Response of Zea Maize and Microorganisms to Soil Pollution with Polycyclic Aromatic Hydrocarbons (PAHs)

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Abstract

Our research aimed to assess the impact of soil pollution with polycyclic aromatic hydrocarbons on the amount of plant biomass produced and the numbers of selected groups of soil microorganisms. The aromatic hydrocarbons supplied to the soil were decomposed to various degrees, but we discovered a considerable decrease in detectable PAHs in soil. In strongly polluted soils the detected amount was the lowest for fluorene, then for benzo(a)pyrene and finally for chrysene. The greatest amounts of maize biomass (the aboveground parts and roots) were assessed on the treatment, where soil revealed elevated PAH content (I). Introducing PAHs to the soil caused quantitative changes in the soil microbiocenotic composition. The numbers of bacteria, fungi and actinomycetes were greater in the soil from treatments polluted with PAHs (I and II) than in the control soil (K).

Introduction

Polycyclic aromatic hydrocarbons (PAHs) constitute a diverse group of compounds with polycyclic structures and different arrangements of benzene rings in the molecule. These compounds may pollute the environment and they might come from fuel-burning processes, and industrial processes such as production of plastics, coke, asphalt or petroleum cracking. Moreover, hydrocarbons enter the environment as a result of abrasion of asphalt surfaces, or tire and waste burning, or its application into the soil [1-4].

Polycyclic aromatic hydrocarbons (PAHs) generally do not occur in the environment as single compounds, more often they are present in several congeners. Their quantitative and qualitative composition may be determined by e.g. the type of material burned or conditions under which the process takes place [2].

Excessive PAH concentrations in soils pose a hazard both to human health and to all biotic elements of the soil ecosystem [5-8].

The majority of PAHs polluting the environment accumulate in soil and the microorganisms living there are exposed to the negative effect [9].

Under favourable soil conditions PAHs may undergo microbiological degradation and, to a lesser degree, physicochemical decomposition [1, 9-11].

The aim of this work was to assess the effect of soil pollution with PAHs on the plant biomass produced and on the number of selected groups of soil microorganisms.

Methods

Experimental Design

The studies were conducted as a pot experiment. Soil material used for the experiments was collected from the (0-20 cm) horizon of the arable field.

Three hydrocarbons; benzo(a)pyrene (BaP), chrysene (Ch) and fluorene (Fl) from the PAH group with diverse physicochemical properties were applied. Due to the fact that generally soil environment is never polluted by single compounds from this group, their mixtures were used in the experiment [1, 6, 12]. Benzo(a)pyrene (BaP), chrysene (Ch) and fluorene (Fl) were added to the soil as liquids in the amount of 100 µg·kg^{-1} and 10,000 µg·kg^{-1} soil d.m. of each substance. An appropriate quantity of PAHs was dissolved in dichloromethane. The experiment comprised: control treatment (K) – soil with natural content of analyzed PAHs, treatment with dichloromethane supplement (0), treatment with 30 µg·kg^{-1} soil d.m. of PAHs (100 µg (BaP) + 100 µg (Ch) + 100 µg (Fl) corresponding to elevated content (I) and the treatment with a supplement of 30,000 µg·kg^{-1} soil d.m. of PAHs (10,000 µg (BaP) + 10,000 µg (Ch) + 10,000 µg (Fl) corresponding to very strong pollution (II) [13].

Soil Material

The research was conducted on the soil of silt sandy loam texture with 26% of < 0.02 mm fraction. The soil material revealed a slightly acid reaction (pH H$_2$O=6.27), hydrolytic acidity (Hh) assessed after the soil extraction with 1 mol·dm^{-3} CH$_3$COONa was 23.9 mmol (+)·kg^{-1} soil d.m. Organic carbon content was 15.99 g C·kg^{-1} and total nitrogen 1.54 g N·kg^{-1} soil d.m. The soil material had natural content of PAHs and heavy metals [13].

