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Effect of oxygen on UV-induced photodegradation of humic acid obtained from composted hulls of *Juglans regia* walnut

D. Sławińska, K. Polewski, P. Rolewski, and J. Sławiński*

Department of Physics, August Cieszkowski Agricultural Academy, Wojska Polskiego 38/42, 60-637 Poznań, Poland

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Abstract. In this paper the physicochemical and spectroscopic properties of humic acid (HA) obtained from composted walnut shell of *Juglans regia* are described. The influence of oxygen on UV-induced photodegradation of HA was investigated using absorption, fluorescence and electron paramagnetic resonance (EPR) spectroscopy. Fluorescence spectroscopy revealed two chromophoric centers. The center featuring long wavelength emission at 510 nm and excitation peaks at 440 and 460 nm appears more resistant to UV and oxygen than the center featuring an emission maximum at 450 nm and excitation peaks at 260 and 350 nm. The latter center can be related to condensed aromatic rings, unsaturated bond systems and electron-withdrawing groups such as carbonyl and carboxyl groups susceptible to oxidation processes. The gradual increase in values of the colour coefficients $Q_{4/6}$, $Q_{2,6/4}$ and $Q_{4/5.2}$ and the concomitant decrease in absorbance for all wavelengths suggested the occurrence of a gradual photodegradation process involving the formation of phenolic and simpler carboxylic acids during irradiation. These modifications would lead to a decrease in molecular weight and lower condensation of HA macromolecules. The high semiquinone radical content measured by EPR spectroscopy is indicative of high concentration of quinoid structures in HA from walnut shells. The sensitivity of non-irradiated samples to the oxygen level also suggested the presence of alcoholic-hydroxyl, phenolic-hydroxyl and carboxylic functional groups susceptible to oxidation in HA.

Keywords: humic acid, walnut hulls, photodegradation

INTRODUCTION

Humic substances (HS) are ubiquitous and play a key role in the soil structure, fertility and nutrient availability, are able to reduce the toxic effects of xenobiotics and plant diseases, and have beneficial effects on plant growth. The chemical composition and humification conditions of plant residues significantly determine the biological and physicochemical properties of HS. The litter and soil under the canopy of European walnut trees *Juglans regia* L. is detrimental for many cultivated plants including apple, asparagus, blackberry, blueberry, cabbage, egg plant, pear, pepper, potato and rhubarb, field crops such as alfalfa, clover, crimson and tobacco, and landscape plants, and flowers (Mandava,1985; Thompson, 1987; Babich and Stern, 1993). This negative effect may be attributed to the fact that many species of walnut trees, especially black walnut (*Juglans nigra*) and *Juglans regia* contain toxic naphthoquinones, among which the most important is 5-hydroksy-1,4-naphthoquinone ie juglone (Fig. 1). Naphthoquinones exhibit a broad spectrum of pharmacodynamic activity, in particular cytotoxicity and genotoxicity. Unripe hulls of walnut fruits have been used for generations in folk medicine and cosmetics, *eg* in the treatment of skin diseases. Fallen residues of walnuts, such as shells, are occasionally used for preparing compost to be used as soil amendment in agriculture. The use of such compost, however, creates problems resulting from the toxic properties of 1.4-naphthoquinones and their derivatives. This problem has not yet been the subject of specific research, which prompted us to undertake experiments to gain more information on the topic.

Humic substances absorb sunlight and may therefore photoinduce the transformation of non-absorbing organic chemicals (Jensen *et al*., 1987; Babich and Stern, 1993; Canonica and Hoigne, 1995). Numerous studies have shown that HS contain polyphenolic compounds with multiple carboxyl, carbohydrate and peptide moieties that can act as

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*Corresponding author's e-mail: polewski@owl.au.poznan.pl

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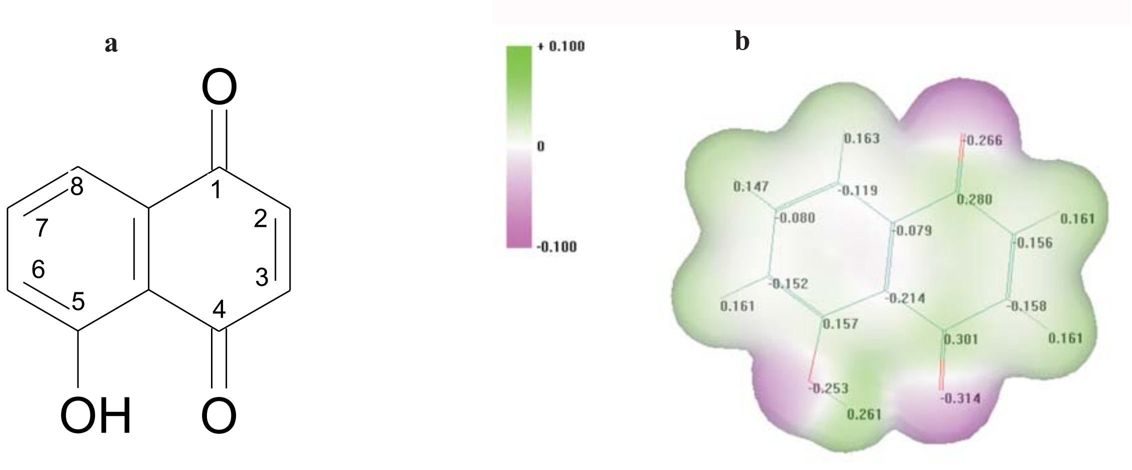

