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Application of geochemical and ecotoxicity indices for assessment of heavy metals content in soils

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Abstract: The research aimed to use chemical, geochemical, and ecotoxicity indices to assess the heavy metals content in soils with different degrees of exposure to human pressure. The research was conducted in southern Poland, in the Malopolska (Little Poland) province. All metal contents exceeded geochemical background levels. The highest values of the I_{geo} index were found for cadmium and were 10.05 (grasslands), 9.31 (forest), and 5.54 (arable lands), indicating extreme soil pollution (class 6) with this metal. Mean integrated pollution index (IPI) values, depending on the kind of use, amounted to 3.4 for arable lands, 4.9 for forests, and 6.6 for grasslands. These values are indicative of a high level of soil pollution in arable lands and an extremely high level of soil pollution in grasslands and forests. Depending on the type of soil use, *Vibrio fischeri* luminescence inhibition was from -33 to 59% (arable lands), from -48 to 78% (grasslands), and from 0 to 88% (forest). Significantly the highest toxicity was found in soils collected from forest grounds.

Introduction

Heavy metals pose a threat to human health and ecological safety (Wei and Yang 2010). This threat arises from emission of metallic elements from industrial plants, electricity boards, traffic routes, and agriculture (Grzebisz et al. 2002, Nicholson et al. 2003, Franc-Uría et al. 2009, Favas et al. 2011, Wuana and Okieimen 2011). Heavy metals accumulate in soil and then penetrate the trophic chain, which leads to destabilization of the proper development of all organisms (Luo et al. 2012). Soil pollution with heavy metals is rarely visible within a short period of time, but is characterized by dangerous ecotoxicological effects which are delayed in time (Garcia-Lorenzo et al. 2009). In particular, the absorptive and buffer properties of soils influence heavy metals accumulation. An important feature which distinguishes heavy metals from other undesired substances is that they do not undergo biodegradation; they only undergo biotransformation as a consequence of complex physico-chemical and biological processes which occur in soil. These processes influence the mobility and availability of heavy metals within the soil-plant-human system. The following play a vital role in the processes of metal binding in soil: reactions, iron and manganese hydroxides, organic matter, clay fraction of soil with a grain diameter above 0.002 mm, oxidation-reduction potential, sorptive capacity, and humidity (Qishlagi and Moore 2007).

Currently, an assessment of soil environment pollution with heavy metals usually consists in determining the heavy metals content by means of chemical analysis. Monitoring of soils in Poland is based mainly on the maximum permissible contents given in the Regulation of the Minister of Environment of 9 September 2002 on soil and earth quality standards (Journal of Laws 2002, no 165, item 1359). However, chemical monitoring alone does not always reveal the real threat connected with the presence of heavy metals in the soil environment. Therefore, a distinct increase in interest in using biological methods to supplement chemical research has been observed in recent years (Boularbah et al. 2005). One of the methods of assessing environmental toxicity which are used in the world is the Microtox® test, which uses luminescent *Vibrio fischeri* bacteria. The test is regarded as the first fast indicator of occurrence of pollutants in the environment (Loureiro et al. 2005, Płaza et al. 2005).

This research aims to assess the risk associated with heavy metals content in soils of different uses and coming from areas with different degrees of exposure to human pressure. The assessment of risk associated with heavy metals content in soils was conducted based on chemical, geochemical, and ecotoxicity indices.

Material and methods

Characteristics of the studied area

The research was conducted in southern Poland, in the northwestern part of the Malopolska (Little Poland) province (Fig. 1). The studied area is characterized by a high diversity of geological structure and types of land use and intensity of industrial development. The main sources of soil pollution with heavy metals include big industrial plants, transportation, the power industry, and burning coal in individual home furnaces. Another factor which influences heavy metals content in soils of the discussed area is the Upper Silesian Industrial Basin, which neighbours the west side, and the transportation of pollutants associated with this region. Additionally, an important source of heavy metals in the northwestern part of the area is the mining-metallurgical activity, which has been conducted for several hundred years and is connected with output as well as the processing of zinc and lead ores (Cabała et al. 2004).

Soil samples

Field investigations were conducted from July to September 2011. Sample collection points were set by the method of equal squares with sides 7.5 km long (Fig. 1) using a GPS device (Garmin 62 s, accuracy +/-2 m). In total 76 points were set. At those points, soil samples were collected from the level of 10 cm with the use of an Eijkelkamp sampler of soil surface layers. Among the collected soil samples, arable lands constituted 32%, grasslands 46%, and forests 22%.

Chemical analyses

All the collected soil samples were dried and then sifted through a sieve with a 2 mm mesh. In order to determine the total content of heavy metals, soil material was digested using the wet method in a closed system in a microwave oven (Multiwave 3000, Anton Paar). The soil material was treated with a 9 cm^3 mixture (1:3 v/v) of concentrated acids, HCl and HNO_3 (Suprapur, Merck). Contents of heavy metals (Cd, Cu, Pb, Zn, Ni, Fe) were determined using a Perkin-Elmer model Optima 7300 DV inductively coupled plasma atomic emission spectrophotometer (ICP-AES) (Tüzen 2003). The soil reaction was determined potentiometrically in a suspension of 1 $mol \cdot dm^{-3}$ KCl, and the organic carbon content was determined by the Tiurin method (Ostrowska et al. 1991). Each sample of the soil material was analysed in two replications. If the analysis results of those replications differed from one another by more than ±5%, another two analyses of that sample were conducted. The quality of the determinations was verified based on the results from heavy metals determinations obtained for the internal standard and on the certified reference material CRM023-050 – Trace Metals – Sandy Loam 7 (RT Corporation). A Microsoft Excel 2007 spreadsheet and the Statistica 10 package were used for the analysis and presentation of the obtained results.

