Environ Monit Assess (2010) 167:257–264
DOI 10.1007/s10661-009-1047-x

Heavy metals in *Patella caerulea* (Mollusca, Gastropoda) in polluted and non-polluted areas from the Iskenderun Gulf (Mediterranean Turkey)

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Received: 18 February 2009 / Accepted: 4 June 2009 / Published online: 20 June 2009
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Abstract The concentrations of Cd, Cu, Zn, Fe, Pb, Ni, and Co were measured in gastropod mollusks *Patella caerulea* in the Mediterranean area. The organisms were collected at two coastal sites in Iskenderun Gulf during winter, spring, summer, and autumn 2008. Samples of the digestive gland, gill, and muscle were analyzed for heavy metals. The aim of study is to determine heavy metal levels in tissues of *P. caerulea* in different locations. Tissues of *P. caerulea* from the polluted site showed metal concentrations appreciably higher than unpolluted organisms. The highest metal levels were registered in the digestive gland of *P. caerulea*. Generally, digestive gland and gills showed higher metal concentrations than muscle. The average concentrations of heavy metals analyzed exhibited the following decreasing order: Fe > Zn > Cu > Ni > Cd > Pb > Co for both stations 1 and 2. Seasonal changes in metal concentrations were observed in the tissues of *P. caerulea* from a polluted and an unpolluted population.

Keywords *Patella caerulea* · Gastropod mollusk · Heavy metals · Mediterranean Sea · Iskenderun Gulf

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Introduction

Heavy metal concentrations in the marine environment are the results of both natural and anthropogenic sources. The accumulation of heavy metals in waters and sediments affects various organisms in the environment (Blackmore et al. 1998; Storelli and Marcotrigiano 2001; Cogun et al. 2005). Mollusks are filter-feeders and thus obtain heavy metals not only from food and water but also from ingestion of inorganic particulate materials. It is well known that mollusks accumulate organic and metallic pollutants at concentrations of the field environment (Bu-Olayan and Thomas 2001; Storelli and Marcotrigiano 2001; Amodio-Cocchieri et al. 2003; Bayen et al. 2004; El-Sikaily et al. 2004).

Bivalve mollusks (Regoli and Orlando 1994; Storelli and Marcotrigiano 2001; Bayen et al. 2004; El-Sikaily et al. 2004) and gastropod mollusks (Bu-Olayan and Thomas 2001; Campanella et al. 2001; Türkmen et al. 2005) were employed as bio-indicator to determine the effect of marine pollution. These organisms were considered as appropriate indicators since they are available all year long and easily collected. Mollusks have been well established as bioindicators for monitoring the concentration of heavy metals in many areas of the Mediterranean (Regoli and Orlando 1994; Storelli and Marcotrigiano 2001, 2005; Amodio-Cocchieri et al. 2003; El-Sikaily et al. 2004).

Patella caerulea and other gastropod mollusks are extensively used in monitoring programs in the marine environment due to their ability to accumulate heavy metals (Bu-Olayan and Thomas 2001; Campanella et al. 2001; Storelli and Marcotrigiano 2005). The gastropod *P. caerulea* is among the commonest inhabitants of rocky shores in the whole Mediterranean basin (Storelli and Marcotrigiano 2005). The small edible herbivorous gastropod has already been employed in campaigns evaluating pollution by metals (Bu-Olayan and Thomas 2001; Campanella et al. 2001). In Turkey, the marine gastropod mollusks *P. caerulea* is abundantly distributed all along the coastal waters of the Mediterranean (Iskenderun Gulf). They were found take up metals from water, food, and inorganic particulate matter.

Several authors have suggested that certain conditions may affect the uptake of some metals; for example, in mollusks, the concentrations of some metals may vary with size of the individual (Sokolowski et al. 2004), salinity (Phillips 1976), and season (Regoli and Orlando 1994; Bu-Olayan and Thomas 2001).

