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Interactions between metals accumulated in the narrow-clawed crayfish *Astacus leptodactylus* (Eschscholtz, 1823) in Dikilitaş Lake, Turkey

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The accumulations of Al, Cd, Cr, Cu, Fe, Ni, Pb and Zn in the exoskeleton, gills, hepatopancreas and abdominal muscles of crayfish *Astacus leptodactylus* (Eschscholtz, 1823) were determined. The strongest correlation observed was between Cr and Ni in the gills ($r = 0.904$); moderate to strong correlations between Al, Cr, Fe, Ni and Cu were also observed in gill tissue. Disregarding the gills, the strongest correlation was found between Cu and Zn in the hepatopancreas ($r = 0.808$); the correlation between these two metals might have been a result of metallothionein activity. The accumulation of Pb was found to correlate with that of Cd in the exoskeleton, Cd and Zn in the gills, Zn and Cu in the hepatopancreas and Cu in the abdominal muscle. None of these correlations were present in lakewater and sediment samples, suggesting that the crayfish metabolism may be responsible for the co-accumulation of metal–metal pairs. As all correlations in non-gill tissues are observed between divalent metals, a shared transporter such as divalent metal transporter 1 might be involved in the accumulation of these metals.

Keywords: correlation; divalent; DMT1; interrelation; metallothionein; multimetal

1. Introduction

Metallic pollutants are commonly introduced to the ecosystem through industrial, agricultural, domestic and natural channels. Unlike other types of contaminants, metals are not easily reduced to non-toxic forms and remain as a health hazard over long periods of time, which facilitates their accumulation through the food chain and renders them particularly dangerous to humans and other top predators. The long effective lifetime of metal pollutants, their ability to concentrate in the higher links of the food chain and the severe effects of even chronic exposure render them particularly important for environmental monitoring efforts.

Crayfish readily accumulate heavy metals and other toxic materials in their tissues, often in amounts that reflect the pollutant concentrations present in the environment. Their longevity, widespread distribution and predisposition towards spending their entire lives in a single

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freshwater source are other factors that increase their reliability as bioindicator species.[1,2] Consequently, crayfish are commonly employed as bioindicators for heavy metal accumulation studies and were chosen as the target species for the present study.[3–5]

Despite the essential roles played by certain metals in plant and animal metabolisms, all metals are toxic over a threshold concentration. Over this threshold, a metal may damage cells and tissues through three principal means: by directly interacting with proteins, stimulating the formation of reactive oxygen species and competing with essential metals.[6] All three interactions may cause damage to the essential or secondary molecules of the cell, which may result in the loss of cellular integrity (e.g. by loss of organelle function, membrane damage, mutations or strand breaks) and eventual cell death.[7] The detrimental effects of metals are typically mitigated through a variety of metal-transport and metal-binding proteins, many of which function on several different metals (albeit with different efficiencies and orders of preference). This relative lack of discrimination allows groups of metals to be transported or stored through the same protein, which creates correlations between their accumulation profiles.

The present study aims to determine the groups of metals (Al, Cd, Cr, Cu, Fe, Ni, Pb and Zn) by analysing the correlations between their accumulations in invertebrates. Interactions between the accumulations of metal pairs are frequently studied in controlled settings; however, fewer studies consider how the sums of these interactions shape metal accumulation in natural environments. Also provided is a discussion on the metabolic pathways underlying the observed trends in metal co-accumulation and the potential factors responsible for the accumulation and transport of multiple metals. The present work is of particular importance for the study of bioindicator organisms, as these species are frequently exposed to multiple metals in their natural environment. It is known that the presence of one metal can alter the uptake of other metals through synergistic and antagonistic interactions.[8] Metal–metal interactions, rather than exposure to each individual metal, may therefore have a significant effect on metal accumulations in the tissues of bioindicator species, which would limit their utility as a means to monitor metal concentrations in the environment. Consequently, a detailed analysis of the complex interactions between metal accumulation trends may both improve our understanding of metal uptake and allow more accurate evaluations of the amount of bioavailable metals present in a given locale.

