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RESISTANCE OF MODEL HUMIC ACIDS TO ULTRAVIOLET-C RADIATION

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Abstract. Increasing penetration of UV radiation into the Biosphere raises the question about the photo resistance of humic substances. Is there any dependence between the photoresitance and chemical composition of their precursors? Humic acids synthesized from pyrocatechol, hydroquinone + tyrosine, purpurogalline and commercial Fluka of natural origin were irradiated with the UV-C radiation. Fluorescence emission spectra, chemiluminescence imaging and absorption spectra of alkali solutions of humic acids before and after various time of irradiation were measured. The most resistant to UV-C radiation was synthetic humic acid from pyrocatechol, and the most labile was humic acid obtained from purpurogalline. Possible causes of the high/low photo resistance of humic acids are shortly considered.

Keywords: humic acids, photo resistance, UV-C radiation, luminescence, chemiluminescence imaging

INTRODUCTION

Humic substances (HS) are the most complex natural dark paramagnetic macromolecular heterogenous substances resulting from the decomposition of organic matter in soils and waters. They consist of polyhydroxylated phenols, phenolic acids and quinones bound with peptides, proteins and carbohydrates. HS represent a mixture of anionic weak polyelectrolytes with molecular weight in the range of 10^3-10^5 Da, high degree of polydispersity and unique biological and physicochemical features. HS can be described as supramolecular associations of relatively small heterogenous molecules linked by weak dispersive forces. They play a multifunctional important role in soil and aquatic environment (Auger, Richard 1996, Auger, Richard *et al.* 1999).

In the last years it was found that HS can act as the sole electron acceptor in the anoxic oxidation of certain organic compounds, e.g. acetates (Chen *et al.* 2003) and ions of heavy metals (Drozd *et al.* 1997). They shuttle electrons between the ultimate electron donor, e.g. bacteria oxidizing contaminants such as cations of copper and iron, dichloroethane, PVC, toluene, nitrobenzene, and subsequently donate those electrons to a terminal electron acceptor. This is because double bonds in quinone groups in HS can act like „on/off‘’ redox switches. Since the electron shuttles are not consumed, high concentrations are not needed to speed up the overall reaction. Therefore, quinoid moieties in HS may be a more important electron acceptor for the bioremedation of contamined environments than previously considered. Soil and water environments rich in HS may be expected to stimulate anaerobic biodegradation of hydrophobic organic and inorganic pollutants, contributing to the intrinsic bioremedation of these habitas.

However, HS absorb a wide spectrum of electromagnetic radiation in the γ, X, UV, visible and IR range. This made them the most important abiotic absorber of the solar radiation acting in a photoactive manner. Such phenomena as fluorescence (Drozd *et al.* 1997) chemiluminescence (Mc Knight *et al.* 2001, Górski and Sławiński 2002), delayed photoluminescence and transient photoinduced-EPR signals (Jezierski *et al.* 2002), as well as HS-photosensitized detoxication of xenobiotics (Lovley *et al.* 1996) prove the generation of electronic excited states and free radicals in HS macromolecules or in their millieu. These reactive species, especially reactive oxygen forms, attack quinone-semiquinone-hydroquinone moieties of HS macromolecules, which leads to irreversible destruction of the quinoid rings and subsequent oxidative degradation of humic acid (HA) macromolecule (Lovley *et al.* 2000). Therefore, the interaction of solar radiation with HS and oxygen, occurring in the surface of soil and aquatic ecosystems, is of high importance for agriculture.

An increasing penetration of UV radiation into the troposphere raises the question about interactions of 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? Qualitative and quantitative studies on the resistance of HS synthetized from different precursors to visible and UV radiation are scarce (Polewski *et al.* 2002; Polewski *et al.* 2005, Sławińska *et al.* 2002). Therefore, in this study we investigate the effect of UVC radiation on model humic acids derived from orto- and para-diphenols. The goal od our study was to compare the photoresistance of certain HA to UV radiation.

