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Response of *Avena sativa*, microorganisms and enzymes to contamination of soil with diesel oil

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ABSTRACT

The scale of the impact of petroleum products on the natural environment is still difficult to determine. This is why it was decided to conduct tests, under the conditions of a pot experiment, for the effects of diesel oil (0, 4, and 8 mL/kg dry matter of soil) on the yield of oat, content of macroelements, as well as the microbiological, biochemical, physicochemical and chemical properties of the soil. The study results showed that diesel oil had an adverse effect on the growth and development of oat, and contents of nitrogen, sodium, calcium and magnesium in the above-ground parts of oat. Diesel oil increased the concentration of the following substances in the soil: naphthalene, phenanthrene, anthracene, benz[a]anthracene, chrysene, benzo[a]fluoranthene, benzo[a]pyrene and benzo[ghi]perylene, organic carbon, total nitrogen, and available potassium, while it decreased the concentration of available phosphorus and magnesium in the soil. Changes in the physicochemical properties of the soil had an unfavourable effect on the microbiological and biochemical properties. As regards the 7 tested enzymes, the most sensitive to diesel oil was catalase, and the least sensitive ones were ß-glucosidase and dehydrogenases. As for 12 various tested microorganisms, bacteria of the *Azotobacter* genus exhibited the highest resistance, while copiotrophic bacteria the lowest.

Keywords: degradation of soil; macronutrient; physicochemical properties of soil; polycyclic aromatic hydrocarbons

The presence of petroleum products in the environment is a major problem as it leads to the gradual degradation of soil in many regions worldwide (Baran et al. 2004, Lebrero et al. 2012), and occasionally to the permanent destruction of the soil, the loss of its fertility, and the disappearance of vegetation cover (Kucharski and Jastrzębska 2006). Within one year, from 0.10–0.25% of the used petroleum products enter into the environment, which accounts for nearly 9 million Mg (Garcia-Lor et al. 2012). The sources of hydrocarbons include: uncontrolled leakages of petroleum substances on the premises of intermediate pumping stations, filling stations or petroleum industry installations; burning of wood and fossil fuels; emissions from vehicles; and the use of sewage sludge (Shrestha et al. 2010). Petroleum substances have great potential for accumulation in the soil environment and they may disturb the homeostasis of the soil (Zhan et al. 2010). The effect of petroleum substances was usually studied for a single parameter i.e. microorganisms (Wyszkowska and Kucharski 2005), enzymes (Wyszkowska et al. 2006, Zhan et al. 2010), the physicochemical properties of soil, the yield of crops, or their chemical composition (Sivitskaya and Wyszkowski 2013), while there are no reports concerning the effects of diesel oil on all these parameters in real time. Only the determination of all these parameters altogether may be a determinant of changes occurring in the soils subjected to the pressure of oil derivatives. Therefore, there is a need for research into the biological parameters of the soil in connection with the physicochemical and chemical properties of the soil as well as the response of the crops. Consequently, a study was initiated with the aim

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to determine the scale of changes occurring in the microbiological, biochemical, physicochemical and chemical properties of the soil contaminated with diesel oil.

MATERIAL AND METHODS

Soil. The test was performed on the topsoil of typical Cambisol (Eutric). According to the classification of the United States Department of Agriculture, it was a soil with a granulometric composition of loamy sand. The content of particular fractions, expressed in g/kg, was as follows: sand (0.05–2 mm) – 755.6; silt (0.002–0.05 mm) – 229.2; and clay (< 0.002 mm) – 15.2. The soil contained, in g/kg: C_{org} – 11.00; N_{tot} – 0.97. Its hydrolytic acidity was 7.80 $mmol_{(+)}$/kg, and the pH value in 1 mol KCl/L was 6.7.

