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ENZYMATIC ACTIVITY AND CONTENT OF POLYCYCLIC AROMATIC HYDROCARBONS (PAHS) IN SOILS UNDER LOW-STACK EMISSION IN LUBLIN

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Abstract. Soils from the Lublin area exposed to low-stack emission from home heating and transport were investigated. Changes in soil enzymatic activity and polycyclic aromatic hydrocarbons (PAHs) content were analysed. Soil samples were collected in July and November 2016 and January 2017. Results of the study show that enzymatic activity and PAHs content depended on types of buildings, study period (intensification of pollutant emission from household heating), traffic volume and atmospheric air movement. Low enzymatic activities and high PAHs content in the soils were observed in the autumn-winter period in the areas with dense single-family housing and located in the vicinity of streets with intense road traffic.

Keywords: urban soils, enzymatic activity, low-stack emission, PAHs

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INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are mutagenic and carcinogenic pollutants that pose a significant human health hazard (Wild and Jones 1995). PAHs identified as the strongest carcinogens include benzo[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, dibenz[*ah*]anthracene, and indeno[1,2,3-*cd*]pyrene (Kanaly and Harayama 2000). Moreover, these xenobiotics disrupt homeostasis of soil environment (Caravaca and Rodán 2003, Dębska *et al.* 2011), contributing to future ecological problems and consequences for the whole urban area. Low-stack emission (transport and home heating) is a significant source of PAH contamination in urban soils. Its environmental impact is further intensified by expansion of artificial surfaces (widely used to cover streets, squares, roofs, etc.) and impairment of complete air change in areas with high-density housing (Adamczewska *et al.* 2000, Bielińska *et al.* 2014).

Enzyme activity reflects anthropogenic changes in the soil environment caused by stressors. It is a precise measure of soil quality that includes both homeostatic capacity of an ecosystem, and a level of its pollution which endangers living organisms (Bielińska *et al.* 2009, Siwik-Ziomek and Lemanowicz 2016). In order to identify a risk of long-term adverse changes in urban soils, the biochemical indicators reflecting the metabolism of soil environment and describing the current eco-chemical soil condition were used.

MATERIALS AND METHODS

The study was conducted in the city of Lublin and encompassed the areas exposed to different levels of low-stack emission (transport, household furnaces). Soil samples were collected from the 0–25 cm layer in three time periods: in July and November 2016, and in January 2017. The soil sampling sites and potential sources of pollution are listed in Table 1.

TABLE 1. CHARACTERISTICS OF SAMPLING SITES

Area	Location	Pollution source
I	the Kalinowszczyzna district – a tower block housing estate called Osiedle 40-lecia; panel buildings	cars, urban buses
II	Stara Kalinowszczyzna – a square called Słomiany Rynek; tenements buildings dating from the early 1900s	cars, urban buses, household heating
III	Lublin–Warsaw exit road, single-family housing	cars, lorries, urban and interurban buses, household heating
IV	Lublin–Zamość exit road	cars, lorries, interurban buses
V	Ogród Saski – an urban park	cars, urban buses, home heating

Representative surface areas on lawns were selected in each study site (Fig. 1). Then samples for further analysis were mixed to obtain an average of the original 5 soil subsamples taken from each surface area. Contents of 16 PAHs were analysed in the soil samples by HPLC with UV detection (254 nm) according to Baran and Oleszczuk (2001) methodology. The activity of four soil enzymes was determined: dehydrogenases (Thalmann 1968), acid and alkalic phosphatases (Tabatabai and Bremner 1969), and urease (Zantua and Bremner 1975). These enzymes play a significant role in transformation of soil organic matter and are highly sensitive to environmental factors.

