SOILS, SEC 3 • REMEDIATION AND MANAGEMENT OF CONTAMINATED OR DEGRADED LANDS • RESEARCH ARTICLE

Effect of soil pollution with polycyclic aromatic hydrocarbons on the properties of humic acids

Magdalena Banach-Szott · Bożena Debska · Ewa Rosa

Abstract

Purpose Interestingly, soil is the component of the natural environment in which most hydrophobic organic pollution including polycyclic aromatic hydrocarbons (PAHs) gets accumulated. The aim of the present paper was to determine the effect of soil pollution with PAHs on the elemental composition, spectral properties, and hydrophobic and hydrophilic properties of humic acids. The research was performed on different types of soil samples that were artificially polluted with selected PAHs (anthracene, pyrene, fluorene and chrysene).

Materials and methods The soil samples were polluted with selected PAHs in an amount corresponding to 10 mg PAHs kg^{-1}. The PAHs-polluted soil samples were incubated for 180 and 360 days at a temperature of 20–25 °C and fixed moisture (50 % of field water capacity). Humic acids (HAs) were extracted from the soil samples prior to the incubation (additionally, soils not polluted with PAHs) and after 180 and 360 days of incubation. For isolated HAs, the following analyses were performed: elemental composition, UV–Vis and IR spectra, susceptibility to oxidation, and hydrophilic (HIL) and hydrophobic (HOB) properties were determined using high-performance liquid chromatography.

Results and discussion The research demonstrated that introducing anthracene, fluorene, pyrene and chrysene to soil samples resulted in a change in some of the quality parameters of humic acids. However, the intensity and the direction of those changes were determined by soil properties. The changes of the parameters, once PAHs were introduced, that did not depend on the soil properties were ΔA_{665u} and ΔA_{465u}

(susceptibility to oxidation at wavelengths of 465 and 665 nm) as well as HIL/ΣHOB. The same tendency in changes in the structure of humic acids, once PAHs were introduced, was also observed based on the Fourier transform infrared spectra pattern.

Conclusions A single pollution of soils with PAHs that leads to changes in the quality parameters of humic acids shows that, as for the soils permanently exposed to pollution with those compounds, significant changes can occur in the properties of humic acids. As a result, it can lead to a change in the functions played by humic acids in the environment.

1 Introduction

More and more often, one realises the problem on soil environment pollution and the importance of soil protection as well as the prevention of soil degradation. The Communication from the Commission to the Council, the European Parliament, the Economic and Social Committee and the Committee of the Regions (COM 179 final 2002) provides eight key threats to soil in the European Union, including a decrease in the content of organic matter and soil contamination. Interestingly, soil is the component of the natural environment in which, finally, most hydrophobic organic pollution, including polycyclic aromatic hydrocarbons (PAHs), gets accumulated. The presence of PAHs in the environment is the source of infection and it poses a direct threat to all of the living organisms in the contaminated area (Maliszewska-Kordybach 1999; Wang et al. 2011). As has been reported in literature (Weissenfels et al. 1992; Pan et al. 2006; Plaza et al. 2009; Guo et al. 2010; Kim and Kwon 2010; Yang et al. 2010; Zhang et al. 2011), an important parameter that determines

M. Banach-Szott · B. Debska (✉) · E. Rosa
Department of Environmental Chemistry, University of Technology and Life Sciences, 6 Bernardynska St., 85-029 Bydgoszcz, Poland
e-mail: debska@utp.edu.pl

the immobilisation of PAHs by soils is the quantity and the quality of organic matter. Humins show a greater PAHs sorption capacity than humic acids, while humic acids bind PAHs more strongly than fulvic acids. However, Pan et al. (2006) stress that PAHs are definitely sorbed faster by humic and fulvic acids than humins. The type of organic matter fraction that PAHs are connected with affects their mobility in the soil profile. Humic acids limit the mobility of hydrocarbons deep down in the soil profile, whereas fulvic acids stimulate that process (Petruzzelli et al. 2002). As was reported by Kim and Kwon (2010), the sorption properties of humic acids towards PAHs increase with an increase in the PAHs hydrophobicity.

As a result of the sorption of PAHs by the organic matter of soils, their mobility and their availability to microorganisms decrease (Bauer and Capone 1985; Cousins et al. 1997). PAHs are strongly bound by organic matter and do not undergo the processes of desorption that persist in soil as the so-called permanent residue—the removal of which is very difficult, even impossible (Seibel et al. 1996; Semple et al. 2003).

The formation of the bonded residue (the so-called permanent residue) is also accompanied by the microbiological decomposition of PAHs which includes the newly processed structures into the structures of the organic matter (White et al. 1998; Northcott and Jones 2001). The structures formed in this way demonstrate properties that are similar to humic acids (e.g. solubility in bases and molecular mass), and they constitute an integral part of the organic substance (Eschenbach et al. 1998; Kastner et al. 1999; Kelsey and Alexander 1997; Launen et al. 1995; Meyer and Steinhart 2001).

It is common knowledge that the properties of the organic matter of the soils (including humic acids) are specific for a given soil type (Orlov 1986; Gonet 1989; Tan 1998; Gonet and Debska 1999; Debska et al. 2012). However, one should note that the properties of the organic matter of the soils can be modified by, e.g. the cultivation method of the land (crop rotation and fertilisation) as well as by other external factors, including pollution (Orlov 1986; Gonet 1989; Gonet and Debska 1999; Debska et al. 2011, 2012). As was reported by Launen et al. (1995), Kelsey and Alexander (1997), Eschenbach et al. (1998), Kastner et al. (1999) and Meyer and Steinhart (2001), PAHs undergo transformations that lead to the formation of structures that are similar to humic acids (HAs) and therefore it is very likely that PAHs can also lead to the changes in the properties (structure) of the molecules of humic acids in soils that have been polluted with those compounds.

The knowledge of the mechanisms of the effects of pollutants that enter the soil, such as, e.g. PAHs, provides a potential for a defence against unfavourable phenomena and therefore it seems to be very important that, in addition to the common

domestic and foreign measurements of the concentrations of PAHs and the factors that affect the rate of their decomposition and bioavailability, the effect of polycyclic aromatic hydrocarbons on the properties (quantity and quality) of humus substances of soils should be determined.

