

Article

Soil Quality and Heavy Metal Pollution Assessment of Iron Ore Mines in Nizna Slana (Slovakia)

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Abstract: Mining activities have resulted in the existence of dumps, which generally present a perpetual danger of moving and transforming toxic elements. The experimental study was carried out in Nizna Slana (Slovakia) where the main source of emission was the iron-ore mining–processing factory focused on siderite mining. Siderite from Nizna Slana is highly ferrous with an increased level of the Mn content. Among the undesirable impurities on the deposit are mainly As, S, Pb, and Zn. According to the environmental regionalization of the Slovak Republic, the surveyed area represents a region with a slightly disturbed environment. The BIOLOG®Eco plates method was used for ecotoxicological evaluation of contaminated soils, where soil enzymes (acidic and alkaline phosphatase and urease) were also monitored in soils and soil contamination was evaluated according to Hakanson (1980). Based on the results obtained, it can be concluded that the content of Hg, Cd, Cr, Cu, As, Fe, Mn, and Mg is above the toxicity level. As, Fe, Mn, and Mg are the most serious pollutants in the area under investigation, and their pronounced excess indicates contamination, where harmfulness and toxicity can be expected. Based on the evaluation of the contamination factor and the degree of contamination, the soils in the emission field of old mining works are very highly to slightly contaminated with heavy metals. The experimental results in the real environment showed that the activity of soil enzymes showed considerable differences, and, regarding the functional diversity of soil microorganisms, we have not seen significant spatial variability.

Keywords: soil ecosystem; heavy metals; BIOLOG®system; enzymes activity; pollution indices

1. Introduction

Soils are biologically active and habitats for living organisms; they are formed by these organisms and without their presence soil development is limited. Soil health, biodiversity, and soil resilience are critically limited in extreme environments and respond very sensitively to anthropogenic impacts [1]. Soil health depends on its biotic component, and the state of biota reflects the current state of soil conditions. The importance of soil fauna (edaphone) as one of the basic components of soil biota within ecosystems is not limited to the decomposition of dead organic matter and the importance of soil fauna is in degradation processes, pedogenesis, and development of soil properties, as well as in their stabilization, including the preservation of soil fertility. In natural as well as anthropogenic ecosystems (e.g., arable land), the individual components of soil fauna react sensitively to a wide range of toxic substances and therefore biological indicators are suitable for early diagnosis of degradation processes [2]. Enzyme activities are currently used as biological indicators of soil quality because they are relatively easy to measure, happen to be environmentally sensitive, and respond rapidly to changes in the soil ecosystem [3]. Many scientific studies have pointed out that enzymatic activity is an important biological indicator of soil quality and soil contamination rates as well as an indicator of soil contamination by heavy metals [4–6]. Heavy metals are toxic on soil microorganisms; they

inhibit microbial activities and change the diversity of microbial communities [7]. Currently, the enzyme catalase, dehydrogenase, urease, phosphatase, etc., are commonly used as bioindicators [8]. Knowledge of how organisms react to direct anthropogenic effects (contamination, erosion) but also indirect (increase in atmospheric carbon dioxide, etc.) is only partial. Available data shows that soil organisms respond to the organic matter content in the soil, as well as to chemical inputs such as heavy metals or organic contaminants, and to any change in physical properties (e.g., compaction). As part of long-term monitoring, it is necessary to consider soil biodiversity at the level of species diversity as well as functional biodiversity [9,10]. The analysis of the metabolic potential by the BIOLOG® system is currently used for analyzing changes in microbial communities [11]. This method is known for its sensitivity and speed; it is used *inter alia* for the ecotoxicological evaluation of contaminated soils [12]. Mining has a negative impact on the environment on a global and regional scale. However, this impact is felt mainly at the local level. Global and regional impacts include the release of greenhouse gases in the extraction and transport of fossil fuels. Locally adverse effects include land take, soil degradation by landfill and extraction, landform changes, groundwater and surface water pollution, atmospheric emissions and seismic effects, and increased levels of noise and dust. Heavy metals in soils are a serious problem in the areas of old environmental burdens, especially after mining and treatment of ores [13]. Heavy metals are considered one of the most important sources of environmental pollution since their significant impact on the ecological quality of the environment has been identified [14]. Increasing concentrations of heavy metals, especially mobile forms, can cause serious environmental problems relating to soil and water biota contamination while posing a serious risk to human health [15,16]. Different pollution indices assess the environmental quality of soil; they provide a comprehensive geochemical assessment of the state of the soil environment, provide an environmental risk assessment, a degree of soil degradation, and help determine whether heavy metal accumulation was due to natural processes or was the result of anthropogenic activities. In addition, pollution indices are very important for monitoring soil quality and ensuring the sustainability of agroecosystems. The commonly used pollution indices for heavy metals in soils are classified into two types: single, respectively individual and integrated, resp. complex pollution index [17]. Currently, the most commonly used are the index of geoaccumulation, contamination factor, ecological risk factor, ecological risk index, degree of contamination, pollution load index, potential ecological risk index, and metal enrichment factor, as shown by numerous studies [17–23]. A detailed review of the pollution indices is given in Kowalska et al. [24].

