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Full Length Research Paper

Seabirds, *Anas crecca* and *Ringa Solitaria* as bioindicators of mercury and methyl mercury contamination, from Arvand River, border between Iran and Iraq

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Seabirds have been used extensively as bioindicators of mercury (Hg) and methyl mercury (MMHg) contamination in the marine environment, although information on seabirds in Arvand river remains limited. In this study, mercury and methyl mercury levels were determined in two bird species from Arvand river, located in the Khuzestan province in the lowlands of southwestern Iran at the head of the Persian Gulf. The order of mercury and methyl mercury concentrations in tissues of bird species were as follows: feather > liver > kidney > muscle. In the migratory bird Iran and Iraq (*Anas crecca*), highest value of mercury 5.2 ($\mu g\ g^{-1}$) and methyl mercury 3.4 ($\mu g\ g^{-1}$) were detected in feather tissue. In the resident bird of Iran (*Ringa Solitaria*), highest value of mercury 4.3 ($\mu g\ g^{-1}$) and methyl mercury 3.1 ($\mu g\ g^{-1}$) were detected in liver tissue. There were significant correlation between mercury and methyl mercury levels and bird size were positive. Comparison between male and female indicated that the average mercury and methyl mercury concentrations in tissues of female birds were found to be significantly higher than those found in the male birds. Also, higher mercury and methyl mercury levels were in tissues of migratory bird species (*Anas crecca*), because mercury levels in these tissues is a reflection of diversity in food items from wider geographical locations.

Keywords Mercury, Methyl mercury, Seabird, Bioindicator, Arvand river

INTRODUCTION

Arvand river located at the head of the Persian Gulf near the citied of Abadan and Khoramshahr in southwestern Iran (Figure. 1). The Arvand river, the border between Iraq and Iran, is the biggest river in the Persian Gulf. It passes three main cities including Al-Basre in Iraq, Abadan and Khoramshahr in Iran. This river is formed by the confluence of Shatt al-Arab in Iraq and Karoon river in Iran. In addition to receiving effluents of more than seven big and small Iranian and Iraqi cities, there are many non-pointed and pointed metals sources along its course (Abdolahpour Monikh *et al.,* 2012; Hosseini *et al.,* 2013). This river is surrounded by many petrochemical units such as Abadan

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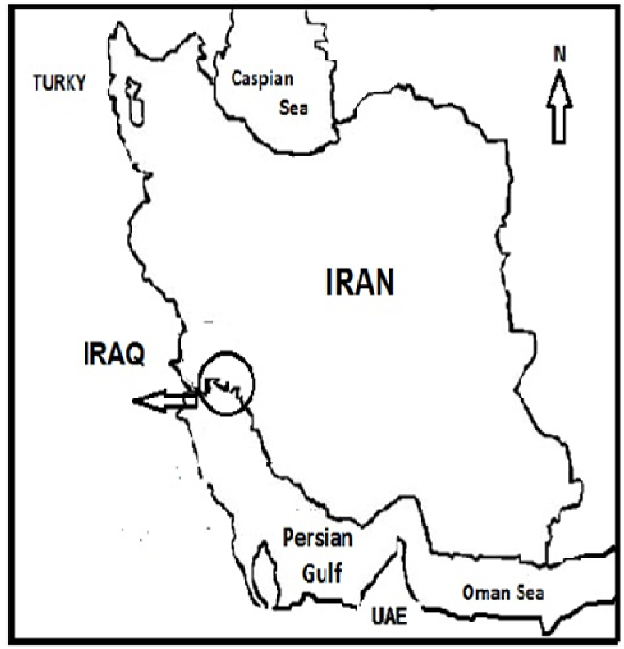

Figure 1 Map of Persian Gulf and study area

petrochemical complex and petroleum refinery. In addition, the Arvand river carries about 48 tons of oil residues to the northwest of the Persian Gulf annually. Other sources of pollution in this area, including wars and invasions, are yet to be methodically investigated (Hosseini *et al.,* 2013).