Experimental Methodology

The pot experiment was conducted for 60 days. 8.6 kg air-dried soil material was weighed into PVC containers and solutions of 3 PAH mixtures in dichloromethane were added. In order to meet the plant nutritional requirements, chemically pure salt solutions containing nitrogen, phosphorus and potassium were added to the soils of (0, I and II) treatments. The amounts of nutrients (N, P and K) introduced per kg of soil were, respectively: 0.12 g N [NH$_4$NO$_3$]; 0.06 g P [Ca(H$_2$PO$_4$)$_2$·H$_2$O]; 0.19 g K [KCl]. The research was carried out in four replications at constant moisture of soil material maintained during the vegetation period at 60% of the soil water capacity. Maize "San" c.v. was cultivated in the experiment - 5 plants per pot grown until harvest time.

After harvesting aboveground parts, roots were picked from the soil block and washed. In order to assess dry weight, the plant material was dried in a dryer with air flow (at 70ºC) until constant weight was reached.

Analytical Methods

Samples of soil material collected separately from each pot were dried at room temperature. Dry material was sieved through a sieve with 1 mm mesh. In the soil material prepared in this way pH was determined in the soil and water suspension by potentiometer, electrolytic conductivity by conductometer, organic carbon content after wet mineralization in potassium dichromate(VI) with Tiurin's method [14]. Selected trace elements were extracted from soil using 0.01 mol·dm^{-3} CaCl$_2$ solution for two hours with soil-solution ratio maintained at 1:10 [15]. The contents of Zn, Cu, Ni, Pb and Cd in the obtained solutions were determined with ICP-AES method using JY 238 Ultrace apparatus.

PAHs were extracted from the soil material by dichloromethane using microwave Mars system at the temperature of 120ºC for 40 minutes [16]. PAHs were determined by means of gas chromatograph coupled with a Varian GC/MS/MS 4000 spectrometer. Non-polar column Factor Four VF-5 MS, 30 m long and with 0.25 mm diameter and split/splitless dispenser (at 320ºC) were applied with the following temperature schedule: 40ºC, 30ºC/min→250ºC, 10ºC/min→320ºC. The analyses were conducted using external standard method with 610 PAH Calibration Mix A standard manufactured by Restek Enterprise. The following 11 compounds were determined in the obtained extracts: naphthalene (Nf), acenaphthylene (Acf), acenaphthene (Ace), fluorene (Fl), phenanthrene (Fen), anthracene (Ant), fluoranthene (Flu), pyrene (Pir), chrysene (Ch), benz(a)anthracene (BaA) and benzo(a)pyrene (BaP). The research presented in this paper demonstrated the content of benzo(a)pyrene (BaP), chrysene (Ch) and fluorene (Fl) and the total of 11 determined PAHs.

Microbiological assessments were conducted in fresh soil material. Samples of the soil material for microbiological analyses were collected to sterile vessels by a sterile botanical knife and subjected to microbiological analysis within 24 hours from the sample collection. Until microbiological analyses the samples were stored in cold storage (at 5ºC). After samples sifting through a sieve with 1 mm mesh, weighed portions (10 g) of material were prepared, which were removed under sterile conditions into 90 cm^3 of physiological solution and shaken for 30 minutes. Quantitative analysis was conducted using serial dilution test. The analyzed material was seeded on selected media and after an appropriate incubation period (Table 1) the units forming colonies were counted and the obtained results were converted into 1 kg of soil dry mass.

Table 1. Conditions of incubation.

Microorganisms	Temperature (ºC)	Time of incubation
Mesophilic bacteria	37	48 hours
Fungi	28	3 days
Azotobacter, Actinomycetes, amylolitic bacteria, nitrifiers and denitrifiers bacteria	28	5 days

Table 2. Some properties of soil.

Objects	pH H$_2$O	Electrolytic conductivity mS·cm^{-1}	Organic C g·kg^{-1} d.m. of soil
K	6.35c	0.086ab	15.18a
0	6.29bc	0.094b	15.84a
I	6.17a	0.073a	15.32a
II	6.27b	0.080ab	15.31a

Means in the same column followed by the same letters are not significantly different ($\alpha < 0.05$).

Amylolytic bacteria, denitrifiers and nitrifier count was determined on liquid media [17-19].

Analyses of the experimental soil material were conducted in four and of initial soil in two replications. A reference sample AG-2 (AgroMAT) was added to each series of the analyzed material and the result was considered reliable if relative standard error (RSD) did not exceed 5%.

Statistics

The obtained results were elaborated statistically according to the stable model where the factor was PAHs pollution level. A one-way ANOVA was used for the statistical computations and the significance of differences was estimated using Tukey test at the significance level $\alpha < 0.05$ [20].