MATERIALS AND METHODS

In this work we attempt to answer, at least partially, the above questions. Therefore we studied the effect of UV radiation on humic acids (HA) - an alkali-soluble fraction of HS of natural and synthetic origin. We used UV-C radiation (predominantly the wavelength of 254 nm) since it provides a wide spectrum of photochemical mechanisms of HA transformations and ensures a rapid, analytically convenient performance of model experiments. Three synthetic HA were prepared: two containing only C, H and O atoms, and another, C, H, O and N atoms. A comercial HA of natural origin and well characterized properties was used for comparison.

Materials and reagents

Model synthetic humic acids (HA) were obtained by the oxidative polymerization/condensation of: 1: 1, 2-dihydroxybenzene (pyrocatechol) (called „PYR”); 2: 1, 4-dihydroxybenzene (hydroquinone) and DL- para.-hydroxyphenylalanine- (tyrosine) (initial molecular ratio 2: 1; (called HQ-TYR)) and 3: 2, 3, 4, 6- tetrahydroxy- 5 H-benzocyclohepten- 5- one (trihydroxybenzotropolone, purpurogallin), called PPG. HA 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/amide forms and eventually C-O-C skeleton. HA-PPG contains C-C and C-O -C skeleton.

For control, a commercial preparation of HA from Fluka AG Bush (called HAF) was used. Elemental composition of the HA is given in Table 1.

As a buffer/solvent the 0.1 molar solution of Na_2CO_3 was used. Other reagents were of analytical purity, from POCh Gliwice. Water was double distilled, from a quartz apparatus. In order to enhance ultra weak chemiluminescence (CL) accompanying photoinduced oxidative degradation of HAs, 5- amino- 2, 3- dihydrophthalazine- 1, 4- dione (luminol)- a chemiluminescent probe (Sigma) was used.

Methods and apparatus

Absorption spectra were measured with a Jasco corporation V-550 spectrophotometer sensitive in the spectral range of 200-1100 nm. Quartz cuvettes with the width of 0.1 and 0.2 cm for the UV and 1 cm for visible range were used. From the measured numerical values of absorbance $A_{\lambda,\ \tau ir}$ colour coefficients Qλi/Qλj, were calculated.

Fluorescence emission spectra were recorded using Shimadzu Corporation Model RF- 5301 PC spectrofluorimeter (220- 900 nm, spectral resolution 2 nm) and 1 cm quartz cuvettes.

Ultra weak chemiluminescence (CL) was recorded with a supersensitive 16 bit charge coupled device (CCD) imaging camera (512 ^{x} 512 pixels Molecular Light Imager 981 „Night Owl", EG&G Berthold) and WinLight EG&G Berthold software. UV-C irradiation of HAs solutions was performed using a low-pressure Hg Philips TuV115 vHO lamp at the distance of 10 cm from sample, giving the total irradiation flux of 113 W m^{-2} (27% at wavelength of 254.7 nm). After the appropriate time, irradiated solutions were immediately poured into dark-stored porcelain cuvettes and placed in the dark chamber of the CCD camera. The time elapse between the end of irradiation and the start of the single photon counting imaging procedure was ca. 3 min. Luminol (final concentration 20 μL) was added to certain samples just after the end of irradiation. During the irradiation, the temperature of solutions was 303+/–2 K. The sampling time for each cuvette was 2.6 min, and the time series of 12 images for the each cuvette (a kinetic curve of the chemiluminescence decay I = f(t)) was obtained. The values of CL intensity I were corrected using a porcelain-incorporated ^{63}Ni radioluminescent standard and pseudo-coloured for better visibility.

RESULTS AND DISCUSSION

Elemental analysis of studied HAs is given in Table 1.

