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GROWTH AND DEVELOPMENT OF SELECTED PLANT SPECIES IN THE PHYTOREMEDIATION OF DIESEL OIL CONTAMINATED SOIL

Effect of soil contamination with diesel oil on the growth of two plant species – faba bean (*Vicia faba* ssp. *minor*) and rape (*Brassica napus* var. *napus*) was assessed. It was demonstrated that the presence of diesel oil initially reduced the number of germinating seeds and then limited their development. Negative effect of the contamination was visible also in relation to the length of the roots and above-ground plant parts. The plants stimulated the process of degradation of diesel oil, however, it occurred only in the initial incubation period.

1. INTRODUCTION

Physical and chemical techniques of soil remediation are very expensive and may be difficult in use especially under conditions of small contamination in large areas. Other option is application of biological methods (bioremediation), i.e. using microorganisms for degradation of pollutants. Also the ability of using plants for environment purification – phytoremediation, has been gaining much interest [1]. In the method, not only the ability of a plant itself to degrade, absorb or neutralize pollutants is important but also the way the plant affects soil microorganisms involved in biodegradation (stimulation or hindrance of their development). Thus such plant species should be used which are able to develop and grow normally on a contaminated area and contribute to a decrease in the content of pollutants in soil and, what is the most important, will stimulate microorganisms to metabolise pollutants.

The idea of using plants to purify environment is nothing new, phytoremediation enables removal of many types of pollutants [2]. Development of this technology pro-

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vides cheap and effective methods of elimination of pollutants with organic compounds, including derivatives of crude oil [3–5]. The process of elimination of petroleum hydrocarbons from soils employs fast-growing trees [6,7], and, above all, different grass species [5, 8], herbs and plants of *Fabaceae* family [2]. Phytoremediation of soil polluted with diesel oil is more difficult in comparison with other petroleum pollution, e.g. with crude oil. Due to a high content of light hydrocarbon compounds toxic to plants [5], remediation of such soils may be not only slower but also less effective [9].

In the present study, the authors examined the possibility of growth and development of rape and faba bean in soil contaminated with diesel oil and influence of their presence on the degree of diesel oil degradation. Among other things, germination and root growth were assessed, being the most important and critical stages in plant development [10], sensitive to soil contamination and used as indicators of soil health [11].

2. MATERIALS AND METHODS

Loamy soil was collected at the depth of 0–15 cm of the humus level. The soil in the place of sampling exhibited mechanical composition of sandy loam of pH (KCl) 6.78, the content of organic carbon was 1.9% and the content of nitrogen – 0.15%. Diesel oil used in the experiment was obtained from a petrol pump of a fuel station. The liquid was clear and bright yellow. Two species of dicotyledonous plants were used in the study – *Vicia faba* ssp. *minor* (faba bean) of the family *Fabaceae* (bean family) and *Brassica napus* var. *napus* (rape) of the family *Brassicaceae* (cabbage family). Faba bean was selected based on literature data [12]. Using rape in the process of phytoremediation would create the possibility of not only elimination of the source of contamination but also would enable to obtain plant biomass that could be used for energy purposes or the production of biofuels.

In order to establish the microbiological balance, the soil was left for 7 days in the dark at a room temperature, and then its 50% capillary water capacity was obtained. The material was divided into 300 g samples and contaminated with diesel oil at the dose of 1% (w/w per dry matter of soil), and then placed in 500 g containers. The following samples were prepared: 0 – soil with diesel oil, I – soil with diesel oil and one of two examined plants (15 seeds): rape (I_a) and bean by (I_b). Rape seeds were placed in pots at the depth of 1.5 cm, while faba bean seeds were placed at a depth of 3 cm. All samples were prepared in three repetitions. The experiment was conducted in the vegetation hall of the West Pomeranian University of Technology in Szczecin. On the 0, 7th, 14th, 28th, 56th, 112th day of incubation the content of compounds susceptible to ether extraction in soil was assessed, the percentage of seed germination and changes in the numbers of plants in the pots of individual objects during the incubation period were determined, and biometric analyses of the length of roots and above-ground parts of the examined plant species were carried out. All the analyses were conducted in triplicate. Research on plants was performed in objects I_a and I_b, while additional triplicate pots with not contaminated soil and a given plant, denoted by K_a for rape and K_b for faba bean, respectively, served as control for plant growth. Based on the measured mean length of roots, the plant resistance index (*RI*) was calculated according to the method described by Hawrot and Nowak [13]:

$$RI = \frac{\text{mean increase in rooth length in soil contaminated with diesel oil}}{\text{mean increase in rooth length in the control object}}$$

The index for the control object amounts to 1, its value higher than 1 stands for plant resistance to contamination.