Results and Discussion

Soil pollution with PAHs did not cause any notable changes to the soil reaction, and the only factor which changed the value of soil pH in the conducted experiments was the introduced mineral fertilization (Table 2). Despite small differences, it caused a significant decline in the soil reaction on treatments (0, I and II) in comparison with the control.

Regardless of the treatment, the determined value of soil electrolytic conductivity was lower than in the soil prior to the experiment outset (0.128 mS·cm^{-1}) (Table 2). The lowest value, however significant, of soil electrolytic conductivity was recorded in the soil with elevated PAHs content (I), where the quantity of maize biomass was the largest. According to Bastida et al. [21] salt concentration in the soil solution is associated not only with their absolute value but with the soil material moistening state, whereas according to Badia [22] it is also linked with the rate of processes mainly involved in mineralization of organic compounds. In the presented research soil moistening was maintained on a steady level (60% of soil water capacity) to provide optimal growing and development conditions for plants, so the cause of decreasing soil electrolytic conductivity may be due to the uptake of minerals from soil by plants.

Table 3. Content of mobile forms of heavy metals in soil (mg·kg^{-1} d.m. of soil).

Objects	Zn	Cd	Cu	Pb	Ni
K	0.64a	trace	0.127a	0.056a	0.061a
0	0.66a	trace	0.110a	0.038a	0.077a
I	0.70a	trace	0.105a	0.041a	0.061a
II	0.65a	trace	0.087a	0.036a	0.056a

Means in the same column followed by the same letters are not significantly different ($\alpha < 0.05$).

The content of organic carbon in soil for individual treatments did not differ significantly (Table 2). The highest content of organic carbon was in the soil from the dichloromethane treatment (0). However, the content was smaller than determined in the soil before the experiment outset. The ecotoxicological effect of xenobiotics in soil environment depends on the soil properties. In the case of soil pollution with hydrocarbons the content of organic matter plays a crucial role [23-25]. Organic mater sorbs a considerable amount of these compounds, on the one hand decreasing their bioavailability but on the other hand increasing persistence of these pollutants in soil environment [26]. In the conducted experiment organic matter contents in the soils of individual treatments were not differentiated, therefore no significant differences in organic C were noted and a decline in this component content in relation to its content in soil before the experiment started resulted from processes of organic substance mineralization.

For a more precise determination of the soil chemical properties, the contents of trace element mobile forms were determined (Table 3). Irrespective of the analyzed element, the amounts were small and the statistical analysis of results did not reveal any significant differences among soils from individual treatments. The quantity of the extracted trace elements, irrespective of experimental treatment, may be arranged in the following decreasing order Zn > Cu > Ni > Pb > Cd. The main determinant of trace element mobility is soil reaction [27]. Soil pH in the experiments was slightly acidic, which may have led to the relatively poor mobility of the analyzed elements.

The processes of organic pollutant decomposition in soil may occur effectively under favourable conditions, especially at proper moisture, temperature, soil pH, aeration and availability of trophic substances [28-30]. The processes of organic pollutant biodegradation are additionally conditioned by the pollutant concentration and dependant on the first stages of transformations as a result of which indirect products of changes may reveal much stronger toxic effects. In the conducted experiment aromatic hydrocarbons added to the soil became decomposed to a various degree (Table 4). A considerable decrease in the content of PAHs supplied to the soil was registered depending on the pollution level. It should be emphasized that the content of the analyzed PAHs was not assessed in the control soil (K) of the object (0) or on the object with polluted soil (I).

Table 4. PAH content in soil (µg·kg⁻¹ d.m. of soil).

Objects	Benzo(a)pyrene (BaP)	Chrysene (Ch)	Fluorene (Fl)	Total 11 PAH
K	0a	0a	0a	109a
0	0a	0a	0a	123a
I	0a	32b	24b	155b
II	1320b	2536c	906c	4944c

Means in the same column followed by the same letters are not significantly different ($\alpha < 0.05$).

