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Original Research

Urease Activity in Soil Contaminated with Polycyclic Aromatic Hydrocarbons

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Abstract

Urease activity was determined in soil contaminated with four polycyclic aromatic hydrocarbons (PAHs): naphthalene, phenanthrene, anthracene, and pyrene in the amount of 0, 1,000, 2,000, and 4,000 mg·kg^{-1} DM soil. Organic materials – cellulose, sucrose, and compost – were applied to the samples in the amount of 0 and 9 g·kg^{-1} DM soil. The experiment was carried out in a laboratory, and soil samples consisted of loamy sand. Soil resistance (RS) and soil resilience (RL) were determined. Soil contamination with PAHs had an adverse effect on urease activity, and naphthalene had the most inhibitory impact on the studied enzyme. Urease activity was significantly determined by the dose of PAH, soil incubation time, and the type of organic material. Soil resistance to PAHs decreased with an increase in contamination levels. The addition of sucrose, cellulose, and compost increased soil's resistance to the toxic effects of naphthalene and phenanthrene. Soil resilience values indicate that polycyclic aromatic hydrocarbons cause long-term impairment of urease activity.

Keywords: PAHs, urease activity, organic materials, soil resistance to PAHs (RS), resilience (RL)

Introduction

Technological, industrial, and economic progress leads to the emission of pollutants into the natural environment, including polycyclic aromatic hydrocarbons (PAHs). PAHs are toxic, carcinogenic, and hydrophobic substances with a tendency to bind to organic soil matter, and they are among the most dangerous contaminants to be introduced into the environment, including soil. PAHs found in soils come from both natural and anthropogenic sources. The natural sources of PAHs include volcanic eruptions, forest fires, and the presence of PAHs in fossil fuels, e.g. petroleum. However, the main cause of soil contamination with PAHs is human activity. The anthropogenic sources of PAHs include wood burning, waste incineration, fuel combustion, pyrolysis of fats and oils, automotive emissions, coal distillation, and sewage sludge application [1, 2]. Significant amounts of PAHs contained in oil derivatives penetrate into the soil environment as a result of accidental and unintentional discharges during production and transportation (oil and fuel oil spills in military airfields, gas stations, pumping stations, refinery installations). In view of the above, PAH doses applied in our study were several times higher than the amounts of naturally occurring PAHs [3, 4].

The sorption of PAHs on organic and inorganic soil colloids can reduce the bioavailability of those organic compounds as substrates for microorganisms. The studied PAHs, naphthalene, phenanthrene, anthracene, and pyrene, are only slightly soluble in water. As shown by their octanol/water partition coefficients, which range from 3.6 for naphthalene to 4.8 for pyrene, they are lipophilic substances. The accumulation of PAHs in the soil environment deteriorates its biological parameters such as enzymatic activity and microbial counts [5-8]. Microorganisms produce urease, an enzyme of the hydrolase class that catalyzes hydrolytic decomposition of urea into ammonia and carbon

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dioxide [9]. Fluctuations in urease activity resulting from the presence of contaminants, including stable organic compounds, can be a sensitive indicator of soil condition [10]. Although the activity of a single soil enzyme (urease) does not provide information on all metabolic processes, it is closely related to soil bioremediation and the content of carbon, total nitrogen and ammonia nitrogen [11]. Urease activity is determined by various factors, including the type of contaminant [12, 13], soil incubation time [5, 14, 15], pH [16], the number of benzene rings in a hydrocarbon [17, 18], and organic carbon content [5, 19]. Organic carbon levels are of particular importance. The supplementation of soil with organic matter reduced the adverse effects of PAHs on urease activity, and the influence of organic matter on the biochemical properties of soil is determined by the type and dose of the applied substance [20].

The aim of this study was to determine the effect of soil contamination with PAHs (naphthalene, phenanthrene, anthracene, and pyrene) on urease activity and to minimize the adverse impacts of PAHs on the analyzed enzyme.

