Electronic Journal of Polish Agricultural Universities is the very first Polish scientific journal published exclusively on the Internet, founded on January 1, 1998 by the following agricultural universities and higher schools of agriculture: University of Technology and Agriculture of Bydgoszcz, Agricultural University of Cracow, Agricultural University of Lublin, Agricultural University of Poznan, Higher School of Agriculture and Teacher Training Siedlee, Agricultural University of Szczecin, and Agricultural University of Wroclaw.



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SPECTROSCOPIC STUDIES ON UVC-INDUCED PHOTODEGRADATION OF HUMIC ACIDS

Danuta Sławińska¹, Krzysztof Polewski¹, Piotr Rolewski¹, Patryk Pluciński,² Janusz Sławiński² ¹Department of Physics, August Cieszkowski Agricultural University of Poznan, Poland ²Department of Radio- and Photochemistry, Institute of Chemistry and Technical Electrochemistry, Poznan, Poland



ABSTRACT

The effect of UV radiation on absorption, fluorescence spectra and dynamics of phototransformation of five different humic acids (HA) of natural and synthetic origin were investigated. Two types of synthetic HA were prepared: one containing only C, H and O atoms, and another with content of C, H, O and N atoms. Natural HA were also diversified with respect to their origin. We used UV-C radiation (predominantly the wavelength of 254 nm) to provide a wide spectrum of photochemical mechanisms.

Fluorescence spectroscopy measurements revealed similar chromophoric centers in different types of HA: at short wavelength ($I_{max,em}$ at $\lambda = 450$ nm for excitation λ_{ex} from 260 – 350 nm) and at longer wavelength ($I_{max,em}$ at $\lambda = 510$ nm for excitation λ_{ex} from 440 – 460 nm). The maximum emission $I_{max,em}$ was always higher for excitation at λ_{ex} 260-350 nm than for λ_{ex} 440-460 nm.

Dynamics of fluorescence intensity accompanying UV-C-induced degradation appeared to be different for both chromophoric centers. Fluorescence emission at short wavelength excitation increased with irradiation time t_{ir} lasting up to 120 min, while for longer excitation wavelength it reached the saturation level or even slightly decreased.

Both synthetic humic acids, regardless whether obtained from pyrocatechol or hydroquinone and tyrosine, appeared to be more resistant to UV-C than the natural ones. The resistance can be parameterized by the rate of increasing fluorescence intensity, i.e. the slope of kinetic curves $I_{em} = f(t_{ir})$, the position of $I_{max,em}$ and induction-like time interval in the initial phase of irradiation.

Key words: humic acids, fluorescence and absorption spectra, UV-C radiation, photodegradation

INTRODUCTION

Increasing penetration of UV radiation into the troposphere raises the question about interactions of humic substances (HS) with solar radiation. How stable are HS in anthropopressure-affected environment? How the origin and structure of HS are related to their stability? What might be possible agricultural and ecological consequences of photodegradation of HS ?

Humic substances (HS) are natural dark paramagnetic, macromolecular, heterogeneous substances with a high degree of polydispersity, extremely complex structure and unique biological and physicochemical features. They occur ubiquitously in soil, aquatic environment and certain sediments. HS due to their complex nature of polyanionic weak electrolytes with molecular weight of $10^3 - 10^5$ Da constitute a class of organic macromolecules difficult to be characterised. However, they are known to contain similar functional groups i.e. C=C, COOH, OH, OCH₃, C=O, NH or NH₂, arrangements such as redox quinone-semiquinone-hydroquinone, charge-transfer planar complexes etc. . Both their dark colour and paramagnetism most likely result from the electronic structure of their core: aromatic subunits, an extended (π -electron) system in the semiquinone moieties, and the electron donor-acceptor nature.

