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CELLULOLYTIC ACTIVITY OF MICROFLORA IN SOIL CONTAMINATED WITH AROMATIC HYDROCARBONS

CELULOLITYCZNA AKTYWNOŚĆ GLEBY ZANIECZYSZCZONEJ WĘGLOWODORAMI AROMATYCZNYMI

Abstract: This study evaluated the influence of contamination with aromatic hydrocarbons on the efficacy of cellulose decomposition with the use of soil cellulolytic microorganisms. In the experiments we used the monoaromatic hydrocarbons from BTEX group (benzene, toluene, ethylobenzene and xylene – a mixture) and polycyclic compounds (naphthalene and anthracene) used in the following concentrations: 100, 1.000 and 10.000 mg · kg^{-1} d.m. of soil.

We found out that usually the presence of aromatic hydrocarbons stimulated the cellulolytic activity of soil microflora – the higher activity was noted in the soil contaminated with PAH (anthracene). The percentage of cellulose decomposition was increased along with the increase of monoaromatic hydrocarbon dosage (except toluene); a similar dependence was not noted for polycyclic aromatic hydrocarbons (PAH).

Keywords: cellulolytic activity, microorganisms, aromatic hydrocarbons

The living organisms are poisoned with substances of different origin. Aromatic hydrocarbons are a very harmful group of compounds, as they have a carcinogenic effect. The disturbance of balance between creation and decomposition of polycyclic aromatic hydrocarbons in the natural environments, including soil, causes the increase of concentration of xenobiotics in ecosystems, and as a result of that, disturbance of biologic balance [1].

The soil microorganisms, thanks to their numerous biologic properties and biochemical abilities, are a necessary factor for metabolism. Participating in different biochemical processes they play an important role in functioning of all of the land ecosystems. The result of their activity is not only mineralization and humification of different organic compounds (including synthesis of humus – the largest bioenergetic potential of soils), but also activation of many mineral compounds, having basic meaning for the life of plants and soil animals [2, 3]. The petroleum hydrocarbons

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introduced to the soil exert an influence on soil respiration, microbial biomass carbon or enzyme activities [4–6].

In the accessible literature there is not too much data concerning the influence of aromatic hydrocarbons on the activity of particular groups of soil microorganisms, which are responsible for stabilization – homeostasis of ecosystem, through composition and decomposition of organic compounds in soil, and also through release and storage of foods [7], while enzymatic reactions are a base of soil metabolism, they decide about the rate and direction of metabolic changes, that take place in the soil [8], have been used to estimate ecosystem functioning and soil fertility [9]. Microbial cellulolytic activities in soil are important indicators of soil health and quality [10, 11].

The aim of this study, considering a very important role of microflora in a proper functioning of soil environment, was evaluation of the influence of chosen monoaromatic hydrocarbons and polycyclic hydrocarbons on the cellulolytic activity of soil microorganisms.

Materials and methods

For our studies we collected some soil samples from Ostoja situated near by Szczecin. It was a clay soil, black ground type. The material was collected from the depth of 0–15 cm of the humus level. After transportation to the laboratory, we dried and ground the soil using the riddle with 2 mm orifices. The material was brought to 60 % MPW. This humidity was constant for the entire time of experiment, in case it was necessary the losses were replenished with distilled water.

The material was divided into small samples, each one of 300 g, and placed in polyethylene containers. Later on, the samples were contaminated with the following doses of hydrocarbons: 100, 1,000 and 10,000 mg $\cdot$ kg^{-1} d.m. of soil. In the experiments we used the monoaromatic hydrocarbons from BTEX group (benzene, toluene, ethylobenzene and xylene – a mixture) and polycyclic aromatic compounds (naphthalene and anthracene). One of the samples, not contaminated with the hydrocarbons was left as a control sample. In order to assess the cellulolytic activity we inserted the cellulose samples into the bags with soil. Each bag had 6 cardboards of the 2 × 2 cm dimensions and the average weight of 350 mg. The soil samples were incubated at room temperature (± 20 ^{o}C).

The level of cellulose decomposition was evaluated based on the differences of weight between the cardboard inserted into the contaminated soil and after finishing of the experiment – a modified method according to Kuzniar [12]. After its taking out, the cellulose cardboard was dried at a temperature of 105 ^{o}C, until receiving of a constant weight. The analysis was carried out on the day of contamination of the soil with hydrocarbons and later after 7, 14, 28, 56 and 112 days of incubation. The study results were analysed statistically using two-way analysis of variance.

Results

Along with the increase of benzene dosage in the studied objects we could observe the increase of activity of cellulolytic microorganisms, but for the dose of 100 and 1,000 mg · kg^{-1} d.m. of soil the value was lower compared with the control object, and it was 16 and 7 %. After use of the highest dose (10,000 mg · kg^{-1} d.m. of soil) we noted the increase of activity of 10 % compared with the control (Fig. 1A).

