Assessment of Toxic Metals Concentration using Pearl Oyster, *Pinctada radiate*, as Bioindicator on the Coast of Persian Gulf, Iran

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ABSTRACT

Background: Persian Gulf is a semi-closed environment which is affected by pollution from heavy metals. Entrance of heavy metals to the water column and binding to sediment particles can affect the benthic organisms that can accumulate these materials in their body. Noticing this ability, mussels are considered as bio-monitoring agents.

Methods: The pearl oyster, *Pinctada radiate*, and sediment samples were collected from Lengeh Port and Qeshm Island. For measuring heavy metals, 0.5g of soft tissue and 1g of shell and sediment were digested by HNO_3 (69%) and hot block digester. The prepared samples were evaluated for Cd, Cu, and Zn using a flame AAS Model 67OG while for Pb a graphite furnace AAS was used.

Results: Higher metal accumulations were observed in soft tissues. Positive correlations between Cd, Pb, Zn, and Cu concentrations in sediments and soft tissues of oyster were observed. The use of soft tissue of *P. radiata* as an indicator showed the highest accumulations of Cd (9.76±0.59) and Zn (3142.60±477.10) in Lengeh Port, but there were no significant differences in Cu and Pb concentrations between the two stations.

Conclusion: The higher concentrations of heavy metals in *P. radiata'* soft tissue in comparison to shell suggested this material as a better heavy metals indicator than shell. Also, the correlation between heavy metals concentration in soft tissue and sediment improve this idea that soft tissue of *Pinctada radiata* can be considered as a biomonitoring agent for toxic metals pollutions. Hence, using this bioindicator showed Lengeh Port as more polluted station than Qeshm Island.

Keywords: Bioindicator, Heavy Metals, Persian Gulf, *Pinctada Radiata*, Shell, Soft Tissue.

INTRODUCTION

Marine pollution is a global environmental problem and the contamination of heavy metals in this ecosystem has attracted much attention during the recent years [1]. The source of the contamination could be natural substances, such as volcanic activities and underground mines, or anthropogenic activities, like industrial and domestic wastewaters which are the main factors causing heavy metals concentration in coastal areas [2]. High levels of essential metals, e.g. Cu and Zn, and small amount of nonessential metals, e.g. Cd and Pb, have toxic effects on aquatic organisms. In addition, due to the stability of heavy metals in nature they can bio-accumulate in food chain and be harmful to human health [3]. Considering the characteristics of the Persian Gulf, like shallow water (average depth 35-40 m), longtime for turnover and flushing water (3-5 years), oil and gas installations, and fishing activities, water quality of this area has been affected over the past years [4]. Hence, having a regular heavy metals monitoring program is important for this region. Bivalve mollusks due to their way of feeding are susceptible to accumulating metals in their tissues from the environment and because of their sedentary nature, wide

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distribution, tolerance to environmental changes and pollution, and easy sampling and identification are commonly known as bioindicators [5]. Bivalve mollusks, like *Solen brevis* [6] and *Saccostrea cuculata* [7, 8], as bioindicator in coastal areas of the Persian Gulf have been successfully used in new studies. *Pinctada radiata*, the Persian Gulf pearl oyster, is one of the native and valuable species of this area which has been used as a heavy metal indicator in several studies [9-11].

The aim of this study was to find out whether soft tissue or shell of *P. radiata* can be used as a biomonitoring material for metals pollution. This oyster was used to compare metal concentrations (Cd, Cu, Pb and Zn) in two stations (Lengeh Port and Qeshm Island) in the intertidal zones of the Persian Gulf.