Experimental Procedures

The effects of four PAHs – naphthalene, phenanthrene, anthracene, and pyrene – on urease activity in soil were determined. Naphthalene was supplied by Przedsiębiorstwo Wielobranżowe B&K, and phenanthrene, anthracene, and pyrene – by Acros Organics. The above compounds have been classified by the US Environmental Protection Agency (US EPA) as hazardous, and contaminated sites have been designated as a priority for remediation. The experiment was performed in three replications. Samples of soil with the texture of loamy sand (100 g air-dry matter each) were placed in 150 cm^3 glass beakers. Sand (particle size 0.05-2 mm), silt (particle size 0.002-0.05 mm), and clay (particle size below 0.002 mm) accounted for 72.42%, 25.31%, and 2.27%, respectively. The following soil parameters were determined: pH_{KCl} – 7.15, extractable acidity – 4.8 $mmol(+)\cdot kg^{-1}$, total exchangeable bases – 279 $mmol(+)\cdot kg^{-1}$, organic C content – 13.1 $g\cdot kg^{-1}$, total N content – 1.1 $g\cdot kg^{-1}$, content of available phosphorus – 459 $mg\cdot kg^{-1}$, potassium – 177 $mg\cdot kg^{-1}$, and magnesium – 27 $mg\cdot kg^{-1}$.

Nitrogen in the form of NH_4NO_3 was added to soil samples in the amount of 100 mg $N\cdot kg^{-1}$ DM to balance the C:N ratio, and the samples were contaminated with naphthalene, phenanthrene, anthracene and pyrene in stable form, in the amount of 0, 1,000, 2,000, and 4,000 $mg\cdot kg^{-1}$ DM soil. The samples were enriched with organic materials: sucrose, cellulose, and compost in the amount of 0 and 9 $g\cdot kg^{-1}$ DM soil. Sucrose was added in the form of granules with a diameter of approximately 2 mm, and cellulose was introduced in microcrystalline form. Disaccharide was supplied by Chempur and cellulose from Acros Organics. As a decomposable disaccharide, sucrose is a source of highly available carbon and energy for microorganisms. Cellulose was used in this experiment due to its high content in straw, post-harvest residues, and plants that are often used as supplementary organic matter to supply additional energy for microorganisms and speed up the decomposition of toxic organic compounds. Compost, a popular organic fertilizer, was used to enrich soil with organic matter and to increase the air and water capacity of soil. The applied compost was characterized by the following parameters: pH_{KCl} – 7.4, extractable acidity – 18.0 $mmol(+)\cdot kg^{-1}$, total exchangeable bases – 464 $mmol(+)\cdot kg^{-1}$, organic C content – 188 $g\cdot kg^{-1}$, total N content – 4.4 $g\cdot kg^{-1}$. Soil samples were incubated for four, eight, and 16 weeks at 25ºC in the dark. Soil moisture was kept constant at 50% capillary water capacity with the use of demineralized water. After incubation, urease activity levels were determined in soil samples in three replications based on the method proposed by Alef and Nannipieri [19, 21]. The results were given in mmol $N\text{-}NH_4\cdot kg^{-1}$ DM $soil\cdot h^{-1}$. Extinction measurements were performed using the Aquarius CE7500 spectrophotometer (CECIL Instruments) at 410 nm wavelength. Soil resistance (RS) and soil resilience (RL) were determined based on the formulas proposed by Orwin and Wardle [22]:

$$RS(t_0) = 1 - \frac{2|D_0|}{(C_0 + |D_0|)}$$

...where: D_0 is the difference between control sample (C_0) and contaminated soil samples after 4, 8, and 16 weeks of incubation (t_0). RL was determined after 16 weeks of incubation.

$$RL \text{ at } t_x = \frac{2|D_0|}{(|D_0| + |D_x|)} - 1$$

...where: D_0 is as above and D_x is the difference between control sample and contaminated soil samples after 16 weeks of incubation. The results were processed statistically by multi-factorial ANOVA and Tukey's test, at a significance level of p=0.01. The coefficients of correlation between the level of soil contamination with PAHs and urease activity were computed in MS Excel. The calculations were performed using Statistica 9.1 [23].

Results

The results of the experiment indicate that soil contamination with PAHs in the presence of organic substances (cellulose, sucrose, and compost) produced different levels of urease activity. All of the tested PAHs reduced urease activity, and their inhibitory effect was magnified with an increase in the degree of contamination. Naphthalene had the most inhibitory effect on soil samples not enriched with organic substances. Regardless of soil incubation time, the addition of naphthalene, phenanthrene, anthracene, and pyrene at a dose of 4,000 $mg\cdot kg^{-1}$ DM soil reduced urease activity by 62%, 52%, 17%, and 14%, respectively, in comparison with the control treatment.

