Assessment of heavy metals contamination in soils in the Beskidy mountain forests and their potential impact on soil enzyme activities and microbial community

Jacek Borgulat (jborgulat@gmail.com)  
Institute for Ecology of Industrial Areas  https://orcid.org/0000-0002-1614-4872

Anna Borgulat  
Central Mining Institute: Glowny Instytut Gornictwa

Aleksandra Nadgórska-Socha  
Uniwersytet Śląski w Katowicach, Instytut Biologii, Biotechnologii i Ochrony Środowiska

Marta Kandziora-Ciupa  
Uniwersytet Śląski w Katowicach, Instytut Biologii, Biotechnologii i Ochrony Środowiska

Research Article

Keywords: heavy metals, CLPP, soil enzymes, soil respiration

DOI: https://doi.org/10.21203/rs.3.rs-355931/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
Abstract

The research was carried out in the Beskid Śląski and Beskid Żywiecki mountains which were affected, among others, by air pollution from the Upper Silesian Industrial Region, the largest industrial zone in Poland. The aim of the study was to assess the heavy metal load in the soils of the studied area and to determine their potential impact on soil metabolism. The research was carried out on 6 permanent sites. For each site, the total content of zinc (Zn), lead (Pb) and cadmium (Cd) was determined for three soil levels (A, B and C). Moreover, the following were determined: total nitrogen, organic carbon, pH and soil moisture and the amount of heavy metals in soil solutions. The metabolic activity of the soil was assessed by measuring: soil enzymes activity, soil respiration and by studying community-level physiological profiling (CLPP) using Biolog ECO-plates. In the case of Pb and Cd their increased content in the topsoil was found, which indicates their anthropogenic origin. Statistical analysis showed that in the case of very acidic forest soil even slightly elevated lead level probably can affect the functional biodiversity of soil microorganisms. The study showed that it is not easy to assess the impact of heavy metals on soil metabolism. Some indicators such as the activity of soil enzymes used individually may not be sufficient to illustrate the changes occurring in the soil environment.

Introduction

Over the years, the Beskidy region was subjected to strong anthropopressure from the surrounding industrial areas (Uzięblo et al. 2012, Klos et al. 2018). It was found that the pollution came mainly from the area of Ostrava and Upper Silesia. This thesis is confirmed by the decrease in the amount of pollutants reaching forest ecosystems, including heavy metals and acidic compounds, noticeable since 2000s, when many industrial plants began to closed down on the mentioned areas (Staszewski & Kubiesa 2008, Staszewski et al. 2008). However, there are only a few studies that focus on the impact of the deposition of air pollutants on the condition of the soil environment in the Beskidy region. Research in this field was also carried out Bierza et al. (2013). Forest ecosystems represent significant global carbon sinks. Consequently, thorough understanding the role and function of soil microorganism in forest ecosystem, and the impact of pollutants on their activity, is essential for predicting and managing C-cycling processes (Žifčáková et al. 2016). It should be remembered that the soil microorganisms is the most important factor conditioning the availability of nutrients for plants, enabling them to grow and develop.

Knowledge about the influence of heavy metals on soil microorganisms is still insufficient. It is known that these elements, if background level is exceeded, can disturb the homeostasis in the soil environment, limiting biodiversity and the number of microorganisms. The harmful effect of metals largely depends on their mobility in the environment and bioavailability, which is influenced by many factors such as environmental acidity, organic matter content and soil granulometric composition (Kaczynska et al. 2014). But it is still difficult to say what concentrations of heavy metals in the soil will have negative effects on microbiota.
The use of biological and biochemical parameters to assess the ecological status of environmental samples provides accurate information (Gryta et al. 2014). One of the commonly used methods in studying the functional diversity of microorganisms is the Biolog EcoPlate technique. This method was used to assess stressing impact on soil environment like pH and salinity (Pankhurst et al. 2001) and also the toxicological impacts of pollutants on it such as hydrocarbons (Nagy et al. 2013) and heavy metals (Boshoff et al. 2014, Feigl et al. 2017). Other monitoring research (Huang et al. 2017) showed that the effect of soil additives on community-level physiological profiles (CLPP) were correlated with the sequencing results like 16S rRNA and ITS rRNA. The research of soil enzymatic activity was the source of information about the condition of soil environment (Telesiński et al. 2019) and also the changes occurring in it (Trasar-Cepeda et al. 2008).

The aim of the study was to determine the content of heavy metals in the soil environment in the Beskid Śląski and Beskid Żywiecki mountains and to assess their impact on the activity of soil microorganisms by using various methods.

**Materials And Methods**

**Site description, sampling, transport and storage of samples**

Samples were collected from 6 sites in the Beskid Śląski and Beskid Żywiecki mountains: SG - Stary Groń, MS - Małe Skrzyczne, KB - Kubalonka, CL - Czarny Las, OR-R - “Oszast” nature reserve, SR-R - “Śrubita” nature reserve (Fig. 1). All sites were located in mixed spruce–fir–beech mountain forests at an altitude of between 600 and 800 meters above sea level. Samples from 2 localisations (SG and MS) were taken near the permanent plots of the Institute of Ecology of Industrial Areas – in Brenna and Salmopol, where other authors (Rrolicka-Kieliszewska et al. 2002) confirmed the concentrations of heavy metals in the soil.

Each site was previously equipped with 5 pairs of vacuum ceramic cup lysimeters for sampling of soil solution. The cups were placed in the A and B soil layers. Soil solutions were collected at least twice a month in the period from April to November 2018. The soil samples were collected at the beginning of November 2018 (when the vegetation period was ended), from small soil pits located near each of ceramic cups of the lysimeters (in total 60 soil samples were taken). In order to assess the content of heavy metals in parent material (C layer), one deeper pit (> 80cm) was made at each of the six locations and the soil was collected for the analysis.

Until the tests were performed soil and water samples were stored in the dark, cold place (T = 4°C). All determinations were performed within one week of the samples' arrival in the laboratory.

**Determination of physicochemical parameters of soil and heavy metals concentrations in soil solutions**

In all the soil samples, the grain size distribution was determined by Casagrande’s hydrometer method modified by Prószyński (PN-ISO 11277: 2009). The following fractions were distinguished: sand (particle size 0.05 -2.0 mm), silt (particle size 0.002–0.05 mm), and clay (particle size below 0.002 mm). After
determining the share of granulometric fractions, individual soil types were determined in accordance with the United States Department of Agriculture (USDA) classification. Dry matter content in soil (soil moisture - SM) was established gravimetrically according to PN-ISO 11465: 1999. Soils pH were established by using glass electrode in a 1:5 (w/w) suspension of soil in water (pH in H₂O) and in in 1 mol L⁻¹ potassium chloride solution (pH in KCl) (PN-ISO 10390: 2005). The amount of organic carbon in soils was assessed by oxidation of organic matter with a mixture of potassium dichromate and sulfuric acid (PN-ISO 14235: 2003). Soil total N was determined by the Kjeldahl method (Bremner & Mulvaney 1982).

