Biota-sediment accumulation factor and concentration of heavy metals (Hg, Cd, As, Ni, Pb and Cu) in sediments and tissues of *Chiton lamyi* (Mollusca: Polyplacophora: Chitonidae) in Chabahar Bay, Iran.

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Abstract

Heavy metals and some organic substances which are drained into the aquatic environments and cannot be decomposed or eliminated will sink into sediments or find their way into different levels of food chain. Bioaccumulation is the process of continuous deposition and aggregation of these substances into the body tissue of living organisms. Mollusks are remarkably appropriate to be used as bio-indicators because of their motionless or almost sessile nature, relatively high abundance, selective absorption of certain ions, and worldwide distribution in marine and inland aquatic habitats. *Chiton lamyi* is a sedentary species from the phylum, Polyplacophora, which is usually abundant on the rocky shores and intertidal zone of marine environments. Sediment and Chiton samples were taken from four stations of Chabahar Bay in autumn 2013. After acid digestion of samples, the concentration of heavy metals (mercury, cadmium, arsenic, nickel, lead and copper) were measured by the graphite furnace atomic absorption apparatus, and the bioaccumulation factor in relation to sediment was calculated. The highest concentration value was calculated as 3.28 for cadmium in the Hafte Tir station. The results of this study indicated that chitons can be used as an appropriate bio-indicator for heavy metals particularly cadmium pollution in the marine environment.

Keywords: Biota-sediment accumulation factor, Heavy metals, Bioaccumulation, Bio-indicator, Mollusks, Chabahar Bay

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Introduction

Nowadays, pollution becomes increasingly prevalent in our daily life. In some areas, it has a fairly negative impact on our health. Among all types of pollution, heavy metals have serious and often irreversible impacts which should never be neglected. Heavy metals are not degradable and transfer into the food chain, and through the bioaccumulation process pass to higher level consumers, resulting in bio-magnification and their concentration exceeds their safe level which cause serious diseases and mortalities among animals and human beings.

To have an environment free of pollution requires consistent monitoring of different pollutants and their sources, regular measurement of concentration of heavy metals, different chemicals and toxic ingredients and setting up a database, followed by analyses of the collected data and implementing appropriate policies to enforce the law and control the contaminants and their sources. The ever growing list of chemical contaminants released into the environment on a large scale are numerous aliphatic and aromatic compounds, heavy metals, radio nucleotides and phthalate esters. Among these innumerable contaminants, pollution by heavy metals in coastal environments has become a global concern because of their toxicity, persistence for several decades in the aquatic environment and bioaccumulation and bio-magnification in the food chains (Valls and Lorenzo, 2002; Gochfeld, 2003; Ebrahimi and Taherianfard. 2011). In order to minimize the hazard risks of different pollutants, the environment protection authorities and other relevant state and local government agents should educate the people to understand the value of recycling reusable waste material, and properly dispose of the toxic and other hazardous waste according to the rules and instructions, or deliver such wastes to special waste processing and management depots. Industrial wastes should also be monitored strictly, and different types of waste should be treated and disposed according to the relevant regulations.

Accumulation of heavy metals by aquatic organisms in many cases can be considered as an irreversible process, which can lead to biological magnification of these metals in higher levels of the food chain. Feeding on living organisms containing high levels of heavy metals, results in successive accumulation of these elements in their body tissues which in turn passes to the next level of the food chain, and is eventually transferred to the human body as the top level consumer (Blackmore, 2007; Yilmaz *et al*., 2007; Monsefrad *et al*., 2012). This is a serious threat to human health (Kumar *et al*., 2008).

Recent developments in Chabahar region such as different industrial and commercial activities encouraged and supported by the establishment of Free Trade Zone, and long-term plans to transform this port into a mega port in

