HUMIC SUBSTANCES IN THE ENVIRONMENT

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Mutual relations between PAHs derived from atmospheric deposition, enzymatic activity, and humic substances in soils of differently urbanized areas

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Abstract

Purpose The aim of this study was to determine the mutual relations between polycyclic aromatic hydrocarbons (PAHs) originated from atmospheric emissions and enzymatic activity and humic substances in soils at differently urbanized area, on an example of the Lublin city, east Poland.

Materials and methods The chosen areas represented three differently urbanized environments: old tenement houses and modern residential blocks, mixture of different building and rural landscape, and typical rural environment with smallholding farms, respectively. On each of the urban, suburban, and rural areas, one representative plot was chosen on fallow lands classified as luvisol derived from loess. The soil samples were collected from the top 25 cm layer. The following properties were determined: pH, organic carbon, total nitrogen, humic and fulvic acids, PAHs content (14 PAHs from US EPA list), and the activities of the following enzymes: dehydrogenases, acid phosphatase, alkane phosphatase, protease, and urease.

Results and discussion Higher contents of organic C and total N were found in the rural soil samples. The share of humic acid was similar in all soils investigated, ranging from 19.38 to 25.27%, while fulvic acid values differ significantly between urban and rural areas. The urban soils indicated much lower share of fulvic acids (9.78–10.99%) than those of rural (29.02–29.32%). Consequently, the values of the $C_{HA}:C_{FA}$ ratio of the urban soil were approximately two times higher than those of the rural soil. The results showed that both the rate of humification and the activity of dehydrogenases, acid phosphatase, alkaline phosphatase, and proteases in the soils increased in the following sequence: urban < suburban < rural.

Conclusions The results showed that an increase of PAHs in the urbanized areas affect other soil properties. The phenanthrene/anthracene and fluoranthene/pyrene ratios pointed to coal combustion as the principal source of PAHs in the investigated soils. The PAH content in the urbanized area inhibit humification processes in the soil and the activity of dehydrogenases, acid phosphatase, alkaline phosphatase, and proteases.

Keywords Enzymatic activity · Humic substances · PAHs · Polycyclic aromatic hydrocarbons · Soil

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1 Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants. PAHs are organic environmental contaminants formed during incomplete combustion of fossil fuels such as coal, oil, and natural gas (Galarneau 2008; Ravindra et al. 2008; Mostert et al. 2010). They represent a large group of organic compounds with two or more fused aromatic (benzene) rings. Low molecular weight PAHs (two and three rings) occur predominantly in the vapor phase in the atmosphere, whereas multi-ringed PAHs (five rings or more) are largely bound to particles. Intermediate molecular weight PAHs (four rings) are partitioned between the vapor and particulate phases, depending on the atmospheric temperature (Ravindra et al. 2008; Mostert et al. 2010). Atmospheric PAHs are continuously deposited to the soil by dry or wet deposition processes. Some of these PAHs are from nearby sources, such as automotive exhaust from adjacent roadways. Other PAHs are from more distant sources and have been carried various distances through the air. Furthermore, PAHs can be added to soils with materials containing these compounds (composts, biochar, spills from agricultural machinery) (Galarneau 2008; Mostert et al. 2010).

PAHs are subjected to many physicochemical and biological processes in soil contributing to a various extent to their loss. However, the majority of the compounds are characterized by a high persistence in the terrestrial environment, due to their low water solubility and high sorption affinity to soil components. According to the literature data (Maliszewska-Kordybach et al. 2008; Luo et al. 2012; Klimkowicz-Pawlas et al. 2017), soil organic matter is generally considered to have a significant impact on PAH concentration. Both amount and composition of soil organic matter affect the sorption of organic contaminants (Ehlers and Loibner 2006; Ni et al. 2008; Dębska et al. 2011; Wang et al. 2012; Klimkowicz-Pawlas et al. 2017). Humic substances constitute the largest part of organic matter and it is considered responsible for the binding of contaminants (Ni et al. 2008; Pignatello 2012). Thus, they may control the processes associated with fate, transport, degradation, and bioavailability of PAHs, affecting the quality of soil.

Additionally, the determination of the quality-related properties can provide important information about the behavior of contaminants in soil. Soil enzyme activities have been suggested as suitable, early and sensitive indicators of soil quality (Baran et al. 2004; Yang et al. 2009; Futa et al. 2016). They are a measure of the soil microbial activity, and therefore, they are strictly related to the nutrient cycles and transformations. Moreover, they also may rapidly respond to the changes caused by both natural and anthropogenic factors and are relatively easy to measure (Yang et al. 2009).

Therefore, for evaluation of the quality of soil contaminated by PAHs, it is important to assess the level of contamination that can be tolerated by soil organisms and plants without any negative impact (Futa et al. 2016). A reliable assessment of soil quality and health can be done by activity tests for a range of enzymes. Mentioned bioindicators reflect the contamination level of the environment, including those hazardous to living organisms.

