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Limpet, *Patella caerulea* Linnaeus, 1758 and Barnacle, *Balanus* sp., as Biomonitors of Trace Metal Availabilities in İskenderun Bay, Northern East Mediterranean Sea

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The use of biological indicators to monitor heavy metal contamination in the marine environment is well documented (Phillips 1977). Although biomonitor organisms may belong to different functional groups, including suspension and deposit feeder bivalves and detritivores, carnivorous and herbivorous gastrapods, the organisms more commonly used as biomonitors are those belonging to the bivalve group, the mussels *Mytilus* spp. (Langston et al. 1998). However, several others, like limpets and barnacles have also been used as sentinel organisms to access the bioavailability of metals in the coastal waters of many parts of the world (Rainbow et al. 1993; Blackmore et al. 1998; Rainbow et al. 2000; Campanella et al. 2001; Cubadda et al. 2001; Bebianno et al. 2003). Along the coast of the East Mediterranean Sea, there are many towns including, İskenderun with an approximate population of 700.000 to 800.000, agricultural lands, and industrial plants (iron-steel plants, beverage, LPG (Liquefied Petroleum Gases) plants, oil transfer docks, other industrial plants and cargo ship's ballast water). As a result, the bay is contaminated directly or indirectly. Although some papers have been published concerning heavy metal levels observed in fish (Canlı and Atlı 2003) and shrimps (Kargın et al. 2003) in northern East Mediterranean Sea environment, this paper presents preliminary results on the levels of cadmium, iron, copper, chromium, cobalt, zinc, lead, nickel, aluminum and manganese in soft tissues of the *P. caerulea* and *Balanus* sp. collected from the İskenderun Bay. These two species occur gregariously on almost all hard substrates along the coast of the bay and samples are easy to collect. Such widespread occurrence facilitates access to reference material in monitoring programs based on each species. The limpets and barnacles can, therefore, be suitable for use as local biomonitors of the sublittoral availabilities of heavy metals, and can be incorporated into the design of intense biomonitoring programs examining marine pollution in the bay. In the present study trace metal concentrations in limpet and barnacle collected at three different sites were investigated with the aim of obtaining background levels for performing intraspecific comparison within the Mediterranean.

MATERIALS AND METHODS

Three sampling stations were established along the 120 km long coastline of the

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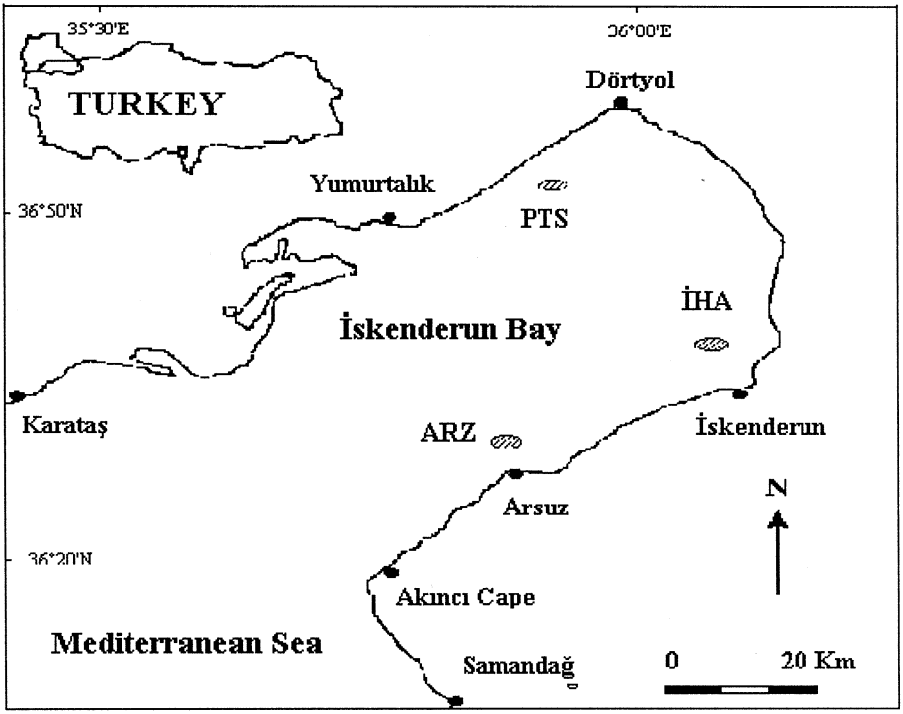

Figure 1. Location map of sampling stations in İskenderun Bay.

