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Assessment of Mercury Concentration in Feathers of Six Species of Waterbirds in Southern Caspian Sea Wetlands

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Abstract
We examined concentrations of mercury (Hg) in the feathers of 72 waterbirds belonging to six species. Mercury concentrations (µg g^{-1} dry weight) in the feathers of waterbirds ranged from 0.11 ± 0.03 (Garganey, *Anas querquedula*) to 0.60 ± 0.15 (*Ardea purpurea,* Purple Heron). Significant differences in Hg concentrations between the six studied species were observed (ANOVA, $p < 0.01$); Purple Heron ≥ Common Teal *Anas crecca* ≥ Northern Shoveler *Anas clypeata* = Common Coot *Fulica atra* > Greater Flamingo *Phoenicopterus roseus* = Garganey. Significant differences in the mean Hg concentrations were found among these species as a function of the feeding method and the trophic level ($p < 0.001$). Mercury concentration in the feathers of waterbirds in the present study were below the thresholds reported to impair reproduction.

1. Introduction

Among the heavy metals, Hg is present in different organic and inorganic forms in nature (Fang & Chen 2010; Ochoa-Acuña *et al.* 2002). Mercury residues in aquatic environments can be converted to methylmercury (MeHg) by aquatic biota. Because of the high affinity of MeHg to sulfhydryl groups of proteins, this heavy metal is rapidly incorporated into the food chain, bioaccumulating in aquatic organisms, and biomagnifying from one trophic level to the next (Ochoa-Acuña *et al.* 2002). Therefore, Hg compounds are nerve toxins with cumulative effects that threaten human, fish and wildlife health.

Birds may be exposed to Hg by direct contact or via ingestion of contaminated water or food (Movalli 2000). Therefore, environmental studying the aquatic birds is important with respect to the Hg contaminations. Moreover, the feathers are useful for measuring Hg contamination in birds because birds sequester heavy metals in their feathers, where the proportion of the body burden is relatively constant for each metal (Burger & Gochfeld 2000a, 2000b). Feathers can play the roles of both storing and eliminating metals. Metal levels in feathers

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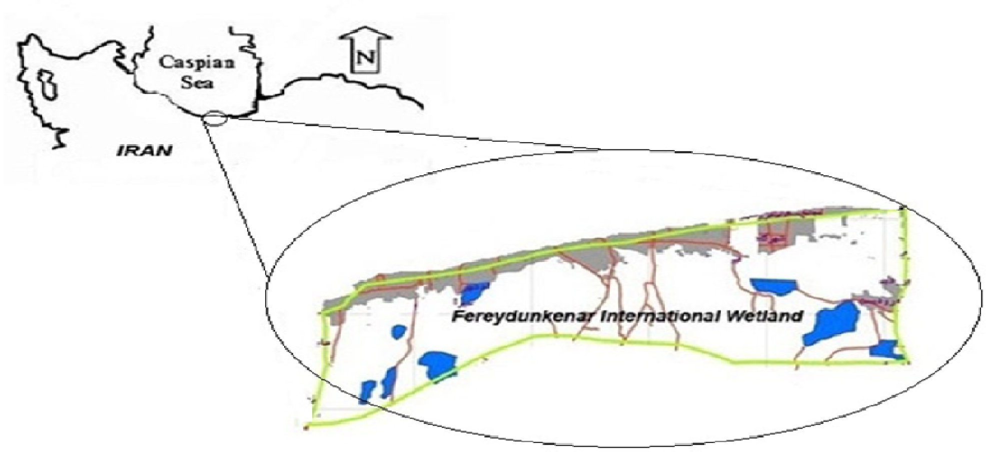

Fig. 1. Geographic position of the Fereydunkenar International wetland in Iran. The blue color shows Fereydunkenar International wetland's logons and areas of collection of samples.

reflect blood levels during the short period of feather growth, when the feather is connected with blood vessels and metals are incorporated in the keratin structure (Dauwe *et al.* 2000). Indeed, feathers can also be used to evaluate external contamination and they thus serve as a useful and a non-destructive tissue for examining exposure in birds.

The aim of this study was to examine Hg concentrations in the feathers of six bird species occupying different levels of the food chain. This study provides the first report on Hg concentration in feathers from Southern Caspian Sea wetlands.

