Role of compost, bentonite and lime in recovering the biochemical equilibrium of diesel oil contaminated soil

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ABSTRACT

The aim of the study was to determine how soil contamination with diesel oil affected biochemical properties of soil and to determine whether the application of compost, bentonite or lime could recover the biochemical equilibrium of soil. The experiments were carried out in a greenhouse. Typical Eutric Cambisols soil formed from sandy loam was polluted with the following amounts of diesel oil: 2.5, 5.0 and 10 cm^3/kg of soil. The results of the tests showed that the contamination of soil with diesel oil at the amount between 2.5 and 10 cm^3/kg of soil disturbed the biochemical balance of soil. Irrespective of the application of compost, bentonite or lime and regardless which plant species was grown, diesel oil significantly (P = 0.01) stimulated the activity of dehydrogenases, urease, and alkaline phosphatase as well as the nitrification of soil. Enrichment of soil with compost, bentonite or lime stimulated the activity of urease, alkaline phosphatase and nitrification. The activity of dehydrogenases, urease and nitrification of soil, in contrast to the activity of acid phosphatase, was higher in soil under spring oilseed rape than in soil under oats. The activity of dehydrogenases, urease, alkaline phosphatase in soil contaminated with diesel oil was positively correlated with the nitrification of soil. The correlation between the activity of acid phosphatase and soil nitrification was negative.

Keywords: diesel oil contamination; activity of dehydrogenases; urease; phosphatases; nitrification; compost; bentonite; lime

Petroleum products that are widespread in the natural environment, contribute to soil degradation by deteriorating soil, air, water and chemical properties (Wang and Bartha 1990, Sztompka 1999). Such contaminants are toxic to soil organisms and to plants (Delille and Pelletier 2002, Wyszkowska et al. 2002a, b) and can transfer to surface and ground waters, threatening thus the human health (Siuta 1995). It is therefore extremely important that contaminated soil should recover its original physical, chemical and biological properties in the shortest possible time. Remediation of soil contaminated with petroleum products is carried out with aeration, optimum soil moisture, mineral and organic fertilization, cultivation of fast-growing plants or introduction of microorganisms (Wyszkowska et al. 2002a, b, Caravaca and Roldán 2003).

Soil enzymatic activities often respond to soil pollution by heavy metals, pesticides or petroleum products (Kiss 1999, Renella et al. 2005). According to Kiss (1999) and Przystas et al. (2000), the activity of soil enzymes is the most appropriate measure of soil biological activity, because the soil enzymes are the basis of soil metabolism and they decide for speed and direction of metabolic transformations that occur in soil. However, the knowledge of the effects of diesel oil on different soil enzyme activities is still poor. The aim of this work was hence to determine the effect of diesel oil contamination of soil on soil dehydrogenases, urease, acid and alkaline phosphatases and nitrification, and to investigate if soil amendment with compost, bentonite and lime could reduce the impact of oil on the studied soil biochemical properties.

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MATERIAL AND METHODS

The experiment was performed in a greenhouse at the University of Warmia and Mazury in Olsztyn (Poland), in polyethylene pots (with 4 replications). Soil material used for the trials was taken from the arable humus soil horizon. Under natural conditions it was typical Eutric Cambisol according to World reference base for soil resources (1998) formed from sandy loam, with the following characteristics: pH in 1M KCl/dm^3 – 5.10; hydrolytic acidity (HA) – 30.8 mmol (H^+)/kg; exchangeable cation bases – Ca^{2+}, Mg^{2+}, K^+ and Na^+ (ECB) – 88.0 mmol(+)/kg; cation exchange capacity (CEC) – 118.8 mmol(+)/kg; base saturation (BS) – 74.1%; C_{org} content – 8.48 g/kg; available phosphorus content – 34.1 mg/kg; available potassium content – 75.2 mg/kg; available magnesium content – 36.7 mg/kg.

The plants tested were Polish cv. Mazowiecki of spring oilseed rape (*Brassica napus* var. *oleifera*) and Polish cv. Borowik of oat (*Avena sativa* L.) as an aftercrop. Prior to placing in the pots, the soil was passed through a sieve with the mesh size 1 cm^2 and mixed with mineral fertilizers, and with diesel oil as well as with compost, bentonite and lime. The concentration of macroelements in these substances (in g/kg) was as follows: compost: P – 2.32, K – 1.33, Mg – 1.47, Ca – 15.86, Na – 0.12; bentonite: P – 0.47, K – 2.43, Mg – 5.03, Ca – 26.72, Na – 12.11; lime: P – 0.10, K – 0.77, Mg – 2.65, Ca – 347.99, Na – 0.07.

