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Comparison of the photoinductive properties of commercial, synthetic and soil-extracted humic substances

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Abstract

The photochemical behavior of a fulvic acid (FA) and several humic acids (HAs) were studied. These synthetic, commercial or soils-extracted humic substances were compared according to their abilities to sensitize the formation of singlet oxygen and to photoinduce the transformation of aromatic compounds such as 2,4,6-trimethylphenol (TMP) and 1,1-dimethyl-3-phenylurea (fenuron). Singlet oxygen was detected by ESR experiments, and quantum yields of singlet oxygen formation were measured by using 1,2-difuranyl-2-hydroxyethanone (furoin) as a probe molecule. The photoinductive capacities were evaluated from the kinetics of TMP and fenuron disappearance. The humic substances that showed the greatest ability to generate singlet oxygen were also most efficient in photoinducing the transformation of TMP and fenuron. The efficiencies were found to increase in the following order: synthetic HAs $\ll$ Podzol HA $<$ Rendzina HA $<$ Aldrich HA $<$ Ranker HA $<$ Ranker FA. Though humic substances formed under the same ecological conditions exhibited similar photoinductive properties, the structural characteristics (IR, elemental analyses, solid-state ^{13}C NMR) showed no apparent correlations with the photochemical behavior.

Keywords: Humic acids; Fulvic acids; Photoinductor; Singlet oxygen; Structural composition

1. Introduction

Photochemical transformations of xenobiotics in the aquatic environment occur either by direct excitation or by the excitation of chromophores acting as sensitizers or precursors in the production of reactive species [1]. Humic substances are organic light-absorbing macromolecules found in natural waters. They are involved in a number of photochemical processes and have been reported to play an important role in the fate of chemicals [2–15]. Photosensitized reactions involving electronic energy transfer from the triplet states of humic or fulvic acids to organic molecules were described [3,7], as well as photosensitized oxygenations via the singlet oxygen pathway [3–6,8,10,13]. Humic substances were shown to photoinduce the transformation of phenolic compounds. The main transient oxidant species involved in these reactions were suggested to be peroxy radicals [2,3,9] or excited triplet states [14,15].

In this work, we compare the photoinductive properties of various humic substances. The comparison is based on the capacity to photosensitize singlet oxygen formation and on the ability to photoinduce the transformation of 2,4,6-trimethylphenol and fenuron. The humic acids (HAs) and fulvic acid (FA) were commercial, extracted from soils or synthesized from phenolic derivatives. To better understand the differences observed in the photochemical properties, we tried to obtain information on the structural composition of the humic substances, which were analyzed by several techniques (elemental analyses, solid-state ^{13}C NMR spectrometry, IR spectrophotometry).

2. Experimental

2.1. Materials

Furoin was recrystallized from water. 2,2,6,6-Tetramethylpiperidone, sodium azide (NaN_3), 1,4-diazabicyclo[2.2.2]octane (Dabco) and 2,4,6-trimethylphenol (TMP) were of the highest grade available and were used as received. Water was purified with use of a Millipore MilliQ device. The commercial humic acid (HA) was purchased from Aldrich Chemical. The synthetic HA, HAP, was obtained, as previously described, by photooxidation at 254

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nm of concentrated solutions of phenol [13]. The synthetic HAs, PCT and DHB, were prepared by the oxidation of an equimolar mixture of catechol and triglycine, and a solution of 2,5-dihydroxybenzoic acid, respectively [16], in a flux of pure oxygen at pH 8 and in the dark. Humic substances were extracted by a standard procedure [17,18] from organic-rich soil horizons of different natural media of Western Europe: the surface (A1) horizons of two mountain acid soils, a Ranker and a Podzol; the spodic (Bh) horizon of the Podzol; the surface (A1) horizon of a forested soil on limestone, a Rendzina.

2.2. *Preparation and irradiation of samples*

The solutions containing the humic substances (0.1 g l^{-1}) and the substrate were buffered at pH 6.5 with phosphates and filtrate on 0.45 μm Millipore prior to irradiation. Solutions were irradiated at 365 nm in a water-cooled reactor using three "black-light" lamps (HPW; 125 W; Phillips). Monochromatic irradiations were also performed at 365 nm with a Bausch and Lomb monochromator equipped with a high-pressure mercury lamp. Ferrioxalate was used as a chemical actinometer. The deoxygenation of solutions was performed by three cycles of freezing–pumping–thawing on a vacuum line.

