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Distribution of Heavy Metals in Different Tissues of the Shrimp *Penaeus semiculatus* and *Metapenaeus monocerus* from the Iskenderun Gulf, Turkey: Seasonal Variations

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Heavy metals occur naturally in sea water in very low concentrations. However in many marine environments heavy metal concentrations exceed natural levels. Investigations of heavy metals in shrimp are an important aspect of environmental pollution control because anthropogenic activities progressively increase the concentrations of metal in the marine environment.

The utility for environmental monitoring studies of marine shrimp as bioindicators of heavy metal pollution has been emphasized by several investigators (Clark et.al.,1987; Darmono,1990; Mizutani et al.,1991; Palmer and Presley,1993; Dallinger,1994). However heavy metal concentrations in these organisms can be influenced by many environmental and biological factors. Seasonal changes of heavy metal levels in seawater influence the accumulation of metal in marine invertebrates (Sivadasan and Nambisan,1988; Howard and Hacker,1990; Regoli and Orlando,1994).

The Iskenderun gulf has been selected for the sampling region because of its state of pollution. Due to industrial effluents, urban and agricultural sewage, important quantities of chemical pollutants are being discharged into the waters of Iskenderun gulf. Recently there have been a large number of studies into heavy metals in marine invertebrates, particularly in heavily polluted areas of Mediterranean sea (Voutsinou-Taliadouri,1982; Regoli and Orlando,1994; Ayaş and Kolonkaya,1996).

This study was undertaken to determine the seasonal variation in levels of Cd, Zn, Fe, Pb and Cu in the muscle, gill and hepatopancreas tissues of two shrimp species *Penaeus semiculatus* and *Metapenaeus monocerus*.

MATERIALS AND METHODS

The shrimp were collected from Iskenderun bay (Figure 1) using cast nets during January, April, July and October of 1999. Immadiately after collection, the shrimp were stored on ice in an instulated box and transported to the laboratory and stored –20^{0}C until dissection. Seasonal variation of metal concentrations in the tissues of *P.semiculatus* and *M.monocerus* were investigated in shrimp of similar size (the mean length and weight of the shrimp were 16.2±0.83 cm and 37.5±1.52 g for *P.semiculatus* and 12.8±0.74 cm and 25.8±1.38 g for *M. monocerus*).

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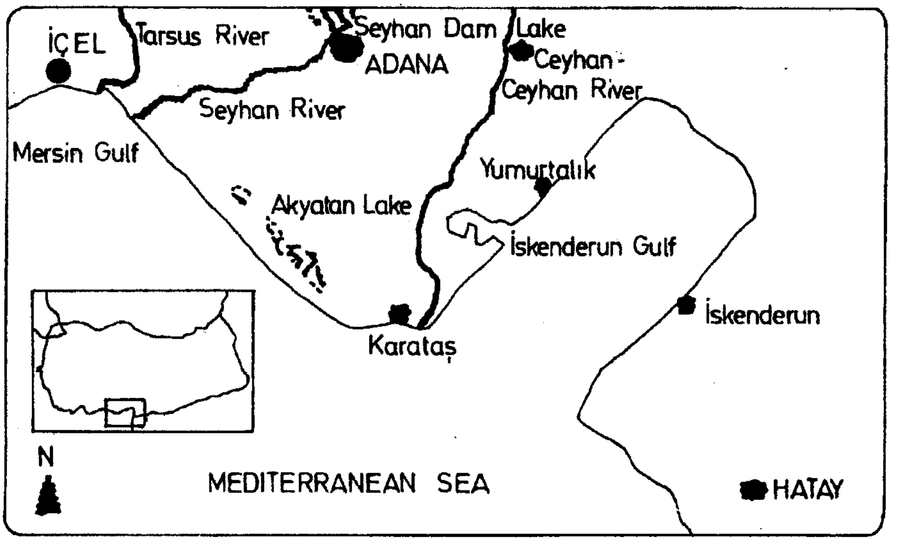

Figure 1. Iskenderun Gulf, Turkey

Prior to analysis, ten shrimp from each species were dissected for their hepatopancreas, gill and muscle tissues . The hepatopancreas, gill and muscle tissues samples were analyzed individually. Sample preparation and analysis were carried out according to the procedure described by Bernhard (1976). The dry weights of each tissue type were determined to be in the following limits: hepatopancreas :512-716, gill :100-184, muscle : 630-960 mg.Tissues were dried at 105^{0}C for 48 hours and digested with concentrated nitric acid and concentrated perchloric acid (2 : 1 v/v) at 120^{0}C for 3 hours (Muramoto,1983). After dilution, metals (Cadmium, Lead, Iron, Zinc and Copper) content of tissues were measured by atomic absorption spectrophotometry (Perkin Elmer AS 3100).

