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Original Research

Assessment of Heavy Metals Contamination and Enzymatic Activity in Pine Forest Soils under Different Levels of Anthropogenic Stress

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Abstract

The aim of this work was to assess heavy metal concentrations and the effects of these metals on soil enzymatic activity in polluted and potentially unpolluted forest areas. The study was performed in typical pine forests located in three heavily polluted (in the immediate vicinity of a zinc smelter, an iron smelter, and a power plant) and three relatively clean sites (a nature reserve, an ecological site, and an unprotected natural forest community) in southern Poland. The research concerned the activity of acid phosphatase, dehydrogenase, β-glucosidase, and urease. In the soils, taken from the top 0-10 cm layer, we also tested the concentrations of heavy metals (Cd, Fe, Pb, and Zn) extracted with 10% HNO_3 and 0.01 M $CaCl_2$, and their pH and organic matter contents. Single pollution index and Nemerow pollution index were also calculated.

Nemerow pollution index indicated serious pollution with heavy metals at two sites. The lowest activity of soil enzymes (acid phosphatase, and in particular β-glucosidase) was found in the site with the highest levels of heavy metals. In this study we found no effect of organic matter on the activity of the selected enzymes. There was a significant effect of pH on the activity of acid phosphatase and β-glucosidase.

Keywords: heavy metals, pollution index, soil enzymes

Introduction

Soil plays a vital role in life organisms, not only for anchorage and source of nutrients but also as a sink to many industrial wastes – most of which are hazardous [1, 2]. Changes in some soil properties may occur very slowly or may only occur when the soil undergoes drastic changes. Such properties are not suitable for estimating soil quality, and properties that respond rapidly to environmental stress must be used [3]. Evaluating the quality and the productivity of the soil is an important part in studying the natural environment [4]. Heavy metals are one of the most significant components of environmental pollution, exerting long-term risky effects on soil ecosystems and negatively influencing biological processes in the soil, hence the need for constant monitoring and regulation of their concentrations in soil [5]. Heavy metals generally affect the growth, morphology, and metabolism of microorganisms that lead to a decrease in the functional diversity of soil ecosystems [6]. Heavy metals can inhibit enzyme activities by interacting with the enzyme substrate complexes, denaturing the enzyme protein and interacting with their active sites [7]. Enzymatic activities are frequently used for determining the influence of various pollutants – including heavy metals – on soil microbiological quality [8, 9].

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The activity of soil enzymes is considered a good bioindicator that reflects the natural and anthropogenic disturbance of the soil and shows a quick response to changes induced in the soil ecosystem. Enzymes are secreted into the soil environment mainly by soil microorganisms, catalyzing biochemical processes crucial for forest soil fertility and productivity in forest ecosystems [4, 10-12]. The evaluation of soil enzyme activity is one of the cheapest and easiest techniques that can be used to evaluate soil pollution [5, 13, 14], being more sensitive to soil contamination and with reactions that are faster in comparison to the monitoring of the chemical and physical properties of the soil. In addition, changes in soil enzyme activity reflect the real impact of stress conditions from contamination on the growth and activity of soil microflora [15]. The aim of this work was to assess the heavy metal concentrations and study the effect of these metals on soil enzymatic activity in polluted and potentially unpolluted forest areas.

Material and Methods

The study was performed in coniferous forest ecosystems located in three heavily polluted sites in the immediate vicinity of the Miasteczko Śląskie zinc smelter (M1), the ArcelorMittal Poland S.A. iron smelter in Dąbrowa Górnicza-Łosień (L2), and the Jaworzno III power plant in Jaworzno (J3), plus three potentially clean sites: Pazurek Nature Reserve in Jaroszowiec Olkuski (P4), the Płone Bagno ecological site in Katowice (PB5), and the unprotected natural forest community in Kobiór (K6). All of the sites were situated in southern Poland in Śląskie or Małopolskie provinces.

Soil samples were collected in May, July, and September 2009 to capture the dynamic variabilities during the 2009 growing season from depths of 0-10 cm. Samples were collected at each site from five random sites within an area of 50 m^2, and soil sub-samples were combined into a composite sample. Soil samples were kept at 4ºC during transport to the laboratory. Soil enzyme activity was determined at field moisture levels in the soil samples, which were sieved through a 2 mm sieve and stored at 4°C prior to microbial analysis. The activity of acid phosphatase, dehydrogenase, and urease were carried out in accordance with the methodology proposed by Schinner et al. [16].