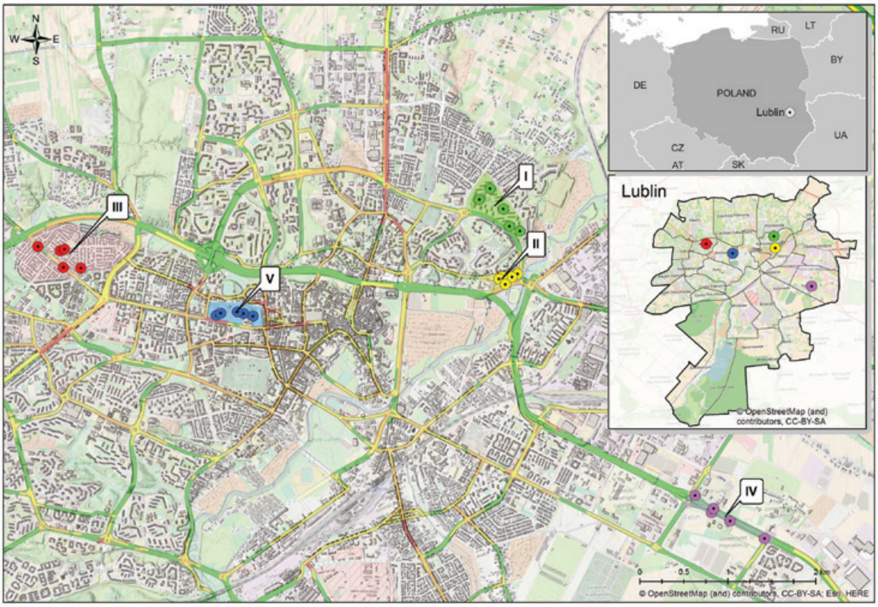

Fig. 1. Site soil sample location overlaid with traffic intensity

I – the Kalinowszczyzna district – a tower block housing estate called Osiedle 40-lecia
II – Stara Kalinowszczyzna – a square called Słomiany Rynek
III – Lublin–Warsaw exit road
IV – Lublin–Zamość exit road
V – Ogród Saski – an urban park

The statistical analysis of the results was performed in the Microsoft Office Excel 2003 spreadsheet and Statistica v. 10PL software. Statistical evaluation of the variability of the results was carried out with the two-way analysis of variance. The significance of the differences between the mean values was verified with Tukey's t-test at a significance level of $p \leq 0.05$. For some parameters, the value of the Pearson correlation coefficient (r) was calculated at $p<0.05$. A maximum 5% dispersion of measurements in the biochemical and chemical analysis was assumed in the study.

RESULTS AND DISCUSSION

Elevated levels of PAHs (Table 2) were observed in the soils taken from the investigated parts of the city in all three sampling periods. According to the classification by Maliszewska-Kordybach (1996), the soils studied can be classified as polluted, with the exception of the soil from the urban park (Ogród Saski) that could be described as slightly contaminated. The highest sum of 16 PAHs was found in the soil sampled in the Kalinowszczyzna district (areas I and II), particularly from Stara Kalinowszczyzna (area II) with its tenement houses built in the early 1900s where household heating most often uses cheap coal of poor quality and, consequently, of low heating value. Another factor influencing the level of soil contamination with PAHs in area II is its location in a land depression (the Bystrzyca River valley) which, alongside the high-density housing, restricts air movement. Moreover, areas I and II are located in the immediate vicinity of a busy arterial road. The total content of PAHs in the soil from area I (Osiedle 40-lecia, a tower block housing estate) ranged between 2,669 and 3,219 $\mu g \cdot kg^{-1}$ and between 3,448 to 4,501 $\mu g \cdot kg^{-1}$ in the soil from area II (Stara Kalinowszczyzna), whereas the PAH content in the soil from the remaining areas examined was several times lower (Table 2). In the soil collected from area III, despite its heavy traffic and dense single-family housing, the total content of PAHs was about two-fold lower than in the soil sampled in areas I and II. This result can be explained by the fact that low-rise buildings that prevail in area III allow particulate matter and gases to be transported longer distances (Kluska and Kroszczyński 2000, Futa *et al.* 2016). This conclusion is further supported by lower concentrations of benzo[*a*]pyrene and benzo[*ghi*]perylene found in the soil from this area compared with areas I and II (Table 2). The above PAHs are adsorbed and emitted into the air on very fine particulate matter and can travel considerable distances (Świetlik *et al.* 1998). Soil contamination with PAHs in area IV (Lublin–Zamość exit road) was similar to that found in the soil from area III. Some differences could result from the lower level of low-stack emission in area IV compared with that collected from area III. The high content of PAHs (1,010–1,203 $\mu g \cdot kg^{-1}$, Table 2) observed in the soil from an urban park (area V), especially in the autumn-winter period, could be partly explained by the lack of protection provided by trees due to a seasonal loss of their foliage. Ogród Saski, the park located in the city centre, is exposed to serious urban air pollution, mainly due to vehicle traffic, while its stand of trees reduces air movement.

