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EVALUATION OF THE SOIL ENZYMES ACTIVITY AS AN INDICATOR OF THE IMPACT OF ANTHROPOGENIC POLLUTION ON THE NORWAY SPRUCE ECOSYSTEMS IN THE SILESIAN BESKID

OCENA AKTYWNOŚCI ENZYMÓW GLEBOWYCH JAKO WSKAŹNIKA WPŁYWU ZANIECZYSZCZEŃ ANTROPOGENNYCH NA FUNKCJONOWANIE EKOSYSTEMÓW LASÓW ŚWIERKOWYCH BESKIDU ŚLĄSKIEGO

Abstract: Activity of soil enzymes is considered as a good indicator of natural and anthropogenic disturbances of the functioning of the soil. Heavy metals can inhibit the activity of enzymes in varying degree, depending on soil properties such as content of clay materials, organic matter and pH of soil solution. The aim of this study was to determine the effect of physicochemical and biological properties of soils on the condition of Norway spruce stands in Silesian Beskid. In the soil samples enzymatic activity of four enzymes (alkaline and acid phosphatase, dehydrogenase, urease) and concentration of three selected heavy metals (Cd, Pb, Zn) and sulfur were determined. The analyses showed no reduced activity of investigated enzymes. Presumably, despite of low pH values of the soil, organic matter contained in the soil is able to effectively bind heavy metal ions, limiting their cycling in the environment. It can be concluded that the condition of spruce stands in Silesian Beskid is not affected by the soil contamination.

Keywords: heavy metals, soil enzymes, Norway spruce, Silesian Beskid

Introduction

Biological processes influencing soil fertility in terrestrial ecosystems are mainly based on the transformation of organic matter. Mostly they are associated with microbes and enzymes secreted by them, and the pace of their respective biogeochemical changes

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in the elements circulation [1]. Evaluation of the quality and the productivity of the soil is an important part in the study of the natural environment. In temperate forest ecosystems, dominated by ectomycorrhizal trees, acid soils accumulating large quantities of organic molecules are predominant. Forest soil fertility and productivity of the forest ecosystems depend on the activity of biochemical processes in the soils, which are catalyzed by enzymes secreted into the soil environment mainly by soil microorganisms [2–4]. Enzymes such as urease usually serve as an index of the soil fertility because their activity correlates with the organic matter content in soil, alkaline and acid phosphatase, which are closely associated with respiratory and biomass of soil organisms, and the dehydrogenase which is an excellent indicator of respiratory activity of soil microorganisms [4, 5]. The activity of soil enzymes is considered as a good bioindicator which reflects the natural and anthropogenic disturbance of the soil [6, 7]. Soil enzymes are inhibited by heavy metals to varying degrees, depending on properties of the soil, such as the contents of clay materials and organic matter, or pH value of a soil solution [8–10]. Yang et al [11, 12] reported that reducing the number of microorganisms in the soil and the inhibition of enzyme action as a result of soil pollution with heavy metals affects the soil fertility.

Forests of the Silesian Beskid Mountains are within the range of pollution impact coming from the north side from the Upper Silesian Industrial District, on the west side from the agglomeration of Ostrava (CZ), and from nearby Bielsko-Biala [13]. Other factors affecting the Silesian Beskid forest are inadequate forestry, worsening climate conditions and the gradation of insects attacking the spruces. As a result of the above-mentioned factors, there is a reduction in vitality of a Norway spruce [14, 15].

The object of the studies on the soil enzymes activity and the contamination of soil with heavy metals and sulphur was to determine the soil condition of Silesian Beskid, and estimate the possible impact of these factors on the weakening of the spruce stands in these mountains.

Material and methods

The material for the analysis was being collected from September to October 2007. Six mountain peaks of the Silesian Beskid have been investigated: Blatnia, Klimczok, Skrzyczne, Soszow, Stozek, Szyndzielnia. Soil samples for the analysis were taken from following depths: 0–10 cm, 10–20 cm and 20–30 cm [16], from five selected points in the entire area of the peak. Soil samples were mixed for each depth and each mount separately.

