

## Changes of Dehydrogenases Activity in Soils Polluted with Diesel Fuel

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### Abstract

Activity of the enzymes can give information on the kind and duration of the effects of pollutants on the metabolic activity of soil. Results of studies on the dehydrogenase activity in soils experimentally contaminated with diesel fuel, with and without modification used in investigation over biodegradation process (including fertilization, stirring and inoculation) were presented in paper. Studies with two rates of pollution were carried out for soils of various organic matter content and different soil particle size grading. Dehydrogenase activity was determined with TTC (2,3,5-triphenyltetrazolium chloride) as a substrate. Increase of dehydrogenase activity was found in the soils that were treated with diesel fuel. Regardless of pollution rate, usually higher activity was recorded in sandy soil. Variance analysis confirmed the highly significant influence of applied modifications on increase of the enzymatic activity in relation to not modified object.

**Key words:** dehydrogenase, soil, microorganisms, diesel fuel

### Introduction

Knowledge of microbial activity of soil is important in evaluating the progress of biodegradation due to crude oil product pollution (Thurmann *et al.*, 1999; Margesin *et al.*, 2000). Activity of enzymes taking part in biological oxidation of organic compounds (Smreczak and Maliszewska-Kordybach, 1998) as well as biomass of viable organisms (Nowak, 1986; Anderson and Domsch, 1978) and microbial counts, are the parameters of activity and quality of soil environment (van Beelen and Doelman, 1997).

Alkane oxidation and breaking the aromatic rings are the most important stages of biochemical transformations of crude oil products. Oxygenases are active in first stage of hydrocarbon oxidation then this process occurs due to dehydrogenase action (Kwapisz *et al.*, 2000). Dehydrogenases are of intracellular character, *i.e.* their activity is closely associated with the number of microorganisms living in the soil. Studies of Smreczak and Maliszewska-Kordybach (1998) revealed that crude oil hydrocarbons caused the decrease of dehydrogenase activity (below 40% of control values), but the inhibition effects were stronger manifested in low-organic-matter soils. Investigations of Margesin and Schinner (1997) confirm the dependence of enzymatic activity on organic matter content, but authors observed the increase of dehydrogenase activity. Moreover, they found slightly elevated activity in soil samples where bacterial inoculate was amended.

This paper presents the results of studies on the changes of dehydrogenase activity in soils polluted with diesel fuel. The influence of pollution rates and modification of bioremediation process in soils of different particle size grading and various organic matter contents was analyzed.

### Experimental

#### Materials and Methods

Two types of soil were used in the studies – sandy and loamy. Material was taken from 0–15 cm depth of humus layer. Size grading of soils was determined applying Cassagrande's method with Prószyński's modification (Ostrowska *et al.*, 1991); total carbon – by Tiurin's method, total nitrogen level – by Kjeldahl's method. Results are presented in Table I. Soils, besides particle size grading, differed also in organic matter content determined by means of digestion method. Sandy soil contained 2.3% of organic matter, loamy one – 5.3%.

Table I  
Soil particle size grading, total carbon and nitrogen contents in studied soils

Soil type	Fraction content [mm]			C <sub>tot.</sub> [%]	N [%]
	1.0–0.1	0.1–0.02	< 0.02		
A. Brown-rusty soil (light loamy silty sand)	61	26	13	1.0	0.09
B. Chernozem (light silty loam)	46	26	28	1.9	0.15

Table II  
Experimental objects

Object	Modifications
K	Control soil
0	Polluted soil, not modified with operations
I	Polluted soil, fertilized, stirred
II 101	Polluted soil, fertilized, stirred, inoculated with BS 101 strain
II 126	Polluted soil, fertilized, stirred, inoculated with BS 126 strain
II 135	Polluted soil, fertilized, stirred, inoculated with BS 135 strain
II BP	Polluted soil, fertilized, stirred, inoculated with strain mixture

Studied soils were preliminarily dried and sieved through 1-mm-mesh screen to remove the mineral parts and mechanical contaminants. Then, applying Kopecky's method (Koćmit *et al.*, 1997), maximum soil water absorption was estimated. Maximum water capacity of soil samples was thus adjusted to 60%. Such humidity was maintained for the whole experiment and possible losses were completed with distilled water.

