Acta Agrophysica, 2001, 51, 113-120

MICROBIOLOGICAL PROPERTIES OF SOIL CONTAMINATED WITH DIESEL OIL

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A b s t r a c t. In the pot experiment the effects of contamination of diesel oil of proper brown soil developed from light loamy sand on microorganisms abundance and activity of soil enzymes were studied. Diesel oil was applied at the following rates: 0, 2, 4 and 6 cm^3 kg^{-1} of the soil. Trial was performed in two experimental series: amended or not amended with straw.

It was found that soil pollution with oil stimulated development of *Azotobacer* sp., oligotrophic, copiotrophic and ammonifiers bacteria; whereas inhibited fungi proliferation, dehydrogenases and urease activities and did not affect actinomycetes number. Diesel oil addition to the soil reduced positive effects of straw on microbiological and biochemical soil properties. Oil pollution adversely affected growth and development of triticale. Straw amendment diminished level of oil toxicity to triticale.

K e y w o r d s: diesel oil, microorganisms, enzymatic activity, soil.

INTRODUCTION

It was reported that soil contaminated with petroleum-derivative compounds including diesel oil, modify its physical, chemical as well as biological properties [2,4,9,10,12,13,16]. Oil may penetrate to the deeper layers of the soil what cause clodding and worsening of all soil properties and finally causes reduction of soil fertility.

Reduced oxygen availability caused negative changes in balance between groups of soil microorganisms. There are conditions which favour microorganisms which are resistant to limited oxygen access and capable to degrade hydrocarbons [1,12,16]. The direction of modifications of soil microflora by oil is determined by the level of contamination. It was also dependent on the kind of the soil [4].

MATERIALS AND METHODS

Experiment was performed in the cold greenhouse in plastic pots in which 2.5 kg of proper brown soil developed from light loamy sand of pH 6.5 (1 M KCl) was placed. Soil was thoroughly mixed with the following rates of macronutrients (in g kg^{-1}): N - 0.15 [$CO(NH_2)_2$], P - 0.1 (KH_2PO_4), K - 0.15 (KH_2PO_4 + KCl), Mg - 0.05 ($MgSO_4 \cdot 7H_2O$) and with diesel oil at the following rates: (in cm^3 kg^{-1} of the soil): 0; 2; 4 and 6. Experiment was carried out in two experimental series with or without barley straw amendment (2.4 g straw kg^{-1} of the soil). Four replications were applied. Tested plant was triticale cv. Gabo (25 plants in pot) harvested at heading. During the whole period of the experiment the moisture level of 60% of maximal water capacity was maintained. After triticale harvesting, soil samples were collected and then following microbial analyses were made: number of oligotrophic (Olig) and copiotrophic bacteria (Cop) - in medium with pepton and meat broth [14], ammonifiers (Am) and immobilizing nitrogen bacteria (Im) - in Winogradski's medium [18], *Azotobacter* sp. (Az) - Fenglerowa's method, [5], actinomycetes (Act) - in Küster and William's medium with actidion and nystatine [15] and fungi (Fun) - in peptono-gluco agar according to Martin's method [11]. Activity of the following soils enzymes were determined: soil dehydrogenases according to Lenhard's method modified by Casidy *et al.* [3]; urease - Gorin and Ching Chang's method [6]; acid and basic phosphatases - according to Tabatabai and Bremner's method [17]. Also following soil parameters were determined: carbon content - Tiurin's method, hydrolytic acidity (Hh) and sum of base cations (S) - Kappen's method [8]. Sorption complex capacity (T) and degree of base saturation (V) also were enumerated.

RESULTS AND DISCUSSION

Contamination of the soil with diesel oil modified soil microorganisms abundance (Table 1). Under the effect of diesel oil, the number of *Azotobacter* sp. and oligotrophic bacteria increased, irrespectively of the other experimental factors (application of straw and plant cover). Similar positive effects of diesel oil on proliferation of ammonifiers and copiotrophic bacteria were observed, excluding straw amended soil left without plant cover. In soil amended with barley straw diesel oil contamination showed adverse effects on the number of nitrogen immobilizing bacteria which could be attributed to deficient of nitrate nitrogen. The number of fungi was decreased irrespectively of the rate of applied diesel oil and in the soil