The activity of acid phosphatase was tested by colorimetric method. The *p*-nitrophenol released by phosphomonoesterase activity was extracted and colored with sodium hydroxide and determined photometrically at λ = 400 nm. The acid phosphatase activity was expressed in µg of *p*-nitrophenol (*p*-Nf) g^{-1} d.m. h^{-1} (d.m. – dry matter). Triphenyltetrazolium chloride was the substrate used for the dehydrogenase activity determination. Triphenyl formazan (TPF) was extracted with acetone and measured photometrically at λ = 546 nm. The dehydrogenase activity was expressed in µg TPF g^{-1} d.m. 16 h^{-1}. The urease activity estimation was based on colorimetric determination of ammonium formation after enzymatic urea hydrolysis. Urease activity was expressed as µgN g^{-1} d.m. Saligenin released from salicin (β-glucosido-saligenin) was determined colorimetrically after coloring with 2,6-dibromochinon-4-chlorimide, at λ = 546 nm for β-glucosidase activity estimation. β-glucosidase activity was expressed as µg saligenin g^{-1} d.m. $3h^{-1}$ [17, 18].

The metal content in the soil was estimated according to the methods of Bouwmann et al. [19] and Ostrowska et al. [20], in air-dried soil samples that had been sieved through a 1 mm sieve. Metals were extracted from the soil with 0.01 M $CaCl_2$ (potentially bioavailable elements) or with 2 M HNO_3 (acid extracted elements). For the $CaCl_2$ extraction, 5g of soil with 50 ml of 0.01 M $CaCl_2$ solution was agitated for 5 h. The HNO_3-extractable fraction was obtained by agitating 10 g of soil sample with 100 ml of 2 M HNO_3 for 1 h. The content of metals in the filtered extracts was measured by inductively coupled plasma-atomic emission spectroscopy (Spectro Analytical Instruments).

The single pollution index and Nemerow pollution index were calculated for acid-extracted elements. The pollution level by a given heavy metal (i) was evaluated with the single pollution index (Pi) calculated as the ratio between the metal concentration (Ci) in a soil sample and permitted standard of the same metal (Si):

$$Pi = \frac{Ci}{Si}$$

[21, 22]

Permitted standard for this study was recommended by the regulation of the minister of the environment about the standards of soil and ground quality (300 mg kg^{-1} Zn, 100 mg kg^{-1} Pb, and 4 mg kg^{-1} Cd) [23].

The overall pollution status of the surface soils by the heavy metals was assessed using the Nemerow pollution index (Pn) [22, 24]:

$$Pn = \sqrt{(P_{ave}{}^2 + P_{max}{}^2)/2}$$

…where P_{ave} is the average of single pollution index of all metals and P_{max} is the maximum value of the single pollution index of all metals. Pollution of the surface soils by the heavy metals was classified into five grades based on the Nemerow pollution index (Pn<0.7 is clean, Pn 0.7-1.0 is the warning limit, Pn 1.0-2.0 is slightly polluted, Pn 2.0-3.0 is moderately polluted, and Pn> 3.0 is seriously polluted) [14].

The results are presented as the means of five replicates of each estimation, together with standard deviation (±SD) of the means. The data was analyzed by ANOVA and the treatments were treated as independent variables. Significant statistical differences of all variables were established using Tukey tests (ANOVA; Statistica 10 software). We also calculated Pearson`s correlation coefficients.

Table 1. pH value and organic matter content [%] in soil of investigated sites (mean values ±SE n = 5). The different letters denote significant differences ($p<0.05$).

	pH	Organic matter
M1	4.27±0.26 b	6.89±3.24 a
L2	4.97±0.20 c	15.42±9.18 a
J3	4.30±0.14 b	26.48±8.50 a
P4	3.67±0.23 a	15.75±1.59 a
PB5	3.46±0.03 a	29.87±8.49 b
K6	3.45±0.10 a	22.26±9.41a

Results

Analysis of the studied soils' acidity indicated that the pH was acidic. The lowest soil pH value was noted at site K6 (3.45), while the highest was observed at site L2 (4.97) (Table 1). In the outer layer of the soil from the analyzed sites, the amount of organic matter ranged from 6.89% at site M1 to 29.87% at site PB5 (Table1).

