

# Effects of Heating Oil on the Count of Microorganisms and Physico-Chemical Properties of Soil

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## Abstract

The aim of our experiment was to determine the effect of heating oil application on the count of microorganisms and some physico-chemical properties of limed and lime-free soil and soil sown with yellow lupine of the Markiz variety and unsown soil.

The results obtained indicate that heating oil deteriorated the physico-chemical properties of the experimental soil (acidification, decrease in the total alkaline exchangeable cations, soil exchange capacity and alkaline cation soil saturation). At the same time, it stimulated, to a different degree, the multiplication of soil microorganisms, especially oligotrophic bacteria (10-fold) and bacteria representing the genera of *Pseudomonas* spp. (8-fold). Lime application and cultivation of yellow lupine had a positive effect on the physico-chemical properties of soil and stimulated the growth of *Azotobacter* spp.

**Keywords:** microorganisms count, heating oil, physico-chemical properties, soil contamination

## Introduction

Heating oil is an energy source used mainly for heating apartments, household facilities and greenhouses [1]. Its transportation by surface, rail or pipeline poses a pollution threat [2, 3, 4].

Heating oil consists mainly of hydrocarbons such as alkanes, cycloalkanes and aromatic hydrocarbons produced during petroleum distillation at 340-370°C. According to the Directive of the Ministry of Finance from 19 December 2001 [5], heating oil must be labelled with a permanent marker and coloured red. N-ethyl-N-[2-(1-isobutoxyetoxy)ethyl]asobenzene-4-amine is used as the marker and is applied at the minimum of 4.6 mg · dm<sup>-3</sup> of the labelled product, while Solvent Red 164 or 19 is used as the colour and is applied at a minimum of 6.3 mg dm<sup>-3</sup> of the labelled product.

Petroleum-derived substances reaching the soil modify its physico-chemical properties. They also affect the biological life of the soil, including the activity of soil

microorganisms as well as the growth and development of plants [6].

The effect of petroleum-derived substances on microorganisms can be positive or negative depending on their systemic classification, ability to use hydrocarbons as an energy substrate as well as sensitivity to toxic substances. Among the microorganisms living in the soil are many bacteria, actinomycetes and fungi, some of which are able to degrade petroleum-derived substances [7, 8] and others very sensitive to these substances. The latter group includes nitrification bacteria [2, 9]. Wyszowska and Kucharski [10] and Kucharski and Wyszowska [11] found a stimulating effect of petroleum-derived substances on copiotrophic, ammonification, oligotrophic bacteria and *Azotobacter* spp.

Hydrocarbons have an unequivocal negative effect on plants. These substances form a thin layer around seeds, limiting access of air and water which, in consequence, impairs their germination [12]. In plants, they reduce the permeability of cellular membranes and disturb the course of the metabolic processes in the cells which inhibits plant growth and development [13].

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On the other hand, plants are used for the bioremediation of soil contaminated with petroleum derived substances or other toxic substances such as heavy metals or pesticides [14, 15, 16, 17]. Plant roots accumulate contaminants and excrete a wide variety of metabolites that stimulate the development of microflora which are active in degradation of substances which have a negative effect on biological life in the soil. They are also the source of enzyme-degrading contaminants [15, 16].

The following factors determine the rate of petroleum-derived hydrocarbons in the soil: temperature, oxygen content, humidity and soil pH [8, 18]. In order to intensify the degradation of petroleum-derived substances, lime is added to contaminated soil [19]. Previous studies have shown that this has a positive effect on the soil structure [20], physico-chemical properties [19] and soil respiration as exhibited by the intense development of soil microorganisms [21].

The aim of the study was to determine the effect of soil contamination with heating oil on the soil physico-chemical properties and the count of microorganisms in limed, lime-free, sown with yellow lupine and unsown soils.

