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Contaminations of Metal in Tissues of Siberian Gull *Larus heuglini*: Gender, Age, and Tissue Differences

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Abstract The objective of the present study was to investigate the levels of metals, namely cadmium, lead, and zinc, in Siberian gull (*Larus heuglini*) (n = 15), in order to: (1) examine the sex and gender related variation in trace metal accumulation, and (2) to determine the significant between metal concentrations in the kidney, liver, and pectoral muscle. The concentrations were different between the tissues of bird as well as among the interaction (sex × age), but this difference (except cadmium in liver and zinc in kidney) between the gender (male and female) and age (adult and juvenile) didn't exist. Results showed that the metal concentrations in the Siberian gull were decreased in sequence of kidney > liver > muscle. The cadmium, lead, zinc concentration overall means they were measured as 2.2 ± 0.7, 8.8 ± 2.5, and 91.1 ± 37. 1 µg/g for kidney, 1.1 ± 0.2, 5.1 ± 0.8, and 68.3 ± 27.8 µg/g for liver, and 0.8 ± 0.1, 3.4 ± 0.6, and 34.4 ± 23.2 µg/g for pectoral muscle, respectively.

Keywords Cadmium · Lead · Seabird · Hara biosphere reserve

Hara biosphere reserve is located in south of Iran in the Straits of Khuran between Queshm Island and the Persian Gulf. Its latitude and longitude coordinates are 26°40′–27°N and 55°21′–55°72′E, respectively. Hara biosphere reserve and other area wetlands have been acknowledged for the important role they play in ecology of migratory birds and as especial habitat for seabirds. The variety of biosphere reserve with its unique mangrove trees provides a diverse habitat for birds like gulls, egrets, herons, pelicans, and plovers. It also serves as a breeding and spawning habitat for fish, shrimp, and other crustaceans, therefore, represents a center of biodiversity in Iran (Mansouri et al. 2012).

Metals, among the various toxic pollutants, are specifically severe in their action because of their toxicity, bioaccumulation, long persistence, and bio-magnification in the food chain (Mansouri et al. 2011a). Metals may enter into the aquatic ecosystem by atmospheric deposition or by weathering of the geological matrix or through anthropogenic sources, such as industrial discharge, sewage, agricultural waste and mining wastes (Ebrahimpour and Mushrifah 2010). One of the most important properties of toxic pollutant is that it can be accumulated in the organs of organisms (Palaniappan and Karthikeyan 2009). Therefore, it is of great importance to know the bioaccumulation potential of a pollutant. Organisms at the top of food chain may accumulate a large amount of metals in their tissue, according to the gender, age, size and feeding habits (Naccari et al. 2009). Hence, the use of indicator species can provide data to monitor the quality of the ecosystem for exposure to contaminants of their biological habits.

Seabirds are susceptible to bioaccumulation of pollutants mainly through the consumption of contaminated food. These species can provide interesting data to relation monitor the quality of the environment (Kim and Koo 2008). Many studies have recommended Seabirds as bioindicators for metals in aquatic systems and local pollution around breeding sites (Burger 2002; Barbieri et al. 2009;

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Mansouri et al. 2012). Because Seabirds such as gulls are high at the top of their food pyramid and can yield information over a large area around each sampling site, not only on bioavailability of contaminants but also on how, where, and when they are transferred within the food web (Battaglia et al. 2005).

Numerous studies have evaluated levels of metals in tissues birds (Kojadinovic et al. 2007; Naccari et al. 2009). Yet, some highly migratory species, such as seabirds, have been largely ignored. In the other hands, there are only a few reports that document metal contamination in southern Iran seabirds (Zolfaghari et al. 2009; Mansouri et al. 2011b, 2012). Hence, our study aimed to further investigate the concentrations of cadmium, lead, and zinc in Siberian gull (*Larus heuglini*) from southern Iran, in order to: (1) examine the sex and gender related variation in trace metal accumulation, and (2) to determine the significant between metal concentrations in the kidney, liver, and pectoral muscle.