As compared with the open Mediterranean Sea, Iskenderun Gulf is more subject to pollution, particularly by heavy metals from industrial, agricultural, and urban origin. Numerous studies on heavy metal pollution from a wide spectrum on anthropogenic sources in various areas of Iskenderun Gulf have been carried out over the years (Kargin 1996; Yilmaz 2003; Cogun et al. 2005; Türkmen et al. 2005; Doygun and Alphan 2006; Firat et al. 2008; Yüce and Alpar 1994).

The selected clean (non-polluted) area, lacking industrial site and quite far away from urbanized Iskenderun Gulf coastline, can be considered virtually uninfluenced by anthropogenic activities. The selected polluted area receives large quantities of untreated industrial, chemical pollutants and domestic sewage due to heavy agricultural and industrial activities. The metal levels in the tissues of the organisms collected at the "clean" station may provide useful background levels which refer to intraspecific comparison within the Iskenderun Gulf (the Mediterranean area).

In this investigation, we examined level of Cd, Cu, Zn, Fe, Pb, Ni, and Co in the tissues of *P. caerulea* in four seasons from a polluted and a relatively uncontaminated area of the Iskenderun Gulf (Mediterranean).

Materials and methods

Specimens of gastropod mollusks *P. caerulea* were collected from two locations (the coasts of Iskenderun and Yumurtalık), the Gulf of Iskenderun during winter, spring, summer, and autumn of 2008. The Iskenderun area (polluted), is located at the eastern coast of Iskenderun Gulf, which is the most industrialized region of turkey. Chemical fertilizer, textiles, oil refinery, and cement factories are among the most important industrial investments in the area (Fig. 1). Immediately after capture, the animals were transferred to the laboratory and frozen at −25°C until analyzed.

Five samples from each sampling station were analyzed in every season in order to determine metal in their tissues. Their digestive gland, gill, and muscle tissues were dissected separately. Tissue samples were dried at 110°C for 48 h. Samples were transferred into a digestion flask containing 2 ml nitric acid and 1 ml perchloric acid (Merck) and digested at 120°C for 3 h (Liang et al. 1999). The digestion was diluted with distilled water appropriately in the range of standards which were prepared from stock standard solution of the metals (Merck). After dilution, metal contents of tissues measured on a PerkinElmer AS 3100 atomic absorption flame spectrophotometry and metal concentration in a tissue was presented as microgram metal/gram dry weight. Detection limit of the spectrophotometer was 0.028 ppm and 90% recovery was obtained during measurement. All calculations refer to dry weight of tissues. The Student t test for comparison of stations or ANOVA for different tissues was followed by the Student–Newmann–Keuls test for determination of significant differences ($p < 0.05$).

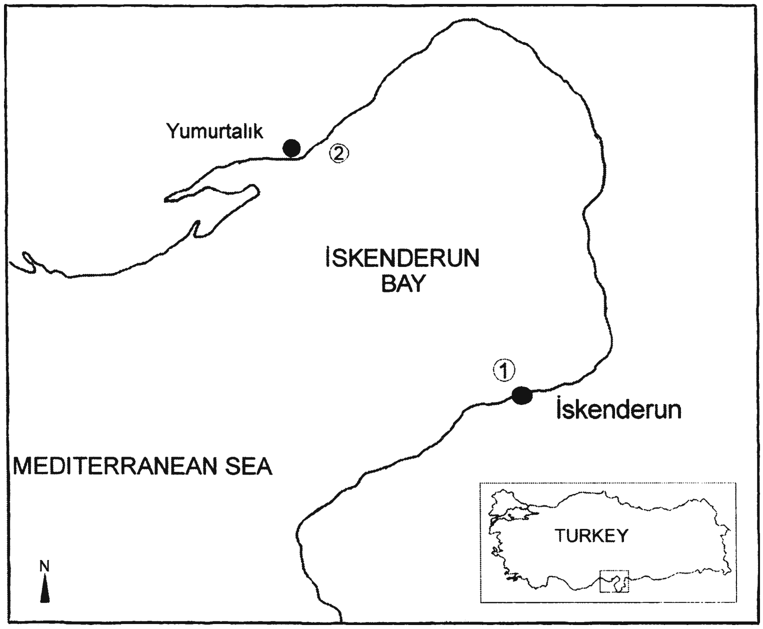

Fig. 1 The study area (Iskenderun Gulf) and sampling stations. *1* Iskenderun, *2* Yumurtalık

