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Seasonal Variation of Heavy Metals in Shrimp *Penaeus kerathurus* (Forskal, 1775) from Izmir Bay, Turkey

Gurel Turkmen
Faculty of Fisheries, Ege University, 35100 Bornova, Izmir, Turkey

Abstract: Seasonal changes in heavy metals (Cd, Cu, Fe, Pb, Zn) concentrations in muscle tissues of both male and female caramote shrimp (*Penaeus kerathurus*) from Izmir bay, Turkey were measured for a year period by using flame and graphite furnace AAS. The relationships in various heavy metal concentrations were compared according to sex and seasons. Heavy metal content varied with type of metals, seasons and sex. The highest concentration of heavy metal in the muscle tissue of *P. kerathurus* for Cd, Pb, Cu, Fe and Zn was registered in Summer months. Mean Fe level in male shrimps was considerably higher (30.19 compared to 20.23 μg g^{-1} dry weight) in October. Regarding Cu, the same case could be observed (27.58 μg g^{-1} dry weight in males compared to 19.02 in females). In the present study, heavy metals accumulated in the shrimp muscle in the order Zn>Fe>Cu>Pb>Cd. Concentrations of metals ranged within the recommended limits for human consumption and not represent a health risk.

Key words: Heavy metals, *Penaeus kerathurus*, seasonal changes, dry weight, Izmir bay

INTRODUCTION

In the marine environment, toxic metals are potentially accumulated in sediments and marine organisms and subsequently transferred to man through the food chain. Thus, it has become increasingly important to determine and assess levels of heavy metals in marine organisms because of nutritional and safety conditions. This is true especially for edible marine organisms as they are a potential dietary source of protein (Pourang and Dennis, 2005). *Penaeus kerathurus* (Caramote shrimp) is distributed in coastal areas of the Marmara, Aegean and Mediterranean seas and it is commercially one of the most important shrimp species in coastal fisheries in Turkey. This species is fished for all along the above mentioned sea coasts because of its large size and excellent taste. It is also a target species for fishermen using trammel nets in Izmir bay in Western Turkey (Turkmen and Yilmazyerli, 2006).

Izmir bay is one of the great natural bays of the Mediterranean. The main urban settlement around this bay is the city of Izmir (88,000 ha and a population of 3,965,232 (2011 census)). The bay has been divided into three sections (outer, middle and inner) according to the physical characteristics of the different water masses (Kontas, 2012). Izmir is an important industrial and commercial centre and a cultural focal point. The streams and hundreds of small domestic discharge outlets, flow to the bay. The main industries in the region include food processing, beverage manufacturing and bottling, tanneries, oil, soap and paint production, chemical industries, paper and pulp factories, textile industries, metal processing and timber processing (Kucuksezgin *et al*., 2006). Since, heavy metal pollution has been incresing in the area, shrimp can accumulate heavy metals from seawater and/or sediment to concentartions that may exceed the levels of safety for consumers. Therefore, it is important to carry out a study to determine heavy metal concentartions accumulated in the shrimp *Penaeus kerathurus*. There have been a large number of detailed studies on heavy metals related to tissue, season or sexes in marine shrimps recently particularly in heavily polluted aereas of Mediterranean and Marmara sea in Turkey (Canli *et al*., 2001; Kargin *et al*., 2001; Cogun *et al*., 2005; Yilmaz and Yilmaz, 2007; Firat *et al*., 2008). However, a few papers have been published to date concerning heavy metal concentrations in *P. kerathurus* in Izmir bay (Kontas, 2012) and Iskenderun bay in Turkey (Balkas *et al*., 1982). But no data are available on heavy metals concentrations related to season or sex differences of *P. kerathurus* from Turkish sea coast. The objective of the present study was to provide information on the seasonal concentration of Cd, Cu, Fe, Pb and Zn in the edible part of shrimp *P. kerathurus* from the Izmir Bay and compare them with other species and previous studies. There is increasing awareness that trace metal concentrations in crustaceans can differ not only among different tissues but also between males and females. To the knowledge, no study to date has focused on analyzing seasonal variation of heavy metals in male and female shrimp of *Penaeus kerathurus*. Additionally, the relationships between seasonal heavy metal concentration and male and female individuals were examined. This data is important as a background for the estimation of the future impact of metal pollutants in this area.

