

# Changes in the Luminescent Properties of Humic Acids Induced by UV Radiation

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Humic acids (HA) are complex, dark, paramagnetic biopolymers, ubiquitous in the soil and aquatic ecosystems. Due to their peculiar properties (multifluorophore system capable of excitation energy transfer, continuous featureless absorption over a wide spectral range, and paramagnetism), HA play an important role as an efficient target for UV solar radiation, O<sub>2</sub>, and O<sub>3</sub>—detrimental environmental factors which affect the Earth's biosphere. Photooxidation of HA causes changes in the absorption and luminescence properties of HA which may be of significant importance for environmental photophysics and photochemistry. We have studied effects of UV irradiation on the degradation of several commercial HA (Fluka, Merck, and Serva). Aqueous, aerated alkaline solutions of HA (0.1–0.4 g/L in 0.006–0.1 M Na<sub>2</sub>CO<sub>3</sub>) were irradiated with an electrodeless Hg (254-nm) lamp in a flow system during several hours. After different times of irradiation, solutions were assayed by means of fluorescence (FI), absorption (UV-Vis), and chemiluminescence (CL) spectroscopy. The data obtained indicate that the free radical-mediated degradative photooxidation of HA macromolecules is accompanied by a very weak, long-lived chemiluminescence (340–800 nm), a gradual decrease in absorbance with characteristic changes in color coefficients  $Q_{416}$ ,  $Q_{271}$ ,  $Q_{274,0}$ , and  $Q_{274,0}$ , and an increase in the intensity of FI emission (340- to 560-nm) and excitation (250- to 400-nm) spectra. Processes undergoing these changes are intrinsically associated with the generation of excited states and reactive oxygen species (ROS<sup>•</sup>, O<sub>2</sub><sup>-</sup>, OH<sup>•</sup>, RO<sub>2</sub><sup>•</sup>, H<sub>2</sub>O<sub>2</sub>, and O<sub>2</sub><sup>•+</sup>; <sup>1</sup>Δ<sub>g</sub>, <sup>1</sup>Σ<sub>g</sub><sup>+</sup>). These processes are expected to play a vital role in the natural environment, e.g., HA-photosensitized decomposition of xenobiotics and solar energy transfer in symbiotic hydrobionts.

**KEY WORDS:** Absorption; fluorescence and chemiluminescence of humic acids; ecological impact of UV radiation; free radical oxidative degradation.

## INTRODUCTION

The increasing penetration of UV radiation to the troposphere due to depletion of the stratospheric ozone layer initiates a variety of detrimental environmental processes. Our environment rapidly becomes more and more enriched in a variety of oxidants such as ozone O<sub>3</sub>, H<sub>2</sub>O<sub>2</sub>, <sup>1</sup>O<sub>2</sub><sup>•+</sup>, nitroperoxyacetates, free radicals (OH<sup>•</sup>,

O<sub>2</sub>, O<sub>2</sub>H<sup>•</sup>, ROO<sub>2</sub>), ions of transition metals, and prooxidative agents such as UV radiation increasingly contaminating many regions of the biosphere. The targets most susceptible to the interaction with these factors are dark paramagnetic biopolymers ubiquitous in the surface layers of waters and soils—humic acids (HA). HA reveal certain unique physicochemical properties: continuous featureless absorption over a wide spectral range, multifluorophore systems capable of excitation energy transfer, paramagnetism, chemical and microbiological resistance, and antioxidative and free radical scavenging activity [1,2]. Macromolecules of HA contain conjugated olefinic, aromatic, phenolic-semiquinone-quinone structures and

