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Original Research

Biochemical Activity of Nickel-Contaminated Soil

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Abstract

The objective of this study was to determine the alleviating effect of cellulose on the biochemical properties of soil contaminated with nickel. Soil samples were contaminated with nickel chloride, and were fertilized with ammonium sulphate and cellulose. The experiment was carried out for 120 days, at a constant temperature and moisture content. The activity of soil enzymes (dehydrogenases, urease, acid phosphatase, alkaline phosphatase, arylsulphatase, β-gluosidase and catalase) was determined on the day the experiment was established, and on days 15, 30, 60, 90 and 120 of the experimental period.

It was found that soil contamination with nickel had a negative impact on the activity of soil enzymes. The sensitivity of the analyzed enzymes to this heavy metal may be presented in the form of the following series: urease > dehydrogenases > alkaline phosphatase > acid phosphatase > catalase > arylsulphatase > β-glucosidase. The adverse effect of nickel on the activity of soil enzymes can be alleviated by soil enrichment with cellulose accompanied by fertilization with ammonium sulphate. The activity of the tested soil enzymes was subject to periodic fluctuations in samples incubated at a constant temperature and under constant moisture conditions.

Keywords: activity of soil enzymes, soil contamination, nickel, organic substance, cellulose

Introduction

Enzymatic activity is one of the indicators of soil fertility, which provides reliable information on soil conditions [1-3]. Key enzymes secreted by soil microbes take active part in the degradation of cellulose and other plant residues, as well as in nitrogen, phosphorus and sulfur transformations [4]. The most important role is played in the soil environment by oxidoreductases (dehydrogenases, catalase) and hydrolases (acid phosphatase, alkaline phosphatase, urease, arylsulphatase, β-glucosidase) [5, 6]. According to Kizilkaya [7], enzymes may serve as a marker of long-term contamination of the soil environment with heavy metals. If present in excessive amounts, these elements, including nickel, contribute to decreasing the rate of most biochemical reactions and the activity of soil enzymes [8, 9]. Nickel is considered to be one of the most dangerous chemical elements, which may cause permanent soil contamination due to its specific physicochemical properties and mechanism of action [10, 11].

Soil enzymatic activity is dependent on total bacterial counts and on the effect of stress factors [12, 13]. Low concentrations of heavy metals in the soil may increase enzymatic activity, which may be irreversibly inhibited by high concentrations of these metals [7, 14]. The negative effect of heavy metals on soil enzymes is both direct and indirect, since heavy metals change soil acidity status, thus affecting the crops and microbial counts. All those elements are associated with soil acidity, which is increased to the highest degree by nickel, copper and zinc compounds [15, 16]. Nickel solubility in soils is positively correlated with acidity, therefore nickel is more available to living organisms in acid soils than in slightly acid or neutral soils [17, 18].

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In the majority of soils in Poland the levels of nickel and other heavy metals do not exceed the maximum allowable concentrations, but in certain areas the amounts of these elements are increased by industrial emissions. Therefore, effective methods for their neutralization in the natural environment should be developed [19, 20]. One such method involves soil amendment with organic substances, which exerts a complex effect on soil properties [21-23]. A significant role is also played by after-harvest residues abundant in cellulose.

The objective of this study was to determine the effect of cellulose on the activity of enzymes in nickel-contaminated soil. In order not to disturb the C:N ratio, the soil was fertilized with ammonium sulphate.

Materials and Methods

The experiment was conducted under laboratory conditions. Prior to the experiment, soil texture was determined by the Cassagrande method modified by Prószyński [24]. The following physicochemical properties of soil were also determined: pH – with a potentiometer, in an aqueous KCl solution at a concentration of 1 mol dm^{-3}; hydrolytic acidity (Hh) and total exchangeable bases (S) by the Kappen method [24]; and the organic carbon content (C_{org}) – by the Tiurin method [24]. Samples were collected from the humus horizon of soil classified under natural conditions as typical brown soil developed from loamy sand, composed of 47% sand, 39% silt and 14% clay. Soil pH_{KCl} was 6.60, hydrolytic acidity was 1.14 $cmol^{+}\ kg^{-1}$ and total exchangeable bases were 7.77 $cmol^{+}\ kg^{-1}$. 1 kg d.m. of soil contained 8.50 g C_{org}.

