The influence of diesel oil contamination on soil microorganisms and oat growth

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ABSTRACT

The effect of diesel oil applied at 0, 2.4, 4.8 and 7.2 ml.kg^{-1} of soil on yield of oat and number of oligotrophic, eutrophic, nitrogen immobilising, ammonifying and cellulolytic bacteria and *Azotobacter* sp., actinomyces and fungi was studied in a pot experiment. Inoculation with *Streptomyces intermedius* spores was used for soil detoxication. The experiment was performed in Eutric Cambisol soil derived from light clay sand. Diesel oil was found to have a negative effect on the growth and development of oat. Inoculation did not attenuate the response of oat to soil contamination with diesel oil, but it had a positive effect on oligotrophic and eutrophic bacteria as well as *Azotobacter* sp., nitrogen immobilising bacteria and fungi. Regardless of sown and unsown soil and inoculation with *S. intermedius* spores, diesel oil stimulated the number of oligotrophic, eutrophic, nitrogen immobilising bacteria and actinomyces. Sowing of oat positively affected microbiological properties of soil, because it had a positive influence on the relation of oligotrophic bacteria and actinomyces to fungi. This positive effect, however, was weakened by diesel oil.

Keywords: diesel oil; oat yield; number of microorganisms

Soil pollution with petroleum and its derivatives is one of the causes of degradation of natural environment. Toxicity of refinery products depends on their physical and chemical properties (Riis et al. 1995, Olson et al. 1999).

Carbohydrates of simple structure and small molecular weight (C5–C11) referred to as light fraction of petroleum, move easily in soil and escape to atmosphere. They dissolve in water more easily than the other carbohydrates. In large concentrations, they are toxic to soil organisms and plants. Physical and chemical activity of carbohydrates decreases with their increased molecular weight. Carbohydrates possessing more than 11 carbon atoms (C12–C42) are less harmful to soil organisms than light fraction carbohydrates. Refinery products may be present in soil in the gaseous state in empty spaces of the aeration sphere not filled with ground water or in the liquid state (Zieńko 1996). These compounds spread in soil as substances floating on the surface of soil solution, carbohydrates dissolved in water, residual contaminants adsorbed on soil molecules or gases (Małachowska-Jutsz et al. 1997). Petroleum-derived substances disperse in soil under the influence of gravitational force and counteractive capillary and sorptive forces. Refinery products may be transferred in soil vertically and horizontally (Olańczuk-Neyman 1994).

Biodegradation of refinery products is one of the methods of combating pollution caused by petroleum-derived compounds (Riis et al. 1995, Margesin and Schinner 1997). This method is particularly useful if a large area in a built-up zone or in the vicinity of a road or railway junction is contaminated; in which case *in situ* decontamination methods must be applied (Tyczkowski 1993). Among the advantages of biodegradation of refinery products are low costs and the fact that soil reclaimed with the help of biological methods retains its properties. A disadvantage, however, is the long time necessary for complete soil reclamation (Tyczkowski 1993). During the process of biodegradation of carbohydrates, a large proportion of contaminants (90–95%) is removed quite efficiently, but the removal of the remaining part is troublesome (Riis et al. 1995).

The aim of our study was to determine modifications of microbiological properties of soil contaminated with diesel oil and to assess the possibility of using actinomyces spores (*Streptomyces intermedius*) for detoxication of such soil.

MATERIAL AND METHODS

Experiments on oat cv. Komes (25 plants per pot) were conducted in a greenhouse. Pots were filled with 2.5 kg of Eutric Cambisol soil derived from light clay sand [organic carbon content (C) – 0.75%; pH in 1 M KCl 6.6; hydrolytic acidity (Hh) 1.16 mmol.100 g^{-1} of soil; total of bases (S) 14.1 mmol.100 g^{-1} of soil; sorptive complex capacity (T) 15.26 mmol.100 g^{-1} of soil; degree of saturation with alkaline cations (V) 92.4%]. Prior to putting into pots, soil was fertilised with the following macroelements, in g.kg^{-1} of soil (expressed as pure component): N – 0.15 [$CO(NH_2)_2$]; P – 0.1 [KH_2PO_4]; K – 0.15 [0.12 g of K as KH_2PO_4 and 0.03 g of K as KCl]; Mg – 0.05 [$MgSO_4$ 7 H_2O]. The fertilisers and diesel oil were added to soil before sowing of oat. Soil was contaminated with the following amounts of diesel oil: 0.0, 2.4, 4.8 and 7.2 ml.kg^{-1} of soil. The experiments involved two series in four replicates. No microorganisms were introduced to soil in

