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Microorganisms in soils with elevated heavy metal concentrations in southern Serbia

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Abstract: Soil chemical properties and total heavy metal concentrations (As, Cd, Cr, Cu, Ni, Pb, Zn, Fe and Mn) and their relationships with some soil microbiological characteristics (such as soil respiration and numbers of specific groups of microorganisms) were evaluated in agricultural soils in southern Serbia. In 42% of samples, slightly elevated concentrations of As, Ni and/or Pb were detected, with the highest values of 43.4, 64.4 and 184.1 mg/kg, respectively. No significant differences were observed in soil microbiological characteristics between soil samples with elevated heavy metals and heavy metals below the maximum allowed concentrations (MAC). There was no negative correlation between any of the heavy metals and any microbiological characteristics, except the total number of fungi and the concentration of Ni (r=-394). Positive correlations between most of the heavy metals and the number of *Azotobacter* were obtained. Correlation analysis and stepwise multiple regression analyses indicated that the chemical properties of the soil are the factors that affected the number of microorganisms the most. The content of $CaCO_3$ significantly contributed to variations in soil respiration (39%), the total number of microorganisms was affected the most by humus (53%), oligonitrophiles by the total N content (49%), while the numbers of actinomycetes and fungi were affected by the pH (48% and 58% of the variation).

Key words: soil quality; heavy metals; microbial community; soil respiration; agroecosystems

INTRODUCTION

Soil, as a non-renewable resource and the basis of our agroecosystems, is essential to sustain human life. The presence of increased heavy metal concentrations in agricultural soils poses some risks for plant production, but also to ecosystems and human health. Certain heavy metals are naturally present in the soil due to geological weathering, but anthropogenic activities can considerably increase their concentrations in soil. Some of the sources of heavy metals in agricultural soils are fertilizers, sewage for irrigation and the deposition of fossil fuel by-products [1].

Increased levels of heavy metals have become one of the major concerns due to their toxicity in biological systems. Soil microorganisms are vital in nutrient cycling, soil organic-matter decomposition, transformation of metals [2]. Elevated levels of heavy metals in soils influence the size of microbial populations and their activities, which may affect soil nutrients and their absorption from the soil by plants [3].

Changes in microbial numbers and activities are proposed as an easy and sensitive indicator of changes in soil physicochemical properties and soil degradation [4-6]. Microorganisms respond quickly to heavy metal effects in the soil and can be good indicators of soil quality [7-9]. Numerous studies have shown the negative effects of metal contamination on soil respiration, enzyme activity, microorganism diversity and microbial community size [5,10-12]. However, toxicity thresholds varied widely in different studies, indicating that the determination of critical metal concentrations in soils for specific groups of microorganisms and processes is very challenging [3,9,13]. On the other hand, there were no differences in microbial biomass, activity of enzymes or presence of some groups of microorganisms between contaminated and uncontaminated soils [9,14].

Numerous chemical and physical characteristics of soils, such as quality and quantity of soil organic matter, soil pH, etc. [4] affect both metal toxicity and

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Table 1. Soil chemical properties.

