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Full Length Research Paper

Mercury (Hg) concentration and distribution in selected tissues of some fish species from Arvand River, south Iran

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In this study, mercury (Hg) levels were determined in three fish species from Arvand river, northwest of the Persian Gulf. It was also our intention to evaluate potential risks to human health associated with seafood consumption. Mercury levels varied with sex and species. Accumulation also differed significantly in certain organs. The order of mercury levels in tissues of the fish species was as follows: liver > gill > muscle. There was a direct relationship between mercury levels in tissues fishes with food habits and habitats. The results of this study show that highest levels of mercury were found in the benthic fish (*E. diacanthus*) followed by benthopelagic fish (*C. chanos*) and pelagic species (*S. argus*). There was a positive correlation between mercury levels tissues with body size of fishes. Highest mercury levels were in tissues of female fishes because they are larger and can eat larger food items. The results confirmed that the levels of mercury in fish were strongly affected by habitat and feeding habits.

Keywords Accumulation, Mercury, Food habitats, Fish, Arvand River

INTRODUCTION

Mercury (Hg) is one of the most hazardous environmental pollutants, due to its toxicity and its accumulation in aquatic organisms. The relative toxicity of mercury depends on its chemical form, methyl mercury being one of the most toxic substances existing in the environment. The consumption of fish is the main route of exposure of humans to

methylmercury (MHg), which represent the main form of mercury in fish due to biomagnification in the marine food chain. (Fitzgerald et al. 2007). Mercury exposure leads to numerous symptoms such as: impaired vision and hearing, dizziness, vomiting, headache, muscular weakness, allergies, depressed immune system, brain damage, but finally can lead to death (Pirrone et al. 2001).

The mercury that contaminates river and wetlands only accumulates in fish after it has been converted to the chemical compound methylmercury. Other forms of

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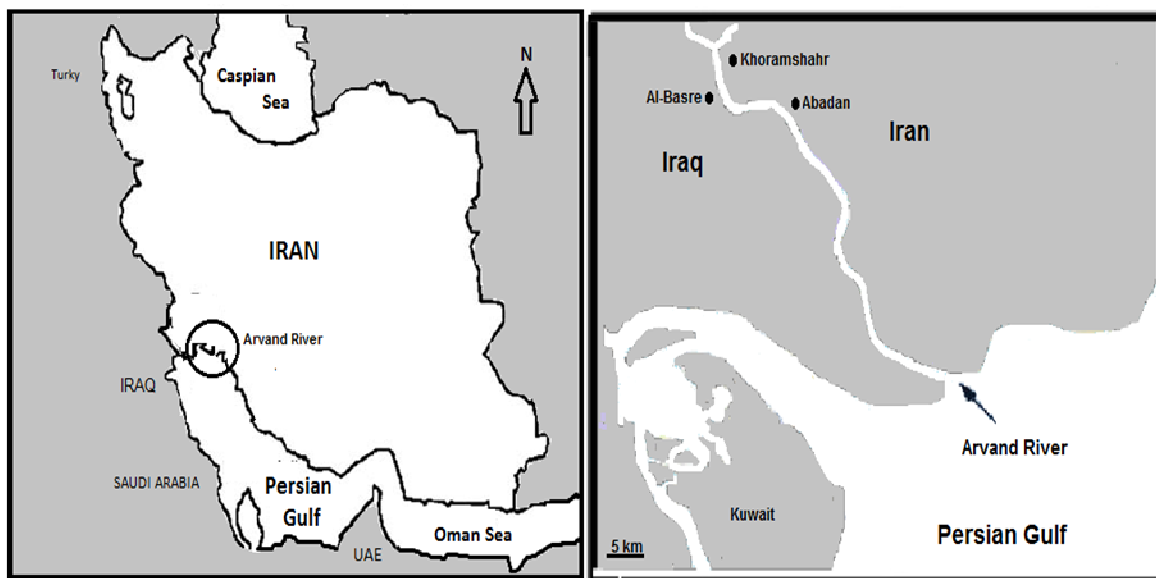


Figure. 1 Map showing the Persian Gulf and study area

mercury do not magnify in concentration up the food chain. Methylmercury is created by bacteria in highly organic portions of aquatic systems, such as the sediment of river and wetlands. The zooplanktons pick up the methylmercury as they filter the water and feed on algae. When small fish eat zooplankton, the methylmercury builds up in their bodies as the fish grow bigger and older. Small fish are eaten by larger fish, and the concentration of methylmercury increases at each step in the aquatic food chain. It is highest in large walleye, northern pike and other predatory fish (Fitzgerald et al. 2007). It's the methylmercury in these fish that poses the greatest threat to human health. Therefore, the people who rely on fish for much of their diet are most at risk from mercury, which can hamper normal development of the central nervous system (Pirrone et al. 2001).