A single rate of fertilization with micro- and macroelements was applied, with the following doses of elements (calculated as elements in mg/kg d.m.): N – 150 [$CO(NH_2)_2$]; P – 30 (KH_2PO_4); K – 70 (KH_2PO_4 + KCl); Mg – 50 ($MgSO_4.7\,H_2O$); Mn – 5 ($MnCl_2.4\,H_2O$); Mo – 5 [$(NH_4)_6Mo_7O_{24}.4\,H_2O$]; B – 0.33 ($H_3BO_3$). After that some pots were enriched with compost (3% relative to soil mass), bentonite (2% relative to soil mass) or lime (in a dose equal to one full hydrolytic acidity – 630 mg Ca/kg of soil). The soil samples thusly prepared were contaminated with diesel oil in the following amounts 0, 2.5, 5.0 and 10 cm^3/kg d.m. of soil. The characteristics of diesel oil were as follows: water content – maximum 220 mg/kg, solid contaminants – maximum 24 mg/kg, sulphur concentration – maximum 0.5 mg/kg, density (temperature 15°C) – maximum 860 kg/m^3, viscosity (temperature 40°C) – 4.5 mm^2/s (PN-EN 590, 1999). The mineral fertilizers, in the form of aqueous solutions, as well as diesel oil and neutralizing substances, wherever appropriate, were introduced to the soil once prior to sowing spring oilseed rape, by mixing the substances with the whole mass of soil per pot. The soil samples prepared (each 9.5 kg) were placed in pots, where their moisture content was brought up to 60% of capillary water capacity. Finally, spring oilseed rape was sown. After emergence 8 plants per pot were left to grow. Immediately after the harvest of the main crop in the flowering stage (58 day of vegetation) and collecting soil samples for biochemical analyses, oat as an aftercrop was sown. After emergence, 15 plants were left in each pot. The harvest of oat was carried in the panicle stage (52 day of vegetation) and soil samples were collected again. During the whole experiment that lasted for 110 days (May–September) constant moisture equal to 60% of capillary water capacity was maintained. The temperature of these months fluctuated from 4.3 to 29.6°C and light regime from 13 hours 19 minutes to 17 hours 16 minutes.

The biochemical analyses involved the determination of:

– Activity of soil dehydrogenases (Deh) with TTC substrate (Öhlinger 1996). A 3% aqueous solution of TTC (2,3,5-triphenyltetrazolium chloride) was used as a substrate of dehydrogenases. Soil incubation was carried out for 24 hours at 37°C. Extinction of TPF (triphenylformazane) thus produced was measured on a spectrophotometer at a wavelength of 485 nm. The results were expressed in cm^3 H_2/d/kg d.m. of soil.
– Activity of urease (Ure) – according to Alef and Nannipieri (1998). The substrate of urease was a 10% aqueous solution of urea. Soil was incubated for 24 h at 37°C. The amount of N-NH_4^+ obtained was determined using Nessler's reagent. Extinction of amidomercury iodide was measured on a spectrophotometer at a wavelength of 410 nm and converted into the amount (mg) of N-NH_4^+/kg d.m. of soil.
– Activity of acid phosphatase (Pac) and alkaline phosphatase (Pal) was measured according to the method described by Alef et al. (1998). The substrate of the phosphatases was 4-nitrophenyl phosphate, disodium salt, hexahydrate. Soil was incubated at 37°C for 1 h (acid phosphatase in pH 6.5 and alkaline phosphatase in pH 11). After incubation extinction of PNP (p-nitrophenol) thus produced was determined on a spectrophotometer at a wavelength of 410 nm. The results were converted to mmols of PNP/h/kg of soil.
– Nitrification of soil according to Kandeler (1996). The substrate was ammonium sulphate. Soil was incubated for 21 days at 25°C and the control samples were kept at –20°C. After incubation

$N\text{-}NH_4^+$ was determined using Nessler's reagent, and $N\text{-}NO_3^-$ – with phenoldisulphonic acid. For extraction of mineral acid a 1% aqueous solution of K_2SO_4 was used. The relation of extractor to soil was 5:1. The measurement of extinction was carried out on a spectrophotometer at a wavelength of 410 nm. The results were calculated as a percentage of nitrified nitrogen during 24 hours.

