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COMPARISON OF THE BIOACCUMULATION POTENTIAL OF SELECTED METALS IN THE TISSUES OF THREE INVERTEBRATE TAXA: *Cepaea*, *Lumbricus* AND *Geotrupes*

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Abstract. The quantity of deposited metals was determined in the tissues of *Cepaea nemoralis*, *Lumbricus terrestris* and *Geotrupes stercorarius*, as well as in plant and soil samples from two locations characterized by different levels of human impact. The bioaccumulation factor (BAF) of metals in the bodies of these invertebrate taxa occupying the same habitats was compared, in relation to their content in *Taraxacum officinale* leaves and in the soil. Analysis of the content of metals in the bodies of selected species belonging to different taxonomic groups demonstrates the usefulness of these invertebrates in biomonitoring.

Key words: *Cepaea nemoralis*, *Lumbricus terrestris*, *Geotrupes stercorarius*, metals, bioaccumulation, monitoring of the environment

INTRODUCTION

The search for useful bioindicator organisms and biomarkers for ecotoxicological testing and environmental monitoring have continued uninterrupted for many years [Heikens *et al*. 2001, Beeby and Richmond 2002, Zödl and Wittmann 2003, Notten *et al*. 2005]. Significant characteristics of a good bioindicator are availability, common occurrence, and the possibility of using analytical methods that can be quickly interpreted and are economically acceptable. Subjects of this type of research have included representatives of numerous invertebrate taxa, including molluscs [Gomot and Pihan 1997, Jordaens *et al*. 2006a, 2006b], oligochaetes [Nahmani and Lavelle 2002, Safwat *et al.* 2002, Zorn *et al.* 2005, Calisi *et al*. 2011] and insects [Finn and Giller 2002, Lagisz and Laskowski 2008]. However, there have been few attempts to compare the possibility of bioconcentration of environmental pollutants in organisms of different invertebrate species living in the same, territorially limited habitats.

The aim of the study was to compare the content of zinc, copper, iron, chromium, lead and cadmium in the bodies of three invertebrate taxa from the same habitats, *Cepaea nemoralis*, *Lumbricus terrestris* and *Geotrupes stercorarius*, in order to confirm the usefulness of these species as bioindicator organisms. An attempt was made to analyse the bioconcentration potential of metals in the tissues of these invertebrates by determining their bioaccumulation factor (BAF).

MATERIAL AND METHODS

Grove snails *Cepaea nemoralis* (Linnaeus, 1758), common earthworms *Lumbricus terrestris* (Linnaeus, 1758) and dung beetles *Geotrupes stercorarius* (Linnaeus, 1758) were collected manually in the first week of July 2015 from two habitats with different degrees of human impact, within the town of Modliborzyce. The sites were selected according to their different pollution levels, following determination of the content of metals in the leaves and soil. The sites were designated as LI, with a lower level of human impact (50°45'29"N, 22°19'5"E), and LII, with a higher level of human impact (50°45'15"N, 22°19'4"E).

In accordance with the methodological notes for ecotoxicological testing, we selected open-land habitats without trees and shrubs and with the most similar possible vegetation. The collection area did not exceed 25 m^2.

Apart from invertebrates, samples of the leaves of common dandelion *Taraxacum officinale* (F.H. Wiggers coll. 1780) and soil were collected. The leaves were stored at –25°C until analysis. The soil was taken from a surface of about 10 cm^2 after removal of the humus layer and cold-stored in closed containers.

Samples of invertebrate tissues, soil and plants were prepared according to a previously described procedure [Kowalczyk-Pecka 2009, Kowalczyk-Pecka and Czepiel-Mil 2011].

The content of metals was analysed by atomic absorption spectrometry at the Central Analytical Laboratory of the University of Life Sciences in Lublin. To compare the amounts of accumulated metals, the results were converted to μg per g dry weight of tissue of the taxa analysed. The content of metals was determined in the soft tissues of the snails and in the soft tissues and shells together, in the bodies of the earthworms and in the bodies of the insects.

The bioaccumulation factor (BAF) was calculated in relation to the content of metals in the plant leaves (T) and the soil (s).

All data were analysed using Statistica software ver. 6.1 (StatSoft 2003). Normal distribution was evaluated using the Kolmogorov-Smirnov test and homogeneity of variance in groups was analysed with Levene's test. The data obtained were analysed statistically using a generalized linear model (GLM) of one-way analysis of variance (ANOVA). Tukey's test was used for multiple comparisons between means, with a significance level of $P < 0.05$.