Fig. 1. Chemical structure of juglone (a) and electrostatic potential distribution calculated with semiempirical AM1 method (b).

sensitizers or precursors for the production of reactive intermediates (Hoigne *et al*., 1989; Wang *et al*., 1995). Some intermediates have been identified as reactive oxygen species such as singlet oxygen, superoxide anion, hydrogen peroxide (Sławinski *et al*., 1978; Cooper *et al*., 1989; Hoigne *et al*., 1989; Aguer *et al*., 2002) or reactive triplet states (Canonica and Hoigne, 1995). For example, peroxyl-type radicals were thought to be responsible for the degradation of phenol derivatives. The photochemical reactivity of HS is complex, and its measurement markedly depends on the experimental conditions under which studies are performed. Important factors are the excitation wavelength and the presence of oxygen.

The objective of this study was thus to examine the physicochemical, especially photochemical properties of humic acid (HA), the most relevant fraction of HS originating from natural sources containing juglone and other naphthoquinones.

MATERIALS

The HA samples were obtained from external (green) shells of mature walnut (*Juglans regia*) composted for a total period of three years and collected once a year. The compost contains a large amount of organic materials, especially polyphenols and quinones. The compost samples (5 kg of a fresh matter) were extracted with 0.1 M Na_2CO_3 in N_2 atmosphere according to IHSS protocol (McCarthy *et al.*, 1985). The extract was centrifuged and the supernatant acidified with HCl to pH≈2 to precipitate the HA fraction (340 g). The precipitate was isolated, washed with diluted HCl and water, and dried at ≅60°C (7.877 g; yield 0.157%). In spectroscopic studies the following buffers were used: 10 mM solution of Na_2CO_3 at pH 10.8 and 0.2 M Na_2HPO_4. Other reagents: Na_2CO_3, Na_2HPO_4 and HCl were of analytical purity from POCh Gliwice (Poland). Water was double distilled from a quartz apparatus.

METHODS

The HA sample was analyzed for elemental composition and by UV-absorption, fluorescence, electron paramagnetic resonance (EPR) and Fourier Transformed Infrared (FTIR) spectroscopy.

Carbon 44.82, hydrogen 4.84, nitrogen 2.48, oxygen 44.06, sulfur 0.23 and ash 3.67% contents of humic acid were measured by combustion in oxygen gas using Vario ELIII Elementar. The content of ash was determined gravimetricly by combustion.

FTIR spectra were obtained in KBr pellets (200 mg KBr + 1.8÷5 mg HA) on a Bruker IFS 113V spectrometer.

The EPR measurements were carried out using a Bruker ESP 300 E spectrometer operating at X-band and equipped with 100 kHz field modulation. The EPR was operated at a peak-to-peak modulation amplitude of 2.0 G, a microwave attenuation of 25 dB, a conversion time of 10.24 ms, and a time constant of 1.28 ms, and with a microwave power of 2 mW. Records of each spectrum were obtained by digital signal averaging and were electronically integrated by standard Bruker data acquisition software. The EPR measurements of irradiated and non-irradiated HA samples were conducted in 0.01 M Na_2CO_3 solutions of 2 mg ml^{-1} HA at pH=10.8 at room temperature using a quartz flat cell and in situ irradiation. Aerated and deaerated HA samples obtained by purging for 40 min with argon were irradiated at room temperature with polychromatic light at 200-600 nm using a high-pressure xenon lamp (Photomax 150 W, Oriel) equipped with quartz lenses with a cutoff filter at 280 nm. The calculation of spin content in HA sample was performed using the spin standard, 4-protio-3-carbonyl-2,2,5,5-tetra-perdeuteromethyl-3-pyrroline-1-yloxy (mHCTPO).

