Growth of peas *Pisum sativum* L. in the presence of diesel and bacteria consortia in peat and sandy soil

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Abstract: Remediation of contaminated soils, using plant-based systems, is known as a very perspective and rapidly developing area of biotechnology. In this respect toxicity of diesel for plants is of great importance. In this work, the results of the greenhouse experiments with soils artificially contaminated by diesel are presented. The seeds of peas were sown into peat and sandy soil mended with 1% and 3% of diesel, as well as inoculum of bacteria consortia, which was previously isolated from oil-contaminated sites. After 85 days, when peas were entering the bloom stage in the control samples, plant biomass was harvested. The presence of 1% diesel in sandy soil totally inhibited the growth of peas, although in peat soil an inhibition effect was about 50 %. Harvested biomass was also tested for moist and dry weight, height, content of chlorophyll a and b. Fermentative activity of soils, i.e. FDA (fluoresceine diacetate hydrolysis), dehydrogenase, urease, as well as respiration were measured. The total microbial number in rhizosphere was 10-100 times higher that in average soil sample. The number of colony forming units on the selective medium with diesel was higher in the samples inoculated with bacteria consortia. The results provided additional information on plant response to diesel contamination in the context of toxicity study and perspectives for phytoremediation.

Keywords: peas, diesel, bacteria consortia, soil fermentative activity, respiration, chlorophyll
Abbreviation: FDA - Fluorescein Diacetate (3',6'-diacetylfluorescein); DHA – Dehydrogenase Activity; TGA Tryptone Glucose Yeast Extract Agar.

1 Introduction

The concept of using plants to remediate contaminated soil has recently become an area of intense scientific study. Plants have been shown to encourage organic contaminant degradation principally by providing an optimal environment for microbial proliferation in the rhizosphere [1-2].
These degradative processes are influenced not only by the rhizosphere microorganisms but also by unique properties of the host plant [3].
Diesel oil is a complex mixture of petroleum hydrocarbons containing everything from volatile, low molecular weight alkanes which are potentially phytotoxic, to naphthalenes which may interfere with normal plant development [4].
If plants can be successfully established on polluted soil, then the plant–microbial interaction in the rhizosphere may provide enhanced breakdown of diesel fuel in vegetated soils as opposed to non vegetated soils. It is apparent that more plants that grow well in contaminated soils need to be identified and screened for use in phytoremediation technologies [5].
Many researchers have studied the phytoremediation of different organic contaminants by using plant species in Europe, North America, Japan. Italian ryegrass, sorghum, maize, alfalfa, rice, Bermuda grass, rice, kudzu, beggar ticks are recognized as phytoremediators [6-10]. Sunflower, southern crabgrass and red clover are recognized as hydrocarbon-tolerant plants [4, 11]. These studies suggested that grass species and *Leguminosae* could be suitable for phytoremediation of petroleum hydrocarbon-contaminated soil.
The aim of this study was to investigate an ability of peas *P. sativum* L. to grow in peat and sandy soil in the presence of diesel.

2 Materials and methods

The greenhouse vegetation experiment was performed with *P. sativum* L. sown in peat and sandy soil contaminated by diesel fuel. The scheme of the

experiments is shown in the Table 1. The hydrocarbon-degrading bacterial consortia added to soil was previously isolated from different contaminated sites.

Table 1. Scheme of the experiment.

Variant No.	Addition of bacterial consortia	Diesel concentration, %
P1 or S1*	-	0
P2 or S2	+	
P3 or S3	-	1
P4 or S4	+	
P5 or P5	-	3
P6 or S6	+	

* P – peat; S – sand.

For each variant set of plastic pods with a volume of 4 L was prepared in 3 replicates. The substrates were fertilized by adding 3 g of Kemira Horti 10-10-20 with microelements fertilizer per pod once for peat substrate (after 45 days from the start of the experiment) and twice for sandy substrate (after 20 and 45 days from the start of the experiment). 20 ml (per one pod) of inoculum with bacterial consortia was added in a concentration of 2.9 x 10^8 cells/mL.

Plant biomass was harvested after 85 days from the start of the experiment, when control plants (P1 and S1) were entering the anthesis stage. Measurement of chlorophyll a and b was performed according to [12].

