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Response of *Avena sativa* L. and the soil microbiota to the contamination of soil with Shell diesel oil

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ABSTRACT

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This study analysed the changes in the development of *Avena sativa* L. cultivated on soil contaminated with diesel oil (DO; 0, 7, 14 and 21 mL/kg of soil dry matter), and in the microbiological, biochemical, chemical and physico-chemical properties of the soil. In addition to basic fertilisation fulfilling the nutritional needs of the oat plant, finely ground barley straw and finely ground charcoal was also applied. The study revealed a highly toxic effect of DO on the growth and development of *Avena sativa* L. The uptake of macro- and micronutrients by the tested plant decreased significantly. The active bacteria were identified based on the analysis of 16S rRNA coding sequences. In objects contaminated with DO, a more rapid development of organotrophic bacteria, actinomyces and fungi was observed, as well as higher activity of dehydrogenases, catalase, urease, acid phosphatase, alkaline phosphatase and β-glucosidase. In the soils contaminated with DO, more C_{org} and available and exchangeable potassium were found than in the non-contaminated soils. DO did not have such a significant effect on the contents of other elements in the soil. The use of charcoal and straw stimulated both the development of microorganisms and the activity of soil enzymes, yet it did not mitigate the adverse effect of DO on the growth or development of the oat plant.

Keywords: petroleum product; pollution; biomonitoring; toxic elements; physico-chemical properties of soil; biostimulation

One of the consequences of technical, industrial and economic progress is an increase in the consumption of petroleum products which, at the same time, results in ever-increasing contamination of the natural environment (Rusin et al. 2015). In Europe, up to 45% of all contaminated habitats are areas subjected to the pressure of oil-derived hydrocarbons (Masy et al. 2016). Oil derivatives are regarded as the most dangerous contaminants of particular components of the natural environment, as they contain many toxic, carcinogenic and teratogenic compounds such as benzene, toluene, ethylbenzene, xylenes (BTEX) and polycyclic aromatic hydrocarbons (PAHs) and exhibit great potential for migration and spread within the environment (Khalilova 2015). Consequently, they accumulate in the soil environment in which they may disturb the homeostasis of the soil (Meckenstock et al. 2016) as they create many physical, chemical and biological properties of the soil (Borowik et al. 2017). Soil contaminated with hydrocarbons loses its structure and the air-water conditions as well as chemical and physical properties are disturbed (Kucharski and Jastrzębska 2005, Kuppusamy et al. 2017). Therefore, it is necessary to search for environmentally-friendly remediation methods (Kumari and Singh 2016). One such method is biostimulation (Kuppusamy et al. 2017) which involves stimulation of the native soil microbiota and the acceleration of processes naturally occurring in soil ecosystems (Martínez Álvarez et al. 2015). Hence, it is necessary to conduct complex studies involving both the soil microbiota and the chemical composition of plants as well as chemical and physico-chemical properties of the soil. A study

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was initiated to determine the scale of changes occurring in the microbiological, biochemical, chemical and physico-chemical properties of the soil contaminated with Shell diesel oil, and subjected to biostimulation. Moreover, the effect of diesel oil on the growth and development of oats was assessed as well as on the uptake of macronutrients and micronutrients by plants. In addition, the efficiency of biostimulation of the autochthonous soil microbiota with straw and charcoal in the restoration of homeostasis of the soil contaminated with diesel oil was determined. A better understanding of the effects of petroleum products on these parameters will be useful soil environment biomonitoring.

MATERIAL AND METHODS

Soil. In the vegetation experiment, soil material was taken from the topsoil layer (humic horizon) of typical brown soils (Eutric Cambisol) originating from the Educational and Experimental Station in Tomaszkowo (north-east part of Poland, 53.7161 N, 20.4167 E), located in the Olsztyn Lakeland. According to the classification of the United States Department of Agriculture, it was a soil with a granulometric composition of loamy sand (sand – 78.08%, silt – 20.46%, clay – 1.46%). The soil was characterised by the following properties: pH in 1 mol KCl/L: 6.58; organic carbon (C_{org}) 12.7 g/kg; total nitrogen (N_{tot}): 0.88 g/kg; hydrolytic acidity (HAC): 8.50 $mmol_+$/kg; total base exchangeable cations (EBC): 105.00 $mmol_+$/kg; cation exchange capacity (CEC): 113.50 $mmol_+$/kg; base saturation (BS): 92.51%.

