

## Use of *Solen brevis* as a Biomonitor for Cd, Pb and Zn on the Intertidal Zones of Bushehr–Persian Gulf, Iran

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**Abstract** The concentrations of Zn, Cd and Pb were determined in tissues (shell and soft tissue) of 144 of bivalve mollusks (*Solen brevis*) and 15 samples of surface sediment collected from three locations in intertidal zones of Bushehr coast, Persian Gulf, Iran in May 2011. The mean concentrations of Zn, Cd and Pb in the sediment samples were 26.2, 1.25, and 21.1  $\mu\text{g/g dw}$ , respectively. The mean levels of Zn, Cd and Pb in the clam samples were 63.3, 0.67, and 4.38  $\mu\text{g/g dw}$  in soft tissue and 10.7, 1.53, and 15.6  $\mu\text{g/g dw}$  in shell, respectively. The degrees of variability (CV %) for Cd and Pb within the shells were lower than for soft tissues, whereas the CV for Zn was lower in the soft tissue than in the shell, indicating that there is more precision (lower CV) in the determination of Cd and Pb in the shells and Zn in the soft tissues. Significant correlation were found between Cd ( $r = 0.63$ ;  $p < 0.05$ ) and Pb ( $r = 0.78$ ;  $p < 0.01$ ) concentrations in the shell of *S. brevis* and their concentrations in the surface sediments. Indeed, Zn concentrations in the soft tissue of *S. brevis* significantly ( $r = 0.63$ ;  $p < 0.05$ ) correlated with Zn concentrations in

surface sediments. The results of this study suggest that the shell of *S. brevis* may serve as a reliable biomonitor for Cd and Pb, and the soft tissue for Zn.

**Keywords** Heavy metals · Sediment · *Solen brevis* · Correlation · Persian Gulf

The Persian Gulf is a semi-enclosed basin that possesses an immense economic wealth and geopolitical importance (Vossoughi et al. 2005). It is generally subject to pollutions such as heavy metals due to a combination of its natural characteristics such as shallow depth, limited circulation, poor flushing and high salinity and temperature (Jaafarzadeh haghghi et al. 2011) and anthropogenic activities, as well as several environmental disasters such as oil spill resulted from different wars of the region (Pourang et al. 2005; Vossoughi et al. 2005). The existence of excess heavy metals in aquatic ecosystems can affect sediments and organisms. Sediments act as an important sink of heavy metals (Sajwan et al. 2008; Kesavan et al. 2010). In addition, sediments are a potential non-point source of heavy metals which may directly influence overlying waters (Houngyi et al. 2009). Heavy metals accumulated in sediment can, over time, be transferred to and accumulated in marine organisms like bivalves (Joksimovic et al. 2011).

Bivalve mollusks have an ability to accumulate heavy metals to various orders of magnitude with respect to the levels found in their environment (Pourang et al. 2010). Mollusks are widely recognized as reliable indicators of bioavailability of metals (Yap et al. 2002b; Liu and Deng 2007; Frías-Espéricueta et al. 2009; María-Cervantes et al. 2009). Two primary aims of the present study were to: (1) determine the concentrations of Zn, Cd and Pb in surface sediments and tissues (shell and soft tissue) of *Solen brevis*

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and (2) investigate the potential of the shell and soft tissue of *S. brevis* as biomonitoring materials for these metals.

## Materials and Methods

The samples were collected from 3 locations at the intertidal zones of Bushehr Province of the Persian Gulf in May, 2011. The sampling sites were selected according to their proximity to areas of industrial, fishing, shipping, and aquaculture activities (Fig. 1).

Forty-eight clams ( $n = 48$ ) of similar size (8–10 cm shell length) were collected from each sampling site, transported to the laboratory in an ice box and stored at  $-10^{\circ}\text{C}$  for later analysis. Five replicates of the surface layer (0–5 cm) of sediments were sampled using a van Veen grab (Hydrobios, India) from each station around the area inhabited by the clams (each replicate consisted of five frequencies). Sediment samples were placed in polyethylene bags, transported to laboratory in an ice box and frozen for further analysis.

