

# Mercury Accumulation in Food Chain of Fish, Crab and Sea Bird from Arvand River

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### Abstract

In this study, concentration of mercury (Hg) was determined in the trophic levels of fish, blue crab and one sea birds 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 concentrations in tissues of the fish species was as follows: liver>gill>muscle, in tissues of the crab was as follows: hepatopancreas>gill>muscle and in tissues of the birds species was as follows: feather>liver>kidney. Therefore, Liver in fish, hepatopancreas in crab and feather in birds exhibited higher mercury concentration than the other tissues. There was a positive correlation between mercury concentrations in fish, crab and birds species with size of its food items. We expected to see higher mercury levels in tissues of female species because they are larger and can eat larger food items. The results of this study show that highest mean mercury levels were found in the birds (*A. crecca*), followed by crab (*P. pelagicus*), pelagic fish (*S. strongylura*) and benthic fish (*E. diacanthus*). Mean value of mercury in fish species, *S. strongylura* were (0.54  $\mu\text{g g}^{-1}$  dry weight), *E. diacanthus* (0.27  $\mu\text{g g}^{-1}$  dry weight), crab *P. pelagicus* (0.74  $\mu\text{g g}^{-1}$  dry weight) and bird species *A. crecca* was (3.4  $\mu\text{g g}^{-1}$  dry weight).

**Keywords:** Accumulation; Mercury; Food chain; Sea bird; Arvand River

### Introduction

Mercury is a highly toxic metal, which, because of its volatility, can be rapidly spread all over the world from its natural and anthropogenic sources. These properties combined with its huge biomagnification in the food chain, make it the metal of the most environmental concern [1,2]. The anthropogenic mercury sources are both diffuse and local. The diffuse sources are mainly the burning of fossil fuel, especially coal, the incineration of municipal solid waste, and the cement production; the local sources consist mainly of effluents from industrial activities, especially in developing countries [3,4].

Most mercury pollution residues in aquatic environments, where mercury is converted to methyl mercury (MeHg) by aquatic biota. Because of the high affinity of MeHg to sulphhydryl 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 [5]. Other forms of mercury do not magnify in concentration up the food chain. Methyl mercury is created by bacteria in highly organic portions of aquatic systems, such as the sediment of river and wetlands. The zooplanktons pick up the methyl mercury as they filter the water and feed on algae. When small fish eat zooplankton, the methyl mercury builds up in their bodies as the fish grow bigger and older. Small fish are eaten by larger fish, and the concentration of methyl mercury increases at each step in the aquatic food chain. It is highest in large walleye, northern pike, and other predatory fish [3]. It's the methyl mercury 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 methyl mercury, which can hamper normal development of the central nervous system. In adults, exposure to methyl mercury can result in damage to the nervous system and organs [4].

It is known that certain forms of mercury can readily accumulate within organisms tissues at much higher levels than those in the water column and in sediment [5]. Mercury is non-biodegradable contaminant that could enter aquatic food chains and consequently

accumulates in organisms positioned in various trophic levels [6]. 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 [6]. Biological and ecological factors such as ecological needs, habitat, feeding habits have significant influences on metals bioaccumulation, bioavailability and therefore on their transference. However, feeding habit plays a significant role in the accumulation of pollutants in organism's tissues [7].

The blue swimming crab *Portunus pelagicus* belongs to the phylum; arthropoda which make up about three quarters of living animal species. For the fauna, the species most studied are the benthic macroinvertebrates, particularly those with low mobility, which accumulate larger concentrations of metals compared to animals that live in open water. Among the most-studied are the crab Portunidae which has important characteristics that allow the study of bioaccumulation: they feed mainly on a wide variety of fish, bivalves, plant, crustaceans and benthic animals, as well as the sediment itself and they have a slow growth rate and long life cycle. Therefore, this crab species is especially appropriate for use in studies of environmental impact by metals pollution.