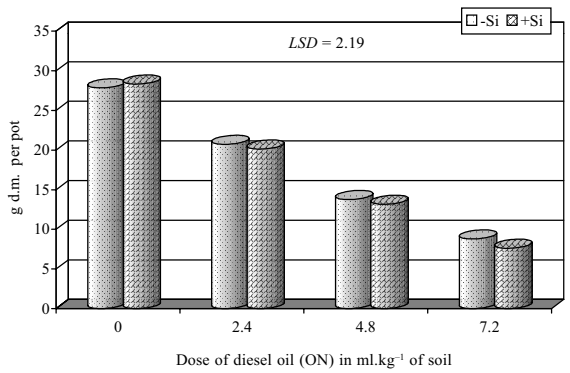

Figure 1. Effect of diesel oil (ON) on oat yield

the first series of the experiments. The aim of the second series was to test the effect produced by increasing doses of diesel oil on soil previously amended with multiplied of *Streptomyces intermedius* at $11.0.10^{13}$ CFU per kg of soil d.m. In addition to this, a series without oat was introduced in order to assess more precisely the influence of oil on autochtonous soil microorganisms.

Streptomyces intermedius was taken from own collection of the Chair of Microbiology. Cells were multiplied on slopes for 7 days at 28°C and then washed off using 3 ml sterile of water solution 0.85% NaCl. Suspension of *S. intermedius* from 60 slopes were poured into a conical flask of 1 dm^3 capacity and mixed. After that, 5 cm^3 of the suspension per pot (2.5 kg of soil) was measured. *Streptomyces intermedius* was cultured on a medium composed of soluble starch – 10.0 g, casein – 0.3 g, KNO_3 – 2.0 g, NaCl – 2.0 g, K_2HPO_4 – 2.0 g, $MgSO_4.7\,H_2O$ – 0.05 g, $CaCO_3$ – 0.02 g, $FeSO_4$ – 0.01 g, agar – 20.0 g, distilled water – to 1 dm^3.

Soil moisture was maintained at constant level of 60% of the capillary water holding capacity for the whole vegetative period of plants (50 days). The yield of oat, harvested at the tasseling stage, was determined. After 14 days and 50 days of the experiment, soil was taken for basic microbiological analyses. The plate method (in three replications) was applied to determine the count of the following microorganisms: organotrophic bacteria (Org) on Bunta and Rovir's medium according to the procedure described by Harigan and McCane (1996), oligotrophic (Olig) and eutrophic (Cop) bacteria on a substrate with peptone and meat extract according to Onta and Hattori (1983), actinomyces (Act) by the method of Künster and Williams with two antibiotics (nystatine and actidione) according to the procedure reported by Parkinson et al. (1971), fungi (Fun) – on glucose-peptone agar according to Martin (1950) and *Azotobacter* sp. by Fenglerowa's method (1965), nitrogen immobilising (Im) and ammonifying microorganisms – on Winogradski's medium (1953).

All the results were analysed statistically with the help of Duncan's test by Statistica (StatSoft, Inc. 2000).

RESULTS AND DISCUSSION

The results of the study clearly show that the soil contamination with diesel oil had a considerable effect on the growth and development of oat (Figure 1). Negative influence of the pollutant was related with the degree of soil pollution. The lowest dose of diesel oil (2.4 $ml.kg^{-1}$ of soil) caused significant inhibition of the growth of the crop (25.6% decrease), but the highest dose (7.2 $ml.kg^{-1}$ of soil) was responsible for a 68.4% decline in the yield. Since the volume of oat yield in the control contaminated objects was unaffected by inoculation with *Streptomyces intermedius* spores, the inoculate was found incapable of relieving the toxic effect produced by oil on the growth and development of oat, which indirectly proves its small efficacy in biodegradation of diesel oil. The statistical analysis of the results revealed a highly significant negative correlation between the dose of diesel oil in soil and oat yield (Figure 2). The correlation coefficient for these two factors in the series without actinomyces spores was –0.99, and –1 for the experiment with *Streptomyces intermedius*.

