Int. J. Environ. Res., 8(4):1315-1322, Autumn 2014
ISSN: 1735-6865

Impact of the Anthropogenisation on the Metal Bioaccumulation and Distribution in the Spiny-Cheek Crayfish (*Orconectes limosus* Raf.) from Lake Gopło, Poland

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Received 4 Feb. 2014; Revised 8 May 2014; Accepted 11 May 2014

ABSTRACT: This study was undertaken to determine the concentrations of metals in the muscle and exoskeleton of the crayfish *Orconectes limosus* in order to compare them with other species of crayfish and crustaceans. In addition, we analyzed the correlation between the concentrations of the same element in the exoskeleton and the meat and different elements accumulated in the same tissue. Crayfish individuals were acquired in spring (May 2012) from Lake Gopło. Individuals with damaged claws were not analyzed. Due to the relatively low amounts of meat that was obtained from the abdomen of individual crayfish, material from individuals with a similar body length was combined; thus, there were ten samples of meat and the exoskeleton obtained on the whole. The muscle samples (about 12 g) were freeze dried in a Finn-Aqua Lyovac GT2 freeze dryer (Finland). Three replicates of each freeze-dried sample were acid digested with 1 ml of concentrated HNO_3 and 1 cm^3 H_2O_2. Metal analyses were performed using an inductively coupled plasma mass spectrometry (ICP-MS) with a Perkin-Elmer Optima 8300 spectrometer. The analyses revealed that the mean metals concentrations ($mg \cdot kg^{-1}$) in the meat of the crayfish and in the exoskeleton were in the following order (Zn (115.571) > Mn (18.825) > Cu (17.226) > Ni (15.472) > Pb (3.535) > Cr (0.769) > Co (0.551) > Cd (0.315) > Hg (0.138)) and (Mn (111.640) > Zn (11.355) > Ni (8.165) > Pb (6.695) > Co (0.595) > Cu (0.575) > Cd (0.379) > Cr (0.195) > Hg (0.0168)), respectively.

Key words: Crayfish, Exoskeleton, Meat, Metals

INTRODUCTION

Aquatic organisms are bathed in solutions that contain metals and therefore, they may take up metals directly in their dissolved form. In a water environment metals are potentially accumulated in the sediments and animals and subsequently transferred along the food chain to humans (Firat *et al*., 2008) Marine and freshwater crustaceans can be used as bioindicators of environmental pollution since they can accumulate these metals and other pollutants (Protasowicki *et al*., 2013). Crayfish are suitable bioindicators of the xenobiotics in freshwater ecosystems due to their rapid bioaccumulation and long retention times. Heavy metals, also referred to as non-essential metals, are not considered as playing any significant role in the metabolism; however, mercury, lead and cadmium are known to be extremely toxic even at relatively low concentrations. Metals can accumulate in water, sediment or organic tissues through physicochemical or biological processes (Pinheiro *et al*., 2013).

Crayfish can be used for monitoring aquatic environments for metal pollution since they are solitary bottom dwellers that keep most of their bodies in contact with surrounding objects and therefore tend to accumulate metals in their tissues (Alcorlo *et al*., 2006). Crayfish are easily recognizable and do not