### Experimental Procedures

This pot experiment was carried out in a vegetation hall in four replications. The experimental soil collected from the humus horizon (0-20 cm) was characterized by the granulometric composition of light clayey sand,  $\text{pH}_{\text{KCl}} - 5.6$  and total content of hydrogen ions of  $13.9 \text{ mmol}(+) \cdot \text{kg}^{-1} \text{ d.m.}$  of soil. The 170 mm high and 160 mm diameter plastic pots contained 3.2 kg of soil fertilized with the following minerals: macroelements expressed as pure component ( $\text{g} \cdot \text{kg}^{-1}$  of soil) - N - 0.20 ( $\text{CO}(\text{NH}_2)_2$ ), P - 0.10 ( $\text{KH}_2\text{PO}_4$ ), K - 0.15 ( $\text{KH}_2\text{PO}_4 + \text{KCl}$ ) and Mg - 0.05 ( $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ ) and microelements ( $\text{mg} \cdot \text{kg}^{-1}$  of soil): Zn - 5.0 ( $\text{ZnCl}_2$ ), Cu - 5.0 ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ), Mn - 5.0 ( $\text{MnCl}_2 \cdot 5\text{H}_2\text{O}$ ), Mo - 5.0 ( $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ ), B - 0.33 ( $\text{H}_3\text{BO}_3$ ). Before transferring into the pots, the soil was also supplemented with heating oil at doses expressed in percentage of soil mass (0.0; 0.25; 0.5; 0.75; 1.0; 1.5) and CaO in the amount of  $0.39 \text{ g} \cdot \text{kg}^{-1}$  of soil. The experiment was carried out in the spring (April, May and June) and lasted for 72 days. The average daily temperature of these months was  $7.8^\circ\text{C}$  (from  $-1.2$  to  $13.0^\circ\text{C}$ ),  $16.2^\circ\text{C}$  (from  $9.8$  to  $21.8^\circ\text{C}$ ) and  $16.1^\circ\text{C}$  (from  $11.3$  to  $23.0^\circ\text{C}$ ), respectively. Throughout the entire experimental period, a constant soil humidity at 60% of capillary water capacity was maintained (using distilled water). After 14 days, the pots were divided into two groups: sown and unsown. The first group was sown with the seeds of yellow lupine Markiz variety (5 plants per pot), immunized with Nitragine (microbiological vaccine containing *Bradyrhizobium* spp. bacteria) -  $2.5 \text{ cm}^3$  per seed, solution of 1 pack of the vaccine in  $1 \text{ dm}^3$  of water. The same amount of Nitragine was also added to the unsown soil.

The experiment lasted 72 days. Soil samples for microbiological and physico-chemical analyses were collected with a Egner-Riehm rod in two terms: on the day of sowing (14<sup>th</sup> day of experiment) and on the day of lupine harvest (blooming phase - 72<sup>nd</sup> day of experiment). Three samples of the entire soil vertical cross-section were taken from each pot, thoroughly mixed and placed in a plastic bag. Soil samples were stored at  $4^\circ\text{C}$  for a maximum of 4 days before they were analyzed in laboratory.

The number of microorganisms was determined with the plate method in three replications. The microorganisms were isolated as follows: 10 g of soil were shaken for 30 minutes with 90 ml sterile physiological saline (0.85% aqueous solution of NaCl). Next, the soil suspension was diluted with physiological saline to obtain respective dilutions: for fungi (Fun) - 1000-fold, for sporulating oligotrophic (Olig<sub>p</sub>) and copiotrophic (Cop<sub>p</sub>) bacteria and *Arthrobacter* (Art) and *Pseudomonas* (Ps) species - 10,000-fold and for oligotrophic (Olig), copiotrophic (Cop) and actinomycetes (Act) - 100,000-fold. The soil suspension for sporulating bacteria was incubated in  $85^\circ\text{C}$  in a water bath for 15 min. 1 ml of soil suspension from each dilution respective to a given microorganism group, was transferred on a sterile Petri-dish and poured over with a cooled sterile selective medium. To determine *Azotobacter* spp., the soil was not diluted but directly placed on a Petri-dish (1g) and poured into a sterile selective medium.

The count of oligotrophic and copiotrophic bacteria was determined on the Onta and Hattory medium [22], the count of *Azotobacter* spp. on a medium according to Fenglerowa [23], the count of *Arthrobacter* spp. and *Pseudomonas* spp. on media according to Mulder and Antheumisse [24], the count of actinomycetes on the Küster and Williams' medium containing two antibiotics: nystatine and actidione [25] and the count of fungi on Martin's medium [26].

The plates with the spread microorganisms were incubated at  $28^\circ\text{C}$  (oligotrophic and sporulating oligotrophic for 21 days, copiotrophic, sporulating copiotrophic and actinomycetes - 7 days, fungi, *Azotobacter* spp., *Arthrobacter* spp. and *Pseudomonas* spp. - 3 days). The grown microorganism colonies were counted with a bacteria colony counter. The results were expressed per 1 kg of soil dry matter.