Toxic influence of refinery products on plants was confirmed by the authors' own investigations and those of other researchers (Rytelewski et al. 1981, Iwanow et al. 1994, Kucharski and Wyszkowska 2001, Wyszkowska and Kucharski 2001, 2000). Rytelewski et al. (1981) attributed reduction in yield of plants grown in petroleum contaminated soil to several events, including high deficiency of available phosphorus and potassium and water deficit, whereas the degrading effect of petroleum-derived com-

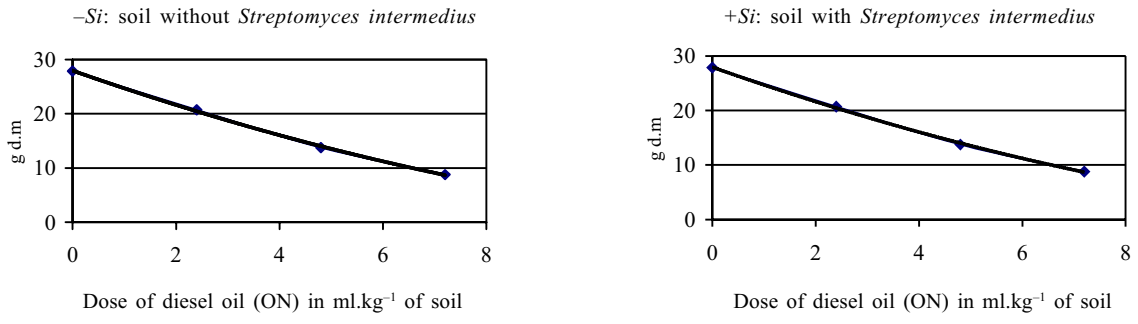

Figure 2. Regression equations for oat yield (g d.m. per pot)

$y = 27.941 - 3.3475x + 0.0937x^2$ $r^2 = -1.00$

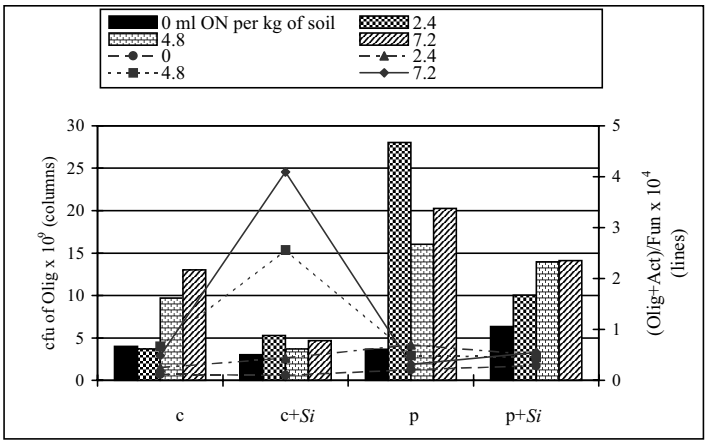

Figure 3. Number of oligotrophic bacteria (cfu) and ratio of the total number of oligotrophic bacteria and actinomyces to fungi in 1 kg of soil d.m.

c – unsown soil without inoculum
p – oat sown soil without inoculum
c+*Si* – unsown soil with *Streptomyces intermedius* inoculum
p+*Si* – oat sown soil with *Streptomyces intermedius* inoculum
ON – diesel oil
LSD for oligotrophic bacteria (Olig) = 5.57
LSD for ratio of the total number of oligotrophic bacteria and actinomyces to fungi (Olig + Act)/Fun = 1.54

ponents consisted not only in causing acute phosphorus deficit but also in generating nitrogen and oxygen deficiency and disturbed water balance. Besides, by penetration deep into soil, diesel oil causes agglomeration of soil and deterioration of chemical, physical and biological soil properties and fertility (Tyczkowski 1993).

Microorganisms play an important role in the flow of energy and biogenous circulation of elements in ecological agrisystems owing to small size of their cells, high metabolic activity and rapid multiplication (Opic 1996). In the experiments reported hereby diesel oil introduced to soil modified the number of all physiological and systematic groups, although the effect observed was not always straightforward (Figures 3–10). Under the influence of diesel oil and regardless of the use of soil (soil unsown or sown with oat) or soil inoculation with *S. intermedius* spores, the average number of oligotrophic (Figure 3), ammonifying (Figure 5), nitrogen immobilising bacteria (Figure 6) and actinomyces (Figure 10) increased.

