously threaten the flora and fauna of the area (3,5). The stability of heavy metals in the environment creates many problems. One of the important results of their stability is biological accumulation in the food chain (6). Iran supports a rich and diverse avifauna with more than 514 species, among which coot (*Fulica atra*) and great cormorant (*Phalacrocorax carbo*) are two species of aquatic birds with great value. These two are palearctic migratory and abundant species in winter in the north and south Wetlands of Iran (7). Coot is the most common aquatic bird that is being hunted in Iran since it serves as a favorite food and can be a commercial species (8). The main reasons for choosing coot as indicator species were being a native bird of abundant numbers, wide range of geographical distribution, great availability, high nutritional

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value, and herbivorous species. Also, all these characteristics mentioned about coot are the same in great cormorant except for great cormorant being a carnivorous species. The main cormorant habitats are inland rivers, lakes, and coasts, but some cormorant groups live near urban areas. Cormorants live all over the world including Europe, Africa, Asia, Oceania, and Northern America (9), so that these species could be a potential global environmental monitor for toxic contaminants. There is, in fact, much worldwide research on heavy metal accumulation in cormorant (10,11). Cormorants have been used as bioindicators for metal contamination due to their specific feeding habits, wide geographical range, and long life-span. However, most of these studies on cormorants have focused on toxic Cd and Hg (2, 12) while other trace

elements have been poorly investigated in these species. In Japan, great cormorants are known as local breeding residents and possible bioindicators for detecting metal exposure in their habitat (13). Aquatic ecosystem health has often been assessed using birds species as indicators of pollution exposure and toxicity. Sea birds may be exposed to significant concentrations of Hg and thus might serve as good monitors of spatial and temporal patterns of Hg presence in marine and coastal ecosystems (2). The main aim of this study was to evaluate feasibility assessment for two important waterbirds in order to determine bio-indicatory index usage and suggest suitable tissue for such studies, especially for coot which is the most commonly hunted bird in the mentioned region.

MATERIALS AND METHODS

Study area

For this study, two wetlands, Anzali and Gomaishan in the south coastal of the Caspian Sea, Iran, were chosen, which are known as national and international wetlands on world scales (Figure 1). Anzali and Gomaishan wetlands are located in 37°28'N, 49°25'E and 54°53'N, 37°9'E and have an average area about 15000 and 20000 ha, respectively (13, 14). Also, these wetlands represent an internationally important wildlife reserve and a sanctuary which is listed under Ramsar Convention. Both of these wetlands are extremely important wintering habitats for a wide variety of waterbirds. In the last decades, these wetlands have been endangered and destroyed by environmental pollution from seven identified sources including rivers, mines, hospitals, and municipal, industrial, commercial, and agricultural wastes. Used oil discharge from ships, illegal construction, drying the wetland, discharge of any wastewater from fish farms, and solid wastes disposal are the other factors which have affected the wetland environment (5). A total of 33 birds, 15 coots and 18 great cormorants, were caught in the South Coast of the Caspian

Sea in the hunting season with legal license from the Department of Environment (DoE), Iran (Table 1).



Figure 1 Sampling sites for birds along the South coast of the Caspian Sea

Table 1 Sex and weight (mean \pm SE g) of the specimens

Species	Sex	Weight(g)
Coot (n=15)	Females (6)	597.5 \pm 15.13
	Males (6)	
	Unknown (3)	
Great cormorant (n=18)	Females (7)	2111.1 \pm 53.41
	Males (11)	

Sample preparation

Samples were brought to the laboratory right away, and birds were dissected immediately. Generally, to determine the concentration of mercury in birds; samples were taken from feather, liver, kidney, and muscle tissues. Liver, kidney, muscle, and feather of these birds were removed and stored at -20° C. Samples were placed inside the freeze-dried for 48 hours to dry. The total mercury concentrations in homogenized samples were determined by the LECO AMA 254 Advanced Mercury Analyzer (USA). In order to assess the analytical capability of the proposed methodology, the accuracy of the total Hg analysis was checked by running three samples of Standard Reference Materials (SRM), National Institute of Standard and Technology (NIST), SRM 1633b, SRM 2709, and SRM 2711 in seven replicates. The recovery fluctuated between 94.8% and 105%.

The detection limit of the method used was 0.001 mg/kg in dry weight.

