The influence of diesel oil contamination on soil enzymes activity

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ABSTRACT

A pot experiment was conducted in order to examine the influence of soil contamination with diesel oil at 0.0, 2.4, 4.8 and 7.2 ml.kg^{-1} on the activity of dehydrogenases, urease, acid phosphatase and alkaline phosphatase. The results indicated that diesel oil contamination of soil strongly inhibited the activity of dehydrogenases and soil urease, but had only a slight effect on the activity of acid phosphatase and alkaline phosphatase. The negative influence of diesel oil on the activity of dehydrogenases and urease was attenuated by soil inoculation with *Streptomyces intermedius* spores. The potential biochemical index of soil fertility computed from the soil enzymatic activity and carbon content was negatively correlated with diesel oil contamination and positively correlated with crop yield. Biochemical properties of soil were improved by oat cultivation.

Keywords: diesel oil; soil enzymes activity

Pollution of natural environment with refinery products has become a serious problem in many countries, including Poland. The full scale of the problem depends on size of the polluted area, depth at which pollutants penetrate soil and chemical composition of polluting substances. Pollution of soil, ground waters and surface waters with fuels and refinery products is most frequently caused by technical failures during transportation and storage or by oil disposal into soil. Products of petroleum industry belong to the most harmful xenobiotics, as many of them are potentially carcinogenic and mutagenous (Wiesel et al. 1993, Gray et al. 1994).

Poland has areas contaminated with xenobiotics, which should all be reclaimed. Decontamination of such soils is most often accomplished with the help of biological methods, the efficacy of which depends, among other factors, on the physical and chemical properties of carbohydrates present in the polluted soil (Riis et al. 1995, Olson et al. 1999), temperature, oxygen content, moisture and reaction (Zieńko 1996) and on biological factors, such as activity of respiration, biomass and activity of enzymes, which is used in eco-toxicological research in long-term studies (Leahy and Colwell 1990).

Soil enzymatic activity, which can be determined quite promptly and precisely, is a reliable indicator reflecting the current biological state of soil. In a short period of time it is possible to attain highly reliable results on the effect of different pollutants on biological life of soil. Such was the aim of our experiment, in which the effect of diesel oil on the activity of dehydrogenases, urease, acid and alkaline phosphatase was determined.

MATERIAL AND METHODS

The experiments were performed in a greenhouse, in pots containing 2.5 kg of Eutric Cambisol soil derived from light clay sand. Oat cv. Komes (25 plants per pot) was grown. A series of pots not sown with oat was also introduced. Prior to putting into pots, soil was contaminated with diesel oil at the following concentrations: 0.0, 2.4, 4.8 and 7.2 ml.kg^{-1} of soil. The effect of soil contamination was experimentally remedied by the application of inoculum composed of *Streptomyces intermedius*, which were added to soil at the amount of 11.0.10^{13} CFU per kg soil d.m. The experiments were conducted in four replicates. A detailed description of the experiments was included in part one of this paper (Wyszkowska et al. 2002).

After two weeks of experiment and after oat had been harvested (at the tasselling stage), the activity of the following enzymes in soil was determined (in three replications): dehydrogenases (Deh) by Lenhard's method modified by Casida et al. (1964), urease (Ure) – according to Gorin and Chine Chang (1966), acid (Pac) and alkaline (Pal) phosphatase – by Tabatabai and Bremner's method (1969). Based on the content of carbon in soil and soil enzymatic activity, potential biochemical index of soil fertility was calculated from the formula (Kucharski 1997): Mw = (Ure.10^{-1} + Deh + Pac + Pal). %C, where: Mw – potential biochemical index, Ure – soil urease activity, Deh – soil dehydrogenases activity, Pac – soil acid phosphatase activity, Pal – soil alkaline phosphatase activity, %C – content of soil carbon.

The results were statistically computed with StatSoft, Inc. (2000) and performed as average from two terms.