Procedure for experiment performance. The study was conducted under monitored conditions in a greenhouse, in pots with a capacity of 3.0 dm^3, in five replications. In the experiment, the variable factors included the degree of soil contamination with diesel oil (DO) in mL per kg of soil: 0, 7, 14 and 21, and the type of substance biostimulating the natural soil microbiota: spring barley straw and charcoal. The diesel oil was purchased at Shell. The density of diesel oil is 845 g/L. It contained polycyclic aromatic hydrocarbons, mainly 3-ring but also the 4–6-ring ones. Detailed characteristics of Shell diesel oil can be found on the website (http://www.epc.shell.com).

Prior to filling the pot, the soil (2.8 kg per 1 pot) was mixed in a polyethylene vessel with macronutrients and, in relevant objects, with diesel oil and biostimulating substances. In all objects, constant fertilisation was applied: N – 112 mg [$CO(NH_2)_2$], P – 39 mg [KH_2PO_4], K – 112 mg [KH_2PO_4 and KCl], Mg – 15 mg [$MgSO_4 \cdot 7\ H_2O$]. After placing the soil in the pots, its moisture content was brought to the level of 60% of the capillary water capacity and oat (*Avena sativa* L.) of cv. Furman was sown (12 seeds 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 oats were harvested at the end of heading: inflorescence fully emerged stage (BBCH 59).

Biostimulating substances. In order to stimulate the multiplication of microorganisms, substances biostimulating the natural soil microbiota, i.e. finely ground barley straw and charcoal were used in the study. The organic carbon content in barley straw was 547.0 g/kg, total nitrogen content was 3.3 g/kg, total phosphorus content was 0.6 g/kg and total potassium content was 2.3 g/kg. The charcoal was made from the beech and hornbeam. 1 kg of DM (dry matter) contained 856.9 g C, 37.0 g N and 49.8 g ash. Doses of straw and charcoal were determined based on the carbon content. They were applied at an amount of 0 and 3 g C/kg of soil DM.

Methods for determining the chemical composition of oats. In the plant material (aboveground part), following the mineralisation in concentrated H_2SO_4 with hydrogen peroxide, the total nitrogen content was determined using the Kjeldahl method; the phosphorus content by the vanadium-molybdenum method; the potassium, calcium and sodium contents by the atomic emission spectroscopy method; and the magnesium content by the atomic absorption spectroscopy method (Sivitskaya and Wyszkowski 2013). Moreover, the contents of Pb, Cd, Cr, Ni, Mn, Zn, Cu, Fe and Co were determined using the flame atomic absorption spectroscopy method (Kosiorek et al. 2016).

Microbiological analysis methods. During the experiment, the counts of organotrophic bacteria (B), actinomyces (Act) and fungi (Fun) were determined twice (on day 30 and 60) in the soil samples from each repetition in three subsequent replications. The nutrient media used were the same as those in a study by Borowik et al. (2017). On their basis, the eco-physiological (EP) diversity indicator was calculated (De Leij et al. 1994). Based on the analysis of 16S rRNA coding sequences, the soil bacteria were identified. The following primers were

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used: B-all Forward GAGTTTGATCCTGGCTCAG and B-all Reverse ACGGCTACCTTACGACTT. Based on the obtained sequences of isolated microorganism nucleotides, a phylogenetic tree was developed in MEGA 7.0 software (Old Main, USA), using the 'neighbour-joining' method.

Determination of the activity of soil enzymes. On day 30 and 60 of the experiment in soil samples, from each repetition in three subsequent replications, the activities of dehydrogenases (Deh), catalase (Cat), urease (Ure), acid phosphatase (Pac), alkaline phosphatase (Pal), β-glucosidase (Glu), and arylsulphatase (Aryl) were determined. The detailed procedure for enzyme determination is described in a study by Borowik et al. (2017).

