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Heavy metal concentration and the occurrence of selected microorganisms in soils of a steelworks area in Poland

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ABSTRACT

This study was aimed to determine the impact of risk element content on the prevalence of the selected microbial groups in industrial soils of one of the steelworks in Poland. The concentration of heavy metals: Cd, Cr, Cu, Hg, Fe, Ni, Pb and Zn along with soil pH and the number of mesophilic bacteria, fungi, actinomycetes and *Azotobacter* was assessed in 20 soil samples. The limit concentrations of Cd, Pb and Zn were exceeded in five sites. However, even in these strongly contaminates sites, the studied microorganisms were abundant. The correlation between the concentration of heavy metals and microbial numbers was very weak and statistically insignificant. High numbers of fungi were observed in the contaminated sites, which was particularly interesting in one of the sites, strongly contaminated with Cd, Pb and Zn.

Keywords: toxic; contamination; microbial community; pollution; industry

Together with rapid worldwide industrialization, heavy metals have become one of the major types of environmental contaminants, particularly in soils (Qin et al. 2012). Metal processing activities, including smelting, are related to environmental contamination with heavy metals, either by their direct discharge, or by the fact that they are located in areas already contaminated, as they have been historically related to metallurgy (Sutkowska et al. 2013). The most difficult problem associated with the contamination of soils with heavy metals is that they cannot be naturally degraded like organic pollutants and they can be accumulated in different parts of the food chain (Šmejkalová et al. 2003). Once heavy metals enter soils, they can be present there for long time, becoming threat not only to crops in the neighborhood of the contaminated area, but also being transferred to plants and thus posing potential threat to human health (Sipter et al. 2008).

Adverse effects of heavy metal contamination of the environment can be reflected not only in the accumulation of metals in plants, but also in the alteration of soil microbial community (Šmejkalová et al. 2003). Chemical analyses provide information only referring to the accumulation of these contaminants in soils, but they will not indicate the consequences they have on soil microbiota composition and metabolism. Microorganisms are the first group that undergoes any direct and indirect effect of heavy metals in the soil environment. Adverse effects of metals on soil microorganisms may cause decreased decomposition of organic matter, reduced soil respiration, decreased microbial diversity, as well as they reduce the activity of some soil enzymes (Tyler 1974). Rajapaksha et al. (2004) in their experiment, in which they compared reactions of various microbial groups to soil pollution with Zn and Cu, showed that the bacteria are much more sensitive to the soil contamination than fungi. These authors also detected varying effect of soil pH on the microbial reaction to soil pollution, in which lower pH enhanced the detrimental effect on bacteria, while this was not observed in the case of fungi.

One of the reasons for the previously mentioned decreased microbial diversity in heavy metal-containing soils is the selection of tolerant strains.

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There is a wide variety of microbial mechanisms to reduce the impact of these xenobiotics on their cells, one of which includes efflux transporters that excrete the excess metal outside the cell (Nies 2003).

Having regard to the importance of the increasing concerns, raised by direct neighbourhood of industrial facilities and environmental pollution related to heavy industry in Upper Silesia, the study was conducted aiming to assess the concentrations of heavy metals in industrial soils of one of the smelter plants in Poland and to determine the number of selected microbial groups in these soils, thus trying to evaluate the effect of risk element concentrations on the abundance of the analysed soil microorganisms. Being aware that some of the soil microorganisms can survive the increased concentrations of risk elements in soils, we were trying to verify whether the long-term effect of high heavy metal content in the examined soils will increase the level of resistance to these xenobiotics in some components of the soil biota.

MATERIAL AND METHODS

The study was conducted based on 20 soil samples collected within one of the smelter plants in Poland. The description of the sampling sites is given in Table 1. The samples were collected four times per year, once per each season, into sterile polypropylene containers from the depth of c.a. 20 cm (ISO 10381-6, 2009). In the laboratory, the samples were passed through a 2 mm sieve and the plate count method was used to enumerate the selected microbial groups: mesophilic bacteria (Trypticase Soy Agar – TSA, 48 h at 37°C), fungi (Malt Extract Agar – MEA, 3 days at 28°C), actinomycetes (Pochon agar, 7 days at 28°C) and *Azotobacter* spp. (Ashby's agar, 5 days at 26°C).

Soil pH was determined in a soil and water (1:2.5) suspension and soil dry matter was determined using the gravimetric method (ISO 10390, 2005, ISO 11465, 1999). The total content of heavy metals: Cd, Cr, Cu, Hg, Fe, Ni, Pb and Zn was determined in dried and mortar-pulverized soils after digestion with *aqua regia* and flame atomization by atomic absorption spectrometry (AAS) using PYE UNICAM SP9/700 spectrometer (Cambridge, UK). Calibration was performed by the method of external standards. The risk element concentrations were analyzed for soil samples collected in the first season, i.e. in spring. The obtained heavy metal concentrations were compared with Polish regulations for the allowed concentrations of heavy metals in soils (Regulation of the Minister of Environment, September 9, 2002 on soil quality standards 2002; further RME 2002).