No of sample	pH KCl	pH H_2O	Humus %	P_2O_5mg/100g	K_2O mg/100g	$CaCO_3$ %	Total N %	Soil type	Land use/Crop
Soil samples with one or more heavy metal concentrations above the MAC (samples>MAC)									
1	6.70	7.80	1.45	49.36	38.60	2.87	0.103	Fluvisol	Orchard/Raspberry
2	6.60	7.80	2.28	44.66	39.36	0.41	0.165	Vertisol	Field/Maize
3	6.10	7.30	2.83	12.52	56.51	0.00	0.164	Vertisol	Field/Wheat
4	6.20	7.50	2.78	10.54	29.22	0.00	0.180	Fluvisol	Field/Cereals
5	6.50	7.50	2.02	25.02	28.80	0.82	0.143	Vertisol	Field/Cereals
6	6.54	7.90	3.91	37.15	53.78	2.05	0.271	Fluvisol	Field/Cereals
7	6.80	7.90	2.00	13.22	35.37	2.46	0.143	Fluvisol	Field/Maize
8	5.90	7.20	2.68	5.31	57.62	0.00	0.192	Vertisol	Field/Cereals
9	6.80	8.10	1.27	12.57	22.91	1.23	0.104	Fluvisol	Field/Cereals
10	5.70	6.70	4.42	37.03	65.17	0.00	0.251	Fluvisol	Orchard/Raspberry
11	5.80	7.00	3.02	3.59	89.59	0.00	0.219	Fluvisol	Field/Clover
Soil samples with all heavy metal concentrations below the MAC (samples<MAC)									
1	4.10	5.20	1.35	0.10	12.15	0.00	0.071	Vertisol	Field/nd
2	6.50	7.00	5.19	58.02	94.71	0.82	0.295	Vertisol	Field/Vegetables
3	5.10	6.30	2.45	0.68	30.62	0.00	0.138	Vertisol	Field/Wheat
4	6.50	7.80	2.38	10.23	48.18	4.10	0.155	Vertisol	Field/Cereals
5	6.60	7.80	3.21	15.24	72.81	3.69	0.211	Fluvisol	Field/nd
6	4.60	6.00	2.01	11.13	41.46	0.00	0.126	Vertisol	Field/nd
7	5.10	6.40	3.63	6.87	44.96	0.00	0.211	Vertisol	Field/Cereals
8	5.20	6.40	2.16	15.76	30.34	0.00	0.127	Vertisol	Orchard/Plum
9	4.20	5.50	2.08	2.74	28.38	0.00	0.124	Fluvisol	Meadow
10	6.50	7.40	1.24	4.29	11.80	0.82	0.084	Vertisol	Field/Maize
11	4.30	5.70	2.59	0.87	27.82	0.00	0.149	Vertisol	Field/Wheat
12	6.60	7.80	3.15	37.96	81.07	3.28	0.199	Vertisol	Orchard/Raspberry
13	4.60	6.00	2.63	0.42	25.65	0.00	0.135	Vertisol	Field/nd
14	5.50	7.00	3.33	16.43	33.60	0.00	0.198	Fluvisol	Field/nd
15	5.10	6.00	3.84	22.44	56.73	0.00	0.206	Fluvisol	Field/Maize
Samples>MAC (n 11)									
Min	5.70	6.70	1.27	3.59	22.91	0.00	0.103		
Max	6.80	8.10	4.42	49.36	89.59	2.87	0.271		
SD	0.41	0.43	0.96	16.50	19.71	1.10	0.050		
Mean	6.33	7.52	2.61	22.81	46.99	0.89	0.170		
Samples <MAC (n 15)									
Min	4.10	5.20	1.24	0.10	11.80	0.00	0.071		
Max	6.60	7.80	5.19	58.02	94.71	4.10	0.295		
SD	0.94	0.87	1.02	16.06	24.35	1.51	0.058		
Mean	5.37	6.55	2.75	13.54	42.68	0.84	0.160		
p	0.0041	0.0024	ns	ns	ns	ns	ns		

MAC – maximum allowed concentrations for total heavy metals in agricultural soils; SD – standard deviation; ANOVA for $p>0.05$, non-significant differences between soils (ns); $p<0.05$ significant differences, $p<0.01$ very significant differences; nd – not determined.

soil microorganisms. The bioavailability of heavy metals depends on different soil properties (pH, cation-exchange capacity (CEC), organic matter, etc.). Microbial communities can also develop resistance to chronic exposure to high heavy metal concentrations [14]. Therefore, due to complex physical and chemical interactions in the soil, the effects of heavy metals on soil microorganisms must be examined together with other soil characteristics.

Increased levels of heavy metals, mainly Ni and Cr, have been found in some parts of Serbia (western Serbia), while elevated concentrations of other metals have been sporadically detected in agricultural soils in different parts of the country [15]. These high metal concentrations were probably influenced by the basic substrate, but anthropogenic influence cannot be excluded [15].

