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Total and Organic Mercury in Liver, Kidney and Muscle of Waterbirds from Wetlands of the Caspian Sea, Iran

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Abstract We measured and compared total and organic mercury in liver, kidney, and muscle of the Great Cormorant (*Phalacrocorax carbo*), mallard (*Anas platyrhynchos*), and coot (*Fulica atra*) from the Caspian Sea wetlands in Iran. For the Great Cormorant organic mercury in liver, kidney and muscle comprised 82 %, 79 % and 58 % of total mercury. In the mallard same values were 46 %, 54 %, and 64 %. For coot total mercury was: 0.1 ± 0.0, 0.1 ± 0.01, 0.03 ± 0.01 in liver kidney and muscle respectively. We detected no organic mercury. In general older birds that feed on higher trophic levels can accumulate more mercury in their tissues.

Keywords Total mercury · Organic mercury · The Great Cormorant · Coot · Caspian Sea · Iran

Mercury (Hg) contamination of the environment has been progressively worsening during the past one and half centuries. Mercury is primarily a neurotoxicant and a nephrotoxicant. In lab animals, Hg carcinogenicity and mutagenicity has been reported (Schurz et al. 2000). Surprisingly, despite the seriousness of the worldwide Hg pollution and high toxicity of this compound, no international legally-binding agreements exist to curb Hg release. Consequently, Hg is now a growing public health concern especially in areas where the population depends heavily on food from the sea (Gerstenberger 2004). Organic forms of Hg (e.g. methyl-Hg) are recognized as contaminants that bioaccumulate in aquatic ecosystems. Organic mercury has been linked to adverse health effects in top carnivores (Jæger et al. 2009). Methylated Hg is the most toxic form of Hg and methylation is done by bacteria in the sediment. Therefore, aquatic ecosystems are most susceptible to methyl-Hg contamination since they are home to large active populations of methylating bacteria (Loseto et al. 2004). We now know that methylation can occur at a faster rate in acidic and oxygen depleted waters which contain more methylating microorganisms.

Wetlands are considered as ''hot spots'' for production of organic forms of Hg since they contain dissolved organic matter and humic acid. Anzali Wetlands (37°28′N, 49°25′E) are an area of about 200 km^2 (Fig. 1). Anzali, and other area wetlands, have been acknowledged for the important role they play in ecology of migratory birds globally. Over a hundred species of waterbirds and twenty species of fish have been spotted in these wetlands. Industrial, agricultural and domestic discharges enter the Eastern part of the Anzali which supports a variety of plant-life (water depth between 0.8 and 1.5 m). Approximately, six million cubic meters of polluted water enter Anzali. The physicochemical parameters of these waters are not regularly monitored (Jafari 2009) which can increase nutrient load and metal content of the Anzali. Temperatures of Anzali range from 4.5°C in February to 27.5°C in August. Gomishan Wetlands (54°53′N, 37°9′E)

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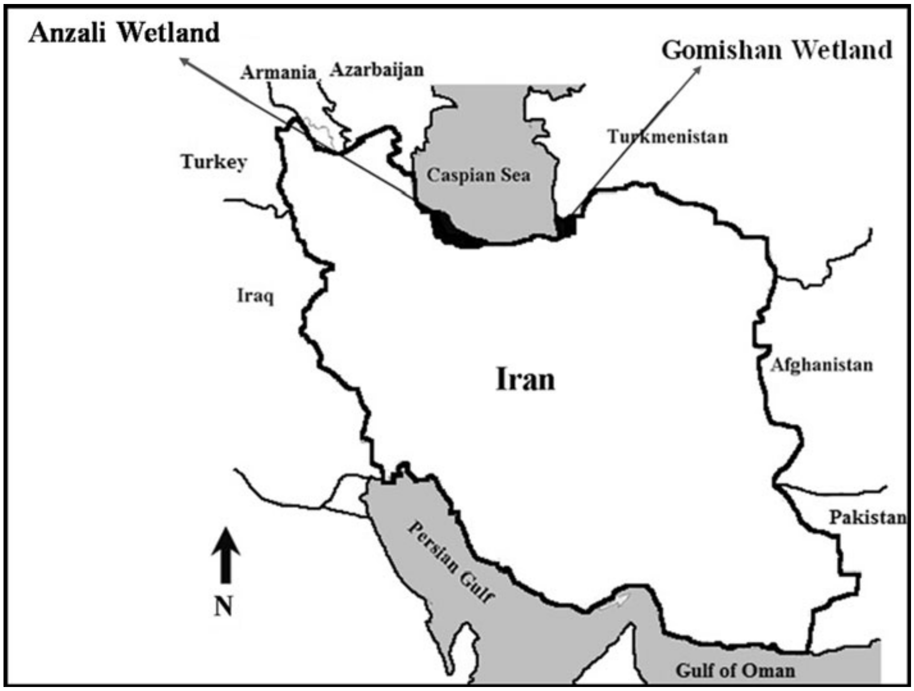