Soil was sifted through a sieve with a diameter of 1 mm and dried to a constant weight, upon which 10 g subsamples from each depth and each uphill were prepared. Soil subsamples were inserted into 100 cm³ of 10 % nitric acid(V) and shaken for one hour. After that the subsamples was filtered. Concentration of three heavy metals (contamination fraction), zinc, cadmium and lead were determined on an atomic absorption spectrometer [17]. Bioavailable fraction of heavy metals in soil samples was determined with similar depth, which were first triturated in a mortar and sieved

through a sieve with a diameter of 0.25 mm. Samples were inserted in 50 cm³ of 0.01 M $CaCl_2$ and shaken for 5 hours, then filtered.

Total sulphur content was determined nephelometrically, according to the method proposed by Ostrowska [17].

Soil pH was determined in H_2O , at a substrate to water ratio of 1:2.5. The measurements were performed by potentiometry method using a SEN 81st TIX electrode.

The content of organic matter in soil was determined by gravimetric method of weight loss during the annealing of the soil sample in a muffle furnace at 550 $^{\circ}$ C [17].

Activity of soil enzymes were carried out in accordance with the methodology proposed by [18]. The activity of acid and alkaline phosphatase were tested by the colorimetric method, where the activity is measured in μ g of *p*-nitrophenol per 1 g fresh weight soil. Dehydrogenase activity was determined by colorimetric method, using the ability of this enzyme to transfer electrons to a synthetic acceptor, *triphenyltetrazolium chloride* (TTC) which in the oxidized form is almost colorless, but in the reduced form gives colored compound *triphenylformazan* (TPF). The activity was measured in μ g of TPF on 1 g fresh weight soil. Urease activity was tested by colorimetric assay based on ammonia formed after the enzymatic hydrolysis of urea, activity is expressed in μ g of N per 1 g fresh weight soil.

Statistical analysis

The results of soil chemical data and enzymes activities were tested for normal distribution (Shapiro-Wilk test) prior to statistical analysis. Statistical comparisons of the six sites were made using Tukey test. Correlation were calculated by Pearson's correlation coefficient.

Results

The amount of organic matter in the outer layer of the soil from the analyzed mountains ranged from 14.1 % in Skrzyczne to 26.6 % in Blatnia. Vertical arrangement of the organic matter content in soil indicated a high concentration of organic matter in the upper layers (0–10 cm) of the tested surface, which was particularly evident on Blatnia (41.2 %) and Szyndzielnia (40.4 %) (Table 1).

Analysis of the studied soils acidity indicated that the pH was acidic. Lowest soil pH value was noted on Klimczok (3.77), while the highest was observed on the Skrzyczne peak (4.22). In all studied sites pH increase with the lowering of soil was observed (Table 1).

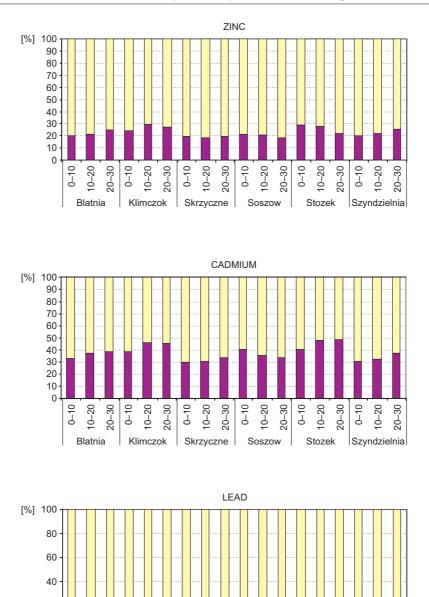
The concentration of sulphur in the outer layer of the soil from the analyzed mountains ranged from 108.8 μ g \cdot g⁻¹ on Skrzyczne to 176.2 μ g \cdot g⁻¹ on Blatnia. Soils gathered at Skrzyczne and Stozek had greater accumulation of sulphur in the 20–30 cm level compared with level 10–20 cm (Table 1).