Toxicity test – Microtox® bioassay

The toxicity of the soil samples was tested using *Vibrio fischeri* bacteria, with an M 500 Analyser (MicrobicsCorporation 1992). The 81.9% Screening Test was carried out. Water extract from the soil was prepared by mixing one volume of the soil with four volumes of redistilled water and shaking mechanically for 24 h (Loureiro et al. 2005). After that time, the samples were centrifuged for 10 minutes at a speed of 3000 rpm and filtered. Measurement of luminescence was carried out before and after incubation of the bacterial suspension with the studied sample (after 15 minutes). Toxicity results were expressed as Percent Effect (PE%). A characteristic feature of *Vibrio* bacteria is the designation of a major part of their metabolic energy to luminescence. Any change in metabolism under the influence of a toxic substance causes a change in the produced luminosity (Ribo and Rogers 1990). Using bacteria for environmental monitoring gives very good results due to the short reaction time of a bacterial colony to the toxic effect of the harmful factor.

Results

Chemical analysis

Selected properties of the soils are provided in Table 1. In the studied area, soils with a very acid reaction were dominant (60%), followed by soils with neutral (34%), slightly acidic (13%), and alkaline (3%) reactions. Soils of forest grounds (FG) showed the lowest pH value; 76% of soils from those grounds had very acid and acid reactions. The studied soils differed significantly in organic C content, depending on the type of use. The lowest organic C content, amounting to 25.94 $g \cdot kg^{-1}$ d.m., was observed in arable lands (AL). Similar organic C contents, amounting on average to 49.08 g and 49.14 g of organic $C \cdot kg^{-1}$ d.m., were determined in soils from grasslands (G) and forest grounds (FG). Iron content was between 2.69 and 21.36 $g \cdot kg^{-1}$ of soil. Significantly the lowest iron content was found in forest soils.

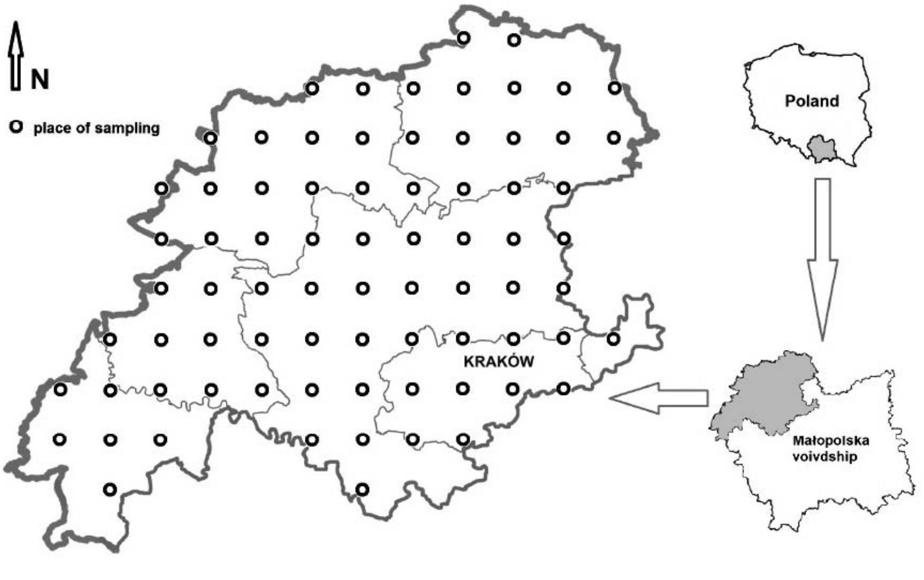

Fig. 1. Places of sampling

Table 1. Selected soil properties from area of north-western Malopolska

Parameters	Arable Lands (AL)	Grasslands (G)	Forest Grounds (FG)
n	24	35	17
pH			
Range	3.90–7.59	3.36–7.36	2.47–7.08
Mean	5.75^{b1}	6.02^{b}	4.67^{a}
SD	1.21	1.19	1.40
Median	5.85	6.65	4.68
C – organic g · kg^{-1} dry mass			
Range	14.0–45.71	15.0–148.47	8.78–109.70
Mean	25.94^{a}	49.08^{b}	49.14^{b}
SD	6.70	31.50	24.57
Median	23.87	40.0	46.23
Fe g · kg^{-1} dry mass			
Range	3.10–21.47	4.61–21.36	2.69–17.75
Mean	12.26^{b}	11.68^{b}	7.99^{a}
SD	4.41	3.66	4.07
Median	12.09	10.79	7.65

[1] Means followed by the same letters in line did not differ significantly at α ≤ 0.05 according to the Tukey test

Based on the research conducted, considerable diversity in heavy metals contents in the soils of the studied area (Table 2) was found. Excluding nickel, generally the highest diversity of heavy metals contents was observed in grassland soils, while the lowest was observed in soils from forest areas. Computed coefficients of variation (CV) for individual metals were as follows: Zn: from 78 (FG) to 278% (G), with a mean of 166%; Cd: from 78 (FG) to 124% (G); Pb: from 48 (AL) to 158% (G); Cu: from 61 (FG) to 128% (G); and Ni: from 40 (G) to 206% (AL). Low values of CV which are below 50% can be indicative of natural heavy metals content in soils, whereas CV values above 50% indicate that their source is anthropogenic. According to this criterion, the metals content in soil was generally connected with human activity.