Mercury is a natural trace component of the aquatic environment and its accumulation in aquatic organisms and is one of the most toxic metals in the environment .The relative toxicity of mercury depends on its chemical form, methyl mercury being one of the most toxic substances existing in the environment. Methyl mercury is created by bacteria in highly organic portions of aquatic systems, such as the sediment of river and wetlands. Methyl mercury is taken up by organisms through the food chain and biomagnifies, resulting in markedly high Hg concentrations in top predators (Hosseini *et al.,* 2015). Methyl mercury is a concern, as it acts as a neurotoxin and can have deleterious effects on organisms (Burger and Gochfeld, 1991). A major concern with MeHg is that it can cross the mammalian placenta and is also incorporated into bird eggs during their formation, posing developmental risks to vertebrate young during critical periods of development (Burger and Gochfeld, 1991). Thus, Hg can affect organisms directly exposed to Hg pollution and, through biomagnification, can affect organisms that are higher up the food chain as well as their future off spring (Baron *et al.,* 1997). Given the potential toxicity of Hg, monitoring of this trace metal in the environment is critical and of international importance.

Since the 1970s, marine birds have served as useful bioindicators for monitoring Hg concentrations and biological (Zolfaghari *et al.,* 2008). The ecology of marine birds is well studied, which has allowed documentation of patterns of Hg bioaccumulation across food webs and trophic levels (Campbell *et al.,* 2005), and at different spatial and temporal scales (Sterry *et al*., 2001). Such studies are possible because marine birds are abundant, widespread, and part of planned sustainable harvests allowing for relatively easy and ethical sampling to occur (Thompson and Furness 1989). Importantly, studies of marine birds offer a number of useful endpoints, including reproductive output in relation to Hg burden at the individual level to changes in demography over periods of decades (Mallory and Braune, 2012). Although marine birds are likely exposed to many different forms of Hg (i.e., elemental Hg, MeHg, etc.), studies indicate that at least 95% of the total Hg in seabirds is MeHg, allowing total Hg to be a useful indicator of the more toxic organic form (Ruelas-Inzunza and Paez-Osuna, 2004).

As birds are ordinarily at the top of the food web, they are valuable for environmental monitoring. Fish-eating birds, as top level carnivores, often have high levels of contaminants (Baron *et al.,* 1997). Mercury accumulation in many piscivorous birds has been frequently reported (Thompson and Furness, 1989; Burger and Gochfeld, 1991; Kim *et al.,* 1996; Monteiro *et al.,* 1999; Saeki *et al.,* 2000; Nam *et al.,* 2005; Houserova *et al.,* 2007) but determination of mercury in birds such as kingfishers is

Table 1 Scientific name , feeding habitat, sex and weight (Mean ± SE g) of the *A. crecca* and *R. Solitaria*

Scientific name	Feeding habitat	Sex	n	Weight
Anas crecca	Piscivorous: consumes mostly fish, also some invertebrates	Females	5	158 ± 5.18
		males	6	131 ± 2.54
Ringa Solitaria	Omnivorous: consumes fish, molluscs, crustacean, and some algae	Females	7	159 ± 1.08
		Males	6	125 ± 0.23

rare and less common (Baron *et al.,* 1997; Zamani *et al.,* 2008; Hosseini *et al.,* 2013). However, since kingfishers have a wide range of distribution, are avid eaters (consuming more than 50% of their body weight each day), and obtain virtually all their food from aquatic systems (Baron *et al.,* 1997) they make for excellent bio monitoring species. The present paper, mercury and methyl mercury levels were determined in different tissues of two sea bird species, *A. crecca* and *R. Solitaria* and effect of sex, size and feeding habitats on accumulation from Arvand river, north part of the Persian Gulf.

MATERIAL AND METHODS

Twenty-four individuals of two bird species were collected from Arvand river. The sea birds were collected from hunters who had shot them during February and March 2014. The collections included migratory bird Iran and Iraq *Anas crecca* (Male = 6, Female = 5) and resident bird of Iran, *Ringa Solitaria* (Male = 6, Female = 7). Table 1 shows scientific name, feeding habitat, sex and mean body weight for the species of kingfisher samples. Birds were thawed for dissection and sexed. Presence of ovary and oviduct was indicative of a female sex, and appearance of male gonads indicated male sex (Dam *et al.,* 2004). The specimens were weighed, stored in polyethylene bags, and kept at -20 °C until they were dissected and analyzed. Samples were thawed and muscle, liver, feather and kidney were separately dissected from the bodies of the specimens. Samples were freez-dried and homogenized (Saeki *et al.,* 2000; Nam *et al.,* 2005; Houserova *et al.,* 2007). Finally they were changed into the powder. Powdered sample and feather sample (finely cut) were directly weighed (50–100 ± 0.1 mg) into the precleaned combustion boats.

