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Assessment of Cadmium, Copper, Lead and Zinc Contamination Using Oysters (*Saccostrea cucullata*) as Biomonitors on the Coast of the Persian Gulf, Iran

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Abstract The levels of Cd, Cu, Pb and Zn in surface sediment, soft tissue and shell of the oyster *Saccostrea cucullata* collected from three locations, in the intertidal zones of Lengeh Port, northern part of Persian Gulf were measured. Results indicated that there were a positive correlation across Zn ($r = 0.58$, $p = 0.025$), Cd ($r = 0.74$, $p < 0.01$) and Cu ($r = 0.80$, $p < 0.01$) levels in the soft tissue of oyster and sediment which supported this fact that the soft tissue of *S. cucullata* can be considered as biomonitoring agent for Cd, Zn and Cu in the Lengeh Port.

Keywords Metals · *Saccostrea cucullata* · Lengeh port · Persian Gulf

Metal pollution of aquatic environments has become a global issue in both the developing and developed world (Boran and Altinok 2010; Shariati et al. 2011). The presence of metals in aquatic ecosystems is the result of two main sources of contamination: natural processes or natural occurring deposits, and anthropogenic activities. During the last few decades, urban and industrial developments along the coastal areas, rivers and estuaries have contributed to the major part of the anthropogenic metal load of the sea and have directly influenced the coastal ecosystems (Azarbad et al. 2010; Yuzereroglu et al. 2010). Metals are a group of the most important pollutants which cause environmental degradation in coastal areas (Raposo et al. 2009). High levels of metals in aquatic ecosystems are regarded as serious pollutants because they can be toxic and get incorporated into the food chain (Alyahya et al. 2011).

Among marine organisms, oysters are a valuable source for protein and vitamin and they are valued as food, and have and important commercial value. Besides, they are an important source for supplying essential elements (Garcia-Rico et al. 2010). They have been employed as biomonitors of environmental pollution by metals around the world (Hamed and Emara 2006; Pourang et al. 2010). One of the attributes, that had let to the use of marine mussels as a biomonitoring agent for metals is that they are commercially important seafood species world wide, other attributes, due to which mussels are often chosen for biomonitoring studies, are that they are sedentary organisms, long lived, easily identified and sampled, reasonably abundant and available throughout the year, tolerant of natural environmental fluctuations and pollution (Bartolome et al. 2010). This study was aimed to recognize relationship between metals (Cd, Cu, Pb and Zn) in surface sediment and their concentrations in soft tissue of *Saccostrea cucullata* from the intertidal zones of Lengeh Port (Persian Gulf) and to investigate whether soft tissue of *S. cucullata* can be considered as a biomonitoring material for metal pollution.

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Materials and Methods

The Persian Gulf is a shallow basin with an average depth of 35–40 m and a total area of around 240 km^2. It joins free international waters through the Strait of Hormuz. The turnover and flushing time have been estimated to be in the range of 3–5 year indicating that pollutants are likely to reside in the Persian Gulf for a considerable time (Pourang et al. 2005). In terms of pollution, the water quality of the Persian Gulf is influenced by various industrial outputs, shipping, fishing and collecting, mining, power plants, and discharging their wastewater directly to the sea or via rivers (Agah et al. 2009).

48 oysters (*S. cucullata*) and 15 surface sediment samples were collected from the Coastal environment (intertidal areas) of Lengeh Port, northern part of Persian Gulf at the same time on October 2010 (Fig. 1). About 16 *S. cucullata* samples of similar sizes were collected from three sampling sites, namely Kong Port, Koohin Area and Bostaneh Port to avoid environmental variations and/or sample bias. Details of the oyster samples which include number of oysters analyzed, shell lengths and site descriptions are given in Table 1.

