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Concentration of potentially carcinogenic PAHs in soils fertilized with sewage sludge

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ABSTRACT

The aim of the study was to determine the effect of urban soil fertilization with different types of sewage sludge on the changes in the concentration of potentially carcinogenic polycyclic aromatic hydrocarbons (PAHs) in soil. The factors in the experiment were: two types of sewage sludge (stabilized dehydrated – S1 and granulated – S2), three sewage sludge doses (0 – control, 14.5 and 29 Mg DM/ha) and 2 years of study. The main objective of this study was the determination of the concentration of the following potentially carcinogenic PAHs: BP – Benzo(g,h,i)perylene, BaA – benzo(a)anthracene, BbF – benzo(b)fluoranthene, Chry – chrysene, BkF – benzo(k)fluoranthene, BaP – benzo(a)pyrene, IP – Indeno(1,2,3cd)pyrene, DBA – dibenzo(a,h)anthracene by using GC/MS. The basic properties of the studied soils were evaluated, that is, dehydrogenase and catalase activity, the total number of bacteria, C:N ratio and organic matter content. Fertilization with sewage sludge had a significant influence on the concentration of individual potentially carcinogenic PAHs in the urban soil samples. Moreover, in the second year, the reduction of the sum of potentially carcinogenic PAHs by approximately 55% after the S1 application and an increase of approximately 6% after the S2 application was observed.

Keywords: Potentially carcinogenic PAHs; Urban soil; Granular and dehydrated sewage sludge

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a major group of organic compounds occurring in the environment. They are composed of two or more fused benzene rings [1,2]. Among all PAHs, the following are classified as probable or possible human and animals carcinogens, even at low levels: BP – benzo(g,h,i)perylene, BaA – benzo(a)anthracene, BbF – benzo(b)fluoranthene, Chry – chrysene, BkF – benzo(k)fluoranthene, BaP – benzo(a)pyrene, IP – indeno(1,2,3cd)pyrene, DBA – dibenzo(a,h)anthracene, with the toxicity equivalency factor for DBA, IP, BbF, BkF, Chry, BaP and BaA, were 1, 0.1, 0.1, 0.01, 0.001, 1 and 0.1, respectively [3–5].

PAHs released in the environment are conditioned by two main sources: natural – forest fires and volcanoes and burning natural vegetation [6] and anthropogenic – industrial processes traffic and fossil fuel combustion [7–9]. The environment acts as a sink for contaminants, which are the major threat for soil due to its capacity of retaining and holding pollutants. Because of this, soil is considered as a steady indicator of the environmental pollution state [2,10]. As noted by Peng et al. [10], long-term accumulation of contaminants may negatively affect soil quality, especially in case of urban soils. However, as suggested by Sieciechowicz et al. [11] urban dusts are carriers of toxic substances and they introduce these compounds into other compartments of the environment,

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such as water bodies, air, plants, etc. This is related not only to urban and street runoff, re-suspension and re-deposition but also to municipal sewage system, which endangers the ecosystem [12]. It is important to mention that the accumulation of PAHs in soil and plants may also lead to the contamination of food chain, and may constitute a potential risk to human health by affecting several systems, for instance, immunological, reproductive, neurological and respiratory [13]. Therefore, the United States Environmental Protection Agency (USEPA) gives indications for controlling the amount of PAHs in major environmental elements, including water, soil, air and plants [3].

According to Singh and Agrawal [14], urban soil has to be constantly protected, because of urban development, high traffic density and incomplete combustion of fuel. Sewage sludge can be used for reclamation treatments [8]. Sludge improves soil condition, enriching it with organic and mineral compounds. It has a significant influence on the number of microorganisms, soil enzymatic activities, and on the development of plants. However, harmful and toxic compounds contained in the sludge, such as heavy metals, PAHs, etc., may influence unfavorably on biological activity and the development of soil microorganisms [14,15]. As reported by Riffaldi et al. [16] soil microorganisms may act as catalysts in the degradation and mineralization of various xenobiotics and in their assimilation or transformation into non-toxic chemicals compounds [17].

The aim of the study was to determine the concentration of potentially carcinogenic PAHs in soils fertilized with different rates of two sewage sludges. Additionally, the objectives were to analyze the persistence of PAHs depending on the sludge dose and some characteristics of studied soils. Moreover the identification of the potential sources of analyzed PAHs in urban soils was carried out.

2. Materials and methods

2.1. Experimental design

The field study was localized on the lawns along the main roads in Białystok (53°08′N, 23°10′E). For the studied soil, two different types of sewage sludge were used: S1 – after-press dewatered sludge from the Treatment Plant in Sokółka and S2 – dry sludge in the form of pellets from the Treatment Plant in Białystok. In the experiment, sewage sludge (0 – control, 14.5 and 29 Mg DM/ha) and two lawns in Bialystok along the main roads (Popiełuszki and Hetmańska Str.) with different traffic intensity and 2 years of study were considered as main factors.