2. Materials and Methods

Fereydunkenar International Wetland of the southern Caspian Sea is composed of Fereydunkenar, Sorkhrud and Azbaran lagoons which were registered in the Ramsar Convention on Wetlands. Fereydunkenar International Wetland (5,427 ha) is located in the southern Caspian Sea (52˚35' to 52˚25' E and 36˚35' to 36˚45' N; Fig. 1). This wetland attracts about one-third of the bird species of Iran (150 species) (Ahmadpour *et al.* 2011). In this area, farmers use chemical fertilizers and pesticides, especially fungicides for planting rice. These practices may have caused an increase in exposure to toxicants and heavy metals in plants and animals of the area.

Bird samples were collected in autumn 2012 throughout the Fereydunkenar International Wetland. Feathers were collected from 72 birds belonging to six species (Table 1). Then the two inner (P 1–2) and the two outer (P 9–10) primary feathers of the right wing were collected from each bird, placed in labeled envelopes, and stored in a light-inhibiting box until transportation to the environmental laboratory of the Faculty of Natural Resources and Marine Sciences for analysis. Before sample preparation, feather samples were washed with tap water, then rinsed with distilled water (3 times) alternating with acetone (3 times) to remove loosely adherent external contamination loosely (Zolfaghari *et al.* 2007). The feather samples were dried in an oven at 60 °C for 24 h (Dauwe *et al.* 2005). Finally, they were cut into pieces of approximately 1 mm and stored in a desiccator until chemical analysis.

The procedure used for measuring Hg concentrations in feathers samples has been described previously (Burger 2002; Dauwe *et al.* 2005), and we made only minor modifications. Between 50 and 100 mg of the homogenized powder of each dried sample was added to 8 ml of concentrated (65%) supra-pure HNO_3 (Merck, Darmstadt, Germany) in a closed cell, polytetrafluoroethylene (Teflon™) lined digestion vessel and incubated for 1 h at 40 ^{0}C in a hot block digester and then the temperature increased to 100°C for 2 h. Samples were then left to cool for half an hour, after which 2 ml of H_2O_2 (30%, Merck, Darmstadt, Germany) was added, and the beaker was returned to the hot block for another hour until any precipitation was fully dissolved. Upon cooling, 5% potassium permanganate ($KMnO_4$) was added to ensure oxidation of all organic mercury compounds. The samples were then heated at 90°C for another 30 min. The samples were cooled and each sample was raised to 25 ml by addition of distilled water. Hydroxylamine hydrochloride was added to reduce excess oxidizing reagents.

The quality assurance and quality control procedures were assessed using standard solution. To read the samples, first the following solutions were prepared: Standard Hg Solutions with concentrations of 100, 200 and 300 ppb (that was prepared from 1000 ppm Standard Hg solution). In order to calibrate the machinery; Nitric acid 1.5% weight in volume (W/V) for preparing standard solutions; Potassium permanganate 5% (KMnO4) for maintaining and fixing the Standard solutions; Mixing the caustic soda solution 1% weight in volume (W/V) with sodium boron hydrate 3% weight in volume (W/V) (as regenerative) for reacting (in the reaction flask) and releasing Hg as vapor form from samples.

The elemental Hg transported into the quartz cell by argon and its concentration were measured in each sample by using atomic absorption tool, the Perkin-Elmer model (AA 700) according to the method of cold vapor technique (United States Environmental Protection Agency 1998). Recoveries of the Hg ranged from 88.6% to 99.2%. In this study, Hg concentrations are expressed as $\mu g\ g^{-1}$ dry weight.

Statistical analyses were carried out using SPSS ver. 18.0. All data were tested using Kolmogorov-Smirnov's test to see whether they fit into a normal distribution. Data were analyzed using parametric procedures after transformed to $\log_{10}$ of Hg concentrations. Duncan's method, along with one-way analysis of variance (ANOVA) was used to evaluate the effects of inter-species affiliation and trophic levels on feather Hg concentrations. A value of $p < 0.05$ was considered statistically significant.

3. Results

Mercury concentrations are shown in Table 1 as means, standard deviation and range on dry basis $\mu g\ g^{-1}$ dry weight. Mercury concentrations in feathers of birds from Southern Caspian Sea wetlands ranged from 0.11 ± 0.03 (Garganey, *Anas querquedula*) to 0.60 ± 0.15 $\mu g\ g^{-1}$ dry weight (Purple Heron, *Ardea purpurea*). There was a significant difference among these species (ANOVA, $p < 0.01$); *post hoc* testing revealed Purple Heron ≥ Common Teal *Anas crecca* ≥ Northern Shoveler *Anas clypeata* = Common Coot *Fulica atra* > Greater Flamingo *Phoenicopterus roseus* = Garganey (Fig. 2). The Purple Heron had significantly higher levels of feather Hg than the other species ($p < 0.01$), ranging from 1.54 to 5.45 times higher than the other species. The Common Teal, Common Coot and Northern Shoveler had intermediate values and the Greater Flamingo and Garganey contained the least concentration of feather Hg.