Prior to plant sowing, the soil was analysed for: pH (exchangeable acidity) with potentiometrical method with the use of aquatic solution of KCl at the concentration of 1M KCl/dm^3; hydrolytic acidity – exchangeable H^+ and Al^{3+} (HA) and exchangeable cation bases – Ca^{2+}, Mg^{2+}, K^+ and Na^+ (ECB) with Kappen method; and the content of organic carbon (C_{org}) with Tiurin method (Lityński et al. 1976). Based on the hydrolytic acidity and exchangeable cation bases, the cation exchange capacity (CEC) and base saturation (BS) were calculated with the following formulas: CEC = ECB + HA, BS = (ECB/CEC) × 100.

The results were processed statistically using a three-factor analysis of variance ANOVA. Additionally, regression equations and determination coefficients were calculated for the degree of soil contamination with diesel oil versus the activity of soil enzymes and nitrification. Finally, Pearson's simple correlation coefficients between the variables tested experimentally were calculated, considering all the three replications for which the biochemical analyses were made (Statsoft, Inc. 2003).

RESULTS AND DISCUSSION

Diesel oil which permeates soil has adverse influence on soil environment, which can be concluded from observations of the upset biochemical equilibrium of soil measured as an activity of dehydrogenases (Table 1), urease (Table 2), acid phosphatase (Table 3), alkaline phosphatase (Table 4) or nitrification (Table 5). The present study demonstrated that the actual effect of diesel oil on the biochemical activity of soil depended on the degree of contamination, application of a substance which neutralized the pollution (compost, bentonite and lime) as well as on the species of cultivated crops, and in consequence, the duration of contamination. Diesel oil, irrespective of the species of crops or addition of neutralizing substances, significantly stimulated the activity of dehydrogenases, urease, alkaline phosphatase as well as nitrification, but inhibited the activity

Table 1. Effect of diesel oil contamination on the activity of dehydrogenases (cm^3 H_2/24 h/1 kg d.m. of soil)

DO dose (cm^3/kg of soil)	Type of substance			
	without substances	compost	bentonite	lime
Spring oilseed rape (*Brassica napus* var. *oleifera*)				
0	6.73 (± 0.09)	7.41 (± 0.11)	4.18 (± 0.05)	7.65 (± 0.05)
2.5	9.52 (± 0.10)	8.75 (± 0.13)	8.37 (± 0.10)	15.77 (± 0.38)
5	9.71 (± 0.10)	9.62 (± 0.15)	11.35 (± 0.19)	20.78 (± 0.58)
10	11.16 (± 0.13)	16.93 (± 0.38)	14.33 (± 0.10)	27.41 (± 0.48)
r	0.91**	0.96**	0.97**	0.98**
Oat (*Avena sativa* L.)				
0	3.08 (± 0.19)	3.32 (± 0.05)	2.31 (± 0.06)	3.70 (± 0.14)
2.5	3.41 (± 0.05)	3.22 (± 0.05)	2.31 (± 0.08)	4.66 (± 0.05)
5	3.51 (± 0.05)	3.51 (± 0.08)	3.70 (± 0.14)	5.19 (± 0.10)
10	5.19 (± 0.38)	4.18 (± 0.05)	4.04 (± 0.19)	6.54 (± 0.77)
r	0.95**	0.95**	0.94**	0.99**
$LSD_{P=0.01}$	a – 0.14, b – 0.14, c – 0.10, a × b – 0.28, a × c – 0.20, b × c – 0.20, a × b × c – 0.39			

± standard deviation; LSD for: a – dose of diesel oil (DO), b – addition of a neutralizing substance, c – plant species; *r* – correlation coefficient significant for: $^{**}P = 0.01$, $^{*}P = 0.05$, $n = 12$

Table 2. Effect of diesel oil contamination on the activity of urease (mg N-NH_4^+/h/1 kg d.m. of soil)

