Bioaccumulation of heavy metals (Ni, V, Cu, Pb) in various tissues of *Metapenaeus affinis* in the Northwest of Persian Gulf

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Abstract

This study was carried out to detect the concentration of heavy metals (Ni, V, Cu, Pb) in the muscle, gills and hepatopancreas tissues of female and male of *Metapenaeus affinis* in Bahrekan Bay. Sampling was conducted in 15 stations in triplicate, on which the samples collected by Trawl net. After separation of tissues, samples were digested by acid digestion with nitric and percloric acid and hydrogen peroxide. The analyses for the detection of heavy metals were carried out by flame atomic absorption spectrophotometer. The highest concentration of metals in gills, hepatopancreas and muscle was related to Cu (in males: 9.06 ±0.15 mg/kg), (in males: 26.80± 0.20 mg/kg), (in females: 16.83±0.76 mg/kg), respectively while the lowest one found for V in gills and hepatopancreas, respectively in male: 1.26± 0.20 mg/kg and (in females: 1.40± 0.10 mg/kg). Ni, V and Pb were no detected in all muscle samples. The alteration process of Cu in both sexes in the selected tissues order as hepatopancreas > muscle > gills. The alteration process of Pb, Ni and V in both sexes in the selected tissues order as hepatopancreas > gills> muscle. Significant differences ($P<0.05$) were recorded between the metal concentrations in all tissues except muscle.

Key words: Heavy metals, *Metapenaeus affinis*, Bahrekan Bay, Bioaccumulation

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Introduction

Heavy metals in the environment are potentially harmful to most organisms at some levels of exposure and absorption (Yoshida, Ikeda & Okuno 2006; Soltani, Kakoolaki & Kisami 2000). Heavy metals are natural components of the earth's crust and they can enter the water and food cycles through a variety of chemical and geochemical processes (Adedeji & Okocha 2011; Biney, Amuzu, Calamari, Kaba, Naeve & Saad 1999). Also anthropogenic

pollutants are the main sources of heavy metal contamination in the ocean (Hamilton & Hafman 2003). Among the contaminants, heavy metals should be highlighted due to the consequences of their bioaccumulation in aquatic ecosystems (Nadmitov, Hong, In Kang, Chu, Gomboev, Janchivdorj, Lee & Khim 2015), in which such as phytoplankton was accumulated by heavy metals (Dashtiannasab, Kakoolaki, Sharif Rohani & Yeganeh 2012). Advancement in technology as well as increase in population have led to environmental concerns relating from indiscriminate dumping of refuse and discharge of industrial effluents, petroleum waste water, and crude oil spills replete with most common heavy metals in our ecosystems (Adedji & Okocha 2011; Jones & Cherian 1990). Heavy metal contamination in on local, regional and global Scales have been intensively studied in recent years, due to the fact that metals are persistent, toxic, tend to bioaccumulation, and they pose a risk to humans and ecosystem (Yahyavi, Afkhami & Khoshnod 2012). In natural life, some trace metals are essential at low levels but toxic at higher concentration. They enter in the human body through food chain causing different diseases and damages to the humans (Yamuna, Saravana, Bhavan & Geraldian 2012; Tabinda, Hussain, Ahmad & Yaser 2010). Heavy metals accumulate in tissues of aquatic animals and hence heavy metals measured in tissues of aquatic animals can reflect past exposures (Yilmaz 2003; Canli & Atli 2003). These metal levels in marine environment should be monitoring studies as bio-indicators of heavy metal pollution has been emphasized by several investigators (Yilmaz & Yilmaz 2007; Pourang, Dennis & Ghourchian 2004). Shrimp, different from most fish in its feeding habit, is a scavenger that feeds on a wide range of materials including debris and other bottom-dwelling animals (Yilmaz & Yilmaz 2007; Canli & Atli 2003). Prawns are an important food source for larger animals from fish to whales. As with other sea food, prawns are high in calcium, iodine and protein but low in food energy. Consumption of prawns from Bays and streams polluted by heavy metals by humans is thought to lead to disorders or diseases (Adedji & Okocha 2011).

In aquatic ecosystems bio accumulation factors is used to quantify chemical accumulation in tissue relative to concentration in water or sediment (Fairbrother, Wenstel, Sappington & Wood 2007; Thomann, Mahony & Mueller 1995). Bioaccumulation of heavy metals is the net accumulation of a metal in the tissue of interest or the whole organism that results from all environmental exposure media,

including air, water, solid phases, and diet (Fairbrother *et al*. 2007).