MATERIALS AND METHODS

Two stations along the intertidal zones of Persian Gulf, one in Lengeh Port and the other near Qeshm Island, were selected (Figure 1).
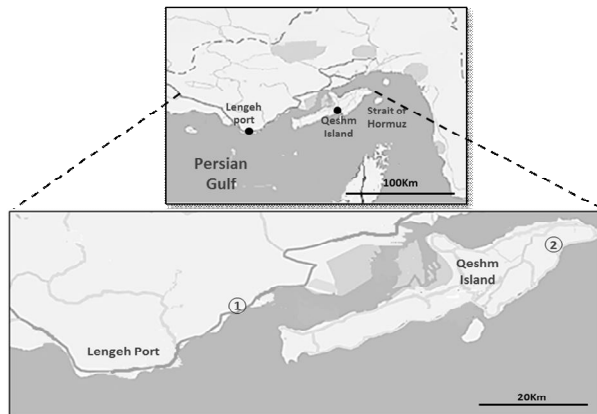
Figure 1. Sampling stations in the Coast of Persian Gulf; 1- Lengeh City, 2- Ramchah Quay.

About 20-46 *P. radiata* samples of similar size were collected from each station in May 2010. In addition to the oysters, five replicates of the top 0–3 cm of surface sediment were also collected from each oyster sampling site. Detailed information on the oyster samples, including the number of oysters analyzed, shell lengths, and site descriptions, are given in Table 1.

The samples were placed in a plastic zip bag and transported to the laboratory in ice. They were, then, stored at -20°C until further analysis. Then the frozen oysters were scattered on paper towels and thawed partially at room temperature before opening. In the laboratory, the soft tissue (ST) of oysters was carefully separated from the shells (SH). Then the SH length of each specimen was measured by caliper. After cleaning shells with a jet of tap water, they were washed with double distilled water (DDW) and 0.5 % of concentrated HNO_3. In the next stage, all oyster samples were dried in an oven at 105°C for 72 hours until reaching constant dry weights (dw). Sediment samples were also dried in an oven at 105°C for 16 hours until reaching constant dry weights (dw). Then SH samples were powdered by a mixer mill and stored in acid-washed polythene bags. From each dried sample, about 0.5 g of ST and 1g of SH and sediments were weighed and placed in a hot block digester and digested with concentrated HNO_3 (AnalaR grade, BDH 69%) first at low temperature for 1 h and then at high temperature (140°C) for at least 3 hours. The digested samples were then diluted to a certain volume with distilled deionized water (DDW).

Table 1. Locations, number of samples analyzed (N), shell length (mm) of oysters, and descriptions of sampling sites of *Pinctada radiata* collected from the coasts of Lengeh Port and Qeshm Island, Persian Gulf.

| No	Location	Latitude(N)	Longitude(E)	N	Shell length Mean(min-max)	Site description
1	Lengeh Port	26°32′59″	54°52′44″	16	40.31(54.4-36.45)	Urban area
2	Qeshm Island	26°53′27″	56°09′37″	16	35.91(59.48-25.52)	Recreational area

After filtration through Whatman No. 1 filter paper, the sample solutions and reagent blanks were analyzed for Pb in a graphite furnace atomic absorption spectrophotometer Model 67OG and for Cd, Cu, and Zn in a flame atomic absorption spectrophotometer Model 67OG. The data are presented in micrograms per gram dry weight basis. In order to avoid possible contamination, all glassware and equipment used were acid-washed. To check for contamination, procedural blanks were analyzed in every five samples [12].The accuracy of the analytical technique was verified by the analysis of the NIST standard reference materials in oyster tissue (SRM-1566b) and estuarine sediment (SRM-1646a). The results showed good precision for the SRM with an overall 95 % confidence level. In addition, the accuracy of the results was good with respect to the certified values. The recoveries were above 90 % for all trace metals measured except for Pb (83%). The t-test was used to determine metals concentration mean differences between the stations sediments and also between soft tissues and shells of oysters. Differences were considered significant only when P-values were lower than 0.05. Prior to analysis, data were inspected for homogeneity of variance (Levene's test). Correlation analyses were performed for mean metals concentrations in sediments and mean metals concentrations in ST and SH. All statistical calculations were carried out using SPSS statistical software (version 16.0).