Experimental design. The trials were set up with five replications and conducted in a greenhouse, in polyethylene pots each with the capacity of 3.5 L. Soil (3 kg per pot) was placed in a polyethylene container, prior to putting into single pots, and polluted with diesel oil (0, 4, and 8 mL/kg). In all objects, one level of macroelement fertilisation was applied (mg/kg soil): N – 100; P – 35; K^+ – 100; Mg^{2+} – 20. Nitrogen was used in a form of $CO(NH_2)_2$; phosphorus – KH_2PO_4; potassium – KH_2PO_4 and KCl; and magnesium – $MgSO_4 \cdot 7\,H_2O$. The experimental plant was oat (*Avena sativa* L.) cv. Kasztan, 12 plants in a pot. For the entire period of the experiment, the moisture content of the soil was maintained at a level of 60% of the capillary water capacity. The oat was harvested at the inflorescence emergence stage (52 BBCH – 20% of inflorescence emerged).

Determination of macroelements in plant. In the plant material, after the mineralisation in concentrated H_2SO_4 with peroxide aqueous solution, the content of total nitrogen was determined using the Kjeldahl method; phosphorus by the vanadium-molybdenum method; potassium, calcium and sodium by the flame photometry method; and magnesium by the ASA method (Sivitskaya and Wyszkowski 2013).

Determination of microorganisms numbers and enzymes activity in soil. During the experiment, the number of microorganisms was determined twice (on day 25 and 50) in the soil samples from each repetition in 3 subsequent replications. The microorganisms in soil determined were: fungi (Fun) and bacteria: oligotrophic (Olig) and oligotrophic sporulating ($Olig_p$), copiotrophic (Cop) and copiotrophic sporulating (Cop_p), ammonifying (Am), nitrate immobilisation (Im), cellulolytic (Cel), *Azotobacter* (Az), *Pseudomonas* (Ps), *Arthrobacter* (Art) and actinomycetes (Act). The nutrient mediums used were the same as those in the study Wyszkowska et al. (2007). On the same dates the activity of dehydrogenases (Deh), catalase (Cat), urease (Ure), ß-glucoidase (Glu), acid phosphatase (Pac), alkaline phosphatase (Pal) and arylsulfatase (Aryl) was determined in soil samples. The methodology for determining the enzyme activity is described in the study by Wyszkowska et al. (2013).

Determination of physicochemical and chemical properties of soil. In the samples of soil passed through a 2 mm sieve were determined: grain-size composition of the soil with laser method using a Mastersizer 2000 m, pH – by potentiometry in 1 mol KCl/L; hydrolytic acidity (HAC) and exchangeable base cations (EBC) – by the Kappen method, organic carbon (C_{org}) content – by the Tiurin method, total nitrogen (N_{tot}) by the Kjeldahl method. The obtained results of tests for the values of HAC and EBC were used for the calculation of the sorption capacity (CEC) and the degree of base saturation of the soil (BS) according to the formula:

$$CEC = EBC + HAC,$$

$$BS = (EBC/CEC) \times 100.$$

Content of available phosphorus (P_{av}) and potassium (K_{av}) were determined by the Egner-Riehm method, and that of available magnesium (Mg_{av}) by atomic absorption spectrometry after extraction by the Schachtschabel method. The methodology for determining the physicochemical and chemical properties is described in Borowik et al. (2014).

The contents of polycyclic aromatic hydrocarbons (PAHs) were also determined using the high-performance liquid chromatography (HPLC) and the GC/MS (SIM) method of extract analysis. The hydrocarbons were extracted in accordance with the standard ISO 13877 (1998E). The contents of 9 PAHs were determined, namely: naphthalene (NAP), phenanthrene (PHE), anthracene (ANT), fluoranthene (FTH), benzo(a)antracene (BaA), chrysene (CHR), benzo(a)fluoranthene (BaF), benzo(a)pyrene (BaP) and benzo(ghi)perylene (BghiP).

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Calculations. The resistance to the action of diesel oil was calculated using the number of microorganisms and the activity of soil enzymes (Orwin and Wardle 2004).