The aim of this study was to determine the relations between the content of PAHs in soils at differently urbanized area and selected soil parameters such as enzymatic activity and humic substances, on an example of the Lublin city, east Poland.

2 Materials and methods

Research area was located in the north-western part of Lublin in Lubelskie Voivodeship (western part of the country, Fig. 1), covered an area of 147 km^2, and featured a relatively high population density; an average density of 2326 persons/km^2 (Rocznik Statystyczny 2015). Research area combines districts of typical urban/industrial character and agricultural areas. The main possible dispersed emission/contamination sources in this region were typical industrial plants located on the outskirts of the city, road transport, airport, power station, and heat and power plant. The transect localization was defined by two factors, firstly, it includes build up areas with an individual coal-based heating system which is a source of PAH emission, and the second transect covers the areas with a different density of built up areas (from high density in an urban zone to low density in the rural zone). Therefore, the transect reflects the gradual density of PAH emission sources starting from the urban zone where we expect the high load of PAHs to the rural zone almost deprived of pollution emitter.

On each of the urban, suburban, and rural areas, one representative plot was chosen on fallow lands located at the higher part of the upland. The soils of all areas were classified as luvisol derived from loess. The samples were collected from the top 25 cm layer, during the first 10 days of March in 2014, 2015, and 2016 year. To ensure that a moderate intensity in the biochemical processes was obtained, the dates of soil sampling were scheduled for a period when the soil was in dynamic equilibrium. From different parts of each representative plot, five combined soil samples were collected.

The collected material was air-dried and sieved through a 2-mm mesh. The following standard properties were determined: pH-potentiometrically in 1 M KCl (ISO 10390), organic carbon content (C_{org})-oxidometrically (ISO 14235), and total nitrogen (Nt) by Kjeldahl method (ISO 13878). Enzymatic activity was determined by dehydrogenase activity (according to Thalmann 1968), acid phosphatase activity, alkaline phosphatase activity (according to Tabatabai and Bremner, 1969), urease activity (according
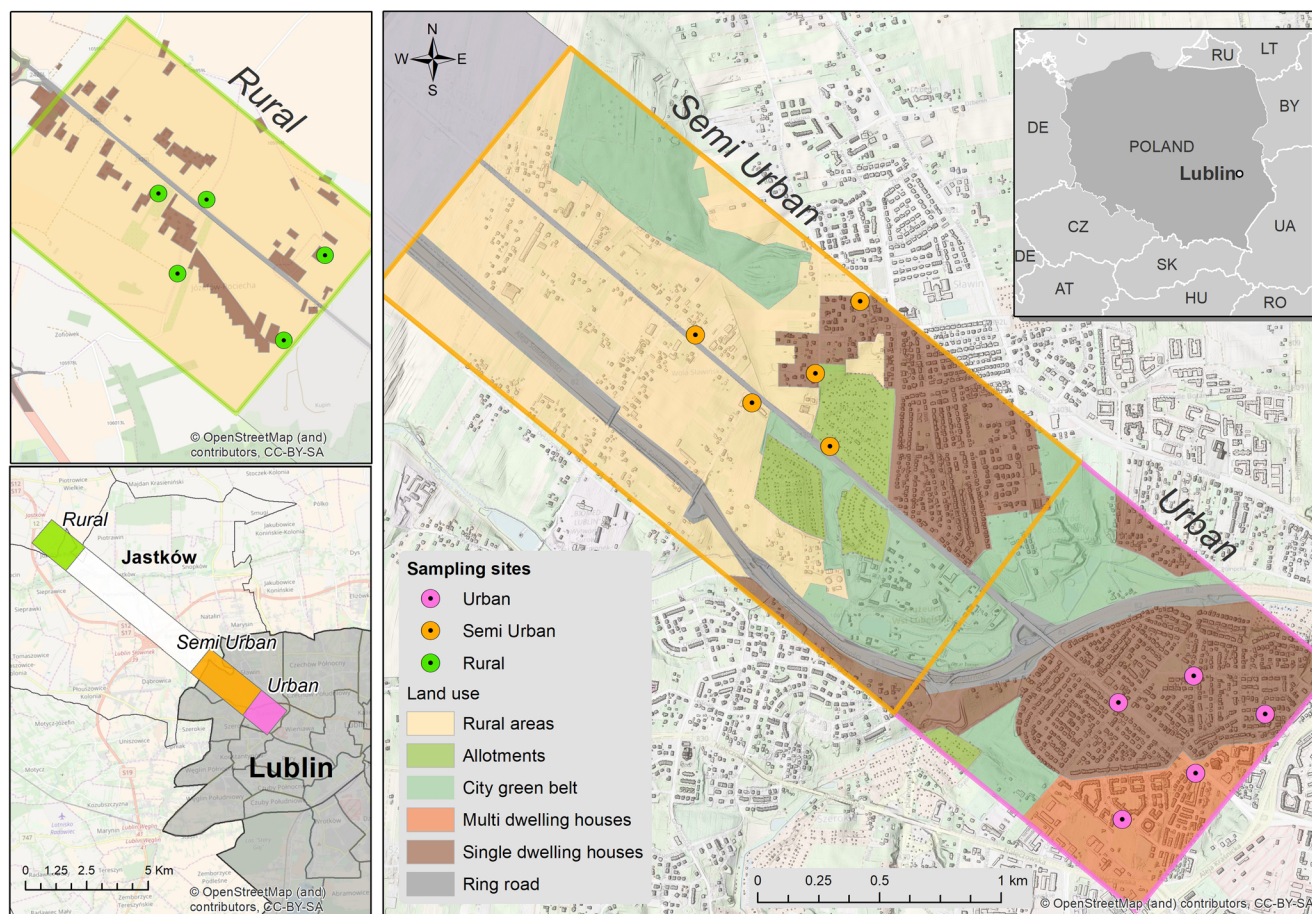
Fig. 1 Research area