Additionally, pH and total content of hydrogen ions (Hh) (the hydrogen ion content in the soil solution and the sorption complex using 0.5 M aqueous solution of  $(\text{CaCOO})_2\text{Ca} \cdot \text{H}_2\text{O}$  with pH 8.2) were determined. The resulting acetic acid was determined by titrating the filtered soil solution with a 0.1 M aqueous solution of NaOH with phenolphthalein as an indicator. The soil samples were also analyzed for total content of alkaline exchange cations (S) and the content of organic carbon ( $\text{C}_{\text{org}}$ ).

Soil acidity (pH) in a water solution of KCl ( $1 \text{ mol} \cdot \text{dm}^{-3}$ ) was determined with a potentiometer. Total content of hydrogen ions (Hh) and total content of alkaline exchange cations (S) were determined with Kappen's method, and the content of organic carbon with Tiurin's method [27].

Table 1. Effect of heating oil on some soil physico-chemical properties.

Heating oil dose in % of soil mass	Organic carbon content C <sub>org</sub> (g kg <sup>-1</sup> of soil d.m.)	pH	Hh	S	T	V %
			mmol(+) kg <sup>-1</sup> of soil d.m.			
0.00	5.95	6.35	8.67	40.69	49.36	82.33
0.25	6.63	6.25	9.75	37.50	47.25	79.25
0.50	7.07	6.17	10.17	36.81	46.98	78.38
0.75	8.30	6.11	10.45	35.38	45.83	77.17
1.00	8.79	5.96	10.69	34.19	44.88	76.10
1.50	9.58	6.04	11.02	33.63	44.64	75.34
r	0.98*	-0.88*	0.92*	-0.93*	-0.93*	-0.94*
LSD	0.56**	0.08**	1.33**	2.26**	n.s.	2.28**

Hh - the total hydrogen ion content; S - the total content of alkaline exchange cations; T - soil exchange capacity, V - alkaline cations soil saturation levels r - correlation coefficient; \*, \*\* - statistically significant differences for P < 0.05 and P < 0.01, respectively; n.s. - insignificant difference

Additionally, the soil exchange capacity (T) and alkaline cations soil saturation levels (V) [27] were obtained.

The obtained results were statistically analyzed with a Duncan test. Polynomial regression equations for the correlation between the number of soil microorganisms and soil contamination with heating oil, as well as the correlation co-efficients between the soil physico-chemical properties and soil contamination with heating oil.

## Results and Discussion

Soil microorganisms play an especially important function in the degradation of organic matter and excreting nutrients for plants as well as in binding atmospheric nitrogen [28]. The count of microorganisms in the soil is determined by a wide variety of climatic and soil factors, including the content of organic matter and clay fraction considered to be of prime importance [1]. Plants have a great influence on both the count of microorganisms [29] and their distribution in the soil. This is the rhizosphere, where bacteria and micorhyse fungi develop intensely with nutrients available [30]. Plant roots also excrete metabolites, which induce the genes responsible for degradation of petroleum-derived substances in the soil [8].

The contamination of the experimental soil with heating oil modified both the physico-chemical properties of the soil and the count of the particular microorganisms.

The hydrocarbons that constitute heating oil are rich in carbon and, naturally, average organic carbon content increased with an increase in the dose of petroleum-derived substance (Table 1). At the same time, the physico-chemical properties of soil deteriorated. This was exhibited by a decrease in pH, the total content of alkaline exchange cations, alkaline cations saturation and soil exchange capacity and by an increase in total content of hydrogen ions. Similar results were found by Caravaca and Roldan

[31] who reported a greater content of organic carbon and lower soil pH in soil contaminated with hydrocarbons than in pure soil.

The application of lime into the soil improved its physico-chemical properties, both in the uncontaminated control pots and the pots contaminated with different doses of heating oil (Table 2). In the limed soil, a slight but significant increase in pH, the total content of alkaline exchange cations, the soil exchange capacity and the soil exchange capacity as well as a decrease in total content of hydrogen ions were observed compared to the lime-free soil regardless of sowing with yellow lupine.

The cultivation of yellow lupine had a similarly positive effect (Table 3). The soil used for the cultivation of lupine had, on average, lower total content of hydrogen ions but higher pH and higher content of alkaline exchange cations, soil exchange capacity and alkaline cations soil saturation.