The mean content of individual heavy metals in soils of arable lands was, in descending order: 107.52 mg Zn; 44.47 mg Ni; 23.23 mg Pb; 12.30 mg Cu; 1.21 mg Cd · kg^{-1} d.m. (Table 2). Compared to the geochemical background (Table 2), the Zn content was exceeded almost three times, Cd six times, Pb one time, Cu two times and Ni seven times. In grasslands the mean metals contents (mg · kg^{-1} d.m.), formed the following series in descending order: Zn (428.04) > Pb (76.35) > Ni (20.93 mg) > Cu (19.60) > Cd (2.38). Compared to their geochemical background levels, on average 11 times more Zn and Cd, four times more Pb, three times more Cu, and two times more Ni were determined in these soils. In forest soils the average contents of zinc, lead, copper, nickel, and cadmium were respectively 149.33, 97.76, 13.32, 13.03, and 2.18 mg · kg^{-1} d.m. Compared to the geochemical background, the zinc content was exceeded almost four times, cadmium ten times, lead five times, copper two times, and nickel three times (Table 2).

While assessing metals contents in the soils, based on maximum permissible values for agricultural soils (Table 2) given in the Regulation of the Minister of Environment of 9 September 2002 on soil and earth quality standards (Journal of Laws 2002, no 165, item 1359), it was found that the permissible content of Zn was exceeded in 18% of studied soils, that of Cd in 12%, that of Pb in 16%, and that of Ni in 2%. Permissible copper content was not exceeded in any of the studied soil samples.

Depending on the type of use, the contents of zinc, cadmium, and copper in the soils formed the following series: G > FG > AL; the content of lead, FG > G > AL; and the content of nickel, AL > G > FG. In the cases of zinc and lead, the differences were statistically significant (Table 2).

Contamination levels of heavy metals

The level of soil contamination with heavy metals was assessed using the geoaccumulation index (I_{geo}), which was introduced by Müller (1969). The geoaccumulation index determines soil pollution with heavy metals by comparing their present and pre-industrial contents. I_{geo} was calculated based on the following formula:

$$I_{geo} = \log_2(C_n/1.5B_n)$$

where:

C_n – concentration of the analysed element

B_n – geochemical background for the analysed element (Table 2)

1.5 – a factor reflecting natural fluctuations in the content of a given element in an environment of minor anthropogenic influences.

Table 2. Content of heavy metals in soils from area of north-western Malopolska (mg · kg^{-1} dry mass)

Parameters	Arable Lands (AL)	Grasslands (G)	Forest Grounds (FG)
n	24	35	17
Zn			
Range	24.7–649.2	26.2–7412.3	20.5–443.0
Mean	107.5^{a}	428.04^{b}	$149.3^{a,b}$
SD	137.9	1224.4	116.6
Median	64.3	89.0	92.3
Cd			
Range	0.42–7.75	0.10–16.89	0.25–5.67
Mean	1.21	2.38	2.18
SD	1.47	3.42	1.70
Median	0.79	0.99	1.69
Pb			
Range	8.92–57.73	8.12–586.41	9.22–309.30
Mean	23.23^{a}	79.35^{b}	97.76^{b}
SD	11.17	116.10	80.12
Median	20.17	28.64	91.36
Cu			
Range	4.00–44.14	3.17–137.98	3.95–38.81
Mean	12.30	19.60	13.32
SD	7.71	24.17	8.17
Median	10.35	12.49	11.17
Ni			
Range	6.66–361.56	6.19–34.05	3.14–26.57
Mean	44.47	20.93	13.03
SD	91.80	8.05	6.38
Median	18.51	19.85	13.95

Background values*				
Zn – 40	Cd – 0.22	Pb – 18	Cu – 6.5	Ni – 6
Norm**				
Zn – 300	Cd – 4	Pb – 100	Cu – 150	Ni – 100

[1] Means followed by the same letters in line did not differ significantly at α ≤ 0.05 according to the Tukey test
* (Kabata-Pendias, Pendias 2001)
** (Journal of Laws of 2002, No. 165, item 1359)

According to Müller (1969) the I_{geo} for each metal is calculated and classified as: uncontaminated ($I_{geo} \leq 0$) – class 0; uncontaminated to moderately contaminated ($0 < I_{geo} \leq 1$) – class 1; moderately contaminated ($1 < I_{geo} \leq 2$) – class 2; moderately to heavily contaminated ($1 < I_{geo} \leq 2$) – class 3; heavily contaminated ($3 < I_{geo} \leq 4$) – class 4; heavily to extremely contaminated ($4 < I_{geo} \leq 5$) – class 5; extremely contaminated ($5 < I_{geo} \leq 6$) – class 6. I_{geo} values for individual metals in soils in arable lands, grasslands, and forests are presented in Table 3. The geoaccumulation indices show diversity depending on the element. From the studied metals, the highest values of I_{geo} were found for cadmium: 10.05 (G), 9.31 (FG), and 5.54 (AL), which is indicative of extreme soil pollution (class 6) with this element, regardless of the kind of land use. The mean value of the geoaccumulation index for zinc was 0.84 for AL, 1.32 for FG, and 2.83 for G. This may provide evidence that soils in arable lands (class 0) are not polluted with this metal, soils under forests (class 2) are moderately polluted, and soils in grasslands are moderately to severely polluted (class 3). Based on the geoaccumulation indices calculated for copper it was established that soils of grasslands (class 4) were severely polluted with this metal and soils of arable lands and forest areas were moderately polluted (class 2). Values of I_{geo} for lead are indicative that soils in grasslands and under forests (class 2) are moderately polluted with this metal and that arable lands (class 0) are not polluted with it. The mean value of the geoaccumulation index for nickel was 2.30 for AL, 1.22 for FG, and 0.55 for G. This indicates that soils in grasslands (class 0) are not polluted with this element, soils under forests (class 1) are moderately polluted or not polluted, and soils in arable lands (class 2) are moderately polluted.