The sampling sites included areas of different environmental backgrounds like fishing boats activities, urban areas, ports, ship repairing operations, industries and shipyard. The top 0–3 cm of surface sediments were collected near the oyster habitats. Five replicates of sediments were sampled from each site. The sediment samples were placed in tightly sealed solvent-rinsed stainless steel containers and oyster samples were placed in a plastic zip bag and transported to the laboratory in ice. The samples were then stored at −20°C until further analysis (Yap et al. 2002). Frozen oysters were scattered on the paper towel and thawed partially at room temperature before opening. In the laboratory, the soft tissues (ST) of oyster were carefully separated from the shell. Then shell length of each individual was measured by caliper. In the next stage all the oysters and sediments samples were dried in an oven at 105°C for 72 h until constant dry weights (dw). Also, the sediment samples were defrosted at room temperature, and then the samples were transferred to an oven and dried at a temperature of 105°C for 24 h. The metal analyses were performed in the 63 µm fraction, because the metals are usually strongly associated with small and fine grain sediments (Yap et al. 2002). About 1 g of each dried sample was 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 h. The digested samples were then diluted to a certain volume with distilled deionized water (DDW). After filtration through Whatman

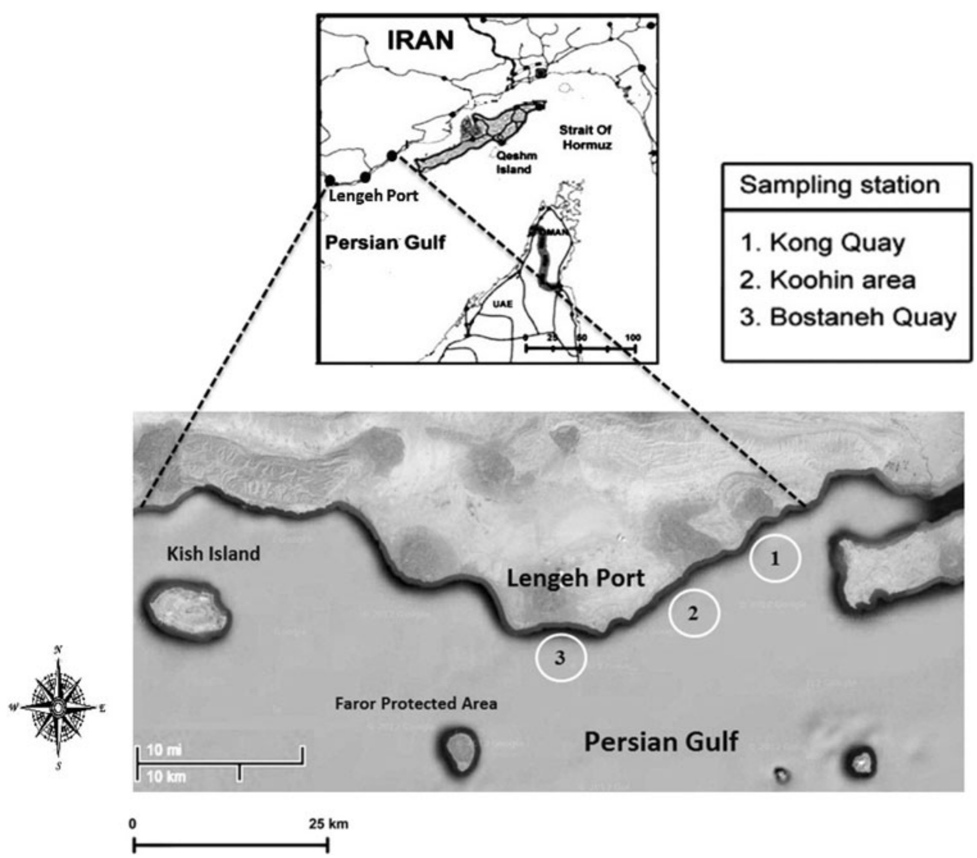

Fig. 1 Sampling stations in the Coast of Lengeh Port, Persian Gulf

Table 1 Positions, number of samples analyzed (N), shell length (mm) of oysters and descriptions of sampling sites of *S. cucullata* collected from the Coast of Lengeh Port, Persian Gulf