Determination of the physico-chemical properties of the soil. In the samples of soil sieved through a 2 mm mesh sieve, both before the establishment of the experiment and after harvesting the plants, the following properties were determined: granulometric composition of soil, the pH value of soil, hydrolytic acidity and exchangeable base cations, organic carbon content, total nitrogen content, and available phosphorus, potassium and magnesium contents. The methodology for determining the physico-chemical and chemical properties is described in a study by Borowik et al. (2017).

Statistical analysis. The study results were statistically processed using the Statistica 12.0 package (StatSoft, Inc. 2015). Since the analysis of η^2 variation demonstrated that the duration of the experiment had no significant effect on microbiological and biochemical properties of the soil, the obtained data were presented in the study as mean values of two dates. For the assessment of the effect of diesel oil and biostimulating substances on the soil microbiota and on the uptake of macro- and micronutrients by the oat plants and the contents of elements in soil, the principal component analysis (PCA) was applied using the multi-dimensional and explorational analyses.

RESULTS AND DISCUSSION

The growth and development of oats was significantly inhibited by diesel oil present in the soil (Figure 1). The content of 7 mL of DO per kg of soil DM decreased the biomass of oats by 31%, while greater amounts of oil, i.e. 14 mL/kg and 21 mL/kg of soil DM decreased the yield by 81% and 89%, respectively. Fertilisation with straw in the objects non-contaminated with DO decreased the biomass of oats by 14% and in the contaminated soil, on average by as much as 41%. In turn, the addition of charcoal to the soil did not modify the growth and development of oats or mitigated the adverse effect of DO. Toxic effects of petroleum products on plants are associated with their physical and chemical properties, which impede the growth of the roots, and the uptake of nutrients. Both is confirmed by literature (Hawrot-Paw and Bąkowska 2014).

The uptake of macronutrients N, P, K, Ca, Mg and Na from the soil by oats is characterised by as much as 97.66% by the first main component (Figure 2a). The value of the coordinates of the end of vectors of the primary variables from PCA1 for all elements ranged from –0.96 (for Na) to –0.99 (for other macroelements N, P, K, Ca, Mg), while for PCA2, from 0.12 (Ca) to –0.26 (Na). The contamination of soil with diesel oil, particularly by doses greater than 7 mL/kg of soil, significantly reduced the uptake of macronutrients N, P, K, Ca, Mg and Na. The reduction in the uptake from the soil contaminated with DO ranged from 49% (Na) to 69% (P and K). The fertilisation of the soil non-contaminated with diesel oil with straw reduced the uptake of macronutrients within the range from 10% (P) to 30% (N). Such effect was not observed for charcoal.

The uptake of micronutrients Cu, Mn, Fe, Zn, Ni, Co, Cr, Pb and Cd was also largely mapped by PCA1 (Figure 2b). The values of the coordinates of the end of vectors of the primary variables of micronutrients from PCA1 ranged from –0.78 (Cr, Co) to –0.98 (Zn, Pb), while for PCA2, they were considerably higher than for macronutrients N,

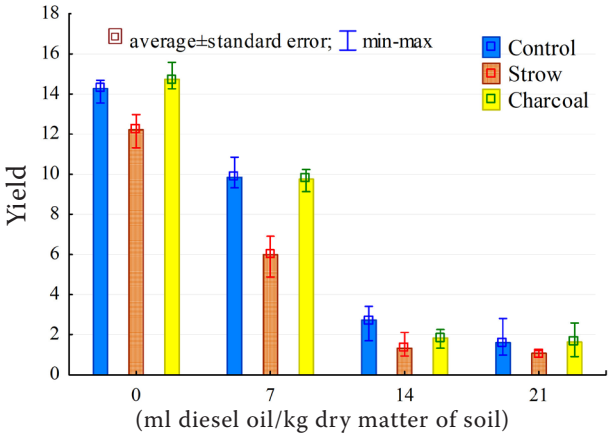

Figure 1. Yield of *Avena sativa* L. (g dry matter per pot)