to Zantua and Bremner 1975), and protease activity (according to Ladd and Butler 1972).

The determination of organic pollutants included 14 individual PAH compounds from the US EPA list—with the exception of naphthalene and acenaphthene. PAHs were isolated by soil extraction with dichloromethane and separated on Spherisorb S5 PAH column with chemically bound C18 phase (Oleszczuk 2007). The concentrations of PAHs were determined by high pressure liquid chromatography (HPLC) with UV detection at 254 nm (Baran and Oleszczuk 2001). The quality control included analysis of certified reference material (CRM 131), laboratory control sample, and solvent blank sample procedure. The recovery for individual compounds from CRM 131 was within 78–85%, and precision expressed as a relative standard deviation (RSD) was $\leq 12\%$. The method detection limit (MDL) for individual PAH compounds fitted on the 0.16 μg kg^{-1} level while the practical quantitation level (PQL) at 0.4 μg kg^{-1}.

Humic substances (C_{HS}), including humic acids (C_{HA}) and fulvic acids (C_{FA}), were separated and measured according to Stevenson (1994).

All determinations were made in three parallel replications. A maximum 5% dispersion (expressed by relative standard deviations) of measurements in the biochemical and chemical analysis was assumed in the study.

2.1 Statistics

Statistica software (Dell Statistica, version 10PL) and Microsoft Office Excel 2003 were used for the statistical analysis. Means were compared by the Tukey's t test at a confidence level of $p < 0.05$. Pearson correlation was used as a measurement of simple association among determined soil parameters. Values of $p \leq 0.05$ were considered statistically significant.

3 Results and discussion

The organic carbon content in the top 25 cm layer of the soil was between 11.4 and 16.8 g kg^{-1} (Table 1), which corresponded to the mean European values according to the European Union criteria (Rusco et al. 2001). The C:N ratios in the urban soils were between 12.5–12.6, while the suburban and rural soils indicated 9.1–9.6 values.

Table 1 Content of organic carbon (C_{org}), total nitrogen (N_t), ratio C:N, and pH

	Year	C_{org} g kg^{-1}	N_t	C:N	pH
Urban area	2014	14.1a	1.1a	12.6a	6.2
	2015	14.5a	1.2a	12.5a	6.4
	2016	14.2a	1.1a	12.6a	6.5
Suburban area	2014	11.4a	1.2a	9.2a	6.1
	2015	11.5a	1.3a	9.1a	6.3
	2016	11.4a	1.2a	9.1a	6.4
Rural area	2014	16.6ab	1.7ab	9.6a	6.0
	2015	16.8ab	1.8ab	9.4a	5.7
	2016	16.7ab	1.8ab	9.2a	5.9

Means followed by the same letters do not differ significantly ($p > 0.05$)

The percentage share of humic substance carbon (C_{HS}) in the organic carbon is considered as one of the parameters of the degree of the humification, referred to as the humification index (Tan 2003). The highest values of C_{HS} were found in the rural soil samples, and the lowest in the urban soil samples (Table 2). The differences in C_{HS} pointed out to lower intensity of humification processes in the urban area, compared to rural areas, which was confirmed also by the data of enzymatic activities (Table 3). The biosynthesis processes and humus degradation are strictly associated with the activity of the soil enzymes. The investigated areas differed also in the final products of the humification processes. The share of humic acid (C_{HA}) was similar in all soils investigated, ranging from 19.38 to 25.27%, while fulvic acids (C_{FA}) share differentiated significantly between urban and rural areas. The urban soils indicated much lower share of fulvic acids (9.78–10.99%) than those of rural (29.02–29.32%). Consequently, the values of the C_{HA}:C_{FA} ratio of the soils in urban area were approximately two times higher than those of the rural area. This may have

Table 2 Percentage share of humic substances, humic and fulvic acids in C_{org} and C_{HA}:C_{FA} ratio

