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Metal (Cd, Pb, Cu, Zn, Fe, Cr, Ni) Concentrations in Tissues of a Fish *Sardina pilchardus* and a Prawn *Peaenus japonicus* from Three Stations on the Mediterranean Sea

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Marine animals require metals such as Cu, Zn and Fe for their metabolism and must acquire from ambient water. Trace levels of these metals occur naturally in seawater, together with toxic metals such as Cd, Pb and Hg. Nevertheless, background levels of these two metal groups in the marine environment increased especially in this century after the growth of industry. Class B metals originated from human activities are one of the major source of metal pollution in sea water.

Class B metals can alter many physiological processes and biochemical parameters either in blood or in tissues including structural deformations in aquatic animals (Heath, 1987; Jana et al 1988; Tulasi et al. 1992; Canli 1995; Canli 1996; Canli and Stagg 1996; Ay et al. 1999). Toxic effects occur after excretory, metabolic, storage and detoxification mechanism are no longer able to match to uptake rate. However, the sublethal effects of these metals are also of great concern as metals accumulate and are transferred in the food-chain to humans.

Class B metal levels in the marine environment should be monitored occasionally to check water quality, animal health and for the quality of public food supplies. In our previous study carried out in the same area (Kalay et. al. 1999), we examined three fish species and found that metal levels in their tissues were elevated. The present study was carried out to investigate some other marine animals in the area that have different ecological needs and biology. Thus, the aim of this study is to examine tissues of the sardine *Sardina pilchardus* and prawn *Peaenus japonicus* for their Class B metal (Cd, Pb, Cr, Ni, Cu, Zn and Fe) loads. Sardine is different from most fish in its feeding habit. It is filter-feeding fish that filter particles in surface waters with the gill rakers and is active swimmer. However, the prawn is a scavenger feeds on a wide range of material, including debris and other bottom-dwelling animals.

MATERIALS AND METHODS

Sardina pilchardus and *Peaenus japonicus* used in this study were sampled at 3 stations from the north-east Mediterranean Sea (Fig 1). Twenty samples for each stations (60 samples in total for each species) were purchased from local fishermen on the spot and brought to the laboratory on ice the same day and were frozen at -25 ^{O}C until dissected. Total length and weight were measured which ranged from 17.8±1.03 cm and 47.9±6.13 g for sardine and 4.01±0.59 cm and 37.3±7.75 g for prawn (carapace length was measured

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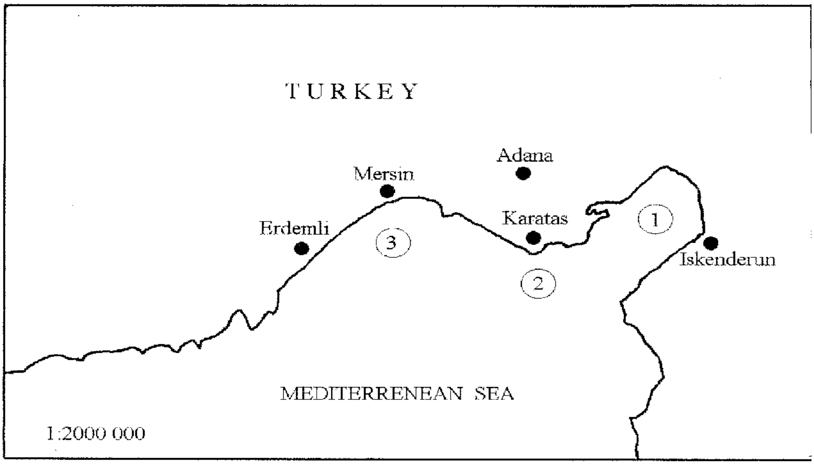

Figure 1. This map shows approximate sampling stations of the animals.

for the prawn). There was no significant difference regarding the size and weight of the animals sampled at various stations (P>0.05).

Gills, liver and muscle tissues (tail muscle and hepatopancreas for the prawn) were dissected out into petri dishes, and dried at 120 ^{o}C. Dry samples were transferred into digestion flasks containing 4 ml perchloric acid and 8 ml nitric acid (Merck) and digested at 120 ^{o}C. The digest was diluted with distilled water appropriately in the range of standards which were prepared from stock standard solution of the metals (Merck). Metal concentrations were measured in a Perkin Elmer AS 3100 flame atomic absorption spectrophotometer and metal concentration in a tissue was presented as μg metal/g dry weight. Accuracy of the atomic absorption spectrophotometer was tested with two samples of reference material (TORT 1 lobster hepatopancreas, National Research Council, Canada). Mean values and standard deviations of reference material measured in this study were within 10% ranges of the reference values.

Statistical Analysis of data was performed out using SPSS statistical package programs. Anova test was used for comparison among stations, Kruskall-Wallis one way Anova was used for data without normal distribution.