Different experimental microorganisms responded differently to soil contamination with heating oil. Oligotrophic, copiotrophic, sporulating oligotrophic and copiotrophic bacteria, actinomycetes and *Pseudomonas* spp. and *Arthrobacter* spp. bacteria used hydrocarbons as an energy source which was confirmed by a significant positive correlation of their count with increasing amounts of heating oil introduced into the soil (Fig. 1). Numerous authors [32, 33, 34] reported the ability of the above-mentioned microorganisms to efficiently degrade petroleum hydrocarbons, since they can utilize carbon contained in their molecules as the sole carbon source. Kucharski and Wyszowska [11] observed the stimulating effect of heating oil present in the soil on the multiplication of oligotrophic and copiotrophic bacteria.

The number of microorganisms in soil contaminated with petroleum-derived substances usually varies over time [35] due to the chemical structure of the petroleum-derived contaminants, their susceptibility to microbio-

Table 2. Effect of heating oil and lime application on some soil physico-chemical properties.

Heating oil dose in % of soil mass	Organic carbon content C <sub>org</sub> (g kg <sup>-1</sup> of soil d.m.)	pH	Hh	S	T	V %
			mmol(+) kg <sup>-1</sup> of soil d.m.			
-Ca						
0.00	6.34	6.32	10.41	38.50	48.91	78.59
0.25	6.88	6.13	11.34	35.75	47.09	75.76
0.50	7.11	5.96	12.19	35.38	47.56	74.33
0.75	8.37	5.83	12.38	33.38	45.75	72.93
1.00	8.70	5.74	12.75	31.50	44.25	71.16
1.50	9.55	5.84	12.94	32.25	45.19	71.30
r	0.98*	-0.84*	0.92*	-0.90*	-0.85*	-0.93*
+Ca						
0.00	5.55	6.39	6.94	42.88	49.81	86.06
0.25	6.38	6.38	8.16	39.25	47.41	82.73
0.50	7.03	6.38	8.16	38.25	46.41	82.44
0.75	8.24	6.39	8.53	37.38	45.91	81.40
1.00	8.87	6.18	8.63	36.88	45.50	81.04
1.50	9.62	6.24	9.09	35.00	44.09	79.37
r	0.98*	-0.76	0.90*	-0.94*	-0.94*	-0.93*
LSD						
a	0.23**	0.02**	0.45**	1.33**	1.85**	1.17**
b	0.14*	0.04**	0.31**	0.64**	0.67**	0.79**
a x b	0.35*	0.11**	0.76**	1.58**	1.65**	1.93**

Hh - the total hydrogen ion content; S - the total content of alkaline exchange cations; T - soil exchange capacity; V - alkaline cations soil saturation levels; -Ca - lime-free soil; +Ca - limed soil, respectively; r - correlation coefficient; a - heating oil dose; b - lime application; a x b - factor interaction; \*, \*\* - statistically significant differences for P < 0.05 and P < 0.01, respectively, n.s. - insignificant difference

logical degradation and varied toxicity to microorganisms [7]. Two or three weeks after soil contamination, the number of microorganisms usually increases and can then decrease significantly with a decrease in the amount of hydrocarbons [36]. Pelletier et al. [37] carried out experiments in the subarctic climate and found a 90% decrease in the concentration of n-alkanes and the lighter fraction of cyclic hydrocarbons from soil contaminated with petroleum over the 6 initial months of the experiment. In this way, microorganisms were deprived of an energy substrate and may modify their number. Long-term studies on the effect of petroleum-derived contaminants on soil indicate, however, that the soil regains its biological efficiency as shown, among others, by the re-occurrence of *Azotobacter* spp. in the soil [38].

In the current experiment, the highest dose of heating oil (amounting to 1.5% of soil mass) had the greatest stimulating effect on the growth of *Pseudomonas* spp. and oligotrophic bacteria. Their count increased 10.4- and 8.1-fold, respectively. The sporulating oligotrophic bacteria were the least stimulated by this pol-

lutant and their count was only 2.2-fold greater than in the uncontaminated control pots.

On the other hand, heating oil had a negative effect on *Azotobacter* spp. bacteria and fungi with the former being especially sensitive to petroleum-derived pollutants. This finding was also reported by Iwanow [38].

