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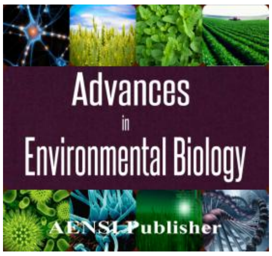

The Study of Bioaccumulation of Heavy Metals (Zn, Cu, Cd, Pb) in (*Metapenaeus affinis*) and (*Litopenaeus vannamei*) in Khouzestan Province, the North of the Persian Gulf

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ABSTRACT

Background: Heavy metal pollution of aquatic environment has become a great concern in recent years. Heavy metals have the tendency to accumulate in various organs of marine organisms, especially fish and shellfish, which in turn may enter into the human metabolism through consumption causing serious health hazards. **Objective:** The major aim of this study was to investigate heavy metal content of edible fish in the Persian Gulf, (*Metapenaeus affinis*) and (*Litopenaeus vannamei*) were collected for the analyses of heavy metals (Zn, Cu, Cd and Pb) in the shell and muscle. 60 samples of *M. affinis* and 80 of *L. vannamei* were collected from stations of Bahrekan in the coast of Hendijan (Khouzestan Province) and Boshehr Province, North of Persian Gulf. Preparation of samples was died and digested using nitric acid and Zn using flame system and Cu, Cd and Pb graphite oven of absorption spectrophotometer were measured. **Results:** Results showed that in the shell and summer season total of metals were higher of the muscles and winter season ($P<0.05$). Results showed all of heavy metals in muscle and shell in *M. affinis* higher than *L. vannamei* ($P<0.05$). Results showed higher mean (± SD) concentrations of Zn were observed in shell and muscle of *M. affinis* (44.61±15.67 and 38.64±12.85 mg/kg, dry weight) and in *L. vannamei* (34.6±12.17 and 26.66±12.00 mg/kg, dry weight) and lower concentrations of Cd were observed. Sequence of concentration of heavy metals in two species and organs were follow Zn> Cu> Pb> Cd. There was also a relationship between sediments and shrimp for accumulation of heavy metals in the two seasons and stations. **Conclusion:** According to the high metal concentration in different parts of shrimps' body, these shrimps can be introduced as a bio-indicator for Pb, Cu, Cd and Zn.

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INTRODUCTION

Heavy metals are natural trace components of the aquatic environment, but their levels have increased due to industrial agriculture, mining activities, industrial activity, shipping accident. Pollution studies in the ROPME area including the Persian Gulf and the Oman Sea are extremely important because this region is shallow, semi-enclosed, and has a very high evaporation rate and poor flushing characteristics [25].

Coastal pollution has been increasing significantly over the recent years and found expanding environmental problems in many developing countries. Urban and industrial activities in coastal areas introduce significant amount of trace metals into the marine environment, causing permanent disturbances in marine ecosystems, leading to environmental and ecological degradation and constitute a potential risk to a number of flora and fauna species, including humans, through food chains [21]. Heavy metals were chosen as suitable pollutants because they are widespread environmental contaminants, from either natural or anthropogenic sources, and are widely believed to be a threat to the health and survival of many marine or aquatic animals, including crustaceans. The contaminated fish and crustaceans from aquatic environment may become a public health concern. Hence, it is important to determine the concentrations of heavy metals in commercial fish and shrimps in order to evaluate the possible risk of human consumption [2].

Penaeid shrimps are economically important crustaceans. They are abundant off tropical and subtropical coasts. The white leg prawn *Litopenaeus vannamei* is a tropical species that is distributed geographically all

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over the world. It is an important commercial crustacean for the Iranian aquaculture industry. It is cultured in extensive, intensive and semi– intensive systems where most post larvae farms are located in zones with high industrial pollution [29].