There was a highly significant positive correlation between the count of oligotrophic bacteria and degree of diesel oil soil contamination (Table 1). For both unsown soil without actinomyces spores and sown soil inoculated with *S. intermedius*, the correlation coefficient was 0.95. Number of these bacteria was respectively 3.3- and 2.2-fold higher in the soil contaminated with the highest dose of oil (7.2 ml.kg^{-1} of soil) in comparison to uncontaminated soil (Figure 3). Soil inoculation with *S. intermedius* resulted in a significantly increased growth of oligotrophic bacteria in unsown uncontaminated soil, but inhibited the effect of diesel oil on multiplication of these bacteria, which may be thought of as a positive outcome, since the biological balance disrupted under the influence of oil was to a certain extent remedied by the inoculate.

Same as oligotrophic bacteria, the number of eutrophic bacteria was positively correlated with the degree of soil contamination with the refinery product (Figure 4, Table 1), except in sown soil with *S. intermedius*, where two concentrations of diesel oil (2.4 and 4.8 ml.kg^{-1} of soil) had a negative effect on the growth of eutrophic bacteria.

Ammonifying bacteria, whose count was significantly positively correlated with soil contamination (Table 1), were found to be highly vulnerable to soil pollution with

Table 1. Correlation coefficients between diesel oil soil contamination and number of microorganisms

Microorganisms	–*Si*		+*Si*	
	unsown soil	sown soil	unsown soil	sown soil
Olig	0.95**	0.45	0.41	0.95**
Cop	0.84**	0.91**	0.82**	0.61*
Am	0.98**	0.90**	0.37	0.93**
Az	0.34	–0.68*	–0.77**	–0.93**
Im	0.96**	0.67*	–0.31	0.72**
Cel	–0.97	0.94**	–0.97**	0.61*
Act	0.84**	0.30	0.55	0.78**
Fun	–0.54*	0.89**	–0.91**	–0.14
(Olig + Act)/Fun	0.81**	0.04	0.98**	0.82**

Olig = oligotrophic bacteria, Cop = eutrophic bacteria, Az = *Azotobacter* sp., Im = nitrogen immobilising bacteria, Am = ammonifying bacteria, Cel = cellulolytic bacteria, Act = actinomyces, Fun = fungi, (Olig + Act)/Fun = ratio of the total number of oligotrophic bacteria and actinomyces to fungi
–*Si* – soil without *Streptomyces intermedius*, +*Si* – soil with *Streptomyces intermedius*
* significance for $p < 0.05$; ** significance for $p < 0.01$; $n = 16$

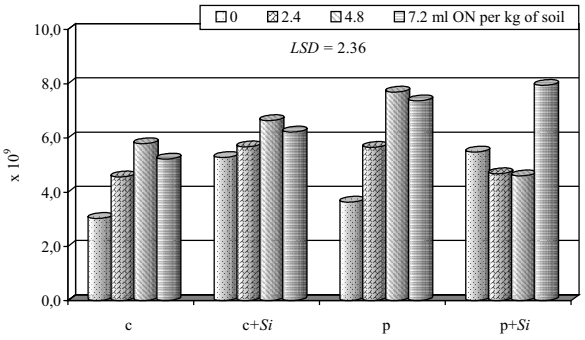

Figure 4. Number of eutrophic bacteria (cfu) in 1 kg soil d.m. (denotations explained in Figure 3)

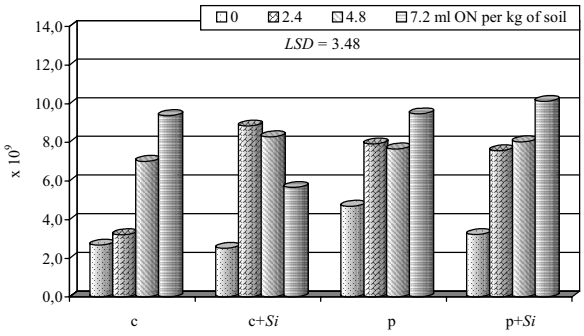

Figure 5. Number of ammonifying bacteria (cfu) in 1 kg soil d.m. (denotations explained in Figure 3)

diesel oil (Figure 5). Diesel oil positively influenced the proliferation of nitrogen immobilising bacteria (Figure 6). The highest significant rise in the number of these bacteria under the influence of oil, regardless of the degree of soil pollution with this contaminant, was recorded for sown soil not inoculated with *S. intermedius*, for example an 11-fold increase in the count of nitrogen immobilising bacteria occurred in the soil contaminated with 4.8 ml of oil per kg of soil. It was only in the soil not inoculated with actinomyces that diesel oil in the amount of 4.8 and 7.2 ml.kg^{-1} of soil did not modify the number of these bacteria.