Statistical analysis. The homogeneity of variance between groups at $P = 0.01$ was determined with the use of the Tukey's test. The obtained data were presented in the study as the mean values for two dates because the analysis of η^2 coefficients demonstrated that the duration of the experiment had no significant effect on the microbiological and biochemical properties of the soil. The statistical analysis was performed using the package Statistica 12.0 (StatSoft, Inc., 2014).

RESULTS AND DISCUSSION

The issue of resistance (RS) of soil to PAHs, based on the number of microorganisms and the activity of soil enzymes, is only addressed in the literature (Lipińska et al. 2014) to a negligible extent, and the relationships between the parameters describing the RS are complex and dependent on specific conditions (Orwin and Wardle 2004). According to Griffiths and Phillipot (2013), the determination of the resistance index allowed the objective determination as to whether a certain ecosystem is stable and able to maintain the right balance. This was the case in our own study (Figure 1). The number of some microorganisms such as oligotrophic, copiotrophic, ammonifying, and nitrogen immobilising bacteria as well as actinomyces, increased. This is indicated by negative resistance indices; the number of certain other microorganisms e.g. cellulolytic bacteria, *Arthrobacter*, oligotrophic spore-forming bacteria, *Pseudomonas*, and fungi (the 2nd dose of diesel oil) decreased. This is indicated by positive RS indices. However, the values were significantly below 1. The microorganisms being most resistant to diesel oil were the bacteria of *Azotobacter* genus. Their RS index nearly approached the value of one, and ranged from 0.91–0.95. The varied response of microorganisms to the tested contamination was associated with their succession (Vázquez et al. 2013) resulting from the death of sensitive microorganisms, and the multiplication of the resistant ones (Wu et al. 2014). Changes to the multiplication of microorganisms in the soil contaminated with diesel oil may result from the fact that hydrocarbons, as the chemical compounds with a various degree of bioresistance, are degraded at different rates, and they have an effect on the soil microbiota in consequence (Semrany et al. 2012).

Diesel oil caused smaller changes to the activity of soil enzymes (Figure 2) than to the number of microorganisms. The RS indices for all enzymes were positive, and their values, except catalase, were higher than 0.75. The following enzymes turned out to be the most resistant to the action of diesel oil: ß-glucosidase (RS = 0.99); dehydrogenases (RS = 0.92–0.98); acid phosphatase (RS = 0.97–0.89); and urease (RS = 0.93–0.84). The least resistant enzyme was catalase (RS = 0.44–0.26). The relatively high resistance of the enzymes to

