

Shells of *Bufo* *echinata* as biomonitoring materials of heavy metals (Cd, Ni and Pb) pollution in the Persian Gulf: with emphasis on the annual growth sections

Pourang N.^{1*}; Bahrami A.²; Nasrolahzadeh Saravi H.³

Received: March 2017

Accepted: January 2018

Abstract

Concentrations of heavy metals (Cd, Ni and Pb) were determined in soft and hard tissues (three separated shell sections) of gastropod *Bufo echinata* as well as surficial sediments collected in October 2015 from two sampling sites located in the sub-littoral zone of Qeshm Island, Persian Gulf. There were significant differences between the sampling sites for concentrations of all the three elements in the shells and sediments. But in terms of the soft tissues, in the case of Ni and Pb significant differences between the sites could be observed. In all the cases, higher levels were observed in the samples from Suza site, which may be mainly due to the proximity of this site to the relevant anthropogenic sources. Comparison of the gained data from this study with the other relevant researches shows that in most cases the levels of the elements in the soft tissues and shells either fell within the range for other world areas or were lower. The observed increasing trends of metals accumulation in the shell sections (from older to younger sections) could be mainly attributed to the gradual increase of relevant anthropogenic pollutants in the study area, especially in Suza pier, during the recent years. Generally, it can be concluded that the shells of *B. echinata* could be possibly employed as a biomonitoring tool for historic metals contamination in northeastern part of the Persian Gulf.

Keywords: Heavy metals, Biomonitoring tool, *Bufo echinata*, Soft and hard tissues, Shell sections, Persian Gulf

1-Iranian Fisheries Science Research Institute, Agricultural Research, Education and Extension, Organization, Tehran, Iran.

2-Bandar Abbas Branch, Islamic Azad University, Bandar Abbas Branch, Iran.

3-Caspian Sea Ecology Research Center, Iranian Fisheries Science Research Institute, Agricultural Research, Education and Extension Organization, Sari, Iran.

*Corresponding author's Email: n.pourang@areeo.ac.ir

Introduction

The increased contamination of aquatic ecosystem by heavy metals is a serious issue affecting water and organisms inhabiting therein (Ghosh *et al.*, 2016). In contrast with organic pollutants, heavy metals cannot be biologically or chemically degraded, thus may either accumulate locally or be transported over long distances (Kathiresan *et al.*, 2014). To assess heavy metal contamination in the aquatic environment different types of organisms may be used. Benthic molluscs are the organisms most often used for the biomonitoring of metal contamination (Pourang *et al.*, 2010). It is well known that molluscs accumulate organic and metallic pollutants at concentrations several orders of magnitude above those observed in the field environment (Ranjan and Babu, 2016). Tissue metal concentration can reflect contamination and molluscs in particular may therefore be sensitive biomonitors of anthropogenic metal inputs (Hendozko *et al.*, 2010). Literature on bivalve molluscs as biomonitoring organisms in the aquatic environment is extensive, but fewer studies have been done on gastropod molluscs, some of which are also considered as useful biomonitors of certain metals (Kesavan *et al.*, 2013). The use of gastropods as biomonitor organisms offer several advantages: a) gastropods have reasonable sizes for analysis and repeatable samplings; b) they have broad geographical distribution; c) they are sedentary or

less mobile than any other organisms such as fishes, and thus accumulate contaminants more efficiently than that of the surrounding waters; d) gastropods exhibit low or undetectable enzyme activities which metabolize pollutants; e) some gastropods are important seafood or source of protein; thus, studying them has significant human health implications (Yap *et al.*, 2010; Youssef *et al.*, 2016). Most metals are generally concentrated many times over within an organism's soft tissue, rather than the shell, and so the accumulation of metals in molluscs has been mainly studied from the standpoint of their accumulation in the soft tissues. However, some studies of the shell material have also been conducted and many authors suggest that shells act as receptor for some metals and can provide a more accurate indication of environmental change and pollution; they exhibit less variability than the living organism's tissue and they provide a historical record of metal content throughout the organism's life time. This record still preserved after death (Huanxin *et al.*, 2000a; Richardson, 2001). Shells of gastropods have been validated as bioindicators of trace elements (McClintock *et al.*, 2014).

The Persian Gulf is a shallow semi-enclosed basin with an average depth of 35 m and a total area of around 240 km² which joins international waters through the Strait of Hormuz. Due to the shallow depths, limited circulation and high salinity and temperature that

characterize the northern part of the Persian Gulf, the impact of the pollutants, especially heavy metals, on the marine environment may be significant (Pourang *et al.*, 2005; Dadolahi Sohrab and Nazarizadeh Dehkordi, 2013).

The main objective of the current investigation was to assess the ability of shells of *Bufo naria echinata*, to be used as a biomonitoring tool for heavy metals in the Persian Gulf.

The other purposes of the research were: (a) to investigate the relationships between levels of heavy metals in the shells, soft tissues, and in sediments from the study areas; (b) to evaluate inter-site differences in accumulation of the elements; (c) to assess the relationships between elements and the biological characteristics of the specimens (length, width and weight); and (d) to compare our results with those obtained by other authors in different areas of the world.