The Bahrekan Bay located in Northwest of Persian Gulf in Iran. That is around 60 km from the Hendijan town (in Khouzestan province). There are many sources of pollution on this coast such as wastes from coastal towns, rivers and oil ships transportations. Also Bahrekan Bay is one of the most important oil fields in Iran (Mohammadi Roobahani et al. 2013). White shrimp is one of the native species in the Persian Gulf and Oman Sea. This species is one of the commercial important shrimp species coastal water of hormozgan province and frist ranking catch that includes about 70 percent of total catch of province.

The presents study has been conducted to determine Ni, V, Cu and Pb accumulation in the muscle, gills and hepatopancreas tissues of female and male sexes of *M. affinis* in Bahrekan Bay located in the north of Persian Gulf.

Material and Methods

Study Area

Study area was in Bahrekan Bay where is located in the Northwest of Persian Gulf in Iran. The Persian Gulf located in the southwest of Iran, between longitudes 48°25' and 56°25" East and latitudes 24°30" and 30°30' North. There is an important fishery wharf in the study area. Also it is one of the most important of oil fields in Iran located in the region. Sampling sites was selected of an area that has a high importance as one of the most significant habitats of shrimp, and in its nearby, fishing activities is the main profession of people.

Sampling stations

Samples of sediments and prawn were collected between (49° 42' 300" – 49° 46' 232"N, 30° 03' 140" - 30° 05' 556") E from 15 coastal localities in Bahrekan Bay (Fig 1). Sediment samples were collected Van Veen grab. The sediment samples were immediately sealed and stored at 4 °C until arriving at the laboratory. In each station, sediment samples were collected according to the standard procedures described in USEPA sediment sampling guide (United States Environmental Protection Agency, 1999). Prawn samples were collected from 15 stations by fishing net (Fig 1).

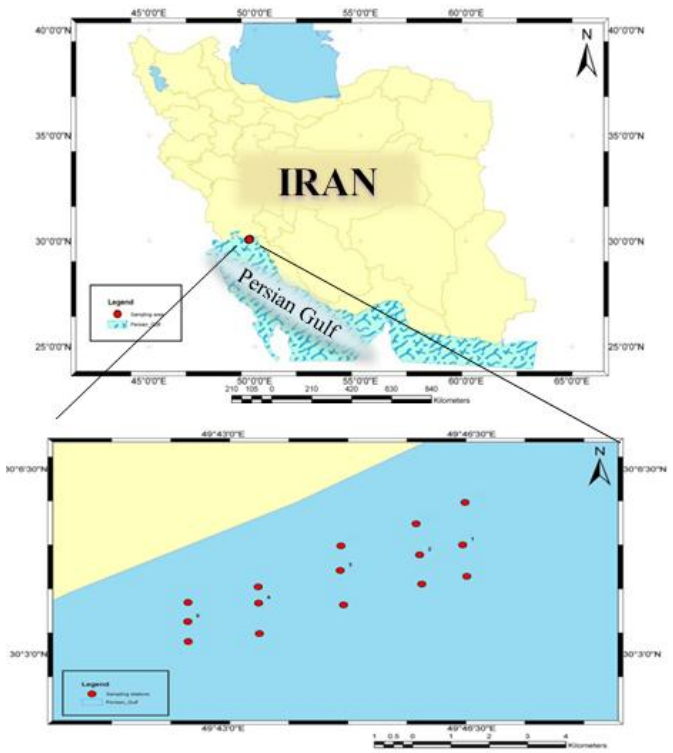

Figure 1 Study area showing the location of the sampling sites

All prawn samples (including 30 males and 30 females) were collected in spring of 2014 with tree repeat at each station by trawl net. In each station, sediment samples were collected according to the standard procedures described in USEPA sediment sampling guide (USEPA 1999). All prawn samples were collected from same stations whole as sediments and were transported to the laboratory in a thermos flask with ice on the same day. Prawn samples were cleaned by deionized distilled water, stored in pre-cleaned plastic, and kept at -20˚C until analysis. Preparation of all samples was carried out according to ROPME (Regional Organization for the Protection of Marine Environment, 1999). In the laboratory muscle, gill and hepatopancreas were dissected, washed with distilled water, weighed, packed in polyethylene bags and stored according to USEPA (1999). Samples of tissues were dried at 65 ˚C for 24 h.