RESULTS AND DISCUSSION

Three taxa commonly occurring in Poland – *Cepaea nemoralis*, *Lumbricus terrestris* and *Geotrupes stercorarius* – were used to confirm the usefulness of invertebrates as bioindicator organisms *in vivo* in their natural habitats.
Clear differences were noted in the content of metals in the dry weight of the tissues as the concentration of these metals in the plants and soil increased (Tables 1 and 2). The amount of accumulated iron, zinc and copper were markedly higher than that of lead, cadmium and chromium in all of the samples (Table 2). This reflects not only the amount of pollutants in the environment, but also differences in the biotransformation processes of each metal and detoxification mechanisms, which in invertebrates are specific for different elements [Yasoshima *et al*. 2001, Nahmani *et al*. 2007, Notten *et al*. 2008, Devi *et al*. 2014]. The small quantity of accumulated metals in the snail shells led to a significant decrease in the total concentration of these elements in the snail bodies. The content of metals in the snails (soft tissues + shells) did not differ significantly from the amounts of these pollutants in the insect tissues (Table 2). The greatest quantity of accumulated metals was noted in the soft tissues of *C. nemoralis* in comparison with the other organisms tested. A high concentration of metals was also found in the bodies of *L. terrestris* (Table 2).

Comparable potential for bioaccumulation of metals in the snails (including the shells) and in insects of the genus *Geotrupes* was confirmed following calculation of the bioaccumulation factor (BAF) in relation to the content of the elements in the plant leaves and soil (Table 3). It was lower, however, than in the case of the tissues of *L. terrestris* and of the soft tissues alone of *C. nemoralis*.

Table 1. Comparison of accumulated metals in the leaves of *Taraxacum officinale* and in the soil in habitats with different levels of human impact, and characteristics of the soil ($\mu g \cdot g^{-1}$ d.w.)

Metal	*T*		S	
	LI*	LII	LI	LII
Zn	16.42 ±1.92	22.74 ±1.99	48.35 ±3.26	283.89 ±9.34
Cu	5.11 ±0.82	8.12 ±0.73	6.85 ±0.43	16.85 ±1.12
Fe	15.91 ±1.26	40.07 ±3.11	3183.08 ±85.97	6325.96 ±87.76
Cr	0.22 ±0.03	0.57±0.03	5.85 ±0.45	7.44 ±0.67
Pb	0.033 ±0.001	0.521 ±0.028	0.63 ±0.04	1.184 ±0.11
Cd	0.027 ±0.003	0.067 ±0.004	0.109 ±0.011	0.164 ±0.013
pH·H_2O of soil			7.3 ±0.03	6.5 ±0.03
Organic matter (%)			18.6 ±0.5	15.8 ±0.6

T – *Taraxacum officinale*; S – soil
* designation of sites explained in 'Material and methods' section; values are given as means ± standard deviation; d.w. – dry weight; n = 20

Table 2. Comparison of amounts of accumulated metals in the bodies of invertebrates from two habitats with different degrees of human impact ($\mu g \cdot g^{-1}$ d.w.)

Metal	CN st	CN + S	LT	GS
		LI*		
Zn	559.15 ±9.34[b]	329.63 ±7.22[a]	443.16 ±8.13[ab]	315.29 ±8.87[a]
Cu	76.07 ±5.83[bc]	45.65 ±4.02[a]	54.74 ±5.99[ab]	47.22 ±3.87[a]
Fe	249.85 ±5.12[ab]	224.67 ±5.64[a]	236.18 ±6.12[ab]	221.99 ±4.49[a]
Cr	0.57 ±0.02[ab]	0.47 ±0.03[a]	0.51 ±0.03[a]	0.44 ±0.02[a]
Pb	0.343 ±0.018[b]	0.277 ±0.014[a]	0.288 ±0.008[a]	0.272 ±0.021[a]
Cd	0.371 ±0.015[b]	0.332 ±0.014[ab]	0.337 ±0.017[ab]	0.307 ±0.013[a]
		LII		
Zn	801.56 ±12.56[bc]	724.87 ±13.65[a]	761.19 ±15.17[ab]	711.56 ±19.77[a]
Cu	94.76 ±4.65[bc]	80.03 ±5.32[a]	87.65 ±4.45[b]	78.92 ±5.95[a]
Fe	395.99 ±9.65[c]	334.21 ±7.43[a]	366.23 ±7.68[b]	328.22 ±7.98[a]
Cr	1.53 ±0.03[b]	1.17 ±0.02[ab]	1.16 ±0.04[ab]	1.04 ±0.03[a]
Pb	0.724 ±0.027[c]	0.672 ±0.011[ab]	0.652 ±0.012[a]	0.650 ±0.021[a]
Cd	0.615 ±0.018[bc]	0.574 ±0.023[ab]	0.594 ±0.042[b]	0.551 ±0.041[a]