Two types of the municipal sewage sludge used in the experiment were analyzed according to the Directive of Ministry of Environment [18]. Both of them fulfilled the requirements for the application of sludge to non-agricultural land reclamation. Table 1 shows physical, chemical and biological properties of the two analyzed sewage sludge types, that is, dewatered after press and dried in the form of pellets.

2.2. Sampling

The samples were taken from 36 plots, which were divided into 18 sectors (5 m^2 each sector, in a completely randomized block design). To determine PAHs and soil physico-chemical parameters, soil samples were taken from each plots at a depth of 0–20 cm from three different places. Subsequently, they were transferred into a specially marked container, which was put in the fridge and transported to the laboratory.

2.3. Physico-chemical parameters of soil samples

The particle size was determined using the Casagrande's areometric method modified by Prószyński according to PN-R-04032 standard [19], which is dedicated to the analysis of agricultural soils. In spring and autumn, pH of soil was evaluated in distilled water in the ratio 1:2.5 (m:v) using a pH-meter, HACH Lange, Wrocław, Poland.

The organic matter content was determined by drying soil samples at 105°C (removing hygroscopic water) and then burned in a muffle furnace at 500°C. The total organic carbon was measured by Tiurin's method described by Ostrowska et al. [20]. The total nitrogen content was analyzed by Kjeldahl method using Gerhardt's Vapodest 50s after mineralization using Kjeldatherm (Gerhardt) block digestion.

2.4. PAHs analyses

Soil samples and two analyzed types of sewage sludge were monitored for 16 PAHs, which are potentially hazardous to human health. The group comprises the following PAHs: BP – benzo(g,h,i)perylene; AN – anthracene; FL – fluorene; ACE – acenaphthene; PH – phenanthrene; ACY – acenaphthylene; PY – pyrene; BaA – benzo(a)anthracene; BbF – benzo(b)fluoranthene; Chry – chrysene; BkF – benzo(k) fluoranthene; FLU – fluoranthene; BaP – benzo(a)pyrene; IP – indeno(1,2,3cd)pyrene; DBA – dibenzo(a,h)anthracene; NA – naphthalene. In our study, from the 16 PAHs, 8 potentially carcinogenic compounds, such as BP, BaA, BbF, Chry, BkF, BaP, IP and DBA were analyzed.

Table 1
Selected physical, chemical and biological properties of sewage sludge before application into soil

Sewage sludge samples	pH	% DM					(%)
		Total nitrogen	N-NH_3	Ca	Mg	Organic matter	Dry matter
After-press dewatered sludge	6.7	4.0	0.10	5.50	0.70	58.4	19.3
Dry sludge in the form of pellets	8.2	4.6	0.18	3.79	0.57	56.9	81.7
Viable helminth ova of *Ascaris* sp., *Trichuris* sp., *Toxocara* sp.	Not detected						
Bacteria of the genus *Salmonella* in 100 g of sludge	Not detected						

All samples were analyzed according to ISO 18287:2008 [21]. The standards of analyzed PAHs were obtained from AccuStandard® Inc., New Haven, USA. All samples were analyzed in triplicate.

The homogenized fresh samples (20 g) were placed in 100 mL flasks and extracted for 1 h on a shaker using 50 mL portion of acetone. Subsequently, 50 mL petroleum ether was added and it was further extracted for 1 h. The extract was decanted and material was flooded with a new portion of petroleum ether (50 mL), extracted for an hour. Subsequently, the extracts were combined. Acetone and other polar compounds were removed by washing the samples twice with 400 mL of deionized water. The remaining organic phase was dried over anhydrous sodium sulfate (anhydrous, ACS reagent, ≥99% Sigma-Aldrich Co., St. Louis, USA) and purified by adsorption chromatography on silica gel (Macherey-Nagel CHROMABOND Columns SiOH, 1,000 mg, pore size 60 Å, particle size 45 µm). 100 µL isooctane was added to pure extracts as a stabilizer and then the extracts were concentrated in an inert gas stream (5.0 purity nitrogen) to a volume of 1 mL. The prepared solutions were analyzed by using a gas chromatograph (GC/MS Triple Quad 7890B, Agilent, Santa Clara, USA) equipped with split/splitless dispenser and capillary column HP-5MS size 30 m × 0.25 mm × 0.25 µm.

2.5. Identification and quantification

PAHs were identified by comparing the retention times and mass spectra of each compound with retention times of reference PAH, using the PAH Mix certified reference material. In order to better evidence the mass spectra, one also compared PAHs with a library of spectra NIST MS search 2.2.

The GC/MS calibration was conducted by running five calibration levels. Calibration curves were developed for each of the 16 PAH compounds and their R^2 values were ≥99%.