Table 1. The effect of organic substance and incubation time on urease activity in soil contaminated with naphthalene (mmol N-NH$_4$·kg^{-1} DM soil·h^{-1}).

Dose (mg·kg^{-1} DM soil)	Type of organic substance			
	control	cellulose	sucrose	compost
4 weeks				
0	5.758 *a*	6.503 *a*	10.701 *a*	8.064 *a*
1,000	4.232 *b*	5.863 *ab*	8.788 *b*	5.692 *b*
2,000	2.541 *c*	5.482 *ab*	4.948 *c*	4.726 *c*
4,000	0.130 *d*	5.059 *b*	4.579 *c*	4.098 *c*
average	3.165	5.727	7.254	5.645
r	-0.996	-0.963	-0.901	-0.894
8 weeks				
0	8.485 *a*	8.935 *a*	11.154 *a*	9.160 *a*
1,000	5.753 *b*	7.107 *b*	9.262 *b*	8.630 *a*
2,000	3.615 *c*	6.386 *c*	6.239 *c*	5.754 *b*
4,000	2.455 *d*	5.347 *c*	5.180 *d*	4.493 *c*
average	5.077	6.944	7.959	7.009
r	-0.937	-0.947	-0.942	-0.951
16 weeks				
0	9.929 *a*	10.068 *a*	11.305 *a*	10.131 *a*
1,000	7.976 *b*	8.194 *b*	11.012 *a*	9.198 *b*
2,000	7.305 *c*	7.509 *c*	10.203 *b*	7.444 *c*
4,000	6.522 *d*	6.412 *d*	7.703 *c*	7.144 *c*
average	7.933	8.046	10.056	8.479
r	-0.918	-0.948	-0.973	-0.916

*homogeneous groups are followed by the same letters;
r – coefficient of correlation

The activity of soil enzymes is largely dependent on the time of soil incubation, as demonstrated by the results of our study where incubation time significantly affected urease activity levels (Tables 1-4). In soil samples not enriched with organic substances and contaminated with naphthalene, urease activity increased 2.5-fold after 16 weeks of incubation in comparison with the initial values noted after four weeks of incubation, regardless of contaminant concentrations (Table 1). Similar correlations were observed in soil samples contaminated with phenanthrene (Table 2), anthracene (Table 3), and pyrene (Table 4), where urease activity increased 1.74, 1.73, and 1.58-fold, respectively.

Cellulose, sucrose and compost had a significant, positive effect on urease activity (Tables 1-4). The most visible improvement was reported in samples treated with sucrose, whereas cellulose and compost produced a similar increase in urease activity. Organic matter also contributes to the growth of microorganisms, thus enhancing the activity of soil enzymes. The addition of cellulose increased urease activity in samples contaminated with naphthalene and phenanthrene by 22% and 17%, respectively. The use of sucrose stimulated urease activity by 36% in samples with naphthalene, by 44% in samples with phenanthrene, by 36% in samples with anthracene, and by 24% in samples with pyrene. The addition of compost also significantly increased urease activity levels in comparison with samples not treated with organic compounds.

Loamy sand was characterized by varied resistance to contamination with PAHs (Figs. 1-4). In the group of samples not enriched with organic matter, the lowest level of resistance was noted in soil contaminated with naphthalene (Fig. 1), where the average value of RS reached 0.396, and the highest level of resistance was reported in response to pyrene contamination (0.816) (Fig. 4). The addition of organic materials led to an increase in average RS values in samples contaminated with naphthalene (Fig. 1) and phenanthrene (Fig. 2), whereas soils containing anthracene

Table 2. The effect of organic substance and incubation time on urease activity in soil contaminated with phenanthrene (mmol N-NH$_4$·kg^{-1} DM soil·h^{-1}).