Materials and methods

Sampling

The samples were collected from two sampling sites (Suza : 26° 45' N, 56° 03' E and Salakh: 26°38'N, 55°50'E) located in the northern part of the Persian Gulf, southern coastal area of Qeshm Island (Fig. 1) in October 2015. Both sites were situated on the muddy sublittoral zone. With regards to the previous relevant studies (Sadeghi Nassaj *et al.*, 2010; Owfi *et al.*, 2011; Ansari *et al.*, 2012), two main criteria were considered in selecting the sites:

a) covering both the relatively polluted and unpolluted areas (Suza and Salakh sites, respectively), b) proper distribution of the species in the selected sites. A preliminary sampling was also performed in June 2015 to make sure about the species distribution in the area. The specimens of *B. echinata* were collected alive from each sampling site by SCUBA divers and among them thirty specimens with the age of ~3 years were selected. The shell dimensions (length and width) were measured using vernier calipers to an accuracy of 0.1 mm. Weight of the specimens were also recorded. Surficial sediments were collected from the sampling sites using Van Veen grab sampler (Hydrobios) with a surface area of 0.1 m². Five sub-samples were collected from each site. The surface layer of the sediment in each undisturbed grab was carefully removed with a plastic spatula and emptied into a clean Nalgene container. The samples were stored at 4 °C.

Preparation of the samples

After collection, the gastropods were transported to the laboratory. The specimens were rinsed with seawater and then placed in about 20 L of constantly aerated clean seawater for 24 h to allow depuration from adhering particles and gut contents (Bat *et al.*, 2000; Abidli *et al.*, 2013). Before the shells were shucked, the external surface was thoroughly cleaned with brush and water to remove all sand, loosely attached biogenic and inorganic

particles to prevent contamination. The soft tissues were removed by shelling the gastropods with a plastic knife on a clean glass working surface. The soft tissues were dried in a freeze drier to a constant weight and homogenized in an agate mortar. Approximately 0.5g of freeze-dried samples were placed in Teflon bombs. Digestion was performed in a microwave digestion system (Milestone, Ethos 1) using high purity concentrated nitric acid (Franco *et al.*, 2002; Usero *et al.*, 2005). Sediment samples were microwave-digested in Teflon bombs using a mixture of HNO₃ and HF according to MOOPAM Instruction (2010). The digested samples were transferred to tightly sealed linear polyethylene containers to avoid adsorption of metals from digested solution and kept at 4 °C prior to further analysis (Pourang *et al.*, 2014). The total organic matter (TOM) in the sediments was determined by drying for 24 h at 60 °C, combusting in a muffle furnace (at 600 °C for 24 h) and calculating differences in weights according to Haque *et al.* (1997). Sediment samples were passed through a 62 µm sieve to separate the coarse (sand and gravel which are retained on the sieve) and fine (clay and silt) fractions (McCave and Syvitski, 1991).

The samples were stored at 4°C. The shells were air dried at room temperature for a minimum of 48 h to constant weight. Each shell was sectioned transversely, across the annual growth lines, into three slabs using a rotating diamond cutting blade. The shell sections were ground using pestle and mortar. The resulting powder was selected, using a plastic sieve with 0.2mm opening size and was stored in desiccator for further analysis. The samples were weighed to 0.5 g and digested with 10 mL of concentrated nitric acid and 3 mL of 30% hydrogen peroxide (Kim and Kim, 2006).

Analytical methods

The trace elements were analyzed by GFAAS (Thermoelectron-M5). All reagents were supra-pure and high-purity water was employed throughout. The accuracy of the determination procedure was assessed by the analysis of certified reference materials (CRMs; mussel tissue: NCSZC 78005 and marine sediment: IAEA-356) in triplicate for each batch of analysis. The digestions were prepared in a similar manner as samples. Recoveries were consistently in the range 94.6–100.5 % (Table 1).

Table 1: Analytical results of certified reference materials (CRMs) in this study.

CRM		Cd	Ni	Pb
Marine sediment (IAEA 356)	Certified value	4.47 ± 0.23	36.9 ± 2.58	347 ± 13.23
	This study	4.23±0.17	36.1±0.12	345±0.01
	Recovery (%)	94.6	97.8	99.4
Tissues (NCS ZC 78005)	Certified value	4.5 ± 0.5	1.03 ± 0.13	1.96 ± 0.09
	This study	4.4±0.1	1±0.01	1.97±0.02
	Recovery (%)	97.7	97	100.5

Concentrations are in µg g⁻¹ dry weight.

Data analysis

Data were tested for normality using the Shapiro–Wilk test. To detect homogeneity of variances Levene’s test was used (Zar, 2010). Data were transformed where necessary. The appropriate transformation estimated using Taylor’s power law (Southwood and Henderson, 2000). A series of independent samples t tests were performed to identify differences between: (a) the sampling sites from soft tissues and shells point of view, (b) the sampling sites from fine fraction and TOM in the sediments aspect, (c) the tissues in accumulation of each trace element and (d) the sampling sites from biological characteristics (length, width and weight) of the specimens point of view. Three Mann-Whitney U tests were used to detect differences between sampling sites with respect to the concentration of each element in sediments. Two Kruskal-Wallis tests were employed to test for significant differences in heavy metal accumulation in sediments. Four one-way ANOVAs were done to examine whether the metals had been accumulated significantly different in the soft and shells. Since highly significant Fs resulted from all ANOVAs, Duncan’s new multiple range tests were used to determine which group means did not differ from one another. Six one-way analysis of variances (ANOVAs) were also conducted to test if significant differences exist between the shell sections in terms of concentration of

each metal (for each sampling site separately), followed by Duncan’s new multiple range test wherever the differences were significant (Steel *et al.*, 1997; Zar, 2010). Pearson’s correlation coefficients were used to examine relationships between the elements as well as between elements and the biological characteristics. Data analyses were performed using new version of statistical packages especially SPSS (Version 23.0).

