Pollution indices and biotests as useful tools for the evaluation of the degree of soil contamination by trace elements

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Received: 14 June 2021 / Accepted: 5 October 2021 / Published online: 15 October 2021
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Abstract

Purpose The aim of the study was the calculation of geochemical, ecological, and ecotoxicological indices for the assessment of risk resulting from the presence of trace metals in soil.

Methods Around 320 soil points were determined for the tests by the regular square-grid method with a square side equal to 7.5 km. A total of 11 indices were used, including 6 geochemical indices, 1 index assessing potential ecological risk, and 4 indices assessing soil ecotoxicity.

Results Two groups of elements were distinguished. The first one included Ni, Cr, and Cu. The calculated geochemical indices generally indicated their natural content and low degree of soil contamination with them and that the elements' sources were connected with natural processes. The second group included Cd, Pb, and Zn. These elements occur in high concentrations in the studied area, which is influenced by both natural and anthropogenic factors. However, contamination with these elements is heterogeneous in the Małopolska and generally observed in its northwestern part. PCA showed that Cd and Pb had the greatest effect on the degree of soil contamination and pose the greatest potential threat to the soil environment. *Heterocypris incongruens* proved to be a more sensitive indicator of soil ecotoxicity compared to plant tests. The potential ecological risk index and biotests indicated that most of the soil samples had low potential ecological risk and low ecotoxicity.

Conclusion The key to the effective assessment of soil contamination with trace elements is the combined use of geochemical, ecological, and ecotoxicological indices, which allows comprehensive monitoring of soil quality.

Keywords Trace elements · Risk assessment · Soil quality · Ecotoxicity

1 Introduction

Due to rapid industrialization and urbanization, soil pollution has become an increasingly important global environmental problem in recent years (Yang et al. 2018; Tóth et al. 2016; El-Sherbiny et al. 2019; Ciarkowska and Gambuś 2020). Trace elements have a special role in soil contamination. Regardless of whether they act as essential nutrients or not, most trace elements, if present in large quantities, are identified in the literature as potentially toxic elements because they migrate over long distances and easily enter trophic chains (Tóth et al. 2016; Wieczorek et al. 2018; Lu

et al. 2021). Trace elements in soils can be considered a threat if their production rate in man-made cycles is faster than from natural sources, they are moved to random locations with greater potential for direct exposure, and the chemical form in which the trace element occurs may make it more bioavailable (Czech et al. 2014; Baran et al. 2018; Lu et al. 2019; Fazekašová and Fasekaš 2020; Lee et al. 2020). A high content of trace elements in soils, especially agricultural soils, affects not only their quality and function, but also food quality and thus human health (Wcisło et al. 2016; Gruszecka-Kosowska et al. 2020; Lu et al. 2021).

Currently, different approaches and methods are used to assess the degree of soil contamination with trace elements. The most commonly used are soil quality standards (limit values relating to the content of a given element) and geochemical indicators, which are primarily aimed at assessing the anthropogenic impact on soil quality and may reveal the possibility of contamination (Kowalewska et al. 2018; Ahmadi et al. 2019; Selzenev et al. 2020). For several years, the ecological risk factor (ER) has also been used to assess the degree

Responsible editor: Dong-Mei Zhou

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of soil contamination with trace elements (Wieczorek et al. 2018; Ciarkowska and Gambuś 2020; Rostami et al. 2021; Lu et al. 2021). This factor allows, in a way, to assess the potential risk for soil organisms that results from the presence of trace elements in the soil.

The aforementioned methods are based on the analysis of the total content of trace elements in the soil and may not always be sufficient to correctly assess the ecological risk associated with their impact on soil ecology or migration in the trophic chain (Baran et al. 2014; Yang et al. 2018). Very often, the assessment of the degree of soil contamination is accompanied by the analysis of the relationship between the trace element content and soil properties. However, the relationship between trace element content and soil properties also does not provide comprehensive information on the degree of soil contamination and on the soil quality (Kowalska et al. 2018). Chemical analyses are necessary to assess soil contamination but provide limited information on the effect of this contamination on the soil environment, i.e., to what extent trace elements in soils are a factor that negatively affects this environment, and therefore whether they can be considered a stress factor for organisms (Baran et al. 2014).

Biotests can be a useful research tool for assessing the ecological effects of contaminations in soil ecosystems (Baran et al. 2014; Adams et al. 2015; Sforzini et al. 2016; El-Alam et al. 2018; Cuske et al. 2017; Domínguez-Rodríguez et al. 2020; Szopka et al. 2021). Biotests use a living organism as an indicator and rely on its exposure to toxic substances present in the sample as well as the qualitative and quantitative assessments of the effects of this exposure (Wierzbicka et al. 2015; Lyubenova and Boteva 2016; Wieczerek et al. 2016; Heise et al. 2020). The advantage of these tests is that it is possible to obtain an assessment based on the actual toxicity of a sample and not just on the potential/predicted toxicity of a component (Adams et al. 2015; Hawrot-Paw et al. 2020). Ecotoxicological tests are effective indicators of soil quality (Oleszczuk et al. 2014). However, results of bioassays are not always easy to interpret, as the tests assess the total toxicity of the sample resulting from the content of and antagonistic/synergistic interactions between substances in the test sample (Baderna et al. 2015; Heise et al. 2020).

The objectives of the study were the (1) calculation of geochemical and ecological indices for the assessment of risk resulting from the presence of trace metals in soil, (2) assessment of the ecotoxicity of soils using biotests Phytotoxkit (*Sorghum saccharatum*, *Lepidium sativum*) and Ostracodtoxkit (*Heterocypris incongruens*), and (3) evaluation of interactions between geochemical, ecological, and ecotoxicological indices for a justified contamination risk assessment.

2 Materials and methods

2.1 Study area and sample collection

The study was carried out in the southern part of Poland, in the Małopolskie Voivodeship. The voivodeship area covers 15,183 km², which is 4.9% of the total area of the country. The voivodeship area is diverse in terms of both nature and land use. The natural diversity, the biggest in the country, is determined by the vertical range of the area — from flat areas of the Sandomierz Basin to high peaks of the Tatra Mountains. A considerable part of the voivodeship is located 500 m above sea level. In terms of land use, the western and central parts of the voivodeship are industrialized areas with the agglomeration of Kraków, whereas the northern, eastern, and southern parts are agricultural areas with two large urban centers — Tarnów and Nowy Sącz (Fig. 1). The main factors affecting the quality of soils in the study area include erosion, mechanical destruction of the soil cover due to urbanization processes, mining activities (extraction of zinc and lead ores), improperly conducted agricultural activities, chemical contamination of soils associated with the emission of pollutants to the environment, emissions of technological pollutants from industry to the air, transport and combustion of solid and liquid fuels, the use of excessive amounts of fertilizers, and storage of municipal and industrial waste (Wieczorek et al. 2018).