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 spectra measured, numerical values of absorbances A $_{,tir}$, colour coefficients Q $_{i/\ j}$ (Senesi *et al.*, 1989; Tan, 2003) and the relative rate of phototransformation $((A_0 - A_{tir}) / t_{ir}$ were calculated. Spectroscopic measurements were made in triplicate for the 240-300 nm range using 0.1, 0.2 and 10.0 mm cuvettes. Values of the colour coefficients $Q_{2.6/4}$ and $Q_{4/6}$, where indexes 2.6, 4 and 6 refer to absorbance measured at 260, 400 and 600 nm, respectively. Additionally, we have introduced a novel colour coefficient $Q_{4/5.2} = A_{400} / A_{520}$ which is specific for HA derived from *Juglans regia* shell nuts as well as for synthetic HA obtained by polymerization of juglone. It reflects the presence of the ionized form of juglone with a characteristic band at 520 nm (to be published). All the colour coefficients were normalized for C content and are presented in Table 1.

A separate irradiation-flow system was designed for measurements of the effect of UV radiation upon absorption and fluorescence spectra. Aliquots of 10^{-4} g L^{-1} of HA in 10 mM Na_2CO_3 were irradiated by electrodeless radiofrequency powered Hg burner emitting 261 nW m^{-2} flux of 254 nm wavelength or 10^{15} photons s^{-1} cm^3 (Sławińska *et al.*, 2002).

Fluorescence excitation and emission spectra were recorded using a Shimadzu RF 5001PC spectrofluorimeter. Fluorescence of the 'dark' and irradiated solvent 10 mM Na_2CO_3 was checked at several excitation wavelengths to evaluate the possibility of photochemical transformations. Solutions of Na_2CO_3 were irradiated with 254 nm; the energy of quanta amounting to 470 kJ mol^{-1} are sufficient to initiate ionization of HCO_3^{1-} / CO_3^{2-} ions and dissolved O_2, and to start photoreactions possibly leading to some artifacts fluorescing in the 260-350 nm range.

Quantum chemistry calculations on the geometry optimisation and partial charge distribution on juglone molecule were performed with the HyperChem suite from Hypercube (Canada). Gas-phase geometries and electronic wave functions of juglone and its free radical forms were fully optimized with the half-electron restricted Hartree-Fock (RHF) approximation (Dewar *et al.*, 1968) using the semiempirical Austin Model 1 (AM1) Hamiltonian (Dewar *et al.*, 1985).

Table 1. Colour coefficients Q $_{2.6/4}$, Q $_{4/5.2}$ and Q $_{4/6}$ changes during irradiation in nitrogen and oxygen atmosphere and initial rates r (min^{-1}) of absorbance decrease at different wavelengths

Irradiation time (min)	Nitrogen			Oxygen			Wavelength (nm)	N_2	O_2
	Q 2.6/4	Q 4/5.2	Q 4/6	Q 2.6/4	Q 4/5.2	Q 4/6		(r 10^{-3}, min^{-1})	
0	2.76	1.54	2.29	2.76	1.54	2.29	260	4.7	20
25	3.03	1.63	2.58	3.43	1.78	2.92	400	4.2	10
40	3.29	1.74	2.84	3.74	1.72	2.58	520	4.0	7
60	3.27	1.80	2.70	5.07	2.00	2.80	600	2.7	4.7
120	3.75	2.00	2.85	4.66	1.50	3.00			

RESULTS AND DISCUSSION

FTIR spectra

Infrared spectroscopy has been widely used for the characterisation of humic substances and can provide information on the structural and functional properties of HA molecules. The FTIR spectra of HA sample are presented in Fig. 2. A broad band around 3400 cm^{-1} is due to O-H stretching of various groups like phenolic and alcoholic. The peak observed at 2940 cm^{-1} is assigned to stretching vibrations of aliphatic CH, CH_2 and CH_3 side chain groups of aromatic rings. 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 CH_2 and CH_3 groups: n (C=C) and asymmetric n (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 CH_3O groups and carbohydrate-like structures. In HA of 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. The relative intensity in absorption maxima of the carbonyl and aliphatic functional groups is comparable to those of aromatic ones. The spectrum presented suggests conjugation of aliphatic compounds with aromatic and quinoid ones, abundant in walnut hulls during humification.

Thus, the FTIR spectrum of HA isolated from walnut compost shows similarity to those of HAs from different types of soil (Stevenson and Goh, 1971; Senesi *et al.*, 1989).