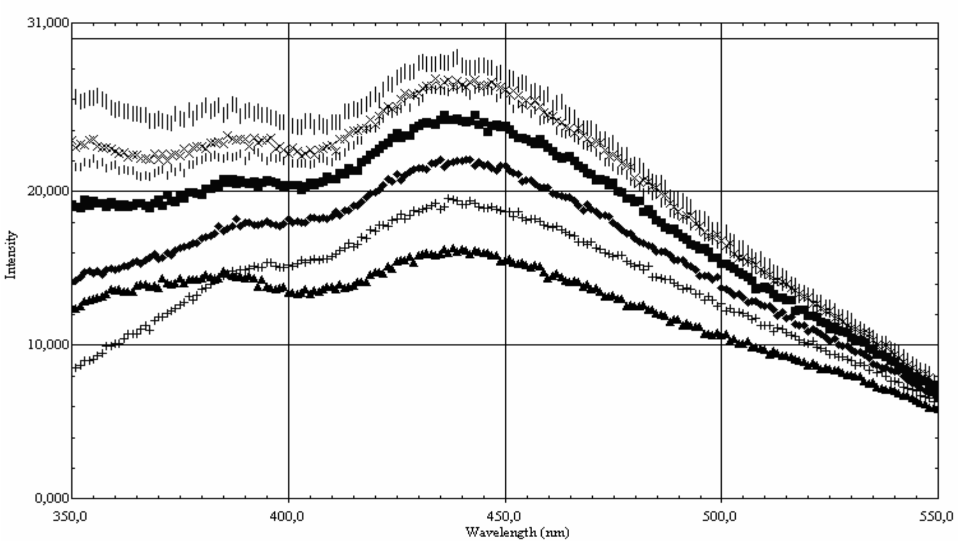
Table 1. Elemental analysis of studied humic acids

HA	Kind of HA	Weight (%)				
		C	H	O	N	S
HAF	NATUR	54,37	4,28	39,84	0.66	0.85
PYR	SYN	57.52	3.47	39.00	0.00	0.00
HQ-TYR	SYN	57.11	3.53	38.49	0.87	0.00
PPG	SYN	54.28	4.29	41.43	0.00	0.00

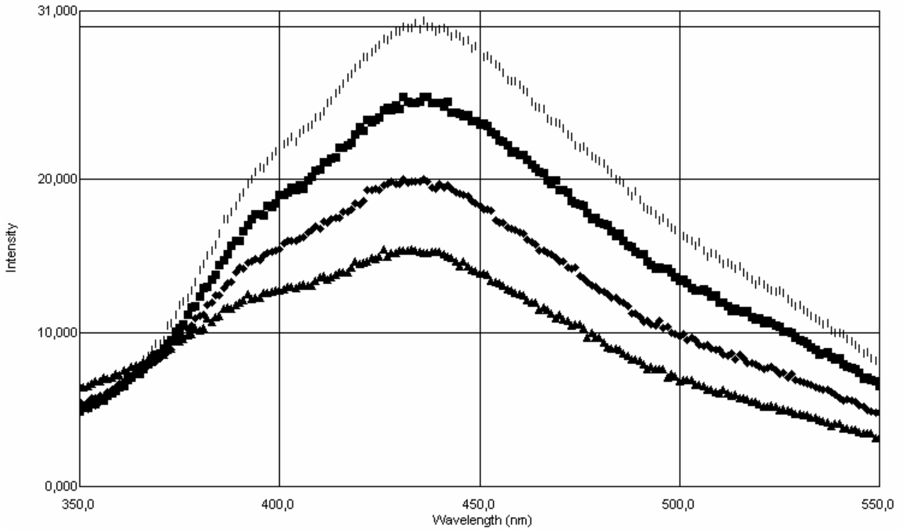
It can be seen that HAF of natural origin and HA synthesized from PPG contain the highest amount of oxygen and hydrogen, and the lowest content of carbon. This might be one of the factors influencing the resistance of HAs to UV radiation and this fact will be compared with other results.

Fluorescence emission spectra are presented in Figures 1 and 2.