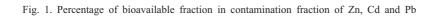
Vertical arrangement of heavy metals distribution indicated their accumulation in the upper layers of all studied surface. The highest concentrations of zinc, cadmium and Wojciech Bierza et al

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Table

		List of res	sults of investigat	ist of results of investigated elements, organic matter an pH values of soil from 6 mountain peaks in Silesian Beskid	c matter an pH v.	alues of soil from (5 mountain peaks i	n Silesian Beskid		
	Depth	Contami	Contamination fraction [µg/g d.m.]	lg/g d.m.]	Bioav	Bioavailable fraction [μg/g d.m.]	g d.m.]	s	Organic	:
	[cm]	Zn	Cd	Pb	Zn	Cd	Pb	[μg/g d.m.]	matter [%]	Hq
	0-10	51.440 ± 3.440	1.055 ± 0.009	430.500 ± 12.000	12.680 ± 0.190	0.515 ± 0.044	6.096 ± 0.749	249.150 ± 19.133	41.222 ± 0.397	3.645
Blatnia	10 - 20	27.160 ± 2.590	0.731 ± 0.107	250.750 ± 16.950	7.382 ± 0.316	0.430 ± 0.002	3.565 ± 0.418	145.833 ± 30.612	21.826 ± 0.140	4.055
	20–30	18.28 ± 0.950	0.584 ± 0.033	155.700 ± 0.400	6.074 ± 0.104	0.369 ± 0.048	3.370 ± 0.088	133.503 ± 7.653	16.752 ± 0.245	4.200
	0 - 10	22.190 ± 0.070	0.557 ± 0.054	283.700 ± 11.800	7.087 ± 0.029	0.347 ± 0.019	3.647 ± 0.090	196.003 ± 8.503	30.230 ± 0.993	3.535
Klimczok	10-20	9.034 ± 0.179	0.243 ± 0.071	95.025 ± 0.665	3.813 ± 0.003	0.208 ± 0.004	0.798 ± 0.093	98.427 ± 9.141	11.523 ± 1.063	3.830
	20–30	9.130 ± 0.031	0.269 ± 0.043	58.880 ± 2.000	3.424 ± 0.067	0.221 ± 0.015	1.976 ± 1.502	87.372 ± 8.291	10.158 ± 0.592	3.940
	0 - 10	30.075 ± 1.365	1.000 ± 0.001	223.000 ± 7.500	7.239 ± 0.082	0.430 ± 0.032	4.296 ± 0.404	166.029 ± 1.488	23.972 ± 0.192	4.015
Skrzyczne	10 - 20	18.955 ± 0.725	0.767 ± 0.004	44.250 ± 0.180	4.385 ± 0.045	0.338 ± 0.004	1.339 ± 0.996	70.578 ± 2.551	9.207 ± 0.362	4.120
	20–30	16.025 ± 2.705	0.754 ± 0.054	47.890 ± 3.910	3.837 ± 0.095	0.373 ± 0.032	2.806 ± 2.197	89.923 ± 6.165	9.234 ± 0.386	4.525
	0 - 10	27.300 ± 2.120	0.543 ± 0.001	213.350 ± 0.350	7.357 ± 0.189	0.371 ± 0.039	3.896 ± 0.841	183.673 ± 14.456	24.277 ± 0.293	3.825
Soszow	10-20	23.495 ± 1.145	0.637 ± 0.029	119.700 ± 4.600	6.057 ± 0.063	0.354 ± 0.025	1.043 ± 0.223	153.486 ± 5.527	18.750 ± 0.590	4.060
	20–30	23.950 ± 0.830	0.739 ± 0.049	82.205 ± 3.445	5.358 ± 0.194	0.372 ± 0.014	2.975 ± 2.526	111.182 ± 4.464	15.454 ± 0.454	4.215
	0 - 10	24.800 ± 0.080	0.576 ± 0.001	187.200 ± 0.000	10.234 ± 1.047	0.389 ± 0.015	7.776 ± 0.718	166.241 ± 9.779	26.984 ± 0.993	3.780
Stozek	10-20	15.125 ± 1.215	0.274 ± 0.035	82.130 ± 2.140	5.806 ± 0.717	0.252 ± 0.004	2.591 ± 0.088	106.080 ± 11.692	14.843 ± 0.757	4.140
	20–30	13.615 ± 0.215	0.247 ± 0.021	67.295 ± 1.185	3.870 ± 0.437	0.232 ± 0.004	1.017 ± 0.132	111.607 ± 19.345	13.919 ± 0.888	4.205
	0 - 10	52.830 ± 10.200	1.241 ± 0.007	502.400 ± 24.900	13.055 ± 0.035	0.544 ± 0.026	2.323 ± 0.284	266.582 ± 8.078	40.444 ± 0.428	3.650
Szyndzielnia 10–20	10-20	24.280 ± 0.700	0.755 ± 0.111	210.450 ± 9.150	6.734 ± 0.293	0.365 ± 0.019	0.623 ± 0.597	153.699 ± 35.077	19.132 ± 1.196	3.895
	20–30	17.035 ± 0.635	0.667 ± 0.019	137.150 ± 0.650	5.935 ± 0.689	0.392 ± 0.011	0.486 ± 0.391	84.184 ± 17.857	15.153 ± 0.437	4.080

