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# Biomonitoring of Heavy Metals from İskenderun Bay Using Two Bivalve Species *Chama pacifica* Broderip, 1834 and *Ostrea stentina* Payraudeau, 1826

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**Abstract**

Bioaccumulation of cadmium, iron, lead, zinc, copper, manganese, nickel, chromium, cobalt and aluminum in the edible parts of two bivalvia species, *Chama pacifica* and *Ostrea stentina*, from İskenderun Bay, the northeastern Mediterranean sea, Turkey was examined at three stations. Mean concentrations of the heavy metals in the examined species were as follows; in *C. pacifica* Cd 7.53; Fe 82.02; Pb 62.34; Zn 419.8; Cu 46.93; Mn 5.79; Ni 22.87; Cr 3.36; Co 33.80; Al 130.9, in *O. stentina* Cd 4.27; Fe 270.6; Pb 6.21; Zn 1002; Cu 64.70; Mn 25.37; Ni 6.92; Cr 9.17; Co 8.67; Al 174.3 mg $kg^{-1}$ dry weight. According to the results of variance analysis, it was observed that the heavy metal concentrations varied significantly by the sampling sites and species. Additionally, the results were compared with other studies and discussed. According to these results, to prevent this heavy metal pollution threat in the bay, taking into consideration the biodiversity in this ecosystem, it is inevitable that protective measurements must be started as soon as possible.

*Key Words:* Heavy metals, İskenderum Bay, bivalvia, *Chama pacifica*, *Ostrea stentina*, biomonitoring.

## Introduction

The ability of bivalve to accumulate heavy metals in their bodies to elevated levels reaching concentrations that are much higher than those of ambient water concentrations makes these organisms useful for assessment purposes. The Mussel Watch project in the USA has been developed using mussels and oysters to monitor spatial and temporal trends of contaminants concentrations in coastal and estuarine regions (O'Conner, 1996). Biomonitors have been defined as species which accumulate trace contaminants in their tissues, responding to that fraction in the environment which is of direct ecotoxicological relevance, i.e. the bioavailable form (Blackmore *et al*., 1998). Biomonitors therefore, should be selected by their adherence to the ideal characteristics (Phillips, 1977). Bivalves have been shown to fulfill many of these characteristics and used to assess the bioavailability of metals in the coastal waters of many parts of the world (Uysal and Parlak, 1992; Egemen *et al*., 1994; Blackmore, 2001; Cohen *et al*., 2001; Hung *et al*., 2001; Sunlu, 2002). Both bivalve mollusc species examined in this study are sessile and filter feeder organisms and live by sticking on stony and rocky substrates. They are abundant along the coast of the entire İskenderun Bay and also have an important role in food web of marine ecosystems. Therefore, they fulfill most of the characteristics of the biomonitor organisms. Although some papers have been published concerning heavy metal levels observed in fish (Türkmen *et al*., 2005a), barnacle and limpet (Türkmen *et al*., 2005b) and shrimps (Kargın *et al*., 2001) on the northeastern Mediterranean sea environment, the present study is the first on the heavy metal bioaccumulation in bivalves species from İskenderun Bay, the northeastern Mediterranean, Turkey.

Along the coast, there are many towns including, İskenderun with an approximate population of 700,000 to 800,000 agricultural lands, industrial plants (iron-steel plants, beverage, LPG plants, oil transfer docks, other industrial plants and cargo ships' ballasts water). Therefore mainly untreated agricultural and municipal wastes and industrial production influence the bay directly or indirectly. The main objective of the present study is to investigate metal contamination in *C. pacifica*, *O. stentina* at three sampling sites with different urbanization degrees in moderately polluted İskenderun Bay.

## Materials and Methods

Three sampling stations were established along the coastline about 120 km long of İskenderun Bay, the northeastern Mediterranean sea of Turkey (Figure 1) from 28 May to 03 July, 2002. These stations are the Arsuz (ARZ), a relatively clean area, İskenderun Harbor Area (IHA) and Petrotrans (PTS), intensively polluted areas by both industrial and domestic sources.

