Assessment of oyster Crassostrea gigas as Biomonitor Agent for Some Metals (Pb and Cu) from Musa Estuary (Persian Gulf)

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Abstract: This study was carried out with the aim of using Saccostrea gigas for biological monitoring heavy metals (Cu and Pb) on the Khor- Musa. Samples of oysters and sediments were collected from the intertidal zone at five different stations in Jan 2011. Sediment and tissue samples were oven dried and acid digested. The metal contents of samples were analyzed using an atomic absorption spectrophotometer. The results showed that Cu and Pb concentrations in the sediment samples are as follows: 17.79 ±1.60 and 6.04 ± 0.95 $\mu g.g^{-1}$dw respectively. The metals concentration in the soft tissues of oyster was determined as 518.5 ±16.36 and 9.58 ±1.14 $\mu g.g^{-1}$dw for Cu and Pb respectively. Similarly, Cu and Pb concentrations in the shells were found to be at 1.94 ± 0.19 and 2.49 ± 0.51 $\mu g.g^{-1}$dw respectively. A Significant correlation was found between Cu and Pb concentration in soft tissue and sediment samples. Similarly correlations between metals contents of sediments and hard tissues were found to be significant,suggesting that the soft tissue of S. gigas should be a useful tool for Cu and Pb monitoring in the study area.

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1. Introduction

Marine pollution is a global environmental problem. Human activities in the coastal area and marine water contribute to the release of various kinds of pollutants such as heavy metals into the marine ecosystems (Censi *et al.,* 2006; Pote *et al.,* 2008). The most important reason for the metal contamination is considered as persistent and due to their toxic properties, could create several problems for different kinds of marine ecosystems and could be accumulating in marine organisms (Wen *et al.,* 2007; Wcislo *et al.,* 2008). Moreover, their accumulation in marine organisms and biomagnification throughout the food chain may be damaging for human health (Valls and Lorenzo, 2002; Gochfeld 2003; Yi *et al.,* 2008). Monitoring of heavy metals in marine environment as particularly coastal region is very important to assess the metal contamination in the marine environment (Fowler *et al.,* 2007; Yin *et al.,* 2008). Traditional monitoring of heavy metals in the aquatic environment involves decisive and comparing the metal in water, sediment and biota. But, each method present it's own problems and limitations (Agrawal, 2005).

The low concentration of metals in the ambient water makes analysis difficult as contamination problems become significant and pre-concentration is required. The usual large temporal variations in metals concentration in water often justify frequent sampling and analysis. Another major disapproval is that information on bioavailability of metals is not provided (Mashinchian Moradi, 2001). The metals concentration in sediment provides a time-integrated estimate of metal levels. However, are significantly affected by particle size, organic content and redox conditions, which cannot be standardized (Tam and Wong, 2000; Santos *et al*., 2005; Mil-Homens *et al*., 2007; El Nemr *et al*., 2007; Karsten *et al*., 2008).

The use of organisms for biomonitoring of heavy metals in marine environment cannot only concentrate metals from water, but also provides a time-integrated estimate on the bioavailable fraction of heavy metals in marine ecosystems (Nicholson & Lam, 2005; Morelli *et al*., 2009). As a result, biomonitoring process has been widely used to monitoring metals in the last two decades (Zelika *et al*., 2003; Nicholson & Lam, 2005; Stanly *et al*., 2008). Different types of organisms may be used for biomonitoring, such as marine algae (Topcuo *et al*., 2003; Besada *et al*., 2009) and filter-feeding mollusks (Mashinchian Moradi, 2001; Zorita *et al*., 2006; Hamed and Emara, 2006). Many studied showed, bivalves do not regulatethe level of some metals within their body (Stanly et al., 2008) and they can deflect the metals contamination from surrounding area. Thus, bivalves to be considered as good

biomonitor agents for heavy metal monitoring in aquatic ecosystems (Elfwing & Tedengreen, 2002; Yap *et al*., 2003; Zelika *et al*., 2003; Nicholson *et al*., 2005; Zorita *et al*., 2006; Vlahogianni *et al*., 2007; Maanan, 2008). Khor-Musa is a complex waterway system located in the north persain Gulf that is consisted ofseveral estuaries, creeks and a main canal. This khor is situated closed to the Imam port one of the biggest ports in Iran and connects this port to the Persian gulf through a 60 Km length canal. There are several sources of anthropogenic pollutants including petrochemical industries, oil transportation and agriculture activities, which produce and discharge substantial amount of contaminant such as heavy metal into the seawater (Zauke *et al*., 1999).