Every soil type studied was divided into two parts: one was polluted with diesel fuel at the rate of 5%, the second – 15% (w/w). Within every pollutant rate, 1.5 kg of soils samples were placed in polyethylene container and modified with fertilization (NPK – 0.1 g N, P<sub>2</sub>O<sub>5</sub>, K<sub>2</sub>O kg<sup>-1</sup> d.m. soil), stirring and inoculation with microorganisms isolated from environments polluted with oil-derivative products-strains, definite as BS 101, BS 126 and BS 135, were described earlier by Hawrot and Nowak (2003). These strains, belonging to *Pseudomonas* (BS 101) and *Bacillus* (BS 126 and BS 135) genus, were characterized by high diesel fuel biodegradation activity (Table II).

Fertilization was applied at the beginning of the experiment and after 3 months of incubation. In order to homogenize and aerate the soil, samples were stirred every 72 hours. The experiment was conducted for 5 months.

Dehydrogenase activity in soil was determined according to Malkomes (1993) method. Determination was made at 540 nm wavelength using UV-VIS spectrometer Lambda Bio (Perkin Elmer) and TTC (chloride 2,3,5-triphenylotetrazole) used as a substrate were applied to measure the activity of dehydrogenase. Soil samples were incubated with TTC in darkness at 30°C for 4 h. Afterwards acetone was added and the samples were stirred for the next 2 h. Then the soil samples were filtered and the optical density was measured. Results were recalculated onto  $\mu\text{g TPF}\cdot\text{g}^{-1}\times\text{d.m.}\times(4\text{ h})^{-1}$ .

Results were analyzed statistically applying variance analysis.

## Results and Discussion

Biodegradation efficiency depends on activity of microorganisms able to biological decomposition of crude oil products.

Dehydrogenase activity determined at the beginning of experiment was 17  $\mu\text{g TPF}$  for sandy and 217 for loamy soil. Slight increasing and decreasing tendencies were observed in control sandy soil throughout the experiment. In loamy soil, three-fold decrease was recorded in the first month of experiment. In sandy soils enzymatic activity is lower than that observed in heavier ones (Kucharski and Niklewska-Larska, 1992; Maliszewska-Kordybach and Masiak, 1988; Ortega-Calvo and Saiz-Jimenez, 1998). The same dependencies were observed in performed experiments, but they changed after the diesel fuel was introduced into the studied soils. Margesin *et al.* (2000) contaminated soil with 5 g $\times$ kg<sup>-1</sup> of diesel oil and observed that activity of soil dehydrogenase increased immediately after oil introduction.

The increase of dehydrogenase activity was observed in sandy soil that was not modified with operations, after diesel fuel amendment at 5% rate. Activity was 309  $\mu\text{g TPF}$ , which caused 485% increase as compared to the control (Fig. 1). However, clear decrease to 28  $\mu\text{g TPF}$  at the end of experiment, which was 5% lower than in control, was recorded at the next months. Similar tendencies were observed in fertilized and stirred object. Two peaks of activity (after 30 and 90 days) were recorded in inoculated objects (except

