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Photodegrading properties of soil humic acids fractionated by SEC-PAGE set-up. Are they connected with absorbance?

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Abstract

Humic acids (HAs) extracted from soils of different origins were fractionated using size exclusion chromatography-polyacrylamide gel electrophoresis set-up. For each HAs, three fractions showing distinct electrophoretic mobilities and molecular sizes (MS) were obtained. Bulk HAs and their fractions (25 mg L^{-1}) were then compared for their ability to photosensitize the degradation of 2,4,6-trimethylphenol upon irradiation at 365 nm. Rate coefficients were deduced from pseudo-first order kinetics. For five of the six series, the rate coefficient varied in the order: high MS < medium MS < low MS. Except for one HA, the general tendency was a linear increase of the rate coefficient with $1–10^{-A}$, where A is the absorbance of HAs or fractions solution at 365 nm. It is an indication that the ratio of photosensitizing to non-photosensitizing chromophores is roughly constant among fractions.

Keywords: Humic substances; Chromophores; Sensitization; Pollutants degradation

1. Introduction

Humic substances (HS) contain a large portion of the total organic carbon in terrestrial and aquatic environments. Increasing interest has been given to HS in the past 25 years as they serve as an effective carbon sink reducing gas emission and greenhouse effect and solar energy absorbant. Due to absorption of solar radiations HS initiate a number of photochemical processes, producing radicals and/or other chemical species able to promote the transformation of organic chemicals (pesticides and other organic pollutants) [1–6]. However, due to the complexity of HS, it still remains difficult to elucidate the exact mechanisms of their photochemical properties and clear up which integral components and chromophores are responsible for those. One way to get a better insight into these properties is to fractionate HS into homogeneous fractions selectively enriched in terms of major components and having different physical–chemical properties and/or functional activity. However, all attempts to find out in HS a specific fraction that would prominently outweigh the others in total photoinductive production were unsuccessful [7–10].

In a previous study, we applied a method of fractionation developed by Trubetskoj et al. to an IHSS soil HA standard (Mollisol-USA), to a Mollisol-Russia HA and to a Vertisol HA. This method based on combination of polyacrylamide gel electrophoresis (PAGE) with size exclusion chromatography (SEC), named SEC-PAGE set-up, allowed to obtain preparative quantities of soil HA fractions with different electrophoretic mobilities and molecular sizes (MS) [11]. We observed that for the three investigated HAs, the ability of fractions to photosensitize the transformation of organic compounds increased when the MS of fractions decreased [12]. In the present work, we extended the study to three other soil HAs which were also extracted and fractionated by SEC-PAGE set-up. Each series composed of the bulk HA and its three fractions were tested for their photodegrading activity on the model compound 2,4,6-trimethylphenol. Results were analyzed by connecting photosensitizing properties with absorption of humic fractions.

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Table 1
Elemental analysis of HAs

Name of the soil HA	C (%)	H (%)	N (%)
Andosol	56.1	5.8	7.3
Calcisol	54.7	n.d.	4.9
Mollisol-Russia	62.5	4.6	5.5
Mollisol-USA	57.4	4.4	3.5
Spodozol	60.7	5.9	4.8
Vertisol	51.7	5.2	5.4

2. Experimental procedures

2.1. Materials

The HAs were extracted using the IHSS extraction procedure (http://www.ihss.gatech.edu) from the top layer (A horizon) of Mollisol (Kursk region, Russia), Spodosol (North Siberia, Russia), Andosol (Aurillac, France), Calcisol (Bologna, Italy), Vertisol (Sierra de la Demanda, Spain). The Elliott soil (Mollisol-USA) standard HA (1S102H) was purchased from IHSS. The elemental composition of HAs in C, H and N are shown in Table 1. Water content was generally around 10% except for Calcisol HA (4.2%). The carbon, hydrogen and nitrogen composition were typical for terrestrial HAs [13]. Water was purified using a Milli-Q (Millipore) device. Disodium hydrogenophosphate and potassium dihydrogenophosphate were 99% purity and supplied by Prolabo (France), 2,4,6-trimethylphenol was purchased from Aldrich (99%). Urea, EDTA and Trisborate reagents have been supplied by Sigma (USA).