About 10 individuals of two bivalve species, *Chama pacifica* and *Ostrea stentina* were sampled at the depths of 2-6 m by snorkeling from each station. To reduce variations in the metal content due to differences in body size, *Ostrea stentina* were collected at shell length of 70-80 mm; *Chama pacifica* at 75-85 mm. After all samples were removed from the substratum with a new stainless steel scraper, they were washed with clean sea water at the point of collection, separated by species, placed in a clean plastic bag and transferred in a cool box to the laboratory at the same day and then frozen at -20 °C until dissection.

For metal analysis, after thawing, the soft body parts of samples were removed with a plastic knife, rinsed, homogenized and weighed, and then individual samples were dried to constant weight at 80 °C in acid-washed Petri dishes for 24 hours. To prepare the samples for analysis, the digestion was performed in a microwave digester (CEM MARS-5 Closed Vessel Microwave Digestion System) using the microwave digestion program listed in Table 1. The completely digested samples were allowed to cool at room temperature, and were filtered through a 0.45 µm membrane filter and diluted to 50 ml in volumetric flasks with double distilled water (Jin *et al*., 1999; Sastre *et al*., 2002). All digested samples were analyzed three times for the metals Cd, Cu, Cr, Pb, Co, Zn, Fe, Ni, Al and Mn using AAS (Varian SpectrAA 220 Fast Sequential Flame Atomic Absorption Spectrometry). The instrument was calibrated with standard solutions prepared from Merck. The analytical blanks were run in the same way as the samples and concentrations were determined using standard solutions prepared in the same acid matrix (Türkmen, 2003). The accuracy and precision of our results were checked by analyzing standard reference material (SRM, Dorm-2). The results indicated a good agreement between the certified and the analytical values, the recovery of elements being partially complete for most of them. All metal concentrations were quoted as mg $kg^{-1}$ dry weight unless otherwise stated. All chemicals and standard solutions used in the study were obtained from Merck and were of analytical grade. Doubled distilled water was used throughout the study. All glassware and other containers were thoroughly cleaned with 10% (w/v) nitric acid solution and finally rinsed with double distilled water several times and air dried prior to use. One-way analysis of variance (ANOVA) and Duncan's test (p=0.05) were used in order to access whether heavy metal concentrations varied significantly between sites and species. The probabilities less than 0.05 ($p<0.05$) were considered statistically significant. All statistical calculations were performed with SPSS 9.0 for Windows.

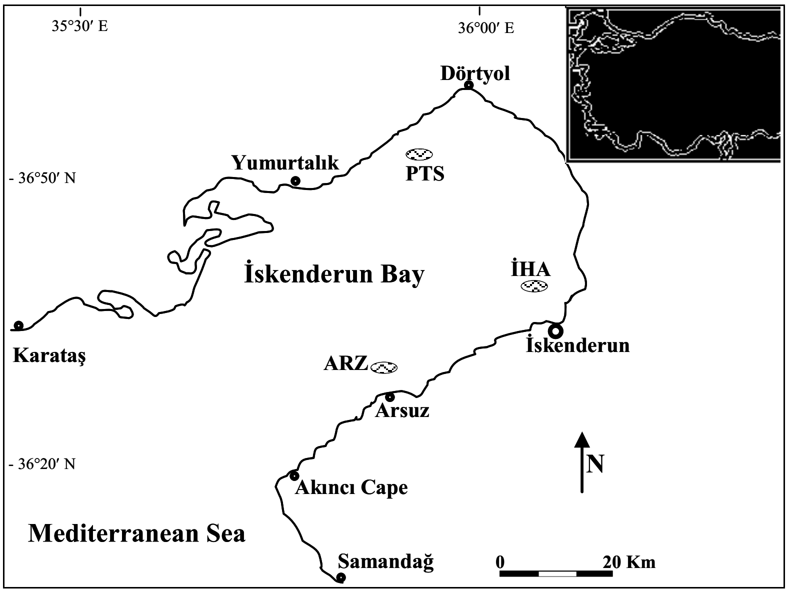


**Figure 1.** Location map of sampling stations in İskenderun Bay.

**Table 1.** Microwave digestion programs for the digestion of two bivalve species with CEM MARS-5

| Step[a] | Power (W) | Ramp (min.) | Press. (psi) | Temp. (°C) | Hold (min.) |
|---|---|---|---|---|---|
| 1 | 600 | 20:00 | 190 | 210 | 10:00 |
| 2 | 600 | 10:00 | 100 | 210 | 0:00 |