EPR spectral analysis

HA are known to contain free radicals in their structure and EPR spectroscopy is used for its measurement (Steelink and Tollin, 1966; Sławińska *et al.*, 1975; Schnitzer and LaVesque, 1979; Senesi, 1990). Walnut HA exhibits a distinct singlet EPR signal with a g-value = 2.0018, linewidth ΔB_{pp} =0.58 mT and a high spin content equal to

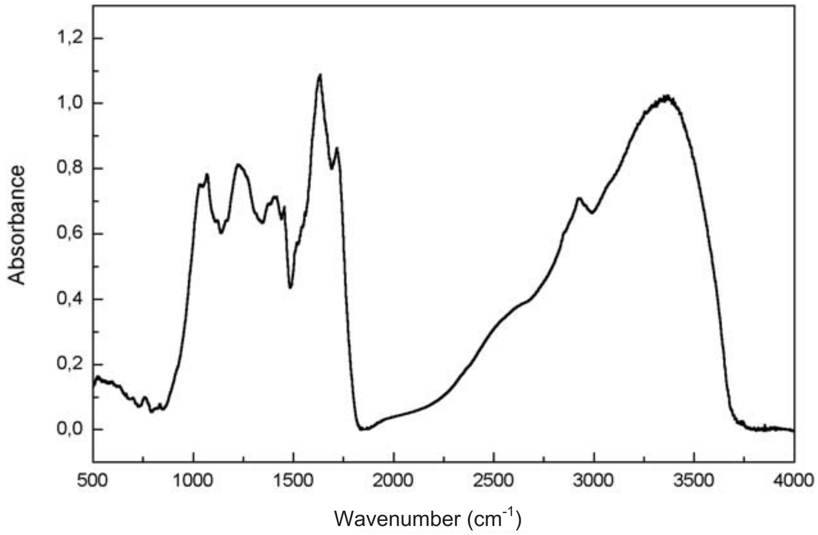

Fig. 2. FTIR spectrum of HA from walnut shells of *Juglans regia*.

6 10^{20} g^{-1}. Figure 3 shows the EPR spectra of dark and irradiated samples in aerated and deaerated atmosphere. The spectra are characterized by an intense, broad single symmetrical line without any hyperfine splitting which generally is an indication of the presence of organic free radicals in HS . Non-irradiated samples in air show a higher level of free radicals as compared to the deaerated sample. The amplitude of photoinduced EPR signal in aqueous solutions depends on the light intensity and its wavelength, as well as on the concentration of oxygen. Usually, high semiquinone content is correlated with high spin content (Sławińska *et al.*, 1975; Schnitzer and LeVesque, 1979). Thus, EPR results are indicative that HA from walnut shells contains a high concentration of quinoid structures. The sensitivity of non-irradiated samples to the oxygen level indicates also the presence of reactive functional groups susceptible to the oxidation process, such as phenolic hydroxyl, alcoholic hydroxyl, aldehyde, diketo and quinone, and unsaturated aliphatic groups. The results of semiempirical quantum calculations (Fig. 1b) reveal regions in the juglone molecule with the highest electron density: 5-OH and 1.4-quinone. If these moieties occur in the HA studied, they would be most probably involved in the redox reactions producing unpaired spins as well as degradative photooxidation.

Absorption spectra analysis

HS exhibit characteristic absorbance spectra, showing strong absorbance in UV range and an almost monotonic decrease toward longer wavelengths (Schnitzer and Khan, 1972; Chen *et al.*, 2002; Tan, 2003). Absorption spectra of

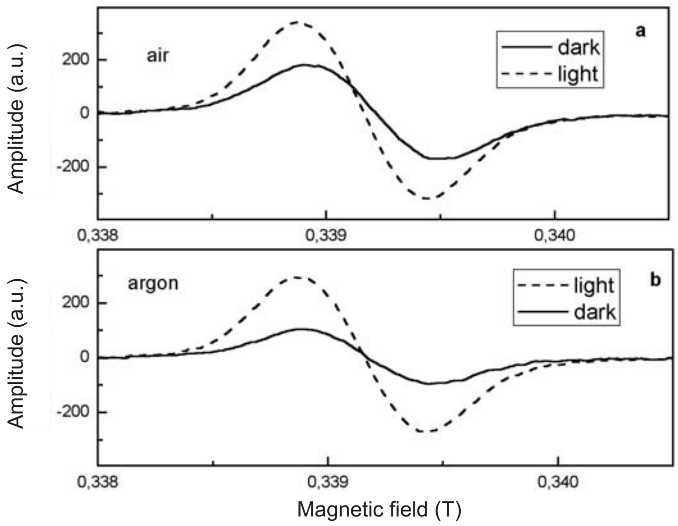

Fig. 3. EPR spectra of 2 mg ml^{-1} of non-irradiated and irradiated HA in Na_2CO_3 buffer in aerated (a) and deaerated (b) conditions.