As birds are ordinarily at the top of the food web, they are valuable for environmental monitoring [8]. Fish-eating birds, as top level carnivores, often have high levels of contaminants [9]. Mercury accumulation in many sea birds has been frequently reported [9-15]

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but determination of mercury in birds such as kingfishers is rare and less common [16]. 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 [16] they make for excellent bio monitoring species.

This study was to determine the levels of mercury in the trophic levels of benthic fish, pelagic fish, crab and sea bird residing in Arvand River, Iran. The second aim was to determine the ability of each species and tissue in mercury accumulation.

## Materials and Methods

### Study area

Sampling sites were selected along the Arvand River, northwest coasts 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-Basre 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 [1,17]. 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 traffic in the river. In addition, the Arvand River carries about 48 tons of oil residues to the northwest Persian Gulf annually [17]. Other sources of pollution in this area, including wars and invasions, are yet to be methodically investigated [1].

Two fish species (*Epinephelus diacanthus* and *Strongylura strongylura*) were caught in October of 2011. The samples placed on ice, immediately transported to the laboratory on the same day and stored at -20°C until analysis [18].

For analysis, muscle and liver of each fish were dissected, freeze-dried and crushed to uniform particle size [19]. It was then drained under folds of filter, weighed, wrapped in aluminum foil and then frozen at 10°C prior to analysis. The tissues were placed in clean watch glasses and were oven dried at 105°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°C for 6 h after adding 2.5 mL each of concentrated HNO<sub>3</sub> and H<sub>2</sub>SO<sub>4</sub> [18,19].

Each species was properly cleaned by rinsing with distilled water to remove debris, planktons and other external adherent. The samples were dissected with sterilized scissors and tweezers to remove samples

Organism	Scientific name	Feeding habitat	Sex	n	Weight
Fish benthic	<i>Epinephelus diacanthus</i>	Benthic predator : consumes mainly benthic invertebrates, detritus and plant	Female	35	72 ± 7.2
			male	40	70 ± 1.3
Fish pelagic	<i>Strongylura strongylura</i>	Consumes mainly benthic fish, also invertebrates, plant and crustacean	Female	42	89 ± 4.9
			male	39	82 ± 1.4
Blue crab	<i>Portunus pelagicus</i>	Fish predator: consumes fish pelagic, detritus and plant	Female	45	98 ± 1.7
			male	51	83 ± 2.3
Sea bird	<i>Anas crecca</i>	Omnivorous: consumes mostly crab, fish, shrimp and marine invertebrates	Female	9	85 ± 3.5
			male	7	69 ± 1.4

**Table 1:** Scientific name, feeding habitat, sex and weight ((Mean ± SE g) of the specimens.

of three tissues (hepatopancreas, muscle and gill), in standardized locations: (i) muscle of the chelar propodus, due to higher metal accumulation was verified (ii) hepatopancreas tissue, which has a particularly high metabolic rate and (iii) gills, because of their osmoregulatory function. It was then drained under folds of filter, weighed, wrapped in aluminum foil and then frozen at 10°C prior to analysis. The tissues were placed in clean watch glasses and were oven dried at 105°C for 1 hour and later cooled in the desiccators.

The birds *Anas crecca* was collected from Arvand River. These birds were collected from hunters who had shot them during October of 2011. The specimens were weighed, stored in polyethylene bags, and kept at -20°C until they were dissected and analyzed. Samples were thawed and liver; feather and kidney were separately dissected from the bodies of the specimens. Samples were freeze-dried and homogenized [20]. 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.