İskenderun Bay, northern East Mediterranean Sea of Turkey in May 2002 (Fig. 1). These stations were the Arsuz (ARZ), İskenderun Harbour Area (IHA) and Petrotrans (PTS). 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. To reduce variations in the metal content due to differences in body size, *Patella caerulea* were collected at shell length of 40-45 mm; *Balanus* sp., at shell heights of 8-10 mm. In the case of both *Balanus* sp. and *Patella caerulea*, fifty samples of each species from each station were taken, and each species separated into five groups, ten samples each, and then these ten samples from each group minced together and a representative sample was then taken. The five samples from each station for each species were then homogenized and weighed, and dried to constant weight at 80 °C in acid-washed petri dishes.

To prepare the samples for analysis, the digestion was performed in a microwave digester (CEM MARS-5 Closed Vessel Microwave Digestion System). The advantages of microwave digestion against the classical methods are the shorter time, less consumption of acid and keeping volatile compounds in the solutions (Krushevska et al. 1993; Gulmini et al. 1994). The completely digested samples were allowed cool to room temperature, filtered and diluted to 25 ml in volumetric flasks with double distilled water. All digested samples were analysed 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 commercial materials. Analytical blanks were run in the same way as the samples and concentrations were determined using standard solutions prepared in the same acid matrix. The accuracy and precision of our results were checked by analyzing standard reference material (SRM, Dorm-2, dogfish muscle obtained from the National Research Council Canada, Ottowa, Ontario, Canada). The results indicated good agreement between the certified and the analytical values (Table 1), 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. The absorption wavelength and detection limits were 228.8 nm and 0.02 ppm for Cd; 324.7 nm and 0.03 ppm for Cu; 425.4 nm and 0.4 ppm for Cr; 232.0 nm and 0.1 ppm for Ni; 217.0 nm and 0.1 ppm for Pb; 213.9 nm and 0.01 ppm for Zn; 240.7 nm and 0.05 ppm for Co; 396.1 nm and 0.5 ppm for Al; 279.5 nm and 0.02 ppm for Mn; 248.3 nm and 0.06 ppm for Fe, respectively. All chemicals and standard solutions used in the study were obtained from Merck and were of analytical grade. One-way analysis of variance (ANOVA) and Duncan's test ($p=0.05$) were used to access whether heavy metal concentrations varied significantly between sites, possibilities less than 0.05 ($p<0.05$) were considered statistically significant. All statistical calculations were performed with SPSS 9.0 for Windows.

Table 1. Concentrations of metals found in SRM, Dorm-2 (SE: standard error).

Metals	Certified ± SE	Observed[a] ± SE	Recovery (%)
Cadmium	0.043 ± 0.008	0.038 ± 0.007	88
Copper	2.34 ± 0.16	2.42 ± 0.24	97
Chrome	34.7 ± 5.5	33.4 ± 1.67	96
Lead	0.065 ± 0.007	0.058 ±0.006	89
Zinc	26.6 ± 2.3	28.4 ± 1.66	107
Nickel	19.4 ± 3.1	17.5 ± 0.88	90
Manganese	3.66 ± 0.34	4.02 ± 0.36	110
Aluminum	10.9 ± 1.7	10.3 ± 0.64	95
Iron	142 ± 10	151 ± 12.6	106
Cobalt	0.182 ± 0.031	0.194 ± 0.016	107

[a] Each value is the average of ten determinations (in mg kg^{-1} dry wt).

