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Correlation between Number of Microbes and Degree of Soil Contamination by Petrol

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Abstract

The aim of this research was to examine the correlation between the presence of microbes in soil and the degree of soil pollution with unleaded and 98 leaded petrol. Detoxication of soils contaminated by refinery products was performed using barley straw. The experiment was conducted in two series: on unsown soil and on soil sown with triticale.

The results of the study have proved that contamination of soil by petroleum compounds stimulates the growth of *Azotobacter* sp., copiotrophic and oligotrophic bacteria, but inhibits the multiplication of fungi and actinomycetes. Fertilisation of soil with barley straw and cultivation of triticale improved microbiological properties of soil. The favourable effect of the treatments was attenuated by leaded and unleaded petrol.

Keywords: leaded and unleaded petrol, number of microbes, organic substance

Introduction

Refinery products, which continually pollute the natural environment, are toxic to micro-organisms, causing changes in their quantitative and qualitative composition. As a result, the biological balance of soil is disturbed

One of the methods used for detoxicating soils contaminated by petroleum products consists of using micro-organisms. Efficiency of biodegradation relies on the adaptability of microbes which use petroleum substances as a source of energy and carbon for living in contaminated soils. Riis et al. [2] have discovered that autochthonous micro-organisms present in contaminated soils are more efficient in degrading petroleum compounds than microbes existing in non-contaminated soils. Provided the right concentrations of carbon, oxygen and nitrogen compounds exist, surface layers of soil can contain from 10 million to 1 billion bacteria per one gram of soil, of which 0.1 to 1.0% bacteria are estimated to be capable of degrading petroleum substances [3, 4].

To what extent these micro-organisms can degrade carbohydrates derived from petroleum depends on their individual characteristics and environmental factors [5].

Although biodegradation of petroleum contaminants may occur both in aerobic and anaerobic conditions, the process is known to proceed more slowly in anaerobic conditions (Bossert and Artha as well as Conney cited in [5])-

The objective of our study was to determine the correlation between the occurrence of micro-organisms in soil and degree of soil contamination with unleaded and 98 leaded petrol. Detoxication of soils polluted by petroleum products was carried out using barley straw.

Methods

The experiments were conducted in plastic pots kept in a greenhouse (in 4 replications). The pots were filled with 2.5 kg typical brown soil from light clay sand [organic carbon content (C) - 0.75%; pH in 1 M KC1 6.5; hydrolytic acidity (Hh) - 1.16 mmol · 100 g^{-1} soil; total exchange bases (S) - 14.1 mmol · 100 g^{-1} soil; sorptive complex capacity (T) - 15.26 mmol · 100 g^{-1} soil; base saturation of soil (V) - 92.4%)].

Prior to filling pots with soil, the following fertilisation treatment was applied in g · kg^{-1} soil (calculated as a pure

