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METAL ACCUMULATION PROFILE IN CRAYFISH TISSUES FROM ÇOMAR STREAM

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ABSTRACT. The accumulation of Cu, Pb, Zn, Ni, Mn, Fe, Cr and Al in the exoskeleton, hepatopancreas, gills and muscle tissues of the *Astacus leptodactylus* (Eschscholtz, 1823) (crayfish) obtained from Çomar Stream were determined by correlation analysis. The strongest correlations were observed between Mn-Cu (r=0.780) and Fe-Cu (r=0.744) in the hepatopancreas. The highest metal concentration was observed in hepatopancreas, the lowest was determined in the muscle tissue. Sequence of metal concentration levels were Al> Fe> Mn> Zn> Cu> Ni> Pb> Cr in exoskeleton and gills, Al> Fe> Mn> Cu> Zn> Ni> Pb> Cr in hepatopancreas and Al> Mn> Fe> Zn> Cu> Pb> Ni=Cr in muscle.

1. Introduction

Heavy metals may originate from volcanoes, rocks and ore minerals. In the last few decades, the effect of the rapid increase in population and anthropogenic activities have led to a significant increase in entrance of the heavy metals to the wetlands. Mining, refining stages and extraction can be counted as anthropogenic source of heavy metals. Heavy metals once released into the aquatic environment and accumulate in the environment and biota without disappearing [1, 2]

Heavy metals are one of the most important environmental pollutant, which has a significant effect on the aquatic environment. They are threatening the ecosystem's stability. Simultaneously essential metals are so important due to yielding as micronutrients to aquatic organisms. Heavy metals are necessary for the optimal yielding as micronutrients to aquatic organisms. working of biochemical and biological activities in organisms. Unlike essential metals, non-essential elements have no known biological activities. Non-essential metals compete with essential metals for membrane protein sites or active enzyme [3, 4]. Unlike other pollutants, metals cannot be easily reduced to non-toxic forms. Therefore, they can be directly

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reach aquatic organisms by means of food chain and they tend to accumulate in sediments. Many aquatic organisms can accumulate significant quantities of metals in their tissues from sediment and nutrients [5, 6].

The behavior of metals in wetlands depends on the composition of the sediment and chemistry of water [7]. Heavy metals may undergo various changes such as dissolution, complexation, absorption and sedimentation, which affect their behavior and bioavailability during their transportation to wetlands [8, 9]. Some aquatic organisms are known as heavy metal bioindicators since their exposure to heavy metals are measurable with levels or effects [10]. It is possible to assess the crayfish as a bioindicator species because of their omnivorous diet, slow movement and limited habitat, having a benthic lifestyle and different body tissues that are large enough to be taken for analysis [11, 12].

Environmental conditions and metabolic pathways may affect accumulating and transporting of the metals in crayfish tissue. The harmful effects of metals are reduced without distinction of metal with various metal binding proteins and metal transport. Therefore, this non-selectivity allows the metal groups to be accepted by the same protein. This situation allows the metal correlations and metal-metal interactions [13]. This study aims to obtain the groups of metals (Cu, Pb, Zn, Ni, Mn, Fe, Cr and Al) by analyzing the correlations between their accumulations in crayfish tissues (gill, hepatopancreas, muscle and exoskeleton) and discuss with metal-metal interactions.

2. Materials and methods

With the help of local fishermen, 20 male crayfish (*Astacus leptodactylus*) samples were taken from Çomar Stream in April 2015. Crayfish samples were brought to the laboratory and stored at -18 °C until dissection. The length and weight parameters were measured before dissection (Table 1). Approximately 1 g of exoskeleton, gill, hepatopancreas and muscle samples were taken from each crayfish. Tissue specimens were digested with nitric acid (HNO_3) at 100 °C for 60 minutes and the metal concentrations were diluted with deionized water [14].