Table 1. Number of microorganisms (cfu) in 1 g of soil dry matter

Oil rate (cm^3 kg^{-1} soil)	Olig* 10^6	Cop 10^6	Im 10^6	Az	Am 10^6	Act 10^6	Fun 10^3
			Bare soil				
0	6.87	2.32	2.32	0.39	1.54	5.52	8.45
2	10.61	6.17	1.21	3.78	2.68	4.95	2.08
4	24.20	9.21	2.10	1.17	3.81	4.39	1.75
6	21.81	12.41	1.66	1.93	3.81	2.67	2.75
			Soil with triticale				
0	10.40	3.83	4.52	5.41	3.83	4.02	6.57
2	7.91	8.67	4.30	5.52	16.91	4.07	6.80
4	25.01	15.68	13.34	10.11	18.20	4.12	5.95
6	21.61	9.03	9.54	100.86	16.04	3.23	5.76
			Bare soil + straw				
0	11.87	9.17	6.94	2.38	5.99	13.85	13.25
2	26.57	9.01	6.45	6.99	5.52	9.40	4.04
4	23.95	7.46	2.91	3.14	3.81	7.34	5.34
6	21.38	9.07	2.65	0.00	5.03	8.63	1.07
			Soil with triticale + straw				
0	16.40	9.06	11.15	1.52	7.95	10.31	14.04
2	32.41	14.17	3.26	2.69	13.71	12.23	4.76
4	30.77	17.36	3.48	1.53	19.46	14.14	4.93
6	23.33	18.46	4.70	88.25	20.85	9.75	6.51
LSD	4.05	2.63	2.32	1.27	4.75	1.65	0.98

*For explanations see "Materials and Methods".

contaminated with the highest rate of the oil (6 cm^3 1 kg^{-1} of the soil) - also the reduction of actinomycetes number was noted. Similar effects of petroleum derivatives on soil actinomycetes and fungi were found by Michalcewicz [12]. However, Borowiec *et al.* [2] reported only effects of oil on actinomycetes number.

Abundance of microorganisms was determined not only by the level of soil contamination but also by the others experimental factors. In soil not amended with straw triticale stimulated proliferation of the following bacteria: oligotrophic, copiotrophic, nitrogen immobilizing, ammonifiers and *Azotobacter* sp. and inhibition of actinomycetes and fungi was observed (Table 2). In the straw amended pots similar effects were noted for oligotrophic, copiotrophic bacteria, nitrogen immobilizing bacteria and ammonifiers. Diesel oil modified ratios of bacteria numbers in soil where triticale was grown to respective number from bare soil. Generally these values increased excluding oligotrophic bacteria, in this case soil contamination reduced the ratio.

Table 2. Ratios of microorganisms numbers between bare soil to soil with triticale

Oil rate ($cm^3\ kg^{-1}$ soil)	Olig 10^6	Cop 10^6	Im 10^6	Az	Am 10^6	Act 10^6	Fun 10^3
			Soil not amended with straw				
0	1.51	1.65	1.95	13.87	2.49	0.73	0.78
2	0.75	1.41	3.55	1.46	6.31	0.82	3.27
4	1.03	1.70	6.35	8.64	4.78	0.94	3.40
6	0.99	0.73	5.75	52.26	4.21	1.21	2.09
			Soil straw amended				
0	1.38	0.99	1.61	0.64	1.33	0.74	1.06
2	1.22	1.57	0.51	0.38	2.48	1.30	1.18
4	1.28	2.33	1.20	0.49	5.11	1.93	0.92
6	1.09	2.04	1.77	88.25	4.15	1.13	6.08

As it was observed for triticale also straw amendment positively affect number of all studied microorganisms, and the only exemption was *Azotobacter* (Table 3). However, the beneficial effects of straw on microorganisms number was diminished by oil contamination.

Table 3. Ratios of microorganisms number between straw amended soil and not amended with the straw

Oil rate ($cm^3\ kg^{-1}$ soil)	Olig 10^6	Cop 10^6	Im 10^6	Az	Am 10^6	Act 10^6	Fun 10^3
0	1.64	2.96	2.64	0.67	2.60	2.53	1.82
2	3.18	1.56	1.76	1.04	0.98	2.40	0.99
4	1.11	1.00	0.41	0.41	1.06	2.52	1.33
6	1.03	1.28	0.66	0.86	1.30	3.12	0.89

Soil contamination by diesel oil affected soil enzymatic activity in other way than the number of microorganisms (Table 4). In bare soil not amended with straw stimulation of the following enzymes activities was noted: dehydrogenases, urease and phosphatases. In soil amended with straw effects of contamination were similar, excluding acid phosphatase which activity was significantly reduced. In the soil with triticale and amended with straw diesel oil adversely affected dehydrogenases, urease and acid phosphatases but in treatment without straw amendment similarly as in other studies [10], negative influence of oil contamination on soil dehydrogenases and urease, whereas positive effect on phosphatases activity was observed.

