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Seasonal Variation and Tissue Distribution of Heavy Metals in Shrimp and Fish Species from the Yumurtalik Coast of Iskenderun Gulf, Mediterranean

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Heavy metals can reach high concentrations in the tissues of shrimp and fish along the foodchain. Due to their position at the least link of foodchain, these organisms are good indicators for the long term monitoring of metal accumulation in the marine environment (Darmono, 1990; Palmer and Presley, 1993; Tom et al., 1999; Kucuksezgin et al., 2001). Monitoring metal pollution in the marine environment is becoming increasingly important with rapid industrial and technological developments. Comparing metal concentrations in fish and shrimp species on a geographical basis as a support for environmental monitoring is also useful.

Heavy metals in marine environment can remain in solution in suspension and precipitate on the bottom or be taken up by organisms. *Solea solea* and *Penaeus semiculatus* are benthic species feeding on organisms living the bottom. Benthic species being bottom dwellers to certain extent, are species that tend to concentrate heavy metals to higher degree than other species (Hamza-Chaffai et al., 1996; Kucuksezgin et al., 2001).

Studies have shown that variations in the metal levels of fish and shrimp might be related to a number factors such as age, size of animals, feeding habits, low dissolved oxygen concentrations, fluctuating temperature, elevated sediment metal loads, salinity, bioavability of chemicals in food and water, physiochemical parameters of aquatic environment (Heath, 1987; Kargin et al., 2001; Eastwood and Couture, 2002).

Heavy metal contents in tissues of marine animals are always monitored. Because the heavy metal levels in tissues of marine organisms reflect past exposure via water of food. Knowledge of the concentrations of metals in tissue of marine organisms is useful for identifying specific organs that may be particularly selective and sensitive regarding the accumulation of heavy metals (Szfer et al., 1990).

Contaminants such as metals are introduced into the Mediterranean sea through rivers or direct discharge of industrial wastes and agricultural and urban sewage. The Yumurtalik region is an area high biodiversity and important site for commercial fisheries (Fig. 1). This region is among some of the most productivity

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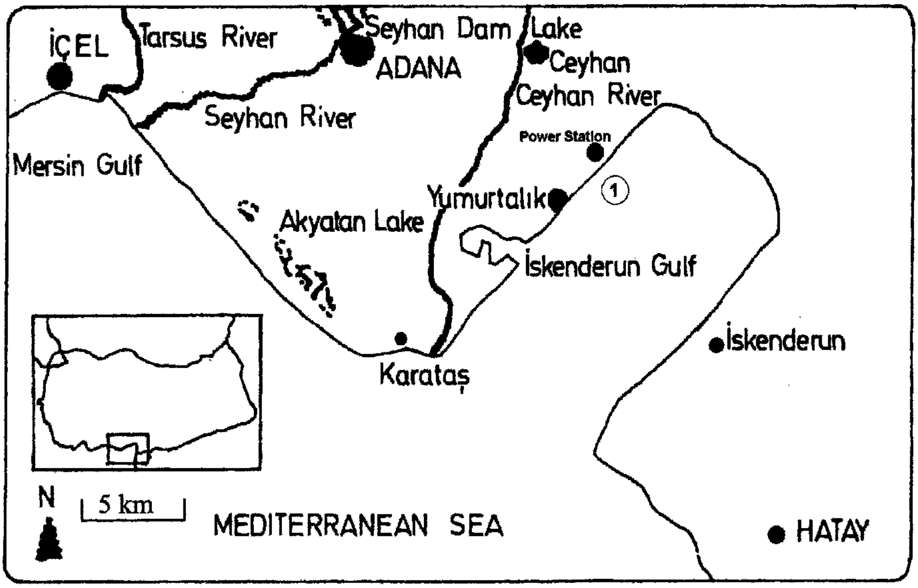

Figure 1. Map of the Yumurtalik area of Iskenderun Gulf, Turkey

in the Mediterranean. In order to investigate the degree and extent of metal contamination in the Yumurtalik area, fish and shrimp must be studied for heavy metal levels. Over the past years, a number of studies have been carried out on the accumulation of heavy metals in organisms of Mediterranean sea (Regoli and Orlando, 1994; Batty et al., 1996; Hamza-Chaffai et al. 1996; Kargin et al., 2001; Cardellicchio et al., 2002). *S. aurata* and *S. solea* (Teleost) have been frequently used as means of evaluating heavy metal pollution in coastal waters. These fish are most popular fish in the Mediterranean countries and have a commercial importance for all population.