RESULTS AND DISCUSSION

Determination of soil biological activity by assessing the count of microorganisms can be affected by a large margin of error, because a large number of microorganisms present in soil can show low activity and vice versa, a small number of soil microorganisms can be

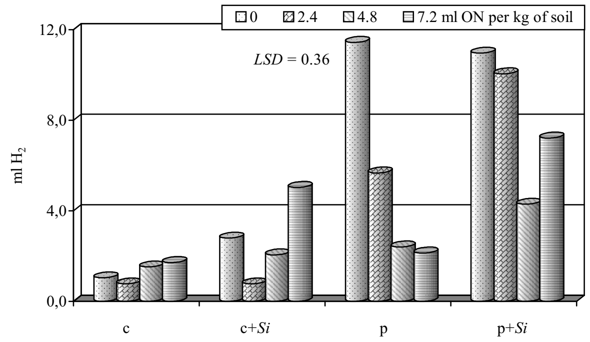

Figure 1. Activity of dehydrogenases in soil (ml H_2 kg^{-1}.h^{-1})

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

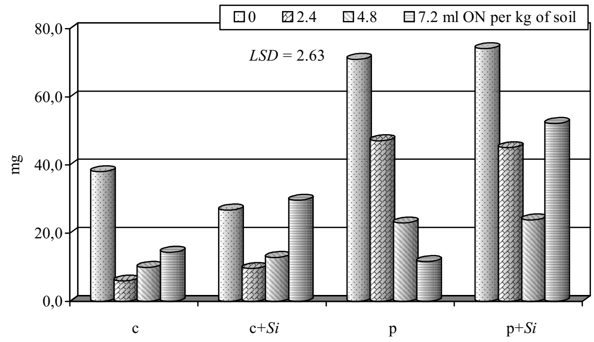

Figure 2. Activity of urease in soil (mg N-NH_4.kg^{-1}.h^{-1}) (denotations explained in Figure 1)

characterised by universal biochemical properties (Kobus 1995, Opic 1996). On the other hand, soil enzymatic activity can serve as good measure of biological activity of soil, which is an aggregate of all transformations of compounds and energy occurring in soil.

Dehydrogenases are the enzymes frequently used for determination of biological properties of soil, as their total activity may be indicative of the activity of all the population of soil microorganisms (Opic 1996). In the authors' own research the activity of dehydrogenases, similar as the number of microorganisms (Wyszkowska et al. 2002), was largely correlated with the degree of soil contamination with diesel oil, inoculum of *Streptomyces intermedius* and soil use (Figure 1). In sown soil diesel oil had an inhibitory effect on dehydrogenases, while the application of inoculate relieved to some extent the outcome of soil pollution. The activity of dehydrogenases was negatively correlated with soil contamination (Table 1). Diesel oil had an extremely toxic effect in sown soil inoculated with actinomyces (r = –0.92), in which the highest dose of the pollutant caused 5.4-fold reduction in the activity of dehydrogenases. In unsown soil, regardless inoculum of *Streptomyces intermedius*, the activity of these enzymes was different, for example it was stimulated by diesel oil added to soil at the amount of 7.2 ml.kg^{-1} of soil.

Urease activity was inhibited by diesel oil, except in unsown soil inoculated with actinomyces, in which the highest dose of the contaminant only stimulated the activity of this enzyme (Figure 2). In the other objects, irrespective of soil use, actinomyces inoculation, and degree of soil contamination, the activity of urease was inhibited. A high negative correlation between diesel oil contamination and urease activity was observed in unsown soil not inoculated with *S. intermedius*, for which the correlation coefficient was –0.99 (Table 1).

Acid phosphatase was more tolerant to soil contamination with diesel oil (Figure 3), even though, same as the enzymes discussed earlier, its activity was negatively correlated with oil contamination in sown soil inoculated with *Streptomyces intermedius* or not inoculated (Table 1). Diesel oil was observed to have a stimulating effect on the acid phosphatase activity only when applied at the concentration of 4.8 ml.kg^{-1} to unsown soil not inoculated with *S. intermedius* and at the 2.4 ml.kg^{-1} to sown inoculated soil. The activity of acid phosphatase, unlike that of the other enzymes, was not modified by the inoculation.