We also compared differences in feather Hg concentrations by trophic levels (Fig. 3). To examine the effect of trophic levels on Hg concentrations, these six bird species were divided in three groups according to their dietary habits: piscivore (Ardeidae), omnivore (Gruidae and Anatidae) and omnivore-Filterfeeding (Phoenicopteridae). There was a significant difference in Hg concentration among these three groups ($p < 0.001$). The mean Hg concentrations in these three trophic levels decreased in the following order: Piscivore > Omnivore ≥ Omnivore (Filterfeeding) ($p < 0.001$) (Fig. 3).

Table 1. Mercury concentrations (μg g^{-1} dry weight) in feather samples of birds of different trophic levels in the Southern Caspian Sea's Wetlands

Bird family [a]	Bird species: Common name	Scientific name	*n*	Type of food (Trophic level)
Gruidae	Common Coot	*Fulica atra*	21	Omnivore
Anatidae	Northern Shoveler	*Anas clypeata*	6	Omnivore
	Garganey	*Anas querquedula*	6	Omnivore
	Common Teal	*Anas crecca*	24	Omnivore
Ardeidae	Purple Heron	*Ardea purpurea*	9	Piscivore
Phoenicopteridae	Greater Flamingo	*Phoenicopterus roseus*	6	Omnivore (Filter-feeding)

[a] Handbook of the Birds of Europe the Middle East and North Africa

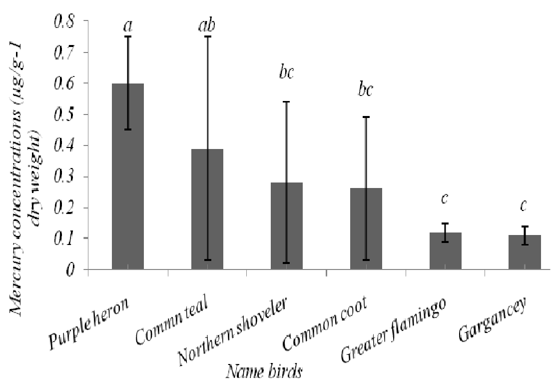

Fig. 2. Comparison of feather Hg concentrations among species. Concentrations with different letters are significantly different ($p < 0.01$, Duncan's method, along with one-way ANOVA). Each point and bar indicates the mean and standard deviation (S.D), respectively

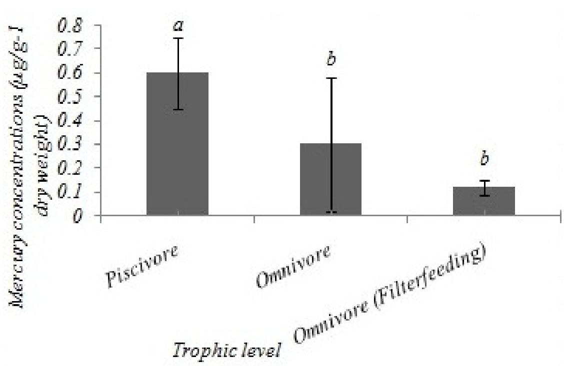

Fig. 3. Effect of trophic level on feather Hg concentrations. Concentrations with different letters are significantly different ($p < 0.001$, Duncan's method, along with one-way ANOVA). Each point and bar indicates the mean and standard deviation (S.D), respectively.

4. Discussion

4.1. Intra-specific comparisons

The Hg concentration in birds can vary between species (Wagemann & Muir 1984) due to differences, molting pattern, season of molting, migration (Thompson & Furness 1989), trophic level and use of different environments (Kim *et al.* 1996). Moreover, it may vary due to differences in physiology between the species; metabolic rates vary inversely with body weight and directly with activities, such as flight and rest (Teal 1969; Welty 1975; Deng *et al.* 2007). Because, Common Teal, Northern Shoveler, Common Coot and Garganey have smaller body size than the Purple Heron (Mansoori 2008), they are expected to have a greater metabolic rate. Greater metabolic rates may cause more rapid accumulation of Hg in birds, though it can also cause a bird to eliminate Hg more rapidly. Our data, in general, support these hypotheses because the concentration of Hg was greater in the Purple Heron than other birds. Molting plays a major role in Hg concentration in birds. When molting is completed, and birds feed on contaminated food, levels of Hg in organs will increase until the next molting period (Dauwe *et al.* 2003; Mansouri *et al.* 2012). All the birds in this study have a slower rate of molting but the Purple Heron molts in winter whereas the Common Teal, Northern Shoveler, Common Coot and Garganey molt in summer. Hence (considering the sampling time in autumn), the Purple Heron had higher levels of Hg in feathers compared with other birds. Also, the difference in the migration chronology of the Purple Heron and other birds in this study, and their use of different emigrational pathways could contribute to the differences in Hg concentration in Purple Heron compared with other birds (Kim *et al.* 1996). The migration of the Purple Heron from the south-west of Iran to the southern Caspian Sea occurs in late spring and it is present in the region until early autumn (Mansoori 2008; Behrooz *et al.* 2009). But the other birds we examined migrate in late summer from northern latitudes to the southern Caspian Sea and are present in the region until early spring. So, considering the sampling time, with Purple Herons in the area longer and using different stopover sites during migration, it is reasonable to presume these account for some of the difference in Hg concentrations in the

