

Mercury levels in selected tissues of shrimp *penaeus merguensis* from persian gulf

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Levels of mercury in tissues of *Penaeus merguensis* from the Persian Gulf, was investigated. This study assessed the relationship between mercury levels in hepatopancreas tissue, muscle and gonads with sex and season. Mercury content varied with type of size and sex. Results showed that hepatopancreas accumulate higher levels of the mercury in comparison to muscle and gonads. There was significant difference ($p < 0.05$) in mercury levels between *P. merguensis* sexes, higher mercury levels found in female than in males. There was significant difference ($p < 0.05$) in mercury levels between different seasons, higher mercury levels found in July (summer season). The variation in mercury levels among the tissues is likely to have resulted from metal bioavailability, changes in tissue composition, organism size, sex and season.

[Key words: Mercury, Levels, Season, *Penaeus merguensis*, Persian Gulf]

Introduction

Mercury is known to bioaccumulate in humans, so bioaccumulation in seafood carries over into human populations, where it can result in mercury poisoning. Mercury is dangerous to both natural ecosystems and humans because it is a metal known to be highly toxic, especially due to its ability to damage the central nervous system¹. In human-controlled ecosystems of fish, usually done for market production of wanted seafood species, mercury clearly rises through the food chain via fish consuming small plankton, as well as through non-food sources such as underwater sediment. This mercury grows in concentration within the bodies of fish and can be measured in the tissues of selected species².

The presence of mercury in fish can be a particular health concern for women who are or may become pregnant, nursing mothers, and young children^{2, 3, 4}. 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 death⁴. Much of the variation in trace metal tissue concentrations in aquatic organisms has been attributed to the variety in size as well as age of

individuals⁵, sex^{6, 7, 8}, feeding habits⁹ and the season of capture^{10, 11}. These mercury levels in marine environment should be monitored regularly to check water quality, animal health and in view of the quality of public food supplies.

Shrimp selected in this study *Penaeus merguensis* is demersal decapod crustaceans of regional economic importance. *P. merguensis* is one of the important representatives of crustacean and a species commonly found in Persian Gulf coasts, Iran¹². Persian Gulf is a body of water in the Middle East between the Arabian Peninsula and Iran. This inland sea is connected to the Gulf of Oman by the Strait of Hormuz¹³. Persian Gulf is a semi-enclosed formation and heavy discharges of the surrounding industries have been ongoing for many decades. Other sources of Persian Gulf pollution include invasions and bombardments that have been staggering in the recent years and are yet to be fully investigated. Large areas of agricultural lands, local fisheries, oil export facilities and a petrochemical plant operate in the general area. Present study was undertaken to determine the seasonal variation in levels of Hg the hepatopancreas, muscle and gonad tissues of both male and female shrimps (*P. merguensis*) and to compare them with the previous studies^{8, 10, 17, 18, 19}.

Materials and Methods

Four sampling sites were selected along the northwest coasts of the Persian Gulf (Fig 1). The first one (S1) was located near the Arvand river's mouth, the second one (S2) was located near Bahrekan estuary's mouth, the third one (S3) was located in Boushehr coasts and the last one (S4) was located near Musa estuary's mouth. *P. merguensis* were collected from Persian Gulf in summer (July) of 2010 and winter (January) of 2011. The four sampling station are shows in Fig. 1. One hundred sixty (80 male and 60 female of shrimp *P. merguensis* individuals were collected. Sampling covered areas of the direct or indirect influence of urban and industrial releases, those located near the mouth of the tributary rivers which carry industrial discharges of pollutants to the offshore waters and a locality not under the influence of industrial or urban releases. The sampling stations were selected to reflect progression of pollution and human activities in the area.

Sampling was performed with shrimp trawl. Samples placed on ice, immediately transported to the laboratory on the same day and stored at -20 °C until analysis¹⁴. For analysis, muscle and hepatopancreas of each shrimp were dissected,

freeze-dried and crushed to uniform particle size²⁰. Sufficient amount of each tissue was digested in mixture of HNO₃ and H₂O₂ by microwave. Mercury concentration was determined by a cold vapor atomic absorption spectrometer (Unicam, model 919). All reagents were of analytical reagent grade, and all chemicals and standard solutions were obtained from Merck. All the plastic and glassware were soaked in nitric acid and rinsed with distilled water before use. The accuracy of method was measured using certified reference materials Dorm-2 (muscle of dogfish, National Research Council of Canada). The recovery means for Hg were 96.7% and 102% respectively.