RESULTS AND DISCUSSION

Mean concentrations and associated standard deviations for various metals in the tissues of *Sardina pilchardus* and *Peaenus japonicus* are summarised in Tables 1-2.

Cd concentrations in both sardine and prawn were elevated and but highest concentrations were found in the gill and liver (or hepatopancreas). Cadmium levels in the hepatopancreas and gill of the prawn were relatively higher than those in fish tissues. Cd and Pb levels in tissues of sardine did not vary significantly between stations. However, concentrations of Pb and Cd in the gill and hepatopancreas of prawn showed significant variations between stations. Cu concentration in some tissues of the animals varied significantly among stations; station 1 generally showing highest concentrations. Highest Cr concentrations were found in the gills. Cr concentrations in some tissues of the animals showed significant

Table 1. Mean concentrations (μg metal/g d.w.) and associated standard deviations of cadmium, lead, copper, chromium, zinc, iron and nickel, in the gill, hepatopancreas and tail muscle of Japanese prawn *Peaenus japonicus* caught from three stations in the north-east Mediterrenean Sea. Results of statistical differences (P) among three stations are also given in these tables. NS=not significant (P>0.05). N=20.

Metal	Station	Gill	Hepatopancreas	Tail Muscle
Cd				
	1	7.67 ± 2.52	4.47 ± 2.17	0.64 ± 0.14
	2	15.10 ± 6.02	16.73 ± 2.47	0.91 ± 0.14
	3	17.86 ± 4.24	15.51 ± 5.54	0.82 ± 0.28
	P	<0.05	<0.05	NS
Pb				
	1	41.12 ± 18.65	6.77 ± 1.14	3.70 ± 1.20
	2	64.43 ± 12.13	14.70 ± 2.30	5.40 ± 1.04
	3	93.63 ± 20.78	14.28 ± 7.25	4.82 ± 1.23
	P	<0.05	<0.01	NS
Cu				
	1	167.0 ± 84.0	336.4 ± 54.3	15.71 ± 5.40
	2	286.3 ± 76.1	270.3 ± 100.6	18.88 ± 6.60
	3	321.4 ± 117.2	585.7 ± 84.3	22.59 ± 6.77
	P	NS	<0.01	NS
Cr				
	1	12.17 ± 2.15	2.08 ± 0.38	1.30 ± 0.40
	2	24.76 ± 10.23	3.74 ± 1.04	0.97 ± 0.14
	3	26.63 ± 13.50	5.75 ± 3.01	1.16 ± 0.45
	P	<0.05	<0.01	NS
Ni				
	1	41.44 ± 5.60	19.03 ± 6.27	3.37 ± 0.47
	2	75.38 ± 25.60	19.78 ± 1.00	2.60 ± 0.58
	3	43.35 ± 12.50	20.56 ± 3.66	2.19 ± 0.71
	P	<0.05	<0.01	NS
Zn				
	1	137.4 ± 47.9	75.1 ± 14.9	24.7 ± 3.47
	2	110.5 ± 21.8	119.8 ± 28.6	18.0 ± 9.41
	3	114.7 ± 16.8	123.5 ± 31.5	22.5 ± 9.94
	P	NS	<0.05	NS
Fe				
	1	651.6 ± 146.5	130.3 ± 18.1	52.3 ± 21.1
	2	1474.5 ± 545.4	197.2 ± 111.2	113.4 ± 35.2
	3	1655.4 ± 831.3	353.6 ± 176.9	65.1 ± 27.7
	P	<0.05	<0.05	<0.05

Table 2. Mean concentrations (μg metal/g d.w.) and associated standard deviations of cadmium, lead, copper, chromium, zinc, iron and nickel, in the gill, liver and muscle of Sardine *Sardina pilchardus* caught from three stations in the north-east Mediterrenean Sea. Results of statistical differences (P) among three stations are also given in these tables. NS=not significant (P>0.05). N=20.