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a wide spectrum of functional groups ( $=C=O$ ,  $-COOH$ ,  $-OH$ ,  $-NH-$ ,  $-NH_2$ ,  $-N=$ ) and chromophores capable of absorbing electromagnetic radiation up to about  $\lambda = 1.5 \mu\text{m}$  [3]. Previous research has shown that the interaction of light with HA and oxygen leads to a transient generation of electronically excited states and free radicals, with concomitant very weak, long-lived chemiluminescence [4–6,11]. It has been shown that reactive oxygen species (ROS;  $O_2$ ;  $OH\cdot$ ;  $RO_2$ ;  $H_2O_2$ , and  $O_2^*$ ;  $^1\Delta_g$ ,  $^1\Sigma_g^+$ ) may be produced during photosensitization, i.e., upon absorption of sunlight by HA and subsequent energy transfer to the ground state  $^3\Sigma_g^-$  of the oxygen molecule. Thus, HA can accelerate photodecomposition of xenobiotics such as oil spills, herbicides, phenols, chlorinated aromatic compounds, azodyes, etc. [1,7]. Moreover, in such a multichromophoric system photophysical energy transfer and absorption processes may be employed by symbiotic hydrobionts, e.g., coral zooanthellae, which produce HA-like pigments to convert short-wavelength light (around 350 nm) to longer-wavelength light (around 470 nm). On the other hand, it has been reported that HA inhibit free radical reactions generating chemiluminescence by the interaction with ROS [2,15]. All these photophysical and photochemical processes occurring in the troposphere and surface layers of the Earth strongly affect biosystems. However, the impacts of UV radiation on aquatic and soil HA are largely unexplored and unexplained. An interaction of the UV solar radiation with surface HA significantly influences processes in various ecosystems, yet our knowledge about the mechanisms of photophysical/photochemical processes in HA and the ecological consequences of these processes is still incomplete. These newly recognized processes have great cognitive and perhaps practical ecological significance [1]. Therefore, we have undertaken experimental investigations on the effects of UV light +  $O_2$  on luminescent properties associated with the photodegradation of HA and their possible ecological implications.

## MATERIALS AND METHODS

We have studied the effect of UV-C radiation on the degradation of several commercial HA (Fluka, Merck, and Serva) used absorption, fluorescence (Fl), and chemiluminescence (CL).

Aqueous solutions of HA (0.1–0.4 g/L in 0.006–0.1 M  $Na_2CO_3$ ) were irradiated with a low-pressure electrodeless Hg lamp ( $4 \cdot 10^{17}$  quanta/s,  $\lambda = 254$  nm) with radiowave excitation (160 kHz) in a flow circulation system [9]. Aliquots of the solution were analyzed by UV-Vis spectrophotometry (Specord UV-Vis; Zeiss),

spectrofluorometry (Shimadzu RF 510), and chemiluminescence spectroscopy [single-photon counting (SPC); PMT QB EMI 9858 and cutoff filters method, LS 100 C Beckmann].

## RESULTS AND DISCUSSION

### Fluorescence

The fluorescence of HA occurs in the excitation/emission wavelength region 250–400/340–580 nm and was measured using the emission/excitation mode. The dependence of the relative fluorescence intensity on the wavelength of excitation and/or emission is sensitive to the origin of the humic substance, even for humic substances from similar sources.

In fluorescence measurements of strongly absorbing HA, the self-absorption effect may be important, and therefore the intensity values were corrected using the following formula:

$$I_0 = \frac{IA}{1 - \exp(-A)} \quad (1)$$

where  $I_0$  indicates the true fluorescence intensity,  $I$  the observed intensity, and  $A$  half of the measured absorbance. There is no considerable difference between values after correction and uncorrected results beyond 400 nm, because the concentration of HA used in the fluorescence measurement was very low (0.0008 g/L), and the self-absorption (inner filter) has an imperceptible effect.

### Fluorescence Emission

The relative intensities of the fluorescence emission of humic substances are dependent on the wavelength of excitation and maxima occurring when the excitation wavelength is at a maximum in the excitation Fl spectrum. The emission spectra show fewer interferences such as Raman peaks but are less specific in that they tend to consist of broad bands rather than a number of peaks or shoulders.