The *Metapenaeus affinis* is the most important shrimps of commercial species in the North Coast of the Persian Gulf in the fishing season time. Since the maximum width of the Gulf is 640 m and the average depth of it is 35 km, the Persian Gulf is a good place to live a large number of Penaeid shrimps family. The time to replace the water in the Gulf is between 3 to 5 years to show there is a lot of time will remain in removing the contaminants. Because of the low depth of the northern part of the Persian Gulf and limited circulation and the high temperature, and salinity, the contaminants of heavy metals remain more in that part. They are in terms of sustainability and the lack of the chemical and biological processes are important [17]. Heavy metals after entering the water ecosystems through the shell and gill tissues enter the organs of aquatic animals and accumulate in the other aquatic food chain [10].

There are several studies in the field of heavy metals accumulation in fishes has been carried out but there are rare studies on shrimps in Iran. Rafiee *et al.* [20] found the amount of mercury, cadmium and lead in the Bahrekan coast, and the estuary of Musa, among *M. affinis*. Razavi *et al.* [22] studied the rate of accumulation of metals (mercury, lead, and cadmium) in the shell and muscles of the Indian white shrimps (*Fenneropenaeus indicus*) in Bahrekan coasts. Reviewed and determined the heavy metals in the Banana shrimps (*Fenneropenaeus merguensis*) released in the Persian Gulf (Bandar Abbas) [12].

Therefore, the present paper aims to highlight the level of selective trace metals (Zn, Cu, Pb and Cd) in the muscle and shell tissue of two commercially important species of shrimps collected from Persian Gulf.

MATERIALS AND METHODS

Description of the Study Site:

This research studies the concentration of heavy metals cadmium, lead, zinc and copper in the muscles and the shell of the *Metapenaeus affinis* as a native species and the culture species *Litopenaeus vannamei* in both summer and winter seasons in Khouzestan and Bushehr Province. 80 samples of *L. vannamei* and surface sediment were collected from the intertidal regions of Nayband Bay (27º 28΄ N; 52º 35΄ E) of Bushehr Province. 60 species of *M. affinis* were caught in the fishing area of Bahrekan (Hendijan) of 5 meters above the sea level (30º 15΄ N; 40º 38΄ E) in Khouzestan Province (Figure 1).

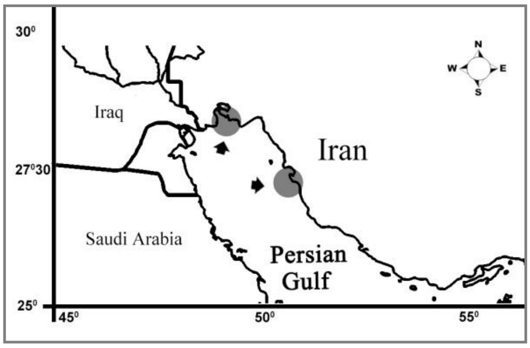

Fig. 1: Map of the study area.

Preparation of Specimen:

After caught, shrimps were kept frozen by storing them in an ice box, and transported to the laboratory as soon as possible. After measuring the physical parameters (weight and length) of each shrimp, they were then filleted by removing the muscle and shell. The samples were then rinsed with purified water to remove foreign particles, and patted dry with paper towels. After that, the samples were frozen, freeze-dried, grounded into powder and homogenized. The procedure for the extraction heavy metals was based on Standard Method 3052 (Microwave-assisted total heavy metal digestion) [27].

Trace Metals Analysis:

A Perkin-Elmer, model 4100 ZL atomic absorption spectrophotometer, equipped with a GTA

Graphite furnace, was used. Pyrolytic-coated graphite tubes with a platform were used and signals were measured as peak areas. All reagents were of analytical reagent grade unless otherwise stated. Double distilled water was used for the preparation of solution. All the plastic and glass ware were soaked in nitric acid for 15 min and rinsed with deionized water before use. The stock solutions of metals (1000 mgl^{-1}) were obtained by dissolving appropriate salts of the corresponding metals (E. Merk) and further diluted prior to use. High purity argon was used as inert gasted prior to use. The samples were solubilized using high-pressure decomposition vessels, commonly known as a digestion bomb. A sample (1g) was placed in to Teflon container and 5 ml of concentrated HNO3 was added. The system was heated to 130 ºC for 90 min and finally diluted to 25 ml with deionized water. The sample solution was clear. A blank digest was carried out in the same way. Zinc, cadmium, lead and copper metals were determined against aqueous standards [28].