2. Materials and methods

The field work was conducted in April 2013, where freshwater crayfish (*Astacus leptodactylus*) were collected from Dikilitaş Lake (Ankara, Turkey). Twenty male specimens of varying sizes were collected and dissected immediately after length and weight measurements; size parameters of the specimens are provided in Table 1. Specimens were transported in plastic storage boxes and stored at -20°C prior to dissection. The exoskeleton, gills, hepatopancreas and abdominal muscles of the animals were collected during dissection and subsequently digested for inductively coupled plasma-mass spectrometry (ICP-MS) analysis following Bernhard.[9] Ten water and sediment samples were collected in a later sampling period in November 2014.

Table 1. Size parameters of crayfish specimens. All collected specimens were male.

	Total length (cm)	Carapace length (cm)	Carapace width (cm)	Total weight (g)
Min.	17.3	6.1	8.3	55.3
Max.	29.1	8.3	5.7	130.8
Mean	24.42	7.35	7.39	93.73
Std. Dev.	3.38	0.72	0.78	24.58

2.1. *Metal analysis*

The concentrations of ^{27}Al, ^{111}Cd, ^{52}Cr, ^{65}Cu, ^{56}Fe, ^{60}Ni, ^{208}Pb and ^{66}Zn were evaluated through ICP-MS. Detection limits for these elements were determined at 3.19, 0.5, 0.9, 0.1, 0.01, 1.4, 0.04 and 0.04 ppb, respectively. Accuracy of measurements was ensured through the use of certified reference material LUTS-1 (non-defatted lobster hepatopancreas) as quality control. ICP-MS measurements were conducted using a Thermo-Scientific X-Series II ICP-MS equipped with a Cetac Asx-260 autosampler accessory. All dilutions were performed using a 2% nitric acid matrix in ultrapure water. Standard curves for all elements were based on the QCS-27 series of elements (High Purity Standards) and covered the typical measurement range for that element in crayfish tissues. A correlation coefficient above 0.99 was obtained for each calibration curve; 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.

2.2. *Statistical analysis*

Correlation analysis was used to determine the trends between metal accumulations in each individual tissue type. Spearman analysis was used as the correlation analysis of choice due to the number of datapoints available. Statistical analyses in the manuscript were performed using SPSS 19.0 (IBM, USA).

3. Results

The present study investigates the extent of heavy metal accumulation in the tissues of *A. leptodactylus* specimens collected from Dikilitaş Lake. Metal concentrations in lakewater and sediment samples were also measured to determine whether metal amounts in crayfish tissues

Table 2. Heavy metal concentrations in environmental (lakewater and sediment) samples.

Stations		Al	Cr	Fe	Ni	Cu	Zn	Cd	Pb
1.	Sediment (µg/g)	17149.14	72.62	9050.03	56.58	10.53	54.61	0.13	9.13
	Water (µg/L)	N.D.	N.D	72.06	5.87	N.D	12.10	N.D.	N.D
2	Sediment (µg/g)	15639.16	65.70	8679.30	59.30	9.96	52.63	0.20	9.77
	Water (µg/L)	N.D.	N.D	73.22	5.91	N.D	12.36	N.D.	N.D
3	Sediment (µg/g)	14129.81	73.46	8990.50	56.80	10.25	47.91	0.15	8.54
	Water (µg/L)	N.D.	N.D	75.56	6.35	N.D	12.63	N.D.	N.D
4	Sediment (µg/g)	14066.90	70.72	9187.52	74.65	14.01	60.03	0.12	10.74
	Water (µg/L)	N.D.	N.D	80.88	6.35	N.D	10.19	N.D.	N.D
5	Sediment (µg/g)	14572.95	60.08	8973.58	60.11	10.96	60.57	0.11	9.05
	Water (µg/L)	N.D.	N.D	74.24	5.95	N.D	13.26	N.D.	N.D
6	Sediment (µg/g)	13746.49	57.19	8581.30	60.37	14.64	56.08	0.78	8.71
	Water (µg/L)	N.D.	N.D	76.42	5.88	N.D	12.21	N.D.	N.D
7	Sediment (µg/g)	13162.00	57.66	8770.16	52.46	11.24	48.09	0.12	8.52
	Water (µg/L)	N.D.	N.D	77.44	5.92	N.D	12.17	N.D.	N.D
8	Sediment (µg/g)	15228.82	56.75	8962.62	55.64	10.64	49.77	0.15	11.79
	Water (µg/L)	N.D.	N.D	76.03	6.13	N.D	12.10	N.D.	N.D
9	Sediment (µg/g)	16505.48	65.01	9874.85	67.44	12.68	90.35	0.10	10.68
	Water (µg/L)	N.D.	N.D	68.53	6.11	N.D	11.49	N.D.	N.D
10	Sediment (µg/g)	15838.21	64.44	9701.74	66.98	12.48	62.49	0.12	9.80
	Water (µg/L)	N.D.	N.D	76.43	6.03	N.D	12.34	N.D.	N.D