2.2. Fractionation

Fractionation of soil HAs by SEC-PAGE set-up (*i.e.* using PAGE for subsequent testing of SEC aliquots from different sections of elution profile) was made as previously reported [11]. The weight distribution of fractions was calculated using the ratio $W_i/\Sigma W_i$, where W_i is the weight of fraction and ΣW_i is the weight of all fractions obtained after SEC-PAGE set-up fractionation of HA.

2.3. Analyses of fractions

The C, H, N-analyses of HAs were performed on a Perkin Elmer CHN Analyzer, series II 2400. Values of elemental analyses were corrected for water and ash contents. Water and ash contents of the samples were measured on a Perkin Elmer Thermal Analyzer. Absorbances at 365 nm of bulk HAs and fractions solutions were recorded on a Cary 3 (Varian) spectrophotometer in a 1-cm quartz cuvette. Humic materials were solubilized under stirring in Milli-Q water buffered at pH 6.5 using phosphate buffers (6.6×10^{-3} mol L^{-1}). Solutions were filtrated on 0.45 μm Millipore filters prior to use.

2.4. Irradiations

HAs or their fractions (50 mg L^{-1}) were first solubilized in water buffered at pH 6.5 using a mixture of disodium hydrogenophosphate and potassium dihydrogenophosphate (6.6×10^{-3} mol L^{-1}). A stock solution of TMP at 10^{-3} mol L^{-1} was prepared independently in pure water. Final solutions containing HAs or their fractions (25 mg L^{-1}) and TMP (10^{-4} mol L^{-1} or 13.6 mg L^{-1}) were prepared by appropriate dilution of stock solutions. Mixtures were filtered on 0.45 μm Sartorius filters (cellulose acetate) prior to use. Eight millilitres of solutions were poured into a Pyrex-glass reactor (14 mm internal diameter) and irradiated in a device equipped with three "black-light" lamps emitting mainly at 365 nm (94% of the radiant energy). This line is present in the solar emission spectrum and has the advantage to be not absorbed by TMP. The absence of direct photolysis of TMP in the experimental device was checked. The photon fluence rate was equal to 5×10^{-8} Einstein cm^{-3} s^{-1}. The reactor was refrigerated by water circulation in a water cooling jacket. The consumption of TMP upon irradiation was determined by HPLC. The HPLC analyses were performed on a Merck chromatograph equipped with a L-6200 model pump, a L-3000 photo diode array detector and a 4.6 mm × 250 mm Spherisorb ODS-2 reverse-phase column (Waters). The UV-detector was set at 278 nm. The mobile phase was a mixture of water–acetonitrile (60:40, v/v). Dark control experiments did not show any decrease in TMP concentration on the time scale of irradiation experiments.

3. Results and discussion

3.1. Humic acids fractionation

Electrophoresis pictures of the six soil HAs are shown in Fig. 1. Three main fractions appeared in the electropherogram: the first fraction did not move into the polyacryamide gel, the

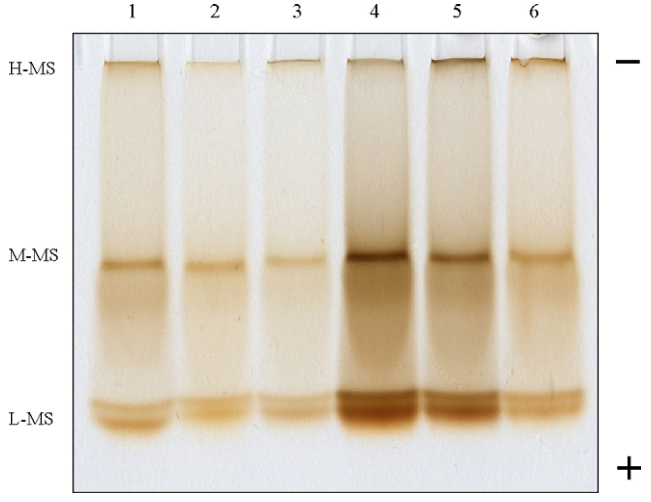


Fig. 1. Electrophoresis picture of 0.15 mg HA from Spodosol (1), Andosol (2), Calcisol (3), Mollisol-Russia (4), Mollisol-USA (5), Vertisol (6) in 10% polyacrylamide gel in the presence of denaturing agents. Here H-MS, M-MS and L-MS are discrete naturally coloured zones of HAs.