Results of recent investigations provided the evidence that HS can no longer be recognized only as a transformer of solar energy into heat, storage of nutrients for plants and microbes, soil-structure-forming factor and plant-growth stimulator. For agriculture, ecology and environmental protection the interaction of solar radiation with the surface of ecosystems constitutes the crucial point. Organics such as amino acids, fatty acids, phenols, carbohydrates, lignins and tannins absorb at wavelengths below 300 nm, and therefore are unlikely to be involved in soil/aquatic photochemistry and photobiology. In contrast to them, HS contain chromophores capable of absorbing a wide spectrum of electromagnetic radiation in X, UV, visible and IR range. Therefore they are the most important solar light absorbers. Apart from providing heat, they can also act as a photoactive agent. Still, such photophysical, photochemical and photobiological functions of HS are barely recognized. Such phenomena as fluorescence , delayed photoluminescence and chemiluminescence of HS , as well as a HS-photosensitised detoxication of xenobiotics in aquatic ecosystems , prove that electronic excited states and free radicals are generated in HS macromolecules or in their photoreaction products.

A variety of methods in fluorescence spectroscopy has been extensively applied as useful means to determine physicochemical characteristics and differentiate both natural and model synthetic HS. Supramolecular structure of HS abundant in spongy microspaces with statistically average dimension (0.24-0.48 nm) resembles a molecular sieve that ensures efficient sorption of low molecular components like organic xenobiotics, herbicides and pesticides, metal ions, etc. Sorption of oxygen molecule which has a diameter of 0.127 nm and easily penetrates into polymer network, and is a biradical, is of particular interest. It was shown that interaction of light with HS produces reactive oxygen species (ROS) which are responsible for sensitised ('photodynamic') processes in surface waters. On the other hand, HS reveal photoprotective role and a high antioxidizing and free radical scavenging capacity. These functions may be of a high importance for agriculture and ecology. Due to their multifunctional physicochemical and biological properties, HS play important manifold roles in the C-cycle, solar energy transformation, metal ions immobilization and fate of xenobiotics and radionuclides.

Taking into account the external superficial distribution aswell as the properties of HS mentioned above, their photoactive function, i.e. interaction with solar radiation, especially within the range of short wavelengths, seems indisputable. The question, however, of possible agricultural and ecological consequences of UV-induced photodegradation of HS remains open. It is a challenging and extremely complex problem, and research in that field is scarce.

In this work we have attempted to answer that question, at least partially. Therefore we compared the effect of UV radiation on absorption, fluorescence spectra and dynamics of phototransformation of five very dissimilar humic acids (HA) of natural and synthetic origin. We used UV-C radiation (wavelength of 254 nm, predominantly) since it provides a wide spectrum of photochemical mechanisms of HA transformations and ensure a rapid, analytically convenient performance of model experiments. Although the UV-C does not penetrate the biosphere at present, the future looms large. The most plausible explanation of this is the following:

UV-C solar radiation is absorbed by the stratospheric O_3 . However, the O_3 concentration in the stratosphere can be diminished be long-term, slow photochemical reactions with flurochloromethanes deposited earlier, and NO_x permanently injected at high altitudes by supersonic planes. Since rains and winds at these altitudes do not occur, it is predictable that the amount of accumulated halomethanes and NO_x will last for about 50 years or more, and catalytic destroy ozone. Therefore, more and more short wave UB-B and long wave UV-C radiation will penetrate the troposphere.

Two types of synthetic HA have been prepared: the most simple models containing only C, H and O atoms, and another ones with C, H, O and N atoms. Natural HA are also diversified with respect to their origin.

MATERIALS AND METHODS

Model synthetic humic acids (HA) were obtained by the oxidative polymerization/condensation of: 1. 1,2dihydroxybenzene (pyrocatechol) (called "PYR") and 2:1,4-dihydroxybenzene (hydroquinone) and β -p.hydroxyphenylalanine (tyrosine) (initial molecular ratio 1:5; called HQ-TYR). PYR contains only the C-C and eventually C-O-C skeleton (no N atoms), while HQ-TYR C-C, C-N heterocyclic and amino/amido forms and eventually C-O-C skeleton. Two other HA were obtained from black meadow soil containing predominant fraction of sand and clay (called HAM) and 3-year composted external (green) shells of matured walnut (*Juglans regia*) (called HAW) collected in October each year. The compost contains a large amount of organic materials, especially polyphenols and quinones. The extraction of the raw material with 0.1 M Na₂CO₃ and isolation of humic fractions was performed in N₂ atmosphere according to IHSS protocol . As a reference, a commercially available HA from Fluka AG Bush, (called HAF) was used.