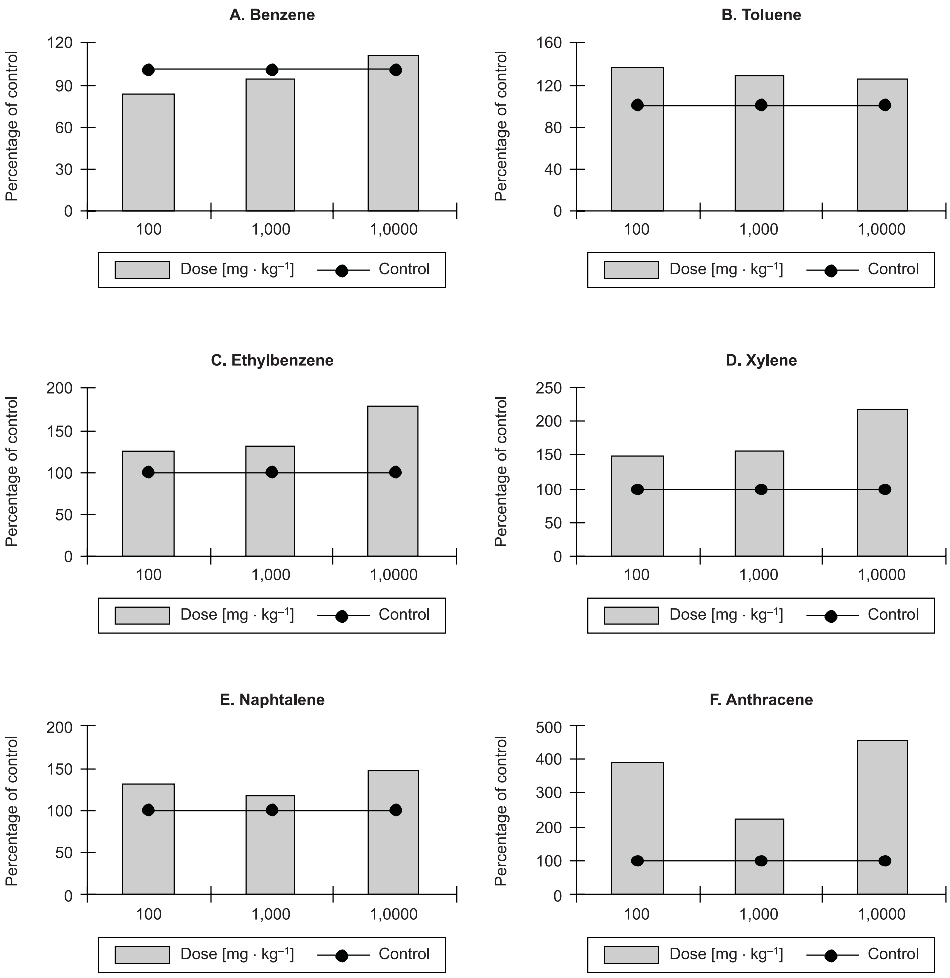

Fig. 1. The influence of hydrocarbons on the cellulolytic activity of soil microorganisms expressed as a control percentage

Toluene implemented to the soil caused increase of activity of cellulose decomposing microorganisms compared with the control, however the activity decreased along with the increase of hydrocarbon concentration (Fig. 1B). Under the dosage of 100 mg · kg^{-1} d.m. of soil the weight loss was 90 mg, while the dose of 10,000 mg · kg^{-1} d.m. of soil caused a loss which was 20 mg lower.

The addition of ethylbenzene caused increase of cellulolytic activity compared with the control, and the higher the dose was the higher the increase was. For the concentration of 10,000 mg · kg^{-1} d.m. of soil the activity was 80 % higher compared to the control, and for the dose of 100 mg · kg^{-1} d.m. of soil – 25 % (Fig. 1C).

After implementation of xylene into the soil we observed a similar reaction. Along with the increase of hydrocarbon's dosage we observed an increasing loss of weight of the cellulose cardboard. In case of xylene we could also observe the increase of cellulolytic activity. For the highest concentration the increase of cellulose decomposing microorganisms was over 100 % compared with the control object (Fig. 1D).

In all of the experiments with naphthalene, just like in case of hydrocarbons from BTEX group, we observed the stimulation of activity of cellulolytic microorganisms, however in this case we did not reveal any dependence between the enzymatic activity and increasing concentration of hydrocarbons – the lowest activity was noted in the object with the dose of 1,000 mg · kg^{-1} d.m. of soil (Fig. 1E). The weight depletion for this dose was 65 mg – which is 15 mg less than in case of the highest dose (the increase of activity for this object was 45 % above the control sample).

