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Seasonal Variation in Copper, Zinc, Chromium, Lead and Cadmium Levels in Hepatopancreas, Gill and Muscle Tissues of the Mussel *Brachidontes pharaonis* Fischer, Collected along the Mersin Coast, Turkey

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Abstract Levels of copper, zinc, cadmium, chromium and lead were determined in the gill, hepatopancreas and muscle tissues of *Brachidontes pharaonis* collected from the Mersin coasts of Turkey. Water and animal samples were collected monthly from the four stations between June 2002 and May 2003 for metal determinations. Metal levels in water samples of Mersin coasts were higher than those measured in other parts of Turkish coasts. Levels of metals in soft tissues of *B. pharaonis* showed an increase towards autumn and winter months which exceeded the levels that are recommended by Turkish Standart Institute's food codex.

Keywords Mussel · Metals · Seasonal levels

Natural processes such as soil erosion and volcanic activities increase the levels of heavy metals in aquatic environments. However, the uses of these metals for industrial and agricultural purposes together with domestic inputs contribute a great deal in this process. Although copper, zinc and iron are essential in trace amounts for biological functioning of organisms, excess amounts of these metals, and metals like cadmium, mercury and lead with no known biological function, cause tissue accumulation, mortality and changes in the structure of aquatic ecosystems (Odzak et al. 2000).

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In previous studies heavy metal accumulation in aquatic invertebrates was shown to be dependent upon the metal (Giusti et al. 1999), biology of the species of concern and on the chemical and physical properties of water (Licata et al. 2004). Since bivalves are suspention feeders, sessile organisms having a wide ecological tolerance and that polluting agents are generally discharged into the littoral zone in which they live, they are used as bioindicators in pollution studies (Geret et al. 2002). Hence heavy metals are readily taken from the environment by this group of organisms, these metals accumulate in their soft tissues, such as hepatopancreas, in excess amounts due to their insufficient regulation mechanisms. Studies carried out by various bivalve species both in laboratory (Geret et al. 2002; Soydemir et al. 2004) and in field (Yap et al. 2004) had shown that high levels of metals can accumulate in pericardial gland, byssus threads and muscle tissue in addition to hepatopancreas and gills.

Mersin coast of Turkey is highly urbanized and under the effect of heavy metal pollution due to agricultural and industrial activities. The mussel, *Brachidontes pharaonis*, is a lessepsien species which shows a wide distribution along Mersin coasts. This study was carried out to determine the levels copper, zinc, cadmium, chromium and lead in gill, hepatopancreas and muscle tissues of *B. pharaonis* to find out the pollution status of the region.

Materials and Methods

B. pharaonis samples were collected monthly between June 2002 and May 2003 from 4 stations in the vicinity of Mersin, namely Karaduvar, Deliçay, Viranşehir and Tece (Fig. 1). Industrial activities were taken into account in selecting Karaduvar and Deliçay, urbanization in Viranşehir and agricultural activities in Tece stations.

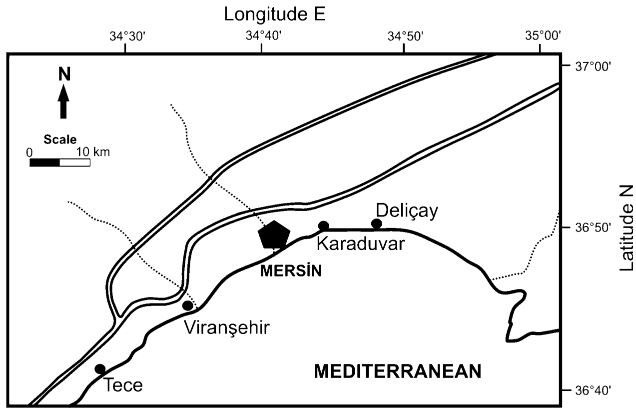

Fig. 1 Location of sampling sites in the Mersin coast

Fifty *B. pharaonis* samples were collected by hand from each station. Animals were placed into cooled containers and brought to the laboratory after adding some seawater from that particular station. The dimensions of the containers were 30 × 40 × 30 cm. The means and the standard deviations of some physical and chemical properties and heavy metal levels (μg/L) of seawater collected from the selected stations are given seasonally in Tables 1 and 2, respectively. Five liters of water samples collected from each station were evaporated at 150°C in 500 mL flasks. After evaporating 5 L of water from one station, the precipitate was dissolved in nitric acid-perchloric acid mixture (2/1; v/v) and was digested at 120°C for at least 8 h until obtaining a clear solution. The remaining digested sample was then transferred to glass flasks and their volumes were adjusted to 50 mL with distilled water (Muramoto 1983).