	Year	C_{HS} (% C_{org})	C_{HA}	C_{FA}	C_{HA}:C_{FA}
Urban area	2014	31.76a	20.77a	10.99a	1.89a
	2015	29.16a	19.38a	9.78a	1.98a
	2016	29.92a	20.12a	9.80a	2.05a
Suburban area	2014	42.08ab	24.36a	17.72ab	1.37ab
	2015	39.86ab	23.95a	15.91ab	1.50ab
	2016	41.06ab	24.11a	16.95ab	1.42ab
Rural area	2014	54.52b	25.21a	29.31b	0.86b
	2015	54.15b	24.91a	29.24b	0.85b
	2016	54.30b	25.27a	29.03b	0.87b

Means followed by the same letters do not differ significantly ($p > 0.05$)

been related to the differences in soil conditions of the rural versus urban environment. Higher values of humification index in soils of urban area are connected with the absence of human interference into the soil structure, favoring protection of soil aggregates and higher water retention (Guimaraes et al. 2013). These conditions limit access to the air and inhibit the mineralization of organic matter. Plowing and other agronomic treatments in arable soils cause the loss of organic matter to varying degrees. Furthermore, accumulated carbon in the topsoil of undisturbed soils remains in a state of dynamic equilibrium with the plant community, which favors the additional protection of its resources, depending on type and age of plant communities (Dinghua et al. 2001; Maryganova et al. 2010). Older trees produce more organic matter, which can be a potential reason for an increased rate of fulvic acids (Antisari et al. 2011). The amounts of humic versus fulvic acids depend on tree species, soil properties as well as weather conditions, which predict the direction of humification processes. Degradation of lignin-rich plant debris may result in an increase in C_{HA}:C_{FA} ratio proportionally to an increase in humic acid content, and thus, soils overgrown with mixed-tree stands may indicate higher C_{HA}:C_{FA} ratio than arable soils (Zhang et al. 1988). Also, the degree of soil contamination with PAHs could affect the properties of the organic matter. Dębska et al. (2011) found that in soil freshly contaminated with PAHs is followed disturbs the equilibrium, resulting in temporary changes in the content of soluble organic matter.

It is well known that enzyme activity depends on with the amount and quality of soil organic matter (Błońska et al. 2017). Non-cultivated soils, dominating in urban areas, contain more organic carbon because roots play a disproportionately important role in the carbon budget in soils (Mazilli et al. 2015). Furthermore, soil organic carbon mineralization is affected by soil aggregation (Fazzle Rabbi et al. 2014; Jia et al. 2015), while physicochemical features of the soil influence the microbial properties (Cheng et al. 2013). You et al. (2014) demonstrated the complex interactions and linkage among plant traits, microenvironment, and soil physiochemical properties, which affect soil organic carbon via microbial regulations. Xie et al. (2017) revealed that the soil physicochemical properties explained 74.5% of the variation in soil enzyme activities.

The enzymatic activity of the analyzed soils varied significantly between differently urbanized areas (Table 3). The intensity and direction of the observed changes depended on the individual characteristics of a given enzyme. The results showed that both the rate of humification and the activity of dehydrogenase, acid phosphatase, alkaline phosphatase, and protease increased in the following sequence: urban < suburban < rural.

The activity of urease was found to be about two times higher in the urban soil than in soils of the suburban and rural areas. Urease, as an extracellular enzyme, can be synthesized

Table 3 Enzymatic activity of soils at differently urbanized area

	Year	DhA	PhacA	PhalA	UA	PA
Urban area	2014	2.48a	1.78a	1.29a	19.92b	4.32a
	2015	1.96a	1.49a	1.10a	15.35b	3.86a
	2016	1.54a	1.16a	1.02a	18.69b	3.12a
	Average	*1.99a*	*1.47a*	*1.13a*	*17.98b*	*3.76a*
Suburban area	2014	5.32b	3.82b	2.95b	17.04b	8.97b
	2015	4.87b	3.44b	2.68b	15.29b	8.32b
	2016	4.36b	3.29b	2.46b	16.11b	7.16b
	Average	*4.85b*	*3.51b*	*2.69b*	*16.14b*	*8.15b*
Rural area	2014	8.59c	6.58b	4.72c	8.73a	12.23c
	2015	8.22c	6.19b	4.49c	7.46a	11.94c
	2016	8.47c	6.74b	4.86c	8.19a	12.89c
	Average	*8.42c*	*6.50b*	*4.69c*	*8.12a*	*12.35c*

Means followed by the same letters do not differ significantly ($p > 0.05$)

DhA dehydrogenases in mg TPF·kg^{-1}·d^{-1}, *PhacA* and *PhalA* acid and alkaline phosphatase in mg PNP·kg^{-1}·h^{-1}, *UA* urease in mg N-NH$_4^+$·kg^{-1}·h^{-1}, *PA* protease in mg tyrosine kg^{-1}·h^{-1}.

only in the presence of substrate (urea). This enzyme is resistant to external factors, and the only factor reducing its activity is deficiency of the urea. Large urban agglomerations are important producers of urea. The sources of urea in the soil of urbanized environment include food waste, animal excretions, fragments of soil fauna tissue, and plant debris. There were no relation between the year of the investigation and the urease activity in the soil.