Purple Heron compared with the other species we examined.

In addition, one of the main reasons for the differences in Hg levels in feathers of birds can be their different trophic levels. Differences in dietary habits may explain most of the variation in the pollutant levels among different bird species (Behrooz *et al.* 2009). In this study, the highest Hg concentration was found in the Purple Heron. The Purple Heron occupies a higher trophic position and feeds predominantly on fish and amphibians, compared to omnivorous birds in this study, which occupy lower levels in food chain, eating primarily plants and invertebrates (insects and worms) (Mansouri *et al.* 2012; Azami *et al.* 2012). Concentrations of Hg in the Greater Flamingo were lower than most birds (see Figs. 2-3). Because they feed mostly on tiny plants and invertebrates in the sediments of wetland (filter-feeding), they are on the lower level of the food chain, and they typically live in ecosystems where they are less affected by anthropogenic pollutants (Zolfaghari *et al.* 2007; Mansoori 2008; Behrooz *et al.* 2009).

4.2. Inter-specific comparisons

In the present study, interspecific differences in Hg concentration was observed within a trophic level. The highest and the lowest Hg concentrations in omnivores were observed in the Common Teal and Garganey, respectively. The Northern Shoveler and Common Coot had the intermediate levels of Hg concentration. The difference in Hg concentration between Common Teal and Garganey Could be related to sex and age differences in these two species (Burger 1993; Kalisinska 2003; Houserova *et al.* 2005; Swaileh & Sansur 2006), as we could not separate the birds according to their age and sex. Mercury concentration in feathers was similar among Common Teal, Northern Shoveler and Common Coot (Fig. 2). We had predicted that larger species could eat larger food items and therefore would accumulate higher levels of Hg in their organs. All of the birds omnivorous species we examined, predominantly eat seeds of aquatic plants and invertebrates (insects and worms). However, the pattern of larger body size = higher Hg body burden was not sustained in the current study where the smallest species had higher Hg levels than the other omnivorous species. Two considerations may explain this finding. First, the lack of relationship between body size and Hg levels may be due to the fact that larger species did not necessarily eat larger prey items. Burger & Gochfeld (2000c) reported that despite eating larger food, there was not any clear relationship between metal levels in feathers and body size. Second, Common Teal migrate to the area earlier than other species and thus may be exposed for a longer period.

4.3. Risk assessment and potential adverse health effects

The mean Hg concentration reported in the present study (0.29 µg g^{-1} dry weight) was lower than reported in feathers of 180 species (2.1 µg g^{-1}, Burger 1993), 18 species of birds in southwest Iran (0.87 µg g^{-1} , Zolfaghari *et al.* 2007); Chilean birds (1.7 µg g^{-1} , Ochoa-Acuña *et al.* 2002); feathers of 18 species in southern Georgia (0.36 µg g^{-1}, Becker *et al.* 2002); and breast feather of 12 species in Midway Atoll (3.53 µg g^{-1}, Burger & Gochfeld 2000c).

Mercury levels of 5 to 65 µg g^{-1} dry weight (Burger & Gochfeld 1997) in feathers are associated with sublethal and reproductive effects. Mercury can also reduce food intake leading to weight loss, progressive weakness in wings and legs with incoordination and difficulty in flying, walking, and standing. In the present study, the Hg levels in the feathers of all birds were not within the effective range of 5 to 65 µg g^{-1} dry weight to cause reproductive effect. It has been suggested that the range for adverse effects in piscivorous waterbirds be 9–20 µg g^{-1} Hg in feathers (Ochoa-Acuña et al 2002). None of the specimens we examined had Hg concentrations within these thresholds.

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