Dry weight of soil and plant (aboveground-part) was determined by sample drying at +105 °C till constant weight. Average soil samples were obtained by mixing 25 g of soil collected from different areas of the microcosm. For microbiological testing and fermentative activity 1g of average soil sample for each test was taken in duplicate. pH and RedOx potential were measured by electrode Hanna pH213. The number of colony forming units was determined using TGA medium (Sifin). Selective M8* medium contained, g/L: Na_2HPO_4 – 60, KH_2PO_4 – 30, NaCl – 5, agar – 15, plant extract – 100, diesel - 10 (pH 6.9). Plant extract was prepared according to [13].Dehydrogenase activity was determined according to [14] by reduction of 2-*p*-iodo-3-nitrophenyl-5-phenyltetrazolium chloride to iodonitrophenylformazan. Soil urease activity was determined by the colorimetric method according to the NH3-N formation in the urea-amended soil sample (after 24 h and 48h incubation at 37 °C) [15]. FDA activity was determined by hydrolysis of fluorescein diacetate [16].

Microbial respiration was determined according to [17] with some modification. The CO_2 released from 30 g of soil after 24 h of incubation at 25°C was trapped in 5 mL of 0.15 mol L^{-1} NaOH and determined by titration with 0.05 mol L^{-1} HCl.

Soil toxicity study was performed using germination test according to EPA 712-C-96-152 [18]. Six species of plants were used: barley *Hordeum vulgare* L., oat *Avena sativa* L., wheat *Triticum sp*., rape *Brassica napus* L., garden cress *Lepidum sativum* L., radish *Raphanus sativus* L.

3 Results and discussion

3.1 Characteristics of plants *P.sativum* grown in peat and sandy soil contaminated with diesel

Plants *P. sativum* grown in soil contaminated with diesel were harvested after 85 days vegetation and characterized by their morphological, physiological and biochemical properties (Table 2).

Table 2. Characteristics of plants *P.sativum* grown in peat and sandy soil contaminated with diesel (Period of vegetation 85 days)

Variants*	Number of plants, %	Height, mm	Aboveground -part dry weight, %	Chlorophyll a, mg/gw	Chlorophyll b, mg/gdw
P1	100	162,84 ± 21,24	41,39 ±6,35	4,05 ±1,26	5,52 ±1,67
P2	100	159,00 ± 10,12	50,95 ± 6,74	3,98 ±1,70	5,50 ±2,33
P3	40	100,07 ± 94,91	37,24 ± 33,23	3,56 ±0,63	4,98 ±0,79
P4	63	143,10 ± 16,66	70,29 ± 10,15	2,68 ±0,60	3,76 ±0,80
P5	53	150,90 ± 50,07	49,33 ± 13,69	3,79 ±1,36	3,76 ±0,80
P6	7	139,66 ±126,93	37,94 ± 34,17	3,32 ±0,11	4,65 ±0,20
S1	93	147,28 ± 16,29	55,73 ± 11,21	5,32 ±3,73	7,40 ±5,14
S2	97	141,23 ± 8,33	51,86 ± 12,97	7,24 ±3,79	9,97 ±5,27

* Variants S3, S4, S5, S6 are not included in the table because of a total inhibition of plant growth in these samples.

There are significant differences in germination of peas in peat and sandy soil. The presence of 1% diesel in sandy soil totally inhibited the growth of peas, although in peat soil an inhibition effect was about 50 %. Addition of bacteria inoculum did not influence this effect.

Peat soil is known to be reach in humin acids, and their functional groups play important role in sorption

processes of organic pollutants [19]. In turn, sand is poor substrate, with practically no sorption properties. This could be a reason why toxic effect of diesel in sandy substrate was more expressive.
Plants' height did not differ significantly among the variants. The highest content of chlorophyll a and b, which may be an indicator of the photosynthetic efficiency, was detected in peas grown in uncontaminated sand (Table 2). Measurement of chlorophyll a has been widely used to assess plant adaptation to an environment, as well as to measure the stress level experienced by a plant [20].

3.2 Physico-chemical properties of soil contaminated with diesel after 85 days vegetation

Comparison of soil physico-chemical properties after 85 days vegetation experiment showed that the noticeable changes have occurred between soils exposed by diesel and without it. Thus, dry weight of peat soil without diesel was higher almost twice, as compared to the samples with diesel, i.e. 66 % and 34 %, correspondingly. The pH level of peat and sandy soil was increased in the diesel-containing samples and varied in the range of 4.6-5.8 for peat and 6.1-7.5 for sandy soil. Redox potential varied in the range of +49 to +117 mV for peat and +6 to -39 mV for sandy soil (Fig.1).