All data were tested for normal distribution with Shapiro-wilk normality test. Significant differences between heavy metals concentration in the samples of various stations, between tissues and between species were determined using One-Way analysis of variance (ANOVA) followed by Duncan post hoc test. The comparisons between muscle and hepatopancreas of shrimp and crab were carried out by t-test.

Table 1 - Sample characteristics (the sampling months, male and female, average wet weights and total lengths of shrimp *Penaeus merguensis* from Persian Gulf

Sex	Season	
	July	January
Male (♂)	85.20 ± 0.40 ^a	90.34 ± 0.03 ^a
	15.01 ± 0.69 ^b	17.22 ± 0.05 ^b
Female (♀)	91.50 ± 0.29 ^a	88.20 ± 0.02 ^a
	16.80 ± 0.45 ^b	16.60 ± 0.05 ^b

^a Weight (gr) (Mean ± SD), ^b Total Length (cm) (Mean ± SD)

Results

Table 1 showed sampling months, sex, the mean body weight and total length as well as the standard deviations for the shrimp samples. Mean concentrations and associated standard deviations of

Hg in the hepatopancreas, gonads and muscle of male and female *P. merguensis* collected for four seasons in the Persian Gulf are given in Table 2. In the *P. merguensis*, concentration of Hg in hepatopancreas tissue of ranged from 0.03 µg g⁻¹ to 0.81, gonads µg g⁻¹ 0.01 µg g⁻¹ to 0.22 and muscle

0.01 to μgg^{-1} 0.14 μgg^{-1} with an average of 0.21 μgg^{-1} . Mercury concentrations varied significantly ($P < 0.05$) for different tissues, estuaries and between sexes. The highest concentration of Hg (0.81 μgg^{-1}) in *P. merguensis* was detected in S4 (Musa estuary). Mercury concentrations varied significantly ($P < 0.05$) between seasons. The highest Hg concentrations were measured in July for both male (0.56) and female (0.81) hepatopancreas tissues. The results show that among the tissues of *P. merguensis*, hepatopancreas accumulated the highest level of Hg for the both sex (Fig. 2). Relationship between the size of the shrimp and the

concentrations of Hg is shown in Fig. 3. Significant correlations ($r = 0.89$) were found between length and concentrations of Hg in *P. merguensis*. Positive correlations between the length of the shrimp and Hg levels can be found for *P. merguensis*. Largest individuals of this species have already gained their full grown size (30 cm). Figure 3 also shows that for a number of species the smallest shrimp have a relatively higher inorganic Hg fraction. These results also show markedly higher concentrations of the Hg in *P. merguensis* from July as compared to those from the January (Fig. 4).

Table 2- Mean concentrations (μgg^{-1}) and their standard deviations (Mean $\pm$ SD) of Hg in the hepatopancreas, gonads and muscle of male and female *Penaeus merguensis* collected for two seasons in the Persian Gulf

Tissues	Sex	July	January
Hepatopancreas	Male (♂)	0.56 $\pm$ 0.02 ^c	0.03 $\pm$ 0.01 ^a
	Female (♀)	0.81 $\pm$ 0.05 ^c	0.09 $\pm$ 0.03 ^b
Gonads	Male (♂)	0.09 $\pm$ 0.02 ^b	0.01 $\pm$ 0.05 ^a
	Female (♀)	0.22 $\pm$ 0.06 ^c	0.05 $\pm$ 0.03 ^b
Muscle	Male (♂)	0.02 $\pm$ 0.05 ^a	0.02 $\pm$ 0.05 ^a
	Female (♀)	0.07 $\pm$ 0.01 ^b	0.04 $\pm$ 0.02 ^a

a, b, c: Different letters show significant differences of Hg concentration between tissues

Discussion

Results showed that mercury concentration in tissues at all sampling stations occurs in descending order of hepatopancreas > gonads > muscle. Patterns of metal accumulations were quite similar for the same sex. The comparison on mercury accumulation between tissues showed that concentrations of the mercury in the hepatopancreas were significantly higher ($P < 0.05$) than muscle. The research same, the comparison on mercury accumulation between all tissues crustaceans show that bioaccumulation of mercury was more in hepatopancreas than other tissues^{15, 19, 21}. Some other researchers also reported that in crustaceans muscle generally contained the lowest amounts of these metals^{15, 22}. Metals are taken up by crustaceans from food and water, distributed throughout crustacean body by blood and eventually accumulated in target organs. Some tissues such as hepatopancreas are considered as target organs for mercury accumulation¹⁹. The very low levels Hg in