Another indicator which is useful for diagnosis of soil pollution with heavy metals is the pollution index (PI) (Wei and Yang 2010). Similarly to the case of the geoaccumulation index, its value is calculated based on the current content of the metal under evaluation and on its geochemical background, according to the following formula:

$$PI = C_n / B_n$$

where:

C_n – concentration of the analysed element

B_n – geochemical background for the analysed element (Table 2)

PI < 1 indicates low soil pollution with a given metal, 1 ≤ PI < 3 indicates average soil pollution, and PI > 3 severe soil pollution (Table 3). In addition, integrated pollution indices (IPI) for soils of different uses were calculated as the mean value of the PIs for the five analysed heavy metals (Gou et al. 2012, Wei and Yang 2010). Depending on the value of IPI, we distinguish soils with low (IPI ≤ 1), average (1 < IPI ≤ 2), high (2 < IPI ≤ 5), and extremely high pollution levels (IPI > 5) (Wei and Yang 2010). On average, the PI value for zinc was lowest in soils of arable lands (2.7), slightly higher in forest soils (3.7), and definitely highest in grasslands (10.8) (Table 3). Soils moderately polluted with zinc were dominant in arable lands; they constituted as much as 88% of the collected soil samples (Fig. 2). Soils moderately polluted with zinc constituted 53% of the soil samples in grasslands, and 47% under forests. The highest share of soils severely polluted with zinc was found in forest areas and was 47%. Regardless of the type of soil use, average values of PI for cadmium were distinctly higher than 3, which is indicative of severe soil pollution with this metal (Table 3). Soils severely polluted with cadmium constituted 79% of the soil samples in arable lands, 77% in grasslands, and 82% under forests (Fig. 2). Mean PI values for lead were within the range of 1.3 (AL) to 5.4 (FG) (Table 3). Soils moderately polluted with lead constituted 58% of the soil samples in arable lands and weakly polluted ones 38%, while severely polluted soils constituted only 4% (Fig. 2). Similarly, in grasslands, soils moderately polluted with lead were dominant. They constituted 43% of samples collected from these areas. Soils weakly and severely polluted with lead each constituted 29% of the soil samples collected from grasslands (Fig. 2). In the case of copper, the mean PI values were 1.9 (AL), 2.1 (FG), and 3.0 (G) (Table 3). In all types of land use, soils moderately polluted with copper were dominant, constituting 71% (AL), 69% (G), and 65% (FG) of the collected soil samples (Fig. 2). The mean PI value for lead was within the range from 2.2 (FG) to 7.4 (AL). Soils polluted with nickel were dominant in arable lands and grasslands; they constituted respectively 79% (AL) and 69% (G) of the collected soil samples (Fig. 2). Soils moderately polluted with this metal were dominant in forest grounds (79% of the soil samples).

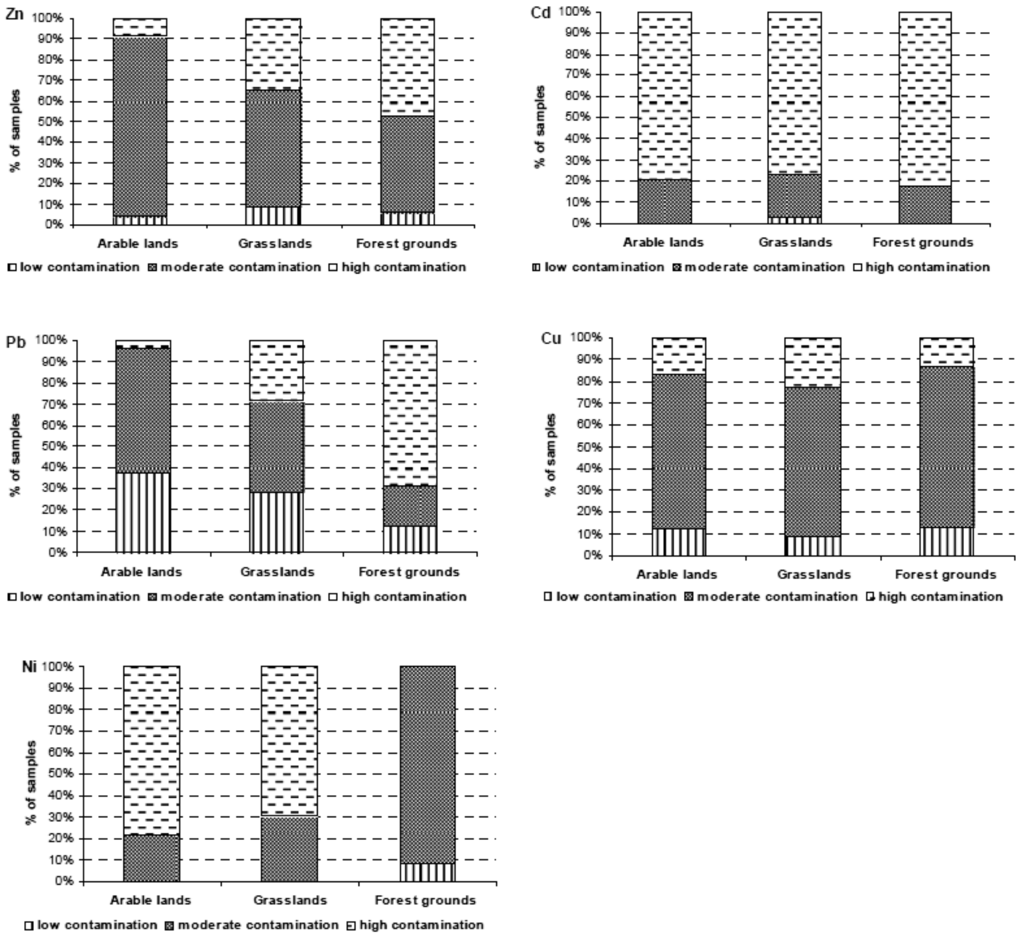

Fig. 2. Pollution characteristic of heavy metals in different functional area

The IPI for heavy metals in the soil samples was within the range from 0.6 to as much as 44. In the studied area, soils with low (IPI ≤ 1), average (1 < IPI ≤ 2), high (2 < IPI ≤ 5), and extremely high (IPI > 5) pollution levels constituted respectively 3, 26, 42, and 29% of the collected soil samples (Fig. 3). Mean IPI values, depending on the kind of land use, amounted to 3.4 for arable lands, 4.9 for forests, and 6.6 for grasslands (Table 3). These values are indicative of a high level of soil pollution in arable lands and an extremely high level in grasslands and forests.

