Environment Protection Engineering

Vol. 31 2005 No. 1

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ENZYMATIC ACTIVITY OF LIGHT SOIL FERTILISED WITH SEWAGE SLUDGE AGAINST A BACKGROUND CONTENT OF POLYCYCLIC AROMATIC HYDROCARBONS

Relationships between enzymatic activity (of dehydrogenase, phosphatase, urease and protease) and the content of polycyclic aromatic hydrocarbons in light soil fertilised with sewage sludge were examined. Soil fertilisation with sewage sludge stimulated activity of the enzymes studied. With time, a gradual decrease in the activity of dehydrogenase, urease and protease was observed. Based on the values of the coefficients of correlation between the activity of the enzymes studied and PAH content it may be concluded that some PAHs can be a source of carbon and energy for microorganisms. Stimulation of the activity of dehydrogenases (in the doses of 75 for anthracene and 150 t/ha for naphthalene, fluorene and benzo[b]fluoranthene), protease (75 t/ha for naphthalene, acenaphthene, fluorene, anthracene, indeno[1,2,3-cd]pyrene and sum of 16 PAHs), phosphatase (150–300 t/ha for over 5 PAHs) and urease (30 t/ha for benzo[a]anthracene, 150 t/ha for benzo[b]fluoranthene and 300 t/ha for naphthalene) was probably related to the enrichment of the environment by the organic matter fractions susceptible to decomposition together with those nutrients necessary for PAH decomposition.

1. INTRODUCTION

Enrichment of soil by sewage sludge is one of the most effective methods of its use in agriculture. About one-third of the sludge generated annually in the countries of the European Union, the United States of America and Canada is utilised in that way [1]. The variety of nutrients which can be found in sewage sludge makes it a valuable source of carbon, nitrogen, phosphorus, potassium, etc. Nutrients in sludge replace or supplement commercial chemical fertilizers, while organic matter from sewage sludge improves soil structure, reduces soil erosion and improves crop yields [2]–[4]. However, heavy metals are also introduced to soils with sewage sludge [5],

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[6]. Moreover, numerous studies have proved that sludge contains a number of organic pollutants [7], [8] which pose a potential danger to human health. Recently the interest in the paths of organic pollutants in soils fertilized with sewage sludge has been grown. Both PAHs [9], [10] and other organic pollutants [11], [12] are of a paramount interest. However, an available information on the paths of PAHs in soils fertilized with sewage sludge still remains sparse.

As a result of the specific conditions generated after the introduction of sewage sludge, organic pollutants, despite their high durability in the soil, can undergo an apparent degradation (formation of bound-residue), dissipation (leaching, volatilisation) or biodegradation. The scope and intensity of this process depend on many factors [1]. A desirable direction of PAH degradation is their microbiological degradation to the forms simpler and less harmful than the parent compounds. However, the above pollutants must be available and non-toxic for microorganisms. The investigations show [13], [14] that a toxic influence of aromatic polycyclic hydrocarbons on microorganisms can really reduce the range of their biodegradation. Introduction of sewage sludge to soil provides nutrients to aid the degradation processes and also increases the "pool" of microorganisms capable of degrading PAH. One of the properties that reflects a general condition of microorganisms is their enzymatic activity. It is considered to be a sensitive indicator often used for assessment of the biodegradation of products originating from crude oil, e.g. polycyclic aromatic hydrocarbons [15], [16].

In the present work, the relationships between enzymatic activity and the content of polycyclic aromatic hydrocarbons in soil fertilised with sewage sludge were studied.

2. MATERIALS AND METHODS

2.1. PLOT EXPERIMENT

The experimental block consisted of six 3×5 m plots established on light soil formed from weak loamy sand. Sewage sludge was introduced to the soil in doses of: 30, 75, 150, 300 and 600 t/ha and mixed with a surface layer of soil to a depth of 20 cm; then wicker (*Salix viminalis*) seedlings were planted. The sludge was prepared in a mechanical-biological sewage treatment plant in such a way that it consisted of communal (70%) and industrial (30%) sewage. In order to stabilize the sewage sludge, it was subjected to mesophilic fermentation.