Sampling preparation

Sediment samples dried at 70 ˚C for 48 h. The dried sediments were grounded into fine powder and were passed from a 63-µm mesh. About 0.5 g of the powdered sample was treated by 5 ml aqua regia. After evaporation, allowed to cool. Then 3 mL of perchloric acid were added. Finally, then samples had been filtered and cooled to

room temperature. The filtrates samples were transferred to 50mL volumetric flasks and brought to volume with 1 N HCl (Chester and Hughes, 1967; Tessier, Campell, & Bisson 1979). The Prawn samples were brought to the laboratory on ice immediately and then frozen at -25°C until dissection. Then samples of different organs have been separated. All of the samples were dried at 60°C for 48 h in laboratory oven.0.5 gr of dried sample were weighted and then digested in acid-cleaned Teflon beaker with 5ml of ultra-pure nitric acid (65%v/v) and placed in an aluminum block for 24h. Then contents of the beakers were evaporated to near dryness on 80°C under the hood. Each beaker removed from the hot plate. 3 cc of H_2O_2 was added to each breaker. Each breaker was replaced on hot plate, heated to 80°C until clear the yellow tint of the solution should disappear are the digest is completed. Digested samples transferred to a graduated plastic test tube and brought up to volume (50ml) (Manual of Oceanographic Observation and Pollutant Analysis Methods 1999). All samples were analyzed three times heavy metals by atomic absorption spectrophotometric.

Statistical Analysis and calculations

To calculate the bioaccumulation factor for each element in muscle, gills and liver tissues of the prawn following equation was used:

$$BAF = Co\ (mg\ g^{-1}\ d.w.)\ /C_s\ (mgg^{-1}\ d.w.)$$

Where *C*o is mean concentrations of metal in the organism and C_s is the mean concentrations of heavy metal in sediments. BAF values indicate relative ability of organisms to absorb selected metals from the ecosystem in which they live (Hendozko, Szefer & Warzocha 2010).

Statistical analysis was done using the methods of two-way variance analysis. Methods for study for examining existence and non-existence of significant difference at the level of 5% between total mean concentration of metals with SPSS software.

Results

Table 1 shows the mean of heavy metals concentration and standard deviations for the *M. affinis* prawn samples. The resulting out came of analyzing the concentration of heavy metals in the species showed that the highest concentration was measured in gills and hepatopancrease for both sexes related to Cu and lowest concentration related to V. Significant differences ($P<0.05$) were recorded between the concentration of heavy metals in different stations. But, no significant differences ($P>0.05$) were analysed between the concentration of

metals in males and females. All metals except of Cu were no detected in 100% of all muscle samples.

The alteration process of Cu, Pb, Ni and V in gills and hepatopancreas for both sexes order as: Cu> Ni> Pb>V. Also in muscle only Cu was detected.

Significant differences ($P<0.05$) were recorded between the concentration of Cu in different tissues prawn samples. The alteration process of Cu in both sexes in the selected tissues order:

hepatopancreas > muscle>gills.

Pb, Ni and V had not detected in muscle and their concentrations order as: hepatopancreas > gills.

Not significant differences ($P>0.05$) were recorded between the concentration of Pb and V for the gills and hepatopancreas tissues. Significant differences ($P<0.05$) were recorded between the concentration of Cu in different tissues. Also mean concentration of Ni had Significant differences ($P<0.05$) between gills and hepatopancreas tissues.

Table 2 shows comparing our results to some other researches in other countries. About comparing to standards values, Zn is less than WHO (2000) and FAO (1983) standard values.

Table 1 Mean concentrations (mg/kg) of Ni, V, Cu, Pb measured from the samples of the gills, hepatopancreas and muscle in male and female of *Metapenaeus affinis*

Metals	Tissues	Sex	Station 1	Station 2	Station 3	Stations 4	Station 5
Ni	**Gills**	Male	4.25±0.05	5.40±0.07	5.35±0.06	5.83±0.20	7.39±0.13
		Female	5.38±0.07	6.29±0.13	7.32±0.12	5.06±0.45	5.56±0.35
	Hepatopancreas	Male	8.76±0.15	8.70±0.10	7.63±0.25	9.16±0.25	8.60±0.45
		Female	7.33±0.20	8.43±0.41	8.70±0.52	8.40±0.43	9.23±0.41
	Muscle	Male	ND	ND	ND	ND	ND
		Female	ND	ND	ND	ND	ND
V	**Gills**	Male	1.26±0.20	1.23±0.05	1.56±0.15	2.23+0.11	2.00±0.26
		Female	1.50±0.40	1.43±0.05	2.06±0.15	1.60±0.36	2.53±0.40
	Hepatopancreas						
		Male	1.43±0.25	2.36±0.15	1.83±0.30	2.53±0.40	2.66±0.40
	Muscle	Female	1.70±0.10	1.40±0.10	1.93±0.37	2.36±0.37	2.73±0.20
		Male	ND	ND	ND	ND	ND
		Female	ND	ND	ND	ND	ND