The basic indicators that are used to evaluate the properties of humic acids are as follows: the elemental composition, including the values of atomic ratios H/C, O/C and N/C, which are determined by drawing on the composition as well as the degree of internal oxidation; the coefficients of absorbance $A_{2/4}$, $A_{2/6}$, $A_{4/6}$ and $\Delta \log K$; the susceptibility to oxidation and the IR spectra (Orlov 1986; Kumada 1987; Gonet 1989; Tan 1998; Gonet and Debska 1999) as well as was shown by e.g. Preuβe et al. (2000) and Debska et al. (2007), the hydrophilic-hydrophobic parameters. According to Wen et al. (2007), it is the polarity and the aromaticity of the humus fractions that are essential in the adsorption processes of PAHs.

The aim of the present paper was to determine the effect of soil pollution with PAHs on the elemental composition, spectral properties, and hydrophobic and hydrophilic properties of humic acids. The research was performed based on the different types of soil samples that had been artificially polluted with selected PAHs (anthracene, pyrene, fluorene and chrysene).

2 Material and methods

2.1 Materials

The research was performed by drawing on soils that were under agricultural use based on the soils sampled in the Kujawy and Pomorze Provinces in the northern part of Poland. The list of soils sampled for the experiment, which is given in Tables 1 and 2, provides their basic properties. Total organic carbon (TOC) and total nitrogen (Nt) were determined by the Vario Max CNS analyser (Elementar, Hannau, Germany). Grain size composition was determined by applying the aerometric method. High-performance liquid chromatography (HPLC, Perkin Elmer, Norwalk, USA) was used to determine the content of PAHs.

Soil samples were polluted with selected PAHs (fluorene, anthracene, pyrene and chrysene) in an amount corresponding to 10 mg PAHs kg^{-1}. The unpolluted and PAHs-polluted soil samples were incubated for 180 and 360 days at a temperature of 20–25 °C and a fixed moisture (50 % of field water capacity). After a specific incubation time, all of the samples were destroyed. The incubation was performed in three repeats for each sampling date. The properties of humic acids were determined for the samples of soils that were unpolluted with PAHs and at 180 and 360 days after adding the PAHs.

Table 1 soil types and basic properties of soil

Sample	Soil	TOC (g kg^{-1})	Nt (g kg^{-1})	TOC/Nt	Grain size composition (%)		
					2–0.05 mm	0.05–0.002 mm	<0.002 mm
Ph1	Phaeozems	16.37	1.43	11.45	69	20	11
Ph2	Phaeozems	25.37	1.91	13.28	67	20	13
Ph6	Phaeozems	21.23	1.75	12.13	73	16	11
Hp8	Haplic Arenosols	5.72	0.42	13.62	93	4	3
Fl9	Fluvisols	34.02	2.69	12.65	55	29	16
Fl10	Fluvisols	8.17	0.63	12.97	82	12	6

2.2 Methods

2.2.1 Extraction of humic acids

Humic acids were extracted from the soil samples prior to the incubation (additionally, soils that were not polluted with PAHs) and after 180 and 360 days of incubation. HAs were extracted and purified according to standard methods using the following procedure:

- Decalcification (24 h) with 0.05 M HCl (1:10 *w/v*). After centrifugation, the residue was washed with distilled water until neutral.
- Extraction (24 h) of the remaining solid with 0.5 M NaOH (1:10 *w/v*) with occasional mixing, followed by centrifugation.
- Precipitation (24 h) of humic acids from the resulting alkaline extract with 2 M HCl to pH=2 and centrifugation.
- Purification of the resulting humic acids as follows: the humic acid residue was treated with a mixture of HCl/HF (950 mL H$_2$O, 5 mL HCl, 5 mL HF) over a 24-h period, followed by centrifugation. This procedure was repeated three times. The humic acids residue was treated with distilled water until a zero reaction to chloride was achieved.

The preparations were lyophilised and powdered in an agate mortar. Ash content in the humic acids preparations was lower than 2 %.

Table 2 Contents of PAHs in the soil samples prior to the experiment

Sample	Fluorene μg kg^{-1}	Anthracene	Pyrene	Chrysene	Sum
Ph1	168	9.01	115	129	421
Ph2	115	10.0	43.2	146	314
Ph6	56.2	0.774	16.9	12.6	86.5
Hp8	3.87	0.128	3.75	0.855	8.60
Fl9	115	6.59	106	42.3	270
Fl10	91.5	0.814	17.0	7.80	117

2.2.2 Characteristics of humic acids

The humic acids that were separated were analysed for the following:

- Elemental composition (Perkin Elmer 2400 CHN analyser). The H/C, O/C, O/H and N/C atomic ratios and ω (internal oxidation degree) were calculated according to the formula:

$$\omega = (2O + 3N - H) : C,$$

where : O, N, H, C−content in atomic%

- UV–Vis absorption spectra (UV–Vis Spectrometer, Lambda 20, Perkin Elmer). VIS spectra were obtained from 0.02 % humic acid solutions in 0.1 M NaOH and UV spectra after fivefold dilution. Absorbance that was measured at 280 nm (A_{280}), 400 nm (A_{400}), 465 nm (A_{465}), 600 nm (A_{600}) and 665 nm (A_{665}) was used to calculate the coefficient values:

$A_{2/4}$—280 and 465 nm absorbance ratio
$A_{2/6}$—280 and 665 nm absorbance ratio
$A_{4/6}$—465 and 665 nm absorbance ratio
$\Delta \log K = \log A_{400} - \log A_{600}$
(Kumada 1987).

- Susceptibility to oxidation with H$_2$O$_2$ by measuring the absorbance of the HAs solution (0.02 % HAs in 0.1 M NaOH and 1.5 % H$_2$O$_2$) at 465 and 665 nm. The susceptibility to oxidation was calculated using the following formula:

$$\%A_x = ((A_0 - A_u)/A_0) \times 100\%$$

where: x is the wavelength (465 and 665 nm), A_0 is the initial absorbance (prior to adding H$_2$O$_2$) and A_u is the absorbance after oxidation (Gonet 1989).

- Infrared spectra (FT-IR Spectrometer, Spectrum BX, Perkin Elmer) over 400–4,400 cm^{-1} were obtained for HAs (3 mg) in KBr (800 mg). Deconvolution was applied using a filter that made the bands of γ=4 narrower and

using the process of smoothing for which the parameter length was $l = 80 \%$ (Cocozza and Miano 2002).