Materials and Methods

Bird samples were collected from November to December 2010 throughout the Hara biosphere reserve. A total number of 15 bird individuals belonging to Siberian gull (*L. heuglini*) were analyzed for cadmium, lead, and zinc concentrations in the kidney, liver and pectoral muscle samples. The collections included Siberian gull with average weight of 675 g, length body of 66 cm, and length wing of 101 cm, respectively. The specimen were killed, weighed, stored in plastic bags, and kept at −20°C until dissection and analysis. Liver, kidney, pectoral muscles were separately dissected from the bodies of the specimens. The samples were dried in a furnace equipped with an air circulation system at 60°C for 48 h, and homogenized using a porcelain mortar. The tissues samples were digested in a nitric acid (HNO_3) and perchloric acid ($HClO_4$) mixture. Tissues were then accurately weighed into 150-mL Erlenmeyer flasks, 10 mL nitric acid (65 %) was added to each sample, and the samples were left overnight to be slowly digested; thereafter, 5 mL perchloric acid (70 %) was added to each sample (Mansouri et al. 2011b). Digestion was performed on a hot plate (sand bath) at 200°C. After that, the digested samples were diluted by 25 mL deionized water. The concentrations of metals were estimated using Shimadzu AA 680 flame atomic absorption spectrophotometer. The accuracy of the analysis was checked by measuring CRM certified reference tissue (DORM-2, NRC Canada). The detection limits for each metal were: cadmium (0.009), lead (0.042), and zinc (0.025). Results for cadmium, lead, and zinc gave a mean recovery of 100 %, 98 %, and 99 %, respectively.

Data analyses were carried out using the statistical package SPSS (Version 16). Concentrations of metals were compared among kidneys, livers, and pectoral muscles in Siberian gull using one-way and two-way analysis of variance (ANOVA). We used a two-way ANOVA for each metal (sex, age, interaction [sex × age]). Data were log transformed to obtain normal distributions that satisfied the homogeneity of variance required by ANOVA. The concentrations of metals in tissues were expressed as microgram per gram dry weight (dw). Values are given in means ± standard deviation (SD).

Results

The mean concentrations of cadmium, lead, and zinc in the kidney, liver, and pectoral muscle of Siberian gull, from Hara biosphere reserve in south of Iran were given in Tables 1, 2, and 3. The cadmium, lead, zinc concentration overall means they were measured as 2.2, 8.8, and 91.1 µg/g for kidney, 1.1, 5.1, and 68.3 µg/g for liver, and 0.8, 3.4, and 34.4 µg/g for pectoral muscle, respectively. Results showed that the metal concentrations in the Siberian gull were decreased in sequence of kidney > liver > muscle.

Cadmium, lead, and zinc concentrations differed significantly in kidneys, livers, and pectoral muscles among tissues in Siberian gull (one-way ANOVA, $p < 0.001$). The results showed that for exception of cadmium in liver and zinc in kidney, there were no significant differences between the male and female (two-way ANOVA, $p > 0.05$). Also, the results showed that for exception of zinc in kidney, there were no significant differences between the adult and juvenile (two-way ANOVA,

Table 1 Cadmium concentrations (mean ± SD µg/g) in Siberian gull from Hara biosphere reserve, Iran

Specie sex/age	No.	Tissues		
		Kidney	Liver	Pectoral muscle
Siberian gull				
Male/adult	4	2.1 ± 0.6	1.4 ± 0.2	0.9 ± 0.1
Male/juvenile	4	2.1 ± 0.8	1.2 ± 0.1	0.7 ± 0.1
Female/adult	3	2.6 ± 0.7	1.1 ± 0.2	0.8 ± 0.1
Female/juvenile	4	2.1 ± 1.1	0.9 ± 0.1	0.8 ± 0.1
Min–max		1.1–3.4	0.7–1.6	0.5–1.1
Overall mean		2.2 ± 0.7	1.1 ± 0.2	0.8 ± 0.1
p value sex		NS	NS	NS
p value age		NS	0.007	NS
p value sex × age		0.001	0.001	0.001

p value for 2-way ANOVA. Interaction term significant as indicated

NS not significant at $p > 0.05$

Table 2 Lead concentrations (mean ± SD μg/g) in Siberian gull from Hara biosphere reserve, Iran