The total content of heavy metals (Zn, Pb and Cd) in soil was determined after ashing the soil in the furnace (450°C) and digesting the ash with aqua regia. Concentrations of the elements were established by inductively coupled plasma optical emission spectrometry (ICP-OES) technique using Thermo Scientific iCAP 6500 equipment. The quality assurance and quality control was performed by analysing the standard samples of the known composition. Cation concentrations (Zn_{ss}, Pb_{ss} and Cd_{ss}) in soil solutions have been determined directly in the samples by also using ICP-OES technique.

**Soil enzymes activity**

Soil enzyme activity was determined in moist soil. The activity of acid and alkaline phosphatase (AcP and AlP), dehydrogenase and urease (Ure) was measured in accordance with the methodology proposed by Schinner et al. (2012).

The activity of both phosphatases was examined by colorimetric method. This method is based on colorimetric estimation of p-nitrophenol (pNP) released when soil is incubated with buffered sodium p-nitrophenyl phosphate (pNPP) solution and toluene at 37°C for 1h. Activity of both enzymes was expressed in µmol pNP g⁻¹ d in soil h⁻¹. Absorbance was measured at a wavelength λ = 400nm. MUB buffer with pH 6.5 for acid phosphatase (AcP) and pH 11 for alkaline (ALP) was used to optimize the reaction. CaCl₂ (0.5 M) and NaOH (0.5 M) were used to brake reaction time.

Dehydrogenase activity (Deh) was determined by the method described by Casida et al. (1964). As a substrate the Triphenyltetrazolium chloride (TTC) was used. TTC was reduced to red-colored triphenylformazan (TPF). Dehydrogenase activity was expressed in µg TPF g⁻¹ d in soil 16 h⁻¹. Absorbance was measured at λ = 546 nm. Urease activity was determined colorimetrically based on determination of ammonium formation after the enzymatic urea hydrolysis at λ = 630 nm and expressed in µg N g⁻¹ d in soil h⁻¹.

**Soil microbial functional diversity - Biolog ECO-plates technique**

Biolog ECO-plates are 96-well plates, containing three replicate sets of 31 different substrates, which are ecologically relevant and structurally diverse compounds. The selected substrates are widely used to assess functional diversity of soil microbial communities (Preston-Mafham et al. 2002, Chojniak et al. 2015).
10 g of soil were shaken in 90 ml of distilled sterile water for 20 min at 25°C. Then 150 µL of each sample was inoculated into each well of Biolog ECO-plates and incubated at 26°C. The determinations have been performed by spectrophotometric measurements of absorbance used by sets of microorganisms of carbon substrates. The oxidation of carbon substrates was read using an automatic microplate reader, by measuring the intensity of the colour change as a result of dye reduction - tetrazolium violet caused by microorganisms (Garland and Millis 1991). 72 hours were the shortest incubation time in which the highest variation between the examined objects was found. The average absorbance - AWCD (Average Well Colour Development) for each soil was calculated based on the absorbance for 31 substrates, less the absorbance for pure water, according to the formula: $AWCD = \Sigma(n_i)/31$, where $n_i$ is the absorbance of each substrate (Hu et al. 2011). The calculated AWCD value was used as a measure of the activity of soil microorganisms. The richness index (Rs) was calculated based on the number of carbon substrates that were used by the microorganisms. The microbial functional diversity index ($H'$) was calculated on the basis of Shannon-Wiener diversity index. In the calculations a number of substrates and the utilisation of an individual substrate by microorganisms were taken into account (Derry et al. 1999, Hu et al. 2011, Klimek et al. 2016).

**Measurements of soil respiration**

After transporting to the laboratory, the soil was sieved (2 x 2 cm) in order to separate stones, invertebrates and plant roots. Then 200 grams of soil were transferred to glass soil chambers and kept at 10°C for 1 day. The chambers were open during the stabilization period. Soil respiration (CO$_2$ efflux) was determined by using infrared gas analyzer LCProplus (ADC Bioscientific, UK) (measurement time − 1 hour, air flow − 200µmol min$^{-1}$).

**Statistical analysis**

The analysis of variance (ANOVA) was used in order to assess differences in soil enzymes activity, soil respiration and indexes of functional diversity of soil bacteria between the sites. As a post hoc analysis the Tukey’s test was performed. The samples were found to differ significantly at the significant level of $p < 0.05$. Relationship between the selected parameters was assessed using the Pearson's correlation coefficient. The following significance levels are indicated in the tables: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. In order to illustrate the relationship between the physicochemical soil parameters and metabolic activity of soil microflora, the principal components analysis (PCA) was used. On the diagrams PC1 and PC2 components are shown, which present the largest variance. To perform the calculations we used a Dell Statistica (data analysis software system), version 13.

**Results And Discussion**

**Soil physicochemical properties and concentration of heavy metals in soil and soil solutions**

Over the years, many authors have found increased values of heavy metals in the Beskidy mountain forests. In the research on soil humus collected in Czech part of Beskidy Mountains bordering the Beskid Śląski and conducted in the early 2000s by Suchara and Sucharova (2002), it was found there was an
increased deposition of trace elements like Fe, Cu, Zn, Cd, As and Pb. The authors emphasized the fact that this area was affected by local sources, mainly steel works and as well as by air-borne pollutants transported long distances from domestic and foreign sources. High content of lead in the Beskid Śląski mountains was also observed by Rrokicka-Kieliszewska et al. (2002) at the beginning of 2000s. In the top layer of soil from MS site these authors recorded 361 mg Pb kg$^{-1}$ dw and near the SG 238 mg Pb kg$^{-1}$ dw were noticed. Now a day, despite the observed decreasing deposition of heavy metals into the environment, the biomonitoring studies performed on bryophytes still showed a greater deposition of trace elements in the Beskid Śląski and Żywiecki mountains compared to the Karkonosze or other forests ecosystems in southern Poland (Kłos et al. 2015, 2018).

Table 1 presents the physicochemical properties of soils and the content of heavy metals in soils A and B layers from the studied locations. On each sites, in both soil layers, the sand content was between 25 and 35%, the clay between 20 and 25% and silt between 75 and 80%. Therefore, all the analysed soil samples (except B layer on the KB) can be classified as silt loam in accordance with the USDA classification. The pH of the studied soils indicates their acidic (pH H$_2$O 4.5−5.5) and very acidic (pH H$_2$O < 4.5) characteristics and seems to be one of the most important factors determining the migration of heavy metals into the soil profile (Table 3).