The scope of the present study is to (i) determine the general soil characteristics (pH, Eh, and SOM) and the content of Hg, Cd, Pb, Ni, Cr, Zn, Cu, As, Fe, Mn, and Mg metals; (ii) investigate the activity of soil enzymes and functional diversity of micro-organisms; (iii) and calculate different single (C_f^i —contamination factor) and integrated (C_d —degree of contamination) pollution indices to assess the soil quality in the area of Nizna Slana (Slovakia), where the main source of pollution was a siderite mining plant.

2. Materials and Methods

2.1. Study Area and Sampling Procedure

Mining and metal processing are responsible for the extensive contamination and pollution of soils and water in the area under investigation. The iron ore mine in Nizna Slana has been one of the most important iron ore producers since the second half of the 17th century. The main production programme was mining and the subsequent treatment of siderite ore (FeCO_3). The decline in mining activities in Slovakia has led to the dismantling of mining plants and the discontinuation of production in 2008. Serious problems are nowadays old mining works, heaps, and tailings ponds, even though they were recultivated in 1996–1999.

The researched area is situated in the southern part of the Slovak Ore Mountains, which is the largest area of the plateau character in Central Europe. The northern part of the territory is dominated

by the highland relief (820–1200 m asl), while the karst plains (450–650 m asl) prevail in the southern part. The territory belongs to the climatic area of warm to cold, soil conditions are relatively homogeneous, cambisols is prevalent on acidic rocks (80%), and fluvisols (10%) and rendzines on dolomitic limestones and dolomites. More than 50% of the land is occupied by permanent grasslands. According to the environmental regionalization of the Slovak Republic [25], the investigated territory of Nizna Slana is in the Rožnava region, which represents the territory of the 2nd environmental quality—it refers to a region with a slightly disturbed environment. The main emission source in Nizna Slana was an iron ore mining and processing plant that focused on siderite mining. The main useful component of the deposit is siderite [26]. Nizhnyan siderite is highly ferrous (33.99% Fe) with an increased level of the Mn content (2.20% Mn). Undesirable impurities in the bearing mainly include As, S, Pb, and Zn. The most important undesirable heavy metal admixture is arsenic [27].

Ten research sites (NS01–NS10) of the study area of Nizna Slana (N48° 43' 29.2" E20° 24' 43.1") were monitored in the agrarian country (Figure 1). Soil samples were collected from the permanent research sites which are used as permanent grasslands and are in the emission field of the iron ore mining and processing plant that focused on siderite mining, right from the A horizon to the depth of 0.05 m to 0.15 m in the years 2017–2019. We used the composite soil sampling method (5 random sampling) to collect soil samples weighing approximately 1000 g from one test site. The procedures were in accordance with state standards [28,29].