The remains of diesel oil were determined using the gravimetric method according to the Polish norm PN-75/C-04573/10 [14] on evaluation of the content of substances extracted with organic solvents.

The obtained results were statistically analysed using Statistica application, and the used method was two-factor analysis of variance taking into account influence of the presence of plants and the incubation period. The relationship was determined using the Pearson linear correlation coefficient, and factor levels were assessed with the use of the Tukey and Newman–Keuls tests, the significance level α was 0.05 for selected parameters.

3. RESULTS AND DISCUSSION

The statistical analysis showed that both the type of the sample (0, I_a, I_b), date of measurement and their interaction had a significant influence on the content of diesel oil in soil, plant growth and development together with the length of roots and above-ground plant parts.

Table 1

Mean content of compounds susceptible to ether extraction in the individual objects of the experiment

Incubation day	Content of compounds susceptible to ether extraction [%]		
	0	I_a	I_b
7	0.546^a	0.606^a	0.477^a
14	0.451^a	0.244^b	0.250^b
28	0.139^b	0.157^b	0.221^b
56	0.128^b	0.151^b	0.175^b
112	0.119^b	0.139^b	0.157^b

Values denoted by superscripts a and b are statistically significantly different on the level $\alpha = 0.05$.

At the beginning of the experiment, the mean content of compounds susceptible to ether extraction in the examined objects amounted to 0.8% – changes during incubation are presented in Table 1.

Małachowska-Jutsz et al. [15] stated that the presence of plants accelerates removal of petroleum hydrocarbon in comparison with removal from soils not grown over with plants – after 16 weeks of the experiment on refinery soil, the authors obtained the greatest degradation (35%) in a sample of contaminated soil supplemented with biopreparation and grass. Changes in the content of compounds susceptible to ether degradation observed in the present study prove constant ongoing degradation of diesel oil, however, the influence of plants on the acceleration of diesel oil degradation in soil was equivocal (Fig. 1). The stimulating influence of the plants, and above all of faba bean, was noticeable only in first weeks of the experiment. After 112 days, the content of compounds susceptible to ether extraction in the soil with rape was higher in comparison with the object not grown over with plants (object 0) by over 17% and by nearly 32% in the soil with faba bean.

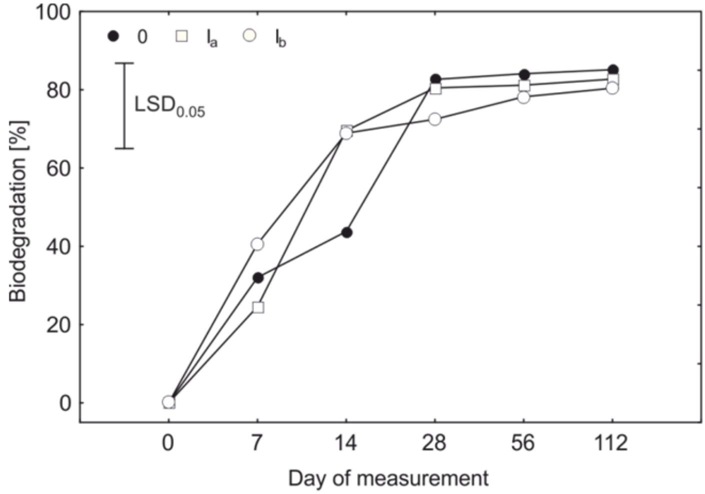

Fig. 1. Influence of plants on diesel biodegradation in soil

The presented results may demonstrate inhibitory influence of plants on the course of degradation of petroleum pollutants in spite of the initial stimulation. The plants may have started production of substances that hindered degradation of pollutants, or changes occurred in soil environment which resulted in lesser activity of microorganism population decomposing petroleum substances in the process of degradation, as in the beginning of the experiment. However, a significant element may be above all the physiological response of the plants to the presence of contamination, decreasing the level of their potential remediation abilities.

