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THE EFFICIENCY OF RHIZOSPHERE BIOREMEDIATION OF SOILS FROM INDUSTRIAL AREAS CONTAMINATED WITH POLYCYCLIC AROMATIC HYDROCARBONS (PAHs)

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Abstract. The effectiveness of rhizosphere bioremediation was tested in a greenhouse, pot study. Two soil materials (PS1 and PS2) collected from industrial areas contaminated with PAHs at the levels of: 12.9 mg Σ13PAHs/kg (PS1) and 22.1 mg Σ13PAHs/kg (PS2) were applied. Phytoremediation potential of four perennial plant species: meadow bluegrass, tall fescue, alfalfa and red clover was investigated. The effect of the rhizosphere bioremediation process was evaluated on the basis of the changes in PAHs residue in planted and unplanted soils (control) and the activity of soil microorganisms (dehydrogenases activity). The cultivated plants did not affect significantly PAHs dissipation in contaminated soils, although after the second year of vegetation the 3–7% decrease of PAHs concentration was observed. It was noticed that plant cultivation affected mainly dissipation of 3-ringed hydrocarbons. The decrease of PAHs concentration in cultivated soils did not correlate with increase of microbial activity.

Key words: rhizosphere bioremediation, polycyclic aromatic hydrocarbons, perennial plants, PAHs degradation.

Abstrakt. W doświadczeniu wazonowym przeprowadzonym w warunkach szklarniowych badano efektywność bioremediacji w ryzosferze. Zastosowano dwa rodzaje materiału glebowego (PS1 i PS2), które pobrano z obszarów przemysłowych, zanieczyszczonych WWA na poziomie: 12,9 mg Σ13WWA/kg (PS1) i 22,1 mg Σ13WWA/kg (PS2). W doświadczeniu uprawiano cztery rodzaje roślin wieloletnich: wiechlinę łąkową, kostrzewę trzcinową, lucernę mieszańcową i koniczynę czerwoną. Efektywność bioremediacji w ryzosferze oceniano na podstawie zmian zawartości WWA w glebach pod uprawą roślin i bez uprawy roślin (gleba kontrolna) oraz aktywności mikroorganizmów glebowych (aktywność dehydrogenaz). Stwierdzono, że uprawa roślin nie wpływała w istotny sposób na rozkład WWA w badanych glebach, niemniej jednak po upływie drugiego sezonu wegetacyjnego oznaczono spadek zawartości tych związków o 3–7%. Spośród badanych związków uprawa roślin wpływała głównie na rozkład 3-pierścieniowych WWA. Nie stwierdzono statystycznie istotnej korelacji pomiędzy spadkiem zawartości WWA a aktywnością mikrobiologiczną badanych gleb.

Słowa kluczowe: bioremediacja w ryzosferze, wielopierścieniowe węglowodory aromatyczne, rośliny wieloletnie, rozkład WWA.

INTRODUCTION

Soils contaminated with polycyclic aromatic hydrocarbons are difficult to bioremediate because PAHs are hydrophobic compounds, poorly soluble in soil solution and strongly absorbed by soil matrix. However, some plants may be able to assist the microorganisms to degrade organic pollutants.

Rhizosphere biodegradation is one of phytotechniques based on natural processes occurring in the rhizosphere zone of plants. Plants may release exudates to the soil environment to help microbial communities to metabolize organic contaminants by inducing enzyme systems of existing bacterial populations, stimulating growth of new species that are able to degrade pollutants,

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and/or increasing soluble substrate concentrations. The most widely used in rhizosphere bioremediation are grasses and papilionaceous plants which posses rich root systems that embrace larger soil volume compared to other plant species. The aim of the study was to evaluate of the effectiveness of rhizosphere bioremediadion as a tool for cleaning up soils contaminated for a long time with polycyclic aromatic hydrocarbons.

