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ACTIVITY OF DEHYDROGENASES IN SOIL CONTAMINATED WITH DIESEL FUEL AND SUBJECTED TO BIOREMEDIATION PROCESS

AKTYWNOŚĆ DEHYDROGENAZ W GLEBIE ZANIECZYSZCZONEJ OLEJEM NAPĘDOWYM I PODDAWANEJ PROCESOWI BIOREMEDIACJI

Abstract: The carried out study referred to evaluation of the activity of dehydrogenases in soil that was contaminated with diesel fuel (5 % w/w). In addition, the influence of modifications used in the process of biodegradation of oil pollutions (agrotechnical measures, biostimulation, bioaugmentation) was evaluated. The experiment was made with *in situ* and *ex situ* methods. It was found that diesel fuel stimulated the activity of dehydrogenases. Also a highly significant effect of applied remediation measures was demonstrated.

Keywords: dehydrogenases, diesel fuel, soil

Petroleum hydrocarbons that get into environment cause in it a number of unfavourable changes. Changes in qualitative and quantitative composition of microorganisms and their activity are a sensitive indicator of the presence of pollutions of that type [1–5]. Many authors point at an important role that can be also played by enzymatic activity, including the activity of dehydrogenases [6–8]. Dehydrogenases, apart from oxygenases, participate in biological oxidation of organic compounds, thus their activity in contaminated soils should increase. However, the response of these enzymes to the presence of petroleum hydrocarbons in environment is not simple, since one can observe both stimulation [9, 10] and inhibition of the activity of dehydrogenases [11, 12] with the concentration of xenobiotic having a significant effect on the response observed [13]. The presented study aimed at evaluation of changes in the activity of dehydrogenases under the influence of diesel fuel and treatments used during remediation of contaminated soil made with *in situ* and *ex situ* methods.

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Materials and methods

Bio-pile experiment (*ex situ* method) was carried out in a vegetation house of the University of Agriculture in Szczecin. In total, 4 piles were prepared, each being a single object: 1) K – control soil, uncontaminated and non-modified, 2) 0 – contaminated soil, non-modified by treatments, 3) I – contaminated soil, nutrient--supplemented and mixed, and 4) II – contaminated soil, modified by nutrient supplementation, mixing and inoculation with a mixture of bacteria cultures isolated from environments polluted with petroleum hydrocarbons – strains, definite as BS 101, BS 126 and BS 135, were described earlier by Nowak and Hawrot [14]. Diesel fuel was introduced into the soil at the concentration of 5 % (w/w). The concrete floor under piles was lined with foil coverings. As the need arose, the pile soil was protected against unfavourable climatic conditions by means of foil coverings. Soil moisture was kept a level of 20 % WHC. Fertilisation was used twice – at the beginning of experiment and after three months – the applied fertiliser doses corresponded to 500 kg $N \cdot ha^{-1}$ (twice, 250 kg $N \cdot ha^{-1}$ each), 120 kg $P_2O_5 \cdot ha^{-1}$ (once), 300 kg $K_2O \cdot ha^{-1}$ (twice, 150 kg $K_2O \cdot ha^{-1}$ each), when converted to 15 cm soil layer. The mixing in respective piles was made every second week. Every 30 days, collective soil samples were collected from a depth of 0–15 cm. In case of object 0, the soil samples were also collected from a depth of 15–30 cm and averaged with the sample from a depth 0–15 cm.

Field experiment (*in situ* method) was set up in the form of microplots with the method of completely randomised blocks in 4 replications. The microcosm were formed, corresponding to bio-pile experiment variants (K, 0, I, II). Diesel fuel was introduced into the soil at the concentration of 5 % (w/w – in count on layer 15 cm depth of plot 1×1 m size). The nutrient supplementation treatment within microcosm I and II was carried out at the beginning of experiment and after 3 months, with application of nitrogen supplementation (100 kg $N \cdot ha^{-1}$), phosphorus supplementation (80 kg $P_2O_5 \cdot ha^{-1}$) and potassium supplementation (100 kg $K_2O \cdot ha^{-1}$). The mixing (by delving) was made every 14 days. The soil was kept under fallow to eliminate the effect of plants of biodegradation efficiency. Every 30 days, a collective soil sample was collected weighing 0.5 kg from a depth of 0–15 cm of each microcosm.

The activity of dehydrogenases in soil was determined by the method according to Malkomes [15]. Measurements were made with a wavelength of λ = 540 nm using a Perkin Elmer UV/VIS Lambda Bio spectrophotometer. Results are given as converted to $\mu g\ TPF \cdot g^{-1} \cdot dm \cdot 4h^{-1}$. The experiment was carried out for 150 days. The obtained results were subjected to statistical analysis using analysis of variance. Within the analysis of variance in the field experiment, effects were combined with error, which allowed for taking into consideration the block variation.

Results

The activity of dehydrogenases determined at the beginning of experiment with bio-piles amounted to 82 μg TPF. In the control pile, it underwent small fluctuations in range 74–125 μg TPF. After soil contamination with diesel fuel, enzymatic activity

clearly increased in all piles, in particular in the third measurement time. In that time, lowest values were recorded in pile 0 (222 μg TPF), while the largest ones in the pile inoculated with a mixture of bacteria cultures (549 μg TPF). In next times, this enzymatic activity was gradually smaller and smaller in all piles. After 150 days of incubation, the values observed in pile 0–146 μg TPF, and in pile I and II – 222 μg TPF and 264 μg TPF, constituted respectively 198, 301 and 359 % when compared with the control (Fig. 1A).

