Polish J. of Environ. Stud. Vol. 15, No. 3 (2006), 501-506

Original Research

Application of the Activity of Soil Enzymes in the Evaluation of Soil Contamination by Diesel Oil

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Received: May 25, 2005
Accepted: January 13, 2006

Abstract

A pot experiment was undertaken to investigate the effect of soil contamination with diesel oil, used at doses of 0, 3, 6, 9, 12 and 24 g kg^{-1}, on the activity of dehydrogenases, urease, acid phosphatase and alkaline phosphatase. An attempt was also made to reduce the negative effects of diesel oil on the biochemical activity of soil by its fertilization with finely ground pinewood sawdust and urea. Enzymatic activity was determined on days 14, 58 and 108 of the experiment.

The experiment demonstrated that soil contamination with diesel oil at amounts from 3 to 24 mg kg^{-1} disturbed the biochemical equilibrium of soil. The contamination stimulated the activity of dehydrogeanses, urease and alkaline phosphatase. In addition, it had adverse influence on the growth and development of oat and maize.

Keywords: soil contamination, diesel oil, enzymatic activity, oat, maize, sawdust

Introduction

Contamination of the natural environment with petroleum-derived compounds poses an extremely serious problem, and products of the petro-chemical industry, including: aliphatic-, ethylenic-, naphthene- and aromatic hydrocarbons are on top of the list of the most dangerous xenobiotics [1]. Components of the petroleum-derived products, mainly aromatic hydrocarbons (benzene, toluene, xylene), display potential carcinogenic and mutagenic activities [2]. Although they can be biodegraded easily in contrast to man-made compounds, they are also dangerous. Diesel oil contains primary hydrocarbons formed by 9-23 carbon atoms per molecule, and the proportion of their aromatic hydrocarbons reaches 45% of the total composition [3].

Contamination of soil with diesel oil tends to upset the biological balance of soil [4-12]. It usually alters the succession of microorganisms [13], which is directly associated with the activity of soil enzymes [9, 11]. In general, it can be said that apart from some quantitative changes which occur under such conditions, the counts of microorganisms grow [6, 12] and the activity of enzymes increases [11]. This, however, does not lead to any improvement in soil fertility. The response of plants to diesel oil contamination is unambiguously negative [14, 15]. This is mainly due to the destructive influence of diesel oil on the soil structure as well as soil air, water and chemical properties [5, 16]. The relevant literature references [4, 13-15, 17-19] report a number of biological assays used for the evaluation of the degree of soil pollution with oil and its bioremediation. A few of these assays take into account the activity of soil enzymes [20-25], but on this subject there is a lack of national investigations. Therefore, it seems useful to undertake research in this field.

The authors' own studies [7, 10, 11] indicate that plants grown as a main crop respond negatively to soil contamination with diesel oil or petrol. In all our previous trials plants were sown to soil directly after contamination treatment. This encouraged us to set up an experiment

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which would enable us to answer the following questions: what is the course of enzymatic activity and how do plants respond to soil contamination with diesel oil when sown 14 days after a contamination event; finally, to what extent can the effect produced by diesel oil be prolonged onto the after crop and whether pinewood sawdust can attenuate the effect produced by soil contaminated with diesel oil. Some earlier studies suggest that straw fertilization can be a means alleviating the effects produced by diesel oil and petrol on biochemical properties of soil and on the growth and development of plants [8, 26]. Sawdust is less readily degradable in soil than straw. Thus, it may be able to maintain favourable air conditions in contaminated soil for a longer time period, which could be of great importance to the enzymatic activity of soil and for plant yields. Since both sawdust and diesel oil are an additional source of carbon for microorganisms, the effects produced by these two factors were tested in conjunction with urea fertilization. This was supposed to prevent a nitrogen deficit in soil.

Thus, the aim of the experiment was to determine the effect of soil contamination with diesel oil on the activity of dehydrogenases, urease, alkaline phosphatase and acid phosphatase, as well as on the yield of oats grown as main crop and maize grown as after crop. Another important aim of the experiment was to verify the suitability of pinewood sawdust for alleviation of negative effects caused by soil contamination with diesel oil.