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migrate. They have a reasonably long life span and hatch many offspring. Moreover, crayfish are in constant physical contact with the sediment and water (Moss *et al*., 2010). The crayfish is a keystone species in ecosystems that is able to tolerate extreme and polluted environments, thus accumulating metals in its tissues (Suárez-Serrano *et al*., 2010). Various factors (for example, environmental, physiological and genetic) may affect the distribution of metals in different tissues of decapod crustaceans (Mouneyrac *et al*., 2001; Turoczy *et al*., 2001; Pourang *et al*., 2004; Thawley *et al*., 2004; Moss *et al*., 2011; Peng *et al*., 2011; Pinheiro *et al*., 2013; Protasowicki *et al*., 2013). As was reported in previous works, in a polluted environment crayfish are able to accumulate a considerable amount of metals in their organs and tissues (Guner, 2007) and that even within the same species of crustaceans, the same metals can preferentially accumulate in different tissues (Tunca *et al*., 2013b). The primary cause is thought to be the concentration of the metal and the duration of the exposure of the animals to the metal. Another reason for this situation is the correlation between elements. Potentially toxic metals may enter an animal's body across the integument, via the gills or through the gut after food consumption (Ahrean *et al*., 2004). The exoskeleton of crayfish may accumulate large amounts of metals owing to chitin. Chitin contains functional groups such as amines and hydroxyls that allow metal binding and removal from solutions (Morris *et al*., 2012). High levels of metals (especially Pb) in the exoskeleton are mainly connected with absorption rather than with bioaccumulation (Alcorlo *et al*., 2006). The abdominal muscle has consistently been found to be the tissue that contains the lowest concentration of metals (Madigosky *et al*., 1991).

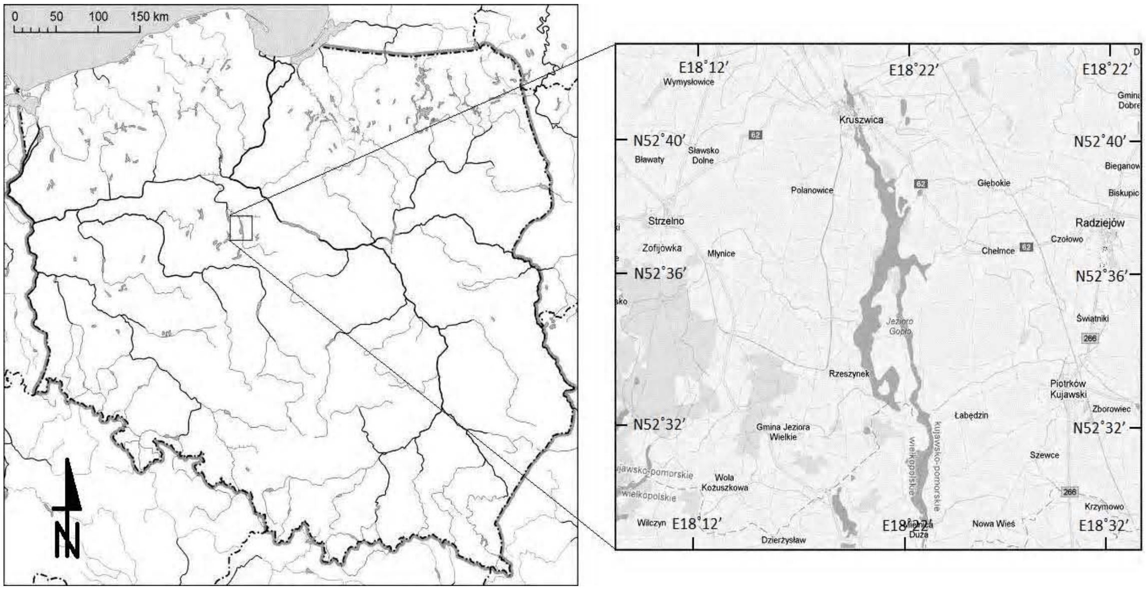
The study was undertaken to determine the concentration of metals in the muscle and exoskeleton of the spiny-cheek crayfish and to compare them with other species of crayfish, as well as with other species of crustaceans. In addition, another aim of the work was to analyze the correlation between the concentrations of the same element in the exoskeleton and the muscle (meat) and the correlation between different elements that had accumulated in the same tissue.