Metal	Station	Gill	Liver	Muscle
Cd				
	1	2.66 ± 0.50	4.52 ± 1.26	1.26 ± 0.12
	2	2.50 ± 0.40	4.81 ± 1.89	1.11 ± 0.43
	3	2.58 ± 0.47	2.94 ± 0.47	0.96 ± 0.29
	P	NS	NS	NS
Pb				
	1	20.65 ± 9.69	18.06 ± 19.14	7.67 ± 1.73
	2	18.81 ± 3.41	19.99 ± 9.98	6.77 ± 2.86
	3	17.66 ± 2.47	11.58 ± 1.44	5.28 ± 1.78
	P	NS	NS	NS
Cu				
	1	9.24 ± 3.43	24.74 ± 7.75	14.53 ± 6.44
	2	6.78 ± 1.48	18.11 ± 6.21	6.04 ± 0.96
	3	5.50 ± 0.99	18.73 ± 1.74	6.50 ± 1.58
	P	<0.05	NS	<0.01
Cr				
	1	3.54 ± 1.00	4.17 ± 1.96	2.15 ± 0.45
	2	17.76 ± 10.14	4.73 ± 1.65	1.22 ± 0.19
	3	3.80 ± 0.73	3.11 ± 0.86	1.38 ± 0.37
	P	<0.05	NS	<0.05
Ni				
	1	9.68 ± 2.09	11.15 ± 5.99	6.20 ± 1.41
	2	27.38 ± 16.97	11.12 ± 4.25	2.47 ± 0.90
	3	8.13 ± 1.16	5.59 ± 0.54	2.34 ± 0.55
	P	<0.01	<0.01	<0.05
Zn				
	1	51.9 ± 14.9	82.8 ± 27.4	42.4 ± 7.84
	2	45.7 ± 8.18	81.2 ± 20.5	26.9 ± 7.78
	3	51.0 ± 10.3	58.8 ± 3.24	24.0 ± 4.77
	P	NS	NS	<0.01
Fe				
	1	222.4 ± 41.9	462.5 ± 70.3	126.4 ± 19.2
	2	267.3 ± 63.2	537.4 ± 145.2	80.0 ± 10.4
	3	202.6 ± 38.7	362.3 ± 34.0	101.9 ± 12.4
	P	NS	<0.05	<0.05

differences among station and generally station 2 showed highest Cr concentrations. Ni concentrations were also elevated in all tissues; the gill showing highest levels. The tissues of prawn also showed highest concentrations of Nil. Ni concentrations tissues of sardine and prawn were significantly different among stations. Zn and Fe with naturally high concentration in fish and prawns also varied among stations. Like other metals, concentrations of Zn and especially Fe in the gill of prawns were higher than in the gills of the fish.

Sardines and prawns from the Northeast Mediterranean Sea showed high metal concentrations in their tissues, especially in the gill and liver (or hepatopancreas) at all stations, although there was no significant differences between sampling stations. In addition, results also showed outstanding differences in metal concentrations between the two animals. As explained in the tables, concentrations of metals in prawn were relatively higher in most cases. This is also true for other fish species (*Mullus barbatus, Mugil cephalus* and *Caranx crysos*) from the same stations (Kalay et al. 1999). The present study also showed that metal concentrations in muscles tissues were lowest. This is accordance with the previous record on metal accumulation in aquatic animals. It is well known that target organs of heavy metals are generally organs like the liver (or hepatopancreas) and the gill that are metabolically active. Therefore these organs accumulate metals in higher levels than the muscle (Heath 1987; Langston 1990; Canli and Furness 1993a and b; Kalay et al. 1999).

Sampling stations chosen were situated in the industrial and agricultural regions of Turkey and received many untreated waste waters by direct inputs and inputs via rivers. Iskenderun is an industrialised city containing a big steel plant (station 1). Mersin is also an industrialised city with 500,000 population (station 3). These two cities also have big harbours, while Karataş is a little town and does not have many industrial plants in its vicinity (station 2). However, results showed that metal concentrations in tissues of the animals at all stations were similar. As such, therefore none of them can be regarded as a cleaner city.

Metal concentrations in the tissues of fishes show a great variation depending on species and catch area. Metal concentrations in fishes from Slovania contained (ppm w.w.) <0.05-0.34 Pb and <0.003-0.05 Cd in the muscle (Doganoc 1995). Concentrations of Cd, Pb and Cr in the muscle of *Mullus barbatus* and *Mullus surmelatus*, collected from Spanish coasts ranged between 0.019-0.187, 0.03-0.803 and 0.023-0.638 µg/g d.w (Medina et al. 1986). These values are lower than those recorded for fish in the present study. However, studies from western Turkish coasts also show high levels of metals in fishes (Uysal and Tuncer 1982). Similarly, Gey (1985) also found high concentrations of Cu, Zn, Pb, Cr and Cd in the tissues of the bass *Dicentrarchus labrax* and sole *Solea vulgaris* from the gulf of Izmir (Turkey). Hornung and Ramelow (1987) reported concentrations of Cd, Cr, Cu and Zn in the muscle of the Eastern Mediterranean fishes (Israel) ranging from 0.01-0.90, 0.03-5.82, 0.12-14.7 and 6.7-58.8 ppm d.w., respectively. These results are similar to the present data. However, Ni, Cu, Pb, Cd and Cr concentrations in the muscle of six marine fishes from Arabian Sea were than those reported in the present study (Ashraf and Jaffar 1989). Perhaps Mediterranean Sea is more polluted as a result of many industrialised countries around it than the Arabian Sea. Windom et al. (1987) found even lower concentrations of Cd, Cu, Pb, Ni in the muscle of fish *Coryphaenoides armatus* sp from the Atlantic and the pacific Oceans. Concentrations of Cd, Cu, Pb, were between 0.025-0.027, 0.034-0.086, 0.012-0.016 and 0.087-0.46 ppm d.w., respectively. These values are very low compared the data obtained in the present study. Romeo (1987) also found relatively low concentrations of Cu, Zn, Cd and Pb in the muscle of *Mugil cephalus* (mullet roe) from the northern coast of Mauritania in the Atlantic Ocean as 2.3, 142, <0.1, <0.5 ppm d.w. respectively. However, this is not surprising as oceans are generally less polluted marine environments than are seas that are generally surrounded by industrialised countries. When present results are compared to our previous work carried out with three fish species (*Mullus barbatus, Mugil cephalus* and *Caranx crysos*) from the same area (Kalay et al. 1999), metal concentrations in the gill and muscle were similar, except Cu in the muscle of *Mugil cephalus*. However, metal concentrations in the liver of sardine were generally higher than the other fish species. This may be related to the feeding behaviour and swimming activity of sardine.

