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Bioaccumulation and distribution of heavy metals (Se, As and Pb) in muscle, gill and hepatopancreas of blue crab *Portunus pelagicus*, Bushehr Coast, Persian Gulf

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Introduction

Heavy metals can be classified as potentially toxic elements such as cadmium, lead and mercury and essential elements such as copper, zinc and iron (Hosseini *et al.,* 2013).Toxic metals naturally occur in aquatic environments in very low concentrations, but their concentration levels have increased due to anthropogenic pollutants (Huang, 2003) over time. Industrial activities as well as agriculture and mining create a potential source of metal pollution in aquatic environments (Fitzgerald *et al*., 2007). Contaminant levels, particularly lead, selenium and arsenic are sufficiently high in some crabs to cause adverse human health effects in people consuming them in large quantities (Gewurtz, 2003).

Accumulation of heavy metals in organisms depends on various biological and environmental factors such as size, age, feeding habit and habitats (Beltrame and Marco, 2010). However, feeding habit plays a significant role in the accumulation of metals in tissues of organisms (Mourente, 1996) because metals have a tendency to be biomagnified through food chains (Dalman *et al*., 2006). Most aquatic food chains begin from invertebrates. However, ability of invertebrates to accumulate contaminants from water varies from species to species. Thus biomagnification of contaminants entering aquatic food chains is significantly affected by invertebrate species. The blue swimming crab *P. pelagicus* is a benthic invertebrate that feeds on crustaceans, fishes, bivalves, plants and benthic animals and they have a slow growth rate and long life cycle (Williams, 1981). Although in other countries, this variety has a special place in nutrition and includes a huge part of industrial hunting, in our country it is treated as incident hunting and it is eaten as a food only in limited areas of the south of the country such as Khuzestan, Hormozgan and Boushehr Provinces. Benthic invertebrates, particularly those with low mobility, accumulate larger concentrations of metals compared to animals that live in open water (Dalman *et al*., 2006). Therefore, this crab species is especially appropriate for use in studies of metal pollution in marine ecosystems.

The Persian Gulf is a shallow and semi-enclosed sea and its environment is changing rapidly. The discovery of oil in this sea led to a massive increase in anthropogenic activities in the area. In general, the agricultural use of fertilizers, herbicides, pesticides, petrochemical and oil industries are the major sources of pollution in this area (Hosseini *et al*., 2013). Therefore, the objectives of this study were to determine the level of heavy metals lead, selenium and arsenic in muscle, gill and hepatopancreas of blue swimming crab *P. pelagicus* and effect of size and food habits on metal bioaccumulation from Khuzestan coast, in the north part of the Persian Gulf, south Iran.

Materials and methods

The crab specimens were collected from Bushehr coast, in the northern part of the Persian Gulf in the south of Iran in May of 2012. The numbers, food habits, carapace width (cm), and weight ranges of the samples are shown in Table 1. Each species was properly cleaned by rinsing with distilled water to remove debris, planktons and other external adherent, and the sex of each individual was determined according to morphological characteristics. Male crabs are bright blue and their abdomen (womb area) is narrow and in the form of a spear, while female crabs are green–brown and have round abdomens (Potter and Lestang, 2000). Studies on food and feeding were carried out following a method adapted by Williams (1981). The dorsal side of the body was cut open and the foregut was removed carefully. The foreguts were preserved in 70 % formalin for a week, prior to being cut open and their contents transferred into petri dishes with distilled water. The food components of the gut contents were separated and identified under a compound microscope. After identification of food items, the crabs were dissected with sterilized scissors and tweezers to remove samples of three tissues (muscle, gill and hepatopancreas), in standardized locations: (i) muscle of the chelar propodus, due to higher metal accumulation verified by Hosseini *et al*.

(2014); (ii) hepatopancreas tissue, which has a particularly high metabolic rate (Mourente, 1996) and (iii) gills, because of their osmoregulatory function (Mourente, 1996). The tissue samples were 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 crab 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_3 and H_2SO_4 (Abdolahpur Monikh *et al*., 2012).

Table 1: Sex, food habit, weight and carapace width of the crab *Portunus pelagicus*.

Sex	Food habit	MW (g)	CW (cm)
Female (*n*=122)	Feed on shrimp, plant and benthic animal	170±0.10	7.2±0.05
Male (*n*=107)	Feed on fish, bivalvia	151±0.02	6.7±0.02

MW; Mean Weight, CW; Carapace Width

Each sample was analyzed for metals by the mineralization method with HNO_3 at 65 percent, according to Abdolahpur Monikh *et al*. (2012). 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 (Abdolahpur Monikh *et al*., 2012). All data were tested for normal distribution with Shapiro-Wilk normality test. The comparisons of metal levels between muscle, gill and hepatopancreas were carried out by t-test. All concentrations are reported in μg g^{-1} dry weight and a probability of $p<0.01$ was set to indicate statistical significance.