Around 320 sampling points were determined for the tests by the regular square-grid method with a square side equal to 7.5 km (Fig. 1). The points in this method are located at equal distances from each other, which makes this method easy to apply in practice. Sampling for the study was attempted at the intersection points of the grid lines (Baran et al. 2018; Wieczorek et al. 2018). Sampling sites were located using a GPS satellite receiver (Garmin 62 s, accuracy ± 2 m). Soil samples were collected from 0–10-cm levels to 40–50-cm levels using Eijkelpamp soil samplers for topsoil and Edelman auger. Plant residues were removed from the soil surface before sampling. Individual soil samples were taken at several dozen locations in a circle of 5–7-m radius. The collected soil material was placed in canvas bags to form an average sample.

The study area was characterized by a great variation in geological structure, in land use, and in the intensity of industrial development. The network of sampling points provided a diversity of soil use. The highest number of soil samples was taken from grassland 126 (39%), followed by forest land 82 (26%), arable land 66 (14%), and wasteland 46 (14%) (Wieczorek et al. 2018).

Fig. 1 Location of soil sampling points

2.2 Chemical analysis

In air-dried soil samples, the following were determined: granulometric composition by Casagrande method modified by Prószyński, pH in 1 mol KCl-dm⁻³ by potentiometric method, cationic sorption capacity, organic carbon by Tiurin method, and nitrogen by Kjeldahl method (Wieczorek et al. 2018). Total trace element contents (Zn, Cd, Pb, Cu, Cr, and Ni) were determined after microwave digestion in a mixture of concentrated HCl and HNO₃ acids (1:3 v/v) on a Perkin–Elmer model Optima 7300 DV inductively coupled plasma atomic emission spectrophotometer (ICP-OES) (Baran et al. 2018). Each soil sample was analyzed in two replicates. If the analysis results of these replicates differed by more than $\pm 5\%$, another two analyses of this sample were performed. The quality of determinations was verified on the basis of the trace element results obtained for the internal

standard and the certified reference material CRM023-050. The analytical results of the quality control samples showed good agreement with the certified value, and the recoveries ranged from 86 to 112%.

2.3 Ecotoxicological analysis

Ecotoxicity of soil samples (0–10-cm level) was tested using three test organisms: *Lepidium sativum*, *Sorghum saccharatum* (Phytotoxkit), and *Heterocypris incongruens* (Ostracodtoxkit). In the Phytotoxkit assay, the measured parameters were seed germination inhibition and root length inhibition of the test plants after their 3-day incubation with soil samples (Baran et al. 2014; Szara et al. 2020). The Ostracodtoxkit assay measured mortality and growth inhibition of *H. incongruens* after a 6-day exposure of the crustacean to test samples (Szara et al. 2020). Biotests were performed

according to the procedure developed by the manufacturer (Ostracodtoxkit 2001; Phytotoxkit 2004). Screening tests were carried out by analyzing undiluted samples. The results obtained were expressed as a test reaction percentage effect (PE). The study assumed the following: the percentage toxic effect of $PE < 20\%$ = no significant negative effect on the organism — the sample is non-toxic; the percentage toxic effect of $20\% \leq PE < 50\%$ — the sample is low toxic; a sample is toxic to an organism if the percentage toxic effect equals $50\% \leq PE < 100\%$; and soil samples for which the percentage toxic effect is $PE = 100\%$ are very toxic (Baran and Wiczonek 2015; Szara et al. 2020).

2.4 Geochemical and ecological indices

The analysis of the level of soil contamination and the potential risk of trace elements in soils was carried out using geochemical and ecological indices (Table S1). It is worth mentioning here that the literature provides no universal indicator to assess the degree of soil contamination with heavy metals, especially at the 0–10-cm level. The 0–10-cm level is the layer of soil called “biofilter,” most exposed to anthropogenic contamination. Additionally, this level is indicated in soil ecotoxicity studies. Seven indices were used in this study, including four single-element indices (geoaccumulation index (I_{geo}), anthropogenic factor (Af), single pollution index (PI), enrichment factor (EF)) and three multi-element indices (pollution load index (PLI), improved Nemerow pollution index (PI_N), potential ecological risk (RI)). Due to their nature, the single-element indices only allow for the assessment of the content of single elements, whereas trace elements in soils usually occur in the form of complex mixtures with significant spatial and temporal variability. Therefore, multi-element indices allow for a comprehensive, holistic determination of the degree of contamination and the risk associated with their occurrence in the environment. Calculations were made according to the formulas in Table S1, assuming the following values of geochemical background (mg/kg) for individual elements: Cd (0.41), Cr (59.5), Cu (38.9), Ni (29), Pb (27), Zn (70), and Fe (12,000) (Kabata-Pendias 2011).

2.5 Statistical and graphical analysis

Basic statistical parameters, such as mean, median, minimum, maximum, standard deviation, and coefficient of variation (CV%), were calculated. To compare the significance of differences between the mean values of single-element indices calculated for the analyzed metals, a one-way analysis of variance was performed, followed by Tukey’s post-hoc test. Possible relationships between data were investigated using Pearson correlation matrix and principal component analysis (PCA). Microsoft Excel 2010 spreadsheet, Statistica

13, and Surfer 8.0 software were used to analyze and graphically present the results obtained.

3 Results and discussion

3.1 Basic properties of soils

The basic soil properties are given in Table 1 and described in detail in previous studies (Baran et al. 2018; Wiczonek et al. 2018). The mean pH value was 4.92 with a median of 4.81 (Table 1). Acidic (41%) and very acidic (29%) soils generally prevailed in the study area. The grain size of the studied soils showed high variation in the sand fraction ($CV\% = 75$) and medium variation in the silt ($CV\% = 40$) and clay ($CV\% = 36$) fractions. The content of sand fraction in soils ranged from 0 to 95%, dust fraction from 0 to 65%, and clay fraction from 0 to 71%. The mean total organic carbon (TOC) content of the soils was 53.47 g/kg dm (0.98–320 g/kg dm). In the studied soil samples, the HA (hydrolytic acidity) value ranged from 6.0 to 477.5 mmol/kg, the BEC (base exchange capacity) value from 44.0 to 970 mmol/kg, and the sorption capacity (CEC) from 87.5 to 1112.7 mmol/kg dm (Table 1). According to the European Soil Bureau, 60% of the studied soils were classified as of medium, 22% as of high, and 18% as of low sorption capacity (Tóth et al. 2008). The iron content was on average 13.38 g/kg (1.56–32.50 g/kg), and the average spatial variation was $CV = 41\%$.

