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INFLUENCE OF INDUSTRIAL CONTAMINATION ON THE CHANGES IN THE ENZYMATIC ACTIVITY OF THE SOILS

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Summary. The enzymatic activity of the soil allows to recognize unfavorable changes in the soil and could be an indicator of the degradation processes. Soil in the neighborhood of 5 different industrial plants located in southeastern Poland was the research subject. The industrial plants were: Machine Factory in Strzyżów (alluvial soil); Thermal-Electric Power Station in Załęż (alluvial soil); Screw Factory in Łańcut (alluvial soil); Magnesite Plant in Ropczyce (gray-brown podzolic soil); and Glassworks in Krosno (brown soil). The soil samples were taken from sides 150-200 m, 250-300 m, and 350-400 m away from the contamination source.

The soil near the Machine Factory had the highest activity of the investigated enzymes (dehydrogenase; phophoatase; urease; protease). The lowest enzymatic activity was observed in the soil around the Thermal-Electric Power Station. The soil from the sites located 150-200 m away from the contamination source had the highest content of lead, zinc, copper, and chromium. The enzymatic activity, as well as the content of heavy metals in the soil were directly proportional to the distance from the contamination source in all the investigated sites.

Keywords: soils, heavy metals, enzymatic activity, soil contamination.

INTRODUCTION

Industrial contamination caused that heavy metals are included in the processes of biogeochemical circulation of elements. This deviates the ecological balance reflecting upon the biodiversity of ecosystems. Heavy metals are major factors in the contamination and devastation of the soil environment due to very little chance of their biodegradation [1, 3, 22]. The result of the activity of heavy metals is related to the physical-chemical properties of the soil, content of humus compounds, and the metal itself [14]. From among the physicochemical properties that regulate the mobilization of heavy metals, the first one to be mentioned is the soil reaction [9]. In Poland, the greatest areas of soils contaminated with heavy metals can be found in highly industrialized regions [4].

The aim of the research was to evaluate the impact of the soil environment contamination with some of the heavy metals (Pb, Cu, Zn, Cr) on the changes in the enzymatic activity of the soil in the regions affected by industrial plants located in southeastern Poland. Soil enzymes are very sensitive to environmental stress [20] which means that the enzymatic activity of the soil is used for evaluating the degree of environment pollution [10]. The activity of soil enzymes is a sensitive indicator that shows the range of soil degradation and allows to recognize the consequences of the environmental processes of soil degradation [8].

Investigated was the activity of the enzymes that are essential for the transformation of organic carbon compounds: dehydrogenase; phosphorus compounds: phosphatase; nitrogen compounds: urease and protease.

EXPERIMENTAL PROCEDURES

The research included the humus horizons of the soils within the range of the activity of the following industrial plants: Machine Factory in Strzyżów (alluvial soil); Thermal-Electric Power Station in Załęż (alluvial soil); Screw Factory in Łańcut (alluvial soil); Magnesite Plant in Ropczyce (gray-brown podzolic soil formed from silt parent material); and Glassworks in Krosno (brown soil developed from boulder clay). Three research spots were established in each of the selected sites, 150-200; 400-450; and 500-550 m away from the pollution emitters in the direction of most winds considering the relief.

The soil samples were taken from the depth of 0-25 cm in the second decade of September, 1998. The activity of the investigated enzymes was analyzed in the soil of natural moisture and the results were converted into dry soil mass. The activity of the following enzymes was measured: dehydrogenase [19], phosphatase [18], urease [23], protease [12]. The physico-chemical properties of the investigated soils were measured using the following methods [13]: lead, copper, zinc, and chromium (total forms – digestion HF and $HClO_4$ and form soluble in 1 M HCl) using the spectrophotometer of atomic absorption (ASA);

soil texture with the Bouyoucose-Casagrande method modified by Prószyński; the reaction was measured potentionmetrically in 1 M KCl; the total of the exchangeable bases (TEB) and cation exchange capacity (CEC) were measured in the NH_4Cl extraction (pH – 8,2) with calculating the degree of the base saturation (BS).