Table 3. Correlations observed between metals in environmental samples.

	Al	Cr	Fe	Ni	Cu	Zn	Cd	Pb
Sediment								
Al	1							
Cr	0.333	1						
Fe	0.552	0.467	1					
Ni	0.127	0.200	0.515	1				
Cu	− 0.333	− 0.382	0.200	0.624	1			
Zn	0.418	− 0.067	0.576	0.806**	0.588	1		
Cd	0.285	0.442	− 0.067	− 0.418	− 0.842**	− 0.576	1	
Pb	0.479	− 0.067	0.455	0.418	0.152	0.442	0.152	1
	Fe	Ni	Zn					
Water								
Fe	1							
Ni	0.292	1						
Zn	− 0.128	− 0.171	1					

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

Table 4. Correlations observed between environmental and tissue samples.

	Sediment	Exo	Gills	Hepa	Muscle
Sediment	1				
Exo	0.357	1			
Gills	0.429	0.976**	1		
Hepa	0.357	1.000**	0.976**	1	
Muscle	0.381	0.905**	0.929**	0.905**	1

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

reflect environmental contamination (Table 2). Metal concentrations were very low in water samples, while Fe and Al were the predominant metals in sediment samples.

Correlations that occur in crayfish tissues but are absent in the lakewater and sediment are indicative of co-accumulation, co-sequestration or other metal–metal interactions that are the main concern of the present study. As such, correlation analyses were also performed for metal concentrations in lakewater and sediment samples (Table 3). Correlations between metal concentrations are limited to Zu–Ni and Cu–Cd interactions in the sediment, as opposed to the broader set of correlations observed in crayfish tissues. In addition, there are few correlations between metal concentrations in crayfish tissues and the sediment (Table 4), while a greater number of correlations are observed between metal accumulations in crayfish tissues. Consequently, metal availability in the environment does not directly translate to tissue accumulation in the present study.

Four tissue types (exoskeleton, gills, hepatopancreas and abdominal muscle) were analysed for the investigation of metal co-accumulation trends. Gills were the site with the greatest correlation strength in general, and displayed the greatest number of significant correlations between metal–metal pairs (Table 5). In addition, metal concentrations were observed to be particularly high in the gills and hepatopancreas (Figure 1). Al, Cr, Cu, Fe and Ni were found to correlate largely with each other, especially in gill tissues, which led to a large number of statistically significant correlations in the gills. A strong correlation between Cu and Zn ($r = 0.808$) was also noted in the hepatopancreas. Pb displayed another interesting trend, in that its accumulation was found to be correlated only with other divalent metals. No significant correlations between specimen size and metal accumulation could be identified.

Table 5. Correlations between metal accumulations in the whole animal and each individual tissue.

