

Resistance of Arylsulfatase to Contamination of Soil by Heavy Metals

Jadwiga Wyszowska*, Karol Wieczorek, Jan Kucharski

University of Warmia and Mazury in Olsztyn, Department of Microbiology,
Plac Lodzki 3, 10-727 Olsztyn, Poland

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Abstract

Contamination of soils by heavy metals is a severe problem because of disturbances caused in the functioning of the soil and the whole ecosystem. Therefore, the aim of our research was to determine the influence of contamination of soil by zinc, copper, nickel, cadmium, and lead on the activity of arylsulfatase. A novel element of the study is a comparison of the response of arylsulfatase to five heavy metals in three soils with two pH ranges: 7.0 and 5.5. The studies were carried out under laboratory conditions. The soils (loamy sand, sandy loam, sandy clay loam), with pH 7.0 and 5.5 and contaminated with zinc, copper, nickel, cadmium, and lead were incubated for 30, 60, and 120 days. After each period, the activity of arylsulfatase was determined. Base on the activity of arylsulfatase, a resistance index of arylsulfatase (RS) to contamination with heavy metals was calculated. The highest activity of arylsulfatase was found in sandy clay loam, while the lowest was found in loamy sand. A significant correlation between the resistance of the enzyme and the level of contamination of the soil with heavy metals was proved. Arylsulfatase was most sensitive to the effects of zinc, copper, and nickel, while cadmium exerted a somewhat weaker influence and lead the least. The enzyme exhibited a higher resistance to the influence of heavy metals in neutral soils than in acidic soils. The toxic effect of heavy metals can be ordered as follows: zinc > copper > nickel > lead > cadmium.

Keywords: enzyme activity, environmental disturbance, soil contamination, trace elements

Introduction

Soil is a non-renewable natural resource. A great increase in living standards contributes to the degradation of soil [1] by worsening its physical, chemical, and biological properties [2, 3]. Contamination of soil by heavy metals is one of the most frequent, and thus one of the greatest, environmental nuisances [4, 5]. The problem of contamination of the soil with heavy metals has consti-

tuted a serious threat to the environment for many years [6]. It is estimated that in Europe formations contaminated with heavy metals [7] amount to 34.8% of degraded soils. Throughout the European continent, contents of zinc in 1 kg of soil oscillated in the range of 4÷2,832 mg, 1÷421 mg copper, 1÷2,565 mg nickel, 0.010÷23.60 mg cadmium, and 1.5÷5,200 mg lead [8]. The amount of heavy metals in the soil is closely correlated with the level of industrialisation. A key part in soil contamination with heavy metals is played by arsenic, cadmium, copper, mercury, nickel, lead, and zinc [8]. Expansive agriculture accelerates the degradation of agricultural areas, manifesting itself by a limitation of their productivity, and therefore

*e-mail: jadwiga.wyszowska@uwm.edu.pl

Table 1. Physico-chemical properties of soils used in the experiment.

Soil type ¹	Soil texture			C _{org} [g/kg]	pH _{KCl}	HAC	TEB	CEC	BS [%]
	[grain diameter, mm]								
	2 – 0.05	0.05–0.002	< 0.002						
	Content [%]								
[mmol(+)/kg DM of soil]									
LS	80	12	8	5.05	7.0	10.50	55.00	65.50	83.97
	80	12	8	5.05	5.5	21.75	47.00	68.75	68.36
SL	72	21	7	7.05	7.0	8.00	111.0	119.0	93.28
	74	16	10	5.75	5.5	25.50	17.00	42.50	40.00
SCL	56	23	21	14.33	7.0	6.00	190.0	196.0	96.94
	56	23	21	14.33	5.5	12.75	107.0	119.7	89.35

¹LS – loamy sand, SL – sandy loam, SCL – sandy clay loam

decreases in the yield and quality of crops [9]. Heavy metals constitute an actual danger for not only the natural environment, but also human health [10]. There are many causes (of an anthropogenic character) of the presence of heavy metals in soils, including wastewater management, using composts consisting of organic municipal waste containing heavy metals [11], utilisation of sewage sludge in agriculture, emissions of combustion gas into the atmosphere followed by deposition in the form of dusts to the soil, the formation of new landfills for municipal and industrial waste and the operation of existing ones [12], and the production and use of plant pesticides and fertilisers [13].

The activity of soil enzymes is considered one of the most important measures of soil quality [14]. Soil enzymes perform as catalysts, accelerating the reactions of metabolic processes that are parts of the cycles of biogenic elements, the biodegradation of pollutants (including limitation of the influence of heavy metals), maintaining homeostasis in the soil. The enzymes also participate in transforming chemical compounds into forms available for higher organisms [15].

More than 95% of total sulfur content in the soil occurs as esters and in organic form. In such a state, this element is unavailable for plants. Mineralisation of sulfur may be carried out by microorganisms, and therefore their role in the sulfur cycle is very important [16, 17].

Enzyme activity, including arylsulfatase activity, is one of the most often used parameters for evaluating soil conditions [18, 19]. Arylsulfatase (EC 3.1.6.1.) is an enzyme belonging to the class of hydrolases [18] responsible for the hydrolysis of sulfate esters containing an aryl. As a result of hydrolysis, sulphate (VI) ions are liberated according to the following equation: $R-C-O-SO_3^- + H_2O \rightarrow R-C-OH + SO_4^{2-} + H^+$. This enzyme plays an important role in the sulfur cycle, and is utilised as a measure for the mineralisation of this element in the soil [13]. Arylsulfatase is a very important enzyme because of its commonness in soil and delivery of sulfur to plants in the oxidation process. The high level of sulfurs VI sulfate and

nitrogen nitrates V in soil inhibit arylsulfatase activity [20]. Activity of arylsulfatase also depends on cultivated species of plants, soil type, soil use, and contamination with heavy metals [21].

