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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 and 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 [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]. The object of the studies on the soil enzymes activity and the contamination of soil with heavy metals and

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sulfur 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 and Szyndzielnia. Soil samples for the analysis were taken from following depths: 0-10, 10-20 and 20-30 cm [11], from five selected points in the entire area of the peak. Soil samples were mixed for each depth and each amount 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 cm^3 of 10% nitric acid(V) and shaken for one hour. After that the subsamples were filtered. Concentration of three heavy metals (contamination fraction), zinc, cadmium and lead were determined on an atomic absorption spectrometer [12]. 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 cm^3 of 0.01 M $CaCl_2$ and shaken for 5 hours, then filtered. Total sulfur content was determined nephelometrically, according to the method proposed by Ostrowska et al [12].

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°C [12].

Activity of soil enzymes were carried out in accordance with the methodology proposed by Schinner et al [13]. The activity of acid and alkaline phosphatase was tested by the colorimetric method, where the activity is measured in μg 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 μg 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 μg 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 was calculated by Pearson's correlation coefficient.

Results and discussion

On all the examined surfaces there was an accumulation of heavy metals and sulfur in the outermost layer of the soil, which gives evidence to the anthropogenic origin of these elements. The similarity in relation to the number of tested parameters showed Szyndzielnia and Blatnia to be the most correspondent, especially when it comes to metal and sulfur content which were also the highest for those two locations. Higher concentrations of sulfur and heavy metals were 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.

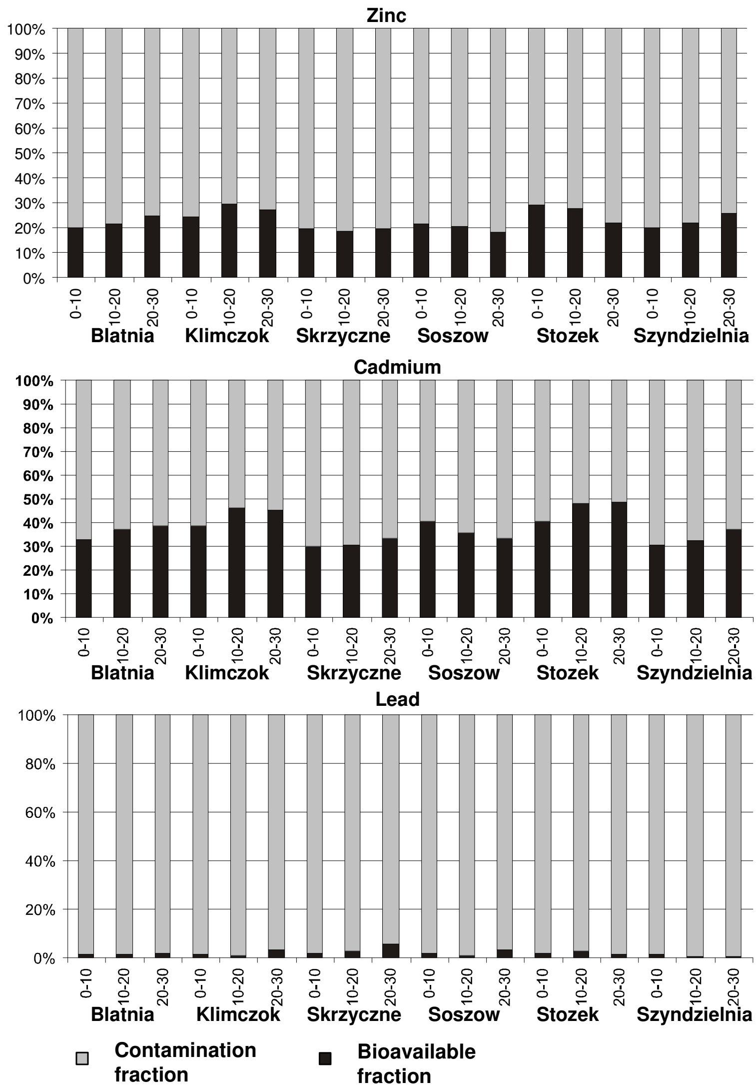

Fig. 1. Percentage of heavy metals (Zn, Cd, Pb) bioavailable fraction in contaminated fraction

Heavy metals and sulfur 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 undergo distinct inhibition [14, 15].

Total dehydrogenase activity is an indicator of the redox system and a measure of respiratory activity of microorganisms. Dehydrogenase is active only within living organisms, and after a cell death their degradation follows quickly. Heavy metals have inhibitory effect on dehydrogenase activity [16-18]. Olszowska [17] 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. 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 in each of the three examined levels.

