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Temporal bio-monitoring of cadmium in barnacle and mussel shells as an indicator of pollution in the central southern coast of Caspian Sea

Robabeh Oskoo[1], Mojtaba S. Taleshi[1*], Mohammad Javad Chaichi[2]

[1] Department of Marine Chemistry, Faculty of Marine and Oceanic Sciences, University of Mazandaran, Babolsar, Iran.
[2] Department of Analytical Chemistry, Faculty of Chemistry, University of Mazandaran, Babolsar, Iran.

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Abstract

The progress of urbanization and industrialization along the southern coast of Caspian Sea can threaten coastal ecosystems because of increasing metal pollution. Cadmium (Cd) is classified as toxic and persistent pollutant in the marine environment, which is also accumulated in many organisms. To obtain contamination levels, the amounts of cadmium in the shell of *Amphibalanus improvisus* (barnacle) and *Mytilaster lineatus* (mussel) were measured as biological monitoring marker of Cd in the central southern coast of the Caspian Sea. Samples were collected seasonally between October 2015 and October 2016 from three stations. The hard tissue of the samples was chemically digested and then the Cd was analyzed by atomic absorption spectroscopy. The results showed that the maximum and minimum Cd concentration for barnacle (32.96 and 1.46 µg/g dry weight) was observed in spring and winter respectively, while the Cd concentration of mussel varied between 8.16 and 0.72 µg/g dry weight. These species can be introduced as a biological indicator due to the accumulation of Cd in their shells with concentrations higher than the water and sediment.

1. Introduction

The Caspian Sea as the largest enclosed body of water is a significant lake in terms of the unique ecosystem (Kosarev and Yablonskaya,1994). There are various sources of the pollution such as municipal waste water, agricultural and onshore industrial and offshore and onshore oil extraction.(Canali *et al*., 1998) Since the Caspian is a closed medium, the contaminants including heavy metals, nutrient wastes and oil pollutants remain in the Sea and its sediments for years. (Elsagh, 2012) However, heavy metals are accounted as very important contaminants (Bundy, 1996). Among effective sources of entering heavy metals into the Caspian Sea, industrial wastewater, municipal wastewater, agricultural wastes,

* **Corresponding Author, E-mail**: ms.taleshi@umz.ac.ir

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mining and metal processing industry can be pointed out. Heavy metals are the most significant persistent pollutants which are not biodegradable and they can accumulate in the food chains. Moreover, their toxic effects expand to further distances away from the source of contamination and cause numerous diseases and damages (Ahmed *et al*., 2010). Cadmium (Cd) is one of the heavy metals, which is considered in prioritized pollutants by United States Environmental Protection Agency (USEPA) (Hendricks, 2006). Cd as a nonessential element is highly toxic to human and aquatic life. High Cd levels in organisms cause calcium (Ca) reduction, vertebrae deformation and fracture. Itai-itai disease is one of the best-known effects of Cd poisoning in human. Kidney dysfunction has been reported in cetaceans when liver concentrations of Cd exceed 20 ppm wet weight (Fujise *et al*., 1988).

Biological monitoring, which is done using appropriate species as bio-indicator, is safe method that represents acceptable information about the bioavailability of contaminants and their potential toxic effects (Amiard *et al*., 2006). Different species including mussels, barnacles, fish (non-migratory species and in particular benthic species) and seaweed have been used for biological monitoring of heavy metal pollution in the marine environment. Among invertebrates, mussels are the most important ones. They are less-mobile, filter-feeding organism and able to accumulate many of the contaminants present in seawater. They are representative of pollution in a region; therefore they are used for monitoring the quality of coastal waters (Maanan, 2008). Barnacles are other sessile invertebrates that have been emerged as biological monitoring (Adjei-Boateng *et al*., 2010; Rainbow, 1995). Barnacles does not migrate compared to the bivalves, therefore, regional marine pollution can be well determined by barnacles. These invertebrates store Cd because of its similarity to Ca in their shells. The inorganic shell structure of mussels and barnacles is calcium carbonate ($CaCO_3$) which exists in crystalline forms of calcite-aragonite. Cd and Ca have very similar atomic size (Yap *et al*., 2004), thus Cd may be taken up through the Ca channels (Banaoui *et al*., 2004). Annually more than 150 tones of Cd are discharged into the Caspian Sea (UNEP, 2006b). There is little information on Cd bioaccumulation and the influence of seasonal changes on Cd content in organisms in the southern coast of the Caspian Sea (Saghali, *et al*., 2014). The aim of this study was to obtain information over different seasons on Cd concentrations in the barnacle and mussel shells, which then were compared to background Cd concentrations in water and sediment. It could indicate whether these species can be used as biomonitors of Cd pollution in the southern coast of the Caspian Sea.

