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Original Research

Correlation Between the Number of Cultivatable Microorganisms and Soil Contamination with Diesel Oil

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Abstract

The effect of diesel oil contamination on the number of soil microrflora in light clay sand and light clay was determined in a pot experiment. The experimental soil was contaminated with the following doses of diesel oil (ON) calculated as maximum water capacity (MWC): 0; 0.5; 1; 1.5; 2; 2.5 and 3%. The lowest dose of diesel oil (0.5% MWC) for the lighter soil was 1.67 g · kg^{-1} d.m. and for heavier soil it was 1.71 g · kg^{-1} d.m. Varied urea fertilization also was applied: 0 and 250 mg N g · kg^{-1} d.m. of soil. For the initial 18 days, the pots were maintained unsown. On day 18, the Juno variety of yellow lupine was planted (7 plants per pot). The yellow lupine plants were harvested at the blooming phase. Soil samples were taken on day 18 and immediately after yellow lupine harvest.

Based on the results, soil contamination with 0.5% to 3.0% MWC of diesel oil was found to disturb the soil microbiological balance. This substance stimulated the development of oligotrophic, copiotrophic, sporulating copiotrophic and Actinomycetales and inhibited the development of *Azotobacter* spp. and cellulolytic bacteria. Fertilisation with urea had a positive effect on the multiplication of the above microorganisms. The number of oligotrophic, copiotrophic bacteria and Actinomycetales was higher in the light clay, whereas the number of sporulating oligotrophic, sporulating copiotrophic and cellulolytic bacteria and fungi was greater in light clay sand soil. Yellow lupine cultivation had a positive effect on the multiplication of sporulating oligotrophic, copiotrophic and cellulolytic bacteria and fungi in both analyzed types of soil. Hydrolytic acidity and organic carbon content were positively correlated, whereas pH, total exchangeable cations and alkaline cation soil saturation were negatively correlated with soil contamination with diesel oil.

Keywords: soil contamination, diesel oil, number of microorganisms, soil physicochemical properties

Introduction

Everyday petroleum derivative products are introduced into the environment and contaminate smaller or larger areas. All these areas should be restored to normal utilization. Increasingly, biological methods are being applied to the decontamination of soil. The bioremedial capacity of hydrocarbons is determined by chain length, number of branches, and the number of aromatic rings. Aliphatic hydrocarbons with small number of carbon atoms in a molecule are relatively easily degradable [1], whereas aromatic, long-chained aliphatic and cyclic hydrocarbons are difficult to break down [2]. These compounds occur in soil in varied forms: floating on the surface of soil solution, water-dissolved hydrocarbons, residual contaminants adsorbed on solid soil particles and gaseous [3, 4].

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Table 1. Some physicochemical properties of the soils used in the experiment.

Type (kind) of soil	Granulometric composition			C [g kg^{-1}]	pH_{KCl}	Hh	S
	1.0-0.1	0.1-0.02	<0.02			[mmol(+) kg^{-1} of soil]	
Proper brown soil, light clay sand	54	33	13	6.2	6.3	10.8	65.5
Proper brown soil, light clay	62	12	26	7.6	6.5	12.4	81.5

C – organic carbon content, Hh – hydrolytic acidity, S – total alkaline exchangeable cations

According to Leahy and Colwell [5], petroleum-derived hydrocarbons are susceptible to microbiological degradation in the following order: n-alkanes > branched alkanes > low-molecular aromatic compounds > cyclic alkanes.

Petroleum-derived compound degradation is determined according to physical and chemical properties of the compounds included in the contaminant, hydrocarbon concentration and their toxicity to microorganisms [6], content of nitrogen, phosphorus and potassium [7, 8, 9], temperature, oxygen content, humidity, pH [10] and biological factors such as quantitative and qualitative composition of microorganisms and enzyme activity [5, 11]. Granulometric composition of the soil under bioremedy is also of great importance because it determines the development of microorganisms [12, 13].

The aim of the study was to determine the correlations between the number of soil microorganisms and soil contamination with diesel oil.