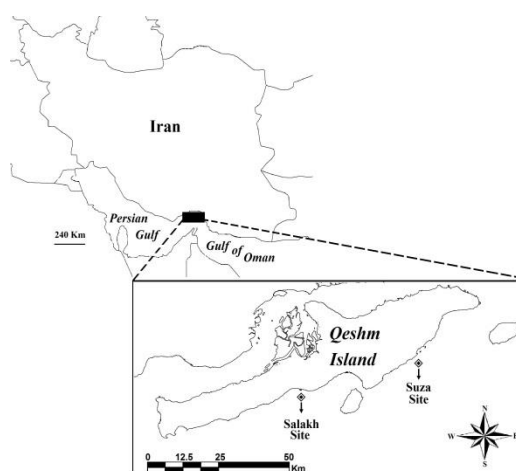


Figure 1: Map of the Persian Gulf showing location of the sampling sites.

Results

Following the conduction of three independent samples t tests (testing the null hypothesis that there were no significant differences between the sampling sites from biological characteristics of the specimens point of view), significant differences could be observed between the sampling sites in length, width and weight of the specimens ($p < 0.05$). Fig. 2 indicates that the biological characteristics of the specimens from Salakh site are higher.

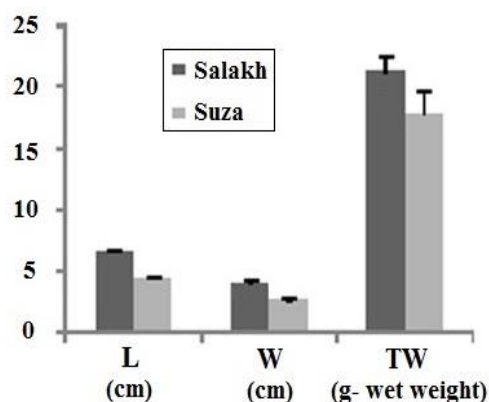


Figure 2: Mean (\pm SD) shells length (L), width (W) and total weight (TW) of *Bufo echinata* specimens from the two sampling sites (Salakh and Suza).

In terms of the elements accumulation in the soft tissues, in the case of Pb and Ni significant differences (independent samples t tests, $p < 0.05$) between the selected sampling sites could be observed, which were considerably higher in the samples from Suza site.

There were significant differences between the sampling sites for concentrations of all the metals in the shells. In all the cases, higher levels were observed in the samples from Suza site (independent samples t tests, $p < 0.001$). At both sampling sites, the levels of all the three elements in the shells were significantly higher than those in the soft tissues (independent samples t tests, $p < 0.05$). The results of Mann–Whitney U tests revealed that

there were significant differences between the sampling sites in the levels of all the three elements in the sediments ($p < 0.05$). The concentrations in Suza site were more than the other site. Percentages of fine fraction ($< 63 \mu\text{m}$) and TOM in the sediments from Suza site (86.21 and 17.86, respectively) were significantly higher ($p < 0.05$) than those in Salakh site (61.54 and 12.47, respectively).

The order of metal contents in the sediments, soft tissues and shells from both sampling sites were similar ($\text{Cd} < \text{Ni} < \text{Pb}$).

The results of the six one-way ANOVAs indicated that there were significant differences between the shell sections in terms of concentration of the metals. Homogeneous groups were identified by Duncan's new multiple range tests. With regards to the results, the following orders were found: Pb (Suza): Section 1 < Section 2 < Section 3; Pb (Salakh): Section 1 < Section 2, Section 3; Ni (Suza): Section 1, Section 2 < Section 3; Ni (Salakh): Section 1 < Section 2, Section 3; Cd (Suza): Section 1 < Section 2 < Section 3; Cd (Salakh): Section 1 < Section 2, Section 3. The similar results can be drawn from Fig. 3.

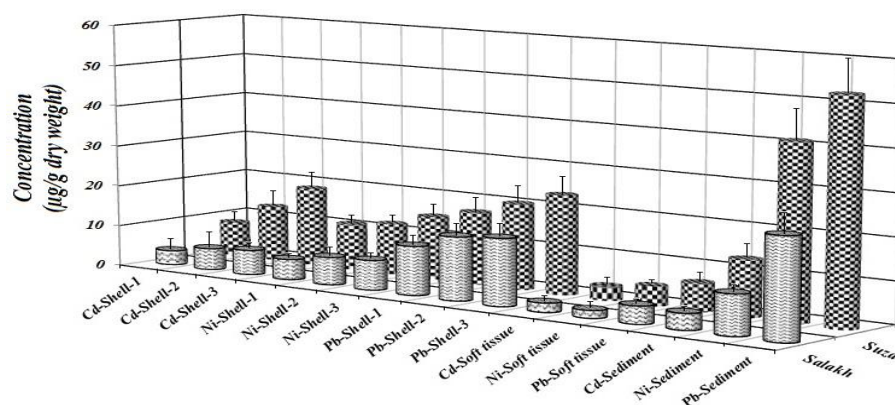


Figure 3: Mean (\pm SD) heavy metal concentrations ($\mu\text{g g}^{-1}$ dry weight) in sediment, soft tissue and the three shell sections of *Bufonaria echinata* specimens from the sampling sites (Suza and Salakh). The numbers indicate the shell sections concerning the relevant years. Cd values are multiplied by 10.