In the laboratory, the clam samples were thawed and washed with tap and double distilled water (DDW) to remove sediment and debris. The soft tissue (ST) was carefully separated from the shell (Sh) using a plastic knife. The samples of soft tissue and shell were dried for 72 h at  $105^{\circ}\text{C}$  in an oven to constant dry weight. Dried samples were ground into fine powder using a pestle. The samples of sediment were dried at  $105^{\circ}\text{C}$  for at least 16 h in an oven to a constant dry weight. Then, the samples were passed through a 0.05 mm stainless steel sieve (Yap et al. 2002b).

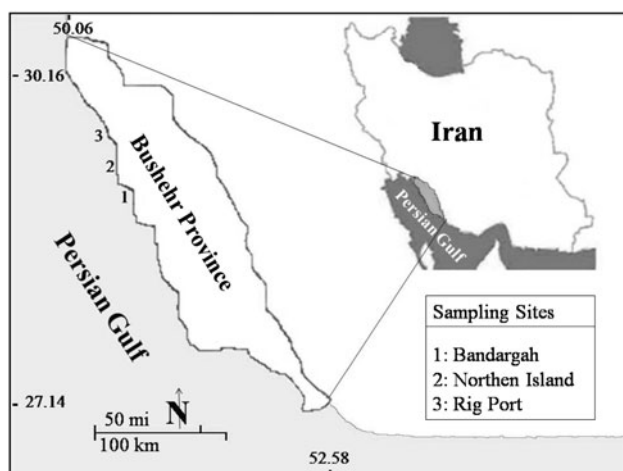
One gram of each sample of soft tissue and shell was digested in concentrated nitric acid (69 %). They were placed in a hot block digester first at  $40^{\circ}\text{C}$  for 1 h and were then digested at  $140^{\circ}\text{C}$  for 3 h (Yap et al. 2002b).

The direct aqua regia method (Yap et al. 2002b) was used for heavy metals analyses in sediment samples. 1 g of each dried sample was digested with a freshly prepared mixture (4:1) of 10 mL concentrated nitric acid (69 %) and perchloric acid (60 %), first at  $40^{\circ}\text{C}$  for 1 h and then at  $140^{\circ}\text{C}$  for 3 h using a hot block digester.

Digested tissue and sediment samples were diluted to 50 mL with DDW and filtered through Whatman No. 1 filter paper. The levels of Zn, Cd and Pb in the filtrate were determined using flame atomic absorption spectrophotometer (Shimadzu Model AA 670 instrument, Japan). The results were expressed in micrograms per gram ( $\mu\text{g/g}$ ) dry weight (dw) basis. The detection limits for each metal were 0.25, 0.05 and  $0.4 \mu\text{g/g}$  dw for Zn, Cd and Pb, respectively. Quantities were estimated using calibration curves obtained from three standard solutions of each metal analyzed. Standard solutions were prepared from 1,000  $\mu\text{g/g}$  stock solution of each metal (Merck Titrisol, Germany).

To check for contamination, all glassware used was acid-washed, and one blank was analyzed after five samples, using the same procedure as for the samples. The accuracy levels of the methods were tested by analyzing IAEA 356 (Austria) and ERM-CE 278 (IRMM; Belgium) Certified Reference Material (CRM) for sediment and mussel tissue, respectively. The analytical values were within the range of certificated values. Recoveries were consistent in the range 91 %–98 % (Table 1).

Normality of data and homogeneity of variances were verified using the Levene test. In order to find the differences among sampling sites (in terms of level of heavy metals in clam and sediment), one-way ANOVA was performed, followed by the least significant difference (LSD) post hoc test. Independent sample *t*-tests were employed to compare mean metal concentrations between soft tissue and shell. Pearson's correlation coefficients were used to test the relationships between the concentrations of metals in sediments with the soft tissue and shell of *S. brevis*. Data analyses were performed using SPSS version 15.0 (SPSS, USA).