Methods

The experiment was carried out in a vegetation hall in plastic pots in four replications. The experimental soil was typical brown formed from light clay sand and typical brown formed from light clay. More detailed characteristics of the experimental soil are given in Table 1. An adequate amount of soil sampled from plough-humus stratum was mixed with mineral fertilisers and transferred to the pots (3.2 kg per pot). Identical fertilization was applied to the entire experiment and was: P - 75 (KH_2PO_4); K - 140 (KH_2PO_4 + KCl); Mg - 40 ($MgSO_4 \cdot 7H_2O$); Zn - 5 ($ZnCl_2$); Cu - 5 ($CuSO_4 \cdot 5H_2O$); Mn - 5 ($MnCl_2 \cdot 4H_2O$); Mo - 5 ($Na_2MoO_4 \cdot 2H_2O$); B - 0,33 (H_3BO_3), mg $\cdot$ kg^{-1} of soil as pure component. Only nitrogen fertilization varied: 0 and 250 mg N $\cdot$ kg^{-1}. Nitrogen was applied as $CO(NH_2)_2$.

In some pots, the soil was contaminated with diesel oil (ON) in the following amounts expressed as maximum water capacity (MWC): 0, 0.5, 1, 1.5, 2, 2.5 and 3%. The lowest dose of diesel oil (0.5% MWC) for the lighter soil was 1.67 g $\cdot$ kg^{-1} d.m. and for the heavier soil it was 1.71 g $\cdot$ kg^{-1} d.m. The diesel oil was characterised by content of water – 220 mg $\cdot$ kg^{-1}, solid contaminants – 24 mg $\cdot$ kg^{-1}, sulphur – 0.5 mg $\cdot$ kg^{-1}, density (15°C) – 860 kg $\cdot$ m^3, viscosity (40°C) – 4.5 mm^2 $\cdot$ s^{-1} [14].

The experiment was carried out for 58 days. Throughout the initial 18 days, the pots were kept unsown. On day 18, soil samples were taken for microbiological and physico-chemical analyses as well as Juno variety yellow lupine was sown (7 plants per pot). Yellow lupine was harvested at the blooming phase. The length of vegetation period was 40 days. On day 40 soil was sampled for analyses. Constant soil humidity at 60% of capillary water capacity was maintained throughout the entire experiment (58 days).

Microbiological analyses were performed on the day of soil sampling and included the determination of the following microorganisms with the use of the platelet method: oligotrophic (Olig) and copiotrophic (Cop) as well as their sporulating forms (Cop_p, $Olig_p$) on peptone and meat extract medium according to Ont and Hattory [15], *Azotobacter* spp. with the Fenglerowa method [16], cellulolytic bacteria (Cel) on Winogradski's medium [17], actinomycetes (Act) with the Küster and Williams method with antibiotics: nysthatin and actidion, according to the description presented by Parkinson et al. [18] and fungi (Fun) on glucose and peptone agar according to Martin [19]. Sporulating oligotrophic and copiotrophic bacteria were determined in the soil pasteurized at 85°C for 15 minutes.

Physicochemical analyses included hydrolytic acidity (Hh) and total alkaline exchangeable cations (S) with Kappen's method [20]. Based on the above results, total exchangeable capacity (T) and alkaline cation (V) saturation were calculated with the following formulas: T= S + Hh; V = S $\cdot$ T^{-1} $\cdot$ 100.

Nitrogen fertilization and date of analysis (on the day of planting and after plant harvest) did not significantly modify the soil physicochemical properties and therefore this paper does not include these results and the physicochemical properties values were gives as mean values.

All laboratory analyses were carried out in three replications. The results were statistically analyzed with ANOVA variance analysis. Additionally, regression equations and coefficients of determination between soil contamination with diesel oil and the number of microorganisms were calculated. Nitrogen fertilization action and term of analysis was missed in the regression analysis. Calculation of regression equations curried out only for two factors: soil type and soil contamination with diesel oil. The effect of nitrogen fertilization and term of analysis on the number of microorganisms was interpreted on the base of ANOVA variance analysis. Pearson's simple correlation coefficients between the number of microorganisms and soil physicochemical properties were calculated based on all replications from microbiological and physicochemical analyses [21].