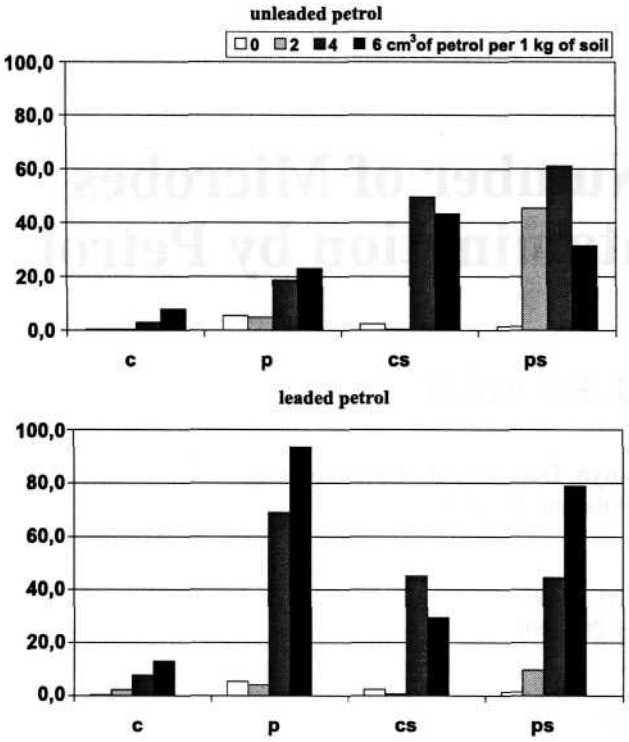


Fig. 1. Number of *Azotobacter* sp. (cfu) in 1 g d.m. of soil, c - unsown soil, not fertilised with straw p - sown soil, not fertilised with straw cs - unsown soil, fertlised with straw ps - sown soil, fertlised with straw

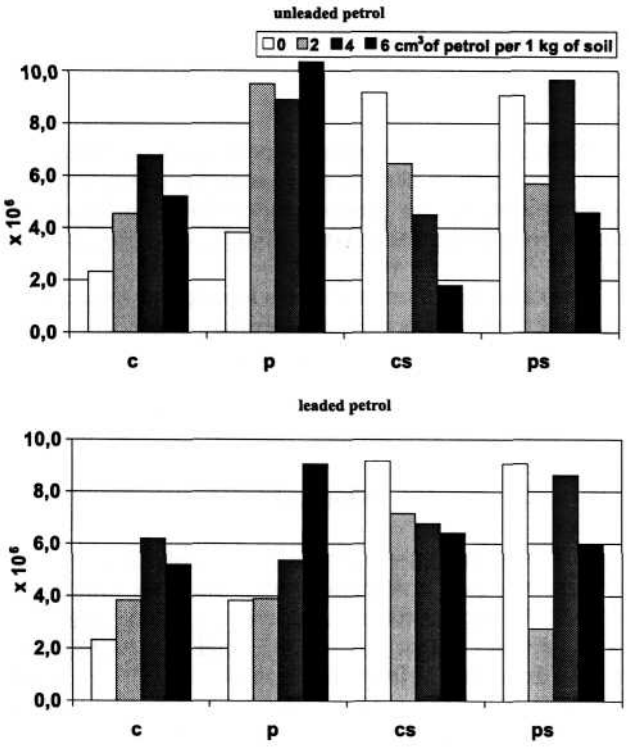


Fig. 2. Number of copiotrophic bacteria (cfu) in 1 g d.m. of soil. * Legend as in Fig. 1.

component): N - 0.15 [$CO(NH_2)_2$]; P - 0.1 [K_2HPO_4]; K - 0.15 [K_2HPO_4 + KCl]; Mg - 0.05 [$MgSO_4$-$7H_2O$]. Mineral fertilisation and unleaded and leaded petrol at 0, 2, 4 and 6 $cm^3 \cdot kg^{-1}$ was applied in a single treatment before the experiment was established. The tests were conducted in two series: without fertilisation and with fertilisation using 2.4 g of finely ground barley straw per kg^{-1} soil. The study was performed simultaneously on unsown soil and soil sown with triticale. The crop tested in the course of the experiments was cv. Gabo triticale (25 plants per pot). During vegetation of triticale plants (49 days) moisture was maintained at 60% of capillary water holding capacity. Soil samples were taken at harvest (triticale in the phase of heading), after which bacteria was counted in sown and unsown soil with the help of the plate method: oligotrophic (Olig) and copiotrophic (Cop) bacteria on a medium with peptone and meat extract according to Onta and Hattori [6], *Actinomycetales* (Act) by the method of Kuster Williams and two antibiotics: nystatin and actidione according to the procedure described by Parkinson et al. [7], fungi (Fun) - on glucose and peptone agar according to Martin [8] and *Azotobacter* sp. with Fenglerowa's method [9], nitrogen immobilizing (Im) and ammonifiers (Am) micro-organisms - on Winogradski's [10] medium.

The pH of the soil was determined using the potentiometric method; hydrolytic acidity and total exchange bases were tested according to Kappen method. The values of hydrolytic acidity and total exchange bases were used to calculate the sorptive complex capacity and base saturation of the soils. The analyses were performed in compliance with the methods quoted by Litynski et al. [11].