Results and discussion

Metal levels in the tissues of *P. caerulea* samples collected from the polluted (Iskenderun) and unpolluted (Yumurtalık) sites in the Iskenderun Gulf are presented in Tables 1, 2, 3 and 4. The results of statistical comparisons of tissue metal levels between two stations are also given in these tables. Iskenderun Gulf is located at the north east part of Mediterranean Turkey. Due to industrial and agricultural activities carried on in the region, this Gulf receives large quantities of untreated or partially treated industrial and domestic sewage. Previous studies, in fact, indicated that

Table 1 Metal concentrations (micrograms per gram of dry weight) determined in the different tissues of *P. caerulea* sampled in winter

	Station	Digestive gland	Gill	Muscle
Co	1	1.00 ± 0.02a	0.66 ± 0.08b	0.25 ± 0.01c
	2	0.81 ± 0.03*a	0.25 ± 0.03*b	0.14 ± 0.06*c
Cd	1	3.28 ± 0.03a	0.59 ± 0.02b	0.50 ± 0.06b
	2	2.26 ± 0.03*a	0.46 ± 0.08*b	0.35 ± 0.01*c
Pb	1	4.13 ± 0.08a	5.83 ± 0.11b	0.70 ± 0.03c
	2	3.22 ± 0.09*a	4.10 ± 0.04*b	0.31 ± 0.02*c
Ni	1	9.36 ± 0.20a	5.97 ± 0.24b	1.60 ± 0.05c
	2	9.21 ± 0.49*a	4.95 ± 0.71*b	0.68 ± 0.01*c
Cu	1	8.83 ± 0.09a	7.62 ± 0.14b	2.90 ± 0.05c
	2	7.89 ± 0.39*a	6.17 ± 0.06*b	1.73 ± 0.17*c
Zn	1	17.10 ± 0.36a	14.0 ± 0.25b	13.7 ± 0.15b
	2	13.26 ± 0.53*a	10.7 ± 0.05*b	9.84 ± 0.68*b
Fe	1	931.2 ± 6.10a	898.8 ± 0.60a	212.9 ± 6.60b
	2	772.5 ± 4.10*a	804.7 ± 2.60*b	168.8 ± 13.7*c

Data are reported as mean ± SD. Letters a, b, and c show differences among tissues. Data shown with different letters are statistically significant at $p < 0.05$

*$p < 0.5$; (Student's *t* test) statistical differences between stations

Table 2 Metal concentrations (μg/g. of dry weight) determined in the different tissues of *P. caerulea* sampled in spring

	Station	Digestive gland	Gill	Muscle
Co	1	2.35 ± 0.02a	0.55 ± 0.02b	0.33 ± 0.02c
	2	1.27 ± 0.03*a	0.21 ± 0.05**b	0.12 ± 0.05**c
Cd	1	2.13 ± 0.03a	0.46 ± 0.04b	0.60 ± 0.01c
	2	1.26 ± 0.02**a	0.26 ± 0.01**b	0.44 ± 0.08*c
Pb	1	1.79 ± 0.05a	4.79 ± 0.26b	0.66 ± 0.01c
	2	0.85 ± 0.01*a	1.80 ± 0.11**b	0.14 ± 0.05**c
Ni	1	7.26 ± 0.08a	3.47 ± 0.01b	0.60 ± 0.09c
	2	4.57 ± 0.19*a	1.33 ± 0.01**b	0.53 ± 0.27*c
Cu	1	9.93 ± 0.01a	8.53 ± 0.06b	5.58 ± 0.18c
	2	8.37 ± 0.28*a	6.20 ± 0.10*b	2.12 ± 0.06*c
Zn	1	26.90 ± 0.60a	15.2 ± 0.94b	11.2 ± 0.60c
	2	14.00 ± 0.90*a	11.5 ± 0.36*b	7.80 ± 0.06**c
Fe	1	637.7 ± 10.0a	903.3 ± 11.8b	83.36 ± 0.52c
	2	222.2 ± 1.62**a	872.0 ± 5.9*b	68.00 ± 1.15*c