Fish, which usually occupies the last levels of aquatic food chains, are considered as the main aquatic pathway for metals to be transferred into human body (Navarro et al. 2006). Biological and ecological factors such as size, sex (Al-Yousuf et al. 2000), ecological needs, habitat, feeding habits (Bustamante et al. 2003) and season (Navarro et al. 2006) have significant influences on metals bioaccumulation, bioavailability and therefore on their transference.

The Persian Gulf is a shallow and semi-enclosed sea that its environment is changing rapidly (Sheppard et al. 2010). The discovery of oil in this sea led to a massive increase in anthropogenic activities in the area. In general, petrochemical and oil industries are the major sources of pollution in this area (Sheppard et al. 2010; Al-Saleh and Shinwari 2002). For instance, this sea has about 800

offshore oil platforms and tolerates the traffic of about 25,000 oil tankers each year (ROPME 1999). According to the results that attained by ROPME, some areas of the sea are at a serious risk of mercury pollution related to petrochemical and oil industries.

In the present paper, mercury levels were determined in three fish species from Arvand river, northwest of the Persian Gulf. Each species was chosen from different layers of the water column to determine the influences of habitats on mercury levels in the species. The second aim was to determine the ability of each species and tissue in mercury accumulation.

MATERIALS AND METHODS

Sampling sites were selected along the Arvand river, northwest of the Persian Gulf (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-Basra in Iraq, Abadan and Khoramshahr in Iran. For people of these cities, the Arvand river is considered as a main resource of seafood and drinking water. 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 (Abdolahpur Monikh et al. 2012). This river is surrounded by many petrochemical units such as Abadan petrochemical complex and petroleum refinery. Also, metals concentration may be due to discharge of sewage and urban effluents and related to the oil tankers

Table 1 Scientific name, trophic level, sex and weight (Mean \pm SE g) of the specimens

Scientific name	Habitats	Food type	Sex	Weight
<i>Epinephelus diacanthus</i>	Benthic	Feed on crustaceans, molluscs and detritus	Male (35)	62.2 \pm 8.2
			Female (29)	76.7 \pm 3.8
<i>Chanos chanos</i>	Bentho-Pelagic	Feeds on shrimp, bivalvia and crab	Male (28)	72.2 \pm 3.4
			Female (34)	68.9 \pm 2.1
<i>Scatophagus argus</i>	Pelagic	feeds on fish, prawns, plant	Male (29)	80.4 \pm 1.4
			Female (34)	86.6 \pm 3.5

Table 2 Mercury concentration ($\mu\text{g g}^{-1}$) in liver, gill and muscle in three fish species

Tissue		<i>E. diacanthus</i>	<i>C. chanos</i>	<i>S. argus</i>
Liver	Mean \pm SE	0.83 \pm 0.07	0.75 \pm 0.11	0.51 \pm 0.03
	Range	0.42–1.26	0.15–1.06	0.09–0.79
Muscle	Mean \pm SE	0.54 \pm 0.05	0.58 \pm 0.04	0.25 \pm 0.31
	Range	0.11–0.76	0.04–0.83	0.05–0.53
Gill	Mean \pm SE	0.69 \pm 0.02	0.63 \pm 0.03	0.42 \pm 0.03
	Range	0.09–1.06	0.07–0.94	0.03–0.81

traffic in the river. 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 agricultural use of fertilizers, herbicides, pesticides, hazardous substance spills from various refineries, wars and invasions, are yet to be methodically investigated (Al-Hello and Al-Obaidy 1997).