Microtox bioassay

Depending on the type of soil use, *Vibrio fischeri* luminescence inhibition was from -33 to 59% (AL), from -48 to 78% (G), and from 0 to 88% (FG) (Table 4). Significantly the highest toxicity was found in water extracts prepared from soils collected from forest grounds. *V. fischeri* luminescence inhibition in these soils reached, on average, 43% and was almost two times higher than in the other soils (Table 4). Numerous researches have established that a toxicity percent effect of PE < 20% is indicative of a lack of a significant toxic effect, whereas

Table 3. Geochemical values of heavy metals in soils, depending on kind of land use

Kind of land use	Metal	I_{geo}, class		Pollution index PI		Integrated pollution index IPI
Arable Lands (AL)	Zn	0.8	0	2.7	M[1]	1.6–14.0 3.4
	Cd	5.5	6	5.5	H[2]	
	Pb	-0.2	0	1.3	M	
	Cu	1.6	2	1.9	M	
	Ni	2.3	3	7.4	H	
Grasslands (G)	Zn	2.8	3	10.7	H	0.6–0.44 6.6
	Cd	10.1	6	10.8	H	
	Pb	1.5	2	4.2	H	
	Cu	3.0	4	3.0	M	
	Ni	0.5	0	3.4	H	
Forest grounds (FG)	Zn	1.3	2	3.7	H	1.9–9.9 4.9
	Cd	9.3	6	9.9	H	
	Pb	1.9	2	5.4	H	
	Cu	1.8	2	2.1	M	
	Ni	1.2	2	2.2	M	

[1] moderate contamination, [2] high contamination

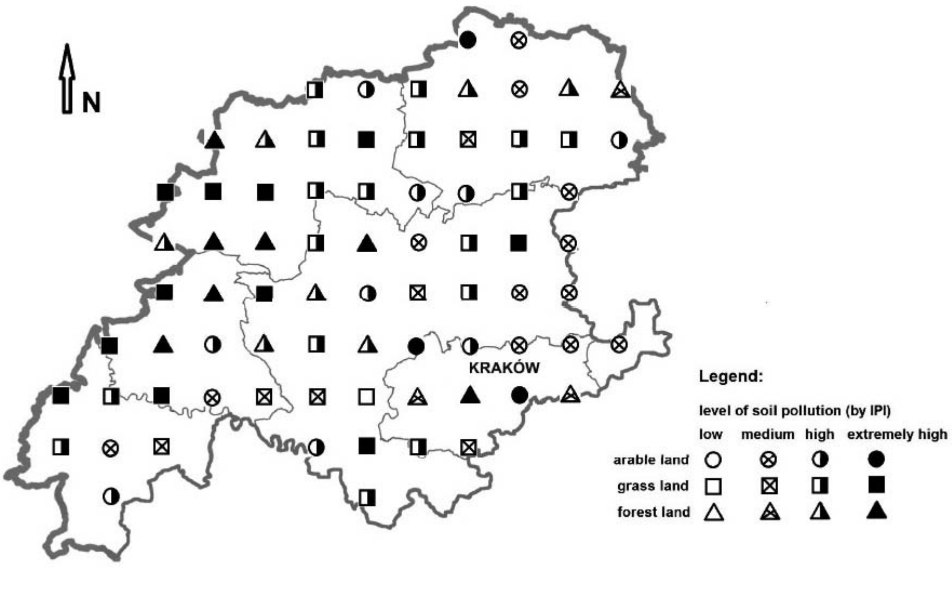

Fig. 3. Assessment of soil pollution with heavy metals based on the integrated pollution index

when the toxicity percent effect is within the range of 20% ≤ PE < 50%, the sample is considered to have low toxicity. Samples whose toxicity percent effect is within 50% ≤ PE < 100% are considered to be toxic (Persoone et al. 2003). Lack of a serious threat (PE < 20%) was shown in 36% of soil samples in arable lands, 43% of those in grasslands, and 15% of those in forest grounds. A low-seriousness threat (20% ≤ PE < 50%) was found in 50% of soil samples collected from arable lands, 43% of those collected from grasslands, and 38% of those collected from forest grounds. Forty-six percent of soil samples collected from forest grounds were characterized by a toxicity percent effect above 50%, which is indicative of a serious threat and significant toxicity of these soils. In arable lands and grasslands, 14% of the collected soil samples had a toxicity percent effect above 50%.