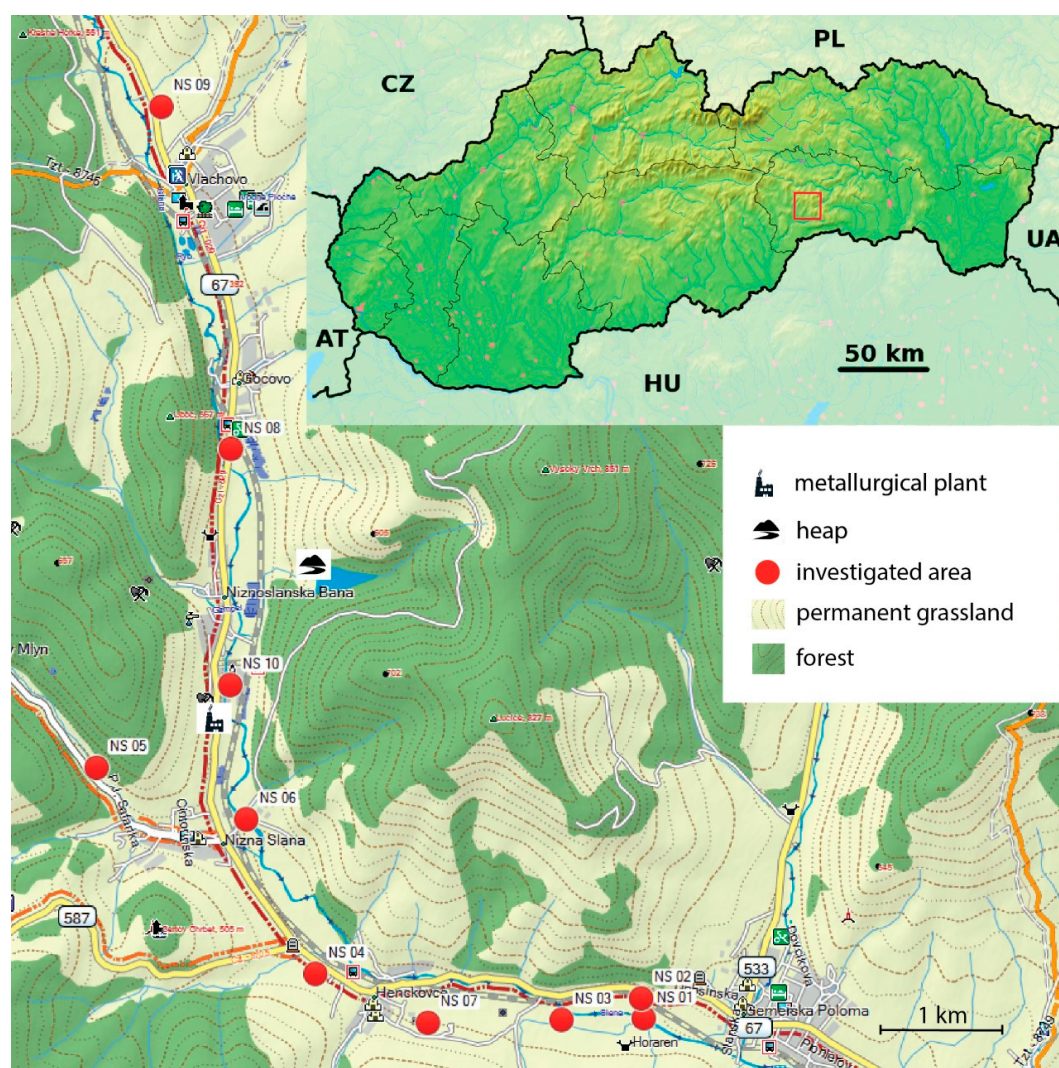


Figure 1. Location of research sites in the investigated areas of Nizna Slana (Slovakia).

2.2. Soil Assays

After homogenization, the soil samples were manually crumbled, dried at room temperature, and obtained (<2 mm) and stored in polyethylene bags until analysis. We studied and evaluated the soil reaction (pH) in the 1M KCl (20 g of soil mixed with 50 ml of 1M KCl) solution using a Mettler Toledo pH meter, redox potential (Eh) using a Redox electrode LE510. The soil organic matter (SOM) content was determined by a dry route on a C / N analyser [30]. Ten heavy metals (Hg, Cd, Pb, Ni, Cr, Zn, Cu, Fe, Mn, Mg) and one metalloid (As) were included in this study, all of them are priority pollutants in the area. The total content of Cd, Pb, Ni, Cr, Zn, Cu, As, Fe, Mn, Mg were determined by X-ray fluorescence spectrometry, while Hg was determined by atomic emission spectrometry by a mercury analyser following the methodology as devised by [30] in cooperation with an accredited geoanalytic laboratory (certificate no. 042/S-004) of the State Geological Institute of Dionýz Štúr. The evaluated values of heavy metals in soils were compared to the limit values of Slovak soils [31].

Urease activity (URE) was measured (5-g soil sample) using the method described by Galstjan [32]. This method is based on the spectrophotometric measure of the ammonia content (at 410 nm), which is created, along with carbon oxide, in a hydrolysis of urea (substratum) catalysed by urease. To determine the content of ammonia by this method, toluene and phosphate buffer (pH 6.7), 2M KCl, Seignet's salt, and Nessler's agent were used. Acid (ACP) and alkaline phosphatase (ALP) was measured using the method described by Chazijev, modified by Grejtovský [33]. The soil (10-g sample) was incubated with phenyl phosphate and the phenol amount was determined by 4-aminoantipyrin spectrophotometric (at 510 nm).

In fresh soil samples (5-g sample), we evaluated the metabolic profiles of microbial communities using Biolog®Eco Plates. Microtiter plates with 31 different organic substrates were incubated for seven days. The data-normalized parameter AWCD (Average Well Colour Development) was calculated according to Garland [34] and the functional diversity of soil microbiological communities was calculated for BIOLOG data by the classical Shannon diversity index (H') [35]:

$$H' = - \sum_{i=1}^s \frac{x_i}{N} \log_2 \frac{x_i}{N}, \quad (1)$$

where s —the number of species, x_i —individuals of one particular species found, N —divided by the total number of individuals found, $i=1$.

Soil contamination was evaluated using the contamination factor and the degree of contamination according to Hakanson [36]:

$$C_f^i = \frac{C_i}{C_n^i}, \quad (2)$$

where C_i is the average heavy metal concentration and C_n^i is the background heavy metal concentration for the soils of the Slovak Republic [37] (Table 1). The following terminology may be used in this risk index approach to get a uniform way of describing the contamination factor by Hakanson [36]: $C_f^i < 1$ low contamination factor (indicating low sediment contamination of the substance in question); $1 \leq C_f^i < 3$ moderate contamination factor; $3 \leq C_f^i < 6$ considerable contamination factor; $C_f^i \geq 6$ very high contamination factor.