These were three specific objectives in this study. The first objective was to determine level of Cu, Cd, Fe, Pb and Zn in tissues of *S. solea, S. aurata* and *P. semiculatus*. The second objective of this study was to investigate whether species conditions and metal concentrations varied seasonally. Finally, in this study a monitoring program is designed to assess the environmental impact of thermic power station put in operation just recently in the Yumurtalik area of Iskenderun Gulf (Mediterranean).

MATERIALS AND METHODS

Specimens were collected in the Yumurtalik coastal area of Iskenderun gulf using cast nets during January, April, June and september of 2002. After collection fish and shrimp were transferred to the laboratory and frozen -20^0C until analyzed. The mean length and weight of the fish and shrimp were 14.7 ± 1.25 cm and 51.4 ± 2.26 g for *S.aurata*, 22.4 ± 1.78 cm and 87.9 ± 1.85 g for *S. solea* and 13.4 ± 1.15 cm and 16.9 ± 1.28 g for *P. semiculatus*.

Before analysis, liver, gill and muscle tissues for each species were dissected separately. Tissue samples were dried at 110^{0}C for two days they reached constant weights in the oven. Samples were transferred into a digestion flask. Nitric acid 2 ml and 1 ml perchloric acid were added and then the digestion flasks were put on a hot plate set to 120^{0}C for three hours (Liang et al., 1999). The remaining digested solution was transferred to 10 ml flasks and diluted to volume with distilled water, and metal concentrations in the samples were measured on a Perkin Elmer AS 3100 atomic absorbtion flame spectrophotometer, calibrated with C-5524 Sigma Standards. Detection limit of the spectrophotometer was 0.028 ppm and 90% recovery was obtained during measurements. In the present study concentrations of the heavy metals (Cd, Cu, Fe, Pb and Zn) were determined in muscle, gill and liver contents of shrimp and fish species. A total of 83 individual fish and shrimp specimens were analyzed, 26 specimens belong to the species *S. aurata*, 31 to *S. solea* and 26 to *P. semiculatus*.

All calculations refer to dry weight of tissues. One-way Analysis of Variance (SNK test) was applied to investigate differences in metal concentrations in seasons and tissues.

RESULTS AND DISCUSSION

The result are shown in Table 1-5 as the avarage and standart error of metal concentrations in the different tissues examined of *S. aurata*, *S. solea* and *P. semiculatus*. Data were analyzed statistically by a series of student Newman Keul's test to identify any differences among monthly (a, b and c) and among tissues (x, y and z) accumulation distributions of the metals. Data shown with different letters are significant at the $P<0.01$ level.

The patterns of heavy metals occurrences in muscle tissues of fish and shrimp samples in order of increasing contents were Zn>Pb>Fe>Cu>Cd fo *S. solea* and *S. aurata*, Zn>Cu>Pb>Fe>Cd for *P semiculatus*. The heavy metal occurrences were in the order Fe>Cu>Zn>Pb>Cd for liver of fish samples, Cu>Zn>Fe>Pb>Cd for liver of shrimp samples. These results showed that the ordes of the metal levels changed between tissues of fish and shrimp.

In all the species, cadmium concentrations in the muscle were low and did not vary much according to the species. Compared to muscle the liver presented higher cadmium concentrations, especially in *P. semiculatus* (37.76 μ/g). Copper concentrations were low in the muscle of both fish. *S.solea* and *P.semiculatus* accumulated copper, particularly in the liver (260.3 and 763.7 μ/g). These values are 8 to 22 times higher than that found for *S. aurata*. The highest lead value analyzed in the liver was found for both fish species in every season, whereas the highest lead levels was observed in the gills of *P. semiculatus*. All the species samples were characterized by high mean zinc and iron concentrations in the liver. Compared to both fish species, in the muscle of shrimp *P. semiculatus* determined higher zinc concentrations in every season. Metal concentration (Cd, Cu, Fe, Pb and Zn) in both shrimp and fish samples were similar. These metals are more concentrated in the livers than in the gills and muscles. Gills present higher levels of metals than in muscles.