**Fig. 1** Map of study area showing location of sampling sites

**Table 1** Analysis of certified reference materials (CRMs) (mean  $\pm$  SD,  $n = 5$ ,  $\text{mg/kg}^{-1}$  dw)

CCRM	Cd	Pb	Zn
Marine sediment (IAEA 356)			
Certified value	$4.47 \pm 0.23$	$347 \pm 13.23$	$977 \pm 18$
Measured value	$4.25 \pm 0.26$	$333 \pm 10.86$	$957 \pm 16.2$
Recovery (%)	95	96	98
Mussel tissue (ERM-CE 278)			
Certified value	$0.348 \pm 0.007$	$2.00 \pm 0.04$	$83.1 \pm 1.7$
Measured value	$0.317 \pm 0.003$	$1.90 \pm 0.05$	$79.2 \pm 1.5$
Recovery (%)	91	95	95

The coefficient of variation (CV) value was calculated to determine the degree of variability of metal concentration in shell and soft tissue (Yap et al. 2003) as follows:

$$CV(\%) = \frac{\text{standard deviation}}{\text{mean}} \times 100$$

## Results and Discussion

Significant differences in heavy metal concentration were apparent between the sampling sites (Table 2), which may have resulted from different anthropogenic activities. The highest concentration of Zn was found in the samples collected from station 2, probably because of the Zn that is associated with oily hydrocarbons (Karbassi et al. 2005) and aquaculture effluents (Mendiguchia et al. 2006; Russell et al. 2011). The high levels of Cd and Pb found in samples from stations 1 and 3 were probably related to oily compounds (Karbassi et al. 2005) and the discharge of effluents from nearby domestic and industrial sources. Indeed, a geological source (Karbassi et al. 2005) and the burning of fossil fuels from boats used for fishing and recreational activities may be other sources of Pb contamination.

Independent sample *t* test showed statistically significant ( $p < 0.001$ ) inter-tissue differences in the accumulation of Zn, Cd and Pb. Considerably higher Zn content was observed in the soft tissue, whereas the shell accumulated higher levels of Cd and Pb (Table 3). Moreover, the ST/Sh ratio for Zn was considerably greater than unity (i.e., 5.5–6.0). The elevated level of Zn in the soft tissue of *S. brevis* compared to Cd and Pb might be due to the major role played by Zn in metabolism (Kamaruzzaman et al. 2010), its binding with metallothioneins (Sajwan et al. 2008), and a possible protective role against the toxicity of

other metals. Joksimovic et al. (2011) suggested that Zn appears to have a protective effect against the toxicities of both Cd and Pb in the soft tissue.

The ratio of ST/Sh for Cd and Pb were less than unity (0.4–0.5 for Cd and 0.25–0.33 for Pb; Table 3). The lower accumulation of these metals in soft tissue than in the shell could be due to the existence of metallothioneins that detoxify and store toxic metals (Podgurskaya et al. 2004; Yap et al. 2006; Cadena-Cárdenas et al. 2009). Boening (1999) and Szefer et al. (2002), reported that the bio-availability of Cd in soft tissue decreases with increasing salinity of the adjacent water. Therefore, the low level of Cd in soft tissue also may be related to the high salinity of the northern part of the Persian Gulf. The high accumulation of Cd and Pb in the shell may be due to the presence of calcium ions in the crystalline structure of the shell, and substitution of Cd and Pb for calcium (Yap and Cheng 2008).

The CV value for Zn in the soft tissue was lower than in the shell (Table 4). Conversely, lower degrees of variability for Cd and Pb were observed in the shell. According to Yap et al. (2003), lower degrees of variability of metal concentration in a specific tissue indicate higher precision in using that tissue as biomonitor for heavy metals.