In this study we evaluated soil chemical characteristics, heavy metal concentrations and soil microbiological characteristics, such as basal soil respiration and the numbers of specific groups of microorganisms and their relationships in soils under intensive agricultural production, in the southern part of Serbia.

MATERIALS AND METHODS

Location description

Soil was sampled from the southeastern part of Serbia, in the area of Pčinja district. In this area, agricultural soils belong to the two types, vertisol and fluvisol [16]. The soils were mainly under cereals (Table 1). Samples were taken in the autumn of 2017, from a depth of 20 cm, and used to analyze the chemical and microbiological properties. Single (composite) soil samples were comprised of 5 subsamples taken from the center and corners of 10x10 m square plots [17]. A total of 26 soil samples was analyzed.

Chemical analysis

Soil pH was established with a glass electrode pH meter in H_2O and 1N KCl (at a ratio of soil:KCl or H_2O of 1:2.5). The AL-method of Egner-Riehm was used to determine the available P and K in the soil. Total soil N (N_{tot}) and total soil C (C_{tot}) were determined by dry combustion with a CNS analyzer (Vario model EL III, Hanau, Germany) and applying ISO standards 13878:1998 and ISO 10694:1995, respectively [18,19]. The humus content was calculated from C_{tot}, determined by a CNS analyzer and $CaCO_3$ and by multiplying with 1.724 (conversion factor). The granulometric composition of the soils was determined by a combined method of sieving and pipetting [20]. The $CaCO_3$ was determined volumetrically with a Scheibler calcimeter. Total heavy metal concentrations (As (arsenic, metalloid), Cd (cadmium), Cr (chromium), Cu (cooper), Ni (nickel), Pb (lead), Zn (zinc), Fe (iron) and Mn (manganese)) were determined by acid digestion (HNO_3) and plasma emission spectrometry (iCAP 6300 ICP, Cambridge, UK) according to ISO 22036:2008 [21]. Quality control, accuracy and precision of the measurement and concentration values were performed using a certified reference material, ERM-CC141 – Loam Soil. The limit of detection (LOD) for each observed metal was as follows in mg/kg: As 0.4, Cd 0.10, Cr 0.3, Cu 0.3, Ni 0.4, Pb 0.6, Zn 0.5, Fe 25.0 and Mn 0.5.

Microbiological analysis

Soil respiration was determined by laboratory incubation with constant temperature and moisture. The respired carbon dioxide was trapped in the NaOH, and the remaining amount of OH^- ions was back-titrated with an HCl solution. Finally, the amount of released CO_2 during the incubation period was calculated [22]. The total number of microorganisms was determined by the plate count method on the agarized soil extract, the number of fungi on the Chapek medium as described previously [2], actinomycetes on the medium according to Krasiljnikov, and oligonitrophiles on the Fyodorov medium [23]. Ammonifiers and *Azotobacter* spp. were determined by the most probable number (MPN) method in the liquid medium with asparagines or mannitol, respectively [24].

Statistics

Statistical differences in chemical and microbial parameters between different groups of soil samples, with and without elevated heavy metal concentrations, were evaluated using one-way ANOVA. In order to determine the relationship between the microbiological properties of soil and other investigated characteristics of soil samples (SPSS 16.0), Pearson's correlation and stepwise multiple linear regression were used.

RESULTS

Soil chemical characteristics and heavy metal concentrations

Soil chemical characteristics and heavy metal concentrations in the investigated area are presented on Tables 1 and 2. The soil samples were divided into two groups: soils containing concentrations of one

Table 2. Heavy metal concentrations in soil.