MATERIALS & METHODS

Lake Gopło is a flow-through container that is located in the southern part of the Kuyavian-Pomeranian Province (fig. 1). The maximum length of Lake Gopło is 25km and the surface of the water exceeds 2,100ha. The greatest depth is approximately 16.6m with 3.6m mean depth. The largest tributary of Lake Gopło is the Noteć River and a few smaller tributaries also flow into it. These flows are significantly overloaded with biogens. The conditions of the catchment cause an unfavorable, i.e. III class, susceptibility to the degradation of the waterbody and this has an adverse effect on the quality of the water. The western part of the lake is a strict nature reserve. According to the limnological classification, Lake Gopło is a eutrophic reservoir and it is a pikeperch type of lake based on the fishing classification. There are 65 species of Rotifera, 34 species of Copepoda and eight species of Cladocera among the zooplankton of Lake Gopło. The main group of benthos is represented by Chironomids (dominated by *Chironomus plumosus*). The white bream (*Abramis bjoerkna*) is predominant among the ichthyofauna. Among the predatory fish, a significant part of the fish stock are eels (*Anguilla anguilla* L.) and pikeperch (*Sander lucioperca* L.). Analyses revealed that the waters of Lake Gopło are qualified as unclassified. The most important element of the catchment of this lake are two sources: 1) agricultural activity, with the direct catchment area of the lake represented by fertile farmlands in which arable lands occupy 70% of the direct catchment area and the remaining areas comprise woods, grasslands and other types of land and 2) the productive and social activities of humans (WIOŚ, 2008). Contaminants come from municipal and industrial waste as well as from dispersed sources. One of the main sources of the biogen supply is also the bed sediment, especially in the northern part of the lake near Kruszwica. High risk can provide numerous centers of the recreation and tourism located near lake. Despite the decline in the amount of mineral fertilizers in the environment, the lake still is threatened by eutrophication.

The spiny-cheek crayfish (*Orconectes limosus* Raf.) was brought to Europe from North America in the late nineteenth century and has replaced the native species – the noble crayfish (*Astacus astacus* L.) and the mud crayfish (*Astacus leptodactylus* Esch.), whose populations were significantly reduced by the plague. *O. limosus* is immune to this plague and does not have a high environmental requirement and easily adapts to new conditions. This species of crayfish can be found in polluted and seriously eutrophic waters (Krzywosz, 1999); therefore, this species has invaded almost the entire area of Polish waters, with the exception of the southeast (Krzywosz, 2004). Crayfish reach their sexual maturity in the second year of life when they have a total length of 5 to 6 cm (Kossakowski, 1961). *O. limosus* is short lived and does not reach a considerable size (12.5 cm according to a report by Leńkowa, 1962) and this species rarely exceeds a total length of 10 cm and rarely lives more than 5 years (Mastyński, 1999).

The crayfish that were used in the experiment were caught in spring (May 2012) from Lake Gopło using



(a) (b)

Fig. 1. Map of Poland (a) and location of Lake Goplo (b)

pond tools. Any crayfish with damaged claws were not taken into account for further analyses. A total of 120 males were collected for the research (Table 1). They were individuals with total lengths that ranged from 9.5 to 11.0 cm. The total length was measured from the rostrum to the end of the telson. Due to the relatively low amounts of meat that was obtained from the abdomen of each individual, the material was combined from individuals of similar sizes (about 8-10 pieces each). For analysis, the meat and exoskeleton were dissected and the muscle samples were freeze dried in a Finn-Aqua Lyovac GT2 freeze dryer (Finland). These samples were acid digested (1 ml of concentrated HNO_3 and 1 cm^3 H_2O_2) and metal analyses were performed on an inductively coupled plasma mass spectrometry (ICP-MS Perkin-Elmer Optima 8300). The content of mercury was determined using an AMA 254 mercury analyzer after thermal decomposition at 700°C,. The Merck Certified ICP Standard was used in the calibration and validation of the standard curves. The concentrations of metals were calculated from linear calibration plots that were obtained by measuring the standard solutions. All determinations were made in triplicate and the dates for the samples of meat were corrected to the oven-dry (105°C) moisture content.