2. Materials and methods

2-1. Animal sampling

Specimens of *Amphibalanus improvisus* Darwin, 1854 (barnacle) and *Mytilaster lineatus* Gmelin, 1791 (mussel) were collected during during four seasons between October 2015 and October 2016 from the coast of the Mazandaran province, in Babolsar, Fereydunkenar and Mahmudabad regions (Fig. 1). Between 50 and 60 individuals of the barnacle (shell diameter 10–15 mm range) and the mussel (shell length 35–50 mm range) collected twice per season

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in the intertidal zone (Table 3). They were then placed in plastic bags and transported to the laboratory where they were stored at –20 °C until analysis (Gavrilovic *et al*., 2010).

2-2. Sediment and seawater

Surface sediment from the station was collected using a plastic scoop. The water samples were collected simultaneously at 30 cm depth above the sediments using 2 liter plastic bottle. A liter of the water samples was filtered through a 0.45 µm Whatman membrane filter (England), acidified with 0.5 ml of concentrated nitric acid and frozen until further analysis (Yap *et al*., 2002).

2-3. Sample preparation and Cd measurement

Barnacles and mussels

The shells of samples were separated from the soft tissue and then oven-dried at 70 °C for 48 hours. The shells were ground in a porcelain mortar. In the next step, 10 ml of concentrated nitric acid and 5 ml of concentrated hydrochloric acid was added to 2 g dry weight of each sample. Complete digestion of the samples was carried out by heating. Finally, the digestion solution was diluted with double distilled water (Yap *et al*., 2002).

Sediment and seawater

Sediment samples were analyzed according to the method described by Yap *et al*. (2002). Samples were dried in an oven at 105 °C for 7 hour and then grounded to pass through a 1 mm sieve. To 2 g of homogenized sample was added a mixture of concentrated nitric acid and hydrochloric acid with a ratio of 3:1. The mixture was heated for 90 minutes and then the solution was filtered before making it up to volume.

Seawater samples were analyzed according to the method of (Katz and Jennis, 1983). Fifty ml of the sample was taken and 5 mL of concentrated nitric acid added, then heated at 100 °C for 1 hour. The remaining solution was made to volume with double distilled water.

The Cd concentration was determined by standard addition with atomic absorption spectroscopy (Analytikjena novAA, 400P model – Germany; acetylene/ air fuel, wavelength 222.8 nm). The preparation and analysis of the samples has been repeated 3 times and the average Cd concentration reported. Accuracy of the method was checked by spiking a shell sample in triplicate with the analyte. The analyte recoveries were within the ±10% range of the spiked values. Concentrations in the shells and sediments are expressed as µg/g (dry weight) and in water as µg/l. Ca in the samples was determined using complexometric titration with EDTA as described by McCornick (1973).

2-4. Statistical Analysis

Statistical analysis was performed on the data generated from the Cd concentration in shells of two the species and their variations between seasons using one-way analysis of variance (ANOVA) using MS-Excel 2013.

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Fig. 1. Sampling area of the central southern coast of Caspian Sea in 2015-2016: S1(Babolsar; 36°42´ N, 52°39´ E), S2 (Fereydunkenar; 36°40´ N, 52°31´ E) and S3 (Mahmudabad, 36°37´ N, 52°15´ E)

3. Results and discussion

The Cd analysis in the barnacle and mussel shells is shown in Table 1. The results showed that seasonal variation affected the Cd concentrations in the both invertebrates. One-way ANOVA showed that for the barnacle shells there was a highly significant differences between the seasons (P<0.001) with Cd concentrations varying from 1.46 µg/g (winter) to 32.96 µg/g (Spring). For the mussel shells the Cd concentrations varied between 0.72 µg/g (Winter) and 8.16 µg/g (Spring) which confirmed that a statistically significant differences (P<0.001) were found between the different seasons. Comparison of the mean Cd concentrations between barnacle and mussel shells over all seasons showed barnacle shells had a significantly higher mean concentration (13.80 µg/g) than the mussel shells (3.85 µg/g). Ca analyses in barnacle and mussel shells are presented in Table 1. Seasonal variations affect the Ca concentration, so that the maximum and minimum for the barnacles (29.0 wt%, dry weight and 14.0 wt%, dry weight) were observed in winter and spring respectively, while the values for the mussels have been 31.4 wt% (dry weight) and 25.1 wt% (dry weight).