Three fish species (*Epinephelus diacanthus*, *Scatophagus argus* and *Chanos chanos*) were collected in the July of 2011. The samples placed on ice, immediately transported to the laboratory on the same day and stored at $-20\text{ }^{\circ}\text{C}$ until analysis (Basset et al. 1981).

For analysis, muscle and liver of each fish were dissected, freeze-dried and crushed to uniform particle size (Dalman et al. 2006). It was then drained under folds of filter, weighed, wrapped in aluminum foil and then frozen at $10\text{ }^{\circ}\text{C}$ prior to analysis. The tissues were placed in clean watch glasses and were oven dried at $105\text{ }^{\circ}\text{C}$ for 1 hour and later cooled in the desiccators. Each sample of fish was homogenized in an acid-cleaned mortar and 2 g were digested in triplicate in a water bath at $60\text{ }^{\circ}\text{C}$ for 6 h after adding 2.5 mL each of concentrated HNO_3 and H_2SO_4 (Basset et al. 1981).

Total mercury was determined by Atomic Absorption Spectrometry Leco AMA-254 (Athanasopoulos 1993). The equipment was calibrated using metal stock solutions (1000 ppm). The recovery means for Hg were 99%, and 102% respectively.

All data were tested for normal distribution with Shapiro-wilk normality test. One-Way analysis of variance (ANOVA) followed by Duncan post hoc test were used to compare the concentration of mercury between species and fish tissues. The comparisons of mercury concentration between muscle, gill and liver of fish species were carried out by t-test. The mercury concentration of each sample is expressed in micrograms of mercury per gram of dry tissue ($\mu\text{g g}^{-1}$) and a probability of $p = 0.05$ was set to indicate statistical significance.

RESULTS AND DISCUSSION

Table 1 shows scientific name, trophic level, sex and mean body weight for the species of fish samples. Mercury concentrations were calculated in micrograms of mercury per gram of dry tissue ($\mu\text{g g}^{-1}$). In order to check the validity of the measurements, reference material (Multi-4, Merck) was used.

Mercury concentrations in the three fish species are shown in Table 2. During current study, mercury concentrations in tested tissues of all the fishes decreased in order liver > gill > muscle (Table 2). Metals are taken up by fishes from food and water, distributed throughout fish body by blood and eventually accumulated in target organs. Some tissues such as liver are considered as target organs for mercury accumulation (Romeo et al.

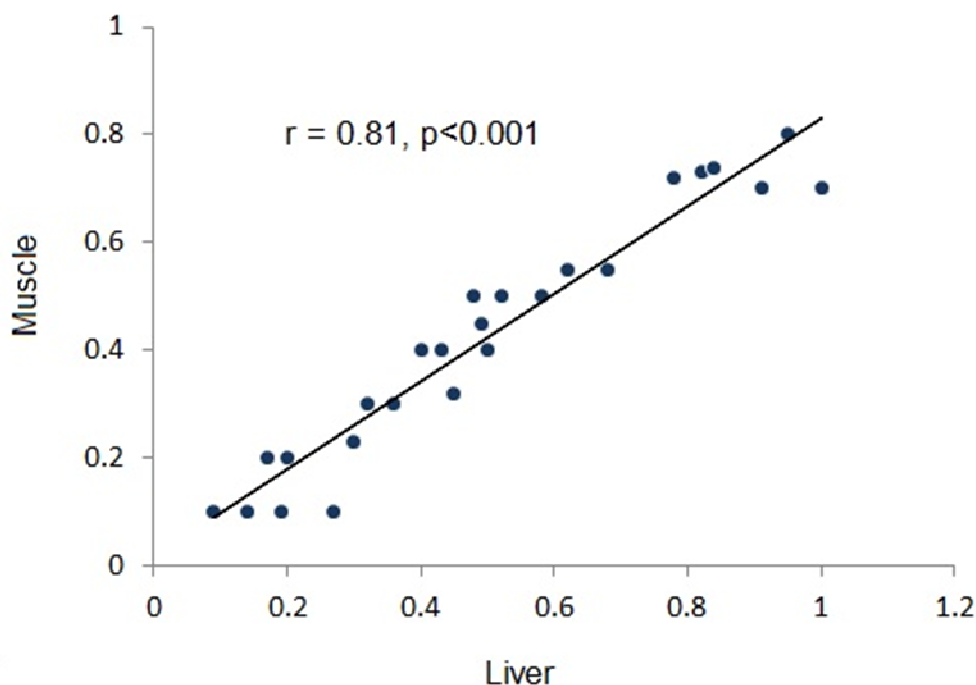


Figure. 2 Relationship of mercury concentrations ($\mu\text{g g}^{-1}$) between liver and muscle of the three species of fish