The mercury and methyl mercury were measured by the LECO AMA 254 Advanced Mercury Analyzer (USA) according to ASTM, standard No. D-6722. The LECO AMA 254 is a unique Atomic Absorption Spectrometer (AAS) that is specifically designed to determine total mercury content in various solids and certain liquids without sample pre-treatment or sample pre-concentration. Designed with a front-end combustion tube that is ideal for the decomposition of matrices, the instrument's operation may be separated into three phases during any given analysis: Decomposition, Collection, and Detection. In order to assess the analytical capability of the proposed methodology, accuracy of total mercury and methyl mercury analysis was checked by running three samples of Standard Reference Materials (SRM), National Institute of Standards and Technology (NIST), SRM 1633b, SRM 2709, and SRM 2711 in seven replicates (Saeki *et al.,* 2000; Nam *et al.,* 2005; Houserova *et al.,* 2007). The recovery means for mercury were 98% and 102% respectively.

The data were tested for normality using a Shapiro–Wilk's test. The data were not normally distributed. Mercury and methyl mercury concentrations in livers, feather, kidneys, and muscles were tested for mean differences among species using One-Way analysis of variance (ANOVA) fallowed by Duncan post hoc test. A Pearson correlation was used to test correlations. All concentrations are reported in $\mu g\ g^{-1}$ wet weight and a probability of $p < 0.01$ was set to indicate statistical significance.

RESULTS AND DISCUSSION

Table 2 and 3 shows the mean and comparison of the mercury and methyl mercury concentration in all tissues of tow sea birds collected from Arvand river. According to these data, mercury concentrations in tested tissues of the birds decreased in order feather > liver > kidney > muscle (Table 2). Mercury concentrations are usually found at the highest levels in feathers followed by liver, kidney and muscle tissues (Baron *et al.,* 1997; Zamani *et al.,* 2008; Hosseini *et al.,* 2013). In birds, feathers play a major role in mercury excretion (Thompson and Furness 1989; Burger and Gochfeld, 1991). Mercury accumulates especially well in bird feather because it has high affinity for the sulfhydryl groups in keratin. Reports indicate that in a variety of wild caught birds, feather mercury levels are consistently greater than liver and kidney levels which in turn exceed those in muscle (Thompson and Furness, 1989; Burger and Gochfeld, 1991; Kim *et al.,* 1996; Monteiro *et al.,* 1999; Saeki *et al.,* 2000; Nam *et al.,* 2005; Houserova *et al.,* 2007).

Once metals enter a bird, they can be stored in internal tissues such as the kidneys and the liver. Many seabirds would demethylate organic Hg in tissues such as the liver and kidneys, and store a large portion of their Hg burdens

Table 2 Mercury concentration (µg g^{-1} dry weight) in tissues of *A. crecca* and *R. Solitaria*

Species	Sex		Muscle	Feather	Liver	Kidney
Anas crecca	Male	Mean ± SE	0.91 ± 0.2	4.3 ± 0.4	2.8 ± 0.6	1.3 ± 0.1
		Range	0.47–2.9	1.1–7.4	0.82–4.1	0.51–3.2
	Female	Mean ± SE	1.3 ± 0.6	5.2 ± 0.1	3.6 ± 0.4	1.6 ± 0.2
		Range	0.75–4.8	1.3–8.6	1.1–6.5	0.92–3.5
Ringa Solitaria	Male	Mean ± SE	0.74 ± 0.4	3.1 ± 0.5	1.9 ± 0.2	0.94 ± 0.11
		Range	0.25–2.3	0.83–6.3	0.65–3.7	0.21–2.7
	Female	Mean ± SE	1.1 ± 0.3	4.3 ± 0.12	2.4 ± 0.15	1.2 ± 0.2
		Range	0.62–2.8	0.71–7.21	0.39–4.6	0.32–4.1

Table 3 Methyl mercury concentration (µg g^{-1} dry weight) in tissues of *A. crecca* and *R. Solitaria*