Table 1

The physicochemical properties of sewage sludge and soil used in the experiment

Properties		Soil	Sewage sludge
Soil texture (%)	1–0.1	86	–
	0.1–0.02	7	–
	<0.02	7	–
pH in KCl		5.8	6.4
CEC (mmol kg^{-1})		48.9	583.2
TEB (mmol kg^{-1})		71.2	607.7
BS (%)		68.7	96.0
Available forms (mg kg^{-1})	P	56	28
	K	87	84
	Mg	49	77
TOC (g kg^{-1})		12.1	277
N_{ogl} (g kg^{-1})		1.2	22.3

CEC – the cation exchange capacity, TEB – the total of the exchangeable bases, BS – the degree of the base saturation, TOC – the total organic carbon, N_{ogl} – the total nitrogen.

The amount of sludge applied was established taking into account fertilising (30 t/ha), melioration (75–300 t/ha), and extreme doses (600 t/ha). The choice of extreme doses was aimed at establishing the degree at which soil becomes polluted with PAHs, and what the background is upon which the durability of these compounds is founded. The physicochemical properties of soil and sewage sludge used in the experiment are summarized in table 1.

2.2. SAMPLE COLLECTION AND PREPARATION

Soil and sewage sludge-amended soil samples (0–20 cm horizon) were collected (after a period of 3 days, 6 months and 18 months after sewage sludge application) with a (5 cm i.d. × 60 cm) stainless steel borer. Six independent samples (replicates) were taken from each plot. Replicates were mixed together and transported to the laboratory in zip-lock bags. Half of each sample, whose content was determined for PAHs, was air-dried in air-conditioned storage rooms (20–25 °C) for 2 d (in darkness), manually crushed and sieved (< 2 mm) prior to chemical analyses. The other half of each sample for enzymatic activity determination were sieved (2 mm) and kept field-moist in a cooler at 4 °C. Samples were analysed within 2 weeks after sampling.

2.3. DETERMINATION OF ENZYMATIC ACTIVITIES

Activity of the following enzymes was determined: dehydrogenase, phosphatase, urease and protease. All assays were carried out in triplicates. Dehydrogenase activity was determined according to the method of THALMANN [17], in which TTC (2, 3, 5-triphenyl tetrazolium chloride) is a terminal acceptor of protons and electrons from organic compounds being oxidized. Potential dehydrogenase activity was assayed by incubating 5 g of moist soil amended with 5 cm^3 of triphenyltetrazolium chloride (TTC) solution (1%, dissolved in Tris-HCl buffer (pH 7.4)) at 37 °C for 96 h. Controls contained only 5 cm^3 of Tris-HCl buffer. The concentration of triphenyl formazan (TPF) produced was estimated colorimetrically (485 nm).

The activities of phosphatases [18] were assayed in 1-g oven-dry equivalents of buffered soil solutions incubated for 1 h at 37 °C after addition of the enzyme-specific substrate solution. The concentration of the product of all reactions, *p*-nitrophenol (PN), was measured colorimetrically (410 nm).

Protease activity was assayed by the method of LADD and BUTTLER [19]. This procedure involves the determination of the content of aromatic amino acids released during the incubation period by using casein as substrate. Soil samples (2 g) were incubated for 2 h in 5 cm^3 of a buffered casein solution (pH 8.1) and 5 cm^3 of Tris-HCl at 50 °C. The aromatic amino acids released were extracted with trichloroacetic acid (TCA) and their concentration was measured colorimetrically (578 nm) using the Folin reagent.

The method of assaying urease activity [20] involved estimation of the content of urea decomposed due to an incubation of soil with urea. Soil samples (10 g) were incubated for 18 h in 10 cm^3 solution of urea (500 mg urea/200 cm^3 water) at 37 °C. After incubation the content of $N–NH_4^+$ was determined by the Nessler method [21].

2.4. DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS

The PAH content was determined using the method of HPLC with UV detection (254 nm) after optimisation of the analytic process with respect to the amount and type of solvent used and extraction time, by means of ultrasonic method [22] and optimisation of the extract purification process by the solid-phase extraction method [23].

In the total procedures (sample preparation, extraction and SPE), the recoveries ranged from 81 to 90% (in soil and sewage sludge-amended soil) and from 72 to 83% (in sewage sludge) for individual PAHs. Only in the case of naphthalene, the recoveries were in the range of 50–60% (in all samples). Precision expressed as relative standard deviation (RSD) was below 21%. Therefore in the concentrations reported here the losses have not been taken into account.

All reported concentrations of PAHs are expressed based on a dry weight of soil (determined by drying the soils for 24 h at 105 °C) and are the average of triplicate extrac-

tion.