– Hydrophilic and hydrophobic properties were determined using liquid chromatograph HPLC Series 200 with a DAD detector by Perkin-Elmer. The separation involved using column X-Terra C18, 5 μm, 250×4.6 mm. The solutions of humic acids were applied in 0.01 mol L^{-1} NaOH at a concentration of 2 mg mL^{-1}; injection of the sample, 100 μL; solvent–acetonitrile–water; solvents flow in the gradient (ratio H_2O:ACN (v/v) over 0–6 min, 99.5:0.5; 7–13 min, 70:30; 13–20 min, 10:90) and detection at a wavelength of 254 nm. The share of hydrophilic (HIL) and hydrophobic (ΣHOB=HOB1+HOB2) fractions in the humic acid molecules and the parameter HIL/ΣHOB were determined based on the areas that were determined under the peaks (Woelki et al. 1997; Preuße et al. 2000; Debska et al. 2007).

The significance of the differences in the parameters, depending on the incubation time, was evaluated by the Duncan's test. Based on the Duncan test results, which did not show significant differences for the parameters evaluated depending on the incubation time for HAs of the soils additionally non-polluted with PAHs, the data presented in Tables 3, 4 and 5 are variants Phaeozems (Ph)1, Ph2, Ph6; Haplic Arenosols (Hp)8; Fluvisols (Fl)9 and Fl10 are an average for three dates (0, 180 and 360 days).

The effect of the management method on the properties of the soils was defined using the cluster analysis method (Fig. 3). The above-mentioned relationships were determined using the statistical software STATISTICA MS.

3 Results and discussion

3.1 Elemental composition of humic acids

One of the basic parameters that are characteristic for the structure of HAs is their elemental composition (Orlov 1986; Gonet 1989; Tan 1998). The elemental composition of the humic acids that were analysed is presented in atomic percent (Table 3). The content of carbon in the molecules of the humic acids ranged from 33.99 to 36.15 %, hydrogen ranged from 40.35 to 44.84 %, nitrogen from 2.37 to 2.98 % and oxygen accounted for 17.08 to 21.09 %. The lowest share of carbon was observed for HAs Fluvisols with a sandy loam texture (sample no Fl9). The humic acids of Haplic Arenosols showed the highest content of hydrogen and the lowest content of oxygen. The share of those elements in the other soils was, in general, similar. Introducing selected polycyclic aromatic hydrocarbons (chrysene, pyrene, anthracene and fluorene), in general, did not result in any significant changes in the elemental composition of the humic acids. One should note the decrease in the share of oxygen and the increase in the share of

Table 3 Elemental composition (atomic percent) of humic acids and the values of atomic ratio and the degree of internal oxidation

Sample	C	H	N	O	H/C	N/C	O/C	ω
Ph1	34.62a	42.49c	2.89b	20.00ab	1.23b	0.084b	0.578ab	0.179a
Ph1V	35.63b	41.74b	2.92b	19.71a	1.17a	0.082b	0.553a	0.180a
Ph1VI	35.71b	40.81a	2.63a	20.85b	1.14a	0.074a	0.584b	0.246b
Ph2	35.63a	41.40ab	2.72a	20.25a	1.16ab	0.076b	0.568a	0.204a
Ph2V	35.26a	41.74b	2.70a	20.29a	1.18b	0.077b	0.576ab	0.197a
Ph2VI	35.89a	40.62a	2.46a	21.03b	1.13a	0.068a	0.586b	0.245b
Ph6	35.59ab	41.23b	2.64ab	20.53a	1.16ab	0.074b	0.577a	0.218a
Ph6V	34.78a	41.76b	2.97b	20.49a	1.20b	0.085c	0.589a	0.234ab
Ph6VI	36.15b	40.35a	2.41a	21.09a	1.12a	0.067a	0.583a	0.251b
Hp8	35.51a	42.86a	2.66a	18.97b	1.21a	0.075a	0.534a	0.086b
Hp8V	35.37a	43.03a	2.79a	18.80b	1.22a	0.079a	0.532a	0.083b
Hp8VI	35.43a	44.84b	2.66a	17.08a	1.26 b	0.074a	0.522a	−0.077a
Fl9	33.99a	42.62a	2.70a	20.69b	1.25a	0.079a	0.609b	0.202b
Fl9V	34.54a	42.54a	2.98a	19.95a	1.23a	0.086b	0.578a	0.184ab
Fl9VI	34.30a	42.82a	2.61a	20.27ab	1.25a	0.076a	0.591ab	0.162a
Fl10	35.84a	41.23a	2.58a	20.35a	1.15a	0.072b	0.568a	0.201b
Fl10V	36.00a	41.40a	2.55a	20.05a	1.15a	0.071ab	0.557a	0.176b
Fl10VI	35.51a	42.21b	2.37a	19.91a	1.19a	0.067a	0.561a	0.133a

Values followed by common letter are not significantly different at the 5 % level

V samples after 180 days of incubation, VI samples after 360 days of incubation

Table 4 spectral properties of humic acids

Sample	A_{280}	A_{400}	A_{665}	$A_{2/4}$	$A_{2/6}$	$A_{4/6}$	$\Delta\log K$	ΔA_{465u}	ΔA_{665u}
Ph1	4.86	1.59	0.200	5.28b	24.28b	4.60b	0.671ab	61.92ab	64.96b
Ph1V	4.24	1.43	0.173	5.27b	24.53b	4.65b	0.700b	66.30b	74.64c
Ph1VI	4.11	1.45	0.209	4.76a	19.67a	4.13a	0.633a	58.45a	60.29a
Ph2	4.69	1.53	0.165	5.69b	28.42b	5.00b	0.730b	63.16b	65.83a
Ph2V	4.56	1.65	0.191	5.13ab	23.85a	4.65ab	0.721ab	64.17b	72.53b
Ph2VI	3.93	1.47	0.190	4.75a	20.71a	4.36a	0.652a	57.15a	62.37a
Ph6	4.70	1.56	0.202	5.00ab	23.23b	4.65b	0.636ab	62.86b	67.67b
Ph6V	4.32	1.35	0.177	5.52b	24.46b	4.43ab	0.657b	64.12b	73.91c
Ph6VI	4.10	1.44	0.229	4.47a	17.88a	4.00a	0.566a	55.59a	60.07a
Hp8	4.35	1.38	0.183	5.16b	23.83b	4.61a	0.629a	67.01b	71.54ab
Hp8V	3.96	1.35	0.197	4.50a	20.10a	4.47a	0.605a	67.73b	75.30c
Hp8VI	3.09	1.07	0.143	4.80ab	21.64ab	4.51a	0.625a	61.93a	66.89a
Fl9	3.87	1.15	0.099	6.47a	38.95b	6.02b	0.800a	70.35b	74.40ab
Fl9V	3.28	1.09	0.098	6.16a	33.61a	5.46a	0.829a	70.74b	83.76b
Fl9VI	2.90	0.891	0.067	6.41a	43.06c	6.72c	0.837a	64.87a	69.96a
Fl10	3.95	1.21	0.126	5.98ab	31.29a	5.23ab	0.735a	69.82ab	76.09a
Fl10V	4.77	1.45	0.156	6.08b	30.51a	5.02a	0.740a	67.71a	80.18b
Fl10VI	3.41	1.11	0.108	5.60a	31.75a	5.67b	0.748a	72.36b	77.16a