MATERIALS AND METHODS

Two different soil materials were used in the study: PS1 — heavy loamy sand (C_{org} = 3.54%, pH_{KCl} = 6.8), and PS2 — light loam (C_{org} = 5.93%, pH_{KCl} = 6.0). Soils were collected from industrial areas highly contaminated with PAHs: PS1 — from Zabrze Makoszowy, PS2 — from Czerwionka (both situated in Silesia district, Poland). Both soils were of agricultural use. PAHs content in PS1 soil was 12.9 mg Σ13PAHs[1]/kg and in PS2 soil — 22.1 mg Σ13PAHs/kg.

The studies included cultivation of four perennial plant species: two grasses: tall fescue (*Festuca arundinacea* Schreb. cv. Terros) Tf and meadow bluegrass (*Poa pratensis* L. cv. Alicja) Bg, and two papilionaceous plants: red clover (*Trifolium pratense* L.) Rc and alfalfa (*Medicago sativa* L. cv. Katon) Af.

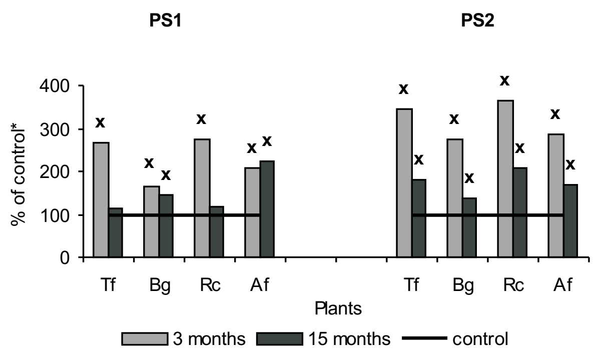
Before the experiment was carried out as a greenhouse pot study, soil material was air dried and sieved through ∅ 1 cm sieve. Then plastic pots were filled with 2.5 kg of soil and watered to the level of 60% of the full water capacity. Plant seeds were sowed and after germination, 10 the most equal seedlings were left in each pot. Every year single nitrate fertilisation was applied (NH_4NO_3) at the level of 33.3 mg N/pot. Plants were cultivated for 15 months and kept during the winter in the cold greenhouse. Unvegetated soils were used as a control. Each experiment (soil/plant) combination was carried out in two replications.

The effect of the phytoremediation process was evaluated on the basis of the determination of PAHs residue changes in planted and unplanted soils after 0, 3, 12, and 15 months. PAHs content in soils was determined according to the procedure described by Klimkowicz-Pawlas and Maliszewska-Kordybach (2003). To assess the activity of soil microorganisms, dehydrogenases activity test was applied (Casida *et al.*, 1964). The measurements were conducted after 0, 3, 12, and 15 months of phytoremediation.

RESULTS AND DISCUSSION

The effect of plants cultivation on dehygrogenases activity in soils PS1 and PS2 is presented on Figure 1. As it was expected, soils under plant cultivation were more microbiologically active (rhizosphere effect), although the observed effect decreased with time.

Higher biological activity of vegetated soils did not correspond to higher rates of PAHs degradation (Fig. 2). In both soils, at the beginning of plant growth the efficiency of phytoremediation was negligible. After longer time period (15 months), the influence of plant cultivation was still statistically insignificant, although small decrease (<3%) in PAHs, content in vegetated soils was noted.



* — control, unplanted soil, 100%

x — statistically different (α ≤0.05) from control

Fig. 1. Relative changes in dehydrogenases activity in vegetated soil as compared to unvegetated; control after 3 and 15 months of phytoremediation

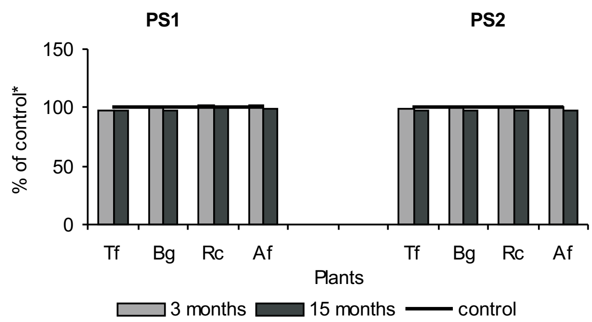


Fig. 2. Relative changes in Σ13PAHs content in vegetated soil after 3 and 15 months of phytoremediation