2.5. DATA ANALYSIS

Statistical analysis was performed with MS Excel 2000 with tool pack (Microsoft), ARStat (Lublin). Significance was set at * – $P < 0.1$ and ** – $P < 0.05$. All reported concentrations of PAHs are expressed based on a dry weight of soil (determined by drying the soils for 24 h at 105 °C) and are the average of triplicate extraction.

3. RESULTS AND DISCUSSION

3.1. CHANGES IN PAH CONTENT

After sewage sludge application, in all variants of the experiment, distinct differences in PAH behaviour were noted, and their scale depended on the experiment variant. During the first six months, a decrease in PAH content was observed in all experimental variants (except for 30 t/ha) (table 2). Over a longer time interval, a slight increase in PAH content was noted. The above phenomenon was observed by many researchers both in relation to PAHs and many other persistent organic pollutants [9], [11], [12], [25]. This is probably related to both quantitative and qualitative transformations in organic matter. Six months after the introduction of sewage sludge, strong bonds between organic matter and PAHs were formed, and hence a decrease in their content was observed (table 2). An increase in the PAH content in the final stage of the experiment was probably due to a partial decomposition of organic matter and release of PAHs adsorbed earlier.

At a sludge dose of 30 t/ha, the content of 2–4-ring PAH at the beginning of experiment (the first six months) was increasing (35–57%), and then it was decreasing to the levels from the beginning of experiment. A continuous decrease of 5- and 6-ring PAH (introduced with sewage sludge to soil) content was noted. At the end of the experiment the content of 5- and 6-ring PAHs decreased by 30 and 14%, respectively, compared to their initial content.

In the case of the remaining doses of sewage sludge (75–600 t/ha), the changes in polycyclic aromatic hydrocarbons content in the soils tested were characterized by distinct similarities (table 2). Only when the dose of 150 t/ha was applied, the amount of 5- and 6-ring PAHs was continuously decreasing from the beginning of the experiment. When the doses of 75, 300 and 600 t/ha were applied, the content of 5- and 6-ring PAHs initially decreased (six months from sludge introduction)

(20–74%), and then increased (by 15–52%) (both in the case of a decrease and an increase, their intensity depended on the sewage dose and the PAH group) (table 2).

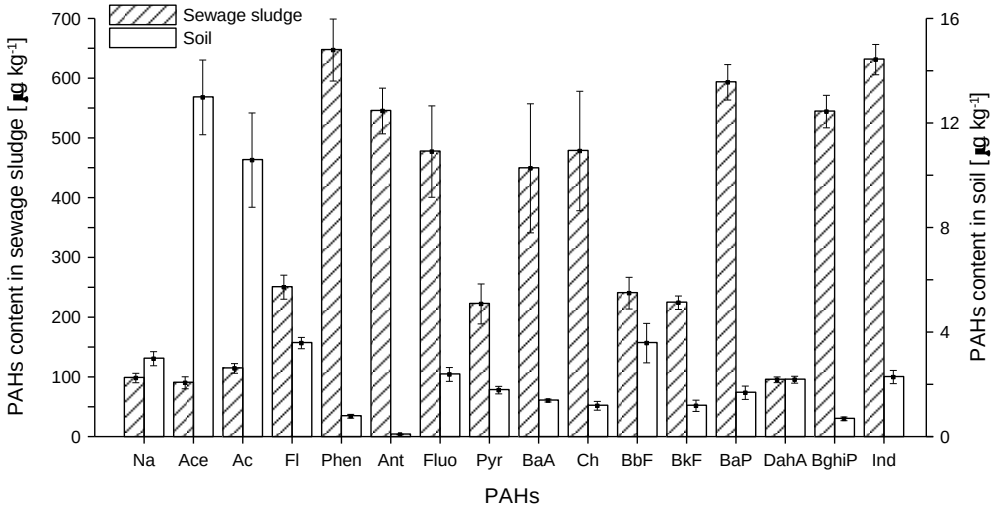

Fig. 1. Content of individual PAH in control soil and sewage sludge used in the experiment. Error bars represent the standard errors of the mean of 3 extractions. The description of abbreviations was introduced under table 2