Experimental Procedures

Experiments were carried out in a greenhouse in polyethylene pots (in 4 replicates). Each of the pots was filled with 3.2 kg of Eutric Cambisols soil formed from light loamy sand (1.0-0.1 mm – 53%; 0.1-0.02 mm – 34%; <0.02 mm – 13%), collected from the ploughing-organic layer. The experimental soil was characterized by the following properties: pH in 1 mol KCl dm^{-3} – 6.00 cation exchange capacity (CEC) – 75.40 mmol(+) kg^{-1}, base saturation (BS) – 86.21%, and the content of $C_{organic}$ – 6.00 g kg^{-1} and N – 0.44 g kg^{-1}.

The soil was passed through a sieve with a mesh diameter of 1 cm^2 and then carefully mixed with diesel oil (DO), pinewood sawdust and macro- and microelements. The rates of diesel oil used for soil contamination were 0, 3, 6, 9, 12 and 24 g kg^{-1}, while pinewood sawdust was added in amounts of 0 and 5 g kg^{-1}. Mineral fertilization rates, converted into pure elements and expressed in mg kg^{-1}, were as follows: N – 125 [$CO(NH_2)_2$], P – 50 (KH_2PO_4), K – 90 (KH_2PO_4 + KCl), Mg – 20 ($MgSO_4 \cdot 7H_2O$), Zn – 5 ($ZnCl_2$), Cu – 5 ($CuSO_4 \cdot 5H_2O$), Mn – 5 ($MnCl_2 \cdot 4H_2O$), Mo – 5 ($Na_2MoO_4 \cdot 2H_2O$), and B – 0.33 (H_3BO_3). Finally, portions of the soil weighing 3.2 kg were put into polyethylene pots. Two plants were used for the trials: cv. Borowik oat (*Avena sativa* L.) grown as the main crop, and cv. Reduta maize (*Zea mays* L.) grown as the after crop.

The experiment was carried out in two series. In the first one, the soil was fertilized with an urea dose of 125 mg N kg^{-1}, in the second one – with 250 mg N kg^{-1} of urea. In the first series, urea was applied prior to oat sowing, whereas in the second series it was applied in two doses: 125 mg N before sowing and 125 mg N in the tillering phase of oat. The soil contaminated with diesel oil and fertilized with micro- and macroelements was adjusted to a moisture content of 60% of capillary water capacity and sustained in that state for 14 days. On day 14, samples were collected for biochemical analyses and oat was sown. After oat sprouting, 15 plants were left in each pot. Immediately after oat harvest, in the phase of pulling out panicles (44 day of vegetation), soil samples were collected for biochemical analyses and maize was sown. In the case of maize, only supplementary fertilization with nitrogen was applied at a dose of 62.5 mg N kg^{-1} soil in those pots in which oat was previously fertilized with the lowest nitrogen dose (125 mg), and at a dose of 125 mg N kg^{-1}, in combinations which were fertilized with a higher nitrogen dose (250 mg) before oat sowing. As in the case of oat, maize was also fertilized with urea. After sprouting, 4 maize plants were left in each pot. On day 50 of vegetation, the plants were harvested and soil samples were collected for analyses. Over the entire experimental period (108 days), the constant moisture content of soil was maintained at the level of 60% of capillary water capacity. The plant material (above-ground parts) collected from each pot, dried at 70°C, weighed and statistically analyzed.

