
BIOACCUMULATION OF SOME ELEMENTS IN THE MILLIPEDE *GLOMERIS HEXASTICHA* (BRANDT, 1833) (DIPLOPODA, GLOMERIDA)

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Abstract

Millipedes are soil invertebrates participating in the decomposition of leaf litter. Previous studies indicated that soil invertebrates were useful in the environmental pollution research dedicated to the content and accumulation of heavy metals. The aim of the present study was to compare the content of some elements in bodies of adult millipedes *Glomeris hexasticha* (Brandt, 1833) collected from a more polluted town called Jaworzno in Upper Silesia, Southern Poland, and from the less polluted Lublin area, Eastern Poland. The content of elements was determined with the ICP method on a VISTA MPX Varian spectrometer. The concentrations of elements in the millipedes from Jaworzno appeared in the following decreasing order: Ca, P, S, Mg, K, Na, Fe, Al, Zn, Cu, Mn, Pb, B, Cr, Cd and Ni, compared to the Lublin area: Ca, P, K, Mg, S, Na, Fe, Al, Cu, Zn, Mn, Pb, B, Cr, Cd and Ni. It was confirmed that the elements such as Al, Zn, Mn, Cr, Cd and Ni in *Glomeris hexasticha* from Jaworzno were in higher concentrations than in specimens from the Lublin area. Reversely, millipedes collected from the Lublin area contained higher concentrations of Ca, P, K, Mg, Na, Fe, Cu and B than those from Jaworzno. The results show that differences in the content and distribution of some elements at two locations with different levels of environmental pollution were clearly reflected in bodies of the millipede *G. hexasticha*.

Keywords: elements, contamination, millipedes, *Glomeris hexasticha*.

**BIOAKUMULACJA NIEKTÓRYCH PIERWIASTKÓW U KROCIONOGA
GLOMERIS HEXASTICHA (BRANDT,1833) (DIPLOPODA, GLOMERIDA)**

Abstrakt

Krocionogi są bezkręgowcami glebowymi, biorącymi udział w dekompozycji ściółki liściowej. Wcześniejsze badania wykazały, że zawartość pierwiastków i akumulacja metali ciężkich u bezkręgowców glebowych stanowią użyteczne dane w badaniach nad zanieczyszczeniem środowiska. Celem pracy było porównanie zawartości niektórych pierwiastków u dorosłych osobników krocionogów *Glomeris hexasticha* (Brandt, 1833) zbieranych z obszaru bardziej zanieczyszczonego Jaworzna na Górnym Śląsku i z mniej zanieczyszczonego obszaru Lublina we wschodniej Polsce. Zawartość pierwiastków oznaczano metodą ICP na spektrofotometrze VISTA MPX VARIAN. U krocionogów z Jaworzna zawartość oznaczonych pierwiastków w kolejności stężeń od najwyższego do najniższego była następująca: Ca, P, S, Mg, K, Na, Fe, Al, Zn, Cu, Mn, Pb, B, Cr, Cd i Ni, a z Lublina: Ca, P, K, Mg, S, Na, Fe, Al, Cu, Zn, Mn, Pb, B, Cr, Cd i Ni. Takie pierwiastki, jak Al, Zn, Mn, Cr, Cd i Ni stwierdzono w większym stężeniu u *Glomeris hexasticha* z Jaworzna niż u osobników z Lublina. Natomiast u krocionogów zebranych z Lublina wykazano większe stężenie następujących pierwiastków: Ca, P, K, Mg, Na, Fe, Cu i B, w porównaniu ze stężeniem u krocionogów z Jaworzna. Wyniki wskazują, że różnice w rozkładzie zawartości niektórych pierwiastków w dwu miejscach Polski o różnym poziomie zanieczyszczenia środowiska, były wyraźnie odzwierciedlone u krocionogów *Glomeris hexasticha*.

Słowa kluczowe: pierwiastki, zanieczyszczenie, krocionogi, *Glomeris hexasticha*.