No of sample	As	Cd	Cr	Cu	Ni	Pb	Zn	Fe	Mn
mg/kg									
Soil samples with one or more heavy metal concentrations above MAC (samples>MAC)									
1	**25.3**	0.49	27.0	27.7	32.4	50.5	107.1	22362	726.4
2	19.5	0.86	53.9	45.2	**57.0**	**104.1**	171.9	31227	796.4
3	**31.1**	0.20	22.9	19.8	20.4	24.9	76.6	24392	577.4
4	**32.4**	1.26	63.0	42.5	**64.4**	**142.3**	226.3	29382	850.4
5	**31.6**	0.20	33.4	20.3	45.7	29.0	56.4	16052	794.9
6	**43.4**	0.69	59.9	49.0	**62.9**	71.1	145.9	32662	864.4
7	**40.3**	0.35	41.9	29.5	45.2	69.5	130.5	22374	624.3
8	12.0	0.14	46.3	34.7	**53.6**	32.2	81.6	24804	728.8
9	**33.2**	<LOD	24.2	16.9	30.0	13.6	43.5	14479	449.5
10	19.5	1.25	60.5	50.7	**51.1**	**184.1**	233.3	30224	794.3
11	**29.6**	0.09	30.4	23.6	38.7	39.9	61.1	16089	785.8
Soil samples with all heavy metal concentrations below MAC (samples<MAC)									
1	4.9	0.11	14.2	8.08	12.3	14.9	25.9	11012	443.3
2	10.3	0.43	31.1	24.6	25.5	30.0	90.7	19292	449.5
3	5.0	0.18	30.2	16.0	27.9	21.8	47.1	18407	458.0
4	4.6	0.22	36.5	17.4	41.0	18.7	50.3	17562	549.9
5	7.4	0.29	42.8	28.8	46.3	28.6	73.9	23402	724.9
6	4.7	0.16	31.7	21.1	21.2	16.7	50.3	19372	293.4
7	5.7	0.20	37.9	19.3	30.6	21.9	56.0	19182	534.9
8	4.3	0.14	30.9	14.4	22.1	13.0	45.1	17527	357.7
9	6.5	0.23	25.2	15.0	24.1	28.3	44.9	15192	853.4
10	13.9	0.41	36.6	22.4	34.5	41.1	93.1	21547	550.4
11	6.6	0.19	36.7	16.3	28.8	29.6	46.5	20792	800.4
12	17.2	0.56	37.9	26.7	43.8	58.2	101.3	20362	668.9
13	5.5	<LOD	23.1	15.7	19.5	22.2	38.2	12939	598.3
14	13.7	0.29	61.5	47.9	47.7	34.5	146.2	34499	998.8
15	8.1	0.13	30.1	19.7	22.8	41.2	75.2	19094	831.8
Samples>MAC (n 11)									
Min	12.0	<LOD	22.9	16.9	20.4	13.6	43.5	14479	449.5
Max	43.4	1.26	63.0	50.7	64.4	184.1	233.3	32662	864.4
SD	9.3	0.44	15.4	12.4	14.1	53.9	66.7	6461	127.3
Mean	28.9	0.55	42.1	32.7	45.5	69.2	121.3	24004	726.6
Samples <MAC (n 15)									
Min	4.3	<LOD	14.2	8.1	12.3	13.0	25.9	11012	293.4
Max	17.2	0.56	61.5	47.9	47.7	58.2	146.2	34499	998.8
SD	4.0	0.13	10.4	9.1	10.6	12.0	31.3	5272	201
Mean	7.9	0.25	33.8	20.9	29.9	28.0	65.6	19345	607.5
p	0.00001	0.0409	ns	0.0098	0.0035	0.0082	0.0089	ns	ns
MAC	25	3	100	100	50	100	300	nd	nd

MAC – maximum allowed concentrations for total heavy metals in agricultural soils; LOD – limit of detection (0.10); SD – standard deviation; ANOVA for $p>0.05$, non-significant differences between soils (ns); $p<0.05$ significant differences, $p<0.01$ very significant differences; $p<0.001$ extremely significant differences; nd – not determined.

or more heavy metals above the maximum allowed concentrations (MAC), and soils with all evaluated metals below the MAC for agricultural soils, according to the national legislation [17].