The degree of the total environmental contamination C_d is defined as the sum of all C_f^i :

$$C_d = \sum_{i=1}^n C_f^i. \quad (3)$$

here C_d is the measure of the degree of overall contamination in a particular sampling site and defined as the sum of all C_f^i . The following terminology may be used for describing the degree of contamination: $C_d < 8$ low degree of contamination; $8 \leq C_d < 16$ moderate degree of contamination; $16 \leq C_d < 32$ considerable degree of contamination; $C_d \geq 32$ very high degree of contamination indicating serious anthropogenic pollution.

The obtained data was processed statistically by means of the Statistica 13 software and PAST 4. One-way ANOVA test confirmed statistical differences among the investigated areas. The level of significance between soil properties was calculated using Spearman's correlation coefficient. The hierarchical cluster analysis and the graphical output of non-metric multidimensional scaling (Nonmetric Multidimensional Scaling—NMDS) were performed using PAST 4. The data was LOG-transformed before the analysis.

The results of background values of potentially toxic elements (Table 1) show considerable heterogeneity caused by geochemical anomalies, the sources of contamination are mainly minerals, their treatment and processing in the past, as well as hazardous waste materials on dumps [37].

Table 1. Background heavy metal concentration (mg kg^{-1}) for the soils (C horizon) of the Slovak Republic.

Locality	Hg	Cd	As	Cu	Pb	Cr	Zn
NS 01	0.34	0.05	16.2	19.0	12.0	79.0	33.0
NS 02	0.34	0.05	16.2	19.0	12.0	79.0	33.0
NS 03	0.34	0.05	16.2	19.0	12.0	79.0	33.0
NS 04	10.60	0.20	30.6	72.0	27.0	94.0	65.0
NS 05	16.24	0.10	24.7	18.0	10.0	56.0	15.0
NS 06	10.60	0.20	30.6	72.0	27.0	94.0	65.0
NS 07	0.13	0.05	9.9	20.0	12.0	102.0	56.0
NS 08	0.17	0.05	15.5	25.0	11.0	65.0	36.0
NS 09	0.20	0.20	17.7	31.0	17.0	94.0	88.0
NS 10	10.21	0.05	41.6	21.0	13.0	116.0	29.0

3. Results and Discussion

The risks of chemical soil degradation in the emission area of the iron ore mines in Nizna Slana are represented by the metallization and acidification of soils. The average values of heavy metals at selected sites in Nizna Slana (Table 2) indicate significant contamination of Hg, Cd, Cr, Cu, As, Fe, Mn, and Mg. The highest measured concentrations exceeded the limit set by Act No. 220/2004 [31] As - 16.0 times, Cu - 4.4 times, Hg - 4.0 times, Zn - 2.9 times, Cd - 2.7 times, Pb - 1.9 times, Cr - 1.8 times, and Ni - 1.4 times. The background values of the Fe content in soils of the Slovak Republic are 2.64 (%). Our research showed that the highest values exceeded the average Fe content up to 6.7 times. In the monitored area, we found significant soil contamination with magnesium which is on average 26 to 53 times higher than the threshold limit. The values of available magnesium in the upper layer of agricultural land in Slovakia are in the range of 200–400 mg kg^{-1} Mg, which represents a high content of this element in the soil [38]. The manganese contents measured show a similar course. The average manganese content in the soil of the Slovak Republic ranges from 0.85 to 112.90 mg kg^{-1} Mn, which indicates a significant spatial heterogeneity of elements, but the mean stock of this element in the soil predominates. In general, Mn is a common element in the earth's crust with a relatively high incidence and its content is not easily influenced by anthropogenic sources [39]. Kabata-Pendias [40] reports a value of 1500 mg kg^{-1} , which shows symptoms of manganese toxicity. Based on the results obtained, it can be concluded that the content of Hg, Cd, Cr, Cu, As, Fe, Mn, and Mg is above the toxicity level. As, Fe, Mn, and Mg are the most serious pollutants in the area under investigation, and their pronounced exceeding indicates contamination, where harmfulness and toxicity can be expected.

Table 2. Measured values of heavy metals in the Nizna Slana region expressed by descriptive statistics.

Parameter	Mean	Min.	Max.	Median	SD	Limit Value *
Hg (mg kg ⁻¹)	1.97	0.38	22.00	0.85	3.95	0.50
Cd (mg kg ⁻¹)	0.73	0.50	1.90	0.50	0.39	0.70
Pb (mg kg ⁻¹)	44.53	23.00	145.00	32.50	30.93	70.00
Ni (mg kg ⁻¹)	43.73	27.00	70.00	46.00	15.05	50.00
Cr (mg kg ⁻¹)	83.15	46.00	123.00	80.00	17.03	70.00
Zn (mg kg ⁻¹)	114.25	69.00	432.00	86.50	95.80	150.00
Cu (mg kg ⁻¹)	94.75	22.00	261.00	67.00	77.80	60.00
As (mg kg ⁻¹)	90.07	23.00	401.00	44.50	98.94	25.00
Fe (%)	5.96	3.19	17.80	4.53	4.07	-
Mn (mg kg ⁻¹)	2800.00	1000.00	10,400.00	1950.00	2383.42	-
Mg (mg kg ⁻¹)	10,585.00	5900.00	21,900.00	10,850.00	3863.63	-

* Act No. 220/2004 Coll. of Laws, SD—Standard deviation.