The response of *Azotobacter* sp. to soil pollution with diesel oil was negative (Figure 7, Table 1). With the exception of two objects (in the unsown soil not inoculated with 7.2 ml of diesel oil per kg and in the oat sown soil not inoculated with 2.4 ml of diesel oil per kg), the number of these cells was negatively correlated with soil contamination with diesel oil. The count of *Azotobacter* sp. grew only in the unsown soil not inoculated with actinomyces and contaminated with the highest dose of oil (7.2 ml.kg^{-1} of soil) and in the sown soil with the lowest dose of the contaminant (2.4 ml.kg^{-1} of soil). Refinery products were particularly toxic in soil sown with oat and amended with *S. intermedius* spores, in which the correlation coefficient between contamination and number of *Azotobacter* sp. was –0.93 (Table 1).

Irrespective of the degree of soil contamination and inoculation with *S. intermedius*, diesel oil in unsown soil always produced a negative effect on the amount of cellulolytic bacteria (Figure 8). A significant negative correlation ($r = –0.97$) between soil contamination with diesel oil and the number of these bacteria was determined for unsown soil (Table 1). Reverse correlation appeared in sown soil not inoculated with actinomyces, in which diesel oil significantly stimulated the growth of cellulolytic bacteria ($r = 0.94$). With regards to the objects with *S. intermedius*, the number of these microorganisms remained stable.

The response of soil fungi to diesel oil was similar to that of cellulolytic bacteria (Figure 9). Diesel oil depressed the number of fungi in unsown soil, especially if it was inoculated with actinomyces. In the latter case, the number of fungi declined 29-fold under the effect of the highest degree of contamination. Objects sown with oat and not inoculated with actinomyces were different as they stimulated the growth of soil fungi.

Actinomyces (Figure 10) responded to soil contamination with diesel oil very much the same as oligotrophic, eutrophic, ammonifying and nitrogen immobilising bacteria did. Regardless of the experimental series and degree of soil contamination, diesel oil stimulated multiplication of actinomyces. Therefore, soil contamination was positively correlated with the count of actinomyces.

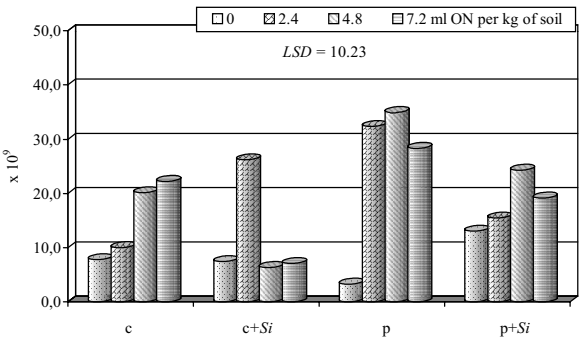

Figure 6. Number of nitrogen immobilising bacteria (cfu) in 1 kg soil d.m. (denotations explained in Figure 3)

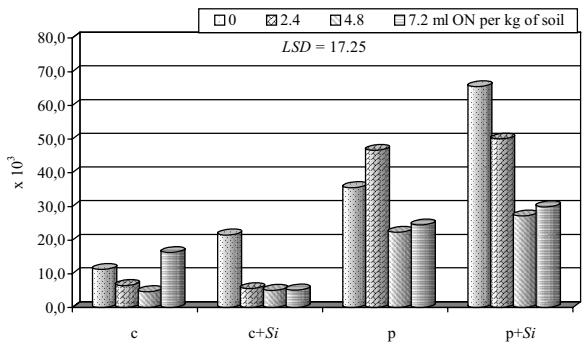

Figure 7. Number of *Azotobacter* sp. (cfu) in 1 kg soil d.m. (denotations explained in Figure 3)

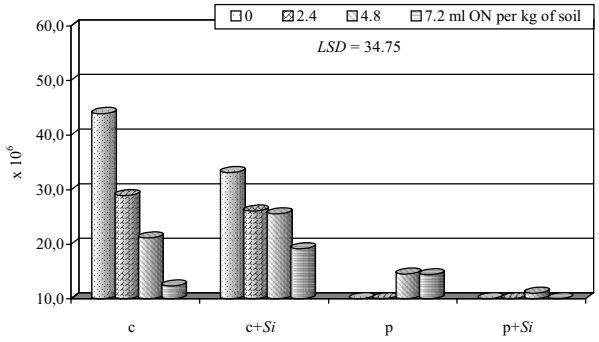

Figure 8. Number of cellulolytic bacteria (cfu) in 1 kg soil d.m. (denotations explained in Figure 3)

