SOIL POLLUTION OF SELECTED PAHS AS A FACTOR AFFECTING THE PROPERTIES OF HUMIC ACIDS

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ABSTRACT

It is well-known that the properties of humus soil substances (including humic acids) are soil-type-specific. However, one shall consider the fact that properties of organic matter of soil can be modified by farming system (crop rotation, fertilisation) as well as other external factors, including pollutants; PAHs. The objective of the paper is to determine the effect of a single-time pollution of soils with high rates of PAHs on the properties of humic acids. The research was performed with the samples of soils representative for the Kujawy and Pomorze Region (*Phaeozems, Luvisol, Haplic Arenosols, Fluvisols*). Soil samples were polluted with selected PAHs; fluorene, anthracene, pyrene and chrysene at the amount corresponding to 100 mg PAHs · kg^{-1}. Treatments, i.e., soils + PAHs, were incubated for 180 and 360 days at the temperature of 20–25 ºC and at constant moisture of 50% of field water capacity. Humic acids were extracted from the soil samples prior to and after 180 and 360 days of incubation. The following analyses were performed for separating humic acids: elemental composition, UV-VIS and IR spectrophotometric analyses, susceptibility to oxidation. Results demonstrated that a single introduction of fluorene, anthracene, pyrene and chrysene at very high rates into soils affects the properties of humic acids. There was mostly recorded a decrease in coefficients of absorbance $A_{2/6}$ and $A_{4/6,}$ an increase in the parameter defining the susceptibility of humic acids to oxidation. There were also noted changes in the pattern of spectra in infrared and the values of the parameter defining the degree of internal oxidation of the humic acids molecules.

Keywords: soils, PAHs, humic acids, elemental composition, UV-VIS, FTIR.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are considered, due to a strong carcinogenic effect and mutagenic properties to represent the group of pollutions most hazardous to human health. Interestingly, soil is the component of the natural environment in which, finally, most hydrophobic organic pollution gets accumulated, including polycyclic aromatic hydrocarbons (PAHs). The PAHs presence in the environment is the source of infection and, as such, it poses a direct threat to all the living organisms in the contaminated area [14, 19]. As reported in the literature [10, 11, 16, 17, 20, 21, 23], an important parameter which determines the immobilization of PAHs by soils is the quantity and the quality of organic matter.

The main factor of organic matter is made up by humus substances being most common organic compounds found in the nature. The compounds participate in all the processes which occur in soil and affect its physical, chemical and biological properties. They are the main link in the seques-

tration of carbon and releasing CO_2 to the air, as well as the detoxication of the environment.

It is well-known that the properties of organic matter of soils (including humic acids) are specific for a given type of soil [5, 8, 9, 15, 18]. However, one shall note that the properties of organic matter of soils can be modified for example by the method of land cultivation (crop rotation, fertilisation) as well as by other external factors, including pollution by PAHs [4, 5, 8, 9, 15].

The aim of the paper is to determine the effect of a single pollution of soils with high rates of PAHs on the properties of humic acids.

MATERIAL AND METHODS

The research was performed drawing on the soils under agricultural use based on the soils sampled in the Kujawy and Pomorze Province, in the northern part of Poland. Their basic properties are listed in Table 1.

Soil samples were polluted with selected PAHs (fluorene, anthracene, pyrene and chrysene), at the amount corresponding to 100 mg PAHs · kg^{-1}. They were not polluted and PAHs-polluted soil samples were incubated for 180 and 360 days at the temperature of 20–25 ºC and fixed moisture (50% of field water capacity). After a specific incubation time, entire samples were destroyed. The incubation was performed in 3 replications for each sampling date.

The properties of humic acids were determined for the samples of soils unpolluted with PAHs and 180 and 360 days after adding PAHs.

Extraction of humic acids

Humic acids (HAs) were extracted from soil samples prior to the incubation (additionally non-polluted with PAHs), and after 180 and 360 days of incubation. Humic acids were extracted and purified 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 till 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 cm^3 H_2O, 5 cm^3 concentrated HCl, 5 cm^3 concentrated 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 [7].