[a] sample weight = 0.1 g; solvent = 7 ml of 70% nitric acid, 3 ml double distilled water in step 1, and 1.5 ml of hydrogen peroxide in step 2

## Results and Discussion

Table 2 summarizes and compares the mean heavy metal concentrations in *Chama pacifica* (*C. pacifica*) and *Ostrea stentina* (*O. stentina*) from three representative sampling sites in İskenderun Bay (mg $kg^{-1}$ dry weight with ± SE). The highest metal levels (except Al and Cr), measured from *C. pacifica* samples, were recorded in the PTS station. The highest Al levels were recorded in IHA station and Cr levels in ARZ station from *C. pacifica* samples. ARZ station showed the minimum of the all metal levels with the exception of Al and Cr, which were the lowest in station PTS and IHA, respectively. A similar pattern was observed in *O. stentina*, except Pb which was the highest in station IHA. These results may show that the most polluted area in the bay may be PTS station. The differences between the mean heavy metal concentrations in stations for both species were statistically significant ($p<0.05$) (Table 2).

Mean concentrations followed the sequence; Zn>Al>Fe>Pb>Cu>Co>Ni>Cd> Mn>Cr for *C. pacifica*, Zn>Fe>Al>Cu>Mn>Cr>Co>Ni>Pb>Cd for *O. stentina,* respectively. In general, both species showed different capacities for accumulating heavy metals. As mg $kg^{-1}$, mean Al (174.3), Cr (9.17), Cu (64.70), Mn (16.36), Zn (1002), Fe (270.6)