The data obtained (Fig. 1) indicate that the intensity  $I_{\text{max,em}}$  of fluorescence emission increases with an increase in irradiation time  $\tau_{\text{ir}}$ , while the position of  $\lambda_{\text{max}}$  445 nm and the spectral bandwidth do not change significantly. These data suggest that the photodegradation produces an increasing amount of similar fluorescent species from HA subunits. Senesi [12] concluded that despite the differences in the spectra, there was a regular occurrence

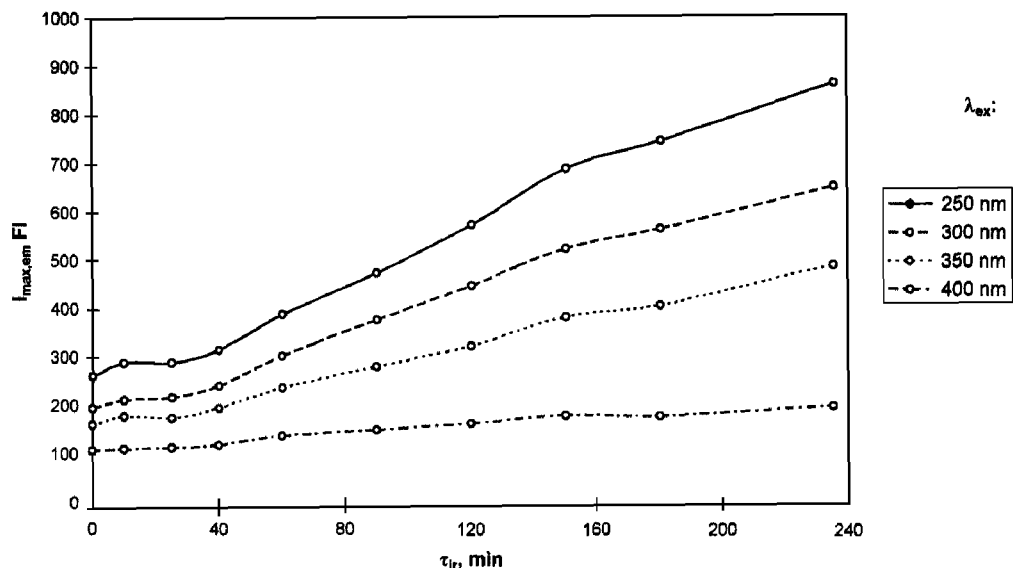


Fig. 1. The influence of the irradiation time  $\tau_{ir}$  on the maximum fluorescence emission intensity at different excitation wavelengths  $\lambda_{ex}$ . The concentration of HA used for the fluorescence measurement was very low (0.0008 g/L).

of some peaks such as at long wavelength, 440–480 nm; intermediate wavelength, 380–400 nm; and short wavelength, 300–360 nm.

#### Fluorescence Excitation

The FI of HA tends to occur mostly in the intermediate- and long-wavelength spectral regions. The intensity of fluorescence excitation (Fig. 2), in the function  $I_{max,ex} = f(\tau_{ir})$ , measured at  $\lambda_{em} = \text{const}$ , tends to increase

with an increase in  $\tau_{ir}$ . Since the slopes of these curves also increase with a decrease in  $\lambda_{em}$ , and the value of  $\lambda_{max,ex}$  increases with the  $\lambda_{em}$ , one can deduce that quenching and excitation energy transfer (EET) processes play a significant role in the spectroscopic behavior of irradiated HA where the concentration reaches 0.4 g/L [10,14].