The concentrations of ^{65}Cu, ^{208}Pb, ^{66}Zn, ^{60}Ni, ^{55}Mn, ^{56}Fe, ^{52}Cr and ^{27}Al were evaluated through ICP-MS. Detection limits for these elements were determined at 0.1, 0.04, 0.04, 1.4, 0.01, 0.01, 0.9 and 0.5 ppb, respectively. Certified reference material LUTS-1 (non-defatted lobster hepatopancreas) was used as quality control. ICP-MS measurements were done using a Thermo-Scientific X-Series II ICP-MS equipped with a Cetac Asx-260 auto sampler accessory. 2% nitric acid in ultrapure water was used for all dilutions. Standard curves for all elements were based on the QCS-27 series of elements (High Purity Standards) and included the measurement

range for that element in crayfish tissues. Each calibration curve had correlation coefficient above 0.99. Calibration curves were redrawn every 40 measurements (twice in total). 10 ppb ^{209}Bi was used as the internal standard. Three replicates were performed for each sample; sampling and washing times were set at 60 s each.

In addition, 10 sediment and 10 water samples were collected. Sediment samples were collected from the surface with Bridge-Ekman grab. Both the stream water and sediment samples were kept at 4 °C prior to analysis. Water samples were acidified with HNO_3 at pH 2 and filtered before analysis. Reference material (CRM-Mess 4 for sediment mg/kg) was used for sediment samples. SPSS (21v) was used for statistical analysis. Correlation analysis was used to determine the trends between metal accumulations in each individual. Spearman analysis was used as the correlation analysis due to the number of available data.

TABLE 1. Size parameters of *Astacus leptodactylus* (crayfish).

	Total length (cm)	Carapace length (cm)	Carapace width (cm)	Weight (g)
Min	16.2	5.9	3.7	47.9
Max	27.3	8.1	6.9	121.6
Mean	22.6	6.7	5.7	86.2
Std. Dev.	2.5	0.6	0.5	22.3

3. Results and discussion

In this study, the degree of heavy metal accumulation in the tissues of crayfish samples collected from the Çomar Stream was investigated (Table 2). Heavy metal concentrations of stream and sediment samples were also measured to determine if the amounts of metal in the crayfish tissue reflect environmental pollution (Table 3). Metal concentrations determined from water samples were observed to be very low. Fe, Al and Mn metals were found as the dominant metals in the sediment samples.

Accumulation on the sediment and water (Table 4) and accumulation in the tissues (Table 5) were examined in terms of the correlation analysis. It was found that there were inter-metal correlations observed in crayfish tissues, which were not found in the sediment. When the correlations between metal concentrations were examined,

the correlations in the sediment were limited with Cu-Pb and Fe-Cr interactions, no correlation was observed in the water samples.

The number of metal correlations in the crayfish tissues was found higher than the sediment. When the correlations between the metals in the tissues and the metals in the sediment were examined, no interaction was observed with the sediment and the metals accumulated in the tissues (Table 6). This can be explained by the fact that the metals in the sediment are not converted directly into tissue accumulation [15].

Exoskeleton, gills, hepatopancreas and muscle were tested for the evaluating of metal-metal interactions. It was determined that the dominant metal was Al for each tissue (Figure 1). Al is a bioavailable and also toxic to freshwater organisms [16-18]. Al did not show any correlation between other metals in the exoskeleton and muscle tissues. This result concerning, Al may be caused by its trivalency. It has similar structure with Fe^{+3}. Therefore, they compete each other for the site of sorption. In addition, previous studies showed that accumulated Al is associated with the gill epithelium. This specific sorption site may be the reason for the lack of correlation for Al [18, 19].