In 15 out of 26 soil samples the total concentrations of all investigated metals (As, Cd, Cr, Cu, Ni, Pb, Zn, Fe and Mn) were below the MAC (Table 2). A total of 11 samples had elevated concentrations

Table 3. Microbiological properties of soils.

No of sample	Soil respiration μgCO_2/g/7 days	Total No of microorganisms x10^6 CFU	Actinomycetes x10^4 CFU	Fungi x10^4 CFU	Ammonifiers x10^5 MPN	*Azotobacter* MPN	Oligonitrophiles x10^5 CFU
Soil samples with one or more heavy metal concentrations above MAC (samples>MAC)							
1	597.52	14.33	8.33	6.67	110.0	95	41.67
2	507.44	39.00	6.00	18.00	140.0	450	14.00
3	1019.84	20.00	4.00	8.00	110.0	25	22.33
4	670.35	12.67	0.67	2.33	110.0	450	55.67
5	676.72	13.00	2.00	9.33	110.0	95	73.33
6	813.13	35.00	11.00	12.67	110.0	450	67.00
7	929.53	24.00	3.67	11.67	140.0	110	58.33
8	406.89	18.00	0.67	7.67	25.0	95	86.00
9	540.01	29.00	0.33	7.33	45.0	45	54.67
10	699.19	28.00	2.00	13.33	45.0	250	73.33
11	617.02	27.67	1.33	27.67	140.0	45	113.33
Soil samples with all heavy metal concentrations below MAC (samples<MAC)							
1	711.47	10.33	0.33	18.67	140.0	9	25.67
2	606.26	43.00	5.67	15.67	140.0	250	59.67
3	588.49	23.33	0.67	14.00	110.0	45	17.33
4	807.55	30.00	11.67	8.00	140.0	150	43.67
5	933.84	19.33	1.00	15.00	15.0	150	72.00
6	506.27	17.67	0.67	19.00	110.0	9	42.33
7	823.31	34.00	0.33	9.00	140.0	25	59.33
8	264.92	27.00	2.00	11.00	140.0	25	53.33
9	531.97	20.33	0.33	23.00	45.0	25	38.00
10	642.39	18.00	22.33	9.67	140.0	250	43.67
11	414.88	8.00	0.67	18.67	45.0	45	33.33
12	619.56	27.00	2.33	8.33	140.0	250	66.00
13	564.39	18.33	0.33	26.33	140.0	25	84.33
14	597.41	18.00	0.33	10.00	110.0	25	76.00
15	806.96	67.00	0.33	16.00	140.0	25	115.33
Samples>MAC (n 11)							
Min	406.89	12.67	0.33	2.33	25.0	25	14.00
Max	1019.84	39.00	11.00	27.67	140	450	113.33
SD	181.85	8.91	3.48	6.82	41.18	175.82	28.01
Mean	679.79	23.70	3.64	11.33	98.64	191.82	59.97
Samples<MAC (n 15)							
Min	264.92	8.00	0.33	8.00	15.0	9	17.33
Max	933.84	67.00	22.33	26.33	140.0	250	115.33
SD	171.44	14.53	6.09	5.58	42.63	95.02	25.14
Mean	627.98	25.42	3.27	14.82	113.00	87.20	55.33
p	ns	ns	ns	ns	ns	ns	ns

MAC – maximum allowed concentrations for total heavy metals in agricultural soils; CFU – colony forming units; MPN – most probable number; CFU/g of dry soil or MPN/g of dry soil; SD – standard deviation; ANOVA for $p>0.05$ non-significant differences between soils (ns).

above the MAC for As, Ni and Pb, out of which 8, 5 and 3 samples had elevated contents of As, Ni and Pb, respectively (Table 2). The concentrations of As in all samples ranged from 4.3 to 43.4 mg/kg (mean 16.8±12.5), with elevated concentrations in 8 samples. The concentrations of Ni ranged from 12.3 to 64.4 mg/kg (mean 36.5±14.3), with elevated concentrations in 5 samples, while Pb was in the range from 13.0 to 184.1 mg/kg (mean 45.5±40.9), with 3 samples with heavy metal values above the MAC. Although below

Table 4. Significant Pearson's correlation coefficients between soil microbiological properties, soil chemical characteristics and heavy metal concentrations.