RESULTS AND DISCUSSION

The alluvial soils, on which the study sites were located, near the Machine Factory, Thermal-Electric Power Station, and the Screw Factory had differentiated physical-chemical properties (Tab. 1). Those soils had different granulometric composition. Generally, silt formations and medium silty loam containing from 25% to 51% of silt and clay. The highest acidity (4.5 – 4.8 pH_{KCl}) was found in the alluvial soils around the Thermal-Electric Power Station and the lowest acidity (6.7 – 7.1 pH_{KCl}) - in the alluvial soils around the Machine Factory. An average content of organic carbon (TOC) in the soil from the Power Station site was about 8-22% lower than the content of that element in the alluvial soil from the Screw Factory and the Machine Factory sites. Great differentiation of the CEC (from 13 to 46 cmol(+)/kg) and the TEB (from 10 to 45 cmol(+)/kg) was found in the investigated soils. In the alluvial soils from the Machine Factory, Screw Factory, and Thermal-Electric Power Station sites, the degree of the BS: over 95%, 83-95%, and 74-75%, respectively.

The gray-brown podzolic soil from the Magnesite Plant site contained on average 53% of silt fractions; 25% of silt and clay; and 21% of sand fractions. Therefore, according to the PN-78/9180-11, it is regular silt. The reaction of that soil in particular study sites ranged from acid to neutral. The content of organic carbon in the 0-25 cm layer was low (from 0.87% to 1.08%). The BS ranged between 63-78%. The soil showed low CEC and TEB (Table 1).

The soil texture of the investigated horizon of the brown soil from the Glassworks site allows to classify it as medium silty loam with a 37-41% clay content. The soil had high acidification (4.0 – 4.5 pH_{KCl}) and improper sorption properties (Tab. 1).

In all the study sites a decrease in the soil pH value with an increasing distance from the industrial plants was observed. The registered differences stayed between 0.2 and 1.7 pH units (Tab. 1). An increased pH value in the soils with greater content of heavy metals was also observed by Januszek [10].

Table 1. Some of the physicochemical properties of the soil of the investigated sites
Tabela 1. Wybrane właściwości fizykochemiczne gleb badanych obiektów

Site	Distance	Percent of particles			pH_{KCl}	TOC	BS	CEC	TEB
		1-0.1	0.1-0.02	< 0.02					
	[metres]	[mm]				[%]		[cmol(+)/kg]	
Thermal-Electric Power Station in Załęż	150-200	18	51	31	4.8	0.78	76.1	13.2	10.0
	300-350	14	50	36	4.6	1.10	74.5	14.0	10.4
	500-550	13	36	51	4.5	1.63	74.9	17.9	13.4
Magnesite Plant in Ropczyce	150-200	24	49	27	6.6	0.87	63.0	8.1	5.1
	300-350	21	54	25	5.5	1.01	70.7	8.7	6.2
	500-550	20	57	23	4.9	1.08	78.5	8.5	6.7
Screw Factory in Łańcut	150-200	28	43	29	6.7	1.21	94.9	25.6	24.3
	300-350	23	44	33	5.6	1.48	86.1	18.9	16.3
	500-550	17	44	39	5.0	1.81	83.6	14.8	12.4
Glassworks in Krosno	150-200	20	39	41	4.5	1.47	67.7	10.2	6.2
	300-350	26	36	38	4.3	1.43	59.2	10.7	6.1
	500-550	28	35	37	4.0	1.10	55.9	11.2	5.4
Machine Factory in Strzyżów	150-200	25	50	25	7.1	1.12	98.6	46.2	45.6
	300-350	17	52	31	6.9	1.23	97.8	29.2	28.6
	500-550	18	39	43	6.7	1.79	96.2	24.7	23.8

Data presented in Tab. 2 and 3 indicates that the content of the analyzed heavy metals in the soil was different depending on the investigated site, the distance from the emitter, the metal itself, and the form of the metal (total or soluble in 1 M HCl). According to the norms given by Kabata-Pendias and Pendias [11], the soils within the range of the pointed out industrial plants showed natural of slightly higher content of Pb, Cu, and Zn. Szerszeń et al. [17] proved on the basis of many years of monitoring the content of heavy metals in the soils around Copper Smelting Plant in Głogów and Legnica, that threat from Copper Smelting Plant did not increase because the inflow of heavy metals to soil had been kept at a certain level. These authors also indicate that does not mean that the state of the soil environment is improving because the heavy metals accumulated in the soil will still be a threat for a long, but hard to estimate, period of time. Kabata-Pendias and Pendias [11] point out that every substance of higher concentration of an element in relation to its natural content, is a possible threat of soil contamination.