All the results were statistically analyzed with the help of Duncan's test.

Presentation and Discussion of the Results

Soil fertility assessment based exclusively on chemical and pedological methods often fails to generate satisfying results due to soil being a specific organism in which micro-organisms and the enzymes they produce catalyse transformations of organic and mineral soil components. Multiplication rate of microbes is related to the content of available compounds of carbon and nitrogen. The following could be good indicators of the biological activity of soil: the number of micro-organisms, their enzymatic activity, the rate of biochemical reactions and content of metabolites produced by microbes [12]. In our experiment the effect of refinery products on micro-organisms was correlated to the type of these products and degree of soil contamination (Figs. 1-8). The presence of leaded or unleaded petrol, regardless of the use of soil (unsown or sown with triticale) or barley straw fertilisation, resulted in an increase in the number of *Azotobacter* sp. (Fig. 1). The number of these bacteria was positively correlated with the degree of soil contamination by refinery products. The highest dose of unleaded and leaded petrol (6 $cm^3 \cdot kg^{-1}$soil) caused the number of *Azotobacter* sp. cells to increase 4 and 17 times in the soil sown with triticale and, correspondingly, 19 and 34 times in the unsown soil. Organic fertilisation also produced some

results. In the soil not sown with triticale barley straw fertilisation attenuated the negative effects of contamination with petrol, although it did not stimulate the growth of *Azotobacter* sp. in the control objects. Similar correlations were observed in the unsown soil fertilised with straw, but only when contaminated with unleaded petrol.

The chemical compounds investigated also had a stimulating influence on the multiplication of copiotrophic bacteria (Fig. 2), but only in the soil not fertilised with straw, irrespective of whether or not it was sown. On the one hand, application of organic matter significantly increased the number of copiotrophic bacteria in the objects not contaminated by petroleum products. On the other hand, in the contaminated unsown soil a negative correlation was noticed between increasing contamination degrees and the multiplication of these bacteria. Unleaded petrol turned out to be particularly toxic. Similar correlations occurred in the soil sown with triticale and fertilised with barley straw, although they were less regular and obvious. Oligotrophic bacteria were more resistant to soil pollution by refinery products (Fig. 3) compared to copiotrophic bacteria. Their response to these contaminants in the unsown soil, regardless of organic fertilisation treatments, was positive. The number of oligotrophic bacteria in the unsown soil remained on a relatively constant level and was only slightly dependent on the contamination of soil by refinery products.

The response of ammonifiers bacteria (Fig. 4) to soil contamination with petrol was correlated to the type of petrol, its dose, straw fertilisation and use of soil. Leaded petrol stimulated the growth of ammonifiers bacteria in the soil fertilised with straw and sown with triticale; the higher the degree of contamination, the stronger the stimulating effect. The number of these bacteria was significantly lower in the soil not fertilised with straw, in which it was not correlated with the degree of soil contamination. Unleaded petrol stimulated the multiplication of ammonifiers bacteria only in the soil sown with triticale, but it had to be added at either 2 or 4 $cm^3 \cdot kg^{-1}$ of soil. The higher dose drastically reduced the number of these bacteria. The negative influence of unleaded petrol on the multiplication of ammonifiers bacteria was seen in the unsown soil fertilised with straw, which may have been due to nitrogen deficiency. Both petrol and straw can be used by micro-organisms as a source of carbon, which may lead to deficiency in the amount of nitrogen. This assumption seems to have been confirmed by the results of our analyses on nitrogen immobilizing bacteria (Fig. 5), the number of which decreased in the objects fertilised with straw in response to the presence of either type of petrol, independent of the way the soil was used. No such effect was observed in the soil enriched with organic matter; on the contrary, the presence of petrol in such soil tended to have a stimulating influence on the bacteria.