100 cm^3 beakers were filled with 100 g of air-dried soil. The variable experimental factors were as follows:

I – soil contamination with nickel: 0 and 400 mg $Ni^{2+}\ kg^{-1}$,
II – nitrogen fertilization: 0 and 150 mg N kg^{-1},
III – cellulose fertilization: 0 and 10 g kg^{-1},
IV – time point of the determination of soil enzymatic activity: experimental day 0, 15, 30, 60, 90 and 120.

Nickel was applied in the form of $NiCl_2 \cdot 6H_2O$, and nitrogen was applied in the form of $(NH_4)_2SO_4$. Each time, following the addition of nickel chloride, ammonium sulphate or cellulose to the beakers, soil samples were mixed thoroughly with the introduced element, and then moisture content was brought to 50% of the capillary water capacity with the use of demineralized water, and samples were incubated at 25ºC. Soil moisture content was monitored on a regular basis.

The experiment was performed in six replications for each of the investigated factors. At every time point (factor IV), six beakers in each treatment were sacrificed and the respective soil samples were separately analyzed for enzyme activities. The remaining beakers filled with soil were sacrificed at successive time points (factor IV).

The activity of soil enzymes was determined at every time point, in the soil from each beaker, as follows: dehydrogenases (EC 1.1) - by the Lenhard method modified by Öhlinger [25], acid phosphatase (EC 3.1.3.2) and alkaline phosphatase (EC 3.1.3.1) - by the method developed by Alef et al. [26], urease (EC 3.5.1.5), arylsulphatase (EC 3.1.6.1), β-glucosidase (EC 3.2.1.21) and catalase (EC 1.11.1.6) – as described by Alef and Nannipieri [27]. On day 0, the analyzes were performed five hours after the introduction of the tested elements into soil samples. Uniform soil moisture content was reached after that time.

The activities of all enzymes, except for catalase, were determined with a Perkin-Elmer Lambda 25 spectrophotometer. The following substrates were used to measure the activity of selected enzymes: 2,3,5-triphenyltetrazolium chloride (TTC) for dehydrogenases, disodium 4-nitrophenyl phosphate (PNPNa) for phosphatases, potassium 4-nitrophenyl sulphate (PNS) for arylsuphatase, 4-nitrophenyl-ß-D-glucopyranoside (PNG) for ß-glucosidase, and urea for urease. Catalase activity was determined by measuring the volume of potassium permanganate used during titration, as a result of hydrogen peroxide decomposition to water and oxygen. The activity of the investigated enzymes was expressed in moles of the obtained product per h and kg d.m. soil, as follows: dehydrogenases – in micromoles of triphenyl formazan (TFF), arylsulphatase, ß-glucosidase and phosphatases – in millimoles of 4-nitrophenol (PNP), urease – in millimoles of NH_4, catalase – in moles of O_2.

The results were processed statistically by Duncan's multiple range test and a four-factorial analysis of variance. Only the values of LSD (least significant difference) for the interactions of all factors (nickel dose x nitrogen rate x cellulose dose x time point of analysis) are given in Figures. Differences were considered significant at $p = 0.01$. A statistical analysis was performed with the use of Statistica software [28]. The results are presented in a separate Figure for each enzyme. The % inhibition of the activity of the tested enzymes was calculated based on the following formula:

$$In = \left(\frac{A}{A_k} - 1\right)100$$

In - % activity inhibition,
A - enzyme activity in contaminated soil,
A_k – enzyme activity in control (non-contaminated) soil.

Results and Discussion

The results of this study show that enzymatic activity was determined by both increased amounts of nickel and cellulose in the soil, and ammonium sulphate fertilization.

The activity of dehydrogenases was significantly stimulated by cellulose over 120 days of the experimental period, while nickel and ammonium sulphate inhibited this activity (Fig. 1). The stimulatory effect of cellulose was greater in non-contaminated soil, compared with nickel-contaminated soil. In nickel-contaminated soil, cellulose exerted a stronger influence on dehydrogenases when applied together with ammonium sulphate, whereas in non-

contaminated soil the effect of cellulose was inhibited by ammonium sulphate. The stimulatory effect of cellulose on dehydrogenases, and the inhibitory effect of nickel and ammonium sulphate, was observed as soon as within five hours after the establishment of the experiment, i.e. at time point 0.