Table 2. Content of total forms of Pb, Cu, Cr, and Zn in the soil of the investigated sites
Tabela 2. Zawartość form całkowitych Pb, Cu, Cr i Zn w glebie badanych obiektów

Site	Distance [metres]	Pb	Cu	Cr	Zn
		[mg/kg]			
Thermal-Electric Power Station in Załęż	150-200	21.1	16.5	35.5	55.6
	400-450	22.4	16.6	37.4	56.2
	500-550	23.1	17.5	38.7	54.7
	$NIR_{0.01}$	r. n.	r. n.	r. n.	r. n.
Mean		22.2	16.8	37.2	55.5
Magnesite Plant in Ropczyce	150-200	17.5	7.0	382.1	39.5
	400-450	16.1	6.1	194.6	37.8
	500-550	15.4	5.5	109.2	36.4
	$NIR_{0.01}$	2.0	1.3	180	r. n
Mean		16.3	6.2	228.6	37.9
Screw Factory in Łańcut	150-200	36.9	41.3	45.8	106.4
	400-450	22.6	16.9	30.9	75.8
	500-550	21.6	20.0	41.1	58.2
	$NIR_{0.01}$	r. n.	9.5	8.5	33.8
Mean		27.0	26.0	39.2	80.1
Glassworks in Krosno	150-200	75.1	13.4	41.6	105.5
	400-450	42.1	11.3	36.5	57.5
	500-550	37.9	9.7	32.3	55.6
	$NIR_{0.01}$	19.6	2.8	8.2	18.5
Mean		51.7	11.4	36.8	72.8
Machine Factory in Strzyżów	150-200	24.6	21.2	36.0	57.9
	400-450	17.6	14.2	29.0	39.8
	500-550	17.1	12.8	26.7	35.8
	$NIR_{0.01}$	3.2	1.8	3.7	4.1
Mean		19.7	16.0	30.5	44.5

r.n. – non significant differences

The greatest content of the analyzed forms of lead was found in the soil from the Glassworks site. In that soil, the average content of total and soluble (in 1 M HCl) forms of Pb was statistically importantly higher (2 and 3 times respectively) than their content in the soil from other study sites. According to Jackowska [9], Pb creates many complex ions. Those complexes are not durable. When the concentration of lead chloride solutions increases, they precipitate as non-soluble sediment. In case of a surplus of chloride ions, this sediment dissolves again.

Table 3. Content of soluble forms of Pb, Cu, Cr, and Zn in the soil of the investigated sites

Tabela 3. Zawartość form rozpuszczalnych Pb, Cu, Cr i Zn w glebie badanych obiektów

Sites	Distance [metres]	Pb	Cu	Cr	Zn
		[mg/kg]			
Thermal-Electric Power Station in Załęż	150-200	10.2	7.7	6.2	19.2
	400-450	10.8	8.4	6.3	19.6
	500-550	9.9	8.4	6.9	18.9
	$NIR_{0.01}$	r. n.	r. n.	r. n.	r. n.
Mean		10.3	8.1	6.4	19.2
Magnesite Plant in Ropczyce	150-200	9.6	2.1	8.6	9.5
	400-450	8.4	1.4	7.4	8.2
	500-550	7.3	1.3	5.5	7.2
	$NIR_{0.01}$	r. n.	0.4	1.1	r. n.
Mean		8.4	1.6	7.1	8.3
Screw Factory in Łańcut	150-200	23.3	18.9	4.7	47.4
	400-450	19.9	10.0	3.2	31.1
	500-550	17.2	7.1	2.5	15.9
	$NIR_{0.01}$	r. n.	7.7	2.0	20.8
Mean		20.1	12.0	3.4	31.4
Glassworks in Krosno	150-200	57.0	6:1	4.1	43.9
	400-450	32.5	4.5	3.3	31.9
	500-550	21.3	4.6	3.0	14.7
	$NIR_{0.01}$	14.7	0.7	0.9	8.3
Mean		36.9	5.0	3.4	30.1
Machine Factory in Strzyżów	150-200	10.5	8.2	5.9	15.2
	400-450	7.6	6.1	4.5	11.5
	500-550	7.1	5.4	4.4	10.9
	$NIR_{0.01}$	1.0	1.0	0.7	4.9
Mean		8.4	6.5	4.9	12.5