CN st – *Cepaea nemoralis* soft tissues; CN + S – *Cepaea nemoralis* + shell; LT – *Lumbricus terrestris*; GS – *Geotrupes stercoravius*
*designation of sites explained in Material and methods section; values are given as means ± standard deviation; d.w. – dry weight; n = 20; Values with different superscript letters in the same row are significantly different ($P < 0.05$)

Table 3. Comparison of the bioaccumulation factor (BAF) of metals in the bodies of invertebrates in relation to their content in the leaves of *T. officinale* and in the soil of the study sites

	CN st		CN + S		LT		GS	
	LI*	LII	LI	LII	LI	LII	LI	LII
Zn *T***	34.05	35.25	20.07	31.88	26.99	33.47	19.20	31.29
Zn s***	11.56	2.82	6.82	2.55	9.17	2.68	6.52	2.51
Cu *T*	14.87	11.67	8.93	9.86	10.71	10.79	8.26	9.72
Cu s	11.11	5.62	6.65	4.75	7.99	5.20	6.89	4.68
Fe *T*	15.70	9.88	14.12	8.34	14.84	9.14	13.95	8.19
Fe s	0.078	0.063	0.071	0.053	0.074	0.058	0.069	0.052
Cr *T*	2.59	2.68	2.14	2.05	2.32	2.04	2.00	1.82
Cr s	0.097	0.205	0.080	0.157	0.087	0.156	0.075	0.139
Pb *T*	10.39	1.39	8.39	1.29	8.73	1.25	8.24	1.25
Pb s	0.540	0.611	0.439	0.567	0.457	0.551	0.431	0.548
Cd *T*	13.74	9.18	12.29	8.56	12.48	8.87	11.37	8.22
Cd s	3.40	3.75	3.05	3.50	3.09	3.62	2.82	3.36

CN st – *Cepaea nemoralis* soft tissues; CN + S – *Cepaea nemoralis* + shell; LT – *Lumbricus terrestris*; GS – *Geotrupes stercoravius*
* designation of sites explained in Material and methods section; ***T* – *T. officinale;* *** s – soil

In the case of iron, chromium and lead, the BAF calculated in relation to the content of the metals in the soil is often several dozen times lower than when calculated in relation to the content of these metals in leaves (Table 3). This may indicate faster transfer of metals to the bodies of the animals by way of plants than via the soil, which is particularly visible in snails, as first-order consumers. The bioaccumulation factors of zinc, copper and cadmium calculated with respect to the content of these metals in leaves was also higher than that calculated as a proportion of the amount of these metals in the soil (Table 3).

The bioaccumulation factor for iron, lead and cadmium calculated with respect to the content of the metals in plant leaves was markedly lower in the tissues of the invertebrates from site II (with a higher level of these metals) than in those from site I (Table 3). A similar tendency was noted for the bioaccumulation factor of zinc, copper and iron calculated in relation to the content of the metals in the soil. This may be linked to differences in the rate of incorporation of these metals into the tissues or to different mechanisms for eliminating them from the body in comparison with other metals.

The BAF calculated for samples from site II and site I differ less than might have been expected after analysing the level of accumulated metals in the tissues of animals from the two locations (Tables 2 and 3).

Metals are important for metabolic processes in animals and plants in very low concentrations [Menta and Parisi 2001], but when the level of exposure is high, defensive (e.g. restricting consumption) and detoxifying mechanisms limiting accumulation are often activated in invertebrates.

RECAPITULATION AND CONCLUSIONS

Differences were observed in the amounts of zinc, copper, iron, chromium, lead and cadmium deposited in the tissues of invertebrates from locations with differing levels of pollutants. The high concentration of metals in the soft tissues of *Cepaea*, *Lumbricus* and *Geotrupes* may be due to direct transfer of metals from the environment by first-order consumers.

The invertebrates studied, which occupy a low level of the food chain, are a useful subject in ecotoxicological research. Their high metabolism rate is of particular importance. A fast reaction in a bioindicator organism makes it easier to find biomarkers of exposure and to reliably verify them.

REFERENCES

Beeby A., Richmond L., 2002. Evaluating *Helix aspersa* as a sentinel for mapping metal pollution. Ecol. Indic. 1, 261–270.

Calisi A., Lionetto M.G., Sanchez-Hernandez J.C., Schettino T., 2011. Effect of heavy metal exposure on blood haemoglobin concentration and methemoglobin percentage in *Lumbricus* *terrestris*. Ecotoxicology 20, 847–854.

Devi P.R., Kombiah P., Sudhakar G.R.L., Babu C., 2014. Purification and characterization of a novel lectin from *Geotrupes stercorarius.* Int. J. Adv. Biotech. Res. 5, 2, 157–162.