	Al	Cr	Fe	Ni	Cu	Zn	Cd	Pb	Length	Weight
All tissues										
Al	1									
Cr	0.609**	1								
Fe	0.511**	0.479**	1							
Ni	0.504**	0.688**	0.694**	1						
Cu	0.190	0.427**	− 0.068	0.295**	1					
Zn	− 0.064	0.232*	− 0.350**	0.041	0.646**	1				
Cd	0.140	0.332**	0.363**	0.399**	0.258*	0.012	1			
Pb	0.569**	0.758**	0.505**	0.704**	0.598**	0.204	0.582**	1		
Length	0.076	− 0.069	0.053	− 0.058	− 0.159	− 0.168	− 0.083	− 0.142	1	
Weight	0.117	− 0.036	0.086	− 0.006	− 0.175	− 0.102	− 0.096	− 0.105	0.821**	1
Exoskeleton tissue										
Al	1									
Cr	0.159	1								
Fe	0.087	0.341	1							
Ni	0.002	0.591**	0.632**	1						
Cu	0.162	− 0.126	− 0.481*	− 0.229	1					
Zn	0.125	0.392	− 0.083	0.075	0.370	1				
Cd	0.339	0.404	0.088	0.007	0.196	0.099	1			
Pb	0.313	0.368	− 0.066	0.081	0.537*	0.308	0.688**	1		
Length	− 0.096	− 0.291	0.074	− 0.167	− 0.313	− 0.358	− 0.179	− 0.262	1	
Weight	− 0.045	− 0.383	− 0.057	− 0.274	− 0.302	− 0.146	− 0.227	− 0.298	0.821**	1
Gills tissue										
Al	1									
Cr	0.708**	1								
Fe	0.887**	0.871**	1							
Ni	0.729**	0.904**	0.889**	1						
Cu	− 0.647**	− 0.623**	− 0.680**	− 0.571**	1					
Zn	0.281	0.364	0.236	0.331	− 0.116	1				
Cd	− 0.286	− 0.097	− 0.323	− 0.007	0.327	0.263	1			
Pb	0.140	0.385	0.182	0.347	0.018	0.702**	0.562**	1		
Length	0.208	0.095	0.242	0.020	0.024	− 0.236	− 0.160	− 0.020	1	
Weight	0.356	0.188	0.343	0.104	− 0.185	0.012	− 0.183	0.072	0.821**	1
Hepatopancreas tissue										
Al	1									
Cr	0.391	1								
Fe	− 0.047	0.178	1							
Ni	0.280	0.236	− 0.232	1						
Cu	0.164	0.287	0.068	0.383	1					
Zn	0.298	0.395	0.269	0.305	0.808**	1				
Cd	− 0.245	0.122	0.034	− 0.014	0.216	0.077	1			
Pb	0.092	0.443	0.220	0.335	0.647**	0.668**	0.393	1		
Length	− 0.150	− 0.256	0.182	− 0.299	− 0.165	− 0.295	− 0.168	− 0.406	1	
Weight	0.000	− 0.158	0.230	− 0.186	− 0.164	− 0.254	− 0.132	− 0.395	0.821**	1
Muscle tissue										
Al	1									
Cr	0.020	1								
Fe	0.141	0.681**	1							
Ni	0.049	0.643**	0.643**	1						
Cu	0.280	− 0.031	− 0.078	0.028	1					
Zn	− 0.273	0.251	0.236	0.124	0.156	1				
Cd										
Pb	0.334	0.131	0.053	0.323	0.757**	− 0.095		1		
Length	0.181	0.131	− 0.056	− 0.042	− 0.232	0.008		− 0.215	1	
Weight	0.178	0.142	0.081	0.166	− 0.239	− 0.155		− 0.024	0.821**	1

4. Discussion

Gills are of fundamental importance for metal accumulation in crustaceans, as metal uptake in aquatic animals is facilitated primarily by respiration and digestion. While the metal in question may ultimately be sequestered in another tissue, it nonetheless must gain a point of entry from the gills, exoskeleton or the gastrointestinal tract, from which it can then be transferred to other tissues. Franchi et al., for example, reported that Cd occurs in free form in the gills and is sequestered (likely with metallothioneins) in the hepatopancreas, suggesting that the free Cd in the gills is later deposited in other tissues.[10] Likewise, Bjerregaard has demonstrated that Cd present in the hepatopancreas is transported there by haemolymph from the gills.[11] As a