Considering the content of hydrocarbons on the object with polluted soil introduced into the strongly polluted soil, the a smallest amount of fluorene (Fl) was assessed, then benzo(a)pyrene (BaP) and finally chrysene (Ch). Despite a smaller molecular mass of chrysene, it was benzo(a)pyrene (BaP) which underwent a faster biodegradation. According to Maliszewska-Kordybach [31] the rate of PAH breakdown in soil is not directly dependant on total count of soil microorganisms, but on the presence of specific microbiological populations capable of PAH breakdown. On the basis of obtained results it may be stated that under conditions of conducted experiments the soil microflora caused a faster biodegradation of benzo(a)pyrene (BaP) than chrysene (Ch). According to the author quoted above [31] a fast decrease in the concentration of lighter PAHs – fluorene (Fl) to the value of 10% of the initial concentration occurred after only 30 days of incubation, but it depended on soil type. Moreover, general PAHs persistence depends on the quantity of organic substance in soil and increases when its amount grows. Relatively fast fluorene (Fl) biodegradation may result from the use of this substance by microorganisms as a potential source of carbon and energy [32].

Analysis of the total content of determined hydrocarbons revealed the greatest number of these compounds in the strongly polluted soil (II) to which application of the studied PAHs to the soil definitely contributed (Table 4). Differences in PAH content between the other treatments were statistically insignificant.

The effect of soil pollution with PAHs on growth and development of plants depends not only on the species but also, as stated by Maliszewska-Kordybach and Smreczak [7] and Totsche and Danzer [26], mainly on the soil properties and primarily on its organic matter concentrations. The quantity of maize biomass produced in the presented investigations on the treatments where the soil was polluted with PAHs was higher than on the control treatment (unpolluted and unfertilized soil) (Fig. 1). Therefore, it may be stated that soil pollution with the analyzed PAHs did not inhibit growth or development of maize. Analysis of maize biomass (the aboveground parts and roots) showed their greatest amounts on the treatment where the soil revealed elevated content of PAHs (I). Also Maliszewska-Kordybach and Smreczak [7] describe the stimulating effect of PAHs on plant yield at the level of these substances in soil below

or slightly above 1,000 µg·kg⁻¹ soil d.m. Kummerowa et al. [33] revealed that PAH concentration not exceeding 10,000 µg·dm⁻³ in a solution may intensify plant biomass increment.

Soil biological activity was assessed on the basis of the selected physiological microorganism groups. A supplement of polycyclic aromatic hydrocarbons to the soil caused quantitative changes in the composition of soil microflora [34]. According to Nowak et al. [35] deposition of oils, greases, petrol or other oil derivatives in the environment leads to impoverishment of its microflora species composition. In the event of oil spills the number of aerobic bacteria decreases dramatically, whereas the number of microorganisms assimilating hydrocarbons and their components increases [36].

Among bacteria *Azotobacter*, which fixes molecular nitrogen from the atmosphere, plays a specific role. In the presented experiments bacteria of this kind were found only in the soil of the control treatment. Besides, soil pollution with PAHs also introduced dichloromethane used as a solvent that negatively affected *Azotobacter* bacteria presence. *Azotobacter* is most sensitive to all unfavourable changes in the soil environment (Table 5). According to Kołwzan [36], research conducted on bioremediation of contaminated lands also revealed a decline in the numbers of nitrogen-fixing bacteria. Research conducted by Jastrzębska [37] demonstrated that increased content of fungicides in soil may have an inhibitory effect on *Azotobacter* bacteria multiplication.

Fungi are mainly saprophytic heterotrophs, which may function even under the most unfavourable conditions. The lowest number of these microorganisms was found in the control soil, the largest number in the strongly PAH-polluted one (Table 5). Smaller quantities of PAHs supplied to the soil did not inhibit the development of this microorganism group. The obtained results confirm relatively considerable fungi resistance to soil PAH pollution and even stimulatory effect of these substances on the numbers of this microorganism group.
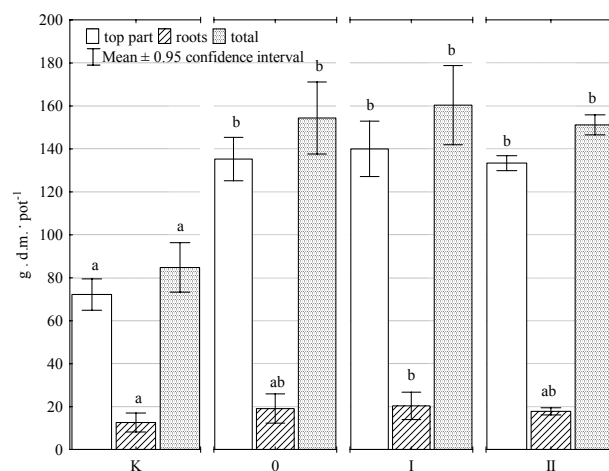

Fig. 1. Biomass of maize.
Means followed by the same letters are not significantly different ($\alpha < 0.05$).