Finally a decrease in a total sum of PAHs was observed in all plots with sewage sludge. The highest decrease, by 46% and 62%, respectively, was found for experiment with 150 and 300 t/ha sewage sludge dose. In the case of the doses of 30, 75 and 600 t/ha, the total content of PAHs decreased by 5%, 17% and 37%, respectively. Figure 2 shows that an introduction of sewage sludge to the soil resulted in clear changes in its enzymatic activity. Soil fertilisation with sewage sludge clearly increased (p = 0.05) the dehydrogenase activity compared to the control. At the beginning of the experiment and six months later, the highest activity of this enzyme was observed in soil fertilised with a sludge dose of 300 t/ha. Dehydrogenase activity has been proposed as a measure of microbial activity in soil [26], although some authors have criticized this approach [27], because the enzyme is affected by numerous factors (soil type, microbial counts, pH, etc.) [28]. Dehydrogenases catalyze biological oxidation–reduction processes in soil organic matter [29]. The above phenomenon was confirmed by our data which showed a considerable decrease in dehydrogenase activity with decreasing the content of organic matter [8].

Urease and protease catalyze the hydrolysis of organic nitrogen to its inorganic form, the former in urea-type substrate and the latter in a simple peptidic substrate. The activity of these enzymes showed a significant (p = 0.01) and continuous increase with increasing sludge doses compared to the control. In soil with the highest sludge dose (600 t/ha), the decrease in their activity was noted. Although the activity of the enzymes mentioned plays an active part in transformations of nitrogen, any clear relationships between the content of nitrogen and the activity of urease and protease were

not observed (figures 2 and 3). Any significant correlations between them were not found either. The decease could be the result of a too high concentration of pollutants in the soil. An inhibiting influence of heavy metals on urease activity [30], [31] and protease activity [32] was demonstrated. Heavy metals may inhibit enzyme activity by masking the catalytically active groups, denaturing effect on protein conformation, or competition with metal ions involved in the formation of enzyme–substrate complexes [33].