Dose (mg·kg^{-1} DM soil)	Type of organic substance			
	control	cellulose	sucrose	compost
4 weeks				
0	5.758 *a*	6.503 *a*	10.701 *a*	8.064 *a*
1,000	4.683 *ab*	6.247 *a*	10.338 *a*	7.017 *b*
2,000	3.924 *ab*	6.125 *a*	10.262 *a*	6.074 *c*
4,000	2.267 *b*	5.319 *a*	8.249 *b*	4.446 *d*
average	4.158	6.048	9.887	6.400
r	-0.998	-0.979	-0.942	-0.998
8 weeks				
0	8.485 *a*	8.935 *a*	11.154 *a*	9.160 *a*
1,000	5.662 *b*	6.515 *b*	9.870 *b*	7.871 *b*
2,000	4.417 *c*	6.164 *b*	9.206 *c*	7.194 *c*
4,000	3.792 *d*	5.651 *c*	8.850 *d*	6.844 *c*
average	5.589	6.816	9.770	7.767
r	-0.887	-0.839	-0.905	-0.904
16 weeks				
0	9.929 *a*	10.068 *a*	11.305 *a*	10.131 *a*
1,000	7.057 *b*	7.455 *b*	10.419 *b*	8.064 *b*
2,000	6.511 *c*	6.780 *c*	9.981 *b*	7.512 *c*
4,000	5.594 *d*	5.765 *d*	9.333 *c*	6.939 *d*
average	7.273	7.517	10.259	8.161
r	-0.882	-0.907	-0.967	-0.882

*explanation as in Table 1

Table 3. The effect of organic substance and incubation time on urease activity in soil contaminated with anthracene (mmol N-$NH_4 \cdot kg^{-1}$ DM soil$\cdot h^{-1}$).

Dose ($mg \cdot kg^{-1}$ DM soil)	Type of organic substance			
	control	cellulose	sucrose	compost
4 weeks				
0	5.818 *a*	7.253 *a*	10.377 *a*	7.751 *a*
1,000	5.001 *b*	5.748 *b*	8.517 *b*	5.452 *b*
2,000	4.603 *b*	5.601 *b*	8.486 *b*	3.751 *c*
4,000	4.339 *b*	5.279 *b*	7.347 *b*	3.137 *d*
average	4.940	5.970	8.682	5.023
r	-0.912	-0.828	-0.917	-0.912
8 weeks				
0	8.854 *a*	8.940 *a*	11.853 *a*	9.678 *a*
1,000	7.755 *b*	7.443 *b*	9.680 *b*	6.556 *b*
2,000	7.625 *b*	6.119 *c*	8.992 *bc*	5.385 *c*
4,000	7.469 *b*	5.762 *c*	8.735 *c*	4.665 *d*
average	7.926	7.066	9.815	6.571
r	-0.805	-0.910	-0.840	-0.882
16 weeks				
0	9.115 *a*	10.755 *a*	12.392 *a*	9.929 *a*
1,000	8.788 *b*	9.643 *ab*	10.340 *b*	8.190 *b*
2,000	8.444 *bc*	9.114 *b*	9.415 *c*	7.593 *c*
4,000	7.971 *c*	9.116 *b*	9.084 *c*	6.963 *d*
average	8.579	9.657	10.308	8.169
r	-0.995	-0.823	-0.872	-0.910

*explanation as in Table 1

(Fig. 3), and pyrene (Fig. 4) were characterized by lower average values of RS than control samples.

PAHs induced permanent changes in loamy sand (Table 5). Despite the presence of additional organic matter in the analyzed samples, negative values of soil resilience (RL) indicate that PAHs exert a long-term, adverse effect on soil. The highest average value of RL in samples not enriched with organic substances was reported in soil contaminated with anthracene (0.280), and the lowest average value of RL was in soil containing phenanthrene (-0.288). The addition of cellulose increased RL values only in samples contaminated with pyrene. The average values of RL in loamy sand enriched with cellulose and contaminated with naphthalene, anthracene, and pyrene decreased, compared with the control treatment. The application of sucrose contributed to an increase in RL values in soil contaminated with naphthalene and phenanthrene, whereas a decrease in the values of RL was noted in soil contaminated with anthracene and pyrene in comparison with control. The addition of compost lowered RL values only in soil samples containing phenanthrene.

Discussion

Soil enzymatic activity, including urease activity, is determined by the quantity of PAHs introduced into the soil environment. The results of this study point to a significant correlation between urease activity and the level of soil contamination with PAHs. A significant decrease in urease activity was noted already at the lowest dose (1,000 $mg \cdot kg^{-1}$ soil DM) of naphthalene, phenanthrene, anthracene, and pyrene. Wyszkowska and Kucharski [24] and Wyszkowska and Wyszkowski [19] demonstrated that gasoline and Diesel oil had an inhibitory effect on urease activity. Similar results were reported by Tejada et al. [20] and Guo et al. [25], in whose work a rapid drop in urease activity levels was noted in soil contaminated with gasoline and petro-

Table 4. The effect of organic substance and incubation time on urease activity in soil contaminated with pyrene (mmol N-$NH_4 \cdot kg^{-1}$ DM soil$\cdot h^{-1}$).