Tissue distribution of required metals, especially that of Cu in decapod crustaceans are much more different those in fish tissues. As a result of moulting seasonal variations of metals in decapod crustaceans may also be much more variable than in fish (Belleli et al. 1988; Engel 1987). Alliot and Frenet-Piron (1990) showed concentrations of Cd, Cu, Pb and Zn in prawn *Palaemon serratus* varied seasonally highest in the summer months. Seasonal, sex and size related variations in metal concentrations in the tissues of the Norway lobster *Nephrops norvegicus* were reported by Canli and Furness (1993a). It was found that concentrations of cadmium, copper and zinc (ppm d.w.) ranged between 1.12-14.25, 25.0-731 and 63.1-242 respectively, highest concentrations being in the hepatopancreas and the lowest in the tail muscle. Distribution of metals in tissues of some other decapod crustaceans was also supported the present data, although tissue concentrations of present data are generally higher (Cuadras et al. 1981; Capelli et al. 1983; Rainbow and Abdennour 1989; Darmano and Denton 1990). However, high concentrations of metals were also measured from contaminated areas. Ray et al. (1981) measured mean cadmium concentrations in the tail muscle, hepatopancreas and gill of the lobster *Homarus americanus* from different stations in the Belladuna Harbour, Canada, between 0.18-0.29, 210-640 and 23-87 ppm d.w. respectively. These values are very high when compared to the present data in the gill and hepatopancreas, but in the tail muscle these data and present data are similar. In the case of Belladuna Harbour which is highly contaminated by a lead smelter shows that habitat plays significant role in metal concentration of marine animals depending on contamination levels. On the contrary heavy metals in decapod crustaceans from North east Atlantic Ocean were considerably low when compared to present study and studies from seas surrounded by countries (Ridout et al. 1989). Khan et al. (1989) also indicated that two population of grass shrimp *Palaemonetes pugio* showed different heavy metal levels and this was related to heavy metal concentrations in their habitat. It was shown that metals in the hepatopancreas of decapod crustaceans come mainly from food whereas in the gill this is not the case as water concentrations of metals play a significant role in metal concentrations of this organ. However for muscle tissue both route may play equal roles (Davies 1981; Canli and Furness 1995).

Present results emphasise that some metal levels were higher than the acceptable values for human consumption set by various health organisations. For example, concentrations of cadmium and lead in the prawn are considerably high compared to daily tolerable cadmium and lead intake from food according to WHO/FAO committee's proposal (Merian 1991). These are approximately 1 μg Cd/kg body weight and 7 μg Pb/kg body weight. It is perhaps fortunate that high concentrations of metals are not in the edible parts of the animals for human consumption. Nevertheless, for the health of animals it may be accepted as a warning signal. Therefore, some measures should be taken to prevent the contamination of the marine environment for human and animal health point of view and studies indicating heavy metal contamination of seafood should be warning for the authorities to take essential measures to save the aquatic environments. For example, Clerk et al. (1995) indicated that concentrations of Hg, Cd, Cu, Zn and Pb in sole from Belgian catches of the North Sea between 1973-1991 showed significant decline in heavy metal concentrations in recent years. Similarly, Uthe et al. (1986) indicated that cadmium levels in Belleduna harbour was as high as to judge dangerous to human consumer that lead to banning of fishing. After some measures were taken the levels in water and lobster tissues fall sharply within 4 years. However, regarding the results of the present study it is difficult to say that heavy metal concentrations are declining when compared data obtained from the prawn at the Northeast Mediterranean Sea.

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