The greatest Cu content (total and soluble in HCl forms) was found in the soil from the Screw Factory site. The content of that element in the soil of other sites did not vary significantly.

In the soil from the Magnesite Plant site, an average content of the total forms of Cr was 228.6 mg/kg and was 6 times higher than in the soil of the other study sites. The range of the medium concentrations of chromium in Polish soils is from 7 to 80 mg/kg depending on the soil type [11]. The content of the soluble (in 1 M HCl) forms of Cr in the soil of that site was only 37% higher than the content of that element in the soil of the other sites. The chemistry of chromium is complex due to the occurrence of its compounds in various levels of oxidation, as well as due to creation of numerous complex ions [21, 23]. In soils, chromium occurs mainly in the Cr^{3+} form and precipitates in the shape of the oxide mixture of Cr^{3+}, Fe^{3+}. The Cr^{3+} compounds are weakly soluble only in very acid solutions, and at pH 5.5 they undergo total precipitation. Cr^{6+} is easily soluble in both acid and alkaline soils [11]. The reaction and the redox potential are main factors affecting the forms and sorption of chromium in soils. Also the organic substance in soil may increase the process of Cr^{6+} reduction to Cr^{3+}. An easy reduction of the soluble Cr^{6+} to the weakly soluble Cr^{3+} in the soil environment favours immobilization of that element [2].

An average content of Zn in the soil of the investigated sites (total and soluble in 1 M HCl forms) stayed within the following range: 37.9-80.1 and 8.3-31.4 mg/kg respectively. The greatest content of the analyzed forms of zinc was stated in the soil from the Glassworks and Screw Factory sites. The lowest content of that element was found in the Magnesite Plant site.

The concentration of Pb, Cu, Cr, and Zn in the soil usually significantly decreased with the distance from the emitter. In the Thermal-Electric Power Station area, no significant influence of the distance on the content of the investigated heavy metals was stated. The content of Pb in the soil from the Screw Factory site and the content of Zn in the soil from the Magnesite Plant site showed weak dependency on the distance from the emission source. The stated differences were not statistically important.

The intensity of the investigated enzymatic processes depended on the source of contamination, distance from the emitter and on individual properties of enzymes (Tab. 4).

The greatest enzymatic activity was found in the soil from the Machine Factory site, while the lowest activity was stated in the soil from the Glassworks site. In the soil from the Glassworks site, the content of Pb and Zn was usually higher than in the other study sites. The inflow of heavy metals into the soil causes changes in the quantity and quality of the composition of the soil microflora, which leads to weakening of the enzymatic activity [4,5]. Causes of the enzymatic activity depression in the soil from the Glassworks site could lie in increased content of Zn in that soil (Table 2, 3). Research carried out by Januszek [10] indicates that zinc is more toxic to microorganisms and soil enzymes than other heavy metals.

