

METAL CONCENTRATIONS IN SELECTED ORGANS OF CRAYFISH – *ORCONECTES LIMOSUS* AND *PACIFASTACUS LENIUSCULUS* FROM MAZURIAN LAKES*

Mikołaj Protasowicki¹, Teresa Własow²,
Monika Rajkowska¹, Małgorzata Polna¹, Alicja Bernad³

¹Chair of Toxicology

West Pomeranian University of Technology

²Department of Ichthyology

University of Warmia and Mazury in Olsztyn

³Regional Veterinary Diagnostic Laboratory, Olsztyn, Poland

Abstract

Crayfish accumulate trace metals, which is why they often serve as environmental indicators. Among heavy metals, Hg, Cd and Pb are considered toxic substances, while Fe, Zn, Cu, Cr, Ni, Mn, V, Al and Li are involved in vital functions, which makes them essential elements in animal organisms.

The study objective was to determine concentrations of the above metals in the abdominal muscles, hepatopancreas and exoskeleton of spinycheek crayfish (*Orconectes limosus* Raff.) and signal crayfish (*Pacifastacus leniusculus* Dana) from Dgał Wielki Lake and Poblędzie Lake in the Mazurian Lake District, Poland.

The analyses were carried out with the use of the following methods: CV AAS (Hg), GF AAS (Cd, Pb) and ICP-AES (Al, Cr, Cu, Fe, Li, Mn, Ni, V, Zn) after wet digestion in HNO₃ and HClO₄ concentrated acid mixture.

Regarding mean concentrations (mg kg⁻¹ wet weight) in crayfish, metals formed the following order: Al (14.8-123.4) > Mn (0.26-91.3) > Fe (0.54-81.1) > Zn (6.80-51.91) > Cu (1.21-4.34) > Ni (0-0.782) > Cr (0.032-0.606) > V (0-0.245) > Li (0.011-0.215) > Pb (0.018-0.079) > Hg (0.004-0.045) > Cd (0.001-0.017).

prof. dr hab. inż. Mikołaj Protasowicki, Chair of Toxicology, West Pomeranian University of Technology, Papieża Pawła VI 3, 71-459 Szczecin, Poland, e-mail: mikolaj.protasowicki@zut.edu.pl

*The study was financed by the University of Agriculture in Szczecin (currently: West Pomeranian University of Technology, Szczecin), grant no. 503-3-3170 and the University of Warmia and Mazury in Olsztyn, grant no. 0804 0809.

Metal concentrations in crayfish organs were varied, and for different metals they decreased in different order: Al, Mn, Cu, Ni, Cr, V and Li – exoskeleton > hepatopancreas > muscles; Zn and Hg – hepatopancreas > muscles > exoskeleton; Fe – hepatopancreas > exoskeleton > muscles, while for lead and cadmium no overall correlation was observed. The comparison of metal concentrations in the organs of spinycheek crayfish from Dgał Wielki Lake and Poblędzie Lake did not prove any statistically significant discrepancies. Also, some minor interspecies differences in the metal content proved insignificant.

According to the European Union regulations, concentrations of toxic heavy metals (Hg, Cd, Pb) in the muscles of both species of the tested crayfish were significantly below the maximum levels.

Key words: metals, crayfish, muscles, hepatopancreas, exoskeleton.

STĘŻENIE METALI W WYBRANYCH NARZĄDACH RAKÓW – *ORCONECTES LIMOSUS* I *PACIFASTACUS LENIUSCULUS* Z JEZIOR MAZURSKICH

Abstrakt

Raki kumulują w swoim organizmie metale, i dlatego są często traktowane jako wskaźnik stanu środowiska. Wśród metali ciężkich za toksyczne uznaje się Hg, Cd i Pb, natomiast Fe, Zn, Cu, Cr, Ni, Mn, V, Al i Li mają udział w funkcjach życiowych, co czyni je niezbędnymi dla organizmu. Celem pracy było określenie stężeń ww. metali w mięśniach odwłokowych, wątrobotrzustce i pancerzu raków przegowatego (*Orconectes limosus* Raff.) i sygnałowego (*Pacifastacus leniusculus* Dana) z jezior Dgał Wielki i Poblędzie (Pojezierze Mazurskie).

Analizy przeprowadzono z użyciem następujących metod: CV AAS (Hg), GF AAS (Cd, Pb) i ICP – AES (Al, Cr, Cu, Fe, Li, Mn, Ni, V, Zn) po zmineralizowaniu próbek w mieszaninie stężonych kwasów azotowego i nadchlorowego.