Determinations of soil enzymatic activity were carried out three times: 14 days after placing soil in the pots, i.e. in the day of sowing oat, then after the harvest of oat, and finally after the harvest of maize (the successive plant). Soil samples to be used for biochemical analyses were sieved in a mesh diameter of 2 mm^2. The biochemical analyses of soil consisted in determining the activities of: soil dehydrogenases (Deh) – with a TTC substrate [27], urease (Ure) – according to Alef and Nannpieri [28], as well as acid phosphatase (Pac) and alkaline phosphatase (Pal) – according to the method described by Alef et al. [29]. A 3% aqueous solution of TTC (2,3,5-triphenyltetrazolium chloride) was used as the substrate of dehydrogenases. Soil incubation was carried out for 24 h at a 37°C. The absorbance of the TPF (triphenylformazane) formed was measured at the wave length of 485 nm. The results were converted into cm^3 H_2 kg^{-1} d.w. soil d^{-1}. The substrate of urease was a 10% solution of urea. The soil was incubated for 24 h at 37°C. The amount of $N\text{-}NH_4$ produced was determined with Nessler's reagent. The absorbance of the ammoniated mercury iodide formed was measured spectrophotometrically at a wavelength of 410 nm and converted into the amount (mg) of the produced $N\text{-}NH_4$ kg^{-1} d. w. soil h^{-1}. The substrate of phosphatases was sodium 4-nitrophenylphosphate (PNPP). The soil was incubated at a temperature of 37°C for 1 h (acid phosphatase – pH 6.5, alkaline phosphatase – pH 11). After incubation, the absorbance of the PNP (p-nitrophenol) formed was measured with a spectrophotometer at the wavelength of 410 nm. The results were expressed in mmoles of the formed PNP kg^{-1} d.w. soil h^{-1}.

The results were elaborated statistically with the use of two- and three-factor variance analysis with ANOVA. In addition, based on biochemical analyses carried out in triplicate, Pearson's simple correlation coefficients were calculated between the plant yield and the activity of soil enzymes [30].

Results and Discussion

Diesel oil significantly increased the activity of soil dehydrogenases (Fig. 1). Its stimulating influence was stronger as the contamination rates increased. Diesel oil produced a stronger effect in soil not sown with plants and maintained in such a state for 14 days, than in soil sown first with oats (main crop) and, after oat harvest, with maize (after crop). It is interesting to note that the stimulation of dehydrogenases was stronger in the soil under maize (after crop) than under oats (main crop). Theoretically, it seems that soil under the successive crop (maize) should reveal weaker effects of diesel oil contamination, as the pollutant had remained in the soil for a longer time period and was therefore more exposed to biodegradation. Fertilization with urea and pinewood sawdust also had a positive effect on the activity of dehydrogenases. Both fertilizers compounded the influence produced by diesel oil on these enzymes.

Diesel oil stimulated the activity of urease (Fig. 2). Analogously to dehydrogenases, urease was under stronger influence of diesel oil during the first 14 days of the experiment. The stimulation became weaker when oat and then maize were grown in the soil. Urease was also strongly stimulated by urea fertilization, but only in the