3.2 Content of heavy metals

The content of heavy metals in soil samples is presented in Table 1. Significant variation was found in the content of heavy metals in soils. The calculated coefficient of variations (CV) for individual metals were as follows: Zn 351%; Cd 171%; Pb 148%; Ni 95%; Cu 77%; Cr 50% (0–10-cm level); Zn 117%; Cd 292%; Pb 196%; Ni 110%; Cu 74%; and Cr 207% (40–50-cm level). In general, 1.05 to 1.71 times higher contents of trace elements were determined in the surface layer compared to the 40–50-cm layer. Significantly higher contents in the surface level compared to the 40–50-cm level were found only for Zn, Pb, and Cr. For cadmium, over 1.30 times higher content was found in the 40–50-cm level compared to the 0–10-cm level. The mean contents of individual heavy metals in the soils were, in descending order, as follows: 122.1 mg Zn; 38.32 mg Pb; 28.43 mg Cr; 23.46 mg Ni; 16.34 mg Cu; 0.93 mg Cd kg dm (0–10-cm level) and 79.82 mg Zn; 22.67 mg Pb; 22.20 mg Ni; 21.61 mg Cr; 14.95 mg Cu; and 1.18 mg Cd kg dm (40–50-cm level) (Table 1). The anthropogenic factor (Af) ranged from 0 to 88.60, with its average expressed by a median, in descending order: Pb 1.75 > Zn 1.51 > Cu 1.26 > Cr 1.20 > Ni 0.97 > Cd 0.88 (Table 1). It is assumed that if $Af > 1$, it indicates that

Table 1 Basic physicochemical properties of studied soils ($n = 320$)

Parameter	Unit	Mean	Median	Minimum	Maximum	CV*
pH	-	4.97	4.81	2.47	7.59	25
Sand*	%	28	21	0	95	75
Silt*		33	34	0	65	40
Clay*		38	39	0	71	36
TOC*	g/kg dm	53.47	44.88	0.98	320	77
Fe	g/kg dm	13.38	12.71	1.56	32.50	41
HA*	mmol (+) /kg	68.3	46.1	6.0	477.5	92
BEC*		300.6	245.8	44.0	970.0	63
CEC*		367.6	320.8	87.5	1112.7	49
Cd	mg/kg dm	0.93	0.49	0.01	16.89	171
Cr	Layer 0–10 cm	28.43 ^{b**}	26.38	3.92	86.05	50
Cu		16.34	13.22	2.77	138.98	77
Ni		23.46	18.76	1.82	271.23	95
Pb		38.32 ^b	22.23	2.95	586.41	148
Zn		122.1 ^b	68.96	8.27	7221.8	351
Cd	mg/kg dm (layer 40–50 cm)	1.18	0.36	0.03	69.95	447
Cr		21.61 ^a	19.88	1.41	184.80	93
Cu		14.95	10.86	1.66	198.65	127
Ni		22.20	18.85	1.20	111.65	62
Pb		22.67 ^a	14.02	2.15	470.80	172
Zn		79.82 ^a	49.83	8.24	5912.00	417
Cd	Anthropogenic factor (Af)	2.33	0.88	0.00	88.55	292
Cr		4.00	1.20	0.00	58.00	176
Cu		1.52	1.26	0.08	8.97	74
Ni		1.36	0.97	0.18	15.82	110
Pb		2.80	1.75	0.05	67.98	196
Zn		1.95	1.51	0.09	24.98	117

* CV, coefficient of variation; sand (2–0.05 mm); silt (0.05–0.002 mm); clay (<0.02 mm); TOC, total organic carbon; HA, hydrolytic acidity; BEC, base exchange capacity; CEC, cation exchange capacity

the site is enriched with trace elements of anthropogenic origin, while if Af is ≤ 1 , the site is considered to have natural element content (Natesan and Seshan 2010). The study revealed that nearly 80% of soils were enriched with Pb, 70% Zn, 66% Cu, 57% Cr, 47% Ni, and 45% Cd of anthropogenic origin. Higher cadmium content at the 40–50-cm level may be related to its high mobility in the soil and leaching into the lower profiles, which leads to a reduction in the element accumulation in the surface layer (Ahmadipour et al. 2014). A very high variation in zinc, cadmium, and lead contents in the soils of the Małopolska region is due to the fact that its northwestern part has high natural contents of these metals in soils. The maximum contents of these metals in soils of the northwestern Małopolska region were, for the 0–10-cm level: Zn 586.41 mg; and Pb 271.23 mg; and for the 40–50-cm level: Zn 5912 mg; Pb 470.80 mg; and Cd 69.95 mg/kg dm. It is related to the historical and current exploitation and processing of zinc and lead ores (Baran et al. 2014; Baran and Wieczorek 2015). The problem of high zinc, cadmium, and lead contents in soils of the Zn-Pb ore mining

and smelting regions worldwide is common (Loureiro et al. 2005, Czech et al. 2014, Lee et al. 2020). The litter and the surface layer of forest soils in the area of Zn-Pb ore mining and smelting often contain more than 10,000 mg Zn to 5,000 mg Pb and up to 100 mg Cd/kg (Cabała et al. 2008). In our previous study, the total metal content in soils sampled in the zinc and lead ore mining and smelting region ranged from 0.29 to 51.91 Cd mg; 7.90 to 3614 mg Zn; and 28.4 to 6844 mg Pb/kg dm (Baran et al. 2014). According to Kabata-Pendias (2011), the maximum heavy metal contents in agricultural soils in some European countries can reach up to 300 mg Zn; 100 mg Pb, Ni, and Cu; and 5 mg Cd. In anthropopressed soils of China, which are used for agricultural purposes, the average trace element contents were 117.22 mg Zn, 31.71 mg Cu, 37.55 mg Pb, 25.53 mg Ni, and 0.43 mg Cd/kg dm. In the study by Gruszecka-Kosowska et al. (2020), the average contents of heavy metals in agricultural soils of southern Poland were Zn 192 mg, Pb 47.1 mg, Cr 19.6 mg, Cu 18.8 mg, Ni 9.91 mg, and Cd 0.03 mg/kg dm.