the tested HA obtained from *Juglans regia* nut hulls have these characteristics (Fig. 4). Such a shape is related with the presence of a variety of aromatic chromophores and other organic compounds. Although the spectra are broad and featureless, there are some regions of wavelength and absorption coefficients log ε that may be used as indicators of the presence of specific structures. The absorptivity around 260 nm and log $\varepsilon = 3.5\text{-}5$ is related to aromatic C=C content because - * transitions in substituted benzenes, benzo- and naphthoquinones, polyphenols and purine bases occur in this spectral region (Ghosh and Schnitzer, 1979). The spectral range of 420-580 nm and log ε = 3-4 is attributed to quinones, hydroxyquinones and semiquinone structures (Thomson, 1987). The 600-700 nm log $\varepsilon \leq 3.0$ is ascribed to polyenes, charge-transfer donor-acceptor complexes and stacking planar aromatic moieties structures (Baes and Bloom, 1990). The changes in the absorption spectrum of HA during irradiation in aerated and deaerated samples are given in Fig. 4. The sample under oxygen shows a more rapid decrease in absorbance than that under nitrogen. The increase in the values of the colour coefficients $Q_{4/6}$, $Q_{2,6/4}$ and $Q_{4/5.2}$ (Table 1) and the permanent decrease in absorbance for all the wavelengths (Fig. 4a and b) indicate a gradual photodegradation process. These observations suggest the formation of phenolic and simple carboxylic acid during irradiation, which leads to a decrease in the molecular weight and a lower condensation degree of HA molecule. Additionally, from the rate of absorbance decrease r (Table 1), it is seen that long wavelength absorbing chromophores (520 and 600 nm) are more resistant to irradiation than those absorbing at shorter wavelengths (260 and 400 nm).

From absorption spectra measured after a given irradiation time, the so called colour coefficients have been calculated. It is well known, according to Senesi *et al.* (1989) and Chen (2002), that these magnitudes are partially correlated with the molecular properties of HS.

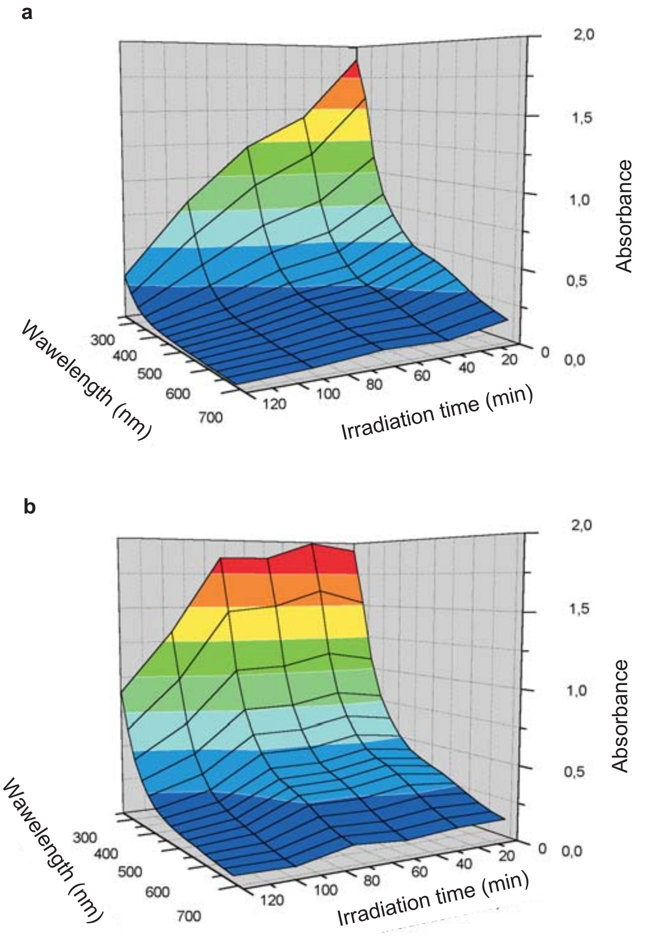

Fig. 4. 3-D presentation of absorbance changes of HA during irradiation in a) O_2 and b) N_2.

Fluorescence spectroscopy analysis

Emission spectra

Fluorescence spectroscopy was used to distinguish between the different components of HA (Miano *et al.*,1988; Miano and Senesi, 1992). As shown in Fig. 5, the emission spectra exhibit two broad bands. When excited between 260 and 360 nm the maximum is at 460 nm, and when excited between 440 and 480 nm the maximum is located at 510 nm. The long wavelength and low intensity fluorescence indicates the presence of condensed aromatic rings and other unsaturated bond systems, a high degree of conjugation and groups such as carbonyl and carboxyl (Chen *et al.*, 2002). The shorter wavelength and higher intensity measurements are associated with a low aromatic content, low molecular weight components and electron donating groups such as