the muscle and gonads in comparison to their hepatopancreas may be related to the content of metallothionein protein in hepatopancreas tissue. Metallothionein protein that plays a significant role in the regulation and detoxification of mercury is produced in high levels in hepatopancreas tissue²¹. This protein contains a high percentage of amino group, nitrogen and sulphur that sequester metals in stable complexes⁶. According to previous studies, in addition to hepatopancreas, muscle can be of the main target organs for mercury accumulation^{23, 24, 25}. 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^{26, 27}. Miei *et al.* (2009) found that in polluted aquatic habitats, hepatopancreas is the main target organ for mercury, while in moderately polluted environment mercury is accumulated in muscle²⁸. There were significant

differences in Hg levels between sexes of *P. merguensis*. We found that Hg levels were larger in tissues of female than the males. Differences in accumulation between the genders have been mainly attributed to differences in diet²⁹, or due to differences in habitat³⁰. Canli and Furness (1993) had investigated influences of gender on the Hg accumulation in muscle and cephalothorax in Norway lobster (*Nephrops norvegicus*)⁷. They showed that Hg level in female tissues was also higher than in males. Our results are in agreement with those of Bu-Olayan *et al.* (1998), who investigated the relationship between Hg level sexes in *T. orientalis* from the Persian Gulf³¹. Both of these studies show that Hg concentrations in females *T. orientalis* are higher than males. Therefore the difference that has been reported in Hg body burdens

in male and female is consistent with our current data of the present study. Therefore the difference in Hg levels between sexes can be attributed to depuration in eggs, sexual dimorphism and niche partitioning of the forage base. Endo *et al.* (2009) have suggested that these differences may reflect slower growth rates of one of the genders, implying that at a specified size, the slower growing gender tends to be older than the faster growing one, and has accumulated Hg over a longer period of time³². Additionally, Gewurtz *et al.* (2011) have shown that higher Hg levels in female fish were due to the increased consumption of food, relative to males, to meet the increasing demands of reproduction³⁰. Other studies showed that length and weight of female crustaceans were higher than of males^{33, 34}.

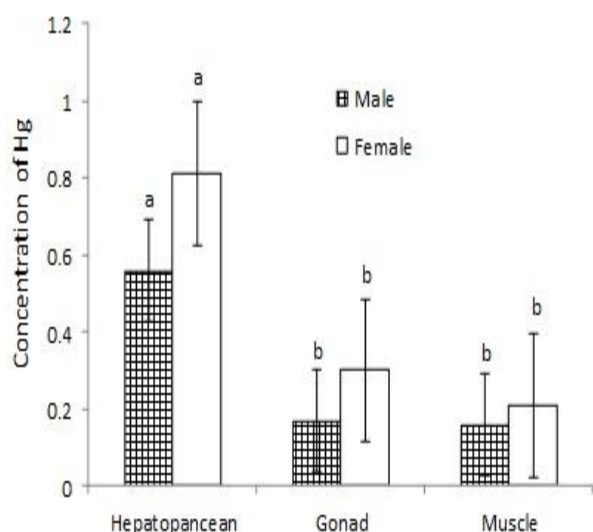


Fig. 2: Comparison of Hg concentrations in the hepatopancreas, gonad and muscle samples of male and female *P. merguensis*

There were significant correlations between total length and Hg concentrations in different tissues of *P. merguensis*. Therefore, we used only total length for prawns, to investigate their relationship with Hg levels in different tissues. The primary biological factors governing the accumulation of mercury include age, weight and numerous field studies have shown that the level of total Hg correlates positively with age, length and weight¹⁹. Storelli *et al.* (2007) found that mercury level increased with size for 7 species of marine fish from the Adriatic Sea²⁵. There was a positive correlation between size and Hg concentrations for 11 of 14 species of marine fish

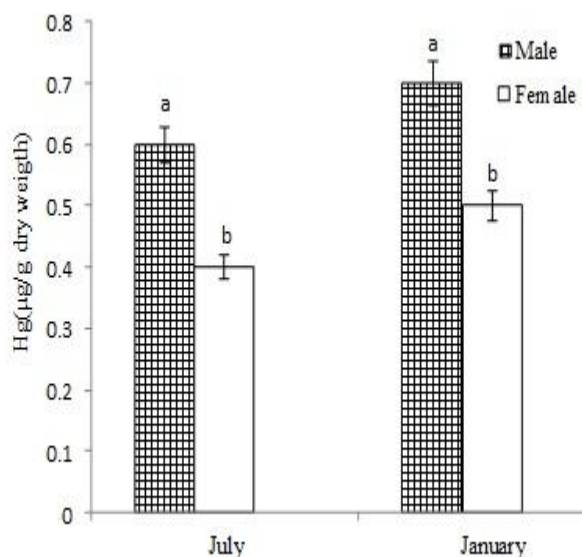


Fig. 4: Comparison of Hg concentrations in the different seasons samples of male and female *P. merguensis*