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

Characteristics of humic acids

The separated humic acids were analysed for:
- elemental composition (Perkin Elmer 2400 CHN analyser, Table 2). The H/C, O/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 (Perkin Elmer UV-VIS Spectrometer, Lambda 20, Table 3). VIS spectra were obtained from 0.02 % humic acid solutions in 0.1 M NaOH and UV-spectra after fivefold dilution. Absorbance was measured at 280 nm (A_{280}), 465 nm (A_{465}) and 665 nm

Table 1. Soil types and basic properties of soil [4, 5]

Sample	Soil	TOC	Nt	TOC/Nt	pH in H_2O	Particle size distribution		
						2-0.05 mm	0.05-0.002 mm	<0.002 mm
		[g · kg^{-1}]				[%]		
Ph2	*Phaeozems*	25.37	1.91	13.28	5.9	67	20	13
Lu5	*Luvisol*	11.74	1.03	11.40	5.9	75	19	6
Hp8	*Haplic Arenosols*	5.72	0.42	13.62	6.4	93	4	3
Fl9	*Fluvisols*	34.02	2.69	12.65	6.4	55	29	16
Fl10	*Fluvisols*	8.17	0.63	12.97	6.3	82	12	6

Explanations: TOC – Total Organic Carbon, Nt – total nitrogen. TOC and Nt determined with the analyser Vario Max CNS (Elementar, Germany).

Table 2. Elemental composition (atomic %) of humic acids and the values of atomic ratio and the degree of internal oxidation

Sample	C	H	N	O	H/C	O/C	ω
	in atomic %						
Ph2 - 0	35.63	41.40	2.72	20.25	1.16	0.568	0.204
Ph2 - 180	35.36	41.40	2.72	20.52	1.17	0.580	0.220
Ph2 - 360	35.81	40.90	2.58	20.72	1.14	0.579	0.231
Lu5 - 0	35.93	40.93	2.56	20.59	1.14	0.573	0.220
Lu5 - 180	37.18	39.88	2.46	20.50	1.07	0.552	0.230
Lu5 - 360	36.33	41.28	2.41	19.98	1.14	0.550	0.163
Hp8 - 0	35.51	42.86	2.66	18.97	1.21	0.534	0.086
Hp8 - 180	35.23	42.61	2.61	19.55	1.21	0.555	0.122
Hp8 - 360	35.01	44.10	2.60	18.29	1.26	0.522	0.008
Fl9 - 0	33.99	42.62	2.70	20.69	1.25	0.609	0.202
Fl9 - 180	34.76	42.23	2.88	20.13	1.21	0.579	0.184
Fl9 - 360	34.32	42.99	2.62	20.07	1.25	0.585	0.146
Fl10 - 0	35.84	41.23	2.58	20.35	1.15	0.568	0.201
Fl10 - 180	35.50	41.78	2.45	20.27	1.18	0.571	0.172
Fl10 - 360	35.39	42.66	2.33	19.62	1.21	0.554	0.101

Explanations: 0, 180, 360 – stands days of incubation.

Table 3. Spectral properties of humic acids

Sample	A_{280}	A_{465}	A_{665}	$A_{2/4}$	$A_{2/6}$	$A_{4/6}$	ΔA_{665u}
Ph2 - 0	4.69	0.826	0.165	5.69	28.42	5.00	65.83 %
Ph2 - 180	5.37	1.052	0.221	5.11	24.29	4.75	71.88 %
Ph2 - 360	3.70	0.705	0.150	5.26	24.70	4.70	60.13 %
Lu5 - 0	5.00	1.06	0.236	4.73	21.16	4.47	67.52 %
Lu5 - 180	4.49	1.11	0.270	4.04	16.65	4.13	70.29 %
Lu5 - 360	4.10	0.839	0.195	4.89	21.05	4.30	70.52 %
Hp8 - 0	4.35	0.843	0.183	5.16	23.83	4.61	71.54 %
Hp8 - 180	3.78	0.931	0.216	4.05	17.52	4.32	74.71 %
Hp8 - 360	3.05	0.629	0.131	4.85	23.27	4.80	66.11 %
Fl9 - 0	3.87	0.598	0.099	6.47	38.95	6.02	74.40 %
Fl9 - 180	3.31	0.555	0.099	5.95	33.29	5.59	84.89 %
Fl9 - 360	2.68	0.408	0.071	6.58	37.75	5.75	74.93 %
Fl10 - 0	3.95	0.661	0.126	5.98	31.29	5.23	76.09 %
Fl10 - 180	4.78	0.871	0.178	5.49	26.88	4.89	78.23 %
Fl10 - 360	2.75	0.532	0.095	5.16	29.00	5.62	69.38 %

Explanations: 0, 180, 360 – stands days of incubation.