DO dose (cm^3/kg of soil)	Type of substance			
	without substances	compost	bentonite	lime
Spring oilseed rape (*Brassica napus* var. *oleifera*)				
0	7.44 (± 0.24)	8.16 (± 0.21)	12.73 (± 0.05)	17.77 (± 0.03)
2.5	7.20 (± 0.16)	8.40 (± 0.24)	28.09 (± 0.07)	20.17 (± 0.02)
5	10.56 (± 0.21)	9.36 (± 0.24)	45.62 (± 0.02)	40.82 (± 0.03)
10	21.85 (± 0.24)	28.09 (± 0.72)	85.48 (± 0.02)	50.90 (± 0.02)
r	0.95**	0.89**	0.99**	0.98**
Oat (*Avena sativa* L.)				
0	5.52 (± 0.02)	8.40 (± 0.07)	13.45 (± 0.02)	11.28 (± 0.02)
2.5	7.44 (± 0.02)	7.92 (± 0.05)	16.33 (± 0.03)	15.85 (± 0.05)
5	7.68 (± 0.02)	11.04 (± 0.07)	22.33 (± 0.03)	16.57 (± 0.04)
10	10.80 (± 0.03)	11,44 (± 0.03)	24.25 (± 0.05)	30.97 (± 0.03)
r	0.99**	0.92**	0.94**	0.97**
$LSD_{P = 0.01}$	a – 0.19, b – 0.19, c – 0.14, a × b – 0.38, a × c – 0.27, b × c – 0.27, a × b × c – 0.54			

Explanations see Table 1

of acid phosphatase. This could be concluded from the equations of regression and significant coefficients of determination calculated for the correlation between the rate of diesel oil and enzymatic and nitrifying soil activity (Figure 1). The stimulating and significant effect produced by diesel oil on the activity of dehydrogenases, urease, alkaline phosphatase and nitrification was particularly evident in the objects polluted with the highest rate of this petroleum product (10 cm^3 diesel

Table 3. Effect of diesel oil contamination on the activity of acid phosphatase (mmol PNP/h/1 kg d.m. of soil)

DO dose (cm^3/kg of soil)	Type of substance			
	without substances	compost	bentonite	lime
Spring oilseed rape (*Brassica napus* var. *oleifera*)				
0	2.87 (± 0.05)	2.87 (± 0.04)	2.15 (± 0.05)	2.19 (± 0.03)
2.5	2.65 (± 0.05)	1.71 (± 0.02)	2.12 (± 0.07)	2.07 (± 0.02)
5	2.36 (± 0.03)	2.60 (± 0.03)	2.07 (± 0.02)	1.90 (± 0.02)
10	2.34 (± 0.02)	2.41 (± 0.02)	1.73 (± 0.02)	1.86 (± 0.02)
r	–0.88**	–0.99**	–0.94**	–0.91**
Oat (*Avena sativa* L.)				
0	2.55 (± 0.02)	2.12 (± 0.07)	1.83 (± 0.02)	1.69 (± 0.02)
2.5	2.38 (± 0.02)	1.32 (± 0.05)	1.78 (± 0.04)	1.52 (± 0.05)
5	1.91 (± 0.03)	1.57 (± 0.07)	1.47 (± 0.03)	1.44 (± 0.04)
10	1.88 (± 0.02)	1.54 (± 0.03)	1.44 (± 0.03)	1.40 (± 0.03)
r	–0.89**	–0.89**	–0.90**	–0.91**
$LSD_{P = 0.01}$	a – 0.02, b – 0.02, c – 0.01, a × b – 0.04, a × c – 0.02, b × c – 0.02, a × b × c – 0.05			

Explanations see Table 1

Table 4. Effect of diesel oil contamination on the activity of alkaline phosphatase (mmol PNP/h/1 kg d.m. of soil)

DO dose (cm^3/kg of soil)	Type of substance			
	without substances	compost	bentonite	lime
Spring oilseed rape (*Brassica napus* var. *oleifera*)				
0	0.46 (± 0.03)	0.62 (± 0.03)	0.91 (± 0.02)	0.98 (± 0.02)
2.5	0.94 (± 0.05)	1.13 (± 0.02)	1.05 (± 0.02)	1.44 (± 0.03)
5	1.03 (± 0.04)	1.17 (± 0.02)	1.42 (± 0.02)	1.50 (± 0.03)
10	1.33 (± 0.03)	1.56 (± 0.02)	1.44 (± 0.02)	1.57 (± 0.07)
r	0.98**	0.99**	0.88**	0.91**
Oat (*Avena sativa* L.)				
0	0.66 (± 0.02)	0.86 (± 0.02)	1.10 (± 0.02)	1.09 (± 0.02)
2.5	0.74 (± 0.02)	1.20 (± 0.03)	1.56 (± 0.02)	1.30 (± 0.03)
5	0.87 (± 0.03)	1.23 (± 0.03)	1.57 (± 0.03)	1.27 (± 0.02)
10	1.52 (± 0.05)	1.32 (± 0.03)	1.61 (± 0.03)	1.76 (± 0.04)
r	0.96**	0.94**	0.89**	0.97**
$LSD_{P = 0.01}$	a – 0.01, b – 0.01, c – 0.01, a × b – 0.03, a × c – 0.02, b × c – 0.02, a × b × c – 0.04			