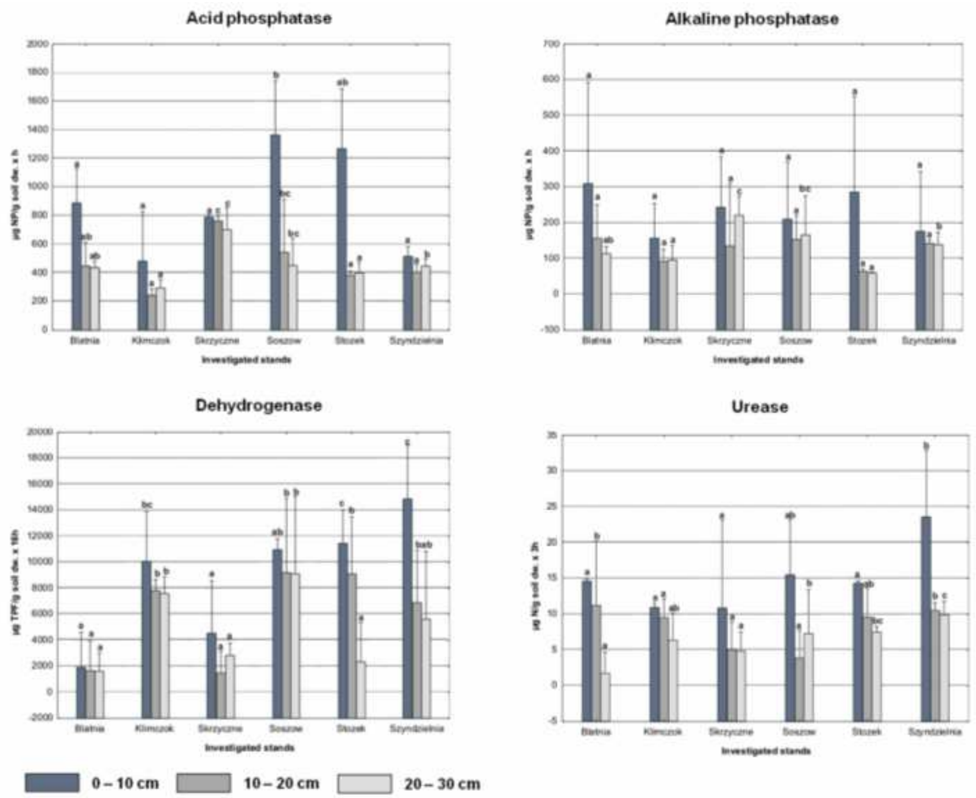

Fig. 2. Activity of investigated soil enzymes. $p < 0.05$, ANOVA TUKEY test - homogenous groups marked with the same letters

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 in the form of complexes with organic matter and humus [19]. Nadgorska-Socha et al [20] found urease activity measured by the amount of the produced nitrogen to be around 75 μg N 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 stood at 6.9 μg N g^{-1} on Skrzyczne to 14.7 μg N g^{-1} on Szyndzielnia. A significant decrease in the activity of this enzyme is not related to the concentration of heavy metals, as evidenced by the positive correlation between the content of bioavailable zinc and cadmium and the amount of nitrogen produced in the layer 0-10 cm and lack of correlation in the other layers.

In soils contaminated by heavy metals, a reduction in activity of phosphatases was observed, which was confirmed by studies in the forest soil in the vicinity of the aluminum smelter [4] and soils treated with heavy metals [18]. In the soils from the test site there was a high activity reported as far as the acid phosphatase is concerned; an enzyme that is associated with the amount of bacteria and fungal biomass in soil [21]. The maximum concentration of p-nitrophenol in this case was 1658.9 μg g^{-1} h^{-1} in soil from Soszow. Besides acid phosphatase among the enzymes that take an active part in the decomposition of organic debris is alkaline phosphatase. In the investigated site concentration of p-nitrophenol in the case of alkaline phosphatase ranged from 115.3 μg g^{-1} h^{-1} on Klimczok to 200.4 μg g^{-1} 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 are crucial. High activity of acid phosphatase is due to low soils pH while alkaline phosphatase shows greater activity in alkaline soils [5].

The obtained results showed that low concentrations of heavy metals in the soils did not affect the activity of soil enzymes significantly.

The main factors controlling the mobility and availability of heavy metals in soil are pH and organic matter content [22, 23]. Soil pH plays a significant role in the occurrence of soluble and bioavailable forms of zinc and lead. The acidity increase of the soil environment occurs among others due to the deposition of sulfur compounds [24]. At the studied localities the sulfur content was relatively low (from 108.8 to 176.2 μg g^{-1}). However, statistically significant negative correlation between sulfur content in the soil and soil pH in the surface layers was found, while in the layers 10-20 cm and 20-30 cm the value of the correlation was statistically insignificant. The observed dependencies indicate the influence of precipitation and accumulation of sulfur 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 [25]. The binding of various metals is varied. The strongest relation is between the soil organic matter and Pb. It is also strong for Cd and Zn [26]. 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 show that despite low soil pH, the organic substance contained in the soil is able to bind heavy metals fairly effectively.

Table 1

The correlation coefficient between soil enzymes and bioavailable Zn, Cd and Pb in soil. Results marked by * are significant at the $p = 0.05$ level

	Alkaline phosphatase	Acid phosphatase	Dehydrogenase	Urease
Zn bioavailable	0.08	0.03	–0.13	–0.04
Cd bioavailable	0.65*	0.6*	–0.18	–0.01
Pb bioavailable	0.35	0.27	–0.05	–0.29

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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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 takich właściwości gleb, 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