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Contamination

fraction

Klimczok

20–30

Skrzyczne

10–20 20–30 0–10

Soszow

Bioavailable

fraction

0-10

0-10

10-20

Szyndzielnia

10-20

Stozek

10-20 20-30 0-10 10-20 20-30 0-10 10-20

Blatnia

lead in the contamination fraction were noted in 0–10 cm levels on Szyndzielnia, accordingly: 52.83 $\mu g \cdot g^{-1}$, 1.24 $\mu g \cdot g^{-1}$ and 502.40 $\mu g \cdot g^{-1}$. Also content of investigated heavy metals in the bioavailable fraction decreased with the increasing soil depth. The highest concentration of zinc (13.05 $\mu g \cdot g^{-1}$), cadmium (0.54 $\mu g \cdot g^{-1}$) and lead (6.82 $\mu g \cdot g^{-1}$) in the bioavailable fraction were recorded in Szyndzielnia (the 0–10 cm level). However, the highest average concentrations of these elements in bioavailable fraction were observed on Blatnia, for zinc it was 9.04 $\mu g \cdot g^{-1}$, for cadmium 0.45 $\mu g \cdot g^{-1}$ and for lead 4.34 $\mu g \cdot g^{-1}$ (Table 1). Percentage of bioavailable fraction at in contamination fraction of Zn, Cd and Pb is given on Fig. 1.

The highest alkaline phosphatase activity was observed on the level of 0–10 cm on Blatnia where the concentration of *p*-nitrophenol was 309.9 μ g · g⁻¹. However, the highest average activity was recorded on Skrzyczne (200.4 μ g · g⁻¹). In soils gathered at Klimczok, Soszow and Skrzyczne *p*-nitrophenol concentration was higher in the 20–30 cm and was respectively: 96.6, 221.6 and 166.3 μ g · g⁻¹, comparing with the concentration in the 10–20 cm level (92.2, 135.4 and 153.8 μ g · g⁻¹). The highest acid phosphatase activity was determined in the level 0–10 cm from Soszow (1658.9 μ g · g⁻¹), the highest average concentration of the secreted *p*-nitrophenol was