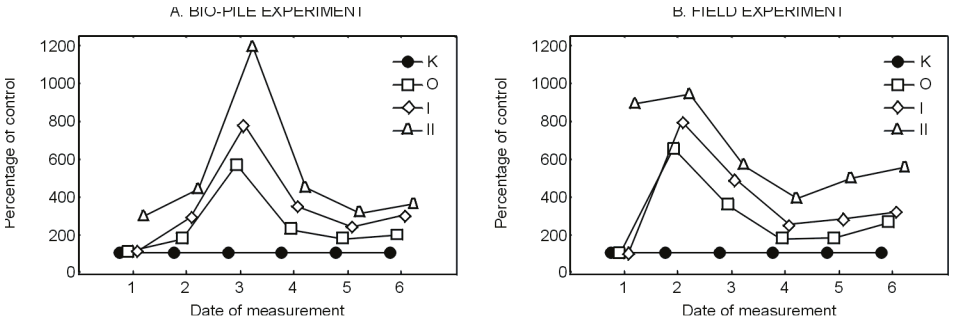

Fig. 1. Effect of soil contamination with diesel fuel in 5 % concentration and treatments used in biodegradation on the activity of dehydrogenases expressed as control percent

In the field experiment, the enzymatic activity determined at the beginning amounted to 26 μg TPF. In the control object this activity increased over 3 times after the third measurement time, to 82 μg TPF, whereupon decreased gradually to a level of 48 μg TPF at the end of experiment. In the soil contaminated with diesel fuel, the activity of dehydrogenases clearly increased. The largest activity in relation to the control was observed after 30 days of incubation, ranging from 656 % in object 0 to 940 % in object II (Fig. 1B). In next days, this activity gradually decreased. After 150 days of incubation, a value of 124 μg TPF was recorded in non-modified object, 150 μg TPF in fertilised and mixed object and 260 μg TPF in inoculated object. During the experiment, the lowest activity was observed in object 0–122 μg TPF, whereas the largest one in object II – 537 μg TPF.

Table 1

Results of statistical analysis for the activity of dehydrogenases in soil in the bio-pile experiment

Factor	Number of independent variables	Mean square sum.	Number of independent variables for error	Mean square sum for error	F value	P value
1	3	445.1544	48	0.467564	952.071	0.00*
2	5	232.0776	48	0.467564	496.354	0.00*
1 × 2	15	26.5867	48	0.467564	56.862	0.00*

Factors: 1 – bioremediation treatment, 2 – measurement period
* = a significant effect of a factor (at $P < 0.05$)

Table 2

Results of statistical analysis for the activity of dehydrogenases in soil in the field experiment

Variation source	Sum of squares	Degrees of freedom	Mean of squares	F value	P value
Blocks	0.89961	3	0.299869		
1	5149.585	3	1716.529	1301.347	0.00*
2	1645.506	5	329.101	249.501	0.00*
1 × 2	463.6986	15	30.91324	23.436	0.00*
Error	91.0137	69	1.31904		

Factors: 1 – bioremediation treatment, 2 – measurement period
* = a significant effect of a factor (at $P < 0.05$)

Discussion and conclusions

Biodegradation efficiency depends on the activity of microorganisms which participate in biological decomposition of petroleum products. The measure of their activity can be the enzymatic activity [16, 17]. Dehydrogenase activity has been suggested as suitable sensitive parameter to organic pollutant contamination among several biological indicators [18]. In our study after introduction of diesel fuel into soil, an increase in the activity of dehydrogenases was found. In the bio-pile soil, the enzymatic activity was higher that in the soil of field experiment. Similar situation was found in fertilised and mixed objects, whereas after soil inoculation a clearly higher activity of dehydrogenases was observed in the bio-pile, maybe the selected bacteria strains introduced into soil brought about an increase in total number of micro-organisms, which in turn affected the increase of respiratory activity, the measure of which is enzymatic activity. Stimulation the microbial activity after addition of nutrient (nitrogen and phosphorus) was observed by Bento et al [19] and Lee et al [20]. Bento et al [19] found the highest dehydrogenase activity after bioaugmentation too. Hawrot et al [10] observed in the study carried out under laboratory conditions that both diesel fuel and modifications applied during their experiment (biostimulation and bioaugmentation) stimulated enzymatic activity, however after 90 days of incubation the values were at a higher level in the inoculated object than in the control one. The study carried out by Margesin and Schinner [9] confirm an increase in the activity of dehydrogenases, in particular in the soil samples into which bacteria inoculate was introduced. Also other authors [21, 22] demonstrated a clearly stimulating effect of diesel fuel. Different results are reported by Claassens et al [11] and Dawson et al [12]. They observed decrease of dehydrogenases activity in soil after petroleum hydrocarbon contamination compared with the uncontaminated soil.

In our study maximum activity of dehydrogenases was found after 60 days of incubation in the bio-pile experiment and after 30 days in the field experiment. Riffaldi et al [23] have recently shown much higher activity in uncontaminated samples than that of the contaminated soil until the 20th day of the experiment, but since the 35th day

he observed increased, probably connected with end of initial adaptation phase microorganisms able to utilize hydrocarbons as a carbon source.

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Abstrakt: Przeprowadzone badania dotyczyły oceny aktywności dehydrogenaz w glebie, którą zanieczyszczono olejem napędowym. Dodatkowo oceniano wpływ modyfikacji stosowanych w procesie biodegradacji skażeń substancjami ropopochodnymi (zabiegi agrotechniczne, biostymulacja, bioaugmentacja). Doświadczenia prowadzono metodą *in situ* oraz *ex situ*. Stwierdzono, że olej napędowy stymulował aktywność dehydrogenaz. Ponadto wykazano statystycznie wysoce istotny wpływ stosowanych zabiegów remediacyjnych.

Słowa kluczowe: dehydrogenazy, olej napędowy, gleba