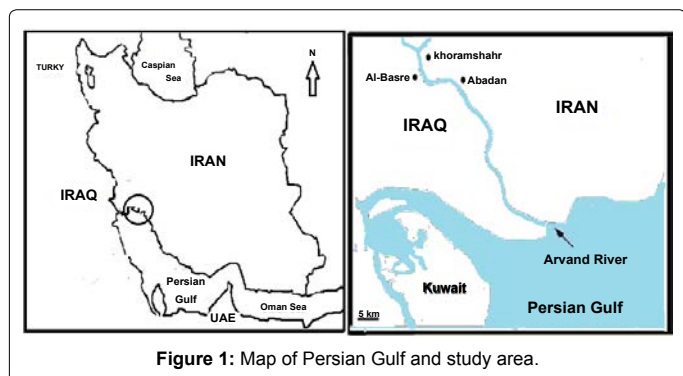
Each sample was analyzed for mercury by the mineralization method with HNO<sub>3</sub> at 65 percent, according to Ruelas-Inzunza J et al. [20]. Analyses were optimized by hollow cathode lamps (LCO), according to the metallic element analyzed, and samples were read using a GBC-932 AA atomic absorption spectrophotometer [21]. The equipment was calibrated using metal stock solutions (1000 ppm). The recovery means for mercury was 102% respectively.

The data were tested for normality using a Shapiro–Wilk’s test. The data were not normally distributed. Mercury concentrations between tissues and species were tested for mean differences among species using One-Way analysis of variance (ANOVA) followed by Duncan post hoc test [22].

## Results and Discussion

Table 1 shows scientific name, trophic level and mean body weight for the species of fish, crab and bird samples. Mercury concentrations were calculated in microgram per gram wet basis (µg g<sup>-1</sup> dry weight). In order to check the validity of the measurements, reference material (Multi-4, Merck) was used.

During current study, mercury concentrations in tested tissues of all the fishes decreased in order liver>gill>muscle and in tissues of crab followed the hierarchical pattern hepatopancreas>gill>muscle (Table 2). Metals are taken up by fishes and crab from food and water, distributed throughout fish body by blood and eventually accumulated in target organs. Some tissues such as liver in fish and hepatopancreas in crab are considered as target organs for mercury accumulation [23]. The



**Figure 1:** Map of Persian Gulf and study area.

Species		Hepatopancreas	Gill	Muscle	Liver	Feather	Kidney
<i>E. diacanthus</i>	Mean ± SE		0.21 ± 0.6	0.12 ± 0.4	0.49 ± 0.4		
	Range		0.09–0.71	0.07–0.73	0.07–0.73		
<i>S. strongylura</i>	Mean ± SE		0.54 ± 0.3	0.32 ± 0.7	0.78 ± 0.7		
	Range		0.28–2.1	0.11–1.2	0.07–0.73		
<i>P. pelagicus</i>	Mean ± SE	1.13 ± 0.1	0.68 ± 0.3	0.41 ± 0.5			
	Range	0.25–1.8	0.15–1.1	0.11–0.91			
<i>A. crecca</i>	Mean ± SE			1.1 ± 0.6	3.6 ± 0.1	5.2 ± 0.4	1.6 ± 0.2
	Range			0.75–4.8	1.1–6.5	1.3–8.6	0.92–4.1

**Table 2:** Mercury concentration ( $\mu\text{g g}^{-1}$ ) in tissues of fish species, blue crab and sea bird species from Arvand River.

very high levels mercury in the liver and hepatopancreas in comparison to other tissues may be related to the content of metallothionein protein in liver tissues. Metallothionein protein that plays a significant role in the regulation and detoxification of mercury is produced in high levels in liver or hepatopancreas tissue [24]. This protein contains a high percentage of amino group, nitrogen and sulphur that sequester metals in stable complexes [23,24]. In general, the accumulation of mercury in the liver or hepatopancreas could be resulted from the abundance of metallothioneins proteins in these tissues in comparison to other tissues. The research same, the comparison on mercury accumulation between all tissues fish show that bioaccumulation of mercury was more in liver than other tissues [18]. Some other researchers also reported that in crustacean bioaccumulation of mercury was more in hepatopancreas than other tissues [25,26].