Statistical analysis

The Statistical analyses were carried out using SPSS software version 17 licensed by Tarbiat Modares University. Data were tested for normality using Shapiro–Wilk’s test. Data were not normally distributed; therefore, a non-parametric test was used for analysis. Mercury concentrations in feather, liver, kidney, and muscle were tested for mean differences between species using Mann–Whitney U test. Probability level of $P > 0.05$ was set to indicate the statistical significance.

RESULTS

Mercury concentrations in tested tissues of all the species decreased in this order: feather > liver > kidney > muscle (Table 2).

Table 2 Mercury concentrations (mg/kg wet weight) in tissues of bird species from the South Coast of the Caspian Sea

Species		Feather	Liver	Kidney	Muscle
Coot (n=15)	Mean \pm SE	0.23 \pm 0.04	0.09 \pm 0.01	0.08 \pm 0.01	0.03 \pm 0.01
	Range	0.06 - 0.53	0.07 - 0.14	0.05 - 0.09	0.01 - 0.08
Great cormorant (n=18)	Mean \pm SE	8.68 \pm 0.68	5.67 \pm 0.19	3.59 \pm 1.77	2.26 \pm 0.58
	Range	5.46 - 16.37	4.46 - 7.64	0.90 - 23.71	0.85 - 11.08
P value		< 0.05	< 0.05	< 0.05	< 0.05

Table 3 Mercury concentrations detected in coots and great cormorants in northern Iran in comparison to mercury concentrations reported in the same species in others studies

Location	Tissue	Mean	SD	References
Coot (Fulica atra)				
Slovakia	leg skeletal muscles ^b	0.01	-	(Gasparik et al., 2010)
Iran	Feather ^b	0.50	0.01	(Zolfaghari et al., 2009)
	Iran Feather ^b	0.23	0.15	This study
	Iran Liver ^b	0.09	0.02	This study
	Iran kidney ^b	0.08	0.02	This study
Iran	Muscle ^b	0.03	0.02	This study
Great cormorant (phalacrocorax carbo)				
Japan and Tokyo	Feather ^b	2.85	0.60	(Saeki et al., 2000)
Japan and Tokyo	kidney ^b	4.05	2.18	(Saeki et al., 2000)
Japan and Tokyo	Liver ^b	3.39	1.39	(Saeki et al., 2000)
Japan and Tokyo	Muscle ^b	1.27	0.35	(Saeki et al., 2000)
Iran	kidney ^c	9.25	-	(Mazloomi et al., 2008)
Iran	Liver ^c	8.32	-	(Mazloomi et al., 2008)
Iran	Feather ^c	4.42	-	(Mazloomi et al., 2008)
Iran	Muscle ^c	2.06	-	(Mazloomi et al., 2008)
Japan	Feather ^a	2.80	2.70	(Nam et al., 2005)
Japan	Kidney ^a	14.00	9.00	(Nam et al., 2005)
Japan	Liver ^a	12.00	9.00	(Nam et al., 2005)
Japan	Muscle ^a	1.10	0.30	(Nam et al., 2005)
Moravia	Muscle ^b	1.00	0.30	(Houserova et al., 2005)
Moravia	Liver ^b	10.00	8.30	(Houserova et al., 2005)
Moravia	kidney ^b	2.70	2.10	(Houserova et al., 2005)
	Iran Feather ^b	8.68	2.90	This study
	Iran Liver ^b	5.67	0.85	This study
	Iran kidney ^b	4.81	2.61	This study
Iran	Muscle ^b	2.26	2.04	This study

a: mg/kg w w, b: mg/kg d w, c: mg/kg undefined

DISCUSSION

In general, mercury concentrations in coot species were lower than cormorants. Also, mercury concentrations in great cormorants of this study were higher than the cormorants of Lake Biwa, Japan and Tokyo (12) and the south-eastern gulf of California region, Mexico (15). In migratory birds, accumulation of Hg depends not only on the degree of pollution in the area of collection, but also on pollution in stopover sites and breeding grounds. We know that many birds that winter in Iran have their breeding grounds in Russia and Eastern Europe (5). Avian mercury concentrations are usually found at the highest levels in feather and in lower degrees in liver, kidney, and muscle tissues (16). In birds, feathers play a major role in mercury excretion (12). Mercury accumulates especially well in feather because of its high affinity for sulfhydryl groups in keratin (17). For a wide range of species, mercury levels of 5 mg/ kg of dry weight in

feathers are associated with reproductive deficits such as lower clutch and egg size, lower hatching rate, decreased chick survival, and decreased overall reproductive success. Mercury even impairs territorial fidelity (18). Generally, larger species that typically live longer and can eat larger food items are expected to have higher levels of pollutants in their bodies. Other contributing factors to such high Hg in the studied birds of the Caspian Sea might be the presence of several polluting industries in this area including local pulp and paper mills and heavy industries in the West Coast of the Caspian Sea in Azerbaijan and the Northern Russian Coast. On the other hand, Hg emissions appear to be increasing globally due to increased coal burning in Asia (19). One important aspect of a comparison among species is to consider whether any species exhibit metal levels that are above those levels known to cause sub-lethal behavioral or reproductive effects.