[1] fluorene+anthracene+phenanthrene+pyrene+fluoranthene+benzo(a)anthracene+chrysene+benzo(b)fluoranthene+benzo(k)fluoranthene+benzo(a)pyrene+dibenzo(a,h)anthracene+benzo(g,h,i)perylene+indeno(1,2,3-cd)pyrene

The loses of Σ13PAHs as related to the initial PAHs concentration in soils were between 3% and 7%, and corresponded to 867 and 699 μg Σ13PAHs/kg for bluegrass, 860 and 660 μg Σ13PAHs/kg for tall fescue, 377 and 211μg Σ13 PAHs/kg for red clover, and 616 and 660 g Σ13PAHs/kg for alfalfa, for soils PS1 and PS2, respectively.

To evaluate the influence of PAHs properties on the rate of their dissipation in soils, contaminants were divided into three groups in relation to the ring number in the particle: 3-ringed compounds which are easily soluble in soil solution, more susceptible to microbial transformation, and more volatile, 4-ringed compounds which are less soluble and degradable with higher affinity to soil organic matter, and 5+6-ringed compounds, hydrophobic with very strong affinity to soil particles.

As it can be seen from Figure 3, plant cultivation in soils affected mainly dissipation of 3-ringed compounds (18–23% of losses in case of bluegrass and alfalfa, respectively). Among 3-ringed PAHs the most degraded was phenanthrene characterised by high water solubility.

Phytoremediation is a technique very hard to control. It is influenced by different factors occurring in the multidimensional soil/plant/pollutant/microorganisms system. Lack of the relationship between parameters describing the soil biological activity and the rate of PAHs dissipation can be assign to the fact that dehydrogenases activity describes the activity of the total microbial population, and not only the specific species active in PAHs degradation processes.

The presented data indicated that effect of plants cultivation on the increase of PAHs dissipation from soils polluted for a long time with these contaminants was very weak. Better results were received in other works (Reilley *et al*., 1996; Frick *et al*., 1999) in which freshly polluted soils were applied. It seems that "aging" of PAHs leading to the lower bioavailability of these compounds is the main factor limiting the effectiveness of phytoremediation. Very low efficiency of phytoremediation process may also be related to the lower abiotic losses of PAHs (volatilisation) from vegetated soils (lower evaporation of water) or/and sorption of PAHs on plant roots which create the additional source of organic matter in soil.

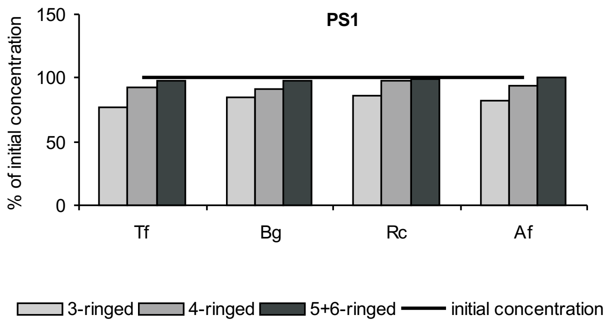


Fig. 3. Losses of 3-, 4- and 5+6-ringed PAHs in soil PS1 after 15 months of phytoremediation as related to the initial PAHs concentration

CONCLUSIONS

Effectiveness of phytoremediation in soils for a very long time polluted with PAHs was low.

The highest influence of plant cultivation on PAHs dissipation was noticed for 3-ringed compounds.

The changes in soil microbial activity did not correlate with the efficiency of phytoremediation.

The low efficiency of phytoremediation was not related to soil properties or plant species.

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