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ELEMENTS CONTENT IN THE MILLIPEDE *OMMATOIULUS SABULOSUS* (LINNAEUS 1758); *ARTHROPODA : DIPLOPODA*

ZAWARTOŚĆ PIERWIASTKÓW U KROCIONOGA PIASKOWEGO *OMMATOIULUS SABULOSUS* (LINNAEUS 1758); *ARTHROPODA: DIPLOPODA*

Słowa kluczowe: krocionóg piaskowy, pierwiastki, metale ciężkie, zanieczyszczenie.
Key words: *Diplopoda*, *Ommatoiulus sabulosus,* elements, heavy metals, contamination.

Celem prezentowanej pracy było określenie zawartości pierwiastków u krocionoga piaskowego – Ommatoiulus sabulosus (Linnaeus 1758). Osobniki były zbierane w Jaworznie (Górny Śląsk) i Chrzanowie (Małopolska) oraz w Lublinie, w Polsce. Zawartość pierwiastków oznaczano metodą ICP na VISTA MPX Varian spektrometrze. Zawartość pierwiastków u krocionoga piaskowego z Jaworzna i Chrzanowa, w kolejności od najwyższego do najniższego stężenia, była następująca: Ca, P, S, K, Mg, Na, Al, Fe, Zn, Cu, Mn, Pb, B, Cr, Cd, Ni, a z Lublina: Ca, P, Mg, S, K, Na, Fe, Al, Zn, Cu, Mn, Pb, B, Cr, Cd, Ni. U wszystkich badanych osobników krocionogów poziom Ca był znacząco wyższy niż innych pierwiastków. U osobników O. sabulosus zebranych z Lublina stwierdzono większą zawartość P, K, Mg i Fe niż u osobników z Jaworzna i Chrzanowa. Zawartość wybranych metali kształtowała się w następującej kolejności: Cu > Pb > Cd. Nie wykazano istotnych różnic w zawartości metali ciężkich w krocionogach z dwóch porównywanych regionów Polski. Wyniki pracy wskazują, że krocionóg piaskowy nie jest bioindykatorem zanieczyszczenia metalami ciężkimi.

1. INTRODUCTION

Millipedes have been recognized as the most widely distributed and abundant organisms in terrestrial ecosystems, as saprophages taking part in decomposition of leaf litter in forests, grasslands and agroecosystems [Hopkin & Read 1992, Kime & Golovatch 2000].

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The diplopods also enrich soil in elements and take part in ecosystem restoration [Tracz & Gromysz-Kałkowska 1987, Tajovsky 2001]. Leaf litter decomposition is significantly reduced by heavy metal pollution [McEnroe & Helmisaari 2001]. The impact of heavy metals on decomposition of organic litter as a result of the harmful effects of metals on microbes and soil fauna is well documented [Baath 1989]. Decomposer community in coniferous forest soil appeared to be quite resistant to heavy metals. Soil contamination decreased soil microarthropods and *Oligochaeta* [Haimi & Siira-Pietikainen 1996, Haimi & Matasniemi 2002]. Upper Silesia (Górny Śląsk) Industrial Region, where Jaworzno is located, and neighbouring Chrzanów (Małopolska) are the largest heavy metal polluted area in southern Poland and is described as an area of ecological state of emergency [Siuta 1987, Pawłowski 1990, www. zazi.iung.pulawy.pl/Images/metale.jpg]. Lublin is situated in the eastern part of Poland, and is less polluted [Bełz 2003, Dechnik et al. 1987, Kozak & Kozak 1987, Lipiński 2003, Sobocińska 2003]. The striped millipede *Ommatoiulus sabulosus* (Linnaeus 1758) occurs throughout Europe. These millipedes are eurytopic, inhabit the open areas of fields, fallows and various plant communities. *Ommatoiulus sabulosus* indicate marked ecological adaptivity in different habitats [Tajovsky 1993, 2001,Voigtländer 1996, Madari et al. 1996]. Annual mass occurrence and migration of *O. sabulosus* has occurred in the regions: Górny Śląsk and Małopolska over 10 years. Millipedes are described as a seasonal pests because huge numbers invade houses, gardens and agroecosystems [Kania & Tracz 2005, Kania 2008, Voigtlander 2005]. The aim of the present study was to examine of the elements content in the bodies of adult millipede *Ommatoiulus sabulosus* from Górny Śląsk and Western Małopolska as well as from the Lublin area.