Results

Based on the results, the effect of diesel oil on the number of soil microorganisms was found to be determined by the soil granulometric composition, contamination, urea fertilization and contamination lingering period (Tables 2, 3). Diesel oil stimulated the multiplication of oligotrophic, copiotrophic, sporulating copiotrophic and actinomycetes and inhibited *Azotobacter* spp. and cellulolytic bacteria (Table 2), regardless of soil type or nitrogen fertilization. However, its effect on the development of fungi was closely related to nitrogen fertilization. In urea-free pots, diesel oil contamination of two types of soil had a significant effect on a decrease in the fungal population ($r = -0.94$ in light clay sand and $r = -0.95$ in light clay), whereas in urea-fertilized pots the contamination stimulated the growth of fungi ($r = 0.80$ in light clay sand and $r = 0.90$ in light clay).

Urea fertilization both modified the direction of diesel oil effect on fungi and strengthened its stimulating effect on oligotrophic and copitrophic bacteria as well as actinomycetes. Urea fertilization stimulated the multiplication of oligotrophic and copitrophic bacteria as well as actinomycetes and fungi, but inhibited the development of cellulolytic bacteria and *Azotobacter* spp., regardless of soil contamination with diesel oil. Urea fertilization had an ambiguous effect on sporulating bacteria. In the lighter soil it decreased the number of sporulating oligotrophic bacteria, whereas in the more compact soil it had a stimulating effect on these bacteria. In contrast, urea fertilization had a negative effect on sporulating copiotrophic bacteria in the more compact soil but in the

Table 2. Effect of diesel oil and nitrogen fertilization on the number of microorganisms in 1 kg d.m. of soil sampled from yellow lupine cultivation.

DO dose, in % MWC	Bacteria							
	oligotrophic x 10^9		oligotrophic sporulating x 10^7		copiotrophic x 10^9		copiotrophic sporulating x 10^7	
	-N	+N	-N	+N	-N	+N	-N	+N
Light clay sand								
0	13.85	20.64	65.15	52.41	22.49	38.21	29.77	30.04
0.5	20.58	23.54	71.51	65.60	24.14	53.55	35.56	40.05
1.0	34.71	30.47	74.54	58.26	36.74	70.18	41.63	40.48
1.5	35.14	49.26	68.92	54.37	42.22	76.27	42.72	41.58
2.0	37.46	68.18	58.83	55.58	45.99	88.42	44.99	41.01
2.5	38.68	70.25	54.47	54.72	48.47	91.65	50.89	60.16
3.0	42.14	77.65	47.30	47.58	50.67	92.46	47.83	47.47
Average	31.79	48.57	62.96	55.50	38.67	72.96	41.91	42.97
Light clay								
0	20.19	23.24	32.43	28.82	23.70	45.83	29.25	32.49
0.5	20.47	53.41	39.32	40.94	40.66	80.83	30.75	36.78
1.0	30.23	56.87	42.30	56.95	42.32	93.38	37.07	37.75
1.5	41.88	56.00	41.07	41.96	50.51	96.51	41.55	38.76
2.0	56.61	60.75	41.81	43.48	44.13	90.02	45.91	33.24
2.5	42.49	63.13	33.50	49.56	40.24	79.93	41.14	29.73
3.0	45.78	69.67	32.63	47.80	32.62	86.97	40.09	29.25
Average	36.80	54.72	37.58	44.22	39.17	81.93	37.97	34.00
LSD	a – 3.68** b – 2.49** c – 2.49** axb – 5.21** axc – 5.21* bxc – 3.52** axbxc – 7.37**		a – 2.92** b – 1.98** c – n.s. axb – 4.13** axc – 4.13** bxc – 2.79** axbxc – 5.85**		a – 3.70** b – 2.50** c – 2.50** axb – 5.23** axc – 5.23** bxc – 3.53** axbxc – 7.41*		a – 2.91** b – 1.97** c – 1.97** axb – 4.12** axc – 4.12** bxc – 2.79** axbxc – 5.83**	

Table 2 continues on next page...