Species	Sex		Muscle	Feather	Liver	Kidney
Anas crecca	Male	Mean ± SE	0.51 ± 0.6	2.6 ± 0.5	1.1 ± 0.5	0.92 ± 0.5
		Range	0.11–1.3	0.6–4.2	0.22–2.4	0.11–1.8
	Female	Mean ± SE	0.92 ± 0.5	3.4 ± 0.7	1.6 ± 0.4	1.2 ± 0.2
		Range	0.25–1.8	0.7–5.3	0.5–3.7	0.12–1.8
Ringa Solitaria	Male	Mean ± SE	0.34 ± 0.2	2.8 ± 0.2	0.83 ± 0.2	0.74 ± 0.01
		Range	0.15–1.6	0.23–5.3	0.65–3.7	0.13–2.2
	Female	Mean ± SE	0.71 ± 0.5	3.1 ± 0.07	1.2 ± 0.04	0.92 ± 0.2
		Range	0.16–1.9	0.31–5.9	0.19–3.9	0.10–2.7

in inorganic form (Zamani *et al.,* 2008; Hosseini *et al.,* 2013). In our samples, liver mercury concentrations were positively correlated with those in kidney of the birds. A positive correlation between mercury concentrations in liver and kidney has been reported for other seabird species (Baron *et al.,* 1997; Zamani *et al.,* 2008; Hosseini *et al.,* 2013). Also, Lewis and Furness (1991) have reported mercury levels in the kidney that were most elevated in relation to the levels in the liver among black headed gull (*Larus ridibundus*) which were given the highest dose of mercury.

The result also showed that there are differences in mercury concentration in the different sexes. The indicated variability of mercury concentration in the different sexes depends on their habitats and feeding habits and food sources (Baron *et al.,* 1997; Zamani *et al.,* 2008; Hosseini *et al.,* 2013).

Kingfisher *A. crecca* feed on fish and also aquatic invertebrates, while *R. Solitaria* eat fish, molluscs, crustacean and some algae found in the river. Mercury can readily accumulate within fish tissues at much higher levels than other organisms (Zamani *et al.,* 2008; Hosseini *et al.,* 2013). Also, fish have been reported as a vector of the transfer of mercury element to top marine predators of the food chains. Therefore, kingfisher *A. crecca* feed more on fish and received higher mercury level in their bodies.

Several studies indicate a difference in metal body burden between male and female birds while other studies report negligible differences in mercury between sexes (Burger and Gochfeld, 1991; Kim *et al.,* 1996; Baron *et al.,* 1997; Zamani *et al.,* 2008; Hosseini *et al.,* 2013).

Since larger organisms generally exhibit higher contaminant level in their bodies and organisms that eat higher organisms also accumulate more contaminants when comparing to organisms that eat a range of different foods or eat smaller organisms. We expected to see higher mercury levels in tissues of female birds because they are larger and can eat larger food items. In general, mercury levels have been shown to increase with size and age of the ingested birds and it tends to be higher in species that occupy higher trophic levels, based on this logic we predicted that there should be higher levels of mercury in the larger predators. Zamani *et al*. (2009) have shown that higher metals levels in female seabirds were due to the increased consumption of food.

We found that mercury values were larger in tissue of female species than the males (Figure. 2). This finding merits future investigation since a larger sample size will allow a more accurate analysis and detection of significant differences if they indeed exist. Other reports indicate that although female birds can get rid of mercury in their eggs, the amount they shed in this way is usually small compared to the amount put into feathers during molt (Burger and Gochfeld, 1991; Kim *et al.,* 1996). Therefore the small difference that has been reported in mercury body burdens in male and female is consistent with our