2. MATERIALS AND METHODS

The millipedes *Ommatoiulus sabulosus* (*Diplopoda: Julida)* were collected from an ecotone of pine forest (*Pinus silvestris*) and sandy grassland of south-eastern part of the Jaworzno area, and western part of Chrzanów as well as from mixed forest with pine and oak (*Pino-Quercetum*) in south part of the Lublin area, Zemborzyce, Poland. Soils of Jaworzno and Chrzanów belong to medium, strong and very strong polluted ones [Pawłowski 1990, www. zazi.iung.pulawy.pl/Images/metale.jpg]. Because of significant soils contamination with heavy metals, most of former agricultural lands are fallows. Soils of Lublin were not indicated excessive concentration of heavy metals [Bełz 2003]. The surface area for sampling was 500 m^2 at each location. The millipedes were captured by hand threefold in three repeats, in 16 May, 26 May and 5 June in 2006, the same for Jaworzno and Chrzanów as well as Lublin. Altogether 18 samples, 9 from each region, were studied. The content of the elements Al, B, Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, Pb, S and Zn, were determined in the bodies of the millipede *O. sabulosus*. Prior to analyses, the adult millipedes were kept in Petri dishes on wet Whatman medium to defecate for two days. Specimens were placed

in a sterile container and distilled sterile water was used for washing for 1 minute, the procedure was repeated four times. Then, the millipedes were killed by immersing in ether, and were air dried at room temperature, in an airy, dry and clean place for a few days. Specimens were dried to constant mass at 30°C in the dryer for 72 hours, according to method described in Falandysz et al. [2007]. Samples of dry material (0.25g – 0.5g), were mineralized in a microwave MARS 5 after addition of 10 ml concentrated HNO_3 and 1 ml of H_2O_2, according to instruction. The content of elements was obtained with the ICP method on a VISTA MPX Varian spectrometer. The measured concentrations of elements in solutions were recalculated to the dry weight, in percentage (%) of dry weight for Ca, P, K, Mg and S, but Na, Fe, Al, Zn, Cu, Mn, Pb, B, Cr, Cd and Ni, were given in $mg \cdot kg^{-1}$ dry weight. Results are the average of three collections, each in three repeats. In collection sites of millipedes measurements of soil pH were taken, using a Takemura Soil Tester DM- 15. Statistical differences in element concentrations of millipedes from the two locations and soil pH were checked by Student's *t* test.

3. RESULTS

Mean level of soil pH from Jaworzno and Chrzanów was acidic (pH = 6.3) and varied between 5.62 and 6.68. Mean level of soil pH from Lublin area was neutral (pH = 6.9), in range from 6.85 to 6.94. Differences between two regions were not statistically significant (Tab. 1).

Table 1. Soil pH at two regions

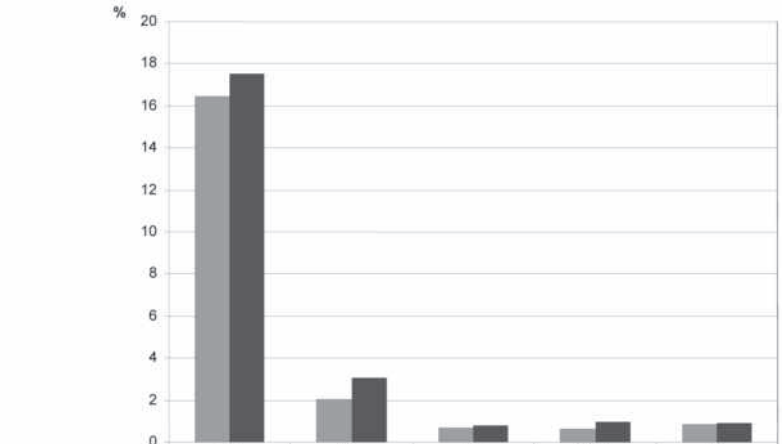
Tabela 1. Wartość pH gleby w dwóch regionach

Location	Soil pH
Jaworzno Chrzanów	range 5.62 – 6.68 average 6.28
	SD 0.46
Lublin	range 6.85 – 6.94 average 6.91
	SD 0.04

Differences are not statistically significant p > 0.05 (różnice nie są statystycznie istotne p > 0.05).

Elements content of *O. sabulosus* from higher to lower concentration in the case of Jaworzno and Chrzanów were the following: Ca, P, S, K, Mg, Na, Al, Fe, Zn, Cu, Mn, Pb, B, Cr, Cd, Ni, and from Lublin area were: Ca, P, Mg, S, K, Na, Fe, Al, Zn, Cu, Mn, Pb, B, Cr, Cd, Ni. In all examined specimens of millipedes the content of Ca was much higher than that of the other elements. *Ommatoiulus sabulosus* collected from Lublin indicated generally higher concentration of elements than the ones from Jaworzno and Chrzanów (Fig. 1, 2, 3). The content of P, K, Mg, Fe and Pb was 1.49; 1.21; 1.57; 2.12 and 1.43 times higher, respectively. The content of sulphur was almost the same in millipedes from the two sampling loca-