Explanations see Table 1

oil/kg). This positive correlation was stronger in the soil under spring oilseed rape (the plant grown as the main crop) than in the soil under oats (an aftercrop). In the control series (without any organic substance or lime added), in the soil samples mixed with 10 cm^3 diesel oil/kg and planted with spring oilseed rape, the activity of dehydrogenases was 1.6-fold (Table 1) higher than in the non-

Table 5. Effect of diesel oil contamination on the activity of nitrification (% nitrified N/day)

DO dose (cm^3/kg of soil)	Type of substance			
	without substances	compost	bentonite	lime
Spring oilseed rape (*Brassica napus* var. *oleifera*)				
0	0.33 (± 0.02)	0.81 (± 0.02)	1.60 (± 0.02)	1.57 (± 0.04)
2.5	0.57 (± 0.03)	0.89 (± 0.05)	2.37 (± 0.02)	3.08 (± 0.05)
5	1.50 (± 0.05)	1.14 (± 0.05)	2.38 (± 0.03)	3.28 (± 0.13)
10	1.70 (± 0.02)	1.87 (± 0.06)	3.82 (± 0.09)	4.02 (± 0.07)
r	0.92**	0.97**	0.97**	0.91**
Oat (*Avena sativa* L.)				
0	1.06 (± 0.08)	1.99 (± 0.05)	2.37 (± 0.06)	2.73 (± 0.08)
2.5	1.40 (± 0.17)	2.60 (± 0.07)	3.25 (± 0.09)	2.45 (± 0.07)
5	2.05 (± 0.08)	4.42 (± 0.06)	3.40 (± 0.08)	2.63 (± 0.12)
10	2.89 (± 0.14)	4.91 (± 0.09)	4.59 (± 0.09)	5.97 (± 0.10)
r	0.99**	0.93**	0.98**	0.86**
$LSD_{P = 0.01}$	a – 0.04, b – 0.04, c – 0.03, a × b – 0.08, a × c – 0.06, b × c – 0.06, a × b × c – 0.11			