Denotations as in Table 3

hydrogen in the HAs of Haplic Arenosols after 360 days of incubation. The increase in the hydrogen content was also observed in the molecules of the HAs of Fluvisols with a fine-sand texture (sample no Fl10). The HAs of Phaeozems that

Table 5 share of hydrophilic and hydrophobic fractions in the molecules of humic acids

Sample	HIL	HOB1	HOB2	ΣHOB	HIL/ΣHOB
Ph1	42.3ab	47.7a	10.0b	57.7b	0.734a
Ph1V	41.5a	50.9b	7.6a	58.5b	0.709a
Ph1VI	45.3b	46.8a	7.9a	54.7a	0.828b
Ph2	41.4a	48.4ab	10.2b	58.6b	0.706a
Ph2V	41.0a	51.1b	8.0a	59.0b	0.694a
Ph2VI	45.2b	47.3a	7.5a	54.8a	0.824b
Ph6	46.1b	45.4a	8.4b	53.9ab	0.857b
Ph6V	42.9a	49.5b	7.6ab	57.1b	0.752a
Ph6VI	46.6b	46.4ab	7.0a	53.4a	0.873b
Hp8	42.6a	48.3b	9.1a	57.4b	0.741a
Hp8V	42.3a	49.7b	8.0a	57.7b	0.733a
Hp8VI	46.0b	42.4a	11.6b	54.0a	0.852b
Fl9	44.1a	46.2b	9.7a	55.9b	0.789a
Fl9V	45.9ab	44.7b	9.5a	54.1b	0.848b
Fl9VI	47.9b	40.8a	11.3b	52.1a	0.919c
Fl10	40.2a	50.0b	9.8a	59.8b	0.672a
Fl10V	40.0a	50.6b	9.4a	60.0b	0.667a
Fl10VI	43.8b	44.5a	11.7b	56.2a	0.781b

Denotations as in Table 3

had been polluted with PAHs demonstrated a lower content of hydrogen and a slightly higher content of oxygen, as compared with the HAs of the unpolluted soils. Interestingly, the slightest changes in the elemental composition of humic acids were noted in the molecules of the HAs of Fluvisols (samples no Fl9 and Fl10).

Much precious information on the structure of HAs is provided by the values of the atomic ratios of the elements that are determined (Orlov 1986; Howard et al. 1998; Gonet and Debska 1999; Debska et al. 2012). It was shown that a decrease in the value of the H/C ratio indicates an increase in the degree of the aromaticity of humic acids, which, in turn, suggests an increase in their degree of humification (maturity). Parameter O/C and O/H as well as ω are indicators of the oxidation of the molecules of humic acids. The higher the value of the O/C ratio, the higher the ω and the higher the degree of humification of humic acids (Gonet and Debska 1999; Howard et al. 1998; Debska et al. 2012). The elemental composition parameters discussed here can, therefore, be used to evaluate the degree of the advancement of the humification process of the soil organic matter, as well as the characteristics of the humus substances that are formed. As was reported by Plaza et al. (2009), the degree of humic acids maturity is a factor that affects the sorption of PAHs.

Introducing selected PAHs into soils changes the quality parameters of humic acids; however, the intensity and the direction of changes were determined by soil type (Table 3). As for the humic acids of Phaeozems, a decrease in the value of the ratios H/C and N/C, as well as the increase in the value

of the degree of internal oxidation was observed. However, no significant changes in the value of the O/C ratio were observed. Only a significant decrease in the value of the degree of internal oxidation was observed for the HAs of Haplic Arenosols. A decrease in value ω was also recorded for the HAs of Fluvisols (samples no Fl9 and Fl10).

As was reported earlier, the humification processes are connected with an increase in the degree of the internal oxidation of the molecules of humic acids (Orlov 1986; Debska et al. 2012). The dependences suggest that for Hp and Fl, introducing PAHs limited the intensity of the transformations of the soil organic matter and/or was a consequence of the adsorption of PAHs molecules or fragments by the molecules of humic acids, which, as a consequence, did not result in any changes in the degree of internal oxidation.

3.2 Spectrometric parameters of humic acids in the UV–Vis range

The important parameters that are used to determine the properties of humic acids and to evaluate the transformations of organic matter in the soils are the spectrometric parameters in the UV–Vis range. Based on many years of research, some relationships between the values of absorbance at the wavelengths of 280, 465 and 665 nm and the structure of humic acids were demonstrated. It is commonly assumed that the value of absorbance at 280 nm defines the content of lignin-type compounds, and the A_{465} value defines the content of substances at the initial stage of decomposition, while A_{665} reflects the content of substances with a high degree of humification. Coefficients $A_{2/4}$, $A_{2/6}$, $A_{4/6}$ and $\Delta\log K$ are important indices of the degree of the advancement of the humification of organic materials and the characteristics of the humus substances that are produced, as well as changes in the properties of the humic acids that occur due to various anthropogenic factors (Kumada 1987; Gonet 1989; Gonet and Debska 1999).

As can be seen from the data presented in Table 4, in general, the lowest values of absorbance at wavelengths of 280 and 400 nm were recorded for the humic acids that were isolated from the soils after 360 days of incubation. Other relationships were recorded at a wavelength of 665 nm. After 360 days of incubation, the highest values of absorbance at that wavelength were reported for the HAs of Phaeozems, while they were lowest for the HAs of Haplic Arenosols and Fluvisols. Drawing on the dependencies that were recorded, one can assume that adding polycyclic aromatic hydrocarbons to Phaeozems accelerates the transformation processes of organic matter (an increase in the value of absorbance at the wavelength of 665 nm), and, as for the other soils types, it slows down and decrease in the value of absorbance at 665 nm.