DO dose, in % MWC	*Azotobacter* spp. x 10^3		Cellulolytic bacteria x 10^6		Actinomycetes x 10^9		Fungi x 10^6	
	-N	+N	-N	+N	-N	+N	-N	+N
Light clay sand								
0	6.46	3.96	48.61	44.27	7.86	6.92	46.84	49.33
0.5	4.25	1.30	48.65	41.10	14.84	10.94	37.79	118.89
1.0	4.65	1.48	49.02	37.20	14.33	12.25	36.21	129.09
1.5	2.03	1.50	49.57	36.05	14.02	14.80	34.90	130.08
2.0	1.65	0.91	52.41	35.91	10.51	17.61	34.81	144.33
2.5	1.85	0.94	45.88	33.09	10.45	24.72	27.84	144.66
3.0	1.87	0.36	43.06	31.02	8.67	25.22	26.38	143.99
Average	3.25	1.49	48.17	36.95	11.52	16.07	34.96	122.91
Light clay								
0	3.74	2.49	44.84	32.84	11.64	8.04	40.82	37.88
0.5	3.37	2.01	35.95	28.90	15.71	16.48	35.51	39.46
1.0	3.31	1.88	36.08	21.53	15.86	18.34	28.14	38.37
1.5	3.34	2.25	30.97	25.11	15.93	18.62	24.80	47.64
2.0	3.03	2.04	28.71	21.78	15.30	17.52	20.06	53.10
2.5	1.76	1.81	26.75	20.61	18.36	19.26	18.99	48.04
3.0	2.00	1.25	25.48	19.61	18.96	22.10	19.63	63.53
Average	2.93	1.96	32.68	24.34	15.97	17.19	26.85	46.86
LSD	a – 0.45** b – n.s. c – 0.30** axb – 0.63** axc – 0.63** bxc – 0.43** axbxc – 0.90*		a – 1.94** b – 1.31** c – 1.31** axb – 2.75** axc – n.s. bxc –1.86 ** axbxc – 3.89**		a – 1.24** b – 0.84** c – 0.84** axb – 1.75* axc – 1.75** bxc – 1.18** axbxc – 2.48**		a – 7.00** b – 4.73** c – 4.73** axb – 9.91** axc – 9.91** bxc – 6.70** axbxc – 14.01**	

LSD for: a – diesel oil dose (DO), b – kind of soil, c – N fertilization, n.s. – non-significant, *significant for p=0.05; **significant for p=0.01

lighter soil it did not have a significant effect on these bacteria.

This positive effect of urea fertilization on the multiplication of the majority of the microorganisms in the soil contaminated with diesel oil can result from an improved C:N ratio. This is confirmed, among others, by an increased content of organic carbon in the contaminated soil (Table 4). According to Margesin and Schinner [8], each additional nitrogen pool has a cushioning effect on these changes, which in turn determine the biodegradation of soil contaminated with petroleum-derived compounds because in such conditions, the assimilable nitrogen deficit can limit the development of microorganisms.

The period of diesel oil lingering in soil and yellow lupine cultivation were important factors determining the number of microorganisms (Table 3). In both analyzed soil types, yellow lupine cultivation had a positive effect on the multiplication of sporulating oligotrophic, copiotrophic and cellulolytic bacteria as well as fungi. In the case of the other microorganisms, the cultivation of this plant did not have an explicit character and their number was more closely related to soil type than to yellow lupine cultivation. The increase in the number of microorganisms in the soil analyzed after yellow lupine harvest could have resulted from the positive effect of both the plant root secretions on the development of microorganisms as well as the positive effect of root systems on soil aeration.

Soil type was a significant factor that modified the number of microorganisms (Fig. 1). The number of oligotrophic and copiotrophic bacteria as well as actinomycetes was significantly higher in light clay than in light clay sand. However, the number of sporulating oligotrophic, sporulating copiotrophic and cellulolytic bacteria as well as fungi obtained was reverse and was higher in the lighter soil. Microbial community response to increasing soil contamination with diesel oil was generally similar in both cases.

The effect of diesel oil on the development of particular microorganism groups was both direct and indirect. The former was exhibited either by a stimulating or in-

Table 3. Effect of diesel oil and analysis date on the number of microorganisms in 1 kg d.m. of soil sampled from yellow lupine cultivation.

