POLISH JOURNAL OF SOIL SCIENCE
VOL. L/2 2017 PL ISSN 0079-2985

DOI: 10.17951/pjss/2017.50.2.291

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THE IMPACT OF HEAVY METALS ON THE SOIL FAUNA OF SELECTED HABITATS IN NIEPOŁOMICE FOREST

Received: 18.07.2017
Accepted: 01.12.2017

Abstract. Pollution of soils with heavy metals is particularly dangerous to living organisms. Invertebrates are sensitive to changes in soil conditions, and, therefore, may be considered invaluable indicators of soil disruptions. This study has been undertaken to determine heavy metal content in the in soil and extracted soil fauna in two types of forest habitats of Niepołomice Forest and to establish their impact on pedofauna – their density, diversity and trophic structure. The investigated sites varied in the soil or humus type, and the composition of forest stand and ground cover. The analysis of the pH value showed that the studied soils had acidic (pH 4.45–4.85). Small differences were observed in the content of heavy metals (lead, cadmium, nickel, zinc and copper) in soil humid forest and fresh mixed forest. It results from the studies that the heavy metal content did not exceed the standards set out for the soils of Poland. The bioconcentration factor shows that both saprophytes and predatory invertebrates collected the largest amounts of zinc and smaller amounts of cadmium, and the remaining metals were collected in the following order: Cu>Ni>Pb. The results obtained show that the tested heavy metals could have an impact on the density and diversity of meso- and macrofauna. Probably soil fauna affected by other factors (soil moisture, organic matter content, type of humus).

Keywords: soil fauna, density, diversity, heavy metal, Niepołomice Forest

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INTRODUCTION

Soil is the key element of geoecosystem, an entity characterized by specific physical, chemical and biological properties which have developed as a result of long-term impact of natural soil-formation processes as well as agricultural and non-agricultural human activities (Haslmayr *et al*. 2016). Processes occurring in the soil are essential to maintain the stability and productivity of land ecosystems. Chemical pollution of the environment, attributable to economic and technological activities of humans, results in various deformations of natural circulation of trace elements in the environment (Gorlach and Gambuś 1991). Pollution of soils with heavy metals is particularly dangerous to living organisms (Laskowski *et al*. 1995, Skwaryło-Bednarz 2006). Heavy-metal-contaminated soil may transfer pollutants to further levels of the trophic chain, i.e. plants, animals and humans, or it may constitute a source of secondary pollution of air and water, therefore, impacting humans directly, without passing through the trophic chain. As opposed to air or water, the soil cleaning process is very slow. Correct assessment of soil pollution with heavy metals and the threats resulting there from is very important to the environment, and, therefore, to living organisms. Excessive quantities of heavy metals pose a significant threat to plants and humans as well as to soil fauna. Invertebrates are sensitive to changes in soil conditions, and, therefore, may be considered invaluable indicators of soil disruptions (Butovsky 2011, Santorufo *et al.* 2012). The presence of trace metals in the soil and plants is an environmental indicator (El-Falakay *et al*. 1991). That is why there is a need to systematically monitor their content in environmental components.

Soil heavy metal content is affected by the chemical composition of the parent rock, and local and transboundary pollutants, transported and washed out with atmospheric precipitation (Kabata-Pendias and Pendias 1999, Van Gestel 2008). Trace elements systematically brought into the soil accumulate in its top layer, because they are bonded by the soil sorption complex and only very slowly move into the depths of the soil profile. In forest litter and in the soil environment, heavy metals demonstrate diverse mobility (Czarnowska 1996). The mobility of Cu and Pb is strongly dependent on the solubility of organic matter (Bergvist *et al.* 1989). Lead is strongly bonded by organic matter of soil top layer and only slightly migrates into the depth of the profile, copper is a little more mobile, and zinc relatively easily moves into the depths of the soil. The total metal content of forest litter is determined, beside the deposition rate, also by the humification conditions of the vegetable material and the balance of metal accumulation and leaching processes (Laskowski *et al.* 1995, Cortet *et al*. 1999, Kabata-Pendias and Pendias 1999).