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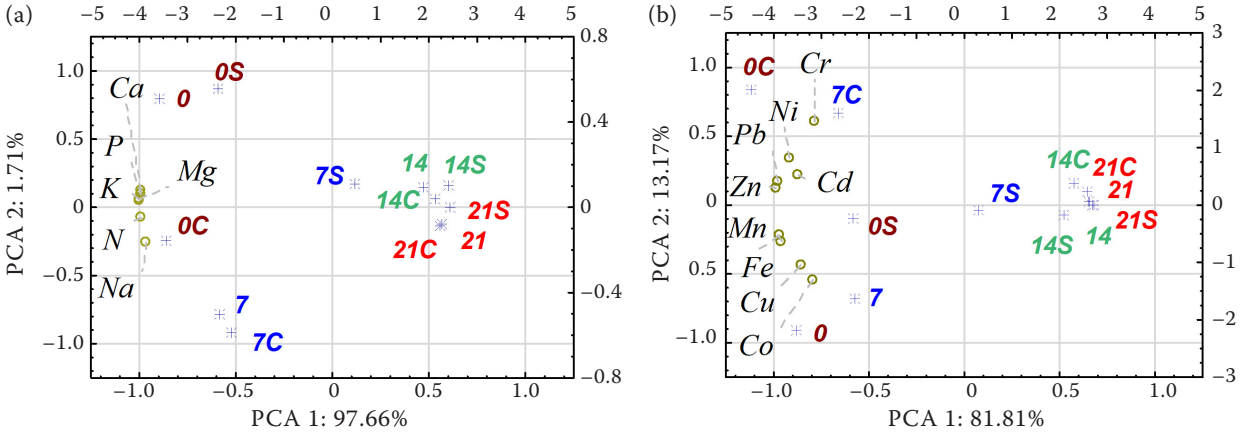

Figure 2. The uptake of (a) macronutrients N, P, K, Ca, Mg and Na and (b) micronutrients Cu, Mn, Fe, Zn, Ni, Co, Cr, Pb and Cd by *Avena sativa* L. 0, 7, 14, 21 – dose diesel oil (mL/kg dry matter of soil); S – straw; C – charcoal; o – the end of the vector of the primary variable; *case

P, K, Ca, Mg, Na and ranged from –0.54 (Co) to 0.61 (Cr). Nevertheless, the location of the cases from the objects contaminated with DO around the positive values of PCA1, and thus on the opposite side of vectors, indicates that the uptake of micronutrients Cu, Mn, Fe, Zn, Ni, Co, Cr, Pb and Cd was also adversely affected by DO.

The fertilisation with straw and charcoal did not change physico-chemical properties of the soil. In the objects non-contaminated with diesel oil, the pH, HAC, EBC, CEC, BS and organic carbon content were at the same level, regardless of the type of fertilisation, compared to the objects contaminated with DO. Therefore, the listed parameters are not included in the manuscript. Figure 3 shows that the C_{org} content increased significantly as the effect of DO on the content of available and exchangeable forms of elements in the soil was less clear. PCA1 mainly characterised the contents of exchangeable and available forms of potassium and C_{org}. The coordinates of the end of vectors of the primary variables with the first main component were very high and ranged from 0.95 to 0.97. PCA2 was to the greatest extent linked to the contents of available phosphorus (a coordinate of –0.62) and exchangeable magnesium (a coordinate of 0.66). The contamination of the soil with DO at an amount of 7–21 mL/kg increased the content of exchangeable and available forms of potassium in the non-fertilised objects by 100–117%, in the objects fertilised with straw by 62–67%, and in the objects fertilised with charcoal by 120–140%. At the same time, the content of exchangeable sodium decreased under the influence of DO. The described changes primarily result from the adverse effect of DO on the yielding of oats as well as the uptake of nutrients (Sivitskaya and Wyszkowski 2013).