DO dose, in % MWC	Bacteria							
	oligotrophic x 10^9		oligotrophic sporulating x 10^7		copiotrophic x 10^9		copiotrophic sporulating x 10^7	
	before sowing	after sowing	before sowing	after sowing	before sowing	after sowing	before sowing	after sowing
Light clay sand								
0	20.14	14.35	5.16	112.40	17.94	42.75	38.03	21.78
0.5	26.07	18.03	7.07	130.03	34.51	43.18	48.95	26.65
1.0	26.90	38.27	7.61	125.19	39.85	67.06	55.13	26.97
1.5	30.97	53.42	7.95	115.33	50.95	67.54	55.84	28.45
2.0	54.59	51.06	9.67	104.73	52.79	81.60	53.56	32.44
2.5	57.35	51.58	11.01	98.17	65.76	74.35	50.88	60.16
3.0	71.41	48.37	10.66	84.23	69.64	73.49	51.44	43.86
Average	41.06	39.30	8.45	110.01	47.35	64.28	50.55	34.33
Light clay								
0	25.20	18.23	7.19	54.06	30.97	38.55	24.88	36.86
0.5	43.57	30.30	12.96	67.30	63.05	58.45	26.27	41.26
1.0	51.79	35.30	14.75	84.50	63.07	72.63	32.04	42.77
1.5	46.92	50.95	14.76	68.27	55.29	91.73	34.89	45.43
2.0	44.76	72.59	18.71	66.58	47.70	86.44	35.30	43.85
2.5	46.78	58.85	24.17	58.89	40.63	79.54	33.57	37.30
3.0	54.64	60.81	24.26	56.18	35.95	83.64	31.26	38.08
Average	44.81	46.72	16.69	65.11	48.09	73.00	31.17	40.79
LSD	a – 3.68** b – 2.49** c – 2.49** axb – 5.21** axc – 5.21** bxc – 3.52** axbxc – 7.37**		a – 2.92** b – 1.98** c – 1.98** axb – 4.13** axc – 4.13** bxc – 2.79** axbxc – 5.85**		a – 3.70** b – 2.50** c – 2.50** axb – 5.23** axc – 5.23** bxc – 3.53** axbxc – 7.39**		a – 2.91** b – 1.97** c – 1.97** axb – 4.12** axc – 4.12** bxc – 2.79** axbxc – 5.83**	
DO dose, in % MWC	*Azotobacter* spp. x 10^3		Cellulolytic bacteria x 10^6		Actinomycetes x 10^9		Fungi x 10^6	
	before sowing	after sowing	before sowing	after sowing	before sowing	after sowing	before sowing	after sowing
Light clay sand								
0	4.25	6.18	51.30	41.58	5.43	9.36	26.10	70.06
0.5	4.64	0.91	44.46	45.27	6.58	19.20	66.94	89.74
1.0	5.76	0.37	43.07	43.15	10.43	16.15	58.23	107.07
1.5	3.33	0.19	41.40	44.22	13.27	15.55	54.23	110.74
2.0	2.38	0.18	38.72	49.59	15.79	12.33	52.27	126.85
2.5	2.79	0.00	34.24	44.72	23.39	11.78	51.07	121.42
3.0	2.22	0.00	30.37	43.70	24.60	9.28	46.40	123.96
Average	3.62	1.12	40.51	44.60	14.21	13.38	50.75	107.12

Table 3 continues on next page...

Light clay								
0	2.43	3.80	38.04	39.64	5.70	13.97	16.93	61.77
0.5	2.32	3.07	33.67	31.16	15.90	16.28	19.57	55.40
1.0	2.55	2.63	32.03	25.59	17.89	16.31	20.21	46.30
1.5	3.20	2.38	25.81	30.26	19.49	15.06	17.61	54.83
2.0	3.37	1.68	23.29	27.19	18.86	13.96	17.40	55.76
2.5	3.18	0.39	22.46	24.90	24.43	13.19	16.24	50.79
3.0	3.25	0.00	21.33	23.75	28.33	12.73	15.16	68.01
Average	2.90	1.99	28.09	28.93	18.66	14.50	17.59	56.12
LSD	a – 0.45** b – n.s. d – 0.30** axb – 0.63** axd – 0.63** bxd – 0.43** axbxd – 0.89**		a – 1.94** b – 1.31** c – 1.31** axb – 2.75** axc – 2.75** bxc – 1.86** axbxc – 3.89**		a – 1.24** b – 0.84** c – 0.84** axb – n.s. axc – 1.75** bxc – 1.18** axbxc – 2.48**		a – 7.00** b – 4.73** c – 4.73** axb – 9.91** axc – 9.91** bxc – 6.70** axbxc – 14.01**	

LSD for: a – diesel oil dose (DO), b – kind of soil, c – use of soil, n.s. – non-significant, *significant for p=0.05; **significant for p=0.01

Table 4. Effect of diesel oil on some properties of soil.