There were significant differences in the content of the metals studied (HNO_3 extracted and $CaCl_2$ extracted) between the polluted and potentially clean sites. A concentration several times lower was determined in the fraction of soil extracted with $CaCl_2$. The highest levels of Cd, Pb, and Zn in both fractions was observed at site M1 (HNO_3, respectively: 25.54 mg kg^{-1}, 1874.99 mg kg^{-1}, and 871.50 mg kg^{-1}; $CaCl_2$-8.59 mg kg^{-1}, 37.20 mg kg^{-1}, and 477.75 mg kg^{-1}). The highest levels of Fe (3584.40 mg kg^{-1} HNO_3 and 61.90 mg kg^{-1} $CaCl_2$) were observed at sites J3, PB5, and L2. Detailed results on the levels of the heavy metals (both fractions) are presented in a paper by Kandziora-Ciupa et al. [25]. Nemerow pollution index ranges from 0.97 in K6 stand to 11.11 in the soil of M1, the most polluted site (Table 2).

Mean activities of the examined enzymes are presented in Figs 1-4. The highest activity of acid phosphatase was observed in the soil collected in May at site L2 (7208.69 µg pNf g^{-1} d.m. h^{-1}), and the lowest was also observed in May at site M1 (803.57 µg pNf g^{-1} d.m. h^{-1}; Fig. 1). The highest mean activity of dehydrogenases in soil was observed in the soil at site L2 (17.05 µg TPF g^{-1} d.m. 16 h^{-1}) in May, and the lowest at site M1 (0.21 µg TPF g^{-1} d.m. 16 h^{-1}) in September (Fig. 2). At all sites the highest dehydrogenase activity was observed at the beginning of the growing season. In September no dehydrogenase activity was observed at site J3. The highest mean activity of β-glucosidase was observed in September at site J3 (7359.91 µg saligenin g^{-1} d.m. $3h^{-1}$), and the lowest in July at site PB5 (376.78 µg saligenin g^{-1} d.m. $3h^{-1}$; Fig. 3). Similar to the activity of dehydrogenase, the highest mean activity of urease was also observed at the beginning of the growing season (L2: 253.56 µg N g^{-1} d.m. $3h^{-1}$), and the lowest in September (M1: 17.29 µg N g^{-1} d.m. $3h^{-1}$; Fig. 4).

Table 2. Single pollution index (SPI) and Nemerow pollution index (NPI) (for acid extracted elements) in soil of investigated sites.

	SPI-Cd	SPI-Pb	SPI-Zn	NPI
M1	4.91	14.02	2.40	11.11
L2	1.40	2.79	1.27	2.35
J3	1.74	4.31	1.33	3.51
P4	0.60	2.18	0.38	1.71
PB5	1.08	2.55	0.35	2.03
K6	0.16	1.27	0.12	0.97

Discussion

Bioavailability is an important factor when evaluating metal toxicity [5]. Total concentration of elements in soil cannot be considered a good indicator of bioavailability [26]. The distribution and abundance of total metal concentrations are useful indicators of the extent of soil contamination [27-29], but risk from metals depends on their bioavailability [29-31].

There was a clear difference between the concentrations of the studied metals in the potentially bioavailable extracted fraction of $CaCl_2$, and HNO_3-

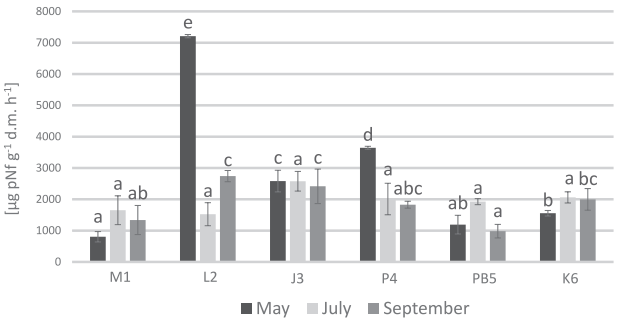

Fig. 1. Acid phosphatase activity in soil of investigated sites (mean values ±SE, n = 5). The different letters denote significant differences between enzyme activity in the same month ($p<0.05$).