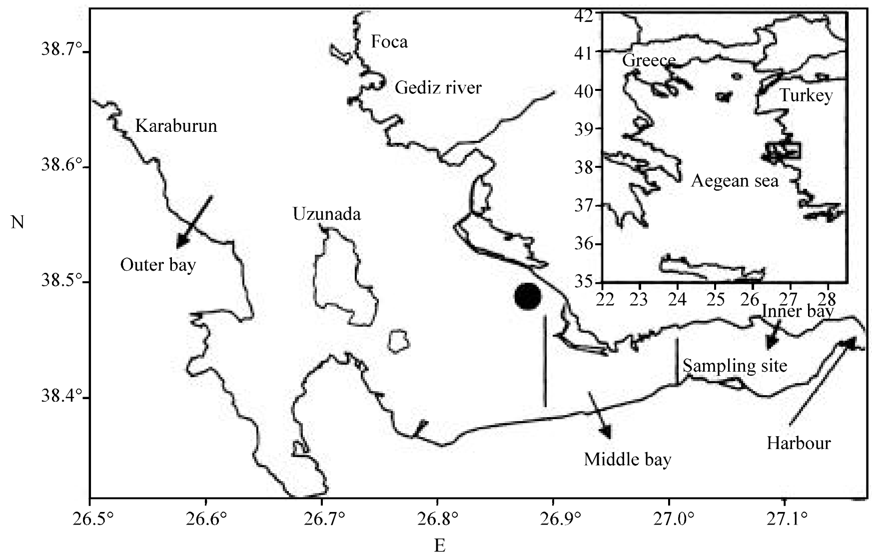

Fig. 1: Izmir bay and location of sampling site

MATERIALS AND METHODS

Caramote shrimps were captured from Izmir bay (Fig. 1) in Summer (July) and Autumn (October) of 2010 and Winter (January) and Spring (April) of 2011 by a commercial shrimp trammel net. Immediately after capture, the shrimp were stored on ice in an insulated box and transferred to the laboratory and frozen -20°C until dissected. Male and female shrimps were grouped in sex and size. Ten samples from each sex were obtained for each season. Total shrimp lenght and weight were measured before dissection. Metal analyses were done in the Central Fishers Research Institute of Ministery of Food, Agriculture and Livestock in Trabzon. For analysis, edible part of each shrimp were dissected, weighed and dried at 120°C until they reached a constant weight. For each sample, between 0.3 and 0.5 g of tissue was weighed and placed in a Teflon digestion vessel, 7 mL of concentrated (65%) nitric acid (HNO_3) and 1 mL of (30%) hydrogen peroxide (H_2O_2) added and digested in a microwave digester (ETHOS 1, Milestone Inc.). The sample in the vessel containing concentrated nitric acid was then subjected to a microwave program as follows: Step 1: 25-200°C for 10 min at 1,000 W; Step 2: 200°C for 10 min at 1,000 W (Digestion Aplication Note: DG-FO-17, ETHOS 1, Milestone Inc.). Digests were diluted with deionized water to 25 mL in acid washed standard flask. Analyses were performed by flame (Fe, Zn) and graphite furnace (Cd, Cu, Pb,) atomic absorption spectrophotometry (GBC 905 model). Following absorption lines were used; Fe 248.3 nm, Zn 213.9 nm, Cd 228.8 nm, Cu 324.7 nm, Pb 283.3 nm. The results were expressed on a dry weight basis (μg g^{-1} dw). Replicates (five) were utilized to analysis of metals concentration. The accuracy of the determination procedure was assessed by the analysis of certified reference material (CRM, Dorm-3) (Fish protein) in triplicate for each batch of analysis. Recoveries were consistently in the range 93.1-104.3%. In order to compare with other shrimp and evaluate human health risk on a wet weight (ww) basis the moisture content (75.8±1.2%) of shrimp species was evaluated and the data was converted to wet weight using conversion factor of 4.13 for muscle. Differences in heavy metal levels between seasons and sexes were compared by one way ANOVA (Kruskal Wallis one way Analysis of Variance). The levels of significance were $p<0.05$.