The aim of our research was to evaluate the possibility of using the activity of arylsulfatase as a microbiological indicator of changes occurring in soil contaminated with heavy metals.

Experimental Procedures

Scope of the Experiment

The scope of our study included determining arylsulfatase activity in loamy sand (LS), sandy loam (SL), and sandy clay loam (SCL) subjected to contamination with Zn²⁺, Cu²⁺, Ni²⁺, Cd²⁺, and Pb²⁺. The soils were collected at the Tomaszkowo Didactic and Experimental Station of the University of Warmia and Mazury in Olsztyn (NE Poland, 53.7161° N, 20.4167° E). It was assumed that the experiments would be carried out in two pH ranges: 7.0 and 5.5; the LS, with a pH of 5.5, was subjected to alkalinizing with 1% aqueous suspension of CaCO₃, while the SCL, with pH of 7.0, was acidified using a 1 M aqueous solution of HCl. In consequence, the desired pH values were obtained in these soils: LS with a pH of 7.0 and 5.5, SCL with a pH of 7.0 and 5.5. A detailed description of the soils is presented in Table 1.

Conditions of the Experiment

The experiments were carried out under laboratory conditions with the following variable factors: a) type of soil texture: loamy sand – LS, sandy loam – SL, sandy clay loam – SCL; b) pH of the soil: 5.5, 7.0; c) heavy metals used: Zn²⁺, Cu²⁺, Ni²⁺, Cd²⁺, Pb²⁺; d) level of contamination of the soil with heavy metals: 0°, I°, II°, III°, IV°, V° (Table 2); e) incubation time of the soil: 30, 60, 120 days. 100 g of air-dried soil was weighed out to each of a

Table 2. Doses of heavy metals used in the experiment (mg/kg DM of soil).

Heavy metal	Soil contamination degree ¹					
	0°	I°	II°	III°	IV°	V°
Zn ²⁺	0	70	200	500	1500	5000
Cu ²⁺	0	25	50	80	100	500
Ni ²⁺	0	25	50	75	150	600
Cd ²⁺	0	0.5	1.5	3	5	10
Pb ²⁺	0	50	100	250	1000	5000

¹0° - natural content, I° - elevated content, II° - weak contamination, III° - medium contamination, IV° - strong contamination, V° - very strong contamination

series of 100 cm³ beakers, then the soil was contaminated with aqueous solutions of: ZnCl₂, CuCl₂, NiCl₂·6H₂O, CdCl₂·2½H₂O, and PbCl₂. For the sake of diversified toxicity of heavy metals, their permissible concentrations are various, and therefore the doses of the metals (Table 2) were defined based on the grades of soil usability for agricultural and non-agricultural purposes valid in Poland and developed by the Institute of Soil Science and Plant Cultivation in Puławy, Poland [22].

Detailed characteristics of the levels of contamination of the soil with heavy metals are presented by Wieczorek et al. [23]. Soil thoroughly mixed with the solution of heavy metals was brought to 50% of maximum water capacity, protected with plastic film, and placed in an incubator with a constant temperature of 25°C. During the whole duration of the experiment (120 days) the humidity level of the soil was checked weekly and water losses were complemented. After 30, 60, and 120 days, a part of the experiment was liquidated and the activity of arylsulfatase was determined. The experiment was carried out in nine repetitions.

Determination of Arylsulfatase Activity

The activity of arylsulfatase (EC 3.1.6.1) was determined according to Alef et al. [24]. Two g of the soil was weighed out to each of a series of 100 cm³ conical flasks, and 4 cm³ of acetate buffer with a pH of 5.8 was added. Then, 1 cm³ of 0.02 M potassium 4-nitrophenylsulfate (PNS) was added to each of the tested samples, and they were incubated for 1 hour at 37°C. After the incubation, 1 cm³ of 0.02 M PNS was added to the standard samples, then 25 cm³ of deionised water was added to every sample, and the suspension was filtered through hardened quantitative filter paper into 100 cm³ conical flasks. Then, 6 cm³ of the filtrate was collected to 20 cm³ test tubes and 4 cm³ of 0.5 M NaOH solution was added. Extinction of the filtrate was measured using a Perkin-Elmer Lambda 25 spectrophotometer ($\lambda = 420$ nm). The activity of arylsulfatase was reported as mM of 4-nitrophenol (PNP) obtained during 1 hour in 1 kg DM of soil.

Calculation of the Resistance Index of Arylsulfatase to Heavy Metals (RS)

Based on the activity of arylsulfatase, a resistance index of the enzyme (RS) to contamination with heavy metals was calculated. The following formula was used for the calculations [25]:

$$RS = 1 - \frac{2|D_0|}{C_0 + |D_0|}$$

...where C_0 is activity of arylsulfatase in the soil with natural contents of heavy metals, and D_0 is the difference between the activity of arylsulfatase in the soil with natural contents of heavy metals (and that in soil subjected to pressure of heavy metals for 30 days). The RS coefficient can range from -1 to +1. Value +1 means that there is no disturbance (full resistance). Lower values indicate a bigger influence of a balance-disturbing factor (lower resistance). Resistance index equal to 0 indicates 100% disturbance, while negative values show a disturbance manifesting itself by an increase in resistance in comparison to a non-contaminated object.

Determination of Physico-Chemical Properties of Soils

Granulometric composition of the soils was determined by densitometry using the Cassagrande method modified by Prószyński; hydrolytic acidity (HAC) of the soils and total exchangeable basic cations (TEB) were determined according to the Kappen method [26]; organic carbon content (C_{org}) was determined using the Tiurin method [27]; pH of the soils was determined by potentiometry, in a 1 M KCl suspension in a ratio of 2.5:1 to the soil [28]. Based on the HAC and TEB values, cation exchange capacity of the soils (CEC) and the degree of saturation of the soils with basic cations (BS) were calculated.