The results of Pearson's correlation coefficients between the biological parameters of the specimens and the mean levels of the elements in the shells showed strong significant negative relationships between the length and concentrations of Pb ($p < 0.01$, $r = -0.83$) and Ni ($p < 0.01$, $r = -0.79$), but no significance correlation between length and Cd concentrations in the shells were observed. In terms of soft tissues, significant negative correlations were detected for Pb with width ($p < 0.05$, $r = -0.70$).

Discussion

Inter-site differences

Sediments

As mentioned previously, levels of all the three elements in sediments from Suza site were considerably higher (compared to the other site). With regards to the relative closeness of Suza site to the potential anthropogenic heavy metal pollution sources (especially industrial and sewage

discharges, waste disposal, leak of oil and its products; (Mashinchian Moradi *et al.*, 2011; Agah *et al.*, 2012), the higher elements levels in sediments from this site would be expected. But the significant differences in the sediments metals concentrations from the sampling sites should not be necessarily attributed to anthropogenic sources. In other words, the relatively high concentrations of the elements in sediments from Suza site might arise from a higher proportion of fine fraction and TOM in this site. Generally, the elemental concentration of sediments not only depends on anthropogenic and lithogenic sources, but also upon the textural characteristic, organic matter content, mineralogical composition, and depositional environment of sediments (Maya, 2005). Trace metals are primarily associated with the fine fractions of surficial sediments. Smaller particles have a larger surface area volume⁻¹ ratio and therefore contain higher concentration of metals. The specific

surface area of sediments is dependent on granulometric parameters and mineral composition (Pourang *et al.*, 2014).

Soft tissues

Since Ni and Pb are usually being considered as environmental indicators for petroleum and its derivatives (Pourang *et al.*, 2005; Youssef *et al.*, 2016), the higher levels of these elements in the soft tissues of the specimens from Suza site may be mainly due to the proximity of this site to the relevant anthropogenic sources in the region, especially in Suza pier, where the elements may be released through incomplete fuel combustion (marine traffic) and boat bilge discharges. In general, the observed differences in Ni and Pb levels in the soft tissues of the specimens at the two sites may be related to one of the following mechanisms: (a) the bioavailability of the metals to the specimens varies with the sites, (b) the animal involves different uptake and retention mechanisms for the metal at two sites (affected by different biotic and/or abiotic factors), and (c) the body size is a factor that is well known to affect metal concentrations in organisms. The two explanations that are generally given are (1) the decreasing surface/volume ratio of an organism with increasing body size, and (2) decreasing metabolic activity in larger organisms. Both parameters would tend to result in decreasing metal uptake with increasing individual size

(Hédouin *et al.*, 2006; Pourang *et al.*, 2014). However, in the present study the mean values of the most biometric parameters (weight and length) at Salakh site were higher, and at the same time Ni and Pb contents in the soft tissues of the specimens from this site were lower. Hence, the decreasing surface volume⁻¹ ratio with increasing body size could also be considered as one of the reasons for the differences between the sites.

Shells

According to Chang *et al.* (2007) trace metal concentrations in shells of mollusc species vary to a wide range even if they belong to the same taxon. The differences between the same species from different regions may be attributable to influential factors that govern the incorporation of trace metals during molluscan shell formation. These influential factors can be summarized as differences in (a) ontogenetic stages, (b) mineralogy (biomineralization) of the shell, and (c) salinity and temperature of different water environments (Huanxin *et al.*, 2000b; Pourang *et al.*, 2014). Since in this study, all the selected specimens were almost the same age, higher concentrations of metals in the samples from Suza site, might be mainly related to the more anthropogenic activities in this area (Mashinchian Moradi *et al.*, 2011; Agah *et al.*, 2012). Moreover, it should be noted that in the present study, we observed significant negative relationships between the shell lengths

and the levels of Pb and Ni, and on the other side the allometric characteristics of the specimens from Salakh site were higher. Therefore, it can be considered as one of the possible reasons for the higher concentrations of metals (especially Pb and Ni) in the shell samples from Suza site. The results are in confirmation with the findings of some other researchers (Hirao *et al.*, 1994; Arai *et al.*, 2003; Yap and Edward, 2010), which reported decreased elemental incorporation in molluscs' shells with size.

Inter-tissue differences

With regards to the higher concentrations of the elements in the shells of *B. echinata* specimens, the incorporation of the elements in the shell matrices may be faster than the soft tissues. Mollusc shell may act as a safe storage matrix for toxic contaminants (such as Cd, Ni and Pb in the present study) which is resistant to soft tissue detoxification mechanisms and not constantly exposed to metabolic processes and therefore have longer biological half-lives (Amin *et al.*, 2006; Yap *et al.*, 2009). On the other hand, the higher concentrations of Cd, Ni and Pb found in the shell could probably be due to the fact that the crystalline structures of the shell matrix have a higher capacity for incorporation of these metals than the soft tissues (Ambekar *et al.*, 2016). According to Simeonova *et al.* (2013) the distinct difference between the shells and different soft tissues in most molluscs

could be also due to the fact that some trace metals are incorporated into the shells through substitution of the calcium ion in the crystalline phase of the shell or are associated with the organic matrix of the shell instead of induction of metallothionein as being found in the soft tissues.