The enzymatic activity (with an exception of urease) in the urban soil was over 3–4 times lower than in the rural soil, and approximately two times lower than in the suburban soil. The observed reduction in the enzymatic activity in the urban soil was associated with increased anthropogenic pressure, expressed by an increase of PAH content in the soil (Table 4). Other studies have also demonstrated that the presence of PAHs inhibits the biosynthesis of numerous enzymes in the soil by microorganisms (Baran et al. 2004; Wyszkowska et al. 2006; Wyszkowska and Wyszkowski 2010). Over the consecutive years during the study, the clear trend of decreasing activity of dehydrogenases, phosphatases, and proteases in the urban and suburban soils was observed.

Differently urbanized areas indicated clear differences in the content of the PAHs in the studied soils (Table 4). The highest content of PAHs was observed in the urban soil. The total content of 14 PAHs determined in this area exceeded 2000 μg kg^{-1}, and was over five times higher than those in the rural soil. For the suburban soil, with the same exposure to atmospheric PAH deposition as the urban soil (dense single-family housing and individual heating, intensity of road traffic), the observed PAH content was approximately 1.5 times lower than in the urban soil. This may be due to the fact that the suburban area was open, allowing pollution emitted there to be moved away by air currents (Futa et al. 2016). This suggestion is confirmed also by the reduced rate of

benzo(*a*)pyrene and benzo(*ghi*)perylene in the suburban soil samples, compared to the urban soil samples, by factors of 3 and 2, respectively. The PAHs are emitted as very fine particulates; thus, they may be effectively transported to long distances (Hwang et al. 2003; Mostert et al. 2010). WHO guidelines (2010) reported PAH concentration found in European soil surfaces (WHO guidelines 2010). Typical concentrations in forest soil range from 5 to 100 μg kg^{-1}. Substantial amounts of PAHs are transferred to forest soil from vegetative litter because the compounds are adsorbed from air onto organic matter such as leaves and pine needles. Rural soil contains carcinogenic PAHs at levels of 10–100 μg kg^{-1}, originating mainly from atmospheric fallout. For both forest and rural soil, values as high as 1000 μg kg^{-1} may occasionally be found (WHO Guidelines 2010). Metropolitan areas have higher PAH concentrations than forest and agricultural areas because of the many sources of fossil fuel combustion. The majority of urban soil concentrations fall in the 600–3000 μg kg^{-1} range. Higher values near areas of heavy transportation and industrialization range from 8 to 336 mg/kg. Values in the order of 1000–3000 μg kg^{-1} are regarded as being in the upper range (WHO Guidelines 2010).

The analysis of individual PAHs demonstrated in all the cases the dominance of low molecular weight PAHs (LMW-PAHs): fluorene, fluoranthene, phenanthrene, and pyrene, which content in the studied soils established approximately 70% of all the determined PAHs (Table 5). The presence of LMW-PAHs in the environment is associated with their emission by various means of transport (Sims and Overcash 1983; Wild and Jones 1995). The high content of these PAHs in the rural area, characterized by low pollution especially connected with transport, may suggest a distant source of pollution, which is confirmed also by other studies (Cousins et al. 1997).

Table 4 The content of PAHs ($\mu g \cdot kg^{-1}$) in years from 2014 till 2016

Year	Urban area 2014	2015	2016	Suburban area 2014	2015	2016	Rural area 2014	2015	2016
Ace	56.9	62.4	70.1	38.1	43.2	47.8	24.3	23.7	25.1
Fl	328.6	340.6	370.5	244.3	258.4	261.9	121.8	122.4	119.7
Phen	401.8	430.2	456.0	219.6	236.8	252.3	58.1	60.2	69.6
Ant	45.6	52.1	55.9	25.9	30.1	34.6	7.9	8.6	9.8
Fluo	369.7	392.5	422.8	321.2	356.8	384.2	57.8	59.3	58.4
Pyr	304.6	321.9	348.2	256.0	272.1	293.6	41.3	42.2	41.4
BaA	136.2	149.8	157.4	49.5	56.8	60.9	6.4	7.6	6.9
Chr	128.3	141.6	152.8	113.9	120.5	134.8	20.9	26.4	22.3
BbF	112.8	130.2	145.6	42.8	46.9	52.3	6.8	7.2	6.1
BkF	58.4	66.0	71.9	30.1	36.8	39.2	4.3	5.2	4.9
BaP	49.6	55.8	61.2	17.9	18.6	19.3	10.8	11.6	11.2
DahA	25.8	27.2	30.5	22.6	25.4	27.8	19.1	18.4	19.8
BghiP	28.4	33.1	35.4	12.8	14.1	15.3	3.8	4.6	4.2
Ind	37.2	40.8	44.6	31.5	36.9	40.1	4.3	5.2	4.9
Σ	2083.9	2244.2	2422.9	1426.2	1553.4	1664.1	387.6	402.6	404.3
Pollution degree[#]	3[0]	3[0]	3[0]	3[0]	3[0]	3[0]	1[0]	1[0]	1[0]