Data are reported as mean ± SD. Letters a, b, and c show differences among tissues. Data shown with different letters are statistically significant at $p < 0.05$

*$p < 0.5$; **$p < 0.1$ (Student's *t* test); statistical differences between stations

agricultural and industrial effluents and harbor operations were the main sources of marine pollution problems in this area (Yilmaz 2003; Cogun et al. 2005; Türkmen et al. 2005; Firat et al. 2008).

In the samples of *P. caerulea* collected at station 1 (polluted), metal concentrations increased in the following order: Co < Pb < Cd < Ni < Cu < Zn < Fe; a similar pattern: Co < Cd < Pb < Ni < Cu < Zn < Fe was observed for samples from station 2 (unpolluted). The comparison between metal concentrations in the samples from the two stations showed all metal concentrations differed significantly in every season. The heavy metals in samples collected in station 1 were statistically higher ($p < 0.5$) for all metals than samples collected from station 2. The Co, Pb, Cd, Ni, Cu, Zn, and Fe values detected in the muscle of *P. caerulea* in station 1 were in the range of 0.15–0.33, 0.13–0.70, 0.41–0.68, 0.53–1.60, 1.38–5.58, 6.50–13.71, and 61.23–212.9, respectively; while Co, Pb, Cd,

Table 3 Metal concentrations (μg/g. of dry weight) determined in the different tissues of *P. caerulea* sampled in summer

	Station	Digestive gland	Gill	Muscle
Co	1	0.95 ± 0.05a	0.35 ± 0.01b	0.20 ± 0.01c
	2	0.66 ± 0.08*a	0.23 ± 0.06*b	0.13 ± 0.03**c
Cd	1	1.42 ± 0.04a	0.49 ± 0.01b	0.68 ± 0.03c
	2	0.63 ± 0.05*a	0.32 ± 0.02*b	0.27 ± 0.01* b
Pb	1	1.46 ± 0.04a	2.36 ± 0.16b	0.13 ± 0.01c
	2	0.91 ± 0.05* a	1.77 ± 0.04**b	0.05 ± 0.01*c
Ni	1	6.42 ± 0.14a	0.94 ± 0.02b	0.53 ± 0.01c
	2	2.21 ± 0.07*a	0.73 ± 0.08*b	0.39 ± 0.03**c
Cu	1	12.0 ± 0.25a	9.18 ± 0.21b	1.38 ± 0.03c
	2	8.87 ± 0.26*a	7.10 ± 0.04*b	1.09 ± 0.01**c
Zn	1	26.43 ± 1.43a	10.5 ± 0.31b	7.48 ± 0.22c
	2	17.30 ± 0.70*a	7.06 ± 0.31*b	4.31 ± 0.35*c
Fe	1	183.4 ± 6.27a	737.4 ± 4.50b	61.23 ± 1.39c
	2	128.0 ± 2.90*a	543.6 ± 13.6*b	36.56 ± 1.25*c

Data are reported as mean ± SD. Letters a, b, and c show differences among tissues. Data shown with different letters are statistically significant at $p < 0.05$

*$p < 0.5$; **$p < 0.1$ (Student's *t* test); statistical differences between stations

Table 4 Metal concentrations (μg/g. of dry weight) determined in the different tissues of *P. caerulea* sampled in autumn