Pod względem średniego stężenia (mg kg^{-1} mokrej masy) metale były uszeregowane w następującej kolejności: Al (14,8-123,4) > Mn (0,26-91,3) > Fe (0,4-81,1) > Zn (6,80-51,91) > Cu (1,21-4,34) > Ni (0-0,782) > Cr (0,032-0,606) > V (0-0,245) > Li (0,011-0,215) > Pb (0,018-0,079) > Hg (0,004-0,045) > Cd (0,001-0,017).

Stężenie metali w narządach raków było zróżnicowane; dla różnych metali zmniejszało się następująco: Al, Mn, Cu, Ni, Cr, V i Li – pancerz > wątrobotrzustka > mięśnie; Zn i Hg – wątrobotrzustka > mięśnie > pancerz; Fe – wątrobotrzustka > pancerz > mięśnie. Nie obserwowano takich zależności dla ołowiu i kadmu.

Porównanie stężeń metali w narządach raka przegowatego z jeziora Dgał i jeziora Poblędzie nie wykazało istotnych statystycznie różnic. Podobnie nieistotne były międzygatunkowe małe różnice w stężeniach metali.

Stężenia toksycznych metali ciężkich (Hg, Cd, Pb) w mięśniach obu badanych gatunków raków plasowały się poniżej najwyższego dopuszczalnego poziomu ustalonego dla środków spożywczych przez Rozporządzenie Komisji WE.

Słowa kluczowe: metale, raki, mięśnie, wątrobotrzustka, pancerz.

INTRODUCTION

Among the native fauna species in Poland, crayfish belong to the largest invertebrates. Until the end of the 19th century, the noble crayfish (*Astacus astacus*) and Danube crayfish (*Astacus leptodactylus*) had been widespread in the European water bodies. But due to their considerable vulnerability to crayfish plague and sensitivity to pollution, at present they are very rare in Poland and under strict protection. These native species were supplanted by the spinycheek crayfish (*Orconectes limosus*), also called American crayfish, imported from the United States of America in 1892, and signal crayfish (*Pacifastacus leniusculus*) introduced to Polish waters in the early 1970s (KOSSAKOWSKI et al. 1978, ŚMIETANA, STRUŻYŃSKI 1999). Having been introduced to Polish waters, these foreign species were unintentionally spread by humans. For example, it is believed that implementation of nylon fishing nets accelerated the phenomenon, because such nets – unlike cotton ones – do not need drying so crayfish caught in nylon nets can be transferred in good condition from one water body to another, even at considerable distances (ŚMIETANA, STRUŻYŃSKI 1999). Besides, foreign species are less susceptible to crayfish plague and they cope better with unfavourable environmental conditions.

Moreover, female signal crayfish lay eggs under the abdomen as early as spring, while female noble crayfish and Danube crayfish do so in autumn, which additionally contributes to quicker expansion of signal crayfish in Europe. The incubation period lasts 6-7 months in native crayfish and just 5-6 weeks in alien ones. Owing to such short incubation, eggs carried under the abdomen of a female are less likely to be lost (ULIKOWSKI, BORKOWSKA 1999). However, signal crayfish do not grow big and an individual 12-centimetre in length is considered very large (ŚMIETANA, STRUŻYŃSKI 1999).

Signal crayfish are equipped with many practical adaptations and reach sizes similar to noble crayfish. Representatives of the species mature one year earlier than the native crayfish and are more productive of offspring; also, their body length increases faster compared to the native crayfish (KRZYWOSZ et al. 1995, KRZYWOSZ, KRZYWOSZ 2001, ŚMIETANA, KRZYWOSZ 2006). However, their introduction is not always successful, especially where spinycheek crayfish are abundant. It should also be remembered that signal crayfish and spinycheek crayfish are a serious threat to noble crayfish, which have become very rare in Poland (GYDEMOR 1996, KRZYWOSZ 2006, KOZUBÍKOVÁ et al. 2008).

Regarding the above, attempts should be made to eliminate the American species from Polish waters. Attaining this goal can be facilitated by the development of fish farming, as farms can preserve native species in their natural environment (BERNAD et al. 2001). It is also supported by Polish legal regulations, stipulating that caught spinycheek crayfish (*Orconectes limosus*

Raff.) and signal crayfish (*Pacifastacus leniusculus* Dana.) individuals should not be released to the same fishing ground, nor to any other water bodies (Dz. U. 2001, 2003).