Like many other estuaries, Khor-Mosa is an important place for fisheries and aquaculture activities. Considerable amount of fish and shrimp are caught from this Khor annually, which are introduced to the markets for human consumption. The presence of high concentrations of heavy metals in aquatic environment could result in metal accumulation by marine organisms(Tuzen, 2003; Uysal et al., 2008) and increase the risk of metal toxicity in the people who consme contaminated seafood. Information on heavy metals concentration in sediment and organisms such as oyster is therefore useful to estimate the level of metals contamination and bioavailability in marine environment(Karadede *et al*., 2004). Although bivalves specially S. gigas are widely distributed in this area, but valuable information about this region has not been published. This study was carried out with purpose using of S. gigas indices biological monitoring of heavy metals in the studied area.

2. Materials And Methods

The study was carried out in the Khor-Musa estuaries. Samples of sediment and oyster were collected from five sampling sites, as indicated in Figure 1. The positions of sampling sites were recorded using GPS (Table 1). All samplings were conducted during Jan 2011. The top 3-5 cm of sediments were collected near the oyster habitats. Three replicates of sediments were sampled from each site. Each sediment sample was placed in an acid-washed polyethylene bag and deep-frozen prior to analysis. Thirty oysters of the same size (5 to 7 cm) were collected from each station. Stainless steel hammer and Rod were used to separate oysters from their surrounding crags. The collected oysters were placed in polyethylene containers and all samples transferred to laboratory by using Icebox. All of debris's were removed from each sample in laboratory. After washing shells with double distilled water, oysters were freezed in -20^{o}c freezer until the next step (Orescanin *et al*., 2006).

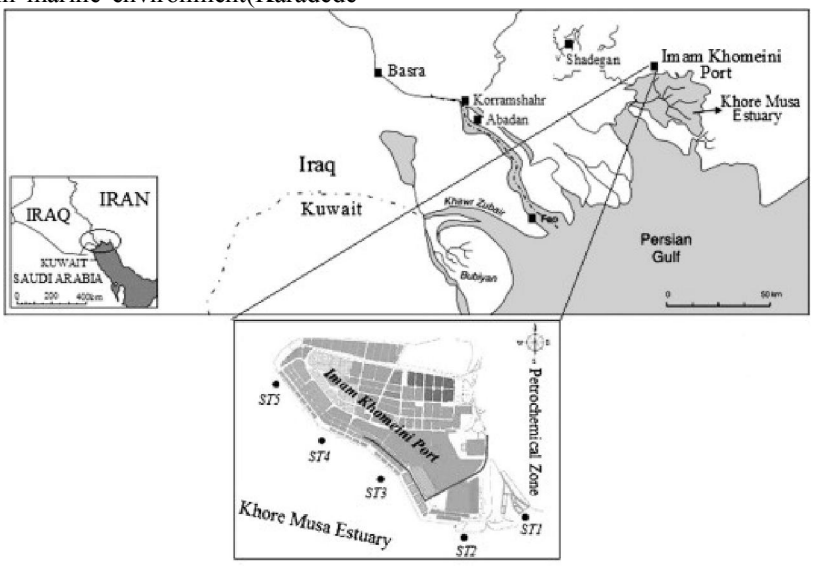

Fig. 1 Map showing study area

3. Sample preparation

In arrange to dry samples, Sediments were oven dried at 105 ^{o}C for at least 16 h until a constant weight was obtained (Delman *et al*., 2006). Then, they were sieved through 63µm stainless steel sieve and shaken vigorously to produce homogeneity. after that they were powdered using glass mortar and stored in polyethylene pillboxes until digestion. Oysters were taken from freezer and were placed in the laboratory to melt their ice. Soft tissues were separated from the shell by using stainless steel knife and both soft and hard tissues were oven dried at 80^{o}C until constant weight was obtained (Yap *et al*., 2002; Orescanin *et al*., 2006). The dried samples

from each station were then pooled together in order to obtain sufficient amount of tissues for metal analysis. They were powdered using glass mortar and were stored in polyethylene pillboxes until digestion. They oyster shells were also washed using percentage 0.5 nitric acid and oven dried. Then they were powdered and stored as the same procedure as for soft tissues (Yap *et al*., 2003).

4. Heavy metals analysis

For the analysis of total Cu and Pb concentrations in sediment, 1g of each dried sample was digested in a combination of concentrated nitric acid (65%, Merck, Darmstadt, Germany) and perchloric acid (60% Merck) in the ratio 4:1, first at low temperature (40^0C) for 1 h and then the temperature was increased to 140^0C for 3 h (Orescanin *et al*., 2006). The metal analysis for both tissues was performed with the same method. 1 g of each dry sample from soft tissue and shell of oyster were digested in pure nitric acid (65% Merck). The samples were predigested first for 1 hour in 40^0C and then digestion was continued for 3 hours in 140^0C. After digestion, samples were cooled in laboratory temperature, diluted to certain volume using double distilled water and filtered by filter paper (Whatman 42µ) (Yap *et al*., 2002). Heavy metals analysis was performed by using GBC model SavantAA ∑ atomic absorption spectrophotometer.

