

CORRELATION PROFILES OF THE ACCUMULATED METALS IN SEAWATER, SEDIMENT AND *Pachygrapsus marmoratus* (Fabricius) TISSUES IN BLACK SEA (ORDU, TURKEY)

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Abstract: This study was performed in order to investigate the interactions of accumulation patterns of some metals (Al, As, Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn) in exoskeleton, gill, hepatopancreas and muscle tissues of the marbled crab *Pachygrapsus marmoratus* (Fabricius) sampled from near streams and domestic discharge points along the coastal region of Black Sea along Ordu in Turkey as well as patterns in sediment and seawater samples of the crab sampling sites. The study area covering a coastal stretch of 28 km along Black Sea lies between the latitudes 41°03'46.42"-41°07'42.35"N and longitudes 37°28'45.63"-37°41'15.29"E. The metal contents of the samples were analysed by the inductively coupled plasma - optical emission spectrometry (ICP-OES) technique. The results showed that the accumulation orders of the metals, in a descending order, were Fe>Al>Mn>Zn>Cr>Pb>Cu>As>Ni>Cd in the sediment, Fe>Mn>Cr>As>Cd in the water, and Al>Fe>Cu>Mn>Zn in crab tissues when evaluated together. In the crab samples, the amount of the accumulation of all metals was ordered as gills>exoskeleton>hepatopancreas>muscle. Al and Fe were the predominant metals in the sediment and crab tissues but Al was not detected in the seawater samples. A correlation test was performed to reveal the interaction of accumulation in the sediment, water and crab tissues. Metal-metal interactions and their co-accumulation was detected by correlation test. These interactions which exist in the crab tissues but not in the seawater and sediment were the main point of this study. Gills and exoskeleton displayed the greatest number of significant correlations between metal-metal interactions. Also, metal concentrations were found to be higher in the gills and exoskeleton. Strong correlations between Mn-Al ($r=0.954$, correlation $p<0.001$), in the exoskeleton, Al-Fe ($r=0.849$, correlation $p<0.001$), Mn-Zn ($r=0.854$, correlation $p<0.001$) in the gills, Al-Zn ($r=0.882$ correlation $p<0.001$) in the hepatopancreas were determined. Moderate correlations between Zn-Cu were found in the hepatopancreas. These metal-metal interactions may have been a result of metallothionein activity. No significant relations were found between metal levels in sediment samples and crab tissues ($p>0.05$). The results also showed that metals present in the seawater and sediment did not directly transform to tissue accumulation. This result showed that metal amounts in the tissues of the *P. marmoratus* did not reflect environmental contaminations and that sediment accumulated higher amounts of metals than seawater and tissues.

Key words: Correlation, metabolic pathways, transferrin, metallothionein.

Özet: Bu çalışma, bazı metallerin (Al, As, Cd, Cr, Cu, Fe, Mn, Ni, Pb ve Zn) Karadeniz (Ordu, Türkiye) kıyısı boyunca dere ve evsel atık bölgelerine yakın noktalardan örnekleme yapılmış mermer yengeci *Pachygrapsus marmoratus* (Fabricius)'un dış iskelet, solungaç, hepatopankreas ve kas dokularındaki birikim ilişkilerini etkileşimlerini araştırmak amacıyla yapılmıştır. Karadeniz boyunca 28 km'lik bir kıyı şeridini kapsayan çalışma alanı 41°03'46.42"-41°07'42.35"N enlemleri ve 37°28'45.63"-37°41'15.29"E boylamları arasındadır. Numunelerin metal içerikleri indüktif eşleşmiş plazma - optik emisyon spektrometrisi (ICP-OES) tekniği ile analiz edilmiştir. Elde edilen sonuçlar birlikte değerlendirildiğinde, sedimentte Fe>Al>Mn>Zn>Cr>Pb>Cu>As>Ni>Cd, suda Fe>Mn>Cr>As>Cd ve yengeç dokularında Al>Fe>Cu>Mn>Zn şeklinde gözlenmiştir. Yengeç örneklerinde, tüm metallerin dokularda birikim miktarının sırası solungaç>dış iskelet>hepatopankreas>kas şeklindedir. Al ve Fe, sediment ve yengeç dokularında baskın metaller iken, deniz suyu örneklerinde Al tespit edilmemiştir. Sediment, su ve yengeç dokularında birikimin etkileşimini ortaya koymak için bir korelasyon testi yapılmıştır. Metal-metal etkileşimleri ve bunların birlikte birikimi korelasyon testi ile tespit edilmiştir. Yengeç dokularında gözlenen, ancak deniz suyu ve sedimentte bulunmayan bu etkileşimler çalışmanın temel noktasını oluşturmuştur. Metal-metal etkileşimleri arasında en fazla anlamlı korelasyon solungaç ve dış iskelette gözlenmiştir. Ayrıca, solungaçlarda ve dış iskeletlerde metal konsantrasyonları daha yüksek bulunmuştur. Dış iskelette Mn-Al ($r=0,954$, korelasyon $p<0,001$), solungaçta Al-Fe ($r=0,849$, korelasyon $p<0,001$), Mn-Zn ($r=0,854$, korelasyon $p<0,001$), hepatopankreasta Al-Zn ($r=0,882$ korelasyon $p<0,001$) arasında kuvvetli korelasyonlar tespit edilmiştir. Hepatopankreasta Zn-Cu arasında ise orta dereceli bir korelasyon bulunmuştur. Bu metal-metal etkileşimleri metallothionein aktivitesinin bir sonucu olabilir. Sediment örnekleri ve yengeç dokuları arasında anlamlı ilişki bulunamamıştır (korelasyon $p>0,05$). Hatta sonuçlar göstermiştir ki deniz suyu ve sedimentindeki metaller, dokudaki birikime direkt dönüşmemiştir. Bu sonuç *P. marmoratus*'un dokularındaki metal miktarının çevresel kontaminasyonu yansıtmadığını göstermektedir ve aynı zamanda sediment sonuçlarının deniz suyu ve yengeç dokularından daha fazla metal birikimi miktarını gösterdiği tespit edilmiştir.