RESULTS AND DISCUSSION

Table 2 summarizes mean heavy metal concentrations (mg kg^{-1} dry weight, Mean±SE) in *Balanus* sp. and *Patella caerulea* from three sites in İskenderun Bay. In *Ostrea stentia*, the highest levels of all other metals except Al and Cr which were maximum in station IHA and ARZ respectively were observed in station PTS. In *Patella caerulea*, the highest levels of all metals were observed in station PTS. In both species, differences between stations were statistically significant ($p<0.05$). On the other hand, mean concentrations in both species were generally minimum in station ARZ. Since industrial plants and residential quarters densely populate the area between IHA and PTS, this situation may explain why station PTS is more polluted than station ARZ. Zinc showed the highest levels in

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

Metals	Station	*Balanus* sp.	*Patella caerulea*
Aluminum	ARZ	7.26±1.46[a]	2.13±0.64[a]
	IHA	21.90±5.11[b]	2.78±0.62[a]
	PTS	15.62±3.93[ab]	3.69±0.73[a]
	Overall	14.93±2.59[x]	2.87±0.40[y]
Chromium	ARZ	7.31±1.55[a]	8.33±1.52[a]
	IHA	5.10±0.89[ab]	4.77±0.98[a]
	PTS	2.38±0.51[b]	6.66±1.49[a]
	Overall	4.93±0.791[x]	6.59±0.82[x]
Copper	ARZ	7.62±1.95[a]	1.58±0.59[a]
	IHA	13.71±2.35[ab]	2.17±0.47[a]
	PTS	19.90±3.80[b]	4.02±0.67[b]
	Overall	13.74±2.02[x]	2.59±0.42[y]
Manganese	ARZ	13.97±2.83[a]	1.39±0.48[a]
	IHA	21.82±3.13[a]	1.81±0.55[a]
	PTS	16.51±3.22[a]	4.31±0.94[b]
	Overall	17.43±1.86[x]	2.50±0.50[y]
Cobalt	ARZ	4.12±1.02[a]	2.31±0.67[a]
	IHA	8.79±1.39[b]	3.85±1.06[a]
	PTS	9.65±1.75[b]	5.15±1.24[a]
	Total	7.52±0.99[x]	3.77±0.63[y]
Nickel	ARZ	5.07±1.11[a]	3.60±1.02[a]
	IHA	6.10±1.24[a]	9.35±1.93[b]
	PTS	38.12±9.94[b]	12.21±1.83[b]
	Overall	16.43±5.14[x]	8.38±1.30[x]
Cadmium	ARZ	6.19±1.38[a]	3.19±1.09[a]
	IHA	10.93±1.58[a]	2.39±0.92[a]
	PTS	22.31±5.58[b]	4.97±1.04[a]
	Overall	13.14±2.07[x]	3.52±0.62[y]
Lead	ARZ	25.47±5.30[a]	4.28±0.86[a]
	IHA	23.14±4.39[a]	7.26±1.27[a]
	PTS	106.91±10.25[b]	14.53±2.01[b]
	Overall	51.84±11.09[x]	8.69±1.39[y]
Zinc	ARZ	46.25±9.45[a]	23.13±3.94[a]
	IHA	98.43±12.52[b]	30.49±5.90[ab]
	PTS	126.24±15.04[b]	46.59±6.71[b]
	Overall	90.31±11.12[x]	33.41±3.99[y]
Iron	ARZ	19.92±2.84[a]	15.34±1.76[a]
	IHA	44.33±8.99[b]	24.55±3.97[a]
	PTS	72.52±9.47[c]	41.20±7.82[b]
	Overall	45.59±7.07[x]	27.03±3.98[y]

[*] Letters a, b and c show differences among stations of same species; x and y between species at same station. Within columns, means with the same letter are not significant, $p>0.05$.

Table 3. Comparison of heavy metal concentrations in *P.caerulea* and *Balanus* sp. with values taken from the open literature.*