Table 4. Enzymatic activity of the soil of the investigated sites
Tabela 4. Aktywność enzymatyczna gleb badanych obiektów

Obiekt	Distance [metres]	ADh	AF	AU	AP
Thermal-Electric PowerStation in Załęż	150-200	0.79	49.3	431.8	16.7
	400-450	0.77	48.9	432.6	16.2
	500-550	0.72	47.9	439.2	16.0
	$NIR_{0.01}$	r. n.	r. n.	r. n.	r. n.
Mean		0.76	48.7	434.5	16.3
Magnesite Plant in Ropczyce	150-200	0.45	18.2	442.4	13.9
	400-450	0.97	22.3	492.6	17.5
	500-550	1.10	39.7	571.3	19.8
	$NIR_{0.01}$	0.02	0.1	3.5	0.1
Mean		0.84	26.7	502.1	17.0
Screw Factory in Łańcut	150-200	1.22	37.3	396.7	15.8
	400-450	0.90	48.9	422.5	18.6
	500-550	0.47	74.2	508.1	20.3
	$NIR_{0.01}$	0.03	0.2	2.1	0.1
Mean		0.86	53.4	442.4	18.2
Glassworks in Krosno	150-200	0.51	26.4	383.6	10.7
	400-450	0.33	24.7	384.9	10.5
	500-550	0.32	21.2	383.2	9.1
	$NIR_{0.01}$	0.02	0.3	r. n.	0.1
Mean		0.38	24.1	383.9	10.1
Machine Factory in Strzyżów	150-200	2.67	54.8	732.5	24.8
	400-450	4.12	95.7	962.1	31.4
	500-550	9.65	132.4	1187.2	37.6
	$NIR_{0.01}$	0.04	0.5	17.5	0.5
Mean		5.48	94.3	960.6	31.2

ADh - activity of dehydrogenase in μg of TPF/1 g of soil/24h; AF - activity of phosphatase in μg of p-nitrophenol/1 g of soil/1h; AU - activity of urease in μg of $N\text{-}NH_4^+$/1 g of soil/24h; AP - activity of protease in μg of tyrosine/1 g of soil /1h.

That author proved that in forest soils one can expect toxic impact of heavy metals on both the microorganisms and soil enzymes even at very low concentration (10-15 mg/kg) of soluble forms of zinc measured in 1 M HCl. After 18 months of incubation of sandy soil with heavy metals, Doelman and Haanstra [5] determined their ecological doses ED_{50} and stated the following order of their effect on limiting the activity of soil enzymes: Zn>Cu>Cd>Ni>Pb>Cr. Changes in the enzymatic activity of the soils in the chosen industrial plant sites under the impact of soil environment contamination with heavy metals clearly depended on the ecological properties of those soils. The activity of the enzymes in the soil from the Screw Factory site was higher than in the soil from the Glassworks site, despite the similar Zn content in the soil of both sites. The scope of the negative impact of the contamination on the soil from the Glassworks area was multiplied by its improper sorption properties (Tab. 1). Siuta [16] stated that the resistance of soil to degradation depended on the CEC and the TEB.

The inhibition of the enzymatic activity of the soil from the Glassworks and the Thermal-Electric Power Station sites was accompanied by strong acidification of those soils (Tab. 1). The soil reaction is the main factor that regulates the assimilability of heavy metals in soils [10]. The exchangeable fractions of metals in soils are easily assimilable by soil microorganisms, which causes changes in the quantity and quality of their composition and leads to a decrease in the activity of the enzymes [4]. Frankenberger and Johanson [6] point out that a great decrease in the enzymatic activity of the soil caused by its acidification could result from the destruction of hydrophobic, ion, and hydrogen bonds, which leads to irreversible loss of the second-row structure of enzymatic protein.

The decrease in the activity of the enzymes in the soil from the Magnesite Plant site was most probably caused by high content of chromium in the soil (Tab. 2). The mobile forms of chromium have a harmful impact on the soil microorganisms by limiting their biological activity [2]. The mechanism of the toxic impact of chromium on the soil microorganisms is based on the reaction with protein, nucleic acid, or nucleotides, which may be the cause of the cell's death or changes in its genetic apparatus [7].

From among all the investigated enzymes, the activity of dehydrogenase was the most sensitive indicator of the soil contamination. In the soil from the Machine Factory site there was a higher (*=0.01) dehydrogenase activity than in the soil from the sites around: Glassworks; Thermal-Electric Power Station; Magnesite Plant; and Screw Factory, respectively: 14, 7, 6.5 and 6 times. The activity of phosphatase, urease, and protease in the soil from the Machine Factory was on average 2 or 3 times higher than the activity of those enzymes in the soil from other study sites. The activity of dehydrogenase in soil is considered a good ecotoxicological index [10].