	Soil respiration	M.Total	Actino	Fungi	*Azotobacter*	Oligo	pH KCl	pH H_2O	Humus	P_2O_5	K_2O	N tot
pH KCl			0.480*	-0.565**	0.561**							
pH H_2O			0.428*	-0.582**	0.544**		0.973**					
Humus		0.534**				0.444*						
P_2O_5		0.412*			0.540**		0.554**	0.458*	0.436*			
K_2O		0.447*				0.464*			0.737**	0.480*		
$CaCO_3$	0.391*						0.637**	0.654**				
N tot		0.507**			0.410*	0.490*			0.951**	0.488*	0.806**	
As					0.426*		0.639**	0.662**				
Cd					0.827**					0.515**		
Cr					0.680**			0.412*	0.418*			0.538**
Cu					0.692**		0.441*	0.483*	0.430*	0.493*		0.564**
Ni				-0.394*	0.749**		0.599**	0.665**				0.450*
Pb					0.694**					0.402*		
Zn					0.744**		0.424*	0.424*		0.488*		0.440*
Fe					0.621**			0.436*	0.388*	0.413*		0.488*
Mn												
	As	Cd	Cr	Cu	Ni	Pb	Zn	Fe	Mn			
As												
Cd												
Cr		0.737**										
Cu	0.459*	0.764**	0.920**						0.459*			
Ni	0.533**	0.677**	0.885**	0.846**					0.533**			
Pb	0.408*	0.938**	0.712**	0.759**	0.655**				0.408*			
Zn	0.477*	0.928**	0.843**	0.902**	0.753**	0.927**			0.477*			
Fe		0.695**	0.893**	0.927**	0.749**	0.639**	0.836**					
Mn		0.400*	0.584**	0.610**	0.612**	0.466*	0.524**	0.566**				

M. Total – Total number of microorganisms; Actino – Actinomycetes; Oligo – oligonitrophiles; *$p<0.05$ significant correlation, **$p<0.01$ very significant correlation

the allowed limits, the concentrations of Cd, Cu and Zn were significantly higher in soil samples, with As, Ni and Pb concentrations above the MAC.

The soil samples were characterized by a slightly acidic or neutral reaction, with a medium level of humus and total N content (Table 1). The available P content varied widely from very poor to high, while the available K content was high to harmful. All soils were of the vertisol or fluvisol soil type, with a clay-loamy texture (data not presented). Considering all the chemical characteristics, only the pH of the soil differed significantly between the soil samples with and without elevated heavy metal concentrations, and it was higher in soils with elevated heavy metal concentrations (Table 1). Positive correlations were detected among most of the evaluated metals (Table 4), as well as with other soil chemical characteristics, such as the pH, N_{tot} and P_2O_5.

Soil microbiological properties

Soil respiration, as well as the number of all investigated microbial groups, did not significantly differ between the samples with heavy metal concentrations above and below the MAC (Table 3). The total microbial number ranged from 8.00-67.00x10^6 to 12.67-39.00x10^6 CFU/g of dry soils in samples with heavy metal concentrations below and above the MAC, respectively. In addition, there were no negative correlations between any of the microbial groups and heavy metals. The only exception was a negative correlation between the total number of fungi and the total Ni concentration (r=-394) (Table 4). Positive correlations between *Azotobacter* and most of the metals were noted. Soil respiration correlated positively with $CaCO_3$, the total number of microorganisms with humus, P, K, and the total N. Actinomycetes correlated

Table 5. Stepwise multiple regression of soil respiration and microbial groups and soil chemical properties ($p < 0.05$).