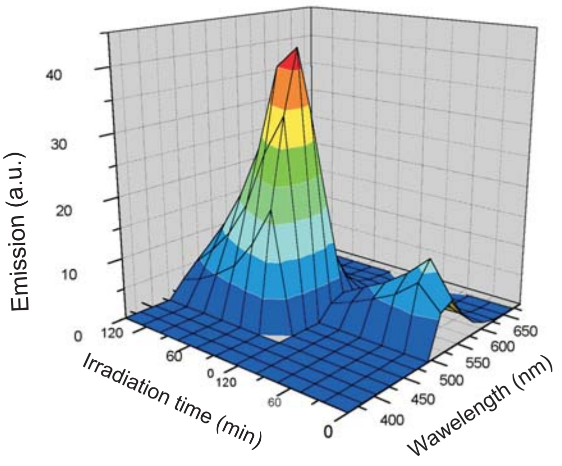

Fig. 5. 3-D emission fluorescence spectrum of HA observed in O_2 at an excitation of 350 nm with the emission maximum at 460 nm (a), and excitation at 440 nm with emission maximum at 510 nm (b).

hydroxyls or methoxyls (Senesi, 1992; Hongve and Dahlback, 1997). The initial increase in the fluorescence intensity followed by its decrease after several hours of irradiation for all λ_{em} and λ_{ex} (Fig. 5) is an additional evidence of deep changes in the chromophore/fluorophore moieties occurring in nitrogen and, more rapidly, in oxygen atmosphere.

Fluorescence kinetics

During irradiation for a given excitation wavelength, neither the position nor the shape of the spectrum undergo distinct changes (Fig. 5). Then, the fluorescence results may be presented as amplitudes of the emission maxima as a function of excitation wavelength and irradiation time (Fig. 6a and b). The common similarity of the kinetics of fluorescence maxima during irradiation in both samples is an initial increase followed by a gradual decrease in the amplitude. The most evident difference is an increase of fluorescence intensity during the first 60 min of irradiation observed in N_2 samples and 40 min in O_2 sample. In N_2 atmosphere, the fluorescence increase shows a gradual growth, whereas in O_2 sample there is a linear-like increase followed by saturation (excitation at 440 nm) or a slow decrease (excitation at 460 nm). Another important feature observed in both samples is the decrease of the long wavelength emission with similar slow rate of disappearance. The main difference is the initial increase of short wavelength emission in N_2 sample compared to the fast decrease of fluorescence in O_2 sample observed with prolonged irradiation. A careful examination of Fig. 6a and b reveals that chromophores excited at 440 and 460 nm exhibit a much slower rate than those excited at 260 and 340 nm. This is a clear indication that long wavelength fluorophores, like quinones, semiquinones, charge transfer complexes or stacking planar structures, are less resistant to UV-C than short wavelength fluorophores such as phenolics or lignin-like moieties.

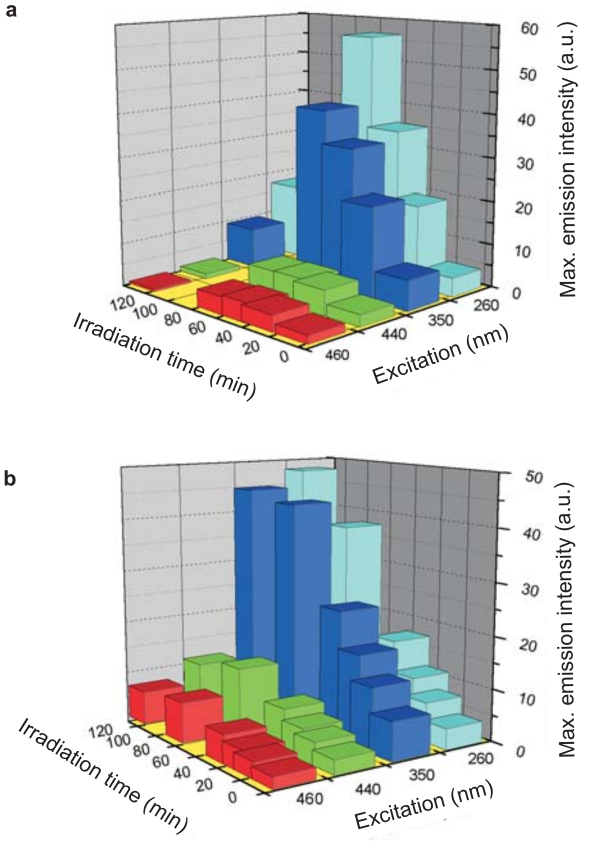

Fig. 6. Kinetics of fluorescence maxima changes of HA excited at different wavelengths during irradiation in the presence of a) oxygen and b) nitrogen.

Less resistant structures are transformed earlier, and after saturation the emission will only decrease during further irradiation. It seems that this process is not influenced by the presence of oxygen, which suggests that the observed emission arises from charge-transfer and/or planar stacking complexes.