In Staszewski et al. (2012) research conducted in 11 mountain National Parks in Poland, the average lead content in the topsoil (0−25 cm) was 0.53 ± 0.72 mg Pb kg$^{-1}$ dw. In our research lead content in soil A layer was diversified and ranged from 38.4 to 146.8 mg Pb kg$^{-1}$ dw. Our results indicates the elevated content of Pb in some regions of the Beskid mountains and shows that the problem of heavy metal burden on the environment exists. According to the standards introduced in September 2016 (Journal of Laws 2016, item 1395), the amount of lead in soil A layer didn't exceed the admissible values (500 mg Pb kg$^{-1}$ dw). However, according to the previously applicable Act (Journal of Laws 2002, item 1359) where permissible level for lead in soil was 100 mg Pb kg$^{-1}$ dw, the lead concentration in the soils, would be exceeded in SG and MS sites. Newerthanless these values are much lower than those obtained in forests localised near urban areas and around industrial plants (Pajak 2016, Borgulat 2017, Rusinowski et al. 2019). For example Pajak (2016) near the zinc smelter in forest areas recorded: 11.5 mg Cd kg$^{-1}$ dw, 709.1 mg Pb kg$^{-1}$ dw and 524.5 mg Zn kg$^{-1}$ dw.

Cadmium was present at the lowest concentration in soil A layer from the Oszast reserve (OR-R) (0.24 mg Cd kg$^{-1}$ dw) and the highest concentration in the Stary Gron (SG) (0.73 mg Cd kg$^{-1}$ dw). In Staszewski et al. (2012) studies concentration of cadmium in Babiogórski National Park in Beskid Żywiecki was 0.48 mg Cd kg$^{-1}$ dw and it was several times higher than in other national parks (0.03−0.13 mg Cd kg$^{-1}$ dw). The quoted and obtained results indicate slightly increased content of this element in soil of some regions of the Beskidy mountains. Both in the case of cadmium and lead (Figs. 3 and 4), it can be seen that the content of these elements in A and B layers was higher than in C – horizon, which indicates the anthropogenic origin of these elements in soil. The obtained results can also be explained by the higher content of organic carbon in the topsoil (Table 1). Zinc content in soil A layer ranges from 31.2 to 78.3
mg Zn kg\(^{-1}\) dw (Table 1). Usually higher values of this element were recorded in soil C layer (59.7–104.9 mg Zn kg\(^{-1}\) dw, Fig. 2). Łaszewska et al. (2007) indicated higher zinc content in the Beskidy area (174–208 mg Zn kg\(^{-1}\) dw). However, it seems that the values given above are typical for the geochemical background of the region.

However, more important than the amount of heavy metals in the soil is their content in dissolved form in soil solutions, because in this form these elements can move in the soil environment and negative affect on it. The content of the selected metals in soil solutions in A layer was in the range of: 10.9–24.7 µg Zn L\(^{-1}\), 6.8–15.8 µg Pb L\(^{-1}\) and 2.4–2.9 µg Cd L\(^{-1}\) and it was higher than in B layer (5.7–21.1 µg Zn L\(^{-1}\), 4.9–10.8 µg Pb L\(^{-1}\), 1.9–2.9 µg Cd L\(^{-1}\)) (Table 2). Nearly 20 years ago, in the same studied area, Staszewski et al. (2008) recorded lower amounts of metals in soil solutions (2.9 µg Pb L\(^{-1}\) and 0.15 µg Cd L\(^{-1}\) on SG and 10.9 µg Pb L\(^{-1}\) and 0.55 µg Cd L\(^{-1}\) on MS). Based on the analysis of the relationship between the content of heavy metals in soil solutions and their concentration in the soil, as well as pH and organic carbon content (Table 3), it was found that pH and organic carbon content largely determine the mobility of these elements in the soil environment. The influence of these factors on the mobility of metals was well documented by others authors (Ramakrishnaiah & Somashekar 2002, Kabala et al. 2014).

**Soil enzymes activity**

Soil enzymes activity (Deh, Ure, AIP) in soil A layer do not differ significantly between the sites (Table 4). In the case of acid phosphatase (AcP), its activity was significantly higher on CL site (1314.1µg pNP g\(^{-1}\) dm h\(^{-1}\)) in relation to other plots (504.5-751.2 µg pNP g\(^{-1}\) dm h\(^{-1}\)). In our research correlation analysis showed that the content of heavy metals in topsoil generally did not affect the obtained results (Table 4). Gucwa-Przepióra et al. (2016) noted that in the area affected by heavy metals contamination, the activity of soil enzymes was lower than in clean areas. In Borgulat’s research (2017) conducted in pine-spruce forest near the Miasteczko Śląskie zinc smelter, the AcP activity in soil A layer was in the range 1.4–13.9 µgp-NP g\(^{-1}\) dm h\(^{-1}\). On sites located in spruce forests subjected to emissions from Huta Katowice metallurgical plants and characterized by a lower degree of degradation, the activity of this enzyme was 5.6–30.6 µgp-NP g\(^{-1}\) dm h\(^{-1}\). In the last century, these two industrial plants were among the biggest emitters of air pollutants in Upper Silesia, and the heavy metals contamination of soil surface near these plants were at very high level. Comparing the quoted results with those obtained in the presented work for AcP (A layer: 504.5-1314.1 µgp-NP g\(^{-1}\) dm h\(^{-1}\) and AIP (A layer: 136.0-415.5 µgp-NP g\(^{-1}\) dm h\(^{-1}\)), one can say that heavy metals probably had no influence on activity of AcP and AIP in soil environment in the Beskidy mountains. However, in the present study it was found that AcP activity in topsoil was positively correlated with soil pH (Table 5). The optimum pH of the activity of acid phosphomonoesterases to be within a range of 4.0 to 6.5 (Rejsek et al. 2012). Herbien and Neal (1990) were found that the optimal pH for phosphatase activity in forest soil was 4.9. At the SG and MS sites, a very acidic soil was found (pH < 4.0), which could have had a negative effect on the AcP activity. The pH close to optimal was found at the CL site where the highest activity of this enzyme was found. Negative correlation was found between urease activity and the amount of lead in the soil. Urease it's an extracellular enzyme implied in the
nitrogen cycle in the forest soil environment to catalyse the transformation of urea into ammonium ion. The negative effect of lead on the activity of soil enzymes, including urease, in forest soils was also found by Pająk et al. (2016) near mining and metallurgical works in Bukowno. However, in the cited studies its content in the soil environment was at a higher level (mean 303.0 mg kg\(^{-1}\) dw, range 14.5–1202.8 mg kg\(^{-1}\) dw). In layer B of soils, lower activity of soil enzymes was found (Table 4) and in the case of dehydrogenase (Deh) its activity statistically differed between the research plots. The determination of dehydrogenase activity (Deh) in soil is an indicator of the intensity of the respiratory metabolism of microorganisms such as soil bacteria and actinomycetes (Januszek et al. 2015). Mocek-Płóciniak (2006) indicates that the dehydrogenases and phosphatase are good biomarkers of soil contamination with zinc and lead. But it should also be mentioned that the inactivation of microbiological processes in the soil occurs at a zinc content of 1000 mg kg\(^{-1}\) dw (Kabata-Pendias & Pendias 1999). In this work, this value did not exceed 100 mg kg\(^{-1}\) dw. It seems that the factor which had the greatest impact on the diversification of the activity of this enzyme in soil B layer was pH and the content of organic carbon in the soil (Table 5).