Table 1. Concentrations of metals (mean ±- SD) registered in the meat and exoskeleton of spiny-cheek crayfish (*Orconectes limosus* Raf.) from Vistula River

Metals	Content of the metals (mg/kg) (mean value ± SD)	
	meat	exoskeleton
Cd	0.315 ± 0.195^a	0.379 ± 0.084^a
Cr	0.769 ± 0.559^a	0.195 ± 0.071^a
Cu	17.226 ± 3.042^b	0.575 ± 0.198^a
Co	0.551 ± 0.161^a	0.595 ± 0.134^a
Zn	115.571 ± 28.584^b	11.355 ± 0.904^a
Ni	15.472 ± 2.589^b	8.165 ± 1.042^a
Pb	3.535 ± 1.786^a	6.695 ± 0.338^b
Mn	18.825 ± 7.440^a	111.640 ± 10.846^b
Hg	0.138 ± 0.058^b	0.0168 ± 0.007^a

The values marked different letters in the row differed statistically ($p<0.05$)

RESULTS & DISCUSSION

The analysis that is presented in Table 1 revealed the accumulation of metals in the meat of the crayfish (in the following order Zn > Mn > Cu > Ni > Pb > Cr > Co > Cd > Hg) and in the exoskeleton (Mn > Zn > Ni > Pb > Co > Cu > Cd > Cr > Hg). The same results were observed for crayfish from the Mazurian Lakes (Protasowicki *et al*., 2013). The trend of increasing metal concentrations in the organs of crayfish is consistent with their content in the lithosphere and hydrosphere (Kabata-Pendias & Pendias, 1999). According to Pinheiro *et al*. (2012), manganese (Mn) accumulation in the mangrove crab (*Ucides Cordatus*) does not differ among tissues, a result that is in contrast with the present study, where the mean content of Mn in the crayfish was 5.93 times higher in the exoskeleton than in the meat, which was statistically significant (Table 1). In the crayfish *A. leptodactylus* from the Aras Dam in Iran, the mean content of Mn was 6.66 times higher in the exoskeleton than in the meat. A high concentration of metal in specific organ may be related to the utilization of that metal, which might indicate that the exoskeleton is involved in the excretion of metals (Naghshbandi *et al*., 2007). A high Mn concentration in the exoskeleton may be related to its limited ability to complement or substitute Mg^{2+}. Additionally, this probably stems from its chemical similarity to Ca^{2+} where Mn is a metal that is able to substitute the calcium in $CaCO_3$, which leads to an accumulation during the calcification of the exoskeleton (Peng *et al*., 2011). In a previous research, 98% of manganese was accumulated in the exoskeleton of the lobster *Homarus vulgaris* (Pourang *et al*., 2004), which shows the importance of this matrix during the immobilization of this metal.

Zinc (Zn) is an essential trace element, although in large amounts it can cause a variety of pathological effects. The concentration of this element is high in the tissues or structures of the crayfish. As Mackevičiené (2002) reported, Zn accumulated in crayfish in the following order: hepatopancreas > exoskeleton > digestive tract > abdominal muscle. Yýlmaz & Yýlmaz (2007) detected the highest Zn concentrations in the hepatopancreas of the green tiger shrimp *Penaeus semisulcatus* De Hann, whereas higher concentrations of this metal were observed in the gills and lower concentrations were found in the muscle. How Pourang *et al*. (2004) stated that a high concentration of Zn in the hepatopancreas appears to be the case among crustaceans. The hepatopancreas plays a central role in the metabolism, storage and detoxification of a number of metals. As the authors indicated, the mean content of Zn in *O. limosus* was statistically significantly higher in the meat than in the exoskeleton (Table 1). The same results were observed in crayfish that were analyzed by Naghshbandi *et al*. (2007) and Protasowicki *et al*. (2013). As was reported in previous works, high concentrations of certain metals in specific organs may be related to its utilization by crayfish. For example, Zn serves as an active center for metalloenzymes and activators of other enzyme systems and it is usually abundant in the hepatopancreas (Peng *et al*., 2011).