The soil contamination assessment, which was calculated on the basis of the contamination factor C_f and the degree of contamination C_d , according to Hakanson [36], for each heavy metal and each sampling site in the monitored area are shown in Table 3.

Based on the average contamination factor (C_f) values, soils at the NS10 site were classified as very highly contaminated with heavy metals in the order of As > Cd > Pb > Zn > Hg > Cu. NS02 was very highly contaminated with Hg > Cd, while NS06 was very highly contaminated with As > Cd > Hg. NS03 and NS08 were very highly contaminated with Cd and Hg. The level of contamination was very high ($C_d \geq 32$) at the collection sites NS10 (72.92) and NS02 (39.57). It was considerably high ($16 \leq C_d < 32$) at NS06, NS03, NS08, and NS01. These findings suggest that agricultural soils in the area under investigation are very highly to slightly contaminated with heavy metals.

The acidification effect is accelerated by an anthropogenic load by acidic emissions of sulphur and nitrogen oxides. The emitted sulphur oxides and nitrogen oxides give rise to acid rain amid the rain and fog. In rainwater, sulphates, nitrates, and chlorides are present, with heavy metals containing Hg, As, Pb, Ni, Cd, and Cr. In addition to the anthropogenic load, the research area is also influenced by geological substrates which consist of acidic rocks, dolomitic limestones, and dolomites. Accordingly, the measured pH values for the soil under investigation range from 4.3 to 7.1 (Table 4), indicating the extremely acidic to alkaline nature of the soil. The acidic pH of the soil is considered to be the most important factor affecting the increased absorption of heavy metals [41,42], while in alkaline soils (pH between 7.1 and 8.1) there is a risk of leaching heavy metals and their bioavailability for the plants below [43].

The soil oxidation–reduction potential (Eh—redox potential) belongs to very important soil parameters. It affects several chemical and biochemical processes in the soil ecosystem. It mainly affects the mobility and toxicity of inorganic and organic contaminants. Positive redox potential values mean oxidation conditions in the soil and negative reductions—if the Eh value falls below 200mV, reduction processes begin to develop in the soil [44]. The soil extracts studied exhibit great variability in the redox potential ranging from −211 to 334 (mV) (Table 4). Soil reduction processes are part of complex chemical processes in soils, including the biochemical processes of energy recovery by soil microorganisms. These include, for example: rotting, peat formation, denitrification, fermentation, and others [45].

Table 3. Ranking order of the soil contamination of the investigated areas according to the C_d -value and the sequence of the contamination factors (C_f^i).

				Contamination Factor (C_f^i)			
		Locality	C_d	Very high $C_f^i \geq 6$	Considerable $3 \leq C_f^i < 6$	Moderate $1 \leq C_f^i < 3$	Low $C_f^i < 1$
Degree of contamination (C_d)	Very high	$C_d \geq 32$	NS 10	As > Cd > Pb > Zn > Hg > Cu Hg > Cd		As > Cu > Pb > Zn > Cr	Cr
			NS 02				
	Considerable	$16 \leq C_d < 32$	NS 06	As > Cd > Hg Cd Hg	Pb Cu > Hg > As Cd > As Cd > Hg	Cu > Zn Pb > Zn Pb > Cu > Zn > Cr Zn > Pb > As > Cu	Cr Cr Cr Cr
			NS 03				
			NS 08				
			NS 01				
	Moderate	$8 \leq C_d < 16$	NS 05		Cd Cd Cd Cd	Zn > Pb > Hg > As > Cr > Cu Pb > Hg > Zn > As Zn > Pb > Hg > As Zn > Pb > Hg > As	Cr > Cu Cr > Cu Cr > Cu
			NS 09				
			NS 04				
			NS 07				
	Low	$C_d < 8$					

Table 4. Measured values of selected soil biochemical parameters in Nizna Slana expressed by descriptive statistics.

Parameter	Mean	Min	Max	Median	SD
pH/KCl	5.7	4.3	7.1	5.5	1.0
Eh (mV)	187.7	−211.0	334.0	223.0	159.1
SOM (%)	3.3	1.4	9.8	2.6	2.4
AWCD	1.1	0.7	1.4	1.0	0.2
H'	3.2	3.0	3.3	3.2	0.1
URE (mg $\text{NH}_4^+\text{-N g}^{-1}$ 24 hod $^{-1}$)	0.6	0.2	1.4	0.6	0.3
ACP ($\mu\text{g P g}^{-1}$ 3 h $^{-1}$)	204.7	67.7	312.3	210.4	73.1
ALP ($\mu\text{g P g}^{-1}$ 3 h $^{-1}$)	165.7	56.3	374.0	144.1	88.8

SOM—Soil organic matter, Eh—Redox potential, AWCD—Average Well Colour Development, H' —Shannon index, SD—Standard deviation, URE—Urease, ALP—Alkaline phosphatase, ACP—Acid phosphatase.