2.3. *Analyses*

High-performance liquid chromatography was carried out on a Waters chromatograph equipped with a photodiode array detector (model 990) and a C18 Lichrosphere column (5 μm; 250 mm × 4 mm) supplied by Merck. UV spectra were recorded on a Cary 118C instrument. ESR experiments were performed using a Brucker ER200D spectrophotometer equippped with a flat quartz ESR cell. Samples were irradiated with a polychromatic xenon–mercury lamp at λ above 345 nm. The ESR measurements were conducted under the following conditions: magnetic field, 3380 ± 25 G; field modulation, 1 Gpp. Solid-state ^{13}C NMR spectra were obtained on a Brucker MSL 300 spectrometer using the cross-polarization technique.

3. Results

3.1. *Detection of singlet oxygen ($^1O_2$)*

3.1.1. *Qualitative experiments*

It was reported that sterically hindered amines such as 2,2,6,6-tetramethyl-4-piperidone react with $^1O_2$ to form a nitroxide radical [19]. When an aqueous solution containing the Ranker FA and 2,2,6,6-tetramethyl-4-piperidone is irradiated, an ESR signal consisting of a triplet ($a_N = 16.1$ G) is observed (see Fig. 1). This signal can be attributed to the 2,2,6,6-tetramethyl-4-piperidone-*N*-oxyl radical. To verify that this radical production takes place via a singlet oxygen reaction, the yields of nitroxide formation were measured in H_2O and in D_2O. They are about five times higher in D_2O than in H_2O. This result confirms the involvement of $^1O_2$ since the lifetime of $^1O_2$ is longer in D_2O than in H_2O [20]. Accordingly, the formation of nitroxide is not observed when $^1O_2$ quenchers, such as sodium azide or Dabco [21], are added to the solution. These qualitative experiments were carried out for several other HAs. The commercial and soil-extracted HAs generate $^1O_2$ whereas HAP, PCT and DHB do not.

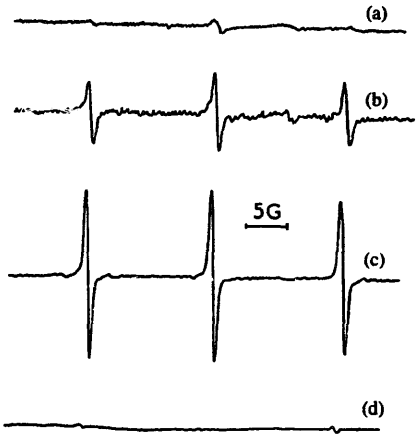

Fig. 1. ESR spectra obtained on irradiation of aqueous solutions containing the Ranker FA (0.1 g l^{-1}) in the presence of 2,2,6,6-tetramethylpiperidone (0.05 M) at pH 10. Spectrum a, before irradiation; spectrum b, after 10 min irradiation (H_2O; gain, 5×10^5); spectrum c, after 10 min irradiation (D_2O; gain, 2.5×10^5); spectrum d, after 10 min irradiation in the presence of sodium azide (3×10^{-3} M).

3.1.2. *Quantitative experiments*

It has been previously shown that furoin (F) reacts with $^1O_2$ ($k_1 = 1.8 \times 10^8$ M^{-1} s^{-1}) leading to 5-hydroxyfuranone (74%) and 1-furanyl-2-dihydroxyethanone (FDE) (44%) [22]. The formation of FDE can be used to characterize and titrate $^1O_2$ since FDE is not produced on the oxidation of F by oxidant species other than $^1O_2$. FDE is formed in small amounts (15%) during the direct photooxidation of F, but this process is slow at 365 nm since the molar extinction coefficient of F is equal to only 60 M^{-1} cm^{-1} and the quantum yield of direct phototransformation is 1.5×10^{-3}.

On the irradiation of air-saturated solutions containing the humic substances (0.1 g l^{-1}) and furoin, FDE is produced. The initial rates of FDE formation measured for various furoin concentrations were corrected for direct photooxidation. The dependences of $(d[FDE]/dt)^{-1}$ on $[F]^{-1}$ are shown in Fig. 2. Assuming the simple mechanism