No	Location	Latitude (N)	Longitude (E)	N	Shell length [mean (min–max)]	Site description
1	Kong Port	26°37′33″	54°59′48″	16	54.16 (38.17–61.15)	Fishing boats activities, domestic discharges, shipyard
2	Koohin Area	26°38′21″	56°01′37″	16	55.92 (35.10–70.20)	Ship repairing operations
3	Bostaneh Port	26°30′17″	54°39′42″	16	56.35 (46.20–68.13)	Fishing boats activities, domestic discharges, shipyard

No 1 filter paper, the levels of Cd, Cu, Pb and Zn in prepared samples were determined using a flame atomic absorption spectrophotometer Model 67OG. The data are presented in micrograms per gram dry weight basis. To avoid possible contamination, all glassware and equipment used were acid-washed. To check for contamination, procedural blanks were analyzed in every five samples. The accuracy of the analytical technique was verified by the analysis of the NIST standard reference materials oyster tissue (SRM-1566b) and estuarine sediment (SRM-1646a). The results show 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 %).

An analysis of variance (ANOVA) was performed to determine any significant differences in metal levels in the ST. Differences were considered significant only when *p* values were lower than 0.05. Prior to analysis data were inspected for homogeneity of variance (Levene test). All statistical calculations were carried out using statistic 5.0 for windows and SPSS statistical software version 17.0.

Results and Discussion

Concentration of metals in surface sediments is presented in Table 2. Among the three stations, levels of Cd, Cu, Pb and Zn ranged from 1.10 to 1.57 $\mu g\ g^{-1}$ dw, 3.45 to 7.50 $\mu g\ g^{-1}$ dw, 96.71 to 195.90 $\mu g\ g^{-1}$ dw and 14.40 to 85.05 $\mu g\ g^{-1}$ dw, respectively. This indicated that the sediment can accumulate these metals in the following order of abundance: Pb > Zn > Cu > Cd.

Analysis of variance (ANOVA), followed by the least significant different (LSD) test, was carried out to compare the metal levels in the oysters from different sites (Table 3).

Based on the results of the one-way ANOVA, concerning the three sampling sites statistically significant differences were observed in the levels of Pb ($F = 71.6$; $df = 2, 45$; $p < 0.01$) and Cd ($F = 6.35$; $df = 2, 45$; $p < 0.01$). On the other hands, there was no significant variation in the Cu ($F = 0.92$; $df = 2, 45$; $p > 0.05$) and zinc ($F = 0.44$; $df = 2, 45$; $p > 0.05$) levels among the sampling sites. LSD test showed a significant difference ($p < 0.05$) at the 95 % confidence level in the Pb between different sampling sites, and level of Cd also revealed a significant variation ($p < 0.05$) among stations 1 and 2, however, the values were not significantly different what was observed at site 3 (Table 3).

Between the different locations (Tables 2 and 3), it can be concluded that samples from site 3 showed the highest levels of Pb (195.90 $\mu g\ g^{-1}$) and Cd (1.57 $\mu g\ g^{-1}$), whereas the lowest level of Pb was observed in the sampling site 1 (96.71 $\mu g\ g^{-1}$) and also for Cd at sampling sites 1 and 2 (1.10 $\mu g\ g^{-1}$). High levels of Pb and Cd in site 3, could be due to various port activities like dumping of cargo ships, fishing boats, shipyard and other coastal activities (peer et al. 2010). Maximum level of Zn were recorded in the samples from site 2 (85.05 $\mu g\ g^{-1}$), as there are no industrial sources nearby this site. However, it is assumed that probably these arise from the weathering of local metal bearing rocks and lateral offshore transport of suspended material or from ship repairing operations in this area (Zulkifli et al. 2010). While the highest sediment Cu content was recorded at site 1 (7.50 $\mu g\ g^{-1}$), the high copper concentration observed are probably caused by the proximity to shipyards and harbors where copper-based, antifouling paints are used and also discharges of waste water in contact with metal surfaces, such as tanks and pipelines (Frias-Espericueta et al. 2009). Meanwhile, the lowest levels of Zn and Cu at the station 3 could probably due to very little contamination by these metals due to land base activities. The comparison between metal levels in the samples from the three sites illustrated that all metal levels differed significantly in sediment samples. The average values of Cd, Cu, Pb and Zn levels in soft tissue (ST) and shell (SH) of *S. cucullata* collected from the sampling sites are listed in Table 4. Metal levels in the ST of *S. cucullata* were compared to the mussel shell, and the ratios of soft tissue/shell (ST/SH) were calculated and results are presented in Table 4.