Dose ($mg \cdot kg^{-1}$ DM soil)	Type of organic substance			
	control	cellulose	sucrose	compost
4 weeks				
0	5.818 *a*	7.253 *a*	10.377 *a*	7.751 *a*
1,000	5.193 *b*	5.776 *b*	9.164 *b*	6.026 *b*
2,000	5.065 *b*	5.567 *b*	8.440 *b*	5.329 *c*
4,000	5.059 *b*	5.303 *b*	7.143 *c*	5.074 *c*
average	5.284	5.975	8.781	6.045
r	-0.77	-0.826	-0.988	-0.863
8 weeks				
0	8.854 *a*	8.940 *a*	11.853 *a*	9.678 *a*
1,000	8.793 *a*	7.230 *b*	9.867 *b*	8.173 *b*
2,000	7.908 *b*	6.748 bc	9.454 *b*	7.379 *c*
4,000	7.784 *b*	6.247 *c*	7.776 *c*	6.940 *d*
average	8.335	7.291	9.737	8.042
r	-0.894	-0.888	-0.965	-0.908
16 weeks				
0	9.115 *a*	10.755 *a*	12.392 *a*	9.929 *a*
1,000	8.672 *b*	10.793 *a*	10.224 *b*	9.222 *b*
2,000	8.082 *c*	9.179 *b*	9.587 *c*	8.812 *b*
4,000	7.683 *d*	7.730 *c*	8.696 *d*	8.081 *c*
average	8.388	9.614	10.225	9.011
r	-0.971	-0.963	-0.916	-0.986

*explanation as in Table 1

leum. Contrary to the findings of our experiment, the stimulating effect of PAHs on the biochemical activity of soil has been postulated by several authors, among them Margesin et al. [12] and Wyszkowska et al. [26], who also observed an increase in urease activity levels in soils treated with PAHs.

In addition to the dose of the introduced contaminant, the enzymatic activity of soil also is determined by incubation time. In this experiment, urease activity levels increased with the time of incubation. In a study investigating the enzymatic activity of soil contaminated with phenanthrene, Zhan et al. [5] reported an increase in urease activity levels on the first 16 days of incubation and the achievement of optimal values on day 16. A drop in urease activity was reported after that period. A significant effect of incubation time on urease activity was noted by Shen et al. [14] and Gianfreda et al. [27]. In the latter study, low levels of urease activity were found in soil contaminated with PAHs over a period of 50 years. An increase in urease activity levels over time, observed in our experiment, can be attributed to microbial adaptive mechanisms. In the process of adapting to new environmental conditions, microorganisms have a higher demand for energy, and they use xenobiotics as an additional source of carbon and energy [18, 28]. The activities of soil enzymes, including urease, are also determined by the properties of hydrocarbons. An important role is played by very low solubility of PAHs in the soil solution as well as by their hydrophobic and nonpolar characteristics, which contribute to strong sorption of organic compounds on humins and humic acids [29]. Soil enzymatic activity may also be stimulated or inhibited depending on the molecular mass of PAHs and the number of benzene rings in their molecules. PAHs with two, three, or four benzene rings (naphthalene, phenanthrene, anthracene, pyrene) are considered to be more readily available to microorganisms, which enhances the activity of soil enzymes. Smreczak and Maliszewska-Kordybach [17] determined the bioavailable fractions of PAHs in soils and observed that hydrocarbons containing four benzene rings were the predominant group of potentially bioavailable PAHs with a 1.5- to 11-fold higher share than PAHs composed of five and six benzene rings.

PAHs undergo abiotic and biotic changes in the soil environment that affect their concentrations. The group of