Results and discussion

Table 2 lists the concentration of metals in all crab samples. The recorded mean concentrations were 0.485 μg g^{-1}Se, 1.89 μg g^{-1} Pb and 0.615 μg g^{-1} As. There were significant differences in Se, Pb or As concentrations detected between different tissues (ANOVA, $p<0.05$). The result demonstrated that metal concentrations in the different organs followed the hierarchical pattern hepatopancreas > gill > muscle (Fig. 1).

Table 2: Mean concentrations (μg g^{-1}) of Se, Pb, and As in different tissues of crab *Portunus pelagicus*.

Metal	Sex		Gill	Hepatopancreas	Muscle
Se	Male	Mean ± SE	0.430 ± 0.05	0.644 ± 0.02	0.531 ± 0.06
		Range	0.088–1.114	0.118–1.404	0.065–0.934
	Female	Mean ± SE	0.612 ± 0.01	0.829 ± 0.07	17.3 ± 0.05
		Range	0.131–1.763	0.163–1.723	0.18–2.84
Pb	Male	Mean ± SE	1.13 ± 0.02	2.2 ± 0.05	0.43 ± 0.03
		Range	0.15–2.84	0.21–3.80	0.24–1.41
	Female	Mean ± SE	1.23 ± 0.05	3.5 ± 0.01	0.71 ± 0.02
		Range	0.18–2.84	0.39–4.14	0.21–1.72
As	Male	Mean ± SE	0.323 ± 0.03	0.761± 0.01	0.253 ± 0.07
		Range	0.087–8.312	0.128–1.254	0.082–0.682
	Female	Mean ± SE	0.725 ± 0.08	1.184 ± 0.06	0.602 ± 0.05
		Range	0.108–1.331	0.129–1.821	0.095–1.119

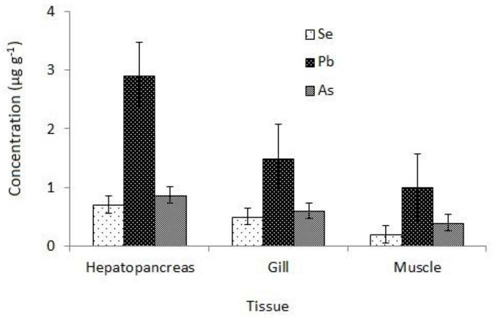

Figure 1: Comparison of Se, Pb and As in different tissues of blue swimming crab *Portunus pelagicus.*

Heavy metal accumulation in crab organs depend on the physiological role of the organs (Sen, A. and Semiz**,** 2007). Some tissues such as hepatopancreas are considered as target organs for metals accumulation (Yilmaz and Yilmaz, 2007). The very high levels of metals in the hepatopancreas in comparison with other tissues 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 metals is produced in high levels in hepatopancreas tissue (Sen and Semiz, 2007). This protein contains a high percentage of amino group, nitrogen and sulphur that sequester metals in stable complexes. In general, the accumulation of metals in the hepatopancreas could result from the abundance of metallothioneins proteins in these tissues in comparison to gills and muscle tissue.

Gills usually reflect the concentrations of metals in the surrounding water (Bustamante *et al*., 2003). 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 crab and its environment (Beltrame *et al*., 2003). However, the muscle tends to accumulate less metal in comparison to the liver and gills.

There were differences in metal concentrations between male and female crabs. Differences in accumulation between the genders have been mainly attributed to differences in diet or differences in habitat (Beckvar *et al*., 1996). The male crab feeds more on fish and bivalvia and female crabs feed more on shrimp, plants and benthic organisms (Hosseini *et al*., 2013). Plants have a relationship with sediments and receive more sediment associated metals. Metals are closely bound to the plant cell wall, slowing its translocation from roots to buds. Because plants are salt-excluders, they hinder the entry of metals through their root system (Beckvar *et al.,* 1996). Also, metals can accumulate within crustacean tissues at much higher levels. Shrimp have been reported as a vector of the transfer of elements to top marine predators of the food chains (Bustamante *et al*., 2003). Therefore, female crabs feed on benthic animals and plants and receive high levels of metals. Therefore, high levels of metals in female crabs can refer to habitat condition as a sedimentary crab because sediments accumulated more metal and benthic organisms are linked to metal exposure.

Since larger organisms generally exhibit higher contaminant level in their bodies (Abdolahpur Monikh *et al*., 2012) and crabs that are higher on the food chain also accumulate more contaminants when compared with crabs that eat a range of different foods or eat smaller organisms. We expected to see higher metal levels in tissues of female crabs because they are larger and can eat larger food items. Gewurtz *et al*. (2011) have shown that higher metal levels in female fish were due to the increased consumption of food, relative to males, to meet the increasing demands of reproduction.