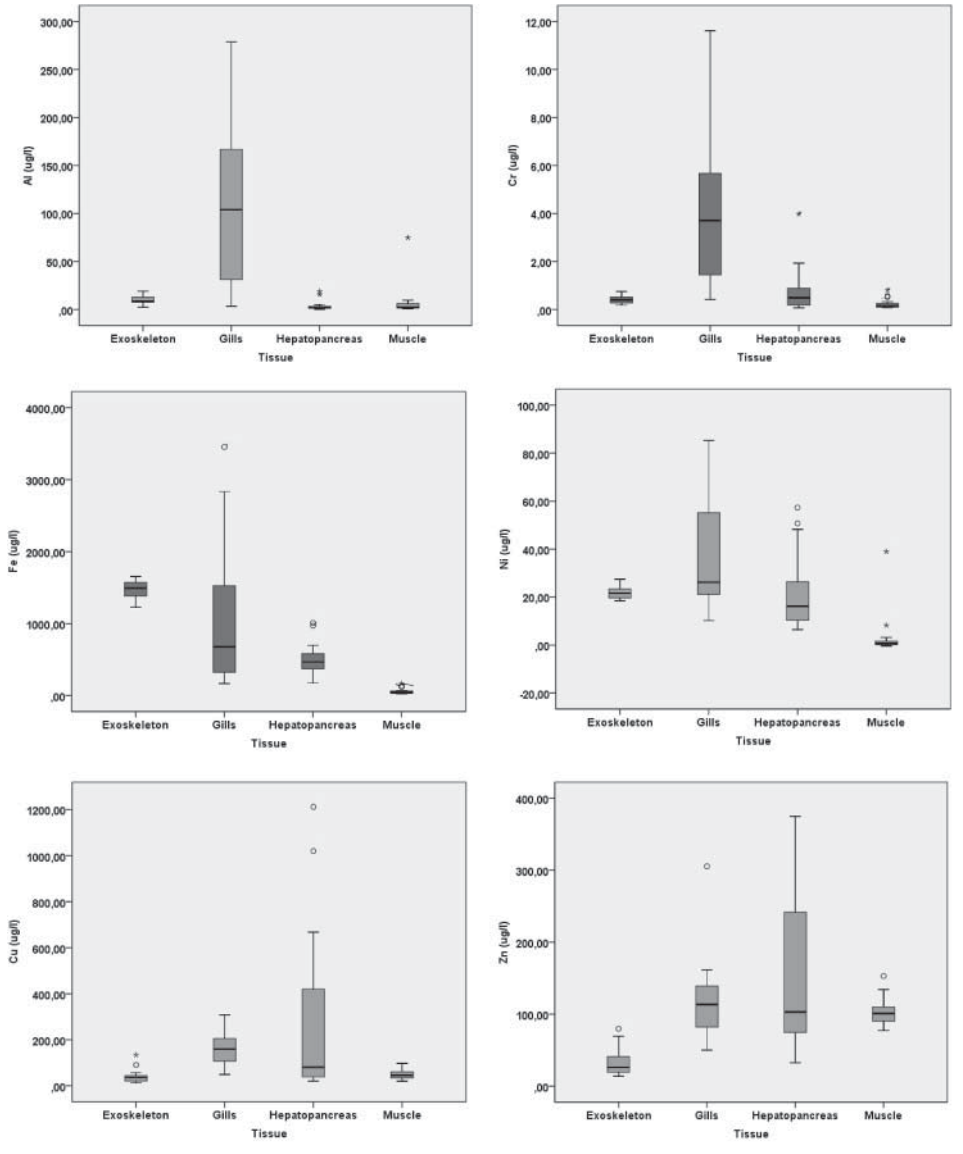

Figure 1. Boxplot comparison of metal accumulations between tissues.