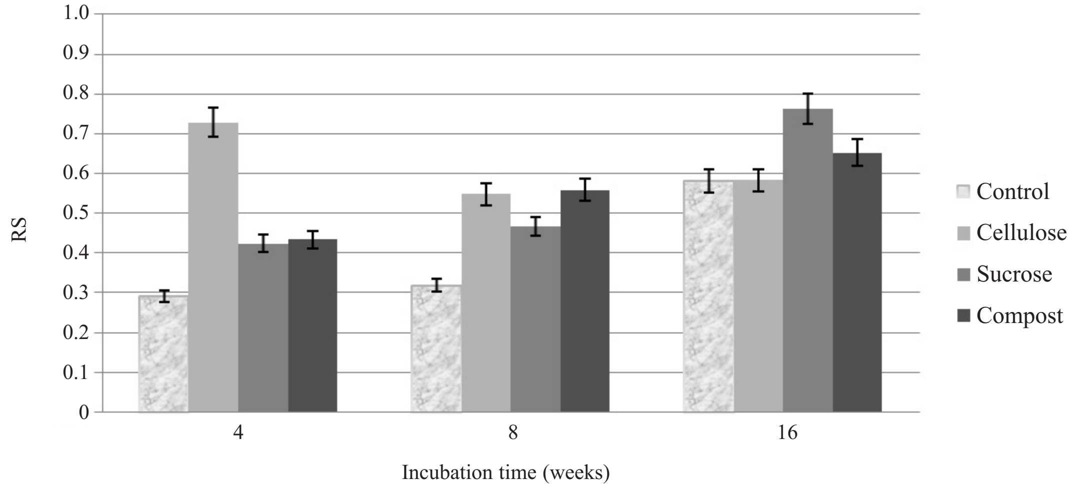

Fig. 1. Mean values of resistance (RS) in soil contaminated with naphthalene; error bars represent SD (n=9).

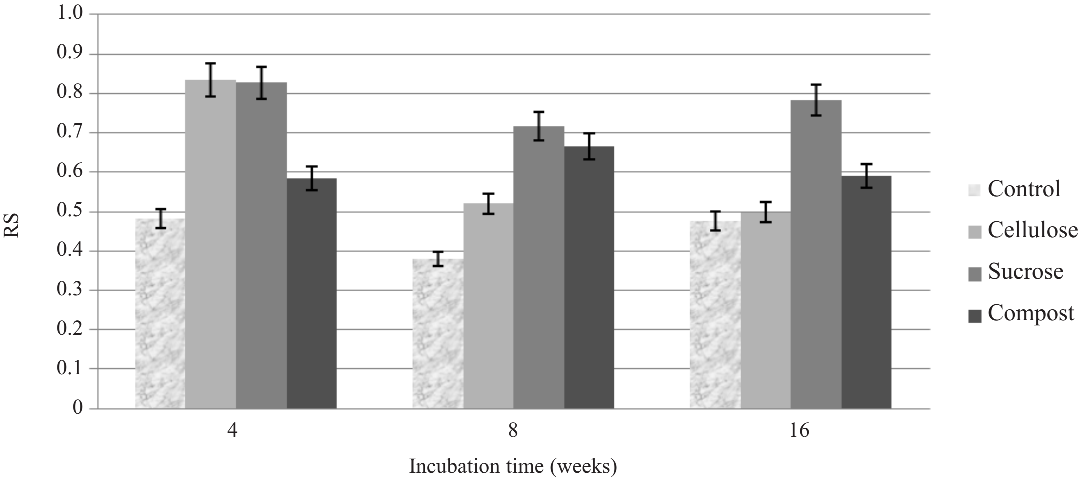

Fig. 2. Mean values of resistance (RS) in soil contaminated with phenanthrene; error bars represent SD (n=9).

abiotic factors includes sorption on mineral and organic colloids, volatilization, and temperature. High moisture content in soil may limit the volatilization of polycyclic aromatic hydrocarbons. PAHs also can be decomposed by soil-dwelling bacteria, Actinobacteria, and fungi whose growth may be suppressed upon first exposure to contaminants. After the period of stagnation, intense microbial metabolism can promote PAH decomposition [14, 18]. Soils contaminated with PAHs are often colonized by microorganisms capable of decomposing those organic compounds. Microbial ability to degrade PAHs may be impaired by adverse environmental factors [30]. For this reason, soil supplementation with additional organic matter is an important consideration. The results of our experiment and other studies [19, 20, 34-37] indicate that the application of organic materials stimulates the biochemical activity of soil. Cellulose, the first of the applied additives, is one of the most popular organic compounds, but it plays a minor role in PAH sorption, as demonstrated by Wang et al. [31] and Jonker [32]. However, cellulose in the amount of 9 g·kg^{-1} soil DM had a beneficial influence on urease activity. Similar results were reported by Wyszkowska and Kucharski [24], who used 2.4 g·kg^{-1} soil DM of barley straw to alleviate the negative effects of petrol in soil. Sucrose (a disaccharide composed of two monosaccharide residues), glucose, and fructose (a highly available organic compound and an additional source of carbon and energy that promotes microbial growth). In the work of Mühlbachovă [33], increased levels of microbial activity were observed in glucose-enriched soil contaminated with PAHs, leading to a 44.6% drop in PAH content. The stimulating effect of compost on the biochemical activity of soil was reported by Cai et al. [34] and Sayara et al. [35, 36]. It should also be noted that the stimulating influence of compost is affected by its type, chemical composition and stability [37].