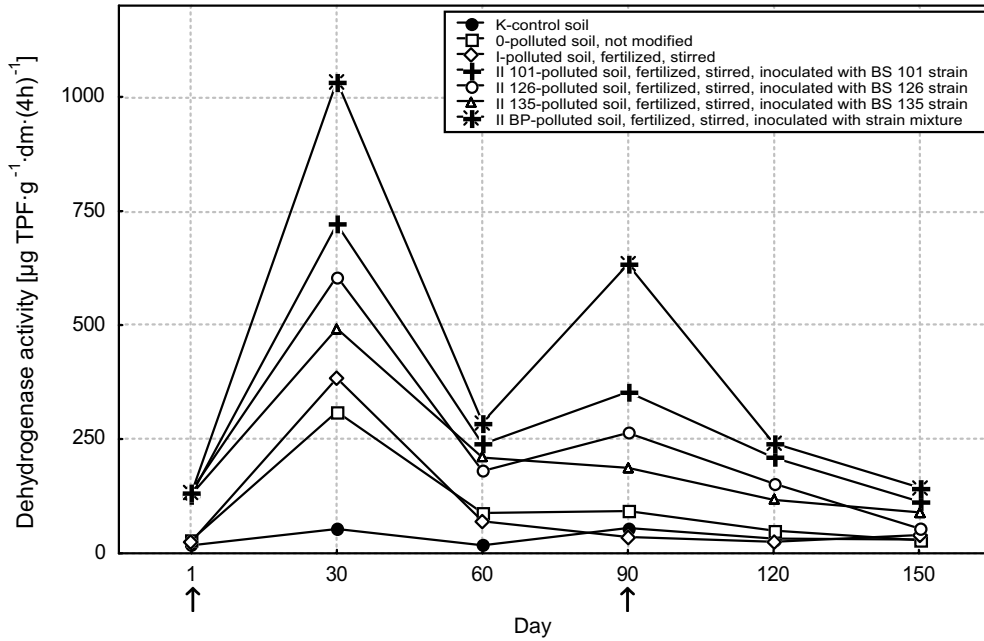


Fig. 1. Influence of sandy soil pollution with diesel fuel at 5% rate and applied modification on dehydrogenase activity (↑ – fertilization)

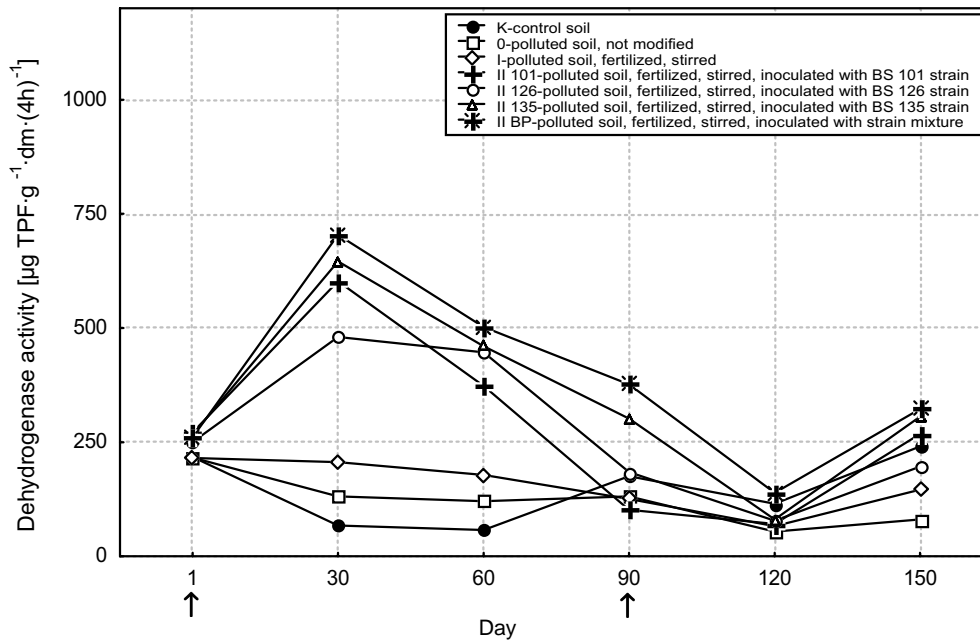


Fig. 2. Influence of loamy soil pollution with diesel fuel at 5% rate and applied modification on dehydrogenase activity (↑ – fertilization)

object inoculated with BS 135 strain). Relatively the highest activity was noted for object inoculated with strain mixture – almost twentyfold more than for control object. At the following months, dehydrogenase activity decreased, practically to values close to these that were determined at the beginning of the experiments. Caravaca and Roldan (2003) also observed higher dehydrogenase activity in the hydrocarbon-contaminated soil than in the control soil.