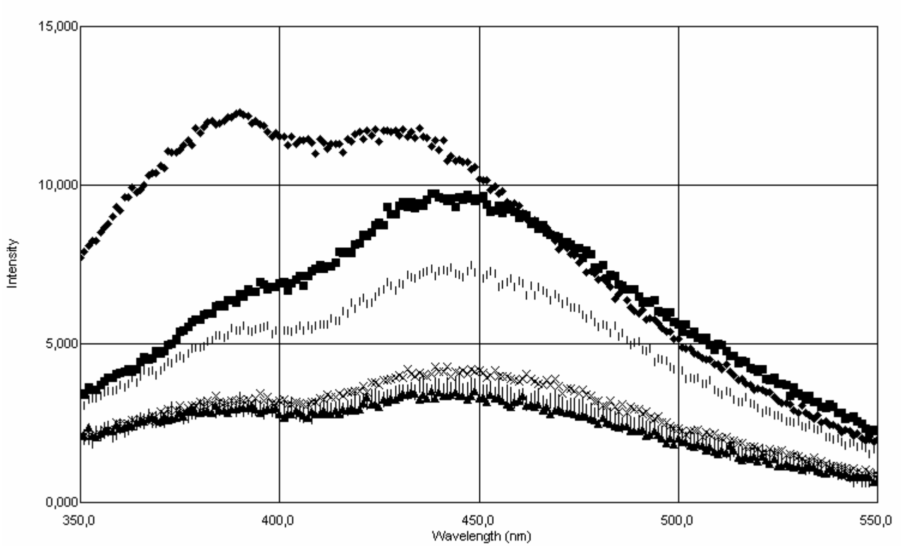
A. Fluka HA 10^{-4} %



B. HA_HQ_TYR 10^{-4}%



C. HA-PPG 8 * 10^{-5}%



D. HA-PYR irradiated for 27h at various concentrations.

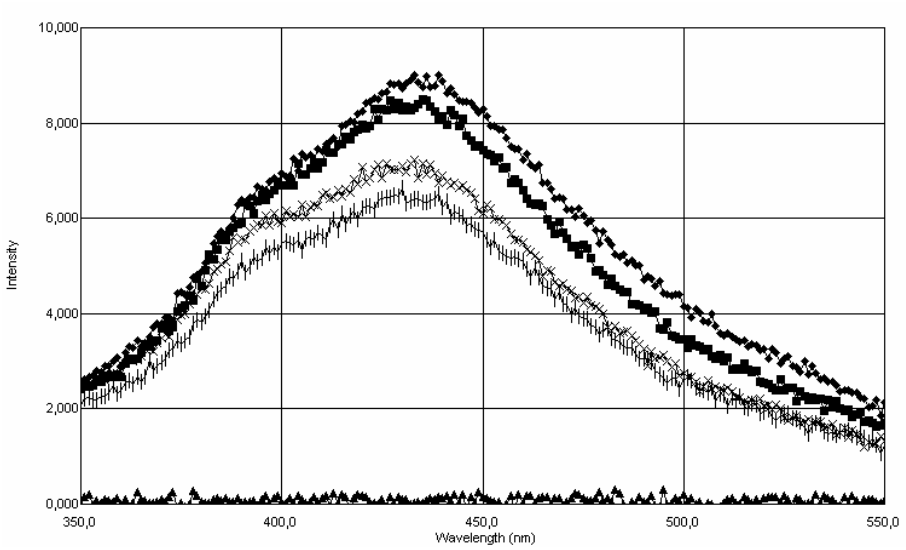


Fig 1. The effect of UV-C irradiation time on the fluorescence emission spectra: For Fig A, B, and C▲-control non-irradiated, ♦- irradiated 10 min, ■- irradiated 20 min, ˡ-irradiated 30 min, + - irradiated 40 min, ×- irradiated 50 min, |- irradiated 60 min. For Fig. D ♦- irradiated 1620 min 10^{-2}%, ■- irradiated 1620 min 10^{-3}%, ×- irradiated 1620 min 10^{-4}%, |-irradiated 1620 min 10^{-5}%, ▲-non-irradiated

From Figures 1 A-D it can be seen that the emission fluorescence spectrum of HA-PPG undergoes the largest changes in its shape, especially in the 350-400 nm region. Smaller, but still clear changes are observed for HAF. The most resistant to UVC appears to be HA PYR, both in respect of the shape and intensity changes. In this case the decrease in the fluorescence intensity increases with dilution of the HA PYR solution. These observations allow to order the resistance of the studied HAs as follows:

HA PYR >HA HQ-TYR > HAF >HA PPG.