Introduction

The coastal areas and estuaries which connect land and sea, provide spawning, nesting and feeding area, and transport nutrient and organic materials for crabs are critically important transition zones (CTZs) (Kasmin 2010). The CTZs fulfil fundamental ecological functions such as decomposition, nutrient cycle and nutrient production. Crabs living in such areas and the sediment present here are important components of these fundamental functions. The most critical function of crabs is achieved by enriching the sediment by processing organic material. Considering the fact that sea pollution is mostly caused by industrial, domestic and agricultural wastes, the major threats to biodiversity in the CTZs appear to be due to anthropogenic effects such as non-traditional pollutants (heavy metals, pesticides, new generation contaminants, drugs, nanoparticles, etc.), species invasions, overfishing, and climate change under the influence of industry, urbanisation and agricultural areas (Kasmin 2010).

CTZs and crabs in marine ecosystems can potentially be exposed to sub-lethal concentrations of a wide range of chemicals originating from anthropogenic sources. Considering the environmental conditions, the most dangerous environmental pollutants are heavy metals. It's a matter of vital importance to understand the existence, accumulation and distribution of heavy metals in marine ecosystems for which heavy metal pollution is a danger. Heavy metal accumulation in crab tissues can be higher than accumulation levels in seawater and sediment (Rainbow 2007), which may imply that there is an acceleration of environmental toxicity. This, in turn, raises the need of certain sensitive monitoring methods to be used to prevent, or least minimize, destructive effects of metal pollutions on marine species (Bresler *et al.* 2003).

There exist some monitoring systems which include chemical analysis of abiotic factors such as seawater and sediment. Although these monitoring systems produce accurate results, it is impossible to provide sufficient data on ecological situation when the number and range of contaminants exceed the ability of the chemical tests applied or when there are possible synergistic effects of contaminants on each other (Phillips & Rainbow 1994).

Metals and metalloids are important environmental pollutants and may show severe acute or chronic effects on living organisms (Ullah *et al.* 2015). Species dispersed in various habitats can play an important role for ecosystems. For instance, any variable that affects crabs can have a major impact on the habitat and the ecosystem (Siddon & Witman 2004, Pandya & Vachharajani 2011, Trivedi *et al.* 2012, Arya *et al.* 2014). Crabs are a vital component of food chain in marine ecosystems, and control ecological function by feeding on both detritus and organic matter (Parsa *et al.* 2014). *Pachygrapsus marmoratus* (Fabricius) is the most common rocky shore inhabitant of the Mediterranean, Black Sea and East Atlantic coasts, and is active both under and above seawater for long periods of time (Cannicci *et al.* 1999).

Organisms need metals in varying quantities. A high concentration of any metal is toxic to organisms. Metal transporters, i.e. transferrin, natural resistance-associated macrophage protein (NRAMP) family, ZIP (ZRT and IRT-like protein) family, etc. are involved in the procurement of metals as in the case of transportation, storage and remobilization of metals to proteins that need metals in their structures (Kramer *et al.* 2007). Metals can accumulate in organisms through different metabolic pathways. These metabolic pathways are not selective about the metal since they can carry more than one metal (Menon *et al.* 2016). The lack of selectivity of the metabolic pathways leads to a competition between the metals and forms the basis of metal accumulation in organisms.