Copper (Cu) is essential for the normal growth and metabolism of all living organisms. This metal is also of key importance for crustaceans and it is a component of the respiratory pigment hemocyanin (Pinheiro *et al*., 2012). The copper accumulation pattern in the marbled crayfish (*Procambarus* sp.) that was analyzed by Soedarini *et al*. (2012) and in *A. leptodactylus* that was examined by Guner *et al*. (2007) have the following pattern: hepatopancreas > gills > exoskeleton > muscle. The authors of this paper show that the mean value of Cu in the *O. limosus* from Lake Gopło was statistically significantly higher in the meat than in the exoskeleton (Table 1.). Studies reported by Naghshbandi *et al*. (2007) demonstrated approximately three times more copper in the exoskeleton than in the meat. The same results were observed by Protasowicki *et al*. (2013) and Tunca *et al*. (2013b). Although gill tissues are in direct contact with the environment and showed a significant decrease in Cu accumulation (Guner, 2007), the exoskeleton may also accumulate large amounts of this metal. Cu accumulation in the exoskeleton may be due to the fact that the exoskeleton is a bio-mineral composite that serves as a structural support and therefore it would take up copper from the water by adsorption. During the post molt, copper is utilized for hemocyanin synthesis and also for hardening of the exoskeleton (Pinheiro *et al*., 2012). The presence of Cu in the exoskeleton of crayfish could have a survival value as a possible elimination mechanism through molting (Guner, 2007).

Nickel (Ni) is a ubiquitous element that is known for its toxicity, persistence and affinity for bioaccumulations, however it is essential at very low concentrations (Kouba *et al*., 2010). Mackevičiené (2002) reported an Ni accumulation in the body structures of *A. astacus* in the following order: exoskeleton > hepatopancreas > muscle > digestive tract. This sequence was very similar to that which was verified in both sexes of *A. leptodactylus* (Tunca *et al*., 2013b), although the Ni accumulation in the gills were in different positions in the males (gills > exoskeleton, hepatopancreas > abdominal muscle) and females (exoskeleton = hepatopancreas = gills) > abdominal muscles. A substantial concentration of nickel in the exoskeleton in these species might indicate that this tissue is involved in the immobilization of this metal (Kouba *et al*. 2010). The lowest amounts of Ni in the abdominal muscle of crustaceans has been observed by some researchers (Pourang *et al*., 2004, Protasowicki *et al*., 2013, Tunca *et al*., 2013a, Tunca *et al*., 2013b). An inverse fact was verified for *O. limosus* in the present study, which showed that the mean concentration of Ni was about two times higher in the meat than in the exoskeleton (Table 1).

Lead (Pb) is a not essential metal for living organisms and is responsible for a large number of adverse effects on biota (Kouba *et al*., 2010). It accumulated in the tissues of the crayfish in the following order: hepatopancreas > digestive tract > muscle > exoskeleton. The hepatopancreas was observed to be the main storage organ for lead in yabby crayfish (*Cherax destructor*) (Bruno *et al*., 2006). This metal is stored in metal-containing vacuoles of hepatopancreatic cells. According to Reinecke *et al*. (2003), the highest concentration of Pb was detected in the gonads of a freshwater crab (*Potamonautes perlatus*), while the lowest concentration was found in digestive system. Seventy percent of the lead in the internal tissues of the Norway lobster (*Nephrops norvegicus*) was located in the gills (Canli & Furness, 1993). The same results were observed by Naghshbandi *et al*. (2007) in *A. leptodactylus*. The concentrations of lead in the crayfish that were analyzed in this study were found in a decreasing order: exoskeleton > muscle (Table 1.). The same results were obtained by Alcorlo *et al*. (2006), Naghshbandi *et al*. (2007) and Protasowicki *et al*. (2013), who concluded that the lead accumulation in crayfish is dose- and time- dependent. Chromium (Cr) is an essential metal, but it can be harmful at high levels. Kouba *et al*. (2010) observed an accumulation of Cr in the tissues of *A. astacus* in the following order: exoskeleton > digestive tract > hepatopancreas > muscle. Jorhem *et al*. (1994) reported that higher concentrations of this metal were found in the hepatopancreas of the signal crayfish (*Pacifastacus leniusculus*) than in the muscle. The same result was observed by Protasowicki *et al*. (2013). Bruno *et al*. (2006) reported that the hepatopancreas of *Ch. destructor* accumulated higher amounts of chromium than the exoskeleton and muscle. This fact was not confirmed in the present study, which shown no statistically significant differences in the mean values of Cr between the meat and exoskeleton (Table 1).