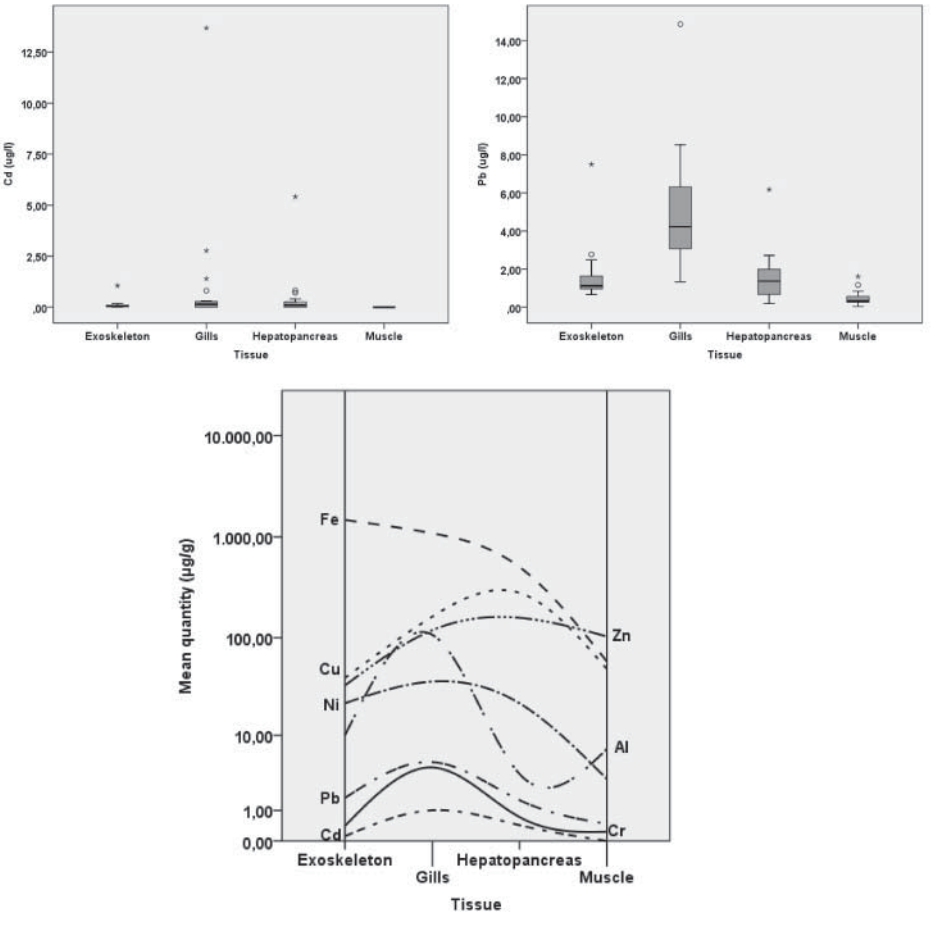

Figure 1. Continued.

result of their direct exposure to the surrounding environment, the gills of crustaceans also tend to accumulate high concentrations of certain metals, such as Cd [12] and Pb.[13]

While the gills were found to accumulate a substantial amount of metals in the present study, it must be noted that bioaccumulation is based on multiple factors and depends heavily on exposure time and metal concentration.[14,15] As such, different tissues may be found to accumulate higher concentrations of the same metal in different studies. For example, different results were obtained in the studies by Tunca et al. and Kurun et al. on the crayfish *A. leptodactylus*: while Tunca et al. found that Mn was accumulated the most in the hepatopancreas of both male and female crayfish, Kurun et al. report the gills as the principal site of accumulation for this metal in both sexes.[16,17]

Sex is another factor altering the accumulation trends of metals and may be particularly important during the reproductive season. Female crayfish of different species were found to accumulate less As, Ni and Cd in their gills when tested during the reproductive season,[16,18] which may suggest that these metals are sequestered within the eggs.[19] Outside the reproductive season, sex has a relatively minor effect on metal accumulation trends, which may [20] or may not [13] be statistically significant.