All analyses were done in triplicate and the results were expressed with standard deviation.

A Van Veen grab was used to collect sediment from the bottom at two stations. In each station, sediment samples were collected at 3 random locations. Samples were kept in per- cleaned glass jars and stored in ice-chest and immediately transferred to laboratory. Any visible materials and shell were removed from the samples. In the lab, sediment samples were freeze dried and ground to powder using a glass mortar to a fine powder and then re-dried for 3 hours and stored in clean plastic vials in a fridge until digestion. Approximately 1g of the sediment samples from each station was digested with 2 ml of HNO and 6 ml of HCl 3 (Merck, Darmstadt, Germany). The remaining digested solution was made up to certain volume with double distilled water [1].

Statistical Analysis:

Statistical analyses were performed using SPSS 20.00 for Windows. All data were tested for goodness of fit to a normal distribution with Kolmogorov-Smirnov´s one sample test. Two-way analysis of variance (ANOVA) was used for comparison of heavy metals concentrations in sell and muscle of shrimp and sediments. Tukey´s Multiple-range test was used for comparison of heavy metals concentrations in shrimp from different sites. Values are expressed in means standard error, mg/kg of dry weight for samples; significant levels were put at P<0.05.

Results:

Mean length, weight and carapace length shrimps presented in Table 1.

Table 1: Mean length and weight of the species examined in present study.

Station	species	The number of samples	Total length± SD (cm)	Carapace length± SD (mm)	Weight± SD (g)
Nayband Bay	*L.vannamei*	80	20.00±15.00	80±26.50	21.00±13.00
Bahrekan	*M. affinis*	60	12.00±6.00	22.00±16.00	11.50±5.50

The concentrations heavy metals in shell and muscle of *M.affinis* and *L.vannamei* are summarized in Table 2 and 3. In the present study, highest concentration of Zn was observed in two shrimps followed by Cu, Pb and Cd (Table 2, 3). In summer, heavy metals levels was higher than winter (P<0.05). Shrimps collected from the Bahrekan station had higher concentration of metals than those from the Nayband Bay station.

Metals levels in shell of two shrimps were high in comparison with muscle and have significant differences (P<0.05). The highest concentration was determined in the shell of *M. affinis* in Bahrekan (Zn: 44.61±15.67 mg/kg d.w) in summer and the minimum concentration in the muscle of *L.vannamei* in Nayband Bay in winter (Cd: 0.001±0.006 mg/ kg d.w).

Table 2: Mean concentration of heavy metals in tissue of *M.affinis*.

Heavy metals (mg/kg dry weight)					
Season	Tissue	Cu	Zn	Pb	Cd
summer	shell	10.00±0.06^{a}	44.61±15.67^{a}	0.20±0.009^{a}	0.15±0.007^{a}
winter	shell	5.50±0.09^{b}	16.33±12.37^{b}	0.08±0.007^{b}	0.10±0.009^{a}
summer	muscle	3.00±0.03^{c}	38.64±12.85^{c}	0.10±0.008^{a}	0.11±0.004^{a}
winter	muscle	1.10±0.04^{d}	10.45±10.33^{d}	0.04±0.005^{b}	0.01±0.003^{b}

Table 3: Mean concentration of heavy metals in tissue of *L.vannamei*.