	Gills	Male	8.16±0.05	9.06±0.15	6.83±0.64	6.23±0.05	8.56±025
		Female	7.26±0.20	8.16±0.11	7.26±0.20	8.40±0.10	7.66±0.40
Cu	**Hepatopancreas**	Male	26.80±0.20	18.86±0.15	18.36±0.45	21.33±0.68	24.36±0.32
		Female	22.80±0.72	19.83±0.76	18.83±0.20	25.86±0.80	19.83±0.80
		Male	11.40±0.45	15.50±0.50	11.86±0.23	15.53±0.47	15.83±0.76
	Muscle	Female	11.83±0.76	17.50±0.50	12.83±0.72	16.83±0.76	15.66±0.35
	Gills	Male	2.73±0.20	3.30±0.26	2.70±0.17	2.50±0.30	3.33±0.15
		Female	1.66±0.05	3.40±0.26	3.70±0.26	5.16±0.25	3.53±0.25
		Male	2.46±0.25	2.66±0.45	2.13±0.25	3.16±0.30	2.86±0.49
Pb	**Hepatopancreas**	Female	2.36±0.37	2.53±0.49	1.93±0.45	3.36±0.30	3.16±0.70
	Muscle	Male	ND	ND	ND	ND	ND
		Female	ND	ND	ND	ND	ND

The concentration of heavy metals in sediment samples of Bahrekan Bay is listed in Table 1. As this table shows, the total concentrations of heavy metals decreased in sediment as pattern of Ni > V > Cu > Pb.

Table 2 Heavy metal concentrations in sediment samples from Bahrekan Bay (mg kg^{-1} d.w.)

Sampling site	**Element**							
	Ni		V		Cu		Pb	
	Average	SD	Average	SD	Average	SD	Average	SD
1	56.33	0.57	29	1.00	10.66	0.57	6.33	1.52
2	67.66	1.52	58.33	17.09	19.33	1.52	11	1.00
3	69.66	0.57	56.66	2.88	32.66	2.51	12.33	0.57
4	90.00	1.00	77.33	2.08	39.66	1.52	15.33	1.52
5	105	4.35	97.66	1.52	52.66	2.08	20.66	1.51
Total	79.53	16.12	63.80	24.61	31	15.40	13.13	5.02

Bioaccumulation factor of trace elements in muscle, gills and hepatopancreas of *M. affinis* in regard to concentrations of metals in sediment are cited in Table 3. As Table 3 Indicates, almost all BAFs in Hepatopancreas are more than gills and then more than muscle. Also BAF of Cu in Hepatopancreas is near to 1. According to Rashed (2001) BAF more than 1, indicates bioaccumulation in an organism (Rashed 2001). It should be noted that Cu is a micronutrient and also have toxic effects.

Table3 Results of BAFs in studied species tissues

Tissue	Sex	Ni	V	Cu	Pb
Gill	Male	0.07	0.02	0.25	0.22
	Female	0.07	0.02	0.25	0.30
Hepatopancreas	Male	0.11	0.03	0.70	0.20
	Female	0.10	0.03	0.69	0.20
Muscle	Male	-	-	0.45	-
	Female	-	-	0.48	-

Other study showed heavy metals concentration (Pb, V and Ni) of edible part of *M. affinis* were significantly lower than other studies on prawn species in Bahrekan bay (Andy, Abdallah & Tayel. 2007; Bin Mookhtar, Zaharin Aris, Munusamy & Mangala 2009; Guhathakurta & Kaviraj 2000; Balkas, Turul & Saliholu 1982; Pourang & Amini 2001). Also the amounts of Cu in the muscle of *M. affinis* was higher than WHO standard levels and was lower than FAO standard levels. Cu concentration in the muscles of *M. affinis* were higher than other speicies of prawn such as *Penaeus monodon, Penaeus Kerathurus* and *Penaeus merguiensis* (Bin mookhtar 2009; Balkas *et al*. 1982; Pourang & Amini 2001).