Anthracene implemented to the soil also stimulated the cellulolytic activity of microorganisms. For the dose of 10,000 mg · kg^{-1} d.m. of soil the activity increased over 350 % compared with the control (Fig. 1F). The weight depletion for this object was almost 200 mg. The lowest activity of microorganisms decomposing cellulose was assessed for the concentration of 1,000 mg · kg^{-1} d.m. of soil – the loss of weight for this dose was 100 mg (the increase of activity was 120 % compared with the control object).

The carried out statistical analysis showed a significant effect of the hydrocarbons dose and incubation days on the cellulolytic activity, but no significant effect of the interaction of both examined factors (Table 1).

Table 1

Results of statistical analysis for the cellulolytic activity of soil microorganisms

Factor	Number of indepen-dent variables	Mean square sum.	Number of independent variables for error	Mean square sum for error	F value	P value
1	5	0.092522	306	0.001524	60.69422	0.00*
2	2	0.019119	306	0.001524	12.54230	0.00*
1 · 2	10	0.001201	306	0.001524	0.78787	0.64

Factors: 1 – measurement period, 2 – dose of hydrocarbons
* – a significant effect of a factor ($p < 0.05$).

Discussion and conclusions

The results of this study illustrate a significant influence of the aromatic hydrocarbons used in the experiments on the activity of the soil microorganisms. Also Zablocka-Godlewska and Buczkowska-Wesolowska [13] assessed that the hydrocarbons implemented to the natural environment like soil, can have a very variable influence on the biologic balance as well as on the quantity and quality changes of microorganisms.

It is assessed that usually the presence of aromatic hydrocarbons in soil stimulated the cellulolytic activity of microorganisms. A similar situation after implementation of the diesel fuel, which is a mixture of hydrocarbons, was observed by Daca et al [14] – the percentage of decomposed cellulose in soil was increased of 40 % compared with the control. Higher decomposition rates of organic matter in the fuel polluted soil than in the uncontaminated was also reported by Griffiths et al [15]. Cellulose is decomposed by aerobic bacteria, anaerobic bacteria, thermo- and mesophylic bacteria, different kinds of fungi, actinomycetales and protozoans, and for most of those organisms cellulose is one of the possible sources of carbon and energy [8]. Therefore such reaction could have been the result of activation of soil microflora influenced by the additional source of carbon.

For benzene, ethylbenzene and xylene the activity increased along with the increase of hydrocarbon concentration. Activities of many soils enzymes are often correlated with organic C content [16, 17], but we did not note any significant dependence after the use of polycyclic aromatic hydrocarbons – the lowest activity to decompose the cellulose was observed for the dose of 1,000 mg $\cdot$ kg^{-1} d.m. of soil. No significant relationship between the concentration of hydrocarbons and cellulose decomposition was observed by Mendelssohn and Slocum [18]. Despite of the dosage of hydrocarbon, in the objects contaminated with naphthalene as well as with anthracene, the cellulose decomposition was more effective than in control sample. Few studies suggest that decomposition should increase with petroleum hydrocarbon addition [16, 19], but other available results of investigation concerning of cellulose decomposition are not synonymous. In the studies of Maliszewska-Kordybach et al [20] the decrease of enzymatic activity due to PAH was observed on the level of 10 mg $\cdot$ kg^{-1} d.m. of soil. Inhibition of cellulolytic activity was observed also by other authors. Małachowska-Jutsz et al [21] found out that the activity of cellulase remained constant for the entire period of experiment and it was about 95–97.5 % of the control sample. The authors found that the activity did not depend of the kind and dosage of xenobiotic. Similar results had also reported Surygala [22] and Przystas et al [8].

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CELULOLITYCZNA AKTYWNOŚĆ GLEBY ZANIECZYSZCZONEJ WĘGLOWODORAMI AROMATYCZNYMI

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Abstrakt: W pracy określano wpływ zanieczyszczenia węglowodorami aromatycznymi na efektywność rozkładu błonnika przy udziale celulolitycznych mikroorganizmów glebowych. W doświadczeniach wykorzystano węglowodory monoaromatyczne z grupy BTEX (benzen, toluen, etylobenzen i ksylen – mieszanina) oraz wielopierścieniowe (naftalen i antracen), które stosowano w stężeniach 0, 100, 1000 i 10 000 g $\cdot$ kg^{-1} s.m. gleby.

Stwierdzono, że na ogół obecność węglowodorów aromatycznych stymulowała celulolityczną aktywność mikroflory glebowej – większą aktywność odnotowano w glebie zanieczyszczonej naftalenem i antracenem. Procent rozkładu błonnika zwiększał się wraz ze wzrostem dawki węglowodorów monoaromatycznych (z wyjątkiem toluenu); podobnej zależności nie odnotowano dla węglowodorów wielopierścieniowych.

Słowa kluczowe: aktywność celulolityczna, mikroorganizmy, węglowodory aromatyczne