The short wavelength emission increase may be ascribed to the formation of simpler structures with higher quantum fluorescence yield. The aromatic core of HA isolated from walnut-compost may be rich in derivatives of naphthoquinones – substances sensitive to degradation reactions initiated by reactive oxygen species, especially by OH radical. Juglone derivatives are known to undergo regioselective oxidation and photooxidation (Couladorous and Strongilos, 2000). Hydroxylation of aromatic subunits leads to the formation of diphenolic and o-ortho or p-quinoid arrangements that easily undergo ring-opening reactions to finally generate low molecular weight aliphatic acids and ketoacids (Jensen-Korte *et al*., 1987; Polewski *et al*., 2002; Sakkas *et al*., 2002). This eventually leads to the disappearance of fluorescence and such a situation is observed for the emission recorded from the oxygenated sample.

CONSLUSIONS

1. A HA fraction was isolated from a compost of *Juglans regia* composted green hulls and characterized for the first time. It appeared to contain a high number of free radicals, probably of semiquinone origin, sensitive to light and O_2.

2. EPR, absorption and fluorescence spectroscopy appear to be a useful method for the characterization of the influence of O_2 on the stability of HA from walnut shells under photoinduced UV degradation.

3. The relative rates of disappearance and colour coefficients calculated from absorption kinetics during irradiation indicate that O_2 accelerates processes leading to the formation of low-molecular weight non-fluorescing products.

4. Fluorescence spectroscopy revealed two chromophoric centers. The one with long wavelength emission at 510 nm excited at 440 and 460 nm appears to be more

resistant to UV and oxygen than the other one, occurring at 450 nm at 260 nm and 350 nm excitation. The latter emission can be ascribed to condensed aromatic rings, unsaturated bond systems and electron-withdrawing groups such as carbonyl and carboxyl groups susceptible to oxidation processes.

5. The data obtained show that in the presence of O_2, the UV-induced photodegradation of walnut HA produces efficiently low-molecular water-soluble products that are less-absorbing in the visible spectral region than the original material.

6. These results may have agricultural implications if a walnut compost-derived HA contains toxic allelopathic phenolics like juglone and its analogues. Then, its UV-stimulated photodegradation to non-toxic products would be a desirable process.

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REFERENCES

Aguer J.P., Richard C., Trubetskaya O., Trubetskoj O., Leveque J., and Andreux F., 2002. Photoinductive efficiency of soil extracted humic and fulvic acids. Chemosphere, 49(3): 259-62.

Amalfitano C., Quezada R.A., Wilson M.A., and Hanna J.V., 1995. Chemical composition of humic acids: a comparison with precursor 'light fraction' litter from different vegetations using spectroscopic techniques. Soil. Sci., 159, 391-401.

Babich H. and Stern A., 1993. In vitro cytotoxicities of 1,4-naphtoquinones and hydroxylated 1,4-naohtoquinones to replicating cells. J.Appl.Toxicol., 13(5): 353-358.

Baes A.U. and Bloom P.R., 1990. Fulvic acid - spectra: influence of solvent and pH. Soil Sci. Soc. Am. J., 54, 1248-1254

Canonica S. and Hoigne J., 1995. Enhanced oxidation of metboxy phenols at micromolar concentration photosensitized by dissolved organic material. Chemosphere, 30: 2365-2374.

Chen J., Gu B., LeBoeuf E.J., Pan H., and Dai S., 2002. Spectroscopic characterization of the structural and functional properties of natural organic matter fractions. Chemosphere, 48, 59-68.

Cooper W. I., Zika R. G., Petasne R. G., and Fischer A.M., 1989. Sunlight-induced photochemistry of humic substances in natural waters: Major reactive species. In: Aquatic Humic Substances: Influence on Fate and Treatment of Pollutants (Eds I. H. Suffet and P. MacCarthy). Am. Chem. Soc. Washington, DC. 333-362.

Couladouros E.A. and Strongilos A.T., 2000. Regioselective oxidation of 3-monosubstituted juglone derivatives. Tetrahedron Lett., 41, 535-538.

Dewar M.J.S., Hasmall J.A., and Vernier C.G., 1968. ?????(Tytuł) J. Am. Chem. Soc., 90, 1953-1957.

Dewar M.J.S., Zoebisch E.G., Healy E.F., and Stewart J.J.P., 1985. AM1. A new general purpose Quantum Mechanical Molecular Model. J. Am. Chem. Soc., 107, 3902-3909.

Drozd J., Gonet S. S., Senesi M., and Weber J., Eds, 1997. The Role of Humic Substances in the Ecosystems and in Environmental Protection. PTHS, Wrocław.

Ghosh K. and Schnitzer M., 1979. UV and visible absorption spectroscopic investigations in relation to macromolecular characteristic of humic substances. J. Soil Sci., 30, 755.