RESULTS AND DISCUSSION

Tissue distribution of heavy metals in *P.semiculatus* and *M. monocerus* are given in Tables 1-5 for each metal respectively. Data were analysed statistically by a series of Student Newman Keul's test to identify any differences among tissues (x,y and z) and among monthly (a and b) accumulation distributions of the metals. Data shown with different letters are significant at the P< 0.01 level. The table also gives an idea of the concentrations of heavy metals in the shrimp occurring in the bay Iskenderun and consumed by the people in this area.

Compared with the other tissues examined, muscle of both species contained the lowest levels of all metals. The hepatopancreas contained the highest levels of Zn, Cu, Fe and Cd followed by the gills in both species in every season. However the highest lead levels were observed in the gills of both species. In general, metal concentration in the tissues of shrimp can be influenced by a number of factors such as metal levels in the sediment, size-dependent differences and seasonal variations in growth rate and uptake rates.

Table 1. Concentration of zinc in various tissues of *Penaeus semiculatus* and *Metapenaeus monocerus* (μg Zn/g dw)

	Tissue	January $\bar{X} \pm S\bar{x}$ *	April $\bar{X} \pm S\bar{x}$ *	July $\bar{X} \pm S\bar{x}$ *	October $\bar{X} \pm S\bar{x}$ *
	Muscle	72.6 ± 1.24 **ax**	70.2 ± 2.10 **ax**	83.6 ± 1.96 **bx**	73.1 ± 1.85 **ax**
P. semiculatus	Hepatopancreas	305.8 ± 3.21 **ay**	305.2 ± 3.76 **ay**	360.4 ± 4.36 **by**	307.4 ± 3.38 **ay**
	Gill	241.2 ± 2.72 **az**	237.5 ± 2.87 **az**	281.5 ± 2.72 **bz**	244.2 ± 2.54 **az**
	Muscle	61.8 ± 1.15 **ax**	60.4 ± 1.34 **ax**	71.3 ± 2.14 **bx**	63.2 ± 1.25 **ax**
M. monocerus	Hepatopancreas	264.1 ± 3.59 **ay**	259.7 ± 3.67 **ay**	295.7 ± 3.75 **by**	266.9 ± 3.97 **ay**
	Gill	168.7 ± 2.98 **az**	165.5 ± 2.86 **az**	212.3 ± 2.85 **bz**	169.2 ± 2.96 **az**

* = SNK : Letters x, y and z show differences among tissues; a and b among months. Data shown with different letters are statistically significant at the $P<0.01$ level.

$\bar{X} \pm S\bar{x}$: Mean ± Standard Error

Table 2. Concentration of iron in various tissues of *Penaeus semiculatus* and *Metapenaeus monocerus* (μg Fe/g dw)

	Tissue	January $\bar{X} \pm S\bar{x}$ *	April $\bar{X} \pm S\bar{x}$ *	July. $\bar{X} \pm S\bar{x}$ *	October $\bar{X} \pm S\bar{x}$ *
	Muscle	51.1 ± 1.72 **ax**	49.7 ± 1.80 **ax**	62.2 ± 1.96 **bx**	52.3 ± 1.45 **ax**
P. semiculatus	Hepatopancreas	235.6 ± 3.01 **ay**	231.2 ± 3.16 **ay**	281.6 ± 3.36 **by**	236.4 ± 3.38 **ay**
	Gill	207.2 ± 2.84 **az**	203.5 ± 2.87 **az**	234.5 ± 2.82 **bz**	209.2 ± 2.66 **az**
	Muscle	62.2 ± 1.75 **ax**	60.7 ± 1.34 **ax**	75.3 ± 2.14 **bx**	64.2 ± 1.85 **ax**
M. monocerus	Hepatopancreas	318.6 ± 3.59 **ay**	312.7 ± 3.67 **ay**	380.7 ± 3.75 **by**	320.9 ± 3.97 **ay**
	Gill	276.7 ± 3.20 **az**	273.5 ± 3.56 **az**	332.3 ± 3.85 **bz**	279.2 ± 3.26 **az**

Abbreviations used as in Table 1.