3.3 Geochemical and ecological indices

Out of over a dozen available geochemical indices for assessing the degree of heavy metal contamination in soils, the six most commonly used were applied in this study. The indices discussed as the first three, i.e., PI, I_{geo} , and EF, are designed for the evaluation of single metal contents, while the next three (PLI, PI_N , RI) enable to make an integrated assessment of soil quality due to the concentration of the whole group of trace elements in it. Assessments of heavy metal contamination of soils of the Małopolska region presented in the study were differentiated and dependent on the geochemical index applied. The basic statistical parameters for individual indices calculated for 320 soil samples from the Małopolska region are presented in Table 2, and their spatial distribution is shown in Figs. 2, 3, 4, and 5.

The first of the calculated indices — single pollution index (PI) — calculated for each of the six analyzed metals, was the highest and most diverse for Pb, Cr, and Cd. As much as 23% of soils were classified as strongly polluted with Cd and Cr and 17.5% with Pb. The evaluation of concentrations of the remaining elements showed a much smaller percentage of samples with the highest degree of contamination (Zn — 7.5%, Cu — 5.0%, and Ni — 2.5%).

Comparison of the PI results between individual metals allowed to discover the significantly highest mean index value for the content of Cr, followed by Cd and Pb. The significantly lowest mean PI values were obtained for Ni and Cu. Statistical analysis also showed large differences between the mean and median calculated for Pb, Cd, and especially for Cr. This indicates an uneven distribution of this parameter, which was confirmed in Fig. 2, showing the spatial distribution of the index in the studied area. Soils from the areas located along the southern border and in the latitudinal strip of land located in the central part of the voivodeship were assessed as strongly contaminated with Cr. The presence of chromium of anthropogenic origin in soils may be due to the large number of tanneries operating in this region (Augustynowicz et al. 2020). The northwestern region, bordering the heavily industrialized Silesia, and part of the Krakow area were characterized by the highest PI values calculated for Cd, Pb, and Zn. In addition, formations from the strip running centrally from the western border of the area to its eastern edges were classified as heavily Cd-contaminated soils. Strong contamination of soils with Pb was also noted in over a dozen small areas scattered throughout the voivodeship.

Table 2 Statistical results of the pollution indices used in the publication

Index	Mean	Median	Minimum	Maximum	SD*
Single pollution index (PI)					
Cd	2.23 ^{c**}	1.51	0.01	17.28	2.26
Cr	2.78 ^d	1.16	0.11	19.68	3.59
Cu	1.41 ^{ab}	1.21	0.08	7.36	0.94
Ni	1.15 ^a	0.95	0.18	9.34	0.78
Pb	2.20 ^c	1.65	0.05	26.78	2.30
Zn	1.71 ^b	1.48	0.09	14.57	1.39
Geoaccumulation index (I_{geo})					
Cd	−0.14 ^{bc}	0.01	−6.79	3.53	1.68
Cr	0.06 ^{cd}	−0.38	−3.77	3.71	1.45
Cu	−0.34 ^{ab}	−0.32	−4.24	2.29	0.86
Ni	−0.58 ^a	−0.66	−3.06	2.64	0.70
Pb	0.14 ^d	0.14	−4.81	4.16	1.08
Zn	−0.06 ^{cd}	−0.02	−4.12	3.28	0.82
Enrichment factor (EF)					
Cd	2.95 ^c	1.17	0.02	84.31	7.33
Cr	0.43 ^a	0.40	0.05	3.41	0.25
Cu	0.39 ^a	0.32	0.06	3.37	0.28
Ni	0.70 ^a	0.59	0.14	4.31	0.39
Pb	1.74 ^b	0.75	0.15	20.93	3.07
Zn	1.86 ^b	0.88	0.21	97.89	6.05
Pollution load index (PLI)	1.48	1.39	0.17	5.67	0.64
Improved Nemerow pollution index (PI_N)	1.29	1.07	0.16	7.55	0.92
Potential ecological risk (RI)	98.0	78.9	7.46	615.7	74.73

*SD, standard deviation (%). **Means followed by the different letters in line indicate significant differences at $p \leq 0.05$ according to the t Tukey test

Fig. 2 Enrichment factor (EF) values calculated on the basis of Cd, Cr, Cu, Ni, Pb, and Zn and local background. Classes of EF: ≤ 1 no enrichment; $> 1-3$ minor enrichment; $> 3-5$ moderate enrichment;

$> 5-10$ moderately severe enrichment; $> 10-25$ severe enrichment; $> 25-50$ very severe enrichment; and > 50 extremely severe enrichment

Fig. 3 Geoaccumulation index (I_{geo}) values calculated on the basis of Cd, Cr, Cu, Ni, Pb, and Zn and local background. Classes of I_{geo} : <0 unpolluted; 0–1 unpolluted to moderately polluted; >1–2 moder-

ately polluted; >2–3 moderately to highly polluted; >3–4 highly polluted; >4–5 highly to extremely polluted; and >5 extremely polluted

Fig. 4 Single pollution index (PI) values calculated on the basis of Cd, Cr, Cu, Ni, Pb, and Zn and local background. Classes of PI: <1 unpolluted, low level of pollution; $1-3$ moderately polluted; and ≥ 3 strongly polluted

Fig. 5 Pollution load index (PLI), improved Nemerow pollution index (PI_N), and potential ecological risk (RI) values calculated on the basis of Cd, Cr, Cu, Ni, Pb, and Zn and local background and share of indi-

vidual classes of complex pollution indices (PLI, PI_N , RI) in different groups depending on the type of land use

The assessment of the degree of contamination of soils with heavy metals on the basis of the geoaccumulation index (I_{geo}) revealed that, similar to the evaluation using the PI index, the studied soils were most contaminated with Cd, Cr, and Pb. The shares of samples assigned to grade 3 (moderately to heavily polluted) or higher were Cd 5.0%, Cr 17%,

and Pb 4%, respectively, while for other metals, the share was below 1%. The I_{geo} values calculated for these elements were also characterized by the highest variability. The index values calculated for different elements were compared, and the highest mean value was found for Pb, differing significantly from the mean I_{geo} values determined for Cd, Cu, and

Ni (Table 2). Due to the high similarity of the geoaccumulation index and single pollution index calculation formulas, the spatial distribution of both indices is very similar (Figs. 2 and 3). However, given the fact that I_{geo} was divided into a larger number of contamination classes, the graphics presenting the distribution of the index values show somewhat more subtle data; they also allow to assess that the soils of the Małopolska region are contaminated to a much smaller degree than it was shown in the evaluation using the PI index. It is worth adding that the PI and I_{geo} values coincide with the aforementioned factor of anthropogenic enrichment (Af) of soils with chromium and lead.