RESULTS AND DISCUSSION

The results show that the concentrations of zinc, iron, copper, lead and cadmium in shrimp muscles were significantly affected by the seasons in Izmir bay except iron and copper for male shrimp ($p<0.05$) (Table1). The highest concentration of heavy metal in the muscle tissue of *P. kerathurus* for Cd, Pb, Cu, Fe and Zn was registered in Summer months. In shrimps, high metal concentrations were observed mainly during the Summer (Mantelatto *et al*., 1999; Kargin *et al*., 2001; Cogun *et al*., 2005). In addittion, Kontas (2012) found that metal concentrations in suspended particulate matter increased during Summer season in Izmir bay. In this study, Zinc, Fe and Cu were the most abundant elements in the different samples analyzed throughout the year whereas Pb and Cd were the least abundant. The order of metal

Table 1: Mean±SD of some body measurements and heavy metal concentrations (μg g^{-1} dw) between seasons and sexes of *P. kerathurus* from Izmir bay

TL-W/Metals	Sex	April	July	October	January
Total Length (cm)	M	14.2±1.5	14.8±1.8	13.1±1.3	13.8±1.4
	F	16.0±2.2	17.8±2.5	15.1±1.8	15.8±2.0
Weight (g)	M	20.9±4.3	22.6±4.0	19.7±4.2	20.2±3.5
	F	34.6±6.6	44.8±7.8	25.9±5.8	26.8±5.6
Cd	M	0.082±0.030^{aA}	0.220±0.074^{bA}	0.082±0.030^{aA}	0.020±0.006^{cA}
	F	0.110±0.035^{aA}	0.188±0.069^{bA}	0.102±0.025^{aA}	0.023±0.007^{cA}
Pb	M	0.42±0.15^{aA}	0.81±0.21^{bA}	0.39±0.18^{aA}	0.36±0.15^{aA}
	F	0.39±0.18^{aA}	0.91±022^{bA}	0.35±0.16^{aA}	0.42±0.19^{aA}
Cu	M	19.11±2.95^{aA}	28.47±3.22^{bA}	27.58±3.28^{bA}	17.93±1.39^{aA}
	F	20.90±3.55^{aA}	26.37±3.34^{bA}	19.02±3.86^{aB}	20.36±3.25^{aA}
Fe	M	20.06±6.06^{aA}	32.47±6.45^{bA}	30.19±5.62^{bA}	21.78±4.51^{aA}
	F	23.97±3.31^{aA}	31.26±8.89^{bA}	20.23±3.06^{aB}	21.18±5.51^{aA}
Zn	M	46.40±4.13^{aA}	63.45±6.26^{bA}	48.01±6.07^{a}	43.09±5.14^{aA}
	F	45.69±4.49^{aA}	60.81±6.41^{bA}	46.94±4.19^{a}	45.31±4.43^{aA}

$^{a-c}$Correspond to the comparison of each group of interest along the seasons; $^{A, B}$Correspond to group comparisons between sexes

concentrations in muscle tissue of both male and female was Zn>Fe>Cu>Pb>Cd. This results can be explained as a result of various biological factors and many decades of intense industrial, anthropogenic and agricultural activties carried out in this area.