The application of lime enhances the soil structure and improves its physico-chemical properties, which was also true in this experiment. However, lime application to the experimental soil did not always significantly modify the count of microorganisms, the average count of copiotrophic bacteria, actinomycetes and *Azotobacter* spp. (due to insignificantly low values, these data were not included in the figures) was greater and the average count of sporulating copiotrophic bacteria was lower in the limed pots than in the lime-free pots (Fig. 2).

Additionally, apart from the stimulating effect of heating oil on oligotrophic bacteria and actinomycetes, the highest dose of petroleum-derived contaminant did not further increase the count of these bacte-

Table 3. Effect of heating oil and yellow lupine on some soil physico-chemical properties.

Heating oil dose in % of soil mass	Organic carbon content C <sub>org</sub> (g kg <sup>-1</sup> of soil d.m.)	pH	Hh	S	T	V
			mmol(+) kg <sup>-1</sup> of soil d.m.			
unsown soil						
0.00	5.82	6.31	9.28	37.88	47.16	80.31
0.25	6.51	6.24	10.22	34.88	45.09	77.29
0.50	7.22	6.16	10.31	35.63	45.94	77.56
0.75	8.42	6.10	10.59	34.50	45.09	76.52
1.00	8.81	5.94	10.78	33.13	43.91	75.36
1.50	9.62	6.02	11.34	32.63	43.97	74.18
r	0.98*	-0.88*	0.95*	-0.91*	-0.86*	-0.94*
sown soil						
0.00	6.08	6.40	8.06	43.50	51.56	84.35
0.25	6.75	6.27	9.28	40.13	49.41	81.20
0.50	6.92	6.18	10.03	38.00	48.03	79.20
0.75	8.18	6.12	10.31	36.25	46.56	77.82
1.00	8.76	5.98	10.59	35.25	45.84	76.84
1.50	9.55	6.07	10.69	34.63	45.31	76.50
r	0.98*	-0.86*	0.88*	-0.93*	-0.94*	-0.91*
LSD						
a	0.23**	0.02**	0.45**	1.33**	1.85**	1.17**
b	0.08**	0.04**	0.39**	0.65**	1.00**	0.66**
a x b	0.22**	0.50**	n.s.	1.60**	n.s.	n.s.

Hh - the total hydrogen ion content; S - the total content of alkaline exchange cations; C<sub>org</sub> - the content of organic carbon; T - soil exchange capacity; V - alkaline cations soil saturation levels; r - correlation coefficient; a - heating oil dose; b - sown soil; a x b - factor interaction; \*, \*\* - statistically significant differences for P < 0.05 and P < 0.01, respectively, n.s. - insignificant difference

ria in the lime-free soil, in contrast to the pots treated with lime.

Soil fertilization by CaO had an effect on the count of soil fungi. Increasing amounts of heating oil introduced to the lime-free soil stimulated the multiplication of fungi, which did not occur in the limed soil. This could have been caused by the slightly lower pH of the lime-free soil being advantageous for the development of fungi.

The lime application had the most positive effect on *Azotobacter* spp., which is a Ca<sup>2+</sup> deficiency sensitive microorganism. In the limed uncontaminated soil, the count of this bacteria was over 100-fold greater than in the lime-free soil (due to insignificantly low values, these data were not included in the figures). Additionally, the presence of lime in the soil samples contaminated with smaller doses of heating oil (0.25 and 0.50 % of soil mass) slightly, but significantly, reduced the toxic effect of oil.

Yellow lupine cultivation did not always modify the count of the tested soil microflora (Fig. 3). This treatment had a positive effect on *Azotobacter* spp. bacteria.

Their count in the uncontaminated but sown pots was many times greater than in the respective pots without lupine. *Azotobacter* spp. was also present in the soil contaminated with the highest dose. The results obtained are in agreement with those reported by Wyszowska and Kucharski [10] based on their study into the count of microflora in the soil contaminated with gasoline, sown with triticale and unsown.

Single and di-cotyledonous plants used in bioremediation of soil not only excrete metabolites which are substrates for the soil microorganisms, but they also have the ability to accumulate petroleum-derived hydrocarbons, especially those containing aromatic rings [30], which decreases their toxic effect on microorganisms.

The cultivation of yellow lupine also increased the count of *Arthrobacter* spp. bacteria in the soil contaminated with 0.5 and 1.5 % (of soil mass) doses of heating oil in comparison to the unsown soil. In the 1.5% contaminated and sown soil, the count of oligotrophic, sporulating oligotrophic and copiotrophic was significantly higher than in respective unsown soil.