**Table 2.** The comparison of the metal concentrations of species and stations

| Metals | Stations | *Chama pacifica* | *Ostrea stentina* |
|---|---|---|---|
| Aluminum | ARZ | $124.0±12.00^{ab}$ | $178.4±17.04^{a}$ |
| | IHA | $179.1±15.56^{b}$ | $229.4±23.76^{a}$ |
| | PTS | $87.74±7.08^{a}$ | $116.0±10.22^{b}$ |
| | Overall | $130.9±11.02^{x}$ | $174.3±13.04^{y}$ |
| Chromium | ARZ | $4.56±0.54^{a}$ | $11.98±1.61^{a}$ |
| | IHA | $2.38±0.26^{b}$ | $7.36±1.14^{b}$ |
| | PTS | $3.13±0.41^{b}$ | $8.17±1.32^{ab}$ |
| | Overall | $3.36±1.51^{x}$ | $9.17±0.85^{y}$ |
| Copper | ARZ | $28.29±2.65^{a}$ | $36.67±2.74^{a}$ |
| | IHA | $42.14±3.72^{a}$ | $69.81±7.53^{b}$ |
| | PTS | $68.43±5.94^{b}$ | $88.61±9.11^{b}$ |
| | Overall | $46.93±3.29^{x}$ | $64.70±4.67^{y}$ |
| Manganese | ARZ | $3.71±0.80^{a}$ | $8.89±1.55^{a}$ |
| | IHA | $5.74±0.97^{ab}$ | $14.80±3.02^{a}$ |
| | PTS | $7.91±1.19^{b}$ | $25.37±3.58^{b}$ |
| | Overall | $5.79±0.64^{x}$ | $16.36±2.03^{y}$ |
| Cobalt | ARZ | $23.68±4.66^{a}$ | $5.11±0.61^{a}$ |
| | IHA | $32.98±4.64^{ab}$ | $8.42±1.00^{a}$ |
| | PTS | $44.74±6.08^{b}$ | $12.47±1.66^{b}$ |
| | Overall | $33.80±3.32^{x}$ | $8.67±0.86^{y}$ |
| Nickel | ARZ | $13.95±2.49^{a}$ | $5.04±0.79^{a}$ |
| | IHA | $21.42±3.71^{ab}$ | $7.38±0.97^{ab}$ |
| | PTS | $33.24±5.86^{b}$ | $8.36±2.44^{b}$ |
| | Overall | $22.87±2.79^{x}$ | $6.92±0.54^{y}$ |
| Cadmium | ARZ | $3.92±0.48^{a}$ | $2.34±0.51^{a}$ |
| | IHA | $8.47±1.35^{b}$ | $3.38±0.59^{a}$ |
| | PTS | $10.12±1.45^{b}$ | $7.11±1.03^{b}$ |
| | Overall | $7.53±0.82^{x}$ | $4.27±0.56^{y}$ |
| Lead | ARZ | $41.19±5.57^{a}$ | $4.64±0.79^{a}$ |
| | IHA | $64.99±6.02^{b}$ | $7.78±0.98^{b}$ |
| | PTS | $81.13±8.48^{b}$ | $6.23±0.89^{ab}$ |
| | Overall | $62.34±4.85^{x}$ | $6.21±0.55^{y}$ |
| Zinc | ARZ | $196.1±18.53^{a}$ | $315.0±34.63^{a}$ |
| | IHA | $433.6±36.12^{b}$ | $926.2±82.69^{b}$ |
| | PTS | $628.3±52.17^{b}$ | $1766±123.65^{c}$ |
| | Overall | $419.8±31.19^{x}$ | $1002±73.01^{y}$ |
| Iron | ARZ | $27.62±2.09^{a}$ | $111.2±12.75^{a}$ |
| | IHA | $81.45±8.35^{b}$ | $201.7±22.17^{a}$ |
| | PTS | $138.3±14.05^{b}$ | $498.5±53.48^{b}$ |
| | Overall | $82.02±6.56^{x}$ | $270.6±19.57^{y}$ |

* Letters a, b and c show differences among stations of same species; x and y between species at same station. Data shown with different letters are significantly different from each other at the $P<0.05$ level.

concentrations appeared considerably higher in *O. stentina* than those determined in *C. pacifica*. On the other hand, mean Co (33.80), Ni (22.87), Cd (7.53), Pb (62.34) concentrations appeared considerably higher in *C. pacifica* than those determined in *O. stentina*. The result of ANOVA indicated that the differences between the mean heavy metal concentrations of both species were statistically significant ($p<0.05$) (Table 2).

The knowledge of heavy metal concentrations in native species is very important with respect to nature management, human consumption of these species and to determine the most useful biomonitor species and the most polluted area. So, the present study examined the concentrations of Cd, Fe, Cu, Zn, Cr, Co, Mn, Ni, Pb and Al in *C. pacifica* and *O. stentina* from three sites in İskenderun Bay. In general, Zn showed the highest accumulation in both species. On the other hand, Cr with 3.36 mg $kg^{-1}$ dry wt. in *C. pacifica* and Cd with 4.27 mg $kg^{-1}$ dry wt. in *O. stentina* showed the lowest accumulation. The present results showed that PTS station was more polluted than the other stations and the least polluted area was ARZ station. This situation might be explained that ARZ is a relatively clean area and both industrial and domestic sources of pollution are intensively between stations IHA and PTS. Effluents of various plants, agricultural activities, motorway and highway with intensive traffic and domestic effluents affect the bay, direct discharge, stream fluency, runoff and atmospheric transportation from ways and chimneys. Although concentrations of Cd, Co, Ni and especially Pb were higher in *C. pacifica* than those in *O. stentina*, Cr, Cu, Fe, Mn, Zn and Al were higher in *O. stentina*. In this situation, it could be said that *O. stentina* is more suitable for biomonitoring of Cr, Cu, Fe, Mn, Zn and Al in the bay. On the other hand, *C. pacifica* might be more suitable for biomonitoring of Cd, Co, Ni and Pb in the bay.