**Functional diversity of soil bacteria and soil respiration**

The negative influence of heavy metals, including lead, on the functional diversity of soil microorganisms assessed by the Biolog ECO-plate technique was reported by many researchers (Roane & Kellogg 1996, Teng et al. 2008, Xie et al. 2016, Kuźniar et al. 2018). This method was used due to the repeatability of the results and the availability of literature data. In this research it was noted that microbes inhabiting the topsoil environment on sites SG and MS, which had a relatively high lead content in the topsoil (A layer), were characterized by statistically significant lower microbial functional diversity index (H’) and low metabolic activity (AWCD) (Table 4). The AWCD index provides information on the whole metabolic activity of microorganisms in the soil environment (Gomez et al. 2004) and reflects metabolic profiles of the soil microbial community, which could be affected by such pollutants as heavy metals (Teng et al. 2008, Fazekašová & Fazekaš 2020). In this research, a large variation of this parameter was found for the A layer between the analysed research plots (30.0-133.0 OD). Both AWCD and H’ correlated negatively with the presence of lead in soils and also with other soil characteristics like pH and content of organic carbon (Table 5). The acidic pH is considered to be one of the most important factors affecting the absorption of heavy metals in soil environment (Zwolak et al. 2019). Our results also show that low soil pH influenced the mobility of heavy metals (Table 3). The higher content of heavy metals in soil solutions could also affect the obtained results. Literature data indicates the influence of the presence of heavy metals on growth, morphology and microbial metabolism, which leads to the decrease in the functional diversity of ecosystems (Hassan et al. 2013). The harmful effect of the presence of heavy metals (mainly lead and zinc) on the metabolism of microorganisms found in soil was demonstrated in the research of Niklińska et al. (2006). However, the authors stated that the low bioavailability of heavy metals may have influenced their results. In the present study high mobility of the heavy metals in soil was found so their potential impact on soil metabolism may indeed have occurred. Other researchers (Kelly and Tate 1998) founded that zinc ions might react with phosphate buffer to form a precipitate, which may result in false positives in the Biolog ECO-plate test, which this study does not seem to confirm.
Other researchers (Chodak et al. 2013) claimed that the use of soil respiration in the studies of the effects of heavy metals on forest soils might be difficult due to confounding influences of other environmental factors. Our results confirm these observations. It should also be emphasized that the content of heavy metals in the tested soil was relatively low, which additionally made it difficult to identify significant differences. In our research no significant relationships were found between the content of heavy metals in topsoil and the rate of soil respiration. The organic carbon (Corg) is a major source of energy for soil microorganisms so the positive relationship between soil respiration (Sres) and its content should not be surprising (Table 5). PCA (Figs. 5 and 6) and the correlation analysis showed that the pH, carbon source, soil moisture and amount of nitrogen were the most essential factors which had influence on soil respiration. Higher intensity of soil respiration was found for A layer (3.3-7.9mg CO$_2$-C kg$^{-1}$ soil h$^{-1}$) than for layer B (0.5-4.0 mg CO$_2$-C kg$^{-1}$ soil h$^{-1}$) which was also influenced by the higher content of organic matter in the topsoil.

**Conclusions**

It seems that Biolog ECO-plates technique is the more sensitive method than soil enzyme analysis and it is more adequate for the assessment of soil environment pollution with heavy metals. Heavy metals may, to some extent, affect soil metabolism of forest ecosystems in the Beskidy, however, acidification is more likely. The above results of the research carried out on the basis of various research methods indicate the need for a comprehensive study of the soil environment and the diversity associated with it. Some commonly used indicators, such as the activity of soil enzymes, may not be sufficient to illustrate the changes occurring in the soil environment, which is not heavily contaminated. According to the Polish standards that were implemented in September 2016 (Journal of Laws 2016, item 1395) lead concentrations in soils do not exceed the admissible values (500 mg·kg$^{-1}$ dw). However, according to the previous Act (Journal of Laws 2002, item 1359) its concentration in the soils would be exceeded in two out of six sites. This information seems to be interesting because the statistical analysis indicate that the lead content could affect the functional diversity of soil microbial communities and indicates the potential impact of this element on the soil environment in the Beskidy mountains. Comprehensive research may provide information about the possible changes in soil metabolism that may affect a decision to make proper use of the researched area.

**Declarations**

**Availability of data and materials** The dataset used and/or analysed during the current study are available from the corresponding author on reasonable request.

**Conflict of interest** The authors declare that they have no conflict of interests.

**Ethical approval and consent to participate** Not applicable.

**Consent to publish** Not applicable.
Authors' contributions

Conceptualization, methodology, sampling, soil analysis (soil physicochemical analysis, soil respiration measurements and CLPP analysis using Biolog ECO-plate technique), data analysis, writing, editing and visualization, Jacek Borgulat; sampling, soil analysis (soil respiration measurements), data analysis, writing, editing and visualization, Anna Borgulat; soil analysis (analysis of soil enzyme activity), data analysis, writing-review, editing and visualization, Aleksandra Nadgórska-Socha, soil analysis (analysis of soil enzyme activity), data analysis writing, editing, Marta Kandziora-Ciupa. Finally, all authors have read and agreed to the published version of the manuscript.

References

Bierza W, Nadgórska-Socha A, Małkowska E & Ciepał R (2012) Evaluation of the Soil Enzymes Activity as an Indicator of the Impact of Anthropogenic Pollution on the Norway Spruce Ecosystems in the Silesian Beskid. Ecol Chem Eng A 19(7):699-717. https://doi.org/10.2429/proc.2013.7(1)003

Borgulat A (2017) Ecophysiological diversification of the selected Vaccinium vitis-idaea L. and Vaccinium myrtillus L. populations within the impact zone of industrial emissions. Dissertation, University of Silesia in Katowice, Poland (in Polish)

Boshoff M, De Jonge M, Dardenne F, Blust R & Bervoets L (2014) The impact of metal pollution on soil faunal and microbial activity in two grassland ecosystems. Environ Res 134:169-180. https://doi.org/10.1016/j.envres.2014.06.024

Bremner JM & Mulvaney C S (1982) Nitrogen-total. In: Page AL, Miller RH, Keeney DR (eds) Methods of soil analysis. Part, vol 2. Chemical and microbiological properties. American Society of Agronomy Inc., Madison, pp 595–624

Casida LE, Klein DA & Santoro T (1964) Soil dehydrogenase activity. Soil Sci 98(6):371-376