The activity of all soil enzymes, except arylsulphatase, was significantly correlated with the first main component (Figure 4). The coordinates from PCA1 ranged from 0.81 (Glu) to 0.98 (Pal). Moreover, a significant positive correlation occurred between these enzymes, whereas the lowest activity was noted in the soil non-contaminated with DO and the highest in the soil contaminated with DO in the amount of 21 mL/kg and fertilised with straw. Fertilisation with straw stimulated the activity of all enzymes except Aryl, which results in more intense degradation of the DO (Margesin and Schinner 1997). The stimulating effect of straw on most enzymes is associated with its positive effect on organotrophic bacteria, actinomyces and fungi, whereas the count of organotrophic bacteria was positively correlated

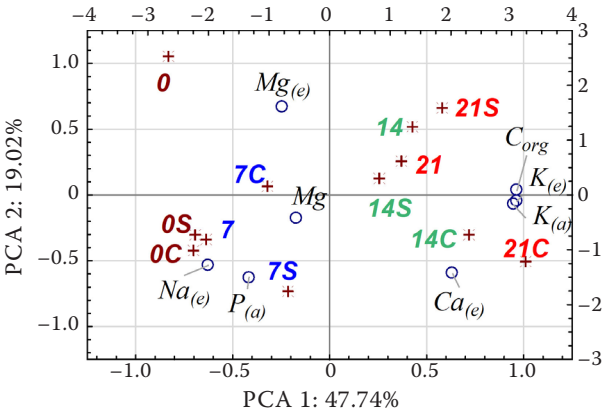

Figure 3. The content of elements in the soil. 0, 7, 14, 21 – dose diesel oil (mL/kg dry matter of soil); S – straw; C – charcoal; a – assimilable; e – exchangeable; o – the end of the vector of the primary variable; *case

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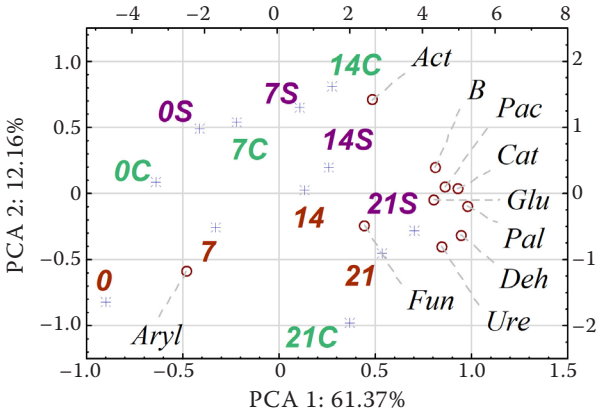

Figure 4. The activity of enzymes and the count of soil microorganisms. 0, 7, 14, 21 – dose diesel oil (mL/kg dry matter of soil); S – straw; C – charcoal; o – the end of the vector of the primary variable; *case

with PCA1 (coordinate value of 0.81), while the count of actinobacteria with PCA2 (coordinate value of 0.71). The count of fungi was, to a small extent, characterised by PCA1 (coordinate 0.44) and by PCA2 (coordinate –0.24). However, their number significantly decreased under the influence of DO, but to a lesser extent than that of bacteria. It follows from both the conducted study and literature (Borowik et al. 2017, Ramadass et al. 2017) that the activity of the tested enzymes well reflects the condition of the microbiota of soils contaminated with DO, even though their responses may be different depending on the type of petroleum product (Wyszkowska et al. 2006).

DO had an adverse significant effect on the diversity of microorganisms, which is indicated by the decreasing eco-physiological diversity indicator along with an increase in the degree of soil contamination (Figure 5). The value of EP for fungi, under the influence of the highest DO dose, decreased by 74%, for actinomyces by 17% and for organotrophic bacteria by 13%. In the contaminated soil, the adverse effect of DO on the diversity of fungi and organotrophic bacteria was mitigated by the fertilisation with straw and charcoal, while DO had no effect on the diversity of actinomyces.

The various responses of soil microorganisms to the contamination with Shell diesel oil is a result of their succession (Vázquez et al. 2013, Wu et al. 2014). It leads to the dominance of microorganisms exhibiting the ability to degrade organic compounds present in DO (Fatima et al. 2015, Borowik et al. 2017). From the samples of the soil subjected to pressure of DO, *Pseudomonas aeruginosa*, *Kocuria palustris*, *Gordonia amicalis*, *Pseudomonas monteilii*, *Bacillus megaterium*, *Bacillus mycoides* and *Bacillus subtilis* subsp. *subtilis* was isolated with 97–100% accuracy score for the adjustment of sequences according to the analysis using the NCBI database (Figure 6). The microorganisms isolated from the soil are probably active in the degradation of oil-derived hydrocarbons (Fatima et al. 2015).

