Pergamon

Chemosphere, Vol. 38, No. 1, pp. 45–50, 1999

0045-6535/99/ $ - see front matter

PII: S0045-6535(98)00166-0

DIFFERENTIATING WITH FLUORESCENCE SPECTROSCOPY THE SOURCES OF DISSOLVED ORGANIC MATTER IN SOILS SUBJECTED TO DRYING

Adam Zsolnay[1*], Erik Baigar[2], Miguel Jimenez[1], Bernd Steinweg[1] and Flavia Saccomandi[3]

[1]GSF-Forschungszentrum für Umwelt und Gesundheit, Institut für Bodenökologie, D-85764 Neuherberg bei München, Germany

[2]Ludwig-Maximilian-Universität, Institut für medizinische Optik, D-80797 München, Germany and

[3]Università di Napoli „Frederico II", Dipartimento di Scienze Chimico-Agrarie, I-8055, Portici (Na), Italy

(Received in Germany 21 January 1998; accepted 17 April 1998)

Abstract

The relative fluorescence, normalised on dissolved organic carbon (DOC), and a humification index, based on the location of the fluorescence emission spectra, were used to investigate the possible sources of the increase in dissolved organic matter (DOM) when a soil is dried. From these 2 parameters it could be seen that air drying resulted in a minor increase of more humified material in DOM while the effect of oven drying was mainly due to cell lysis. ©1998 Elsevier Science Ltd. All rights reserved

Introduction

There are normally two major sources for DOM in soils. One is the mature organic matter of the soil's matrix (SOM), which is more or less humified. Any change in the state of a soil's pore space, such as from drying, can conceivably alter the amount of this material, which is available in the DOM pool [1]. The second source is freshly introduced material, such as from rhizoexudation or cell lysis, and is not humified. Both of these groups of organic compounds play different ecological roles, reflecting their different chemical structures [1]. A natural or anthropogenic perturbation can cause a quantitative change in DOM. For example, it is well known that air drying modestly, but significantly, increases the amount of DOM, which can be extracted from a soil, while oven drying results in a massive increase [1-2].

Fluorescence has been used to characterise the organic matter in aqueous systems [3-4] and humic substances [5-6] but has not been adequately used for this purpose in the research of soil and sediment DOM. Two basic assumptions are made in the approach presented here: (1) fresh water soluble extracellular material fluoresces per unit of organic carbon quantitatively to a lesser degree than the water soluble

humified material in soil or sediment [7] and (2) the fluorophore containing material fluoresces qualitatively differently in DOM from different sources.

Experimental Section

The spectra were obtained with a simple Hitachi F-2000 Fluorescence Spectrophotometer using 1 cm cuvettes. A typical spectrum is shown in Fig. 1. The pH of a solution can have a strong effect on fluorescence spectra [8-9]. Therefore, in comparison studies, the pH must be held constant. All measurements were done at pH 2, since the samples needed to be acidified for the DOC analyses. The 2 sharp peaks in Fig. 1 were caused by the scattering of the 254 nm excitation light. This gave a usable emission range between 300 and 480 nm. The optical density of the samples at the excitation wavelength was determined with a Hitachi U-2000 Spectrophotometer, and the fluorescence emission corrected by being multiplied with the factor e^A, where A is the absorbance in cm^{-1}. This correction was found to be adequate for a large range of fluorescence measurements. Also, Yang and Zhang [9] reported that emission spectra were less susceptible to concentration errors than excitation and synchronous spectra. Nevertheless, samples were diluted when the absorbance was greater than 0.1 cm^{-1}.

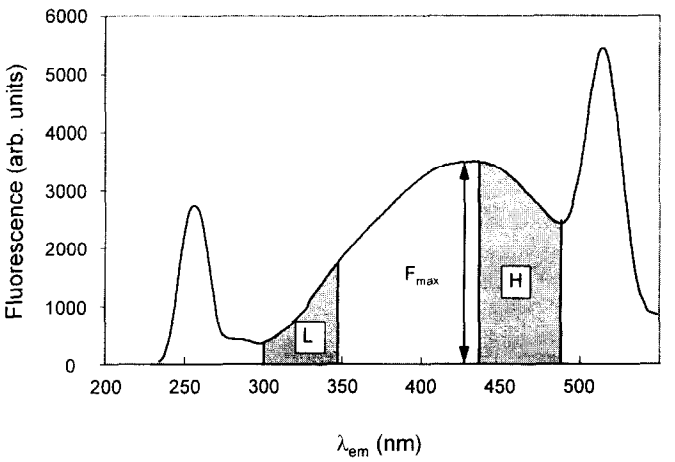

Fig. 1. Typical fluorescence emission spectrum of an aqueous soil extract. The excitation wavelength was 254 nm. „L" represents the lower quarter (330-345 nm) of the usable spectrum, while „H" represents the upper quarter (435-480 nm). See text for details.

The relative fluorescence was defined as being the fluorescence at maximum emission (F_{max} in Fig. 1) divided by the DOC concentration. DOC was measured with a Shimadsu TOC-5050 Total Organic Carbon Analyzer using high temperature, catalytic oxidation. Carbonates were removed by sparging the acidified (pH 2) solutions. In order to avoid arbitrary units, the relative fluorescence was standardised with quinine sulphate dihydrate [10]. A solution of 2.6 mg quinine sulphate dihydrate in 1 litre 0.1 *M* H_2SO_4 was

used, and its emission maximum determined at 450 nm (excitation at 254 nm). This value was then divided by the relative fluorescence. The resulting standardised relative fluorescence therefore has the units mg (quinine sulphate dihydrate) g^{-1} (DOC).