Soil organic matter (SOM) is one of the main factors controlling the physical, chemical, and biological properties of soil and plays an important role in soil hygiene (immobilization of heavy metals and organic pollutants) [46]. Recent research has unequivocally confirmed the key role of organic carbon in the sorption of inorganic and organic contaminants [47], and the presence of organic substances can inhibit metal absorption from soil solution and reduce metal mobility and bioavailability [43,48,49]. In the study area, SOM values ranged from 1.4 to 9.8%, indicating that the soil humus supply is moderate to very good.

The monitored soil pH, Eh, and SOM parameters influence the release dynamics and possible mobility of heavy metals, as pointed out in the work of [50], and are considered critical parameters regulating the fate of pollutants in the environment [51]. The results of the studied biochemical soil parameters expressed in descriptive statistics are shown in Table 4.

The presence of metals in concentrations above certain thresholds affects the microbiological balance of soils and may reduce their fertility. Soil pollution due to heavy metal contamination is a serious problem because it is toxic and its bioaccumulation capacity is very dangerous for its effects on the food chain [19]. The microbial activity expressed by the activity of soil enzymes is an important biological factor that makes it possible to assess the quality and functionality of the soil ecosystem. Soil enzyme activity is used as a reliable biological indicator to assess soil contamination. Heavy metal toxicity is usually evaluated by inhibiting the action of soil enzymes [52,53]. Currently, no quantitative standard of soil enzyme activity has been set to assess the level of heavy metal soil pollution. Generally, high enzyme activity represents good soil quality, while low activity may be related to the toxicity of pollutants to biological processes [8,54]. The urease enzyme (URE) plays an important role in the N cycle [55], it is loosely present in soil and is more often bound to soil organic matter or clay particles. Its soil stability is mainly influenced by the soil depth, soil type, heavy metal content, pH, among others [56]. Soil urease activity was in the range of 0.2–1.4 mg $\text{NH}_4^+\text{-N g}^{-1}$ 24 hod $^{-1}$. The highest urease activity values were found at the sites furthest from the source of pollution (NS03), and the measured heavy metal values did not exceed the legal limit values except for Hg and As. Acid phosphatases (ACP) and alkaline phosphatases (ALP) are the key enzymes that hydrolyse organic phosphate to the inorganic form, thereby increasing the supply of soil phosphorus. Therefore, they play a major role in soil phosphorus cycling [57,58]. Experimental results in the real environment showed that ACP and ALP activity fluctuated considerably. ACP activity ranged from 67.7–312.3 ($\mu\text{g P g}^{-1}$ 3 h $^{-1}$), while ALP activity also showed marked differences (56.3–374.0 $\mu\text{g P g}^{-1}$ 3 h $^{-1}$). At the same time, the results of many experimental studies suggest that inhibition of soil enzymes has diminished over time, sudden exposure of microorganisms to heavy metals results in a significant reduction in enzymatic activity, and microorganisms later adapt to polluted environments and thereby help enzymatic activity to recover usually [8,9].

We used a BIOLOG®Eco plate to analyse changes in microbial communities. This method is known for its sensitivity and speed; it has been used inter alia for the ecotoxicological evaluation

of contaminated soils [12]. The Biolog system uses 31 different carbon sources to create a metabolic profile of microorganisms [34]. To calculate the Shannon diversity index [35], we used the absorbance in samples examined after 168 hours. The AWCD (Average Well Colour Development) is an important and sensitive indicator reflecting the metabolic profiles of the soil microbial community, especially to toxic heavy metals in soils [59]. The values of the metabolic activities of the microorganisms (AWCD) ranged from 0.7 to 1.4 and the diversity at the sites studied in the Nizna Slana region was ranged from 3.0 to 3.3, which corresponds to typical values reported in most environmental studies (from 1.5 to 3.5, rarely reaching 4.5). Studies suggest that the soil ecosystem in a deteriorated environment is unstable and its function impaired. Greater diversity stabilizes the ecosystem's functional properties, which are more stable, productive, and more resistant to stress factors and disorders [60]. Xie et al. [61] points out the clear inhibitory effect of an increasing level of metal content on the functional activity of soil microorganisms. Khan et al. [9] confirms the inhibitory effect of heavy metals on soil microorganisms, especially at high Cd and Pb concentrations. According to Konopka et al. [62], even a short-term response to heavy metal contamination is manifested by a significant reduction in microbial activity. Yin et al. [63] find in their research a decrease in the diversity of the microbial community due to long-term heavy metal pollution. In view of the above, it can be assumed that in accordance with the findings of Zhao et al. [48] and Xu et al. [64], heavy metals are fundamental factors affecting the diversity of microbial communities. At the same time, the authors pointed out that when the community was exposed to heavy metal pollution, the diversity suddenly decreased, but on the contrary resistant microorganisms adapted to new habitats and increased in large quantities, thereby resulting in a change in the structure of the microbial community.