(A_{665}) and was used to calculate coefficient values:

$A_{2/4}$ – 280 nm and 465 nm absorbance ratio,
$A_{2/6}$ – 280 nm and 665 nm absorbance ratio,
$A_{4/6}$ – 465 nm and 665 nm absorbance ratio [12];

- susceptibility to oxidation with H_2O_2 by the measurement of HAs solution absorbance (0.02% HAs in 0.1 M NaOH and 1.5% H_2O_2, Table 3) at 665 nm. The susceptibility to oxi-

dation was calculated from the following formula:

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

where: x – wavelength (665 nm),
A_0 – initial absorbance (prior to adding H_2O_2),
A_u – absorbance after oxidation [8];

- infrared spectra (Perkin-Elmer FT-IR Spectrometer, Spectrum BX) over 400–4400 cm^{-1}

were obtained for HAs (3 mg) in KBr (800 mg); deconvolution was applied, with a filter generating the bands of $g = 4$ narrower, and using the process of smoothing, for which the length parameter was $l = 80\%$ [3].

The results were processed with the analysis of variance with the use of the Tukey test at the significance level of $\alpha = 0.05$, for the two-factor experiments without reps, applying the ANALWAR 5.2 statistics software (Author: Prof. Franciszek Rudnicki), with the following factor design: factor I – time; factor II – soil type. The results of the analysis of variance for parameters, for which a significant LSD value was recorded for factor I given in Table 4.

RESULTS AND DISCUSSION

One of the basic parameters characteristic for the structure of humic acids (HAs) is their elemental composition. The elemental composition of the humic acids analyzed is presented in atomic percentage (Table 2).

The content of carbon in the molecules of humic acids ranged from 34.32 to 37.18%, hydrogen from 39.88 to 44.10%, nitrogen from 2.33 to 2.88% and oxygen accounted for 18.29–20.72%. The basic factor differentiating the content of respective elements was the sampling site. Introducing selected PAHs (chrysene, pyrene, anthracene, fluorene) into soil samples only slightly modified the degree of internal oxidation (Tables 2 and 4).

Humic acids (HAs) from *Luvisol*, *Haplic Arenosols* and *Phaeozems* isolated from the soil samples after 180 days of incubation showed higher values of the degree of internal oxidation, as compared to the HAs of unpolluted soils. As for the HAs of *Fluvisols* (sample Fl9, Fl10) a reverse dependence was observed. After 360 days of incubation, only the humic acids of *Phaeozems* showed higher values of the degree of internal oxidation, as compared to the HAs of initial soil. With that in mind, one can assume that introducing chrysene, pyrene, anthracene and fluorene to soils modifies the properties of humic acids connected with their degree of oxidation, however the direction of changes depends on soil properties.

Spectrometric parameters in the UV-VIS range are important parameters used for characterising the properties of humic acids and evaluating the transformations of organic matter in the soils. Based on years' 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. The values of absorbance are used to calculate the ratios of absorbance $A_{2/4}$, $A_{2/6}$, and $A_{4/6}$. Coefficients $A_{2/4}$, $A_{2/6}$ and $A_{4/6}$ are important indices of organic materials humification advancement and the characteristics of the humus substances produced, as well as changes in the properties of humic acids which occur due to various anthropogenic factors [8, 9, 12]. Among the humic acids of the initial soils (additionally non-polluted with PAHs), the highest values of $A_{2/4}$, $A_{2/6}$ and $A_{4/6}$ were recorded for the HAs isolated from the *Fluvisols* with the texture of fine sand (sample no Fl9, Table 3).

The lowest values of the coefficients of absorbance were recorded for the HAs of the *Luvisol* (Lu5). Providing the soils with very high rates of PAHs (100 mg · kg^{-1}) decreased value $A_{2/4}$, $A_{2/6}$ and $A_{4/6}$ of humic acids isolated after 180 days of incubation (Table 4).