Zn being an essential element for normal growth and metabolism of animals, exhibited highest accumulation in the shrimp muscle when compared with the other four metals. Zn concentrations in the muscle ranged from 38-73 μg g^{-1} dw. Although, there were no significantly differences in the zinc levels of different sexes, zinc cncentrations varied significantly between seasons in the studied shrimp samples (Table 1) ($p<0.05$). The high concentration of Zn is associated with the activity of enzymes played a role in the enzymatic processes of in aquatic animals (Cogun *et al*., 2005). In general, zinc concentrations in muscle tissues were much higer than other metals in shrimps (Table 2). Iron, an oligo element plays a vital role in the enzymatic and respiratory processes of aquatic animals. The iron contents of shrimp changed from 20.06-32.4 μg g^{-1} dw. The lowest ($p<0.05$) iron content was found in the Spring and Winter. Iron concentrations did not vary significantly in muscles of both sexes except in October. Copper is an essetial trace metal for animal metabolism but at high levels is a very toxic substance to aquatic life. Copper was present at relatively high concentrations in the muscule tissue with only significant differences between males and females in the Autumn (Table 1). The high levels of this element can be due in part to its high capacity of hemocyanin for binding Cu to a respiratory pigment present in large amounts in the gills of crustaceans (Paez-Osuna and Tron-Mayen, 1995). The available evidence leads us to postulate that *P. kerathurus* males and females differ in their physiological requirements for this metal. The high concentrations detected in males were probably due to the greater development of their gonads in relation to males during a phase characterized by an accelerated metabolic rate with somatization of metal absorption. Gonads maturation in males and females of *P. kerathurus* at Izmir bay occurs during Spring and Summer with a high incidence in Summer and involves the simultaneous increase of organic constituents and ovary mass (Turkmen and Yilmazyerli, 2006). A similar pattern was described for *Pleoticus muelleri* studied in San Jorge Gulf, Argentina by Jeckel *et al.* (1996) with copper concentrations tending to increase in the male gonad in parallel to increases of these trace metals in the digestive gland indicating that their levels in the male reproductive system reflect at least partly, their levels of intake. However, Rainbow (1998) reported that decapods usually regulate their body concentrations of the essential elements Zn, Cu and Fe to approximately constant levels while they are net accumulators of the non-essential elements Cd and Pb.

Lead is a neurotoxin that causes behavioral deficits in aquatic organisms' and cause decreases in survival, growth rates and metabolism. There is often little accumulation of Pb in marine and freshwater species (Mitra *et al*., 2012). Lead is higher concentration can occur in aquatic organisms close to anthropogenic sources. Most of the lead appears to be accumulated in the gill and liver and lower conentrations of the element were found in the muscle tissue in shrimp (Cogun *et al*., 2005). Highest concentrations in both of male and female of lead were observed in July (Table 1). Cadmium is regarded as a priority pollutant because of its toxicity to organisms in the aquatic environment. The concentrations of Cd in *P. kerathurus* showed a wide variability, being the highest in Summer displaying a similarity between in Spring and Authum and being the lowest in Winter season (Table 1).

Metal concentrations in *P. kerathurus* of Izmir bay were compared to previous studies performed in the same and other areas of the world (Table 2). Uysal and Tuncer and Kontas (2012) studied fish and shrimp of the Izmir

Table 2: Comparison of heavy metal concentrations in *P. kerathurus* and shrimp species (belonging to family Penaeidae) from Turkey and different areas of the world as well as maxium allowable concentrations of several heavy metals in shrimp for human consumption