A significant correlation ( $r = 0.63$ ,  $p < 0.05$ ) was found between Zn level in soft tissue and Zn level in surface sediments (Fig. 1). Jamil et al. (1999) found significant correlation between Zn in soft tissues of three clam species *Anodonata anatina*, *Unio pictorum*, *U. tumidus* and surface sediments in the Danube Delta, Romania. However, Yap et al. (2002b) reported insignificant correlation between Zn level in sediment and soft tissue of *Perna viridis* due to the

**Table 2** Means  $\pm$  SD ( $\mu\text{g/g dw}$ ) of heavy metals in the surface sediment samples collected from intertidal zones of Bushehr–Persian Gulf, Iran

Station	Zn	Cd	Pb
1	23.4 $\pm$ 1.18	1.29 $\pm$ 0.04	24.3 $\pm$ 0.57
2	28.5 $\pm$ 2.03	1.17 $\pm$ 0.02	18.8 $\pm$ 1.06
3	26.7 $\pm$ 1.14	1.29 $\pm$ 0.04	20.3 $\pm$ 0.73

**Table 4** Comparison between coefficients of variation (%) of heavy metal concentrations in total soft tissue and total shell of *S. brevis*

Metal	n	CV (%)
Zn-soft tissue	53	9.77
Zn-shell	53	35.9
Cd-soft tissue	53	21.5
Cd-shell	53	12.1
Pb-soft tissue	53	43.7
Pb-shell	53	26.6

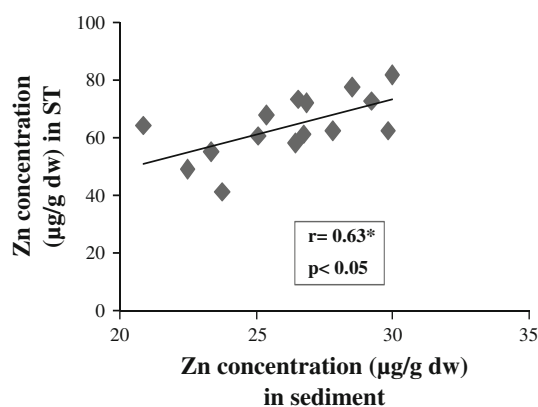
**Table 3** Means  $\pm$  SD ( $\mu\text{g/g dw}$ ) of heavy metals in the soft tissue and shell of *S. brevis* collected from intertidal zones of Bushehr–Persian Gulf, Iran

Station	Zn <sub>(ST)</sub>	Zn <sub>(Sh)</sub>	Zn <sub>(ST/Sh)</sub>	Cd <sub>(ST)</sub>	Cd <sub>(Sh)</sub>	Cd <sub>(ST/Sh)</sub>	Pb <sub>(ST)</sub>	Pb <sub>(Sh)</sub>	Pb <sub>(ST/Sh)</sub>
1	56.8 $\pm$ 8.12	9.73 $\pm$ 1.02	5.80 $\pm$ 0.48	0.79 $\pm$ 0.11	1.69 $\pm$ 0.14	0.47 $\pm$ 0.07	6.19 $\pm$ 1.39	18.6 $\pm$ 3.14	0.33 $\pm$ 0.05
2	69.3 $\pm$ 8.46	11.5 $\pm$ 0.97	6.08 $\pm$ 0.57	0.53 $\pm$ 0.08	1.37 $\pm$ 0.13	0.39 $\pm$ 0.06	3.21 $\pm$ 1.18	12.3 $\pm$ 3.32	0.25 $\pm$ 0.04
3	64.0 $\pm$ 9.83	10.8 $\pm$ 2.51	5.59 $\pm$ 0.90	0.77 $\pm$ 0.08	1.54 $\pm$ 0.13	0.50 $\pm$ 0.05	3.74 $\pm$ 1.13	15.9 $\pm$ 3.33	0.25 $\pm$ 0.05