Statistical analysis of the results was conducted using the Statistica v. 10 software (StatSoft, Tulsa, USA) – Spearman's correlation (*R*) was applied to verify the relationship between the number of microorganisms and soil pH, and heavy metal concentration. One way analysis of variance was used to test the significance of differences in the microbial abundance between the examined soil samples and seasons of the year.

RESULTS AND DISCUSSION

The conducted analyses of heavy metal concentration in soils showed that five out of the twenty examined sites were characterized by the

Table 1. Characteristics of the sampling sites

Soil No.	General characteristics	Mean soil pH	Moisture range (%)
1	metal processing facilities	6.78	5.16–13.12
2	product transportation	7.2	2.18–16.83
3	fallow land	7.86	9.70–15.55
4	metal processing facilities	7.25	8.20–16.78
5	industrial waste collection and processing	8.07	9.25–15.34
6	industrial waste collection and processing	7.82	8.30–19.41
7	metal processing facilities	7.35	0.95–19.30
8	metal processing facilities	7.46	3.87–19.12
9	metal processing facilities	7.79	7.68–20.55
10	industrial waste collection and processing	7.93	7.43–16.67
11	metal processing facilities	8.14	6.07–13.40
12	metal processing facilities	7.65	3.15–10.90
13	metal processing facilities	7.71	2.20–14.72
14	product transportation	7.79	0.96–13.44
15	energy production facilities	7.86	1.30–16.18
16	fallow land	7.63	5.03–13.26
17	product transportation	7.92	8.30–35.60
18	energy production facilities	7.68	1.83–12.70
19	product storage	7.77	3.32–14.54
20	product storage	7.55	4.70–14.75

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exceeded limits (RME 2002) – Table 2. However, only the concentrations of lead (three sites), zinc (four sites) and cadmium (one site) were exceeded. This contamination is much smaller than that recorded in the previous study conducted in the Krakow ArcelorMittal steelworks, where apart from lead, zinc and cadmium, the limits for two other heavy metals were exceeded (i.e. Cu and Cr – limits exceeded in eight sites out of twenty) (Lenart and Wolny-Koładka 2013). But similarly to the mentioned studies, very high concentrations of zinc were recorded in two sites, and the admissible values were exceeded. In general, it can be stated that except for two sites, the recorded concentrations of heavy metals are comparable to those reported by other authors in studies conducted in industrial areas in Poland, including those affected by steel plants. For instance, Cuske et al. (2013) in their study on the effect of the zinc smelter in Oława (south-western Poland) found that the admissible concentration of zinc was exceeded in 9 out of 12 examined sites and the concentration of lead was exceeded in 10 sites. The highest concentration of zinc in their study reached 2.404 mg/kg and the highest concentration of lead was 1.948 mg/kg of soil. Our values of Cd, Cr and Ni were also similar to those reported by Sutkowska et al. (2013) in their analyses of soil contamination induced by historical zinc smelting in Jaworzno. For example, the concentrations of chromium in the study by Sutkowska et al. (2013) ranged from 21.91–119.32 mg/kg, compared to the range of 5–130 mg/kg recorded in our study. The nickel concentration, as ob-

Table 2. Concentration of heavy metals in soil. The limit values according to the Regulation of the Minister of Environment (2002) are given in brackets and the metal contents which exceed the limit values are given in bold type (RME 2002)

Soil No.	Cd (15)	Cr (500)	Cu (600)	Fe (–)	Hg (30)	Ni (300)	Pb (600)	Zn (1000)
1	2.9	11	14	7550	< LOD	5.6	83	240
2	4	33	45	12 950	< LOD	5.4	230	420
3	2.6	39	24	26 700	0.011	36	76	340
4	2.5	36	23	21 100	< LOD	37	**620**	400
5	31	54	60	165 400	< LOD	37	**1540**	**4940**
6	**65**	65	150	210 000	< LOD	68	**4740**	**105 800**
7	3.9	37	57	30 800	< LOD	7.5	91	400
8	8.6	68	33	136 000	0.018	38	450	800
9	5.2	130	480	164 800	< LOD	70	91	800
10	4.7	44	8.2	21 600	< LOD	2.4	81	230
11	5.5	44	6.7	11 100	< LOD	25	69	420
12	6.1	44	130	56 000	< LOD	38	250	760
13	7.2	29	15	53 000	< LOD	27	160	180
14	7.2	4.3	7.2	5900	< LOD	5.1	200	**1200**
15	0.62	15	12	112 500	< LOD	9.5	50	130
16	6.8	10	13	151 000	< LOD	6.3	190	160
17	4.6	5	6.7	9200	< LOD	4.3	130	400
18	4.1	17	46	179 500	< LOD	6.7	160	**320 300**
19	4.1	35	160	7600	< LOD	4.4	700	500
20	0.73	17	29	8500	< LOD	5	96	200
Mean	8.87	36.87	65.99	69 560	0.0015	21.91	500	21 931
Median	4.65	35.50	26.50	28 750	0.0000	8.50	160	410
SD	14.62	28.75	108.40	71 500	0.0046	21.26	1057	74 054
MAD	4.22	1.37	39.49	40 810	0.0015	13.41	340.35	21 521