Soil resistance (RS) and soil resilience (RL) are indicators of soil's ability to resist or recover its healthy state in response to destabilizing influences. Those indices describe the stability of the soil complex, namely its ability to function in environmental conditions that were modified by human activity. Soil resistance is determined by the magnitude of changes caused by contamination, whereas soil resilience is its response to environmental stressors.

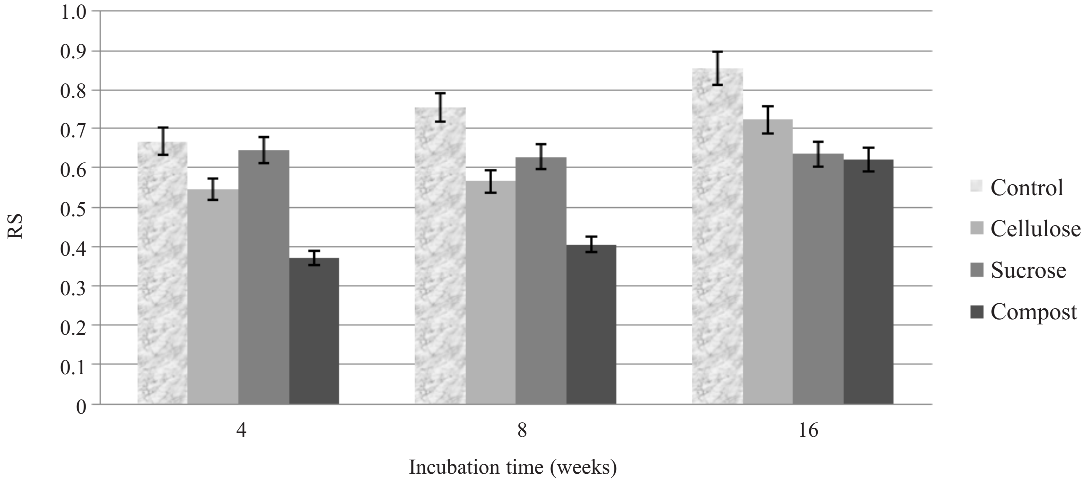

Fig. 3. Mean values of resistance (RS) in soil contaminated with anthracene; error bars represent SD (n=9).

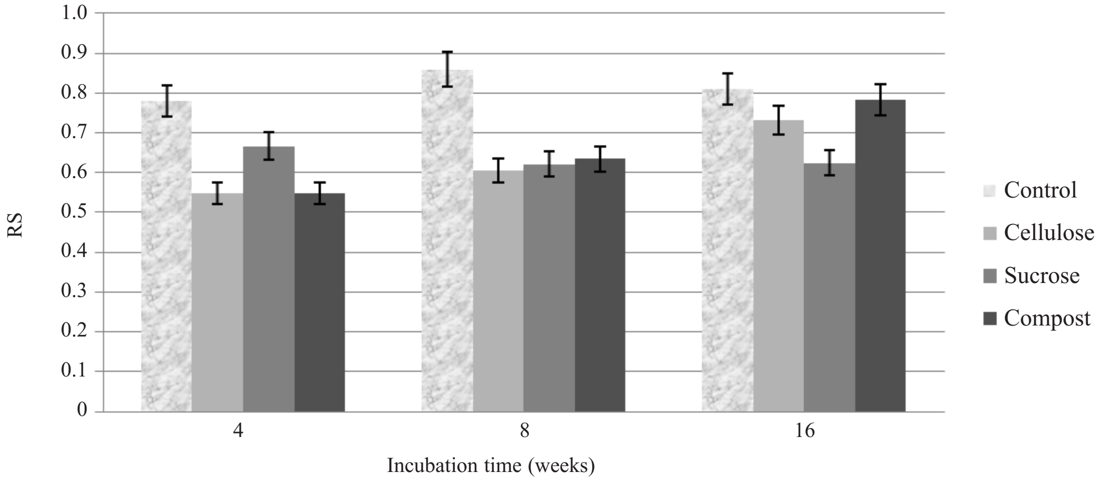

Fig. 4. Mean values of resistance (RS) in soil contaminated with pyrene; error bars represent SD (n=9).