The Cd analysis in sediment and seawater in the study are shown in Table 1. The results showed the Cd concentration varied between 2.92 µg/g (dry weight) and 2.63 µg/l respectively, in the sediment and seawater.

Cd maximum level in foodstuffs such as bivalve mollusks is 1.0 µg/g wet weight (EFSA, 2006). The results show a significant Cd levels in barnacles and mussels. Comparison of the Cd concentrations between the both invertebrates and the seawater and sediment indicated that this metal is strongly accumulated by the invertebrates from their surrounding environment. The high accumulation of Cd shows a high pollution in the central southern coast of Caspian Sea. The study area is accounted as an important agricultural area of Mazandaran province; the use of chemical fertilizers on farms is very high. After the consumption of fertilizers (phosphate fertilizers with Cd contamination), they enter rivers from several ways like wind blow, washing farm soil due to rain and agricultural sewage which lead to contamination of seawater and subsequently seashells. Our finding is supported by Yap *et al*. (2003) who have reported the concentration of heavy metals in mussel shells

(*Perna viridis*) along Malaysian coast, the mean Cd levels in shell of oysters was found 10.11 µg/g. They showed that the oyster shell is a suitable Cd bioindicator, which is corresponded with the results of the current study. As shown in the Fig. 2, the barnacle shells have more ability to monitor Cd than mussel. Although two species studied are filter-feeders, the dietary preferences may affect the Cd bioavailability to each species (Philips and Rainbow., 1988). Seasonal variations can contribute to change the chemical and physical factors of seawater. On the other hand, these factors can involve in absorption of heavy elements by organisms (Norris, 2001). Generally, accumulation of heavy metal because of metabolism and high rate of excretion in warm seasons must be less than the cold seasons unless environmental factors increase the bioavailability of contaminants. This study indicates different results so that the Cd level in the samples during warm seasons are more than the cold seasons (Fig. 1). The seasonal variation of Cd in the barnacles is also much more than the mussels. Similar results found by monitoring Cd concentration in oysters in northern coast of Persian Gulf, which showed that the highest accumulation of heavy metal were in the spring and summer (Khoshnood *et al*., 2013). It seems that seasonal variations in the concentration of heavy metals vary by environmental conditions. Salinity is also one of the most essential factors on the bioavailability of heavy metals (Momboya, 2007). Since Cd in coastal water forms strong complex with chloride ions, thus the level of Cd complexation should be inversely related to chloride concentration in coastal water. The chloro-complexation of Cd decreases in low salinity and increase free (hydrated) Cd ions which can be accumulated with organisms (Langost, 1986). In warm seasons, the salinity of the southwestern coastal waters of the Caspian Sea is 0.2 psu less than cold seasons (Jamshidi and Yousefi, 2013). As a result, low salinity during warm seasons increases Cd bioavailability in the barnacle and mussels.

Table 1. Seasonal means ± standard deviation (SD) of Cd (µg/g, dry weight) and Ca (wt%, dry weight) concentrations measured during different seasons in the shells of barnacle and mussel, seawater and sediments in the southern coast of the Caspian Sea in 2015-2016.

Season	Cd (µg/g, dry weight)	Ca (wt%, dry weight)
	Barnacle	
Spring (Apr. & May)	33.0 ± 3.1	5.7±14.0
Summer (July & Aug.)	18.2 ± 3.0	2.8±18.0
Autumn (Oct. & Nov.)	2.6 ±1.3	4.3±23.1
Winter (Jan. & Feb.)	1.5 ± 0.4	1.4±29.0
All seasons mean ± S.D.	13.8 ± 14.9	6.4±21.0
	Mussel	
Spring (Apr. & May)	8.2 ± 0.2	5.1±25.1
Summer (July & Aug.)	1.1 ± 5.0	3.8±29.4
Autumn (Oct. & Nov.)	1.5 ± 0.7	4.2±30.1
Winter (Jan. & Feb.)	0.7 ± 0.5	6.3±31.4
All seasons mean ± SD	3.9 ± 3.4	2.7±29.0
Seawater (µg/L)	2.6 ± 0.1	Not measured
Surface sediment (µg/g, dry mass)	2.9 ± 0.1	Not measured