Determination of Heavy Metal Contents in the Soil

Determination of the heavy metal contents in soils was carried out using an AAS-30 apparatus from Carl Zeiss Jena, after extraction with a 1 M solution of hydrochloric acid [29, 30]. The contents of metals were determined at the following wavelengths: 214 nm for zinc, 328 nm for copper, 232 nm of nickel, 228 nm for cadmium, and 284 nm for lead (Table 3).

Statistical Calculations

The data were processed statistically using the ANOVA variance analysis module [31]. By carrying out Tukey's test at the significance level of $p = 0.01$, homogeneous groups were defined. Pearson linear correlation coefficients between the arylsulfatase activity and RS index, and the degrees of contamination of the soil with heavy metals were calculated.

Table 3. Measured concentrations of available content of heavy metals (mg/kg DM of soil).

Heavy metal	Soil type ²	pH	Soil contamination degree ¹					
			0°	I°	II°	III°	IV°	V°
			mg/kg DM of soil					
Zn ²⁺	LS	7.0	10.8	79.5	208.4	503.2	1450.3	4916.7
		5.5	10.8	81.2	212.6	513.3	1479.3	5015.0
	SL	7.0	16.6	81.7	209.0	504.1	1461.2	4920.6
		5.5	11.6	80.9	213.2	515.4	1485.6	5020.0
	SCL	7.0	13.0	82.8	213.8	503.2	1460.1	4921.5
		5.5	13.0	82.8	214.3	515.0	1484.3	5019.8
Cu ²⁺	LS	7.0	2.5	24.5	44.8	74.1	87.7	473.5
		5.5	2.5	24.9	45.9	77.3	93.4	487.9
	SL	7.0	3.8	25.8	46.1	75.4	89.0	474.8
		5.5	1.2	26.2	47.2	78.6	94.7	489.2
	SCL	7.0	3.6	25.6	45.9	75.2	88.8	474.6
		5.5	3.6	25.4	45.7	75.0	88.6	474.4
Ni ²⁺	LS	7.0	3.2	23.4	44.6	69.8	140.0	524.4
		5.5	3.2	24.3	46.8	71.2	151.2	567.6
	SL	7.0	4.6	24.6	45.8	71.0	141.2	525.6
		5.5	4.5	25.4	47.9	72.3	152.3	568.7
	SCL	7.0	3.7	24.1	45.3	70.5	140.7	525.1
		5.5	3.7	24.8	47.3	71.7	151.7	568.1
Cd ²⁺	LS	7.0	0.3	0.8	1.6	3.3	5.2	10.0
		5.5	0.3	0.8	1.7	3.4	5.3	10.2
	SL	7.0	0.3	0.8	1.7	3.2	5.3	10.4
		5.5	1.0	1.4	2.6	4.3	6.4	11.3
	SCL	7.0	0.2	0.7	1.6	3.1	5.2	10.3
		5.5	0.2	0.8	1.7	3.2	5.2	10.4
Pb ²⁺	LS	7.0	12.0	55.7	108.3	247.2	953.6	4921.0
		5.5	12.0	59.6	112.3	255.8	983.5	4978.5
	SL	7.0	16.0	59.2	111.8	250.7	957.1	4924.5
		5.5	9.3	62.8	114.5	259.0	986.7	4981.7
	SCL	7.0	11.9	63.4	114.0	254.9	961.3	4928.7
		5.5	11.9	65.3	114.9	259.8	997.2	4995.1

¹0° - natural content, I° - elevated content, II° - weak contamination, III° - medium contamination, IV° - strong contamination, V° - very strong contamination ²LS – loamy sand, SL – sandy loam, SCL – sandy clay loam

The activity and resistance of arylsulfatase to contamination of the soil with heavy metals were also presented in the form of graphs of primary component analysis (PCA). Based on the analysis of the effect measure η^2 by variance analysis (ANOVA) percentage shares of all variable factors affecting the activity of arylsulfatase were defined.

Results

Activity of Arylsulfatase

In the paper the results for arylsulfatase activity were shown in the form of average values of the three experiment dates, because the η^2 variance analysis indicated that the

Table 4. The analysis of the effect measure η^2 in soils contaminated with Zn^{2+} , Cu^{2+} , Ni^{2+} , Cd^{2+} , and Pb^{2+} .

Factor ¹	Zn^{2+}	Cu^{2+}	Ni^{2+}	Cd^{2+}	Pb^{2+}
	[%]				
a	5.54	3.57	4.08	1.65	13.97
b	41.52	51.42	30.37	25.58	5.55
c	2.59	1.75	11.31	13.56	3.87
d	7.25	2.64	3.22	0.77	10.26
ab	1.72	1.48	4.30	4.28	30.67
ac	0.03	0.21	0.25	0.52	0.47
bc	3.77	2.00	17.59	16.53	4.49
ad	0.78	0.31	0.21	0.60	4.65
bd	15.05	7.02	9.06	10.71	2.11
cd	6.32	8.62	5.21	6.93	1.43
abc	0.31	0.65	0.52	0.46	1.31
abd	1.22	1.39	0.53	0.40	5.85
acd	0.35	0.87	0.45	0.27	3.54
bcd	12.37	17.15	11.30	15.45	1.24
abcd	0.80	0.69	1.31	1.28	9.98
Error	0.38	0.24	0.29	1.02	0.62

¹a - soil contamination degree, b - soil type, c - soil pH, d - soil incubation period

duration of incubation of the soil affected the activity of the enzyme to the least degree (from 0.77 to 10.26%). The following factors affected the activity of the enzyme to higher degrees: the value of pH of the soil (from 1.75 to 13.56%), the type of soil texture (from 5.55 to 51.42%), and the degree of contamination of the soil with the heavy metal (from 1.65 to 13.97%) (Table 4).

The activity of arylsulfatase was the highest in SCL, next in SL, and the lowest in LS in uncontaminated soils, probably due to differences in organic carbon and exchangeable basic cations content and sorption capacity of soil. An increase of arylsulfatase activity was observed with the increasing value of these physicochemical parameters.