$$HA \xrightarrow{h\nu} HA^* \xrightarrow{O_2} HA + {}^1O_2$$

$${}^1O_2 + F \xrightarrow{k_1} \text{products}$$

$${}^1O_2 \xrightarrow{k_d} {}^3O_2$$

the rate law of FDE disappearance can be written

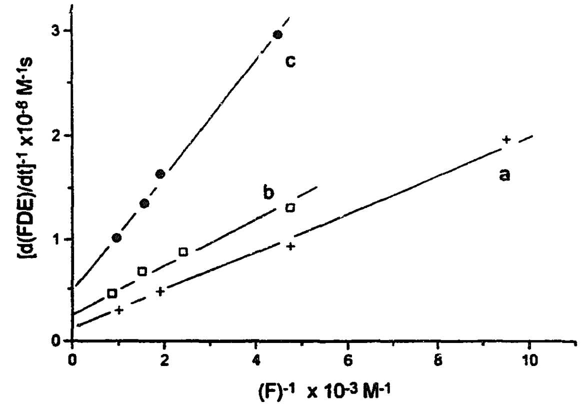

Fig. 2. Dependence of $(d[FDE]/dt)^{-1}$ on $[F]^{-1}$. (a) Ranker HA; (b) Ranker FA; (c) Rendzina HA (air-saturated solutions; $\lambda_{exc} = 365$ nm).

$$\frac{d[FDE]}{dt} = -\frac{d[F]}{dt} \times 0.44 = 0.44 I_a \Phi_{^1O_2} \left(\frac{k_1[F]}{k_1[F] + k_d} \right)$$

where I_a is the light intensity absorbed by the humic substance, $\Phi_{^1O_2}$ is the apparent quantum yield of singlet oxygen formation, and k_d is the rate constant of singlet oxygen deactivation in water ($k_d = 2.25 \times 10^5$ s^{-1}). In accordance with this relationship, the plots of Fig. 2 are linear. Values of slopes and intercepts are reported in Table 1. Calculated ratios of slope over intercept are in good agreement with the expected value of k_d/k_1 (1.25×10^{-3} M). Values of $\Phi_{^1O_2}$ were deduced from experimental results of the intercept, using the values for I_a reported in Table 1.

Significant differences in the ability to photosensitize the formation of $^1O_2$ are observed. The Ranker humic substances exhibit the greatest ability to produce $^1O_2$ whereas the Podzol HA shows the smallest. The commercial HA represents the average.

3.2. Transformation of 2,4,6-trimethylphenol (TMP) and fenuron induced by excitation of the humic substances

The kinetics of TMP disappearance in irradiated oxygen-saturated solutions of the HAs are shown Fig. 3. The initial rate of TMP consumption increases in the following order

$$\text{HAP} \ll \text{PCT} \ll \text{Podzol} < \text{Rendzina} < \text{Aldrich} < \text{Ranker HA} < \text{Ranker FA}$$

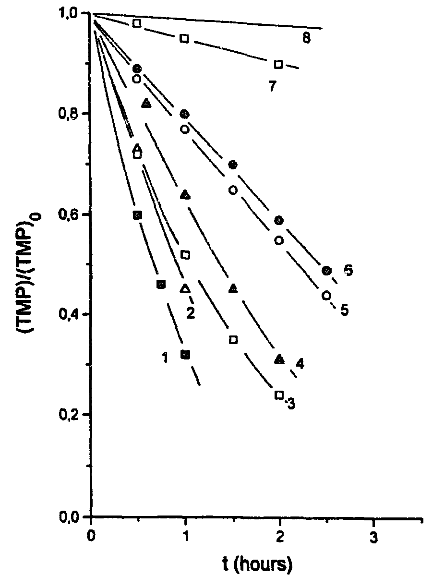

Fig. 3. Kinetics of TMP disappearance in irradiated oxygen-saturated solutions of HAs (pH 6.5; $[TMP] = 1.2 \times 10^{-3}$ M; $m_{HA} = 0.1$ g l^{-1}). 1, Ranker FA ($OD^{365} = 0.17$); 2, Ranker FA ($OD^{365} = 1.2$); 3, Aldrich HA ($OD^{365} = 1$); 4, Rendzina HA ($OD^{365} = 0.67$); 5, Podzol Bh HA ($OD^{365} = 0.88$); 6, Podzol Al HA ($OD^{365} = 0.66$); 7, PCT or DHB ($OD^{365} = 1.0$); 8, HAP ($OD^{365} = 1.0$).