Analysis of the results of enrichment factor (EF) calculations — the last of the indices designed to assess single metal contamination of soils — showed that only less than 15% of the investigated soils were enriched with Cd at a moderate or higher level ($EF > 3$); and for Pb and Zn, it was less than 10%. Only one soil enriched moderately with Cr, Cu, or Ni was recorded for each metal. Among all studied trace elements, the indices calculated for Cd showed the significantly highest mean value of EF (2.95) and variability ($SD = 7.33$). The mean EF values calculated for Pb and Zn were at a very similar level and significantly higher than the mean values obtained for Cr, Cu, and Ni (Table 1). On the basis of developed maps (Fig. 4), it was established that soils proven to be significantly enriched in Cd, Pb, and Zn were located mainly in the northwestern part of the studied region and within the Kraków area. It is worth mentioning that the assessment of soil contamination based on EF did not show any contamination of soils with Cr, as was the case for the indicators discussed earlier.

The next three geochemical indices belong to the group of complex indices, taking into account the content of many trace elements. Their common feature was that the pollution indices (PI) of single elements were applied in each case for their calculation. However, the use of different calculation formulas (Table S1) gave different assessment results. The characteristic feature of the pollution load index (PLI) is that it indicates mainly how many times the natural content of the studied metals was exceeded. The Nemerow pollution index (PIN) value is strongly dependent on the most polluting element in the soil. The potential ecological risk (RI), on the other hand, additionally takes into account the toxicity of individual metals. The results of the chemical degradation assessment of soils in Małopolska based on the aforementioned indices were different (Table 2, Fig. 5). The estimation based on PLI showed that soil contamination in Małopolska is a serious problem as only 19% of the samples were classified as unpolluted. The parameter values were the least variable and ranged from 0.17 to 5.67. On the other hand, the evaluation of soils based on the PIN index showed that soils with some degree of contamination (grades 3–5) predominated in the study area, but their share was below

55%. Analysis of the RI results allowed us to conclude that this index was the most diverse among the three comprehensive indices. The assessment of the degree of degradation, especially when compared to the evaluation based on the PLI index, was much more favorable, as soils classified as of low ecological risk constituted 85.3% of all the examined samples, while those most dangerous (of very high ecological risk) are only 0.3%. Considering the results of multi-element indices, taking into account the soil use, it can be noticed that, as a rule, forest soils had the highest degree of degradation (Fig. 5). Figure 5, which presents the spatial distribution of index values, confirms the earlier findings obtained during the analysis of indices for individual elements, i.e., that the soils most degraded by heavy metals are located in the northwestern and central parts of the voivodeship.

The applied principal component analysis (PCA) made it possible, firstly, to compare the indices with each other; secondly, it indicated the differences and similarities between the indices; and allowed to determine 3 groups of elements (Table 3). PCA (PC1, PC2, PC4) confirmed the aforementioned similarity between the two indices, i.e., I_{geo} and PI for the analyzed elements. These indices are calculated on the basis of geochemical background and have been quite widely used for many years

Table 3 Principal component analysis (PCA) for all indices

Indices	PC1	PC2	PC3	PC4
Ig_Zn	0.159	0.256	0.259	0.722
Ig_Cu	0.319	0.784	−0.041	−0.016
Ig_Ni	0.058	0.587	0.485	−0.406
Ig_Cd	0.839	−0.003	−0.098	−0.030
Ig_Pb	0.509	0.413	0.124	0.234
Ig_Cr	−0.260	0.779	−0.138	0.282
PI_Zn	0.188	0.113	0.281	0.720
PI_Cu	0.299	0.755	0.037	−0.042
PI_Ni	0.184	0.533	0.492	−0.463
PI_Cd	0.923	−0.050	0.170	0.025
PI_Pb	0.538	0.324	0.306	0.084
PI_Cr	−0.315	0.720	−0.119	0.348
EF_Zn	0.064	−0.050	0.627	0.156
EF_Cu	0.227	0.068	0.615	0.102
EF_Ni	−0.060	0.052	0.686	−0.171
EF_Cd	0.500	−0.035	0.632	0.195
EF_Pb	0.464	−0.016	0.614	0.242
EF_Cr	0.033	0.004	0.395	0.157
PLI	0.540	0.750	0.221	0.227
PI _N	0.860	0.228	0.196	0.162
RI	0.921	0.151	0.223	0.055
Total variance (%)	33.539	14.959	9.437	8.432
Cumulative (%)	33.539	48.498	57.935	66.367

Loadings > 0.5 are shown in bold; $n = 320$

in assessing the degree of soil contamination with trace elements. The disadvantage of both indices is that they do not take into account the influence of heavy metals on the biological properties of soils and do not distinguish between the sources (natural, anthropogenic) of trace elements (Kowalska et al. 2018). The PC analysis also confirmed the differences between the I_{geo} , PI (PC1, PC2, PC4), and EF (PC3) indices mentioned previously. EF, although based similarly to I_{geo} and PI on the geochemical background, is a standardized index and distinguishes between natural and anthropogenic sources of trace elements; to calculate it, it is necessary to know, apart from the geochemical background of the element under study, the reference value for the element which is characterized by a low frequency of variation (Kowalska et al. 2018). It is noteworthy that PCA showed three groups of elements. PC1 represents the group of elements Pb and Cd, with which soils were polluted to the highest degree; all the 3 single-element indices revealed contamination with these elements, and this contamination was visible (dispersed) throughout the whole voivodeship, obviously with high intensity in the northwestern part of the Małopolska region. PC2 represents the group of elements Ni, Cu, and Cr, for which the calculated indices showed their usually natural level and low degree of contamination, and their sources are rather connected with natural processes. The locally elevated Cr content in soils in the latitudinal strip in the central part of the study area is of some concern (Figs. 3 and 4). PC4 represents Zn, which occupied an intermediate position as regards the degree of soil contamination in the studied area. The PCA analysis also revealed similarities among the multi-element indices. A significantly positive correlation was demonstrated between the typical geochemical parameters PLI and PI_N and ecological parameter ER (Table 3). Furthermore, PC1 explained 33.359% of the total variance, and the main components of the first factor were I_{geo} Cd, I_{geo} Pb, PI Cd, and PI Pb and PLI, PI_N , and ER (positive charges > 0.50) (Table 3). This suggests that Cd and Pb have the greatest impact on the degree of soil contamination and pose the greatest potential threat to soil organisms.