Heavy metals (mg/kg dry weight)					
Season	Tissue	Cu	Zn	Pb	Cd
summer	shell	7.00±0.02^{e}	34.11±12.17^{e}	0.20±0.004^{e}	0.10±0.004^{e}
winter	shell	3.50±0.01^{f}	10.38±9.34^{f}	0.02±0.001^{f}	0.01±0.009^{f}
summer	muscle	4.50±0.01^{g}	26.66±12.00^{g}	0.10±0.001^{e}	0.01±0.004^{f}
winter	muscle	1.00±0.01^{h}	5.19±3.11^{h}	0.01±0.003^{f}	0.001±0.006^{f}

Within each column of the metal of each species, means with the same letter are not significantly different according to Duncan’s Multiple Range Test at 5% level.

In case of Cu and Zn concentrations significant differences were observed between the organs and season (P<0.05).

Concentration metals in sediment in two station and seasons observed in Table 4, 5. In the case of sediment, the concentration of Cd, Zn, Pb and Cu in the sediment of Bahrekan Bay was significantly higher than that in the Bahrekan (P<0.05). The highest concentrations of all the four heavy metals in sediments were found at station of Bahrekan.

Table 4: Concentration of metals (mean± S.D) µg g^{-1} dry weight in sediments collected from Bahrekan.

Season	Heavy metals			
	Cd	Zn	Pb	Cu
summer	1.00±0.05^{a}	164.04±52.22^{a}	45.83±18.48^{a}	85.00±23.33^{a}
winter	0.30±0.07^{b}	98.74± 35.66^{b}	20.00±13.66^{b}	52.01±15.44^{b}

Table 5: Concentration of metals (mean± S.D) µg g^{-1} dry weight in sediments collected from Nayband Bay.

Season	Heavy metals			
	Cd	Zn	Pb	Cu
summer	0.80±0.04^{c}	45.00±12.45^{c}	20.04±10.90^{d}	25.66±14.00^{c}
winter	0.08±0.02^{d}	29.04±11.00^{d}	14.55± 5.33^{d}	15.00±13.23^{d}

Within each column of the metal of each species, means with the same letter are not significantly different according to Duncan’s Multiple Range Test at 5% level.

Comparing heavy metal contents of shrimp species with sediments of stations, their coefficients of correlation (r) were calculated and the correlation between the metals was determined. In summer for the levels of Zn, Cu, Pb and Cd, inter-metal relationships positively correlated in the environmental matrices ($r=0.84$, $P\leq 0.05$, $n=4$), but in winter inter-metal relationships appear to be different and not positively correlated ($r=0.64$, $n=4$, $P\geq 0.05$).

Discussion:

According to the Priority List of Hazardous Substances established by the Agency for Toxic Substances and Disease Registry [3], the descending order of heavy metals threatening to human health were As> Pb> Cd> Ni> Zn> Cr> Cu> Mn. These toxic metals consequently accumulate in fish muscle, threatening human health through the consumption of contaminated aquatic animals. Aquatic animals have been successfully used as accurate indicator organisms for environmental monitoring programmes because they possess numerous advantages, which include: (1) they are typically present in all aquatic systems; (2) there is extensive life-history and environmental response information available for most species; (3) aquatic animals communities usually include a range of species that represent a variety of trophic levels and include foods of both aquatic and terrestrial origin; (4) they are comparatively stable and therefore provide a long-term record of environmental stress; (5) they contain many life forms and functional guilds and thus are likely to cover all components of aquatic ecosystems affected by anthropogenic disturbance; and (6) they are both sedentary and mobile and thus reflect stressors within one area as well as providing scientists to give a broader assessment of effects [13]. Therefore, the elucidation of heavy metal levels in the aquatic animal species investigated in this study provided an indication of the current environmental conditions of the Bahrekan and Nayband.

Levels of Zn and Cu have been found to be higher than Pb and Cd and it could be explained because of these metals play a role in the enzymatic and respiratory processes of in aquatic animals [8]. Results showed that shell in accumulation of heavy metals have effective than muscle and in *M.affinis* higher than *L.vannamei*. The levels of heavy metal in aquatic animals vary in various species and different aquatic environments. Trace element concentrations varied markedly among species. These variations are presumably due to individual samples being of different size categories, from different ecological niches, and from different trophic levels. Possibly, species also have different metabolic requirements for specific trace element [15]. The present study exhibited significant spatial variation in metal level amongst the species, which may be due to variations in environmental conditions.