In the objects with contaminated soil, 100% of seeds germinated only after 2 weeks when compared to the control object. Diesel could provide a physical barrier limiting water and oxygen intake inside the seeds [2]. Crude oil derivatives may not only delay seed germination but also limit the development of roots and reduce plant height, decrease their biomass or completely prevent the development [16–18]. A decrease in the number of plants in individual pots was noted during incubation (Table 2). Some plants, especially rape, were characterized by exceptionally small leaves with symptoms of chlorosis, short and thin stems, poorly developed root structure, and died during the experiment. Such a plant response could have resulted from changes in water and air relationships in the root zone, accumulation of petroleum compounds in plant tissues and their dehydration [19]. Limited development of roots may also decrease the content of cytochrome P450, whose presence in root saps may increase oil degradation [1].

Table 2

Mean number of plants in pots
of the individual objects of the experiment

Day of measurement	Number of plants [%]			
	Rape *Brassica napus* var. *napus*		Faba bean *Vicia faba* ssp. *minor*	
	K_a	I_a	K_b	I_b
7	100^{a}	96^{ab}	100^{a}	98^{ab}
14	100^{a}	100^{a}	100^{a}	100^{a}
28	100^{a}	93^{ab}	100^{a}	100^{a}
56	100^{a}	93^{ab}	97^{ab}	96^{ab}
112	77^{c}	84^{bc}	93^{ab}	93^{ab}

Values denoted by superscripts a and b are statistically significantly different on the level $\alpha = 0.05$.

The contamination dose has a significant influence on plant response. According to Ogbo [20], even 1% of diesel oil in soil may result in limited germination, growth and development of plants. However, effect of pollutants on plants, i.e. their response, depends also on plant species. The results obtained in the present study demonstrate that the examined plants showed diverse sensitivity to the presence of diesel oil. Roots and above-ground parts of rape developed more poorly in contaminated soil than those of faba bean. Great sensitivity of rape (*Brassica napus*), when compared to alfalfa (*Medicago sativa*) was confirmed also by Włodkowic and Tomaszewska [21]. In the study by Hawrot-Paw and Hreczuk [12], among 14 plant species used in the toxicity test, only three showed resistance to 5% dose of diesel oil: faba bean (*Vicia faba*, var. *Amulet*), white mustard (*Sinapis alba*) and alfalfa (*Medicago sativa*, var. *Alba*). In the present study faba, bean developed more poorly in contaminated soil until the 28th day in the case of roots, and until the 56th day in the case of above-ground parts (Fig. 2), while the parameters determined for rape were lower than in the soil of the control object for the whole incubation period. Adverse effect of diesel oil on the development of seedlings and roots has been confirmed also by other authors [22].