During the extraction, no internal standard was added, while the control of the method was conducted by simultaneous performing the executed analytical procedure for a certified reference material sample – Clean Soil Reference Material EDF-5183 (CERILLIANT Analytical Reference Standards). In this study, external standard calibration was performed by using calibration curves for individual compounds. Calibration solutions were prepared from the standard 16 PAHs mixture (Z-014G, AccuStandard, New Haven, USA). The recovery of PAHs individual compounds was between 85% and 91%. The limit of detection (LOD) was calculated by determining the ratio and values of signal-to-noise. The LOD for PAHs was 0.0004 mg/kg and the limit of quantification reached 0.001 mg/kg. The signal-to-noise ratio was 0.00033. In the validation procedure, it was assumed that LOD = 3S/N, while LOQ = 4LOD.

2.6. Microorganisms analyses

Soil samples from the rhizosphere level were analyzed three times (April, July and October in 2 years) and were prepared according to Galimska-Stypa et al. [22]. A serial 10-fold dilution (10^{-1}–10^{-6}) for each of the samples was prepared and each dilution was used to inoculate petri dishes. The total bacteria number was determined using 10% tryptic soy agar medium after the incubation for 72 h at 28°C. The Gram-negative bacteria was determined using 10% tryptic soy agar medium with 0.1% solution of crystal violet after incubation at 28°C for 72 h [22].

The mean number of colonies in triplicate was presented as a colony-forming unit (CFU) in grams of roots and soil dry matter (DM).

2.7. Analyses of enzyme activities

Dehydrogenase activity (DHA) in soil was determined according to Casida et al. [23], modified by Tabatabai [24]. One mL of 3% 2,3,5-triphenyltetrazolium chloride aqueous solution was added to a 6-g soil sample and 2–4 mL distilled water was added to all the samples. The samples were incubated for 20 h at 37°C. 20 mL of methanol was added to each sample and shaken vigorously, then filtered. The TPF – triphenylformazan was extracted and the reddish color absorbance was measured with a HACH – spectrophotometer UV-VIS DR 5000 at l = 485 nm. The obtained results were expressed in µmol TPF/g DM/h.

The catalase activity (CAT) in soil was determined by manganometric method described by Johnson and Temple [25] in the presence of 3% H_2O_2 as a substrate. To 2 g of air-dried soil, 40 mL of distilled water and 5 mL of substrate were added to each sample, shaken vigorously and 1.5 M H_2SO_4 was added, then filtered and it was titrated with 0.1 mol/L $KMnO_4$. The catalase soil activity was calculated as an amount of H_2O_2 decomposed by 1 g DM of soil during 1 min and were expressed in µmol H_2O_2/g DM min.

2.8. Statistical analysis

The influence of area localization, dose and type of sewage sludge was estimated by one-way variance analysis. The significant differences between means were evaluated by Tukey test at $p < 0.05$. The effect of studied parameters was assessed by performing Pearson correlation (at $p < 0.05$) by using Statistica 13.0.

3. Results and discussion

3.1. Concentration of PAHs in sewage sludge before application into soil

According to the literature data, sewage sludge applied to soil may raise significantly the level of PAHs in treated soil [26,27]. In our study, the sum of 16 PAHs in the tested sludge reached lower values than the European Union-suggested PAHs limit for the sludge (6 mg/kg DM), which is considered safe for application to soils [28]. In the tested sewage sludge applied to the urban soil, the concentration of 16 PAHs was 0.0024 mg/kg of DM for S1 and 3.11 mg/kg of DM for S2. Moreover, the sum of potentially carcinogenic PAHs for the two analyzed types of sewage sludge: S1 and S2, was 0.0007 and 1.582 mg/kg of DM, respectively (Table 2). According to Hua et al. [26], the PAHs concentration in sewage sludge, particularly potentially carcinogenic aromatic hydrocarbons, depends mainly on the quantity and type of industrial wastewater flowing into the treatment plants. Agricultural production dominates in the Podlaskie voivodeship (North East Poland) and there are no industrial plants

Table 2
Concentration of analyzed PAHs in the tested sewage sludge used in the experiment

PAHs in mg/kg of DM	S1	S2
BaA	0.00013	0.2485
Chry	0.00015	0.2885
BbF	0.00007	0.2910
BkF	0.00005	0.2821
BaP	0.00004	0.2362
IP	0.00013	0.0247
DBA	0.00008	0.0490
BP	0.00003	0.1615
Sum Σ8 PAHs	0.0007	1.5815
Sum Σ16 PAHs	0.0024	3.1075

S1 – after-press dewatered sludge; S2 – dry sludge in the form of pellets; DM – dry matter.

in this region. Moreover, sediments formed in the municipal treatment plants are characterized by low PAHs content in the sewage sludge. Therefore, untreated sewage sludge from small wastewater treatment plants situated in non-industrial areas is not an additional source of soil contamination and is a valuable substrate for soil fertilization in urbanized areas.