It has been previously shown [23] that the rate of TMP disappearance in irradiated solutions of the Aldrich HA can be described by the following rate law

$$-\frac{d[TMP]}{dt} = I_a \Phi_m \frac{[TMP]}{[TMP] + 8.3 \times 10^{-3} m_{HA} + 3.0 \times 10^{-4}}$$

where $I_a \Phi_m$ is the upper limit value of the rate of TMP consumption, I_a is the light absorbed by HA, and m_{HA} is expressed in grams per liter. Oxidant species produced by excitation of the HA not only react with TMP, but also disappear in other processes. HA itself competes with TMP for reaction with the oxidant species. Hence, to evaluate ϕ_m, the rate of TMP disappearance was measured at low HA concentration (3 mg l^{-1} or less) and high TMP concentration (6×10^{-4} M or above). Under these conditions, the rate of TMP consumption was zero order with respect to the TMP concentration. Values

Table 1
Quantum yields of singlet oxygen formation upon irradiation at 365 nm of HAs and FA in the presence of furoin

Sample	Slope (s)	Intercept (M^{-1} s)	Slope/intercept (M)	I_a (E l^{-1} s^{-1})	$\Phi_{^1O_2}$ [a]
Ranker FA	2.5×10^4	2.6×10^7	9.5×10^{-4}	1.6×10^{-5}	5.5×10^{-3}
Ranker HA	2.0×10^4	1.2×10^7	1.6×10^{-3}	5.3×10^{-5}	3.6×10^{-3}
(Commercial Aldrich HA)	–	–	–	–	2.0×10^{-3}
Rendzina HA	5.7×10^4	5×10^7	1.4×10^{-3}	4.2×10^{-5}	1.1×10^{-3}
Podzol A1 or Bh HA	1.8×10^5	1.4×10^8	1.3×10^{-3}	4.2×10^{-5}	3.9×10^{-4}

[a] The uncertainty in the $\Phi_{^1O_2}$ values is about 20%.

Table 2
Kinetic parameters of TMP consumption

Sample	ϕ_m^a
Ranker FA	0.024
Ranker HA	0.013
Aldrich HA	0.0069
Rendzina HA	0.0036
Podzol Al or Bh HA	0.0020

[a] The uncertainty in the ϕ_m values is about 15%.

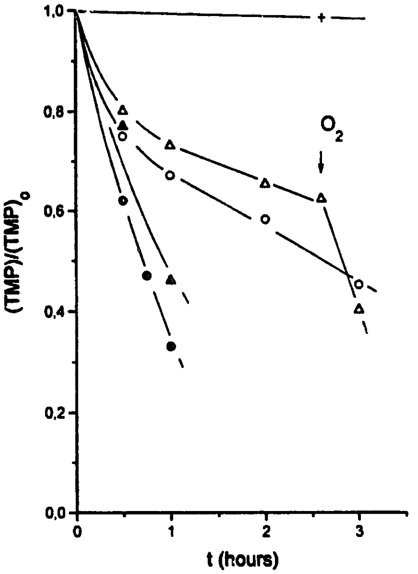

Fig. 4. Influence of oxygen concentration on the kinetics of TMP disappearance (pH 6.5; [TMP] = 1.2×10^{-3} M, m_{HA} = 0.1 g l^{-1}). Ranker FA: ○, air-saturated solution; ●, oxygen-saturated solution. Ranker HA: +, under vacuum; △, air-saturated solution; ▲, oxygen-saturated solution.

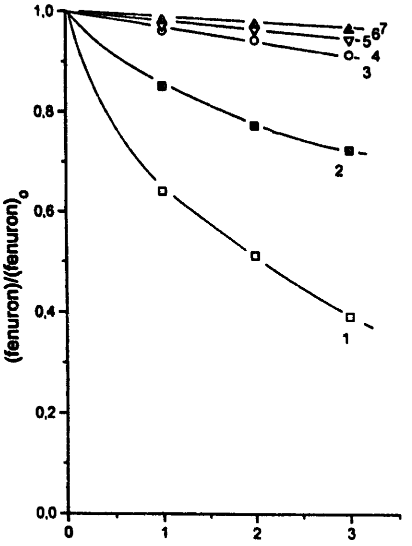

Fig. 5. Kinetics of disappearance of fenuron in irradiated air-saturated solutions of HAs (pH 6.5; [fenuron]) = 2×10^{-4} M; m_{HA} = 0.1 g l^{-1}). 1, Ranker FA (OD^{365} = 0.17); 2, Ranker HA (OD^{365} = 1.2); 3, Aldrich HA (OD^{365} = 1); 4, Rendzina HA (OD^{365} = 0.67); 5, Podzol Bh HA (OD^{365} = 0.88); 7, PCT or DHB (OD^{365} = 1.0).

of Φ_m are reported in Table 2. It appears that Φ_m and $\Phi_{^1O_2}$ increase in the same order.