The analysis of the individual PAHs demonstrated that in all cases (areas, sampling dates) hydrocarbons composed of 2–4 aromatic rings were dominant: naphthalene, fluorene, phenanthrene, fluoranthene, and pyrene accounted for about 70% of all the determined PAHs (Table 3). Observations of other authors (Sims and Overcash 1983, Wild and Jones 1995, Zerbe *et al.* 1995) suggest that

TABLE 2. THE CONTENT OF PAHS IN SOILS OF LUBLIN ($MG \cdot KG^{-1}$)

PAHs	Area														
	I			II			III			IV			V		
	Sampling periods														
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Na	214	230	257	255	309	346	121	157	161	107	140	143	184	238	257
Ace	63	70	81	72	97	118	51	84	92	32	49	56	24	39	46
Ac	101	115	123	118	151	169	62	93	115	34	52	64	46	63	78
Fl	323	352	373	711	785	824	191	224	262	232	284	315	143	172	186
Phe	416	442	463	358	412	457	148	239	255	132	179	205	82	95	112
Ant	31	46	54	49	70	86	12	24	27	12	16	18	7	13	16
Fln	454	575	606	525	672	694	209	298	348	174	192	206	80	126	159
Pyr	511	532	548	583	607	623	256	267	302	198	248	267	84	92	106
BaA	116	139	161	190	315	342	42	51	56	37	45	50	8	12	15
Ch	132	142	157	178	213	238	101	124	133	48	59	63	36	43	78
BbF	129	140	151	145	169	189	32	38	45	27	33	38	8	11	17
BkF	54	63	71	76	98	104	18	29	38	23	37	49	6	14	18
BaP	44	51	60	62	85	98	10	16	19	8	12	15	12	28	34
DahA	21	28	32	25	38	45	14	26	30	11	20	24	20	39	46
BghiP	26	32	37	54	79	92	7	12	15	6	12	15	5	8	11
Ind	34	40	45	47	62	76	19	38	42	7	15	16	9	17	24
Σ16PAHs	2669	2997	3219	3448	4162	4501	1293	1720	1940	1088	1393	1544	754	1010	1203

PAHs: Na – naphthalene, Ace – acenaphthylene, Ac – acenaphthalene, Fl – fluorene, Phe – phenanthrene, Ant – anthracene, Fln – fluoranthene, Pyr – pyrene, BaA – benz[*a*]anthracene, Ch – chrysene, BbF – benzo[*b*]fluoranthene, BkF – benzo[*k*]fluoranthene, BaP – benzo[*a*]pyrene, DahA – dibenz[*a,h*]anthracene, BghiP – benzo[*ghi*]perylene, Ind – indeno[*1,2,3-cd*]pyrene

Area: I – Osiedle 40-lecia (housing estate), II – Słomiany Rynek, III – Lublin–Warsaw exit road, IV – Lublin–Zamość exit road, V – Ogród Saski (an urban park)

Sampling periods: 1 – July 2016, 2 – November 2016, 3 – January 2017 the presence of 2–4 ring PAHs in the environment is linked to low-stack emission. The high content of PAHs in the soil of the urban park (area V), where the low-stack emission is limited, could indicate the remote source of pollution, which is confirmed by other publications (Cousins *et al.* 1997, Bielińska *et al.* 2011).