order to upgrade its capacity to receive big cargo and commercial ships, development of petrochemical and oil refinery factories, as well as the extension of fisheries, shrimp farming and agricultural activities, would result in the increase of various pollutants and contaminants entering the sea water. At present the main sources of pollutants entering the sea are coastal urban and industrial sewage, agricultural effluents, different cargo ships, oil tankers and other vessels' blast water, bilge water discharge, ship wastes discharge, oil and dredging, and exchange of pollution through the water currents of Persian Gulf and Oman Sea (Amini and Miraki, 2006). As a result the heavy metal concentration in the water column will increase and make them sink into the bottom sediments. Analysis of these sediments can provide valuable information on different contaminants and heavy metal pollution (Cundy *et al*., 2003; Jha *et al*., 2003) Aquatic organisms, especially sedentary crustaceans and shellfishes such as mollusks, clams and oysters have been used as bio-indicators to measure the concentrations of heavy metals of different aquatic habitats of the world. Examples of some of these studies are: Demina *et al.* (2009) in hydrothermal fields of the Guayamas Basin (Gulf of California); Turkmen *et al*. (2005) in Iskenderun Bay, Egypt; Salman (2006) in Euphrates River, Ghana; Liu *et al*. (2007) in China; Shanmugam *et al*. (2007) in India; Turkey; Ohimain *et al*. (2008) in the Niger Delta; Gaber *et al*. (2008) in lake Timsah, Iraq; Netpae and Phalaraksh, (2009) in Bung Boraphet Reservoir, Thailand; Amisah *et al*. (2009) in Volta estuary, and Gheytassi (2013) in Chabahar Bay, Iran. In this study we used the chiton to investigate the heavy metal pollution in Chabahar Bay.

Materials and methods

Study area

Four different stations were studied along Chabahar coasts from Great Sea to Tiss port (Fig. 1). The position and the name of each station and the main activities in each station are shown in Table 1.

Sample collection

Sediment and Chiton samples were taken from the intertidal zone in autumn 2013. Fifteen chitons of the same size were collected from each station (Fig. 2). Sediment samples were taken from the closest location to chiton sampling location, and kept in polyethylene containers. Then all samples were transferred to the laboratory. Chiton samples were frozen at -20°C and sediment samples were refrigerated at 4°C.

Samples preparation

For measurement of cadmium, copper, nickel, lead and arsenic metals in chiton, the tissue samples were prepared according to Gheytassi (2013) method.

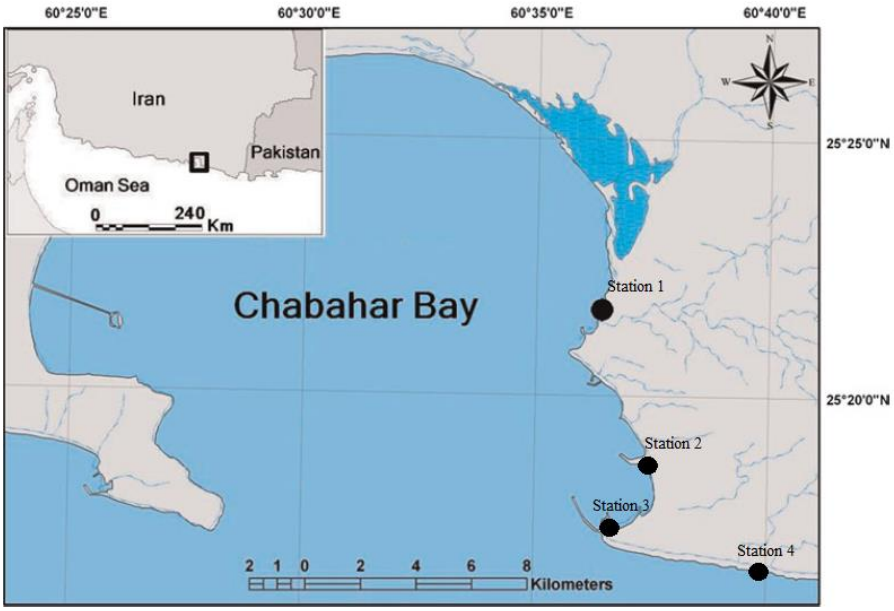

Figure1: Sampling locations in Chabahar Bay.

Table 1: Information related to sampling stations of the present study in Chabahar Bay.

Station	Geographical position	Current activities in the area
Station 1: Tiss	60º 36' 3.06" E 25º 21'10.46" N	The fishing port, entertainment and recreation
Station 2: Chabahar Maritime University	60º 37' 27.58" E 25º 18' 42.20" N	Social, urban
Station 3: Haft Tir Port	60º 37' 27.58" E 25º 17' 34.27" N	The mooring of various buoys, loading and unloading ships and fishing activities
Station 4: Great Sea	61º 3' 10.47" E 25º 12' 5.22" N	Entertainment and recreation, direct access to the Oman Sea

Figure 2: A view of the *Chiton lamyi* in a small rock pool in Chabahar Bay intertidal zone.