Specie sex/age	No.	Tissues		
		Kidney	Liver	Pectoral muscle
Siberian gull				
Male/adult	4	9.1 ± 3.6	4.7 ± 0.9	3.3 ± 0.7
Male/juvenile	4	9.7 ± 2.7	4.7 ± 0.6	3.2 ± 0.5
Female/adult	3	7.8 ± 1.2	5.5 ± 0.9	3.3 ± 0.4
Female/juvenile	4	8.8 ± 3.3	5.5 ± 0.9	3.8 ± 0.8
Min–max		5.5–13.2	4.1–6.6	2.7–4.4
Overall mean		8.8 ± 2.5	5.1 ± 0.8	3.4 ± 0.6
p value sex		NS	NS	NS
p value age		NS	NS	NS
p value sex × age		0.001	0.001	0.001

p value for 2-way ANOVA. Interaction term significant as indicated

NS not significant at $p > 0.05$

Table 3 Zinc concentrations (mean ± SD μg/g) in Siberian gull from Hara biosphere reserve, Iran

Specie sex/age	No.	Tissues		
		Kidney	Liver	Pectoral muscle
Siberian gull				
Male/adult	4	129.3 ± 36.1	72.3 ± 7.7	30.2 ± 8.1
Male/juvenile	4	93.2 ± 26.2	89.5 ± 50.7	56.6 ± 40.8
Female/adult	3	90.2 ± 23.9	58.1 ± 11.5	28.6 ± 8.8
Female/juvenile	4	51.3 ± 19.6	53.4 ± 17.6	22.1 ± 10.3
Min–max		28.8–158.1	35.1–147.9	12.4–103.1
Overall mean		91.1 ± 37. 1	68.3 ± 27.8	34.4 ± 23.2
p value sex		0.023	NS	NS
p value age		0.032	NS	NS
p value sex × age		0.001	0.001	0.001

p value for 2-way ANOVA. Interaction term significant as indicated

NS not significant at $p > 0.05$

$p < 0.001$), while the results indicated showed that there were significant differences between the interaction [sex × age] (two-way ANOVA, $p < 0.001$) (Table 4).

Discussion

Cadmium was examined because it concentrates in the marine prey consumed by gulls. Gulls are top trophic level carnivores and are especially susceptible to contaminants because they are long-lived. Therefore, they have been used as bioindicators of environmental contaminants such as metals (Barbieri et al. 2009). DiGiulio and Scanlon (1984) measured cadmium levels in the liver of 13 species of wild ducks. Mean concentrations ranged from 3.1 to 0.64 μg/g dw. Mean cadmium concentrations reported by Barbieri et al. (2009) in the kidney and liver of the *Puffinus gravis* were higher than our own results for Siberian gull. Scheuhammer (1987) reported background levels of cadmium at <3 and <8 μg/g dry weight in liver and kidney, respectively. In this study, the mean concentrations of cadmium in kidney (2.2 ± 0.7 μg/g dw) and liver (1.1 ± 0.2 μg/g dw) were at lower background levels (Table 1). Threshold cadmium concentrations hazards to wild birds have been relatively well established (Rattner et al. 2000), and low cadmium concentrations associated with embryo toxicity and impaired hatching success (Furness 1996). Once absorbed, cadmium is retained in the body and usually only a small proportion is excreted. Cadmium causes sublethal and behavioral effects at lower concentrations than mercury and lead (Eisler 1985). Concentrations of cadmium in the kidney can fall considerably after cadmium induced tubular disfunctions consequent to high exposure (Battaglia et al. 2005). Cadmium levels are often higher in biota near heavily and industrialized sites or near sites with historical evidence of mining pollution than in biota in more pristine areas (Nelson Beyer and Meador 2011). In this study the concentration of cadmium was higher in kidney than liver and pectoral muscle. Concentrations of cadmium in birds are generally reported to be highest in kidneys, lower in liver, and very low in muscle (Thompson 1990). According to Furness and Monaghan (1987), cadmium concentration is always highest in the kidney, where it is thought that a specific metal-binding protein (metallothionein) generally renders the metal harmless. Barbieri et al. (2009) have also reported high accumulation of the metal in the kidney of *Puffinus gravis* than in liver.