INTRODUCTION

Soil dwelling arthropods are distinguished by a great diversity of species. Millipedes are soil invertebrates which participate in the decomposition of leaf litter. Leaf litter decay is significantly reduced by heavy metal pollution (MCENROE, HELMISAARI 2001). Heavy metals have been found to cause reductions in the abundance of soil decomposers and in the diversity of animal communities (HAIMI, SIIRA-PIETIKAINEN 1996, GRELE et al. 2000). Environmental pollution field studies have been conducted on terrestrial isopods, centipedes, millipedes, earthworms, insects and snails (CZARNOWSKA 1980, HOPKIN et al. 1985, DALLINGER et al. 1992, DALLINGER 1994, PAOLETTI et al. 1991, GRELE et al. 2000, HOBBELEN et al. 2004, NAKAMURA et al. 2005, WARCHAŁOWSKA-ŚLIWA et al. 2005, KOWALCZYK-PECKA 2009, KANIA 2010). It has been stated that heavy metal deposition in millipedes depends on environmental pollution (HOPKIN et al. 1985, KÖHLER et al. 1995, NAKAMURA, TAIRA 2005). *Glomeris hexasticha* is the south-eastern and central European millipede species of soil macrofauna. Specimens of *Glomeris hexasticha* (Brandt,1833) were collected from a more polluted town called Jaworzno, which lies in Upper Silesia (southern Poland), an area in the state of ecological emergency (PAWŁOWSKI 1990, www.zazi.iung.pulawy.pl/Images/metale.jpg) and from the less polluted Lublin area, in the eastern part of Poland (DECHNIK et al. 1987, KOZAK, KOZAK 1987). Soils around Lublin do not indicate excessive concentrations of heavy metals, with their levels similar to natural ones (BELZ

2003, LIPIŃSKI 2003). The aim of the present study was to compare concentrations of some elements in bodies of adult millipedes *Glomeris hexasticha*, from two locations: Jaworzno and Lublin in Poland.

MATERIAL AND METHODS

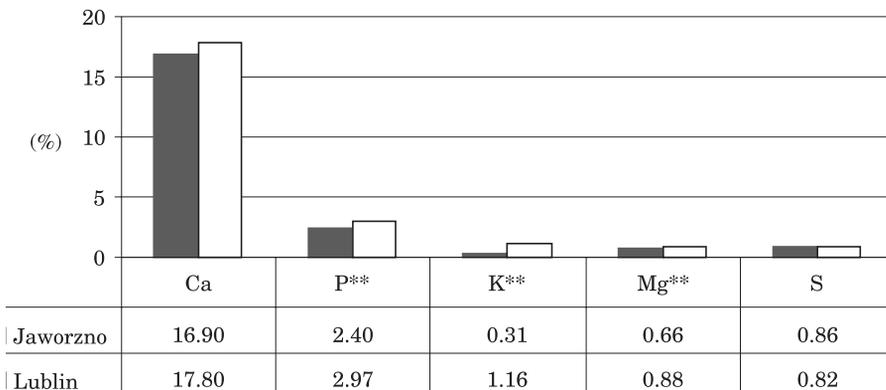
Soils in Jaworzno are moderately, heavily and very heavily polluted. Soils with the content of Cd, Cu, Pb above the upper threshold and with a very high concentration of Zn have been found in there (PAWŁOWSKI 1990). It has been reported that Cd, Cu, Pb and Zn in soils in the south-eastern part of Jaworzno are up to 25.7 mg kg⁻¹, 119 mg kg⁻¹, 403 mg kg⁻¹ and 1920 mg kg⁻¹, respectively [www.bip.jaworzno.pl]. The soil pH in the northern part of Jaworzno exceeds 8 but falls below 7 in southern areas of the town. Acid soils are mainly south of the town [www.bip.jaworzno.pl]. Soils in the Lublin area are characterized by very good properties, for example 92% of soils have a natural content of heavy metals (BELZ 2003, LIPIŃSKI 2003). The content of heavy metals in soils in allotment gardens in Lublin demonstrate their slight contamination (BOŻEK, KRÓLIK 2009). The soil pH (7.1-7.8) in Lublin is neutral and alkaline (SOBOCIŃSKA 2003). The millipedes *Glomeris hexasticha* were collected from an ecotone of a pine forest (*Pinus silvestris*) and sandy grassland in the south-eastern part of Jaworzno (Upper Silesia), as well as from a mixed forest with pine and oak in Zemborzyce, south of in Lublin, Poland. The surface area for sampling was 500 m² at each location. In May and June 2006, millipedes were captured manually, three times and in three replicates, at ten-day intervals, both in Jaworzno and Lublin. In total, 18 samples, 9 from each region, were studied. In 2008, the content of the elements Al, B, Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, Pb, S and Zu was determined in the bodies of *Glomeris hexasticha*. In a laboratory, the millipedes were kept in glass vessels and fed with plant litter. For sample preparation, adult millipedes were kept for two days in Petri dishes on wet Whatman medium to defecate. Next, the specimens were placed in a sterile container and washed with distilled sterile water for 1 minute. The procedure was repeated four times. Then, the millipedes were euthanized by immersing in ether, after which they were dried to dry mass by keeping in an airy place at room temperature for a few days. Finally, they were preserved until analyses. Prior to analyses, the samples were dried to constant mass in a dryer set at 30°C for 72 hours, according to the method described in FALANDYSZ et al. (2007). Samples of dry material (0.25 g-0.5 g) were added 10 ml concentrated HNO₃ and 1 ml of H₂O₂, and mineralized in a MARS 5 microwave, according to instruction. Concentrations of the elements were determined with the ICP method on a VISTA MPX Varian spectrometer. They were measured in solutions and then recalculated to dry weight, as a percentage (%) of dry weight for Ca, P, K, Mg and S. However, Na, Fe, Al,