For this 1 g of dried powder of tissue samples was acid-digested with 7 mL of 65% HNO_3 and 3 mL of 37% HCl for 24 h at room temperature (Gheytassi, 2013), whereas for determination of mercury the tissue samples were processed in Teflon with10 mL of 65% HNO_3 according to Anderson and Meyers (2000). Sediment samples were digested with 30 mL of ($1HNO_3$:3HCl) acid solution, for the measurement of cadmium, copper, nickel, lead and arsenic. For determination of mercury 2 g of sediment was digested in Teflon with 10 mL of 37% HCl and 65% HNO_3 (1:3) (Anderson and Meyers, 2000).

After preparation, samples were refrigerated in polyethylene containers until they were injected into the apparatus (Yap *et al*., 2002). It should be noted that samples for the analysis of mercury were injected into the apparatus a few days after preparation (Feldman. 1974). Diluted nitric acid (25% v) was used as blank samples based on Kumar and Achyuthan (2007). Each sample was analyzed in triplicate to ensure the precision of each measurement. Then concentration of heavy metals was measured by Graphite furnace atomic absorption apparatus model YOUNG LIN 8020 made in southern Korea. The analytical figures of merit for Pb, As, Cd, Hg, Ni and Cu determinations by GFAAS are shown in Table 2.

Table 2: Figures of merit for Pb, As, Cd, Hg, Ni and Cu determinations by GFAAS.

Parameter	Pb	As	Hg	Cd	Ni	Cu
LOD (µg/mL)	0.056	2.28	0.15	0.04	1	0.7
Linear working range (µg/mL)	0.056-50	2.38-30	0.15-10	0.04-8	1-80	0.7-80
R	0.9989	0.9988	0.9996	0.9999	0.9997	0.9955
% RSD	2.29	4.9	6.5	4.9	8.6	2.3

LOD = limit of Detection
R = Linear correlation coefficients
RSD = Relative Standard Deviation

It is also notable that the accuracy of this analytical method in every measurement was adjusted by injection of standard solution of the respective element.

Statistical analysis

The SPSS and Excel software were used for statistical analysis. All data were tested for normal distribution first (Shapiro-Wilk), then two-way analysis of Variance (ANOVA) was conducted using SPSS software to find out any significant differences between metal concentrations in samples from different stations (with 95% confidence level). Then the related graphs were plotted with Excel software. The Tukey HSD test was also used to compare the means of different stations.

Results

The results of this survey revealed the overall pattern of heavy metals in the

sediment and Chiton samples as Ni>Cu>Pb>As>Cd>Hg, and Cu>Ni>Pb>As>Cd>Hg, respectively. The order of stations in terms of pollution for Chiton was 1>2>3>4 and for sediments was 4>2>1>3, respectively. The results of each individual heavy metal concentration at different stations are illustrated in Figs. 3 to 8.

In order to express whether chiton was able to adsorb or accumulate metals from the sediments, the Biota-Sediment Accumulation Factor for each heavy metal at different stations was calculated (Szefer *et al*., 1999). The results are given in Table 3.

$$\text{BSAF}=\frac{\text{Metal's concentration in organism}}{\text{Metal's concentration in surrounding sediment}}$$

According to BSAF values, organisms are classified into three groups;

1. If BSAF>2, the organism is macro-concentrator.
2. If 1<BSAF<2, the organism is micro-concentrator.
3. If BSAF<1, in this case the organism is de-concentrator and releasing the metal in sediment (Dallinger, 1993).

According to Bohac (1999), macro-concentrators can be particularly suggested as suitable bio-monitors (or bio-monitoring organ/material). Therefore, it is useful to recommend mollusks as potential bio-monitors to determine the relationship between the concentration of a given metal in the organism and its bioavailability form the associated sediments (Berandah *et al*., 2010).

Comparison of heavy metals concentration in this study (sediment) with their standard amounts is shown in Table 3. The Cu concentration which was measured from LAL, ISQGs and ERL was low, whereas its concentration was higher in ERM, PEL and HAL. The measured amounts of As were lower than its mean in sediments.

The comparison of the average heavy metal concentrations (Cu, Cd, Hg and As) in our chiton samples with their standard concentrations in some mollusks which have been offered by some international organizations (WHO, FAO, USFDA and NHMRC) are presented in Tables 4 and 5.

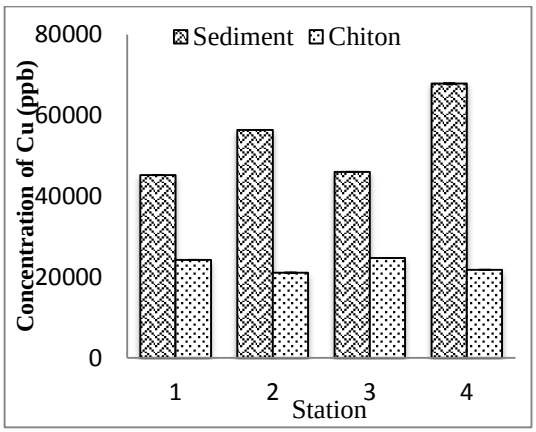

Figure 3: Concentration of Cu in the Chiton and sediment samples from different stations.