Leaded petrol had an unquestionably negative effect on the number of fungi (Fig. 6), regardless of the degree of soil pollution, soil use or organic matter fertilisation. A similar response of fungi to contamination by unleaded petrol was recorded only in the control object not fertilised with straw and not sown with triticale. No such unfavourable effect of unleaded petrol on fungi was observed in the soil of the other objects. The number of

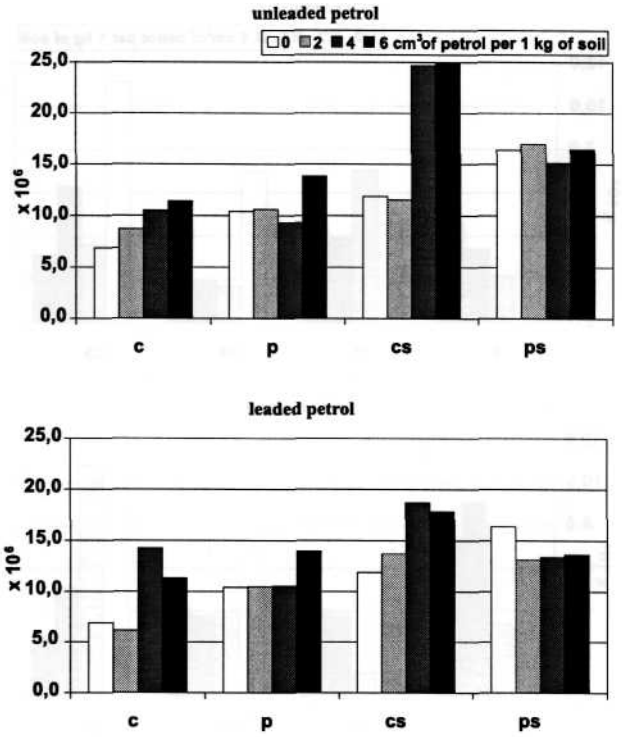
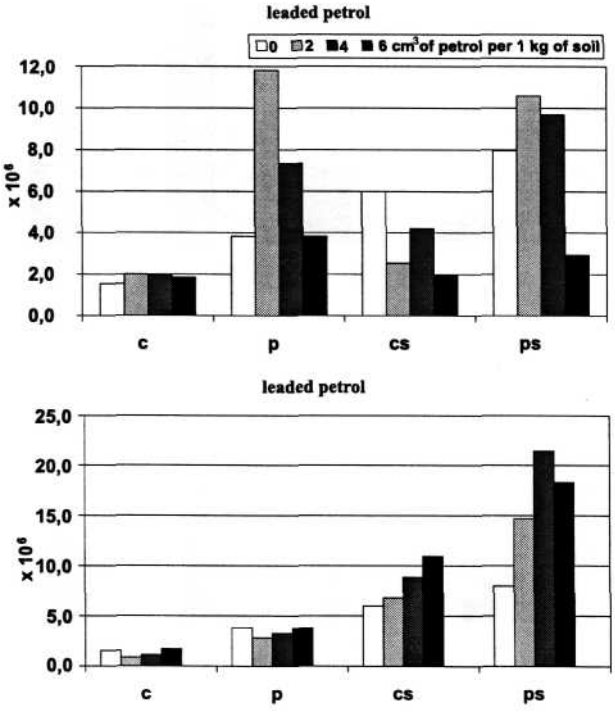


Fig. 3. Number of oligotrophic bacteria (cfu) in 1 g d.m. of soil. * Legend as in Fig. 1.

Fig. 4. Number of ammonifiers bacteria (cfu) in 1 g d.m. of soil. * Legend as in Fig. 1.