The studies performed with the aim of determination of metal levels in Black Sea region mostly used various fish species as an indication of accumulation of metal pollution in living organisms. In one of these study, Filazi *et al.* (2003) evaluated levels of Cu, Pb, Cd, Cr and Ni in liver and muscle tissues of *Mugil auratus* Risso (syn. *Chelon auratus* (Risso)) collected from Sinop-Icliman, Black Sea, Turkey and found that the highest level of accumulation was of Pb. Tüzen (2003) determined concentrations of Pb, Cd, Fe, Cu, Mn and Zn in the fish species *Alosa caspia* (Eichwald), *Engraulis encrasicolus* (L.), *Trachurus trachurus* (L.), *Sarda sarda* (Bloch), and *Clupea sprattus* (L.) sampled from the middle Black Sea Region and found that levels of the essential metals in the samples were higher than those of the non-essential metals. Altas & Büyükgüngör (2007) investigated the heavy metals levels in water samples of the Middle Black Sea region of Turkey from May 2000 to October 2001 and determined that Cd and Cu levels generally and Pb and Zn levels sometimes exceeded the levels reported in the Marine General Quality Criteria, while Ni concentrations were at desired levels. Makedonski *et al.* (2017) were investigated Cd, As, Hg, Pb, Zn and Cu levels in edible parts and gills of seven Bulgarian fish species ((*Sprattus sprattus* (L.), *Trachurus mediterraneus ponticus* Aleev (syn. *Trachurus mediterraneus* (Steindachner)) *Neogobius melanostomus* (Pallas), *Alosa pontica* (Eichwald) (syn. *Alosa immaculata* (Bennett)), *Sarda sarda*, *Pomatomus saltatrix* (L.), *Mugil cephalus* L. collected from north-east coast of Black Sea and found that the metal concentrations were highest in the gills for all fish species, showing that metal accumulation varied between fish species, tissues and the metals analysed.

In the present study, the marbled crab *Pachygrapsus marmoratus* (Fabricius) sampled from different streams and domestic discharge points along the coastal region of Black Sea in Ordu, Turkey was investigated for accumulation levels of Al, As, Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn and in exoskeleton, gill, hepatopancreas and muscle tissues. The sediment and seawater samples from the crab sampling sites were also analysed for the same purpose. *Pachygrapsus marmoratus* was chosen as the

study material because it has previously been reported as a bioindicator for metal accumulation (Álvaroa *et al.* 2016). The sampling localities were selected from the coastal region of Ordu where no former study has been performed addressing metal accumulation levels. The results were analysed based on metal-metal interactions and co-accumulations and the obtained data was discussed with regard to the effects of metabolic pathways in co-accumulation.

Materials and Methods

Sample collection

The samples included in the study were obtained in February 2015 from 8 different stations along the coastal region of Black Sea in Ordu in Black Sea Region of Turkey. Stations were selected near from streams (Bolaman, Elekçi, Ilica and Çalış) and domestic discharge points near human settlements (Fig. 1).

Table 1. Latitude and longitude of the sampling stations.

Station	Latitude	Longitude
S1	41°03'46.42"N	37°28'45.63"E
S2	41°02'18.59"N	37°29'33.06"E
S3	41°01'17.57"N	37°32'13.97"E
S4	41°02'01.92"N	37°35'02.09"E
S5	41°02'57.34"N	37°36'35.45"E
S6	41°04'51.87"N	37°37'44.11"E
S7	41°06'30.37"N	37°39'06.73"E
S8	41°07'42.35"N	37°41'15.29"E

The study area covered a coastal stretch of 28 km along Black Sea (Figure 1, Table 1). The 10 male crab samples from each location (N=80) were randomly collected directly by hand picking. Only male individuals were studied to prevent the results will not affect from gender. 2 surface seawater and 2 surface sediment

samples from each station (N=16) were also sampled from the crab sampling sites.

Metal Analysis

Male crabs were immediately transported live to Toxicology Laboratory of Ankara University in frosted plastic storage boxes and stored at -20°C until the analyses performed. All samples were dissected immediately to obtain the tissues for metal analysis. Approximately 2g of tissue samples were taken from hepatopancreas, muscles, exoskeleton and gills through dissection and all were digested for the inductively coupled plasma/optical emission spectroscopy (ICP-OES) (Agilent, 5110) analysis. Tissue samples were placed in porcelain crucibles, dried at 50-60°C for 24h and then weighed to determine their net dry weights. Approximately 1g of each tissue sample was used for the measurements. 3mL of nitric acid (HNO₃) (Merck) were added to the dried tissue samples. The tissues were kept at 100°C temperature for 20min on a heating table to achieve a thorough digestion (Núñez-Nogueira *et al.* 2013). After the samples were cooled, 5mL of distilled water (pure water) was added on each. The samples were then filtered with a 0.45µm pore size glass microfiber Whatman filter paper with the help of a syringe.