Table 4. Results of analysis of variance of selected parameters of humic acids

Factor		ω	$A_{2/4}$	$A_{2/6}$	$A_{4/6}$	ΔA_{665u}
Mean - Factor I	0	0.183	5.606	28.73	5.066	71.076
	180	0.186	4.928	23.726	4.736	76.000
	360	0.130	5.348	27.154	5.034	68.214
Mean - Factor II	Ph2	0.218	5.353	25.803	4.817	65.947
	Lu5	0.204	4.553	19.620	4.300	69.443
	Hp8	0.072	4.687	21.540	4.577	70.787
	Fl9	0.177	6.333	36.663	5.787	78.073
	Fl10	0.158	5.543	29.056	5.247	74.567
LSD - Factor I		0.053	0.512	2.025	0.324	5.340
LSD - Factor II		0.083	0.799	3.162	0.505	8.335

Explanations: 0, 180, 360 – stands days of incubation.

In the soils, between 180 and 360 days of incubation, there occur processes leading to the formation of humic acids with higher values $A_{2/4}$, $A_{2/6}$ and $A_{4/6}$, as compared to HAs after 180 days of incubation. Despite the increase in the values of the above ratios of absorbance, after 360 days of incubation, the HAs of the soils polluted with PAHs recorded, in general, still lower values $A_{2/4}$, $A_{2/6}$ and $A_{4/6}$, as compared to the HAs of non-polluted soils.

The structure of humic acids can be also indirectly inferred from the changes in the value of absorbances which occur as a result of the reaction of oxidation with hydrogen peroxide of humic acids [8]. According to Gonet [8], the aliphatic part of the HAs molecules showed to be more susceptible to oxidation, while the aromatic part is more resistant. The changes in the value of absorbance at 665 nm of humic acids which occur as a result of oxidation with H_2O_2 are given in Table 3. The values of parameter ΔA_{665u} (susceptibility to oxidation determined at the wavelength of 665 nm) recorded for the HAs of the initial soils (with no PAHs added) ranged from 65.83 (Ph2) to 76.09% (Fl10). Highest values of ΔA_{665u} were recorded for the humic acids isolated from the soil samples additionally polluted with PAHs after 180 days of incubation. For the HAs of *Phaeozems* (Ph2), *Haplic Areonosols* (Hp8) *and Fluvisols* (Fl10), the values of ΔA_{665u} after 360 days of incubation were significantly lower, as compared to the HAs of non-polluted soils. Humic acids of *Fluvisols* with the texture of fine sand (sample no Fl9) after 360 days of incubation showed value ΔA_{665u} similar to the HAs of non-polluted soil. On the other hand, the HAs of *Phaeozems* demonstrated the lowest values ΔA_{665u} after 360 days of incubation. Therefore, introduction of chrysene, pyrene, fluorene and anthracene to soils resulted in changes in the structure of humic acids, which must have increased the share of unsaturated aliphatic structures, which, in turn, decreased the resistance of the molecules of humic acids to oxidation [6, 8].

The changes in the structure of the molecules of humic acids obtained once PAHs were introduced into soils are confirmed by the spectra produced in the IR range. Spectra of HAs *Phaeozems* and *Fluvisols* (Fl9, Fl10) isolated from the samples of soils additionally polluted with high values of PAHs, after 180 days of incubation, as compared to the HAs of the original soil, demonstrated a higher band intensity of the frequency of about 1510 cm^{-1}, 1460 cm^{-1}, 1420 cm^{-1}, 1260 cm^{-1} and the peaks in the range 1130–1000 cm^{-1} (Figure 1, due to a similar pattern of HAs spectra, the present paper includes spectra for a single variant only).