Location	Species	Cd	Cu	Fe	Pb	Zn
Bay of Izmir, Turkey[1]	*Penaeus kerathurus*[**]	0.20	6.14	4.45	0.96	14.62
Mediterranean, Turkey[2]	*Penaeus kerathurus*[**]	0.03	7.4	3.1	0.34	13.2
Bay of Izmir, Turkey[3]	*Penaeus kerathurus*[**]	-	4.75	6.70		10.80
Present study	*Penaeus kerathurus*[*]	0.103±0.077	22.03±4.50	25.76±7.10	0.50±0.27	49.96±8.67
Present study	*Penaeus kerathurus*[**]	0.025±0.018	5.33±1.09	6.24±1.72	0.12±0.07	12.10±2.10
Mar Menor, Spain[4]	*Penaeus kerathurus*[**]	0.5	12.0	12.2	0.4	22.2
Mediterranean[5]	*Penaeus kerathurus*[**]	-	5.2±2.7	-	-	22±16
Amrovikos Gulf, Greece[6]	*Penaeus kerathurus*[*]	-	19.0-25.2	-	-	-
Mediterranean[7]	*Penaeus kerathurus*[**]	0.16	1.5	-	0.46	-
Mediterranean, Spain[8]	*Penaeus kerathurus*[**]	0.03-0.05	-	-	0.05-0.79	-
Lesina Lagoon, Italy[9]	*Penaeus kerathurus*[*]	0.16	15.83	-	0.59	50.60
Sea of Marmara, Turkey[10]	*Parapenaeus longrostris*[*]	0.77±0.31	24.5±3.85	56.20±34.82	4.82±2.08	40.42±11.24
Ýskenderun Bay, Turkey[11]	*Penaeus semisulcatus*[*]	2.7-5.0	27.9-41.9	8.7-16.9	15.4-28.6	50.1-63.1
Mediterranean, Turkey[12]	*Penaeus japonicus*[*]	0.64-0.91	15.71-22.59	52.3-113.4	3.70-5.40	18.0-24.7
Ýskenderun Bay, Turkey[13]	*Metapenaeus monoceros*[*]	0.5-1.1	20.6-30.7	60.7-75.3	10.3-21.6	60.4-71.3
Mexico[14]	*Penaeus californiensis*[*]	ND	18.2	28	-	74
Sundurbans, India[15]	*Penaeus indicus*[*]	ND-4.50	14.50-63	-	8.5-12	20.50-87.50
Persian Gulf[16]	*Penaeus merguiensis*[*]	0.07±0.03	17.86±11.53	17.72±9.72	-	40.20±15.21
Persian Gulf[16]	*Metapenaeus affinis*[*]	0.11±0.06	17.37±9.89	22.15±16.84	-	46.06±15.22
Bay of Bengal[17]	*Penaeus monodon*[*]	0.2-0.3	12.2-21.3	9.1-15.7	0.8-1.3	24.2-35.7
Pacific coast, Mexico[18]	*Penaeus vannamei*[*]	0.57	23.3	180.1	-	60.6
Persian Gulf, Iran[19]	*Penaeus semisulcatus*[**]	0.133±0.010	3.418±0.711	-	-	8.977±0.959
Mediterranean, Algeria[20]	*Parapenaeus longrostris*[*]	0.65	117.75	142.50	1.27	98.17
Gulf of Mexico[21]	*Penaeus setiferus*[*]	3.64	17.6	102.1	4.22	106
Gulf of California[22]	*Litopenaeus stylirostris*[*]	0.66	25.4	-	5.3	57.8
Chittagong Bangladesh[23]	*Metapenaeus monoceros*[*]	0.7	33.1	43.0	3.6	40.8
Arabian sea[24]	*Penaeus japonicus*[**]	0.47	-	837	-	7.11
Saronikos gulf, Greece[25]	*Parapenaeus longrostris*[**]	0.05	6.30	7.40	2.27	13.40
Ubatuba bay, Brazil[26]	*Xiphopenaeus kroyeri*[*]	2.09	48.97	-	34.49	67.83
WHO[19**]		0.2	10	-	-	1000
European Commission[27**]		0.5	-	-	0.5	-
UK (MAFF)[19**]		0.2	20	-	2.0	50
IAEA-407[28*]		0.19	3.28	146	0.12	67.1
FDA[29**]		3	60	-	1.5	200
Turkish Food Codex[30**]		0.5	20	-	0.5	50

[1]Uysal and Tuncer (1982); [2]Balkas *et al.* (1982); [3]Kontas (2012); [4]Leon *et al.* (1982); [5]UNEP/FAO (1986); [6]Panayotidis and Florou (1989); [7]Medpol (1992); [8]Pastor *et al.* (1994); [9]D'Adamo *et al.* (2008); [10]Kurun *et al.* (2007); [11]Çogun *et al.* (2005); [12]Canli *et al.* (2001); [13]Kargin *et al.* (2001); [14]Paez-Osuna and Tron-Mayen (1995); [15]Mitra *et al.* (2012); [16]Pourang and Amini (2001); [17]Hossain and Khan (2001); [18]Paez-Osuna and Fernandez (1995); [19]Pourang *et al.* (2005); [20]Abdennour *et al.* (2000); [21]Vazquez *et al.* (2001); [22]Frias-Espericueta *et al.* (2007); [23]Khan and Alam (1994); [24]Tariq *et al.* (1993); [25]Voutsinou-Taliadouri (1982); [26]Mantelatto *et al.* (1999); [27]European Commission (2011); [28]IAEA-407 (2003); [29]FDA (2009); [30]Turkish Food Codex (2008); [*]$\mu g\ g^{-1}$ dry weight; [**]$\mu g\ g^{-1}$ wet weight; ND: Not Detected