Ace acenaphthlene, *Fl* fluorene, *Phen* phenanthrene, *Ant* anthracene, *Fluo* fluoranthene, *Pyr* pyrene, *BaA* benzo(*a*)anthracene, *Chr* chrysene, *BbF* benzo(*b*)fluoranthene, *BkF* benzo(*k*)fluoranthene, *BaP* benzo(*a*)pyrene, *DahA* dibenzo(*a,h*)anthracene, *BghiP* benzo(*ghi*)perylene, *Ind* indeno(1,2,3-*cd*)pyrene

[#] Pollution degree (Kabata-Pendias et al. 1995) 1[0] uncontaminated soil—increased content (ΣPAHs 200–600 $\mu g \cdot kg^{-1}$) 3[0] contaminated soil (ΣPAHs 1000–5000 $\mu g\ kg^{-1}$); the values apply to "mean" soil, containing up to 2% of organic matter ($mg \cdot kg^{-1}$)

The PAH emission profile for a given source depends on the processes producing the PAHs. During low temperature processes (e.g., wood burning), low molecular weight PAHs are usually formed, whereas high temperature processes, such as the combustion of fuels in engines, emit higher molecular weight PAH compounds (Mostert et al. 2010). At high

Table 5 Percentage share of particular PAHs in years from 2014 till 2016

Year	Urban area 2014	2015	2016	Suburban area 2014	2015	2016	Rural area 2014	2015	2016
Ace	2.7	2.8	2.9	2.6	2.8	2.9	6.3	5.9	6.2
Fl	15.8	15.2	15.3	17.1	16.7	15.7	31.4	30.4	29.6
Phen	19.3	19.2	18.8	15.4	15.2	15.2	14.9	14.9	17.2
Ant	2.2	2.3	2.3	1.8	1.9	2.1	2.0	2.1	2.4
Fluo	17.7	17.5	17.6	22.4	22.9	23.1	14.9	14.8	14.4
Pyr	14.6	14.3	14.4	17.9	17.6	17.6	10.6	10.5	10.2
BaA	6.5	6.7	6.5	3.5	3.6	3.6	1.6	1.9	1.7
Chr	6.2	6.3	6.3	7.9	7.8	8.1	5.6	6.5	5.6
BbF	5.4	5.8	5.9	3.0	3.0	3.1	1.9	1.8	1.5
BkF	2.8	2.9	2.9	2.1	2.4	2.4	1.1	1.3	1.2
BaP	2.4	2.5	2.5	1.6	1.2	1.2	2.8	2.9	2.8
DahA	1.2	1.2	1.3	1.6	1.6	1.7	4.9	4.6	4.9
BghiP	1.4	1.5	1.5	0.9	0.9	0.9	0.9	1.1	1.1
Ind	1.8	1.8	1.8	2.2	2.4	2.4	1.1	1.3	1.2

Ace acenaphthlene, *Fl* fluorene, *Phen* phenanthrene, *Ant* anthracene, *Fluo* fluoranthene, *Pyr* pyrene, *BaA* benzo(a)anthracene, *Chr* chrysene, *BbF* benzo(b)fluoranthene, *BkF* benzo(k)fluoranthene, *BaP* benzo(a)pyrene, *DahA* dibenzo(a,h)anthracene, *BghiP* benzo(ghi)perylene, *Ind* indeno(1,2,3-cd)pyrene

Table 6 The molecular diagnostic ratios of Phen/Ant and Fluo/Pyr for evaluation of PAHs contamination source

Year	Phen/Ant			Fluo/Pyr		
	2014	2015	2016	2014	2015	2016
Urban area	8.8	8.2	8.1	1.2	1.2	1.2
Suburban area	8.4	7.8	7.3	1.2	1.3	1.3
Rural area	7.3	7.0	7.1	1.4	1.4	1.4

Phen/Ant ratio < 10—pyrogenic source of PAHs; Fluo/Pyr ratio > 1.0—petrogenic source of PAHs

temperatures, organic compounds are cracked to reactive radicals, which react to form stable PAHs during pyrosynthesis. These PAHs are less alkylated and their molecules contain more aromatic rings than petrogenic PAHs (Hwang et al. 2003). PAHs are emitted as a mixture, and the relative molecular concentration ratios are considered to be characteristic of a given emission source (Abdel-Shafy and Mansour 2016; Santos et al. 2017). PAH diagnostic ratios show intrasource variability but intersource similarity. The search for PAH emission sources using diagnostic ratios should proceed with the determination of the ratios for each emission source present in the area investigated. Most diagnostic ratios involve pairs of PAHs with the same molar mass and similar physicochemical properties (Galarneau 2008; Tobiaszewski and Namiesnik 2012). According to Tobiaszewski and Namiesnik (2012), the Fla/(Fla + Pyr) and IcdP/(IcdP + BghiP) ratios are more conservative than Ant/(Ant + Phe) and BaA/(BaA + Chr), which are particularly sensitive to photodegradation. The Ant/(Ant + Phe) ratio is sensitive to environmental changes and its values for the identification of particular processes lie within a narrow range, which makes it hard to use. Gschwend and Hites (1981) and Ravindra et al. (2008) suggest that Phe/Ant and Fla/Pyr ratios are most often used as a semiquantitative environmental forensic technique to distinguish between petrogenic (oil-based) and pyrogenic (combustion-based) sources of PAHs. The