DO dose, in % MWC	pH (1 mol KCl·dm^{-3})	Hh	S	T	V %	C g · kg^{-1} gleby
		mmol(+) · kg^{-1} gleby				
Light clay sand						
0	6.45	10.41	62.75	73.16	85.78	6.69
0.5	6.41	10.59	62.25	72.84	85.46	7.61
1.0	6.40	11.25	62.38	73.63	84.72	8.03
1.5	6.38	11.44	62.00	73.44	84.43	8.53
2.0	6.35	11.91	62.00	73.91	83.89	8.66
2.5	6.33	12.66	60.63	73.28	82.73	8.87
3.0	6.28	12.94	60.13	73.06	82.29	9.56
Average	6.37	11.60	61.73	73.33	84.18	8.28
Light clay						
0	6.61	12.00	79.38	91.38	86.87	8.19
0.5	6.55	12.09	78.63	90.72	86.67	8.78
1.0	6.43	12.28	78.25	90.53	86.43	9.68
1.5	6.33	12.47	77.50	89.97	86.14	10.05
2.0	6.33	13.22	77.50	90.72	85.43	10.89
2.5	6.36	13.13	76.88	90.00	85.42	11.21
3.0	6.25	13.59	75.63	89.22	84.76	11.72
Average	6.41	12.68	77.68	90.36	85.96	10.07
LSD a b a x b	 0.03** 0.02** 0.04**	 0.20** 0.14** 0.29**	 0.74** 0.50** n.s.	 n.s. 0.49** n.s.	 0.29** 0.20** 0.41**	 0.02** 0.01** 0.03**

r – correlation coefficient LSD for: a – diesel oil dose (DO), b – kind of soil, n.s. – non-significant, *significant for p=0.05; **significant for p=0,01; Hh – hydrolytic acidity, S – total alkaline exchangeable cations, T – total exchangeable capacity, V – alkaline cation saturation, C - organic carbon content

habiting effect of this petroleum-derived compound on the development of the particular microorganisms and the latter was exhibited through the modification of soil physicochemical properties, particularly the content of organic carbon and acidity (Table 4). The content of carbon in the soil increased with increasing soil contamination with diesel oil. At the same time, soil hydrolytic acidity increased and pH decreased. This obviously affected the alkaline cation saturation of soil. The above correlations are confirmed by Pearson's simple correlation coefficients as well as previous studies [22, 23]. It is interesting that in both types of soil (light clay sand and light clay) the number of the majority of microorganisms was highly significantly or significantly correlated with the soil basic physico-chemical properties (Table 5).

Based on the results, the number of microorganisms was modified by yellow lupine cultivation, urea fertilization, a period of diesel oil lingering in the soil as well as likely by specific and mutual effect of the native microflora living in soil contaminated with diesel oil (Table 5). Such conclusions