Sediment samples were taken superficially from the same coordinates with a Bridge-Ekman grab sampler from sampling stations and samples were put in plastic containers. Both seawater and sediment samples were kept at 4°C prior to analysis. The water samples were acidified with HNO₃ at pH 2 and filtered before analysis. The samples were read in triplicate. Prior to analysis, the sediment samples were combined in a FRITSCH tungsten carbide mortar, mixed with the binder material (Wachs) at ICP-OES measurements. The sediment and seawater samples were analyzed by ICP-OES. It was service procurement from Cinar Engineering Consulting Co. for the reading of the samples. Reference materials (CRM-Mess 4 for sediment and LUTS-1 for tissues) were also analysed to avoid any error with samples. The reference material values and control samples results displayed good harmony between each other.

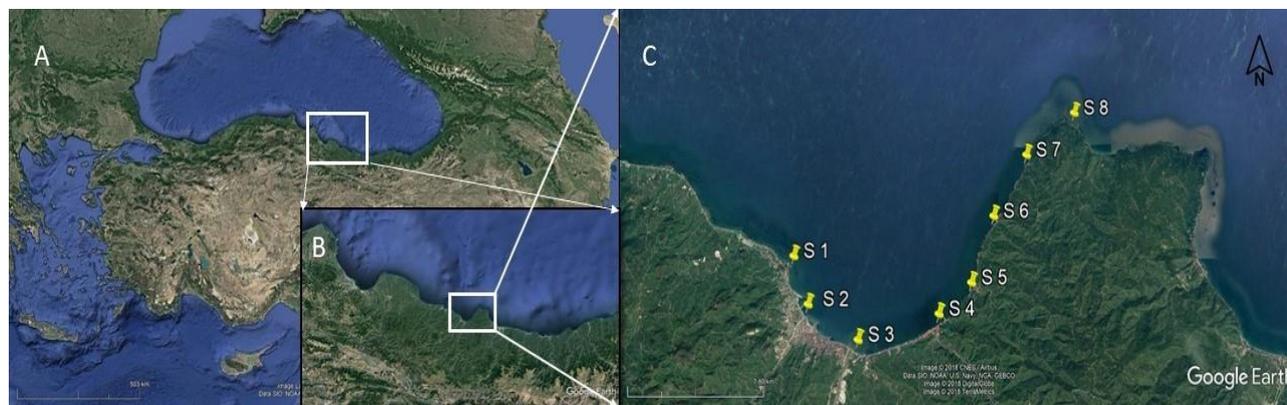


Fig. 1. The map showing the sampling locations. (A) Turkey, (B) Ordu province (C) Sampling Stations

Statistical Analyses

All statistical analyses were performed by the SPSS 21.0v. software. Prior to analysis, all results were subjected to the Kolmogorov–Smirnov test to observe the normality of data distribution. Data sets that fit a parametric distribution were analysed by the Pearson's correlation test, whereas those which did not fit a parametric distribution were analysed by the Spearman's correlation rank test. Mann-Whitney U-test test was used to investigate the accumulation differences in non-parametric data.

Results

The results showed that among the tested metals, only Al, Cu, Fe, Mn and Zn were detected in the crab tissues. When the accumulation levels of these metals were evaluated in all the tissues as a whole, the highest accumulation value was determined for Al followed by

Fe, Cu, Mn and Zn (Table 2). The correlation analysis results revealed different correlation values for metal pairs for each tissue (Table 3). The correlations were strong and very strong for most of the pairs. The tissues were examined to investigate the metal co-accumulation tendency (Table 3). Gills and exoskeleton were found to show the greatest number of significant correlations of metal-metal interactions (Table 3). Strong correlations between Mn-Al ($r=0.954$, $p<0.001$), in the exoskeleton, Al-Fe ($r=0.849$, $p<0.001$) and Mn-Zn ($r=0.854$, $p<0.001$) in the gills and Al-Zn ($r=0.882$, $p<0.001$) in the hepatopancreas were noted.

The results showed that metal concentrations were found to be very lower in seawater samples than sediment samples (Table 4). The predominant metals in the sediment were Al and Fe.

Table 2. Mean values of metal concentrations in crab tissues ($\mu\text{g/g}$ dry weight).

Tissues	Al	Cu	Fe	Mn	Zn
Hepatopancreas	123.4±12.9 (25.5-325.9)	12.1±3.2 (1.7-35.6)	9.1±1.4 (1.3-13.8)	2.2±1.3 (1.1-4.1)	4.2±2.1 (1.4-7.9)
Exoskeleton	711.6±28.4 (74.3-1658)	14.3±5.2 (4.7-42.2)	79.2±11.7 (10.2-161.8)	6.4±2.3 (1.8-10.7)	6.5±2.3 (4.2-8.8)
Muscle	20.9±12.7 (6.0-35.7)	7.4±1.7 (1.04-26.02)	4.0±1.1 (0.6-8.2)	1.7±0.5 (0.5-3.6)	2.0±0.7 (0.6-3.04)
Gills	806.0±100.6 (97.4-1674.8)	17.8±8.6 (10.8-23.6)	120.0±12.5 (9.8-241.7)	11.5±3.1 (3.9-20.9)	10.9±4.9 (8.4-15.3)

Table 3. Correlations of accumulation levels of metal pairs in crab tissues.