3.2. Concentration of potentially carcinogenic PAHs in soil after application of sewage sludge

PAHs are very widespread in soil, which results from their persistence [2]. As noted by Riccardi et al. [29], the accumulation of PAHs in soil is associated with their molecules. One of the most important factors affecting the movement of PAHs in soil is the size of soil particles and the size of the sorbent. Moreover, as suggested by Abdel-Shafya and Mansour [30], the octanol–water partitioning coefficient and solubility as well as soil conductivity also have influence on the PAHs mobility in soil. Urban soil incorporate various building wastes, which results in low humus and water content as well as in the low biological activity of these soil. In addition, intensive car traffic contributes to gradual chemical degradation of soil, leading to sustainable deterioration of its properties [9]. In this study, the sum of 16 PAHs in urban soil at selected locations before application of the sludge was approximately 1.8 mg/kg DM for Hetmańska Str. and 3.1 mg/kg DM for Popiełuszki Str. The potentially carcinogenic PAHs concentration in soil depended on the type of sewage sludge and location of experimental areas. However, the dose of sludge had no effect on their concentration (Figs. 1–3). A higher concentration of BaA, Chry, BbF and BP was observed for S2 as well as on the plots at Popiełuszki Str. (Figs. 1 and 3).

In the first year after the S1 application, the sum of potentially carcinogenic PAHs in soil ranged from 0.7 (for the objects fertilized with 29 Mg DM/ha at Hetmańska Str.) to 3.7 mg/kg DM (for the objects fertilized with 14.5 Mg DM/ha at Popiełuszki Str.). In the next year of the study, the sum of potentially carcinogenic PAHs was 0.6 on the control plots at Hetmańska Str., while on the control plots at Popiełuszki Str., it amounted to 6.7 mg/kg DM. Moreover, in the first year

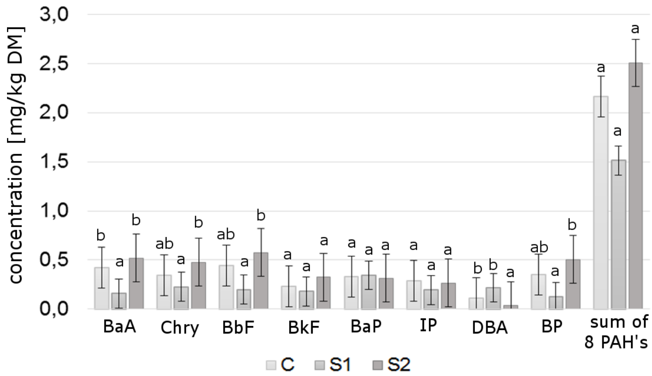

Fig. 1. Influence of two different types of sewage sludge (S1 and S2) on the potentially carcinogenic PAHs concentration in soil (the same letters mean non-significant differences between two kinds of sludge, evaluated by Tukey test at $p < 0.05$). C – without sewage sludge; S1 – after-press dewatered sludge; S2 – dry sludge to form pellets.

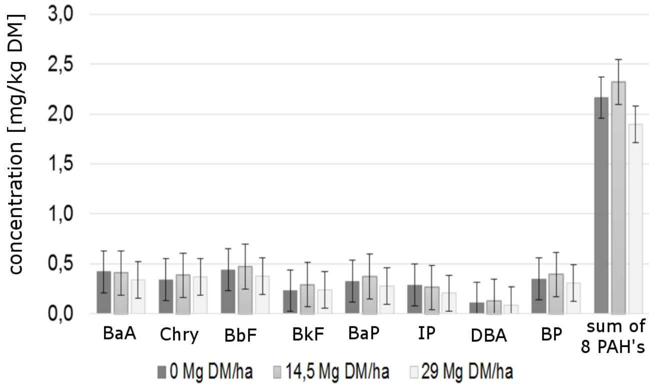

Fig. 2. Concentration of the potentially carcinogenic PAHs in soil depending on sewage sludge dose (0, 14.5 and 29 Mg DM/ha) (no letters mean non-significant differences between three dose of sludge, evaluated by Tukey test at $p < 0.05$).

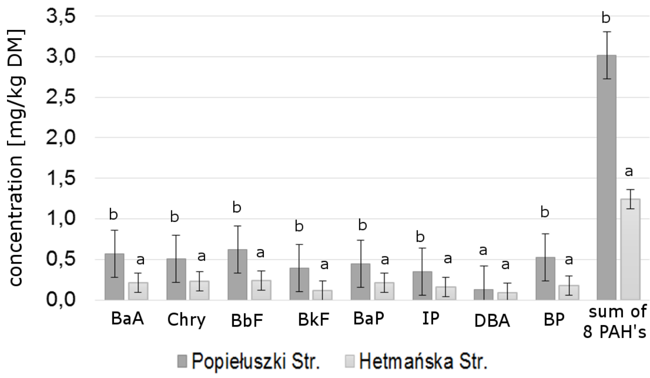

Fig. 3. Concentration of the potentially carcinogenic PAHs in soil depending on localization (Popiełuszki and Hetmańska Str.) (the same letters mean non-significant differences between two localizations, evaluated by Tukey test at $p < 0.05$).

after the application of S2 into soil, the sum of potentially carcinogenic PAHs reached values from 1.0 (for the control plots at Hetmańska Str.) to 5.3 mg/kg DM (for the objects fertilized with 29 Mg DM/ha at Popiełuszki Str.). In the second year, after the S2 application, the sum of potentially carcinogenic PAHs was 0.46 (for the objects fertilized with 29 Mg DM/ha at Hetmańska Str.) to 7.2 mg/kg DM (for the objects fertilized with 14.5 Mg DM/ha at Popiełuszki Str.). Among all analyzed potentially carcinogenic PAHs, in both years of study, benzo(a)pyrene was the most dominant compound in the samples collected from the plots where S1 had been applied. However, in the plots where S2 had been applied benzo(b)fluoranthene was the most dominant compound in the samples in both years of study, with the highest values of chrysene in the first year and benzo(a)anthracene in the second year (Fig. 4).