Table 1. Concentration of lead in various tissues of *Solea solea*, *Sparus aurata* and *Penaeus semiculatus* (µg Pb/g dw)

	Tissue	January $\overline{X} \pm S\overline{x}$ *	April $\overline{X} \pm S\overline{x}$ *	June $\overline{X} \pm S\overline{x}$ *	September $\overline{X} \pm S\overline{x}$ *
Solea solea	Muscle	14.0 ± 2.72 **ax**	17.2 ± 1.63 **ax**	26.6 ± 1.89 **bx**	17.8 ± 1.14 **ax**
	Gills	29.5 ± 3.64 **ay**	33.9 ± 3.74 **ay**	44.9 ± 3.29 **by**	36.3 ± 2.85 **ay**
	Liver	38.2 ± 4.23 **az**	42.5 ± 3.86 **az**	62.8 ± 4.87 **bz**	51.3 ± 4.73 **cz**
Sparus aurata	Muscle	13.6 ± 2.15 **ax**	17.6 ± 1.63 **ax**	22.1 ± 1.85 **bx**	15.6 ± 1.78 **ax**
	Gills	18.5 ± 1.34 **ay**	23.5 ± 2.84 **ay**	34.1 ± 2.84 **by**	24.7 ± 1.35 **ay**
	Liver	29.9 ± 3.14 **az**	33.0 ± 2.67 **az**	45.5 ± 4.66 **bz**	36.8 ± 3.64 **az**
Penaeus semiculatus	Muscle	15.4 ± 1.44 **ax**	16.2.± 0.97 **ax**	28.6 ± 2.15 **bx**	16.5 ± 1.76 **ax**
	Gills	159.0 ± 6.32 **ay**	168.6 ± 5.74 **ay**	317.5 ± 7.46 **by**	179.6 ± 4.58**cy**
	Liver	58.4 ± 3.75 **az**	50.9 ± 2.25 **az**	127.1 ± 5.36 **bz**	54.3 ± 3.86 **az**

* = SNK : Letters x, y and z show differences among tissues; a,b and c among months. Data shown with different letters are statistically significant at the $P<0.01$ level. $\overline{X} \pm S\overline{x}$: Mean ± Standard Error

Table 2. Concentration of cadmium in various tissues of *Solea solea*, *Sparus aurata* and *Penaeus semiculatus* (µg Cd/g dw)

	Tissue	January $\overline{X} \pm S\overline{x}$ *	April $\overline{X} \pm S\overline{x}$ *	June $\overline{X} \pm S\overline{x}$ *	September $\overline{X} \pm S\overline{x}$ *
Solea solea	Muscle	2.1 ± 0.62 **ax**	2.5 ± 0.47 **ax**	3.5 ± 1.65 **bx**	2.7 ± 0.64 **ax**
	Gills	4.8 ± 0.87 **ay**	5.2 ± 0.84 **ay**	9.5 ± 1.89 **by**	6.1 ± 1.77 **ay**
	Liver	7.8 ± 1.24 **az**	8.1 ± 1.15 **az**	13.7 ± 2.83 **bz**	9.3 ± 1.24 **az**
Sparus aurata	Muscle	2.4 ± 0.88 **ax**	2.2 ± 0.44 **ax**	3.2 ± 1.35 **bx**	2.1 ± 0.97 **ax**
	Gills	4.2 ± 0.95 **ay**	3.8 ± 0.50 **ay**	6.2 ± 1.07 **by**	4.7 ± 1.18 **ay**
	Liver	6.7 ± 1.24 **az**	5.9 ± 1.25 **az**	9.7 ± 2.08 **bz**	6.3 ± 2.06 **az**
Penaeus semiculatus	Muscle	3.2 ± 1.14 **ax**	2.7.± 0.87 **ax**	5.0 ± 0.86 **bx**	3.0 ± 0.74 **ax**
	Gills	15.8 ± 2.45 **ay**	14.3 ± 2.74 **ay**	24.8 ± 2.85 **by**	13.3 ± 2.15 **ay**
	Liver	20.2 ± 2.17 **az**	21.6 ± 2.36 **az**	37.4 ± 2.32 **bz**	23.3 ± 2.54 **az**

All tissues metal levels were significantly higher in the June than the other months in both fish and shrimp samples. Statistically significant ($P<0.01$) seasonality in the concentrations of the five metals in all species were observed. In *S. solea* the highest monthly mean concentrations of Pb (44.89), Cd (9.45), Cu (17.79), Fe (130.9) and Zn 107) µg g^{-1} were observed in June. The lowest monthly mean concentrations of Pb (29.65), Cd (4.27), Cu (10.33), Fe (90.17) and Zn (78.81) µgg^{-1} in January (Table 1-5). The ratio between the seasonal mean maximum and minimum concentrations was greatest for cadmium and least for zinc in *S.solea* and *P. semiculatus*.