	All tissues	Fe	Cu	Zn	Al
Cu		.604**			
<i>p</i>		.000			
Zn		.576**	.760**		
<i>p</i>		.000	.000		
Al		.840**	.560**	.582**	
<i>p</i>		.000	.000	.000	
Mn		.431**	.483**	.435**	.487**
<i>p</i>		.001	.000	.001	.000

Exoskeleton	Fe	Cu	Zn	Al
Cu	.628*			
<i>p</i>	0.007			
Zn	.654**	.700**		
<i>p</i>	.008	.004		
Al	.664**	0.496	.714**	
<i>p</i>	.007	.060	.003	
Mn	.752**	.588*	.758**	.954**
<i>p</i>	.001	.021	.001	.000

Gills	Fe	Cu	Zn	Al
Cu	.571*			
<i>p</i>	.026			
Zn	.618*	.764**		
<i>p</i>	.014	.001		
Al	.849**	0.481	.549*	
<i>p</i>	.000	.070	.034	
Mn	.679**	.725**	.854**	.690**
<i>p</i>	.005	.002	.000	.004

Hepatopancreas	Fe	Cu	Zn	Al
Cu	.782**			
<i>p</i>	.001			
Zn	.618*	.782**		
<i>p</i>	.014	.001		
Al	.632*	.679**	.882**	
<i>p</i>	.011	.005	.000	
Mn	-0.504	-0.175	-0.375	-0.439
<i>p</i>	.056	.533	.168	.101

Muscle	Fe	Cu	Zn	Al
Cu	-.150			
<i>p</i>	.593			
Zn	-0.271	.729**		
<i>p</i>	.328	.002		
Al	.575*	.048	-.100	
<i>p</i>	.025	.864	.723	
Mn	-.150	.445	.421	.343
<i>p</i>	.594	.096	.118	.211

*The mean difference is significant at the 0.05 level ($p < 0.05$).

**The mean difference is significant at the 0.01 level ($p < 0.01$).

Table 4. Metal concentrations in sediment ($\mu\text{g/g}$) and seawater ($\mu\text{g/L}$) samples.

Sediment	Al	As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn
S1	12560.2	12	0.08	18.9	25.8	15432	316.2	12.8	20.8	30.9
	14499.4	8	0.12	19.1	20	12774.2	400.6	14	17.6	81.3
S2	13002.2	23.7	0.09	38.9	28.3	10937.8	432.2	17.3	24.7	79.8
	9050.4	12.3	0.11	41.1	10.1	31156.8	554	12.1	20.9	59.6
S3	8931.3	22.8	0.18	33	16.1	21537.6	602.3	16.1	27.3	81.2
	7415.9	14.8	0.22	22.8	15.1	12830.8	552.1	14.1	23.5	57.8
S4	14992.9	14	0.05	17.5	10.7	21823.9	389	14.7	26.3	76.5
	12767.9	7	0.15	7.5	14.3	10190.7	507.2	16.1	12.1	106.5
S5	14322.8	12.8	0.1	14.2	12.3	18348	332.5	20	20.6	27.4
	14295.6	6	0.1	12.2	15.1	10456	381.9	15.2	17.6	77
S6	11657.8	21.3	0.19	21.3	18.9	20962.6	496.9	14	25.2	37.9
	12268	14.7	0.21	30.1	12.1	20136.2	476.5	12	14.4	67.3
S7	17891.7	12.1	0.08	32.4	22.1	23656.9	666.8	13.3	27.3	101.7
	6850.7	26.3	0.12	34	16.3	22247.7	484.4	13.1	18.5	89.7
S8	8449.5	17.2	0.31	17.1	25.5	18932.8	382.3	17.2	18.4	62.3
	14578.5	22	0.09	13.1	22.9	22766.2	498.7	18	31	57.3
Mean \pm SD	12095.9 \pm 3035.5	15.4 \pm 6.01	0.14 \pm 0.07	23.33 \pm 9.9	17.9 \pm 5.5	18386.9 \pm 5664.2	467.1 \pm 94.8	15 \pm 2.2	21.6 \pm 4.99	68.4 \pm 22.3
Seawater	Al	As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn
S1	N.D.	1.2	1.3	0.7	N.D.	658.7	145.8	N.D.	N.D.	N.D.
	N.D.	0.6	1.1	0.5	N.D.	564.9	211.8	N.D.	N.D.	N.D.
S2	N.D.	1.3	0.3	1.7	N.D.	892.6	159.3	N.D.	N.D.	N.D.
	N.D.	0.5	0.7	1.1	N.D.	816.8	91.7	N.D.	N.D.	N.D.
S3	N.D.	0.8	1.1	0.8	N.D.	554.2	102.9	N.D.	N.D.	N.D.
	N.D.	0.6	0.7	1	N.D.	359.3	90.9	N.D.	N.D.	N.D.
S4	N.D.	0.9	0.3	0.3	N.D.	743.6	103	N.D.	N.D.	N.D.
	N.D.	1.7	0.7	0.5	N.D.	721.4	117.6	N.D.	N.D.	N.D.
S5	N.D.	0.8	0.9	0.9	N.D.	1021.6	129.5	N.D.	N.D.	N.D.
	N.D.	0.6	0.7	0.7	N.D.	518.2	108.7	N.D.	N.D.	N.D.
S6	N.D.	1.1	0.3	0.2	N.D.	903.2	192.3	N.D.	N.D.	N.D.
	N.D.	0.5	0.5	0.6	N.D.	579.8	78.7	N.D.	N.D.	N.D.
S7	N.D.	0.8	0.6	0.8	N.D.	432.2	129	N.D.	N.D.	N.D.
	N.D.	0.8	0.6	0.4	N.D.	442	126	N.D.	N.D.	N.D.
S8	N.D.	0.9	0.3	0.3	N.D.	506.3	120.2	N.D.	N.D.	N.D.
	N.D.	1.1	0.5	0.5	N.D.	602.1	100	N.D.	N.D.	N.D.
Mean \pm SD	N.D.	0.9 \pm 0.3	0.7 \pm 0.3	0.7 \pm 0.4	N.D.	644.8 \pm 183.6	125.5 \pm 35.4	N.D.	N.D.	N.D.