1999). The very high levels mercury in the liver for fish species in comparison to their muscle may be related to the content of metallothionein protein in liver tissue. Metallothionein protein that plays a significant role in the regulation and detoxification of mercury is produced in high levels in liver tissue (Sen and Semiz 2007). This protein contains a high percentage of amino group, nitrogen and sulphur that sequester metals in stable complexes (Romeo et al. 1999; Sen and Semiz 2007). In general, the accumulation of mercury in the liver could be resulted from the abundance of metallothioneins proteins in these tissues in comparison to muscle. The research same, the comparison on mercury accumulation between all tissues fish show that bioaccumulation of mercury was more in liver than other tissues (Sen and Semiz 2007; Abdolapur Monikh et al. 2012). Some other researchers also reported that in fish muscle generally contained the lowest amounts of these metals (Al-Saleh and Shinwari 2002; Pourang et al. 2005; Gewurtz et al. 2011).

Gills usually reflect the concentrations of metals in surrounding water (Sen and Semiz 2007). This organ is directly in contact with water and suspended materials, thus could absorb different substances from the surrounding environment. They also serve a variety of physiological functions such as osmoregulation and gas exchange. Due to these functions, gills have remarkable

influences on the exchange of toxic metals between a fish and its environment (Sen and Semiz 2007).

According to previous studies, in addition to liver, muscle can be of the main target organs for mercury accumulation (Kovekovdova and Simokon 2002; Pethybridge et al. 2010). For muscle, this is possibly attributed to the tendency of mercury to react with the sulfhydryl groups of methionine and cysteine proteins that are at high levels in the muscle (Boening et al. 2000; Houserova et al. 2006). Mieiro et al. (2009) found that in polluted aquatic habitats, liver is the main target organ for mercury, while in moderately polluted environment mercury are accumulated in muscle. The absence or low value of mercury level in some tissues may indicates that the tissues are not the target organs for mercury accumulation or may be due to the major functional differences in their body (Sen and Semiz 2007).

In our samples, liver mercury concentrations were positively correlated with those concentration in muscle of the three species of fishes ($r = 0.81$ $P < 0.001$, Figure. 2). A positive correlation between mercury concentrations in liver and muscle has been reported for other species (Pourang et al. 2005; Abdolapur Monikh et al. 2012).

In the present study, significant differences were observed among the species. In general, different species showed different levels of mercury accumulation. The

differences in mercury concentration in various fish species could considerably be attributed to the differences in feed habits and habitats (Caussy et al. 2003; Yilmaz and Yilmaz 2007).

In present study, we considered three groups of fish including, benthic carnivorous (*E. diacanthus*), benthopelagic carnivorous (*C. chanos*) and pelagic omnivorous (*S. argus*) as candidate biological indicators for evaluating the effects of trophic levels and habitats on mercury accumulation. *E. diacanthus* lives in close association with sediment and feeds mainly upon crustaceans, molluscs, detritus and shrimp. *C. chanos* is a benthopelagic species that feeds on shrimp, bivalvia and crab. *S. argus* is a pelagic omnivorous species that feeds on crustaceans, fish, prawns, plant and other invertebrates.

Fishes that are high on the trophic level might be expected to accumulate higher levels of bioaccumulative heavy metals (Bustamante et al. 2003; Yi et al. 2008). Benthic fishes are close to bottom sediment and receive more sediment-associated mercury than pelagic fishes. Ratkowsky et al. (1975) studied mercury contamination of Derwent Estuary in Australia. They found that there is a relationship between the frequency of high concentrations of mercury in fish tissues and feeding habits of the fish. According to Yi et al. (2008), heavy metals concentrations in food chain increase in the following order: benthic invertivores > piscivores > zooplanktivores > phytophagic fishes > phytoplanktivores > carnivorous fishes. Thus, in terms of mercury accumulation, the expected ranking for fish species in our study is benthic fish > benthopelagic fish > pelagic fish. However, our results indicate that habitat affects mercury accumulation rather than feeding habit. This finding does not mean that the role of feeding habit on mercury accumulation in fish is low, but it shows that, in general, for these species the effects of habitat are more than feeding habit. Because in current study, benthic fish (*E. diacanthus*) was more contaminated than benthopelagic and pelagic species (*C. chanos* and *S. argus*).