Introducing selected PAHs into Phaeozems, in general, did not result in significant changes in the value of absorbance coefficients $A_{2/4}$, $A_{2/6}$, $A_{4/6}$ and $\Delta\log K$ after 180 days of incubation; after 360 days of incubation, the HAs of Phaeozems showed significantly lower values of the coefficients as compared with the HAs of the original soils. As was demonstrated earlier (Kumada 1987; Gonet and Debska 1999), a decrease in the values of the coefficients of absorbance is connected with an increase in the degree of the 'maturity' of the molecules of humic acids. As for the humic acids of the Haplic Arenosols that were isolated from the samples of soil polluted with PAHs, the $A_{2/4}$ and $A_{2/6}$ values were the only ones that were lower as compared with the HAs of the soil that was unpolluted with PAHs. As for the humic acids of Fluvisols with a sandy loam texture (sample no Fl9), significant differences in the value of coefficients $A_{2/6}$ and $A_{4/6}$ were found during the incubation. The lowest values of the coefficients were reported after 180 days of the decomposition of the PAHs that had been introduced into the soil and the lowest was after 360 days of incubation. For the HAs of Fluvisols with a fine-sand texture (sample no Fl10), the values of coefficients $A_{2/4}$, $A_{2/6}$, $A_{4/6}$ and $\Delta\log K$ for the humic acids of the soil polluted with PAHs were similar to the HAs of the original soil.

The structure of humic acids can also indirectly be inferred from the changes in the value of absorbances that occur as a result of the reaction of oxidation with the hydrogen peroxide of humic acids (Gonet 1989). According to Gonet (1989), the aliphatic part of the HAs molecules appeared to be more susceptible to oxidation, while the aromatic part appeared to be more resistant. The changes in the value of the absorbance of humic acids, which occur as a result of oxidation with H_2O_2, are given in Table 4.

The values of parameter ΔA_{465u} (susceptibility to oxidation as determined at a wavelength of 465 nm) that were recorded for the HAs of the original soils (with no PAHs added) ranged from 61.92 (Ph1) to 70.35 % (Fl9). The values of parameter ΔA_{665u} (susceptibility to oxidation as defined at a wavelength of 665 nm) were higher than ΔA_{465u}, and they ranged from 64.96 % (Ph1) to 76.09 % (Fl10). Introducing PAHs into soils, in general, resulted in a significant increase in the susceptibility to oxidation that was measured at 665 nm (the values recorded after 180 days of incubation).

In the period from 180 to 360 days, the process of the transformations of the organic matter of soil occurs as a result of which humic acids with significantly lower values of ΔA_{465u} and ΔA_{665u} were observed as compared with the values that were recorded for HAs after 180 days of incubation. It should also be stressed that the values ΔA_{465u} and ΔA_{665u} that were recorded after 360 days of incubation were frequently lower than the values that were recorded for the HAs of the original soil samples. Therefore, introducing PAHs (chrysene, pyrene, fluorene and anthracene) to soils resulted in

changes in the structure of humic acids, which must have resulted in an increase in the share of unsaturated aliphatic structures and/or simple aromatic structures, which, in turn, results in a decrease in the resistance to oxidation of the molecules of humic acids.

3.3 Analyses of the Fourier transform infrared (FTIR) spectra of humic acids

The changes in the structure of the molecules of humic acids that were obtained once PAHs were introduced into soils were confirmed by the spectra produced in the IR range (Fig. 1).

Generally, the IR spectra of the humic acids that were isolated from the samples of soils that were additionally polluted with PAHs, after 180 days of incubation, as compared with the HAs of the original soil, demonstrated, e.g. a higher band intensity of the frequency of about 1,540, 1,510, 1,460, 1,420 and 1,260 cm^{-1}, and the peaks are in the range of 1,130–1,000 cm^{-1} (Fig. 1).

The band at around 1,540 cm^{-1} is determined by the presence of amine groups (deformation vibration), the 1,520–1,500 cm^{-1} band is characteristic for aromatic compounds (C=C). The band in the range of wavenumbers 1,460–1,440 cm^{-1} correspond to the deformation vibration of groups –CH_3 and =CH_2, the band 1,420–1,400 cm^{-1} points to the presence of groups O–C–O in esters and C=O in carboxyl acids and OH groups. The 1,380–1,320 cm^{-1} band is connected with the presence of groups of COO and CH bonds in the aliphatic chain. In the 1,280–1,200 cm^{-1} range, there were bands of the C–O groups of phenols, carboxyl acids, esters and ethers and the bands in the range 1,130 and 1,080–1,030 cm^{-1} point to the presence of polysaccharides. They can also be related to the presence of groups of OCH_3 that occur in lignins and OH in alcohols (Tan 1998; Orlov 1986; Gonet 1989; Cocozza and Miano 2002). The intensity of the above-mentioned absorption bands decreased from the 180th
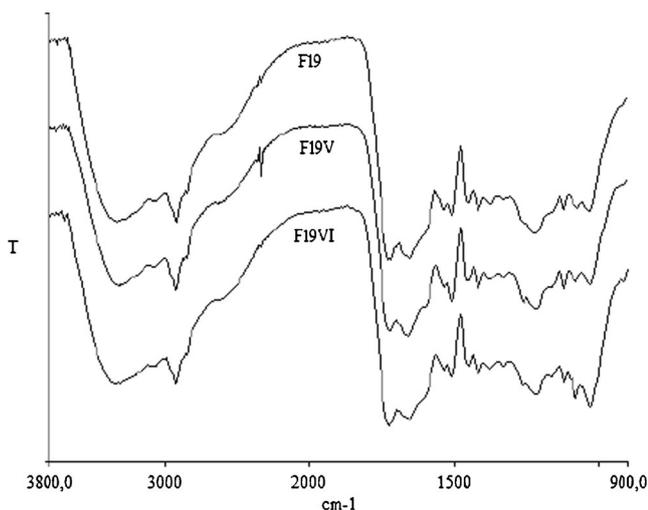
to 360th day of incubation. However, the humic acids that were isolated after 360 days of incubation, as compared with the HAs of the original soils, showed a slightly higher share of simple aromatic structures (a higher intensity of the 1,510 cm^{-1} band) as well as a higher band in the range of wavenumbers 1,460–1,030 cm^{-1}. The IR spectrum pattern definitely indicates the effect of the PAHs that were introduced into the soil on the structure of humic acids.