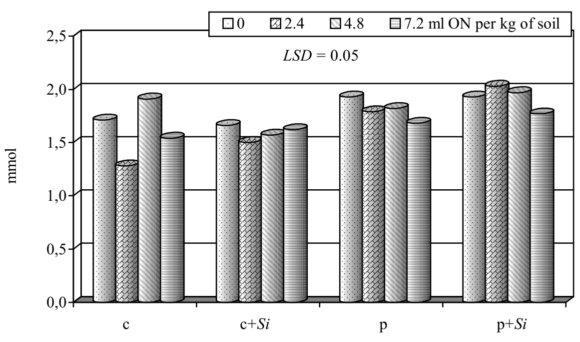

Figure 3. Activity of acid phosphatase in soil (mmol PNP.kg^{-1}.d^{-1}) (denotations explained in Figure 1)

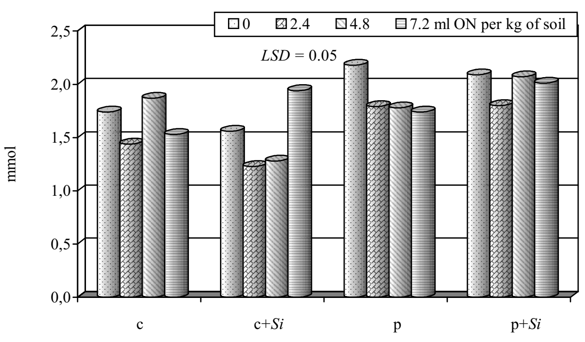

Figure 4. Activity of alkaline phosphatase in soil (mmol PNP.kg^{-1}.d^{-1}) (denotations explained in Figure 1)

Table 1. Correlation coefficients between diesel oil contamination of soil and activity of enzymes

Kind of enzymes	–*Si*		+*Si*	
	unsown soil	sown soil	unsown soil	sown soil
Dehydrogenases	0.84**	–0.92**	0.60*	–0.74**
Urease	–0.58*	–0.99**	0.17	–0.53*
Acid phosphatase	0.08	–0.89**	–0.07	–0.64*
Alkaline phosphatase	–0.11	–0.82**	0.49	0.06

–*Si* – soil without *Streptomyces intermedius* inoculum
+*Si* – soil with *Streptomyces intermedius* inoculum
* significance for $p < 0.05$; ** significance for $p < 0.01$

Table 2. Ratio of the activity of enzymes in soil with *Streptomyces intermedius* inoculum to soil without inoculum

Dose of oil ($ml.kg^{-1}$ of soil)	Dehydrogenases	Urease	Acid phosphatase	Alkaline phosphatase
0	1.10	0.93	0.99	0.93
2.4	1.68	1.03	1.15	0.94
4.8	1.62	1.12	0.95	0.92
7.2	3.19	3.13	1.05	1.21
$LSD_{p = 0.05}$	0.12	0.10	0.02	0.04

In addition, the activity of alkaline phosphatase was dependent on the experimental series and degree of soil contamination with diesel oil (Figure 4). Same as in the case of dehydrogenases, urease and acid phosphatase activity, the negative effect of diesel oil was particularly strong in sown soil not inoculated with *S. intermedius*, while in soil inoculum with actinomyces only one dose of oil decreased the activity of alkaline phosphatase, with the other concentrations leaving it on a relatively stable level. Less obvious results were obtained on the activity of this enzyme in unsown soil, in which it was not connected, with the dose of diesel oil.

In uncontaminated soil the activity of all the enzymes analysed was weakly influenced by the *S. intermedius* inoculum (Table 2). However, inoculation clearly had a favourable effect by reducing the negative role of die-

Table 3. Ratio of activity of enzymes in oat sown soil to unsown soil

Dose of oil ($ml.kg^{-1}$ of soil)	Dehydrogenases	Urease	Acid phosphatase	Alkaline phosphatase
		–*Si*		
0	11.00	1.87	1.13	1.25
2.4	7.36	7.84	1.40	1.24
4.8	1.58	2.31	0.95	0.95
7.2	1.25	0.81	1.09	1.14
		+Si		
0	3.93	2.77	1.16	1.34
2.4	13.05	4.66	1.35	1.46
4.8	2.09	1.85	1.25	1.62
7.2	1.43	1.76	1.09	1.04
$LSD_{p = 0.05}$				
A	0.58	0.09	0.04	0.02
B	0.41	0.07	0.03	0.01
$a \times b$	0.82	0.13	0.06	0.02

a – for diesel oil dose, *b* – for *Streptomyces intermedius* inoculum (denotations explained in Table 1)