Chodak M, Gołębiewski M, Morawska-Płoskonka J, Kuduk K & Niklińska M (2013) Diversity of microorganisms from forest soils differently polluted with heavy metals. Appl Soil Ecol 64:7-14. https://doi.org/10.1016/j.apsoil.2012.11.004

Chojniak J, Wasilkowski D, Plaza G, Mrozik A & Brigmon R (2015) Application of Biolog Microarrays Techniques for characterization of functional diversity of microbial community in phenolic-contaminated water. Int J Environ Res 9(3):785-794. https://doi.org/10.22059/ijer.2015.965

Decree of the Minister of Environment of 1 September 2016 on the conduct of the assessment of contamination of the surface of the earth (Journal of Laws, item 1395)

Decree of the Minister of Environment of 9 September 2002 soil quality standards and earth quality standards (Journal of Laws, no. 165, item 1359)
Derry AM., Staddon WJ, Kevan PG & Trevors JT (1999) Functional diversity and community structure of micro-organisms in three arctic soils as determined by sole-carbon-source-utilization. Biodivers Conserv 8(2):205-221. https://doi.org/10.1023/A:1008893826597

Fazekašová D & Fazekaš J (2020) Soil quality and heavy metal pollution assessment of Iron ore mines in Nizna Slana (Slovakia). Sustainability 12(6):2549. https://doi.org/10.3390/su12062549

Feigl V, Ujaczki É, Vaszita E, Molnár M (2017) Influence of red mud on soil microbial communities: Application and comprehensive evaluation of the Biolog EcoPlate approach as a tool in soil microbiological studies. Sci Total Environ 595:903-911. https://doi.org/10.1016/j.scitotenv.2017.03.266

Garland JL & Millis AL (1991) Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilization. Appl Environ Microbiol 57:2351-2359. https://doi.org/10.1128/AEM.57.8.2351-2359.1991

Gomez E, Garland J & Conti M (2004) Reproducibility in the response of soil bacterial community-level physiological profiles from a land use intensification gradient. Appl Soil Ecol 26(1):21-30. https://doi.org/10.1016/j.apsoil.2003.10.007

Gryta A, Frąc M & Oszust K (2014) The application of the Biolog EcoPlate approach in ecotoxicological evaluation of dairy sewage sludge. Appl Biochem Biotechnol 174(4):1434-1443. https://doi.org/10.1007/s12010-014-1131-8

Gucwa-Przepióra E, Nadgórnska-Socha A, Fojcik B & Chmura D (2016) Enzymatic activities and arbuscular mycorrhizal colonization of Plantago lanceolata and Plantago major in a soil root zone under heavy metal stress. Environ Sci Pollut Res 23(5):4742-4755. https://doi.org/10.1007/s11356-015-5695-9

Hassan W, Akmal M, Muhammad I, Younas M, Zahaid KR & Ali F (2013) Response of soil microbial biomass and enzymes activity to cadmium (Cd) toxicity under different soil textures and incubation times. Aust J Crop Sci 7(5):674-680.

Herbien SA & Neal JL (1990) Soil pH and phosphatase activity. Commun Soil Sci Plant Anal 21(5-6):439-456. https://doi.org/10.1080/00103629009368244

Hu J, Lin X, Wang J, Dai J, Chen R, Zhang J, Wong MH (2011) Microbial functional diversity, metabolic quotient, and invertase activity of a sandy loam soil as affected by long-term application of organic amendment and mineral fertilizer. J Soils Sediments 11(2):271-280. https://doi.org/10.1007/s11368-010-0308-1

Huang N, Wang W, Yao Y, Zhu F, Wang W & Chang X (2017) The influence of different concentrations of bio-organic fertilizer on cucumber Fusarium wilt and soil microflora alterations. PLoS One, 12(2):e0171490. https://doi.org/10.1371/journal.pone.0171490
Januszek K, Blonska E, Dluga J & Socha J (2015) Dehydrogenase activity of forest soils depends on the assay used. Int Agrophys 29(1):47-59. https://doi.org/10.1515/intag-2015-0009

Kabala C, Karczewska A & Medynska-Juraszek A (2014) Variability and relationships between Pb, Cu, and Zn concentrations in soil solutions and forest floor leachates at heavily polluted sites. J Plant Nutr Soil Sci 177(4):573-584. https://doi.org/10.1002/jpln.201400018

Kabata-Pendias A & Pendias H (1999) Biogeochemistry of trace elements. PWN, Warszawa, Poland

Kaczynska G, Lipinska A, Wyszkowska J & Kucharski J (2014) Response microorganisms to soil contamination with heavy metals. J Centr Eur Agric 15(3):302-314. https://doi.org/10.5513/JCEA01/15.3.1491

Kelly JJ & Tate III RL (1998) Use of BIOLOG for the analysis of microbial communities from zinc-contaminated soils. American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America. 27(3):600-608. https://doi.org/10.2134/jeq1998.00472425002700030018x

Klimek B, Sitarz A, Choczyński M & Niklińska M (2016) The effects of heavy metals and total petroleum hydrocarbons on soil bacterial activity and functional diversity in the Upper Silesia industrial region (Poland). Water Air Soil Pollut 227(8):1-9. https://doi.org/10.1007/s11270-016-2966-0

Kłos A, Bochenek Z, Bjerke J W, Zagajewski B, Ziółkowski D, Ziembik Rajfur M, Dolhańczuk-Śródka A, Tømmervik H, Krems P Jerz D, Zielińska M (2015) The use of mosses in biomonitoring of selected areas in Poland and Spitsbergen in the years from 1975 to 2014. Ecol Chem Eng S 22(2):201-218. https://doi.org/10.1515/eces-2015-0011

Kłos A, Ziembik Z, Rajfur M, Dolhańczuk-Śródka A, Bochenek Z, Bjerke JW & Zieleńska M (2018) Using moss and lichens in biomonitoring of heavy-metal contamination of forest areas in southern and northeastern Poland. Sci Total Environ 627:438-449. https://doi.org/10.1016/j.scitotenv.2018.01.211

Kuźniar A, Banach A, Stępiewska Z, Frąc M, Oszust K, Gryta A, Kłos M & Wolińska A (2018) Community-level physiological profiles of microorganisms inhabiting soil contaminated with heavy metals. Int Agrophys 32(1):101-109. https://doi.org/10.1515/intag-2016-0096

Łaszewska A, Kowol J, Wiechuła D & Kwapuński J (2007) Bioaccumulation of metals in selected species of medicinal plants in the Silesian and Żywiec Beskids. Problemy Ekologii 11:285-291 (in Polish)

Mocek-Plóciniak A & Sawicka A (2006) Effect of copper and lead on the number of microorganisms in the soils in the neighbourhood of the Legnica Copper Smelter. Zeszyty Naukowe Uniwersytetu Przyrodniczego we Wroclawiu 89(546):259-270 (in Polish)