In the present study, it was found that concentration of Zn in two the shrimp species were relatively higher compared to concentration of other metals in the same animals. Zn being an essential element for normal growth and metabolism of animals, exhibited highest accumulation in the shrimp shell when compared with the other three metals. It is generally believed that fin and shell aquatic animals are actively regulate Zn concentrations in tissues and that therefore Zn tissue levels do not reflect the changes in Zn concentrations in the environment [23]. Cu is an essential trace metal for animal metabolism but at high levels is a very toxic substance to aquatic life. The main sources of Cu in the coastal waters are antifouling paints and this metal entered into the water body through industrial effluents containing $CuSO_4$ used in metal plating and fishing operations. Contaminated food probably represents a more important source of copper than water and thus burdens in fish and shell fishes cannot be consistently related to ambient pollution levels in water. Despite the existence of a number of detoxifying and storage systems for Cu, it is the most toxic metal after mercury and silver, to a wide spectrum of marine life, hence its value in antifouling preparations [16]. Hence, the relatively high levels of these metals can be attributed to their essentiality.

It is well documented that levels of heavy metals vary in aquatic animals depending on factors such as habitat, migration, age, ecological needs, size, length fish, metabolism and feeding activities [26]. In study of Askari Sari *et al*. [4] results showed that the distribution patterns of heavy metals (Zn, Cd, Cu, Fe, Hg, Mn) in tissue of *Liza abu* of north of Persian Gulf follows the order: gill> liver> muscle. Heavy metal concentrations were higher in the gill and liver, when compared with muscle. Absorption of metals on to the shell and gill surface, as the first target for pollutants in water, could also be an important influence in the total metal levels of those. Studies have shown that muscle is not an active tissue in accumulating heavy metals. This may reflect the low levels of metal-lothionein, low molecular weight binding proteins, in the muscle**.** Metals that enter the body via food are carried by the blood bound to 256 proteins, where they move first move into the liver and gradually into the muscle tissues [32].

The findings of other studies are summarized in the Table 5, and are compared with the concentrations reported in this study and elsewhere in the world, which reflects the adverse impact of industrialization and urbanization on the biotic community. It is interesting to note that in some cases, mean levels of trace elements in edible tissues of shrimps from a selected region may be different under different storage conditions. The metal concentrations in this study were below the permissible limits for human consumption of international guidelines except Cd (Table 6). Cadmium is regarded as a priority pollutant because of its toxicity to organisms in the aquatic environment. The comprehensive analysis showed that Cd in two shrimps muscle represents the potential risk to human health. Since the study areas is still an important region for export activities and mari-culture production in Iran, it is necessary to control heavy metal levels in water, sediment and aquatic animals. Quality control of shrimp pond water by monitoring input and output water, and assessing and controlling the metal contents in cultivated shrimps and associated sediments should be regularly conducted in the future.

Table 6: A comparison of heavy metals concentrations (ppm in dry weight) in crustaceans collected from different parts of the world.