In the SCL with a pH of 7.0, a significantly higher activity of arylsulfatase was observed than in the other soils. In the SL with a pH of 7.0, the activity was twice as high as in the SL with a pH of 5.5, while the situation was opposite in the case of LS with a pH of 7.0 and LS with a pH of 5.5 (Table 5).

In the LS with a pH of 7.0, Zn^{2+} inhibited the activity of arylsulfatase in the range from 11 to 77%, Cu^{2+} – from 15 to 62%, Ni^{2+} – from 9 to 26%, and Cd^{2+} – from 12 to 26%. Contamination with Pb^{2+} did not reduce arylsulfatase activity, but on the contrary increased it. A I° contamination of the soil with this element caused a 300% increase in the arylsulfatase activity in comparison to the

soil with a natural Pb^{2+} content, II°-IV° a 98% increase, and VI° a 65% increase. The effect of Zn^{2+} and Cu^{2+} in the LS with a pH of 5.5 contributed to a decrease of arylsulfatase activity in the range from 31 to 94% and from 22 to 74%, respectively. Contamination with Pb^{2+} did not affect the activity of the enzyme unequivocally adversely, as the pollutant amount of 50 mg/kg DM of soil led to an increase in the arylsulfatase activity by 12%, and doses of 5,000 mg/kg DM of soil contributed to a 13% decrease in enzyme activity. At I° of contamination in the LS with a pH of 5.5, the effect of Ni^{2+} was more unfavourable than in the LS with a pH of 7.0. Higher doses of this metal decreased the arylsulfatase activity in the LS with a pH of 5.5 more than in the LS with a pH of 7.0. The effect of Cd^{2+} was significantly weaker in the LS with a pH of 5.5 than in the LS with a pH of 7.0, in which arylsulfatase activity was limited to 2-10% compared to 0°.

Arylsulfatase activity decreased in the SL with a pH of 7.0: from 36 (I°) to 93% (V°) in the case of Zn^{2+} , and from 23 (I°) to 35% (V°) in the case of Pb^{2+} (in comparison with the non-contaminated soil). Doses of 25 mg Cu^{2+} and 25 mg Ni^{2+} /kg DM of soil led to an increase in enzyme activity by 19% and 15%, respectively. Higher doses of Cu^{2+} and Ni^{2+} acted adversely – at V° they decreased arylsulfatase activity to 72% and 25%, respectively. Cd^{2+} had a more inverse effect than Zn^{2+} and Pb^{2+} , irrespective of its dose. A dose of 0.5 mg/kg DM of soil contributed to an increase of enzyme activity by 57%, and the highest dose – 20 mg/kg DM of soil – by 15% in comparison to soil with a natural amount of Cd^{2+} . In spite of this, arylsulfatase activity decreased together with an increase in the degree of contamination of the soil with this element.

The influence of pH of the soil affected the activity of arylsulfatase more clearly in the SL contaminated with Ni^{2+} and Cd^{2+} , where the presence of these metals in the SL with a pH of 5.5 adversely influenced the arylsulfatase activity to a larger extent. The value of the pH of the soil affected the activity of the enzyme in the SL contaminated with Zn^{2+} , Cu^{2+} , and Pb^{2+} to a smaller extent than the degree of contamination of the soil with these elements.

The introduction of Zn^{2+} , Ni^{2+} , Cd^{2+} , and Pb^{2+} to the SCL with a pH of 5.5 disturbed the action of arylsulfatase to a larger extent than their introduction to the SCL with a pH of 5.5. The activity of the enzyme decreased most greatly under the influence of contamination with Cd^{2+} and Pb^{2+} . Contamination of soil with Cd^{2+} and Pb^{2+} above I° decreased the arylsulfatase activity only slightly. In the case of Cu^{2+} , the response of the enzyme depended most strongly on the dose of the metal. I° and II° of contamination with Cu^{2+} increased the arylsulfatase activity in the SCL with a pH of 5.5, while contents below 50 mg/kg DM of soil decreased it more than in the SCL with a pH of 7.0. The effect of Zn^{2+} , Ni^{2+} , and Pb^{2+} was significantly stronger in the acidified soil, while the decrease in the arylsulfatase activity by contamination with Pb^{2+} was only slightly smaller in the SCL with a pH of 7.0 than in the SCL with a pH of 5.5.

As results from Fig. 1 show, primary components explain 93.34% of the original variables. These variables

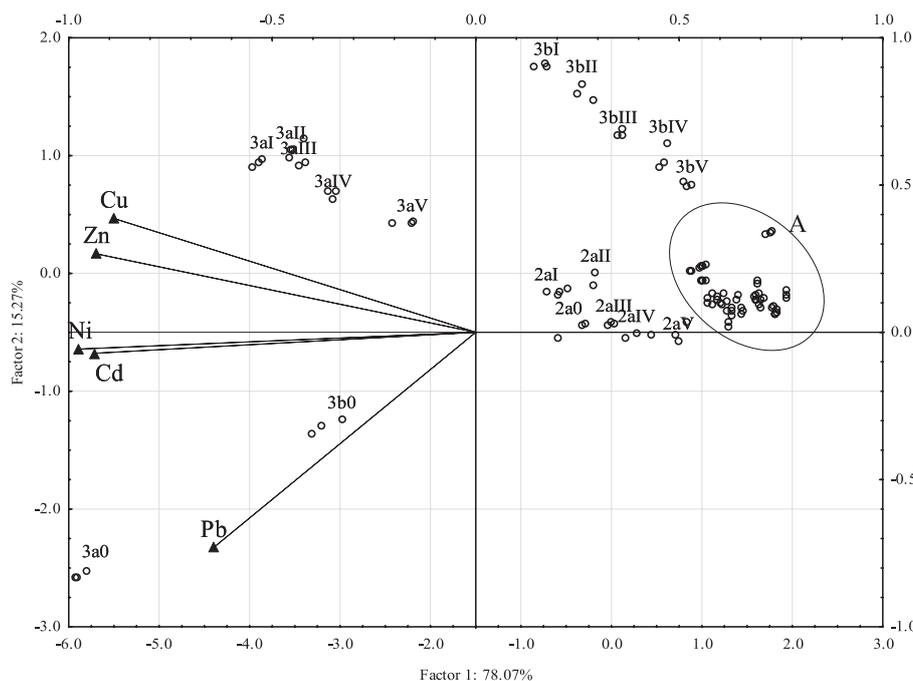


Fig. 1. Arylsulfatase activity in soils contaminated with Zn^{2+} , Cu^{2+} , Ni^{2+} , Cd^{2+} , and Pb^{2+} , presented with principal component analysis.