Table 5. Resilience (RL) of soil contaminated with PAHs.

Dose ($mg \cdot kg^{-1}$ DM soil)	Type of organic substance			
	control	cellulose	sucrose	compost
naphthalene				
1,000	-0.123 *a*	-0.491 *a*	0.734 *a*	0.435 *a*
2,000	0.101 *b*	-0.429 *b*	0.678 *a*	0.108 *b*
4,000	0.246 *c*	-0.433 *b*	0.259 *b*	0.140 *b*
average	*0.074*	*-0.451*	*0.557*	*0.228*
phenanthrene				
1,000	-0.455 *a*	-0.821 *a*	-0.418 *a*	-0.327 *a*
2,000	-0.301 *a*	-0.793 *a*	-0.501 *a*	-0.136 *b*
4,000	-0.107 *a*	-0.568 *a*	0.108 *b*	0.062 *c*
average	*-0.288*	*-0.727*	*-0.270*	*-0.133*
anthracene				
1,000	0.427 *a*	0.150 *a*	-0.049 *a*	0.138 *a*
2,000	0.286 *b*	0.003 *b*	-0.223 *b*	0.262 *c*
4,000	0.127 *c*	0.092 *a*b	-0.044 *a*	0.217 *b*
average	*0.280*	*0.082*	*-0.105*	*0.206*
pyrene				
1,000	0.170 *a*	0.950 *a*	-0.282 *a*	0.418 *a*
2,000	-0.156 *b*	0.033 *b*	-0.183 *a*	0.368 *a*
4,000	-0.307 *b*	-0.216 *c*	-0.066 *a*	0.183 *b*
average	-0.097	0.255	-0.177	0.323

*homogeneous groups are followed by the same letters

In scientific literature, the resistance and resilience of soils contaminated with PAHs has never been investigated based on urea activity levels. The patterns of interactions between RS, RL, and the time of soil contamination may vary [22]. In our study, interactions were observed between RS and the dose of PAH, soil incubation time, and the type of organic substance added to soil. In samples without organic additives, the lowest resistance value was noted in soil contaminated with naphthalene (0.396), and the highest in soil samples containing pyrene (0.816). RS values range from 0 to 1, where 1 denotes no effect of a given contaminant on the analyzed soil. The lower the RS value, the lower the soil resistance and the stronger the effect of toxic compounds. Both the RS values and urease activity levels observed in our study are indicative of strong adverse effects of naphthalene, which could result from rapid decomposition of this compound, followed by the release of its toxic metabolites. High soil resistance to pyrene is most probably associated with the sorption of this hydrocarbon on organic colloids, which considerably reduces its bioavailability to soil microbes. RL values range from -1 to 1, where 1 denotes the ability of soil to fully recover its healthy state in response to destabilizing influences. In samples without organic additives, the highest average resilience value (0.280) was noted in soil contaminated with anthracene, and the lowest in soil treated with phenanthrene (-0.288). Negative resilience values suggest that PAHs exert long-term effects on urease activity, which could be partially attributed to the loss of selected microbial species [38]. Our results cannot be compared with the findings of other authors because there are no published sources in this area. Therefore, further studies are needed to investigate the ranges of RS and RL values in soils contaminated with PAHs, based on the activity of soil enzymes other than urease.

Conclusions

1. Soil contamination with PAHs had an adverse influence on urease activity. Naphthalene had the most inhibitory effect and pyrene the least inhibitory effect on the activity levels of the analyzed enzyme.
2. Urease activity levels in soil contaminated with naphthalene, phenanthrene, anthracene, and pyrene were significantly determined by hydrocarbon dose, soil incubation time, and the type of the applied organic substance. Urease activity decreased with an increase in contamination levels, whereas an increase in urease activity was observed over incubation time.
3. All of the tested organic substances (cellulose, sucrose, compost) had a positive effect on urease activity levels, and sucrose was the most effective additive.
4. Soil resistance decreased with an increase in soil contamination with PAHs. The addition of cellulose, sucrose and compost contributed to an increase in RS values in soil samples contaminated with naphthalene and phenanthrene in comparison with the control treatment.

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