The results revealed that the mean levels ($\mu g\ g^{-1}$ dw) of Cd, Cu, Pb and Zn in the oyster ST from 3 sites in ranged

Table 2 Metals levels (μg g $^{-1}$ dw) in the sediment samples collected from the Coastal intertidal zones of Lengeh Port, Persian Gulf

Locations	Replicate	Pb	Zn	Cd	Cu
	1	148.01	41.32	1.10	4.26
	1	158.91	41.28	1.12	4.00
Kong Port	1	96.71	27.05	1.10	7.50
	1	159.33	35.07	1.16	5.51
	1	150.64	36.61	1.14	4.04
	2	148.42	85.05	1.20	3.97
	2	137.17	31.35	1.10	6.00
Koohin Area	2	148.44	37.64	1.10	4.45
	2	165.22	30.52	1.20	6.82
	2	166.12	37.57	1.12	4.42
	3	175.35	14.40	1.50	4.27
Bostaneh Port	3	182.47	28.57	1.50	3.45
	3	176.70	17.92	1.57	3.97
	3	195.90	18.01	1.54	4.04
	3	173.47	18.15	1.50	3.60
Mean ± SE		158.86 ± 6	33.37 ± 4.36	1.26 ± 0.05	4.69 ± 0.31

Table 3 The separation of significant mean difference Pb and Cd levels (μg g $^{-1}$ dw) in the oyster ST from the Coast of Lengeh Port, Persian Gulf, using LSD test (Least significant difference)

	Station 1	Station 2	Station 3
Pb			
Station 1	1.00	24.06*	13.53*
Station 2		1.00	37.59*
Station 3			1.00
Cd			
Station 1	1.00	1.76	7.50*
Station 2		1.00	5.7*
Station 3			1.00

* There is significant difference at the 0.05 level

from 10.28 to 12.03 and 294.10 to 345.80, 20.64 to 58.23, and 735.60 to 760.40, respectively. Average levels of in the oyster SH ranged from 3.50 to 6.55 and 138.50 to 306.90, 117.10 to 130.60, 114 to 199.10, respectively. This illustrated that the soft tissue of *S. cucullata* can accumulate these metals in the following order of abundance: Zn > Cu > Pb > Cd. However, this pattern of metal concentration differed from that observed in the shell which had the following order of abundance: Cu > Zn > Pb > Cd. These results indicated that the accumulation of Cd, Cu, Pb and Zn in the ST and the SH of *S. cucullata* was slightly different. Applying the *t* test (%5 significant level) for comparing the means of two categories (ST and SH of oysters), it was concluded that levels of Cd, Cu, Pb and Zn showed significant differences ($t = 20.4$, $df = 94$, $p < 0.01$) for Cd, ($t = 5.69$, $df = 94$, $p < 0.01$) for Cu, ($t = 27.02$, $df = 94$, $p < 0.01$) for Pb and ($t = 45.3$, $df = 94$, $p < 0.01$) for Zn. Based on the results mentioned above, the Cd and Cu levels were found about 2 times higher in the ST than those in the SH of oysters. The Zn levels in the ST were 3–6 times higher than its levels in the SH of oysters. The Pb levels in the SH were 3–5 times higher than those in the ST of *S. cucullata*. These results indicated that the soft tissue had higher affinities for Cd, Cu and Zn than the shell of *S. cucullata*. The high concentration of Cd, Cu, and Zn found in the oyster soft tissue could be attributed to the Cd, Cu, and Zn in mollusks