Fig. 1 Sampling sites for the Great Cormorant (*Phalacrocorax carbo*), mallard (*Anas platyrhynchos*), and coot (*Fulica atra*) along the south coast of the Caspian Sea in Iran. The two studied wetlands (Anzali and Gomishan) are indicated in *black*

consist of a series of narrow, brackish lagoons that lay on the eastern side of the Caspian Sea. Gomishan wetlands extend to Turkmenistan where it becomes a protected area for wildlife conservation. The average depth of Gomishan is 100 cm (max 250 cm) and the temperatures range between 4.5°C in February to 30.5°C in July. East to Gomishan is a vast area of low-lying plains with salt-tolerant vegetation. The recent rise of almost 2 meters in the Caspian Sea level has flooded these plains and increased the size of Gomishan to ~200 km^2.

Wetlands affect concentration, speciation, and transport of Hg (Burger 1993). Normally, methyl-Hg amounts to <1 % of the Hg in marine and freshwaters. But since methyl-Hg is the form that bioaccumulates in the food web, ≥95 % of the total mercury in top predators is in methylated form (Wiener and Spry 1996). Trophic-level relationships have the most serious effect on Hg in organisms. Species that occupy higher trophic levels accumulate more Hg (Siegers et al. 2011). It is hypothesized that, in birds, feather-molt and egg production eliminate toxic methyl-Hg from the body. The Great Cormorant, mallard, and coot are common waterbirds with varying diets that occupy different ecological niches. Great Cormorant is a carnivore that feeds on fish. The mallard and coot, are omnivorous. The cormorant is highest on the trophic scale, and the mallard and coot occupy lower positions. In general, species that are higher on the food chain accumulate higher levels of contaminants (Siegers et al. 2011). Current literature on Hg in waterbirds tissues suggests that the inorganic Hg, from demethylation in the liver, remains in the internal tissues (Bond and Diamond 2009b). This hypothesis has been tested in some studies, however, additional studies across more species, and comparisons with percentage organic-Hg in internal tissues, are required to validate the theory that inorganic Hg remains in the internal tissues. Selecting bird species with significantly different diets would allow examining differences in Hg accumulation in internal tissues; which is suggested to be an ideal way for determining efficiency of demethylation (Monteiro and Furness 2001). We aimed to examine the proposed hypothesis that the immobile inorganic Hg from demethylation remains in the internal tissues and tried to relate that information to the bird's diet.

Materials and Methods

In March 2009, under license of Environmental Protection Agency of Gilan and Gorgan, a total of 51 birds were randomly shot and removed from the two wetlands. Specimens from Anzali (n = 23) and Gomishan (n = 28) included Great Cormorant (n = 18); mallard (n = 18); and coot (n = 15). Birds were immediately transported to the laboratory and their weigh and length were recorded. Sexual dimorphism in mallards allowed phenotypic identification of the sexes. In the Great Cormorant and coot, sex was determined by identifying the sex organ during dissection. Bird carcasses were dissected and sexed by identifying the sex organ; ovaries and testes that are located on top of the kidneys were identified by gonad inspection. On the same day, liver, kidney, and pectoral muscle, were removed and kept in clean plastic bags at −20°C for later use. Sample preparation followed Spalding et al. (1994) with some modifications. All soft tissues were weighed once before drying and wet weight was recorded. Then, samples were placed in freeze-dryer for 48 h to dry because the mercury analyzer requires dry material for analysis. Dry samples were then homogenized by Polytron Homogenizer for uses in mercury analyzer. Values are reported in mg/kg wet weight.