Crayfish may be used as an environmental indicator because their tissues tend to accumulate metals, including heavy ones (ANDERSON et al. 1997b). Among heavy metals, mercury, cadmium and lead are toxic and not essential to organisms. Other metals from the same group, namely iron, zinc, copper, chromium, nickel, manganese and vanadium, are essential to organisms because of positive roles they play in many vital functions. Also aluminium and lithium, which belong to heavy metals, are essential to animals. An extensive review of the distribution and role of trace elements in the environment has already been provided by KABATA-PENDIAS, PENDIAS (1999). Situations when toxic metal concentrations exceed the tolerable level or when the essential metals exceed concentrations physiologically needed to maintain a biochemical balance pose a threat to animal health and life.

Bioaccumulation of metals in crayfish bodies may impair the immune system, reproduction, heart rhythm, breathing processes, regeneration and moulting processes; it may also cause changes in pigmentation, increase glucose concentration, change pH of digestive juices and induce histopathological changes in the hepatopancreas (MEYER et al. 1991, REDDY et al. 1994, STYRISHAVE, DEPLEDGE 1996, ANDERSON et al. 1997b, AHERN, MORRIS 1999, RODRIGUEZ et al. 2003, WARD et al. 2006, WOODBURN et al. 2011). The hepatopancreas, an organ involved in the processes of nutrient absorption and storage as well as detoxification, is particularly prone to bioaccumulation of metals. They are stored in metal-containing vacuoles, as demonstrated by a study on lead accumulation carried out in laboratory conditions (ANDERSON et al. 1997a).

The objective of this study was to test concentrations of metals, mainly heavy ones, in tissues (exoskeleton, hepatopancreas and muscles) of spinycheek crayfish (*Orconectes limosus* Raff.) and signal crayfish (*Pacifastacus leniusculus* Dana). A hypothesis was formulated that metal concentrations in particular organs of crayfish depended on which lake given crayfish originated from (Dgał Wielki or Poblędzie Lake) and on the species.

MATERIAL AND METHODS

Study area

The lakes where the crayfish were caught are located in the Mazurian Lake District, Poland. Dgał Wielki Lake is a flow-through lake with an area of 94.5 ha, maximum depth 17.6 m, average depth 5.3 m, maximum length 1,300 m, maximum width 1,120 m and the shoreline length of 5,200 m. The lake has a developed shoreline, hard sandy bottom with muddy sediments

in its bays. The lake's direct catchment area comprises 35% of poor arable land, 30% of pastures and fallows, 25% of coniferous forest and almost 10% of farm buildings. This mesotrophic lake is inhabited by such fish as roach, pike, bream, perch, sheatfish, eel, sturgeon and bighead carp.

Pobłędzie Lake is half the size of Dgał Wielki. It has an area of 57.6 ha, maximum depth 15.4 m and an average depth of 5.9 m. Pobłędzie is a flow-through lake located in a protection zone of the Puszcza Romnicka Landscape Park. Perch and pike are dominant species in its ichthyofauna, but eels are absent. The lake is distinguished by relatively low trophy, although it cannot be classified as an oligotrophic water body (KRZYWOSZ, KRZYWOSZ 2002).

Material

The tested material consisted of 40 individuals, including 30 individuals of spinycheek crayfish (*Orconectes limosus* Raff.) and 10 individuals of signal crayfish (*Pacifastacus leniusculus* Dana). Spinycheek crayfish were caught in two lakes: Dgał Wielki ($n=15$, 12 males and 3 females) and Pobłędzie ($n=15$, 9 males and 6 females); signal crayfish ($n=10$, 9 males and one female) were caught only in Pobłędzie Lake. The crayfish were caught on 28th September 2003, then transported to a laboratory, weighed and measured (Table 1). The following organs were dissected: abdomen muscles, hepatopancreas and exoskeleton. Because of the small weight of spinycheek crayfish, the material from three individuals of the same gender was aggregated for analysis. The samples were tightly packed, frozen and stored for analyses at -30°C .