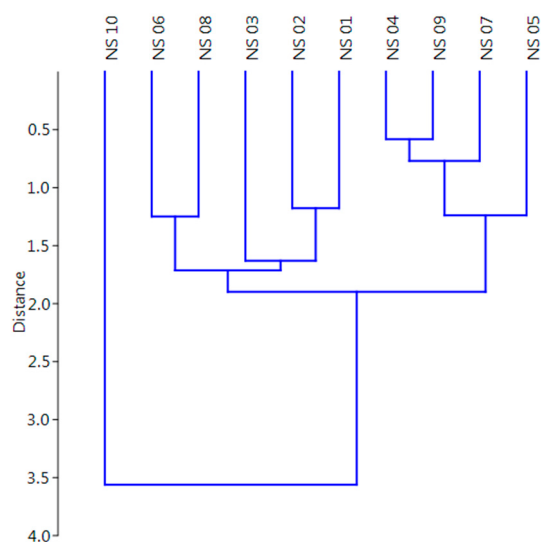
Spearman's correlation analysis between selected biochemical parameters and heavy metals in soil samples is shown in Table 5. AWCD has a significantly positive correlation with Cd, Zn, Fe, and Mn. Significant positive correlation was found between H' and Cd, H' and Zn. The results of several studies suggest that the total bioactivity, richness, and diversity of microorganisms decreased with increasing heavy metal concentrations because microorganisms differ in their sensitivity and heavy metal toxicity [65,66]. In our study, we did not find the expected negative correlations between AWCD and H' and heavy metal works compared to other studies. Probably in our research area, the development of metal-resistant microbial populations has enhanced, as stated in his work [66]. The URE enzyme is positively correlated with Zn, Mn, and Mg. The results of other studies show [6] that URE appears to be more sensitive to pollution stress than phosphatase which was not confirmed in our study. Positive correlations were found between the enzyme ALP and Hg, Cd, Ni, Cu, Mn, and SOM. On the other hand, the ACP enzyme correlated negatively with Zn and Mg. It follows that ACP was more affected by the conditions of soil contaminated, in our case, ACP was inhibited by Zn and Mn, as also indicated in their work Santos et al. [67]. The soil reaction (pH) is correlated significantly with Hg, Pb, and Cu. Similar results were obtained by Yang et al. [68] who found that Zn, Pb, Cu, Cd, As and Hg were positively correlated with pH, which may suggest that pH influenced the distributions of these metals in soils. However, the concentrations of Cd, Ni, Cr, As, Fe, Mn, and Mg were not correlated with pH, demonstrating that this parameter was not important to the Cd, Ni, Cr, As, Fe, Mn, and Mg distribution. Redox potential (Eh) is correlated negatively with Zn, Mn, and Mg. Cao et al. [51] report that the solubility of metals (Pb, Cd, and Zn) directly correlates with changes in pH and Eh. Soil organic matter (SOM) is correlated positively with Ni, Cr, Zn, Cu, and Mn in our research which suggests that Ni, Cr, Zn, Cu, and Mn increase with increasing organic matter in the soil. The result is the formation of soil organic matter and heavy metal ion compounds, thereby reducing the bioavailability and mobility of heavy metals [47,69]. No significant correlation was found for arsenic. It can be argued from the above-mentioned research that the contamination of the soil environment by arsenic is related not only to anthropogenic effects, but also to geochemical effects, as confirmed by Čurlík and Ševčík [37] in their study.

Table 5. Spearman's correlation between selected biochemical soil parameter and heavy metals.

Parameter	AWCD	H'	URE	ACP	ALP	pH	Eh	SOM
Hg	0.26	0.35	0.38	−0.10	0.72 **	0.72 **	−0.52	0.55
Cd	0.77 *	0.77 *	0.57	−0.10	0.69 **	0.62	−0.49	0.57
Pb	0.51	0.54	0.22	0.19	0.35	0.71 **	−0.07	0.22
Ni	0.51	0.35	0.55	−0.24	0.69 **	0.08	−0.52	0.65 **
Cr	0.30	0.18	0.24	0.21	0.60	−0.08	−0.39	0.66 **
Zn	0.70 **	0.64 **	0.74 **	−0.65 **	0.56	0.20	−0.77 *	0.66 **
Cu	0.54	0.39	0.44	−0.44	0.66	0.75 **	−0.58	0.82 *
As	0.58	0.42	0.42	−0.39	0.39	0.49	−0.27	0.37
Fe	0.71 **	0.52	0.37	−0.25	0.32	0.24	−0.22	0.38
Mn	0.63 **	0.56	0.68 **	−0.36	0.76 **	0.36	−0.65 **	0.70 **
Mg	0.47	0.46	0.72 **	−0.66 **	0.37	0.01	−0.65 **	0.32
pH	0.19	0.14	0.04	−0.24	0.38	-	−0.19	0.50
Eh	−0.58	−0.68 **	−0.86 *	0.37	−0.80 *	−0.19	-	−0.77 *
SOM	0.56	0.47	0.50	−0.30	0.77 *	0.50	−0.77 *	-