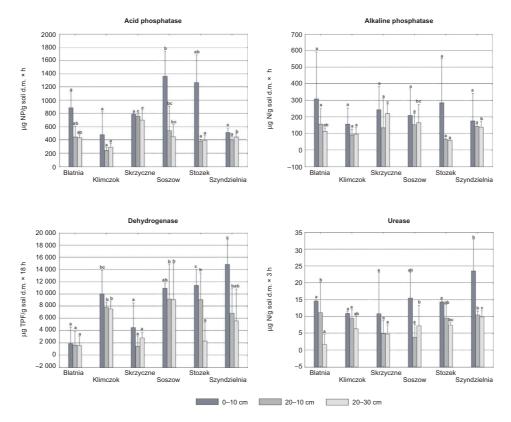


Fig. 2. Activity of investigated soil enzymes. p < 0.05, ANOVA Tukey test – homogenous groups marked with the same letters

		20–30 cm	-0.04	-0.01	-0.29	-0.38
	Urease	10–20 cm	0.30	0.01	0.23	0.35
n soil.		$0-10~\mathrm{cm}$	0.59*	0.60*	-0.28	0.63*
nd Sulphur i	e	20–30 cm	-0.13	-0.18	-0.05	-0.40
n, Cd, Pb ai <i>0.05</i> level	Dehydrogenase	0-10 cm 10-20 cm 20-30 cm	-0.01	-0.40	-0.28	0.35
The correlation coefficient between soil enzymes and bioavailable Zn, Cd, Pb and Sulphur in soil Results marked by * are significant at the $p = 0.05$ level	De	0-10 cm	0.24	0.07	-0.23	0.17
	Acid phosphatase	10–20 cm 20–30 cm	0.03	0.60*	0.27	-0.09
		10–20 cm	-0.01	0.41	0.02	-0.27
		$0-10~\mathrm{cm}$	-0.23	-0.37	0.40	-0.47*
	Alkaline phosphatase	20–30 cm	0.08	0.65*	0.35	-0.24
		10–20 cm	0.30	0.62*	0.12	0.41
		$0-10~\mathrm{cm}$	0.24	0.29	0.38	-0.10
			Zn bioavailable	Cd bioavailable	Pb bioavailable	Sulphur

Table 2

determined also on Soszow (885.5 $\mu g \cdot g^{-1}$). In soils derived from Szyndzielnia and Klimczok *p*-nitrophenol concentration was higher in the 20–30 cm level and was respectively 295.5 and 448.4 $\mu g \cdot g^{-1}$ in relation to the concentration of the substance in level 10–20 cm where it was 241.7 and 405.7 $\mu g \cdot g^{-1}$. The interesting fact was that there were small differences in acid phosphatase activity.

Dehydrogenase activity was measured by the content of TPF produced within 16 hours of incubation. At all the sites except for Blatnia the activity of this enzyme was very high. A very high concentration of TPF was characteristic for level 0–10 cm from Szyndzielnia (14872.0 $\mu g \cdot g^{-1}$), where the average number of TPF produced was also the highest (9135.0 $\mu g \cdot g^{-1}$). Soils from Skrzyczne and Klimczok were characterized by greater activity of dehydrogenase in the level of 20–30 cm compared with level 10–20 cm.

Urease activity was measured by the content of nitrogen produced in 3 hours. The highest enzyme activity was determined on Szyndzielnia (23.6 μ gN \cdot g⁻¹), where the average concentration of the produced nitrogen was also the highest (14.7 μ g \cdot g⁻¹). A higher level of urease activity was observed in level 20–30 cm (7.28 μ g \cdot g⁻¹) compared with level 10–20 cm (3.9 μ g \cdot g⁻¹) for the Soszow soils. Results for all four enzymes activity are given in Fig. 2.

Discussion

At all the examined surfaces an accumulation of heavy metals and sulfur in the outermost layer of the soil has been stated, which gived evidence to the anthropogenic origin of these elements. The similarity in relation to the many of tested parameters showed for Szyndzielnia and Blatnia to be the most correspondent, especially in content of metal and sulphur. Higher concentrations of sulphur and heavy metals was linked to the north-west winds that bring pollution from above Bielsko-Biala. Similarity was equally often disclosed by Soszow and Stozek because of their close positions.