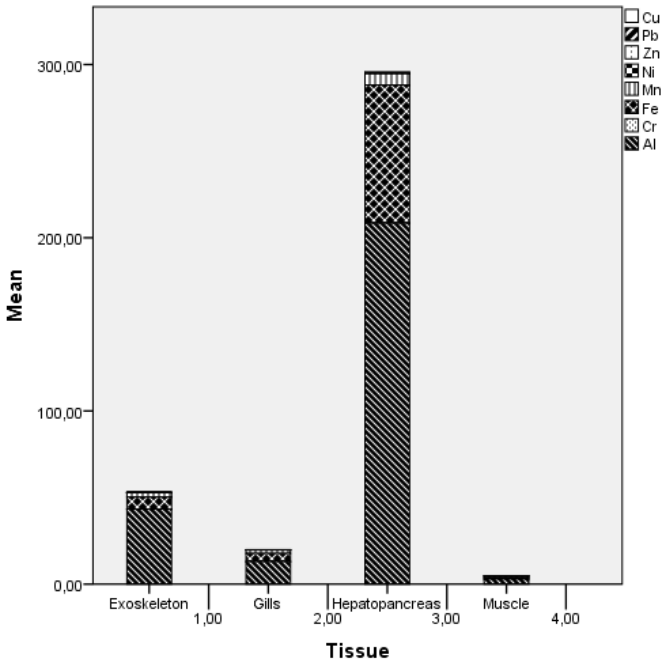

FIGURE 1. Graphical representation of metal accumulations between tissues (µg/g).

Literature information of the accumulated trends of heavy metal in *Astacus leptodactylus* were briefly listed in table 7. According our findings, primary sorption site was determined as hepatopancreas and it was in line with literature findings (Figure 2). Hepatopancreas was the site with the most correlation strength in general. Also it was found the greatest number of significant correlations between metal-metal pairs in the hepatopancreas (Table 5). It was probably due to metabolizable elements generally storage in the digestive gland [20]. It was found that hepatopancreas (digestive gland) combines variety of the functions of the vertebrate liver, pancreas and intestines [21]. Hepatopancreas is the main site of metal-storage, metal-detoxication, nutrient absorption and metal-secretion. Metal secretion property is due to the metallothionein protein family. Metallothionines (MT) are protein rich in cysteine amino acids that play an important role in the homeostasis and detoxification of metals [22]. MT is the most prominent protein group that has high affinity to metals and selectively binds metal ions at very low intracellular concentrations [23]. The highest correlations were found between Cu-Mn (r=, 780**) and Cu-Fe (r=, 744**) in the hepatopancreas tissue. It has been supported by many cell culture studies, where MT's are induced by two divalent cations (such as Cu^{+2}, Fe^{+2}, Mn^{+2}) [24]. Therefore, these divalent cation correlations in hepatopancreas may be explained by metallothionein activity. Naghshbandi et al. [25] analyzed accumulated metals in the crayfish (*A. leptodactylus*) tissue from the Aras Dam (Iran). Similar to our study, they found that the most accumulated metal was in the hepatopancreas tissue.

The highest correlation in the exoskeleton was found between the Zn-Fe (r=, 658**). Transferrin protein plays an important role in the transport of Fe metal in tissues. This protein also facilitates the transport of Zn metal [26]. This two-metal correlation may be explain by the transferrin protein. Correlations of the Zn-Mn-Fe in the exoskeleton may explain a metabolic function associated with new shell of crustaceans just before molt [27].

Gills are an important tissue in metal uptake and accumulation in crustaceans. In life of aquatic organisms, respiratory and digestive pathways are facilitators in metal accumulations. There must be an entrance to the body, and the gill and the exoskeleton are the entry points to the aquatic organisms. In our study, the accumulations and correlations in the gills are quite low. This may be explained by the fact that (1) the water, which the organisms are obtained, is not contaminated by metals and (2) the accumulated metals are already transported to the hepatopancreas for detoxification. The heavy metal analysis of the water and sediment shows that the stream is not polluted by heavy metals. In addition, the accumulation in the hepatopancreas compared to other tissues and the presence of more metal correlations is the indication that the metal has transport to this tissue. There are