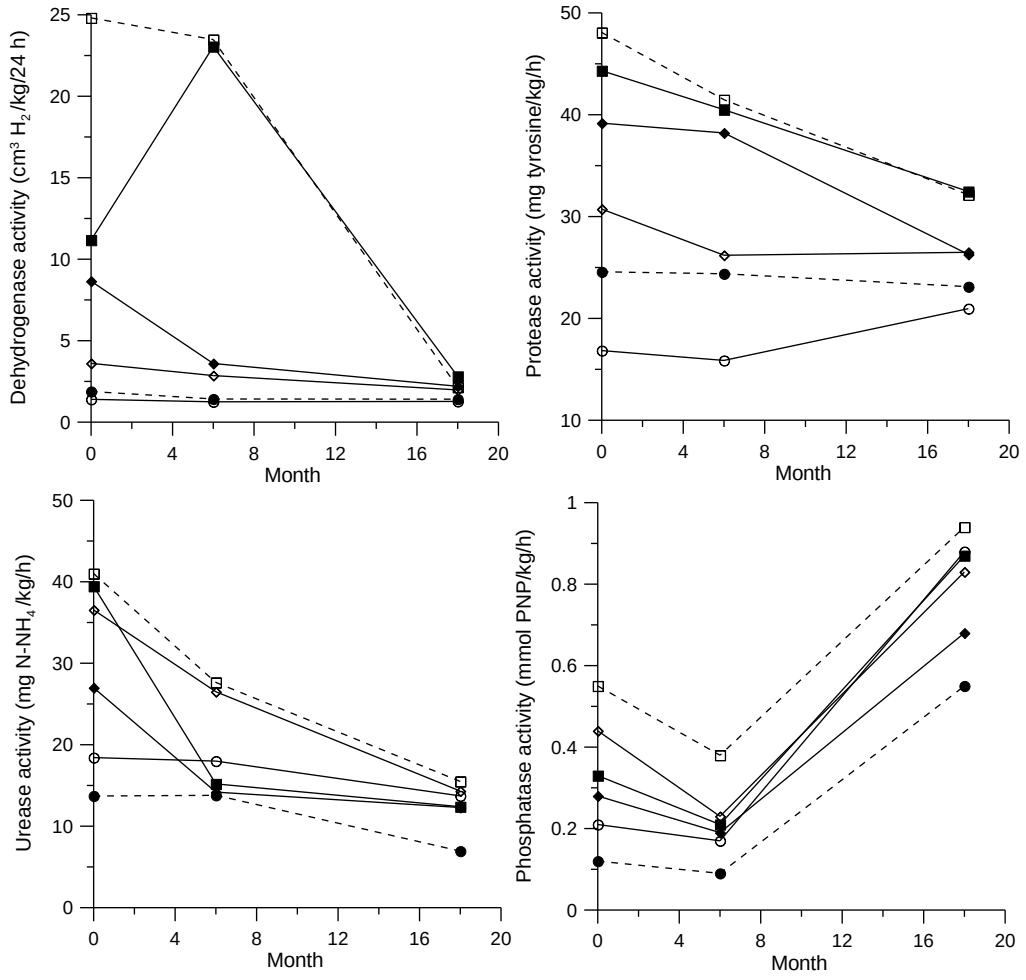

Fig. 2. Enzymatic activity of sewage sludge-amended soil during research period.
Sewage sludge dose: (○) control; (●) 30 t/ha; (◊) 75 t/ha; (♦) 150 t/ha; (□) 300 t/ha; (■) 600 t/ha

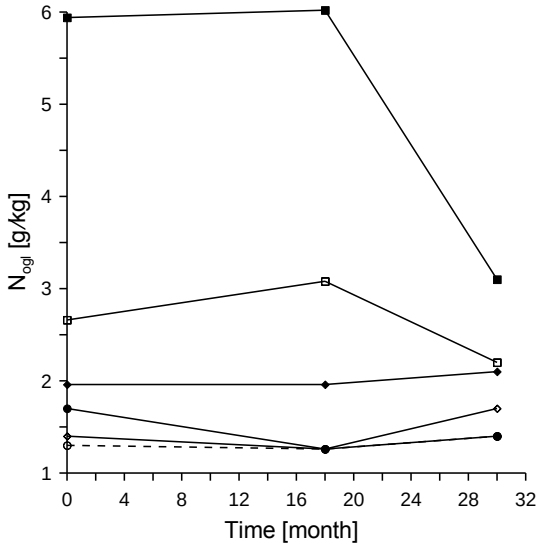

Fig. 3. Changes of total organic nitrogen content (N_{ogl}) during research period.
Sewage sludge dose: (○) control; (●) 30 t/ha; (◊) 75 t/ha; (♦) 150 t/ha; (□) 300 t/ha; (■) 600 t/ha

The level of heavy metals (Pb, Zn, Cu) determined in sewage sludge-amended soil was so high [34]–[36] that it could have an adverse effect on microorganisms [37], [38]. This effect, however, is dependent on many factors such as pH or content of organic matter [33]. The problem of relationships between heavy metals and enzymes was described in detail elsewhere [34]–[36]. DICK [39] suggested that an excessive amount of metabolites such as NH_4^+ can also inhibit to some extent the urease activity as a result of soil mineralisation. During the present experiment, we observed a continuous and significant decrease in urease activity (in all experimental variants) and in protease activity at sludge doses $\geq$ 150 t/ha. A constant protease activity at the sludge doses of 30 and 75 t/ha can result either from the strong bonding of enzymes to soil colloids suggested by some authors [40] which protects the enzyme from denaturation or, as mentioned above, from the excess of metabolites.

While studying the influence of fertilisation with sewage sludge on enzyme activity, MARTENS and co-workers [41] put forward the hypothesis that after an initial stimulatory effect of a new substrate, the production of high level of enzyme is inhibited by a feedback mechanism due to an adequate supply of energy. It might also be possible that there is an increase in the amount of abiotic enzymes being stabilized in microhabitats and being capable of acting. In such a case, microorganisms would no longer need to excrete large amounts of hydrolytic exoenzymes to soil solution to decompose substrates [39].

Phosphatase is a very important enzyme in agriculture since it catalyses the hydrolysis of organic phosphorus to its inorganic form which can be assimilated by plants [42]. All treatments led to a significant increase in soil phosphatase activity compared to the control

(figure 2). After 6 months, its marked decrease was noted. The introduction of considerable amount of available phosphorus with the sewage sludge could be responsible for the reduction in the phosphatase activity observed, as it could have limited the enzyme synthesis [43]. In the final stage of the experiment, a distinct increase in the phosphatase activity was observed (figure 2) (most probably related to a decrease in the content of available phosphorus) which exceeds its initial value in all experimental variants.