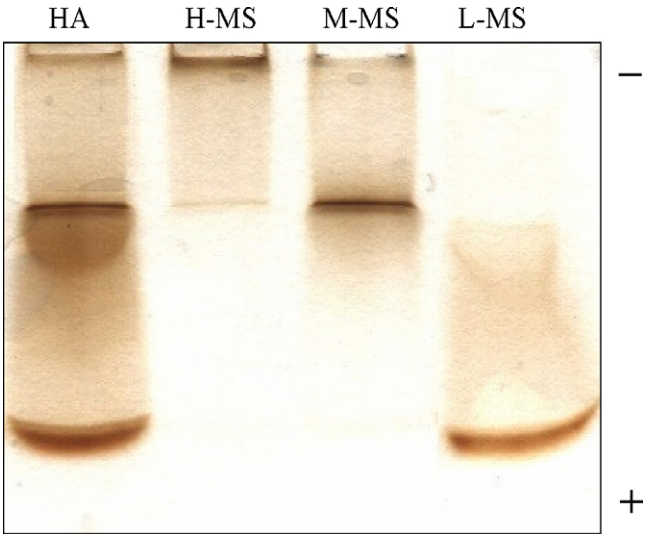


Fig. 2. Electrophoretic analysis of the bulk Mollisol-Russia HA and H-MS, M-MS and L-MS fractions, obtained after set-up SEC-PAGE.

second fraction formed narrow band in the mid part of the gel, and the third fraction corresponded to several bands with similar electrophoretic mobility in the bottom of the gel. For better clarity and based on the chromatographic behaviours, we named H-MS (instead of A [12]) the first fraction that showed the highest MS, M-MS (instead of B) the second fraction that showed medium MS, and L-MS (instead of C + D) the third fraction that showed the lowest MS. Electrophoreses of individual fractions obtained by SEC-PAGE set-up from Mollisol-Russia are shown in Fig. 2. The other soil HA samples investigated gave similar electropherograms. The weight contents of fractions are given in Table 2. The difference to 100% corresponded to the remaining fraction and was mainly constituted by a mixture of M-MS and L-MS fractions.

3.2. Absorbance of fractions

Absorbances of HAs and fractions solutions for a 14-mm path-length (reactor internal diameter) are given in Table 3. Bulk HAs gave absorbances within the range 0.21–0.62. Absorbances of H-MS fractions varied between 0.07 and 0.21, those of M-MS between 0.18 and 0.45, and those of L-MS between 0.33 and 0.48. For a given HA, the absorbance of fractions increased as the MS decreased. High and medium MS fractions were always less absorbing than bulk HAs, while, depending on the soil origin, L-MS fractions were more, equally or less absorbing than corresponding bulk HA. These data show that absorbing centres are not randomly distributed among fractions, but preferentially contained in the lower MS fractions.

Table 2
Relative amounts in weight of individual fractions of HAs

Name of the soil HA	H-MS fraction (%)	M-MS fraction (%)	L-MS fraction (%)
Andosol	27 ± 3	18 ± 2	25 ± 2
Calcisol	21 ± 2	19 ± 2	36 ± 2
Mollisol-Russia	24 ± 3	19 ± 2	36 ± 4
Mollisol-USA	21 ± 3	26 ± 2	30 ± 3
Spodosol	34 ± 3	23 ± 2	24 ± 2
Vertisol	21 ± 2	26 ± 3	30 ± 3

Table 3
Photosensitized transformation of TMP (10^{-4} M) in the presence of HAs or their fractions (25 mg L^{-1})