3.4 Ecotoxicity assessment

Table 4 shows the results of the Phytotoxkit and Ostracod-toxkit assays. Quite high values of coefficients of variation ($CV = 70\text{--}285\%$) were obtained in the assays, which indicates a large variation in the response of organisms to chemicals contained in the tested soil samples. The very high values of the coefficient of variation for *Heterocypris incongruens* mortality result from the fact that 245 soil samples showed zero mortality of ostracods, and 23 samples showed 100% mortality. The assays showed a similar response of the test plants; however, slightly greater inhibition of germination and root growth was shown for *Lepidium sativum* than for *Sorghum saccharatum*. The percentage inhibition of seed germination of *Lepidium sativum* ranged from 0 to 100%, and of *Sorghum saccharatum* from 0 to 87%, with a mean value of 15% and 14%, respectively. The percentage inhibition of root growth of *L. sativum* was 27% (–40 to 100%) and of *S. saccharatum* 21% (–54 to 95%). The mortality of *H. incongruens* was between 0 and 100% with a mean value of 9%, and the percentage inhibition of its growth ranged from –64 to 100% with a mean of 33% (Table 4). The mean values for the tested parameters indicated the lack or low toxicity of the soils to the test organisms. Most of the soil samples tested were non-toxic to the test plants. On average, they constituted for *L. sativum* 42% (root growth inhibition) and 70% (germination inhibition) and for *S. saccharatum* 50% and 72% of all tested soil samples, respectively. Low toxicity ($20\% \leq PE < 50\%$) was shown for *L. sativum* in 38% (for root growth inhibition) and 26% of samples (for germination inhibition) and for *S. saccharatum* in 28% and 20% of tested samples, respectively (Fig. 6). Toxic and very toxic samples accounted for a total of 19% (root growth inhibition) and 4% (germination inhibition) for *L. sativum* test reactions, and 22% and 8% for *S. saccharatum*, respectively. For the mortality parameter of *H. incongruens*, non-toxic samples represented 91% of all soil samples collected, low-toxic samples 2%, and very highly toxic samples 7%. For the second parameter, i.e., growth inhibition of *H. incongruens*, low-toxic samples constituted 38%, non-toxic 35%, toxic 17%, and very toxic 11% of all samples tested (Fig. 6). In general,

Table 4 Biological properties of soil samples

Parameter	Unit	Mean	Median	Minimum	Maximum	CV
<i>L. sativum</i>	GI%	15 ^a	10	0	100	96
	RGI %	27 ^b	25	–40	100	72
<i>S. saccharatum</i>	GI%	14 ^a	10	0	87	15
	RGI %	21 ^b	17	–54	95	76
<i>H. incongruens</i>	M%	9 ^a	0	0	100	285
	LI%	33 ^c	32	–64	100	70

GI, germination inhibition; RGI, roots growth inhibition; M, mortality; LI, length (growth) inhibition, minimum and maximum value; CV, coefficient of variation

CV – coefficient of variation GI – germination inhibition, RGI – roots growth inhibition, M – mortality, LI – length (growth) inhibition, minimum and maximum value

Fig. 6 Percentage toxic effect (PE) of the soils: **a** inhibition of *Lepidium sativum* root growth, **b** inhibition of *Sorghum saccharatum* root growth, and **c** inhibition of *Heterocypris incongruens* growth.

Effect: no toxic (PE < 20%), low toxic ($20\% \leq \text{PE} < 50\%$), toxic ($50\% \leq \text{PE} < 100\%$), and very toxic (PE = 100%)

the highest number of samples toxic and very toxic to the test organisms was determined for soils collected from forest land, and in total, they accounted for 11% (germination inhibition) and 34% (root growth inhibition) for *L. sativum*, 11% (germination inhibition), and 20% (root growth inhibition) for *S. saccharatum* and 26% (mortality) and 57% (growth inhibition) for *H. incongruens* of all samples collected on this land. Our previous study also showed higher ecotoxicity of forest soils, and the inhibition of *A. fischeri* luminescence in these soils averaged 43% and was nearly 2 times higher than in other soils collected from arable land and grasslands (Baran and Wieczorek 2015). Germination and root growth are two critical stages in the development of plants particularly sensitive to environmental contamination; however, in this study, greater toxicity was shown for *H. incongruens* (LI% growth inhibition) compared to the response of test

plants. Our results are consistent with those of other authors (Vasícková et al. 2016; Urbaniak et al. 2020; Szara et al. 2020), in which invertebrates were shown to be more sensitive to the presence of trace elements in soil-sediment or sediment-waste mixtures than plants. In the study by Szopka et al. (2021), crustacean-based bioassays (*Thamnocephalus platyurus*, *H. incongruens*) were more sensitive to the presence of high arsenic contents in soils than the Microtox microbial test. The higher sensitivity of *H. incongruens* was probably due to direct contact of the medium with the test organism, as the main route of exposure of *H. incongruens* is the oral route (Oleszczuk 2008; Cooman et al. 2015; Szara et al. 2020). On the other hand, the reason for reduced plant sensitivity in the Phytotoxkit assay may be the separation of plant roots from the test soil with filter paper, which is performed according to the test procedure (Van der Vliet et al.

2012). In addition, plants are generally less sensitive to trace elements in soils than invertebrates. Most of the taken-up trace element ions are retained in the plant root system by structural elements of the cell wall. Efficient detoxification of metals in the root cells and their limited translocation through the xylem to the aboveground part result in a high accumulation of metals in the root and a low accumulation in the aboveground part (Wierzbicka et al. 2007; Baran 2013). Also, some studies have shown that germination is relatively little affected by the presence of contaminants in the soil (Baran and Antonkiewicz 2017). Many plants germinate in a contaminated environment but later stop growing; therefore, the root growth parameter is more sensitive and useful in assessing the ecotoxicity of different samples (An 2004; Ali et al. 2004; Gomes et al. 2012). Roots are the first part of the plant exposed to toxic substances that inhibit root growth, which is associated with a decrease in cell mitotic activity (Fusconi 2006). The present study also showed statistically significantly greater root growth inhibition of the test plants compared to germination inhibition (Table 4). Many studies have also shown that monocotyledonous plants are less sensitive than dicotyledonous plants to contaminants present in the media (Wierzbicka et al. 2015; Szara et al. 2020). The study did not show statistically significant differences between the responses of the test plants. However, when looking at the spatial distribution of soil phytotoxicity measured by root length inhibition, differences between the responses of the test plants can be observed (Fig. 6). For *S. saccharatum*, more toxic samples were found in the north-western part of the study area, while for *L. sativum*, more toxic samples were found in the central and eastern parts of the voivodeship. On the other hand, studies by other authors on soils contaminated with heavy metals revealed greater sensitivity of monocotyledonous *S. saccharatum* compared to the dicotyledonous *Salix alba* (Badarna et al. 2015; Cuske et al. 2017).