This conclusion is supported by the following findings:

- (i) the positions  $\lambda_{em \max}$  are independent of the irradiation time  $\tau_{ir}$ ,

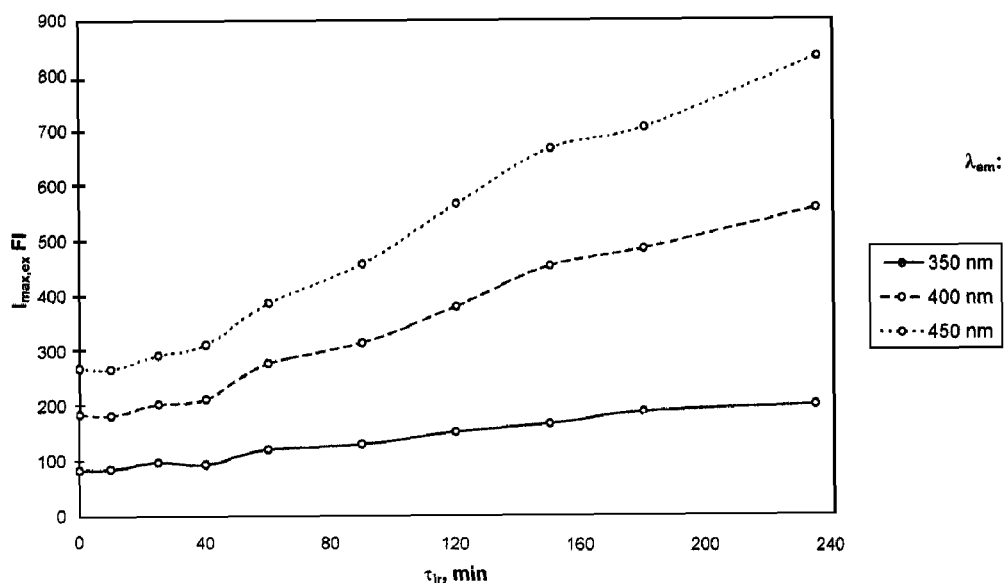


Fig. 2. The influence of the irradiation time  $\tau_{ir}$  on the maximum fluorescence excitation intensity at different emission wavelengths  $\lambda_{em}$ . The concentration of HA used for the fluorescence measurement was 0.0008 g/L.

- (ii)  $\lambda_{em\ max}$  are slightly dependent on the  $\lambda_{ex}$ ,
- (iii) the excitation spectra do not look like the absorption spectra, and
- (iiii) the quantum yield of fluorescence is low ( $<10^{-2}$ ) but increases slightly with an increase in  $\tau_{ir}$ .

In such large structures, intramolecular EET processes between different chromophores which are bound together may occur at all concentrations. For every fluorophore in HA, there may be many nonfluorescent chromophores. From our previous [4–6,11] and literature data [10], it follows that the energies of the first S and T states are in the range of 23,000–27,000  $\text{cm}^{-1}$ . Since this range covers the energies of ( $^1\text{O}_2^*$ )<sub>2</sub> in combined states  $^1\Delta_g$  and  $^1\Sigma_g^+$ , a sensitized EET from HA\* to  $\text{O}_2$  may occur, with subsequent formation of ROS and photodegradation of HA.

### UV-Vis Absorption Spectrophotometry and Color Coefficients

The important indicators of HA degradation are the color coefficients,  $Q_{ij}$ , where  $Q$  is the ratio of absorbance at selected wavelengths, and indices  $i$  and  $j$  indicate the absorption wavelength.

Values of the color coefficient  $Q_{ij}$ , which reflect the condensation degree of the aromatic nuclei of HA and are correlated with the chromophore and/or auxochrome concentration, molecular weight, and intrinsic free radical concentration, change with the  $\tau_{ir}$ . It follows that the molecular size decreases due to the degradation of HA macromolecules and production of low molecular weight end products [8].