Correlation coefficient analysis

A linear dependency between individual heavy metals in soil may be a result of their geochemical connections, and it can also inform about their mobility and sources of origin (Guo et al. 2012). In order to identify these relations in the studied soils, linear correlation coefficients between individual pairs of metals were calculated (Table 5). In the studied soils, the following pairs of metals were found to be strongly positively correlated: Zn with Pb, Cd, and Cu; Cd with Pb and Cu; and Pb with Cu. Strong linear correlations between individual pairs of heavy metals confirm that they are of similar origin, usually connected with human activities and particularly with output as well as processing of zinc and lead ores. Approximately 20% of the studied area is used by this type of industry. While analysing mutual dependencies between contents of different metals, iron was treated in a different way because the correlation between the content of iron and that of other elements may make it possible to distinguish soils with a natural content of an element from soils where this content is enriched as a result of human activities (Presley et al. 1992). Lack of a correlation between the content of iron and other heavy metals may point to their anthropogenic origin. The research did not reveal a significant correlation between Fe and Zn, Cd, or Pb, which confirms their anthropogenic origin (Table 5). Relatively high I_{geo} and PI values were also noted for these metals (Table 3). A significant positive correlation of copper and nickel with iron was found, which may point to a lack of soil pollution with these metals. The research revealed a strong relation between the contents of Zn, Cd, Pb, and Cu and the C-organic content (Table 5).

Apart from the assessment of correlation between heavy metals, an analysis of correlation between contents of metals, C-organic, pH, and results of toxicity tests on *V. fischeri* was carried out (Table 5). Positive values of the correlation coefficients indicate a connection between metal content in soil and toxicity for organisms, whereas negative values mean that an increase in metal concentration does not cause an increase in sample toxicity. The obtained results indicate a positive correlation between the contents of zinc, cadmium, and lead (statistically significant for Pb) and *V. fischeri* luminescence

Table 4. Toxicity of soils from area of north-western Malopolska to *Vibrio fischeri*

Parameters	Arable Lands (AL)	Grasslands (G)	Forest grounds (FG)
n	24	35	17
Luminescence inhibition 15 min – PE%			
Range	-33–59	-48–71	0–88
Mean	23^{a1}	22^{a}	43^{b}
SD	25	29	25
Median	22	24	45

[1] Means followed by the same letters in line did not differ significantly at α ≤ 0.05 according to the Tukey test

Table 5. Correlation analysis for heavy metals content in soils, and pH, C – organic and toxicity results

Parameters	Zn	Cd	Pb	Cu	Ni	Fe	pH	C-org
Cd	0.8***	-	-	-	-	-	-	-
Pb	0.7***	0.8***	-	-	-	-	-	-
Cu	0.8***	0.6***	0.5***	-	-	-	-	-
Ni	0.02	0.01	0.05	0.03	-	-	-	-
Fe	0.2	0.1	-0.1	0.4*	0.3*	-	-	-
pH	0.4**	0.2	-0.1	0.3*	-0.2	0.2	-	-
C-org	0.6***	0.6***	0.7***	0.5***	-0.6	-0.05	-0.02	-
V. Fischeri	0.13	0,2	0.2*	-0.1	-0.01-	-0.1	-0.5***	0.2

Significant at *** p ≤ 0.001, ** p ≤ 0.01, * p ≤ 0.05

inhibition (Table 5). The contents of copper and nickel in soils did not influence the increase of inhibition of bacterial luminescence. A negative correlation dependency was noted between *Vibrio fischeri* luminescence inhibition and pH (Table 5), which is indicative that substratum toxicity decreases with increases in pH. This is in accordance with other authors' research, where it was proved that solubility (and consequently bioavailability) of heavy metals increases at low soil pH (acid and very acid soils) (Venditti et al. 2000). However, it is important to remember that despite a significant influence of reaction of the substratum on the assimilability of heavy metals by soil organisms, the total content of an element has the greatest influence on their uptake. As mentioned above, approximately 20% of the studied area is exposed to the direct influence of industrial activities connected with output as well as processing of zinc-lead ores. These ores can be found in ore-bearing dolomites, which are a source of not only zinc, lead, and cadmium but also calcium and magnesium, which in turn have an alkaline effect on the environment (Cabała et al. 2008).

Conclusions

The research revealed occurrences of elevated, and sometimes high, contents of heavy metals in soils. This is evidenced by high values of I_{geo}, IP, and IPI (Table 3, Figs. 2 and 3). The Zn and Pb ore mining and processing industry, neighbourhoods of metallurgical plants, municipal and industrial landfill sites, and neighbourhoods of urban and communication areas are all sources of pollution of the studied soils. Apart from the above-mentioned anthropogenic factors, attention should be drawn to the fact that some soils in northwestern Malopolska (Little Poland) have a naturally high content of heavy metals because these soils were formed from bedrocks containing considerable amounts of metals (Cabała and Teper 2007).

According to numerous research studies, the influence of particulates emitted by Zn, Pb, and Cu plants is particularly negative (Lee and Kao 2004, Venditti et al. 2000). This problem touches many Zn-Pb ore mining and metallurgical regions all over the world (Cabała and Teper 2007, Cabała et al. 2008). Zn-Pb ore mining and metallurgical regions in Poland are also characterized by very high contents of Zn, Pb, and Cd. The litter and surface layer of forest soils in the Zn-Pb ore mining and metallurgical regions often contain over 10 000 mg Zn, up to 5 000 mg Pb, and up to 100 mg $Cd \cdot kg^{-1}$ (Cabała and Teper 2007, Cabała et al. 2008). Also, in the present author's own research, the highest contents of these three heavy metals were found in soil samples from the vicinity of Bukowno, which is directly exposed to the influence of mining-metallurgical plants that process zinc-lead ores (Fig. 3).