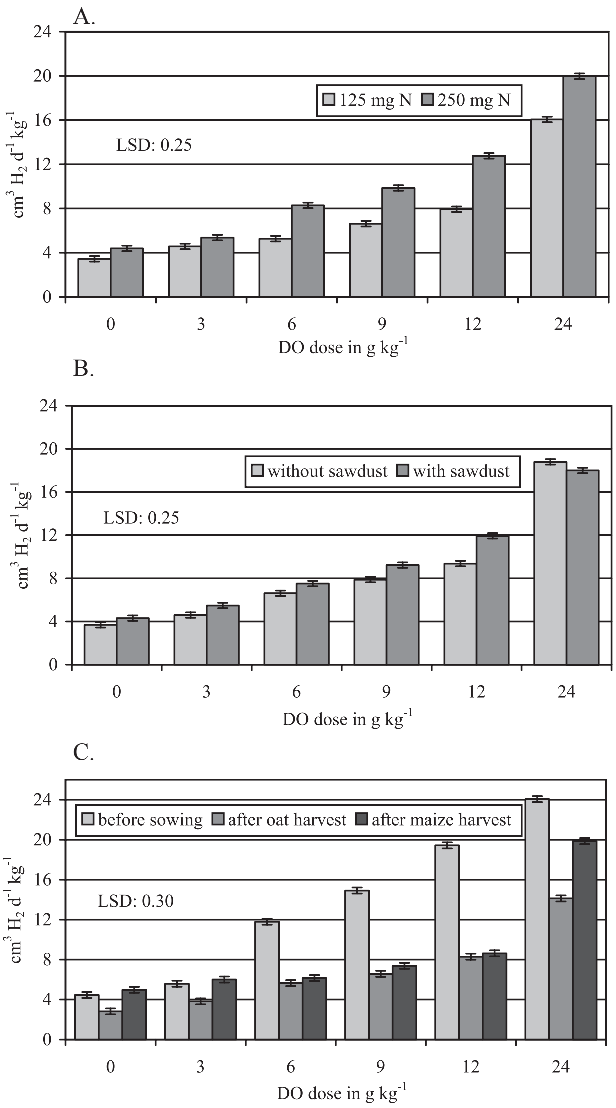

Fig. 1. The soil dehydrogenases activity depending on diesel oil and urea doses after the crops (A), diesel oil doses and sawdust fertilization after the crops (B), diesel oil doses and term of analysis (C).

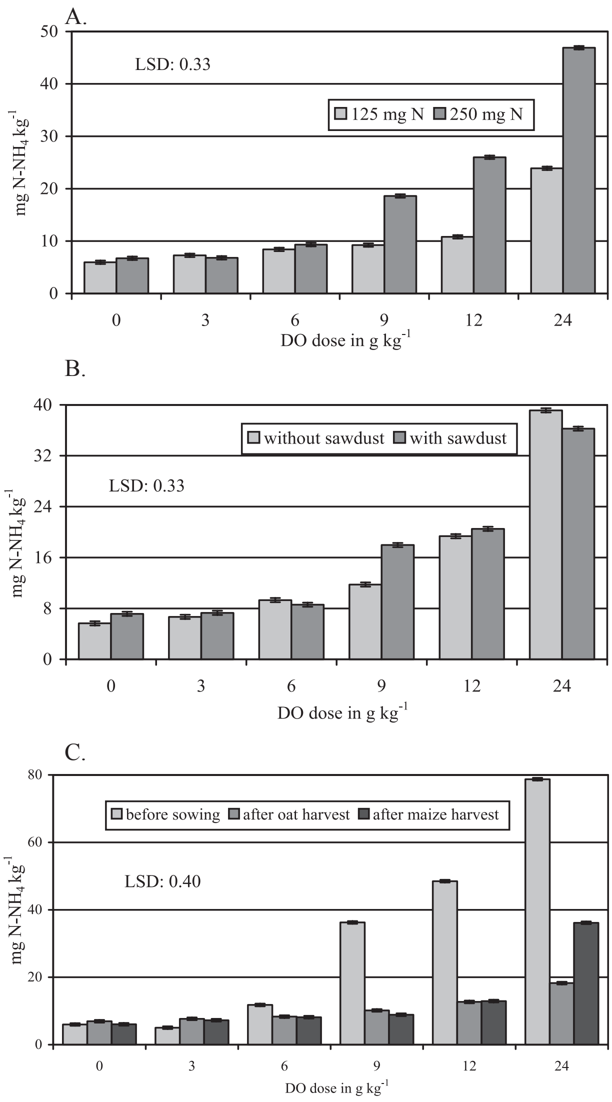

Fig. 2. The soil urease activity depending on diesel oil and urea doses after the crops (A), diesel oil doses and sawdust fertilization after the crops (B), diesel oil doses and term of analysis (C).

soils contaminated with the highest rates of diesel oil (9-24 g/dm^3). Pinewood sawdust added to soil did not affect urease as strongly as urea. The impact produced by pinewood sawdust appeared only in the soil contaminated with 9 g diesel oil per 1 kg^{-1} soil, where the activity of urease was stimulated, and in the soil polluted with 24 g of diesel oil per 1 kg^{-1}, where the enzyme was inhibited.