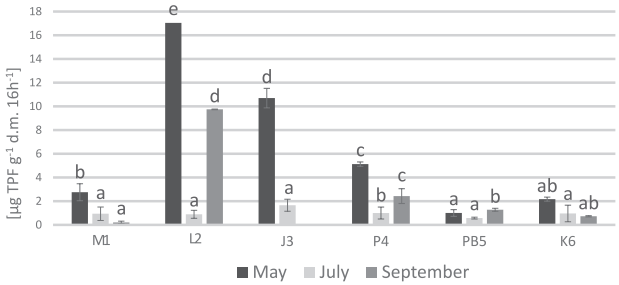

Fig. 2. Dehydrogenase activity in soil of investigated sites (mean values ±SE, n = 5). The different letters denote significant differences between enzyme activity in the same month ($p<0.05$).

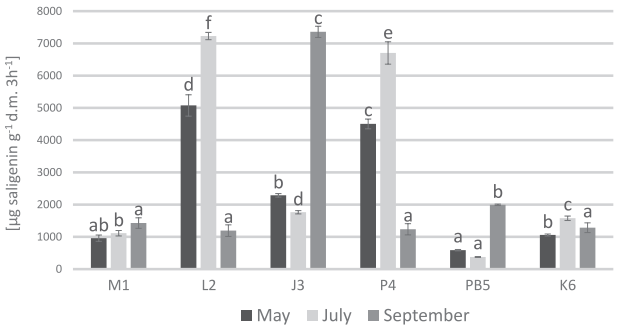

Fig. 3. β-glucosidase activity in soil of investigated sites (mean values ±SE, n = 5). The different letters denote significant differences between enzyme activity in the same month ($p<0.05$).

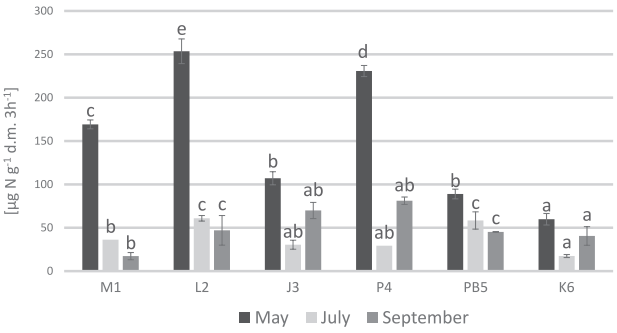

Fig. 4. Urease activity in soil of investigated sites (mean values ±SE, n = 5). The different letters denote significant differences between enzyme activity in the same month ($p<0.05$).

extracted fractions at all the studied sites [25]. The order of metals in the soil fraction extracted with HNO_3 was as follows: Fe>Pb>Zn>Cd and for the $CaCl_2$ extracts it was Zn>Cd>Pb>Fe. In most cases, soils from polluted sites had a higher concentration (often above the permissible concentrations for soil according to Environmental Regulation 2002 [23]) of heavy metals (both fractions) than in areas potentially free of contaminants. A similar correlation was reported by Kafel et al. [32]. Also, Nemerow pollution indices indicated serious pollution with heavy metals at sites M1 and J3, moderate pollution at L2 and PB5, slight pollution at P4, and the warning limit at K6.

Changes in physico-chemical properties of forest soils under the influence of industrial pollution are not adequate indicators of soil degradation. A more sensitive indicator is change in soil enzyme activity that catalyzes biochemical processes crucial for the forest soil fertility and productivity in the forest ecosystem [33]. Phosphatases in soil ecosystems are believed to play critical roles in P cycles through the stimulation of conversions of organic phosphor compounds into inorganic phosphates, directly available to plants and soil organisms [34-36]. In this paper the lowest activity of acid phosphatase was observed at sites M1 and PB5. There was a negative correlation between the activity of the acid phosphatase and the level of bioavailable forms of Cd, Fe, and Zn

Table 3. The correlation coefficients between heavy metal content ($CaCl_2$ fraction) and pH value in studied soil samples and enzyme activity ($p<0.05$); NS: not significant.

	Cd	Fe	Pb	Zn	pH
acid phosphatase	-0.32	-0.29	NS	-0.28	0.33
dehydrogenases	NS	-0.34	NS	NS	NS
β-glucosidase	-0.28	-0.42	NS	NS	0.39
urease	NS	NS	NS	NS	NS

(Table 3). In a study on soil enzymatic activity in forests in areas affected by nitrogen production plants, Bielińska and Domżał [37] also showed an adverse effect of Cd and Zn on the activity of phosphatases. Balyaeva et al. [38], Nadgórska-Socha et al. [39], and Effron et al. [40] also observed a decrease in the activity of acid phosphatase under the influence of heavy metals. Dehydrogenases are intracellular enzymes involved in the microbial metabolism of oxygen [15]. In this paper we found no decrease in the activity of dehydrogenase at sites more burdened by trace elements despite many authors [e.g., 41-43] showing the highly inhibitory effect of heavy metals on the activity of dehydrogenases. We only observed the negative correlation between the activity of these enzymes and the level of available fractions of Fe (Table 3) at site PB5, where the level was the highest.