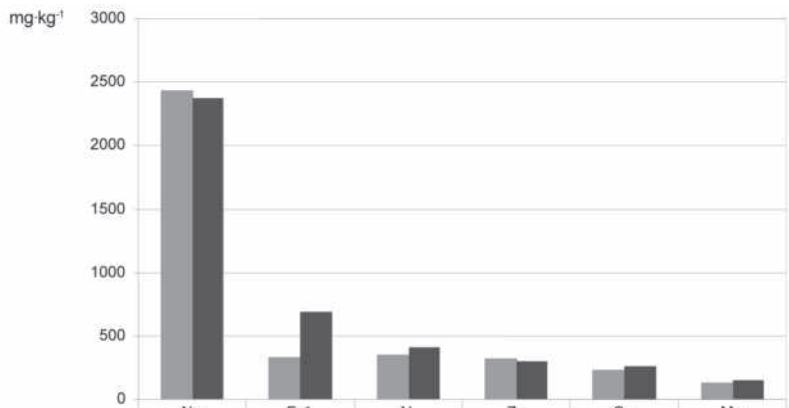
tions. In particular, copper was found at remarkably high concentration in the *O. sabulosus*. The hazardous metal concentrations in all examined specimens were in the order of Cu> Pb> Cd (in mg·kg^{-1} dry weight): 228 > 17.52 > 1.98 from Jaworzno and Chrzanów, and 258 > 25.08 > 2.21 from Lublin, respectively (Fig. 2, 3).



	Ca	P*	K*	Mg*	S
Jaworzno, Chrzanów	16,46	2,05	0,67	0,6	0,85
Lublin	17,5	3,06	0,81	0,94	0,91

* Differences statistically significant p ≤ 0.05 (* różnice statystycznie istotne p ≤ 0.05).

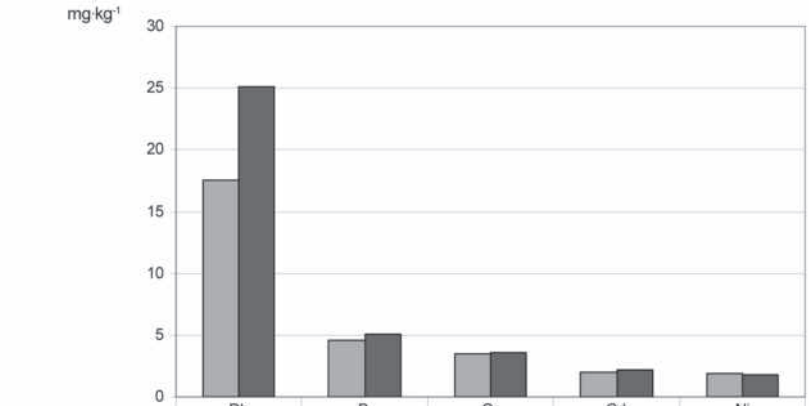
Fig. 1. Elements content (% dry weight) in the body of *Ommatoiulus sabulosus*
Rys. 1. Zawartość pierwiastków (% suchej masy) w ciele *Ommatoiulus sabulosus*



	Na	Fe*	Al.	Zn	Cu	Mn
Jaworzno, Chrzanów	2435	324	345	321	228	128
Lublin	2368	686	406	302	258	154

* Differences statistically significant p ≤ 0.05 (* różnice statystycznie istotne p ≤ 0.05).

Fig. 2. Elements content (mg·kg^{-1} dry weight) in the body of *Ommatoiulus sabulosus*
Rys. 2. Zawartość pierwiastków (mg·kg^{-1} suchej masy) w ciele *Ommatoiulus sabulosus*



	Pb	B	Cr	Cd	Ni
Jaworzno, Chrzanów	17,52	4,57	3,47	1,98	1,92
Lublin	25,08	5,13	3,58	2,21	1,83

Fig. 3. Elements content (mg·kg^{-1} dry weight) in the body of *Ommatoiulus sabulosus*
Rys. 3. Zawartość pierwiastków (mg·kg^{-1} suchej masy) w ciele *Ommatoiulus sabulosus*

Ommatoiulus sabulosus is not bioindicator of heavy metal pollution.