3.5 Correlation and PCA analysis

Correlation analysis and principal component analysis (PCA) allowed us to find interesting relationships between the studied parameters (Table 2 and Table 5). The correlation relationship between individual heavy metals in the soil may result from their geochemical connections and inform about their similar sources (Guo et al. 2012). In the investigated soils, significantly positive correlations were found between the following metal pairs: Zn and Cd ($r=0.376$, $p \leq 0.05$); Zn and Pb ($r=0.326$, $p \leq 0.05$); Zn and Cu ($r=0.582$, $p \leq 0.05$); Cu and Cr ($r=0.425$, $p \leq 0.05$); Cu and Ni ($r=0.252$, $p \leq 0.05$); Cu and Pb ($r=0.125$, $p \leq 0.05$); Cu and Cd ($r=0.250$, $p \leq 0.05$); Ni and Cr ($r=0.541$, $p \leq 0.05$); and Cd and Pb ($r=0.788$, $p \leq 0.05$). The study also revealed significantly

Table 5 Principal component analysis (PCA) for all parameters

Parameters	PC1	PC2	PC3	PC4
<i>L. sativum</i> GI	−0.104	−0.016	0.554	−0.173
<i>L. sativum</i> RGI	−0.146	0.099	0.540	−0.186
<i>S. saccharatum</i> GI	−0.326	0.203	0.019	0.039
<i>S. saccharatum</i> RGI	−0.245	0.194	0.037	−0.001
<i>H. incongruens</i> M	−0.099	−0.011	0.734	0.007
<i>H. incongruens</i> LI	−0.089	−0.100	0.583	0.133
Zn	0.110	0.565	0.008	0.239
Cu	0.599	0.477	−0.020	0.114
Ni	0.618	0.048	−0.002	0.061
Cd	−0.114	0.675	−0.024	0.405
Pb	−0.143	0.584	0.222	0.424
Cr	0.832	0.068	0.040	−0.051
Fe	0.827	0.105	−0.042	−0.212
Sand	−0.395	0.029	−0.005	0.824
Silt	−0.062	−0.054	0.083	−0.819
Clay	0.678	0.008	−0.074	−0.472
pH	−0.151	0.449	−0.629	−0.182
TOC	0.176	0.312	0.633	0.151
Hh	0.193	−0.089	0.777	−0.009
S	0.028	0.780	−0.247	−0.233
CEC	0.091	0.766	−0.005	−0.240
Total variance (%)	17.193	15.289	13.746	7.873
Cumulative (%)	17.193	32.482	46.227	54.100

Loadings > 0.5 are shown in bold; $n = 320$

positive correlations between Zn ($r=0.561$, $p \leq 0.05$), Cu ($r=0.271$, $p \leq 0.05$), Ni ($r=0.298$, $p \leq 0.05$), and Pb ($r=0.337$, $p \leq 0.05$) in the 0–10- and 40–50-cm layers. For Cd ($r=0.064$, $p \leq 0.05$) and Cr ($r=0.101$, $p \leq 0.05$), the values of correlation coefficients were also positive but not statistically significant. The significantly positive correlations between the element contents indicate that their partly natural and anthropogenic sources and routes of distribution to soils are similar. When analyzing the interrelationships between the content of trace elements, iron was treated in a separate way because the correlation between the content of iron and the content of another trace element may make it possible to distinguish soils with a natural element content from those enriched as a result of human activity. The lack of significant correlations between iron and other heavy metal contents may indicate their anthropogenic origin (Baran and Wiczorek 2015; Gruszecka-Kosowska et al. 2020). In the studied soils, a significantly positive correlation was found between iron and Cu ($r=0.454$, $p \leq 0.05$) and Ni ($r=0.378$, $p \leq 0.05$) and Cr ($r=0.708$, $p \leq 0.05$) contents, which rather confirms their natural origin. However, due to the intensive anthropopressure of the study area and the large natural background of metals (especially for Zn, Cd, and Pb), it can be assumed

that the relationships between the analyzed elements in the studied soils are disturbed and may give unreliable information about their sources.

In the studied soils, a significantly positive correlation was found between the TOC content and sorption capacity (CEC) and the content of most analyzed heavy metals (Zn, Cu, Cd, Pb, Cr). A significantly positive correlation was also determined between pH and the content of Zn, Cu, and Cd. The granulometric composition of soils also had a significant and differentiated effect on the content of the analyzed metals. The sand fraction correlated significantly negatively with Cu, Ni, and Cr and positively with Cd and Pb, while opposite relationships were found for the clay fraction (Table 2). The silt fraction was significantly negatively correlated with Cd and Pb contents in soils. Significant correlations were also discovered between the Cd and Pb contents and the response of *S. saccharatum* and between the Pb content and toxicity of soils to *H. incongruens* (Table 2). Significantly negative correlations were found for Ni and germination inhibition of *L. sativum*, for Cu and germination inhibition of *S. saccharatum*, and growth inhibition of *H. incongruens* (Table 2). Significantly positive values of correlation coefficients indicate a relationship between the content of a given trace element in the soil and its toxicity to organisms, while negative values mean that the element did not increase the ecotoxicity of the sample. Our previous study on soils in the area directly affected by zinc and lead ore mining and smelting showed significantly positive correlations between metal (Cd, Zn, Pb) content and *A. fischeri* luminescence inhibition and *S. saccharatum* root growth inhibition (Baran et al. 2014). Garcia-Lorenzo et al. (2014) also demonstrated a positive correlation between total trace elements (As, Cu) and the response of *L. sativum* and *S. alba*; however, *S. saccharatum* was not sensitive to the presence of metals in the tested sediment. Another publication by Garcia-Lorenzo et al. (2009) also showed a positive correlation between heavy metal content in soils from mining impact areas and *A. fischeri* luminescence inhibition and the inhibition of germination and root growth of test plants.

It is worth noting that, due to the high variability of the studied parameters, the calculated correlation coefficient values in the present study indicated low or very low correlations ($0 < r < 0.3$, very low correlation; $0.3 \leq r < 0.5$ low correlation) between the investigated parameters.