Carbon, hydrogen, nitrogen and sulphur contents of each humic acid were measured by combustion in oxygen gas using Vario ELIII Elementar. Values of the colour coefficient $Q_{2.6/4}$ and $Q_{4/6}$, where indexes 2.6, 4 and 6 refer to absorbance measured at 260, 400 and 600 nm, respectively, were calculated for equal C content and are presented in Table 1.

Symbol of HA	Origin	Content, %					Ratio		Color coefficient	
		С	Н	0	N	S	H/C	O/C	Q _{2.6/4}	Q _{4/6}
PYR	synthetic	57.52	3.47	39.01	0.00	0.00	0.06	0.68	2.94	2.22
HQ-TYR	synthetic	57.11	3.53	38.49	0.87	0.00	0.06	0.67	2.74	2.55
HAM	meadow soil	30.89	4.29	61.06	3.31	0.45	0.14	1.98	6.00	4.58
HAW	walnut compost	44.82	4.84	47.63	2.48	0.23	0.11	1.06	2.73	3.14
HAF	commercial Fluka AG	52.12	5.10	41.03	0.63	1.12	0.10	0.79	4.07	2.93

Table 1. Elemental analysis and color coefficients of the studied humic acids (HA)

Humic acids (HA) derived from: PYR - pyrocatechol;

HQ-TYR -hydroquinone and tyrosine;

HAM - black meadow soil;

HAW - composted external (green) shells of matured walnut (Juglans regia)

Infrared FTIR spectra were obtained in KBr pills (200 mg KBr + $1,8\div5$ mg HA) on Bruker IFS 113V spectrometer. All IR spectra display similar bands, but at different intensities. The band for C=O vibration at 1720 cm⁻¹ is as distinct and strong in intensity as the band at 1625-1630 cm⁻¹ for carboxylate anion. The band at ca 1400 cm⁻¹ can be assigned to the deformation modes of CH₂ and CH₃ groups: v (C=C) and asymmetric v (C-O) stretch of the --COO⁻ group. Aromatic conjugated COOH groups, quinone and amide groups can also produce 1620 cm⁻¹ peaks. Peaks at 1020-1050 cm⁻¹ may originate from CH₃O groups and carbohydrate-like structures. In humic acids of the natural origin containing sulfur, the weak absorption maxima within the range from 900 to 450 cm⁻¹ might be assigned to the deformation S-H vibration, tiophenols and sulfonamide stretching vibration.

As a buffer/solvent the 0.01 molar solution of Na_2CO_3 at pH 10.9-11.2 was used. Other reagents were of analytical purity from POCh Gliwice. Water was double distilled from a quartz apparatus.

Absorption spectra were measured with a Top Sensor System from Ocean Optics (Netherlands) equipped with a H-source, quartz fiber and a photodiode array detector sensitive in the spectral range 220-800 nm. Quartz cuvettes 0.1 and 0.2 cm wide for UV and 1 cm wide for visible range were used. From the measured numerical values of absorbances $A_{\lambda,tir}$ colour coefficients $Q_{\lambda i/\lambda j}$ and the relative rate of phototransformation ((A₀ - A)t_{ir})_{λ} / t_{ir} were calculated.

Spectroscopic measurements were made in triplicate for the 240-300 nm range using 0.1, 0.2 and 10.0 mm cuvettes.

Fluorescence excitation and emission spectra were recorded with Shimadzu RF 5001PC spectrofluorimeter. Fluorescence of the 'dark' and irradiated solvent 0.01 M Na₂CO₃ was checked at several excitation wavelengths. The reason for this auxiliary measurement is justified by the possibility of photochemical transformations. Solutions of Na₂CO₃ were irradiated with 254 nm (the energy of quanta amounted to 470 kJ/mol). Such energy might be sufficient to initiate ionization of HCO_3^{1-} / CO_3^{2-} ions and dissolved O₂, and start photoreactions leading possibly to some products fluorescing in 260-350 nm range.