The influence of the oxygen concentration on the reaction was studied (see Fig. 4). In deoxygenated medium, the rate of TMP consumption is small. The initial rate of TMP disappearance is almost the same in initially air-saturated solutions ($[O_2] = 2.6\times10^{-4}$ M) as in oxygen-saturated media ($[O_2] = 1.3\times10^{-3}$ M). However, in the former case, the rate of TMP transformation is drastically reduced as soon as the amount of TMP that has disappeared reaches 2.6×10^{-4} M. The rate of transformation increases again when oxygen is supplied. In oxygen-saturated solutions, the amount of TMP that can be transformed seems unlimited, suggesting that the chromophores that are responsible for the formation of the reactives species are regenerated under oxygenating conditions.

The rate of TMP disappearance is reduced by less than 10% when sodium azide (10^{-3} M) is added to the solutions. This result indicates that the consumption of TMP through a singlet oxygen reaction is a minor pathway.

The transformation of fenuron induced by excitation of the humic substances was also studied (Fig. 5). Significant differences in photoinductive properties are observed and once more the two Ranker humic substances are the best photoinductors.

4. Analyses

4.1. Elemental analysis

Data reported in Table 3 show that the Ranker FA has the highest H/C ratio, indicating that this compound is less aromatic than the other humic substances. Its oxgen content is also high compared with that of the other natural compounds. The synthetic HAs PCT and HAP are the most aromatic. Nitrogen contents are low in the case of Podzol and Aldrich. The mineral composition is generally 2% or less with the exception of the Podzol Bh HA. The result obtained in this latter case is probably due to the inefficiency of acid reprecipitation during purification. The synthetic HAs contain mineral elements introduced during the synthesis. The copper contents are not negligible. For the soil and commercial samples, aluminum, silicon and iron are the major mineral elements.

4.2. Infrared spectroscopy

The IR spectra of all the soil and commercial humic substances are qualitatively identical, indicating that the major chemical groups present in each of these samples are similar. In the Ranker FA, the band at 1050 cm^{-1} corresponding to the C–O stretching vibration of polysaccharide-like substances is more intense than in the other samples [14]. This is in good agreement with the fact that the Ranker FA has the greatest H/C and O/C ratios (Fig. 6).

Table 3
Elemental composition (%) and the main atomic ratios of the humic substances

Sample	C	H	O	N	S	H/C	O/C	Al	Si	Fe	Cu	Mn	Mineral compounds
Ranker FA	40.2	5.7	46.7	3.2	1.0	1.7	0.87	0.57	0.10	0.072	0.005	0.005	0.75
Ranker HA	46.2	4.6	–	4.7	–	1.2	–	0.63	1.1	0.37	0.001	0.015	2.1
Aldrich HA	41.9	4.1	50.4	0.55	–	1.2	0.90	0.70	–	1.3	–	–	–
Rendzina HA	47.5	4.7	40.9	4.0	0.42	1.2	0.65	0.11	0.22	0.20	0.009	0.002	0.54
Podzol A1 HA	49.0	5.0	38.8	1.9	0.94	1.2	0.60	0.20	0.70	0.12	0.004	0.005	1.0
Podzol Bh HA	36.2	3.9	–	1.6	0.31	1.3	–	2.8	4.5	2.3	0.008	0.004	9.6
PCT	46.6	4.1	38.3	10.4	0.61	1.0	0.62	0.005	0.10	–	0.02	0.009	0.15
DHB	32.2	3.2	–	–	–	1.2	–	0.005	0.10	–	0.004	0.004	0.14
HAP	63.1	4.5	32.4	–	–	0.85	0.38	–	–	–	–	–	–

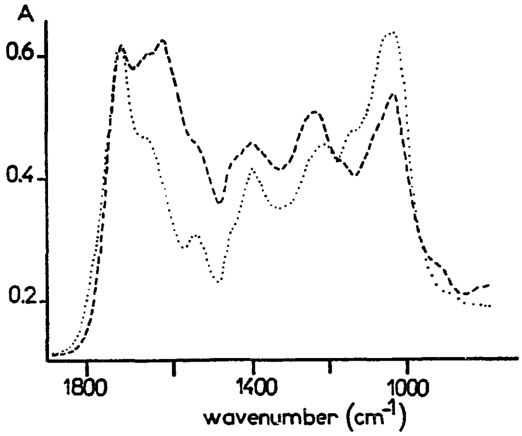

Fig. 6. IR spectra of (- - -) the Ranker HA, and (· · ·) the Ranker FA.