Heavy metals and sulphur accumulated in soils, not only modify their properties, but also severely affect the soil microorganisms and change the soil enzymatic activity. Processes such as nitrification and the pace of the organic matter decomposition are undergoing distinct inhibition [19–20].

The main factors controlling the mobility and availability of heavy metals in soil are pH and organic matter content [21, 22]. Soil pH plays a significant role in the occurrence of soluble and bioavailable forms of zinc and lead. However, according to Keller and Hammer [23] and Pueyo et al [24] the contents of bioavailable forms of cadmium in the soil is less dependent on pH. The acidity increase of the soil environment occurs among others due to the deposition of sulphur compounds [25]. At the studied localities the sulphur content was relatively low. However, statistically significant negative correlation between sulphur content in the soil and soil pH in the surface layers was found. In the layers 10–20 cm and 20–30 cm the value of the influence of precipitation and accumulation of sulphur compounds on pH decrease. The factor which largely determines the enzymatic activity of the soils, is their content of

organic matter. Soil organic matter has a large absorptive surface, and many functional groups (carboxyl, thiol and phenolic) that are capable of efficient binding of heavy metals in the form of complexes [26]. The binding of various metals is different. The strongest relation can be observed between the soil organic matter and the Pb, but also strong for Cd and Zn [27]. In the investigated soils the concentration of lead in bioavailable fraction was minimal, which was indicating strong binding by organic matter. A smaller but still significant was the degree of binding of cadmium and zinc. The results also showed that despite low soil pH, the organic substance contained in the soil was able to bind heavy metals fairly effectively.

In soils contaminated by heavy metals reduction in activity of phosphatases was observed, which was confirmed by studies in the forest soil in the vicinity of the aluminum smelter and soils treated with heavy metals [4, 28, 29]. In the soils from the test sites a high activity of the acid phosphatase was reported as far as the acid phosphatase was concerned an enzyme that is associated with the amount of bacteria and fungal biomass in soil [30]. The maximum concentration of p-nitrophenol in this case was 1658.9 $\mu g \cdot g^{-1} \cdot h^{-1}$ in soil from Soszow. Alkaline phospahate is another enzyme which takes an active part in the decomposition of organic debris. Nowak et al [28] found decreased activity of this enzyme in soils contaminated with zinc and cadmium. In the investigated site concentration of p-nitrophenol in the case of alkaline phosphatase ranged from 115.3 $\mu g \cdot g^{-1} \cdot h^{-1}$ on Klimczok to 200.4 $\mu g \cdot g^{-1} \cdot h^{-1}$ on Skrzyczne. Statistical analysis did not show a negative correlation between the concentration of metals in the bioavailable fraction and the activity of both phosphatases in the 0-10 cm layer, while in the 10-20 cm layer there was a positive correlation observed for cadmium content and the activity of alkaline phosphatase. In addition, in the 20-30 cm layer a positive correlation between the concentration of cadmium and acid phosphatase activity was reported. These results suggest that soil pH for these enzymes is crucial. High activity of acid phosphatase is due to low soils pH while alkaline phosphatase shows greater activity in alkaline soils [5]. In addition, Acosta--Martinez and Tabatabai [31] postulated that the cadmium content in soil has a greater effect on the activity of alkaline phosphatase than acid phosphatase.