soil type: 1 - loamy sand, 2 - sandy loam, 3 - sandy clay loam; soil pH: a - pH 7.0, b - pH 5.5; soil contamination degree: 0 - 0°, I - I°, II - II°, III - III°, IV - IV°, V - V°

A: 1a0 - 1aV, 1b0 - 1bV, 2b0 - 2bV

are negatively correlated with the first primary component. The strongest positive correlation was found between the degrees of soil contamination with Ni^{2+} and Cd^{2+} , with a slightly weaker correlation between soil contamination with Zn^{2+} and Cu^{2+} . A different effect on the activity of arylsulfatase was found between Cu^{2+} and Pb^{2+} and between Zn^{2+} and Pb^{2+} . The influence of heavy metals on the arylsulfatase activity was the highest in the SCL with a pH of 7.0, then in the SCL with a pH of 5.5, SL with a pH of 7.0, SL with a pH of 5.5, LS with a pH of 5.5; the smallest was in the LS with a pH of 7.0.

RS Index

Arylsulfatase exhibited a higher resistance in the LS with a pH of 7.0 contaminated with Zn^{2+} , Cu^{2+} , and Ni^{2+} , while a higher resistance of the enzyme was found under the influence of contamination with Cd^{2+} and Pb^{2+} in the LS with a pH of 5.5 (Table 6). Contamination with Ni^{2+} did not significantly affect the resistance of arylsulfatase in the LS with a pH of 7.0, while resistance increased in the case of contamination of the same soil with Pb^{2+} .

In the SL, the resistance of arylsulfatase was more diversified. An acidic reaction decreased the resistance of arylsulfatase more strongly in the soil contaminated with Cd^{2+} and Pb^{2+} , with a neutral reaction in the soil contaminated with Zn^{2+} and Cu^{2+} . Acidification of the SL contaminated with Ni^{2+} in doses of 25 and 600 mg/kg DM of soil affected arylsulfatase resistance more adversely than on the SL with a pH of 7.0.

Irrespective of the doses of Ni^{2+} , Cd^{2+} , and Pb^{2+} , the resistance of arylsulfatase was by 75-91% lower in the SCL with a pH of 5.5 than the resistance in the non-contaminated soil. Increasing the doses of these metals did not increase the resistance of the enzyme significantly in comparison with I°. In the SCL with a pH of 7.0, these metals did not exhibit such a high reduction of the RS index in relation to the non-contaminated sample (from 8 to 38%). Contamination of SCL with a pH of 5.5 using Zn^{2+} in the amount of 70 mg/kg and Cu^{2+} in a dose of 25 mg/kg DM of soil caused a significant disturbance of the arylsulfatase resistance (52% and 82% decrease, respectively). The introduction of these metals corresponding to degrees II°-IV° did not reduce the resistance to such a large extent. Irrespective of the pH of the soil, contamination of the SCL with Zn^{2+} from II° to V° led to a gradual decrease in the resistance of arylsulfatase, while the same was obvious in the case of contamination with Cu^{2+} only at V°.

As results from Fig. 2 show, primary components explain 71.95% of the original variables. In the same soils, Ni^{2+} , Cd^{2+} , and Pb^{2+} affected the resistance of arylsulfatase similarly. On the other hand, a different influence on the resistance of arylsulfatase was found in the soil contaminated with Zn^{2+} . No correlation between Cu^{2+} and Ni^{2+} , Cd^{2+} and Pb^{2+} , and Zn^{2+} and Cu^{2+} was proven. The resistance of arylsulfatase was reduced most strongly by contamination with Cu^{2+} , then by Cd^{2+} , Ni^{2+} , and Zn^{2+} , and most weakly by Pb^{2+} . Also, a stronger influence of heavy metals on arylsulfatase resistance was found in acidic soils than in neutral soils, particularly in the SCL with a pH of 7.0.

Table 5. Activity of soil arylsulfatase in soils contaminated with Zn²⁺, Cu²⁺, Ni²⁺, Cd²⁺, and Pb²⁺ (mM PNP/kg/h DM of soil).