In loamy soil, in not modified object, dehydrogenase activity was higher up to 60<sup>th</sup> day than in control object by 95–108% (Fig. 2). At the subsequent months, it significantly decreased and the obtained values were by 25–66% lower than for control. In fertilized and stirred object, dehydrogenase activity gradually decreased during studies. In all inoculated objects, similarly as in sandy soil, the increase of dehydrogenase activity was found, but in loamy soil, it referred only to the first month after pollution. At that moment, the

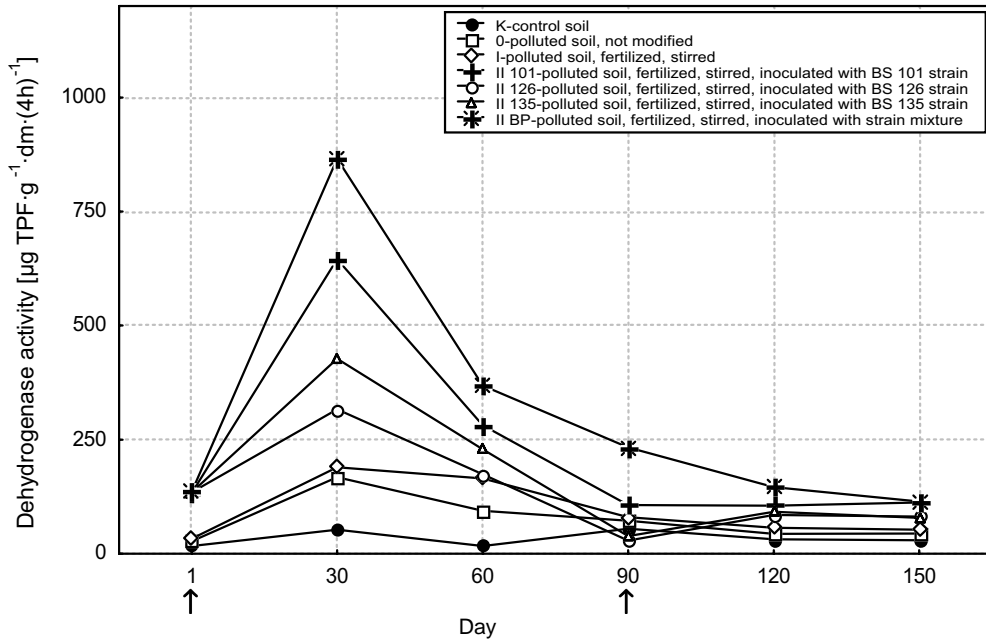


Fig. 3. Influence of sandy soil pollution with diesel fuel at 15% rate and applied modification on dehydrogenase activity (↑ – fertilization)