SOM—Soil organic matter, Eh—Redox potential, AWCD—Average Well Colour Development, H' —Shannon index, URE—Urease, ALP—Alkaline phosphatase, ACP—Acid phosphatase, * $p < 0.05$, ** $p < 0.01$

Using eight biochemical parameters and 11 heavy metals as variables, cluster analysis was used to describe the difference in soil monitoring points. The results indicated that the points were categorized into three groups (Figure 2). Cluster 1 includes a metallic contaminated point (NS10), while Cluster 2 includes predominantly metallic but also acidified contaminated points (NS01, NS02, NS03, NS08, NS09). Cluster 3 includes metallic and acidified contaminated points (NS04, NS05, NS07, NS09). Cluster analysis showed the diversity of the sites examined. Statistical evaluation of the investigated sites was processed by nonmetric multidimensional scaling (NMDS), while using all examined biochemical parameters and heavy metals. The most important variables are pH, urease (URE), and Cr, which correlate with the horizontal axis, and these variables had a significant impact on diversity (Figure 3).

**Figure 2.** Dendrogram of the soil-monitoring points from the hierarchical cluster analysis using biochemical parameters and heavy metals as variables.

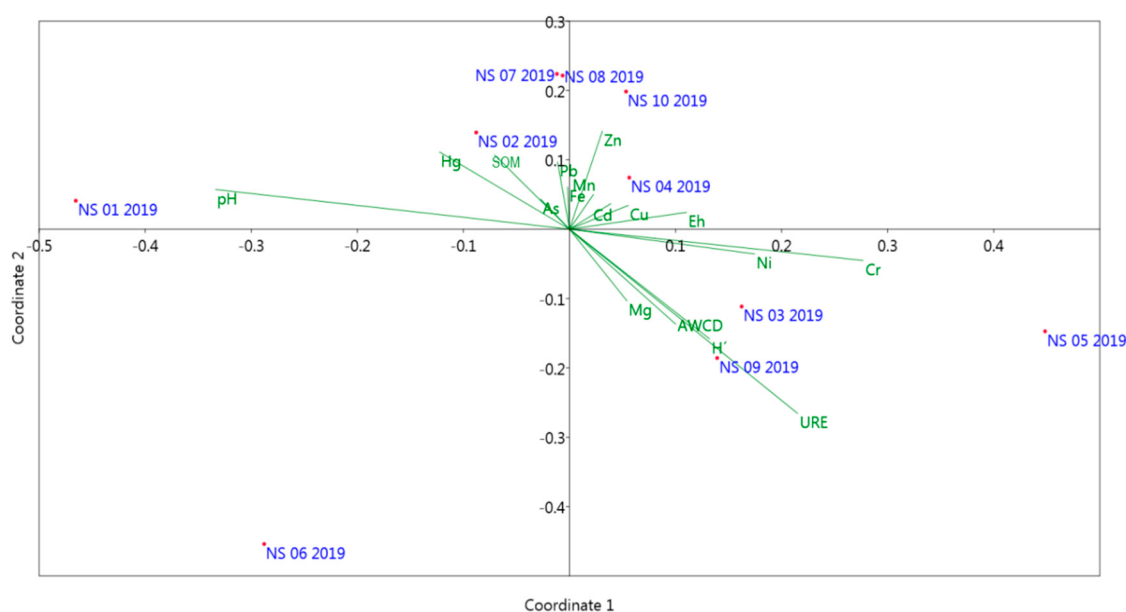


Figure 3. Graphical output of non-metric multidimensional scaling (Nonmetric Multidimensional Scaling—NMDS), SOM—Soil organic matter, Eh—Redox potential, AWCD—Average Well Colour Development, H' —Shannon index, URE—Urease, ALP—Alkaline phosphatase, ACP—Acid phosphatase.

4. Conclusions

The experimental study was carried out in Nizna Slana (Slovakia), where the main emission source was an iron ore mining and processing plant that focused on siderite mining. The decline in mining activities in Slovakia has led to the dismantling of mining plants and closure of production in 2008. Serious problems are nowadays old mining works, heaps, and tailings ponds. Based on the results obtained, it can be concluded that the content of Hg, Cd, Cr, Cu, As, Fe, Mn, and Mg is above the toxicity level. As, Fe, Mn, and Mg are the most serious pollutants in the area under investigation, and their pronounced exceeding indicates contamination, where harmfulness and toxicity can be expected. Based on the evaluation of the contamination factor and the degree of contamination, the agricultural soils in the emission field of old mining works are very high to slightly contaminated with heavy metals. The experimental results in the real environment showed that the activity of soil enzymes showed considerable differences and regarding the functional diversity of soil microorganisms, we have not found significant spatial variability, and the level of the functional diversity reached the average value of 3.2. The results of this study showed the complexity of the effect of heavy metal contamination on soil biological activity and the sustainability of the soil ecosystem.

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