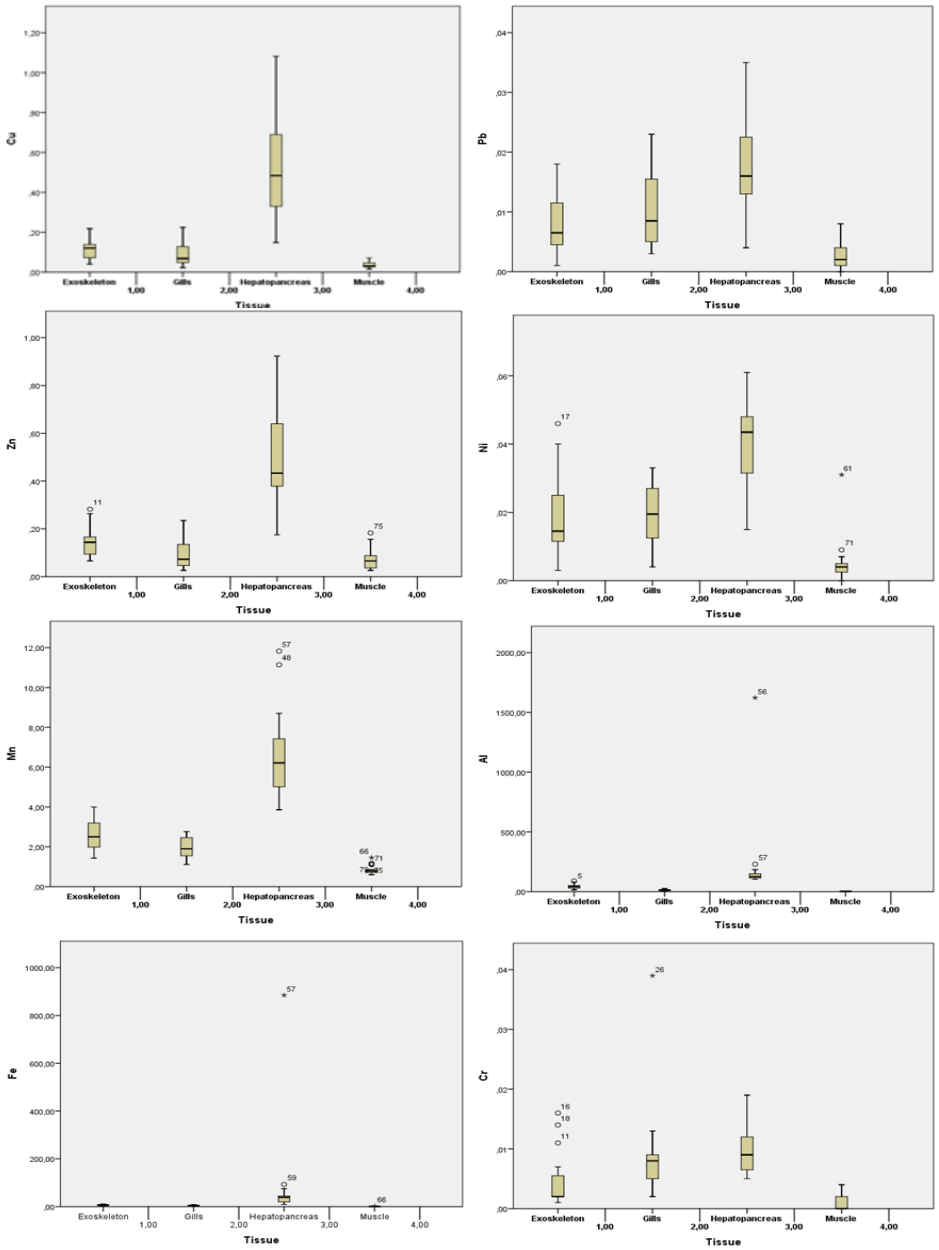

FIGURE 2. Boxplot comparison of metal accumulations between tissues (μg/g).

TABLE 2. Metal concentrations in crayfish tissues (dry weight µg/g). Data are given as Mean±SE.