analysis of the results indicated that the PAHs in analyzed soils originated from both petrogenic and pyrogenic sources. Phe/Ant ratio from 7.0 to 8.8 (below 10) indicates on pyrogenic source of hydrocarbons while Fla/Pyr ratio from 1.2 to 1.4 suggests that they originate from petrogenic sources (Table 6).

The total rates of PAHs characterized by the strongest carcinogenic and mutagenic effects (benzo(a)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, dibenzo(a,h)anthracene, indeno(1,2,3-cd)pyrene) in the urban soils were between 20.1 and 20.9% of the total content of all analyzed PAHs, while in the suburban and rural soils, these rates were significantly lower, ranging from 13.3 to 14.4% (Table 7).

The content of the sum 14 PAHs indicated significantly negative correlation with the content of C_{HS}, and contained in them C_{FA}, while the relations with C_{HA} were not significant. Similarly, negative correlations between contaminants and the soil enzymes such as dehydrogenases, acid phosphatase, and proteases were observed (Table 8). This may indicate a significant effect of hydrocarbons on the reduction of microbial activity and related with that fulvic fraction content—the most sensitive to change soil conditions and dependent on microbiological activity. The detailed role of the organic matter fractions in the transformation and accumulation of organic pollutants in the soil is described by Kuppusamy et al. (2017). Furthermore, Pitnatello and Xing (1996), Cornelissen et al. (2003), and Oleszczuk (2007) showed a clear correlation between the organic carbon of anthropogenic origin and PAH sorption in the soil. Positive correlations between the organic carbon, total nitrogen content, C:N ratio, C_{HS}, C_{HA}, fulvic C_{FA}, and activity of most of soil enzymes (excluding urease) are clear and do not require any additional comment.

Our research has shown that the individual PAH indicated significant negative correlation with soil parameters and the activity of dehydrogenase and protease (Table 9). The content of C_{FA} indicated a significantly negative correlation with about 70% of 14 PAHs, while the content of C_{HS} and the activity of acid phosphatase showed a significantly negative

Table 7 The percentage share of carcinogenic PAHs in ∑14PAHs

	Urban area			Suburban area			Rural area		
	2014	2015	2016	2014	2015	2016	2014	2015	2016
BaA	6.5	6.7	6.5	3.5	3.6	3.6	1.6	1.9	1.7
BbF	5.4	5.8	5.9	3.0	3.0	3.1	1.9	1.8	1.5
BkF	2.8	2.9	2.9	2.1	2.4	2.4	1.1	1.3	1.2
BaP	2.4	2.5	2.5	1.6	1.2	1.2	2.8	2.9	2.8
DahA	1.2	1.2	1.3	1.6	1.6	1.7	4.9	4.6	4.9
Ind	1.8	1.8	1.8	2.2	2.4	2.4	1.1	1.3	1.2
Σ 6PAHs	20.1	20.9	20.9	14.0	14.2	14.4	13.4	13.8	13.3

BaA benzo(a)anthracene, *BbF* benzo(b)fluoranthene, *BkF* benzo(k)fluoranthene, *BaP* benzo(a)pyrene, *DahA* dibenzo(a,h)anthracene, *Ind* indeno(1,2,3-cd)pyrene

Table 8 Correlation coefficients between investigated soil properties (n = 12)

	N_t	C:N	C_{HS}	C_{HA}	C_{FA}	DhA	PhacA	PhalA	PA	UA	Σ14PAHs
C_{org}	0.96	0.93	0.59	0.69	0.73	0.82	0.88	0.92	0.78	n.s.	n.s.
N_t		0.99	0.78	0.86	0.86	0.89	0.94	0.98	0.83	0.67	n.s.
C:N			0.82	0.91	0.88	0.88	0.93	0.98	0.81	0.74	n.s.
C_{HS}				0.97	0.97	0.89	0.87	0.86	0.83	0.92	− 0.58
C_{HA}					0.95	0.87	0.87	0.91	0.79	0.94	n.s
C_{FA}						0.97	0.95	0.93	0.93	0.82	− 0.59
DhA							0.99	0.95	0.99	0.67	− 0.64
PhacA								0.98	0.97	0.66	− 0.58
PhalA									0.90	0.72	n.s.
PA										0.57	− 0.73
UA											n.s.