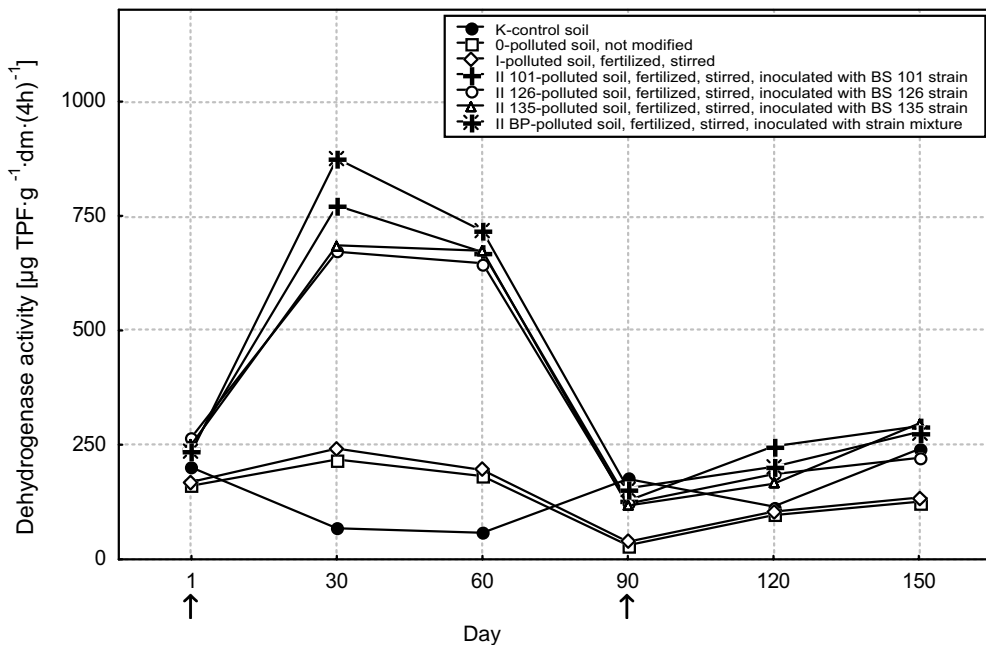


Fig. 4. Influence of loamy soil pollution with diesel fuel at 15% rate and applied modification on dehydrogenase activity (↑ – fertilization)

lowest activity (481 µg TPF) was recorded in object II 126, the highest (704 µg TPF) in object II BP. Values gradually decreased at the following measurements.

Dehydrogenase activity also significantly increased after treatment the sandy soil with diesel fuel at 15% rate, although not to the same extent as that observed at lower rate. Result of measurement in not modified object after the month of incubation revealed 168 µg TPF, *i.e.* 317% as compared to non-polluted soil (Fig. 3). In subsequent months, the activity decreased to 45 µg TPF in 6<sup>th</sup> measurement (149% as compared to control). Activity also increased in fertilized and stirred object and in inoculated objects at 30<sup>th</sup> day after pollution. In general decreasing tendency found was then maintained till the end of experiment.

In loamy soil polluted with 15% diesel fuel, similarly as at lower rate, initial great stimulation of enzymatic activity was observed. For not modified object after the increase up to 218 µg TPF at the second

Table III  
Statistical analysis results for dehydrogenase activity (P<0.05)

Factor	Number of independent variables	Mean square sum.	Number of independent variables for error	Mean square sum for error	P value	F value
Sandy soil						
1	5	8905.544	318	28.00486	11.79042	0.00**
Loamy soil						
1	5	8259.508	318	25.97330	16.56479	0.00**

Factors: 1 – modification

\*\* = highly significant action of factor

month of measurement, the activity decreased to 31 µg TPF at the fourth month-decrease by 82% as compared to the control (Fig. 4), and then it increased again approaching 125 µg TPF at the end of incubation time. Almost the same tendencies of activity were observed for soil with fertilization and stirring, but there were higher values by 4–30% than those determined for not modified object, on average. The highest dehydrogenase activity stimulation was recorded for inoculated objects after 30 days of incubation (672–875 µg TPF). Activity significantly decreased (by almost 1000%) in every object between 60<sup>th</sup> and 90<sup>th</sup> day of measurement. The values reached only 67–88% of control ones.

Regardless the contamination dose, usually higher dehydrogenase activity was determined in sandy soil. Fertilization and stirring slightly increased the activity of dehydrogenases in relation to the control object, but significant changes were observed in objects with inoculation.

Variance analysis confirmed highly significant effect of pollution rate and operations applied during biodegradation on the dehydrogenase activity in studied soils (Table III).

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