TABLE 3. COMPOSITION (%) OF INDIVIDUAL PAHS IN THE SOILS UNDER INVESTIGATION

PAHs	Area														
	I			II			III			IV			V		
	Sampling periods														
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Na	8.0	7.7	8.0	7.4	7.4	7.7	9.4	9.1	8.3	9.8	10.1	9.3	24.4	23.6	21.4
Ace	2.4	2.3	2.5	2.1	2.3	2.6	3.9	4.9	4.7	2.9	3.5	3.6	3.2	3.9	3.8
Ac	3.8	3.8	3.8	3.4	3.6	3.8	4.8	5.4	5.9	3.1	3.7	4.1	6.1	6.2	6.5
Fl	12.1	11.7	11.6	20.6	18.9	18.3	14.8	13.0	13.5	21.3	20.4	20.4	19.0	17.0	15.5
Phe	15.6	14.7	14.4	10.4	9.9	10.2	11.4	13.9	13.1	12.1	12.8	13.3	10.9	9.4	9.3
Ant	1.2	1.5	1.7	1.4	1.7	1.9	0.9	1.4	1.4	1.1	1.1	1.2	0.9	1.3	1.3
Fln	17.0	19.2	18.8	15.2	16.1	15.4	16.2	17.3	17.9	16.0	13.8	13.3	10.6	12.5	13.2
Pyr	19.1	17.8	17.0	16.9	14.6	13.8	19.8	15.5	15.6	18.2	17.8	17.3	11.1	9.1	8.8
BaA	4.3	4.6	5.0	5.5	7.6	7.6	3.2	3.0	2.9	3.4	3.2	3.2	1.1	1.2	1.2
Ch	4.9	4.7	4.9	5.2	5.1	5.3	7.8	7.2	6.9	4.4	4.2	4.1	4.8	4.3	6.5
BbF	4.8	4.7	4.7	4.2	4.1	4.2	2.5	2.2	2.3	2.5	2.4	2.5	1.1	1.1	1.4
BkF	2.0	2.1	2.2	2.2	2.4	2.3	1.4	1.7	2.0	2.1	2.7	3.2	0.8	1.4	1.5
BaP	1.6	1.7	1.9	1.8	2.0	2.2	0.8	0.9	1.0	0.7	0.9	1.0	1.6	2.8	2.8
DahA	0.8	0.9	1.0	0.7	0.9	1.0	1.1	1.5	1.5	1.0	1.4	1.6	2.7	3.9	3.8
BghiP	1.0	1.1	1.1	1.6	1.9	2.0	0.5	0.7	0.8	0.6	0.9	1.0	0.7	0.8	0.9
Ind	1.3	1.3	1.4	1.4	1.5	1.7	1.5	2.2	2.2	0.6	1.1	1.0	1.2	1.7	2.0
Σ16PAHs	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

The level of soil contamination with PAHs in areas I and II was similar, however, differences were found in contents of particular hydrocarbons, i.e.: fluorene, phenanthrene, pyrene, and benz[*a*]anthracene (Table 3). Greater percentage of benz[*a*]anthracene in the soils from area II noted in sampling time 2 and 3 indicates increased emission from household heating where coal is burned. One method of identifying sources of soil contamination with PAHs is to calculate the ratio between selected hydrocarbons (Oleszczuk and Baran 2005). In order to determine likely PAH sources, phenanthrene/anthracene and fluoranthene/pyrene ratios are most often used (Gschwend and Hites 1981, Oleszczuk and Baran 2005). The data analysis showed that coal burning in the autumn-winter period is the main source of PAHs in the soils of area I, II, III and V, which is reflected in the values of phenanthrene/anthracene ratios. The values did not exceed 10 (they ranged from 5.3 to 9.9), and in the case of the fluoranthene/pyrene ratios were higher than 1.

TABLE 4. PHENANTHRENE/ANTHRACENE (PHE:ANT) AND FLUORANTHENE/PYRENE (FLN:PYR) RATIOS

Area	Phe:Ant			Fln:Pyr		
	Sampling periods					
	1	2	3	1	2	3
I	13.4	9.6	8.5	0.8	1.1	1.1
II	7.3	5.8	5.3	0.9	1.1	1.1
III	12.3	9.9	9.4	0.8	1.1	1.1
IV	11.0	11.2	11.4	0.8	0.7	0.7
V	11.7	7.3	7.0	0.9	1.3	1.5

The concentrations of the most carcino- and mutagenic PAHs (i.e. benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, dibenz[*a,h*]anthracene, indeno[*1,2,3-cd*]pyrene) did not exceed 20% of the total PAH content, ranging from 8.5% to 12.7% in the soil from areas III, IV and V, and from 14.8% to 19.0% in the soil from areas I and II (Table 5).