Diesel oil stimulated the activity of alkaline phosphatase in soil (Fig.3). The activity of alkaline phosphatase in the non-contaminated soil and in the soil polluted with 3 g of diesel oil per kg^{-1} of soil was the highest when maize was grown as the after crop. In the other objects, the stimulatory influence of diesel oil on alkaline phosphatase was weaker in the soil under maize. The activity of alkaline phosphatase was also stimulated by the fertilization treatments with urea and sawdust, but only in the soils contaminated with diesel oil from 6 to 24 g kg^{-1}. In the soil polluted with the lowest amount of diesel oil (3 g kg^{-1}), both urea and sawdust had negative influence on alkaline phosphatase.

The response of acid phosphatase to soil contamination with diesel oil was quite the reverse to the above (Fig. 4). Acid phosphatase was initially stimulated by diesel oil, which was revealed by the determinations completed on day 14 of the experiment. However, later on its activity began to decline, and the decrease was bigger at higher levels of contamination. The negative effect of diesel oil contamination was stronger in the soil under the successive plant (maize) than under the main crop (oat). A very strong and positive effect on the activity of acid phosphatase was produced by urea fertilization, in contrast to the application of pinewood sawdust, which depressed the activity of this soil enzyme.

To recapitulate, it can be claimed that the effect of diesel

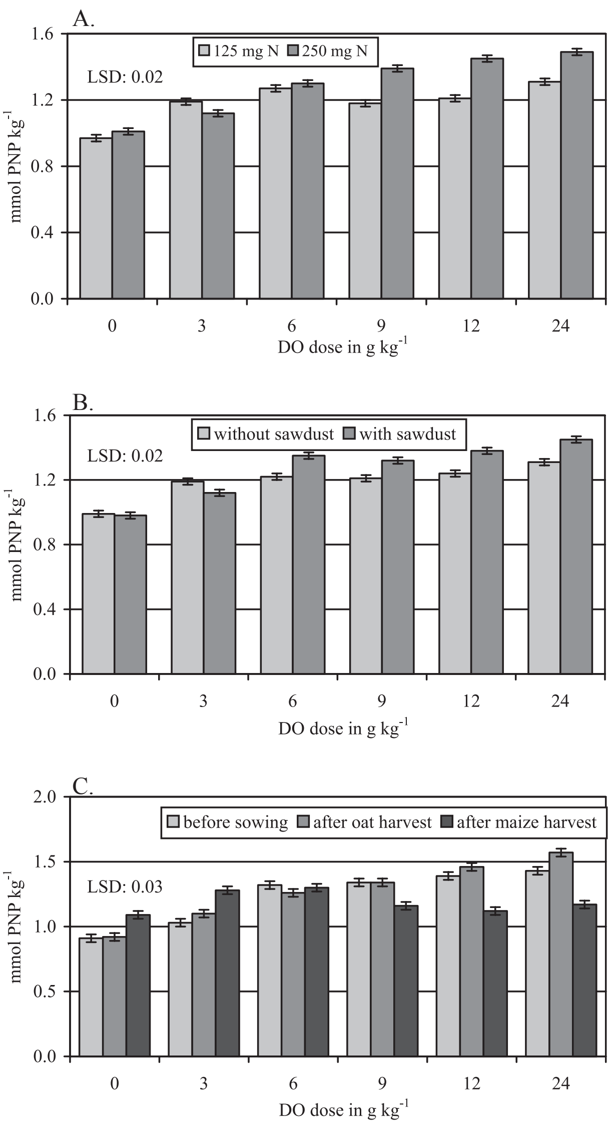

Fig. 3. Soil alkaline phosphatase activity depending on diesel oil and urea doses after crops (A), diesel oil doses and sawdust fertilization after crops (B), diesel oil doses and terms of analysis (C).