Table 4 Statistical Analysis of metal concentrations in the tissues of Siberian gull

	One-way ANOVA					
	Cadmium		Lead		Zinc	
	F value	*p* value	*F* value	*p* value	*F* value	*p* value
Siberian gull	27.35	<0.001	36.32	<0.001	10.88	<0.001

p significance level

Lead has been the most extensively studied metal in birds. Lead background concentrations in wild bird livers and kidney were <6 µg/g dw; exceeded 6 µg/g dw was the range of concentrations associated with exposure to lead, and >20 and >30 µg/g dw was considered a toxic level in kidney and liver, respectively (Clark and Scheuhammer 2003). In this study, lead concentrations in kidney (8.8 ± 2.5 µg/g dw) and liver (5.1 ± 0.8 µg/g dw) were less than the toxic. Evans et al. (1987) found lead concentrations of about 4 µg/g in livers and 7 µg/g in the kidneys of dunlins. Jager et al. (1996) reported that 10 µg/g dw lead in kidneys corresponds to environmental contamination. In this study, kidney lead concentrations were lower than 10 µg/g dw in Siberian gull. Lead concentrations in Siberian gull kidney and muscle from the present study were higher than those in Black-crowned Night Heron and Grey heron from Korea (Kim and Koo 2008) and Common buzzards from Sicily, Italy (Naccari et al. 2009). Lead has been responsible for acute incidents of bird poisoning (Bull et al. 1983). It is emitted into the environment by both natural and man-made sources. Generally, human activities and the developments in petrochemical industry, oil refineries, lead Qeshm factories, Al-Mahdi aluminum factories, and ship transportation in south coasts of Iran may be noted as the prime pollution sources and the main factor in increasing these metals (Mansouri et al. 2011b).

Zinc concentrations in Siberian gull liver from the present study were lower than those in Great knot and Terek sandpiper from Korea (Kim et al. 2009) but higher than those of Black-crowned night heron and Grey heron from Korea (Kim and Koo 2008). Essential elements, such as zinc, are necessary for the metabolism but can however cause adverse effects when their concentration in the organism becomes excessive. Although the mechanisms are incompletely understood, zinc has been shown to be effective in suppressing toxic effects of cadmium (Sandstead 1977) and some of the symptoms of head poisoning (Thawley et al. 1978). Its higher concentration may, impair physiological functions in birds, as well as contributing to a decline in species populations (Ferreira 2010). In this study, levels of zinc were higher in both the kidney and the liver than pectoral muscle. In birds, the threshold level of zinc toxicosis was 1,200 µg/g (Gasaway and Buss 1972). In this study, zinc concentration in Siberian gull was far below the level associated with zinc toxicosis. In general, birds with higher cadmium concentrations are known to show high zinc in tissues as well (Kim et al. 2009). Honda et al. (1990) reported zinc concentrations varied widely among species, depending on the cadmium concentrations. This may have resulted from the induction of metallothionein synthesis by high accumulation of cadmium leading to greater binding of zinc and hence increased zinc uptake for essential cellular functions. Also, Hutton and Goodman (1980), and Kim et al. (2009) reported a relationship between cadmium and zinc levels for kidney and liver of birds.

In regards to sex-dependent and age-dependent metal body burden in birds, there is conflicting data. Several studies in literature indicate a difference in metal concentrations between sexes and ages (Burger 1993; Swaileh and Sansur 2006) while other studies report no differences in metals between sexes and ages (Gochfeld and Burger 1987; Zaccaroni et al. 2003). Many studies have shown that cadmium concentrations are higher in adults than in juveniles or immatures (Lock et al. 1992; Furness 1996). In the other hands, there is gender related variation in the capability of eliminating trace metals. Females can sequester metals in eggs which provides them with a unique route of elimination, not available to males (Burger 1993; Mansouri et al. 2011b). Nevertheless, there is reason to expect that metal levels may vary in those species that have sexual size dimorphism or have differential diets. Some of these differences may reflect differential feeding habits between males and females, or different metal dynamics (Gochfeld 1997). Metallothionein, a metal-binding protein, shows some gender differences that may explain the differences in metal accumulation (Millána et al. 2008). In this study, we did not observe any significant differences between the metal content of kidney, liver, and pectoral muscle (except zinc in kidney and cadmium in liver) from adult and juvenile Siberian gull. These results suggest that there is no age-related difference in the Siberian gull. Also, in the present study, for exception of zinc in kidney, there was no evidence of significant differential accumulation between males and females, this may indicate that both sexes utilise similar foraging strategies (Hindell et al. 1999).

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