The aim of this study was to determine the content of the heavy metals Pb, Cd, Ni, Zn and Cu in the soil of selected habitats of Niepołomice Forest and the fauna inhabiting them, and also to determine the effect of these metals on the density, diversity and trophic structure of the fauna studied.

MATERIALS AND METHODS

Niepołomice Forest is a forest complex located in the western part of Sandomierz Basin, approx. 25 km east of Kraków, in the fork of Wisła and Raba rivers. It is located on the territory of Małopolskie province. Total area of Niepołomice Forest is approx. 115 km^2, including 110 km^2 of wooded areas. The Forest consists of several forest habitat types: humid forest, fresh forest, mixed fresh forest, mixed wet forest, alder forest, ash-alder forest, mixed fresh coniferous forest, mixed bog coniferous forest and mixed humid coniferous forest (Gruszczyk 1981).

The research was conducted in the western part of Niepołomice Forest, in the mixed fresh forest and in the humid forest in the northern part of Niepołomice Forest.

Mixed fresh forest occupies 921.18 hectares, which represents 8% of total area, and it occurs mostly in the western part of the main Forest complex. It grows on acidic brown soils and podzols. The forest stand is largely mixed, and it contains pine (*Pinus sylvestris* L.), sessile oak (*Quercus sessilis* Ehrh.), pedunculate oak (*Quercus robur* L.), silver birch (*Betula verrucosa* Ehrh.), small-leaved linden (*Tilia cordata* Mill.), aspen (*Populus tremula* L.), beech (*Fagus silvatica* L.), spruce (*Picea excelsa* (Lam.) Lk). and fir trees (*Abies alba* Mill). The understory in mixed fresh forest, with the exception of solid beech stands, is quite well developed. It is comprised of: alder buckthorn (*Frangula alnus* Mill.), spindle (*Evonymus europaea* L.) and tree saplings. The undergrowth in mixed fresh forest is comprised of species with very broad ecological amplitude. The species include species typical to mixed coniferous forest as well as deciduous forest. The species composition and the quantitative composition of the undergrowth is often indicative of how fertile the soil is.

The humid forest occupies an area of 2200.76 hectares and represents 22% of the total area of Niepołomice Forest. It grows on brown soils and dystric gleysols. The humid forest is dominated by pedunculate oak (*Quercus robur* L.) with black alder (*Alnus glutinosa* (L.) Gaertn.), small-leaved linden (*Tilia cordata* Mill.) and hornbeam (*Carpinus betulus* L.) (Table 1).

The soil samples were taken in older age classes (III – age from 41 to 60 years and in the age class with stand over 120 years) in the spring and summer periods of the 2013–2014 vegetation season. With the use of Morris square frame (25 cm by 25 cm) a series of samples was taken on the selected localities. The frame was thrust into the soil on the depth of 10 cm. Each series consisted of 16 tests on the surface of around 1 m^2. Pedofauna was scampered away by employing the dynamic method in the modified Tullgren apparatus. After marking the select mesofauna, its density and diversity were analysed. In soil samples the following taxonomic groups were identified: *Enchytreidae*, *Lumbricidae*, *Aranea*, *Acarina*, *Pseudoscorpionidae*, *Symphyla*, *Chilopoda*, *Diplopoda*, *Protura*, *Diplura*, *Col-*

TABLE 1. COMPARISON OF STUDIED HABITAT TYPES OF THE FOREST

Habitat type	MFF	HF
sort of soil	brown-acidic and podsolic	brown and dystric gley sols
sort of humus	moder, moder mull	mull
the composition of forest stand	*Pinus sylvestris* L., *Quercus sessilis* Ehrh., *Quercus robur* L., *Alnus glutinosa* L., *Betula verrucosa* Ehrh.,	*Quercus robur* L., *Alnus glutinosa* L., Gaertn, *Fraxinus excelsior* L., *Tilia cordata* Mill., *Ulmus minor* Mill, *Carpinus betulus* L.
the composition of ground cover	*Stellaria holostea* L., *Milium effusum* L., *Hepatica nobilis* Mill., *Galium Schultesii* Vest, *Melica nutans* L., *Lilium martagon* L.	*Impatiens noli-tangere* L., *Urtica dioica* L., *Aegopodium podagraria* L., *Asperula odonata* L., *Carex brizoides* L.