S. solea and *P semiculatus* are suggested here to serve as a suitable pollution bioindicator species. These species abundantly found year round on sandy

Table 3. Concentration of copper in various tissues of *Solea solea*, *Sparus aurata* and *Penaeus semiculatus* (µg Cu/g dw)

	Tissue	January $\overline{X} \pm S\overline{x}$ *	April $\overline{X} \pm S\overline{x}$ *	June $\overline{X} \pm S\overline{x}$ *	September $\overline{X} \pm S\overline{x}$ *
Solea solea	Muscle	5.2 ± 0.72 **ax**	4.7 ± 0.97 **ax**	8.3 ± 1.18 **bx**	5.8 ± 1.24 **ax**
	Gills	10.3 ± 2.24 **ay**	6.8 ± 1.27 **by**	17.8 ± 2.28 **cy**	11.2 ± 2.10 **ay**
	Liver	209.2 ± 5.84 **az**	193.1 ± 3.74 **bz**	260.3 ± 5.72 **cz**	215.7 ± 4.28 **az**
Sparus aurata	Muscle	3.8 ± 1.11 **ax**	3.4 ± 1.15 **ax**	5.8 ± 1.09 **bx**	4.7 ± 0.97 **ax**
	Gills	6.3 ± 1.08 **ay**	6.3 ± 1.23 **ay**	9.1 ± 1.16 **by**	6.1 ± 1.24 **ay**
	Liver	24.4 ± 2.81 **az**	20.8 ± 2.84 **az**	32.5 ± 3.65 **bz**	23.2 ± 2.36 **az**
Penaeus semiculatus	Muscle	27.9 ± 2.14 **ax**	28.8.± 1.24 **ax**	41.9 ± 2.63 **bx**	30.4 ± 1.28 **ax**
	Gills	247.6 ± 4.75 **ay**	216.0 ± 3.64 **by**	299.4 ± 5.18 **cy**	250.5 ± 5.63 **ay**
	Liver	676.0 ± 5.27 **az**	433.6 ± 5.15 **bz**	763.7 ± 7.36 **cz**	681.6 ± 7.32 **az**

Table 4. Concentration of zinc in various tissues of *Solea solea*, *Sparus aurata* and *Penaeus semiculatus* (µg Zn/g dw)

	Tissue	January $\overline{X} \pm S\overline{x}$ *	April $\overline{X} \pm S\overline{x}$ *	June $\overline{X} \pm S\overline{x}$ *	September $\overline{X} \pm S\overline{x}$ *
Solea solea	Muscle	23.3 ± 2.10 **ax**	22.7 ± 1.69 **ax**	33.8 ± 1.28 **bx**	23.2 ± 1.24 **ax**
	Gills	78.8 ± 2.83 **ay**	80.5 ± 3.72 **ay**	107.7 ± 3.25 **by**	82.2 ± 4.15 **ay**
	Liver	134.8 ± 3.78 **az**	114.8 ± 4.23 **bz**	160.1 ± 5.74 **cz**	116.5 ± 3.72 **bz**
Sparus aurata	Muscle	21.6 ± 1.08 **ax**	21.1 ± 2.86 **ax**	28.7 ± 1.78 **bx**	22.7 ± 1.34 **ax**
	Gills	72.4 ± 2.84 **ay**	73.1 ± 2.84 **ay**	90.2 ± 3.71 **by**	74.3 ± 3.15 **ay**
	Liver	112.9 ± 3.76 **az**	110.7 ± 3.73 **az**	145.3 ± 4.76 **bz**	115.2 ± 3.36 **az**
Penaeus semiculatu s	Muscle	50.4 ± 2.24 **ax**	51.5.± 1.79 **ax**	63.1 ± 2.26 **bx**	50.1 ± 1.94 **ax**
	Gills	162.2 ± 3.84 **ay**	163.5 ± 2.18 **ay**	209.1 ± 5.84 **by**	171.3 ± 3.37 **ay**
	Liver	273.6 ± 4.74 **az**	290.6 ± 3.76 **az**	331.1 ± 6.23 **bz**	268.2 ± 3.44 **az**