Table 5. Changes in number of fungi, actinomycetes and total number of bacteria in soil.

	Azotobacter cfu·g⁻¹ d.m. of soil	Fungi cfu·10³·g⁻¹ d.m. of soil	Actinomycetes cfu·10⁴·g⁻¹ d.m. of soil	Bacteria cfu·10⁵·g⁻¹ d.m. of soil
K	124	44a	143a	123a
0	0	829c	180a	1315c
I	0	413b	522c	1110c
II	0	1263d	374b	660b

Means in the same column followed by the same letters are not significantly different ($\alpha < 0.05$).

The Actinomycetales are microorganisms revealing both features of bacteria and filamentous fungi. The smallest number of these microorganisms was assessed in the control soil and in the soil from treatment with added dichloromethane (Table 5). A significant reduction of the Actinomycetales, in relation to the number determined in the soil of treatment with elevated PAH content, was found in strongly polluted soil.

Soil pollution with the analyzed PAHs did not cause any major changes in starch-decomposing bacteria activity or in the activity of bacteria participating in nitrogen transformations (Table 6). Microorganisms little sensitive to oil derivatives are bacteria from *Baccilus* and *Pseudomonas* genera and a majority of Actinomycetales. The other microorganisms resistant to toxic reactions of oil-derivative compounds are bacteria from *Arthrobacter, Micrococcus, Flavobacterium, Vibrio, Aeromonas, Acinetobacter* and *Mycobacterium* genera, as well as *Candida, Fusarium, Penicillium, Aspergillus, Saccharomyces, Rhizopu* and *Geotrichum* fungi, whereas many genera of Actinomycetales participating in hydrocarbon degradation include *Actinomycetes, Nocardia* and *Streptomycetes* [38].

The total number of bacteria was larger in the soil with mineral fertilization and dichloromethane (0) and with PAHs (I and II) than the quantity assessed in the control soil (K) (Table 5). According to Siuta [39] in order to increase microorganism number, it is necessary to supply other mineral nutrients, such as: nitrogen, phosphorus, sulphur, calcium, magnesium and potassium. Fertile soils contain all indispensable components, whereas in polluted soils the ratio of organic carbon amounting to the quantity of phosphorus and nitrogen is radically distorted. A big deficit of the latter elements caused by an excess of carbon causes microorganisms to be unable to utilize fully the energy contained in hydrocarbons. This is due to the fact that protein synthesis is crucial for microorganism development, which cannot take place without nitrogen, phosphorus and sulphur participation.

Conclusions

1. Aromatic hydrocarbons introduced into the soil were broken down to varying degrees, still a considerable decrease in the content of analyzed PAHs was registered.
2. In strongly polluted soils, the detected amount was the lowest for fluorene, then for benzo(a)pyrene and finally for chrysene.

Table 6. The occurence of some microorganisms in soil.

Objects	Bacteria		
	Amylolytic	Nitrifiers	Denitrifiers
K	10⁻⁵a	10⁻⁶a	10⁻⁶a
0	10⁻⁶a	10⁻⁶a	10⁻⁶a
I	10⁻⁶a	10⁻⁶a	10⁻⁶a
II	10⁻⁶a	10⁻⁶a	10⁻⁶a

Means in the same column followed by the same letters are not significantly different ($\alpha < 0.05$).

3. PAHs supplied to the soil did not reduce maize biomass increment. The largest amount of plant biomass (the aboveground parts and roots) was registered on the treatment where the soil revealed elevated PAH concentrations (I).
4. Introduction of polycyclic aromatic hydrocarbons to the soil caused quantitative changes in soil microbiocenotic composition. The number of bacteria, fungi and actinomycetales was higher in the soils of treatments polluted with PAHs (I and II) than in the control soil (K). No Azotobacter bacteria presence was noted in PAH-polluted soil.

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