4. DISCUSSION

Soil pH of Jaworzno in northern part of town exceeds 8 and decreased in south direction to less than 7. Acidic soils exist mainly in southern vicinity of Jaworzno (www.bip.jaworzno.pl). According to Adrianek & Skowronek [2005] in Upper Silesia acidic soils dominate, which is confirmed with data of soil pH in Jaworzno and Chrzanów (Tab. 1). In the Lublin area neutral soil pH dominate (Tab. 1), which is in accordance with previous data of soil pH (7.1 – 7.8) in the Lublin area [Sobocińska 2003]. As a result of acidity, the reduction of saturation of soil exchange ions with loss of Ca and Mg takes place [Turski 1987]. Soil of Upper Silesia occur to lack absorption forms of Ca, Mg, P and K, affecting the decrease of availability of elements for plants, and soil fauna [Adrianek & Skowronek 2005]. This was reflected in *Ommatoiulus sabulosus* (Fig. 1). In millipedes from Jaworzno and Chrzanów, the content of P, K and Mg were lower than in specimens from Lublin, and all differences were statistically significant (Fig. 1). Medium and high contents of P, K, Mg and B were found in soils of the Lublin area [Dechnik et al. 1987], which is in accordance with the elements content of *O. sabulosus* from Lublin. Concentrations of Ca, P, K, Mg and B were higher in the total body of millipedes from Lublin than it was in the specimens from Jaworzno and Chrzanów (Fig. 1). The alkaline soil pH may be affected by amounts of Ca, Mg, K on the millipede *Oxidus gracilis* [Nakamura & Taira 2005]. Ca is a major element of millipede exoskeleton [Tracz & Gromysz-

Kałkowska 1987, Nakamura et al. 2005, Nakamura & Taira 2005], which is in accordance with the present study on *O. sabulosus* (Fig. 1). The results show that the content of Fe in soils from two regions was reflected in the body of *O. sabulosus* (Fig. 2). Millipedes from Lublin were contained more of Fe than those from Jaworzno and Chrzanów. High concentration of Fe is characteristic of most soils in the Lublin area, but from twofold to threefold lower content of Fe indicated in soils of the Upper Silesia [Kabata-Pendias 1981]. The lower content of Fe in millipedes from Jaworzno and Chrzanów may result from blocking Fe metabolism by heavy metals [Adrianek & Skowronek 2005], which is caused by competition between heavy metals and iron at the same places in chelates. Heavy metals oust iron from centre activity of enzymes and take possession of its place. Heavy metals in excessive concentrations disturbed the circulation of Fe in soils ecosystems [Kabata-Pendias and Pendias 1999]. The copper content in the millipede from Lublin area was 258 $mg \cdot kg^{-1}$dry weight and in specimens from Jaworzno and Chrzanów was 228 $mg \cdot kg^{-1}$dry weight, respectively. In soils of Lublin range of Cu was 1–91 $mg \cdot kg^{-1}$ dry weight [Wiatr et al. 1997], and in soils of Jaworzno, range of Cu was 1–119 $mg \cdot kg^{-1}$ dry weight [www.bip.jaworzno.pl]. In *O. sabulosus* from Lublin the concentration of Cu was 1.13 times greater than in specimens from Jaworzno and Chrzanów. High concentration of copper in *O. sabulosus* (Fig. 2) is in accordance with previous studies on millipedes [Hopkin et al. 1985, Hunter et al. 1987, Heikens et al. 2001, Hobbelen et al. 2004, Nakamura et al. 2005]. Millipedes have been shown to accumulate cadmium [Hopkin et al. 1985, Hunter et al. 1987], which is comparable to Cd content in *O. sabulosus*. The hazardous heavy metal concentrations in millipedes, such as *Glomeris marginata, Polydesmus denticulatus, Oxidus gracilis* [Read & Martin 1990, Nakamura & Taira 2005] were in the order of Cu> Pb> Cd, which is in accordance with present study on *O. sabulosus*. Interestingly, the content of elements such as Ca, K, P, Mg, Fe and Cu in soils from two regions of Poland [Kabata-Pendias 1981, Dechnik et al. 1987, Wiatr et al. 1997, Sobocińska 2003, Adrianek & Skowronek 2005, www.bip.jaworzno.pl] was reflected in the body of analysed millipedes. Different metal accumulation strategies of soil invertebrates are a consequence of varying detoxification mechanisms [Gräff et al. 1997]. Diplopods possess effective mechanisms to bind and to detoxify potentially toxic metals in tissue. Metals can be bound to proteins or precipitate in forms of salts in granules. Granules consist of Ca, P, Zn, Mn, Cu, Mg, Fe, K, Si and Pb [Kőhler et al. 1995]. According to Pobozsny [1985] the concentration of lead in the millipedes differs from species to species. The detoxification mechanism of metals in millipedes may be explanation for lacking of significant differences between content of heavy metals in *O. sabulosus* collected from two locations, more and less contaminated. Obtained results show that *O. sabulosus* is not bioindicator of heavy metal pollution on the contrary to studies on millipedes as biological indicators [Hunter et al. 1987, Nakamura et al. 2005].

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