Mussels collected from each station were divided into five groups, each group containing ten mussels and used as replicates. Their lengths and total weights were determined. A total of 2,400 mussels having 35.0 ± 5.0 mm shell length and 4.2 ± 0.7 g weight were used during the experiments. Gill, hepatopancreas and muscle tissues, dissected from the mussels in each group, were pooled and used for Cu, Zn, Cd, Cr and Pb determinations in these tissues.

The dissected tissues were placed in Petri dishes and oven dried at 150°C until they came to a constant weight. Dried tissue samples were transferred to digestion flasks and a nitric acid (HNO_3, %65, sp gr: 1.40, Merck) and perchloric acid ($HClO_4$, %60, sp gr: 1.53, Merck) mixture (2/1; v/v) was added. They were then digested on a hotplate adjusted at 120°C for 8 h (Muramoto 1983). The digested samples were transferred to polyethylene tubes and their total volumes were adjusted to 5 mL. Concentrations of trace metals (Cu, Zn, Cd, Cr and Pb) in water and in tissues were determined using an inductively coupled plasma-atomic emission spectrometer (ICP-AES, Perkin Emler Optima 3100 XL). Before each metal analysis session, ICP-AES was calibrated by standard addition methods and freshly prepared metal salt (Merck) solutions. Addition of blank solutions on reagents and equipment showed no significant contamination. Data were analyzed statistically using analysis of variance and Student Newman Keul's Procedure (SNK) (Sokal and Rohlf 1969).

Results and Discussion

Seasonal variations in the levels of heavy metals of hepatopancreas tissue of *B. pharaonis* collected from the four sampling stations are given in Fig. 2.

Levels of Cd, Cr and Cu in the hepatopancreas of the samples collected from Karaduvar station increased beginning from summer months to autumn and decreased during spring months. Lead and zinc levels reached their maximum levels in autumn months ($p < 0.05$). The same was true for the levels of Cd, Cu and Zn at Tece station samples, the highest Pb and the lowest Cr levels were measured in winter months ($p < 0.05$). Hepatopancreas levels of Cd, Cu and Zn of Deliçay station samples were higher in autumn and spring compared with summer and winter months.

Table 1 Seasonal variations in some physical and chemical properties of seawater in the stations selected

Physical-chemical properties of seawater	Seasons			
	Summer	Autumn	Winter	Spring
	X ± SEM	*X* ± SEM	*X* ± SEM	*X* ± SEM
pH	8.16 ± 0.08	8.09 ± 0.05	8.19 ± 0.05	8.27 ± 0.04
Temperature (°C)	28.00 ± 1.6	25.50 ± 0.4	15.23 ± 0.9	19.59 ± 0.16
Salinity (ppt)	36.40 ± 0.9	36.22 ± 0.5	34.32 ± 0.7	35.65 ± 0.6
Conduct. (μS/cm)	52.30 ± 2.4	52.60 ± 2.1	47.40 ± 3.2	48.60 ± 1.9
Dis. oxygen (mg/L)	6.28 ± 0.21	7.12 ± 0.25	8.56 ± 0.32	7.62 ± 0.24

X ± *SEM* mean ± standard error of mean

Table 2 Seasonal variations in heavy metal levels (μg/L) of seawater collected from the selected stations

Metals (ppm)	Seasons			
	Summer	Autumn	Winter	Spring
	X ± SEM	*X* ± SEM	*X* ± SEM	*X* ± SEM
Cu	1.07 ± 0.12	1.47 ± 0.15	1.60 ± 0.17	1.03 ± 0.12
Zn	6.27 ± 0.63	8.37 ± 0.45	9.65 ± 0.60	6.07 ± 0.43
Cd	0.47 ± 0.07	0.57 ± 0.09	0.97 ± 0.09	0.43 ± 0.07
Cr	0.57 ± 0.09	0.67 ± 0.09	0.93 ± 0.09	0.53 ± 0.06
Pb	0.10 ± 0.003	0.13 ± 0.03	0.17 ± 0.03	0.09 ± 0.003