Table 1

Body weight and length (from the rostrum to the end of telson) of spinycheek crayfish (*Orconectes limosus* Raff.) and signal crayfish (*Pacifastacus leniusculus* Dana.) from Dgał Wielki Lake and Pobłędzie Lake, Poland

Species	Lake	Gender*	n	Weight (g)		Length (cm)	
				min-max	mean \pm SD	min-max	mean \pm SD
Spinycheek crayfish	Dgał Wielki	m	12	20.36-32.94	25.68 \pm 3.69	8.5-10.6	9.2 \pm 0.6
		f	3	10.56-25.26	17.24 \pm 7.44	7.3-9.9	8.6 \pm 1.3
	Pobłędzie	m	9	16.17-34.87	25.24 \pm 5.66	7.8-10.2	8.7 \pm 0.8
		f	6	21.64-31.37	25.85 \pm 3.31	8.7-10.5	9.6 \pm 0.6
Signal crayfish	Pobłędzie	m	9	67.80-114.90	90.79 \pm 16.85	12.0-13.5	12.7 \pm 0.5
		f	1	-	47.63	-	11.3

* m – males, f – females

Analytical methods

Mercury determination. The samples of the muscles and hepatopancreas were digested in mixture of concentrated nitric acid and perchloric acid (7:3), as in ADRIAN (1971), while the samples of the exoskeleton were mineralized for 12 hours in concentrated nitric acid in an incubator at 70°C. After dissolution with deionized water, the samples were filtered to a volumetric flask and made up to 100 ml. The content of mercury was determined by cold vapour atomic absorption spectrometry (CV AAS) in a Bacharach Coleman MAS 50D apparatus, after reducing mercury ions with 5 ml 10% SnCl₂ solution.

Determination of other metals. Tissue samples were placed in test tubes and mineralized in a mixture of concentrated nitric acid and perchloric acid (4:1) in a heating block. The block temperature was gradually increased from 50°C to 200°C in order to avoid untimely evaporation of the acids and carbonization of the residue. The dry residue was diluted in 2 ml of 15% HNO₃, transferred to a volumetric flask and made up to 20 ml with deionized water.

Cadmium and lead were determined using graphite furnace atomic absorption spectrometry (GF AAS) in a Perkin Elmer ZL 4110 apparatus, while the other chemical elements (Al, Cr, Cu, Fe, Li, Mn, Ni, V, Zn) were determined using inductively coupled plasma atomic emission spectroscopy (ICP AES) in a Jobin Yvon JY-24 apparatus.

RESULTS AND DISCUSSION

It is common knowledge that the content of elements in aquatic organisms depends on their environmental concentrations and their involvement in metabolism (ANDERSON et al. 1997b). Average concentrations (mg kg⁻¹ wet weight) of the analysed metals in the three selected organs of crayfish were as follows: Hg – 0.004-0.045; Cd – 0.001-0.017; Pb – 0.018-0.079; Cu – 1.21-4.34; Zn – 6.80-51.91; Cr – 0.032-0.606; Ni – 0-0.782; Fe – 0.54-81.1; Mn – 0.26-91.31; V – 0-0.245; Li – 0.011-0.215 and Al – 14.8-123.4 (Table 2). With regard to the concentrations, the metals formed the following order: Al > Mn > Fe > Zn > Cu > Ni > Cr > V > Li > Pb > Hg > Cd.

The observed order of increasing metal concentrations in crayfish organs is consistent with their content in the lithosphere and hydrosphere provided by KABATA-PENDIAS, PENDIAS (1999); it is also consistent with the results obtained by MACKEVIČIENĖ (2002).

Mercury, cadmium and lead are explicitly toxic substances, although their low concentrations are tolerated by organism. The other metals belong to the group of elements essential for life; they have to be supplied to a body but their excess is harmful.

Mercury, cadmium and lead are found in animal tissues even when there is no environmental pollution. In increased concentrations, the metals inhibit enzymes, bind to amino acids, hemoglobin, RNA and DNA, cause histopathological changes in the hepatopancreas and exhibit mutagenic and carcinogenic activity (ANDERSON et al. 1997b, KABATA-PENDIAS, PENDIAS 1999, KOUBA et al. 2010).

KOUBA et al. (2010) discussed the content of these metals in the abdominal muscles and hepatopancreas of some species of crayfish caught in various regions of the world, based on a publication covering the period from the early 1970s until 2007. Metal concentrations (mg kg^{-1} wet weight) in the organs of crayfish caught in unpolluted areas were varied: 0.010-0.274 mercury in the muscles and 0.048-0.420 in the hepatopancreas; cadmium <0.004-0.116 and 0.02-0.72 respectively, and lead 0.012-2.14 and 0.008-1.66, respectively. This indicates that accumulation of mercury, cadmium and lead in the crayfish from the Mazurian Lakes (Table 2) lied within the lower range of the results obtained by the above authors.