Nagy ZM, Gruiz K, Molnár M & Fenyvesi É (2013) Comparative evaluation of microbial and chemical methods for assessing 4-chlorophenol biodegradation in soil. Period Polytech Chem Eng 57(1-2):25-35. https://doi.org/10.3311/PPch.2167
Niklińska M, Chodak M & Laskowski R (2006) Pollution-induced community tolerance of microorganisms from forest soil organic layers polluted with Zn or Cu. Appl Soil Ecol 32(3):265-272. https://doi.org/10.1016/j.apsoil.2005.08.002

Pająk M (2016) The content of zinc, lead and cadmium in bay bolete (Xerocomus badius (fr.) E.) collected from a strongly polluted forest complex. Ecological Engineering 2016(49):221-226 (in Polish) https://doi.org/10.12912/23920629/64530

Pankhurst CE, Yu S, Hawke BG & Harch BD (2001) Capacity of fatty acid profiles and substrate utilization patterns to describe differences in soil microbial communities associated with increased salinity or alkalinity at three locations in South Australia. Biol Fertil Soils 33(3):204-217. https://doi.org/10.1007/s003740000309

PN ISO 14235. 2003. Soil Quality - determination of organic carbon by sulfochromic oxidation

PN-ISO 10390. 2005. Soil Quality - Determination of pH

PN-ISO 11277. 2009. Soil quality - Determination of particle size distribution in mineral soil material - Method by sieving and sedimentation

PN-ISO 11465:1999. Soil Quality - Determination of dry matter and water content on a mass basis. Gravimetric method

Preston-Mafham J, Boddy L & Randerson PF (2002) Analysis of microbial community functional diversity using sole-carbon-source utilisation profiles–a critique. FEMS Microbiol Ecol (1):1-14. https://doi.org/10.1111/j.1574-6941.2002.tb00990.x

Ramakrishnaiah H & Somashekar RK (2002) Heavy metal contamination in roadside soil and their mobility in relations to pH and organic carbon. Soil Sediment Contam 11(5):643-654. https://doi.org/10.1080/20025891107014

Rejsek K, Vranova V, Pavelka M, Formanek P (2012) Acid phosphomonoesterase (EC 3.1. 3.2) location in soil. J Plant Nutr Soil Sci 175(2):196-211. https://doi.org/10.1002/jpln.201000139

Roane TM & Kellogg ST (1996) Characterization of bacterial communities in heavy metal contaminated soils. Can J Microbiol 42(6):593-603. https://doi.org/10.1139/m96-080

Rokicka-Kieliszewska B, Rudawska M, Staszewski T, Kurcynska E, Karlinski L (2002) Ectomycorrhizal associations in Norway spruce stands influenced by long lasting air pollution (Silesian Beskid Mountains, Poland). In Long-term air pollution effect on forest ecosystems, Zvolen (Slovak Republic), 30 Aug-1 Sep 2002. Forest Research Institute

Rusinowski S, Szada-Borzyszkowska A, Zieleźniak-Rusinowska P, Małkowski E, Krzyżak J, Woźniak G, Sitko K, Szopiński M, McCalmont JP, Kalaji HM & Pogrzeba M (2019) How autochthonous
microorganisms influence physiological status of Zea mays L. cultivated on heavy metal contaminated soils? Environ Sci Pollut Res 26(5):4746-4763. https://doi.org/10.1007/s11356-018-3923-9

Schinner F, Öhlinger R, Kandeler E & Margesin R (2012) Methods in soil biology. Springer Science & Business Media

Staszewski T & Kubiesa P (2008) Fate of air pollutants in spruce and beech stands on permanent plots in Brenna–the Silesian Beskid. Beskydy 1(1):77-84

Staszewski T, Kubiesa P, Łukasik W & Szdzuj J (2008) Lead and cadmium content in forest and coniferous ecosystems in Poland. In: Hławiczka S (ed) Metale ciężkie w środowisku. Ekonomia i Środowisko, Białystok, Poland pp 74-89 (in Polish)

Staszewski T, Łukasik W & Kubiesa P (2012) Contamination of Polish national parks with heavy metals. Environ Monit Assess 184(7):4597-4608. https://doi.org/10.1007/s10661-011-2288-z

Suchara I & Sucharová J (2002) Distribution of sulphur and heavy metals in forest floor humus of the Czech Republic. Water Air Soil Pollut 136(1-4):289-316. https://doi.org/10.1023/A:1015235924991

Telesiński A, Krzyśko-Łupicka T, Cybulska K, Pawłowska B, Biczak R, Śnieg M & Wróbel J (2019) Comparison of oxidoreductive enzyme activities in three coal tar creosote-contaminated soils. Soil Res 57(8):814-824. https://doi.org/10.1071/SR19040

Teng Y, Luo YM, Huang CY, Long J, Li ZG, Christie P (2008) Tolerance of grasses to heavy metals and microbial functional diversity in soils contaminated with copper mine tailings. Pedosphere 18(3):363-370. https://doi.org/10.1016/S1002-0160(08)60026-0

Trasar-Cepeda C, Leirós MC & Gil-Sotres F (2008) Hydrolytic enzyme activities in agricultural and forest soils. Some implications for their use as indicators of soil quality. Soil Biol Biochem 40(9):2146-2155. https://doi.org/10.1016/j.soilbio.2008.03.015

Uziębło AK, Barc A, Kubiesa P & Staszewski T (2012) Changes of the structure of forest phytocoenosis after clear-cutting of the declining spruce stand and evaluation of management methods in the forests of the Silesian Beskid Mts. (Western Carpathians). Roczniki Bieszczadzkie 20(1):44-65 (in Polish)

Xie Y, Fan J, Zhu W, Amombo E, Lou Y, Chen L & Fu J (2016) Effect of heavy metals pollution on soil microbial diversity and bermudagrass genetic variation. Front Plant Sci 7:755. https://doi.org/10.3389/fpls.2016.00755

Žifčáková L, Větrovský T, Howe A & Baldrian P (2016) Microbial activity in forest soil reflects the changes in ecosystem properties between summer and winter. Environ Microbiol 18(1):288-301. https://doi.org/10.1111/1462-2920.13026
Tables