The same conclusion follows from the rate of fluorescence changes (Fig. 2), since HA PPG and HAF reach the maxima of fluorescence emission in the shortest time of irradiation. It is also evident that UV-C radiation causes stronger changes

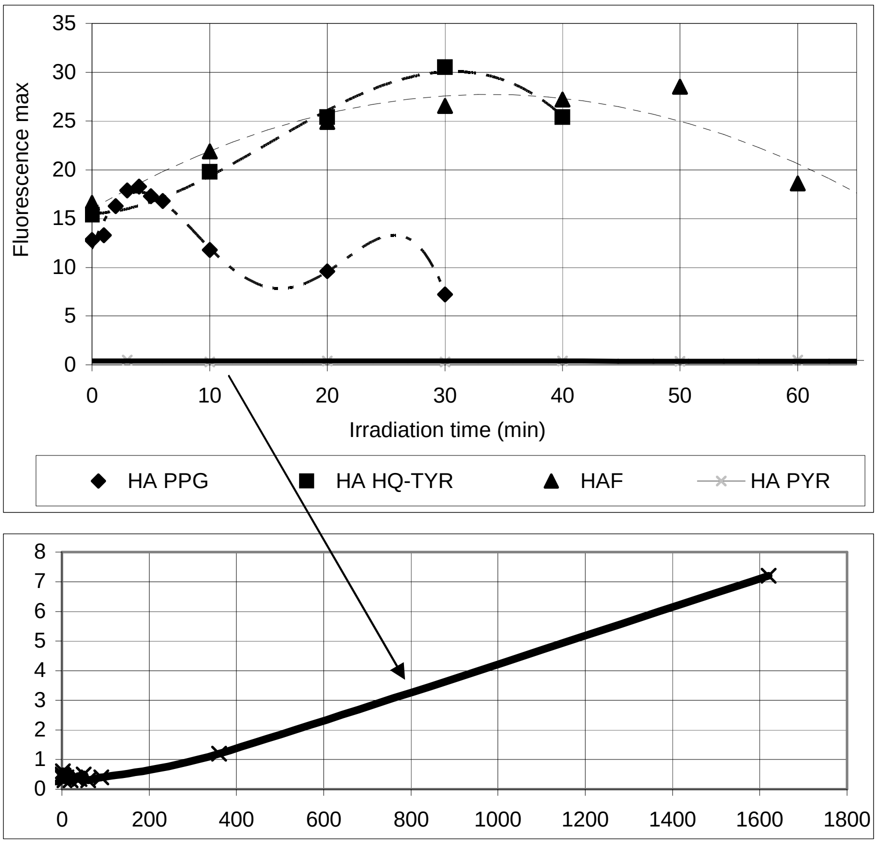


Fig. 2. Kinetics of the maximum intensity of fluorescence emission of studied HA irradiated with UV-C in 0.1 M Na_2CO_3. These kinetic curves $I_{max} = f(t_{ir})$ are obtained from the maximum fluorescence spectra presented in Figure 1 (A-D)

in the short-wave 350-450 nm range than in the longer spectral region, and these changes follow the consecutive pattern of kinetics. An unequivocal interpretation of this phenomenon is not possible at this time and requires further well designed experiments.

Chemiluminescence (CL) images (Fig. 3) show a low level of emission from HAs irradiacted with UV-C for different time periods without and with luminol. For control, images of the Na_2CO_3 solution are also presented. A majority of samples