Gills usually reflect the concentrations of metals in surrounding water [7]. 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 [27]. However, the muscle tended to accumulate less mercury in comparison to the liver and gills. This finding may reflect the low concentration of metallothioneins in the muscle tissue [7].

Mercury concentrations in tested tissues of the bird decreased in order feather>liver>kidney (Table 2). Mercury concentrations are usually found at the highest levels in feathers followed by liver, kidney and muscle tissues [11,28]. In birds, feathers play a major role in mercury excretion [13]. Mercury accumulates especially well in bird feather because it has high affinity for the sulfhydryl groups in keratin [28].

Once metals enter a bird, they can be stored in internal tissues such as the kidneys and the liver [28]. Many seabirds would demethylate organic Hg in tissues such as the liver and kidneys, and store a large portion of their Hg burdens in inorganic form [13]. Reports indicate that in a variety of wild caught birds, liver mercury levels are consistently higher than kidney levels which in turn exceed those in muscle [8,11,15]. This suggested markedly with studies which have reported levels of mercury in the kidney was higher than in the liver tissue [13,14,29].

Lewis and Furness [29] 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 ratio of levels in the kidney and liver may, therefore, be an indicator of mercury poisoning. Higher kidney to liver ratios may indicate elevated mercury levels [29].

The result also showed that there are differences in mercury concentration in the different species. The indicated variability of metal

concentration in the different species depends on their habitats [30] and feeding habits and food sources [31].

In present study, we considered two groups of fish including, benthic fish (*E. diacanthus*) and pelagic species (*S. strongylura*) as candidate biological indicators for evaluating the effects of trophic levels and habitats on metal accumulation. *E. diacanthus* lives in close association with sediment and feeds mainly upon benthic organism, detritus and tiny invertebrates. *S. strongylura* is a pelagic species that feeds on fish and mollusca. The blue swimming crab *P. pelagicus* feed mainly on a wide variety of fish, bivalves and crustaceans.

Also, we considered one species of bird *Anas crecca* that eat mainly aquatic crustacean and also fish. Therefore, *E. diacanthus* feeds on sediment and benthic organism, *S. strongylura* species feeds on *E. diacanthus*, blue crab *P. pelagicus* feed on both fish and bird *Anas crecca* feed on fishes and crab species.

Despite of being benthic and non-migratory fish, *E. diacanthus* indicated high mercury accumulation in their tissues. Because *E. diacanthus* feed on sediment and benthic organism and is close to bottom sediment and receive more sediment-associated mercury. Ratkowsky et al. [32] 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. [22], heavy metals concentrations in food chain increase in the following order benthic species followed by other species.

The high concentration of mercury in the blue crab species, *P. pelagicus* may be related to fish eating habits of the species. The diet of *P. pelagicus* consists of benthic fish. Fishes have been reported as a vector of the transfer of mercury element to top marine predators of the food chains [7,22].

The highest concentration of mercury was detected in tissues of bird *A. crecca*. Because organisms that are high on the trophic level might be expected to accumulate higher levels of bioaccumulative metals [7,22]. Thus, in terms of mercury accumulation, the expected ranking in our study is pelagic fish<benthic fish<blue crab<bird. Therefore, this finding could confirm that mercury concentration is heavily controlled by habitat and feeding habits [33].

Since larger organisms generally exhibit higher contaminant level in their bodies [18,33] 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 crab (Figure 2) 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 crab and it tends to be higher in species that occupy higher trophic levels [34], based on this logic we predicted that there should be higher levels of