The influence of distance from the contamination source on changes in the activity of the investigated enzymes was differentiated depending on the site and individual properties of the enzymes (Tab. 4). In the soil from the Glassworks site, the activity of dehydrogenase, phosphatase, and protease decreased significantly with the distance from the emitter. No significant differences were only found in the case of urease. Similar tendencies were observed in the soil from the Thermal-Electric Power Station, but the registered differences were not statistically important. Alleviation of the results of the harmful activity of heavy metals in the study sites closest to the contamination sources could have occurred in result of the increased pH value of the soils containing more heavy metals (Tab. 1) This suggestion is confirmed by the research carried out by Olszowska [15]. The enzymatic activity of the soil from the Machine Factory site, which had stable reaction, significantly increased with the distance from the emitter. The activity of the investigated enzymes in the site located at 500-550 m away from the Factory was several times higher than their activity in the site closest to the emitter (150-200 m). Also in the soil from the Magnesite Plant site (with high content of Cr) the activity of the enzymes increased significantly with the growing distance from the emitter. Similar changes in the intensity of the activity of the investigated enzymes, except dehydrogenase, were registered in the soil from the Screw Factory site (with higher content of Zn). The observed changes in the enzymatic activity of the investigated soils result from the differentiated ecological properties of those soils, as well as great variety of microorganisms, variety of their reactions, resistance, and sensitivity to various natural and antropogenic factors.

CONCLUSION

1. The enzymatic activity of the soils around the selected industrial plants usually decreased along with increasing content of Pb, Cu, Cr, and Zn in the soil.
2. Changes in the activity of the investigated enzymes (dehydrogenase, phosphatase, urease, protease) under the influence of soil environment contamination with heavy metals clearly depended on the ecological properties of those soils.
3. The highest enzymatic activity was observed in the soil from the Machine Factory site.

4. The depression in the enzymatic activity was stated in the soil from the Glassworks site. The scope of the negative impact of contamination on the soil from that area was multiplied by its improper sorption properties and high acidity.
5. High content of chromium in the soil from the Machine Factory decreased its enzymatic activity.
6. The activity of dehydrogenase was the most sensitive indicator of soil contamination from among all the investigated enzymes.
7. The influence of distance from the contamination source on changes in the activity of the investigated enzymes was different depending on the site, soil quality, and individual properties of the enzymes.

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WPŁYW ZANIECZYSZCZEŃ PRZEMYSŁOWYCH NA ZMIANY AKTYWNOŚCI ENZYMATYCZNEJ GLEB

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Streszczenie. Zbadano zawartość wybranych metali śladowych (Pb, Cu, Zn i Cr) i aktywność enzymatyczną gleb z rejonów oddziaływania pięciu zakładów przemysłowych zlokalizowanych na terenie południowo-wschodniej Polski. Celem podjętych badań była ocena wpływu zanieczyszczeń środowiska glebowego niektórymi metalami śladowymi na zmiany aktywności enzymatycznej gleb. Enzymy glebowe są bardzo wrażliwe na stresy środowiskowe, w związku z czym aktywność enzymatyczna gleby jest stosowana do oceny stopnia zanieczyszczenia środowiska. Aktywność enzymatyczna gleb w rejonach wytypowanych zakładów przemysłowych zmniejszała się na ogół wraz ze wzrostem zawartości Pb, Cu, Cr i Zn w glebach. Zmiany aktywnosci badanych enzymów (dehydrogenaz, fosfataz, ureazy i proteazy) pod wpływem zanieczyszczeń środowiska glebowego metalami ciężkimi uzależnione były wyraźnie od właściwości ekologicznych tych gleb. Aktywność dehydrogenaz, spośród wszystkich badanych enzymów, była najbardziej czułym wskaźnikiem zanieczyszczenia gleb.

Słowa kluczowe: gleby, metale śladowe, aktywność enzymatyczna, zanieczyszczenia gleb.