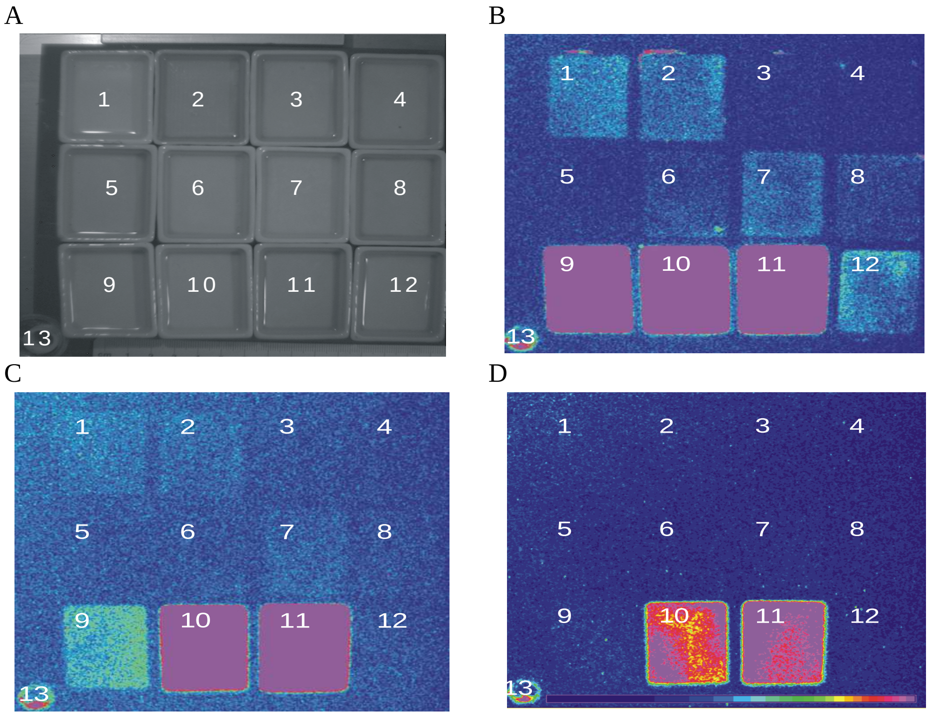


Fig. 3. Single photon counting imaging of humic acids chemiluminescence presented as recorded with CCD camera „Night Owl" Molecular Light Imager.

1. Na_2CO_3; 2. HAF; 3. HA HQ-TYR; 4. Na_2CO_3 irradiated 20 min; 5. Na_2CO_3 irradiated 60 min; 6. HAF irradiated 60 min; 7.HA HQ-TYR irradiated 60 min, 8. HAF irradiated 20 min; 9. Na_2CO_3 irradiated 60 min + luminol; 10. HAF irradiated 60 min + luminol; 11. HA HQ-TYR irradiated 60 min + luminol; 12. HA HQ-TYR irradiated 20 min, 13 radioluminescent standard ^{63}Ni.

The pseudo scale is shown in right lower corner, the dark violet and magenta corresponding to the lowest and highest intensity of UVC-induced chemiluminescence, respectively. The acquisition time of every image was 30 min and the total time of measurement was 332 min. Chemiluminescent images are shown only after 0 min, 151 min, 332 min from those samples which gave CL intensity stronger than the background exhibit very weak CL intensity, close to the level of background, with the signal-to-noise (S/N) ratio of about 2.8. The emission level from 0.1 M Na_2CO_3 alone was higher (ca. 4-5 ± 5 counts per s). This is obvious as HAs are strong absorbers of light (self-absorption effect) and, more important, scavengers of reactive oxygen species (ROS). ROS are spontaneously formed in alkali solutions saturated with dissolved O_2. Irradiated HAs show a slightly higher emission than nonirradiated. As can be seen from Figure 3, the images are very weak for samples without luminol.