MFF – mixed fresh forest, HF – humid forest

lembola, *Coleoptera* (imago and larvae), *Diptera* larvae (families: *Cecidomyiidae*, *Dolichopodidae*, *Scatopsidae*, *Bibionidae*, *Rhagionidae*, *Muscidae*, *Tipulidae*, *Chironomidae*, *Sciaridae, Therevidae)*, *Hymenoptera (Formicidae)*, *Homoptera*, *Heteroptera*, *Thysanoptera*, *Isopoda*, *Gastropoda*, *Lepidoptera*, *Dermaptera*, *Psocoptera.* Soil fauna were separated into trophic groups – saprophages, predators, phytophages – in which heavy metals were marked.

The heavy metal content in soil and soil fauna was determined by FAAS after previous mineralization of the soil and animal test materials. For this purpose, animals and soil samples were dried at 105°C to obtain a dry weight. After obtaining the dry weight, each trophic group was weighed. Also, 2 g of dried soil from each location was weighed. The soil and soil organisms underwent the mineralization process in a Velp ScientificaDK-20 mineralizer in concentrated nitric acid at 120°C until the tissue was completely dissolved. Then, the resulting solutions were poured into measuring flasks filled with distilled water up to 10 ml. In the solutions thus prepared, the heavy metal content was determined content – cadmium, lead, nickel in a spectrophotometer. The heavy metal content in soil and soil fauna was determined by Buck Scientific 200A Flame Atomic Absorption Spectrophotometer. Moisture content and pH of the investigated soils were determined as well.

RESULTS AND DISCUSSION

The investigated sites varied in the soil and humus type, as well as the composition of forest stand and ground cover (Table 1). The analysis of the pH value showed that the studied soils had acidic in the 4.45–4.85 pH range (Table 2). In this case the pH value could become a factor affecting a particular increase in the metal mobility in the studied soils, because low pH value of the soil promotes heavy metal assimilation (especially Pb and Cd) by soil organisms. With a very strong fall in the pH value, the biological activity of the soil decreases and leads to the accumulation of thick layers of "raw" humus (Jelaska *et al.* 2007). Moisture in both types of studied forests was slightly different. In the mixed fresh forest soil, the moisture content was ca. 5% higher than in humid forest (Table 2).

TABLE 2. GENERAL CHARACTERISTICS OF FOREST SOILS

Selected parameters	MFF		HF	
	Mean (Min–Max)	SD	Mean (Min–Max)	SD
Soil pH	4.45 (3.9–5.05)	0.59	4.83 (4.61–5.09)	0.24
Area temperature °C	22.7 (19.8–25.3)	2.71	18.8 (15.2–22.3)	2.90
Soil temperature °C	16.47 (14.3–18.2)	1.92	13.39 (11.1–16.7)	2.95
Soil moisture %	21.71 (19.92–24.15)	2.18	26.09 (22.1–30.3)	3.68

Min – minimum, Max – maximum, SD – standard deviation

These results indicate that concentrations of analyzed heavy metals do not exceed boundary values defined in the Regulation of the Minister of Environment on soil quality standards and earth quality standards for group B soils, including the forest, tree-covered and shrub-covered soils, wastelands and built-up and urbanized areas, however, excluding industrial areas, mining grounds and transport areas (Regulation 2002).

Mean metal concentrations ranged from 7.7% Cu, 12.3% Zn, 13.8% Ni to 45.9% Pb of their allowable values. The higher contents of cadmium, lead and nickel were recorded in soil of HF, whereas zinc and copper content were predominant in MFF (Table 3). Cadmium, nickel and lead belong to toxic elements, very strongly affecting the environment, leading to the change of biological properties of the soil (Kabata-Pendias and Pendias 1999, Jelaska *et al*. 2007).