3.3 Biochemical properties of soil contaminated with diesel after 85 days vegetation

Soil respiration is known as an appropriate index of soil microbial activity. Many authors have reported an increase of soil respiration after application of hydrocarbons probably due to the fact that soil microorganisms resisting hydrocarbon toxicity can degrade these new sources of carbon [21-23]. These data are in a good agreement with results obtained in our experiments. Thus, the presence of diesel stimulated microbial respiration in concentration dependent manner (Fig.2). Besides, microbial respiration rates were found higher in variants with the presence of bacterial consortia compared to variants without it. It attributes to both, peat and sandy soil (Fig.2).
Data on respiration are in a good agreement with data on the total microbial count, which was increased in the samples containing diesel. Besides, the total microbial count in rhizosphere was 10-100 times higher that in average soil sample. The number of colony forming units on the selective medium with diesel was higher in the samples inoculated with bacteria association. This effect was more pronounced in the samples with sandy soil.

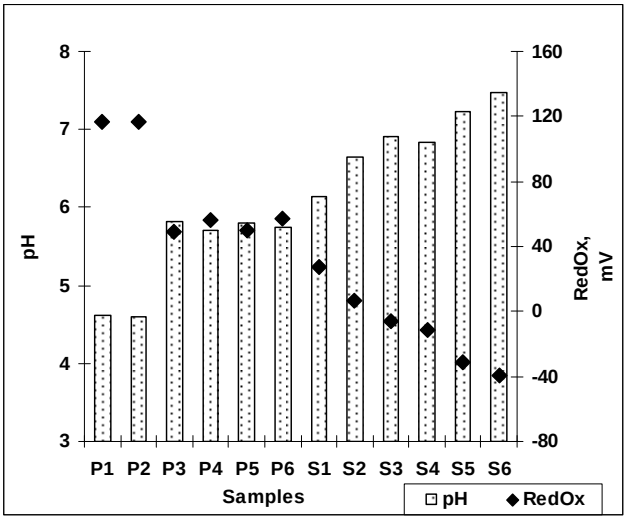

Fig.1. pH and redox potential of the analyzed soil.

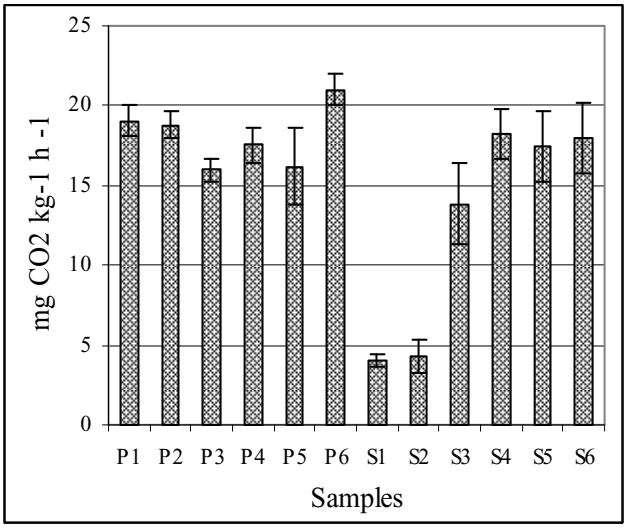

Fig.2. Microbial respiration in the analyzed soil.

It is important to note, that organic contamination such as petroleum hydrocarbons, resulted in the stimulation of microbial activity due to the added carbon, but causes an imbalance in the soil C: N ratio. This may result in immobilisation of soil nitrogen by the microbial biomass, leaving none available for plant growth. Peas fix atmospheric nitrogen to produce their own source of nitrogen for growth therefore they may prove more successful at growing on petroleum hydrocarbon contaminated soil. In support of this statement, species of *Leguminosae* have been found to be the most abundant reinhabitors of petroleum hydrocarbon contaminated sites [24]. Dehydrogenase activity typically occurs in all intact, viable microbial cells. Thus, its measurement is usually related to the presence of viable microorganisms and their oxidative capability [25].

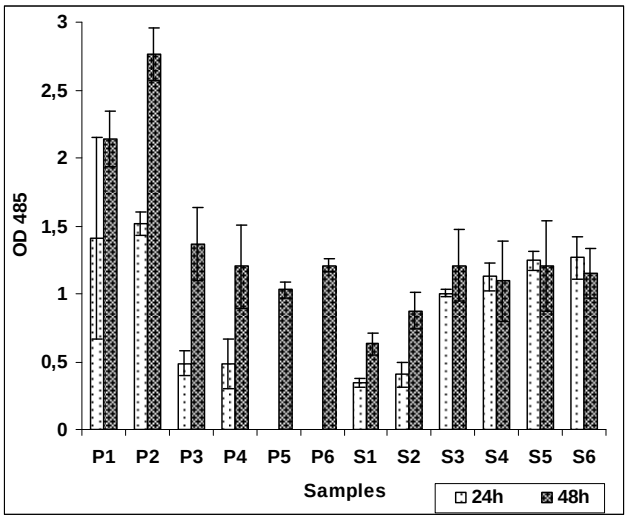

Fig.3. DHA activity of the analyzed soil.