N.D.: Not Detected.

Correlation analyses were also performed to reveal metal-metal accumulation interactions, if any, in seawater and sediment samples (Table 5). Some correlations determined in the crab tissues but not in seawater and sediment samples were considered as indicators of metal-metal interactions and co-accumulation. Significant correlations in the sediment samples were among Mn-Pb ($r=0.743$, $p=0.035$), As-Pb ($r=0.916$, $p=0.01$), As-Fe ($r=0.790$, $p=0.02$), Cr-Mn ($r=0.738$, $p=0.37$), Al-Pb ($r=-0.874$, $p=0.005$) and Al-Mn ($r=-0.714$, $p=0.047$). The level of correlation between metal concentrations in crab tissues and the sediment samples were also tested but no significant correlation was determined whereas significant correlations were observed between concentration values in crab tissues (Table 6). The results showed that

metals present in the seawater and sediment did not directly transformed to accumulations in crab tissues.

Discussion

The accumulation orders of metals were determined as $\text{Al} > \text{Fe} > \text{Cu} > \text{Mn} > \text{Zn}$ in crab tissues, $\text{Fe} > \text{Mn} > \text{Cr} > \text{As} > \text{Cd}$ in the seawater and $\text{Fe} > \text{Al} > \text{Mn} > \text{Zn} > \text{Cr} > \text{Pb} > \text{Cu} > \text{As} > \text{Ni} > \text{Cd}$ in the sediment. Al and Fe were found to be predominant metals in the sediment but Al was not detected in the water samples. This may be related to the fact that sediment particles tend to absorb metals in the water, and this metal with high molecular weight tend to precipitate at the bottom (Table 2, 4).

Metal concentrations in the sediment and seawater samples were measured to determine whether accumulation levels in crab tissues signify environmental contamination. Compared to the crab tissues and

seawater, there were higher levels of metal concentrations in the sediment. This can be attributed to the fact that the sediment in wetlands assumes a storage role in terms of heavy metal accumulation. It has been reported that sediment accumulates metals at higher amounts compared to water and tissues (Mendil & Uluözlu 2007, Yıldız & Yener 2010, Oner & Celik 2011, Kır & Tumantozlu 2012, Fikirdeşici Ergen *et al.* 2015).

Although a positive strong correlation between As-Pb ($r=0.916$, $p=0.001$) and a negative strong correlation between Al-Pb ($r=0.874$, $p=0.005$) were determined in sediment samples, there was no correlation among the metals in seawater samples. This means that the metal concentrations in the sediment were not affected by the changes in the seawater samples. This can also be explained by the fact that metals are under the influence of processes such as absorption and desorption (Hung *et al.* 2001).

Among the investigated metals, only Fe, Cu, Zn, Al and Mn were detected in crab tissues. The amount of accumulation of these metals in crab tissues revealed an order as gills>exoskeleton>hepatopancreas>muscle (Table 2) most probably because gills and exoskeleton tissues are in contact with the external environment (Tunca *et al.* 2013a). The accumulation level of metals in the muscle tissue is less than the other tissues. This can be

explained by the limited occurrence of metal binding proteins in muscle tissue (Guner 2007). Al was determined at high levels in all tissues. Gills in particular accumulated Al at higher levels compared to other tissues. The levels of Al accumulation in hepatopancreas and muscle were lower than in the gills and exoskeleton as reported in previous studies (Alexopoulos *et al.* 2003, Kurun *et al.* 2010, Tunca *et al.* 2013b). The gills are the primary sites for the uptake of soluble metals from the aquatic environment (Walton *et al.* 2010). Gills were found to be the prime site of Al accumulation in this work. There was higher accumulation level of Al in the tissues ($p<0.05$) since it is as an essential element (Woodburn *et al.* 2011) and also essential metals were reported to be transported to other organs by the gills (Nott 1991, Guner 2007). The results of these studies also showed that gills play an important role in Al intake and this intake cause excessive mucus secretion.