S.	*Areas*	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn	R.
P.c.	Favignana Island	1.7-11.8	-	0.1-1.01	0.47-3.79	-	-	-	0.06-2.18	2.2-19.1	1
P.l.		2.0-6.6	-	0.1-0.90	1.42-3.90	-	-	-	0.0-1.02	5.8-22.8	
P.a.	Portuguese coast	1.7-6.5	-	-	3.5-6.2	-	-	-	-	154-298	2
B.a.	Hong Kong	0.69-9.45	0.04-4.77	0.62-3.32	52.4-1810	313-1470	14.5-95.4	1.25-98.9	0.40-9.08	2860-23300	3
T.s.		2.30-10.2	0.03-0.83	0.72-4.49	1.53-21.2	160-511	10.5-74.4	0.41-3.41	0.52-4.47	2040-8310	
B.i.	Gulf of Gdansk	18.7-23.6	-	-	42.5-86.2	6740-22230	187-307	39.1-59.4	92-130	-	4
B.a.	Fujian Province	5.02-11.9	-	2.13-5.77	34.4-182	-	-	-	3.05-7.41	1467-6965	5
B.u.		5.90-8.70	-	2.50-6.62	61.3-90.7	-	-	-	4.27-8.57	1163-2966	
T.s.		16.8-19.9	-	11.1-18.2	15.0-16.6	-	-	-	10.6-11.3	5059-13599	
B.a.	Fujian Province	0.87-5.50	-	-	29-2204	816-3126	38.8-277	-	-	1521-10000	6
T.s.		4.43-5.07	-	-	12.4-13	700-1033	49.6-54.6	-	-	2243-2630	
B.	İskenderun Bay	6.2-22.3	4.1-9.7	2.4-7.3	7.6-19.9	19.9-72.5	13.9-21.8	5.1-38.1	23.1-107	46.3-126	7
P.c.	İskenderun Bay	2.39-4.97	2.31-5.15	4.77-8.33	1.58-4.02	15.3-41.2	1.39-4.31	3.6-12.2	4.28-14.5	23.1-46.6	7

* Values represent the ranges expressed as mg kg^{-1} dry wt., S.: sample, *P.c.*: *Patella caerulea*, *P.l.*: *Patella lusitanica*, *P.a.*: *Patella aspera*, *T.s.*: *Tetraclita squamosa*, *B.i.*: *Balanus improvisus*, *B.a.*: *Balanus amphitrite*, *B.u.*: *Balanus uliginosus*, B.: *Balanus* sp., R.: references, 1: Cubadda et al. 2001, 2: Bebianno et al. 2003, 3: Rainbow and Blackmore 2001, 4: Rainbow et al. 2000, 5: Rainbow et al. 1993, 6: Blackmore et al. 1998, 7: this study.

both species, and; Fe accumulated at the second highest levels. All mean heavy metal levels in *Balanus* sp. were higher than those in *P. caerulea* except for chromium. Differences between species were significant except chromium and nickel ($p<0.05$). *Balanus* sp. have proved more successful as a biomonitor of metals than *P. caerulea* in the İskenderun Bay, providing a constant picture of metal bioavailability. The mean concentrations of Cd, Zn and Pb in both species from the bay were higher than the legal limits proposed for mollusks by the Republic of Turkish (Anonymous 2002).

Table 3 compares the results of the present study with values taken from the open literature. Cd and Cu concentrations in the limpets, *P.caerulea*, collected from different sites along the İskenderun Bay are within the ranges of those reported for other limpet species in the Favignana island which is an uncontaminated area, but Cr and Zn concentrations appeared to be much higher than those in that area (Cubadda et al. 2001). Generally, although the levels of Co, Cr and Pb reported from barnacles in Hong Kong coastal waters were lower than ours, Cu, Fe, Mn and Zn levels were higher than ours (Rainbow and Blackmore 2001). On the other hand, heavy metal levels of barnacles in the Gulf of Gdansk were higher than İskenderun Bay (Rainbow et al. 2000). This situation may indicate that the Gulf of Gdansk is more polluted than İskenderun Bay.

This study is the first on barnacles and limpets in this area and these data are important as a background for the estimation of the future impact of heavy metal concentrations in this region of the Eastern Mediterranean Sea. In the future, in İskenderun Bay, increases of heavy metal pollution caused by particular inputs and local conditions may cause increasing of pollution in the Eastern and Middle Mediterranean Sea. Although industrial and agricultural activities are increasing rapidly and unconsciously in this area, the necessary measures to stop the associated inputs of contaminants are not taken.

The main goal of the present study was to provide background levels against which to perform intraspecific comparison within the Mediterranean area. These data clearly indicate that both gastropod species are very efficient heavy metal accumulators. Zinc showed the highest levels among the accumulated heavy metals in both Balanus sp and *P. caerulea*. The very high Cd obtained for the species of the genus *Patella* are remarkable as these species are consumed as seafood in many Mediterranean countries.

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