Age and size are other factors affecting heavy metal accumulation in a broad range of organisms. While size was not found to correlate with the accumulation of any metal in the

present study, such correlations are frequently reported in the literature.[21] Negative correlations between size and metal presence are typically attributed to the growth-hindering effect of toxic metals, while positive correlations suggest that the animal is continuously accumulating metals during the course of its growth, and that the rate of growth is slower than the rate of metal accumulation per gram of tissue.[22,23] The latter trend is reversed in animals that grow at rates faster than the rate of metal accumulation, resulting in negative correlations between size and metal accumulation.[24,25] Smaller or immature animals may also feed more frequently than larger or adult conspecific, which may lead to greater metal accumulation in smaller animals and result in negative correlations between size and bioaccumulation rates.[26] Moulting status has also been associated with changes in metal accumulation trends, and the moulted exoskeleton itself may be used to reduce metal burden.[27]

The strongest correlation in the gills was observed between Ni and Cr ($r = 0.904$), which was also the strongest correlation in any tissue. We have observed a strong correlation between Ni and Cr in the gills of crayfish in a prior study,[21] and similar effects were reported in other animals and plants, for example, two oyster species (*Crassostrea hongkongensis* and *Crassostrea sikamea*),[28] the macrophyte *Phragmites australis* [29] and the nile tilapia *Oreochromis niloticus*.[30] Weng and Wang reported that the correlation between Ni and Cr were present in the environment as well as the two species of oyster, suggesting that the correlation is not a function of the animals' metabolic processes, but a result of the fact that accumulation in bioindicator species reflects the metal amounts in their environment.[28,31] However, we found no correlation between Ni and Cr in lakewater or sediment samples, indicating that the observed effect is not a reflection of metal profiles in the environment. Instead, this correlation might be a result of shared metabolic pathways between these metals, such as the use of Zn transport mechanisms by Cd [32] or the Cd^{+2} – binding property of the divalent cation transporter IRT1, which ordinarily sequesters cations such as Fe^{+2}, Mn^{+2} and Co^{+2}.[33]

In addition to the Cr–Ni interaction, Al, Cr, Fe, Ni and Cu were observed to correlate with each other under every two-metal combination ($r = 0.708$ for Al–Cr, 0.887 for Al–Fe, 0.729 for Al–Ni, 0.647 for Al–Cu, 0.871 for Cr–Fe, 0.904 for Cr–Ni, 0.623 for Cr–Cu, 0.889 for Fe–Ni, 0.680 for Fe–Cu and 0.571 for Ni–Cu). These correlations comprise the majority of interactions in gill tissue; Pb–Zn and Pb–Cd were the only other correlations observed. It is notable that all metals in the earlier-mentioned group are transported by transferrins, a protein family that until recently has been considered to be limited to chordates, but has been reported from the crayfish *Pacifastacus leniusculus* (pacifastin heavy chain gene) and the crab *Cancer magister* (crab iron-binding protein).[34–36]

Transferrin plays an important role in the transport of Fe in particular,[37] but also facilitates the transport of Al^{3+}, Cr^{3+}, Cu^{2+}, Ga^{3+}, Ni^{2+}, Ti^{4+} and Zn^{2+}.[38] Of these elements, Ga and Ti were not included in this study, but associations can be observed between the accumulations of Fe, Al, Cr, Cu, Ni and Zn. The correlations between Fe, Al, Cr, Cu and Ni in gill tissue were noted earlier; while Zn is not present in this group, correlations between Zn and Cr, Fe and Cu were observed in other tissues. Transferrin has been reported to show different preferences for different metals in a concentration-dependent manner.[38] As such, the negative correlations shown between Cu and Al, Cr, Ni and Fe might be explained by competitive effects over transferrin binding. In addition, it must be noted that some of the metal–metal correlations observed in the gills are not present in other tissues. As gills serve as the main site of metal entry and are rich in blood vessels, the effect of a blood-borne transfer protein such as transferrin might be more pronounced in the gills than in other tissues, where other transport and chelation agents might have stronger influences. A lack of these agents, such as that found in abdominal muscle,[39] might also alter correlation results.