< LOD – not detected above the limit of detection: Hg 0.009; SD – standard deviation; MAD – median absolute deviation

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served by Sutkowska et al. (2013), ranged from 2.15–36.16 mg/kg, while in this study the minimum concentration of this heavy metal was 2.4 and the maximum concentration was 70 mg/kg of soil (Table 2).

The collected soil samples were in most cases characterized by neutral pH, with the maximum value of 8.14, recorded in the site No. 11 and the minimum of 6.78 in No. 1 (Table 1). It has been confirmed that near-neutral pH is the most preferable by the majority of soil microorganisms and it results in the largest and most diverse composition of bacterial populations (Lal 2005).

Mesophilic bacteria were the most abundant in the site No. 3, i.e. 6.6 × 10^6 colony forming units (CFU)/g of soil and the smallest number of these microorganisms was observed in the site No. 13, i.e. 3.8 × 10^5 CFU/g. The site No. 3 was also characterized by the greatest number of fungi. The smallest number of fungi was observed in the site No. 8. The number of actinomycetes ranged from 1.7 × 10^6 CFU/g (Site No. 16) to 2.9 × 10^4 CFU/g (site No. 14). On the other hand, *Azotobacter* spp. was not recorded in three sites (No. 1, 6 and 19), while its greatest prevalence was recorded in the site No. 7 (960 CFU/g, Table 3). Of all the mentioned sites, only one (No. 14), where the smallest number of actinomycetes was recorded, was characterized by the exceeded concentration of heavy metals – in this case zinc (Table 2). The spatial differences in the numbers of the examined microorganisms are statistically insignificant (F values for bacteria, fungi, actinomycetes and *Azotobacter* are 1.00, 0.93, 1.07 and 1.12, respectively; $P < 0.05$). Also the Spearman's correlation coefficient between the number of the studied microbial groups and the heavy metal concentrations, as well as soil pH did not reveal strong, statistically significant relationships, except for average negative correlation

Table 3. Mean prevalence of the analyzed microorganisms in the tested soil samples (colony forming units/g)

Analyzed microorganism	Soil No.				
	1	2	3	4	5
Mesophilic bacteria	1 750 000 (818 700)	1 032 000 (1 093 300)	6 630 000 (8 268 300)	1 290 000 (1 039 900)	980 000 (1 433 100)
Fungi	45 300 (50 100)	74 000 (90 400)	298 000 (445 800)	80 000 (123 900)	86 000 (122 300)
Actinomycetes	597 000 (732 800)	213 000 (238 800)	455 000 (485 700)	227 000 (249 200)	64 000 (101 500)
Azotobacter spp.	0 (0)	140 (200)	150 (180)	660 (700)	10 (10)
	6	7	8	9	10
Mesophilic bacteria	637 000 (725 500)	1 876 000 (867 000)	1 814 000 (1 844 200)	4 656 000 (5 319 900)	674 000 (1 078 000)
Fungi	177 000 (306 300)	21 000 (15 700)	9000 (8500)	17 500 (7500)	13 400 (17 000)
Actinomycetes	38 800 (44 600)	136 000 (150 000)	131 000 (119 000)	209 000 (313 700)	33 800 (64 700)
Azotobacter spp.	0 (0)	960 (1800)	60 (60)	20 (30)	10 (25)
	11	12	13	14	15
Mesophilic bacteria	2 142 000 (2 580 500)	977 000 (719 400)	380 000 (284 800)	897 000 (984 900)	1 649 000 (1 724 400)
Fungi	192 000 (354 400)	46 000 (55 600)	23 600 (21 200)	41 100 (34 700)	40 000 (23 000)
Actinomycetes	139 000 (163 700)	38 000 (34 600)	59 500 (110 900)	29 400 (27 200)	112 000 (116 700)
Azotobacter spp.	10 (15)	10 (30)	15 (30)	110 (200)	180 (340)
	16	17	18	19	20
Mesophilic bacteria	3 346 000 (5 814 200)	2 097 000 (2 477 500)	1 467 000 (816 400)	614 000 (671 300)	4 800 000 (6 586 300)
Fungi	31 500 (30 100)	59 000 (8 200)	27 000 (21 300)	38 500 (17 800)	13 300 (11 600)
Actinomycetes	1 745 000 (3 150 000)	175 000 (154 300)	116 000 (121 000)	45 500 (46 900)	46 200 (79 900)
Azotobacter spp.	70 (140)	170 (190)	380 (430)	0 (0)	60 (100)