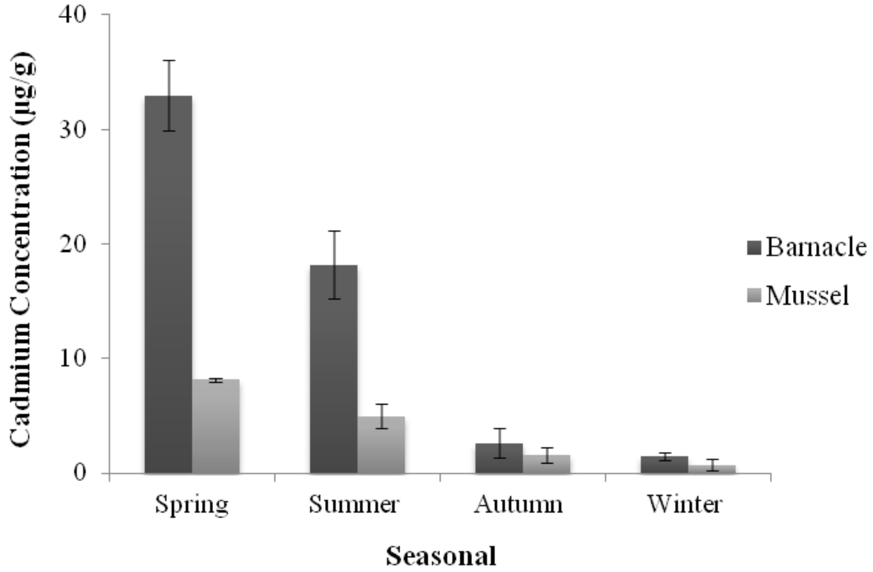

Fig. 2. Seasonal comparison of Cd in barnacles and mussels of the southern coast of Caspian Sea in 2015-2016

Cd accumulation in hard tissue can be related to crystal structure and calcareous material of the tissue. Variations in Ca concentration may partially account for Cd at different salinities, where greater Cd uptake at low salinity could be due to decreased competition with Ca (Wright, 1977). When Ca uptake changed, the absorption of Cd may be changed. As shown in Fig. 2, Ca absorption in the samples during warm seasons is less than cold seasons, for this reason the barnacles and mussels show more bioavailability to Cd during warm seasons. In addition to low salinity, low Ca concentration contributes to more Cd absorption by the both invertebrates.

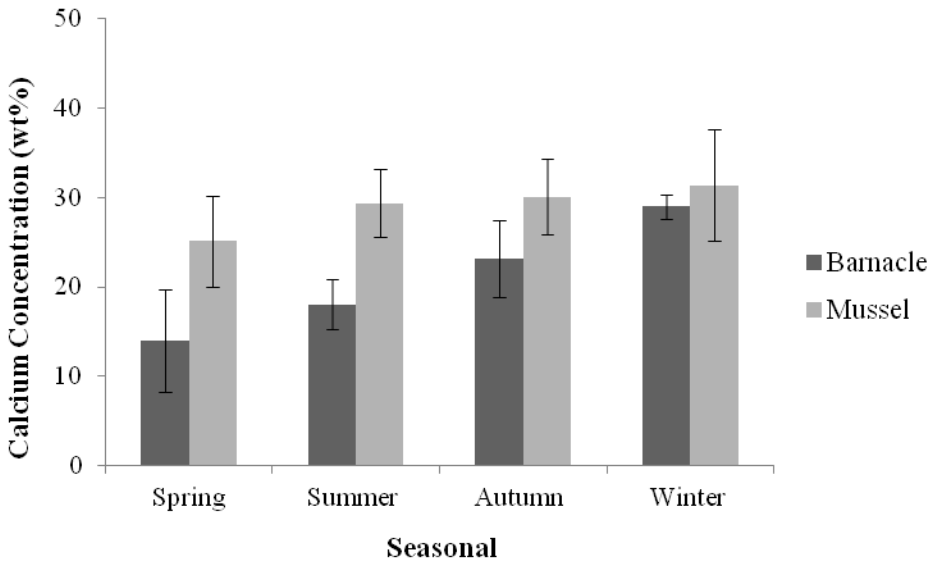

Fig. 3. Seasonal comparison of Ca in barnacles and mussels of the southern coast of Caspian Sea in 2015

4. Conclusion

A comparison of Cd concentrations in sediment and seawater with those in the invertebrate species shows that this trace metal can accumulate to levels that may cause a threat to some of the marine organisms. The Cd accumulation in shells of the investigated species show that the highest Cd concentration is in warm seasons and the Cd concentration in barnacles (32.96 µg/g) is much more than mussels (8.16 µg/g). The overall results of this work may suggest that, higher-level ecological effects along the coast of the Caspian Sea can be warranted by long term monitoring of barnacle and mussel shells.

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