It should be pointed out that the accumulation of lead in forest litter is a long-lasting process, hence the found lead concentrations may result from the total pollutant amounts from deposition lasting many years. Many authors emphasise that lead accumulates in the humus layer of the soil profile (Brożek *et al.* 2003, Jelaska *et al.* 2007). According to Cieśla *et al.* (1994), a higher accumulation of lead in the studied forest soils may be caused not only by the acidic pH, but also by a higher organic carbon content. The enrichment of the top layers of the forest soils in Pb, Zn and Cu may prove their anthropogenic origin. Most probably, this soil environmental condition is affected by the inflow of gaseous and particulate pollutants transported by wind mainly from over the industrial conurbation.

The analysed metals content in the studied soils correlated with the abundance and the biodiversity of meso- and macrofauna, including the *Diptera* larvae. The highest density (9,682 individuals per sq m) and diversity (22 taxonomic group) were recorded in fresh mixed forest MFF, where Cd, Pb and Ni were recorded in low amounts (Tables 3–4). The trophic structure of pedofauna was also tested. In both types of forest habitat dominated by soil saprophages, whose share was 86.7% in humid forest and 92.2% in mixed fresh forest (Table 4). Table 5 includes heavy metal concentrations in soil invertebrates extracted from soil samples collected from the mixed fresh forest and humid forest.

TABLE 3. CONTENT OF HEAVY METALS IN FOREST SOILS (mg·kg^{-1} D.M.)

Metal	MFF				HF				limit values[1]
	Mean	Min	Max	SD	Mean	Min	Max	SD	
Cd	0.66	0.48	0.80	0.13	0.89	0.39	1.74	0.47	4
Pb	42.32	25.30	55.91	13.49	45.89	8.61	88.59	27.89	100
Ni	5.38	4.89	5.41	0.47	13.84	5.75	44.84	13.45	100
Cu	11.63	5.47	20.12	6.39	8.23	5.09	22.92	7.89	150
Zn	43.25	22.15	62.00	16.58	42.25	17.66	85.44	18.56	350

[1] Limit values for the heavy metal content set out in the Minister of Environment Regulation on the soil quality standards and earth quality standards for group B – (Polish Journal of Laws 2002 No. 165, item 1359 of 4 October 2002)

TABLE 4. DENSITY, DIVERSITY AND TROPHIC STRUCTURE OF PEDOFAUNA IN DIFFERENT HABITAT TYPES OF THE FOREST

Parameters	MFF	HF
Density of pedofauna (sp.no. per m^2)	9682 (3714–18004)	4750 (3875–6815)
Density of *Diptera* larvae (sp.no. per m^2)	228 (60–440)	272 (71–540)
Diversity (number of taxonomic group, number families of *Diptera*)	22 10	15 8
Trophic structure of pedofauna (%)		
Saprophages	92.2 (89.2–94.4)	86.7 (80.7–92.8)
Predators	6.4 (4.2–8.0)	10.8 (3.7–18.9)
Phytophages	1.4 (0.5–3.2)	2.5 (0.6–4.3)

Accumulation of all heavy metals, except Pb, in soil animal organisms was significantly higher than in the soil, and varied depending on the types forest (Tables 3, 5). This is confirmed by other studies of Chrzan *et al.* (2013). Zn, attaining an average concentration of 428–940 mg·kg^{-1} d.m of fauna, exceeding 10-20 times the soil values, showed the greatest difference (Table 5). Contents of Cd, Ni, Cu and Zn were higher in the soil fauna in the wet forest than in the mixed forest. In contrast, Pb concentration was higher in both saprophages and predators in mixed forest (44.7–59.9 dm mg·kg^{-1}). Similar concentration of Pb was noted by Rożen *et al.* (2004) in Niepołomice Forest.

Values of cadmium in soil animals were higher than in the soil itself. This element was accumulated in large quantities in both saprovores (3.2–5.3

mg·kg^{-1}) and predators (3.9–16.7 mg·kg^{-1}), however, differences in its content between trophic groups were not statistically significant.Cadmium was accumulated in the highest amounts in predators in humid forest. Much higher cadmium concentration was observed by Rożen *et al*. (2004) in *Enchytraeidae* in oak-hornbeam and in mixed oak-pine stands in Niepołomice Forest. (32.33 ± 23.22 mg·kg^{-1} dry mass). Predators accumulated more heavy metals (Cd, Pb, Ni, Cu and Zn) than saprophages in both forest (Table 5). Also Chrzan *et al*. (2013) showed a higher content of Pb, Ni and Cu in predatory invertebrates in the Wolski Forest. The largest concentration of pollutants is usually discovered in predators in the higher trophic levels of the food pyramid (Walker *et al.* 2002, Hedde *et al*. 2012).