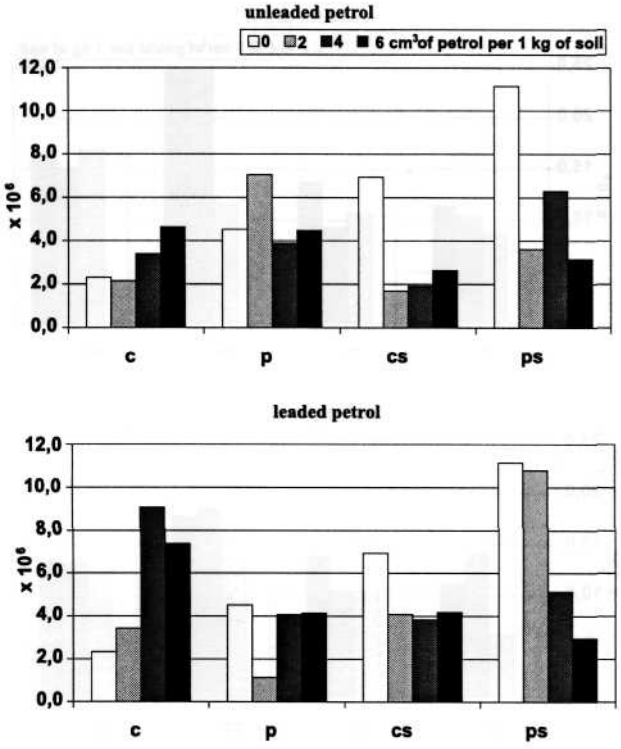


Fig. 5. Number of nitrogen immobilizing bacteria (cfu) in 1 g d.m. of soil.
* Legend as in Fig 1

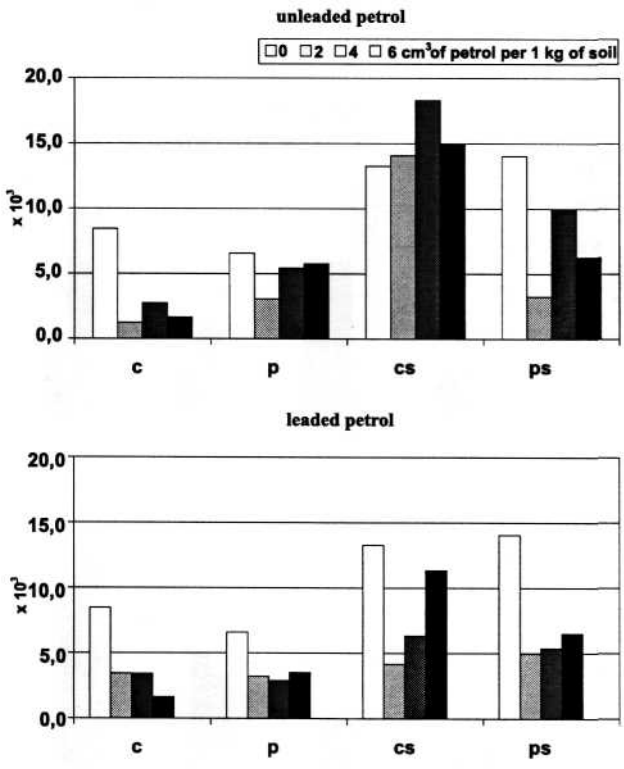


Fig. 6. Number of fungi (cfu) in 1 g d.m. of soil
* Legend as in Fig. 1.

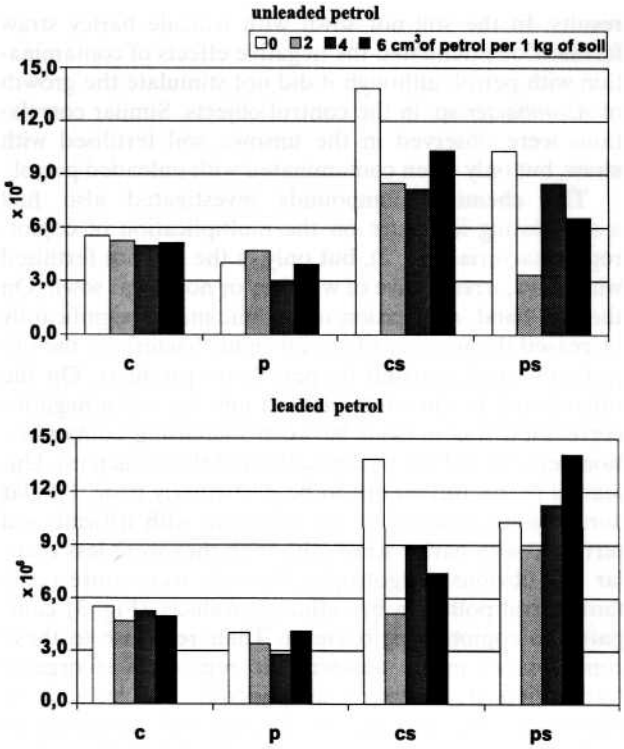


Fig. 7. Number of actinomycetes (cfu) in 1 g d.m. of soil.
* Legend as in Fig. 1.