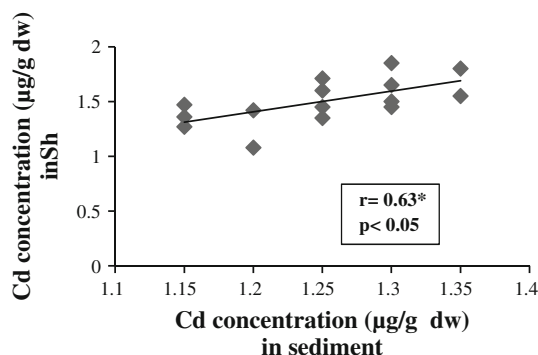
ability to regulate Zn from its soft tissue. Owing to its regulative mechanism in the soft tissues of *P. viridis*, Yap et al. (2004a) proposed the use of mussel shells as a bio-monitoring material for Zn since they found positive correlations of Zn between mussel shells and surface sediment.

Concentrations of Cd and Pb in the shell correlated significantly with their respective concentrations in the sediment (Figs. 2, 3). For Cd, the correlation coefficient,  $r$ , was 0.63 ( $p < 0.05$ ), and for Pb,  $r = 0.78$  ( $p < 0.01$ ). This finding supports previous research by Yap et al. (2003) which linked relationships of Cd and Pb concentrations between in the shell of mussel and sediment. Kanakaraju et al. (2008) found significant negative correlation for Cd in shell of *Solen* spp. with sediments at Maura Tebas, Malaysia ( $r = -0.51$ ,  $p = 0.000$ ), possibly due to low bioavailability of Cd from sediment and water in that particular environment (Fig. 4).

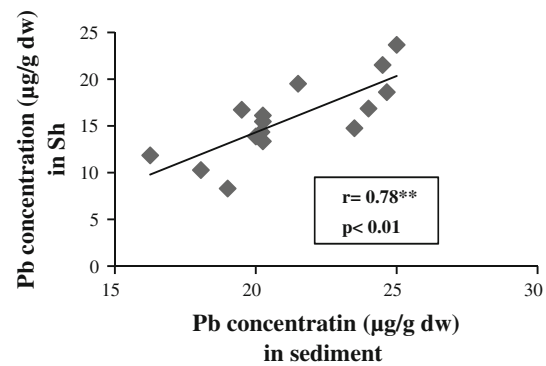
The basic concept of biomonitoring is that the concentrations of pollutants in the organism reflect those of its environment (Yap et al. 2002b; Ravera et al. 2003). Since significant correlations were observed between heavy



**Fig. 2** Correlation of Zn concentrations between surface sediment and soft tissue of *S. brevis* ( $n = 15$ )



**Fig. 3** Correlation of Cd concentrations between surface sediment and shell of *S. brevis* ( $n = 15$ )



**Fig. 4** Correlation of Pb concentrations between surface sediment and shell of *S. brevis* ( $n = 15$ )

metals in sediments and in the tissues of *S. brevis*, it could be assumed that the bioavailability of metals to *S. brevis* might be affected by the changes in sediment chemistry. Absorbed heavy metals could be desorbed from sediments when environmental conditions change and cause a secondary pollution of heavy metals to organisms (Houngyi et al. 2009).

Although present findings suggested the clam can be positively employed as a good biomonitor, *S. brevis* from different geographical areas in Bushehr–Persian Gulf should be tested for its genetic structure in order to confirm its genetic similarity (Yap et al. 2002a) in addition to other important criteria for a good biomonitor such as good accumulation capacity (Yap et al. 2004b).

In the present study concentrations of Zn, Cd and Pb in clams and surface sediments showed significant differences mainly associated with geographical locations. The significant correlations between concentrations of Zn, Cd and Pb in tissues of *S. brevis* with their corresponding concentrations in sediments, suggested that the soft tissue could be a more suitable biomonitor for Zn and the shell for Cd and Pb.

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