Zn, Cu, Mn, Pb, B, Cr, Cd and Ni were given in mg kg⁻¹ dry weight. At the sites where millipedes were collected, measurements of soil pH were taken with a Takemura Soil Tester DM-15. Statistical differences in elemental concentrations found in bodies of millipedes from the two locations and in soil pH were checked by Student's *t* test.

RESULTS AND DISCUSSION

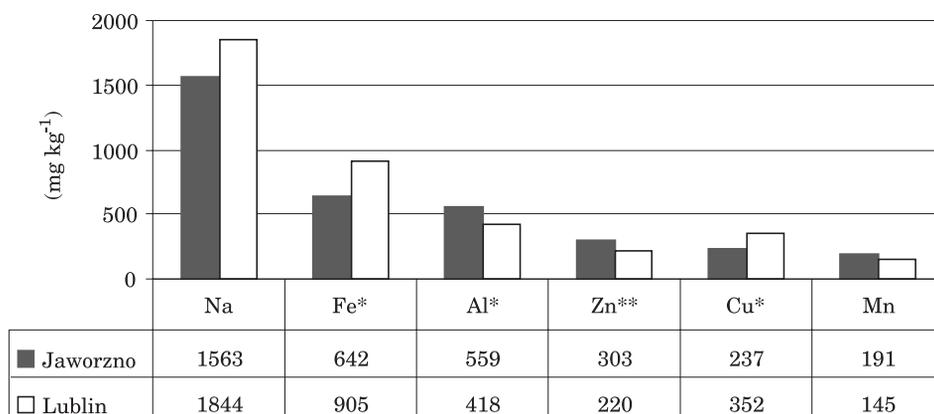
The content of the elements such as Al, B, Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, Pb, S and Zn was determined in whole bodies of the millipede *Glomeris hexasticha*, collected from Jaworzno and from the Lublin area. In the samples from Jaworzno the elements appeared in the following decreasing order: Ca, P, S, Mg, K, Na, Fe, Al, Zn, Cu, Mn, Pb, B, Cr, Cd, Ni; in millipedes from the Lublin area, the decreasing order was Ca, P, K, Mg, S, Na, Fe, Al, Cu, Zn, Mn, Pb, B, Cr, Cd, Ni. In all the specimens, the content of Ca was the highest, on average 16.9% (from Jaworzno) and 17.8% (from Lublin), respectively (Figure 1). Millipedes from Lublin had higher concentrations of the following elements: Ca, P, K, Mg, Na, Fe, Cu and B, than those from Jaworzno. *Glomeris hexasticha* from Upper Silesia showed higher quantities of Al, Zn, Mn, Cr, Cd and Ni, than individuals of the same species collected from the Lublin area. The differences were statistically significant in the case of P, K, Mg and Zn, Fe, Al, Cu (Student's *t* test) – Figures 1, 2.