RESULTS

Mean concentrations of metals in soft tissue (ST) and shell (SH) of oysters are given in Table 2. Applying the t-test (5% significant level) for comparing the means of metals in ST and the SH of *P. radiata* collected from two stations indicated that in both stations the levels of Cd, Pb, and Zn showed significant differences (P<0.001) between ST and SH and the higher accumulation of these metals were related to ST, whereas Cu concentration did not show a significant difference between ST and SH in both stations (P>0.05).

Correlations between the mean metal levels in ST of *P. radiata* with respect to elemental levels in sediment are illustrated in Figure 2. The significant positive correlation (r=0.80, P<0.01) was observed in Cu levels between the ST of oyster and sediments (Figure 2a). The same positive correlation (r=0.98, P<0.01) was found for Pb accumulation between ST and sediment (Figure 2b). Also, a significant positive correlation (r =0.94, P<0.01) was observed among Cd levels in sediment and soft tissue of oyster (Figure 2c). Figure 2d revealed that Zn in total soft tissues of *P. radiata* was significantly correlated with the total Zn in the sediment (r=0.87, P<0.01).
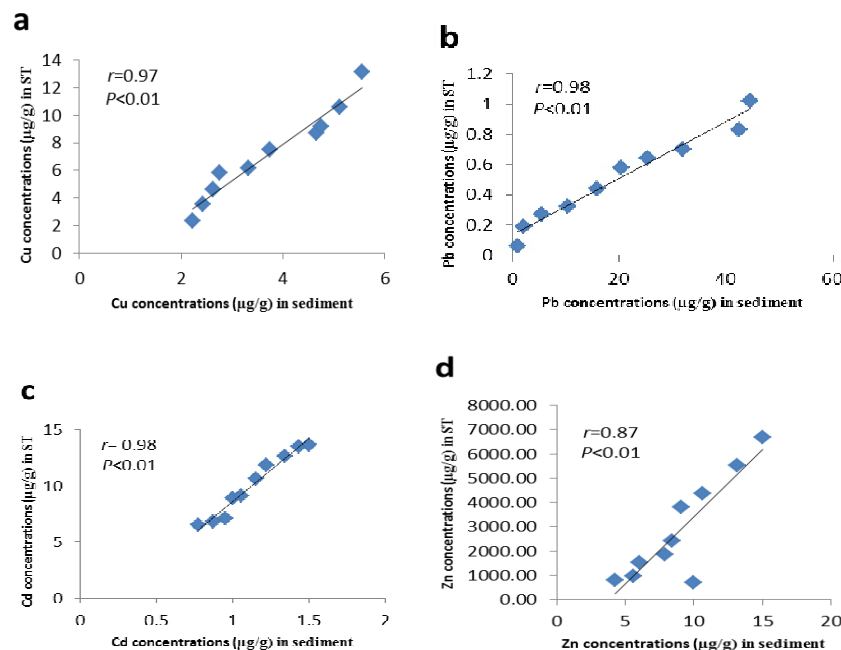
Figure 2. Pearson's correlation coefficients (*r*) between Cu, Cd, Zn, and Pb levels in sediment samples and the soft tissue (ST) of oysters from the coast of Persian Gulf.

Table 2. Mean concentrations±SE ($\mu g\ g^{-1}dw$) of Cd, Pb, Zn, and Cu in total soft tissue (ST) and total shell (SH) of *Pinctada radiata* collected from the intertidal coast of Lengeh Port and Qeshm Island, Persian Gulf.

Stations	Cd_{SH}	Cd_{ST}	Pb_{SH}	Pb_{ST}	Zn_{SH}	Zn_{ST}	Cu_{SH}	Cu_{ST}
Lengeh Port	0.01	9.76	0.08	0.37	5.47	3142.60	4.22	6.02
Qeshm Island	0.01	5.95	0.21	0.49	2.86	769.34	5.33	6.49

Noticing the previous results of this study, the soft tissue of *P. radiata* is a better material for indicating heavy metals contaminations, so in the next step of this study, the mean concentrations of Pb, Cd, Zn, and Cu in the ST were compared between two stations to identify the most polluted area. The mean concentrations of metals in ST of two stations samples are shown in Table 2. Independent sample t-test indicated that accumulations of Cd and Zn in ST samples significantly differed (P<0.001) between the two stations. Higher Cd and Zn concentrations were observed in Lengeh Port but there were no significant differences in Pb and Cu accumulations (P>0.05) in ST samples between the two stations.