Soil type ²	Soil pH	Soil contamination degree ¹						r ³
		0°	I°	II°	III°	IV°	V°	
Zn ²⁺								
LS	7.0	0.130 ^a	0.116 ^b	0.114 ^b	0.063 ^c	0.043 ^d	0.030 ^e	-0.840 ^{**}
	5.5	0.241 ^a	0.167 ^b	0.114 ^c	0.066 ^d	0.025 ^e	0.015 ^e	-0.896 ^{**}
SL	7.0	0.417 ^a	0.267 ^b	0.232 ^b	0.157 ^c	0.043 ^d	0.031 ^d	-0.912 ^{**}
	5.5	0.228 ^a	0.173 ^b	0.133 ^c	0.077 ^d	0.040 ^e	0.037 ^e	-0.940 ^{**}
SCL	7.0	1.151 ^{ab}	1.158 ^a	1.093 ^{bc}	1.081 ^c	0.851 ^d	0.663 ^e	-0.212
	5.5	0.840 ^a	0.691 ^{bc}	0.731 ^b	0.628 ^c	0.362 ^d	0.225 ^e	-0.606 ^{**}
Cu ²⁺								
LS	7.0	0.130 ^a	0.110 ^a	0.099 ^{ab}	0.073 ^{bc}	0.071 ^{bc}	0.050 ^c	-0.717 ^{**}
	5.5	0.241 ^a	0.187 ^b	0.169 ^b	0.135 ^c	0.122 ^c	0.062 ^d	-0.855 ^{**}
SL	7.0	0.417 ^b	0.497 ^a	0.484 ^a	0.374 ^b	0.317 ^c	0.118 ^d	-0.594 ^{**}
	5.5	0.228 ^a	0.226 ^a	0.192 ^b	0.173 ^{bc}	0.163 ^c	0.131 ^d	-0.634 ^{**}
SCL	7.0	1.151 ^b	1.270 ^a	1.147 ^b	1.092 ^b	1.072 ^b	0.780 ^c	-0.174
	5.5	0.840 ^b	1.230 ^a	0.862 ^b	0.734 ^c	0.670 ^c	0.578 ^d	-0.412 ^{**}
Ni ²⁺								
LS	7.0	0.130 ^a	0.118 ^{ab}	0.105 ^{bc}	0.100 ^c	0.095 ^c	0.096 ^c	-0.465 ^{**}
	5.5	0.241 ^a	0.212 ^b	0.211 ^{bc}	0.196 ^{bcd}	0.192 ^{cd}	0.190 ^d	-0.397 ^{**}
SL	7.0	0.417 ^b	0.481 ^a	0.386 ^{bc}	0.361 ^{cd}	0.334 ^{de}	0.313 ^e	-0.529 ^{**}
	5.5	0.228 ^a	0.234 ^a	0.202 ^b	0.185 ^b	0.180 ^b	0.122 ^c	-0.598 ^{**}
SCL	7.0	1.151 ^a	0.878 ^b	0.882 ^b	0.853 ^b	0.849 ^b	0.759 ^c	-0.221
	5.5	0.840 ^a	0.276 ^b	0.233 ^{bc}	0.181 ^c	0.172 ^c	0.200 ^{bc}	-0.662 ^{**}
Cd ²⁺								
LS	7.0	0.130 ^a	0.114 ^{ab}	0.106 ^{bc}	0.095 ^c	0.099 ^{bc}	0.096 ^c	-0.497 ^{**}
	5.5	0.241 ^a	0.237 ^{ab}	0.218 ^{ab}	0.213 ^b	0.211 ^b	0.216 ^{ab}	-0.208
SL	7.0	0.417 ^b	0.653 ^a	0.604 ^a	0.553 ^{ab}	0.541 ^{ab}	0.481 ^{ab}	-0.015
	5.5	0.228 ^{ab}	0.243 ^a	0.225 ^{ab}	0.216 ^{ab}	0.229 ^{ab}	0.209 ^b	-0.079
SCL	7.0	1.151 ^a	0.913 ^b	0.890 ^b	0.899 ^b	0.868 ^b	0.873 ^b	-0.152
	5.5	0.840 ^a	0.254 ^{bc}	0.271 ^b	0.207 ^{cd}	0.207 ^{cd}	0.187 ^d	-0.586 ^{**}
Pb ²⁺								
LS	7.0	0.130 ^c	0.255 ^a	0.258 ^a	0.257 ^a	0.258 ^a	0.215 ^b	0.192
	5.5	0.241 ^b	0.270 ^{ab}	0.261 ^{ab}	0.286 ^a	0.254 ^b	0.210 ^c	-0.062
SL	7.0	0.417 ^a	0.320 ^{bc}	0.297 ^{bc}	0.340 ^b	0.328 ^b	0.273 ^c	-0.348 [*]
	5.5	0.228 ^b	0.246 ^{ab}	0.270 ^a	0.269 ^a	0.248 ^{ab}	0.216 ^b	-0.035
SCL	7.0	1.151 ^a	0.400 ^b	0.335 ^{cd}	0.325 ^{cd}	0.353 ^{bc}	0.302 ^d	-0.534 ^{**}
	5.5	0.840 ^a	0.180 ^b	0.147 ^d	0.180 ^b	0.161 ^c	0.162 ^c	-0.591 ^{**}

** significant for p = 0.01, * significant for p = 0.05, n = 53

The same letters in rows are assigned to homogenous groups (p < 0.01)

¹ 0° - natural content, I° - elevated content, II° - weak contamination, III° - medium contamination, IV° - strong contamination, V° - very strong contamination

² LS - loamy sand, SL - sandy loam, SCL - sandy clay loam

³ correlation coefficient between the soil contamination with heavy metal and arylsulfatase activity

Table 6. Arylsulfatase resistance index (RS) in soils contaminated with Zn²⁺, Cu²⁺, Ni²⁺, Cd²⁺, and Pb²⁺ in 30 days of incubation.