Table 3. Concentration of copper in various tissues of *Penaeus semiculatus* and *Metapenaeus monocerus* (µg Cu/g dw)

	Tissue	January	April	July	October
		$\overline{X} \pm S\overline{x}$ *	$\overline{X} \pm S\overline{x}$ *	$\overline{X} \pm S\overline{x}$ *	$\overline{X} \pm S\overline{x}$ *
	Muscle	31.2 ± 1.72 **ax**	30.1 ± 1.28 **ax**	42.2 ± 1.86 **bx**	32.3 ± 1.45 **ax**
P. semiculatus	Hepatopancreas	862.6 ± 4.01 **ay**	854.2 ± 4.16 **ay**	962.6 ± 4.36 **by**	863.4 ± 4.33 **ay**
	Gill	258.3 ± 2.15 **az**	253.5 ± 2.82 **az**	342.6 ± 2.81 **bz**	260.3 ± 2.26 **az**
	Muscle	22.1 ± 1.15 **ax**	20.6 ± 1.04 **ax**	30.7 ± 1.14 **bx**	22.5 ± 1.05 **ax**
M. monocerus	Hepatopancreas	626.3 ± 3.49 **ay**	618.7 ± 3.27 **ay**	814.5 ± 4.25 **by**	627.2 ± 3.95 **ay**
	Gill	201.4 ± 2.70 **az**	196.8 ± 2.76 **az**	251.8 ± 2.15 **bz**	202.5 ± 2.51 **az**

Abbreviations used as in Table 1.

Table 4. Concentration of lead in various tissues of *Penaeus semiculatus* and *Metapenaeus monocerus* (µg Pb/g dw)

	Tissue	January	April	July	October
		$\overline{X} \pm S\overline{x}$ *	$\overline{X} \pm S\overline{x}$ *	$\overline{X} \pm S\overline{x}$ *	$\overline{X} \pm S\overline{x}$ *
	Muscle	6.3 ± 0.64 **ax**	5.7±0.58 **ax**	8.5 ± 0.96 **bx**	6.8 ± 0.72 **ax**
P. semiculatus	Hepatopancreas	13.7 ± 1.10 **ay**	12.2 ± 1.36 **ay**	19.7 ± 2.13 **by**	13.9 ± 1.26 **ay**
	Gill	18.9 ± 1.72 **az**	17.3 ± 1.75 **az**	26.2 ± 2.24 **bz**	18.6 ± 1.84 **az**
	Muscle	11.3 ± 0.86 **ax**	10.3 ± 0.94 **ax**	21.6 ± 1.12 **bx**	12.1 ± 0.92 **ax**
M. monocerus	Hepatopancreas	52.3 ± 1.34 **ay**	50.2 ± 1.62 **ay**	76.2 ± 2.30 **by**	53.1 ± 1.86 **ay**
	Gill	72.9 ± 1.86 **az**	69.9 ± 2.15 **az**	101.2 ± 2.15 **bz**	73.2 ± 1.98 **az**

Abbreviations used as in Table 1.

Table 5.Concentration of cadmium invarious tissues of *Penaeus semiculatus* and *Metapenaeus monocerus* (µg Cd/g dw)

	Tissue	January	April	July	October
		$\overline{X} \pm S\overline{x}$ *	$\overline{X} \pm S\overline{x}$ *	$\overline{X} \pm S\overline{x}$ *	$\overline{X} \pm S\overline{x}$ *
	Muscle	1.1 ± 0.42 **ax**	1.0 ± 0.56 **ax**	1.9 ± 0.66 **bx**	1.1 ± 0.53 **ax**
P. semiculatus	Hepatopancreas	8.2 ± 1.15 **ay**	7.9 ± 1.26 **ay**	12.7 ± 1.71 **by**	8.3 ± 1.10 **ay**
	Gill	2.5 ± 0.76 **az**	2.4 ± 0.93 **az**	4.5 ± 1.14 **bz**	2.6 ± 0.94 **az**
	Muscle	0.6 ± 0.34 **ax**	0.5 ± 0.43 **ax**	1.1 ± 0.78 **bx**	0.7 ± 0.44 **ax**
M. monocerus	Hepatopancreas	3.4 ± 0.76 **ay**	3.3 ± 1.17 **ay**	5.6 ± 1.32 **by**	3.5 ± 1.12 **ay**
	Gill	2.1 ± 0.85 **az**	2.1 ± 0.95 **az**	3.1 ± 1.16 **bz**	2.2 ± 1.24 **ay**

Abbreviations used as in Table 1.

In this study, copper was present at relatively high concentrations in the tissues of both species. The high levels of this metal may be related with essential role of this metal in the production of the respiratory protein, haemocyanin (Bryan,1968; Frenet and Alliot,1985; Paez-Osuna et.al.,1995). Zinc and Iron were present in large quantities in the tissues both *P. semiculatus* and *M. monocerus*. These metals play a role in the enzymatic and respiratory processes (Bryan,1968).In general, cadmium and lead have been found at lower levels in the tissues of both species in every season. A number of studies show that the concentrations of cadmium and lead in the aquatic organisms depend mainly on their environmental levels (Amiard et.al.,1987; Heath,1987; Bryan and Langston,1992).