According to the literature, the addition of sludge to soil may influence the accumulation and persistence of PAHs [13,14]. In the present study, the addition of sludge to urban soil in the first year increased the sum of potentially carcinogenic PAHs by approximately 15% (for the objects fertilized with 14.5 mg DM/ha) and 6% (for the objects fertilized with 29 mg DM/ha) as compared with the control plots. Whereas, in the second year after the application of sludge, it was observed that the concentration of potentially carcinogenic PAHs in urban soil decreased in comparison with the control plots by approximately 6% (for the objects fertilized with 14.5 mg DM/ha) and 27% (for the objects fertilized with 29 mg DM/ha) (Fig. 4).

As noted in the studies by Lipińska et al. [31], the rate of PAHs biodegradation in soil depends on physicochemical and biological parameters and on the conditions in which organic compounds are decomposed, that is, availability of nutrients, presence of oxygen, pH and temperature. Table 3 shows selected properties of the studied soil after the application of two different types of sewage sludge. For all collected soil samples, the pH values ranged from 6.9 to 8.0, which shows that at each point alkalinity of soil was observed. The ratio of C:N at the control plots had a wide range, which in the first year was from 3.5 to 14.5 and in the following year

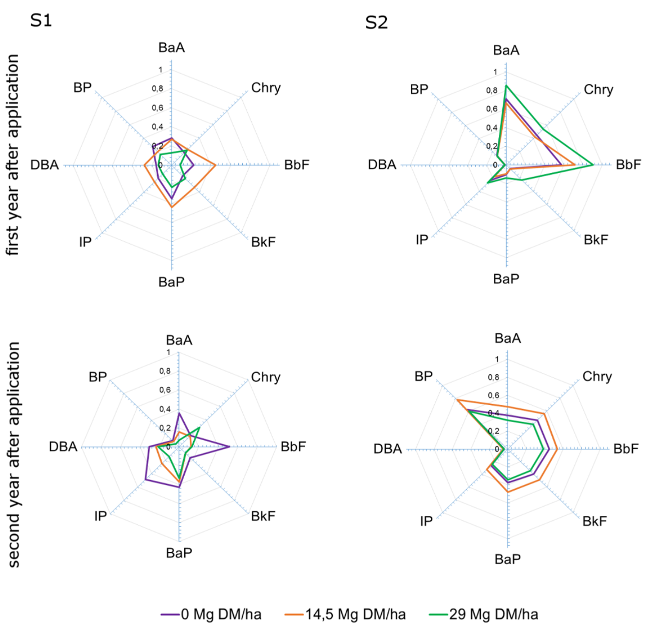

Fig. 4. Potentially carcinogenic PAHs (mg/kg DM) according to the year, dose and type of sludge.

from 3.7 to 13.7. Basing on the granulometric particle size, it was found that testing urban soil could be classified as light soil because the content of sand was ranged from 41% to 67%, the content of silt was ranged from 22% to 52% and clay content range was from 4.1% to 14%.

After the S1 application, the content of organic matter was ranged from 3.0% (for the control plots in the second year) to 7.7% for the plots with a double dose of sludge in the first year, while the plots fertilized with S2 ranged from 3.8% (for the control plots in the first year) to 10.1% in the second year after being fertilized with a single dose of sludge (Table 3). The sludge S1 and S2 influenced positively the growth of the total number of bacteria and the number of Gram-negative bacteria. Moreover, the usage of S1 and S2 also increased dehydrogenase and CAT in soil as shown in Table 3.

3.3. Identification of PAHs sources using diagnostic ratios

Diagnostic indicators allow for determining the sources of PAHs contamination in soil, water and air. The usage of PAHs ratios enables for the determination of the diversity of diesel and gasoline combustion emission [32], different oil processing products and biomass burning processes, including grass fires [33]. As reported by Manoli et al. [34], the PAH emission profile for a given source depends on the PAHs production processes. The type and amount of PAHs in soil depend on nearby sources, such as automotive exhaust gases and combustion of wood or hard coal in individual houses. In addition, some PAHs may originate from distant sources carried by air, including benzo(a)pyrene [35]. In this study, the equations representing a ratio of lnP/(lnP + BP) and BaA/(BaA + Chry) were used to identify pyrolytic sources [36]. A ratio of BaP/BP provides more precise information about the sources of traffic emissions.