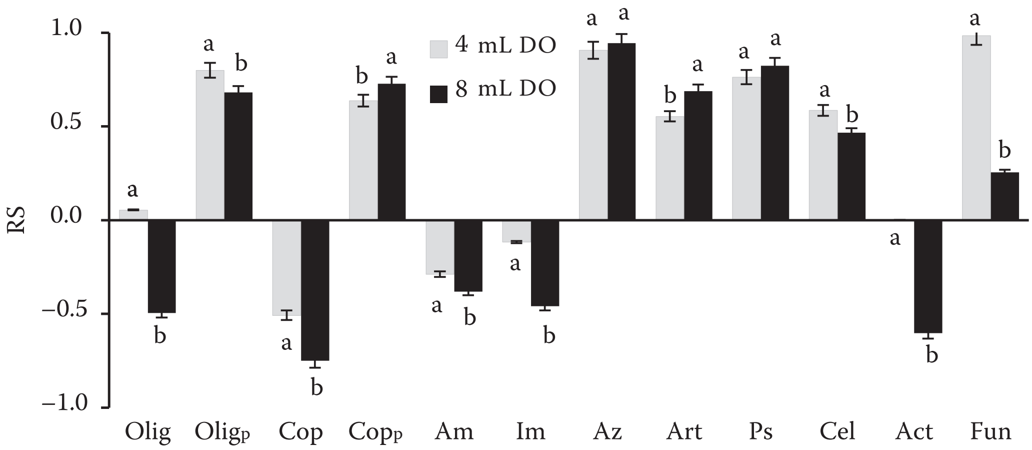

Figure 1. Resistance (RS) of soil microorganisms to diesel oil (DO). The same letters in microorganisms are assigned to homogeneous groups ($P = 0.01$). Bacteria: Olig – oligotrophic; $Olig_p$ – oligotrophic sporulating; Cop – copiotrophic; Cop_p – copiotrophic sporulating; Am – ammonifying; Im – nitrate immobilisation; Az – *Azotobacter;* Art – *Arthrobacter*; Ps – *Pseudomonas*; Cel – cellulolytic; Act – actinomycetes; Fun – fungi

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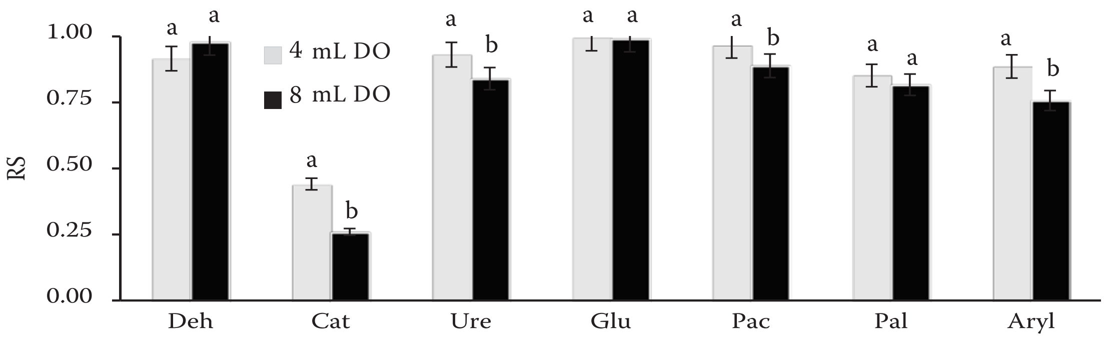

Figure 2. Resistance (RS) of soil enzymes to diesel oil (DO). The same letters in enzymes are assigned to homogeneous groups (*P* = 0.01). Deh – dehydrogenases; Cat – catalase; Ure – urease; Glu – ß-glucoidase; Pac – acid phosphatase; Pal – alkaline phosphatase; Aryl – rylsulfatase

diesel oil (DO) may result from the increased development of certain groups of microorganisms and their heightened activity, since diesel oil might be a good nutrient substrate for certain microorganisms (Kucharski and Jastrzębska 2005, Wyszkowska and Kucharski 2005).

The soil contaminated with diesel oil contained more organic carbon, total nitrogen and available potassium, and less available phosphorus and magnesium (Table 1). In addition, the sorption capacity of the contaminated soil was higher than that of non-contaminated soil, and the hydrolytic acidity changed slightly. Changes in the contents of aromatic hydrocarbons in the soil were greater than in the physicochemical properties (Table 2). The content of all tested PAHs was significantly higher in the soil contaminated with diesel oil than in the non-contaminated one. The high percentage of 2- and 3-ring PAHs in the total PAH content, which was noted in this study, was consistent with the observations of Nganje et al. (2007). According to Wyszkowski and Ziółkowska (2013), the varied content of PAHs in the soil depends on the physicochemical properties, mainly the content of organic substances.

The soil contaminated with hydrocarbons loses its structure, and the water-air balance of the soil as well as its chemical and physicochemical properties deteriorate (Kucharski and Jastrzębska 2005, Wyszkowska and Kucharski 2005). In our study, changes to the physical and chemical properties had an adverse effect on the size and quality of the yield of the above-ground parts of oat. In the objects with 8 mL/kg soil, the growth inhibition, chlorosis of the leaves, and deformation of the root system were noted. Under the influence of the action of diesel oil, both the yield of oat and contents of nitrogen, sodium, calcium and magnesium changed significantly in the above-ground parts of oat. Only the contents of potassium and phosphorus in oat increased. The adverse effect of diesel oil on the plants may be probably explained by the fact that hydrocarbons from diesel oil restrict the transpiration and respiration of the plants, and reduce the permeability of cellular membranes (Pezeshki et al. 2000, Kucharski and Jastrzębska 2006). There is no doubt that the crops cultivated in soils contaminated with diesel oil are exposed to the direct toxic action of this product (Wyszkowska et al. 2006) as