Name of the soil (origin)		k[a] in 10^{-4} s^{-1}	A_{365}
Andosol (France)	Bulk HA	2.4	0.29
	High MS fraction	1.0	0.13
	Medium MS fraction	1.1	0.21
	Low MS fraction	3.5	0.37
Calcisol (Italy)	Bulk HA	1.7	0.21
	High MS fraction	1.0	0.07
	Medium MS fraction	1.3	0.18
	Low MS fraction	3.2	0.33
Mollisol (Russia)	Bulk HA	3.3	0.62
	High MS fraction	1.7	0.29
	Medium MS fraction	1.7	0.45
	Low MS fraction	2.8	0.48
Mollisol (USA)	Bulk HA	3.3	0.52
	High MS fraction	1.7	0.21
	Medium MS fraction	2.2	0.29
	Low MS fraction	3.6	0.38
Spodosol (Russia)	Bulk HA	2.0	0.36
	High MS fraction	1.8	0.17
	Medium MS fraction	1.6	0.25
	Low MS fraction	1.2	0.40
Vertisol (Spain)	Bulk HA	3.3	0.42
	High MS fraction	0.6	0.14
	Medium MS fraction	1.7	0.34
	Low MS fraction	5.2	0.42

Rate coefficient (k in s^{-1}) and absorbance at 365 nm for a 14 mm of path-length corresponding to the internal diameter of the reactor.
[a] R^2 values were comprised between 0.998 and 0.98.

3.3. Photosensitized transformation of 2,4,6-trimethylphenol

The irradiation of mixtures containing TMP and each of the six HAs or their fractions led to the consumption of TMP. Typically, the plot of $\ln (TMP)/(TMP)_0$ *versus* time was linear indicating a pseudo-first order kinetics (see Fig. 3 in the case of Andosol samples). From the slope of linear plots, we got the rate coefficient, k, using the relationship:

$$\ln(TMP)/(TMP)_0 = k \times t$$

The k values and correlation coefficients R^2 are reported in Table 3. The k values varied within the range 0.6×10^{-4} to 5.2×10^{-4} s^{-1}. For five of the six series (Mollisol-Russia, Andosol, Calcisol, Vertisol, and Mollisol-USA), k varied in the order:

$$\text{H-MS} < \text{M-MS} < \text{L-MS}$$

and the k value was 1.5–10-fold higher for the L-MS than for the H-MS fraction. For Andosol, Calcisol and Vertisol, the L-MS fraction exhibited a higher k value than the corresponding bulk

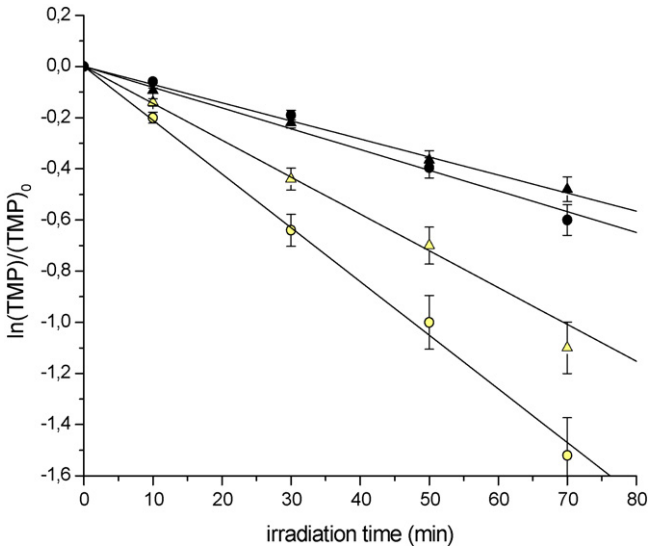


Fig. 3. Logarithmic decay of TMP (10^{-4} M) in the case of Andosol HA samples: bulk HA (△), H-MS fraction (▲), M-MS fraction (●) and L-MS fraction (◯).

HA. In the other cases, it was close or lower. For the Spodosol series, however, k value was slightly lower for L-MS than for M-MS and H-MS.

3.4. Connection of rate coefficient with absorbance

In order to determine whether the rate coefficient was connected to the amount of light absorbed by the HAs or their fractions, we plotted k *versus* $1–10^{-A}$. When one considers all the data, one gets an increase of k with $1–10^{-A}$ as a general tendency (see Fig. 4). For each individual series, we tested a linear plot (see Fig. 5). Values of slopes and correlation coefficients are given in Table 4. The best correlation coefficients were obtained for Andosol (0.870), Calcisol (0.815), and Mollisol-USA (0.765). Mollisol-Russia and Vertisol gave R^2 values of 0.660. Spodosol clearly diverged with a R^2 value equal to 0.130. Data of L-MS fractions were in four cases among six above the mean straight line. The slopes varied within a narrow range, between 3.7×10^{-4} and 5.6×10^{-4} s^{-1}.