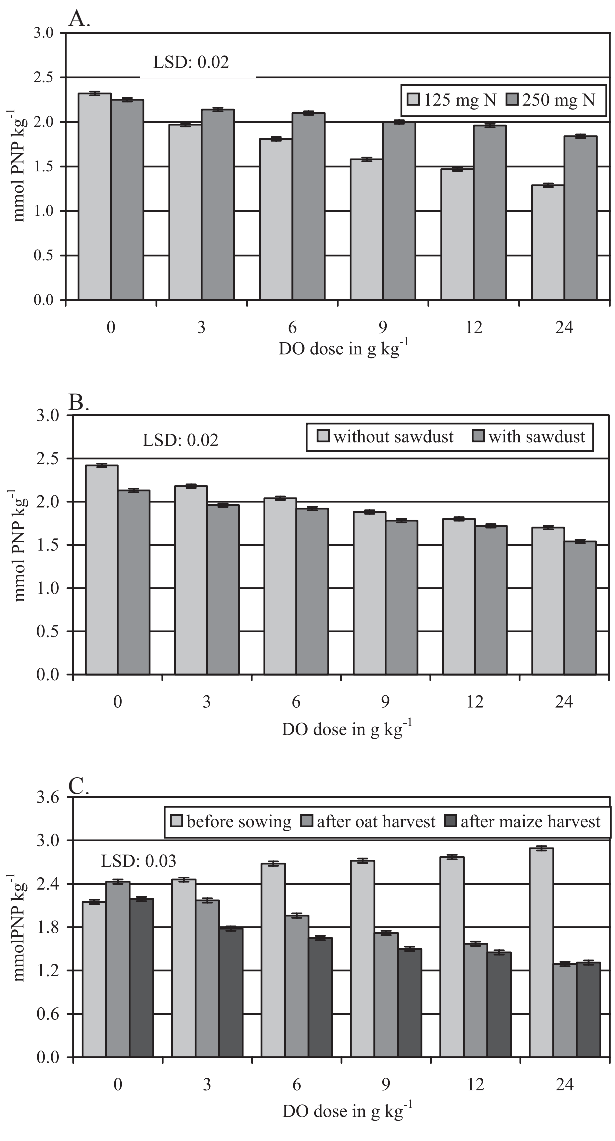

Fig. 4. Soil acid phosphatase activity depending on diesel oil and urea doses after crops (A), diesel oil doses and sawdust fertilization after crops (B), diesel oil doses and terms of analysis (C).

oil on the examined soil enzymes was highly significant. Diesel oil stimulated dehydrogenases, urease and alkaline phosphatase, but inhibited acid phosphatase. In general, the influence produced by diesel oil tended to grow weaker with time. This seems only logical, as diesel oil could at least in part volatilize to the atmosphere and also undergo biodegradation and phytoremediation [21]. Diesel oil volatilization to the atmosphere could be stronger than natural soil because it was a potted experiment with the amount of soil in pots limited. Machałowska-Jutsz et al. [31] observed that in the first weeks soil contamination with diesel oil caused a significant increase in the activity of amylases, proteases and dehydrogenases, while in the following weeks it contributed to a depression in their activity. The stimulating influence of diesel oil on dehydrogenases, urease and alkaline phosphatase has also been confirmed by the authors in their previous studies [11]. They were, however, much shorter in duration and as a result corresponded more closely to the data obtained for the soil analyzed on day 14 of the present experiment. In addition, they did not involve urea or wood sawdust fertilization as experimental factors. Here, increased urea fertilization turned out to be particularly important, as it produced a beneficial effect on the unbalanced carbon-to-nitrogen ratio. The soil polluted with the highest rate of diesel oil was recorded to have the C:N ratio reaching 16.70 (these data are not included in the figures). The same issue is mentioned in the research conducted by Margesin and Schinner [32].