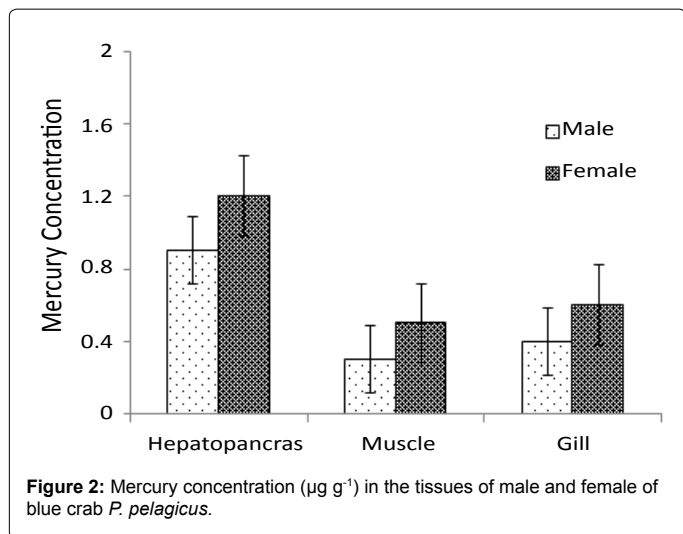


Figure 2: Mercury concentration ( $\mu\text{g g}^{-1}$ ) in the tissues of male and female of blue crab *P. pelagicus*.

mercury in the larger predators. Gewurtz et al. [26] have shown that higher mercury levels in female crustacean were due to the increased consumption of food.

Several studies indicate a difference in metal body burden between male and female birds [35,36] while other studies report negligible differences in mercury between sexes [13,14,28]. We found that mercury values were larger in tissue of male of the species than the females (Figure 3). 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 [35,36]. Therefore the small difference that has been reported in Hg body burdens in male and female is consistent with our current data. Zamani et al. [28] have shown that higher mercury levels were in female bird because they are larger and can eat larger food items.

Overall, these four species (fish, crab and bird) feed at comparable trophic levels and exhibit similar foraging behavior. The fish and crab species are (residents of Arvand River) only have access to local food, but bird *A. crecca*, which is migratory, can obtain food from other regions. We conclude that mercury concentrations in fish and crab species reflect mercury contaminant in Arvand river, but mercury burden of bird *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.

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. The results of this study show that highest mean mercury level were found in the bird *A. crecca*, followed by blue crab, benthic fish and pelagic fish. Barbosa et al. [24] 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 benthic, followed by piscivorous pelagic and herbivorous pelagic. They concluded that mercury is biomagnified through the food web. Cheng et al. [23] 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.

### Correlation

There was significant correlation between mercury concentrations in fish and crab tissues ( $r=0.81$ ,  $P<0.01$ , Figure 3a). It is suggested that significant correlation between mercury concentration in fish and crab tissues may be related to high variability of mercury levels in the fish.

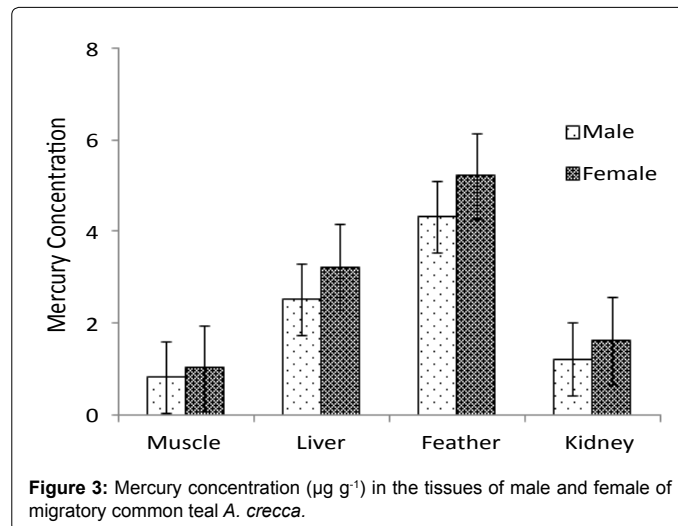


Figure 3: Mercury concentration ( $\mu\text{g g}^{-1}$ ) in the tissues of male and female of migratory common teal *A. crecca*.