bay and found concentrations ($\mu g\ g^{-1}$ ww) of Cd, Cu, Fe, Pb and Zn in the edible muscle of *P. kerathurus* at 0.20, 6.14-4.75, 4.45-6.70, 0.96 and 14.62-10.80, respectively. In the investigation, metal concentrations (considering dry weight) of, except for Cu, Fe and Zn were lower than these. The mean Cd value [(0.103 $\mu g\ g^{-1}$ dw) (concediring, 0.024 $\mu g\ g^{-1}$ ww)] is lower than the 0.20 $\mu g\ g^{-1}$ ww reported by Uysal and Tuncer. The results for higher concentrations of copper, iron and zinc than other metals (Cd and Pb) in shrimp were similar to findings previously recorded for shrimp other waters of the world. The levels of almost all the metals measured in this study are relatively lower than those recorded in shrimp from other region of Turkey and the world (Table 2).

The information on the differences between the metal content of the sexes is more limited than for the other factors so far considered. The faster growing sex (usually the female) can be expected to contain lower concentrations of metals but not necessarily a smaller total body burden (Pourang *et al*., 2005). In this study, highly significant differences between sexes in Fe and Cu contents could be detected in October (Table 1). Mean Fe level in male shrimps was considerably higher (30.19 compared to 20.23 $\mu g\ g^{-1}$ dw). Regarding Cu the same case could be observed (27.58 $\mu g\ g^{-1}$ dw in males compared to 19.02 in females). Frenet and Alliot (1985) reported that bioaccumulation factor of copper in the prawn Palaemonetes varians was greater in males and juveniles. Paez-Osuna and Tron-Mayen (1995) observed sex based differences in concentrations of Zn, Ni, Cu and Cd in some tissues of shrimp *Penaeus californiensis*. In the case of Zn and Cu, the higher concentrations were observed in muscle of males.

Almost all metals measured in this study are relatively lower than the values recorded in shrimp species from other regions of the world. The findings of other studies are shown in the Table 2 and are compared with the concentrations reported in this study and elsewhere in the world. Patterns of distribution of trace elements in edible tissues of aquatic organisms are very important from different aspects, especially from health of sea food consumers' point of view. Considering limit value for human consumption of Cd, Cu, Fe, Pb and Zn, these concentrations ranged within the recommended limits for human consumption and not represent a health risk (WHO, European Commission, UK (MAFF), FDA, Turkish Food Codex) (WHO, 2006, Table 2). Comparing the present data with guidelines and limits, it can be seen that the mean values of Cu and Pb in the muscle for both sexes were only higher than levels of IAEA. Cd concentrations in the species ranged from 0.02-0.20 $\mu g\ g^{-1}$ dw. Cd concentration in *P. kerathurus* was below the local legislative limit of 0.5 $\mu g\ g^{-1}$ ww and within EU regulations for crustaceans. Cu concentrations throughout the study ranged from 17.93-27.58 mg $\mu g\ g^{-1}$ dw. The lowest and highest Cu level was detected in January and October month. The Cu concentrations found in the species were below the local legislative limit of 20 $\mu g\ g^{-1}$ ww. The Turkish legislation establishes maximum levels for two of the metals studied, above which human consumption is not permitted; 0.5 $\mu g\ g^{-1}$ ww for Pb and 50 $\mu g\ g^{-1}$ ww for Zn.

CONCLUSION

The knowledge of heavy metal concentrations in shrimp are very important with respect to nature management, human consumption of these species and to determine the most useful biomonitor species and the most polluted area. Information on the distribution pattern of toxic heavy metal pollutants in aquatic environment becomes important so as to know the accumulation of such pollutants in the organisms and final transfer to man through sea foods. A comparison of the present results with data reported for shrimp from other marine environments in general heavy metal concentrations in the shrimp samples are not higher in Izmir bay. Based on the sample analyzed, metal concentartions found in the muscluces proved to be the acceptable values for human consumption.

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