5. Data analysis

All data were analyzed statistically by using SPSS version 16. The data were tested for normal distribution first. After ensuring normal distribution of data, the One-way analysis of Variance (ANOVA) was used to find any significant difference between metals concentration in samples. If significant difference was observed, Tukey post hoc test was used to determine different kinds. The Pearson's correlation coefficient was applied to determine the relationship and the significant levels between any two variables.

Table 1. Position of stations along Khor-Musa

station	longitude	latitude
St1	49º 04' 25" E	30º 25' 36" N
St2	49º 04' 26" E	30º 25' 15" N
St3	49º 03' 44" E	30º 26' 02" N
St4	49º 02' 32" E	30º 26' 76" N
St5	49º 02' 01" E	30º 27' 15" N

6. Results

The concentration of Cu and Pb in sediment and different tissues of oyster *S. gigas* are agreed in tables 2. Cu and Pb concentrations in sediment from five different stations were measured 17.12 to 21.87 $\mu g.g^{-1}$ and 5.4 to 8 $\mu g.g^{-1}$ respectively. This indicated, the order of metal concentrations in the sediment of abundance: Cu > Pb. Similarly, the metal concentrations ranged in the shell were obtained 1.67 to 2.18 $\mu g.g^{-1}$ and 2.12 to 3.15 $\mu g.g^{-1}$ for Cu and Pb respectively. However, this order of metal accumulation was different from that found in the soft tissue. Which had the following order of abundance: Pb > Cu. 476 to 64.43 $\mu g.g^{-1}$and 6.2 to 16.2 $\mu g.g^{-1}$ were measured for Cu and Pb in soft tissue of oyster in different stations. Cu and Pb concentration in soft tissue was found to be higher than shell.

Results indicated the pattern of metals accumulation in the sediment and soft tissues of oyster were more and less similar (Cu>Pb). While, the pattern of metals accumulation in shell was Pb>Cu. Cu concentration was found to be higher in the soft tissues. Unlike Cu, the concentration of Pb in the shell was about 2 times higher than the soft tissues.

The assessment for the potential use of the total soft tissue and shell of S. gigas as a biomonitoring tool for Cu and Pb was based on the following. The relationship between metals concentration in the different tissues of oyster and those in the sediment are presented in table 3. significant correlation was found for Cu and Pb between the sediment and the total soft tissue of oyster ($P<0.05$, $R=0.87$, 0.92 respectively). A significant correlation was found for Cu and Pb concentrations between the shell of oyster and the sediment ($P<0.05$, $R=0.59$, 0.87 respectively).

Table 2. concentration of Cu and Pb ($\mu g.g^{-1}$ dry weight) in sediment and different tissues of *S. gigas* in khor- musa(Persian Gulf)

matrix	location	Cu	Pb
sediment	St1	21.87±2.12	8±1.54
	St2	17.46±1.89	5.6±0.97
	St3	17.12±1.62	5.7±1.2
	St4	17.86±1.45	5.5±0.43
	St5	14.64±0.96	5.4±0.61
Soft tissue	St1	604.43±23.14	16.2±1.25
	St2	503.62±19.76	9.3±1.18
	St3	476±14.65	6.9±0.77
	St4	512±12.87	6.2±0.65
	St5	496.45±11.41	9.3±1.87
Shell	St1	2.18±0.32	3.15±0.65
	St2	1.98±0.19	2.14±0.43
	St3	2.16±0.21	2.71±0.31
	St4	1.67±0.14	2.37±0.76
	St5	1.73±0.12	2.12±0.41

Table 3. The Correlation between heavy metal concentrations in sediment and different tissues of *S.gigas* in khor- musa (Persian Gulf)

		Cu	Pb
Soft tissue	Sediment	R=0.87*	R=0.92*
Shell	Sediment	R=0.59*	R=0.87*

*Levels of significance are indicated as $P < 0.05$.

7. Discussion

Heavy metals concentration in sediment and different tissues of oyster were measured in Khor-Musa along the north coasts of the Persian Gulf. Results showed the order of metals accumulation in the sediment and soft tissue of oyster are similar to Cu > Pb. While, the order of metal accumulation in the shell of oyster is like to Pb > Cu. Oyster is a non–migrant species of long life, has a world– wide distribution, a reasonable size and easy to sample, and an ability to concentrate numerous pollutants. Oysters accumulate metals such as copper and lead and can tolerate very high metal concentrations, without apparent detrimental effects (Lin&Hsie, 1999) and accumulate trace metals in proportion to the integrated ambient availabilities (Soto–Jimenez& Paez–Osuna, 2001). Under normal conditions, as much as 387 L of water is pumped through the gills in a single day (Ingle& Whitfield,1968) and therefore oysters accumulate a large amount of heavy metals by the ingestion of phytoplankton and organic particles as well as direct uptake from solution. So they could be used as biomonitors provide integrated measures of the supply of heavy metals available to them in an environment, accumulating the heavy metals taken up from all sources such as from water and from food (Phillips& Rainbow, 1993).