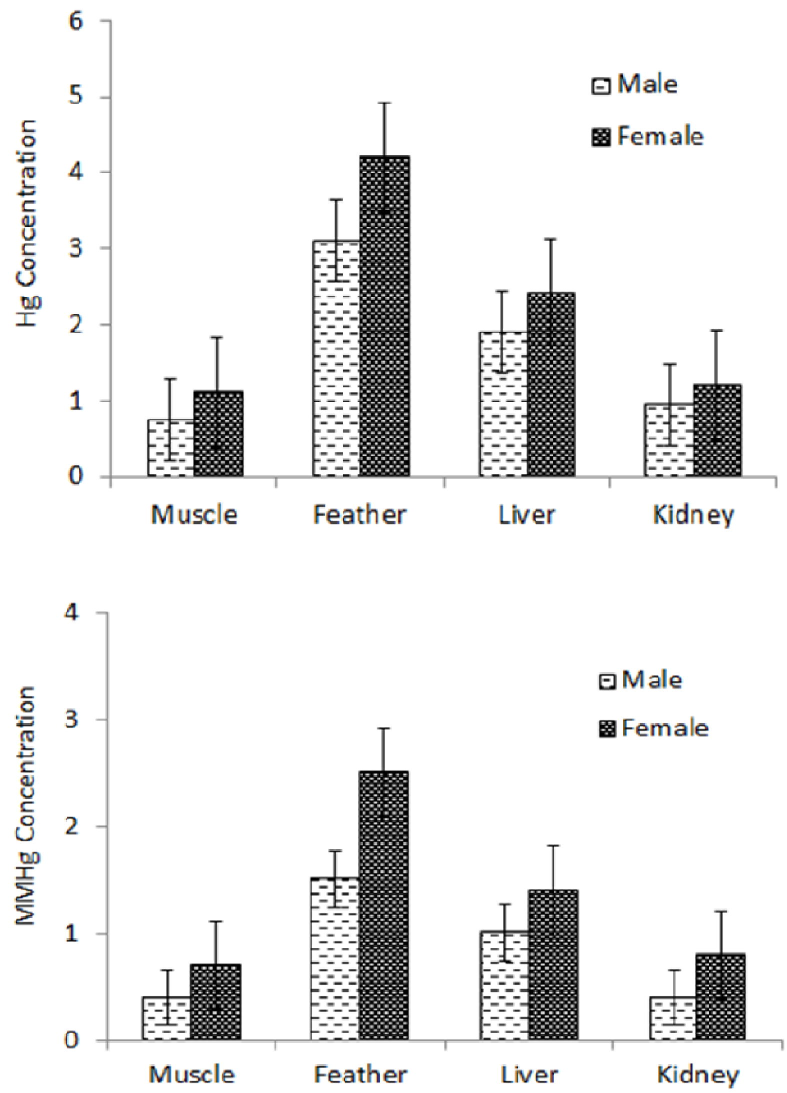

Figure. 2 Comparison of mercury and methyl mercury concentrations (µg g-1) between different tissues of male and female bird species

current data. Zamani *et al.* (2009) have shown that higher mercury levels were in female bird because they are larger and can eat larger food items.

The bird species that are residents of Arvand river (*R. Solitaria*) only have access to local food, and mercury concentrations in these tissues reflect mercury contaminant in Arvand river, but kingfisher *A. crecca*, which is migratory, can obtain food from other regions. We conclude that mercury concentrations in the kingfisher *A. crecca* is a reflection of diversity in food items from wider geographical locations with perhaps much higher mercury pollution than water of Iran and Iraq. Therefore, *Anas crecca* could be considered as suitable biomonitor agents for mercury contamination in the study area.

There were few significant correlations among contaminated in different tissues of birds. That is, knowing that one contaminated was high (or low) did not predict what the other contaminated would be. There were positively correlated between Hg and MeHg level in liver (r = 0.78) of birds (Figure. 3). Significant correlation between Hg and MeHg concentrations reflect beginning the detoxification process and proceeding of Hg–MeHg complex, detoxification process is under the influence of species type, tissue properties, chemical form of Hg and MeHg and the exact mechanism are not apparent (Burger and Gochfeld, 1991; Kim *et al.,* 1996). Positive correlation was found between the two contaminated in birds and highest correlation was in liver tissue. But this correlation

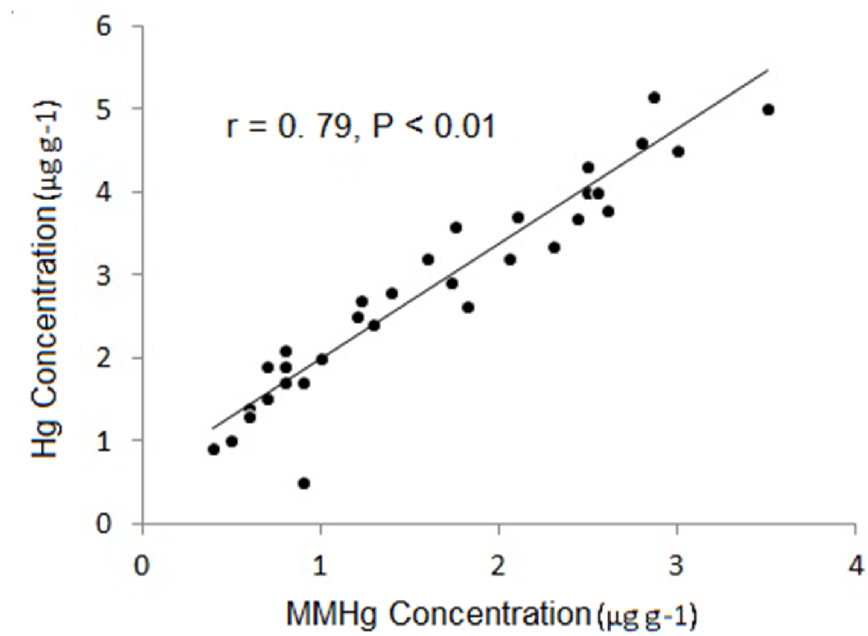

Figure. 3 Relationship of Hg and MMHg concentrations (µg g-1) between different tissues of bird species