Differences between the shell sections

With regards to the results (Fig. 3), the observed increasing trends of metals accumulation in the shell sections (from older to younger sections) could be mainly attributed to the gradual increase of relevant anthropogenic pollutants in the study area, especially in Suza pier, during the recent years (Mashinchian Moradi *et al.*, 2011; Agah *et al.*, 2012).

Generally, the heavy metals levels in different parts of the mollusc's shell could be explained on the basis of calcification in molluscs occurring within the extrapallial fluid, which is secreted by the mantle. The composition of the extrapallial fluid might be significantly altered with respect to seawater due to the influence of mantle metabolic activity on the transport of metals through the mantle, to the contributions of metals and carbon from metabolic source or to organic complexation. Additionally, heavy metals are not necessarily incorporated into the calcite crystal structure but they can also be adsorbed onto the skeletal organic matrix or entrapped as a separate mineral phase (Yap *et al.*, 2010; Marin *et al.*, 2012).

Comparison with published data

Table 2 summarizes the calculated mean and SD values as well as data on metals contents in the soft tissues and shells of different gastropod species from the literature.

The widest and narrowest range of variations can be observed in the case of Pb and Cd, respectively. These data indicate that the results of the present study are comparable to those reported previously for *B. echinata* and other gastropods from other geographical regions. It can be seen that, in most cases, the levels of all the metals in this investigation either fell within the range for other areas or are lower. However, it should be noted that some of the values mentioned in the table correspond to the relatively polluted sampling sites.

The interspecies differences in accumulation of the metals in the soft tissues, which can be seen clearly in the table may arise from different feeding habits, the differences in the aquatic environments concerning the sources of

contaminants, growing rates of the species, and some other factors. Thus, the differences between metal accumulations in similar tissues of different species are probable (Amin *et al.*, 2006; Türkmen and Ciminli, 2007). On the other side, according to Chang *et al.* (2007) trace metal concentrations in shells of mollusk species vary to a wide range even if they belong to the same taxon. The differences between different species from different regions may be attributable to influential factors that govern the incorporation of trace metals during molluscan shell formation. These influential factors can be summarized as differences in (a) taxonomies and ontogenetic stages, (b) mineralogy (biomineralization) of the shell, (c) salinity and temperature of different water environments, and (d) detoxification mechanism and the crystalline structures of the shell matrix among species (Huanxin *et al.*, 2000b; Heungtae and Kim, 2006).

Table 2: Comparison of mean (\pm SD) of heavy metal concentrations ($\mu\text{g g}^{-1}$ dry weight) in soft tissue and shell of *Bufo echinata* with other gastropod species reported in the literature.

Species	Geographical area	Cd	Ni	Pb	Tissue	Reference
<i>Phorcus turbinatus</i>	Eastern coast of Algeria, Gulf of Skikda	3.51 \pm 0.58	2.66 \pm 0.66	0.79 \pm 0.17	Soft tissues	Boucetta <i>et al.</i> , 2016
<i>Cipangopaludina chinensis</i>	Saint-Augustin Lake, Québec, Canada	0.6	1.05	1.0	Soft tissues	Tornimbeni <i>et al.</i> , 2013
<i>Littorina littorea</i>	Southern part of West Bengal (India)	BDL	73.81	25.51	Shell	Ghosh <i>et al.</i> , 2016
<i>Littorina littorea</i>	Southern part of West Bengal (India)	BDL	26.19	BDL	Soft tissues	Ghosh <i>et al.</i> , 2016
<i>Littorina scabra scabra</i>	Southern part of West Bengal (India)	BDL	83.34	20.41	Shell	Ghosh <i>et al.</i> , 2016
<i>Littorina scabra scabra</i>	Southern part of West Bengal (India)	5.50	22.40	53.20	Soft tissues	Ghosh <i>et al.</i> , 2016
<i>Canarium (Gibberulus) gibbosus</i>	Egyptian Red Sea coast	-	5.55	0.33	Shell	El-Sorogy <i>et al.</i> , 2013

Table 2 continued:

<i>Nerita albicilla</i>	Egyptian Red Sea coast	-	6.57	188	Shell	El-Sorogy <i>et al.</i> , 2013
<i>Nerita oryzae</i>	West coast of India , north of Mumbai , Popharan	BDL	0.11	0.05	Shell	Ambekar <i>et al.</i> , 2016
<i>Nerita oryzae</i>	West coast of India , north of Mumbai , Popharan	0.03	0.08	BDL	Soft tissues	Ambekar <i>et al.</i> , 2016
<i>Nerita lineata</i>	Johor Malaysia	4.18	19.29	59.84	Shell	Amin <i>et al.</i> , 2006
<i>Nerita lineata</i>	Johor Malaysia	1.24	5.57	19.75	Soft tissues	Amin <i>et al.</i> , 2006
<i>Nerita lineata</i>	Dumai Indonesia	4.14	20.73	44.43	Shell	Amin <i>et al.</i> , 2006
<i>Nerita lineata</i>	Dumai Indonesia	0.71	5.08	9.35	Soft tissues	Amin <i>et al.</i> , 2006
<i>Nerita lineata</i>	Peninsular Malaysia, Sungai Janggut, Selangor	3.13	3.54	2.86	Shell	Yap and Cheng, 2013
<i>Nerita lineata</i>	Peninsular Malaysia, Sungai Janggut, Selangor	0.55	0.42	0.36	Muscle	Yap and Cheng, 2013
<i>Hemifusus pugilinus</i>	West coast of India	0.399±0.017	-	0.261±0.016	Shell	Kupekar and Kulkarni, 2014
<i>Hemifusus pugilinus</i>	West coast of India	0.228±0.009	-	0.182±0.009	Soft tissues	Kupekar and Kulkarni, 2014
<i>Faunus ater</i>	East and West Coast of Peninsular Malaysia, Kesang Laut	4.97±0.19	24.53±1.23	57.80±1.50	Shell	Yap <i>et al.</i> , 2010
<i>Faunus ater</i>	East and West Coast of Peninsular Malaysia, Kesang Laut	0.57±0.06	1.81±0.62	2.75±1.35	Muscle	Yap <i>et al.</i> , 2010
<i>Telescopium telescopium</i>	North East coast of Andhra Pradesh, India	3.12±0.96	10.4±1.6	54.2±4.5	Shell	Ranjan and Babu, 2016
<i>Cerithidea obtusa</i>	North East coast of Andhra Pradesh, India	0.36±0.07	1.22±0.33	21.36±4.8	Shell	Ranjan and Babu, 2016
<i>Cerithidea cingulata</i>	North East coast of Andhra Pradesh, India	0.01±0.007	0.34±0.12	38.6±0.54	Shell	Ranjan and Babu, 2016
<i>Bufonaria Rana</i>	Southeastern waters of Hong Kong	0.38±0.06	1.21±0.18	0.49±0.05	Soft tissues	Blackmore and Rainbow, 2000
<i>Bufonaria echinata</i>	West coast of India	0.390±0.025	-	0.251 ±0.009	Shell	Kupekar and Kulkarni, 2014
<i>Bufonaria echinata</i>	West coast of India	0.395±0.017	-	0.172 ±0.023	Soft tissues	Kupekar and Kulkarni, 2014
<i>Bufonaria echinata</i>	Persian Gulf, southern coastal area of Qeshm Island, Suza site	0.31±0.05	4.74±0.51	6.76±0.78	Soft tissues	Present study
<i>Bufonaria echinata</i>	Persian Gulf, southern coastal area of Qeshm Island, Suza site	1.36±0.19	13.01±1.26	20.74±2.33	Shell	Present study
<i>Bufonaria echinata</i>	Persian Gulf, southern coastal area of Qeshm Island, Salakh site	0.29±0.06	1.83±0.55	4.21±1.04	Soft tissues	Present study
<i>Bufonaria echinata</i>	Persian Gulf, southern coastal area of Qeshm Island, Salakh site	0.49 ±0.09	6.28±1.53	14.38±3.11	Shell	Present study

* BDL= Below Detectable Level

The results of this study suggest that the shell of *B.echinata* is a suitable candidate to be used as biomonitoring tool for metal contamination in

northeastern part of the Persian Gulf. However, in order to provide better insight in this regard, several consecutive years data on the heavy

metals levels in water and sediments from the study area should be collected and compared with those of the relevant shell sections. The present work also represents a database for future research and monitoring programs in the area.

Acknowledgements

The authors express their gratitude to the personnel of the Persian Gulf and Oman Sea Ecological Research Center especially M.S. Mortazavi and E. Kamali for their assistance in the samples preparation and some parts of the analyses.

References

- Abidli, S., Lahbib, Y., Rodriguez Gonzalez, P., Ignacio García Alonso, J. and Trigui El Menif, N., 2013.** Imposex and butyltin burden in *Bolinus brandaris* (Mollusca, Gastropoda) and sediment from the Tunisian coast. *Hydrobiologia*, 714, 13-24.
- Agah, H., Hashtroudi, M.S. and Baeyens, W., 2012.** Trace metals and major elements in sediments of the northern Persian Gulf. *Journal of the Persian Gulf*, 3(7), 45-58
- Ambekar, A.A., Kubal, P.V., Prakash, C. and Sivaperumal, P., 2016.** A study on heavy metals in marine gastropod (*Nerita oryzarum*: recluz, 1841) at nuclear power plant site, Tarapur, Maharashtra, India. *International Journal of Recent Scientific Research*, 7(6), 11806-11813.
- Amin, B., Ismail, A., Arshad, A., Yap, C.K. and Kamarudin, M.S., 2006.** A comparative study of heavy metal concentrations in *Nerita lineata* from the intertidal zone between Dumai Indonesia and Johor Malaysia. *Journal of Coastal Development*, 10(1), 19-32.
- Ansari, Z., Seyfabadi, J. and Owfi, F., 2012.** Intertidal species diversity of southern coastline of Qeshm Island, based on dominant biotope. *Journal of Natural Environmental, Iranian Journal of Natural Resources*, 65(1), 13-29.
- Arai, T., Maeda, M., Yamakawa, H., Kamatani, A., Ohji, M. and Miyazaki, N., 2003.** Uptake and elimination of trace metals in shells of abalones *Haliotis* spp. *Bulletin of Environmental Contamination and Toxicology*, 71, 75-82.
- Bat, L., Gönlügür, G., Andaç, M., Öztürk, M. and Öztürk, M., 2000.** Heavy metal concentrations in the sea snail *Rapana venosa* (Valenciennes, 1846) from Sinop coasts of the Black Sea. *Turkish Journal of Marine Sciences*, 6, 227-40.
- Blackmore, G. and Rainbow, P.S., 2000.** Trace metals in the sublittoral epibenthic *Bufo naria rana* (Gastropoda: Bursidae) from the southeastern waters of Hong Kong. The marine flora and fauna of Hong Kong and Southern ChinaV (ed. B. Morton). Hong Kong University Press, pp. 435-454.