DISCUSSION

The results of our study show the significant difference in heavy metals concentrations between soft tissue and shell of oysters. The higher concentrations of Cd, Pb, and Zn were observed in soft tissue but Cu concentration did not show any differences. Filter-feeder oyster uptake dissolved metals ions in solution or in particular form, via food by filtering large volumes of water each day [13]. There are many ways to maintain homeostasis of essential metals and detoxification of non-essential metals in bivalves' tissues including binding metals to low-molecular weight proteins, like metallothioneins and storing them in lysosomes or metal-containing granules, so that they can accumulate metals in their tissues several times higher than their ambient water [14]. This could be the cause of more Cd, Pb, and Zn accumulations in ST than SH as mentioned by other authors [7, 15]. Unlike other metals, copper accumulation did not show a significant difference between ST and SH of oyster

samples. Cu is an essential metal for organisms survival due to its involvement in many enzymes structures, like cytochrome c oxidase, the critical enzyme in eukaryotes cellular respiration [16]. Moreover, in mollusk, copper is the main metal in hemocyanin building that does the gas exchanges [17]. Thus this metal absorbs and regulates within oyster tissues and do not accumulate except in sever pollutions; this result was in agreement with the findings of Serafim and Bebianno [18] and Geret *et al*. [19] who observed no significant concentrations of Cu in oyster tissue. In general, comparing metals accumulation in ST and SH indicated that ST had higher affinities for metals than SH of *P. radiata*; therefore, it was examined as an indicator in next step of this study.

The results of the correlation test between heavy metals concentration of sediment and ST indicate the high correlation for all metals. Sediments act as sinks for toxic metals that enter the coastal areas from anthropogenic sources like industrial and urban run-off; these contaminated sediments can be ingested by suspended filter-feeder oysters and become a source of metals uptake [20]. Our results are in agreement with Franklin *et al*. [3] who found significant correlations between Cu in caecum organ of *C. capucinus* and sediment. Maria-Cervantes *et al*. [21] found significant correlations between Pb in soft tissue of *H. trunculus* and sediment. The similar results have been observed in the soft tissue of *S. cucullata* and sediments [7]. The same results have been reported by Diaz Rizo *et al*. [22] who found significant correlations between Zn levels in total soft tissue of *C. rhizophorae* and Zn levels in the sediment. According to the correlations between Cu, Cd, Pb and Zn levels in sediments and ST of *P. radiata*, this

species can be suggested as a biomonitoring agent for coastal zone of Persian Gulf.

The comparison of the ST metals concentrations between the two stations showed higher accumulations in Lengeh Port samples. The higher mean concentrations of metals Zn (3142.60 μg g^{-1}) and Cd (9.76 μg g^{-1}) in Lengeh port samples can be related to ship transport activities, and municipal and industrial waste disposal in this area. Heidari Chaharlang et al. [7] also found high levels of Zn, Cd and Cu in soft tissue of S. cucullata in this region. Observing no significant difference for Cu concentrations between soft tissue samples from two stations can be attributed to the high regulation of this metal in oysters which discussed above. The same results for Pb concentrations in soft tissue samples can support this idea that mussel's shell is a better indicator for this metal because the crystalline structures of the shell have a higher capacity for incorporation of this metal than the soft tissue as suggested by other authors [7, 15].

CONCLUSION

In conclusion, the results of this study showed that the soft tissue of P. radiata accumulates more Zn, Cd, Pb, and Cu than its shell. The high correlation in Zn, Cd, Pb and Cu accumulations among oyster's soft tissue and sediment supports this idea that the P. radiata soft tissue can be used as a biomonitoring agent for these metals in the Persian Gulf area. Using soft tissue as an indicator showed the higher concentrations of Zn and Cd in Lengeh Port than Qeshm Island.

ACKNOWLEDGMENTS

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