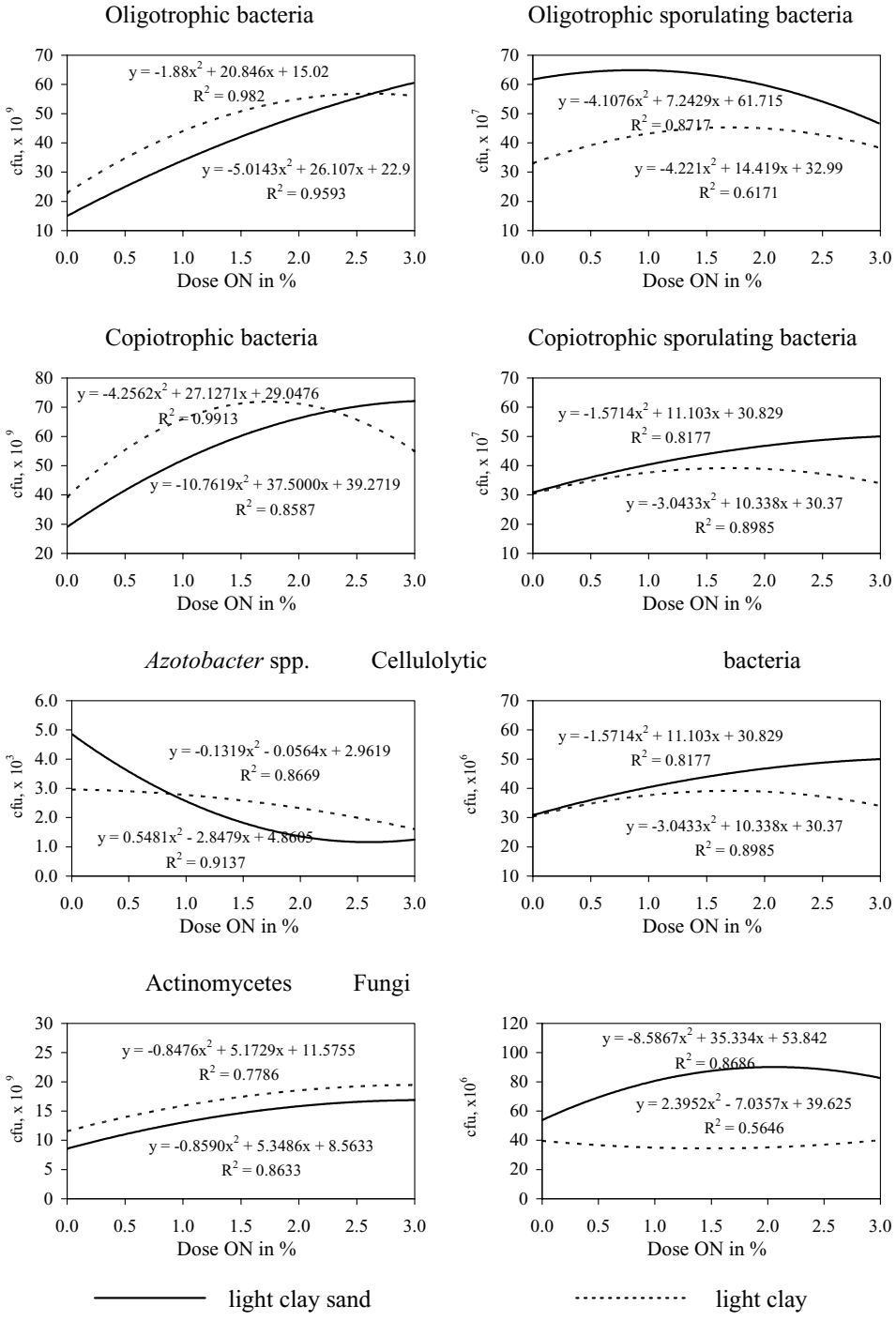

Fig. 1. Correlation between soil contamination with diesel oil and an average number of soil microorganisms.

Table 5. Pearson's simple correlation coefficients between variable factors.

Variable	Olig	$Olig_p$	Cop	Cop_p	Az	Cel	Act	Fun
Light clay sand								
Olig	1.00	-0.52**	0.10	1.00	0.17	-0.16	-0.20	0.13
$Olig_p$	-0.52**	1.00	0.24**	-0.52**	-0.45**	0.23*	0.03	0.32**
Cop	0.10	0.24*	1.00	0.10	-0.62**	-0.50**	0.35**	0.69**
Cop_p	1.00	-0.52**	0.10	1.00	0.17	-0.16	-0.20	0.13
Az	0.17	-0.45**	-0.62**	0.17	1.00	0.14	-0.31**	-0.41**
Cel	-0.16	0.23*	-0.50**	-0.16	0.14	1.00	-0.46**	-0.39**
Act	-0.20	0.03	0.35**	-0.20	-0.31**	-0.46**	1.00	-0.01
Fun	0.13	0.32**	0.69**	0.13	-0.41**	-0.39**	-0.01	1.00
pH	-0.61**	0.75**	-0.14	-0.61**	-0.17	0.42**	-0.12	-0.04
Hh	0.30**	-0.11	0.56**	0.30**	-0.52**	-0.22*	0.14	0.28**
S	0.32**	-0.82**	-0.37**	0.32**	0.55**	-0.18	-0.01	-0.38**
T	0.44**	-0.90**	-0.19	0.44**	0.39**	-0.26*	0.04	-0.30**
V	-0.10	-0.26*	-0.59**	-0.10	0.64**	0.09	-0.10	-0.38**
C	0.29**	0.15	0.63**	0.29**	-0.47**	-0.36**	0.28**	0.43**
Light clay								
Olig	1.00	0.52**	0.22*	1.00**	-0.06	0.06	-0.14	0.30**
$Olig_p$	0.52**	1.00	0.45**	0.52**	-0.26*	-0.09	-0.14	0.61**
Cop	0.22*	0.45**	1.00	0.22*	-0.52**	-0.28**	-0.09	0.67**
Cop_p	1.00**	0.52**	0.22*	1.00	-0.06	0.06	-0.14	0.30**
Az	-0.06	-0.26*	-0.52**	-0.06	1.00	0.27**	0.25*	-0.28**
Cel	0.06	-0.09	-0.28**	0.06	0.27**	1.00	-0.43**	0.07
Act	-0.14	-0.14	-0.09	-0.14	0.25*	-0.43**	1.00	-0.25*
Fun	0.30**	0.61**	0.67**	0.30**	-0.28**	0.07	-0.25*	1.00
pH	0.29**	0.56**	0.04	0.29**	-0.06	0.49**	-0.62**	0.43**
Hh	-0.16	-0.11	0.10	-0.16	-0.29**	-0.59**	0.34**	-0.18
S	-0.39**	-0.68**	-0.60**	-0.39**	0.49**	0.15	0.13	-0.66**
T	-0.43**	-0.71**	-0.58**	-0.43**	0.44**	0.03	0.20	-0.70**
V	-0.12	-0.32**	-0.44**	-0.12	0.52**	0.51**	-0.16	-0.27**
C	0.16	-0.03	0.19	0.16	-0.26*	-0.42**	0.44**	0.07