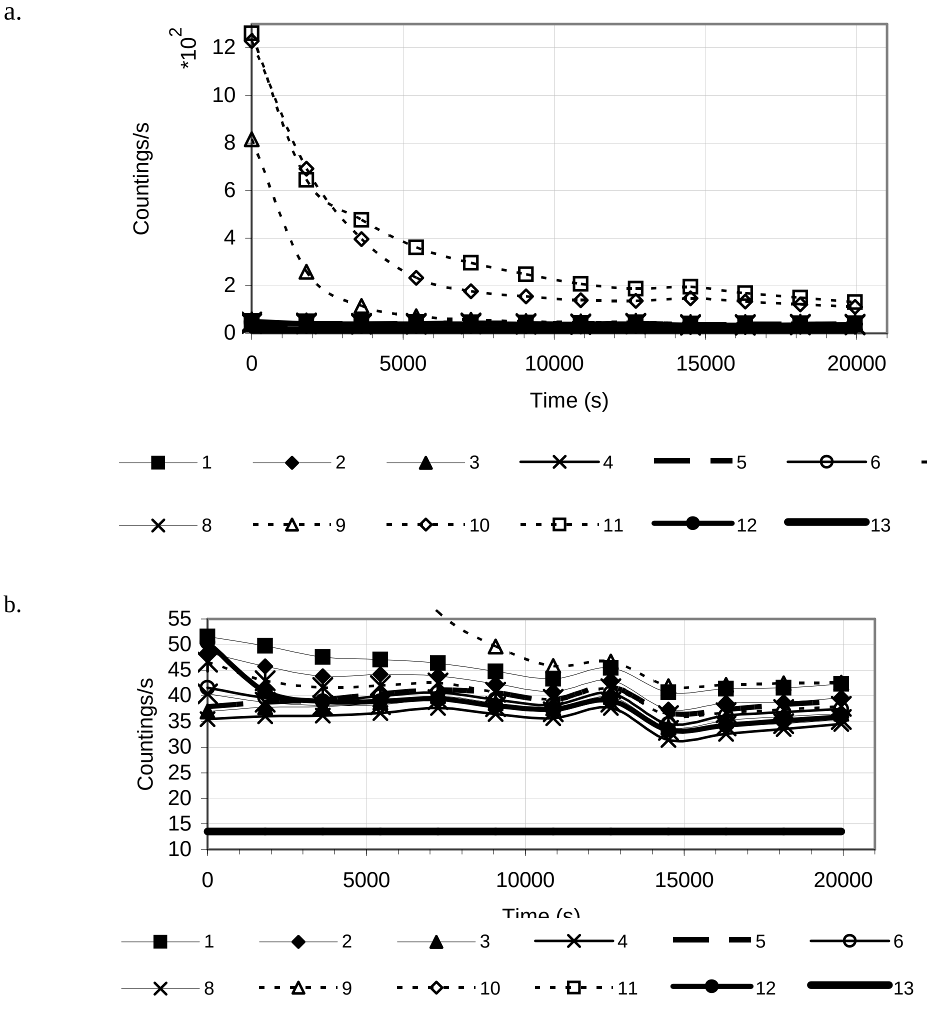


Fig. 4. Kinetic curves of chemiluminescence intensity I = f(t) of HAs after UV-C irradiation. Explanations as in Fig3; (b) – magnified portion of (a)

Absolute values of chemiluminescence intensity are ranging from 35-50 counts/s for irradiated HAs (without luminol) to 816-1228 counts/s in the case of luminol addition after irradiation. It means that UV-C induces long lasting very slow reactions producing ROS and gradually degrading HA.

Figure 4 shows the kinetics of the UVC-induced delayed CL and reveals the free radical mechanism of HAs degradation. One can conclude that the use of chemiluminescent probes (enhancers) such as luminol, its derivatives and lucigenin, permits successful monitoring of extremely slow oxidative degradation reactions of HS initiated by UV radiation.

Colour coefficients calculated from the absorption spectra of control (non irradiated) and irradiated 30 min HAs are shown in Table 2.

Table 2. Colour coefficients of HA control (non-irradiated) and irradiated for 30 min

King of HA	Non-irradiated		irrad.30 min	
HA	Q2,7/4	Q4/6	Q2,7/4	Q4/6
HA FLUKA	2,632	2,842	3,844	2,4375
HA HQ-TYR	3,102	6,532	6,458	5,41
HA PPG	2,216	5,84	3,45	2,51
HA PYR	2,494	3,374	2,40*	3,38*
HA PYR**	2,494	3,374	3,60**	4,41**

Absorption spectra of the studied HA are typical of HA (Sławinska *et al.* 2002, Pluciński 2002), except for HA PPG which shows local maxima, probably due to a low condensation /polymerization degree of the benzotropolone rings. UV irradiation decreases the values of absorbance, particularly in the long wavelength region of 450-700 nm. The ratio $Q_{\lambda i} / Q_{\lambda j}$ is widely used as a “colour coefficient” for characterization of absorption properties of HS (Auger 1996, Auger *et al.* 1999).