The diagnostic ratios of BaP/BP vs. InP/(InP + BP) and BaA/(BaA + Chry) vs. InP/(InP + BP) indicate various sources of contamination with potentially carcinogenic PAHs: a ratio of lnP/(lnP + BP) < 0.20 – the source of a petroleum, a ratio within the range 0.20–0.50 – a liquid fossil fuel (crude and vehicle oil) source of combustion, and a coefficient > 0.5 – sources of grass, wood or coal combustion [33]. A BaP/BP indicator of >0.6 means a source of primarily traffic emissions and BaP/BP ratio of <0.6 indicates that no traffic emissions are involved [37]. As shown in Fig. 5, the ratio of InP/(InP + BP) was ranged between 0.08 and 0.98 (66.6% of the 24 samples exceed the level of 0.50 for S1 and 50% of the 24 samples exceed 0.50 for S2), which is also indicative of fossil fuel combustion as a main source of contribution in particular from coal combustion. In this study, the ratio of BaP/BP, ranged from 0.02 to 1.4 for S1 – 4 out of 24 samples reached higher values than 0.60, but for S2 – 22 out of 24 samples reached higher values than 0.60 showing that the major source of potentially carcinogenic PAHs observed in urban soil was influenced by traffic emissions. Furthermore, on the control plots, it was observed that the main sources of potentially carcinogenic PAHs in soil was both traffic emissions and coal combustion as shown in Fig. 5.

Moreover, the next ratio for a BaA/(BaA + Chry) < 0.20 indicates the sources of petroleum, a coefficient > 0.35 – sources of combustion, with values between 0.20 and 0.35 – combustion and petroleum sources [33,37]. In the present research, it can

Table 3
Selected properties of studied soil after application of two different type of sewage sludge

Parameters		Types of sewage sludge	Year	Dose of sewage sludge (Mg DM/ha)						Range	Mean
				0		14.5		29			
				Minimum–maximum	Mean	Minimum–maximum	Mean	Minimum–maximum	Mean		
pH H_2O	–	S1	I	7.6–7.7	–	7.4–7.8	–	7.3–7.6	–	7.3–8.3	–
			II	7.7–7.9		7.6–8.0		7.6–7.8			
		S2	I	7.1–7.5	–	7.3–7.5	–	7.2–7.4	–	6.9–7.7	–
			II	7.0–7.7		6.9–7.6		6.9–7.5			
C:N	–	S1	I	3.5–6.2	4.6 ± 1.12	4.6–6.4	5.3 ± 0.80	3.9–6.2	5.1 ± 1.12	3.5–11.1	5.7
			II	3.7–11.1	4.8 ±3 .27	6.7–9.3	7.6 ± 1.23	4.8–9.2	6.8 ± 1.85		
		S2	I	10.7–14.5	12.4 ± 1.8	11.3–12.7	11.8 ± 0.63	9.0–11.8	10.8 ± 1.2	6.2–18.7	11.3
			II	8.3–13.7	11.7 ± 2.1	6.6–18.7	12.2 ± 3.6	6.2–11.3	9.2 ± 0.2		
Sand	%	S1	I	53–67	60.7 ± 6.13	57–68	61.7 ± 4.8	56–65	60.5 ± 4.20	53–68	60.9
			II								
		S2	I	41–57	48.5 ± 5.7	43–54	47.0 ± 3.9	54–56	54.9 ± 0.53	41–57	50.1
			II								
Silt	%	S1	I	22–35	27.5 ± 5.57	23–32	27.8 ± 4.03	26–31	28.3 ± 2.63	22–35	27.9
			II								
		S2	I	38–52	46.1 ± 5.5	40–51.1	46.6 ± 4.3	41–43	41.9 ± 1.1	38–52	44.9
			II								
Clay	%	S1	I	11–13	11.7 ± 0.96	9–11	10.5 ± 1.0	9–14	11.3 ± 2.10	9–14	11.2
			II								
		S2	I	4.7–6.4	5.4 ± 0.64	4.1–9.2	5.6 ± 2.4	4.1–4.8	4.5 ± 0.33	4.1–9.2	5.2
			II								
Organic matter	%	S1	I	4.5–4.8	4.6±0.16	5.1–6.3	5.7 ± 0.52	5.1–7.7	6.4 ± 1.04	3.0–7.7	4.9
			II	3.0–4.1	3.6 ± 0.6	3.9–5.3	4.4 ± 0.62	4.9–5.2	4.9 ± 0.17		
		S2	I	3.8–5.7	4.9 ± 0.84	5.7–6.5	5.9 ± 0.37	6.2–8.5	7.5 ± 1.1	3.8–10.1	6.5
			II	4.0–6.9	5.6 ± 0.95	5.1–10.1	6.9 ± 1.6	7.2–9.5	8.1 ± 0.8		
Total number of bacteria	CFU × 10^7/g DM	S1	I	7.1–30.8	18.5 ± 10.75	9.6–42.7	26.7 ± 15.5	12.7–37.9	25.5 ± 11.07	1.4–42.7	14.3
			II	1.6–7.0	3.6 ± 2.33	1.4–6.2	3.9 ± 2.05	3.3–12.7	7.7 ± 3.8		
		S2	I	1.9–10.8	6.3 ± 4.5	7.0–17.7	11.6 ± 5.0	8.5–22.9	15.1 ± 6.1	1.9–55.0	14.0
			II	2.4–26.0	9.9 ± 8.1	2.7–25.4	13.6 ± 7.8	3.6–55.0	27.7 ± 18.5		
Gram-negative bacteria	CFU × 10^6/g DM	S1	I	1.8–8.2	5.2 ± 2.9	3.1–7.5	5.8 ± 2.3	8.0–16.4	11.3 ± 4.4	0.9–16.4	6.6
			II	1.9–13.0	6.0 ± 4.9	0.9–14.9	6.1 ± 5.9	1.6–13.1	5.3 ± 4.9		
		S2	I	0.3–3.7	2.1 ± 1.3	4.0–8.8	6.0 ± 2.3	1.8–20.1	8.3 ± 7.3	0.3–20.1	4.8
			II	1.0–4.0	2.1 ± 0.9	0.7–11.5	4.6 ± 3.9	1.1–17.0	5.4 ± 4.7		
DHA activity	μmol TPF/g DM 20 h	S1	I	0.15–0.28	0.21 ± 0.05	0.22–0.35	0.28 ± 0.06	0.18–0.39	0.25 ± 0.10	0.03–0.68	0.35
			II	0.17–0.55	0.38 ± 0.15	0.03–0.68	0.45 ± 0.29	0.21–0.67	0.50 ± 0.21		
		S2	I	0.1–0.41	0.29 ± 0.13	0.09–0.40	0.27 ± 0.13	0.06–0.34	0.20 ± 0.12	0.04–0.87	0.27
			II	0.04–0.35	0.24 ± 0.1	0.05–0.37	0.21 ± 0.13	0.22–0.87	0.41 ± 0.22		
CAT	μmol H_2O_2/g DM min	S1	I	3.01–4.26	3.7 ± 0.55	2.25–4.26	3.6 ± 0.91	3.0–4.5	3.7 ± 0.80	2.25–5.52	4.0
			II	3.76–5.52	4.8 ± 0.74	2.26–5.27	3.9 ± 1.24	3.5–4.5	4.1 ± 0.43		
		S2	I	6.26–7.8	7.1 ± 0.64	8.0–10.5	9.2 ± 1.05	7.0–8.8	7.9 ± 0.9	5.9–16.9	8.2
			II	6.1–8.4	7.3 ± 0.8	6.8–15.3	8.6 ± 2.7	5.9–16.9	9.1 ± 3.3		