Microbial groups (Dependent variable)	Soil properties (Predictor)	R^2
Soil respiration	$CaCO_3$	0.391
Total microorganisms	Humus	0.534
Actinomycetes	pH KCl	0.480
Fungi	pH H_2O	**0.582 neg**
Azotobacter	Cd	0.827
	Cd, pH KCl	0.874
Oligonitrophiles	N_{tot}	0.490

R^2 – square of the coefficient of multiple correlation which indicates the proportion of the variance in the dependent variable that is predictable from the independent variable (predictor), **neg** – negative influence

positively with soil pH, while fungi correlated negatively with soil pH. Oligonitrophiles correlated positively with humus, K and total N (Table 4). Stepwise multiple regression analysis was used to investigate and model the relationships between the chemical and microbiological properties of soil samples, with the aim of identifying soil factors with the highest influence. Regression analyses of microbial groups and soil properties confirmed that the numbers of actinomycetes and fungi were mostly influenced by the pH, soil respiration by the $CaCO_3$, the total number of microorganisms by humus, while oligonitrophiles by the total N (Table 5). The number of *Azotobacter* was under positive influence of Cd, and to a lesser extent the pH value.

DISCUSSION

In this study, elevated concentrations of one or more heavy metals were detected in 42% of the investigated parcels under intensive agricultural production. In most samples (30%), total As concentrations were elevated above the MAC, Pb concentrations above the MAC were found in only 8% of samples, while Ni was slightly increased in 20% of the samples. Natural concentrations of As in soils are usually from 1 to 40 mg/kg, but can be much higher due to pesticide application or waste disposal [25]. In our research, the maximum detected As value was 43.4 mg/kg. Most solid foods contain low levels of As because plants (with some exceptions) hardly take up As from the soil, which indicates a low probability of food contamination due to soils exhibiting a small increase in As concentration, as observed in this research [26]. Pb concentrations in soil are usually in the range from 1 to 30 mg/kg, while the MAC is 300 mg/kg for soils with pH>5.5 [27]. Plants grown in soil with elevated Pb usually accumulate Pb in roots, while lower concentrations are expected in shoots [28]. The maximum total Ni concentration of 64 mg/kg detected here can be considered slightly increased, since the MAC of total Ni for agricultural soils is 50 mg/kg [17]; elsewhere in the world, the MAC can range from 20-60 mg/kg to 100 or 200 mg/kg [29]. Taking this into account, the soils with elevated heavy metal concentrations investigated here can be considered slightly contaminated.

In the group of samples with increased total concentrations of As, Ni, and Pb, the total concentrations of Cd, Cu, and Zn were also significantly higher. In addition, significant positive correlations were detected between almost all examined heavy metals. Elevated concentrations of metals were usually present in fluvisol soils; out of 11 fluvisol soils, elevated concentrations of heavy metals were present in 7. This can indicate the geological origin of heavy metals in some soils [15] and the low probability of inclusion of these metals in the food chain. However, further investigation of available heavy metal concentrations in soil and their concentrations in plants should be conducted.

In our research, there were no differences in soil respiration, nor in the number of investigated groups of microorganisms between soil samples with and without elevated concentrations of heavy metals. Soil respiration is generally considered an important indicator of soil health because it indicates the level of microbial activity, soil organic matter content and its decomposition [30]. In previous research, As contamination had little influence on soil respiration and no significant toxicity effect at 1200 mg/kg [31]. In the same study, no toxic response was observed for 500 mg/kg and more of Pb in soils. On the other hand, a negative correlation of soil respiration and different enzyme activities in agricultural soils with increased heavy metal concentrations (Pb, Ni, Cu, Cr, Cd, Co) was detected [11]. However, in this study, the concentrations of As, Pb and Ni were much higher than in our study. Ramsey et al. [32] reported that *in situ* soil respiration correlated negatively and linearly to the concentrations of As, Cd, Cu, Pb and Zn, but only when they were much higher than the background levels that influenced changes in the structures of the microbial communities. Previously, significant decreases in the total number of bacteria, fungi, actinomycetes and asymbiotic nitrogen fixers was detected in the contaminated site where the concentrations of As and Pb were much higher when compared to our research, and high levels of Hg and Zn were also determined (As 1558, Pb 270, Hg 109, Zn 165 mg/kg of dry soil) [33]. In our study, the only weak negative correlation was observed between the number of fungi and total Ni concentrations. Similar to our research, Lenart-Boroń and Wolny-Koładka [34] did not detect any effects of elevated concentrations of some heavy metals, mainly Zn and Pb, on the numbers of some microbial groups (mesophilic bacteria, *Azotobact*er, actinomycetes, fungi) in soils. Niemeyer et al. [10] observed a negative correlation between soil respiration and total concentrations of Pb, but not with Ni; in addition, ammonifiers correlated negatively with Cu, Fe, Mn, Zn, Cd, Cr, Pb, Ni.