	Cu	Pb	Zn	Ni	Mn	Fe	Cr	Al
Exo	0.11±0.05	0.01±0.005	0.14±0.06	0.02±0.01	2.59±0.79	6.99± 2.1	0.004±0.004	43.23±18.8
Gills	0.09±0.05	0.01±0.006	0.10±0.07	0.02±0.009	1.95±0.52	4.67±1.8	0.001±0.008	13.07±5.3
Hepato	0.53±0.25	0.02±0.008	0.49±0.19	0.04±0.01	6.56±2.02	79.78±185.7	0.001±0.004	208.36±325.9
Muscle	0.04±0.02	0.003±0.002	0.07±0.04	0.001±0.006	0.84±0.21	0.78±0.35	0.001±0.001	2.98±1.11

TABLE 3. Heavy metal concentrations in sediment and stream water samples.

Stations		Cu	Pb	Zn	Ni	Mn	Fe	Cr	Al
1	Sediment µg/g	9.93	10.83	48.53	29.32	610.86	9827.6	50.14	29398.49
	Water µg/L	ND	ND	42.41	18	11.87	490.21	ND	23.01
2	Sediment µg/g	9.87	10.87	49.79	30.98	585.66	9210.29	50.39	24136.06
	Water µg/L	ND	ND	34.88	15.3	16.83	299.3	ND	22.72
3	Sediment µg/g	9.94	10.18	48.78	29.31	573.19	9601.68	51.06	27147.61
	Water µg/L	ND	ND	48	22.96	12.65	394.34	1.14	21.27
4	Sediment µg/g	9.1	10.98	49.52	39.22	605.66	8930.69	54.98	25700.93
	Water µg/L	ND	ND	39.15	16.63	21.75	298.46	1.1	ND
5	Sediment µg/g	9.2	11.05	49.31	28.15	601.27	8625.32	52.06	23914.24
	Water µg/L	ND	ND	33.27	25.63	25.15	196.94	ND	ND
6	Sediment µg/g	9.41	10.96	49.32	27	645.96	9827.6	49.75	25189.74
	Water µg/L	ND	ND	34.37	32.34	14.66	492.5	ND	ND
7	Sediment µg/g	10	10.85	50.21	27.9	648.63	9205.14	51.53	29153.98
	Water µg/L	ND	ND	41.07	22.78	21.66	495.03	ND	ND
8	Sediment µg/g	9.65	10.92	49.79	38.89	624.88	9430.74	50.44	26201.14
	Water µg/L	ND	ND	38.24	25.15	11.81	387.42	ND	23.54
9	Sediment µg/g	9.11	10.93	50.73	29.84	623.94	9000.28	51.15	25370.33
	Water µg/L	ND	ND	56.44	15.53	17.59	193.94	ND	ND
10	Sediment µg/g	9.22	10.83	49.11	29.69	597.39	9490.82	49.91	25090.5
	Water µg/L	ND	ND	31.34	15.58	12.8	393.84	ND	21.49

ND: Not detected

TABLE 4. Correlations observed between environmental samples (sediment-water) metals.

Sediment		Cu	Pb	Zn	Ni	Mn	Fe	Cr	Al
	Cu	1							
	Pb	**-,723***	1						
	Zn	-0,17	0,39	1					
	Ni	-0,418	0,097	0,255	1				
	Mn	0,055	0,304	0,492	-0,273	1			
	Fe	0,523	-0,625	-0,537	-0,31	0,024	1		
	Cr	-0,273	0,426	0,353	0,224	-0,006	**-,857****	1	
	Al	0,152	-0,45	0,024	0,115	-0,079	-0,14	0,261	1
Water		Zn	Ni	Fe	Mn				
	Zn	1							
	Ni	-0,224	1						
	Fe	0,006	0,37	1					
	Mn	-0,564	0,103	-0,139	1				

*Correlation is significant at the 0.05 level (2-tailed).
**Correlation is significant at the 0.01 level (2-tailed).

TABLE 5. Metal correlations in each tissue.

