Biochemical Processes in Oil-Contaminated Soil in Relation to Phytoremediation

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Abstract –The usefulness of biochemical data in monitoring phytoremediation efficiency was studied. The oxidation enzymes activity and soil respiration were sensitive to soil oil pollution and plants cover.Catalase and dehydrogenase activity of oil-contaminated soil were stimulated by Zea mays* L. *vegetation, whereas the effect of Viciafaba L. was less significant.

Key words – soil, biochemical parameters, biological activity, phytoremediation, oil-contamination, soil respiration, oxidizing enzymes, catalase, dehydrogenase.

I. Introduction

Nowadays soil quality dramatically decreased all over the world. And oil hydrocarbons are the most frequently occurring environment contaminant, a typical side-effect of industrial activity. One of the safe and cost-effective treatments of oil-contaminated soil is phytoremediation. It is based on biochemical activity of soil organisms [[9]]. Despite extensive chemical technology application for monitor level of soil pollution little is known about biochemical processes during phytoremediation, including oxidation enzymes activity and CO_2 evolution by soil. The assessment of biological activity via biochemical analyses is a sufficient and valid tool to monitor oil pollution. Biological parameters reflect entire response of living system. This integral parameter can be more useful monitoring tool to predict potential threats to environment than chemical data alone.

The major task of this study is to prove the usefulness of soil biochemical parameters to monitor the efficiency of plant maize *(Zea mays* L.) and legume (*Vicia faba L.*) as remediation agents.

II. Materials and methods

The experiment was performed in field conditions during 19.04.13– 30.08.13. It consisted of sixmicrofield plots (1,2×0,6 м^2) and treated as follows:

Controls

C – uncontaminated soil, C+Z – uncontaminated soil planted with *Zea mays* L.; C+Vuncontaminated soil planted with*Faba bona*.

Contaminated with oil

O – soil contaminated with oil at the dose 10 l/m^2 and thoroughly mixed to the depth 15 cm after 2 months of contamination, O+Z – soil contaminated with oil (the same way) and planted with *Zea mays* L. O+V – soil contaminated with oil (the same way) and planted with *Faba bona* L.Plants were sown after 2 months of initial soil contamination by oil and tillage in order to evolve evaporation of most toxic hydrocarbons.

The soil samples were collected from the depth 3-5 cm before plants sowing and after 30 days plants vegetation: 90th day after pollution for *Z. mays* and 97th – for *V. fabaL.* (since the interval in emerging). Soil samples were air-dried in a dark room, mixed well, sieved through 1 mm sieve before analysis.

The following soil biological analyses were performed with three replicates.Soil respiration (SR): by CO_2 fixing in NaOH solution and alkali excess was titrated with HCl [[5]]. Dehydrogenase activity (DH): by a colorimetric method, using 2,3,5-triphenyltetrazoliumchloride (TTC) as substrate. Catalase activity (CAT): by aerometric method usinghydrogen peroxides substrate [[3]]. Total petroleum hydrocarbons content was estimated by combustion [[8]].

III. Results and discussion

During initial period of pollution volatile hydrocarbons, which are the most toxic, rapidly evaporate. It causes decrease in oil content in soil. After 2 months period residual oil content was 10% of dry soil weight – in the top 5 cm of oil spill, whereas 7,5% – after soil mixing.

TABLE I

SOIL BIOCHEMICAL PARAMETERS BEFORE PLANS VEGETATION (2 MONTHS AFTER CONTAMINATIN BY OIL)

Treatment	Soil respiration (CO_2 μg/g×h)	Catalase activity (ml O_2 /min×g)	Dehydrogenase activity (optical density of formazan solution)
C	1,8±0,09	0,43±0,02	0,254±0,01
O	5,5±0,2	0,34±0,02	0,112±0,02