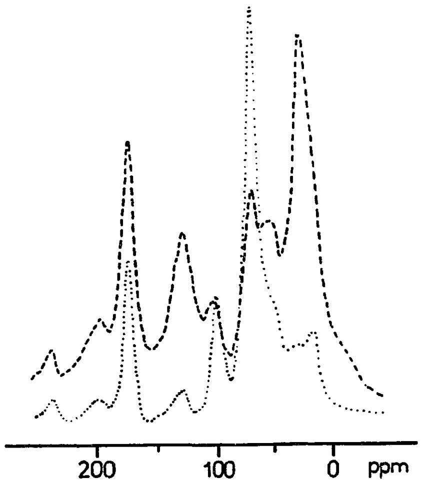

Fig. 7. ^{13}C NMR spectra of (- - -) the Ranker HA, and (· · ·) the Ranker FA.

4.3. ^{13}C NMR spectroscopy

The ^{13}C NMR spectra of both Ranker samples are shown in Fig. 7. They are quite different. The HA contains more saturated aliphatic carbon atoms (0–50 ppm) and aromatic carbon atoms (50–100 ppm) than the FA for which the strongest peak corresponds to carbohydrate carbon atoms (50–100 ppm). The NMR spectra of the other HAs are very similar to that of Ranker HA except that the relative intensity of the bands are slightly different. A broad signal due to carboxylic functions (165–185 ppm) is present in all the spectra as well as a shoulder corresponding to non-carboxylic carbonyl carbon atoms (185–200 ppm).

5. Discussion

The photoinductive properties and the efficiencies of sensitizing the formation of singlet oxygen depend on the origin of the humic substances. The HAs which show the greatest ability to generate singlet oxygen also photoinduce the most efficiently the transformations of 2,4,6-trimethylphenol and of fenuron. The efficiencies increase in the following order

Podzol HA < Rendzina HA < Aldrich HA
< Ranker HA < Ranker FA

The synthetic HAs are not able to produce $^1O_2$ and they photoinduce the transformation of TMP inefficiently.

The differences in photoreactivities could result from the presence of variable amounts of key chromophores. Data on structural characteristics can be analyzed in this way. The Ranker FA which is the best photoinductor is poor in aromatic groups (high H/C ratio) and rich in carbohydrate (high O/C ratio) compared to the other HAs. However, the Podzol HAs that are less efficient than the other soil humic substances have the same H/C ratios as Rendzina, Aldrich and Ranker HAs. As a consequence, this parameter cannot be correlated with the photochemical properties. Due to the lack of some data relating to oxygen content, no conclusions can be drawn concerning the O/C parameters. The mineral element contents are also not correlated with the photochemical behavior.

It was suggested that the reactive species involved in the transformation of phenolic compounds could be aromatic ketones in the excited triplet state [14,15]. The NMR spectra indicate that carbonyl groups are present in each of the soil or commercial humic substances, in good agreement with the literature [24]. However, the corresponding signals are too weak to be quantified.

The differences in photoreactivities could be also explained by the presence, in variable amounts, of deactivators of excited states. It was shown that paramagnetic metallic cations deactivate the excited states of HAs [10,25]. Elemental analyses indicate the presence of iron, copper and manganese in the humic substances. Even though the Ranker FA is poor in metallic cations and the Podzol Bh HA is rich in them, no correlation between the amounts of these elements and the photoreactivity can be found. However, the efficiency to quench the excited states should depend not only on the amount but also on the localization and the chemical form of the metals in the macromolecules.

The photochemical behavior of humic substances results from complex phenomena and depends on many factors. It is interesting to stress that the two Ranker humic substances exhibit the best photoinductive properties. This suggests that the ecological conditions for the generation of humic substances influence their photochemical reactivity through structural characteristics that are not well understood.

In conclusion, the commercial and soil-extracted humic substances photosensitized the production of singlet oxygen and photoinduced the transformation of TMP and fenuron, whereas synthetic HAs exhibited very poor photoreactivity. The humic substances which showed the greatest ability to generate singlet oxygen also photoinduced the most efficiently the transformation of TMP and fenuron. The reasons why soil humic substances have different photoreactivities are probably numerous and related to still unknown structural characteristics of these substances.

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