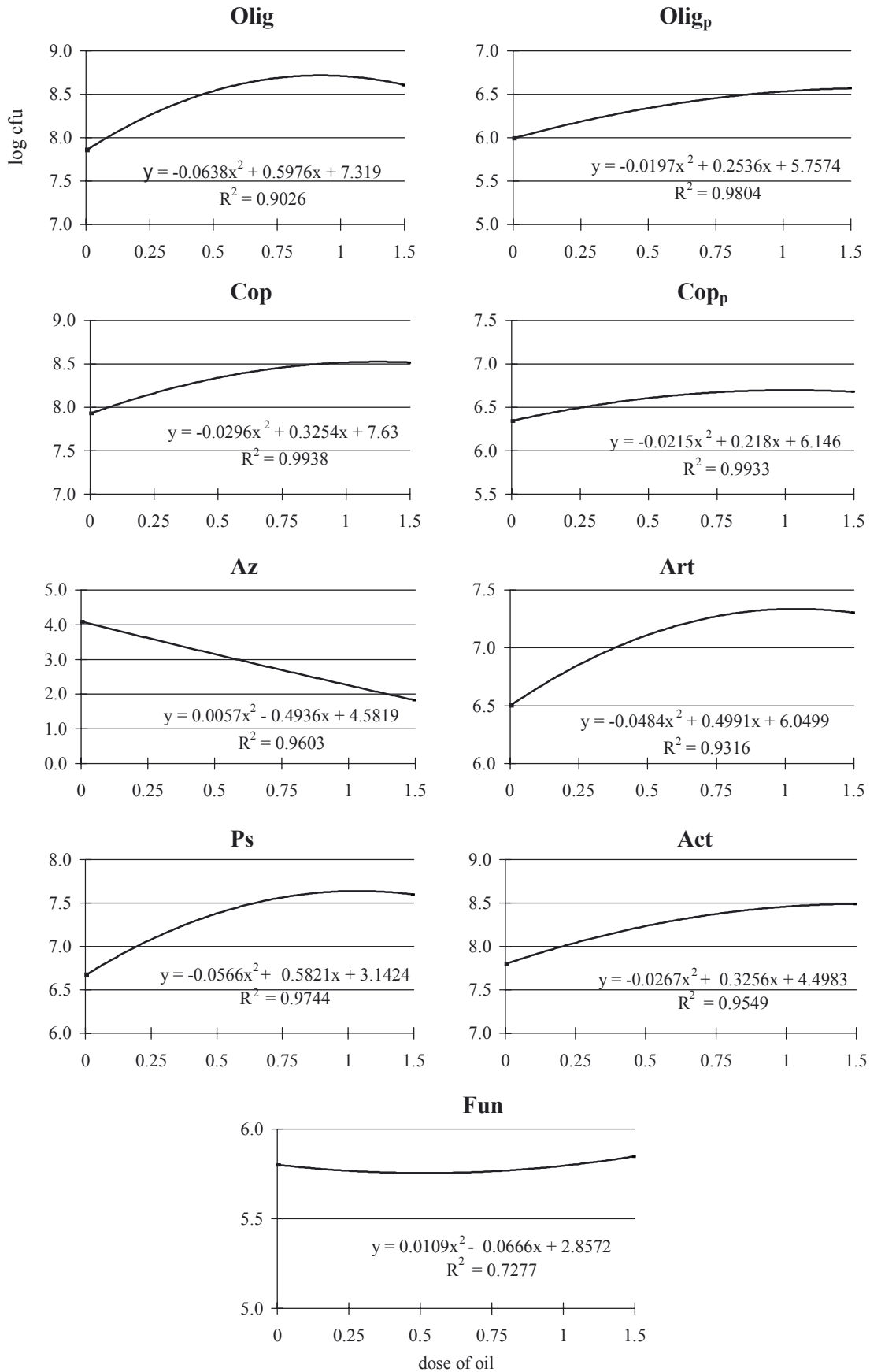


Fig. 1. Effect of heating oil on the average count of soil microorganisms in 1 kg of soil d.m.