be seen that the BaA/(BaA + Chry) ratio was between 0.06 and 0.89. In most soil samples, it was higher than 0.35, which suggests a main mixed source of combustion (Fig. 5).

3.4. Analysis of correlation

As noted by Riccardi et al. [29], PAHs occurring in soil begin to associate with its particles. In turn, one of the most important factors influencing the movement of PAHs in soil is the size of pores and the sorbent particle [17]. Table 4 shows the coefficients correlation of potentially carcinogenic PAHs content in soil between selected parameters of soil. Among analyzed potentially carcinogenic PAHs, only DBA was significantly positively correlated with soil pH ($r = 0.56$) for $p < 0.05$. In turn, the C:N ratio showed significantly positive correlation with BaA, Chry, BbF and BP (respectively, $r = 0.36$, $r = 0.32$, $r = 0.36$ and $r = 0.29$), whereas a negative correlation was observed for DBA $r = –0.40$ in the $p < 0.05$. Based on these results, was observed negative correlations between the sand in soil and BaA ($r = –0.40$), Chry ($r = –0.54$), BbF ($r = –0.44$), BkF ($r = –0.57$), BaP ($r = –0.36$), IP ($r = –0.31$) and BP ($r = –0.69$), as well as between clay and BaA ($r = –0.45$), Chry ($r = –0.53$), BbF ($r = –0.37$), BkF ($r = –0.27$) and BP ($r = –0.39$), significant at $p < 0.05$. The content of DBA was positively correlated with sand and clay (respectively, $r = 0.27$ and $r = 0.64$) and negatively with silt and organic matter (respectively, $r = –0.44$ and $r = –0.38$, for $p < 0.05$). Beside, silt was positive correlated with BaA ($r = 0.49$), Chry ($r = 0.61$), BbF ($r = 0.48$), BkF ($r = 0.53$), IP ($r = 0.26$) and BP ($r = 0.65$) for $p < 0.05$ (Table 4). According to Singh and Ward [38], due to their hydrophobic nature, PAHs show a high affinity for organic phases of soil and specific mineral surfaces, in particular clay minerals [39]. Numerous studies of the correlation of the partition coefficient with soil properties have found that the organic carbon content usually yields the most significant correlation [29].