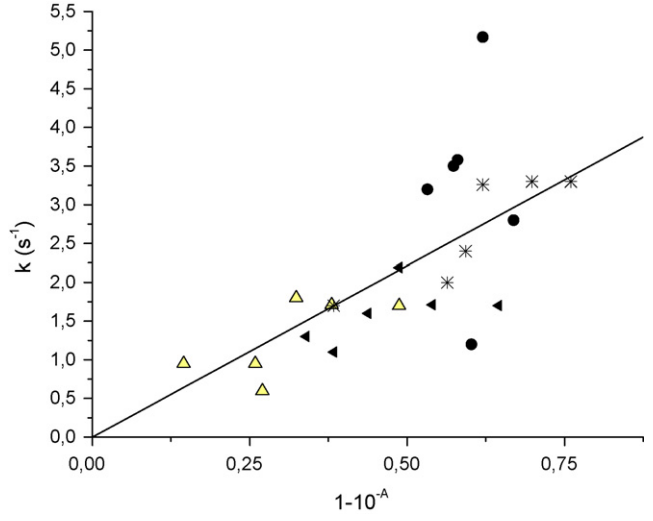
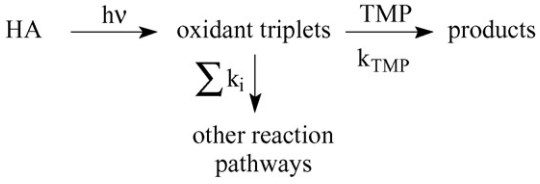


Fig. 4. Plot of rate coefficients *vs.* $1–10^{-A}$ for all series. Bulk HAs (*), H-MS fraction (△), M-MS fraction (◄) and L-MS fraction (●). The straight line corresponds to the better linear plot for bulk HAs.

Variations of k values with $1–10^{-A}$ can be also investigated considering bulk HAs alone or fractions of same MS. For bulk HAs, the plot was satisfactorily linear with $R^2 = 0.775$ (see Fig. 4), while for fractions, R^2 values were small, less than 0.5. If one takes for basis the straight line defined for bulk HAs, one observes that data of H-MS and M-MS fractions were generally below the line indicating that they were less photosensitizing than bulk HAs at comparable absorbance. Data of L-MS fractions were apart of the straight line, well above for Andosol, Calcisol, Vertisol and Mollisol-USA, close to it for Mollisol-Russia and below for Spodosol.

3.5. Discussion

The irradiation of HA generates several types of oxidant species (radicals, singlet oxygen, triplet excited states) susceptible to degrade TMP. For simplification and using results of a work under progress, let us make the hypothesis that, under the chosen experimental conditions, TMP is mainly oxidized by triplet excited states according to the following scheme:



where k_{TMP} is the rate constant of reaction of TMP with the oxidant triplets and $\sum k_i$ is the sum of the apparent monomolecular rate constants of disappearance of the oxidant triplets in the absence of TMP. The TMP consumption was found to follow a pseudo-first order kinetics. The rate law can be thus written:

$$r_{TMP} = k \times [TMP] = I_a \times \Phi \times k_{TMP} \times \tau \times [TMP]$$
$$= I_a \times \alpha \times [TMP] \quad (1)$$

where I_a is the amount of light absorbed by the chromophores generating the oxidant triplets, Φ, the quantum yield of formation of the oxidant triplets and τ is the lifetime of the oxidant triplet $\approx 1/\sum k_i$.