Essential elements (Cu, Zn, Cr, Ni, Fe, Mn, V, Li, Al) are present in crayfish bodies as a result of their physiological functions. Besides the data on three toxic metals, KOUBA et al. (2010) also provided results of studies on the content of essential elements, namely: Cu, Zn, Cr and Ni. Their content (mg kg^{-1} wet weight) in the abdominal muscles and hepatopancreas of crayfish caught in unpolluted water bodies ranged within: copper 0.594-1.44 and 2.07-117.0, zinc 1.06-25.5 and 5.02-62.0, chromium <0.02-0.62 and 0.03-0.18, and nickel <0.08-0.97 and 0.25-0.93, respectively. The results of our study (Table 2) seem to be in agreement with the results provided by KOUBA et al. (2010). Similar data were also reported in publications of other authors (FINERTY et al. 1990, ANDERSON et al. 1997b, MOELLER et al. 2003), and some of them additionally provided data on metal content in exoskeleton (MACKEVIČIENĖ 2002, POURANG, DENNIS 2005).

There are fewer publications on iron, manganese, vanadium, lithium, and aluminium in crustaceans. The content of these metals in crayfish organs varied depending on where they were caught and on the species. Concentrations detected in this study (Table 2) did not differ significantly from those reported by other authors for crayfish caught in unpolluted areas. According to KURUN et al. (2010), iron concentrations (mg kg^{-1} wet weight) in crayfish ranged within 67.77-73.41 in muscles and 68.07-75.34 in hepatopancreas. Taking into account the proportion of dry weight in the tested organs, these concentrations did not differ from iron content determined in our study (Table 2). Similar concentrations of iron level were found for whole crayfish (MOELLER et al. 2003).

MACKEVIČIENĖ (2002) reported the manganese content (mg kg^{-1} wet weight) in crayfish at the level of 5.56 ± 3.87 in muscles, 118 ± 11 in exoskeleton, and 56.00 ± 8.81 in hepatopancreas. Similar concentrations of the element in crayfish muscles and hepatopancreas were also determined by KURUN et al. (2010).

Table 2

Metal concentrations in the hepatopancreas, exoskeleton and abdominal muscles of spinycheek crayfish (*Orconectes limosus* Raff.) and signal crayfish (*Pacifastacus leniusculus* Dana.) from Lake Dgaj Wielki and Lake Pobędzie, Poland

Species	Lake	Organ*	Content ** (mg kg ⁻¹ wet weight)												
			Hg	Cd	Pb	Cu	Zn	Cr	Ni	Fe	Mn	V	Li	Al	
Spinycheek crayfish	Dgaj Wielki n = 5	h	0.035 ±0.011	0.002 ±0.002	0.025 ±0.018	1.70 ±0.29	21.28 ±8.92	0.032 ±0.045	0.156 ±0.147	20.4 ±15.1	4.16 ±1.70	0.000	0.011- ±0.009	52.3- ±20.1	
		e	0.007 ±0.007	0.006 ±0.006	0.057 ±0.023	2.92 ±0.33	6.80 ±1.07	0.606 ±0.198	0.782 ±0.092	6.8 ±4.5	35.76 ±8.81	0.245 ±0.033	0.215 ±0.021	123.4 ±8.1	
		m	0.028 ±0.034	0.002 ±0.001	0.022 ±0.015	1.21 ±0.41	10.66 ±4.21	0.040 ±0.034	0.000	0.54 ±0.78	0.260 ±0.134	0.000	0.013 ±0.003	26.6 ±14.1	
	Pobędzie n = 5	h	0.030 ±0.011	0.002 ±0.001	0.018 ±0.018	11.86 ±11.34	20.71 ±10.78	0.072 ±0.053	0.084 ±0.081	32.9 ±17.7	9.12 ±4.76	0.005 ±0.012	0.020 ±0.010	47.2 ±29.0	
		e	0.004 ±0.001	0.004 ±0.002	0.079 ±0.058	3.98 ±0.92	7.51 ±3.04	0.446 ±0.087	0.749 ±0.246	7.9 ±9.2	70.78 ±20.08	0.242 ±0.013	0.189 ±0.031	106.3 ±11.4	
		m	0.009 ±0.007	0.003 ±0.002	0.033 ±0.025	2.77 ±1.04	14.96 ±1.02	0.092 ±0.037	0.000	2.38 ±0.84	0.76 ±0.30	0.000	0.018 ±0.014	14.8 ±9.1	
Signal crayfish	Pobędzie n = 10	h	0.045 ±0.017	0.017 ±0.019	0.046 ±0.021	3.18 ±1.12	51.91 ±28.47	0.120 ±0.061	0.379 ±0.298	81.1 ±45.4	6.54 ±5.50	0.015 ±0.013	0.030 ±0.012	47.2 ±25.3	
		e	0.020 ±0.026	0.012 ±0.009	0.025 ±0.063	4.34 ±0.53	8.85 ±3.33	0.374 ±0.215	0.518 ±0.083	5.2 ±5.8	91.31 ±60.38	0.182 ±0.038	0.149 ±0.021	99.4 ±10.3	
		m	0.026 ±0.045	0.001 ±0.001	0.023 ±0.010	2.02 ±0.68	9.97 ±5.07	0.060 ±0.030	0.016 ±0.032	1.29 ±1.58	0.430 ±0.306	0.000	0.025 ±0.006	34.0 ±11.2	