Explanations see Table 1

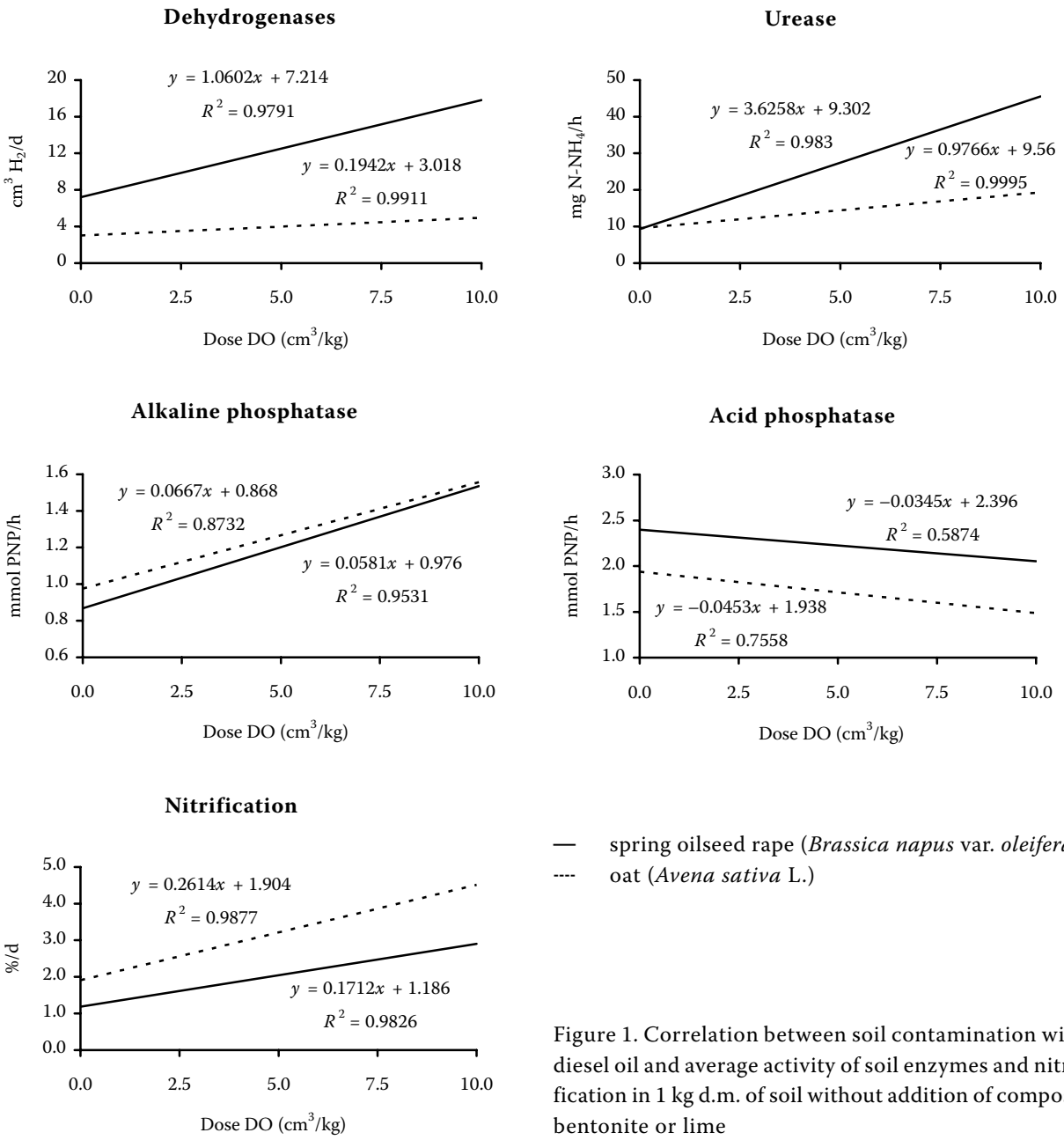

Figure 1. Correlation between soil contamination with diesel oil and average activity of soil enzymes and nitrification in 1 kg d.m. of soil without addition of compost, bentonite or lime

contaminated objects; the activity of urease and alkaline phosphatase was 2.9-fold higher (Tables 2 and 4), whereas the nitrification was as much as 5.2-fold higher (Table 5). In the soil under oats the values were 1.7, 2.0, 2.3 and 2.7, respectively. The highest activity of dehydrogenases among the objects with spring oilseed rape was found in those containing the highest rate of diesel oil. Compared to the non-contaminated objects, the increase was 2.3-fold (compost), 3.4-fold (bentonite) and 3.6-fold (lime). Similar and significant correlations were found for urease activity. In soil samples under the main crop grown with compost and polluted with 10 cm^3 diesel oil, the activity of this soil enzyme was 3.4-fold higher than in non-contaminated soil samples. The difference in the activity of urease between contaminated and non-contaminated soil samples treated with bentonite was 6.7-fold and with lime – 2.9-fold.

A very important factor, which significantly modified the enzymatic activity of soil under either of the test plant species, was an addition of compost, bentonite or lime (Figure 2). On average, irrespective of the degree of contamination, all components introduced to soil in order to mollify possible negative effects of diesel oil stimulated the activity of dehydrogenases, urease, alkaline phosphatase and nitrification. This positive and