**Table 1** Physicochemical properties of soils and the content of heavy metals in soil and soil solutions

| Site  | Layer | Soil type | Corg (%) | N (%) | SM H₂O KCl | pH | Zn (mg kg⁻¹ dw) | Pb (mg kg⁻¹ dw) | Cd (mg kg⁻¹ dw) |
|-------|-------|-----------|----------|-------|------------|----|----------------|----------------|----------------|
| SG    | A     | silt loam | 6.0      | 0.74  | 3.9        | 3.3  | 69.9           | 102.5          | 0.73           |
|       | B     | silt loam | 1.6      | 0.18  | 2.7        | 4.3  | 3.7            | 87.7           | 22.9           |
| MS    | A     | silt loam | 7.9      | 0.49  | 3.9        | 3.2  | 36.6           | 146.8          | 0.37           |
|       | B     | silt loam | 3.1      | 0.19  | 3.5        | 4.4  | 3.8            | 32.5           | 45.1           |
| KB    | A     | silt loam | 8.7      | 0.35  | 4.4        | 3.6  | 31.2           | 62.5           | 0.50           |
|       | B     | silty clay loam | 2.2  | 0.14  | 4.6       | 3.8  | 25.0           | 25.8           | 0.36           |
| CL    | A     | silt loam | 2.8      | 0.35  | 4.8        | 3.9  | 72.0           | 62.3           | 0.53           |
|       | B     | silt loam | 1.4      | 0.20  | 5.0        | 4.2  | 63.2           | 25.5           | 0.45           |
| OR-R  | A     | silt loam | 2.1      | 0.16  | 4.7        | 3.9  | 78.3           | 48.7           | 0.24           |
|       | B     | silt loam | 0.4      | 0.16  | 5.0        | 4.2  | 96.8           | 19.3           | 0.25           |
| SR-R  | A     | silt loam | 2.7      | 0.27  | 5.0        | 4.0  | 72.4           | 38.4           | 0.48           |
|       | B     | silt loam | 0.7      | 0.16  | 5.5        | 4.2  | 78.9           | 21.9           | 0.45           |

*a*United States Department of Agriculture (USDA) classification

Abbreviations: C<sub>org</sub>- organic carbon, SM- soil moisture

**Table 2** Concentrations of soluble forms of heavy metals in soil solutions

| Site  | Layer | Zn<sub>SS</sub> (µg L⁻¹) | Pb<sub>SS</sub> | Cd<sub>SS</sub> |
|-------|-------|----------------|--------------|----------------|
| SG    | A     | 23.0 ± 14.3 | 2.9 | |
|       | B     | 21.1 ± 7.9  | 2.8 | |
| MS    | A     | 24.7 ± 15.8 | 2.8 | |
|       | B     | 16.8 ± 10.8 | 2.9 | |
| KB    | A     | 19.3 ± 10.4 | 2.4 | |
|       | B     | 15.8 ± 8.1  | 2.9 | |
| CL    | A     | 12.4 ± 9.5  | 2.5 | |
|       | B     | 13.0 ± 6.6  | 2.5 | |
| OR-R  | A     | 12.9 ± 7.0  | 2.4 | |
|       | B     | 11.5 ± 5.7  | 2.1 | |
| SR-R  | A     | 10.9 ± 6.8  | 2.5 | |
|       | B     | 5.7 ± 4.9   | 1.9 | |

**Table 3** Pearson’s correlation coefficient between selected parameters (*n* = 30; *p*<0.05, **p**<0.01, ***p**<0.001)
| Layer   | Zn | Pb | Cd | pH_{H2O} | Corg  |
|---------|----|----|----|----------|-------|
| Layer A |    |    |    |          |       |
| Zn_{SS} | 0.82*** | -0.79*** | 0.78*** |          |       |
| Pb_{SS} | 0.19 |    |    | -0.86*** | 0.77*** |
| Cd_{SS} | 0.10 | -0.56** | 0.34 |          |       |
| Layer B |    |    |    |          |       |
| Zn_{SS} | 0.18 |    |    | -0.72*** | 0.47*  |
| Pb_{SS} | -0.34 |    |    | -0.67*** | 0.72*** |
| Cd_{SS} |    | -0.41* | -0.64** | 0.62**   |       |

Abbreviations: SS– soluble forms of heavy metals in soil solutions

Table 4 Soil enzymes activities, metabolic functional diversity indices of soils microbial communities and soil respiration for selected sites in Beskidy mountains. The same letters indicate homogeneous groups (n = 5, p<0.05, ANOVA, post hoc Tukey’s test)
### Soil enzymes activity

|          | SG\(^a\) | MS\(^a\) | KB | CL | OR-R | SR-R |
|----------|-----------|-----------|----|----|------|------|
| **A layer** |           |           |    |    |      |      |
| Deh \((\mu g \text{TTPF g}^{-1} \text{16h}^{-1} \text{dm})\) | 1.0a (±0.3) | 0.7a (±0.3) | 1.1a (±0.4) | 0.7a (±0.5) | 0.8a (±0.6) | 0.4 a (±0.1) |
| Ure \((\mu g \text{N g}^{-1} \text{dw})\) | 32.5a (±5) | 38.0a (±4.6) | 38.0a (±0.9) | 44.8a (±9.5) | 39.5a (±3.2) | 38.5a (±12.3) |
| AlP \((\mu g \text{pNP g}^{-1} \text{dm h}^{-1})\) | 221.5a (±110.9) | 268.0a (±42.9) | 415.5a (±223.2) | 407.5a (±183.9) | 136.0a (±82.2) | 374.5a (±260.6) |
| AcP \((\mu g \text{pNP g}^{-1} \text{dm h}^{-1})\) | 615.5a (±151.1) | 504.5a (±47.9) | 569.1a (±58.4) | 1314.1b (±371.5) | 619.5a (±442.3) | 751.2a (±84.9) |
| **Biolog ECO-plates** |           |           |    |    |      |      |
| H\(^\prime\) | 2.65a (±0.05) | 2.70a (±0.01) | 3.15b (±0.05) | 3.10b (±0.27) | 3.10b (±0.02) | 3.20b (±0.09) |
| Rs | 21.0ab (±0.9) | 19.0a (±1.8) | 29.0c (±0.9) | 25.0bc (±5.5) | 24.0abc (±0.9) | 26.5bc (±1.4) |
| AWCD \((\text{OD})\) | 46.0ab (±7.3) | 30.0a (±8.2) | 66.0ab (±15.5) | 133.0c (±35.6) | 126.5c (±5.9) | 81.0b (±17.3) |
| **Soil respiration** |           |           |    |    |      |      |
| Sres \((\text{mg CO}_2\text{-C kg}^{-1} \text{soil h}^{-1})\) | 7.9b (±0.2) | 4.1a (±0.8) | 4.1a (±0.9) | 3.3a (±0.3) | 4.9a (±0.9) | 3.4a (±0.7) |