In particular, Cu was found in a remarkably high concentration in *G. hexasticha*. The Cu content was 352 mg kg⁻¹ dry weight in millipedes from the Lublin area and 237 mg kg⁻¹ dry weight in specimens from Jaworzno.



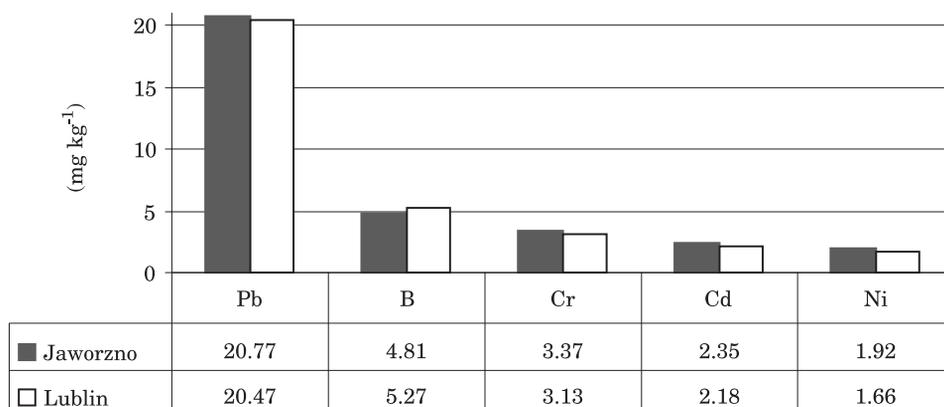
** differences statistically significant $p \leq 0.01$

Fig. 1. Content of elements (% dry weight) in *Glomeris hexasticha*



* differences statistically significant $p \leq 0.05$ ** differences statistically significant $p \leq 0.01$

Fig. 2. Content of elements (mg kg^{-1} dry weight) in *Glomeris hexasticha*



differences are not statistically significant

Fig. 3. Content of elements (mg kg^{-1} dry weight) in *Glomeris hexasticha*

Concentrations of hazardous metals determined in all the examined specimens of *G. hexasticha* were in the order of $\text{Cu} > \text{Pb} > \text{Cd}$ (in mg kg^{-1} dry weight): $237 > 20.77 > 2.35$ from Jaworzno and $352 > 20.47 > 2.18$ from Lublin, respectively (Figure 2, 3). Interestingly, the levels of S and Pb were almost the same in the millipedes from both sampling sites, while the potassium concentration in specimens from the Lublin area was 3.74-fold higher than in the millipedes from Jaworzno (Figures 1, 3). The soil from Jaworzno was acidic ($\text{pH} = 6.28$) and the soil from the Lublin area was neutral ($\text{pH} = 6.91$) – Table 1.