Only a weak emission in the 330-520 nm region was recorded for excitation wavelengths 260 and 350 nm. The maximum emission intensity was less than 10 arbitrary units. It is important to note that emission intensity was decreased after 60 min of irradiation by a factor of 2–3. Thus, the contribution of the solvent fluorescence is negligent. Since the fluorescence of humic substances is sensitive to concentration, the dependence of fluorescence spectra on the concentration of HA was measured in the range $10^{-6} - 10^{-3}$ g/dm³. The minimum fluorescence quenching was found in the range $5 \cdot 10^{-5} - 10^{-4}$ g/dm³ and applied in experiments.

The source of UV-C irradiation was a home-made low-pressure Hg burner without electrodes; it was powered by a 140 MHz radio-wave generator, 40 W, and emitted 85 % of the total output power in the resonance wavelength 254 nm (261 mW/m²). The output power corresponds to 254 nm photon flux equal to 10^{15} photons/ s cm³. Solutions of tested HA and 0.01 molar Na₂CO ₃ were circulated in a flow system at the rate of 60 ml/min. The volume of 25 ml of the irradiated solutions, with pH 10.30 at temperature 25°C, was circulated under aerobic conditions. The temperature of irradiated solution was kept constant (+/-2 °C). The irradiation system is described in details elsewhere. After the sufficient time of irradiation (t_{ir}), the aliquots of 5 ml of solution were taken for analysis.

RESULTS AND DISCUSSION

The effect of irradiation on absorption spectra and kinetics

The results of spectrophotometric and fluorometric measurements are presented as 3-dimensional spectra (3D spectra), which reduces significantly the number of pictures needed for different irradiation times, excitation and emission wavelengths.

Absorption spectra of HA are featureless and decline exponentially in absorbance from high to low frequencies. 3D spectra for control (not irradiated) and irradiated solutions of HA show absorbancy as a function of wavelength and irradiation time (Fig. 1a-e). In general, absorbancy values (A), for all wavelengths λ , tend to decrease gradually with the increase of t_{ir} . However, synthetic HA PYR and HQ-TYR after a short t_{ir} 5-15 min exhibit a 3-8% increase in absorbancy (compared to the initial A-values). Also the rate ((A₀ - A) t_{ir}) λ / t_{ir} of synthetic HA phototransformation is positive for the irradiation time-interval 5-15 min, which indicates a transient increase in absorbancy. This may be a result of photopolymerization/condensation of lower molecular fractions (subunits) of HA. The correlated transient decrease of the colour coefficient Q_{4/6} values supports this interpretation. The model HA were synthesized under mild conditions and their condensation/aromacity degree is rather low. It means that the substances may contain low-molecular subunits for which UV-C radiation-induced polymerisation can occur. Such interpretation seems to be supported by incorporation of simple organic molecules into MA macromolecule observed during Fenton-photoreaction.





For longer irradiation times t_{ir} the rate values $((A_0 - A)t_{ir})_{\lambda} / t_{ir}$ are positive and $Q_{2.6/4}$ and $Q_{4/6}$ colour coefficients increase that indicates the progress of photodegradation.

Other HA of natural origin exhibit a gradual decrease in absorbancy for all tested wavelengths and t_{ir} and an increase in colour coefficient $Q_{2.6/4}$ and $Q_{4/6}$. The data confirm a gradual increase of the amount of phenolic and simpler carboxylic acids in irradiated solutions and a decrease of the molecular weight and condensation degree of the HA macromolecule's core.

Kinetic changes of absorbance $A_{\lambda} = f(\lambda)$ presented in Fig 1a-e provide information about resistance of certain chromophores to UV-C radiation. The analysis of kinetic curves $(A_0-A_{ir})_{\lambda} / A_0 = f(t_{ir})$ at wavelengths 600, 400 and 260 nm leads to a general conclusion that long-wave absorbing chromophores ($\lambda = 600$ nm) are more susceptible to photodegradation than short-wavelength absorbing chromophores. The resistance of irradiated HA clearly depends on their origin. The most resistant are synthetic HA, particularly the PIR. Their photodecomposition is delayed by an initial phase of photocondensation/polymerization and has some 'induction time'. It is important to realize that HAs of natural origin contain traces of metals that might exert a catalytic effect upon the rate of photodegradation.