Olig – oligotrophic bacteria, $Olig_p$ – oligotrophic sporulating bacteria, Cop – copiotrophic bacteria, Cop_p - copiotrophic sporulating bacteria, Az – *Azotobacter* spp., Cel – cellulolytic bacteria, Act – actinomycetes, Fun – fungi, Hh – hydrolytic acidity, S – total alkaline exchangeable cations, T – total exchangeable capacity, V – alkaline cation saturation, C - organic carbon content; Correlation coefficient for p=0.05; **significant for p=0.01; n = 84

were drawn from the calculated Pearson's simple correlation coefficients between the number of individual microorganism groups in typical brown soil formed from light clay sand and typical brown soil formed from light clay.

The correlations between soil contamination with diesel oil and the number of microorganisms observed in this experiment agree with those previously obtained by the authors [11, 22, 23] as well as by Galas et al. [24], Łebkowska et al. [25] and Margesin and Schinner [8]. An increase in the number of oligotrophic and copiotrophic bacteria as well as actinomycetes can probably be explained by the large population of species exhibiting

the ability to utilise petroleum hydrocarbons as a carbon source. The bacteria most active in petroleum-derived compounds degradation include: *Achromobacter, Acinetobacter, Flavobacterium, Nocardia, Flavobacterium, Arthrobacter*, *Pseudomonas, Acetobacter, Xantomonas*, *Bacillus*, *Micrococcus*, *Corynebacterium* [5, 26, 27, 28]. Also, fungi representing: *Aspergillus, Penicillium*, *Pichia, Torulopsis, Cladosporium*, *Trichoderma, Fusarium*, *Candida, Rhododorula* exhibit the ability to degrade petroleum-derived compounds [5, 24, 29].

Conclusions

1. Soil contamination with diesel oil doses from 0.5% to 3.0% MWC disturbed the soil microbiological balance. This substance stimulated the growth of oligotrophic, copiotrophic and sporulating copiotrophic bacteria as well as actinomycetes, but inhibited *Azotobacter* spp. and celulolitic bacteria. Urea fertilization strengthened the positive effect of the contamination on the above-mentioned microorganisms.
2. The number of oligotrophic and copiotrophic bacteria as well as actinomycetes was greater in the light clay soil, whereas the number of sporulating oligotrophic, sporulating copiotrophic and cellulolytic bacteria as well as fungi was greater in the light clay sand.
3. Yellow lupine cultivation had a positive effect on the multiplication of sporulating oligotrophic, copiotrophic and cellulolytic bacteria as well as fungi in both analyzed soils.
4. Hydrolytic acidity and organic carbon content were positively correlated, whereas pH, total exchangeable cations and alkaline cation saturation were negatively correlated with soil contamination with diesel.

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