In the first phase of irradiation, the ratio of  $Q_{4/6}$  to  $Q_{2.7/4.0}$ , which is a conventional measure of the polymerization degree or aromaticity of HA, gradually and slightly increases (Fig. 3). The color coefficient  $Q_{2.7/4.0}$  characterizes the degradation of the phenolic/quinoid core of HA to simpler carboxylic aromatic compounds, since the spectral range of about 270 nm reflects the absorption of basic constituents of HA: phenols, quinones, phenolic acids, purines, pyrimidines, and indoles. The increase in the color coefficient values indicates a degradation of long-wavelength absorbing chromophores such as, e.g., CT complexes, quinoid and semiquinoid arrangements, and aromatic and/or heterocyclic zwitteranions (e.g., aminochromes), etc. In the second phase of irradiation (Fig. 3), the  $Q_{4/6}$  value decreases slightly, while  $Q_{2.7/4.0}$  increases linearly with time. The decrease in the  $Q_{4/6}$  value is probably caused by competitive secondary reactions, e.g., a cross-linking involving photodimerization of complex aromatic structures.

### Chemiluminescence

The chemiluminescence signal of UV-C-irradiated HA is presented as a function of irradiation time  $\tau_{ir}$  in Fig. 4. The intensity values were corrected (self-absorption effect) using formula (1). The illustrated function follows a consecutive degradation reaction, and it is evident that photodegradation of the HA +  $\text{O}_2$  + sodium carbonate solution produces CL about seven times stronger than the sodium carbonate +  $\text{O}_2$  alone does. These data prove that photooxidation of HA generates excited species which are deactivated by luminescence. The spectral distribution of the photoinduced CL covers 340–800 nm, with two broad emission bands centered around 490 and 634 nm. The broad emission bands indicate that various emitters contribute to the CL and their excitation energy ranges from 1.2 to 4 eV. The enhancement of CL intensity in  $\text{D}_2\text{O}$  solution by a factor of about 4.5, inhibition by azide  $\text{N}_3$ , and predominant contribution of red luminescence (634–780 nm) to the total emission [4–6,11] suggest that the EET from excited chromophores in HA and/or in their degradation products with  $\text{O}_2$  molecules are strongly absorbed within the spongy structure of the biopolymer, leading to the formation of  $\text{O}_2^*$  excimers and ROS.

### CONCLUSIONS

The action of UV-C (254 nm) upon HA initiates complex free radical-mediated degradative oxidation reactions intrinsically associated with the generation of electronically excited states, low molecular weight fluorescent species, chemiluminescence, and free radicals. These reactions are exergonic enough to produce photons with energies lower than 350 kJ/mol ( $\lambda$  longer than 330 nm) [5]. Therefore, in the initial phase of the light-HA interaction, transient CL signal enhancements are observed, as well as a small increase or decrease in the color coefficients. A long-time interaction of the UV with HA always leads to a decrease in CL and an increase in  $Q_{4/6}$ . These data suggest that the aromatic hydrophobic core of the HA colloidal micelle undergoes gradual degradation to more hydrophilic low molecular weight acidic compounds. The general conclusion from our research points to the important, yet unrecognized role of the interaction of light with soil/aquatic HA. The generation of electronically excited states with energy levels which surpass the energetic effects of enzymatic reactions occurring in these ecosystems puts forth a new interdisciplinary problem. Excited species have a potential to trigger some critical processes which are kinetically slowed