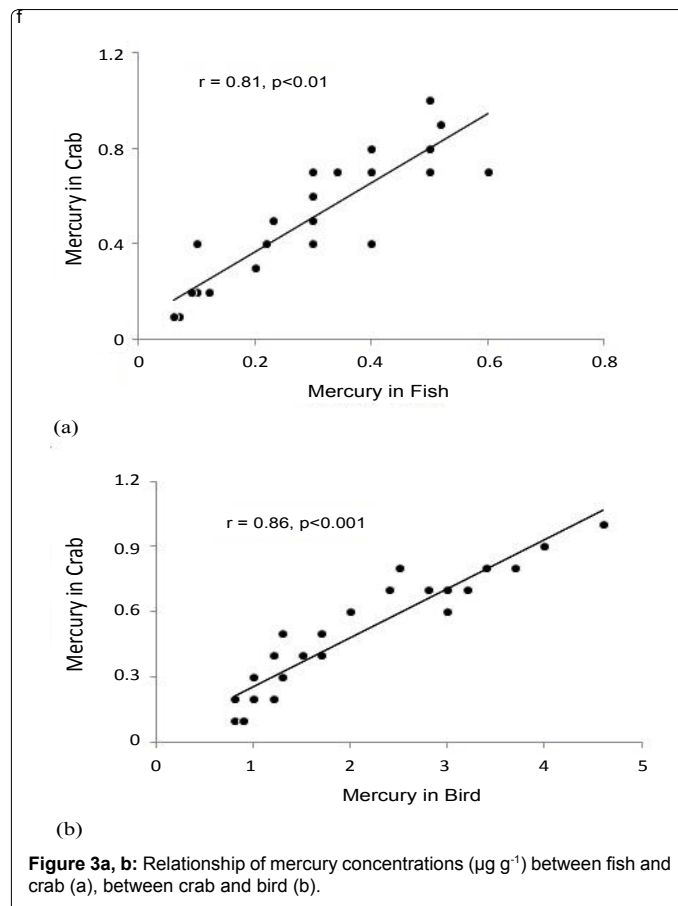


Figure 3a, b: Relationship of mercury concentrations ( $\mu\text{g g}^{-1}$ ) between fish and crab (a), between crab and bird (b).



*E. diacanthus* is benthic species and is more common in the bottom sediment and receive more sediment associated metals. Also, there was significant correlation between mercury concentrations in crab and bird tissues ( $r=0.86$ ,  $P<0.001$ , Figure 3b). Therefore, this finding could be due to the differences in their ecological niches and feeding habitats. Therefore, *Anas crecca* could be considered as suitable biomonitor agents for mercury contamination in the study area.

## Conclusion

Our results indicated that the levels of mercury varied among species and tissues. Mercury can be transferred to higher trophic level by biomagnification. Also, mercury concentrations in high trophic level depend on the organisms of lowest trophic level. Therefore, the results of this study show that highest mean mercury level were found in the bird (*A. crecca*), followed by blue crab (*P. pelagicus*), benthic fish (*E. diacanthus*) and pelagic fish (*S. strongylura*). There was a positive correlation between mercury concentrations in organisms with size of food items. Therefore, we expected to see higher mercury levels in tissues of female species because they are larger and can eat larger food items. The results confirmed that the concentration of mercury in organisms strongly affected by habitat and feeding habit, however, the influences of habitat appears to be more than feeding habit. The fish species are traditional food for fishermen and traditional coastal communities, where it is an important source of protein. From a public health standpoint, measurement of mercury levels in the tissues of fishes is highly dangerous for human health. Therefore, studies of mercury concentrations in coastal areas are relevant and useful for monitoring the health of environmental compartments, maintenance of biodiversity, and for assuring the quality of life, mainly for humans. Finally, there is a need to develop and refine bird model to serve as a sentinel of ecosystem health, to help provide early warning indications for possible human exposure.

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