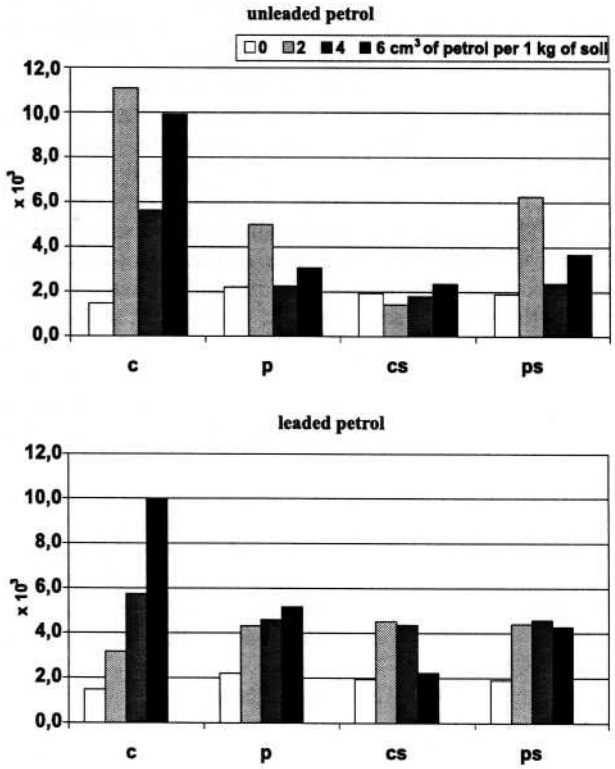


Fig. 8. Ratio of oligotrophic bacteria and actinomycetes to fungi $(\frac{Olig + Act}{Fun})$ in 1 g d.m. of soil.
* Legend as in Fig. 1.

Table 1. Some physical and chemical properties of soil sown with triticale.

Dose of petrol ($cm^3 \cdot kg^{-1}$ soil)	Hh mmol · 100 g^{-1} of soil		S mmol · 100 g^{-1} of soil		T mmol · 100 g^{-1} of soil		Vs %	
	-Pb	+Pb	-Pb	+Pb	-Pb	+Pb	-Pb	+Pb
soil not fertilized with straw								
0	1.05	1.05	8.65	8.65	9.70	9.70	89.18	89.18
2	1.05	1.10	8.80	8.60	9.85	9.70	89.34	88.66
4	1.19	1.01	8.52	8.40	9.71	9.41	87.00	89.24
6	1.01	1.10	8.25	8.10	9.26	9.20	89.09	88.04
$\bar{x}$	1.08	1.07	8.41	8.44	9.49	9.50	88.67	88.79
soil fertilized with straw								
0	1.19	1.19	8.20	8.20	9.39	9.39	89.18	87.35
2	1.05	1.19	8.45	8.55	9.50	9.74	89.34	87.80
4	1.49	1.31	7.75	8.35	9.24	9.66	86.98	86.42
6	1.63	1.84	7.55	7.70	9.18	9.54	89.09	80.73
$\bar{x}$	1.34	1.38	7.99	8.20	9.33	9.58	88.67	85.58
LSD*	a – 0.02; b – 0.02; c – 0.01; a x b – 0.03; a x c – 0.02; b x c – 0.02; a x b x c – 0.04		a – 0.16; b – 0.19; c – 0.13; a x b – 0.33; a x c – 0.23; b x c – 0.04; a x b x c – 0.46;		a – 0.15; b – 0.017; c – 0.12; a x b – 0.30; a x c – 0.21; b x c – 0.07 a x b x c – 0.42;		a – 0.79; b – 0.91; c – 0.65; a x b – 1.58; a x c – 1.12; b x c – 1.29; a x b x c – 2.24;	