collected in the western Aleutians (Bering Sea/North Pacific)³⁵. Additionally, because larger crustacean specimens have been exposed a longer time compared of smaller and thus bioaccumulated higher mercury concentrations (usually size is used as a surrogate for age). Larger specimens are also more dependent to bed (benthic) and less to surface (pelagic) in comparison to smaller specimens and thus are longer present in polluted sediment^{36, 37, 38, 39}. For a number of species the smallest crab have a relatively higher inorganic Hg fraction. This may be linked to changes in feeding behaviour during their growth (from detritus and phytoplankton to small

prey) and relatively more direct uptake of inorganic Hg. However, total mercury level may not always correlate with size due to differences associated with diet and residence time in a contaminated habitat⁴⁰. There was significant difference ($P < 0.05$) between the level of Hg during different seasons. Relatively high concentrations were observed in prawns collected during July. Seasonal variation may effect metal concentration in body organism. This variation could result in internal biological cycle in organism or variation in bioavailability of metal in environment. Temperature, food availability and water could increase metal concentration in summer than winter⁴¹. Such this condition was happened during present study and Hg levels in different tissues showed higher in summer season compared

to winter. In other words, most levels of metals in body organism is in the form methyl metal which is soluble in fatty tissues, thus seasonal reproduction could be cause increase heavy metals in summer season^{34, 41}. Similarly in the present study, more Hg levels uptake was showed during summer season. According to different studies the heavy metal levels in invertebrates showed higher in summer season^{15, 42, 43}. It was revealed that invertebrates all show similar seasonal patterns in metal concentrations it would seem likely that environmental factors (discharges to the estuary, pH, salinity, suspended matter, etc.) are having a greater overall influence on seasonality than biological factors (metabolism, reproduction, fluctuations in tissue weight, etc.).

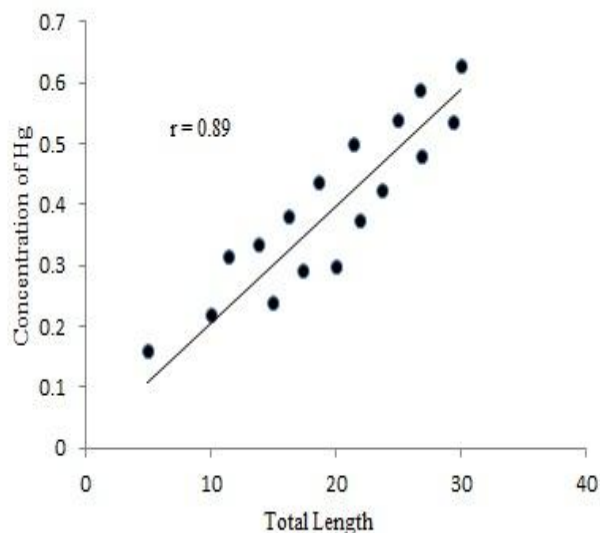


Fig. 3: Relationship between size (cm) and Hg concentration ($\mu\text{g/g-1}$) in *P. merguensis*

The seasonal spawning of gametes from many benthic invertebrates changes the relative proportion of the body tissue weight within different organ types⁸. Steenkamp *et al.* (1994) reported that significant differences were detected in various months for most tissues (except the gills) in crab, *Potamonautes warreni*, sampled from Natalspruit River (South Africa)⁴⁴. Hosseini *et al.* (2012) showed that in blue swimming crab *Portunus pelagicus*, heavy metals exhibited seasonality¹⁵. The present study shows a significant seasonal variation ($P < 0.05$) in the concentration of the studied Hg in the selected tissues. Gametes generally contain heavy metals at a concentration level below the average for all of the organs of the individual and therefore spawning may substantially increase the

mean value of the tissue metal concentration of the body as a whole⁴⁵. In this study, higher concentrations were observed in female shrimp collected summer season, which coincide with the peak temperature of the seawater in the studied region. Remarkably high accumulation of most of the mercury were found in all the species from S4 (Musa estuary) and S1 (Arvand river). Musa estuary is surrounded by more than 19 petrochemical units such as chlor-alkali plant and superphosphate plant. The fact that total concentration of some petrochemical-related metals such as Hg in the northwest of Persian Gulf decreased with distance from Musa estuary is a strong evidence reflects that the Hg in this estuary were sourced from petrochemical activities, and not from background