- Boucetta, S., Beldi, H. and Draredja, B., 2016.** Seasonal variation of heavy metals in *Phorcus (Osilinus) turbinatus* (Gastropod, Trochidae) in the Eastern Algerian coast. *Global Veterinaria*, 17(1), 25-41.
- Chang, F., Li, G.C., Haws, M. and Niu, T.H., 2007.** Element concentrations in shell of *Pinctada margaritifera* from French Polynesia and evaluation for using as a food supplement. *Food Chemistry*, 104, 1171–1176.
- Dadolahi Sohrab, A. and Nazarizadeh Dehkordi, M., 2013.** Heavy metals contamination in sediments from the North of the Strait of Hormuz. *Journal of the Persian Gulf*, 4 (11), 39-46.
- El-Sorogy, A., El Kammar, A., Ziko, A., Aly, M. and Nour, H., 2013.** Gastropod shells as pollution indicators, Red Sea coast, Egypt. *Journal of African Earth Sciences*, 87, 93–99.
- Franco, J., Borja, Á., Solaun, O. and Pérez, V., 2002.** Heavy metals in molluscs from the Basque Coast (Northern Spain): results from an 11-year monitoring programme. *Marine Pollution Bulletin*, 44(9), 973–976.
- Ghosh, I., Mitra, A., Rudra, T., Pramanick, P. and Biswas, P.P., 2016.** Bioaccumulation pattern of heavy metals by gastropods: A case study from lower Gangetic Delta. *Journal of Environmental Science, Computer Science and Engineering & Technology*, 5(1), 95-102.
- Haque, A.M., Szymelfenig, M. and Węslawski, M., 1997.** Spatial and seasonal changes in the sandy littoral zoobenthos of the Gulf of Gdańsk. *Oceanologia*, 39, 299–324.
- Hédouin, L., Metian, M., Teysié, J.L., Fowler, S.W., Fichez, R. and Warnau, M., 2006.** Allometric relationships in the bioconcentration of heavy metals by the edible tropical clam *Gafrarium tumidum*. *Science of the Total Environment*, 366, 154–163.
- Hendozko, E., Szefer, P. and Warzocha, J., 2010.** Heavy metals in *Macoma balthica* and extractable metals in sediments from the southern Baltic Sea. *Ecotoxicology and Environmental Safety*, 73, 152–163.
- Heungtae, K. and Kim, J.G., 2006.** Heavy metal concentrations in the mollusc gastropod, *Cipangopaludina chinensis malleata* from Upo Wetland reflect the level of heavy metals in the sediments. *Journal of Ecology and Field Biology*, 29(5), 453-460.
- Hirao, Y., Matsumoto, A., Yamakawa, H., Maeda, M. and Kimura, K., 1994.** Lead behavior in abalone shell. *Geochimica et Cosmochimica Acta*, 58, 3183–3189.
- Huanxin, W., Lejun, Z. and Presley, B.J., 2000a.** Bioaccumulation of heavy metals in hydrocarbon and artificial radionuclide data. *Environmental Science and Technology*, 17, 490–496.

- Huanxin, W., Lejun, Z. and Presley, B.J., 2000b.** Bioaccumulation of heavy metals in oyster (*Crassostrea virginica*) tissue and shell. *Environmental Geology*, 39, 1216–1226.
- Kathiresan, K., Saravanakumar, K. and Mullai, P., 2014.** Bioaccumulation of trace elements by *Avicennia marina*. *Journal of Coastal Life Medicine*, 2(11), 888–894.
- Kesavan, K., Murugan, A., Venkatesan, V. and Kumar, V., 2013.** Heavy metal accumulation in molluscs and sediment from Uppanar estuary, southeast coast of India. *Thalassas*, 29(2), 15–21.
- Kim, H.T. and Kim, J.G., 2006.** Heavy metal concentrations in the mollusc gastropod, *Cipangopaludina chinensis malleata* from Upo Wetland reflect the level of heavy metals in the sediments. *Journal of Ecology and Field Biology*, 29(5), 453–460.
- Kupekar, S. and Kulkarni, B.G., 2014.** Accumulation of heavy metals in intertidal gastropod shells used as bioindicator from Uran coast (West coast of India). *International Journal of Scientific and Engineering Research*, 5(10), 1542–1550.
- Mashinchian Moradi, A., Jalali Mosallam, M. and Fatemi, M.R., 2011.** A survey on the accumulation of heavy metals as indicator of oil pollution index (Vanadium and Nickel) in bivalve rock oyster (*Saccostrea cucullata*) in Qeshm Island coasts. *International Journal of Marine Science and Engineering*, 1(1), 51–58.
- Maya, K., 2005.** Studies on the nature and chemistry of sediments and water of Periyar and Chalakudy rivers, Kerala, India. Department of Marine Geology and Geophysics, School of Marine Sciences, Cochin University of Science and Technology, Kochi - 682 016, 160P.
- Marin, F., Le Roy, N. and Marie, B., 2012.** The formation and mineralization of mollusk shell. *Frontiers in Bioscience*, 4, 1099–1125.
- McCave, I.N. and Syvitski, J.P.M., 1991.** Principles and methods of particle size analysis. In Syvitski, J. P. M. (Ed.), Principles, methods and application of particle size analysis. New York: Cambridge University Press. pp. 3–21
- McClintock, J.B., Amsler, C.D., Amsler, M.O., Duquette, A., Angus, R.A., Hall-Spencer, J.M. and Milazzo, M., 2014.** Trace elements in shells of common gastropods in the near vicinity of a natural CO₂ vent: no evidence of pH-dependent contamination. *Biogeosciences Discussions*, 11, 5215–5237.
- MOOPAM, 2010.** Manual of oceanographic observations and pollutant analysis methods. Kuwait: Regional Organization for Protection of the Marine Environment (ROPME). 543P.