3.2. RELATIONSHIP BETWEEN ENZYMATIC ACTIVITY AND PAH CONTENT

After its introduction to soil, organic matter in sewage sludge (16%–65%) better stimulates the soil sorption than organic pollutants, including PAHs [1], [44]. It is known that sorption limits considerably the availability of these compounds to microorganisms [35], [45], which reduces their biological decomposition. On the other hand, adsorption of pollutants decreases their toxicity to soil organisms. The present results and also results reported by other authors [1], [9] prove that after the introduction of organic pollutants with sludge to soil, their presence can be limited. Often an increase in the activity of many enzymes as well as soil microbial biomass and soil respiration can be observed in the conditions thus created [5], [37], [41], [47].

Table 3

Correlation between individual PAH/sum of PAHs and dehydrogenase activity in sewage sludges-amended soil

PAHs	Dose of sewage sludges					
	0	30 t/ha	75 t/ha	150 t/ha	300 t/ha	600 t/ha
Na	0.84	–0.01	0.91	0.98*	0.56	–0.18
Ace	–0.97*	–0.61	0.43	0.49	0.15	–0.66
Ac	0.92	–0.43	0.83	0.96	0.39	–0.45
Fl	–0.65	–0.97	–0.75	0.99**	0.44	–0.60
Phen	–0.87	–0.25	0.90	0.86	0.43	–0.45
Ant	–0.56	–0.49	0.99**	0.95	0.54	–0.22
Fln	–0.61	0.81	0.36	0.81	–0.34	–0.41
Pyr	–0.88	–0.73	–0.72	0.74	0.38	–0.52
BaA	–0.89	0.67	0.85	0.85	0.49	–0.16
Ch	–0.96	–0.83	0.83	0.70	0.46	–0.20
BbF	0.71	0.08	0.93	1.00**	0.48	–0.21
BkF	–0.89	1.00**	0.38	0.67	0.43	–0.22
BaP	–0.94	0.48	0.88	0.78	0.47	–0.60
DahA	0.67	0.99**	0.28	0.03	0.49	–0.18
BghiP	–0.73	0.57	0.75	0.84	0.23	–0.74
Ind	–0.40	0.7	0.35	0.97	0.47	–0.58
Σ PAH	–0.67	–0.35	0.63	0.92	0.41	–0.37

* $P \leq 0.1$; ** $P \leq 0.05$. The description of PAH abbreviations under table 2.

Table 4

Correlation between individual PAH/sum of PAHs and urease activity in sewage sludges-amended soil

PAHs	Dose of sewage sludges					
	0	30 t/ha	75 t/ha	150 t/ha	300 t/ha	600 t/ha
Na	0.87	0.52	0.94	0.93	0.98*	0.56
Ace	–0.89	–0.09	0.49	0.30	0.79	0.06
Ac	0.42	0.12	0.87	0.88	0.92	0.30
Fl	–0.99**	–0.68	–0.80	0.96	0.94	0.12
Phen	–0.98*	0.30	0.93	0.75	0.93	0.30
Ant	–0.97	0.05	0.98*	0.87	0.97	0.52
Fln	–0.98*	0.37	0.43	0.68	0.40	0.34
Pyr	–0.98*	–0.26	–0.77	0.59	0.91	0.22
BaA	–0.97	0.96*	0.81	0.72	0.96	0.58
Ch	–0.91	–0.41	0.87	0.54	0.95	0.54
BbF	0.07	0.60	0.95	0.99**	0.95	0.53
BkF	–0.97*	0.82	0.31	0.50	0.93	0.52
BaP	–0.93	0.88	0.91	0.63	0.95	0.13
DahA	0.00	0.78	0.35	0.24	0.95	0.56
BghiP	–1.00**	0.05	0.79	0.71	0.83	–0.07
Ind	–0.91	0.26	0.42	0.91	0.95	0.16
Σ PAH	–0.99**	0.21	0.68	0.83	0.92	0.38

* $P \leq 0.1$; ** $P \leq 0.05$. The description of PAH abbreviations under table 2.

In our experimental set-up, we observed a negative influence of PAHs (expressed by high negative correlation coefficients) in control soil on the dehydrogenase (statistically significant for acenaphthylene) (table 3) and urease activities (statistically significant for 6 PAHs and sum of 16 PAHs) (table 4). A stimulating influence of PAH on phosphatase and protease was observed (expressed by high positive correlation coefficients) (table 5 and 6). The control soil was a light soil with a low nutrient content (table 1). Its content of organic carbon was too low (11.2 g/kg) [8] to "protect" microorganisms in such conditions. Moreover, a poor content of nutrients can also limit the activity of microorganisms and increase a negative effect of PAHs.