Despite of being benthic and benthopelagic species, *E. diacanthus* and *C. chanos* indicated different mercury accumulation in their tissues. These differences may be resulted from the variation in their diet and accumulation strategies.

The concentration of mercury in the benthopelagic fish, *C. chanos* with respect to the other species may be related to crustaceans eating habits of the fish. The diet of *C. chanos* consists of crab, shrimp and bivalvia. Crustaceans have been reported as a vector of the transfer of mercury element to top marine predators of the food chains (Bustamante et al. 2003).

Finally, among the three species, the benthic species accumulated higher concentrations of the mercury, due to the greater exposure to mercury enriched sediment and interactions with benthic organisms (Huang 2003; Yi et al. 2008). Therefore, this finding could confirm that mercury concentration is heavily controlled by habitat, feeding

habits, capacity of metal accumulation and kind of species (Bustamante et al. 2003; Agah et al. 2009).

There have been several studies on accumulation of mercury in fish species, yet few studies have taken into account the effect of sexual changes with respect to the mercury accumulation and distribution among tissues. We found that mercury values were larger in female tissues than in males. Differences in accumulation between the genders have been mainly attributed to differences in diet, differences in habitat (Beckvar et al. 1996). The male fishes feeds mainly on fish, plant and bivalvia and females on crab, shrimp, fish, detritus and benthic organisms. Benthic organisms have relationship with sediment and receive more sediment associated metals. Therefore, this finding could be due to the differences in their ecological niches and the relationship with sediments. It is known that certain forms of mercury can readily accumulate within fish tissues at much higher levels than those in the water column and in sediment (Beltrame and Marco 2010).

Since larger fishes generally exhibit higher contaminant levels in their bodies (Agah et al. 2009; Abdolapur Monikh et al. 2012) and fishes that eat higher organisms also accumulate more contaminants when comparing to fishes that eat a range of different foods or eat smaller organisms. We found that mercury values were larger in tissues of female fishes 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 fish and it tends to be higher in species that occupy higher trophic levels (Phillips et al. 1980), based on this logic we predicted that there should be higher levels of mercury in the larger predators. Gewurtz et al. (2011) have shown that higher mercury levels in female fish were due to the increased consumption of food.

Overall, these three species feed at comparable trophic levels and exhibit similar foraging behavior. The two species (*E. diacanthus* and *S. argus*) are residents of Arvand river and only have access to local food, but *C. chanos*, which is migratory, can obtain food from other regions. We conclude that mercury concentrations in two species (residents of Arvand river) reflect mercury contaminant in Arvand river, but mercury burden of *C. chanos* is a reflection of diversity in food items from wider geographical locations with perhaps much higher mercury pollution than water of river and Persian Gulf.

Consequently, mercury can be transferred to higher trophic level by biomagnification. This finding confirms that mercury have the ability to biomagnified through the aquatic food chains. In addition, their concentrations in high trophic level depend on the organisms of lowest trophic level. Mercury bioaccumulation in aquatic systems varies considerably with food-chain structure and length (Misztal-Szkudli et al. 2011). The results of this study show that highest mean mercury levels were found in the carnivorous fishes, followed by omnivorous and herbivorous. Barbosa et al. (2003) studied the

biomagnification of mercury in a marine food web in Rio Negro, Brazil. They found that mercury concentrations varied widely in all species; however, they showed a trend that depended on fish feeding strategies. The highest mean level was found in the piscivorous species, followed by detritivorous and herbivorous. They concluded that mercury is biomagnified through the food web. Cheng et al. (2011) investigated mercury biomagnification in some food webs in the aquaculture pond ecosystem of the Pearl River Delta, China. They reported that the concentrations of mercury in the high trophic levels of the food web depend on those concentrations in lower trophic levels.

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