Various consumption advisories on animals due to Hg concentration exist, and restricted international standards normally range between 0.3 and 0.5 mg/kg wet weight (W.W) (20,21,22). In the present study, mercury levels in great cormorants feather were higher than the threshold level suggested for adverse effects. Once metals enter a bird, they can be stored in internal tissues such as the kidneys and liver (23, 24). Many seabirds would demethylate organic Hg in tissues such as the liver and kidneys and store a large portion of their Hg burdens in an inorganic form (12). In our samples, liver mercury concentrations were higher than those of kidney in both species. Reports indicate that in a variety of wild caught birds, liver mercury levels are consistently greater than kidney levels which, in turn, exceed those in muscles (25,26). In this study, mercury concentrations in muscles of both species were lower than other tissues. Mercury concentrations in tissues showed significant differences between the two species (Table 2). In comparison to other investigations, it seems that pollution of Caspian basin birds is approximately 10 times less than other flyways for the same species in Europe and Asia, though the greatest concentration of mercury exists in Japanese birds and habitants (27) (Table 3). Although increasing environmental pollution by heavy metal contaminants has been reported in recent

years in almost every industrialized nation in South Asia (28), the major sources of contamination in the Caspian Sea include the agricultural use of fertilizers, herbicides, pesticides, hazardous substance spills from various refineries, and adjacent countries petrochemical factories. Although mercury concentrations in the birds were not the same in the two species and different tissues, statistical analysis did not show a significant difference between male and female birds in the tissues of the two species.

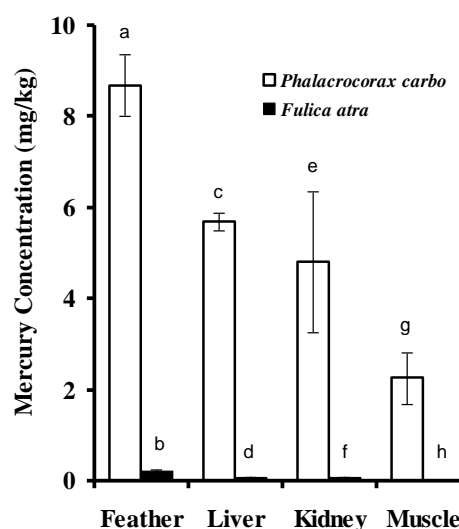


Figure 2 Mercury concentrations in the tissues of two birds in the north of Iran

CONCLUSION

There are several reports on mercury concentrations in different bird species of Iran (5,24,29,28,30) but there are not any reports on coots as the most hunted birds of Iran. Also, previous studies have not included total mercury comparisons between this bird species with others in the northern wetlands of the Caspian Sea. Therefore, the present study investigated mercury levels in two species of birds to determine the usefulness of these species as bio-indicators from the Caspian Sea. Mercury concentrations in great cormorant tissues were higher than coots that can be due to their specific feeding habit, long life-span, and greater weight. Mallards are easily

recognizable, show distinct sexual dimorphism, are long-lived and abundant, and have wide geographical ranges which meet the requirements of good bio-monitors for European countries (31), but for Iran, coots are the most hunted birds that are used for edible reasons and for game hunting. Therefore, they seem to provide the acceptable material for the bio-monitoring approach. Our data can contribute to the development of management programs for understanding the ecotoxicological status of the Caspian Sea and its coastal environment. In addition, our results can help determine restriction standards for use by people. Great cormorants and coots can be used as bio-indicators for metal contamination in the wetlands of the north of Iran.

ACKNOWLEDGMENTS

We would like to thank A. Kazemi, A. Khalili and E. Abdolazade for field assistance, S. Ezatti and M. Abdollahi for guidance in writing this manuscript and R. Zamani-Ahmadmahmoodi for help with the statistical analysis. This study was funded by Tarbiat Modares University of Iran (TMU).

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