TABLE 5. PERCENTAGE OF CARCINOGENIC PAHS IN TOTAL CONTENT OF PAHS

PAHs	Area														
	I			II			III			IV			V		
	Sampling periods														
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
BaA	4.3	4.6	5.0	5.5	7.6	7.6	3.2	3.0	2.9	3.4	3.2	3.2	1.1	1.2	1.2
BbF	4.8	4.7	4.7	4.2	4.1	4.2	2.5	2.2	2.3	2.5	2.4	2.5	1.1	1.1	1.4
BkF	2.0	2.1	2.2	2.2	2.4	2.3	1.4	1.7	2.0	2.1	2.7	3.2	0.8	1.4	1.5
BaP	1.6	1.7	1.9	1.8	2.0	2.2	0.8	0.9	1.0	0.7	0.9	1.0	1.6	2.8	2.8
DahA	0.8	0.9	1.0	0.7	0.9	1.0	1.1	1.5	1.5	1.0	1.4	1.6	2.7	3.9	3.8
Ind	1.3	1.3	1.4	1.4	1.5	1.7	1.5	2.2	2.2	0.6	1.1	1.0	1.2	1.7	2.0
Σ6PAHs	14.8	15.3	16.2	15.8	18.5	19.0	10.5	11.5	11.9	10.3	11.7	12.5	8.5	12.1	12.7

Enzymatic activity in the soil studied clearly varied depending upon the area and sampling time. The level and direction of the change was influenced by properties of the enzyme analyzed (Table 6).

TABLE 6. ENZYMATIC ACTIVITY OF SOILS

Area*	SP**	DhA	PhacA	PhalA	UA
I	1	5.11	6.23	4.86	11.24
	2	4.22	5.38	4.21	10.53
	3	4.76	6.19	4.78	9.42
	$\bar{x}$	4.69	5.93	4.61	10.39

Area*	SP**	DhA	PhacA	PhalA	UA
II	1	4.51	5.26	3.81	14.58
	2	3.45	4.03	3.05	12.72
	3	4.28	5.07	3.83	10.90
	$\bar{x}$	4.08	4.78	3.56	12.73
III	1	6.42	7.31	5.92	13.12
	2	5.32	6.02	4.85	11.84
	3	5.73	7.15	5.86	9.88
	$\bar{x}$	5.82	6.82	5.54	11.61
IV	1	6.93	7.49	6.48	10.35
	2	5.49	6.28	5.19	9.21
	3	6.12	7.32	6.12	8.76
	$\bar{x}$	6.18	7.03	5.93	9.44
V	1	8.46	9.67	7.62	14.91
	2	6.89	7.31	5.47	13.87
	3	8.11	9.45	7.38	12.34
	$\bar{x}$	7.82	8.81	6.82	13.70
$LSD_{0.05}$ for:	Area	1.39	1.57	0.98	3.19
	Sampling periods	1.16	1.21	0.67	1.88

*Explanations in Table 2.
**Sampling periods
DhA – Dehydrogenases in mg $TPF \cdot kg^{-1} \cdot d^{-1}$
PhacA – Acid Phosphatase and PhalA – Alkaline Phosphatase in mg $PNP \cdot kg^{-1} \cdot h^{-1}$
UA – Urease in mg $N\text{-}NH_4^+ \cdot kg^{-1} \cdot h^{-1}$

Soils from areas I and II showed the lowest activity of dehydrogenases, acid phosphatase and alkaline phosphatase (Table 6). The decreased enzyme activities in both areas resulted from intensive low-stack emission, which was the source of PAHs in the soils (Table 2). The 16 PAHs content had a significantly negative correlation with the activity of most of the studied soil enzymes: dehydrogenases, acid phosphatase and alkaline phosphatase (Table 7). The data presented by numerous authors (Maliszewska-Kordybach and Smreczak 1997, Baran *et al.* 2004, Wyszkowska *et al.* 2006, Bielińska *et al.* 2014, Lipińska *et al.* 2014) confirm particularly high sensitivity of dehydrogenases and phosphatases to soil contamination with PAHs. Their activity may be used as an indicator of soil pollution with these xenobiotics (Bielińska *et al.* 2014). Areas III and IV, where PAH levels were within the same range, exhibited similar activities of dehydrogenases and phosphatases in the soil (Table 2). The highest activity of these enzymes was stated in the soil from the park (area V) and it was 1.5–2 times higher than in the soils collected in areas I and II (Table 6). Relatively high enzymatic activity of the soil from the park could be connected with the entry of fresh organic matter to soil environment. It should be emphasised that fresh organic matter not only activates metabolism of microorganisms but also is favourable for decomposition of organic pollutants (Junter *et al.* 2002).