geological sources. Besides, some main creeks of this estuary flow into two crowded and industrialized cities and consequently receive huge amounts of domestic effluents and urban wastewaters⁴⁶. The Arvand 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 course⁴⁶. For shrimp species, Kargin *et al.* (2001) reported concentrations of heavy metals in the muscle and hepatopancreas of *Penaeus semiculatus* and *Metapenaeus monocerus* collected from the Iskenderun Gulf, Turkey. Concentrations of Hg in shrimps from the Persian Gulf were lower than those reported by Kargin *et al.* (2001)¹⁰. The concentrations of Hg in *Penaeus semisulcatus* of the northern east Mediterranean Sea¹⁹ were lower than those in the Persian Gulf shrimps, whereas the concentration of Hg in Yilmaz and Yilmaz (2007) study was higher than present finding. Ozden (2010) studied heavy metals concentration in *Parapenaeus*

longirostris from Marmara Sea⁴⁷. The levels of Hg in the shrimps from the Persian Gulf were higher than those in Marmara Sea shrimps. According to field and experimental studies, tissue distribution and accumulation of mercury in crustacean varies widely depending on size, sex, growth stage, molting, migration, season of sampling, metal bioavailability, hydrodynamics of the environment, changes in tissue composition, reproductive cycle, habitat, feeding habits, capacity of metals accumulation and kind of species^{19, 40}. Despite of being benthic and non-migratory species indicated different mercury accumulation in their tissues. These differences may be resulted from the variation in their diet and accumulation strategies. Generally, the benthic species accumulated higher concentrations of the Hg, due to the greater exposure to Hg enriched sediment and interactions with benthic organisms^{47, 48}. Biota caught from the mouth of Musa estuary was found to containing high level of Hg in their tissues.

Table 3. Comparison of mean levels of mercury ($\mu\text{g g}^{-1}$) in tissues of various crustacean species from different parts of the world and some available standards

Species	Tissues	Hg	Location	Reference
<i>P. merguensis</i>	H	0.81	Persian Gulf	This research
<i>P. merguensis</i>	G	0.27	Persian Gulf	This research
<i>P. merguensis</i>	M	0.16	Persian Gulf	This research
<i>P. semisulcatus</i>	H	0.51	Persian Gulf	[46]
<i>P. merguensis</i>	H	0.48	Persian Gulf	[46]
<i>P. pelagicus</i>	H	0.22	Persian Gulf	[15]
<i>P. pelagicus</i>	M	0.35	Persian Gulf	[15]
<i>P. pelagicus</i>	E	0.41	Persian Gulf	[15]
<i>P. merguensis</i>	H	0.45	Ligurian Sea	[34]
<i>Mantis shrimp</i>	M	0.075	Ligurian Sea	[34]
<i>Pink shrimp</i>	H	0.44	USA	[36]
<i>Pink shrimp</i>	M	0.047	USA	[36]
Australia MPC (1987)	0.5			[49]
Denmark (1987)	0.3			[49]
OEHHA Califoornia	0.3			[50]
EC (2001)	0.5-1			[50]
FAO* (2002)	1			[51]
WHO* (1996)	1			[52]

H (Hepatopancreas), M (Muscle), G (Gonad), E (Exoskeleton)

In relation to other species and other conditions, concentration of mercury in crustacean in this study was compared with other studies around the world (Table 3). The mean concentration of Hg in hepatopancreas of crustacean showed almost higher

than other tissues during present study and other studies around the world. However, in this study, concentration of Hg in tissues of crustacean was compared WHO (1996), Australia MPC (1987), Denmark (1987), EC (2001) and FAO (2002)

standard values. The concentration of Hg in tissues of *P. merguensis* showed that higher than other conditions and higher than Australia MPC (1987), Denmark (1987) and EC (2001). The concentration of Hg in all stations showed lower than WHO and FAO. The concentrations of Hg in the tissues of *Penaeus merguensis* in July were generally higher than the levels issued by Australia MPC (1987), Denmark (1987) and EC (2001).

Conclusion

However, as only a few species were analysed, with a limited size range and at a limited amount of stations, it is clear that further research is required in order to assess the ecological state of the Iranian marine environment regarding Hg pollution. From a

public health standpoint, metal concentrations found in the edible part of some species were relatively higher than the tolerance levels for human consumption. Thus, people how consume crustacean from this area should eat a diversity of crustacean to avoid consuming harmful quantity of non-essential metals. The mean concentration of Hg in hepatopancreas of shrimp showed almost higher than other tissues, these are usually not used as food.

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