substrate along the Turkey Mediterranean coast, in polluted and non-polluted sites. Levels of metals in these species reflect the known pollution status in different field sites. Higher levels of all metals were found in the tissues of benthic *S. solea,* compared with that of pelagic *S. aurata* throughout year. Differences in five metal concentrations in fish may be attributable to many factors such as, feeding habits, habitats, metabolic activity and behavior of two species. Deep-sea carnivor shrimp are though to accumulate heavy metals in their tissues via the food chain. In this study, level of cadmium, lead, copper, iron and zinc were significantly higher tissues in *P. semiculatus* when compared with two fish species.

In aquatic animals, different organs show different capacities for accumulating metals. The present data showed metal concentrations in the liver and gill were highest in all the species in every season. Metal concentrations seem to be associated with organ function. Liver and gills were chosen as target organs for assessing metal accumulation. The concentrations of metals in gills reflect the

Table 5. Concentration of iron in various tissues of *Solea solea*, *Sparus aurata* and *Penaeus semiculatus* (µg Fe/g dw)

		January	April	June	September
	Tissue	$\bar{X} \pm S\bar{x}$ *	$\bar{X} \pm S\bar{x}$ *	$\bar{X} \pm S\bar{x}$ *	$\bar{X} \pm S\bar{x}$ *
Solea solea	Muscle	9.9 ± 1.26 **ax**	9.2 ± 1.21 **ax**	15.9 ± 1.84 **bx**	9.4 ± 1.12 **ax**
	Gills	90.2 ± 3.24 **ay**	91.3 ± 3.72 **ay**	130.9 ± 2.82 **by**	92.7 ± 2.76 **ay**
	Liver	236.2 ± 4.82 **az**	240.5 ± 4.82 **az**	300.7 ± 4.28.**bz**	271.5 ± 5.18 **cz**
Sparus aurata	Muscle	7.9 ± 1.15 **ax**	8.5 ± 1.20 **ax**	14.5 ± 1.23 **bx**	9.1 ± 1.34 **ax**
	Gills	75.2 ± 3.68 **ay**	77.3 ± 2654 **ay**	105.1 ± 2.78 **by**	79.6 ± 2.87 **ay**
	Liver	326.3 ± 4.82 **az**	331.2 ± 4.82 **az**	363.0 ± 4.21 **bz**	330.6 ± 4.92 **az**
Penaeus semiculatus	Muscle	8.7 ± 1.05 **ax**	9.1.± 0.79 **ax**	16.9 ± 3.76 **bx**	9.8 ± 1.24 **ax**
	Gills	92.6 ± 2.48 **ay**	95.4 ± 2.15 **ay**	134.1 ± 3.16 **by**	109.5 ± 4.15 **cy**
	Liver	114.9 ± 3.61 **az**	112.3 ± 4.12 **az**	167.4 ± 4.84 **bz**	135.4 ± 4.23 **cz**

concentrations of metals in waters, whereas the concentration in liver represents storage of metals (Romeo et al., 1999). Induction of metallothionein in the liver is the main form of storage and detoxification of metals in fish and shrimp (Heath, 1987; Darmono and Denton, 1990; Schumacher et al., 1992; Mantelatto et al., 1999). The gills are responsible for the water flow and exposed to large water mass and thus are expected to have high metal concentrations (Swaileh and Adelung, 1994). Muscle tissues could be less responsive to recent uptake or excretion of heavy metals than other tissues. In this study, the lowest levels of metals were observed in the muscle tissues of shrimp and fish species in every season.

Cadmium concentrations in the liver are slightly variable according to species in this study. Cadmium is particularly concentrated in the liver and gill of *S.solea* and *P. semiculatus*. Liver cadmium concentrations may be reflective of recent accumulation may be a useful indicator of recent exposure. Cadmium could originate from water, sediment and food. High levels of cadmium were already in shrimp and benthic fish marine environment (Frenet and Alliot, 1985; Dietz et al., 1996; Kargin, 1996; Mantelatto et al., 1999; Romeo et al., 1999; Kargin et al., 2001).