Organic matter introduced with sewage sludge limited the toxicity of PAHs to microorganisms by forming difficult-to-access bindings of PAH–organic matter (e.g. in the bound-residue). The microorganisms present in the sewage sludge-amended soil were able to "use" the nutrients and to degrade PAHs which were released as the result of organic matter mineralisation.

Studies by MALISZEWSKA-KORDYBACH [46] showed that the introduction of organic fertiliser to the soil polluted with PAHs stimulated decomposition of the latter

in the initial phase. Other authors have also reported a distinct increase in enzymatic activity in soil strongly polluted with PAHs and then enriched with horticultural compost [47], straw [48] or manure [49].

Table 5

Correlation between individual PAH/sum of PAHs and protease activity in sewage sludges-amended soil

PAHs	Dose of sewage sludges					
	0	30 t/ha	75 t/ha	150 t/ha	300 t/ha	600 t/ha
Na	–0.72	0.71	1.00**	0.59	0.83	0.69
Ace	0.75	0.08	0.70	–0.26	0.50	0.23
Ac	–0.17	0.59	1.00**	0.49	0.70	0.46
Fl	0.99**	–0.48	0.98*	0.66	0.74	0.29
Phen	0.89	0.69	0.97	0.27	0.73	0.46
Ant	1.00**	0.97	1.00**	0.48	0.81	0.66
Fln	1.00**	0.01	0.94	0.17	0.03	0.50
Pyr	0.88	0.27	0.89	0.06	0.69	0.39
BaA	0.87	0.98*	0.96	0.23	0.77	0.71
Ch	0.76	0.58	0.87	0.01	0.75	0.68
BbF	0.19	0.74	0.97	0.71	0.77	0.67
BkF	0.87	0.68	0.84	–0.04	0.73	0.66
BaP	0.80	0.65	0.91	0.11	0.79	0.30
DahA	0.26	–0.07	–0.23	0.72	0.77	0.69
BghiP	0.97	0.47	0.95	0.22	0.57	0.11
Ind	0.99**	–0.00	1.00**	0.55	0.76	0.33
Σ PAH	0.99**	0.32	0.99**	0.39	0.71	0.53

* $P \leq 0.1$; ** $P \leq 0.05$. The description of PAH abbreviations under table 2.

Stimulation of the activity of dehydrogenases (in the doses of 75 for anthracene and 150 t/ha for naphthalene, fluorene and benzo[b]fluoranthene), protease (75 t/ha for naphthalene, acenaphthene, fluorene, anthracene, indeno[1,2,3-cd]pyrene and sum of 16 PAHs), phosphatase (150–300 t/ha for over 5 PAHs) and urease (30 t/ha for benzo[a]anthracene, 150 t/ha from benzo[b]fluoranthene and 300 t/ha for naphthalene) (tables 3–6) in the soil supplemented with sewage sludge, despite an increasing PAH content with an increasing sludge dose in the soil, was probably related to the enrichment of the environment with the organic matter fractions susceptible to decomposition together with the nutrients necessary for PAH decomposition.

Table 6

Correlation between individual PAH/sum of PAHs and phosphatase activity in sewage sludges-amended soil

PAHs	Dose of sewage sludges					
	0	30 t/ha	75 t/ha	150 t/ha	300 t/ha	600 t/ha
Na	–0.80	–0.98*	–0.36	0.79	0.99**	0.97
Ace	0.83*	–0.67	0.33	0.97	0.93	0.71
Ac	–0.29	–0.81	–0.21	0.86	0.99**	0.86
Fl	1.00**	–0.07	0.07	0.73	1.00**	0.75
Phen	0.94	–0.90	–0.34	0.95	1.00**	0.86
Ant	0.99**	–0.76	–0.79	0.87	1.00**	0.96
Fln	1.00**	0.43	0.40	0.98*	0.64	0.88
Pyr	0.94	–0.53	0.02	0.99**	0.99**	0.81
BaA	0.93	–0.85	–0.98*	0.97	1.00**	0.97
Ch	0.84	–0.39	–0.20	1.00**	1.00**	0.96
BbF	0.06	–0.99**	–0.40	0.68	1.00**	0.96
BkF	0.93	–0.14	–0.92	1.00**	0.99**	0.96
BaP	0.87	–0.95*	–0.28	0.99**	1.00**	0.75
DahA	0.13	–0.08	0.47	–0.71	1.00**	0.97
BghiP	0.99**	0.70	–0.07	0.97	0.96	0.61
Ind	0.97	0.53	0.41	0.82	1.00**	0.78
Σ PAH	1.00**	–0.86	0.10	0.91	0.99**	0.90

* $P \leq 0.1$; ** $P \leq 0.05$. The description of PAH abbreviations under table 2.