Essential metals such as zinc, copper and manganese are regulated in crustaceans whereas non essential metals such as cadmium appears not to be regulated (Bryan,1976;).The studies on copper and zinc in *Palaemonetes varians* (Amiard et.al.,1987), *Crongon crongon* (Amiard et.al.,1985) and *Humarus americanus* (Chou et.al.,1988) indicate a marked ability on the part of crustaceans to regulate internal copper and zinc concentrations. In this study, Zn, Fe and Cu were the most abundant metals in the different tissues analyzed throughout the year, whereas Cd and Pb were the least abundant both in *P. semiculatus* and *M.monocerus*. Cu and Zn are apparently an essential trace metal for crustaceans (Voutsiou-Taliadouri,1982; Rainbow and White,1989). In addition, the high concentrations of Cu, Fe and Zn may be related to the use of these metals by factories of Iskenderun.

The level of a given metal were also significant differences between the tissues throughout the year both in *P.semiculatus* and *M. monocerus* .Except for lead, highest concentrations of each metal, in the present study were observed in the hepatopancreas and the lowest in the muscle. This is not surprising, since the hepatopancreas is the main regulatory organ in crustacean species and as such it would be the prime site for metal storage and detoxification in these animals (Lyon et.al.,1983; Bagatto and Alikhan,1987; Khan et.al.,1989; Thaker and Haritos,1989; Darmono and Denton,1990; Mantelato et.al.1999). Heavy metals mainly accumulate in metabolicaly active tissues . The hepatopancreas and gill tissues were highly active in the uptake and storage of heavy metals. Crustaceans (shrimp) respond to heavy metal exposure by producing metallothionein, particularly in hepatopancreas (Darmono1990; Howard and Hacker,1990; Dallinger,1994). High levels of metals found in the hepatopancreas of *P.semiculatus* and *M.monocerus* is possibly due to binding of the metals to metallothionein proteins. In both species the highest Pb concentrations were observed in the gills. The gill was at tissue which were active and passive exchanges occur between the animal and aquatic environment. First high levels of metals accumulate in the gill tissues by absorption and adsorption (Heath,1987). The lowest levels of metals was observed in the muscle tissues of both species in every season. Similar results were reported for a number of shrimp species (Khan et.al.,1989; Darmono,1990; Darmono and Denton,1990). There were significant differences in metal levels in the tissues of *P.semiculatus* and *M.monocerus* in every season. Differences in heavy metal concentration in shrimps may be attributable to many factors such as, feeding habits, metabolic activity and behavior of two species. Similar finding was reported by Darmono and Denton (1990 in the tissues of *Penaeus merguiensis* and P*enaeus monodon*.

Invertebrates, particularly crustaceans, were very sensitive to heavy metals (Thorp and Lake,1974). Shrimps from environments contain very low levels of metals. However they can accumulate substantial quantities of these metals under polluted conditions. For example Palmer and Presley (1993) reported that *Penaeus aztacus* rapidly accumulate Hg when confined to a contaminated area. Higher metal concentrations were found in the tissues of *Palamonetes varians* caught in a polluted area. (Frenet and Alliot, 1985).

In both species, levels of all metals were significantly higher in tissues in the summer when compared with other seasons This is probably due to the use of synthetic fertilizers, pesticides and the greater human activity in the gulf during summer. Similar increases in metal concentration were observed during summer months in the Mediterranean invertebrate (*Palaemon serratus, Mytilus galloprovincialis*) (Alliot and Frenet-Piron, 1990; Regoli and Orlondo, 1994) and Mediterranean fish *(Mullus barbatus, Sparus aurata*) (Voutsinou-Taliadouri, 1982; Kargın, 1996) this was related to the increased metabolism due to high temperatures (Voutsinou-Taliadouri, 1982). Alliot and Frenet-Piron (1990) found that metal levels increased in water during summer and also in shrimps. Each metal presents a peak of pollution in water every year in July and August, then the metal levels decreased during winter, going back to a normal level. In this study, metal levels increased in both shrimp species during July, then the metal levels decreased during October and January.

Heavy metal concentrations (Cu, Fe, Zn, Pb, Cd) in the muscle of shrimps from Iskenderun gulf were similar to those reported for other relatively unpolluted areas of the Mediterranean (Voutsinou-Taliadouri, 1982; Alliot and Frenet-Piron, 1990).In general, our data were within the ranges seen in other regions of the Mediterranean (Table 6), and these data were important as a background for the estimation of the future impact of heavy metals in this region of the Eastern Mediterranean.

Table 6. Heavy metal concentrations (μg/g) in shrimps in other regions of the Mediterranean.

Region	Zn	Cu	Cd	Pb	Fe	Reference
France	105.0	60.1	0.025	0.5	62.2	Alliot and Frenet 1990
Greece	12.8	7.4	0.055	0.31	8.9	Voutsinou-Taliadouri 1982
Turkey	14.6	6.1	0.20	0.96	4.5	Uysal and Tuncer 1982

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