Lead is non-essential element and higher concentrations can occur in aquatic organisms close to anthropogenic sources. Tissues lead concentrations are variable according to species. High levels of lead found in the gills of *P. semiculatus* are possibly due to high concentrations of this metal in the ambient water. High level of lead in the gills, indicated that the shrimp accumulates the metal from the water, primarily across the gill filaments. Most of lead appears to be accumulated in the gill and liver, and lower concentrations of the element were found in the muscle.

Copper levels are known to vary widely among aquatic organisms. Copper concentrations are higher in the liver of *P. semiculatus* samples relative than those measured in *S. solea* and *S. aurata*. In shrimp species, copper appears to be under close physiological regulation as generally stated for this essential element (Bryan, 1976; Amiard et al., 1987). In this study, the highest value 260.3 µg Cu

g^{-1} was found in the liver of *S. solea*. A high liver copper storage related to a high hepatic metallothionein level (Romeo et al., 1999).

In general, zinc concentrations in muscle tissues were much higher than other metals in both fish and shrimp. Similarly, high zinc concentrations were found in the muscle of different fish species (Hamza-Chaffai, 1996). In this study, the relatively higher zinc concentration in the tissues of fish and shrimp species may be due to the role that zinc plays as an activator of numerous enzymes present in the liver.

Iron, an oligo-element, plays a vital role in the enzymatic and respiratory processes of aquatic animals (Frenet and Alliot, 1985). The present study shows significant variations in the concentration of iron in tissues of fish and shrimp species. Liver showed higher iron concentrations followed by gill and muscle. Similar high iron values have been reported previously for *P. semiculatus* (Kargin et al., 2001) and *S. aurata* (Kargin, 1996).

Local differences in geology or anthropogenic sources are some parameters that may affect the presence of metals. Local productivity and differences in food composition are other parameters that can affect the accumulation of metals. In addition to this, year to year variations, migrations and differences and other factors may introduce variance to the expected pattern (Dietz et al., 1996). Concentrations of heavy metals in tissues of fishes and shrimp from different region of Mediterranean showed great variations. Metal concentrations of the tissues in the present study were lower than in fish and shrimp from other regions of Mediterranean (Regoli and Orlando, 1994; Hamza-Cfaffai et al., 1996; Kargin, 1996; Kargin et al., 2001; Kucuksezgin et al., 2001).

There is a seasonal effect on the metal concentrations of fish and shrimp which might reflect variations in these species metabolic activity. Seasonal differences for all metals in liver, gill and muscle of three species were observed in this study. Variation in metal concentrations with season has been well-documented in different studies from freshwater and marine environment (Regoli and Orlando, 1994; Kargin, 1998; Mantellato et al., 1999; Foster et al., 2000; Kargin et al., 2001; Eastwood and Couture, 2002). It is reported to be due to varying seasonal growth rate, reproductive cycle, water salinity and temperature. Larson et al. (1985) indicated that many of physiological parameters in fish are subjected to seasonal variations. In all species, high metal concentrations were observed mainly during the summer month. This was related to in the spring season using of metal content fungicides, pesticides and fertilizers in agriculture in this area. Similar increases in metal concentrations were observed during summer months in the Mediterranean shrimp (Alliot and Frenet-Piron, 1990; Kargin et al., 2001) and Mediterranean fish (Hornung and Ramelow, 1987; Hamza-Chaffai et al., 1996; Kargin, 1996; Kucuksegin et al., 2001).

The proposed limit values for human consumption of fish reach approximately 0.2. µg Cd/g , 10.0 µg Cu/g and 150.0 µg Zn/g wet weight set by the Australian National Health and Medical Research Council (Sharif et al., 1991) When the mean muscle metal levels of *P. semiculatus*, which has higher metal levels than *S. aurata* and *S. solea*, are expressed in terms of wet weight (ww.) the following values were obtained; 0.9 µg Cd/g ww, 4.9 µg Pb/g ww, 8.3 µg Cu/g ww and 14.1

μg Zn/g ww. Hence cadmium in the muscle tissues of these species exceeds, while copper and zinc are within the above given consumption levels.

Finally, in this study we have identified a range of metal concentrations in fish and shrimp from Yumurtalik area of Iskenderun gulf. These data important as a background for the estimation of the future impact on metal concentrations in organisms of thermic power station put in operation just recently in this area.

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