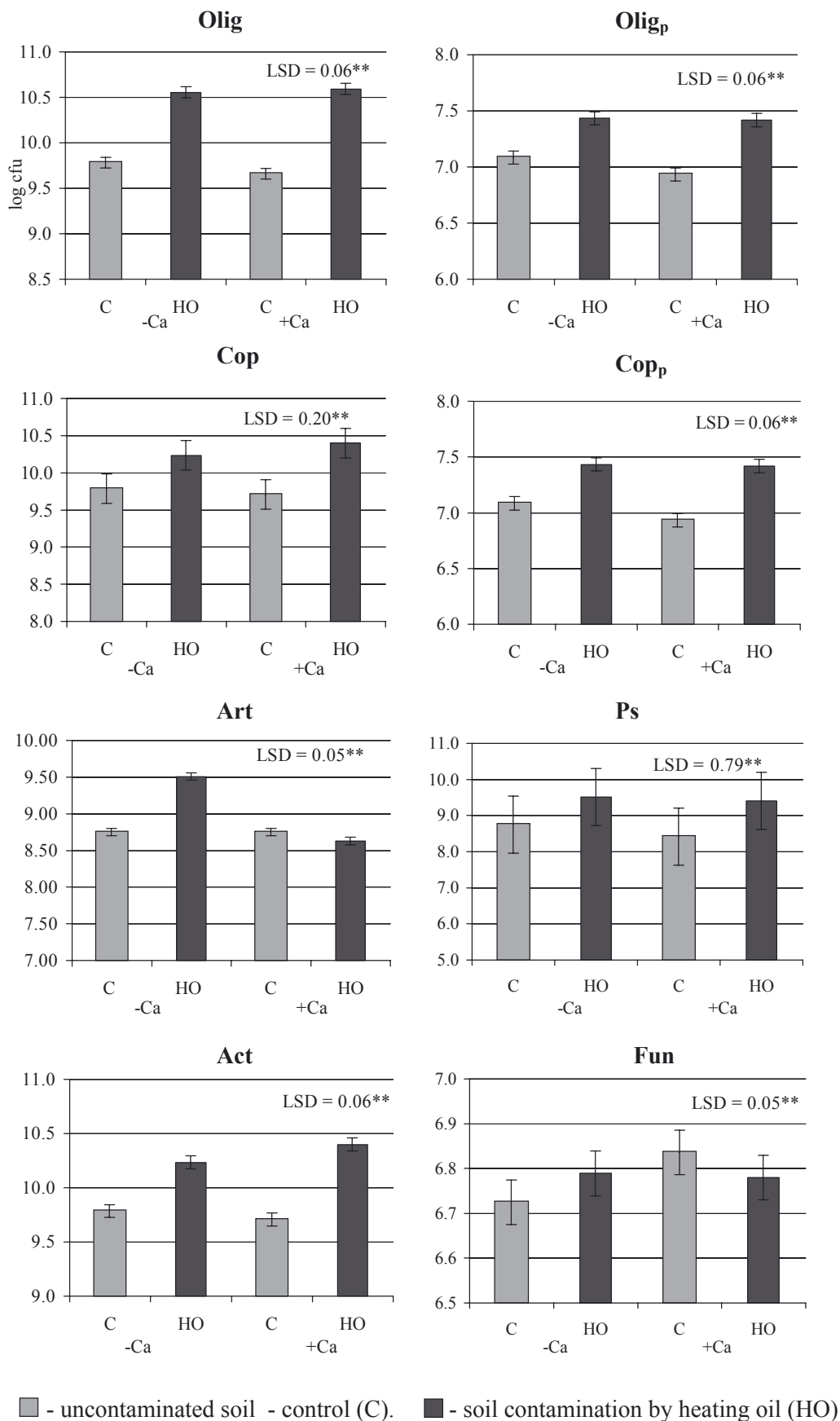


Fig. 2. Effect of heating oil and lime application on the count of soil microorganisms in 1 kg of soil d.m. -Ca – lime-free soil, +Ca – limed soil, \*\* statistically significant differences for P< 0.01.