*h – hepatopancreas; e – exoskeleton; m – abdominal muscle; ** mean ± standard deviation

The study of FINERTY et al. (1990) showed that the vanadium content (mg kg^{-1} wet weight) in organs of crayfish caught in unpolluted areas was 0.000-0.946 in abdominal muscles and 0.000-0.554 in the hepatopancreas. The same authors observed several-fold higher concentrations in muscles (2.190-5.134 mg kg^{-1}) and the hepatopancreas (3.577-7.271 mg kg^{-1}) of crayfish caught from polluted areas.

Lithium occurs in all waters as hydroxide usually combined with colloidal suspension but rarely as an ion. The element also appears in all animal tissues, and is particularly abundant in parenchymatous organs (KABATA-PENDIAS, PENDIAS 1999). The available literature lacks publications on the lithium content in crustaceans.

Recent studies have proven that aluminium impairs functions of the hepatopancreas of crayfish (WOODBURN et al. 2011) and may also have an adverse effect on the immune system (WARD et al. 2006). The growing interest in this chemical element is stimulated by the fact that when environmental acidification increases, the bioavailability of aluminium increases as well. According to the reports published so far, the aluminium content (mg kg^{-1} wet weight) in crayfish muscles and the hepatopancreas ranged within 0.00-44.59 and 0.00-74.34, respectively (FINERTY et al. 1990, KURUN et al. 2010).

The crayfish organs tested in our study presented various degrees of metal bioaccumulation. According to the decreasing metal concentrations, the following order of organs was observed: Al, Mn, Cu, Ni, Cr, V and Li – exoskeleton > hepatopancreas > muscles; Zn and Hg – hepatopancreas > muscles > exoskeleton; Fe – hepatopancreas > exoskeleton > muscles. For lead and cadmium, no regular pattern of their concentrations in the particular organs was observed. The content of these metals depended on the species and on where they were captured (Table 2). Thus, among the three tested organs the highest metal concentration was observed in the exoskeleton or the hepatopancreas, being usually the lowest in muscles, an observation confirmed in publications by other authors (MACKEVIČIENĖ 2002, POURANG, DENNIS 2005, NAGHSHBANDI et al. 2007, KOUBA et al. 2010). Lead, cadmium, zinc and mercury were exceptions. The lead and cadmium content was dependent more on the catching location and the species than on the organ. The zinc and mercury concentrations in muscles were higher than in the exoskeleton, but lower than in the hepatopancreas. A similar relationship for zinc was reported by POURANG and DENNIS (2005).

A high content of metals in the hepatopancreas results from its function. It is a well-known fact that this organ is involved in absorption and storage of nutrients, and in detoxification processes, which makes it prone to bioaccumulating metals (ANDERSON et al. 1997b, POURANG, DENNIS 2005). High levels of metals in the exoskeleton may be due to the absorption of metals from water (ANDERSON et al. 1997a, b) or the fact that it is the route for both absorption and excretion of metals from the organism (MACKEVIČIENĖ 2002). Some of the detected differences in metal concentrations in crayfish

organs between the species and the catching areas proved to be statistically insignificant (Table 2). However, significant differences were demonstrated in some earlier research (KOUBA et al. 2010). Our results allow us to conclude that metal concentrations in the lakes where the crayfish had lived were within the range of natural background.

Abdominal muscles of crayfish are treated as food, therefore it is essential to compare the determined concentrations of mercury, cadmium and lead with the legally set maximum limits. In Poland, which is a member state of the European Union, concentrations of the three toxic heavy metals are regulated by Commission Regulation (EC) No 1881/2006, which sets threshold levels for certain contaminants in foodstuffs (Dz.U. 2006). This Regulation stipulates that the content of mercury, cadmium and lead in meat of crustaceans shall not exceed 0.50 mg kg^{-1} wet weight. Concentrations of these metals in the meat of the tested crayfish were several-fold lower, hence it should be safe for the health of potential consumers.