This study also aimed to investigate relationships between metal concentrations in hepatopancreas and crab size. Results showed that there was a positive relationship between crab size and the contaminant levels in most cases (Fig. 2). It is well known that one of the most important factors that plays a significant role in the accumulation of contaminants in marine organisms is the metabolic activity (Hosseini *et al*., 2015). It is also accepted that contaminants' accumulation in organisms was controlled by uptake, detoxification, and elimination mechanisms. The metabolic activities of *P. pelagicus* differ from size to size. The diet of *P. pelagicus* changes with the size of crabs and seasonally probably because it could be able to capture live swimming organisms such as fish for food (Williams, 1981; Hosseini *et al*., 2013). P. pelagicus is a bottom-feeding carnivore preying on a wide variety of sessile and slow moving organisms. The diet of juvenile *P. pelagicus* is small bivalves, decapods, and algae, while the basic diet components of adult *P. pelagicus* are fish, bivalve, and shrimp (Hosseini *et al*., 2013). Prasad and Neelakantan (1988) were also of the opinion that adult *P. pelagicus* are opportunistic omnivores with a preference for animal food in conjunction with a definite predatory propensity. Therefore, the differences in metal concentration in various crab size could be attributed to the differences in feeding habits.

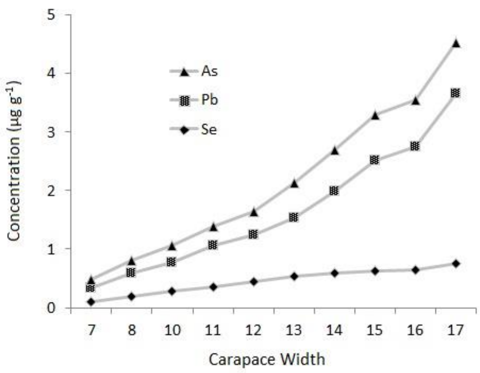

Figure 2: Relationship between heavy metals levels with size of blue crab *Portunus pelagicus.*

There were few significant correlations among metals with the highest sample size (Table 3). That is, knowing that one metal was high (or low) did not predict what the other metals would be. Selenium and lead were the only metals that were positively correlated for crab species with the highest sample sizes. Significant correlation between selenium and lead concentrations reflects the beginning of the detoxification process and proceeding of Se–Pb complex. Detoxification process is under the influence of species type, tissue properties, chemical form of selenium and lead and the exact mechanism is not apparent. Positive correlation was found between the two metals in crabs and highest correlation was in liver of *P. pelagicus*. But this correlation was not statistically significant in other tissues of crab.

Table 3: Correlations among metals in different tissues of crab *Portunus pelagicus.*

	Gill	Muscle	Hepatopancreas
Selenium with			
Arsenic	0.27 (0.002)	0.36 (0.002)	0.78 (0.001)
Lead	0.42 (0.02)	0.35 (0.002)	0.324 (0.02)
Arsenic with			
Lead	0.28 (0.003)	Ns	0.33 (0.001)

An overview of metal concentrations in marine fish and crustacean in different marine environments from the Persian Gulf is presented in Table 4. The mean selenium level in the blue crab in this research was higher than the mean selenium concentration in *P. pelagicus* and *P. semisulcatus* collected from the Khuzestan coast, but was lower than other studies in the Persian Gulf. Comparing our results with literature data from different parts of the Persian Gulf indicates that the lead and arsenic levels in this study were lower than the levels of two metals in all studies, expect in shrimp *P. merguiensis* and *M. stabbbingi* from the Bushehr coast.

The blue swimming crab *P. pelagicus* is traditional food for fishermen and traditional coastal communities, where it is an important source of protein. Although the muscle is most often consumed, in some places (e.g., south Iran state), it is common to add cassava flour to previously cooked hepatopancreas. According to the values of Table 4, the metals values in all tissues are not very high, with the exception for hepatopancreas. Therefore, measurement of contamination levels in the tissues of *P. pelagicus* is suitable for human health. Finally, studies of metal 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.

Table 4: Comparison of the metal levels (μg g^{-1}) in tissues of various crustacean and fish species from different parts of the Persian Gulf .

Species	As	Pb	Se	Location
Portunus pelagicus	0.82	2.34	0065	Persian Gulf (Khuzestan)
Penaues semisulcatus	0.71	3.12	0.12	Persian Gulf (Khuzestan)
Metapenaues affinis	0.75	1.94	1.78	Persian Gulf (Khuzestan)
Penaeus semisulcatuse	0.68	1.39	2.65	Persian Gulf (Khuzestan)
Penaeuse merguiensis	0.08	0.027	1.25	Persian Gulf (Bushehr)
Metapenaeus stabbbingi	0.11	0.13	2.62	Persian Gulf (Bushehr)
Metapenaeus affinis	1.21	3.12	1.54	Persian Gulf (Bushehr)
Parapenaeupsis stylifera	0.65	1.78	2.89	Persian Gulf (Bushehr)
Otolithes ruber	0.88	1.95	1.76	Persian Gulf (Khuzestan)
Psettodes erumei	0.95	1.86	0.44	Persian Gulf (Khuzestan)
Carcharhinus dussumieri	1.07	1.58	2.45	Persian Gulf (Bushehr)
Carcharhinus dussumieri	1.49	1.83	1.42	Persian Gulf (Dayir)
Carcharhinus dussumieri	1.53	2.45	0.84	Persian Gulf (Genaveh)
Euryglossa orientalis	1.61	1.69	0.97	Persian Gulf (Hormozgan)

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