Table 3 compares our results with values in bivalve species from other sampling areas. Our results were compared with the other bivalve species because there is no data available about heavy metal concentrations of *C. pacifica* and *O. stentina*. In both species, although Ni (agree with Iberian Peninsula) and Pb levels obtained from this study are higher than those from Mexico Bay (Presley *et al*., 1989), Iberian Peninsula (Carballeira *et al*., 2000), Florida Bay (Oliver *et al*., 2001), Mazatlan Bay (Soto-Jimanez *et al*., 2001), Basque coast (Franco *et al*., 2002), especially Cu, Fe, Al, Mn and Zn (except Iberian Peninsula for Mn and Zn) levels are lower than those in these areas. Concentrations of Cd obtained from the present study are higher than those in Mazatlan Bay, Basque coast and Iberian Peninsula, but they are similar to those in Florida and Mexico Bay. Similarly, Cr levels are higher than those in Mazatlan bay and Iberian Peninsula. This situation indicates that the differences between heavy metal levels of the areas compared depend on species and regional properties. The mean concentrations of Cd, Cu, Zn and Pb in both species from the bay were higher than the legal limits proposed for molluscs by the Republic of Turkey (Anonymous, 2002). On the other hand, although the levels of Cd, Co and Pb in *C. pacifica* were higher than the legal limits proposed by Nauen (1983) and EPA (2002), the levels of Cd, Cr and Pb in *O. stentina* were high.

The results of this study show that the bivalves from the bay follow normal patterns of heavy metal content variability, related to differences between stations and species. The metal concentrations measured reflect a clear influence of anthropogenic activities. Additionally, the effective exposure of organisms to different metals may be influenced by either changes in metal speciation or relative distribution of metals between particles of different sizes and densities. According to these results, to prevent this heavy metal pollution threat in İskenderun Bay, taking into consideration the biodiversity in this ecosystem, it is inevitable that sustainable controls and measurements must be regulated as soon as possible. Urban wastewater entering collection systems shall be subject to secondary treatment before discharge. Therefore, future studies should reveal lower metal concentrations in bivalves.

**Table 3**. Comparison of present results with values in bivalve species from the other sampling areas

| *Sampling area* | Heavy metal concentrations, mg $kg^{-1}$ dry wt. | | | | | | | | | | R* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Cd | Co | Cr | Cu | Fe | Mn | Ni | Pb | Zn | Al | |
| Florida Bay | 0.49-12.9 | - | 0.29-11 | 63-2013 | 232-586 | 11.4-38 | 0.39-13.4 | 0.23-13.3 | 925-9077 | 129-614 | 1 |
| Mazatlan Bay | 2.3 | - | 0.99 | 86.9 | 2560 | 18.8 | 5.41 | 2.3 | 1161 | - | 2 |
| Mexico Bay | 4-6 | - | - | 150-250 | 290-370 | 14-18 | 1.8-2.2 | 0.6 | 1880-2150 | - | 3 |
| *Basque Coast* | 2.91 | - | 2.80 | 417 | - | - | 3.96 | 3.25 | 3084 | - | 4 |
| Iberian Peninsula | 0.3-2.7 | 2.3-11.2 | 3.1-25.8 | 12-292 | 102-860 | 2.3-12.4 | 5.2-23.8 | 2-26.5 | 190-997 | - | 5 |
| İskenderun Bay | | | | | | | | | | | |
| -*Chama pacifica* | 7.53 | 33.8 | 3.36 | 46.9 | 82.0 | 5.79 | 22.9 | 62.3 | 419 | 130 | 6 |
| -*Ostrea stentina* | 4.27 | 8.67 | 9.17 | 64.7 | 270 | 16.4 | 6.92 | 6.21 | 1002 | 174 | 6 |

*R: references, 1: Oliver *et al*., 2001; 2: Soto-Jiménez *et al*., 2001; 3: Presley *et al*., 1989; 4: Franco *et al*., 2002; 5: Carballeira *et al*., 2000; 6: This study.

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