Urease activity, similar to dehydrogenase activity, was subject to fluctuations during the experiment (Fig. 2) – it reached the highest level on day 15 of soil incubation, and then decreased significantly. However, differences between the values of urease activity recorded over the experimental period did not follow a regular pattern. Urease activity, just like dehydrogenase activity, was adversely affected by nickel and ammonium sulphate. Ammonium sulphate fertilization resulted in a slight, although significant, drop in urease activity, whereas nickel contamination resulted in a twofold decrease in its activity. The negative effect of nickel on urease was alleviated by cellulose introduced into the soil, and almost completely neutralized when cellulose was applied together with ammonium sulphate.

In contrast to the activity of dehydrogenases and urease, the activity of acid phosphatase (Fig. 3) and alkaline phosphatase (Fig. 4) was the highest on day 30 of soil incubation. The effect of all experimental factors (nickel, cellulose, ammonium sulfate) on phosphatases was much slighter than on dehydgrogenases and urease. Nickel contamination caused a 28% and 39% decrease in the activity of acid phosphatase and alkaline phophatase, respectively. In treatments fertilized with ammonium sulphate, soil amendment with cellulose alleviated the inhibitory effect of nickel on acid phosphatase to 6% and entirely eliminated the negative effect of this element on alkaline phosphatase. When applied alone, ammonium sulphate adversely affected the activity of phosphatases.

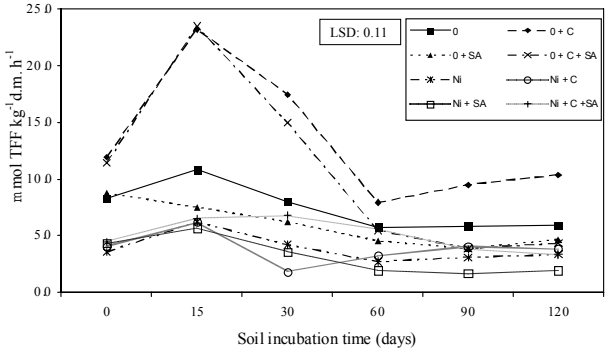

Fig. 1. Effect of soil contamination with nickel on the activity of dehydrogenases (mmol TFF kg^{-1}d.m. h^{-1}).
*0 – objects not polluted with nickel;
Ni – objects polluted with nickel;
C – cellulose;
SA – ammonium sulphate.

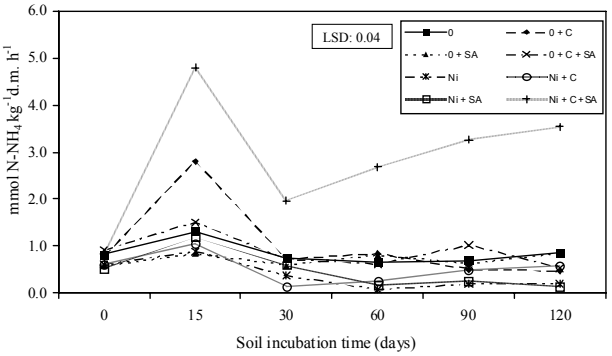

Fig. 2. Effect of soil contamination with nickel on the activity of urease (mmol N-NH_4 kg^{-1}d.m. h^{-1}).
*explanations as in Fig. 1.

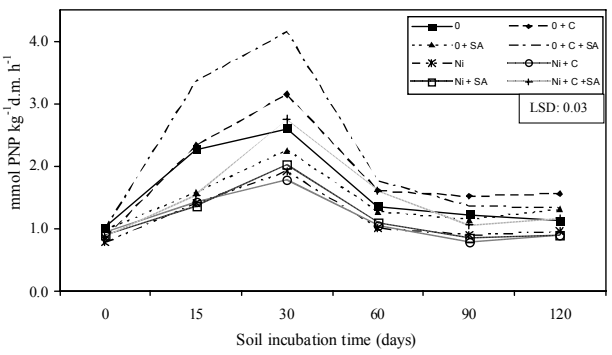

Fig. 3. Effect of soil contamination with nickel on the activity of acid phosphatase (mmol PNP kg^{-1} d.m. h^{-1}).
*explanations as in Fig. 1.