Concentration of total mercury in homogenized samples was determined by the LECO AMA 254 Advanced Mercury Analyzer (USA). The detection limit of the instrument was between 5 µg/kg and 5 mg/kg. In order to assess the analytical capability of the method, 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. Recovery varied between 94.8 % and 104 %. The detection limit of the method used was 0.001 mg/kg in dry weight. The result of total Hg for comparing with organic Hg was calculated on the basis of wet weight.

For determination of the organic mercury, we used the method of Carbonell et al. (2009) with some modifications. Briefly, 0.5 g of wet tissue was hydrolyzed with 10 mL of HCl (37 %). Sample was shaken on a vertical shaker for 5 min, and then 10 mL of toluene (99.9 %) and 0.5 g NaBr was added. All samples were allowed to shake for another 20 min, and then centrifuged at 2,400 rpm for 20 min. The supernatant, containing organo mercury species was collected in glass tubes. The combined organic extracts were subjected twice to back extraction with 6 mL of 1 % (v/w) L-cysteine aqueous solution to strip organic mercury from toluene. Then, 200 µL of aqueous phase was immediately analyzed with advanced mercury analyzer (AMA 254; EPA. 1998). For preparation of L-cysteine solution 1 % (v/w) 1 g of L-cysteine, 0.78 g of sodium acetate, and 12 g of sodium sulfate, were dissolved in 100 mL of distilled water. Accuracy and precision of the above mentioned method was tested against with standard sample of methyl mercury chloride (Sigma-Aldrich Co). Recovery varied between 98.2 % and 101.8 %.

Social Sciences software package (SPSS version 17) was used for all statistical analysis. Data were tested for normality using Shapiro–Wilk's test. Data were normally distributed; therefore a parametric test was used for analysis. ANOVA was used for comparison of mercury concentration (total and organic) among different tissues and the same comparison in different species. Pearson correlation and linear regression were used to determine relationship between organic and total Hg in different tissues. Mercury concentrations in liver, kidney, and muscle were tested for mean differences between species using Mann–Whitney U test. Probability level of $p \leq 0.05$ was set to indicate statistical significance.

Results and Discussion

Table 1 summarizes organic and total-Hg concentrations (mg kg^{-1} wet weight) in tissues of the Great Cormorant (*Phalacrocorax carbo*), mallard (*Anas platyrhynchos*), and coot (*Fulica atra*) from Anzali and Gomishan wetlands of the Caspian Sea in Iran. Organic mercury was below the limit of detection in the coot, the mean percent organic mercury to total mercury in the Great Cormorant was liver > kidney > muscle. The ratio for the mallard was muscle > kidney > liver (Fig. 2). High methyl mercury concentrations in birds like cormorants that feed high on trophic levels, lead to high kidney lipid content and consequently higher methyl mercury levels in the liver. However, birds that feed on lower trophic level, lipid is more concentrated in the muscle tissue. Similar finding in fish have been reported by Kannan et al. (1998). Organic Hg levels were significantly correlated with total mercury concentrations in both species (r = 0.82, $p = 0.05$). The slope of the regression of total against organic Hg, representing the percentage methyl mercury, was positive. In the Great Cormorant (r = 0.89, $p = 0.05$, slope of the

Table 1 Total and organic mercury (mg kg^{-1} wet weight) in liver, kidney and muscle of the Great Cormorant, mallard and coot from Anzali and Gomishan wetlands of the Caspian Sea in Iran

Tissue	Species	Total-Hg Mean ± SEM	Organic-Hg Mean ± SEM
Liver	Great Cormorant (n = 15)	5.67 ± 0.23	4.64 ± 0.19
	Mallard (n = 18)	0.28 ± 0.03	0.13 ± 0.01
	Coot (n = 15)	0.01 ± 0.09	Limit of detection
Kidney	Great Cormorant (n = 15)	0.58 ± 3.59	0.45 ± 2.85
	Mallard (n = 18)	0.26 ± 0.03	0.14 ± 0.02
	Coot (n = 13)	0.08 ± 0.01	Limit of detection
Muscle	Great Cormorant (n = 15)	2.26 ± 0.53	1.32 ± 0.32
	Mallard (n = 18)	0.01 ± 0.11	0.01 ± 0.07
	Coot (n = 15)	0.01 ± 0.03	Limit of detection