PAH influence on the metabolic activity of microorganisms depends on the amount of pollutants introduced to the environment as well as on soil pH, humidity, temperature and oxygen content [15], [16], [50], [51]. It was observed [37] that a certain part of organic matter introduced to the soil with sewage sludge underwent decomposition within a few months. Hence, it should be assumed that a decrease in dehydrogenase, protease and urease activity, depending on the PAH content, in the case of higher sludge dose (600 t/ha), was the result of releasing an excessive amount of PAHs (via mineralisation of organic matter) which began to be toxic to microorganisms present in the experimental set-up. Only in the case of phosphatase, the increasing sludge doses did not influence its activity.

4. CONCLUSION

After sewage sludge had been introduced to the soil, an increase in the PAH content in the fertilised soil was observed. This increase was proportional to the sewage dose applied. After 18 months, the highest decrease in the PAH content was observed in the fields with a sewage sludge dose of 300 t/ha which probably testifies to the favourable conditions for their decomposition. This fact was confirmed by the high coefficients of

correlation between phosphatase activity and PAH content which allows the statement that microorganisms that release these enzymes actively degrade PAHs.

Soil fertilisation with sewage sludge stimulated activity of all the enzymes tested. This was associated with an increase in nutrient (N, P, K) and also organic matter content, which stimulated microorganisms to produce the enzymes studied. With time, a gradual decrease in dehydrogenase, protease and urease activity was noted. This may be due to the degradation of organic matter and the release of pollutants adsorbed (both inorganic and organic) which inhibit microbiological activity. An increase in the phosphatase activity can prove that PAHs do not exert any negative influence on the activity of this enzyme, on the contrary (as confirmed by data collected in the experimental plot), they can stimulate decomposition.

The coefficients of correlation between the activity of the enzymes studied and PAHs content revealed that some PAHs can be a source of carbon and energy for microorganisms, but these compounds are slowly released as a result of the biological desorption processes in the soil. The results obtained testify to the multidirectional influence of sewage sludge on the soil environment related mainly to the amount of pollutants introduced to the soil. The problems discussed require further detailed studies that will take into account the soil types and a differentiated chemical composition of sewage sludge used for soil fertilisation.

ACKNOWLEDGEMENTS

Financial support from the State Committee for Scientific Research (KBN, Warsaw), Project no. P06H 062 20, is gratefully acknowledged. Patryk Oleszczuk is granted by the Foundation for Polish Science (FNP).

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AKTYWNOŚĆ ENZYMATYCZNA LEKKIEJ GLEBY UŻYŹNIONEJ OSADEM ŚCIEKOWYM W ZALEŻNOŚCI OD STĘŻENIA WIELOPIERŚCIENIOWYCH WĘGLOWODORÓW AROMATYCZNYCH

Badano zależność między aktywnością enzymatyczną dehydrogenaz, fosfataz, urazy i protezy a zawartością wielopierścieniowych węglowodorów aromatycznych (WWA) w lekkiej glebie użyźnionej osadem ściekowym. Osad ściekowy stymulował aktywność badanych enzymów. Z upływem czasu obserwowano stopniowy spadek aktywności dehydrogenaz, ureazy i proteazy. Wyliczone współczynniki korelacji między aktywnością badanych enzymów a zawartością WWA wykazały, że niektóre WWA mogą być źródłem węgla i energii dla mikroorganizmów. Dodatek osadu ściekowego miał stymulujący wpływ na aktywność dehydrogenaz (dawka 75 t/ha dla antracenu i 150 t/ha dla naftalenu, fluorenu i benzo[b]fluorantenu), proteazy (75 t/ha dla naftalenu, acenaftenu, fluorenu, antracenu, indeno[1,2,3-cd]pirenu i sumy WWA), fosfataz (150–600 t/ha dla 5 WWA) oraz ureazy (30 t/ha dla benzo[a]antracenu, 150 t/ha dla benzo[b]fluorantenu i 300 t/ha dla naftalenu). Obserwowane zjawisko mogło być związane z wprowadzeniem materii organicznej stymulującej razem ze składnikami odżywczymi degradację WWA.