β-glucosidase (cellobiase) is a common – and the most important – soil enzyme [37]. It takes part in the mineralization of cellulose, catalyzes the reactions of cellobiose degradation into two glucose molecules, and cleaves glucose molecules from the non-reducing ends of cello-oligosaccharides [44]. β-glucosidase is a useful indicator of soil quality and its activity may indicate changes in the level of organic carbon long before measurements by other methods [36]. Obtained results show a negative correlation between the concentration of bioavailable forms of Cd and Fe and the activity of β-glucosidase (Table 3). The mean activity of β-glucosidase in soils at the examined sites was significantly less at sites M1 (with the highest level of bioavailable fractions of cadmium), and PB5 and K6 (the highest levels of bioavailable fractions of Fe).

Many authors [45, 46] confirm the negative effects of trace metals on the activity of β-glucosidase. Jiang et al. [47] also confirm a decrease in the activity of β-glucosidase induced by an increase in the level of cadmium in soil near a copper mill. The decrease in the activity of urease (an extracellular enzyme hydrolyzing C-N bonds in some amids and urea) seen in this study is not entirely tantamount to increased soil pollution. The lowest mean activities of this enzyme were observed at site M1 (the highest levels of trace metals in the soil) while low activities were observed at less polluted sites (e.g., K6 – the lowest soil pH). Also, Madejón et al. [48], Castaldi et al. [49], and Friedlová [15] found no correlation between the increased concentrations of trace metals and urease activity. Some studies demonstrated significant relationships between

soil enzymes and other soil characteristics, but those relationships largely depended on the species of enzyme and the environmental variable [50, 43]. A factor that may largely determine soil enzyme activity is its organic matter. In the present study, we found no statistically significant correlation between the activity of the enzymes and organic matter content in the soil. This could be related to the low level of humic substances in the total organic matter, and consequently limited availability of easily assimilable carbon, which determines the development of soil bacteria that produce enzymes [51]. Effron et al. [40] reported that enzyme activity was sensitive to changes in pH. We found a positive correlation between the activity of acid phosphatase and soil pH, and the same for β-glucosidase.

According to Acosta-Martinez and Tabatabai [52], Madejón et al. [48], and Makoi and Ndakidemi [35], β-glucosidase is also very sensitive to changes in pH (optimum pH range is 6.2-7.8), which is confirmed in this study by an increase in the activity of this enzyme at sites with the highest soil pH (L2 and J3) and decrease β-glucosidase activity at sites with the lowest pH (PB5 and K6) (Table 3, Fig. 3).

Despite the fact that a relationship between urease activity and pH levels was not significant, we observed that activity of this enzyme was lowest at sites with the lowest soil pH (below 3.5, as for PB5 and K6 – Table 1 and Fig. 4) while optimum pH for urease amounts to 6-7 [53]. Weakening of enzymatic activity in soil with the increase of soil acidity is the effect of destroying ion and hydrogen bonds in the enzyme's active center [54, 53].

Because they derive from living organisms, soil enzymes are season-dependent macromolecules [5]. In this work, in most cases the higher activity of the enzymes was noticed in the spring and summer. Also, Zhang et al. [55] found that there were seasonal differences in the effect of heavy metals on soil enzymes, with the effect of the heavy metals more obvious in spring and summer than in autumn [5].

Conclusions

The examined sites are characterized by diverse levels of soil pollution. Single pollution and Nemerow indexes indicated that two sites (M1 and J3) were severely contaminated with heavy metals. None of the examined sites can be classified as clean. Soil pH had the highest influence on enzyme activity; low soil pH could be one reason for low urease and β-glucosidase activities at less-polluted sites. Less activity of urease and β-glucosidase in low pH soil could mean decreasing their effectiveness on chemical reactions. The effects of heavy metals on enzyme activity varied considerably among the elements and enzymes. Soil enzymatic activity decreased significantly with increasing contamination by heavy metals – especially acid phosphatase and β-glucosidase. Therefore, enzyme activity analysis and factors influencing them seem to be a useful tool for monitoring forest soil quality under heavy metal pollution.

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