TABLE 7. CORRELATION COEFFICIENTS BETWEEN THE ACTIVITY OF THE EXAMINED ENZYMES AND CONTENT OF Σ16PAHS (N=30).

	DhA	PhacA	PhalA	UA
Σ16PAHs	-0.872	-0.838	-0.8657	n.s.

*significant at $p<0.05$; n.s. – not significant

No clear link between the study area and urease activity was found in the study period (Table 6). Urease is resistant to external factors and can even increase its activity in extreme conditions (Stępniewska and Samborska 2002, Li *et al.* 2015). As an extracellular enzyme, urease is synthesised only in the presence of its substrate, urea, and its availability is the only factor limiting urease activity (Carbrera *et al.* 1994). Large urban agglomerations are particularly significant urea producers. The sources of urea in urban soils are, among others things, animal excreta, household food waste, fragments of tissues and cells of soil micro-, meso- and macrofauna, plant residues, and, in the case of parks, intentionally added organic fertilisers (Russel and Wyczółkowski 2005). Urease activity can be used to assess the level of soil anthropogenisation (Russel and Wyczółkowski 2005).

Changes of biochemical processes in the specified sampling periods differed depending on enzyme properties (Table 6). The lowest activities of dehydrogenases, acid phosphatase and alkalic phosphatase in all studied soils were observed in the 2nd sampling period (the second half of November). Activities of these enzymes in winter (the 3rd sampling period) were comparable to those stated in summer (the second half of July). Many authors (*i.a.* Burton and Beauchamp 1994, Díaz-Raviña *et al.* 1995) indicate relatively high soil enzymatic activity in winter and directly after thawing of the soil. The literature data describe a complex mechanism of sorption and desorption of enzymes following the freezing and thawing of the soil (Burton and Beauchamp 1994, Bielińska *et al.* 2014a). The increase in enzyme activity as a result of freezing and thawing of the soil may be linked to destruction of soil aggregates. Bacteria frequently form the core of soil micro-aggregates. Winter increase in soil enzymatic activity is attributed to accumulation of enzymes released by dying soil organisms and to desorption and reactivation of enzymes accumulated earlier in the soil (Bielińska *et al.* 2014a). The lowest activity of urease was observed in the winter time (Table 6). It could be connected with different mechanisms regulating immobilisation of this enzyme in soils (Januszek 1999), and reduced availability of the substrate for urease in this season. In the study on influence of soil freezing and thawing on activity of selected enzymes, Januszek (1999) showed decrease in urease activity as the result of its desorption.

CONCLUSIONS

1. The results indicate that both enzyme activity and content of PAHs in soil depend on a type of housing, study period (intensification of pollutant emission from household heating), traffic volume and atmospheric air movement.

2. The level of soil contamination with PAHs within the studied areas of Lublin is high and dominance of 2–4 ring aromatic hydrocarbons proves that the low-stack emission is the main source of soil pollution with PAHs.

3. Coefficients calculated on the basis of phenanthrene/anthracene and fluoranthene/pyrene ratios indicate that carbon combustion is the main source of PAHs in the soil studied in the autumn-winter period.

4. Low enzymatic activities and high PAHs contents in the soils were noticed in the autumn-winter period in the areas with dense single-family housing and located in the vicinity of streets with intense road traffic. PAHs pollutants generated in open areas by low-stack emission are transported over longer distances, which was reflected by their lower contents in the soils.

5. The enzymatic activity measurements in the soils allow environmental risk assessment connected with low-stack emission.

6. PAHs durability in the soil contributes to their accumulation, indicating the need of monitoring the soils of Lublin in the areas exposed to low-stack emission.

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