Soil type ²	Soil pH	Soil contamination degree ¹					r ³
		I°	II°	III°	IV°	V°	
Zn ²⁺							
LS	7.0	0.930 ^a	0.889 ^b	0.342 ^c	0.211 ^d	0.127 ^e	-0.934 ^{**}
	5.5	0.919 ^a	0.323 ^b	0.215 ^c	0.075 ^d	0.015 ^e	-0.903 ^{**}
SL	7.0	0.298 ^a	0.215 ^b	0.131 ^c	0.033 ^d	0.028 ^d	-0.958 ^{**}
	5.5	0.525 ^a	0.248 ^b	0.241 ^b	0.104 ^c	0.089 ^d	-0.893 ^{**}
SCL	7.0	0.864 ^a	0.576 ^b	0.450 ^c	0.174 ^d	0.090 ^e	-0.978 ^{**}
	5.5	0.476 ^c	0.734 ^a	0.669 ^b	0.368 ^d	0.144 ^e	-0.659 ^{**}
Cu ²⁺							
LS	7.0	0.866 ^a	0.792 ^b	0.322 ^d	0.449 ^c	0.289 ^e	-0.787 ^{**}
	5.5	0.805 ^a	0.560 ^b	0.390 ^c	0.348 ^d	0.193 ^e	-0.944 ^{**}
SL	7.0	0.451 ^a	0.448 ^a	0.388 ^b	0.376 ^b	0.078 ^c	-0.774 ^{**}
	5.5	0.547 ^d	0.920 ^a	0.651 ^b	0.545 ^d	0.565 ^c	-0.306
SCL	7.0	0.972 ^a	0.659 ^c	0.692 ^b	0.973 ^a	0.321 ^d	-0.573 [*]
	5.5	0.176 ^c	0.859 ^c	0.958 ^b	0.990 ^a	0.632 ^d	0.505 [*]
Ni ²⁺							
LS	7.0	0.991 ^a	0.702 ^b	0.708 ^b	0.671 ^c	0.701 ^b	-0.621 [*]
	5.5	0.822 ^{ab}	0.816 ^b	0.828 ^a	0.833 ^a	0.817 ^b	0.133
SL	7.0	0.804 ^a	0.782 ^b	0.755 ^c	0.676 ^d	0.601 ^e	-0.447
	5.5	0.603 ^d	0.903 ^b	0.954 ^a	0.871 ^c	0.267 ^e	-0.372
SCL	7.0	0.925 ^a	0.924 ^a	0.825 ^b	0.826 ^b	0.618 ^c	-0.848 ^{**}
	5.5	0.159 ^a	0.115 ^b	0.119 ^b	0.095 ^c	0.155 ^a	-0.136
Cd ²⁺							
LS	7.0	0.898 ^a	0.697 ^b	0.573 ^c	0.607 ^d	0.623 ^c	-0.640 ^{**}
	5.5	0.893 ^c	0.927 ^a	0.912 ^b	0.894 ^c	0.839 ^d	-0.374
SL	7.0	0.811 ^d	0.852 ^c	0.933 ^b	0.994 ^a	0.941 ^b	0.514 [*]
	5.5	0.285 ^d	0.364 ^c	0.454 ^b	0.280 ^d	0.531 ^a	0.496
SCL	7.0	0.819 ^a	0.829 ^a	0.832 ^a	0.716 ^c	0.776 ^b	-0.441 [*]
	5.5	0.180 ^d	0.236 ^b	0.206 ^c	0.253 ^a	0.257 ^a	0.773 ^{**}
Pb ²⁺							
LS	7.0	-0.271 ^a	-0.288 ^b	-0.264 ^a	-0.310 ^c	-0.317 ^c	-0.293
	5.5	0.161 ^b	0.154 ^b	0.105 ^d	0.118 ^c	0.372 ^a	0.494
SL	7.0	0.638 ^b	0.637 ^b	0.655 ^a	0.623 ^c	0.657 ^a	0.116
	5.5	0.443 ^a	0.334 ^d	0.367 ^c	0.367 ^c	0.388 ^b	0.001
SCL	7.0	0.651 ^d	0.729 ^b	0.769 ^a	0.758 ^a	0.686 ^c	-0.586 [*]
	5.5	0.133 ^b	0.088 ^c	0.140 ^{ab}	0.132 ^b	0.147 ^a	0.492

**significant for p = 0.01, *significant for p = 0.05, n = 14

The same letters in rows are assigned to homogenous groups (p<0.01)

¹0° - natural content, I° - elevated content, II° - weak contamination, III° - medium contamination, IV° - strong contamination, V° - very strong contamination

²LS - loamy sand, SL - sandy loam, SCL - sandy clay loam

³correlation coefficient between the soil contamination with heavy metal and resistance index

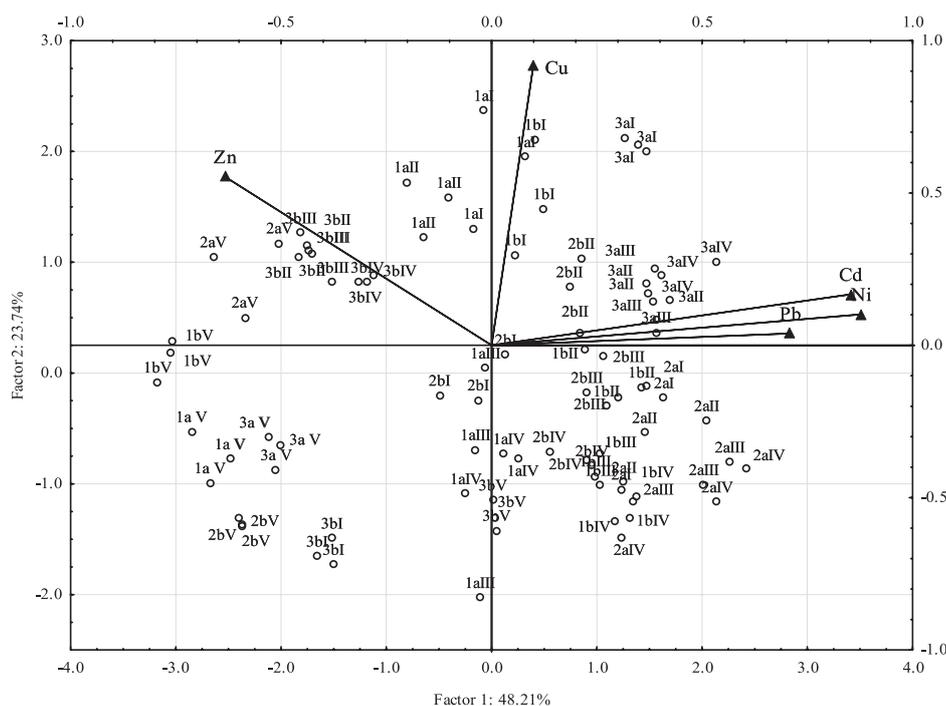


Fig. 2. Arylsulfatase resistance index in soils contaminated with Zn^{2+} , Cu^{2+} , Ni^{2+} , Cd^{2+} , and Pb^{2+} , presented with principal component analysis.