Another correlation of note is that between Cu and Zn in the hepatopancreas ($r = 0.808$). This is the strongest correlation seen in non-gill tissues, and has also been reported in previous

studies (e.g. by Nakayama et al. on *Cherax quadricarinatus* and Tunca et al. on *A. leptodactylus* [30,40]). The hepatopancreas is a major site of heavy metal sequestration in crustaceans, and metallothioneins are one of the most prominent metal sequestrating protein families. Found in all invertebrate phyla and almost all vertebrates, metallothioneins are rich in cysteine and can selectively bind metal ions under very low intracellular concentrations.[41] IB and IIB metals such as Cu, Cd, Zn, Ag and Hg are the main targets of metallothioneins.[42,43] Their main function is to prevent metals from participating in unwanted reactions by binding to and neutralising them, and they play important roles in the transfer and storage of both non-essential metals and excess concentrations of essential metals. As such, they serve as a countermeasure against metal-related toxic effects. Metallothioneins are present in high concentrations in the hepatopancreas, and assist this organ in detoxifying hazardous metals.[44] Metallothionein production in the hepatopancreas is linked to the Cu and Zn accumulation in this tissue, which is in line with the Cu–Zn correlation observed. The lack of similar correlations in other tissues might result from the fact that non-hepatopancreas tissues produce lesser amounts of metallothioneins.

Pb^{+2} displays another interesting set of correlation trends. Almost all correlations of Pb involve divalent cations: Cu and Cd in the exoskeleton, Cd and Zn in gills, Zn and Cu in the hepatopancreas and Cu in the abdominal muscle. Due to its similarity to Ca^{+2}, Pb^{+2} has been proposed to enter into cells by using calcium transport mechanisms,[45] which has also been supported by experimental evidence.[46] All Pb correlations observed in the present study were with other divalent cations (though the reverse is not true, not all divalent cations correlated with Pb in every tissue). The transport of divalent metals in vertebrates is facilitated by many proteins, one of which is the divalent metal transporter, DMT1 (DCT1 or Nramp1).[41] Homologues of this protein are known in invertebrates.[47,48] While DMT1 is primarily an iron transporter,[49] other divalent metals such as Cu^{+2}, Zn^{+2}, Cd^{+2} and Pb^{+2} can also be transported by the protein.[50] The positive correlations between Pb and other divalent metals suggest that the presence of a divalent cation transporter might be effective in Pb sequestration. However, the lack of correlations between Pb and other divalent metals in some tissues suggests that other transport mechanisms might be active (e.g. the earlier-mentioned Ca^{+2}-based entry pathways, which would not be apparent in the present study as Ca was not among the metals tested).

5. Conclusion

Metal–metal interactions have been observed to play a substantial role in the metal accumulation profiles of crayfish tissues. As none of the tissue correlations were present in freshwater and sediment samples, the observed effects are attributed to metabolic interactions between the metals, rather than an artefact of metal availability in the environment. These metabolic interactions are primarily related to the similar sizes, structures and functions of the interacting metals. The most pronounced among these features is valency, as metals with equal valence states are observed to display either synergistic (positive correlations) or antagonistic (negative correlations) trends in accumulation. This effect is likely caused by the fact that many proteins involved in metal transport and sequestration are not particularly selective and may bind to any metal sharing a common valence state. Valence-based correlations in the present study include interactions between Pb and Cd, Cu and Zn. All four metals have a dominant valence state of $+2$ and are known to be transported by divalent metal transporters such as DMT1.

In addition, non-essential metals are known to share metabolic pathways with their essential counterparts, allowing them to accumulate in tissues or display toxic effects in proteins that use metallic coenzymes. Given the importance of transport and storage proteins in metal sequestration, shared pathways may create substantial correlations in metal accumulation rates. Correlations observed between Al, Cr, Cu, Fe and Ni in the present study can be attributed to

shared transport pathways, as these metals are all known to be transported by transferrin. In addition, correlations between these metals were especially prominent in the gills, where the effect of transferrins (which are commonly found in the blood or haemolymph) would be more pronounced.

While the importance of these pathways and their effects on metal accumulation are well-recognized, the present study further demonstrates that metal–metal interactions may skew accumulation rates in natural environments and should therefore be taken into account for future bioaccumulation and biomonitoring studies.

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