X ± *SEM* mean ± standard error of mean

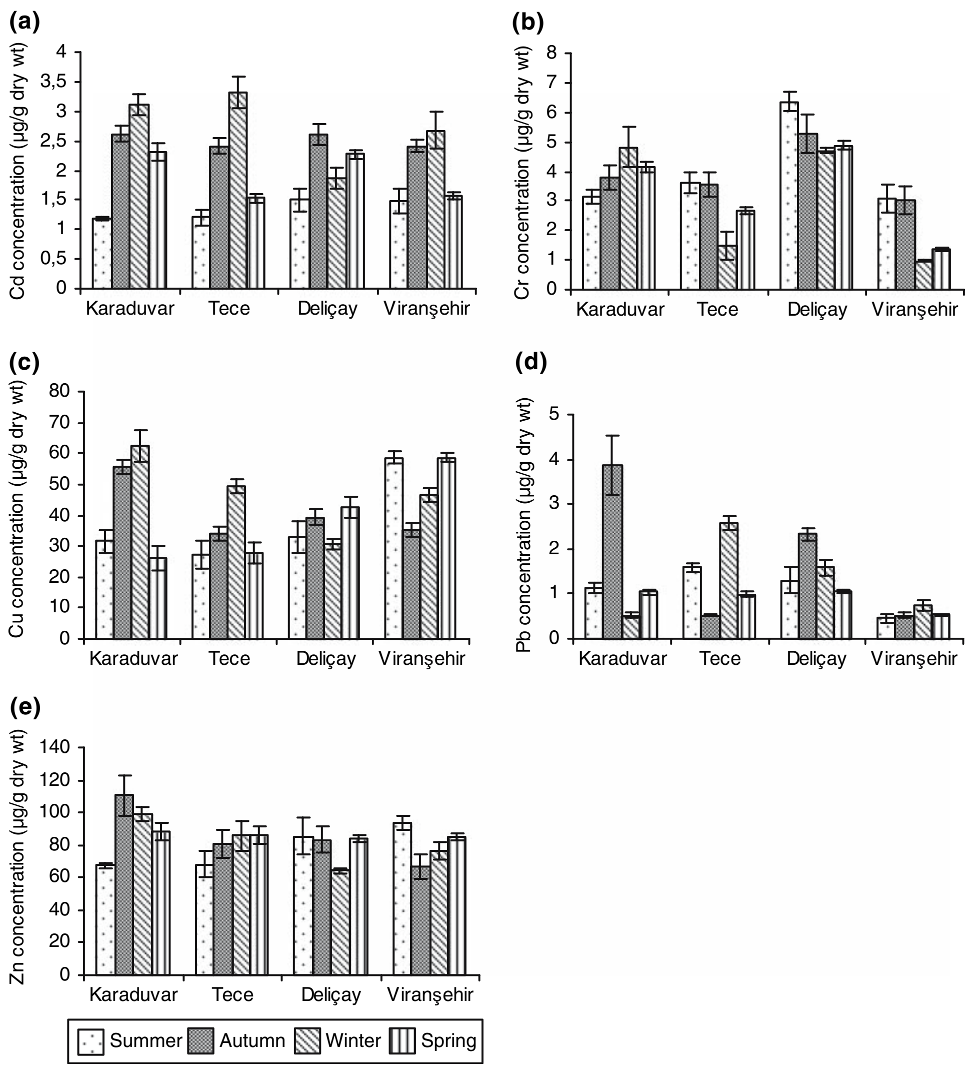

Fig. 2 Seasonal variation in the levels of (μg metal/g d.w.) Cd (**a**), Cr (**b**), Cu (**c**), Pb (**d**) and Zn (**e**) in hepatopancreas tissue of *B. pharaonis* collected from the stations selected