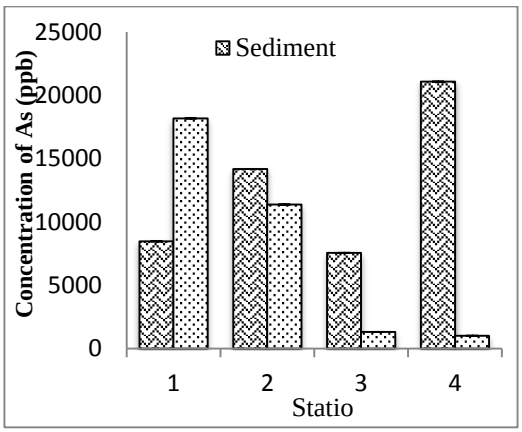

Figure 4: Concentration of As in the Chiton and sediment samples from different stations.

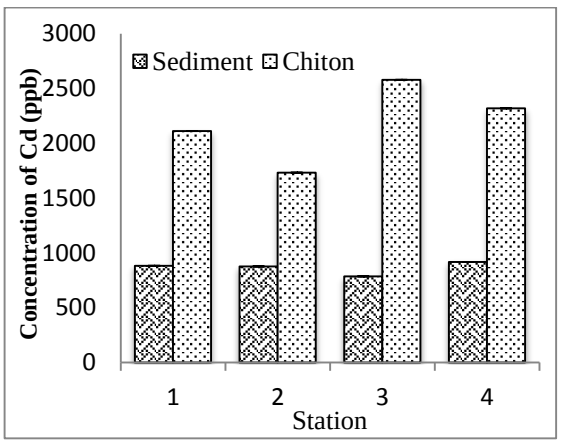

Figure 5: Concentration of Cd in the Chiton and sediment samples from different stations.

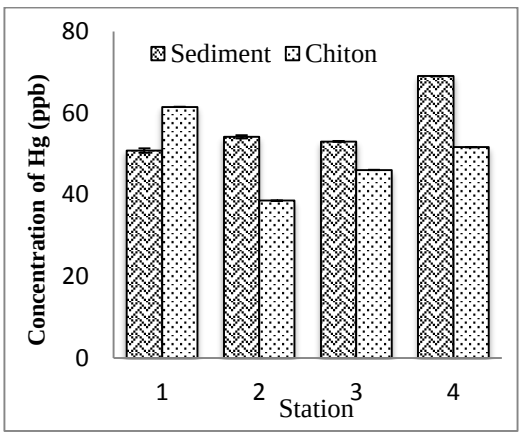

Figure 6: Concentration of Hg in the Chiton and sediment samples from different stations.

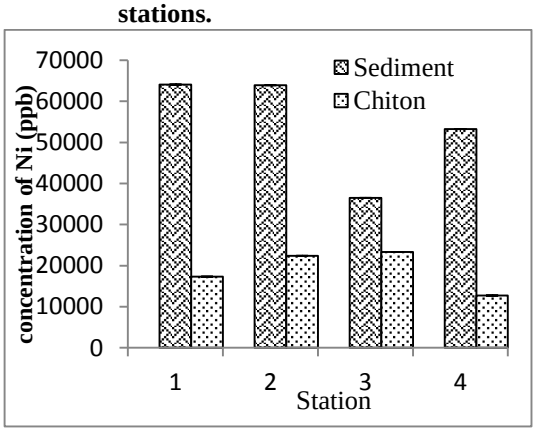

Figure 7: Concentration of Ni in the Chiton and sediment samples from different stations.

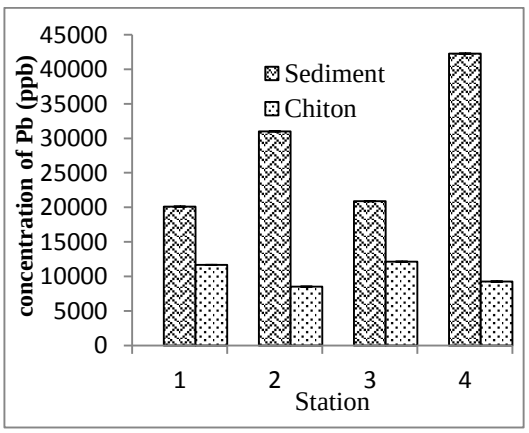

Figure 8: Concentration of Pb in the Chiton and sediment samples from different stations.