Table 4. Enzymatic activity per 1 g of soil dry matter

Oil rate ($cm^3\ kg^{-1}$ soil)	Dehydrogenases ($mm^3\ H_2$)	Urease (μg hydrolized urea $1\ h^{-1}$)	Phosphatase (μmol p-nitrophenol)	
			acid	basic
		Bare soil		
0	1.19	51.02	1.37	0.91
2	0.96	34.73	1.20	0.96
4	1.77	65.90	1.51	1.44
6	3.22	136.75	1.60	1.77
		Soil with triticale		
0	3.37	165.54	1.28	1.34
2	2.09	135.39	1.37	1.65
4	2.14	119.54	1.36	1.54
6	2.04	112.96	1.22	1.54
		Bare soil + straw		
0	1.06	75.30	1.87	1.12
2	1.41	51.38	1.65	1.26
4	1.84	97.08	1.47	1.50
6	1.43	145.69	1.44	1.49
		Soil with triticale + straw		
0	3.68	229.63	1.60	2.00
2	3.19	214.08	1.43	1.98
4	2.86	199.91	1.38	2.04
6	2.53	143.92	1.38	1.95
LSD	0.08	1.8	0.03	0.05

Growing of triticale favourably affected biochemical properties of the soil (Table 5). The increase of the activities of all studied enzymes excluding acid phosphatase was found. The positive influence of triticale root system on soil enzymatic activity was inhibited by the contamination by diesel oil, especially in the treatment with the highest oil rate. Straw addition also increased soil enzymatic activity and in this case oil contamination, irrespectively of pollutant rate, reduced straw beneficial effect which was manifested as an increase of phosphatases activity. Oil at the rate of 6 $cm^3\ kg^{-1}$ of the soil inhibited also straw beneficial influence also on activities of dehydrogenases and urease (Table 6). However, favourable influence of straw amendment on microbiological and biochemical soil properties was limited by oil contamination. Comparison of triticale yield obtained from soil amended and not amended with straw, indicates that toxic effects of oil was diminished by straw amendment (Table 7). It may be explained by better development of microbial communities and

Table 5. Ratios of enzymatic activities between soil with triticale to bare soil

Oil rate (cm^3 kg^{-1} soil)	Dehydrogenases (mm^3 H_2)	Urease (μg hydrolized urea 1 h^{-1})	Phosphatase (μmol p-nitrophenol)	
			acid	basic
Soil not amended with straw				
0	2.83	3.24	0.93	1.47
2	2.18	3.90	1.14	1.72
4	1.21	1.81	0.90	1.07
6	0.63	0.83	0.76	0.87
Soil straw amended				
0	3.47	3.05	0.86	1.79
2	2.26	4.17	0.87	1.57
4	1.55	2.06	0.94	1.36
6	1.77	0.99	0.96	1.31

Table 6. Ratios of enzymatic activities between soil amended and not amended with the straw

Oil rate (cm^3 kg^{-1} soil)	Dehydrogenases (mm^3 H_2)	Urease (μg hydrolized urea 1 h^{-1})	Phosphatase (μmol p-nitrophenol)	
			acid	basic
Soil without straw amendement				
0	1.04	1.41	1.31	1.39
2	1.51	1.56	1.20	1.24
4	1.20	1.60	0.99	1.19
6	0.75	1.16	1.00	1.04

Table 7. Triticale yield (g of dry matter pot^{-1})

Oil rate (cm^3 kg^{-1} soil)	Soil without straw amendment	Soil amended with straw	Mean
0	25.62	21.47	23.55
2	12.25	15.85	14.05
4	6.35	10.14	8.25
6	2.85	6.34	4.60
Mean	11.77	13.45	12.61
LSD*	a = 1.25 b = 0.92 a x b = 1.85		

* a - rate of diesel oil; b - straw amendment; a x b - interaction.

more intense oil biodegradation, because analysed physicochemical properties: hydrolytic acidity, base saturation and sorption complex saturation of the soil were close in soil amended and not amended with straw (Table 8). Organic carbon content was

Table 8. Some physicochemical properties of the soil

Oil rate ($cm^3\ kg^{-1}$ soil)	C (%)	Hh	S	T	V (%)
		me/100 g soil			
Soil without straw amendment					
0	0.68	1.05	8.65	9.70	89.18
2	0.88	1.28	8.40	9.68	86.82
4	0.84	1.36	8.75	10.11	86.53
6	0.83	1.36	8.85	10.21	86.66
Soil amended with straw					
0	0.82	1.19	8.20	9.39	87.35
2	1.01	1.14	9.00	10.14	88.78
4	0.84	1.35	8.90	10.25	86.83
6	1.08	1.45	8.15	9.60	84.90

differentiated and diesel oil contamination increased hydrolytic acidity of the soil, irrespectively of straw application.

Response of triticale to soil contamination was similar to response to dehydrogenases and yield is positively and significantly correlated to activity of this enzyme [7].

CONCLUSIONS

1. Soil contamination by diesel oil stimulated *Azotobacer* sp., oligotrophic, copiotrophic and ammonifiers bacteria but inhibited fungi proliferation and did not affect number of actinomycetes.

2. Activities of soil dehydrogenases and urease in soil contaminated by diesel oil are considerably lower than in uncontaminated soil and is highly correlated to triticale yield.

3. Soil contamination by diesel oil adversely affected growth and development of triticale. Negative effects of diesel oil might be diminished by straw amendment.

4. Diesel oil reduced positive effects of straw on biochemical and microbiological properties of the soil. It contributed also to soil acidification.

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