* a - type of contamination, b - dose of the compound, c - organic substance supplement.
-Pb - unleaded petrol; +Pb - leaded petrol
axb, a x e , b x c and a x b x c - LSD of factors interaction.

actinomycetes (Fig. 7) was mainly stimulated by straw fertilisation, and it was in the straw fertilised objects that modifications in their numbers occurred. Unleaded petrol caused a decline in the number of actionycetes, whereas leaded petrol decreased their number in the control soil and unsown soil, but in the soil sown with triticale it resulted in an increase in the number of these micro-organisms. In the soil not fertilised with straw the number of actinomycetes remained on a relatively stable low level and was not modified by the contamination of soil with refinery products.

The ratio of the number of bacteria and actinomycetes versus the number of fungi expresses the microbiological properties of soil more fully than the number of each group considered separately [12]. Higher values of this ratio are indicative of a stronger growth of bacteria and actinomycetes but a weaker development of fungi, an observation which was confirmed by the results of our experiments (Fig. 8). It is commonly believed that the higher the ratio, the more fertile the soil. This may be true about unpolluted soils, but if the biological balance of soil is disturbed by the anthropogenic factor, drawing any conclusions on the fertility of soil from the ratio between the total number of bacteria and actinomycetes to fungi has an illusory value and, as such, is absolutely useless. Leaded petrol raised the value of the ratio, even though it had a negative effect on the yielding of triticale [13]. Unleaded petrol produced a similar, albeit less obvious, effect on the mutual relations between the bacteria, actinomycetes, and fungi.

The influence of refinery products on the development of particular groups of micro-organisms may be either direct or indirect. Direct influence lies in the stimulating or inhibiting effect of the components of petroleum products on the development of micro-organisms, while indirect influence is produced as a result of changes in physical and chemical properties of soil, including soil seration. Neither type of petrol, leaded or unleaded, changed significantly all the properties of soil. Hydrolytic acidity (Tab. 1), for instance, altered only in response to the highest doses of petrol (4 and 6 $cm^3 \cdot kg^{-1}$ of soil) in conjunction with straw fertilisation. A change in the total exchange base cations in the sorptive complex was also observed in such objects. This, however, had hardly any effect on the sorptive complex capacity and degree of base saturation.

Straw fertilisation did not deteriorate the physical properties of soil, stimulating at the same time the development of most of the micro-organisms (Tab. 2). Nevertheless, it affected negatively the multiplication of the *Azotobacter* sp. bacteria. Straw was found to have a stimulating effect on the growth of copiotrophic and nitrogen immobilising bacteria as well as actinomycetes. The stimulation was, however, attenuated by leaded or unleaded petrol. Straw fertilisation applied to contaminated soil stimulated the multiplication of *Azotobacter* sp. and fungi, and in the objects polluted by leaded petrol it also stimulated ammonifiers bacteria and inhibited oligotrophic bacteria. Unleaded petrol influenced ammonifiers bacteria in a similar manner to leaded petrol, affecting oligotrophic bacteria.

Apart from soil fertilisation with straw, another factor

Table 2. Ratio of the number of microbes in straw fertilised soil to unfertilised soil.

Dose of petrol ($cm^3 \cdot kg^{-1}$ soil)	Olig		Cop		Im		Az		Am		Act		Fun	
	-Pb	+Pb	-Pb	+Pb	-Pb	+Pb	-Pb	+Pb	-Pb	+Pb	-Pb	+Pb	-Pb	+Pb
0	1.64	1.64	2.96	2.96	2.64	2.64	0.67	0.67	2.60	2.60	2.53	2.53	1.82	1.82
2	1.48	1.61	0.87	1.28	0.58	3.27	8.66	1.62	0.95	5.88	1.20	1.72	4.01	1.38
4	2.01	1.29	0.90	1.33	1.13	0.68	5.09	1.17	1.50	6.87	2.07	2.49	3.44	1.86
6	1.63	1.25	0.41	0.87	0.64	0.62	2.42	1.02	0.87	5.34	1.86	2.36	2.84	3.48