The above considerations clearly indicate that the contamination of the soil with Shell diesel oil disturbed the metabolic profile of the soil, and that only monitoring of the plants' responses taking account of the uptake of macro- and micronutrients from the soil in combination with microbiological and biochemical indicators provides complete information on the health status of soils.

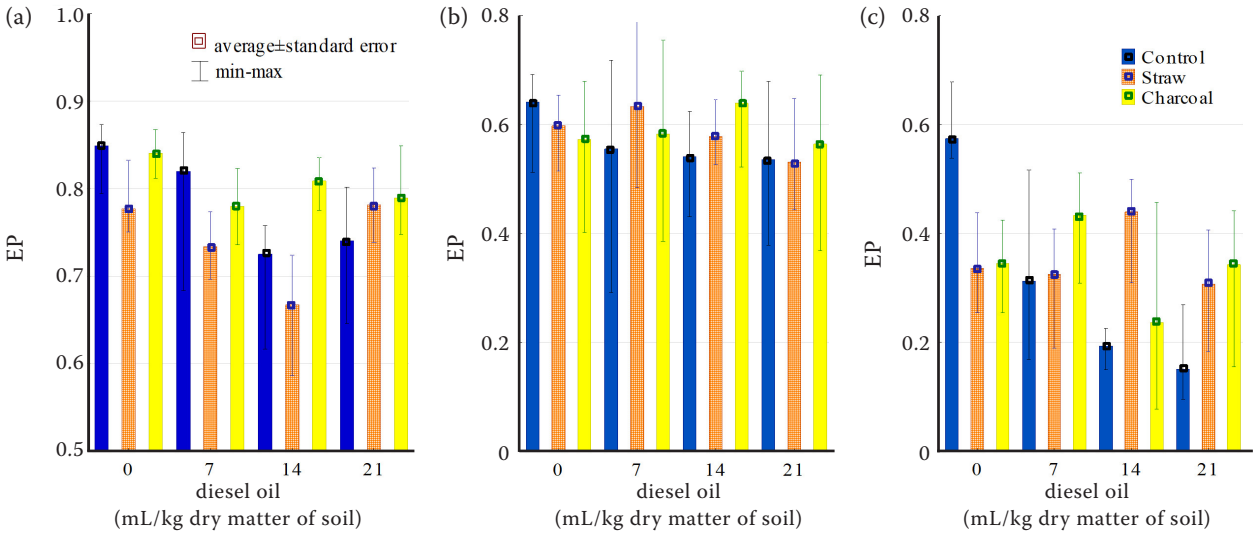

Figure 5. The colony eco-physiological diversity (EP) indices for (a) organotrophic bacteria, (b) actinomyces and (c) fungi

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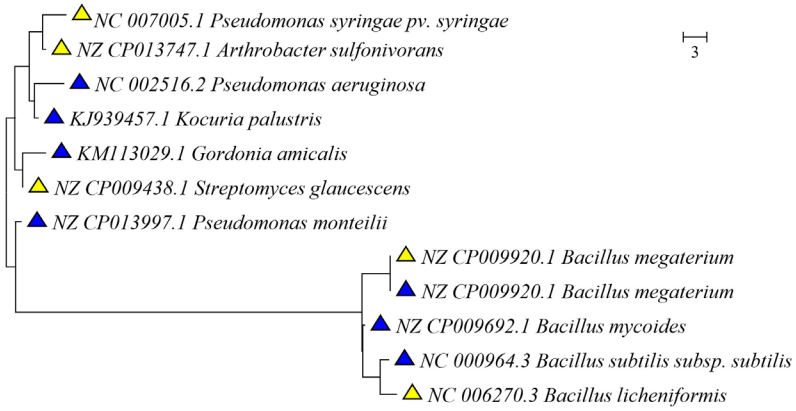

Figure 6. Bacteria isolated from the soil

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