Table 1. The effect of diesel oil (DO) on the physicochemical properties of the soil

mL DO/kg DM soil	C_{org}	N_{tot}	P_{av}	K_{av}	Mg_{av}	pH_{KCl}	HAC	EBC	CEC	BS
	(g/kg DM soil)		(mg/kg DM soil)				$mmol_+$/kg DM soil			(%)
0	11.00[c]	0.94[b]	46.80[a]	70.78[b]	22.65[a]	6.70[a]	10.50[a]	119.67[b]	130.17[b]	91.93[a]
4	11.55[b]	0.97[b]	42.88[b]	68.60[b]	16.27[b]	6.70[a]	10.50[a]	124.33[a]	134.83[a]	92.21[a]
8	12.95[a]	1.01[a]	43.71[b]	95.84[a]	16.77[b]	6.60[a]	10.13[b]	125.33[a]	135.46[a]	92.53[a]

The same letters in columns are assigned to homogeneous groups (*P* = 0.01). DM – dry matter; C_{org} – organic carbon; N_{tot} – total nitrogen; P_{av} – available phosphorus, K_{av} – available potassium; Mg_{av} – available magnesium; HAC – hydrolytic acidity; EBC – exchangeable base cations; CEC – calculation of the sorption capacity; BS – base saturation

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Table 2. The effect of diesel oil (DO) on the contents of polycyclic aromatic hydrocarbons in the soil (μg/kg DM)

mL DO/kg DM soil	NAP	PHE	ANT	FTH	BaA	CHR	BaF	BaP	BghiP	Σ
0	1.15[c]	13.16[c]	1.17[c]	17.02[c]	2.16[c]	8.19[c]	2.71[c]	9.12[c]	7.41[c]	62.09
4	17.13[b]	16.24[b]	4.23[b]	29.71[b]	7.32[b]	14.91[b]	6.82[b]	11.37[b]	8.69[b]	116.42
8	38.25[a]	21.36[a]	9.67[a]	75.89[a]	14.56[a]	21.37[a]	8.57[a]	15.61[a]	9.73[a]	215.01

The same letters in columns are assigned to homogeneous groups (*P* = 0.01). DM – dry matter; NAP – naphthalene; PHE – phenanthrene; ANT – anthracene; FTH – fluoranthene; BaA – benzo(a)antracene; CHR – chrysene; BaF – benzo(a)fluoranthene; BaP – benzo(a)pyrene; BghiP – benzo(ghi)perylene

Table 3. The effect of diesel oil (DO) on the yield of oat (g DM/kg DM soil) and the content of macroelements in the plant (g/kg DM)

mL DO/kg DM soil	Yield	N_{tot}	P	K	Na	Ca	Mg
0	7.474[a]	29.680[a]	4.431[a]	17.543[b]	2.208[a]	15.936[a]	3.911[a]
4	5.910[b]	28.545[b]	4.500[a]	24.735[a]	1.800[b]	15.675[a]	3.390[b]
8	5.393[c]	21.368[c]	4.610[a]	23.592[ab]	1.208[c]	13.520[b]	2.536[c]

The same letters in columns are assigned to homogeneous groups (*P* = 0.01). DM – dry matter

well as to the indirect action through the changes of the physical and chemical properties of the soil (Kucharski and Jastrzębska 2005, Wyszkowska and Kucharski 2005).

The results presented in this study clearly indicate that the combination of microbiological, biochemical, physicochemical and chemical parameters of the soil with the simultaneous use of the responses of plants offers a chance to comprehensively assess the quality of soil under the pressure of diesel oil.

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