Table 3: Comparison of BSAF in different stations.

Elements	Ni	Cu	As	Pb	Hg	Cd
Station 1	0.27	0.53	2.14	0.58	1.21	2.39
Station 2	0.35	0.37	0.8	0.27	0.71	1.98
Station 3	0.64	0.54	0.17	0.27	0.87	3.28
Station 4	0.24	0.32	0.04	0.27	0.75	2.52

Table 4: Comparison of the heavy metal concentration in some international organizations with the present study (mg/kg).

Standards metals	USEPA[1], 1996 (Bowen, 1979)		Environment Canada standard (CCME[2], 1999)		American standard (Long *et al.*, 1995)		The present study
	HAL[3]	LAL[4]	ISQGs[5]	PEL[6]	ERL[7]	ERM[8]	
Cu	270	2	18.70	108	34	270	53.91
Ni	50	3	15.9	42.8	20.9	51.6	54.45
Pb	218	2	30.20	112	7.46	218	28.57
As			**The mean in sediment 16.4**				12.82
Hg	0.71	0.01	0.13	0.7	0.15	0.71	0.05
Cd	9.60	0.04	0.70	4.20	1.20	9.60	0.86

1. United State Environmental Protection Agency.
2. Canadian Council of Ministers of the Environment.
3. Highest Alert Level.
4. Lowest Alert Level.
5. Interim Sediment Quality Guidelines.
6. Probable Effect Level.
7. Effect Range Low.
8. Effect Range Medium.

Table 5: Comparison of the heavy metals concentration in some international organizations with *Chiton lamyi* (µg/g).

metals / standards	Cu	As	Hg	Cd	Ni	Pb	Reference
WHO[1]	10	--	0.5	0.2	0.2	--	Shulkin *et al*., 2003
FAO[2]	30	--	0.5	0.75	0.5	0.5	Shulkin *et al*., 2003
US FDA[3]	100	--	0.5	--	0.8	1.7	Liu and Kueh, 2005
NHMRC[4]	30	--	1	2	--	--	Chen and Chen, 2003
Chiton lamyi	22.96	7.97	0.049	2.18	18.95	10.37	the present study

1. World Health Organization.
2. Food and Agriculture Organization.
3. United States Food and Drug Administration.
4. National Health Medical Research Council.

Discussion

According to study of Peer *et al*. (2011), significant differences in the concentration of heavy metals in different coastal environments, indicate that the origin of these pollutants are not from a single spot, but they are derived from different sources (Peer *et al*. 2011). The main sources responsible for the heavy metal pollution in coastal areas are the anthropogenic activities such as the urban untreated sewage and wastewater, industrial effluents resulting from ship repairing factories, electroplating and textiles factories, fuel discharge of ships, chemical industries and erosion caused by weathering of the mountains (Zhou and Hao, 2007). Cadmium is a heavy metal which particularly might be present in higher

concentrations in coastal areas, resulting from human activities (Sadige, 1992). High levels of mercury in one region can be due to the existence of power plants (with fossil fuel), large coastal oil storage tanks, and small scale industrial and agricultural activities (Hoseini *et al*., 2011). Chemical fertilizers, rat poison and pesticides which are used in agriculture contain high levels of arsenic. More seasonal rivers enter into station 1 of our study area which can have a crucial role in washing and draining these pollutants into the sea.

Geological studies on the seabed of the Oman Sea have shown that the seabed composition is mostly of ophiolite, with more nickel sulphide content which is the natural source of nickel in the coastal areas, rather than the anthropogenic activities (Leblanc and Ceuleneer, 1991). Such information could be used to establish some baselines in the area. The Study of De Mora *et al*. (2004) in southern coasts of the Oman Sea also proved that some metals like the Ni have natural geological sources in this region. In our study area the Haft Tir Quay zone to Beheshti port are subject to heavy metal pollution resulted from the human activities such as shipping, fishing and cannery industries which can increase the Pb concentration in coastal areas.

Generally, considering the calculated values for the BSAF in *Chiton lamyi*, which varied from 0.04 to 3.28, between different stations and heavy metals, it can be concluded that chiton had a more active role in the accumulation and fixing of heavy metals, compared to the sediments. It is also notable, that in all stations, *Chiton lamyi* showed higher BSAF for cadmium. Therefore chitons can be used specifically for the measurement of cadmium in the aquatic environments.

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