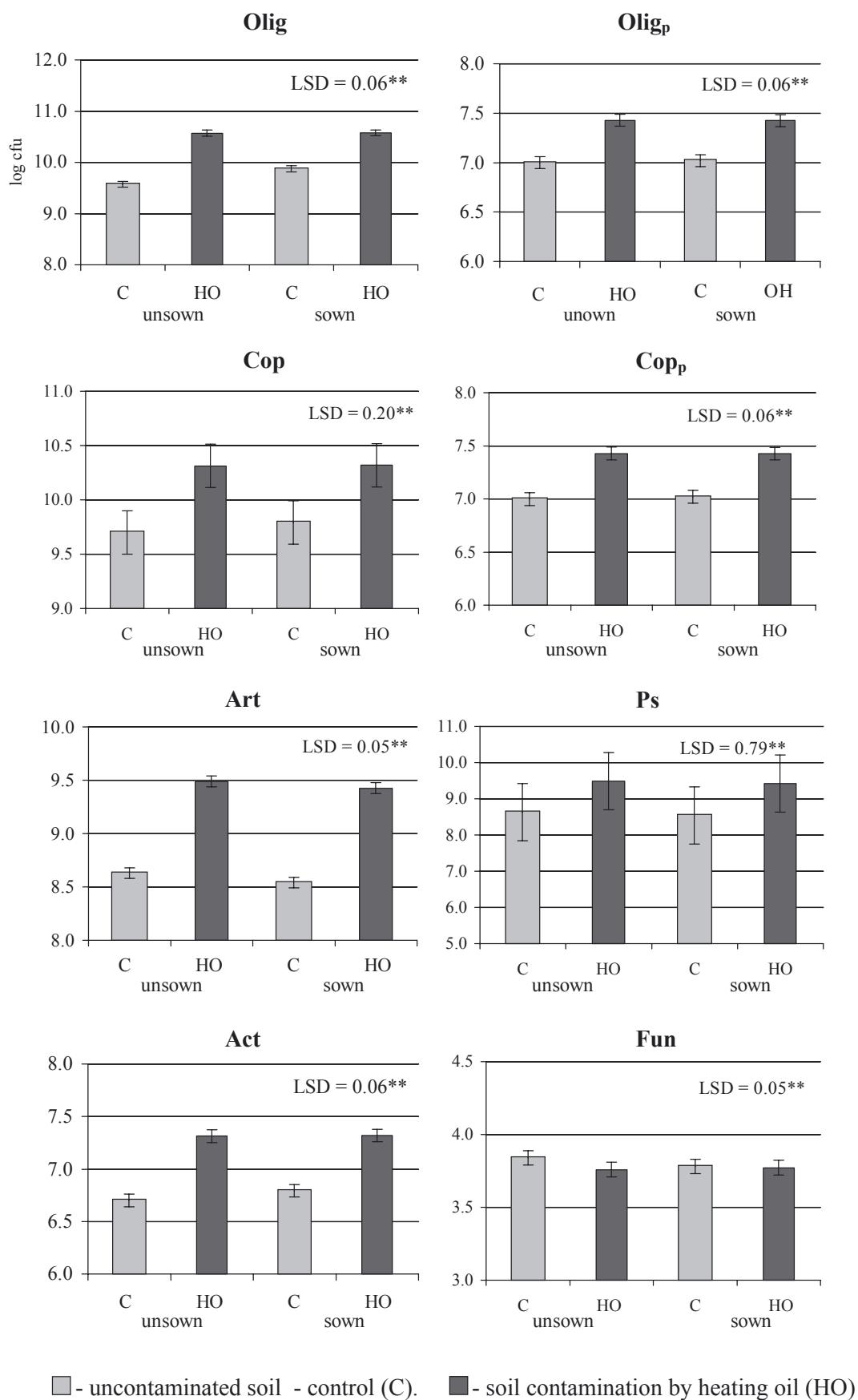


Fig. 3. Effect of heating oil and yellow lupine on the count of soil microorganisms in 1 kg of soil d.m. unsown – unsown soil, sown – sown soil \*\* - statistically significant differences for  $P < 0.01$



### Conclusion

1. Soil contamination by heating oil can contribute to soil biological imbalance, both indirectly through the modification of its physico-chemical properties and directly through the effect on microorganisms. Therefore, investigating the character of these changes can facilitate the removal of heating oil contaminating the soil.
2. Soil contamination by heating oil deteriorated its physico-chemical properties, i.e. increased its acidity and decreased the total content of alkaline exchange cations, soil exchange capacity and alkaline cations soil saturation.
3. Heating oil had a varied effect on the examined soil microorganisms stimulating the multiplication of oligotrophic, copiotrophic, sporulating copiotrophic and oligotrophic bacteria, actinomycetes and *Pseudomonas* spp. and *Arthrobacter* spp. bacteria.
4. Soil fertilization by lime and cultivation of yellow lupine improved the physico-chemical properties of the soil and favoured the development of oligotrophic, copiotrophic and *Azotobacter* spp. bacteria.

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