In the general case of photochemistry, the rate of phototransformation of a given compound is proportional to the amount of light absorbed by it: $I_a = I_0 \times (1–10^{-A})$, where I_0 is the photon

Table 4
Plots of k *vs.* $1–10^{-A}$, slopes and correlation coefficients

Samples	Slope in 10^{-4} s^{-1}	R^2
Andosol series	4.9	0.870
Calcisol series	5.2	0.815
Mollisol-Russia series	3.8	0.664
Mollisol-USA series	5.1	0.765
Vertisol series	5.6	0.664
Bulk HAs	4.4	0.775

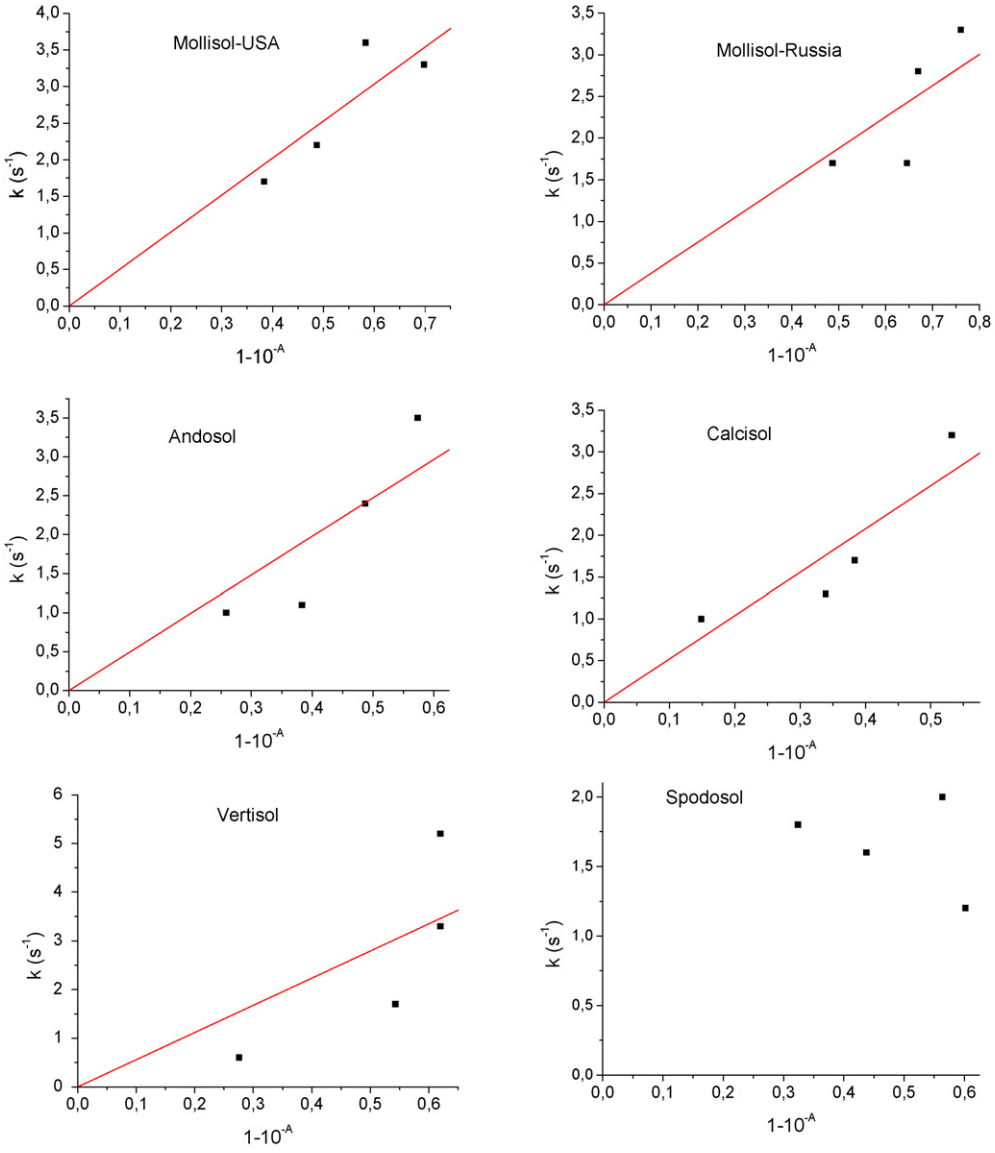


Fig. 5. Plots of rate coefficients *vs.* $1–10^{-A}$ for each series.