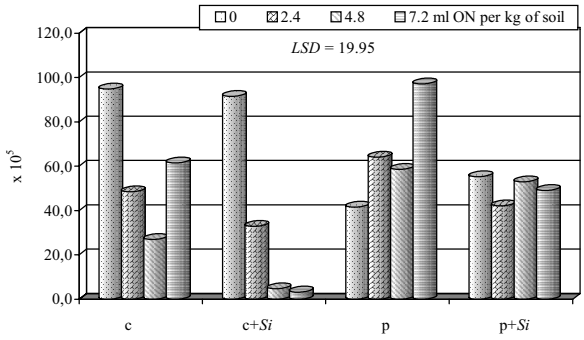

Figure 9. Number of fungi (cfu) in 1 kg soil d.m. (denotations explained in Figure 3)

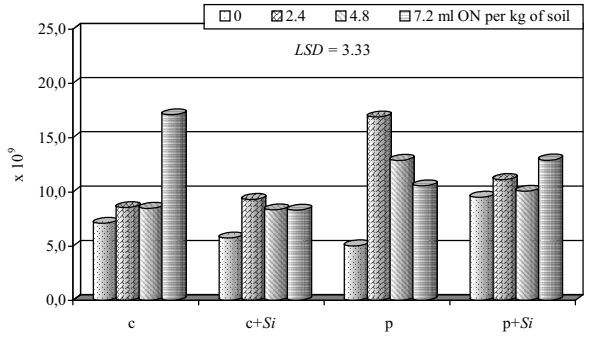

Figure 10. Number of actinomyces (cfu) in 1 kg soil d.m. (denotations explained in Figure 3)

Analysis of fluctuations in the number of particular groups of soil microorganisms may be informative in terms of direction and range of transformation of the arable soil layer. Myśków (1981) claimed that the ratio of the number of bacteria and actinomyces to the number of fungi reflected more fully the microbiological properties of soil than the count of each group of microorganisms separately. The usefulness of this ratio, however, finds no confirmation in this paper because the ratio of the total count of oligotrophic bacteria and actinomyces to the number of fungi increased and reached particularly high levels in unsown soil inoculated with *S. intermedius* (Figure 3), even though the effect of diesel oil on yield of oat was clearly negative. This would suggest that the above index should be treated with great caution when assessing soil fertility.

It is interesting to observe the interaction between the inoculum of *S. intermedius* and the groups of microorganisms studied (Table 2). The inoculum was favourable to oilgotrophic, eutrophic, *Azotobacter* sp., and nitrogen immobilising bacteria and to actinomyces and fungi, but it affected adversely cellulolytic and ammonifying bacteria. The influence of the *S. intermedius* inoculum was weakened by the introduction of diesel oil to soil.

The effect of growing oat was beneficial to most microorganisms, and *Azotobacter* sp. in particular (Table 3). The count of all groups of microorganisms, except cellulolytic bacteria and fungi in uncontaminated objects, was higher in sown versus unsown soil. Such an effect produced by root secretions on microorganisms was also noticed in diesel oil contaminated soil, with the number of fungi increasing under these extreme conditions.

Fluctuations in the count of microorganisms were caused by oat cultivation, inoculation of soil with *S. intermedius* spores and, perhaps, by specific mutual interactions of autochtonous microorganisms in soil polluted with diesel oil as well as the use of this contaminant as a source of carbon and energy (Margesin and Schinner 1997).