soil type: 1 - loamy sand, 2 - sandy loam, 3 - sandy clay loam; soil pH: a - pH 7.0, b - pH 5.5; soil contamination degree: 0 - 0°, I - I°, II - II°, III - III°, IV - IV°, V - V°

Discussion of Results

Soil enzymes play the important role of catalysts in processes occurring in the soil, biogeochemical cycles of biogenic elements, maintaining soil homeostasis, or in the biodegradation of pollutants. Enzymatic activity is also used for evaluating the influence of pollutants on the biological quality of soils [32]. Products of reactions catalysed by them may be growth substrates for plants and microorganisms [15]. Enzymes are a sensitive indicator of biological changes occurring in the soil [33]. Determination of activity of the soil enzymes and utilisation of their activities as parameters of quality is well-grounded for many reasons. The most important of them include: strong correlation with properties of the soil, such as organic matter, activity of microorganisms, and granulometric composition; faster response to changes in parameters of the soil; and comparatively simple and fast determination methods. Arylsulfatase is an enzyme strongly correlated with the activity of microorganisms [13], as well as the organic matter content in the soil [34].

The intensity of the hydrolysis of sulfate esters is negatively correlated with the contents of lead and copper in the soil, and to a smaller extent with the contents of zinc, nickel, and cadmium [35]. Nevertheless, an increase in contamination of the soil environment with zinc and cadmium may adversely affect arylsulfatase activity. Our research also proved a significant negative correlation between the content of zinc and arylsulfatase activity.

An inhibiting influence of heavy metals on the activity of arylsulfatase was observed, similarly as in the pre-

sented results, also in soil textures contaminated with copper [36]. Moreover, arylsulfatase activity depended on the type of soil texture: it is higher in loamy soils than in sandy soils [37].

Based on Tables 1 and 5, a distinct dependence of arylsulfatase activity on the physico-chemical properties of the soil may be ascertained. Decidedly, a higher activity of the enzyme was found in the SCL, in which the flammable parts content was approximately twice as high as in the other soils. A higher content of clay and silt fraction means an increase in the buffering properties of the soil textures, evident in the form of diversified C_{org} contents and cation exchange capacity. Thanks to this, the buffer capacity of the SCL is many times higher than that of sandy soils. Thus, the same load of Zn^{2+} , Cu^{2+} , Ni^{2+} , Cd^{2+} , and Pb^{2+} may affect the enzymatic activity in different soils to various degrees. A lower sensitivity of arylsulfatase in the soils with a higher content of SCL clay and silt fraction results from the fact that a part of the total enzyme content has probably been stabilised by soil colloids, and therefore protected from the influence of metals. In formations with conditions more favourable for the growth of microorganisms, the adverse effects of heavy metals may be significantly less noticeable and more brief than in poorer soils. Similarly as in our studies, the dependence between the activity of soil enzymes and the type of the soil texture was ascertained by Kolesnikov et al. [38].

Acidification of the soil may additionally intensify the effect of the pressure of heavy metals [39]. Low reaction of the soils may contribute to an increase in the mobility of heavy metals, and thus also to their availability to

soil microorganisms. This fact was confirmed in our studies, based on the differences in arylsulfatase activity in the SCL and SL with a pH of 7.0, and in the same soils with a pH of 5.5. Simultaneously, the artificial shift of pH of the soil textures contaminated with Zn^{2+} , Cu^{2+} , Cd^{2+} , and Pb^{2+} toward the alkaline reaction may also inhibit arylsulfatase activity [15], which was reflected in our studies by the differences between the activity of the enzyme in loamy sand with a pH of 7.0 (alkalised soil) and in that with a pH of 5.5.

Prolonged contamination of the soil with zinc not only decreases the activity of arylsulfatase, but also leads to a reduction of its resistance [40, 41]. However, the reaction of the soil has a significant influence on the resistance of the enzyme. Acidic soils contaminated with metals – with enhanced availability of these elements – disturb the action of arylsulfatase much more efficiently. As Fig. 2 shows, arylsulfatase was more sensitive to the influence of Zn^{2+} , Cu^{2+} , Ni^{2+} , Cd^{2+} , and Pb^{2+} both in moderately and highly contaminated soils with acidic reaction. At the same time, the effect is more pronounced in sandy formations than in loamy formations [36]. One may suppose that as a consequence of the significant dependence between the activity of arylsulfatase and its resistance, the addition of organic substances (i.e., cellulose, compost, manure, or bark) to soil contaminated with heavy metals may contribute to an improvement of both parameters [42].

Conclusions

Contamination of the soil with Zn^{2+} , Cu^{2+} , Ni^{2+} , Cd^{2+} , and Pb^{2+} in amounts exceeding their natural contents adversely affects arylsulfatase activity. The activity of arylsulfatase depends on the type of soil texture to the largest extent, then on the degree of soil contamination, pH of the soil, and to the smallest extent on the time of incubation of the soil.

The activity of arylsulfatase is significantly affected by the physico-chemical properties of the soil: content of silt fraction, content of organic carbon, and cation exchange capacity of the soil. Soils with a higher content of clay and silt fraction are characterised by a higher resistance of arylsulfatase to contamination with Zn^{2+} , Cu^{2+} , Cd^{2+} , and Pb^{2+} than sandy soils.

Soils with acidic reaction contaminated with Zn^{2+} , Cu^{2+} , Ni^{2+} , and Cd^{2+} are characterised by a lower resistance of arylsulfatase than soils with a neutral reaction.

The adverse effect of heavy metals on the activity and resistance of arylsulfatase may be ordered as follows: zinc > copper > nickel > lead > cadmium. Measurement of arylsulfatase activity is a valuable marker of soil quality because its activity is correlated positively with cation exchange capacity and negatively with heavy metals contamination.

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