Total dehydrogenase activity is an indicator of the redox system and a measure of respiratory activity of microorganisms. Dehydrogenase are active only within living organisms, and after a cell death their degradation follows quickly. Therefore, the dehydrogenase activity indicates the presence of physiologically active microorganisms [3, 32]. Heavy metals have inhibitory effect on dehydrogenase activity [33–35]. Olszowska [34] found a negative correlation between dehydrogenase activity and the content of Zn, Cd and Pb in soils of pine stands located in the vicinity of the impact of lead and zinc smelter. Kieliszewska-Rokicka [4] obtained a similar result in soils from nearby aluminum smelters. In the investigated soils, dehydrogenase activity was high, and there was a lack of negative correlation between enzyme activity and the content of heavy metals in the bioavailable fraction and sulphur in each of the three examined levels.

The activity of urease, which catalyzes the hydrolysis of urea to ammonia and CO_2 , is related to the pace of change in soil nitrogen. This enzyme is accumulated in the soil

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in the form of complexes with organic matter and humus [36]. Nadgorska-Socha et al [37] found urease activity measured by the amount of the produced nitrogen to be around 75 μ gN \cdot g⁻¹ in the soil located under the direct influence of heavy metal emitter. In the investigated soils, the urease activity was expressed as a concentration of secreted nitrogen which was at the level of 6.9 μ gN \cdot g⁻¹ on Skrzyczne to 14.7 μ gN \cdot g⁻¹ on Szyndzielnia. A significant decrease in the activity of this enzyme was not related to the concentration of heavy metals, as evidenced by the positive correlation between the content of bioavailable zinc and cadmium in 0–10 cm layer and lack of correlation in the other layers. Low activity of urease may be due to the sensitivity of this enzyme in the acidic soils.

The obtained results showed that low concentrations of heavy metals in the soils did not affect the activity of soil enzymes significantly. Similar conclusions were reached by Dar [38], who after adding cadmium concentration of 10 μ g · g⁻¹ to the soil did not report any significant changes in the activity of soil enzymes. Furthermore, it was proved that small concentrations of lead have stimulating effect on soil enzymes [39]. Also, Shah and Dubey [40] noted the increase in protease activity in the soil after adding sediment containing small amounts of cadmium (50–100 μ M).

Conclusions

The studies of the soils from the site of Silesian Beskid showed no impact of anthropogenic contaminants (heavy metals and sulfur) on the activity of soil enzymes. High enzyme activity demonstrates the viability of soil microorganisms, proper circulation of biogenic elements such as phosphorus and nitrogen. Therefore, it can be concluded that the causes of Beskid spruce extinction have different backgrounds.

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OCENA AKTYWNOŚCI ENZYMÓW GLEBOWYCH JAKO WSKAŹNIKA WPŁYWU ZANIECZYSZCZEŃ ANTROPOGENNYCH NA FUNKCJONOWANIE EKOSYSTEMÓW LASÓW ŚWIERKOWYCH BESKIDU ŚLĄSKIEGO

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Abstrakt: Aktywność enzymów glebowych uważana jest za dobry wskaźnik naturalnych i antropogennych zaburzeń w funkcjonowaniu gleby. Metale ciężkie w różnym stopniu mogą hamować działanie enzymów, w zależności od właściwości gleb, takich jak zawartość materiałów ilastych, gliny, materii organicznej czy wartości pH roztworu glebowego. Celem pracy było określenie wpływu właściwości fizykochemicznych i biologicznych gleb na kondycję drzewostanów świerkowych w Beskidzie Śląskim. Próbki glebowe zbadano pod względem aktywności enzymatycznej (fosfataza kwaśna i zasadowa, dehydrogenaza, ureaza) oraz koncentracji trzech wybranych metali ciężkich (Cd, Pb, Zn) i siarki. Analizy nie wykazały obniżonej aktywności badanych enzymów. Prawdopodobnie mimo niskich wartości pH gleby, zawarta w niej materia organiczna efektywnie wiąże metale ciężkie, ograniczając ich obieg w środowisku. Można stwierdzić, że stan drzewostanów świerkowych w Beskidzie Śląskim nie ma związku z zanieczyszczeniem gleb na tym terenie.

Słowa kluczowe: metale ciężkie, enzymy glebowe, świerk pospolity, Beskid Śląski