- Owfi, F., Rabbaniha, M., Ansari, Z., Rahimi, M. and Toosi, M., 2011.** Ecological classification of intertidal habitats (Iranian coasts of the Persian Gulf & Oman Sea), using by CMECS Model. INOC- XI International Symposium, Bogor-Indonesia. pp. 206-215.
- Pourang, N., Nikouyan, A. and Dennis, J.H., 2005.** Trace element concentrations in fish, surficial sediments and water from northern part of the Persian Gulf. *Environmental Monitoring and Assessment*, 109(1-3), 293-316.
- Pourang, N., Richardson, C.A. and Mortazavi, M.S., 2010.** Heavy metal concentrations in the soft tissues of swan mussel (*Anodonta cygnea*) and surficial sediments from Anzali Wetland, Iran. *Environmental Monitoring and Assessment*, 163, 195–213.
- Pourang, N., Richardson, C.A., Chenery, S.R.N. and Nasrollahzede, H., 2014.** Assessment of trace elements in the shell layers and soft tissues of the pearl oyster *Pinctada radiata* using multivariate analyses: a potential proxy for temporal and spatial variations of trace elements. *Environmental Monitoring and Assessment*, 186(4), 2465-85.
- Ranjan, T.J.U. and Babu, K.R., 2016.** Heavy metal risk assessment in Bhavanapadu Creek using three potamidid snails - *Telescopium telescopium*, *Cerithidea obtusa* and *Cerithidea cingulate*. *Journal of Environmental and Analytical Toxicology*, 6(4), 1-7.
- Richardson, C.A., 2001.** Molluscs as archives of environmental change. *Oceanography and Marine Biology: An Annual Review*, 39, 103-164.
- Sadeghi Nassaj, S.M., Nabavi, S.M.B., Yavari, V., Savari, A. and Maryamabadi, A., 2010.** Species diversity of macrobenthic communities in Salakh Region, Qeshm Island, Iran. *World Journal of Fish and Marine Sciences*, 2(6), 539-544.
- Simeonova, P., Simeonov, D., Spassov, L. and Simeonov, V., 2013.** Determination and statistical interpretation of toxic metals content in mollusks and snails from Black Sea. *Bulgarian Journal of Chemistry*, 2(3), 105-114.
- Southwood, T.R.E. and Henderson, P.A., 2000.** Ecological methods (3rd ed.). Oxford: Blackwell Sciences. 592P.
- Steel, R.G.D., Torrie, J.H. and Dickey, D.A., 1997.** Principles and procedures of statistics. A Biometrical Approach, (3rd ed.). McGraw-Hill: Publisher. 672P.
- Tornimbeni, O., Galvez, R., Triffault-Bouchet, G., Dassylva, N. and Roberge, S., 2013.** Heavy metal concentrations in *Cipangopaludina chinensis* (Reeve, 1863) and relationships with sediments in Saint-Augustin Lake, Québec City (Qc, Canada). *Annales de Limnologie - International Journal of Limnology*, 49, 21–29.

- Türkmen, M. and Ciminli, C., 2007.** Determination of metals in fish and mussel species by inductively coupled plasma atomic emission spectrometry. *Food Chemistry*, 103(2), 670–675.
- Usero, J., Morillo, J. and Gracia, I., 2005.** Heavy metal concentrations in molluscs from the Atlantic coast of southern Spain. *Chemosphere*, 59, 1175–1181.
- Yap, C.K., Kamarul, A.R. and Edward, F.B., 2009.** Heavy metal concentrations (Cd, Cu, Ni, Pb, Fe and Zn) in different soft tissues and shells of *Pholas orientalis* collected from Sekinchan and Pantai Remis, Selangor. *Malaysian Applied Biology Journal*, 38(1), 21–27.
- Yap, C.K. and Edward, F.B., 2010.** Distribution of heavy metals in the different parts of *Cerithidea Obtusa* and the relationships between metal distribution and allometric parameters of the snail. *Environment Asia*, 3(2), 38–48.
- Yap, C.K., Hisyam, M.N.D., Edward, F.B., Cheng, W.H. and Tan, S.G., 2010.** Concentrations of heavy metal in different parts of the gastropod, *Faunus ater* (Linnaeus), collected from intertidal areas of Peninsular Malaysia. *Pertanika Journal of Tropical Agricultural Science*, 33, 45–60.
- Yap, C.K. and Cheng, W.H., 2013.** Distributions of heavy metal concentrations in different tissues of the mangrove snail *Nerita lineata*. *Sains Malaysiana*, 42(5), 597–603.
- Youssef, M., El-Sorogy, A., El-Sabrouy, M. and Al-Otaiby, N., 2016.** Invertebrate shells as pollution bioindicators, Gebel El-Zeit area, Gulf of Suez, Egypt. *Indian Journal of Geo-Marine Sciences*, 45(5), 687–695.
- Zar, J.H., 2010.** Biostatistical analysis, 5th Edition, Prentice-Hall. New Jersey: Inc. Englewood Cliffs. 944P.