Fluorescence spectra and the kinetics of irradiation-induced fluorescence changes

The wavelengths of the emission maxima are dependent on the excitation wavelength λ_{ex} , since HAs present a multichromophoric (fluorophoric) system. In such systems every fluorophore may be immersed in many non-fluorescent chromophores. Such a situation would explain the very low quantum efficiency of HAs, typically « 0.01. We measured fluorescence emission spectra in the range 330-640 nm at four excitation wavelengths λ_{ex} 260, 350, 440 and 460 nm. Generally, all investigated HAs at the concentration 10^{-4} g/dm³ when excited at 260 and 350 nm, exhibit emission maxima around 445 ± 5 nm. Excitation at 440 nm and 460 nm results in emission centered around 510 ± 5 nm. During the UV-C irradiation the shape of spectra does not change significantly, but amplitude increases with the increase of t_{ir}. Fig. 2a and 2b show 3D emission spectra at various time of irradiation and excitation wavelength. It appears that for a given λ_{ex} neither position and shape of the spectrum undergo distinct changes, to which amplitude is subjected. Therefore, fluorescence emission maxima (amplitudes) may be presented in Fig. 3 a-e as a function of λ_{ex} and t_{ir} .









Figure 3. Changes of the fluorescence emission maximum of 0.1 mg/ml of humic acids of different origin as the function of irradiation time t_{ir} and excitation wavelength λ_{ex} . Legend as used in Figure 1. Fluorescence intensity I is given in arbitrary units.

General regularities of the kinetics of fluorescence emission $I_{em,max} = f(t_{ir})$ in the initial stage of photodegradation can be observed. The first and most evident of then is an increase of fluorescence intensity with prolonged irradiation time t_{ir} for all tested HAs. However, the chromophores excited at 440 and 460 nm exhibit much slower increase rate in the fluorescence intensity - even a small decrease after reaching the maximum is noticeable, than the short-wavelength ones excited at 260 and 350 nm. One of possible explanations of such diversity may be based on the assumption that the long wavelength fluorophores (e.g. quinones, semiquinones, charge transfer (CT) complexes and/or stacking planar structures) are less resistant to UV-C than short wavelength fluorophores, such as phenolics or lignin-like moieties. Less resistant moieties are transformed earlier and therefore the level of fluorescence intensity is saturated and can only decrease later.

Higher intensities of fluorescence emission and a prolonged time of its increase at shorter λ_{ex} may be ascribed to formation of simpler structural components with higher quantum yield of fluorescence, or to the destruction of the surrounding non-fluorescent moieties. The aromatic core of a humic macromolecule can be a rich source of substrates for degradation reactions initiated by reactive oxygen species. These reactions can lead to hydroxylation of aromatic subunits and subsequent ring-opening reactions that finally generate aliphatic ketoacids. Photodegradation products detected after UV-C ($\lambda = 253.7$ nm) irradiation of humic material and water were low molecular-weight mono- and dicarboxylic acids ($C_2 - C_4$) and aldehydes ($C_8 - C_{14}$), series of n-alkanes and a group of alcohols . Photodegradation of relatively resistant HS to compounds of lower molecular weight and higher hydrophobicity during UV-C irradiation is likely to enhance microbiological growth in terrestrial and aquatic ecosystems. Both transient hydroxylated aromatics and keto/hydroxyacids have the higher quantum yield of fluorescence than conjugated unsaturated systems capable of a high degree of resonance, internal energy conversion into heat, i.e. a low quantum yield of fluorescence (e.g. non-fluorescent graphite-like structure). Such interpretation would be in agreement with the contents of functional groups in HAs and results of previous studies on chemiluminescence of humic acids.

The precursors of long wavelength fluorophores emitting at 510 nm might include quinoid, CT and stacking planar structures. Such structures are known to absorb light at 420-500 nm and are sensitive to nucleophillic attack of ROS. Therefore, a lower contents of such structures would result in their faster consumption (transformation to fluorescing intermediates) and final rearrangement to low molecular products fluorescing at shorter wavelengths. Such a model would explain faster and longer fluorescence increase at 445 nm ($\lambda_{ex} = 260$ and 350 nm). Kinetic bars of I_{em,max} shown in Fig 3a-e are coherent with the interpretation quoted above.