3.4 The hydrophilic and hydrophobic nature of humic acids

Humic acids are built from structures with both hydrophobic and hydrophilic properties. The hydrophilic and hydrophobic properties determine, e.g. the solubility and the susceptibility of humic acids to biodegradation and affect their sorption potential as well, e.g. towards PAHs (Wen et al. 2007).

By applying the HPLC method, one can divide the molecules of humic acids into fractions that are hydrophilic and hydrophobic in nature (Fig. 2). Fractions of a retention time of 3.0–8.0 min show greater hydrophilic properties, whereas the fractions of a retention time between 12.0 and 25.0 min become more and more hydrophobic (Woelki et al. 1997; Preuße et al. 2000; Debska et al. 2007, 2010, 2012). In the humic acids that were analysed, the share of the hydrophobic fraction was slightly higher than the share of the fraction containing compounds with hydrophilic properties (Table 5). After 360 days of incubation in the molecules of the humic acids of the soils, the share of hydrophilic fractions was higher and the share of hydrophobic fractions was lower, as compared with the share of those fractions in the molecules of HAs prior to the start of incubation. The changes in the share of the respective fractions resulted in changes in the value of the


Fig. 1 Selected FTIR spectra of humic acids
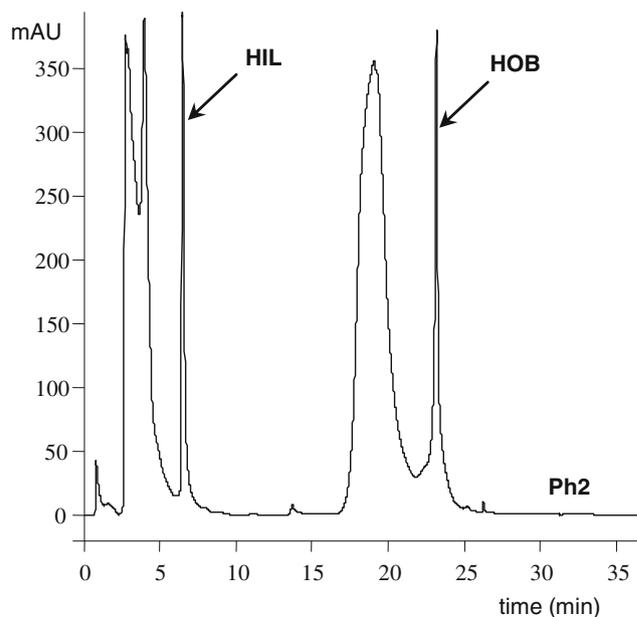
Fig. 2 Selected RP-HPLC chromatograms spectra of humic acids (Fl9)

HIL/ΣHOB ratio. Parameter HIL/ΣHOB, as shown by, e.g. Debska et al. (2007, 2010, 2012), is connected with the degree of organic matter humification; the value of the parameter increases with an increase in the degree of the maturity of the molecules of humic acids. In this experiment, after 180 days of incubation, the values of the HIL/ΣHOB ratio were similar to the values of the ratio of the HAs of the original soil samples. Only the HAs of Fluvisols with a sandy loam texture (sample no Fl9) showed higher values of the HIL/ΣHOB ratio than the HAs of the original soil sample; the HAs of Phaeozems sampled at Orlinek (Ph6) showed lower values. For most soils, the highest values of parameter HIL/ΣHOB were reported for the HAs that were isolated after 360 days of incubation. The changes in the value of the HIL/ΣHOB ratio were the result of an increase in the share of hydrophilic fractions and a decrease in the share of hydrophobic fractions. However, it should be stressed that, as for the HAs of Phaeozems (Ph1, Ph2 and Ph6), the decrease referred especially to the hydrophobic fraction with a longer retention time (HOB2) and in the humic acids of Haplic Arenosols and Fluvisols, the share of the HOB1 fraction decreased (the hydrophobic fraction with a shorter retention time; Fig. 2). Additionally, an increase in the share of the hydrophobic fraction with a longer retention time (HOB2) was observed in the HAs of Haplic Arenosols and Fluvisols. Such dependences suggest that the process of the transformations of PAHs entering the soil, on the one hand, depends on their properties and, on the other hand, is determined by the soil properties (Guo et al. 2010; Yang et al. 2010; Debska et al. 2011).