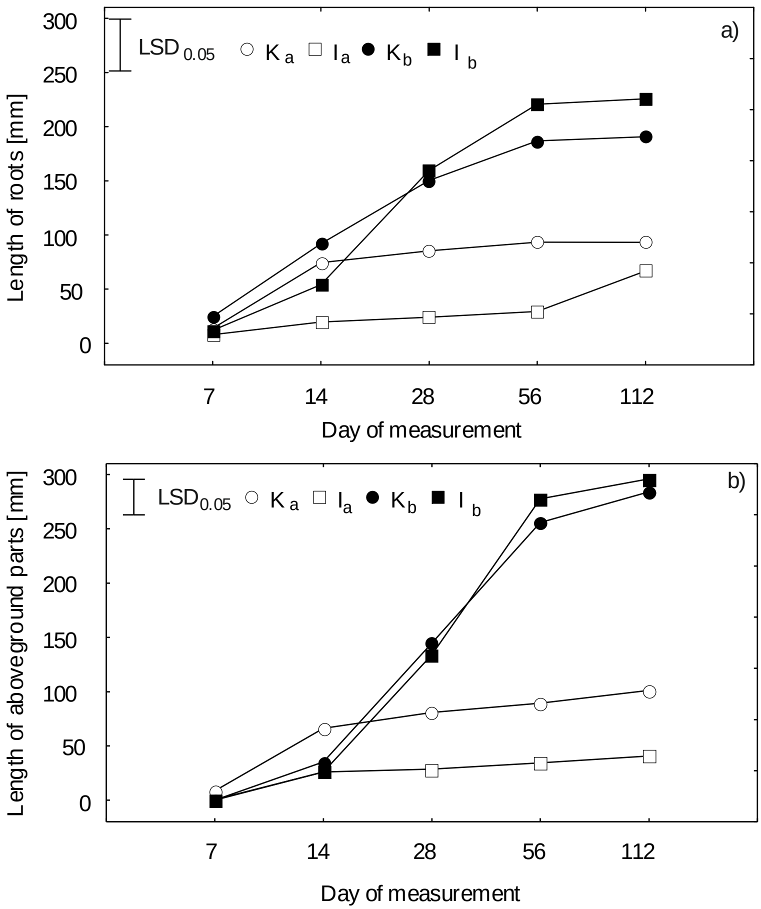

Fig. 2. Results of biometric measurements of the examined plants in relation with the length of roots (a) and above-ground parts (b)

A significant negative correlation between the content of compounds susceptible to ether extraction and length of plant roots and above-ground parts was demonstrated (Table 3). Also other authors, such as Kaimi et al. [3] and Kechavarzi et al. [4] found a relationship between the growth of ryegrass roots and degradation of diesel oil. In the presented experiment, a difference in tolerance of the both species to diesel oil was noted. In the case of rape, the resistance index ranged from 0.263 to 0.716 (Table 4).

The resistance index on the 7th day of the experiment amounted to 0.484 for faba bean, and in the subsequent dates its constant increase was observed. Maximum values of the indexes, demonstrating resistance of faba bean to the presence of contamination in soil, were determined on the 56th and 112th day of the experiment.

Table 3

Pearson's linear correlation coefficient *r* for the content of compounds susceptible to ether extraction and selected biometric parameters of the examined plants

Variable	Rape *Brassica napus* var. *napus*	Faba bean *Vicia faba* ssp. *minor*
Length of roots	–0.577	–0.796
Above ground plant parts	–0.931	–0.746

Table 4

Resistance index *RI* for the plants used in the study

Day of measurement	Rape *Brassica napus* var. *napus*	Faba bean *Vicia faba* ssp. *minor*
7	0.584	0.484
14	0.263	0.588
28	0.314	1.062
56	0.281	1.184
112	0.716	1.181

The type of contaminating hydrocarbons and the type of soil have a significant influence on plant response. According to Labud et al. [23], diesel oil exhibits stronger phytotoxic properties against a range of plants than other fuels, e.g. petrol. It has been confirmed in the study by Radwan et al. [24]. Plants of Vicia faba species were resistant to the presence of even 10% dose of crude oil in sandy soil. The observed relationships may be influenced by the composition of diesel oil, which may differ depending on the origin of crude oil, refining process and the presence of components that are added in refineries in the end stages of its production. Absorption of petroleum hydrocarbons by soil colloids present in loamy soil on the one hand may limit its adverse effect, and on the other hand it may cause the appearance of their toxicity only after some time from the moment of contamination [23].

Higher content of ether extracts of plants in buildings may be the result of phytostabilisation of pollution. In the reduction of migration and bioavailability proteins and enzymes produced by the plants and exuded into the rhizosphere may be involved [25] and may also relate to organic pollutants, including petroleum derivatives.

4. CONCLUSIONS

• Germination of seeds and growth and development of plants in soil contaminated with diesel oil depends on a plant species. Introduction of petrol into soil adversely affected mainly rape, limiting also the development of rape roots and above-ground parts. Based on the resistance index, greater tolerance was noted for faba bean.

• Plant species used in the present study, taking into account their response to contamination and the content of compounds susceptible to ether extraction determined in soil, are not the best candidates to be used in the process of phytoremediation of soils contaminated with diesel oil.

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