Values of standard deviation are given in parentheses

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between soil pH and the number of actinomycetes ($R = -0.47$). This is also reflected by the fact that the two sites, where the highest concentrations of heavy metals were recorded, i.e. site No. 5 and 18, were at the same time characterized by relatively high numbers of all studied microbial groups. This is particularly interesting in the site No. 6 in which, even though the recorded numbers of mesophilic bacteria and actinomycetes were low and no *Azotobacter* spp. colonies were observed, still the number of fungi was very high. In the discussed site very high concentrations of cadmium, lead and zinc were recorded and these elements are considered to have strong inhibitory effect on soil microorganisms (Kabata-Pendias and Pendias 1999, Wyszkowska et al. 2013). The fact that of all examined microbial groups, fungi seemed to be the least affected by the high concentrations of heavy metals is however not unprecedented, as fungi together with yeasts, are considered to be the most tolerant and thus versatile group of soil microorganisms (Iram et al. 2013). Fungal cell wall contains different materials which proved to be efficient metal biosorbents (Gavrilescu 2004). Due to their properties, some fungal species, such as *Aspergillus niger* or *Mucor rouxii* have been successfully investigated for their usefulness in adsorption of heavy metals and therefore metal removal from various environments (Javaid et al. 2011, Joshi and Sahu 2014).

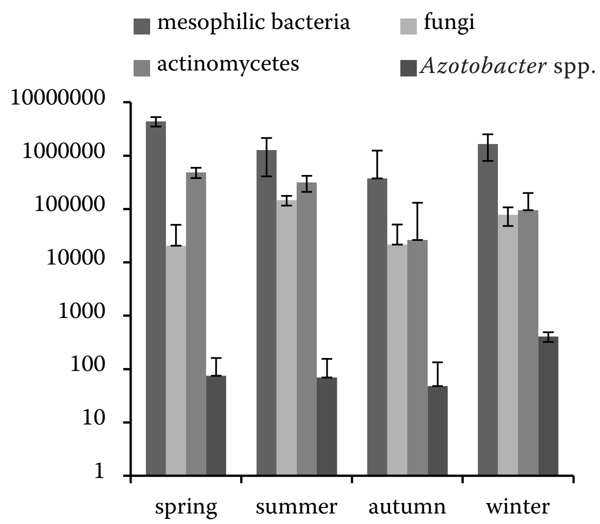

Figure 1. Seasonal variation in the number of studied microorganisms. The values presented are means of all 20 samples. To allow the comparison of values, the data are presented on the logarithmic scale (colony forming units/g)

In terms of seasonal variation, the majority of the studied microorganisms were the most abundant in spring or summer, only the number of *Azotobacter* spp. was the greatest in winter (Figure 1). However, Ram et al. (2013) observed a similar trend in the prevalence of these microorganisms in the topsoil (0–20 cm) layer of neutral and alkaline soils, as the population of these microorganisms was the greatest in winter months. The seasonal differences between the numbers of microorganisms were statistically significant for bacteria ($F = 7.87$; $P < 0.05$), fungi ($F = 3.35$; $P < 0.05$) and *Azotobacter* spp. ($F = 2.74$; $P < 0.05$), but they were insignificant for actinomycetes ($F = 1.60$; $P < 0.05$).

In conclusion, the results of the conducted study showed that despite increased concentrations of some heavy metals, particularly zinc and lead, the recorded contamination did not have a significant impact on the numbers of the selected microbial groups in soils. Our study showed that fungi occurred very frequently in soils with strongly increased concentrations of heavy metals, especially cadmium, lead and zinc. It was proved that these microbes may possess various resistance mechanisms even to toxic heavy metal concentrations and that these properties may be applied in the removal of heavy metals from different environments. Therefore, it would be advisable to conduct further studies, aimed to verify whether the concentration of heavy metals in sites abundantly inhabited by fungi decreased in time together with the assessment of changes in microbial composition of these soils.

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