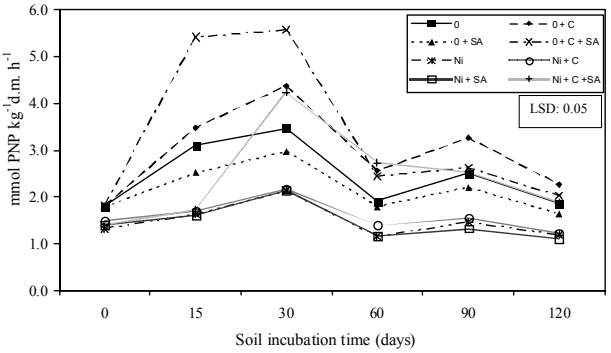

Fig. 4. Effect of soil contamination with nickel on the activity of alkaline phosphatase (mmol PNP kg^{-1} d.m. h^{-1}).
*explanations as in Fig. 1.

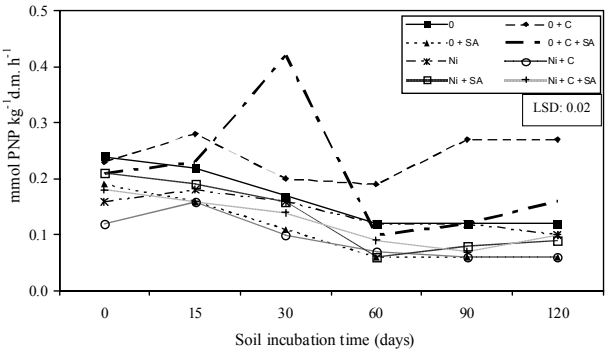

Fig. 5. Effect of soil contamination with nickel on the activity of arylsulphatase (mmol PNP kg^{-1} d.m. h^{-1}).
*explanations as in Fig. 1.

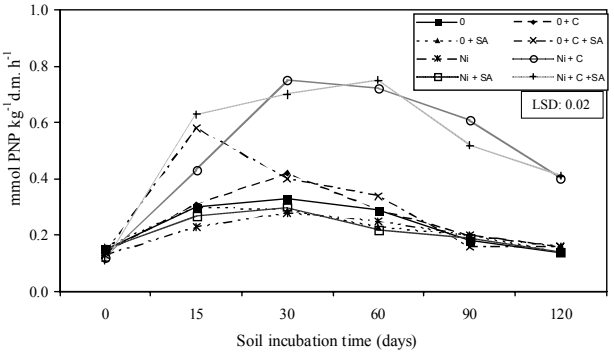

Fig. 6. Effect of soil contamination with nickel on the activity of β-glucosidase (mmol PNP kg^{-1} d.m. h^{-1}).
*explanations as in Fig. 1.

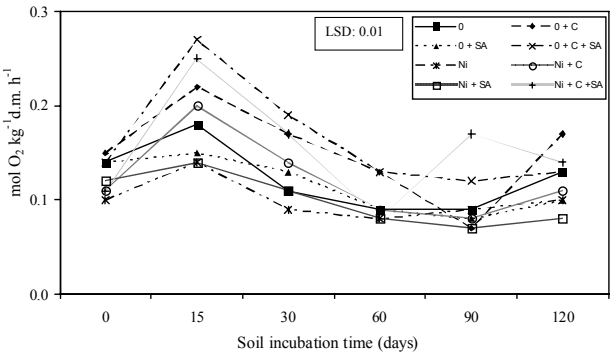

Fig. 7. Effect of soil contamination with nickel on the activity of catalase (mol O_2 kg^{-1}d.m. h^{-1}).
*explanations as in Fig. 1.

Arylsulphatase activity (Fig. 5) was dependent on nickel contamination to an even lower degree than the activity of phosphatases. On average, this element inhibited arylsulphatase activity by 18%. However, from days 30 to 90 of soil incubation, nickel did not decrease arylsulphatase activity, and the above average value was determined by activity levels measured at the other three time points. The protective effect of cellulose was not observed with respect to this enzyme.

β-glucosidase activity (Fig. 6) was inhibited by nickel in 9% on average. Ammonium sulphate exerted a similar influence on this enzyme, while cellulose caused a highly significant (over twofold) increase in β-glucosidase activity, regardless of the levels of ammonium sulphate fertilization. The activity of this enzyme varied over the experimental period – it remained at a higher level from days 15 to 60, and then decreased gradually, as confirmed by measurements performed on days 90 and 120.