	Station	Digestive gland	Gill	Muscle
Co	1	0.62 ± 0.07a	0.32 ± 0.05b	0.15 ± 0.06c
	2	0.13 ± 0.08**a	0.13 ± 0.01**a	0.05 ± 0.03**b
Cd	1	2.93 ± 0.08a	1.25 ± 0.02b	0.41 ± 0.03c
	2	1.36 ± 0.04**a	0.36 ± 0.01**b	0.24 ± 0.03** c
Pb	1	2.51 ± 0.09a	4.12 ± 0.19b	0.27 ± 0.01c
	2	1.15 ± 0.06*a	1.63 ± 0.10**b	0.11 ± 0.08* c
Ni	1	4.76 ± 0.38a	1.76 ± 0.08b	1.35 ± 0.02b
	2	2.71 ± 0.02**a	1.07 ± 0.02*b	1.05 ± 0.03** b
Cu	1	7.83 ± 0.24a	4.87 ± 0.28b	2.48 ± 0.18c
	2	3.74 ± 0.05**a	2.12 ± 0.06**b	1.20 ± 0.05*c
Zn	1	12.86 ± 0.46a	9.80 ± 0.06b	6.50 ± 0.31c
	2	7.58 ± 0.17*a	6.31 ± 0.01**b	3.70 ± 0.20*c
Fe	1	138.5 ± 1.00a	598.0 ± 1.30b	150.9 ± 9.50c
	2	115.5 ± 2.10*a	541.5 ± 5.90*b	68.30 ± 4.20*c

Data are reported as mean ± SD. Letters a, b, and c show differences among tissues. Data shown with different letters are statistically significant at $p < 0.05$

*$p < 0.5$; **$p < 0.1$ (Student's *t* test); statistical differences between stations

Ni, Cu, Zn, and Fe levels in station 2 were in the range of 0.05–0.14, 0.05–0.31, 0.24–0.44, 0.39–1.05, 1.09–2.12, 3.70–9.84, and 36.56–168.8 μg g^{-1} d.w., respectively.

In the present study, we considered three different tissues with the aim of identifying their role as sites of specific metal accumulation. Compared with the other tissues examined, the digestive gland of *P. caerulea* contained the highest levels of metals in both stations. In the present study, the highest concentrations of Co, Cd, Ni, Cu, Zn, and Fe were determined, for each sampling, in the digestive gland, while Pb was always observed gills of *P. caerulea*. Seasonal differences for all metals in the three tissues of *P. caerulea* were observed in this study.

Gastropod mollusks and bivalve mollusks are good indicators for the long-term monitoring of metal pollution in the marine environment. These species are easy to identify and to sample, are available all year round, and are present in almost all coastal areas of Mediterranean Sea (Conti and Cecchetti 2003). *P. caerulea* is herbivorous; it can be supposed that metal levels in their soft tissues are substantially influenced by metals accumulated in the algae on which they graze.

The ability of mollusks to accumulate heavy metals in their tissues in well known. Mollusks are filter-feeding organisms characterized by low biotransformation capacities. They accumulate the bioavailable fraction of the contaminants present in the water and the metal content of their tissues reflect generally the profile of the pollution (Storelli and Marcotrigiano 2001). As shown in the tables, in most cases, the digestive gland exhibited the highest concentrations of heavy metals. Some researchers (Regoli and Orlando 1994; Frias-Espericueta et al. 1999; Nicholson and Szefer 2003) reported that in mollusks, the digestive gland performs a central role in metabolism, storage, and detoxification of a number of metals. The digestive gland accumulated several metals in considerably higher concentrations than the whole tissues. The digestive gland in considered to play a major role in the accumulation and detoxification of metals in mollusks compared with the gill and muscle tissues.

In *P. caerulea* collected from two stations, the levels of Fe, Zn, and Cu have been found to be highest in the all tissues in every season while Co, Cd, Pb, and Ni have been found at lower levels. Zn, Fe, and Cu are essential elements and play important roles in growth, cell metabolism, and survival of most animals including mollusks. Hence, the relatively high levels of these metals can be attributed to their essentiality (Pourang et al. 2005). A capacity to regulate the internal concentrations of these essential elements as has been reported for some mollusks (Amiard-Triquet et al. 1986). Zn and Fe were the two dominant metals among all the seven studied metals in both stations 1 and 2. Zn and Fe which are usually recorded in high concentration in fish tissues (Kargin 1996), in crab and shrimp tissues (Firat et al. 2008), also exhibited high concentrations in mollusk tissues (Nicholson and Szefer 2003).