Olig - oligotrophic bacteria; Cop - copiotrophic bacteria, Im - immobilizing nitrogen bacteria; Az -*Azotobacter* sp.; Am - ammonifiers bacteria; Act - actinomyces; Fun - fungi -Pb - unleaded petrol; +Pb - leaded petrol

Table 3. Ratio of the number of microbes in soil sown with triticale to unsown soil.

Dose of petrol ($cm^3 \cdot kg^{-1}$ soil)	Olig		Cop		Im		Az		Am		Act		Fun	
	-Pb	+Pb	-Pb	+Pb	-Pb	+Pb	-Pb	+Pb	-Pb	+Pb	-Pb	+Pb	-Pb	+Pb
soil not fertilized with straw														
0	1.51	1.51	1.65	1.65	1.95	1.95	13.87	13.87	2.49	2.49	0.73	0.73	0.78	0.78
2	1.22	1.70	2.09	1.02	3.26	0.33	13.30	1.81	5.88	3.29	0.89	0.73	2.44	0.94
4	0.88	0.74	1.31	0.87	1.14	0.45	6.08	8.83	3.79	2.90	0.61	0.53	1.97	0.85
6	1.21	1.23	1.98	1.74	0.97	0.56	2.90	7.13	2.05	2.20	0.76	0.83	3.47	2.14
soil fertilized with straw														
0	1.38	1.38	0.99	0.99	1.61	1.61	0.64	0.64	1.33	1.33	0.74	0.74	1.06	1.06
2	1.47	0.96	0.88	0.38	2.14	2.64	113.50	12.61	4.19	2.17	0.40	1.71	0.23	1.19
4	0.61	0.71	2.13	1.27	3.28	1.34	1.23	0.99	2.30	2.42	1.04	1.26	0.54	0.85
6	0.66	0.76	2.54	0.93	1.20	0.71	0.73	2.67	1.47	1.68	0.63	1.91	0.42	0.57

Legend as in Tab. 2.

which stimulated multiplication of micro-organisms in soil was triticale cultivation (Tab. 3), except actinomycetes irrespective of fertilisation, fungi in soil not fertilised with straw and *Azotobacter* in fertilised soil. Contamination of soil by petrol inhibited the stimulating effect of secretions produced by roots of triticale plants on the growth of oligotrophic and nitrogen immobilizing bacteria. The effect of soil being polluted with petrol on the other groups of microbes was less explicit and differed from object to object, depending on the straw fertilisation treatment and type of petrol.

It is claimed that those micro-organisms which use refinery products as sources of carbon and energy play an important role in the degradation of petroleum compounds. This assumptions finds confirmation in our own studies as well as those of other authors [4,14,15,16,17]. The negative influence of refinery products on fungi and actinomycetes was determined by Michalcewicz *et al.* [17], and Kucharski and Wyszkowska [18]. In his other experiments, Michalcewicz [12] discovered differences in the response of fungi to the fungistatic activity of diesel oil. Two strains of fungi were most strongly affected by the refinery product: *Fusarium oxysporum* and *Bottytis cinerea,* followed by *Paecilomyces liliancinus.* The effect of refinery products on the growth of *Azotobacter* sp. is not clear. Our results seem to coincide with the experiments reported by Bieszkiewicz *et al.* [5], but Iwanow *et al.* [20] found *Azotobacter* sp. and nitrifying bacteria to be highly vulnerable to soil contamination by petrol.

Conclusions

1. Contamination of soil by refinery products stimu lated the growth of *Azotobacter* sp., copiotrophic and oligotrophic bacteria, but inhibited the multiplication of fungi and actinomycetes.

2. Soil fertilisation with barley straw and cultivation of triticale affected favourably the microbiological proper ties of soil. This positive effect was attenuated by the presence of leaded and unleaded petrol.

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