Catalase activity (Fig. 7) was inhibited by both nickel and ammonium sulphate, on average by 20% and 7%, respectively. The negative impact of ammonium sulphate was even stronger in nickel-contaminated soil. The effect of cellulose on catalase was highly positive. When applied alone (without ammonium sulphate), this polysaccharide substantially alleviated the adverse influence of nickel. When applied in combination with ammonium sulphate, cellulose completely eliminated the inhibitory effect of this heavy metal. Catalase activity, similar to the activity of the other tested enzymes, fluctuated over the experimental period, reaching the highest value on day 15 of soil incubation.

The sensitivity of the analyzed enzymes to nickel may be presented in the form of the following series: urease > dehydrogenases > alkaline phosphatase > acid phosphatase > catalase > arylsulphatase > β-glucosidase. This series indicates that enzymatic activity decreased in nickel-contaminated soil, as follows: urease – by 54%, dehydrogenases – by 49%, alkaline phosphatase – by 39%, acid phosphatase – by 27%, catalase – by 20%, arylsulphatase – by 18%, and β-glucosidase – by 9%. Our results are partly consistent with the findings of Kandeler et al. [16], who also demonstrated that urease is more susceptible to the negative effect of nickel than phosphatases and arylsulphatase. Similar results, with regard to dehydrogenases and catalase, were obtained by Kizilkaya et al. [7]. However, these authors observed a completely different effect of nickel on urease. In their study nickel stimulated the activity of urease, which is natural provided that this element is present in soil in quantities sufficient to support microbial growth, since it is contained in the active center of urease.

The activity of all enzymes was stimulated by cellulose added to the soil. When applied together with ammonium sulphate, this carbohydrate exerted a particularly beneficial influence and limited the negative impact of nickel to the highest degree. Cellulose was more effective when combined with ammonium sulphate because it allowed control of nitrogen deficiency in soil that resulted from more intensive growth of micoorganisms [29].

Cellulose not only improved the biochemical properties of non-contaminated soil, but also significantly alleviated the negative effect of nickel on particular enzymes, which can be explained by a beneficial influence exerted by this polysaccharide on the entire spectrum of soil biological properties [30]. According to MacCarty [22], organic substance may be a factor preventing soil contamination through effective immobilization of heavy metals. The complex process of binding heavy metal cations may occur by way of adsorption, comprising the formation of salts, chelate compounds, complex compounds and heterocyclic bonds [17, 20]. On the other hand, chelate compounds may enhance the mobility of some heavy metals, including nickel.

Tejada et al. [31] also pointed out a positive role of organic substance in neutralizing the adverse effect of nickel on soil enzymatic activity. These authors found that under laboratory conditions the negative effect of soil contamination with nickel on the activity of urease, BBA-protease, alkaline phosphatase, β-glucosidase and arylsulphatase may be alleviated by the application of poultry dung and cotton burr compost. Chaudhuri et al. [15] also demonstrated that the supply of organic matter to soil can diminish the negative effect of heavy metals on the activity of dehydrogenases, urease, acid phosphatase and arylsulphatase. In the present study, cellulose added to the soil

together with ammonium sulphate completely eliminated the adverse influence of nickel on urease, β-glucosidase, catalase and alkaline phosphatase, and decreased the negative effect of this heavy metal on the activity of dehydrogenases and acid phosphatase – by 16% and 22%, respectively. However, cellulose had no protective effect on arylsuphatase.

The results of this study indicate that a more significant role in improving soil tilth is played by effective management of after-harvest residues and straw that contain cellulose as the predominant carbohydrate.

Conclusions

1. Soil contamination with nickel at a dose of 400 mg Ni^{2+} kg^{-1} d.m. has a negative impact on the activity of soil enzymes.
2. The sensitivity of the analyzed enzymes to nickel may be presented in the form of the following series: urease > dehydrogenases > alkaline phosphatase > acid phosphatase > catalase > arylsulphatase > β-glucosidase.
3. The adverse effect of nickel on the activity of soil enzymes can be alleviated by soil enrichment with cellulose accompanied by fertilization with ammonium sulphate.
4. The activity of enzymes varies even in soil incubated at a constant temperature and under constant moisture conditions. The highest activity of dehydrogenases, urease, arylsulphatase and catalase was reported on day 15 of the experiment, while the highest activity of acid phosphatase, alkaline phosphatase and β-glucosidase was observed on day 30.

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