Reference	Cu	Zn	Cd	Pb	Species
Movahed *et al*. [17]	0.45±0.03	1.7±0.03	10±0.35	1.4±0.15	*Ferropenaeus indicus*
Movahed *et al*. [17]	0.47±0.03	1.28±0.03	9.60±0.27	2.10±0.15	*Penaeus semisulcatus*
Movahed *et al*. [17]	0.36±0.03	1.35±0.02	9.80±0.33	2.80±0.31	*Litopenaeus vannamei*
Movahed *et al*. [17]	0.44±0.03	1.23±0.03	10.0±0.75	2.20±0.25	Sea shrimps
Rahouma *et al*. [24]	39.37-56.09	29.18-46.34	0.04±0.76	0.07±0.59	*Acetes* sp.
Bat *et al*. [5]	5.85-14.77	18-36	0.228±0.481	0.291±0.491	*Crangon crangon*
Rafiee *et al*. [20]	-	-	0.28	0.63	*Metapenaeus affinis*
Rafiee *et al*. [20]	-	-	0.24	0.55	*Metapenaeus affinis*
Rafiee *et al*. [20]	-	-	0.19	0.41	*Metapenaeus affinis*
Rafiee *et al*. [20]	-	-	0.045-0.12	0.072-0.164	*Fenneropenaeus indicus*
Javaheri Baboli and Velayatzadeh [12]	1.26±0.20	13.80±0.70	0.175±0.006	0.414±0.012	*Fenneropenaeus merguiensis*
Mitra *et al*. [16]	8-13	10-32	0-1	0-3.67	*Metapenaeus brevicornis*
Pourang *et al*. [18]	20.3	47.3	0.31	-	*Fenneropenaeus merguiensis*
The present study	1.10-10.00	10.45-44.61	0.01-0.15	0.04-0.20	*Metapenaeus affinis*
The present study	1.00-7.00	5.19-34.11	0.001-0.10	0.01-0.20	*Litopenaeus vannamei*

Table 7: The tolerable values of some heavy metals in the fish (mg kg^{-1}).

References	Heavy metals				
	Pb	Cd	Cu	Zn	Standards
MAFF [14]	2	<0.2	20	50	The food safety
WHO [30]	0.5	0.2	10	100	World Health Organization WHO
Zheng *et al*. [31]	0.3	2	20	50	FAO
FDA [11]	5	1	-	-	The food and drug FDA-America
Darmono and Denton [9]	1.5	0.05	10	150	The Australian national health and medical research
MAFF[14]	2	0.2	20	50	The Ministry of agriculture, fisheries and food
Movahed *et al*. [17]	1	0.1	20	50	Iran National Institute of standard

Pb is a neuro-toxin that, cause behavioral deficits in aquatic organisms and decreases in survival, growth rates and metabolism [6]. There is often little accumulation of Pb in marine and freshwater species. Consequently lead is not a threat to fisheries resources except at extreme pollution [7]. The most toxic of the heavy metals is Pb, which finds its way in coastal waters through the discharge of industrial waste waters, such as from painting, dyeing, battery manufacturing units and oil refineries *etc*. Antifouling paints used to prevent growth of marine organisms at the bottom of the boats and trawlers also contain lead as an important component. These paints are designed to constantly leach toxic metals into the water to kill organisms that may attach to bottom of the boats, which ultimately is transported to the sediment and aquatic compartments. When compared with the recommended value of World Health Organization [30] in context to consumption of prawn (0.5 ppm for Pb), the concentrations in two the shrimp species from stations were below this level.

In the case of Pb and Cd, the reverse case can be observed, namely their levels were lower relative to the other studied elements (Table 5). The relatively low accumulation of these elements may be due to existence of developed systems to excrete toxic metals in crustaceans.

Most shrimps are benthic organisms living on the bottom of oceans or seas. They generally live among algae and sea grass, under stones and shells, in the cracks of rocks and corals on hard surfaces, and in shallow holes on soft surfaces and they are omnivores that consume foraminifer, polychaeta, crustacean, algae species, and detritus. It could be said that sediment geochemistry has a very important role in the accumulation of metal in shrimps because of feeding behaviors of shrimps besides creating a living environment for shrimps. When the metal contents of shrimps are examined, it is seen that there are differences among species. It is thought that these differences are caused by the genetic variations among species, different feeding habits of species and differences in their living environments [19].

Conclusion:

Bioaccumulation patterns of metals in shellfish muscle can be utilized as effective indicators of environmental metal contamination. According to many researchers, some shellfishes by virtue of their mobile nature are not fair indicator of aquatic contamination, but their regular consumption by human beings makes it absolutely necessary to monitor their different organs, particularly the muscles. The present study is therefore important not only from the safety point of view of human health, but also from the quality point of view as many of these shellfish species have high export value.

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