TABLE 5. CONTENT OF HEAVY METALS IN SAPROPHAGES AND PREDATORS IN FOREST SOILS (mg·kg^{-1} D.M.)

Trophic groups		SAPROPHAGES		PREDATORS	
Metal		MFF	HF	MFF	HF
Cd	Mean	3.24	5.34	3.90	16.72
	Min	2.43	4.23	2.82	15.39
	Max	4.75	6.13	4.95	17.75
	SD	1.07	0.81	1.06	1.21
Pb	Mean	44.66	20.84	59.54	44.61
	Min	21.49	18.92	40.98	38.47
	Max	62.21	23.63	81.89	55.33
	SD	17.10	2.02	18.23	9.31
Ni	Mean	4.91	4.73	22.56	27.39
	Min	3.54	3.68	12.56	26.21
	Max	6.28	5.98	37.69	37.69
	SD	1.94	0.95	8.84	6.34
Cu	Mean	24.33	21.26	30.99	48,53
	Min	16.36	12.15	18.38	35.75
	Max	37.37	34.23	61.53	70.57
	SD	10.99	9.42	20.42	19.17
Zn	Mean	428.29	729.08	940.32	824.42
	Min	338.58	569.05	554.36	698.31
	Max	503.16	859.05	1576.71	952.15
	SD	83.28	120.28	555.25	126.93

Mobility of potentially toxic trace elements in soil invertebrates was assessed with the bioconcentration factor (BCF). The potential risk may be expressed through the accumulation index representing the ratio between the average concentration of the element in the body to its content in the soil. In Table 6 presented BCF for predatory and saprophage invertebrates. Bioconcentration factor shows that both soil saprophages and predacious invertebrates accumulated the largest amounts of zinc and cadmium, while much less of copper, nickel and lead. Heavy metal toxicity depends on the roles they play in the metabolic processes of the organisms and their susceptibility to bioaccumulation. Zinc is crucial for proper functioning of all cells of an organism and is present in many enzymes responsible, *inter alia*, for metabolism of proteins, carbohydrates, and fats. Zinc content in organisms is high. According to Heikens *et al*. (2001), the relationship between total heavy metal soil content and the internal metal content in invertebrates tends to be strong in the order of Pb>Cd>Cu, Zn. It has been speculated that in terrestrial invertebrates Cu and Zn can be regulated to a certain degree, resulting in a constant body concentration over a range of soil concentrations (Heikens *et al.* 2001, Santorufo *et al*. 2012). Cadmium also has accumulated in large quantities in the soil fauna, the bioconcentration factor ranged from 6.0 to 18.8 in humid forest (Table 6).

TABLE 6. BIOCONCENTRATION FACTOR VALUES (BCF) OF THE POTENTIALLY TOXIC TRACE ELEMENTS IN SAPROPHAGES AND PREDATORS

Metal	SAPROPHAGES		PREDATORS	
	MFF	HF	MFF	HF
Cd	4.91	6.04	5.91	18.79
Pb	1.05	0.38	1.41	0.97
Ni	0.91	0.34	1.67	1.98
Cu	2.09	2.58	2.66	3.89
Zn	9.90	17.26	21.74	19.51

Potentially, contamination with cadmium imposes a serious threat to environments, as the element is easily accumulated, both biologically and anthropogenically, and therefore may be incorporated into trophic chain.

CONCLUSIONS

1. Content of the heavy metals in the forest soils did not exceed the limit values for forest land according to the Regulation of the Minister of Environment on standards for soil quality and earth quality standards of 04.10.2002.
2. Soil invertebrates accumulated the most of Zn and Cd.
3. Predatory invertebrates in more quantity accumulated all heavy metals in both types of forest than saprophytes.
4. The density and biodiversity of pedofauna have an influence next to heavy metals probably other factors (soil humidity, organic matter content, humus type).

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