Levels of Cr and Pb at this station reached their maximum levels in summer and autumn months, respectively ($p < 0.05$). There was a gradual increase in levels of Cu and Zn the hepatopancreas of Viranşehir mussel samples from autumn towards summer ($p < 0.05$). While no seasonal variation was observed in the levels of Pb at this station ($p > 0.05$), there was an increase in the levels of Cr and a decrease in Cd levels ($p < 0.05$). With the exception of Pb, metal levels in the gill tissues of *B. pharaonis* sampled from Karaduvar and Tece stations increased from summer towards autumn and decreased in spring months ($p < 0.05$) (Fig. 3). Metal levels in the gill tissues of

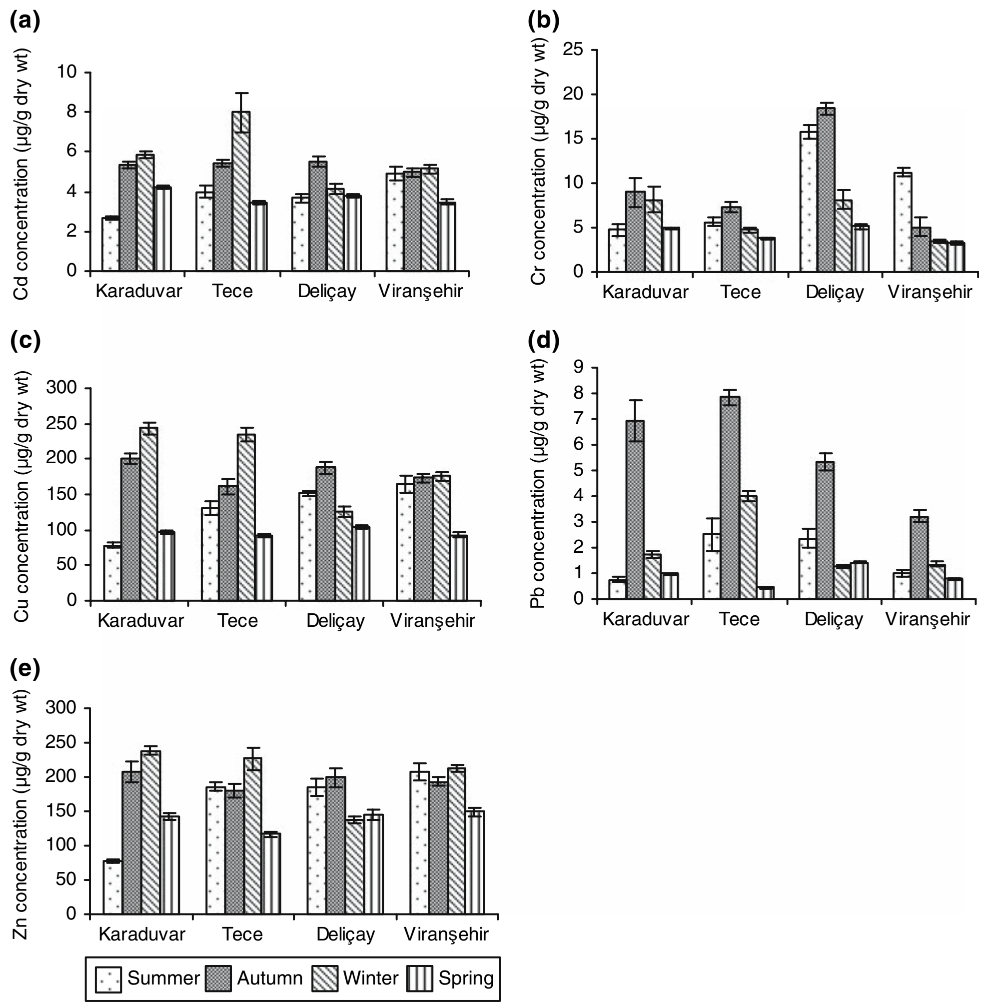

Fig. 3 Seasonal variation in the levels of (µg metal/g d.w.) Cd (**a**), Cr (**b**), Cu (**c**), Pb (**d**) and Zn (**e**) in gill tissue of *B. pharaonis* collected from the stations selected

Deliçay samples were high during summer, increased towards autumn and decreased during spring months ($p < 0.05$). There was no significant difference ($p > 0.05$) in the levels of Cd, Cu and Zn in gill tissues of Viranşehir samples among summer, autumn and winter months decreasing towards spring. The highest levels of Pb and Cr at this station were observed in autumn and summer, respectively ($p < 0.05$).