Some contaminated areas may have unusual communities of fauna, shaped by their varied resistance to pollutants (HOPKIN et al. 1985). Previous studies indicated that elemental content and accumulation of heavy metals

Table 1

Location	Soil pH at two locations		
	range	average	SD
Jaworzno	5.62 - 6.68	6.28	0.46
Lublin	6.85 - 6.94	6.91	0.04

Differences are not statistically significant

in invertebrates were useful for studies on environmental pollution caused by harmful products from industries (READ et al. 1998, NIJMA 1998, HEIKENS et al. 2001, WARCHAŁOWSKA-ŚLIWA et al. 2005). Excessive amounts of various chemicals interfere with complex processes occurring in soils and may lead to the degradation of soils, primarily because of two factors: a change in soil acidity or alkalinity and accumulation of trace elements, particularly metals (PAWŁOWSKI 1990). The soil properties such as reaction and elemental concentrations modify assimilation of some elements by soil organisms. According to ADRIANEK and SKOWRONEK (2005), acidic soils prevail in Upper Silesia, the fact which is confirmed by our results on soil pH determinations in Jaworzno; the mean pH was 6.28, within the range 5.62-6.68 (Table 1). South of Lublin, in Zemborzyce, the mean soil pH was 6.91, within the range 6.85-6.94 (Table 1), which supports previous data on soil pH in the Lublin area (SOBOCIŃSKA 2003). In acidic soils, saturation with soil exchange ions with loss of Ca^{2+} and Mg^{2+} is lower, and potentially toxic metals Al^{3+} and Mn^{2+} travel to the soil solution (SIUTA 1987, TURSki 1987, ADRIANEK, SKOWRONEK 2005).

Acidification of soils activates considerable amounts of Al, whose excess affects the plant growth and development as well as the human health (MICHAŁEK 1997). Al^{3+} ions, which appear in the soil solution at pH = 5.5-7.0, are accessible to plants. Absorbed by plants, Al^{3+} ions enter the trophic chain, showing links with soil fauna. This explains why the specimens of *G. hexasticha* from Jaworzno showed a higher concentration of aluminum than millipedes from the Lublin area (Figure 2). Acidic soils of Upper Silesia are poor in absorbable forms of Ca, Mg, P and K, which translates into the inferior availability of elements to plants and soil fauna (ADRIANEK, SKOWRONEK 2005). In contrast, medium and high concentrations of P, K, Mg and B were found in soils of the Lublin area (DECHNIK et al. 1987), which manifested themselves in bodies of *Glomeris hexasticha*. Particularly, 3.74-fold more potassium was found in *G. hexasticha* individuals from Lublin than in the ones from Jaworzno (Figure 1). Such results indicated lesser environmental contamination of the Lublin area, because excessive concentration of potassium were indicated in plants from areas which are not industrially polluted (SIUTA 1987). Millipedes *G. hexasticha* and *O. sabulosus* from Lublin (KANIA 2010) contained more Fe than those from Jaworzno (Figure 2). High levels of Fe content are characteristic for most soils in the Lublin area (KABATA-PENDIAS 1981). This is confirmed by KOWALCZYK-PECKA (2009), who showed high concentrations of Fe in samples of soil and in snail tissues from

Lublin. The lower Fe content in millipedes from Jaworzno may have resulted from the Fe metabolism being blocked by heavy metals (ADRIANEK, SKOWRONEK 2005), due to the competition between heavy metals and iron for the same places in chelates. Heavy metals in soils of Upper Silesia appear in quantities above the norm (PAWŁOWSKI 1990, www.zazi.iung.pulawy.pl/Images/metale.jpg). The most severely contaminated with heavy metals are acidic soils in the south-eastern part of Jaworzno (www.bip.Jaworzno.pl), which is where the millipedes for our analyses were collected. The acid reaction of soils increases assimilation of some elements by soil organisms (SIUTA 1987). The differences in the distribution of elements, particularly toxic metals, between the two locations with different soil pH were distinctly manifested by differences in the elemental composition of millipedes. The elements such as Al, Zn, Mn, Cr, Cd and Ni in *G. hexasticha* from Jaworzno were in higher concentrations than in *G. hexasticha* from Lublin (Figures 2, 3).