In the present study, concentrations of heavy metals showed location-dependent variation. The heavy metal concentrations were quite different between stations 1 and 2, bioaccumulation being lower in station 2. Heavy metal concentrations (Fe, Zn, Cu) in the tissues of *P. caerulea* from the unpolluted (station 2) area were similar to those reported for other relatively unpolluted areas of the Mediterranean (Campanella et al. 2001; Cubadda et al. 2001). The concentrations of metals in *P. caerulea* tissues generally present elevated levels from individuals collected in station 1 of Iskenderun Gulf. The elevated metal concentration in *P. caerulea* from station 1 is a reflection of a heavily polluted area.

In the collected *P. caerulea* samples, high concentrations of Fe, Zn; Cu, and Ni were observed in the samples collected from station 1 in comparison with the samples collected from station 2. This is the result of the exposure of station 1 to heavy discharge of pollutants from numerous industrial processes. Among these industries are iron-steel plant, oil refineries, organic, and inorganic chemicals. Highly elevated levels of Zn, Fe, Cu, Ni, Pb, Cd, and Co in station 1 also observed in *P. caerulea* from Mediterranean coastal waters near heavily industrialized areas (Conti and Cecchetti 2003; Feldstein et al. 2003; Türkmen et al. 2005).

In this study, differences in Cd, Co, Ni, Pb, Cu, Zn, and Fe contents in *P. caerulea* undoubtedly shows a lower degree of contamination than severely polluted sites found in other areas of Mediterranean. High concentrations of heavy metals were also observed in *P. caerulea* from the Ionian Sea (Storelli and Marcotrigiano 2001), Mediterranean Sea and Red Sea (Campanella et al. 2001; Feldstein et al. 2003), and Tyrrhenian coastal areas (Conti and Cecchetti 2003).

In this study, seasonal variation in concentrations of Cd, Co, Ni, Pb, Cu, Zn, and Fe was found in *P. caerulea* at two separate locations. The highest concentration of heavy metal in tissues of *P. caerulea* was registered in winter and spring months. In mollusks, high metal concentrations were found to occur in winter and spring than in the summer and autumn months (Regoli and Orlando 1994; Frias-Espericueta et al. 1999; Sokolowski et al. 2004). Seasonal fluctuations of tissue metal concentrations in mollusks may be affected by various environmental (physicochemical conditions of water) and biological factors (physiological state of organism; Sokolowski et al. 2004).

P. caerulea is a commonly consumed seafood in many Mediterranean countries. Therefore, the investigation of heavy metal concentrations in the tissues of this species may provide useful information on the transfer of potentially toxic elements from abiotic compartments (water, sediment) to higher consumers, including man (Campanella et al. 2001). The usefulness of gastropod mollusks as bioindicators for the detection of metal pollution is confirmed. However, the effect of some biological variables on metal concentrations in gastropod mollusks may be different, according to the sampling period and to the level of metal pollution of the area under investigation.

Conclusions

P. caerulea has considerable potential as cosmopolitan biomonitors of heavy metals in the Mediterranean. This sedentary species are available in every season all over the Mediterranean coastal area and is easy to sample and identify. In both station tested, *P. caerulea* from station 1 was found subjected with more concentrations of heavy metals when compared to heavy metal concentrations from station 2. The cause for such pollution in station 1 is due to the rapid industrial activities and domestic discharges into the sea in the recent past. Among the metals analyzed, Cu, Zn, and Fe were the most abundant in the different tissues while Cd, Co, Ni, and Pb were the least abundant in *P. caerulea*. The maximum concentrations of heavy metals in the digestive gland of *P. caerulea* were observed in both stations 1 and 2. Seasonal variation in the concentrations of Cd, Co, Ni, Pb, Cu, Zn, and Fe was found in samples collected at two separate locations. Generally high concentrations of heavy metals occurred during the winter and spring months. Finally, it is suggested that *P. caerulea* may be used successfully to monitor heavy metal availability in Iskenderun Gulf.

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