Seasonal variations in the levels of metals in muscle tissue of *B. pharaonis* are given in Fig. 4. Muscle metal levels of Karaduvar samples showed an increase towards winter and a decrease in spring months ($p < 0.05$). Cr and Pb levels of muscle tissue at Tece station showed a gradual decrease from summer towards spring while Cd levels reached to its maximum at winter months. Muscle metal levels of Deliçay samples showed a decrease towards winter and an increase towards spring except Cd, which reached to its maximum levels during autumn. Muscle tissue Cr and Pb levels of mussels collected from Viranşehir station showed a decline beginning from summer months. Cu and Zn levels were high in spring and summer months while Cd levels were highest in winter ($p < 0.05$). Metal levels showed differences between the tissues at the station studied. The following relationship was found in accumulating metals between the tissues studied;

Gill > Hepatopancreas > Muscle.

Metal levels of seawater at the selected stations were higher than those given by Tubitak (1989) for Turkish coastal waters (Cd: 0.1; Cr: 0.3; Cu: 0.5; Pb: 0.03; Zn: 4.9 µg/L) which might suggest input of these metals into coastal waters of Mersin through various human activities and distributed by coastal currents.

Heavy metal accumulation in invertebrates (Depledge 1989) and fish (Gautam 1989) is dependant upon the physical and chemical properties of water, such as pH, temperature, salinity, conductivity and dissolved oxygen levels, at the selected stations were measured seasonally. It was shown that increase in temperature and decrease in salinity increase the filtration rate in bivalves (Kramer et al. 1989). Wong et al. (2000) suggested that the increase in the levels of Cd, Cr and Pb during the winter