Table 4 Comparing between results of this study with different shrimp species (mg/kg)

Species	**V**	**Ni**	**Pb**		**Cu**	**References**
Penaeus indicus	-	0.0002	23		-	Andy *et al.* (2007)
Penaeus monodon	0.254	۰,۱۲۲	13.30		7.21	Bin mookhtar *et al*. (2009)
Penaeus monodon	-	-	32.12		-	Guhathakurta & Kaviraj (2000)
Penaeus Kerathurus	-	-	0.34		7.4	Balkas *et al*.(1982)
Penaeus merguiensis	-	0.07	-		17.77	Pourang & Amini (2001)
M. affinis	-	-	-	15.53	Male	**Our Study**
				12.83	Female	
	0.5	0.3	0.5-1.5		10	WHO (2000)
	0.5	-	۰,۵		20	FAO (1983)

Discussion

Prawn is an important source of food for human and also an important part of natural food chain. The concentration of heavy metals in different tissues of *M. affinis* showed the potential effects of Ni, V, Cu and Pb on the prawn themselves and organisms that consume them. The results showed the presence of Ni, V, Cu and Pb in all gills and hepatopancreas tissues.

As we expected, significant differences were found in different tissues in view of accumulation of the selected metals. In this study the results of measuring Cu, Pb, Ni and V at both sex showed that the concentrations of Cu in comparison with other metals had the highest concentration in both sex and Pb, Ni and Cd were no detected in 100% of all in prawn muscle samples. Also, the comparison of our results with the international standards showed that the concentration of Cu in the prawn samples was less than the authorized rang of FAO standards, but it is more than WHO standard. Fortunately, our results absconded the Pb, Ni and V accumulation in muscle tissue. Also the results indicated a statistical variation between the metals levels in the analyzed gills, hepatopancreas and muscle tissues, as also registered by Pourang & Amini (2001).

Cu is an essential element and plays important roles in growth and cell metabolism of most animals. Hence, the relatively high levels of these metals may be blue-blooded to their essentiality. The relatively low accumulation of this metal can be because the existence of developed systems to excrete toxic elements in crustaceans (Simkiss & Taylor 1995). Our study showed that hepatopancreas tissue accumulate the heavy metals more than the other tissues of *M. affinis.* Some of researchers reported that in crustacean, hepatopancreas is the organ of metal storage and detoxification (Bliss 1983; Dall & Moriarty 1983; Anderson, Preslan., Jolibois, Bollinger & George 1997; Pournag & Amini 2001).

With regarding to BAF of Cu result of hepatopancreas tissue, which was close to 1.0, it must be considered that may be other tissues show a potential to accumulate other metals. Concentration of heavy metals is suggested to be determined in other prawn species tissues in the Bahrekan Bay.

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تجمع بیولوژیکی فلزات سنگین نیکل، وانادیوم، مس و سرب در بافت های متفاوت میگوی سر تیز *Metapenaeus affinis* در شمال غربی خلیج فارس

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چکیده

این مطالعه جهت تعیین غلظت فلزات سنگین نیکل، وانادیوم، مس و سرب در بافت های ماهیچه، آبشش و هپاتوپانکراس دو جنس نر و ماده گونه *Metapenaeus affinis* در ساحل بحرکان انجام شد. نمونه برداری در ۱۵ ایستگاه با ۳ تکرار و به وسیله تور ترال صورت گرفت. پس از جداسازی بافت ها، نمونه ها به وسیله اسید نیتریک، پرکلریک اسید و آب اکسیژنه هضم اسیدی شدند. قرایت فلزات سنگین توسط دستگاه جذب اتمی انجام شد. بیشینه غلظت فلزات به ترتیب در آبشش، هپاتوپانکراس و ماهیچه مربوط به فلز مس با میانگین غلظت (جنس نر: ۰/۱۵± ۹/۰۶)، (جنس نر: ۰/۲۰± ۲۶/۸۰)، (جنس ماده: ۰/۷۶± ۱۶/۸۳) میلی گرم بر کیلوگرم در حالی که میانگین کمینه غلظت مربوط به وانادیوم در هر سه بافت به ترتیب در آبشش و هپاتوپانکراس (جنس نر: ۰/۲۰± ۱/۲۶)، (جنس ماده: ۰/۱۰± ۱/۴۰) میلی گرم بر کیلوگرم تعیین گردید. نیکل، وانادیوم و سرب در هیچ یک از نمونه های ماهیچه قرایت نگردیدند. روند تغییر غلظت مس در هر دو جنس به ترتیب به صورت هپاتوپانکراس> ماهیچه> آبشش می باشد. روند تغییرات غلظت سرب، نیکل و وانادیوم در دو جنس و در بافت های انتخابی به صورت هپاتوپانکراس> آبشش> ماهیچه می باشد. بین غلظت فلزات در تمامی بافت ها به جز بافت ماهیچه، اختلاف معنی دار مشاهده گردید ($P<0.05$).

کلمات کلیدی: فلزات سنگین، *Metapenaeus affinis* ، ساحل بحرکان، تجمع بیولوژیکی

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