Besides heavy metals, microbial communities are also influenced by other environmental factors [11]. One of the most important factors is soil pH [35, 36]. With pH decrease, the solubility of metals and their bioavailability increase. In our study, in samples with elevated metal concentrations, the pH was significantly higher as compared to the other samples; therefore, a lower availability of metals was expected and consequently a lower influence on microorganisms. Previously, soils with higher pH values were less sensitive to the toxicity of some metals in terms of soil-respiration response [31]. In our research, there was a positive correlation between soil respiration and $CaCO_3$ concentration. In addition, regression analyses indicated $CaCO_3$ as the most important factor that significantly accounts for variation in respiration (39%). A lower reduction in basal respiration in soils contaminated with Pb, Zn and Cu was detected in carbonate- compared to non-carbonate-containing soils [31], which indicates that respiration depends on soil type and present pollutants.

Positive correlations of *Azotobacter* numbers and all heavy metals (except Mn) were observed, including those with concentrations above the MAC (As, Ni, Pb) (Table 4). In addition, regression analysis of all soil parameters showed that *Azotobacter* was positively affected by increased Cd concentrations (82% variation explained). In previous studies, some heavy metals, such as As, Cd or Hg, positively influenced the number of bacteria, oligonitrophiles or fungi, but their concentrations were far below the MAC [37]. It was shown that the activity of certain soil enzymes increases in the presence of low concentrations of certain heavy metals, but that high metal concentrations inhibit their activity [38]. In our research, the investigated soils were either uncontaminated or only slightly contaminated, which could explain the established positive correlation between heavy metals and *Azotobacter*.

The total number of microorganisms and oligonitrophiles correlated with soil chemical properties, mainly with the available nutrients (humus, P, K, total N). The humus content significantly contributed to the variation in the total number of microorganisms (53%) and the total N to oligonitrophiles (49%). There was a significant positive correlation between the pH and the number of actinomycetes and *Azotobacter*, while a negative correlation was observed between the soil pH and the number of fungi. Additionally, the numbers of actinomycetes and fungi were affected most by the pH (48% and 58%, respectively). Similarly, the number of fungi correlated negatively with soil pH (r=-0.707), while the number of actinomycetes correlated positively with soil pH (r=0.520) [39]. Previous studies suggested that the changes in soil microbiological properties were related to soil properties (organic matter, P content) rather than to heavy metal concentrations [40]. In addition, some findings suggested that native soil bacterial populations might have adapted to altered soil conditions, which can also be the case in our study [41]. It is noted that long-term exposure of microbial communities to heavy metals can influence their tolerance and adaptation to soil contamination. Soil organic matter is considered the main factor for high microbial enzyme activities in such soils and mitigation of heavy metal toxicity [42].

In this study, negative effects of slightly elevated heavy metal concentrations on soil respiration and numbers of selected microbial groups were not detected. Soil chemical characteristics were shown to be factors that influence soil microbiological properties the most. Due to long-term presence of heavy metals in soil, microorganisms may have developed tolerance to their increased concentrations. The results of the study point out that heavy metal effects should be considered in the context of different soil properties, but further research should assess their contribution.

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