fluence rate delivered by the emitting source and A is the absorbance of the compound of interest at the excitation wavelength. The case of HAs is, however, more complex, because all absorbing constituents do not necessarily show photosensitizing properties. Only a part of them might be involved in photosensitizing reactions so that the absorbance at 365 nm is written:

$$A = A^{\mathrm{PC}} + A^{\mathrm{NPC}} \tag{2}$$

where A is the total absorbance, A^{PC}, the absorbance of photosensitizing chromophores, and A^{NPC} is the absorbance of non-photosensitizing chromophores. In the rate expression (1), I_{a} corresponds to the amount of light absorbed by the photosensitizing chromophores and is equal to:

$$I_{\mathrm{a}} = \frac{I_0 \times A^{\mathrm{PC}}}{A} \times (1 - 10^{-A}) \tag{3}$$

As a result we would get:

$$k = \frac{I_0 \times A^{\mathrm{PC}}}{A} \times (1 - 10^{-A}) \times \alpha \tag{4}$$

Hence, under given illumination conditions, k is expected to depend on the absorbance of HA at the excitation wavelength, on the ratio A^{PC}/A and on the kinetic characteristics of the oxidant triplets (Φ and τ). Let us now consider the case of fractions. Each of them contains a part of the absorbing centres initially present in bulk HA. Spectral data show that these parts vary from a fraction to the other. SEC-PAGE fractionation did not yield a random distribution of chromophores among fractions, but it concentrated absorbing centres in the lower MS fractions. For performing photosensitizing tests, we chose to work at constant concentration of fractions and of HAs (25 mg L^{-1}) and thus at different absorbances. In order to have access to the product $A^{\mathrm{PC}}/A \times \alpha$, it is necessary to correct k values for $1–10^{-A}$ or to plot k *versus* $1–10^{-A}$ according to Eq. (4). When one proceeds this

way, one observes that, except in one case, k shows the tendency to increase linearly with $1–10^{-A}$. It indicates that the product $A^{PC}/A \times \alpha$ is relatively constant among fractions and bulk HA of a same series. At the condition that the parameter α does not change very much from a fraction to the other, this result indicates that the ratio of photosensitizing to non-photosensitizing chromophores is roughly constant.

For Andosol, Vertisol and Calcisol series, L-MS samples layed clearly above the mean straight line. This deviation from the linearity indicates that concentration of chromophores is not the only factor influencing the photosensitizing properties of fractions. In particular, localisation of photosensitizing chromophores might be determining. Indeed, oxidant triplets must be accessible to the probe. It seems logical that reactive species are in average more accessible to TMP in L-MS than in H-MS fractions because these latter are of much bigger size than L-MS and are thus more susceptible to contain hydrophobic region in which oxidant species would be embedded.

In the case of bulk HAs, we observed that the increase of k with $1–10^{-A}$ was satisfactorily linear. It would indicate that the ratio A^{PC}/A does not vary very much among all tested HAs. It is a quite amazing result if we take into account the great variety of soil from which HAs have been extracted. The fact that the slopes obtained for the five series varied in a very narrow range is a confirmation of these similarities.

The Spodosol series did not behave as the other series from the photochemical point of view in the sense that L-MS fraction was less photosensitizing than the other fractions although more absorbing. We have no clear explanation for this peculiar behaviour. It is possible that some photosensitizing chromophores were lost from the L-MS fraction during the purification procedure and in particular during dialysis that was performed using membrane with a cut-off of 5000. But this explanation does not match with the fact that L-MS is as absorbant as the L-MS fractions of the other series. As an alternative, the L-MS fractions might contain species scavenging excited states such as paramagnetic cations [14]. The Spodosol soil top layer (A horizon) from which HA was extracted, unlike from all other soils investigated, is enriched up to 50% sesquioxides of Al and Fe [15]. This might influence the photochemical reactivity. This phenomenon needs further investigations.

4. Conclusions

The SEC-PAGE fractionation of soil humic acids yielded fractions showing distinct absorption and photosensitizing properties. As a general rule, absorption and ability of fractions to photosensitize the transformation of 2,4,6-trimethylphenol increased as their molecular size decreased. The analysis of kinetic data revealed that photosensitizing properties are satisfactorily correlated with the amount of light absorbed by the humic material. Fractions of low, medium and high MS would contain the same types of chromophores but in different amounts. By giving information on these parameters, photochemical studies may help to improve the knowledge on humic substances structure.

Acknowledgement

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