Comparison of the colour coefficient ratios $Q_{2,7/4}$ and $Q_{4/6}$ before and after 30 min irradiation shows that the HA synthetized from PPG undergoes fast changes while the HA PYR is the most resistant. Prolonged irradiation always leads to the bleaching of the solution; values of the color coefficients $Q_{4/6}$ decrease after irradiation, while $Q_{2\ 7/4}$ increase (Tab. 2).

This can only be due to the photodecomposition of long-wave absorbing chromophores such as semiquinones and quinones, and to the formation of short-wavelength absorbing compounds, mostly polyphenols (λ_{max} 250-270 nm). Irradiated solutions have slightly lower pH than those before irradiation, e.g. from $pH_0 = 11.47$ to pH = 10.99 for HQ-Tyr and pH_0 = 11.32 to pH =11.28 for KHF after 30 min of irradiation. One has to take into account that 0.1M Na_2CO_3 that decreases ΔpH. This indicates the formation of simpler compounds with -COOH groups. Thus, highly polymerized model HA are transformed to more hydrophilic fulvic acids.

This observation correlates with the fast decrease of fluorescence of PPG–HA (Fig. 2) and with previous literature data (Sławińska *et al.* 2002). Moreover, the lowest resistance of PPG–HA may be due to the highest content of oxygen (Tab. 1) and abundance of = C = O and –O–H groups in tropolone moieties. On the other hand, PYR–HA containing orthoquinone groups is the most resistant to UV-C radiation. This discrepancy suggests that other factors related to the molecular structure and chemical composition may be involved in the resistance of HAs to the UV radiation.

CONCLUSIONS

1. The data presented above lead to a general conclusion that UV-C irradiation of HAs generates electronic excited states and free radicals as indicated by fluorescence and chemiluminescence.
2. In aerated solutions the degradative oxidation reactions transform HAs into more hydrophilic and acidic products like fulvic acids and low-molecular aromatic and aliphatic acids. Such character of changes has negative implications on the environmental and agricultural functions of HS.
3. Elucidation of the photoresistance of HAs is a serious problem related to the following aspects: 1) precursors (substrates) of HAs, 2) physicochemical conditions of their synthesis (humification), 3) molecular and supramolecular properties of synthetized HA, and 4) environmental physicochemical conditions of the interaction between HA and UV radiation (aerobic/anaerobic medium, pH, concentration of HAs and temperature). Therefore, more advanced studies on the above dependencies are necessary.

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WYTRZYMAŁOŚĆ MODELOWYCH KWASÓW HUMUSOWYCH NA PROMIENIOWANIE UV-C

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Abstract. Promieniowanie UV coraz to bardziej przenikające przez Biosferę stawia pytanie o fotowytrzymałość substancji humusowych. Czy istnieje zależność między fotowytrzymałością a składem chemicznym prekursorów kwasów humusowych. Zsyntetyzowano kwasy humusowe z pyrokatecholu, hydrochinonu + tyrozyna, purpurogaliny oraz użyto dla porównania naturalnych kwasów Fluka. Były one napromieniowywane UV-C. Mierzono widma emisji fluorescencji, absorpcji oraz chemiluminescencję roztworów alkali kwasów humusowych przed i po różnych czasach napromieniowania. Najbardziej odporny na promieniowanie UV-C był syntetyczny kwas humusowy otrzymany z pyrokatecholu, natomiast najbardziej labilny był kwas humusowy otrzymany z purpurogaliny. Możliwe powody wysokiej/ niskiej fotowytrzymałości kwasów humusowych na UV-C są krótko omówione.

Słowa kluczowe: kwasy humusowe, fotowytrzymałość, promieniowanie UV-C, widma luminescencji, obrazowanie chemiluminescencji