Table 4. Potential biochemical index of soil contaminated with diesel oil

Kind of enzymes	–*Si*		+*Si*	
	unsown soil	sown soil	unsown soil	sown soil
0	6.22	16.99	6.52	16.80
2.4	3.07	10.47	3.35	13.79
4.8	4.72	6.23	4.64	8.04
7.2	4.67	5.04	8.67	12.15
Correlation coefficient	–0.27	–0.95	0.45	–0.70
$LSD_{p = 0.05}$	*a* – 0.10; *b* – 0.07; *c* – 0.07; *a* × *b* – 0.15; *a* × *c* – 0.15; *b* × *c* – 0.10; *a* × *b* × *c* – 0.21			

a – for diesel oil dose, *b* – for *Streptomyces intermedius* inoculum, *c* – for soil use (denotations explained in Table 1)

sel oil as an inhibiting factor towards dehydrogenases activity, regardless of the degree of soil contamination, and partly towards urease activity.

The activity of dehydrogenases, urease and acid and alkaline phosphatase was modified to a greater extent by the cultivation of oat than by the application of *S. intermedius* inoculum (Table 3). Oat grown in uncontaminated soil stimulated the activity of all the soil enzymes analysed, regardless of inoculation, which may have been caused by the positive influence of root secretion on microbiological and biochemical soil properties. The favourable effect of oat on dyhedrogenases activity was weakened by diesel oil in soil not inoculated with *S. intermedius*, which was proved by the negative correlation between soil contamination with diesel oil and the activity of all these enzymes in soil sown with oat versus unsown soil. The ratio of the activity of acid and alkaline phosphatase was narrowed (less than 1) only in soil not inoculated with actinomyces and contaminated with oil at 4.8 $ml.kg^{-1}$ of soil, that of urease activity – at the highest degree of contamination.

Changes in the activity of enzymes, especially dehydrogenases and urease, observed in the course of our experiments, coincide with those reported in earlier studies on the effect of refinery products on biological properties of soil (Wyszkowska and Kucharski 2000, Kucharski and Wyszkowska 2001). Lower activity of dehydrogenases in soil was also notices by Małachowska-Jutsz et al. (1997), who suggested that a decline in dehydrogenases activity was probably caused by diesel oil, which eliminated from soil those microorganisms that were less tolerant to petroleum derivatives. Galas et al. (1997) reported undesirable depression in the activity of these enzymes in cells of *Bacillus* sp. and *Micrococcus* sp.

Determination of microbiological and biochemical activity of soil, in which the activity of particular enzymes is accounted for, allows us to draw conclusions about soil fertility. More complete information on biochemical transformations in soil can be obtained on the basis of the potential biochemical index of soil fertility (*Mw*), which comprises the activity of dehydrogenases, urease, acid and alkaline phosphatase as well as the organic carbon content .The potential biochemical index of soil fertility computed in the present study (Table 4) decreased with degrees of soil contaminated with diesel oil, like the yield of oat in presented in another paper (Wyszkowska et al. 2002).

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ABSTRAKT

Vliv kontaminace motorovou naftou na půdní enzymatickou aktivitu

V nádobovém pokusu jsme sledovali vliv kontaminace půdy motorovou naftou v množství 0; 2,4; 4,8 a 7,2 ml.kg^{-1} na aktivitu dehydrogenáz, ureázy, kyselé fosfatázy a alkalické fosfatázy. Kontaminace půdy motorovou naftou měla silný inhibiční účinek na aktivitu dehydrogenáz a půdní ureázy, ale pouze slabý účinek na aktivitu kyselé fosfatázy a alkalické fosfatázy. Negativní účinek motorové nafty na aktivitu dehydrogenáz a ureázy zmírnila inokulace půdy sporami *Streptomyces intermedius*. Potenciální biochemický index půdní úrodnosti, vypočtený na základě půdní enzymatické aktivity a obsahu uhlíku, naznačil negativní korelaci s kontaminací motorovou naftou a pozitivní korelaci s výnosem plodin. Pěstování ovsa zlepšilo biochemické vlastnosti půdy.

Klíčová slova: motorová nafta; půdní enzymatická aktivita

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