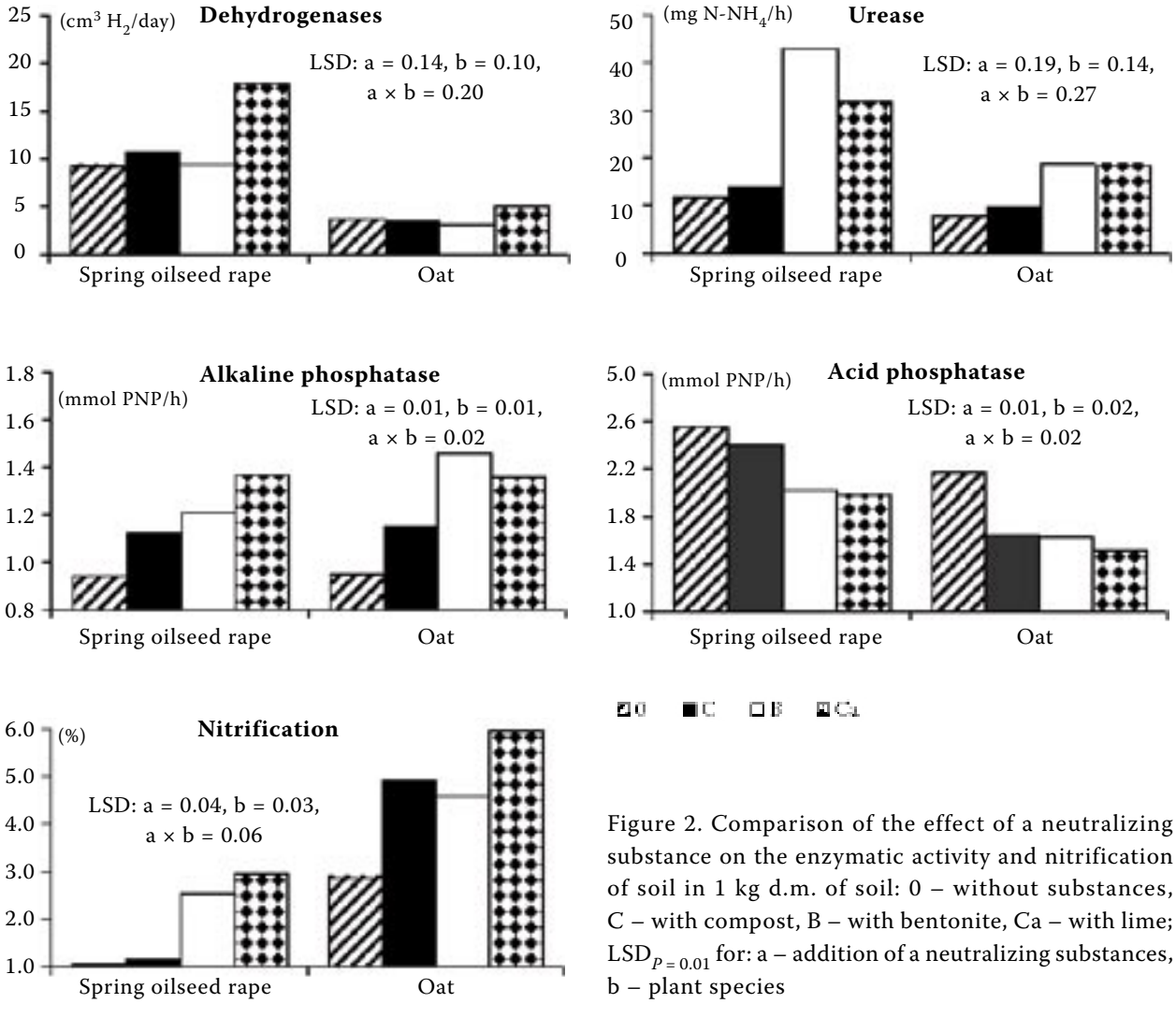

Figure 2. Comparison of the effect of a neutralizing substance on the enzymatic activity and nitrification of soil in 1 kg d.m. of soil: 0 – without substances, C – with compost, B – with bentonite, Ca – with lime; $LSD_{P=0.01}$ for: a – addition of a neutralizing substances, b – plant species

significant influence of compost, bentonite or lime was the strongest in increasing nitrification. Obviously, the effect of the neutralizing substances varied depending on the rate of diesel oil added to soil. Liming only, both in the non-contaminated and in contaminated sites, had a consistently beneficial effect on the activity of dehydrogenases, urease, alkaline phosphatase and nitrification in soil samples under spring oilseed rape and oats. Also bentonite stimulated the activity of urease, alkaline phosphatase and nitrification in soil under both plant species. In the limed and bentonite-treated series, the highest and significant (circa 5-fold) increase in nitrification was observed in the pots under spring oilseed rape either non-contaminated or polluted with the lowest rate of diesel oil, and that in the activity of urease (2.4-fold) – in the soil samples under both crops regardless the rate of diesel oil. The results obtained after an application of compost were less consistent.

When analysing the effect of soil contamination with diesel oil, irrespective of the duration of contamination (date of analysis), a highly significant increase in the activity of dehydrogenases, urease, alkaline phosphatase and nitrification along with a depressed activity of acid phosphatase were observed as the amount of the xenobiotic substance added to soil increased (Tables 1–5). The activity of soil enzymes in the pots polluted with diesel oil remained on an elevated level throughout the whole experiment. Nonetheless, among all the soil samples, regardless the rate of the contaminant, the activity of dehydrogenases, urease and acid phosphatase was higher in the soil planted with spring oilseed rape, while the nitrification was superior in the soil under oats (Figure 1).