CONCLUSIONS

1. With regard to the concentration in crayfish organs, the following order of metals was observed: $\text{Al} > \text{Mn} > \text{Fe} > \text{Zn} > \text{Cu} > \text{Ni} > \text{Cr} > \text{V} > \text{Li} > \text{Pb} > \text{Hg} > \text{Cd}$.

2. The tested crayfish organs presented different degrees of metal bioaccumulation; regarding concentrations of metals, the following order of organs was observed: the exoskeleton > hepatopancreas > muscles for Al, Mn, Cu, Ni, Cr, V and Li; hepatopancreas > muscles > exoskeleton for Zn and Hg; hepatopancreas > exoskeleton > muscles for Fe. No such explicit correlations were found for Pb and Cd.

3. Some differences in the metal content in crayfish organs between the species and catching areas proved to be statistically insignificant.

4. The concentrations of toxic metals in the abdominal muscles of the tested crayfish were several-fold lower than the maximum permissible levels, which means that the meat was safe for the health of potential consumers.

REFERENCES

- ADRIAN W. 1971. *A new digestion method for biological material utilizing pressure*. At. Absorpt. Newsl., 10(4): 96.
- AHERN M.D., MORRIS S. 1999. *Respiratory, acid-base and metabolic responses of the freshwater crayfish *Cherax destructor* to lead contamination*. Comp. Biochem. Physiol., 124A(2): 105-111.
- ANDERSON M.B., PRESLAN J.E., JOLIBOIS L., BOLLINGER J.E., GEORGE W.J. 1997a. *Bioaccumulation of lead nitrate in red swamp crayfish (*Procambarus clarkii*)*. J. Hazard. Mater., 54: 15-29.

- ANDERSON M.B., REDDY P., PRESLAN J.E., FINGERMAN M., BOLLINGER J.E., JOLIBOIS L., MAHESHWARUDU G., GEORGE W.J. 1997b. *Metal accumulation in crayfish Procambarus clarkii, exposed to a petroleum – contaminated Bayou in Louisiana*. Ecotox. Environ. Safe, 37: 267-272.
- BERNARD A., WŁASOW T., SOBCZAK J. 2001. *Assessment of the health of freshwater crayfish in relation to the standard BN-73/9147-17*. Biul. Nauk. UWM, 13: 157-158. (in Polish)
- Dz.U. 2001. *Ordinance of the Minister of Agriculture and Rural Development of 12.11.2001 on fishing and farming conditions, fish farming and other organisms living in the water*. Journal of Law No 138, item 1559. (in Polish)
- Dz.U. 2003. *Ordinance of the Minister of Agriculture and Rural Development of 17.01. 2003 amending regulation on fishing and farming conditions, fish farming and other organisms living in the water*. Journal of Law No 17, item 160. (in Polish)
- Dz.U. 2006. *Commission Regulation (UE) No 1881/2006 of 19.12.2006 setting maximum levels for certain contaminants in foodstuffs*. Official Journal of the European Union. (in Polish)
- FINERTY M.W., MADDEN J.D., FEAGLEY S.E., GRODNER R.M. 1990. *Effects of environs and seasonality on metal residues in tissues of wild and pond-raised crayfish in Southern Louisiana*. Arch. Environ. Con. Tox., 19: 94-100.
- GYDEMOR R. 1996. Signal crayfish, *Pacifastacus leniusculus*, as a vector for *Psorospermium haeckli* to noble crayfish, *Astacus astacus*. Aquaculture, 148: 1-9.
- KABATA-PENDIAS A., PENDIAS H. 1999. *Biogeochemistry of trace elements*. Wyd. Naukowe PWN, Warszawa. (in Polish)
- KOSSAKOWSKI J., MNICH M., KOSSAKOWSKI G. 1978. *The first introduction of the crayfish Pacifastacus leniusculus Dana into Polish waters*. Freshw. Crayfish, 4: 195.
- KOUBA A., BUŘIČ M., KOZÁK P. 2010. *Bioaccumulation and effects of heavy metals in crayfish*. Water Air Soil Pollut., 211: 5-16.
- KOZUBÍKOVÁ E., PETRUSEK A., ĎURIŠ Z., MARTIN M.P. DIÉGUEZ-URIBEONDO J., OIDTMANN B. 2008. *The old menace is back: Recent crayfish plague outbreaks in the Czech Republic*. Aquaculture, 274: 208-217.
- KRZYWOSZ T. 2006. *Evaluation of changes in the abundance of three catchable crayfish species in Lake Płędzie (Northern Poland)*. Arch. Pol. Fish., 14(1): 131-140.
- KRZYWOSZ T., CHYBOWSKI L., ULIKOWSKI D. 1995. *Signal crayfish in Poland – history, present, prospects*. Kom. Ryb., 1: 5-8. (in Polish)
- KRZYWOSZ T., KRZYWOSZ W. 2001. *Preliminary observations of an american crayfish Pacifastacus leniusculus (Dana) population at a new site in the Mazurian Lake District*. Arch. Pol. Fish., 9(2): 273-278.
- KRZYWOSZ T., KRZYWOSZ W. 2002. *Observations of the signal crayfish Pacifastacus leniusculus (Dana) in a lake in the eastern Suwałki Lake District*. Arch. Pol. Fish., 10(2): 255-267.
- KURUN A., BALKIS N., ERKAN ., BALKIS H., AKSU A., ERŞAN M.S. 2010. *Total metal levels in crayfish Astacus leptodactylus (Eschscholtz, 1823), and surface sediments in Lake Terkos, Turkey*. Environ. Monit. Assess., 169: 385-395. DOI 10.1007/s10661-009-1181-5
- MACKEVIČIENĖ G. 2002. *Bioaccumulation of heavy metals in noble crayfish (Astacus astacus L.) tissues under aquaculture*. Ekologija (Vilnius), 2: 79-82.
- MEYER W., KRETSCHMER M., HOFFMAN A., HARISCH G. 1991. *Biochemical and histochemical observations on effects of low level metal load (lead, cadmium) in different organ systems of freshwater crayfish, Astacus astacus L. (Crustacea Decapoda)*. Ecotox. Environ. Safe, 21:137-156.
- MOELLER A., MACNEIL S.D., AMBROSE R.F., QUE HEE S.S. 2003. *Elements in fish of Malibu Creek and Malibu Lagoon near Los Angeles, California*. Mar. Pollut. Bull., 46: 424-429.