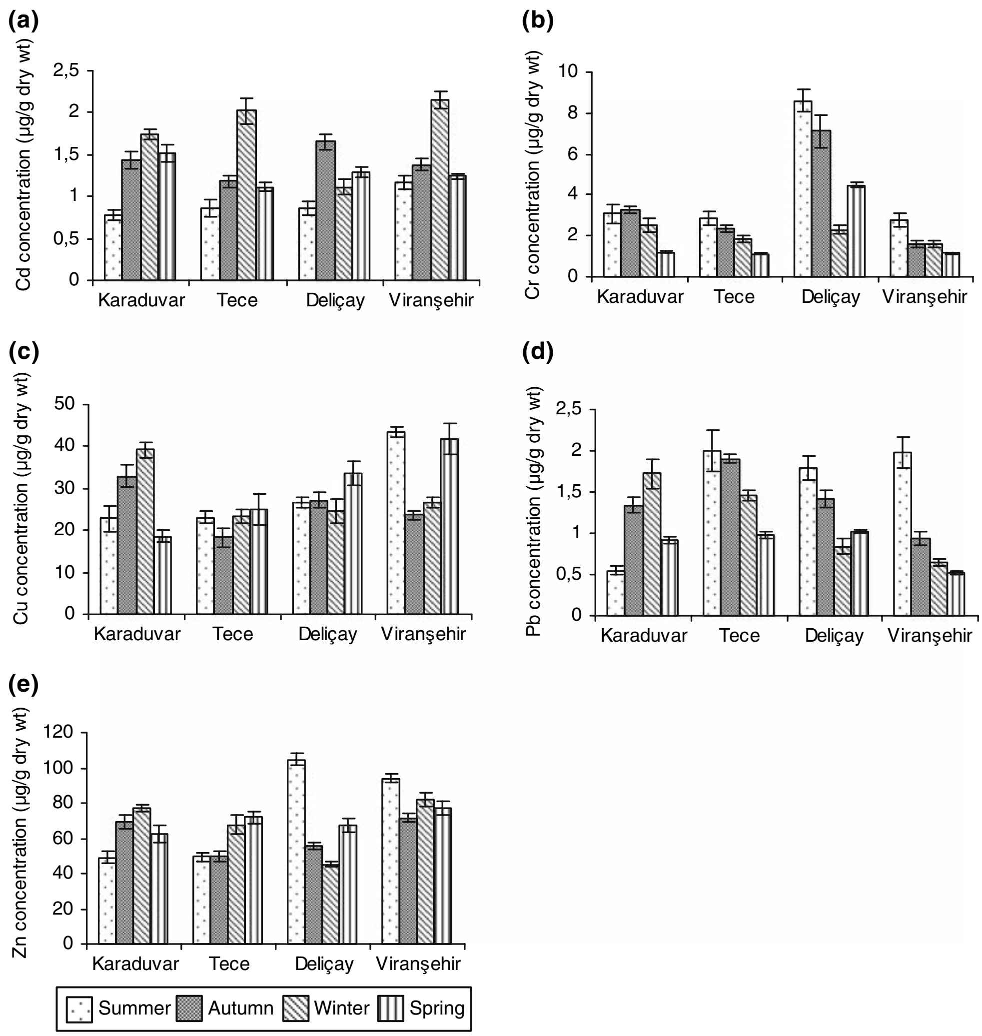

Fig. 4 Seasonal variation in the levels of (µg metal/g d.w.) Cd (**a**), Cr (**b**), Cu (**c**), Pb (**d**) and Zn (**e**) in muscle tissue of *B. pharaonis* collected from the stations selected

months in *Perna viridis* sampled from Tolo harbor (Hong-Kong) was due to the decrease in temperature and salinity due to precipitation. The levels of Cd in hepatopancreas, gill and muscle tissues of *B. pharaonis* was shown to increase from summer to winter months in Karaduvar, Tece and Viranşehir stations. Hence decrease in temperature and salinity might be the basis for the increased tissue levels of Cd during winter months. On the other hand the increase in Cd levels in soft tissues of Deliçay station might be due to the increased flow rate of Deliçay stream during this season.

Heavy metal levels in mollusks changes depending on season. Seasonal differences in the levels of Cd, Cu, Zn, Pb, Cr, Ni, Hg, As and Ag were shown to occur in scallops and oysters, being at higher levels in autumn and winter compared with spring and summer (Franco et al. 2002). The levels of Zn, Cu, Cr, Pb and Cd showed an increasing trend towards autumn and summer months which might be due to an increase in metabolic activity during this pre-reproduction period.

Metal accumulation in soft tissues of aquatic invertebrates varies with the metal of concern. The following relationship was found on the accumulation of heavy metals in *M. edulis* sampled from five stations from northeastern England; Fe > Zn > Cu > Pb > Mn > Ni > Cr > Ag ≥ Cd (Giusti et al. 1999). The same relationship was found, except chromium, in accumulating heavy metals in hepatopancreas, gill and muscle tissues for *B. pharaonis*. The high levels of Cr found in tissues at Deliçay station were probably were due to Cr industry carried out in this region. Zn and Cu levels were high in the tissues studied, which might be explained by selective binding capacity of these metals to membrane vesicles. Moreover, the presence of elevated urban, agricultural and industrial inputs in this site might cause the increase the Zn and Cu levels at this station.

Lead accumulation in soft tissues of *B. pharaonis* during autumn at Karaduvar station was probably due to the petroleum refinery nearby and to the concentrated fishery activities carried out in the region. High levels of Cd at the other station might be explained by the presence of manure, lime and glass industry in Mersin. Levels of Cu, Zn, Cd, Cr and Pb in soft tissues of *M. galloprovincialis* (Bat et al. 1999), *M. galloprovincialis* and *Rapana venosa* (Topçuoğlu et al. 2002) collected from Black Sea and *M. galloprovincialis* (Gey and Mordogan 1988) collected from Aegean Sea coasts of Turkey were found to be significantly lower than the levels measured in *B. pharaonis* sampled from Eastern Mediterranean in the present study.

Differences among tissues, seasons and sampling stations in Cu, Zn, Cd, Cr and Pb levels in tissues of *B. pharaonis* collected from four stations along Mersin coast were probably due to variation in metabolic activity of tissues studied, seasonal variations of chemical and physical properties of seawater and different pollutant inputs of the stations selected.

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