Cobalt (Co) is also an essential metal for many organisms (Tunca *et al*., 2013a). Similar to chromium, the mean contents of cobalt in the meat and exoskeleton of the crayfish from Lake Gopło did not differ statistically significantly and these values were very similar (Table 1). The low levels of Co in the tissue can be explained by the fact that Co bioaccumulation is inhibited when other metals are present, especially Ni, Cu, Zn and Mn, due to the competitive interactions between these cations (Tunca *et al*., 2013a). Cadmium (Cd) is a non-essential element that has teratogenic, carcinogenic and highly nephrotoxic effects on living organisms (Kouba *et al*., 2010; Tunca *et al*., 2013a). Many researchers have reported that cadmium is taken up and accumulated by crayfish, both from the surrounding water and along with food (Kouba *et al*., 2010). The hepatopancreas is the main organ for the accumulation and detoxification of Cd in crayfish (Kouba *et al*., 2010), lobsters (Barrento *et al*., 2008), crabs (Turoczy *et al*., 2001) and shrimps (Pourang *et al.*, 2004). As numerous studies of crustaceans have shown, cadmium was accumulated in the following order: hepatopancreas > gills > muscle (Chambers, 1995), hepatopancreas > exoskeleton > muscle (Bruno *et al*., 2006) and hepatopancreas > alimentary tract > blood > exoskeleton (Madigosky *et al*., 1991). The mean content of Cd in the meat and exoskeleton of the crayfish from Lake Gopło did not differ statistically significantly and these values were very similar (Table 1). The same results were observed by Protasowicki *et al.* (2013).

Crayfish take up mercury (Hg) and methylmercury (MeHg) from water and food, and the latter represent approximately 90% of the total mercury in crayfish (Kouba *et al*., 2010). In those animals, mercury is accumulated largely in muscle, which is confirmed in *O. limosus* (Table1) in which the mean concentration in this tissue comprised eight times that registered in exoskeleton. These data are in sharp contrast with Northern Clearwater crayfish (*Orconectes propinquus*) that were fed pellets dosed with Hg and MeHg. The relative levels of mercury accumulation in various organs were: hepatopancreas > gills > exoskeleton > abdominal muscle and for the methylmercury: gills > abdominal muscle > hepatopancreas > exoskeleton (Kouba *et al*., 2010). As Turoczy *et al*. (2001) reported, the highest concentrations of mercury were found in the muscle tissue of the king crab (*Pseudocarcinus gigas*). As mercury is not an essential element, these results suggest that this metal may accumulate in these tissues as the crabs grow. The aim of this study was to investigate of the correlation between different metals in the meat and exoskeleton (Table 2 and 3, respectively). The correlation coefficients between metal pairs in decapod crustaceans show species and location dependent differences (Pourang *et al*., 2004). In the meat of *O. limosus* form Lake Gopło. the strongest positive and statistically significant correlations were observed between Pb-Cd and Mn-Hg, while negative correlations (also significant) were observed between

Ni-Mn and Ni-Hg (Table 2). Tunca *et al.* (2013a) observed a strong positive correlation between Pb and Cd in the meat of *A. leptodactylus* (correlation coefficient 0.917). A positive correlation for Cd and Pb in different shrimp species form the Gulf of Fonseca (Central America) was evaluated by Carbonell *et al.* (1998) and Madigosky *et al.* (1991) analyzed their content in the crayfish *Procambarus clarkii*. This fact is possible due to the elimination of metals by ecdysis in which the exoskeleton can be ingested and its minerals/metals incorporated into the new shell by the crayfish. These strong correlations indicate that these metals have the same accumulation mechanism. Negative correlations between metals suggest that there may be competition between these elements (Pourang *et al.*, 2004).