Table 4 Mean concentrations (μg g^{-1} dw) of Cd, Pb, Zn and Cu in total soft tissue and total shell and comparison with the ratios of soft tissue/shell of *S. cucullata* collected from the intertidal Coast of Lengeh Port, Persian Gulf

Stations	Cd_{SH}	Cd_{ST}	Cd_{ST}/Cd_{SH}	Pb_{SH}	Pb_{ST}	Pb_{ST}/Pb_{SH}	Zn_{SH}	Zn_{ST}	Zn_{ST}/Zn_{SH}	Cu_{SH}	Cu_{ST}	Cu_{ST}/Cu_{SH}
1	3.5	12.03	3.60	125.4	44.7	0.36	141	760.4	6.24	170.8	331.9	2.58
2	5.2	10.28	1.96	117.1	20.64	0.18	114	735.6	6.93	138.5	294.1	2.48
3	6.55	11.11	1.45	130.6	58.23	0.45	199.1	748	4.04	306.9	345.8	1.33

SH shell

ST soft tissue

ST/SH ratio of soft tissue to shell

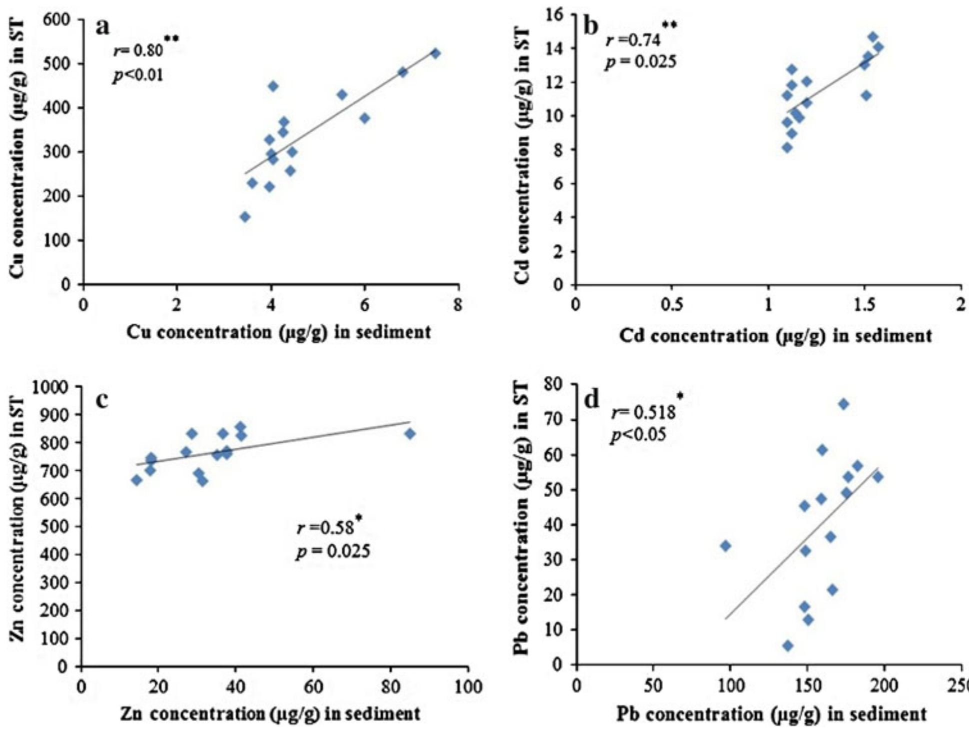

Fig. 2 Pearson's correlation coefficients (*r*) between Cu, Cd, Zn and Pb levels in sediment samples and the soft tissue (ST) of oysters from the Coast of Lengeh Port-Persian Gulf