Possible mechanisms of the UV-C induced degradation of HAs

Taking into account energy of photons ($E = hc/\lambda$) of the low-pressure Hg burner, emitting at $\lambda = 254$ nm (E = 470 kJ/mol) and aqueous air-saturated solutions, several reaction mechanisms can be envisaged.

The emitted energy can dissociate covalent bonds such as C-C, C-H, O-H, C-O and ionize susceptible moieties of HA macromolecule, producing free radicals R⁻ and e⁻:

HA
$$e^{\text{nergy}} \rightarrow \bullet \text{HA}^+ + e^-$$

 $\mathrm{e}^- + \mathrm{H}_2 \mathrm{O} \rightarrow \mathrm{e}^-_{\mathrm{aq}}$

 $e_{aq}^- + O_2 \rightarrow H_2O + \bullet O_2^-$

In Na₂CO₃ solutions containing dissolved CO₂ additional formation of superoxide ion-radical can take place:

$$CO_2 + e_{aq}^- \rightarrow \bullet CO_2^-$$

 $\bullet \mathrm{CO}_2^- + \mathrm{O}_2 \to \mathrm{CO}_2 + \bullet \mathrm{O}_2^-$

Dismutation of generates hydrogen peroxide:

$$\bullet O_2^- + \bullet O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$$

which reacting with produces a highly reactive hydroxyl radical in the Haber-Weiss cycle:

 $\texttt{H}_2\texttt{O}_2 + \bullet \texttt{O}_2^- \rightarrow \bullet \texttt{OH} + \texttt{OH}^- + \texttt{O}_2$

Hydroxyl radical hydroxylates organic molecules at a diffusion-controlled rate, initiating degradation reactions. Moreover, primary produced radicals R• produce organic unstable peroxides ROO•. All the ROS can attack susceptible moieties of HA macromolecule, which finally leads to its gradual breakdown.

CONCLUSIONS

Absorption and fluorescence spectroscopy have appeared to be useful for the characterization of resistance to UV-C-induced degradation and its dynamics of different HA's.

Fluorescence spectroscopy measurements revealed similar chromophoric centers in different types of HA: at short wavelength ($I_{max,em} \lambda = 450$ nm for excitation wavelengths λ_{ex} from 260 nm to 350 nm) and at longer wavelength $I_{max,em} \lambda = 510$ nm (for excitation wavelengths λ_{ex} from 440 to 460 nm). The maximum $I_{max,em}$ was always higher at excitation for wavelengths from 260nm to 350 nm than for excitation in the range 440-460 nm.

Dynamics of fluorescence intensity accompanying UV-C-induced degradation appeared to be different for both chromophoric centers. The fluorescence emission at short wavelengths increased with prolonged t_{ir} (up to 120 min), while for long wavelengths it reached the saturation level or even slightly decreased.

Synthetic humic acids, PIR and HQ-TYR, with C content (ca 57%) higher than the natural ones (31-52%), are more resistant to UV-C than natural HA. The resistance can be parameterised by the rate at which fluorescence intensity increases, i.e. the slope d I_{em} / d t_{ir} of kinetic curves $I_{em} = f(t_{ir})$, the position of $I_{max, em}$ and induction-like time interval in the initial phase of irradiation.

ACKNOWLEDGMENT

This work has been supported by grant 3 PO49 016 22 from the State Committee for Scientific Research, Poland.

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Danuta Sławińska, Krzysztof Polewski, Piotr Rolewski, Department of Physics August Cieszkowski Agricultural University of Poznan ul. Wojska Polskiego 38/42, 60-637 Poznan, Poland phone/fax (48 61) 848 7496, 7495 e-mail dslawins@wodcock.au.poznan.pl

Patryk Pluciński and Janusz Sławiński Department of Radio- and Photochemistry Institute of Chemistry and Technical Electrochemistry Poznan University of Technology Poznan, ul. Piotrowo 3, 60-965 Poznan, Poland

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