However, the most important and direct test for soil contamination is the plant assay, which proved unambiguously that diesel oil was strongly toxic to oats (grown as the main crop) [Fig.5]. Under the effect of diesel oil rates from 6 to 24 g kg^{-1}, the growth of oats was nearly completely inhibited, and this adverse effect could not be reversed either by urea fertilization or by the application of pinewood sawdust to the soil. The influence of diesel oil on the after crop (maize) was much weaker. Under the influence of diesel oil added in the rates from 3 to 9 g kg^{-1}, a significant increment in the mass of maize plants was observed, and toxic impact was produced only by the highest contamination rates (12 and 24 g kg^{-1}). The yield of maize was positively affected by urea and pinewood sawdust fertilization. The weaker influence of diesel oil produced on maize than on oats could also have resulted from its partial biodegradation [18].

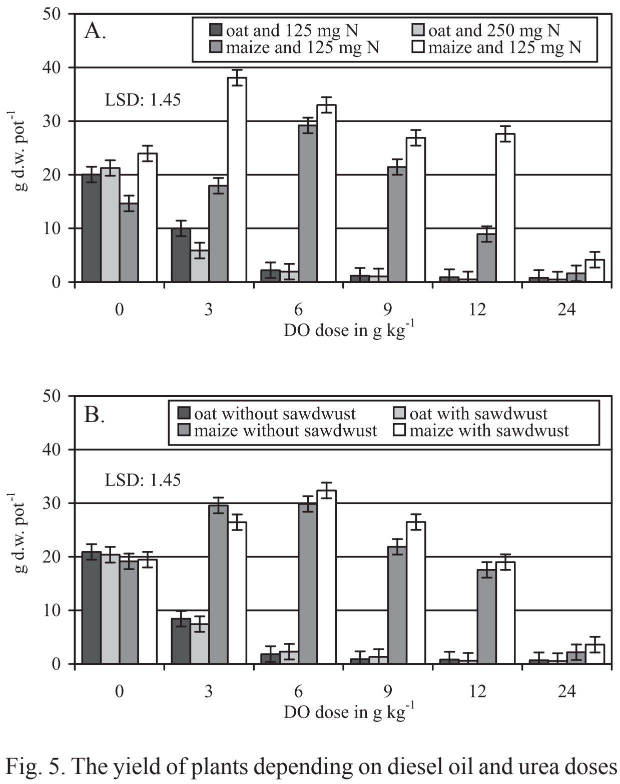

Fig. 5. The yield of plants depending on diesel oil and urea doses (A), diesel oil doses and sawdust fertilization (B).

Diesel oil was toxic to plants [18]. Its influence on the oats and maize grown also was due to physical and chemical soil properties [5, 7, 16]. In this context, the response of maize (after crop) to lower levels of diesel oil contamination appears to be optimistic, and so does the alleviating influence of urea fertilization and pinewood sawdust application on maize grown in the soil most strongly polluted with diesel oil (12 and 24 g kg^{-1}). Nonetheless, the yields of the crops obtained suggest that the activity of soil enzymes is not a good indicator of soil contamination with diesel oil. This is made evident by the significantly negative correlation between the yield of plants and activity of dehydrogenases (-0.92) and urease (-0.71) as well as the lack of significant, positive correlation between the yield of plants and activity of acid phosphatase (0.40) and alkaline phosphatase (0.24).

Conclusions

1. Soil contamination with diesel oil at doses ranging from 3 to 24 g kg^{-1} unbalanced the biochemical properties of soil. Irrespective of sawdust and urea fertilization for 108 days, that substance was observed to stimulate the activities of dehydrogenases, urease and alkaline phosphatase.
2. Soil enrichment with organic matter in the form of pinewood sawdust, as well as intensified fertilization with urea exerted a positive effect on the enzymatic activity of soil.
3. Soil contamination with diesel oil at doses ranging from 3 g to 24 g kg^{-1} had a negative impact on the growth and development of oat, and that applied at doses of 12-24 g kg^{-1} – on the growth and development of maize.
4. Soil supplementation with sawdust and nitrogen attenuated the negative impact of diesel oil on the yield of maize (the successive plant), yet it failed to reduce their impact on oats (the main plant).
5. The activity of dehydrogenases and urease in the soil contaminated with diesel oil was negatively correlated with the yields of oats and maize.

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