Metal uptake and accumulation can be facilitated by respiration and digestion in aquatic animals. Metals have a point of entry from the environment to the animal's body. Gills and exoskeleton are the primary point of entry and they revealed the greatest number of significant correlations of metal-metal interactions in the present study (Table 3).

Table 5. Correlations between metal pairs in seawater and sediment samples.

Sediment	Cu	Pb	Zn	Ni	Mn	Fe	As	Cd	Cr
Pb	0.494								
<i>p</i>	.213								
Zn	0.072	0.431							
<i>p</i>	.866	.286							
Ni	-0.096	-0.084	-0.156						
<i>p</i>	.820	.843	.713						
Mn	0.012	.743*	0.643	-0.455					
<i>p</i>	.978	.035	.086	.257					
Fe	0.323	0.647	0.571	-0.287	0.643				
<i>p</i>	.435	.083	.139	.490	.086				
As	0.524	.916**	0.479	-0.096	0.611	.790*			
<i>p</i>	.182	.001	.230	.820	.108	.020			
Cd	0.17	0.624	-0.282	0	0.282	0.169	0.567		
<i>p</i>	.687	.099	.499	1.000	.499	.689	.143		
Cr	0.395	0.563	0.357	-0.611	.738*	0.667	0.443	0.056	
<i>p</i>	.333	.146	.385	.108	.037	.071	.272	.895	
Al	-0.443	-.874**	-0.238	0.18	-.714*	-0.548	-0.695	-0.62	-0.69
<i>p</i>	.272	.005	.570	.670	.047	.160	.056	.101	.058

Seawater	Mn	Fe	As	Cd
Fe	0.214			
<i>p</i>	.610			
As	0.012	0.073		
<i>p</i>	.977	.864		
Cd	0.108	-0.241	-0.442	
<i>p</i>	.798	.565	.273	
Cr	0.22	0.39	-0.522	-0.173
<i>p</i>	.601	.339	.185	.682

*The mean difference is significant at the 0.05 level ($p < 0.05$).

**The mean difference is significant at the 0.01 level ($p < 0.01$).

Table 6. Correlations between metal levels in crab tissues and the sediment samples.

	Hepatopancreas	Exoskeleton	Muscle	Gills
Exoskeleton	.818**			
<i>p</i>	.004			
Muscle	.842**	.927**		
<i>p</i>	.002	.000		
Gills	.903**	.794**	.782**	
<i>p</i>	.000	.006	.008	
Sediment	.073	-.220	-.134	.152
<i>p</i>	.841	.542	.712	.674

Exposure time and metal concentrations are some of the important reasons for bioaccumulation (Anderson *et al.* 1997). Therefore, different tissues in an organism or the same tissues in different individuals of the same organism can accumulate high concentrations of the same metal. So, it is possible to find examples for that different tissues accumulate same metals. For instance, Chagas *et al.* (2009) found that hepatopancreas was the main tissue to accumulate Mn, whereas in the present study, the results indicated gills as the principal site of accumulation for Mn. The strongest correlation was found between Mn-Al ($r=0.954$, $p<0.001$) in the exoskeleton. Moderate correlation was determined between Mn-Al ($r=0.690$, $p=0.004$) in the gills but not in hepatopancreas and muscle (Table 3). This suggests that Mn and Al can have the same natural origin and there are similarities between the adsorption chemistries of these metals. In addition, the correlation between Mn-Al might be due to the transportation of these metals by transferrin protein because common use of metabolic pathways can lead to strong correlations in tissues (Cohen *et al.* 1998). However, negative moderate correlation between Mn-Al ($r=-0.714$, $p=0.047$) was determined in sediment samples which points out that the sighted effect is not a reflection of metal background in the environment (Table 5). This might be related with the fact that there is an antagonism between these metals during absorption and it may be related with the competitive effects over transferrin binding. Transferrin has an important role especially in the transport of Fe (Chua *et al.* 2007) but it also plays a role in transports of Al^{3+} , Cr^{3+} , Cu^{2+} , Ga^{3+} , Ni^{2+} , Ti^{4+} and Zn^{2+} (Quarles *et al.* 2011). A positive strong correlation between Mn-Zn ($r=0.854$, $p<0.001$) in the gills and a moderate correlation ($r=0.758$, $p=0.001$) in the exoskeleton were found while there were no correlations between these metals in the hepatopancreas and muscle. Bervoets *et al.* (2001) found the same strong correlation between Mn-Zn in the gills of the *Gastrosteus aculeatus* L.