binds to metalothionein, proteins of low molecular weight that decrease metal toxicity; however, they would not reduce the potential risk for consumption (Cadena-Cardenas et al. 2009). Cd is non-essential element, and not needed for oyster growth and may even be deleterious (Huanxin et al. 2000), some animals such as bivalve mollusks are well known for their ability to accumulate metals and other substances in their tissues. For example, oysters and mussels can accumulated in their tissues Cd levels up to 100,000 times higher than the levels observed in the water where they live (Avelar et al. 2000). Conversely, it is relevant that Zn and Cu are essential elements and play important roles in growth, cell metabolism and survival of most animals including bivalves and they are greatly enriched in oyster tissue over the levels in water or even sediment without apparent detrimental effects (Priya et al. 2011; Vázquez-Sauceda et al. 2011). On the other hand, the shells of *S. cucullata* accumulated more Pb than the soft tissues. The high levels of Pb found in the oyster shell could be probably due to crystalline structures of the shell matrix which have a higher capacity for incorporation of this metal than the soft tissue (Yap et al. 2008). Therefore, it seems that oyster has no tendency to accumulate the high level of Pb in its body. Our results are consistent with the results found by Peer et al. (2010) and Yap et al. (2003) who found the SH of oyster was a concentrating organ of lead contamination in coastal waters.

Correlations between the average metal levels in ST of *S. cucullata* with respect to elemental levels in sediment are illustrated in Fig. 2. The significant positive correlation ($r = 0.80$, $p < 0.01$) was observed in Cu level between the ST of oysters and sediments (Fig. 2a). This result was in agreement with that reported byYap et al. (2002) who found significant correlations between Cu in total soft tissues of *P. viridis* and sediment. Figure 2c revealed that Zn in total soft tissues of *S. cucullata* was significantly correlated with the total Zn in the sediment ($r = 0.58$, $p = 0.025$). Noorhaidah et al. (2010) reported significant ($p < 0.05$) correlations between Zn levels in different soft tissues of *T. telescopium* and some geochemical fractions of Zn in the sediment. Also, correlation between the ST of *S. cucullata* and sediment through positive, was slightly low ($r = 0.51$, $p = 0.048$) for Pb (Fig. 2d). Apeti et al. (2005) found that there was low relationship ($r = 0.20$) between total Pb in sediment and Pb in total soft tissue of *C. virginica*. In addition, significant positive correlation ($r = 0.74$, $p < 0.01$) was observed among Cd levels in sediment and soft tissue of oyster (Fig. 2b). Similar results have been observed for other marine bivalves where Cd levels in soft tissues are related to those in sediments (Vázquez-Sauceda et al. 2011). This is due to the ingestion of sediment particles by filter feeding mollusks, which is an important mechanism for incorporation of cadmium into the oysters. Based on the results mentioned above, the ST of *S. cucullata* can be a useful biomonitoring agent for Zn, Cd and Cu.

Based on the results obtained above, high levels of zinc, cadmium and copper in the soft tissues of *S. cucullata* relative to those in the shells elucidate *S. cucullata* concentrates zinc, cadmium and copper in the soft tissue, while the shells of *S. cucullata* accumulated more Pb than the soft tissues of *S. cucullata.* Also, results of this study revealed that there are several reasons for the prevalent use of the soft tissue of *S. cucullata* as a biomonitoring agent for Zn, Cd and Cu levels in the Persian Gulf. The reasons being the observed: the observed higher concentration of Zn, Cd and Cu in the oyster ST than the SH of *S. cucullata* and also the highest correlation in Zn, Cd and Cu accumulations among oysters ST and sediments.

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