The content of Zn in *G. hexasticha* reflected the environmental state of pollution, with an abnormally high concentration of Zn in the soils lying in the south-eastern part of Jaworzno (www.bip.Jaworzno.pl). The higher Zn content in *G. hexasticha* from Upper Silesia than from Lublin coincided with a higher Zn concentration in *Tetrix tenuicornis* (Orthoptera, Insecta) collected from Upper Silesia (WARCHAŁOWSKA-ŚLIWA et al. 2005) and with high Zn quantities in *Glomeris marginata* from a contaminated grassland in England (HOPKIN et al. 1985). Differences in the Zn concentration between environmental habitats were also reflected in bodies of the millipede *Oxidus gracilis* (NAKAMURA et al. 2005). In contrast, HEIKENS et al. (2001) reported that Zn remained on a constant level in invertebrates.

Copper is an essential metal for invertebrates and, to a certain degree, can be regulated by several species, so that its concentration in animal bodies is rather constant over a range of soil concentrations (HEIKENS et al. 2001). High Cu concentrations have been indicated in millipedes (HOPKIN et al. 1985, HUNTER et al. 1987, HEIKENS et al. 2001, NAKAMURA et al. 2005, KANIA 2010) which was confirmed by the present study (Figure 2). The higher content of Cu in the millipedes from Lublin than that from Jaworzno reflected its medium content in soils of Lublin (SOBOCIŃSKA 2003), where the range of Cu was 1-191 mg kg⁻¹ dry weight (WIATR et al. 1997), compared to 1-119 mg kg⁻¹ dry weight in soils of Jaworzno (www.bip.Jaworzno.pl). In *G. hexasticha* specimens from Lublin, the concentration of Cu was 1.8-fold higher than in soil, and in the millipede individuals from Jaworzno, the concentration of Cu was double the one in the soil. However, the concentration of Cu in the millipede *Chamberlinius hualienensis* was 4-11-fold higher than in soil (NAKAMURA et al. 2005).

Millipedes have been shown to accumulate cadmium (HOPKIN et al. 1985, HUNTER et al. 1987), which is comparable to the Cd content in *G. hexasticha* reported herein and in *O. sabulosus* (KANIA 2010). Cadmium is one of the heavy metals which is potentially toxic at low levels (KABATA-PENDIAS 1981).

According to HOPKIN et al. (1985), the net assimilation of lead in millipedes during the passage through the gut is very small, for example the pill millipede *Glomeris marginata* did not assimilate significant amounts of lead even when it foraged on badly polluted sites. Therefore, the content of Pb in millipedes from both locations examined herein is nearly identical (Figure 3). However, according to POBOZSNY (1985), lead concentrations in millipedes differs from species to species. There is strong evidence that many soil animal species have adapted to heavy metals.

Different metal accumulation strategies of soil invertebrates are a consequence of diverse detoxification mechanisms (GRAFF et al. 1997). Diplopods possess effective mechanisms to bind and detoxify potentially toxic metals in tissues (KÖHLER et al. 1995). The detoxification mechanism of metals in millipedes may explain the absence of significant differences between concentrations of heavy metals in *G. hexasticha* collected from more (Upper Silesia) and less contaminated (Lublin area) locations in Poland.

The concentrations of hazardous heavy metal in millipedes such as *Brachyiulus pusillus*, *Cylindroiulus latestriatus*, *C. brittanicus*, *Brachydesmus superus*, *Julus scandinavicus*, *Polydesmus denticulatus*, *Oxidus gracilis* and *Ommatoiulus sabulosus* (HOBBELEN et al. 2004, NAKAMURA, TAIRA 2005, KANIA 2010) were in the order of Cu>Pb>Cd, which agrees with the results obtained in the current study on *Glomeris hexasticha*.

CONCLUSIONS

1. Because of the local environmental pollution, samples from Jaworzno had a higher content of toxic metals such as Al, Zn, Mn, Cr, Cd, Ni than the ones from Lublin.

2. Concentrations of the following elements: Ca, P, K, Mg, Na, Fe, Cu, and B in bodies of *G. hexasticha* reflected their concentrations in soils at the two locations.

3. The results show that differences in the distribution of some elements between the two locations, due to the differences in the degree of environmental pollution, were clearly reflected in *G. hexasticha*.

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