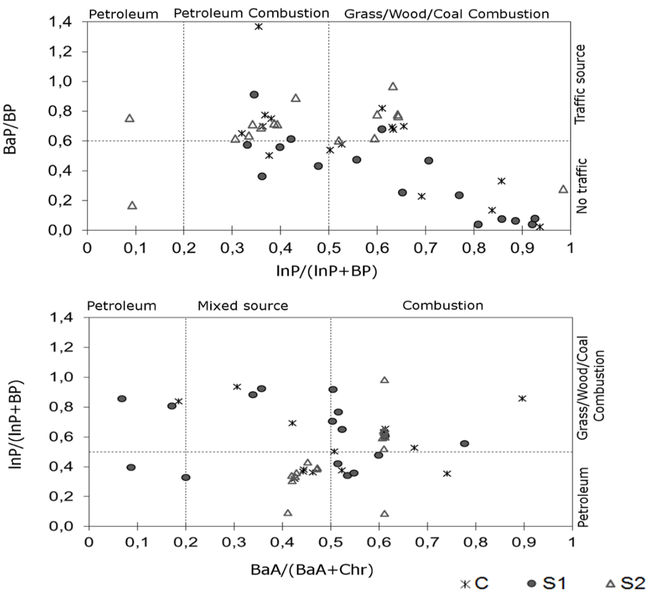

Fig. 5. Diagnostic ratios of BaP/BP vs. InP/(InP + BP) and BaA/(BaA + Chry) vs. InP/(InP + BP) contamination from different sources of potentially carcinogenic PAHs in urban soil. C – without sewage sludge; S1 – after-press dewatered sludge; S2 – dry sludge to form pellets.

There are many reports of PAHs degradation under the influence of microorganisms; however, in natural conditions, it is a slow process in comparison with the rate of PAHs degradation under controlled conditions [16,40]. Microorganisms in soil contaminated with PAHs can use the organic substrate as a source of carbon and energy for the production of their own biomass. The analysis gave a positive correlation of CAT with BaA, Chry, BbF and BP (respectively, $r = 0.32$, $r = 0.36$, $r = 0.31$ and $r = 0.31$) and a negative one with DBA $r = –0.50$, significant at $p < 0.05$. DHA was negatively correlated with BP ($r = –0.36$) (Table 4).

As reported by Chandra et al. [40], one way of decomposing hydrocarbons is by transferring them into the cell via bacteria. This process takes place through the formation of a surfactant, so called emulsan or emulsifier (biosurfactant), glycolipids, lipopeptides, phospholipids, neutral fats, polysaccharides or fatty acids. Gram-negative bacteria have cell membranes which contain lipophilic components such as lipopolysaccharides or steroids. There were negative correlations between the Gram-negative bacteria and Chry ($r = –0.28$) and a positive correlation with BkF ($r = 0.26$) for $p < 0.05$ (Table 4).

Table 4
Correlation of coefficients of potentially carcinogenic PAHs content in soil, and microbiological activity, granulometric parameters, pH and C:N ratios

Compound	pH H_2O	C:N	(%)				CFU/g DM		DHA	CAT
			Sand	Silt	Clay	OM	Total number of bacteria	Gram-negative bacteria		
BaA	–0.17	0.36*	–0.40*	0.49*	–0.45*	0.12	–0.15	–0.14	–0.23	0.32*
Chry	–0.25	0.32*	–0.54*	0.61*	–0.53*	0.24	–0.14	–0.28*	–0.20	0.36*
BbF	–0.18	0.36*	–0.44*	0.48*	–0.37*	0.17	–0.14	0.02	–0.16	0.31*
BkF	–0.23	0.19	–0.57*	0.53*	–0.27*	0.24	–0.06	0.26*	–0.23	0.23
BaP	0.15	–0.03	–0.36*	0.23	0.11	–0.07	–0.18	0.03	–0.12	–0.08
IP	0.11	0.19	–0.31*	0.26*	–0.04	–0.05	–0.19	–0.11	–0.05	0.04
DBA	0.56*	–0.40*	0.27*	–0.44	0.64*	–0.38*	–0.04	0.16	0.16	–0.50*
BP	–0.24	0.29*	–0.69*	0.65*	–0.39*	0.29*	–0.51	0.01	–0.36*	0.31*

*Significant correlations for $p < 0.05$.
OM, organic matter.

4. Conclusions

1. Fertilization with sewage sludge influenced significantly the concentration of individual potentially carcinogenic PAHs in the urban soil samples. Moreover, in the second year, a reduction of the sum of potentially carcinogenic PAHs by approximately 55% after the S1 application and an increase of approximately 6% after the S2 application was observed.
2. The type of sewage sludge had significant influence on the concentration of BaA, Chry, BbF and BP. A higher concentration was observed for granular sludge (S2).
3. The analysis of the correlations indicates that the concentration of potentially carcinogenic PAHs such as BaA, Chry, BbF and BP in urban soil influenced significant CAT, while the Chry inhibited Gram-negative bacteria and DHA activity was inhibited by BP.

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Conflict of interest

The authors declare that they have no conflict of interest.

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