On the basis of the obtained results it was found that cadmium constituted the greatest threat. On estimating the pollution of soils based on I_{geo}, it was established that the soils were extremely polluted (class 6) with this metal. Numerous researches have proven that cadmium is very mobile in the soil environment and shows potentially high toxicity for living organisms, even at low contents (An 2004). Cadmium content varied in the analysed soils from 0.10 to 16.89 $mg \cdot kg^{-1}$ of soil d.m. Jung and Thornton (1997) showed that the cadmium content in soils collected from areas influenced by Zn and Pb ore mining (Korea) was at the level of 40 $mg \cdot kg^{-1}$ d.m. In soils from areas of China in agricultural use and subjected to human pressure, the mean cadmium content reached 0.43 mg, and mean contents of other metals amounted to 117.22 mg Zn, 31.71 mg Cu, 37.55 mg Pb, and 25.53 mg $Ni \cdot kg^{-1}$ d.m. (Venditti et al. 2000, Wei and Yang 2010). Mean contents of heavy metals in soils in agricultural use in Spain amounted to 0.38 mg Cd, 57.8 mg Zn, 19.6 mg Pb, and 21.6 mg $Cu \cdot kg^{-1}$ of soil d.m. and formed the series Zn > Ni > Cu > Pb > Cd (Wei and Yang 2010). Mean contents of Zn, Cd, and Pb in the studied soils were higher and reached 228.3 mg Zn, 1.92 mg Cd, and 65.78 $Pb \cdot kg^{-1}$, whereas the mean contents of Cu and Ni were lower, amounting to 15.07 mg Cu and 26.14 mg $Ni \cdot kg^{-1}$. The above values formed the series Zn > Pb > Ni > Cu > Cd. Pollution of soils with zinc, cadmium, and copper according to the type of land use can be arranged in descending order: G > FG > AL; for lead pollution the series follows the order FG > G > AL, and for nickel pollution, AL > G > FG. According to Kabata-Pendias and Pendias (2001), maximum heavy metals contents in agricultural soils in some European countries may amount to up to 300 mg Zn, 100 mg Pb, Ni, and Cu, and 5 mg Cd.

To summarize, geochemical indicators are in fact useless for diagnosis of soil pollution with heavy metals. This study did not examine the usefulness of Microtox as a tool to assess the toxicity of soils. Heavy metals content in soils in agricultural use may constitute a potential source of threat to plants and underground waters. In consequence, metals can enter the food chain and constitute a threat to human health. Therefore, this problem requires constant control and monitoring (Guo et al. 2012). Due to the considerable strength of binding of most heavy metals by the soil sorptive complex, heavy metals usually become immobilized in the soil surface layer, which means that the state of pollution remains a problem for a long time, even after eliminating the source of emissions.

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References

[1] An, Y.J. (2004). Soil ecotoxicity assessment using cadmium sensitive plants, *Environmental Pollution*, 127, pp. 21–26.

[2] Boularbah, A., Schwartz, Ch., Bitton, G. & Boularbah, J.L.M. (2005). Heavy metals contamination from mining sites in South Morocco: 1. Use of a biotest to assess metal toxicity of tailings and soils, *Chemosphere*, 63, pp. 802–810.

[3] Cabała, J. & Teper, L. (2007). Metalliferous constituents of rhizosphere soils contaminated by Zn-Pb mining in southern Poland, *Water, Air and Soil Pollution*, 178 (1–4), pp. 351–362.

[4] Cabała, J., Żogała, B. & Dubiel, R. (2008). Geochemical and geophysical study of historical Zn-Pb ore processing waste dump areas (Southern Poland), *Polish Journal of Environmental Studies*, 17 (5), pp. 693–700.

[5] Favas, P.J.C., Pratas, J., Gomes, M.E.P. & Cala, V. (2011). Selective chemical extraction of heavy metals in tailings and soils contaminated by mining activity: Environmental implications, *Journal of Geochemical Exploration*, 111, pp. 160–171.

[6] Franco-Uría, A., López-Mateo, C. Roca, E. & Fernández-Marcos, M.L. (2009). Source identification of heavy metals in pastureland by multivariate analysis in NW Spain, *Journal of Hazardous Materials*, 165, pp. 1008–1015.

[7] Garcia-Lorenzo, M.L., Martinez-Sanchez, M.J., Perez-Sirvent, C. & Molina, J. (2009). Ecotoxicological evaluation for the screening of areas polluted by mining activities, *Ecotoxicology*, 18, pp. 1077–1086.

[8] Grzebisz, W., Cieśla, L., Komisarek, J. & Potarzycki, J. (2002). Geochemical assessment of heavy metals pollution of urban soils, *Polish Journal of Environmental Studies*, 11, 5, pp. 493–499.

[9] Guo, G., Wu, F., Xie, F. & Zhang, R. (2012). Spatial distribution and pollution assessment of heavy metals in urban soils from southwest China, *Journal of Environmental Sciences*, 24(3), pp. 410–418.

[10] Jung, M.C. & Thornton, I. (1997). Environmental contamination and seasonal variation of metals in soils, plants and waters in the paddy fields around a Pb–Zn mine in Korea, *Science of The Total Environment*, 198, pp. 105–121.

[11] Kabata-Pendias, A. & Pendias, H. (2001). *Trace elements in soils and plants*, 3rd ed. CRC Press, Boca Raton, FL, 2001.

[12] Lee, Ch.-S. & Kao, M.-M. (2004). Distribution of forms of heavy metals in soils contaminated by metallurgical smelter emissions, *Journal of Environmental Science and Health*, 39(3), pp. 577–585.

[13] Loureiro, S., Ferreira, A.L.G., Soares, A.M.V.M. & Nogueira, A.J.A. (2005). Evaluation of the toxicity of two soils from Jales Mine (Portugal) using aquatic bioassays, *Chemosphere*, 61, pp. 168–177.

[14] Luo, Ch., Yang, R., Wang, Y., Li, Y., Zhang, G. & Li, X. (2012). Influence of agricultural practice on trace metals in soils and vegetation in the water conservation area along the East River (Dongjiang River), South China, *Science of The Total Environmen*t, 431, pp. 26–32.