Finn J.A., Giller P.S., 2002. Experimental investigations of colonisation by north temperate dung beetles of different types of domestic herbivore dung Appl. Soil Ecol. 20, 1–13.

Gomot A., Pihan F., 1997. Comparison of the bioaccumulation capacities of copper and zinc in two snail subspecies (*Helix*). Ecotoxicol. Environ. Safe. 38, 85–94.

Heikens A., Peijnenburg W.J.G.M., Hendriks A.J., 2001. Bioaccumulation of heavy metals in terrestrial invertebrates. Environ. Poll. 113, 385–393.

Jordaens K., De Wolf H., Vandecasteele B., Blust R., Backeljau T., 2006a. Associations between shell strength, shell morphology and heavy metals in the land snail *Cepaea nemoralis* (Gastropoda: Helicidae). Sci. Total Environ. 363, 285–293.

Jordaens K., De Wolf H., Van Houtte N., Vandecasteele B., Backeljau T., 2006b. Genetic variation in two land snails, *Cepaea nemoralis* and *Succinea putris* (Gastropoda, Pulmonata), from sites differing in heavy metal content. Genetica 128, 227–239.

Kowalczyk-Pecka D., 2009. Rola naturalnej populacji A*rianta arbustorum* (Gastropoda: Pulmonata) w transferze i bioakumulacji metali ciężkich w ekosystemach zurbanizowanych. Ochr. Środ. Zas. Natur. 41, 22–31.

Kowalczyk-Pecka D., Czepiel-Mil K., 2011. Synantropijne ślimaki nieoskorupione z rodzaju Arion i Deroceras (Gastropoda: Pulmonata) jako biokoncentratory metali ciężkich. Ochr. Środ. Zas. Natur. 49, 126–134.

Lagisz M., Laskowski R., 2008. Evidence for between-generation effects in carabids exposed to heavy metals pollution. Ecotoxicology 17, 59–66.

Menta C., Parisi V., 2001. Metal concentrations in *Helix pomatia, Helix aspersa* and *Arion rufus*: a comparative study. Environ. Poll. 115, 205–208.

Nahmani J., Lavelle P., 2002. Effects of heavy metal pollution on soil macrofauna in a grassland of Northern France. Eur. J. Soil Biol. 38, 297−300.

Nahmani J., Hodson M.E., Black S., 2007. A review of studies performed to assess metal uptake by earthworms. Environ. Poll. 145, 402–424.

Notten M.J.M., Oosthoek A.J.P., Rozema J., Aerts R., 2005. Heavy metal concentrations in a soil-plant-snail food chain along a terrestrial soil pollution gradient. Environ. Poll. 138, 178–190.

Notten M.J.M., Walraven N., Beets C.J., Vroon P., Rozema J., Aerts R., 2008. Investigating the origin of Pb pollution in a terrestrial soil-plant-snail food chain by means of Pb isotope ratios. Appl. Geochem. 23, 1581–93.

Safwat H., Shakir H., Weaver R.W., 2002. Earthworm survival in oil contaminated soil. Plant Soil 240, 127–132.

Yasoshima M., Matsuo M., Kuno A., Takano B., 2001. Studies on intake of heavy metals by Bradybaena similaris, land snails, by XAFS measurement. J. Synchrotron Rad. 8, 969–71.

Zorn M.I., van Gestel C.A.M., Eijsackers H., 2005. The effect of *Lumbricus rubellus* and *Lumbricus terrestris* on zinc distribution and availability in artificial soil columns. Biol. Fertil. Soils 41, 212–215.

Zödl B., Wittmann K.J., 2003. Effects of sampling, preparation and defecation on metal concentrations in selected invertebrates at urban sites. Chemosphere 52, 1095–1103.

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PORÓWNANIE POTENCJAŁU BIOAKUMULACJI WYBRANYCH METALI W TKANKACH TRZECH TAKSONÓW INVERTEBRATA: *Cepaea*, *Lumbricus* I *Geotrupes*

Streszczenie. Określano wielkość depozytu sześciu metali w tkankach *Cepaea nemoralis*, *Lumbricus terrestris* i *Geotrupes stercorarius* oraz w próbkach roślin i gleby pochodzących z dwóch lokalizacji o różnym stopniu antropopresji. Porównano współczynnik bioakumulacji (BAF) metali w ciele taksonów bezkręgowców zajmujących te same siedliska w stosunku do ich zawartości w liściach *Taraxacum officinale* i w glebie. Analiza zawartości metali w ciele wybranych gatunków należących do różnych grup taksonomicznych dowodzi użyteczności wybranych bezkręgowców w biomonitoringu.

Słowa kluczowe: *Cepaea nemoralis*, *Lumbricus terrestris*, *Geotrupes stercorarius*, metale, bioakumulacja, monitoring środowiska