3.5 General consideration

In order to document the changes in the properties of humic acids due to polycyclic aromatic hydrocarbons, cluster analysis was performed for the HAs of the original soil samples (Fig. 3a) after 180 (Fig. 3b) and 360 days of incubation (Fig. 3c). The objects with similar properties are located on the dendrograms in the homogenous groups. The humic acids of the soils that were analysed prior to the introduction of PAHs occur in two major groups on the dendrograms (Fig. 3a). The first one is made up of the HAs of Fluvisols (Fl9 and Fl10), and the second one is made up by the HAs of the other soils. However, it should be noted that the greatest similarity is recorded for the HAs of the Phaeozems that are marked with the symbols Ph1 and Ph2, which is understandable since these soils were sampled quite close to each other. The dendrogram also shows that the quality parameters of the HAs of Haplic Arenosols are closer to the parameters that were reported for the HAs of Phaeozems than for the HAs of Fluvisols. The dendrogram pattern (Fig. 3b) differed from that reported for the HAs of the original soil samples (Fig. 3a) for the humic acids after 180 and after 360 days of incubation
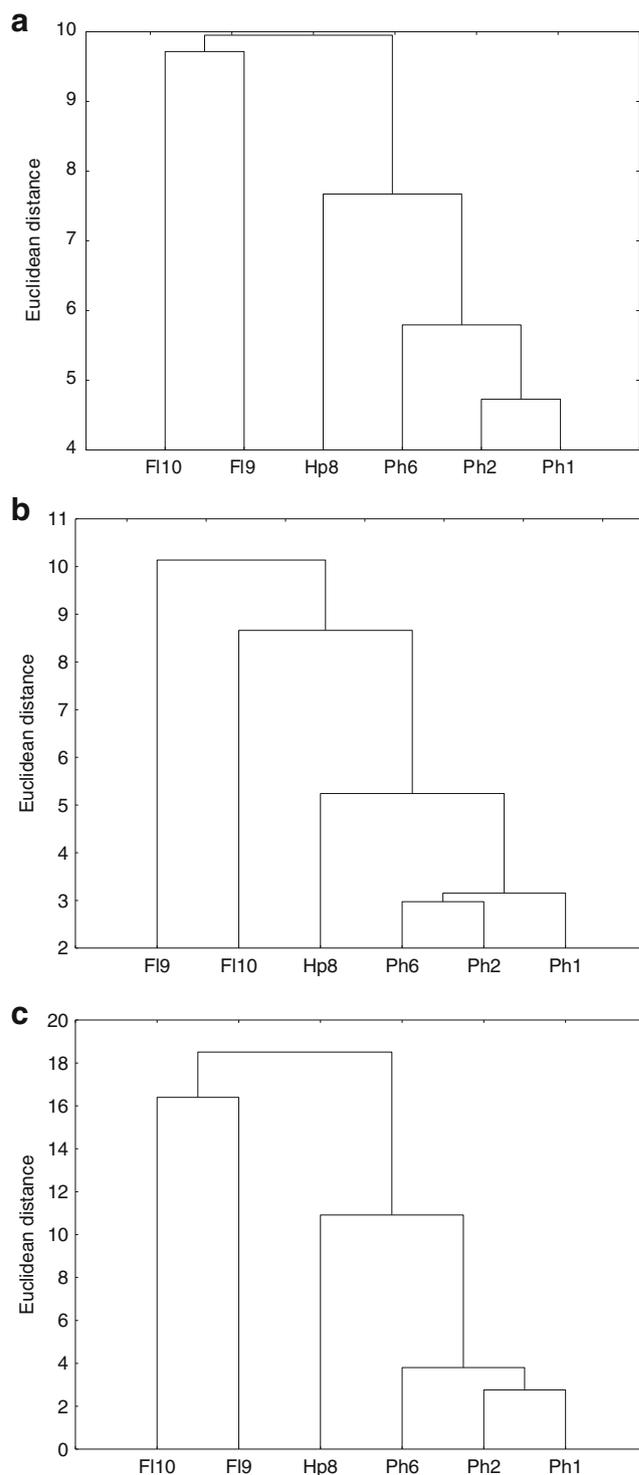
Fig. 3 Cluster analysis of humic acids determined based on the following parameters: H, O, ω, $A_{2/4}$, $A_{2/6}$, $A_{4/6}$, ΔA_{465u}, ΔA_{665u}, HIL, HOB1, HOB2, HIL/ΣHOB. **a** The HAs of the soils additionally polluted with PAHs before incubation. **b** The HAs of soils after 180 days of incubation. **c** The HAs of soils after 360 days of incubation

(Fig. 3c). The pattern of the dendrogram produced after 360 days of incubation, on the other hand, was similar to the one recorded for the HAs of the original soil samples;

however, the objects were more distant from each other. The relationships clearly demonstrate that after having introduced the PAHs, the properties of humic acids change, which is due to the role that is played by organic matter in the processes of the immobilisation of polycyclic aromatic hydrocarbons.

The changes in the humic acid properties due to PAHs are the result of the sorption of the PAHs by the organic matter of the soils, a part of which does not undergo the processes of desorption (Seibel et al. 1996; Semple et al. 2003). PAHs that are permanently connected with organic matter, which are exposed to the effect of microorganisms, undergo the processes of transformation and they are included in the structures of the organic matter (Northcott and Jones 2001; White et al. 1998). The properties of the compounds are similar to those of humic acids and are an integral part of the organic substance (Eschenbach et al. 1998; Kastner et al. 1999; Kelsey and Alexander 1997; Launen et al. 1995; Meyer and Steinhart 2001).

The significantly greater changes in the properties of humic acids that were recorded 180 days after introducing PAHs than after 360 days demonstrate the share of undecomposed or slightly decomposed PAHs in the structure of the HAs after 180 days. After 360 days of PAHs decomposition in the soil, humic acids, the properties of which are, in general, similar to the HAs of the original soil samples, are produced. It can be that after 1 year of PAHs persisting in soils, they undergo considerable transformations and become an integral part of the organic matter.

However, the relationships that were recorded for HAs 180 days after introducing PAHs definitely demonstrated that PAH-type soil pollutions significantly modify the properties of humic acids, the basic component of organic matter, which can be essential for soil that is exposed to a permanent threat of pollution with high concentrations of PAHs. Without a doubt, investigating the properties of soils that have been exposed to the threat of polycyclic aromatic hydrocarbons, the effect of those compounds on the properties of organic matter should be considered.

4 Conclusions

The research showed that introducing anthracene, fluorene, pyrene and chrysene into the soil samples resulted in a change in some of the quality parameters of the humic acids that were isolated from the soil samples that were incubated for 180 and 360 days. However, the intensity and the direction of those changes were determined by the soil properties.

The parameters the changes of which did not depend on the soil properties after the introduction of PAHs included ΔA_{665u} and ΔA_{465u}, HIL/ΣHOB. Similarly, irrespective of the soil type, in the molecules of humic acids after 180 days of

incubation, as compared with the HAs of the original soil samples, the following were found:

– Higher values of parameter ΔA_{665u}, which defines the susceptibility to chemical oxidation of the molecules of humic acids at a wavelength of 665 nm.
– A higher share of simple aromatic structures (FTIR spectra).

After 360 days of incubation, the HAs showed:

– Lower ΔA_{465u} values.
– Higher values of the HIL/ΣHOB ratio.
– The share of simple aromatic structures was still higher, as compared with the HAs of the original soil samples; however, it was clearly lower than in the HAs after 180 days of incubation.

A single pollution of soils with PAHs that led to changes in the quality parameters of humic acids shows that for the soils permanently exposed to pollution with those compounds, significant changes in the properties of humic acids can occur. As a result, it can lead to a change in the functions played by humic acids in the environment.