[15] Micó, C., Peris, M., Sánchez, J. & Recatalá, L. (2006). Heavy metal content of agricultural soils in a Mediterranean semiarid area: the Segura River Valley (Alicante, Spain), *Spanish Journal of Agricultural Research*, 4(4), pp. 363–372.

[16] Microbics Corporation, 1992. *Microtox Manuale Toxicity Testing Handbook*. Carlsbad, CA, USA, 1992.

[17] Müller, G. (1969). Index of geo-accumulation in sediments of the Rihne River, *Geojournal*, 2, pp. 108–118.

[18] Nicholson, F.A., Smith, S.R., Alloway, B.J., Carlton-Smith, C. & Chambers, B.J. (2003). An inventory of heavy metals inputs to agricultural soils in England and Wales, *Science of The Total Environment*, 311, pp. 205–219.

[19] Ostrowska, A., Gawliński, S. & Szczubiałka, Z. (1991). Methods of analysis and assessment of soil properties and plant, first ed., Institute of Environmental Protection, pp. 333. (in Polish)

[20] Persoone, G., Marsalek, B., Blinova, I., Törökne, A., Zarina, D., Manusadzianas, L., Nalecz-Jawecki, G., Tofan, L., Stepanova, N., Tothova, L. & Kolar, B. (2003). A practical and user-friendly toxicity classification system with microbiotests for natural waters and wastewaters, *Environmental Toxicology*, 18(6), pp. 395–402.

[21] Płaza, G., Nałecz-Jawecki, G., Ulfig, K. & Brigmon, R. (2005). The application of bioassays as indicators of petroleum – contaminated soil remediation, *Chemosphere*, 59, pp. 289–296.

[22] Presley, B.J., Taylor, R.J. & Boothe, P.N. (1992). Trace metal concentration in sediments of Eastern Mississippi Bigh, *Marine Environmental Research*, 33, pp. 267–282.

[23] Qishlaqi, A. & Moore, F. (2007). Statistical analysis of accumulation and sources of heavy metals occurrence in agricultural soils of Khoshk River Banks, Shiraz, Iran, *American-Eurasian Journal of Agricultural & Environmental Sciences*, 2(5), pp. 565–573.

[24] Ribo, J.M. & Rogers, F. (1990). Toxicity of mixtures of aquatic contaminants using the luminescent bacteria bioassay, *Toxicity Assessment*, 5, pp. 135–152.

[25] Regulation of the Minister of Environment of 9 September 2002 on soil and earth quality standards. Journal of Laws of 2002, No. 165, item 1359.

[26] Tüzen, M. (2003). Determination of heavy metals in soil, mushroom and plant samples by atomic absorption spectrometry, *Microchemical Journal*, 74, pp. 289–297.

[27] Venditti, D., Durecu, S. & Berthelin, J. (2000). A multidisciplinary approach to assess history, environmental risks, and remediation feasibility of soils contaminated by metallurgical activities. Part A: Chemical and physical properties of metals and leaching ability, *Archives of Environmental Contamination and Toxicology*, 38, pp. 411–420.

[28] Wei, B. & Yang, L. (2010) A review of heavy metal contaminations in urban soils, urban road dusts and agriculture soils from China, *Microchemical Journal*, 94, 99–107.

[29] Wuana, R.A & Okieimen, F.E. (2011). Heavy metals in contaminated soils: A review of sources, chemistry, risks and best available strategies for remediation, ISRN Ecology, 2011.

Zastosowanie wskaźników geochemicznych i ekotokysycznych w ocenie zawartości metali ciężkich w glebach

Celem badań było wykorzystanie wskaźników chemicznych, geochemicznych i ekotoksycznych w ocenie zawartości metali ciężkich w glebach o zróżnicowanym stopniu narażenia na antropopresję. Badania prowadzono na terenie Polski Południowej w województwie Małopolskim. Punkty poboru prób wyznaczono metodą równych kwadratów o boku kwadratu równym 7,5 km przy wykorzystaniu urządzenia GPS (Garmin 62s, dokładność +/- 2 m). Łącznie wyznaczono 76 punktów w których pobrano próby glebowe z poziomu 0–10 cm. Średnia zawartość poszczególnych metali ciężkich w glebach gruntów ornych (GO) wyniosła w kolejności malejącej: 107,52 mg Zn; 44,47 mg Ni; 23,23 mg Pb; 12,30 mg Cu; 1,21 mg Cd · kg^{-1} s.m. Na użytkach zielonych (UZ) średnia zawartość metali tworzyła szereg malejący (mg · kg^{-1} s.m): Zn (428,04) > Pb (76,35) > Ni (20,93 mg) > Cu (19,60) > Cd (2,38). W glebach leśnych (L) przeciętna zawartość cynku wyniosła 149,33 mg, ołowiu 97,76 mg, miedzi 13,32 mg, niklu 13,03 mg oraz kadmu 2,18 mg · kg^{-1} s.m. Spośród badanych metali największe wartości wskaźnika I_{geo} wykazano dla kadmu: 10,05 (UZ); 9,31 (L); 5,54 (GO), co świadczy o ekstremalnym zanieczyszczeniu (klasa 6) gleb tym pierwiastkiem, niezależnie od rodzaju użytkowania terenu. Średnie wartości zintegrowanego współczynnika zanieczyszczenia wyniosły: 2,8 (GO); 5,3 (L) oraz 6,4 (UZ). Wartości te świadczą o wysokim stopniu zanieczyszczenia gleb na gruntach ornych oraz ekstremalnie wysokim na użytkach zielonych i lasach. Inhibicja luminescencji *Vibrio fischeri* w zależności od sposobu użytkowania gleb wyniosła od -33 do 59% (GO), od -48 do 78% (UZ) oraz od 0 do 88% (L). Istotnie największą toksycznością charakteryzowały się gleby pobrane z użytków leśnych.