ns not significant at $p > 0.05$

correlation with the 50% of the determined PAHs. These results confirm findings on sensitivity of dehydrogenase and phosphatase to soil pollution with PAHs (Baran et al. 2004; Wyszkowska et al. 2006; Wyszkowska and Wyszkowski 2010). Obtained results show that the activity of dehydrogenase and protease may be a valuable indicator of soil degradation by contaminants, such as PAH compounds (Bielińska et al. 2012). Individual enzymes have different requirements regarding optimal conditions for the environment where they remain active, and they demonstrate varied resistance and sensitivity to environmental stress. This confirms again that determination of the activity of soil enzymes provides a

Table 9 Correlation coefficients between the individual PAH content and soil properties (n = 12)

	C_{HS}	C_{FA}	DhA	PhacA	PA
Ace	n.s.	n.s.	− 0.58	n.s.	− 0.68
Fl	n.s.	n.s.	− 0.59	n.s.	− 0.66
Phen	− 0.60	− 0.65	− 0.69	− 0.61	− 0.77
Ant	− 0.62	− 0.64	− 0.65	− 0.58	− 0.72
Fluo	n.s.	− 0.58	− 0.64	− 0.59	− 0.74
Pyr	n.s.	− 0.58	− 0.62	n.s.	− 0.72
BaA	− 0.68	− 0.71	− 0.71	− 0.62	− 0.78
Chr	− 0.60	− 0.64	− 0.68	− 0.59	− 0.76
BbF	− 0.60	− 0.64	− 0.68	− 0.60	− 0.77
BkF	− 0.60	− 0.63	− 0.65	− 0.58	− 0.73
BaP	n.s.	− 0.59	− 0.62	n.s.	− 0.71
DahA	n.s.	n.s.	− 0.62	n.s.	− 0.68
BghiP	− 0.62	− 0.62	− 0.61	n.s.	− 0.68
Ind	n.s.	n.s.	− 0.59	n.s.	− 0.63

Ace acenaphthlene, *Fl* fluorene, *Phen* phenanthrene, *Ant* anthracene, *Fluo* fluoranthene, *Pyr* pyrene, *BaA* benzo(*a*)anthracene, *Chr* chrysene, *BbF* benzo(*b*)fluoranthene, *BkF* benzo(*k*)fluoranthene, *BaP* benzo(*a*)pyrene, *DahA* dibenzo(*a*,*h*)anthracene, *BghiP* benzo(*ghi*)perylene, *Ind* indeno(1,2,3-*cd*)pyrene, *ns* not significant at $p = 0.05$

reliable evaluation of soil quality (Futa 2017; Li et al. 2015). Obtained results indicated that all discussed soil parameters showed a significantly negative correlation with the following PAHs: phenanthrene, anthracene, chrysene, and those indicating the strongest carcinogenic and mutagenic properties: benzo(*a*)anthracene, benzo(*b*)fluoranthene, benzo(*k*)fluoranthene. The inhibiting effects of PAHs are associated with their toxicity to microorganisms, type of enzyme, characteristics of individual compounds, and their quantity in the soil environment (Lipińska et al. 2014; Wyszkowska et al. 2015). The adaptation of soil microorganisms to environmental pollution with PAHs is also important (Rodrigues-Blanco et al. 2010).

Results obtained in the vicinity of Czechowice-Dziedzice refinery in Poland, where the total PAH content was over 25,000 μg kg^{-1}, showed a very low dehydrogenase activity, at the range of 0.32–0.46 mg TPF kg^{-1} day^{-1} (Małachowska-Jutsz and Miksch 2000). Also, soils of the industrialized area of Upper Silesia, characterized by a very high content of PAHs, exceeding 18 g kg^{-1}, indicated very low activity of dehydrogenases, acid phosphatase, alkaline phosphatase, urease, and proteases, in the range of 0.57–0.63 mg TPF kg^{-1} day^{-1}, 5.12–7.96 mg PNP kg^{-1} h^{-1}, 3.98–6.89 mg PNP kg^{-1} h^{-1}, 2.95–9.29 mg N-NH4^{+} kg^{-1} h^{-1}, and 1.23–1.56 mg tyrosine kg^{-1} h^{-1}, respectively (Bielińska et al. 2012). Suburbs of the city of Zamość, where the total PAH content in soils did not exceed 100 μg kg^{-1}, indicated several times higher activity of these enzymes (Bielińska et al. 2012), which were similar to the data presented in this study.

4 Conclusions

1. Anthropogenic factor in the urbanized areas contributes to an increase of polycyclic aromatic hydrocarbons (PAHs) and affect other soil properties.

2. The phenanthrene/anthracene and fluoranthene/pyrene ratios pointed to coal combustion as the principal source of PAHs in the investigated soils.

3. The PAHs originated from the urbanized area inhibit humification processes in the soil and the activity of dehydrogenase, acid phosphatase, alkaline phosphatase, and protease.

4. Negatively correlated amounts of PAHs and activities of dehydrogenase and protease indicate that enzymatic activity can be used as an indicator of the soil pollution with PAHs.

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