A strong correlation was determined between Al-Fe in the gills ($r=0.849$, $p<0.001$) whereas a moderate correlation was determined between Al-Fe in other tissues. All correlations between Al and Fe were found to be positive. Kurun *et al.* (2010) reported similar correlations between Al and Fe accumulations in the crayfish *Astacus leptodactylus* (Eschscholtz). This result may be due to one or more of the following items: (1) similarities of Fe and Al in metal adsorption kinetics, (2)

similar chemical properties of Fe and Al, (3) the major protein, transferrin which plays a major role in the Fe transport and Al absorption (Moshtaghi & Taher 1993). The correlation results of Al accumulation with Cu and Zn are remarkable in hepatopancreas since hepatopancreas is the central place for accumulation of various metals and detoxification in decapod crustaceans (Tunca *et al.* 2013a).

Moderate correlation between Zn and Fe accumulations in hepatopancreas ($r=0.618$, $p=0.014$), exoskeleton ($r=0.654$, $p=0.008$), and gills ($r=0.618$, $p=0.014$) were determined whereas there was no correlation between Zn and Fe accumulations in muscle. Zn is an important essential metal for decapods and serves as a cofactor for the enzymatic systems. Additionally, metalloenzymes use Zn as the active core (Alcorlo *et al.* 2006). A moderate correlation was determined between Cu and Fe accumulations in hepatopancreas ($r=0.782$, $p=0.001$), exoskeleton ($r=0.628$, $p=0.007$) and gills ($r=0.571$, $p=0.026$) whereas there was no correlation present between Cu and Fe accumulations in muscle. The exoskeleton serves as a structural support and has biomineral composite, so it tends to accumulate copper from the water environment (Soedarini *et al.* 2012). Gills, the respiratory organs of crabs, were indicated to be sensitive to changes in copper concentrations in seawater. Copper in the water binds to hemocyanin by respiration and then circulated to all organs of the crustacean.

Some of the metal-metal correlations found in gills, exoskeleton and hepatopancreas were not present in the muscle tissues. A metal should gain an entry point from the gills and the exoskeleton to be transported to another tissue. For this reason, high correlations in these tissues are expected. Furthermore, especially gills are primary site of metal entry because they are rich in blood vessels. Gills are under effects of a blood-borne transfer protein such as transferrin so they may be related to metal-metal interactions more than other tissues. Another reason may be that the binding proteins are more prominent in the gills (Guner 2007). The lack of correlations in the muscle tissue can be explained by the lack of these proteins in the muscle tissue.

A moderate correlation between Zn-Cu was found in all tissues but the correlation in hepatopancreas was slightly higher ($r=0.782$, $p=0.001$). Metallothioneins are one of the most important metal sequestering protein families and hepatopancreas is a main site of metal sequestration in crustaceans. Metallothioneins plays an important role in preventing undesirable reactions in the transfer of IB and IIB metals such as Zn, Cu and Hg (Naji *et al.* 2014). For this reason, they are important proteins in preventing metal toxicity. If they are found in hepatopancreas at high concentrations, they help detoxify dangerous metals (Pourang *et al.* 2005). The production of the metallothionein in hepatopancreas is correlated with Zn and Cu accumulation in this tissue. Bochenek *et al.* (2008) found relationships between tissues (kidney, liver, gill and muscle) concentrations of Zn and Cu of the

Rutilus rutilus (L.) and the sediment. In the present study, no correlation between Zn-Cu was determined in sediment samples.

When the correlations among tissues were examined, it was determined that the highest correlation was between muscle-exoskeleton ($r=0.927$, $p<0.001$) and gills-hepatopancreas ($r=0.903$, $p<0.001$). These results were similar to study of the Fikirdeşici Ergen *et al.* (2015). There was also a high correlation between hepatopancreas-muscle and gills, and moderate correlations between gills-muscle and hepatopancreas (Table 6).

Conclusion

In conclusion, as a result of examining the metal accumulation profiles of crab tissues, it can be concluded that metal-metal interactions and metabolic pathways play important roles in accumulation of specific metals. Metal concentrations in the sediment and seawater samples showed that the metal amounts and correlations in the crab

tissues did not reflect any environmental contamination. Very low metal concentrations were found in seawater samples. No correlation was found between metal concentrations in crab tissues and the sediment samples whereas significant correlations were determined among the crab tissues, meaning that metals present in the seawater and the sediment did not directly transform into accumulation in crab tissues. In order to examine the existing metal deposits in an aquatic environment and to have an understanding of the metal pollution, it is absolutely necessary to conduct analysis on the sediment. It was concluded that the sediment sample results reflected reality more than those of the seawater and the tissues analysed in this study.

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