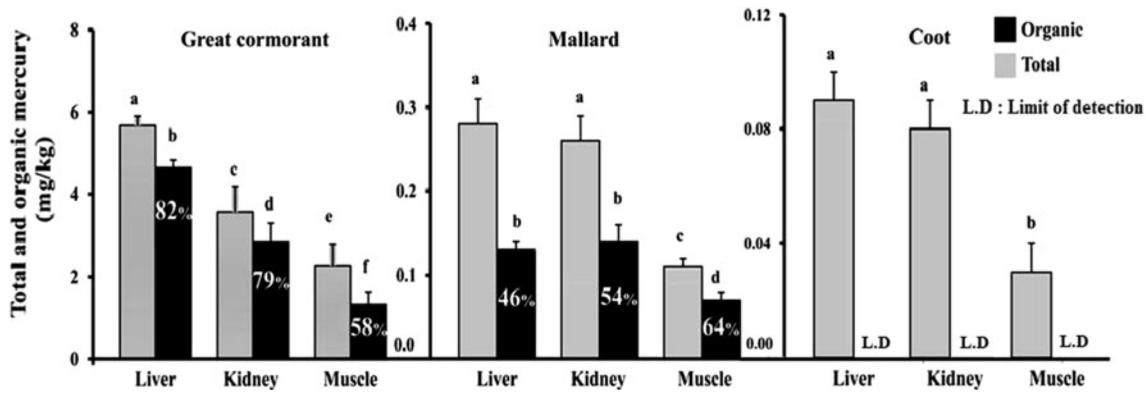

Fig. 2 *Bar graphs* indicate a comparison between total and organic mercury (mg kg^{-1} wet weight) in liver, kidney, and muscle of the Great Cormorant, mallard, and coot from Anzali and Gomishan wetlands of the Caspian Sea in Iran. *Different letters* indicate significant differences at $p \leq 0.5$

regression line = 0.79), and mallard (r = 0.75, $p = 0.05$, slope of the regression line = 0.56) we found a significant relationship when species were examined individually. Percent of methyl-Hg ranged between 82 % in the liver of the Great Cormorant and 46 % in mallard liver (Fig. 2). There were significant differences in the amount of organic mercury among species. Independent samples T Test gave this result (F = 47.2, $p = 0.00$). We found a significant positive correlation between weight and length in the Great Cormorant ($R^2 = 0.85$, $p = 0.00$) and mallard ($R^2 = 0.48$, $p = 0.01$). In the coot there was a significant negative correlation ($R^2 = 0.07$, $p = 0.34$; Table 2; Fig. 2). Several factors have exacerbated the widespread contamination of the world's ecosystems with mercury compounds. They include continuous release of large quantities of Hg into the environment, and absence of any legally-binding agreements to curb Hg release. As a result, worldwide Hg is a growing public health concern. Our interest in Hg in birds of the Caspian originated from the fact that many of these birds are consumed by the local people and consumption of contaminated food can have significant effect on public health in the area. Many studies have indicated that bioaccumulation of Hg in top consumers, including humans; can lead to physiological adverse effects including neurological and reproductive impairments (Rutkiewicz et al. 2011). Current literature suggests that Hg accumulates in liver and kidney of waterbirds. Our study also corroborates with these earlier findings. Mercury was significantly higher in liver followed by kidney and muscle tissue of the three birds we examined. Similar to previous findings, we found a relationship between trophic level and Hg in internal tissues. The coot, feeding on the lowest trophic level, had no detectable methyl-Hg and total Hg was primarily found in liver and kidney. Cormorant and mallard that feed higher on the trophic chain had significantly more Hg.

Table 2 Correlations between total and organic mercury in muscle, kidney, and liver of the Great Cormorant and mallard from Anzali and Gomishan wetlands of the Caspian Sea in Iran

Values	Species		
	Muscle	Kidney	Liver
Great Cormorant			
Correlation			
r	0.48	0.72	0.68
p	0.03	0.01	0.02
Mallard			
Correlation			
r	0.75	0.82	0.89
p	0.01	0.01	0.00