References

Bauer JE, Capone DG (1985) Degradation and mineralization of the polycyclic aromatic hydrocarbons anthracene and naphtalene in intertidal marine sediments. Appl Environ Microbiol 50(1):81–90

Cocozza C, Miano T (2002) Structural resolution of metal-humic acids interactions through deconvolution FT-IR spectroscopy. In: Davis G, Ghabbour EA (eds) Proceedings of the 11th IHSS Meeting. IHSS, Boston, USA, pp 264–266

COM 179 final (2002) Communication from the Commission to the Council, the European Parliament, the Economic and Social Committee and the Committee of the Regions: Towards a Thematic Strategy for Soil Protection, Brussels

Cousins IT, Kreibich H, Hudson LE, Lead WA, Jones KC (1997) PAHs in soil: contemporary UK data and evidence for potential contamination problems caused by exposure of samples to laboratory air. Sci Total Environ 203:141–156

Debska B, Drag M, Banach-Szott M (2007) Molecular size distribution and hydrophilic and hydrophobic properties of humic acids isolated from forest soil. Soil Water Res 2(2):45–53

Debska B, Banach-Szott M, Dziamski A, Gonet SS (2010) Chromatographic characteristics (HPLC, HPSEC) of humic acids

of soil fertilised with various organic fertilisers. Chem Ecol 26(S2): 49–57

Debska B, Banach-Szott M, Rosa E, Drag M (2011) Effect of soil pollution with PAHs on organic matter properties. Pol J Soil Sci 44(2):97–103

Debska B, Drag M, Tobiasova E (2012) Effect of post-harvest residue of maize, rapeseed, and sunflower on humic acids properties in various soils. Pol J Environ Stud 21(3):603–613

Eschenbach A, Wienberg R, Mahro B (1998) Fate and stability of non-extractable residues of ^{14}C PAH in contaminated soils under environmental stress conditions. Environ Sci Technol 32:2585–2590

Gonet SS (1989) Properties of humic acids of soils of varied fertilisation. Dissertation, UTA Bydgoszcz (in Polish)

Gonet SS, Debska B (1999) Properties of humic acids produced during decomposition of plant residues in soil. Rostlinna Vyroba 45(10): 455–460

Guo X, Luo L, Ma Y, Zhang S (2010) Sorption of polycyclic aromatic hydrocarbons on particulate organic matters. J Hazard Mater 173: 130–136

Howard PJA, Howard DM, Lowe LE (1998) Effects of tree species and soil physico-chemical conditions on the nature of soil organic matter. Soil Biol Biochem 30(3):285–297

Kastner M, Streibich S, Beyrer M, Richnow HH, Fritsche W (1999) Formation of bound residues during microbial degradation of [^{14}C]-anthracene in soil. Appl Environ Microbiol 65(5):1834–1842

Kelsey JW, Alexander M (1997) Declining bioavailability and inappropriate estimation of risk of persistent compounds. Environ Toxicol Chem 16(3):582–585

Kim SJ, Kwon JH (2010) Determination of partition coefficients for selected PAHs between water and dissolved organic matter. Clean Soil Air Water 38(9):797–802

Kumada K (1987) Chemistry of soil organic matter. Elsevier, Amsterdam

Launen L, Pinto L, Wiebe C, Kiehlmann E, Moore M (1995) The oxidation of pyrene and benzo[a]pyrene by nonbasidiomycete soil fungi. Can J Microbiol 41(6):477–488

Maliszewska-Kordybach B (1999) Persistent organic contaminants in the environment; PAHs as a case study. In: Block JC, Goncharuk VV, Baveye P (eds) Bioavailability of organic xenobiotics in the environment. Kluwer, Dordrecht, pp 3–37

Meyer S, Steinhart H (2001) Fate of PAHs and hetero-PAHs during biodegradation in a model soil/compost-system: formation of extractable metabolites. Water Air Soil Pollut 132(3–4):215–231

Northcott GL, Jones KC (2001) Partitioning, extractability and formation of nonextractable PAH residues in soil. Part I. Compound differences in aging and sequestration. Environ Sci Technol 35:1103–1110

Orlov DS (1986) Humus acids of soils. Balkema, Rotterdam

Pan B, Xing BS, Liu WX, Tao S, Lin XM, Zhang XM, Zhang YX, Xiao Y, Dai HC, Yuan HS (2006) Distribution of sorbet phenanthrene and pyrene in different humic fractions of soils and importance of humin. Environ Pollut 143(1):24–33

Petruzzelli L, Celi L, Cignetti A, Marsan FA (2002) Influence of soil organic matter on the leaching of polycyclic aromatic hydrocarbons in soil. J Environ Sci Health B 37(3):187–199

Plaza C, Xing B, Fernandez JM, Senesi N, Polo A (2009) Binding of polycyclic aromatic hydrocarbons by humic acids formed during composting. Environ Pollut 157:257–263

Preuße G, Friedrich S, Salzer R (2000) Retention behavior of humic substances in reversed phase HPLC. Freseniuz J Anal Chem 368: 268–273

Seibel F, Heidenreich S, Frimmel FH (1996) Interaction of humic substances and polycyclic aromatic hydrocarbons (PAHs) during the biodegradation of PAHs. Acta Hydrochim Hydrobiol 24(6):260–266

Semple KT, Morriss AWJ, Paton GI (2003) Bioavailability of hydrophobic organic contaminants in soils: fundamental concepts and techniques for analysis. Eur J Soil Sci 54(4):809–818

Tan KH (1998) Principles of soil chemistry. Dekker, New York

Wang W, Huang M, Kang Y, Wang H, Leung A, Cheung K, Wong M (2011) Polycyclic aromatic hydrocarbons (PAHs) in urban surface dust of Guangzhou, China: status, sources and human health risk assessment. Sci Total Environ 409:4519–4527

Weissenfels WD, Klewer HJ, Langhoff J (1992) Adsorption of polyclic aromatic hydrocarbons (PAHs) by soil particles: influence on biodegradability and biotoxicity. Appl Microbiol Biotechnol 36(5): 689–696

Wen B, Zhang JJ, Zhand SZ, Shan XQ, Khan SU, Xing B (2007) Phenanthrene sorption to soil humic acid and different humin fractions. Environ Sci Technol 41:3165–3171

White JC, Quinones-Rivera A, Alexander M (1998) Effect of wetting and drying on the bioavailability of organic compounds sequestered in soil. Environ Toxicol Chem 17:2378–2382

Woelki G, Friedrich S, Hanschmann G, Salzer R (1997) HPLC fractionation and structural dynamics of humic acids. Freseniuz J Anal Chem 357(5):548–552

Yang Y, Zhang N, Xue M, Tao S (2010) Impact of soil organic matter on the distribution of polycyclic aromatic hydrocarbons (PAHs) in soils. Environ Pollut 158:2170–2174

Zhang Y, Li F, Wang J (2011) Determination of retardation effect of SOM on aqueous leaching of polycyclic aromatic hydrocarbons using confocal laser scanning Microscope. Int J Environ Res 5(4):999–1008