Hoigne J., Faust B.C., Haag W.R., Scully F.E.J., and Zepp R.G., 1989. Aquatic humic substances as sources and sinks of photochemically produced transient reactants. In: Aquatic Humic Substances: Influence on Fate and Treatment of Pollutants (Eds I. H. Suffet and P. MacCarthy). Am. Chem. Soc., Washington, DC, 363-381.

Hongve D. and Dahlback A., 1997. On the efficiency of solar UV radiation on degradation of aquatic humic substances. In: The Role of Humic Substances in the Ecosystems and in Environmental Protection (Eds Drozd *et al.*). PTSH-Polish Soc. Humic Substances Wrocław, 595-602.

Jensen-Korte U., Anderson C., and Spiteller M., 1987. Photodegradation of pesticides in the presence of humic substances. Sci. Total Environ., 62, 335-40.

Mandava N.B., 1985. Chemistry and biology of allelopathic agents. In: The Chemistry of Allelopathy (Ed. A.C. Tomson). Am. Chem. Soc., Washington DC, 33-54.

McCarthy P., Malcom R., Hayes M.H.B., Swift R., Schnitzer M., and Campbell W.L., 1985. IHSS communication to members: outline of humic and fulvic acids extraction procedure. ??? Wydawnictwo?

Miano T.M. and Senesi N., 1992. Synchroneous excitation spectroscopy applied to soil humic substances chemistry. Sci. Total Environ., 117.

Miano T.M., Sposito G., and Martin J.P., 1988. Fluorescence spectroscopy of humic substancces. Soil Sci. Soc. Am. J., 52, 1016.

Piccolo M., Ed., **1996.** Humic Substances in Terrestrial Ecosystems. Amsterdam, Elsevier.

Polewski K., Sławińska D., and Sławiński J., 2002. The role of ortho- and parasemiquinones in the chemiluminescence and antioxidazing activity of humus substances. In: Bioluminescence and Chemiluminescence. Progress and Current Applications (Eds Satnley P.E., Kricka L.J.). Word Scientific, New Jersey-London-Singapore, 161-164.

Rozanowska M., Ciszewska J., Korytowski W., and Sarna T., 1995. Rose-bengal-photosensitized formation of hydrogen peroxide and hydroxyl radicals. J. Photochem. Photobiol. B: Biology, 29, 71-77.

Sakkas V.A., Lambropoulou D.A., and Albanis T.A., 2002. Study of chlorothalonil photodegradation in natural waters and in the presence of humic substances. Chemosphere, 48(9): 939-45.

Schnitzer M. and Khan S.U., 1972. Humic Substances in the Environment. Marcel Dekker, New York.

Schnitzer M. and LaVesque M., 1979. Electron spin resonance as a guide to degree of humification of peats. Soil Sci., 127, 140.

Senesi N., 1990. Application of ESR spectroscopy in soil chemistry. In: Adv. Soil. Sci. (Ed. B.A. Stewart). Springer Verlag Heidelberg, 77-80.

Senesi N., 1992. Binding mechanisms of pesticides to soil humic substances. Sci. Total Environ., 123-124: 63-76.

Senesi N., Miano T.M., Provenzano M.R., and Brunetti G., 1989. Spectroscopic and compositional comparative characterization of IHSS reference and standard fulvic and humic acids of varoius origin. Sci. Total Environ., 81/82, 143.

Sławińska D., Polewski K., Rolewski P., Pluciński P., and Sławiński J., 2002. Spectroscopic Studies on UVC-induced Photodegradation of Humic Acids. Electr. J. Polish Agric. Univ. 5(2); www.ejpau.media.pl/series/volume5/issue2/environment/index.html, 5(2).

Sławińska D., Sławiński J., and Sarna T., 1975. Effect of light on EPR spectra of humic acids. J. Soil Sci., 26, 93-99.

Sławiński J., Puzyna W., and Sławińska D., 1978. Chemiluminescence in the photooxidation of humic acids. J. Photochem. Photobiol., 28, 75-81.

Steelink C. and Tollin G., 1966. Biological polymers related to catechols: electron paramagnetic resonance and infrared studies of melanin, tannin, lignin, humic acid and hydroxyquinones. Biochim. Biophys. Acta, 112, 337.

Stephenson F.J. and Goh K.M., 1971. Infrared spectra of humic acids and related substances. Geochim. Cosmochim. Acta, 35, 471.

Tan K.H., 2003. Humic Matter in Soil and the Environment. Principles and Controversies. Marcel Dekker Inc., New York, Basel.

Thompson R.H., 1987. Naturally Occurring Quinones III. Recent advances. Chapman and Hall, London.

Wang C.X., Yediler A., Peng A., and Kettrup A., 1995. Photodeegradation of phenantrene in the presence of humic substances and H_2O_2. Chemosphere, 30, 501-510.