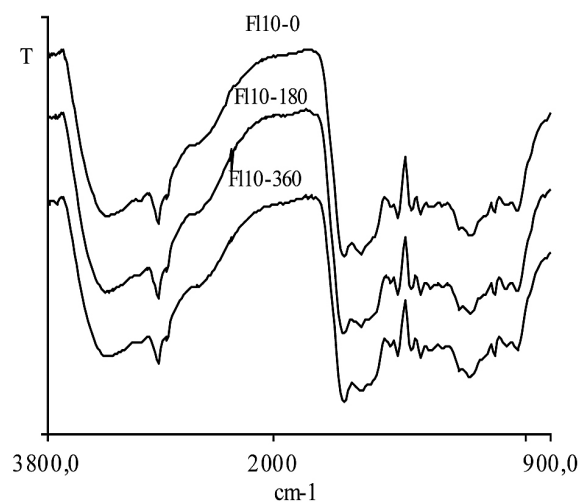**Figure 1.** FT-IR of humic acids of *Fluvisol*

The band at 1520–1500 cm^{-1} is characteristic for aromatic compounds (C=C). The band in the range of 1460–1440 cm^{-1} corresponds to the deformation vibration of groups –CH$_3$ and =CH$_2$, the band 1420–1400 cm^{-1} points to the presence of groups O-C-O in esters and C=O in carboxyl acids, and OH groups. In the 1280–1200 cm^{-1} range there occur bands of C–O groups of phenols, carboxyl acids, esters and ethers, and the bands in the range 1130 cm^{-1} and 1080–1030 cm^{-1} point to the presence of polysaccharides. They can also be related to the presence of groups OCH$_3$ which occur in lignins and OH in alcohols [8, 3, 15, 18].

The intensity of the above absorption bands decreased from the 180 to 360th day of incubation. However, the humic acids isolated after 360 days of incubation, as compared to the HAs of the original soils, show a slightly higher share of simple aromatic structures (a higher intensity of the 1510 cm^{-1} band), as well as higher band in the range of 1460–1030 cm^{-1}. Spectra of HAs of *Luvisol* and *Haplic Arenosols* isolated from the samples of soils additionally polluted with high values of PAHs, after 180 and 360 days of incubation, as compared to the HAs of the original soil showed a similar pattern (Figure 2).

The spectrum pattern of the humic acids investigated confirms, on the one hand, the effect of PAHs on the properties of humic acids, and, on the other hand, points that the process of transformations of pollutants introduced into soil can differ and it also depends on soil properties.
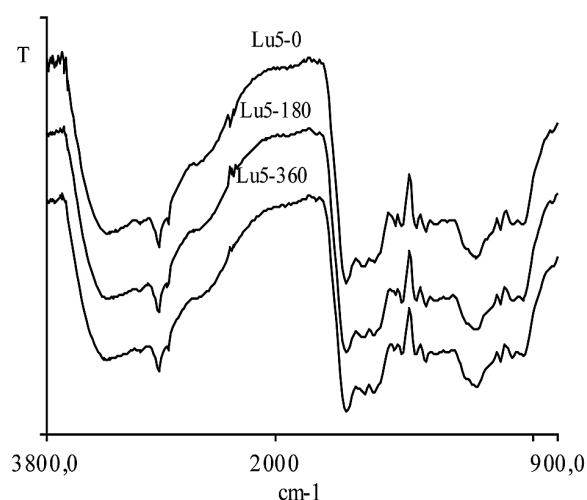
Figure 2. FT-IR of humic acids of *Luvisol*

The present results (elemental composition, spectrometric parameters in the UV-VIS and IR range then) coincide with earlier literature reports [1, 2, 11, 13, 17, 20, 22] which stressed a considerable share of organic matter, including humic acids, in the PAHs sorption processes. However, one shall also remember that the compounds formed in the process of PAHs decomposition can be bound by the fractions of organic matter (humic acids, fulvic acids, humins).

CONCLUSION

In the humic acids isolated from the samples of soils additionally polluted with PAHs at the dose 100 mg · kg^{-1}, as compared with the HAs of the non-polluted soils, the following were observed:
- a change in the parameter ω – the degree of internal oxidation;
- an increase in the intensity of absorption bands in the 1520–1000 cm^{-1} range, higher in all the HAs spectra isolated after 180 days than after 360 days. The band connected with an increase in the share of simple aromatic and aliphatic structures in the molecules of humic acids;
- an increase in the susceptibility to oxidation recorded for the humic acids isolated after 180 days of incubation.

The results point to the need of considering while investigating the properties of soils in the areas exposed to the effect of PAHs, the role of those compounds in the organic matter properties development.

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