Cu is an essential metal for oysters. They are use the Cu to make haemocyanin for respiratory pigments (Launestein et al., 2002; Caussy et al., 2003؛ Conner & Launestein, 2005). While, Pb is not essential element for oysters (Boening, 1999). Meanwhile, The shell matrix has a higher capacity for incorporation of these metals than the soft tissue (Ballan-Dufrancias *et al*., 2001; Lauenstein *et al*., 2002). Gillikin et al., (2005) were compared the concentration of heavy metals between shell and soft tissue of Mercenaria mercenaria in Carolina. They were found, heavy metals concentration in the shell has not changed during 1949 to 2002. They were suggested a little change in metals concentration in the shell may be lack of effect of physiological processes. In addition, the increase of Pb in the shell of Oyster may be due to crystalline structure of the shell. The Pb ion is then to several times tendency to bind with carbonate ions in the calcareous structure (Babukutty & Chako, 1995). Thus, the high concentration of Pb in the shell of oyster may be due to replace of Pb ions in the calcareous structure of the shell. Biomonitors organisms accumulate the heavy metals from ambient bioavailable sources of the trace metal over a period (Rainbow, 2006). Thus, the accumulated heavy metals in difference tissue of biomonitor organism are a measure of the total integrated bioavailability of those metals to that organism at studied area along the previous period (Saed, 2001). Some mollusks including oysters are used for biomonitoring programs in marine ecosystems. The oyster S. gigas like other mollusks is suspension feeder and can uptake they heavy metals from suspended sediment (Coles et al., 1997; Caihuan & Wang, 2001). This oyster has a wide distribution in the Khor- Musa and may be a suitable bioindicator organism for heavy metals in this area. The correlation between Cu and Pb concentration in the shell and total shell of oyster S. gigas and those in ambient sediment was studied. The stronger correlation coefficients were found between heavy metals concentration in the sediment and shell and soft tissue, but this correlation between sediment and soft tissue is stronger than shell. Thus, the soft tissue of oyster is more suitable as an monitor of Cu and Pb contamination in this area and the shell of S. gigas should be a useful tool for indicator of all studied metals in the study area.

Shulkin *et al*., (2003) were found a significant correlation between Cu, Zn, Pb, Cd and Ni in the soft tissues of both mussel (Crenomytilus grayanus), oyster (Crassostrea gigas) and in their ambient sediment. They were suggested these mollusks can be use mainly for the monitoring of low and moderate contaminated in Japanian coasts.

Yap *et al*., (2002) were studied the correlation between Cd, Cu, Pb and Zn concentration in the soft tissue of P. veridis and surface sediment in Malasia coasts. They were found a significant correlation between Pb and Ni in soft tissue and their ambient sediment. But, no significant correlation was found for Cu and Ni in this area. In according these results they are concluded, the soft tissue of mussel could be a useful tool for Cd and Pb in Malaysia coasts. A significant correlation was found for Cu, Zn, Pb, Cd and Ni in soft tissue of mussel (Crenomytilus grayanus), oyster (Crassostrea gigas) and ambient sediment in northern coast of the Japan. So the soft tissue of these mollusks was found to be a good tool for these metal biomonitoring in this studied area (Shulkin *et al*., 2003). Szefer *et al*., (2002) were found a significant relationship between Hg, Cd, Pb, Ag, Cu, Pb, Cr, Co, Mg and Fe in the soft tissue and byssus of Metilus edulis and surface sediment in Polish coasts in Baltic Sea. They were suggested, the

soft tissue and byssus of M. edulis could be suitable for these metals biomonitoring in this area.

8. Conclusion

This study was showed the pattern of metals accumulation in the soft and hard tissue of S. gigas is different. The difference pattern of metals accumulation in the soft tissue could be related to the biological role of that metal in the body.

The stronger correlation coefficients were obtained for metals concentration between sediment and soft tissue of oyster. The significant correlation in the total soft tissue of S. gigas and sediment suggested, it to be generally a more sensitive and precise biomonitoring material for heavy metals than the shell of S. gigas. Soft tissue of S. gigas for Pb and Cu was useful tool for biomonitoring of total metals studied in Persian gulf.

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