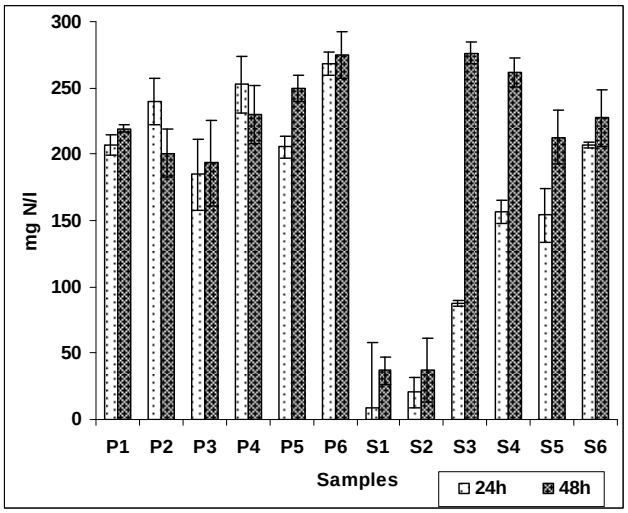

Fig.4. Urease activity of the analyzed soil.

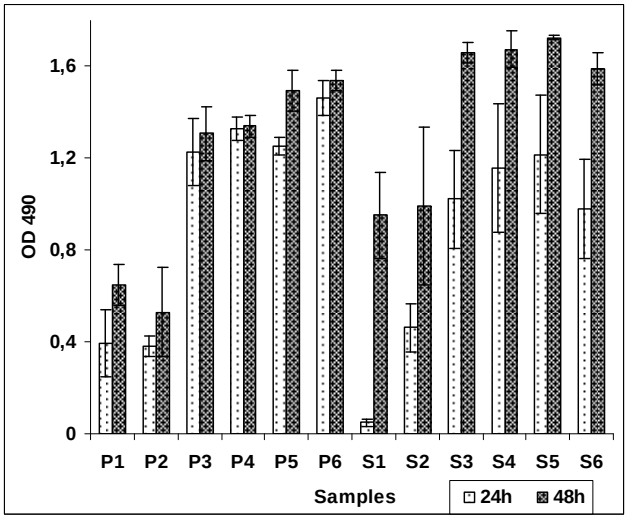

Fig.5. FDA-hydrolyzing activity of the analyzed soil.

In this study it was found that dehydrogenase activity was partly inhibited by diesel in peat soil, in turn, partly stimulated by diesel in sandy soil (Fig.3). Addition of bacterial consortia in the samples without diesel (P2 and S2) resulted in an enhanced DHA activity (Fig.3).

Urease activity differed significantly in the samples with sandy soil. Thus, the presence of 1% and 3% diesel resulted almost 5-fold increase of urease activity (Fig.4). FDA activity was significantly lower in uncontaminated variants of peat and sand and as compared to diesel-containing soil (Fig.5).

3.4 Soil toxicity assessment by germination test

Germination tests showed much higher toxicity of diesel-containing sandy soil compared to diesel-contaminated peat (data are not shown). Seeds of all six plant species (barley, oat, wheat, rape, garden cress, and radish) have germinated in peat. In sandy soil seeds of barley and garden cress were the most susceptible to diesel, and germination either was not detected or was bad. There was an expressed effect of bacterial consortia on seed germination in sand – seeds of garden cress, barley, rape germinated much better in bacteria-containing soil compared to diesel-contaminated soil alone.

4 Conclusions

In this study a model system "plant-soil-microorganism-diesel" was investigated using different approaches. Toxicity of diesel for *P.sativum* grown in sandy soil was considerably higher as compared to peat soil. Bioaugmentation resulted in an enhanced microbial respiration and fermentative activity in soil, as well as decreased soil toxicity determined by germination test (for cress, barley and rape) after 85 days vegetation experiment. The results obtained in this study indicated to the noticeable differences in soil physico-chemical, biochemical and plant physiological properties between samples with different soil type. These data provided the additional information on plant response to diesel contamination in the context of toxicity study and perspectives for phytoremediation. Environmental protection by using nature's own resources would be both economically and environmentally beneficial.

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