In the exoskeleton of *O. limosus* form Lake Gopło the strongest positive and statistically significant correlations were observed between Pb-Ni, while negative correlations (also significant) were observed between Co-Cd and Cr-Hg (Table 3). Tunca *et al.* (2013a) reported a strong negative correlation of Pb and Ni in exoskeleton samples. Analyses of the correlation trends of the different metals in the meat and exoskeleton are presented in Table 4. There were no statistically significant correlations of individual metals concentrations between muscle and exoskeleton. Tunca *et al.* (2013a) reported negative associations of Ni concentrations between muscle and the gills and exoskeleton, which indicates that Ni is mostly absorbed by the gills or exoskeleton.

Table 2. Matrix of correlation rates among different metals of meat samples of spiny-cheek crayfish (*Orconectes limosus* Raf.) from Vistula River

Cr	-0.085							
Cu	0.119	-0.169						
Co	0.119	0.205	-0.088					
Zn	-0.397	-0.214	-0.071	0.147				
Ni	0.337	-0.875	-0.463	0.611	0.372			
Pb	**0.817**	0.013	0.170	0.259	-0.014	0.479		
Mn	-0.374	0.474	0.365	-0.345	-0.099	**-0.726**	-0.273	
Hg	0.169	0.174	0.528	-0.541	-0.149	**-0.668**	0.161	**0.669**
	Cd	Cr	Cu	Co	Zn	Ni	Pb	Mn

Table 3. Matrix of correlation rates among different metals of exoskeleton samples of spiny-cheek crayfish (*Orconectes limosus* Raf.) from Vistula River

Cr	0.025							
Cu	-0.631	-0.251						
Co	**-0.671**	-0.504	0.548					
Zn	-0.216	-0.201	-0.202	0.059				
Ni	-0.016	0.353	0.255	-0.242	-0.423			
Pb	-0.356	0.265	0.389	-0.122	0.009	**0.713**		
Mn	-0.415	-0.175	0.303	0.339	-0.084	-0.128	-0.245	
Hg	0.281	**-0.741**	0.036	0.115	0.327	-0.254	-0.062	-0.051
	Cd	Cr	Cu	Co	Zn	Ni	Pb	Mn

Table 4. Matrix correlation rates in nine metals between different tissues (meat and exoskeleton) of spiny-cheek crayfish (*Orconectes limosus* Raf.) from Vistula River

		Exoskeleton								
		Cd	Cr	Cu	Co	Zn	Ni	Pb	Mn	Hg
Meat	Cd	-0.072	**-0.637**	0.0945	0.610	0.074	0.018	-0.182	-0.109	0.348
	Cr	0.190	0.083	-0.191	-0.283	0.367	0.129	0.505	**0.671**	0.218
	Cu	0.186	-0.225	-0.417	0.087	-0.079	-0.055	-0.455	0.120	-0.233
	Co	0.379	0.302	-0.174	-0.561	-0.168	0.169	0.127	**-0.726**	-0.263
	Zn	0.073	-0.129	-0.229	-0.106	-0.089	-0.627	-0.352	0.115	0.056
	Ni	0.114	0.056	-0.072	-0.162	0.232	-0.394	-0.182	-0.442	0.106
	Pb	0.051	**-0.654**	-0.039	0.539	0.076	-0.065	-0.273	-0.153	0.397
	Mn	0.055	0.005	-0.155	-0.137	0.133	-0.012	0.072	0.175	-0.072
	Hg	-0.114	0.267	0.262	-0.027	-0.450	0.385	0.235	0.574	-0.191

CONCLUSION

The concentration of metals in the meat and exoskeleton of crayfish *O. limosus* from Lake Gopło were similar to the ranges that were observed in other crustaceans (crabs, shrimps or lobsters). The different accumulations of metals in the meat and exoskeleton may be largely associated with the specific pathway of metal metabolism. The specimens collected in Lake Gopło are safe for human consumption because reduced contamination levels were found. The concentration of metals in the meat of crayfish was lower than the maximum levels set by law (Commission Regulation).

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