The study presented in this paper revealed some strong and significant correlations between the activity of dehydrogenases, urease, acid phosphatase and alkaline phosphatase and the rate

Table 6. Pearson's simple correlation coefficient between diesel oil dose and enzymatic and nitrifying activity

Variable	Deh	Ure	Pac	Pal	Nitr
Spring oilseed rape (*Brassica napus* var. *oleifera*)					
DO	0.68**	0.65**	–0.34**	0.76**	0.57**
Deh		0.59**	–0.46**	0.77**	0.77**
Ure			–0.63**	0.65**	0.85**
Pac				–0.68**	–0.73**
Pal					0.78**
Oat (*Avena sativa* L.)					
DO	0.66**	0.57**	–0.47**	0.67**	0.63**
Deh		0.55**	–0.40**	0.49**	0.36**
Ure			–0.63**	0.84**	0.74**
Pac				–0.76**	–0.66**
Pal					0.74**

DO – diesel oil, Deh – dehydrogenases, Ure – urease, Pac – acid phosphatase, Pal – alkaline phosphatase, Nitr – nitrification; correlation coefficient significant for $^{**}P = 0.01$, $n = 48$

of diesel oil, as well as the nitrification of soil (Table 6). Such relationships are confirmed by correlation coefficients calculated between the activity of particular enzymes and the nitrification of soil. The activity of dehydrogenases, urease and alkaline phosphatase was highly significantly positively correlated with the nitrification of soil, while the correlation between the latter and the activity of acid phosphatase was highly significantly negative.

To conclude, by enriching soil contaminated with diesel oil with organic matter, bentonite or lime it was possible to produce a positive influence on the biological activity of soil, which was demonstrated as an improved activity of dehydrogenases, urease, alkaline phosphatase and nitrification. These neutralizing substances added to soil increased its tolerance to eco-toxic effects of diesel oil, a petroleum product.

Soil that is richer in humus absorbs more diesel oil and it deforms its structure to a greater extent than in the case of light soil. In more compact soils maintaining constant supply of oxygen is more difficult, and for this reason the activity of dehydrogenases in our experiment was higher in the objects without compost. This effect was observed in the soil samples under spring oilseed rape as well as oats. A weaker positive influence of compost on the enzymatic activity of humus-rich soil can derive from an excessively broad C:N ratio (Margesin and Schinner 1997). Organic substance added to soil improves absorbance of petroleum products, which can undergo oxygenation in a very limited manner and have a weaker influence on the biological life of soil (Małachowska-Jutsz et al. 1997).

By analyzing the role of all components added to soil, it can be concluded that soil liming depressed only the activity of acid phosphatase, whereas compost and bentonite had an adverse effect on the activity of alkaline phosphatase and dehydrogenases (in most of the pots). An elevated activity of dehydrogenases, urease, alkaline phosphatase and higher nitrification in the limed series can probably be explained by an increased multiplication of bacteria and actinomyces in soil, when multiplication of fungi was decreased (Galas et al. 1997). Besides, the amounts of components available to plants rise, which is of importance for plant growth and development (Wyszkowski et al. 2004, Wyszkowski and Wyszkowska 2005). A significant effect of bentonite in restricting the effects of soil contamination with diesel oil may have resulted from the chemical properties of this substance as well as its permeability. Bentonite added to soil forms a compact barrier that prevents petroleum products from reaching deeper horizons of a soil profile.

The biochemical activity of most of the parameters observed was higher in the soil under spring oilseed rape grown as the main crop than in the soil under oats as the aftercrop; it may have resulted from a more intense growth of microorganisms

and their activity, since diesel oil can be potentially a good nutrient substrate for some microorganisms (Krahl et al. 2002, Sarkar et al. 2005). The research carried out by Małachowska-Jutsz et al. (1997) demonstrated that soil contamination with petroleum products improved the activity of amylases, proteases and dehydrogenases in the first weeks of the trials, while in the subsequent weeks it generally led to a depressed activity of these enzymes.

The relationships between the degree of soil contamination with diesel oil and the activity of dehydrogenases and urease, which occurred in the present trials, find confirmation in the previous studies (Wyszkowska et al. 2002b, Wyszkowska and Kucharski 2004). Also Galas et al. (1997) and Xu and Johnson (1997) determined that the level of activity of dehydrogenases was largely a function of the concentration of hydrocarbons in a medium.

To summarize, by enriching soil contaminated with diesel oil in organic matter, bentonite or lime it was possible to produce a positive influence on the biological activity of soil, which was demonstrated as an improved activity of dehydrogenases, urease, alkaline phosphatase and nitrification. These neutralizing substances added to soil increased its tolerance to eco-toxic effects of diesel oil, a petroleum product.

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