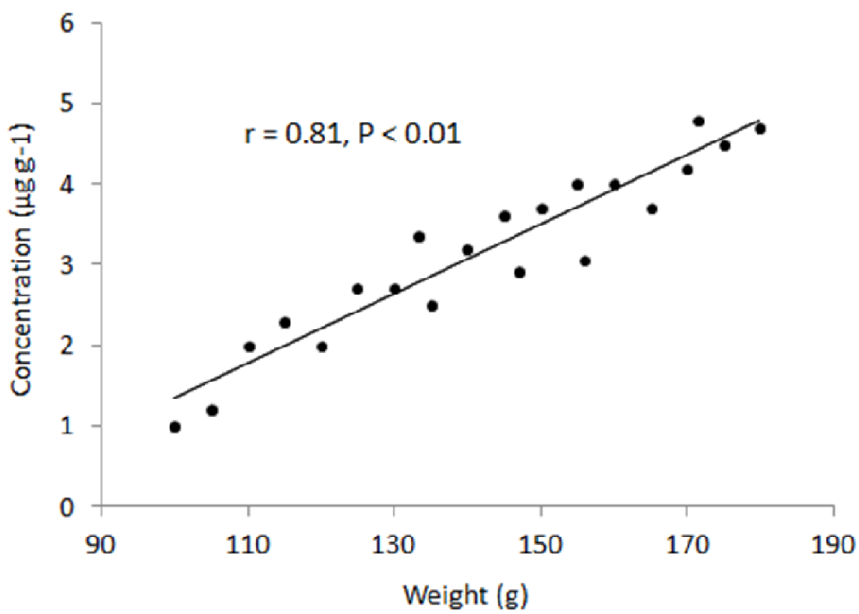

Figure. 4 Relationship of mercury concentrations (µg g-1) and weight of bird species

was not statistically significant between contaminated in other tissues of birds.

This study also aimed to investigate relationships between pollutants level in tissues and bird size and weight (Figure. 4). Results showed that there were positive relationship between bird weight and the contaminant levels in most cases. It is well known that one of the most important factors that play a significant role in contaminants accumulation in marine organisms is the metabolic activity (Baron *et al.,* 1997; Zamani *et al.,* 2008; Hosseini *et al.,* 2013). It is also accepted that contaminants' accumulation in organisms were controlled by uptake, detoxification, and elimination mechanisms (Baron *et al.,* 1997). The metabolic activities of seabirds differ from size to size (Baron *et al.,* 1997). The diet of seabirds changes with the size of these and seasonally probably because it could be able to capture live organisms such as fish for food (Baron *et al.,* 1997). The diet of juvenile two seabirds is small bivalves, decapods, and algae, while the basic diet components of adult these are more fish and shrimp (Baron *et al.,* 1997). The increase in Hg and MeHg concentration with growth of seabirds may be related to fish and crustaceans eating habits of the seabirds. Fish and crustacean have been reported as a vector of the transfer of mercury element to top marine predators of the food chains. Also, larger organisms generally exhibit higher contaminant level in their bodies (Zamani *et al.,* 2008) and birds that are higher on the food chain also accumulate more contaminants when comparing to birds that eat a range of different foods or eat smaller organisms.

CONCLUSION

Little data has been reported in the scientific literature for Arvand river of Iran. Here we report the average levels of Hg and MMHg in the tissues of two seabird species in river. Our results indicated that the levels of mercury varied among species and tissues. The results confirmed that the concentrations of mercury in organisms were strongly affected by habitat and feeding habit. Mercury concentrations in tissues of *R. Solitaria* reflect mercury contaminant in Arvand river, but kingfisher *A. crecca*, which is migratory, can obtain food from other regions .Our results can be used in environmental risk assessments that aim to protect wild birds of the region. Furthermore, our results enable us to evaluate differences within and across specific geographical areas, contributing to future efforts to interpret, yet to come, findings from the region and to identify locales with severe trends in mercury contamination effects on birds and other biota.

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