Most research on the effect of refinery products on microorganisms has been conducted *in vitro* under laboratory conditions (Tong et al. 1998). However, reports on the influence of petroleum derivatives in natural environment, including microorganisms, are scarce. The effect of diesel oil on fungi determined by Michalcewicz (1995), Kucharski and Wyszkowska (2001) and Wyszkowska and

Table 2. Ratio of the number of microorganisms in sown soil with *Streptomyces intermedius* inoculum to soil without inoculum

Diesel oil (ml.kg^{-1})	Olig	Cop	Az	Im	Cel	Am	Act	Fun	(Olig + Act)/ Fun
0	1.22	1.62	1.86	1.85	0.82	0.78	1.26	1.08	1.16
2.4	0.48	1.01	1.05	0.98	0.87	1.47	0.80	0.67	1.00
4.8	0.69	0.83	1.19	0.56	0.97	1.11	0.86	0.67	2.59
7.2	0.57	1.12	0.86	0.52	0.97	0.84	0.77	0.33	5.74
LSD	0.27	0.67	0.43	0.24	0.77	0.77	0.28	0.38	1.13

Denotations explained in Table 1

Table 3. Ratio of the number of microorganisms in oat sown soil to unsown soil

Diesel oil (ml.kg^{-1})	Olig	Cop	Az	Im	Cel	Am	Act	Fun	(Olig + Act)/ Fun
				–Si					
0	0.92	1.20	3.16	0.42	0.14	1.75	0.71	0.44	1.75
2.4	7.55	1.24	7.15	3.23	0.33	2.46	1.97	1.32	2.80
4.8	1.65	1.33	4.77	1.73	0.69	1.09	1.52	2.17	0.74
7.2	1.55	1.41	1.50	1.27	0.96	1.01	0.62	1.58	0.65
				+Si					
0	2.09	1.04	3.04	1.75	0.24	1.28	1.65	0.60	2.99
2.4	1.89	0.83	8.90	0.59	0.28	0.86	1.20	1.28	1.13
4.8	3.79	0.69	5.34	3.82	0.47	0.97	1.21	11.25	0.18
7.2	3.00	1.28	5.78	2.70	0.48	1.79	1.55	15.39	0.13
LSD									
a	1.78	n.s.	4.11	0.78	0.35	0.78	1.49	7.93	0.68
b	1.03	0.69	2.37	0.45	0.20	0.45	0.86	4.58	0.42
a × *b*	2.52	1.69	5.81	1.11	0.49	1.11	2.11	11.21	0.99

a – for diesel oil dose, *b* – for *Streptomyces intermedius* inoculum
Denotations explained in Tables 1 and 2

Kucharski (2001), although varied, and was usually negative, resembling the results obtained in this paper. The authors' own research as well as that by Iwanow et al. (1994) showed that *Azotobacter* sp. was not tolerant to soil contamination with diesel oil. The growth of the other microorganisms could be explained by the fact that many species of microorganisms are capable of degrading refinery products. The following genera of bacteria decompose carbohydrates most efficiently: *Achromobacter*, *Actinetobacter*, *Arthrobacter*, *Flavobacterium*, *Nocardia*, *Flavobacterium*, *Pseudomonas*, *Acetobacter*, *Xantomonas*, *Bacillus*, *Micrococcus*, *Corynebacterium* (Bieszkiewicz et al. 1998, Tong et al. 1998).

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ABSTRAKT

Vliv kontaminace motorovou naftou na půdní mikroorganismy a růst ovsa

V nádobovém pokusu jsme sledovali vliv motorové nafty aplikované v množství 0; 2,4; 4,8 a 7,2 ml.kg^{-1} půdy na výnos ovsa a na počty oligotrofních a eutrofních bakterií, bakterií působících imobilizaci dusíku, amonifikaci, celulolytických bakterií a bakterií *Azotobacter* sp., a dále na počty aktinomycet a hub. K detoxikaci půdy jsme použili inokulaci sporami *Streptomyces intermedius*. Pokus jsme prováděli na eutrické kambizemi vyvinuté na lehké jílovitopísčité půdě. Zjistili jsme, že motorová nafta má negativní vliv na růst a vývoj ovsa. Inokulace neoslabila reakci ovsa na kontaminaci půdy motorovou naftou, ale pozitivně ovlivnila oligotrofní a eutrofní bakterie i *Azotobacter* sp., bakterie působící imobilizaci dusíku a houby. Bez ohledu na to, zda byla půda osetá či neosetá, a na inokulaci sporami *S. intermedius*, motorová nafta měla stimulační účinek na oligotrofní a eutrofní bakterie, bakterie působící imobilizaci dusíku a na aktinomycety. Osetí půdy mělo pozitivní účinek na mikrobiologické vlastnosti půdy, protože byl pozitivně ovlivněn početní vztah mezi oligotrofními bakteriemi a aktinomycetami na jedné straně a houbami na straně druhé. Tento kladný účinek byl však oslaben působením motorové nafty.

Klíčová slova: motorová nafta; výnos ovsa; počet mikroorganismů

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