Exoskeleton	Cu	Pb	Zn	Ni	Mn	Fe	Cr	Al
Cu	1,000							
Pb	,150	1,000						
Zn	,224	**,465***	1,000					
Ni	-,216	,205	,120	1,000				
Mn	,331	,244	**,490***	,381	1,000			
Fe	,105	,402	**,658****	,176	**,501***	1,000		
Cr	,147	,405	,110	-,144	,080	,201	1,000	
Al	,044	-,250	-,061	,307	,314	,140	-,022	1,000
Gill								
Cu	1,000							
Pb	,184	1,000						
Zn	-,272	,045	1,000					

Ni	,125	**,503***	,339	1,000				
Mn	,380	-,005	,358	,348	1,000			
Fe	**,464***	,418	-,198	,239	,374	1,000		
Cr	,170	,068	-,215	,243	,109	,009	1,000	
Al	**,505***	,358	,304	,388	**,565****	,374	,034	1,000
Hepatopancreas								
Cu	1,000							
Pb	,216	1,000						
Zn	**,495***	,142	1,000					
Ni	**,529***	,398	,136	1,000				
Mn	**,780****	**,484***	,441	**,467***	1,000			
Fe	**,744****	,171	**,615****	**,463***	**,675****	1,000		
Cr	,418	,013	,270	,317	,402	,286	1,000	
Al	**,478***	,106	,352	,065	**,451***	,362	,170	1,000
Muscle								
Cu	1,000							
Pb	-,265	1,000						
Zn	**,536***	-,020	1,000					
Ni	,096	-,008	,327	1,000				
Mn	-,106	,073	,238	,267	1,000			
Fe	**,464***	,308	,243	-,032	-,335	1,000		
Cr	-,249	,085	,259	-,086	,202	-,398	1,000	
Al	,201	,174	,158	,268	-,005	,415	-,386	1,000

TABLE 6. Correlations between sediment and tissue samples.

	Exoskeleton	Gill	Hepatopancreas	Muscle	Sediment
Exoskeleton	1,000				
Gill	,951**	1,000			
Hepatopancreas	,950**	,935**	1,000		
Muscle	,935**	,925**	,932**	1,000	
Sediment	,033	-,005	-,002	,056	1,000

**Correlation is significant at the 0.01 level (2-tailed).

TABLE 7. Accumulation trends of heavy metals for crayfish in literature.

Al	G>M>E>H	Tunca et al. [18]
As	H>G>E>M	
Cr and Cu	G>H>E>M	
Mn	H>E>M>G	
Fe, Mn and Cd	E>H>G>M	Naghshbandi et al. [25]
Cu	H>G>E>M	
Zn	H>G>M>E	
Pb	E>G>M>H	
Zn	H>E>M	Mackeviciene [31]
Cu and Ni	E>H>M	
Mn and Cr	E>H>M	
Pb and Cd	H>M>E	
Al, Mn, Cu, Ni, Cr, V and Li	E> H > M	Protasowicki et al. [34]
Fe	H>E>M	
Zn and Hg	H>M>E	
Al	G>M>H	Kurun et al.[35]
SCd	M>H=G	
Cu	H>G>M	
Fe and Mn	G>H>M	
Cu, Pb, Zn, N, Mn, Fe, Cr and Al	H>E>G>M	This Study

E:exoskeleton, G: gills, H: hepatopancreas, M: abdominal muscle

4. Conclusion

When the accumulation in tissues was examined, the highest accumulation was observed in hepatopancreas while the lowest metal concentrations and correlations between metals were observed in the muscle tissue. The highest correlations were found between Cu-Mn (r=, 780**) and Cu-Fe (r=, 744**) in the hepatopancreas tissue. The accumulation rates in the selected tissues and environment samples (sediment and water) were found very low. Also according to the correlation tests performed to reveal the accumulation profile, metal-metal interactions observed in the tissues were not found in the sediment and stream water. Metal-metal interactions are one of the most important methods to reveal the metal profile of a region.

Consequently, the crayfish tissues, water and sediment in Çomar Stream were found not contaminated by heavy metals.

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