The principal component analysis (PCA) confirmed the aforementioned relationships and also allowed indicating other interesting relationships between the content of trace elements in soils, physicochemical properties of soils, and ecotoxicity (Table 5). PCA identified four main factors explaining 54.110% of the total variance of the data set. PC1 explained 17.193% of the total variance, and the main components of the first factor were clay fraction and Cu, Ni, Cr, and Fe contents (positive loads > 0.50). The second

factor (PC2) accounted for 15.289% of the total variance and was dominated by Zn, Pb, and Cd contents and the sorption capacity of soils. The third factor (PC3) explained 13.746% of the total variance and was related to soil toxicity to *L. sativum* and *H. incongruens* and pH, Hh, and TOC content. The fourth factor (PC4) explained only 7.873% of the total variance and was related to sand and silt fractions (Table 5). In the study, the determined principal components represent both natural (PC1, PC2, PC3, PC4) and anthropogenic (PC2, PC3) factors determining the soil element content and ecotoxicity. The PC results confirmed the presence of significantly positive correlations between the elements. The PC analysis also allowed to distinguish two groups of trace elements, which is related to their similar sources and distribution routes. PC1 represents the group of Ni, Cu, and Cr (whose content in the studied soils was generally low) at the level not posing any threat to the soil ecosystem, which was also confirmed by the calculated geochemical and ecological indices. Positive correlations were also found (PC1) between contents of clay and Fe fractions and contents of Cu, Ni, and Cr, which may suggest their natural sources; and in the case of Cr, local and point sources. PC2 represents a group of Zn, Cd, and Pb, which are present in high concentrations in the studied area, which is connected with both natural (high geochemical background related to the presence of zinc and lead ores in the area) and anthropogenic (mining and processing of zinc and lead ores) factors. These elements, especially Cd and Pb, according to the calculated geochemical and ecological indices, were responsible for contamination of soils in the Małopolska region and posed a high ecological risk to the soil ecosystem. PC2 also confirmed the influence of the cationic sorption capacity on the content of trace elements, especially Cd, Pb, and Zn. The sorption capacity of soils is an important parameter, as it is crucial in limiting the migration and bioavailability of trace elements (Baran et al. 2014; Boguta and Sokołowska 2020). Our previous studies showed a high capacity of the investigated soils to bind Zn, Cd, and Pb (Baran et al. 2018; Wieczorek et al. 2018). It was also demonstrated that, despite the high content of mobile forms of elements extracted with $0.01 \text{ mol/dm}^3 \text{ CaCl}_2$, the solubility (mobility) of metals from soil samples collected in the northwestern Małopolska (zinc and lead ore mining region) was generally low or moderate. This was related to the fact that the ores occur in ore-bearing *Diplopora* dolostones, which are a source not only of lead and cadmium, but also of calcium and magnesium carbonate, which affect the binding of metals into stable carbonate minerals. PC3 revealed the effects of pH and TOC on soil ecotoxicity to the test organisms. Both parameters are important physicochemical factors determining the conditions for growth and development of organisms in soils and have a significant effect on the accumulation, mobility, and bioavailability of trace elements in soils (Son et al. 2007; García-Lorenzo et al. 2014;

Kim et al. 2015; Cuske et al. 2017). The study by Son et al. (2007) showed a reduction in cadmium toxicity to *Paronychurus kimi* (Lee) in soils with increased organic matter content and pH values. Also, the publication by García-Lorenzo et al. (2014) revealed a positive correlation between the pH value and the response of the test plants in the Phytotoxkit assay, suggesting that acidic pH increases the medium toxicity to plants. In our study, the effect of pH is also particularly interesting, as the analyzed soils were mostly acidic and very acidic. Under pH conditions below 5.5, acidic and very acidic soils increase the mobility and thus the bioavailability of trace elements, and there may be an excessive activity of aluminum ions toxic to organisms. In the tested soils, the pH may have increased the bioavailability and toxicity of Cd and Pb to organisms (Wieczorek et al. 2018). For these elements, a significantly positive correlation was found with the test organisms' response. In addition, many studies highlight the high sensitivity of *H. incongruens* to the acidic pH of soils/bottom sediments (Riuz et al. 2013), which may also explain the higher toxicity of the tested soils to the crustacean in relation to the test plants. Furthermore, the positive correlation of soil ecotoxicity with organic matter content may suggest the effect of other substances accumulated in soils on the response of test organisms. Organic matter is the most important soil sorption factor and thus influences the bioavailability and toxicity of other pollutants (PAHs, pesticide residues, dioxins) not investigated in this study but affecting the toxicity of soils.

Tu sum up, the key to the effective assessment of soil contamination with trace elements is the combined use of geochemical, ecological, and ecotoxicological indices, which allows comprehensive monitoring of soil quality. In the present study, a total of 11 indices were used, including 6 geochemical indices, 1 index assessing potential ecological risk, and 4 indices assessing soil ecotoxicity. This approach made it possible not only to assess the content of trace elements, but also to evaluate their potential and actual risk to organisms.

4 Conclusion

1. With the use of the different indices, two groups of elements were distinguished. The first one included Ni, Cr, and Cu. The calculated geochemical indices generally indicated their natural content and low degree of soil contamination with them and that the elements' sources were connected with natural processes. The second group included Cd, Pb, and Zn. These elements occur in high concentrations in the studied area, which is influenced by both natural (high geochemical background related to the presence of zinc and lead ores in this area) and anthropogenic (mining and processing of zinc and

lead ores) factors. However, contamination with these elements is heterogeneous in the Małopolska region and generally observed in its northwestern part.

2. PCA showed that Cd and Pb had the greatest effect on the degree of soil contamination and pose the greatest potential threat to the soil environment. This was confirmed by the observed significant correlation between the Cd and Pb contents and the response of *S. saccharatum*, as well as between the Pb content and soil toxicity to *H. incongruens*. *H. incongruens* (length inhibition) proved to be a more sensitive indicator of soil ecotoxicity compared to plant tests. It must be emphasized that most of the soils were not or at most low toxic to plants. In the case of *H. incongruens* growth inhibition, low-toxic and non-toxic samples represented 72%, toxic 17%, and very toxic 11% of the tested samples. The calculated potential ecological risk index (RI) also indicated that nearly 85% of the samples had low potential ecological risk.
3. The study demonstrated the importance of other soil properties on the content of trace elements in soils and their ecotoxicity. A large role in the ecotoxicity of soils in relation to the test organisms was shown for the pH, and it is worth noting that acidic and very acidic soils predominated in the study area.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s11368-021-03091-x>.

Acknowledgements The studies were financed by grant no. N N305 107640 provided by the National Science Centre, Poland, and from the subsidy for science granted by the Polish Ministry of Education and Science.

Author contribution Jerzy Wieczorek: investigation, resources, conceptualization, writing — original draft, and visualization. Agnieszka Baran: investigation, conceptualization, methodology, formal analysis, writing — review and editing, and funding acquisition.

Funding The studies were financed by grant no. N N305 107640 provided by the National Science Centre, Poland, and from the subsidy for science granted by the Polish Ministry of Education and Science.

Availability of data and materials Additional data is available on request.

Declarations

Ethics approval Not applicable.

Consent to participate Informed consent was obtained from all individual participants included in the study.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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