Birds of this study were collected at the end of wintering period (end of February). No molting occurs at this time. Molting is the most effective way of reducing Hg body burden in birds. In addition, these birds had been spending the winter months with relatively low activity in these wetlands. Low activity reduces metabolism; increased metabolism can reduce metal body burden (Kannan et al. 1998). Consequently, the levels of Hg we report in these birds must approach the maximum possible at this time in these species. Muscle contained the least organic Hg in all birds; this can be attributed to high levels of oxygen in the muscle tissue as a result of bird's inactivity during winter months. We found a significant positive correlation between bird's length and weight in both cormorant and mallard ($r^2 = 0.85$; $r^2 = 0.48$), but in the coot this correlation was negative ($r^2 = 0.07$); suggesting that larger birds are a better indicative of such correlations. In general, current literature supports the hypothesis that older birds that feed on higher trophic levels can accumulate more mercury in their tissues. No other associations were found in this study. To put the Hg pollution in the wetlands of the Caspian Sea in Iran, into a global perspective, we compiled

Table 3 A review of the current literature on organic and total mercury levels in muscle, liver, and kidney of birds from other geographical locations in comparison to this study (organic Hg/total Hg*100 is the value in the column depicted as "organic/total %")

Species name		Hg in muscle		Hg in liver		Hg in kidney		References
Scientific	Common	Total	Organic/total %	Total	Organic/total %	Total	Organic/total %	
Podiceps cristatus	Great crested grebe	2.3	86	7.2	76	4.7	53	Houserova et al. (2007)
Phalacrocorax carbo	Great Cormorant	3.4	31	42.2	42	7.2	40	
Phalacrocorax brasilianus	Neotropic cormorant	1.5	47	–	–	–	–	Ruelas-Inzunza et al. (2009)
Anas clypeata	Shoveler	0.5	100	–	–	–	–	
Pelecanus occidentalis	Brown pelican	2.8	94	–	–	–	–	
Anas discors	Blue-winged teal	0.8	26	–	–	–	–	
Aythya affinis	Lesser scaup	1.1	61	–	–	–	–	
Clangula hyemalis	Oldsquaw	1.5	100	31.4	7	6	63	Kim et al. (1996)
Diomedia epomophora	Royal Albatross	2.4	46	58.1	19	14.9	24	
Diomedea nigripes	Black-footed Albatross	15.1	46	309	7	37.4	17	
Diomedea immutabilis	Lysan Albatross	–	–	38.9	30	–	–	
Procellaria aequinoctialis	White-chinned Petrel	2	45	29	28	18.8	23	
Fulmarus glacialis	Northern Fulmar	1.4	64.3	14	22	6.7	25	
Sula leucogaster	Brown Booby	3.8	76	7.2	51	6.5	55	
Larus argentatus	Herring Gull	0.8	75	4.2	29	3.6	36	
Sterna paradisaea	Arctic Tern	0.9	100	4.9	27	3.6	58	
Phalacrocorax carbo	Great Cormorant	2.7	58	5.7	82	0.6	79	This study
Anas platyrhynchos	Mallard	0.01	64	0.28	46	0.28	54	
Fulica atra	Coot	0.01	L.D	0.01	L.D	0.01	L.D	

L.D limit of detection

Table 3. Table 3 contains a review of the current literature on organic and total mercury levels in muscle, liver, and kidney of birds from other geographical locations (Organic Hg/Total Hg*100 is the value in the column depicted as "organic/total %"). Total Hg in our study sites seem to be lower than what has been reported elsewhere.

Confirming previous findings that trophic level in birds affects Hg body burden; we found that the Great Cormorant and mallard, which occupy higher trophic levels, had higher Hg than the coot. Some waterbirds are thought to be able to demethylate organic mercury into less toxic inorganic form (Scheuhammer et al. 2007). Inorganic Hg remains in the liver since it is nonmoving but the toxic form is excreted during feather molt. To validate the theory that "the inorganic Hg remains in the internal tissues", measuring Hg concentrations in internal tissues of more species of waterbirds has been emphasized in the literature (Bond and Diamond 2009a). Essentially, most Hg found in liver, kidney, and muscle was organic (L: 82 %–46 %, K: 79 %–54 % and M: 58 %–64 %) was organic; with no interspecific differences in percentage organic Hg. Regression models which were analyzed to detect if there are any correlations between weight, length, total and organic Hg in liver, kidney, and muscle, indicated no significant correlations. We found no significant correlations between these parameters. There was also no interspeific differences in percentage organic Hg in birds of this study.

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