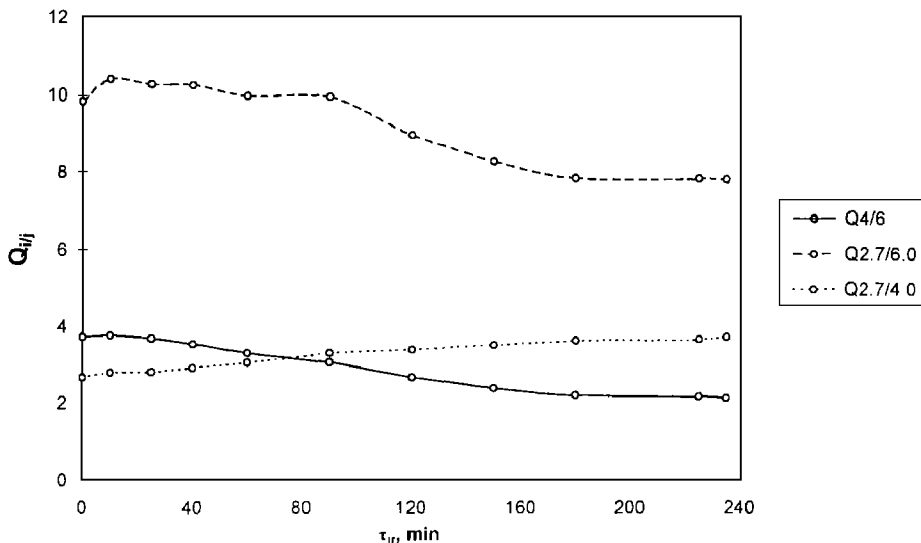


Fig. 3. The effect of the irradiation time  $\tau_{ir}$  on the color coefficients,  $Q_{ij}$  of Fluka humic acid, where  $Q$  is the ratio of absorbances, and indexes  $i$  and  $j$  indicate the absorption wavelength.

down because of a large activation energy  $E_a$ . Expected effects of the oxidative degradation reactions and generation of ROS and excited species from HA initiated by increasingly effective antropogenic environmental factors such as UV radiation may include the following processes: (a) a decrease in the protection capacity of HA against UV and ionizing radiation and peroxyradicals, (b) an alteration of the photodetoxication or photostabilization of xenobiotics sorbed by HA, (c) an alteration

of the activity of soil/surface water microorganisms, (d) changes in the sorption properties and accumulation capacity of HA with respect to plant nutrients, and (e) increased solubilization of soil and rock constituents resulting in changes in the dynamics of soil processes, e.g., podsolization.

Whether these processes play a significant ecological role remains to be explained in further research. The results obtained may be considered a contribution to fur-

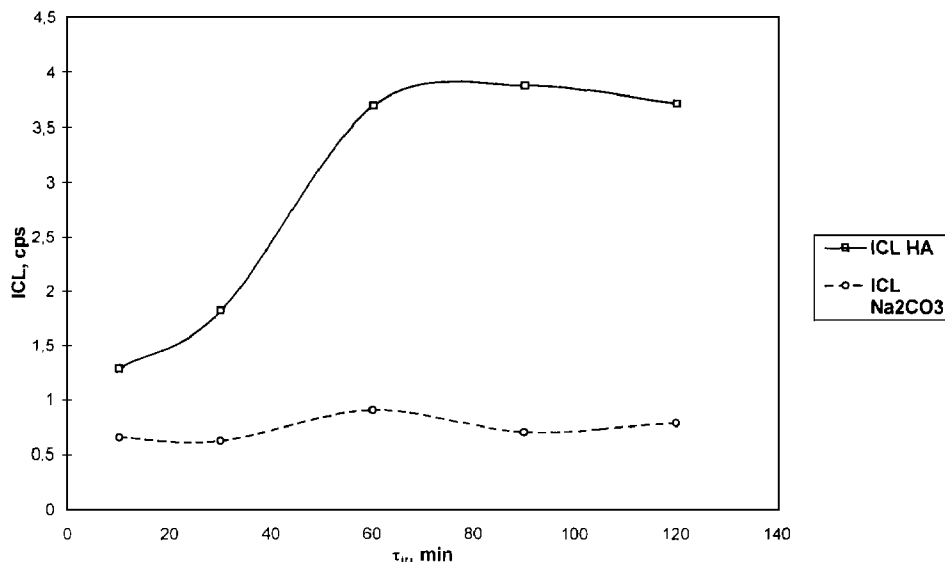


Fig. 4. Kinetics of photoinduced chemiluminescence of Fluka humic acids (0.02%) irradiated in 0.1 N  $\text{Na}_2\text{CO}_3$  at 23°C. The integral light intensity ICL (counts per second; cps) measured at a constant flow rate as a function of the irradiation time  $\tau_{ir}$  is shown.

ther application of the Jablonski diagram to the electronic levels of luminescence of biophysically vital HA macromolecules.

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