### B layer

|          | SG\(^a\) | MS\(^a\) | KB | CL | OR-R | SR-R |
|----------|-----------|-----------|----|----|------|------|
| **Soil enzymes activity** |           |           |    |    |      |      |
| Deh \((\mu g \text{TTPF g}^{-1} \text{16h}^{-1} \text{dm})\) | 0.7d (±0.2) | 0.4bc (±0.1) | 0.5cd (±0.1) | 0.3abc (±0.1) | 0.1a (±0.1) | 0.1ab (±0.1) |
| Ure \((\mu g \text{N g}^{-1} \text{dw})\) | 18.8a (±6.3) | 21.4a (±6.3) | 23.0a (±9.2) | 32.1a (±1.5) | 21.4a (±6.4) | 27.1a (±9.1) |
| AlP \((\mu g \text{pNP g}^{-1} \text{dm h}^{-1})\) | 196.5a (±49.1) | 182.2a (±70.7) | 208.4a (±105.6) | 172.9a (±61.4) | 78.9a (±22.9) | 210.2a (±83.6) |
| AcP \((\mu g \text{pNP g}^{-1} \text{dm h}^{-1})\) | 332.2a (±61.2) | 266.2a (±38.4) | 435.6a (±120.9) | 484.9a (±285.4) | 430.2a (±75.1) | 379.3a (±135.9) |
| **Biolog ECO-plates** |           |           |    |    |      |      |
| H\(^\prime\) | 1.3c (±0.1) | 0.9ab (±0.2) | 1.2bc (±0.1) | 1.1bc (±0.1) | 1.1bc (±0.1) | 0.9a (±0.1) |
| Rs | 23.0d (±1.8) | 13.8a (±2.3) | 17.7abc (±3.7) | 18.8bcd (±0.2) | 19.7cd (±0.3) | 14.7ab (±1.2) |
| AWCD \((\text{OD})\) | 67.3b (±6.0) | 49.5ab (±13.6) | 67.1b (±17.8) | 52.3ab (±15.6) | 73.6b (±8.9) | 28.9a (±0.7) |
**Soil respiration**

| Sres (mg CO$_2$-C kg$^{-1}$ soil h$^{-1}$) | 2.4b (±0.8) | 4.0c (±0.8) | 2.5b (±0.8) | 2.3b (±0.5) | 0.5a (±0.2) | 0.9a (±0.1) |

$^a$Sites with elevated lead concentration

Abbreviations: Deh- dehydrogenase, Ure- urease, AlP- alkaline phosphatase, AcP- Acid phosphatase, H'- microbial functional diversity index, Rs- richness index, AWCD- average well colour development, Corg- organic carbon, Sres- soil respiration

### Table 5 Pearson correlation coefficient between selected parameters ($n=30$; *p<0.05, **p<0.01, ***p<0.001)

#### A layer

|          | Deh | Ure | AlP | AcP | H' | Rs  | AWCD | Sres  |
|----------|-----|-----|-----|-----|----|-----|------|-------|
| pH$_{H2O}$ | -0.16 | 0.42* | 0.10 | 0.44* | 0.88*** | 0.63** | 0.78*** | -0.54** |
| pH$_{KCl}$ | -0.26 | 0.40 | 0.05 | 0.35 | 0.87*** | 0.61** | 0.81*** | -0.56** |
| Corg     | 0.37 | -0.28 | 0.06 | -0.34 | -0.75*** | -0.49* | -0.65** | 0.56** |
| N        | 0.32 | -0.41* | 0.22 | -0.04 | -0.69*** | -0.45* | -0.49* | 0.71*** |
| SM       | 0.19 | -0.02 | 0.12 | 0.01 | -0.22 | -0.14 | -0.10 | 0.41* |
| Zn       | -0.13 | -0.08 | -0.08 | 0.37 | 0.09 | -0.16 | 0.50* | 0.17 |
| Pb       | 0.18 | -0.42* | 0.05 | -0.23 | -0.73*** | -0.65** | -0.54** | 0.24 |
| Cd       | 0.22 | -0.08 | 0.20 | 0.16 | -0.34 | -0.07 | -0.24 | 0.68*** |
| Zn$_{SS}$ | 0.38 | -0.57** | 0.20 | -0.27 | -0.61** | -0.44* | -0.58** | 0.36 |
| Pb$_{SS}$ | 0.07 | -0.32 | -0.13 | -0.28 | -0.79*** | -0.61** | -0.71*** | 0.33 |
| Cd$_{SS}$ | -0.10 | -0.38 | 0.22 | -0.14 | -0.53** | -0.48* | -0.43* | 0.23 |

#### B layer

|          | Deh | Ure | AlP | AcP | H' | Rs  | AWCD | Sres  |
|----------|-----|-----|-----|-----|----|-----|------|-------|
| pH$_{H2O}$ | -0.71*** | 0.45* | -0.08 | 0.23 | -0.28 | -0.14 | -0.24 | -0.66*** |
| pH$_{KCl}$ | -0.81*** | 0.40 | -0.25 | 0.17 | -0.23 | -0.09 | -0.18 | -0.64** |
| Corg     | 0.54** | -0.10 | 0.35 | -0.16 | -0.10 | -0.28 | 0.02 | 0.94*** |
| N        | 0.10 | 0.10 | 0.19 | 0.01 | -0.15 | 0.07 | -0.13 | 0.33 |
| SM       | 0.40 | -0.14 | 0.25 | -0.28 | -0.24 | -0.24 | -0.10 | 0.79*** |
| Zn       | -0.31 | -0.10 | -0.30 | 0.01 | 0.20 | 0.47* | 0.04 | -0.40 |
| Pb       | 0.05 | -0.08 | 0.19 | -0.25 | -0.23 | -0.44* | -0.03 | -0.40 |
| Cd       | -0.08 | 0.40 | 0.40 | 0.30 | -0.42* | -0.25 | -0.61** | 0.01 |
| Zn$_{SS}$ | 0.72*** | -0.30 | 0.17 | 0.09 | 0.43* | 0.34 | 0.28 | 0.51* |
| Pb$_{SS}$ | 0.39 | -0.31 | 0.23 | -0.18 | 0.06 | -0.18 | 0.15 | 0.70*** |
| Cd$_{SS}$ | 0.60** | -0.24 | 0.28 | -0.15 | 0.28 | 0.18 | 0.19 | 0.70*** |

Abbreviations: Deh- dehydrogenase, Ure- urease, AlP- alkaline phosphatase, AcP- Acid phosphatase, H'- microbial functional diversity index, Rs- richness index, AWCD- Average Well Colour Development, Corg- organic carbon, Sres- soil respiration, SM- soil moisture, SS- soluble forms of heavy metals in soil solutions
**Figures**

*Elevated Pb concentration in soil upper layer (>100 mg Pb kg⁻¹ dw)*

**Figure 1**

Localisation of sampling sites. SG - Stary Groń, MS - Małe Skrzyczne, KB - Kubalonka, CL - Czarny Las, OR-R - "Oszast" nature reserve, SR-R - “Śrubita” nature reserve.

![Figure 1](image1.png)

**Figure 2**

The content of heavy metals in the soil (mean ± SD)
Figure 3

The content of heavy metals in the soil (mean ± SD)

Figure 4

The content of heavy metals in the soil (mean ± SD)

Figure 5
Principal Component Analysis (PCA) of soil physicochemical properties and indicators of soil microbiological activity of soils collected from mixed forests in the Beskidy mountains.

Figure 6

Principal Component Analysis (PCA) of soil physicochemical properties and indicators of soil microbiological activity of soils collected from mixed forests in the Beskidy mountains.