- NAGHSBANDI N., ZARE S., HEIDARI R., RAZZAGHZADEH S. 2007. *Concentration of heavy metals in different tissues of Astacus leptodactylus from Aras Dam of Iran*. Pakistan J. Biol. Sci., 10(21): 3956-3959.
- POURANG N., DENNIS J.H. 2005. *Distribution of trace elements in tissues of two shrimp species from the Persian Gulf and roles of metallothionein in their redistribution*. Environ. Int., 31: 325-341.
- REDDY P.S., DEVI M., SAROJINI R., NAGABHUSHANAM R., FINGERMAN M. 1994. *Cadmium chloride induced hyperglycemia in the red swamp crayfish, Procambarus clarkii: Possible role of crustacean hyperglycemia hormone*. Comp. Biochem Physiol., 107C(1): 57-61.
- RODRIGUEZ P., MEDESANI D., RODRIGUEZ E. 2003. *Inhibition of molting by cadmium in the crab Chasmagnathus granulata*. Aquat. Toxicol., 64(2): 155-164.
- ŚMIETANA P., KRZYWOSZ T. 2006. *Determination of the rate of growth of Pacifastacus leniusculus, in Lake Pobłędzie using polymodal length frequency distribution analysis*. Bull. Fr. Peche Piscic., 380-381: 1229-1244.
- ŚMIETANA P., STRUŻYŃSKI W. 1999. *Crayfish – an outline of the general situation in the country*. Mag. Przem. Ryb., 2: 80-81. (in Polish)
- STYRISHAVE B., DEPLEDGE H. 1996. *Evaluation of mercury-induced changes in circadian heart rate rhythms in the freshwater crab, Potamon potamios and the crayfish Astacus astacus as an early predictor of mortality*. Comp. Biochem. Physiol., 115A(4): 349-356.
- ULIKOWSKI D., BORKOWSKA I. 1999. *The mating of spinycheek crayfish (Orconectes limosus Raf.) – in autumn or spring?*. Kom. Ryb., 3: 4-5. (in Polish)
- WARD R.J.S., MCCROHAN C.R., WHITEK.N. 2006. *Influence of aqueous aluminium on the immune system of the freshwater crayfish Pacifastacus leniusculus*. Aquat. Toxicol., 77: 222-228.
- WOODBURN K., WALTON R., MCCROHAN C., WHITE W. 2011. *Accumulation and toxicity of aluminium-contaminated food in the freshwater crayfish, Pacifastacus leniusculus*. Aquat. Toxicol., 105: 535-542.