TABLE II

SOIL OXIDATIVE ACTIVITY AFTER PHYTOREMEDIATIONOF SOIL CONTAMINATED WITH OIL

Treatment	Catalase activity (ml O_2 /min×g)		Dehydrogenase activity (optical density of formazan solution × 10^3)	
	Z. mays	*V. faba*	*Z. mays*	*V. faba*
C	0,35±0,05 a	0,42±0,03 c	66±0,01	106±0,002 g
C+Pl	0,4±0,01 a	0,4±0,01 c	102±0,01	75±0,01h
C+Plrh.	0,48±0,06	0,36±0,02 c	244±0,04	77±0,01h
O	0,17±0,03	0,35±0,03 d	225±0,02	106±0,01g
O+Pl	0,32±0,03 b	0,36±0,05 d	358±0,02	91±0,002
O+Pl rh.	0,33±0,03 b	0,35±0,07 d	313±0,01	134±0,02

Means with the same latter in row are not significantly different ($p<0,05$)

C – uncontaminated soil (control), C+Pl – uncontaminated soil from root zone of plants, C+Pl rh. – uncontaminatedsoil from rhizosphereof plants, O – oil

contaminated soil., O+Pl– oil contaminated soil from root zone of plants, O+Pl rh. – oil contaminated soil from rizosphere of plants.

As can be seen from Table I oil pollution definitely effected on soil biochemical processes. Respiration of oil contaminated soil is three times as large as for uncontaminated soil. Soil microbial respiration represents the sum of all metabolic processes producing CO_2 as result of an uptake of O_2 by metabolically active organisms living in soil [[10], [9]]. The rate of SR reflects both the amount and the quality of the C source [[5]]. In this case oil can be used as a sole source of organic C by microorganisms consequently resulting in intensification of SR. The enhanced levels of SR for polluted soil were observed next two months in planted and unplanted trials. Elevated meanings of SR probably indicate intensive oil consumption and degradation.

Soil enzymes are the catalysts of important metabolic processes including the decomposition of organic inputs and the detoxification of xenobiotic compounds [[6], [7]]. Oil contamination caused a slight inhibition of catalase activity and more notable decrease of dehydrogenase activity compered to control. This result can be attributed to toxic effect of oil. CAT exists in the soil and organisms widely. Its role is to catalyze the decomposition of hydrogen peroxide, which is harmful to the organisms. Meanwhile a high activity of CAT is important regarding its antioxidant function. A low CAT activity can indicate adverse conditions for oil degradation. Dehydrogenases are located only in intact living cells and its activity is negatively related to some toxic compounds [[9]]. Its activity also positively correlates with amount of microorganisms. The inhibited DH activity probably is related to decrease microbial abundance.

But after one month of plants vegetation the soil biochemical processes have shown a tendency to restoration (Table II). The differences also were observed between the same trials in one week range. For instance CAT activity increased in twice during one week, whereas DH restored to control level. Fluctuations can reflects dynamic in microbial populations and be caused by temperature changes. The limitation of biological parameters is it high liability and sensitivity to environmental factors [[7]]. Its can cause inconstancy of data. For more accurate explanation the physical and chemical parameters must be considered.

The data indicate that there is connection between enzyme activity and plants cultivation. CAT activity in trial C+Z was significantly affected by plans *Z. mays*, while no difference was observed for trial C+F. At the same time DH activity was enhanced after plant cultivation in trial C+Z, in trial C+V– only in rhizosphere. Furthermore the highest activity of both enzymes was observed in rhizosphere soil samples for polluted soils. This positive effect of plants can be due to the release of exudates and lysates of plant roots. It could stimulate the microbial growth and influence the diversity of microorganisms especially in rhizosphere [[1]]. It was proved that *Zea mays* L. and *Vicia faba* L. caused a higher oil dissipation and stimulation of microbial growth compared to unplanted soil. These two plants are recommended as phytoremediation agents [[2], [2]]. In fact biochemical data shows support of phytoremediation value of these two plants, but mention should be made of slight efficiency of *V. faba*.

Conclusion

The results support positive effect of *Z. mays* on soil biochemical activity, but undermined efficiency of *V. faba*. Nowadays it is still problematic to use biological parameters for environment protection. Nevertheless these parameters are promising and require further investigations. Thus the assessment of biochemical activity in soil is sufficient during phytoremediation processes.

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