The first assumption stated in the Introduction was tested by comparing the relative fluorescence of aqueous soil extracts to those of 2 extremely different DOM pools obtained from the same soils: (1) the extracellular material released by cell lysis and (2) the fulvic acids. Soil samples were taken from the Ap horizon of 3 soils near Munich. Two were mineral soils, a sandy and a silty loam, with 10 and 11 mg g^{-1} organic carbon, respectively. The other was a clay loam muck with an organic carbon content of 110 mg g^{-1}. They were all at 60% of their maximum water holding capacity. Soil DOM was extracted with a 4 m*M* $CaCl_2$ solution (2:1, volume:oven dry mass equivalent) for 10 min in an overhead shaker, followed by centrifugation at 6000 rpm for 10 min and filtration through a 0.4 μm pore polycarbonate filter [1]. In order to obtain DOM from lysed cells, a portion of each soil was also extracted after having been fumigated with $CHCl_3$ using the method reported by Vance *et al.* [11]. In addition DOC and fluorescence spectra of fulvic acid samples, which were obtained with the usual NaOH-pyrophosphate method [12], were determined.. All extracts were done in triplicate. The results for the silty loam are shown in Fig. 2. As was expected, fumigation, which lysed the biomass, released relatively weakly fluorescing material.

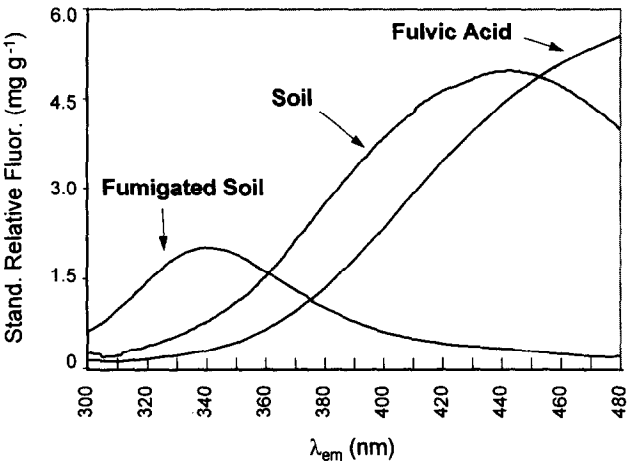

Fig. 2. The standardised relative emission spectra of the silty loam extracts in mg of quinine sulphate dihydrate equivalents per g DOC (see text for details).

The basis of the second assumption in the Introduction was that as fluorescing molecules become more condensed (lower H/C ratios), their emission spectra will tend to shift toward longer wavelengths. The theoretical background is given in Haken and Wolf [13] and can be confirmed by the analysis of literature data (e.g. [14-16]) as well as by the results presented in Fig. 2. Since humification can be considered to be

associated with a decrease in the H/C ratio [12, 17] and with a resulting shift to longer emission wavelengths [18], a humification index was developed on the basis of the location of the emission spectra. The area in the upper quarter („H" in Fig. 1, 435-480 nm) of the usable emission peak was divided by the area in the lower usable quarter („L" in Fig. 1., 300-445 nm). Since this parameter was based on internal measurements, no adjustments were needed either for DOC or optical density.

In order to investigate the effect of drying on DOM, aliquots of the sampled soils were either air dried at 20° or oven dried at 105° C. Afterwards, they were extracted in the same manner as reported above, and the results of the fluorescence measurements of the aqueous extracts of the treated soils were added to those obtained from the field fresh soils, the fumigated soils, and the fulvic acids. A cluster analysis was done on the entire data set after the values had been standardised to zero mean and unit variance. The data were clustered (ellipses) in Fig. 3 when their average squared Euclidean distance was less than 5% of the maximum distance present.

Results and Discussion

Air drying resulted in a minor shift into what can be considered the humified region. Therefore, the conclusion is that this treatment contributed to DOM mainly from increased extraction of soil organic material rather than from biomass lysis. Oven drying resulted in a strong shift into the region indicative of cell lysis. Therefore, one can conclude that the chief source of DOM released by this treatment was destroyed biomass. The fumigated material from the mineral loams differed from that of the fumigated muck samples. This could indicate either a different biomass in the soils or, what is more likely, that different pore structures enabled different lysed biomass fractions to be extracted after fumigation. The treatment of oven drying was so extreme that this difference was eliminated, and the fluorescence properties of DOM from the mineral loams could not be distinguished from those of the muck. Although soils are not oven dried under natural conditions, they can become far more desiccated than through air drying in the laboratory because of the extraction of water by the roots and because of the drying of the soil surface by sun and wind.

Some caution must be used in interpreting results based on net data, such as those presented here. It is obvious that only overall processes can be seen. It is certainly possible that oven drying also releases ecologically relevant humified material into the DOM pool, but the amount of this material is relatively small compared to that introduced from non-humified sources.

In summary a characterisation approach, based on fluorescence, was used to determine the source of DOM released through drying. This approach is rapid and economical compared to other analytical techniques such as FTIR or NMR. It also has the advantage that the samples do not have to be concentrated or separated. Theoretically, this type of analysis with the use of optical fibre technology can also be applied for non-destructive *in situ* studies. Although not all inclusive, it nevertheless provides a tool to investigate

the possible sources of DOM released as a result of perturbations. This approach is also being used to follow the effect of increased atmospheric CO_2 on DOM (unpublished research) and to predict the ability of DOM to co-transport hydrophobic compounds. It could also be used to follow the effects of other perturbations, such as temperature changes, heavy metal contamination, desertification etc.

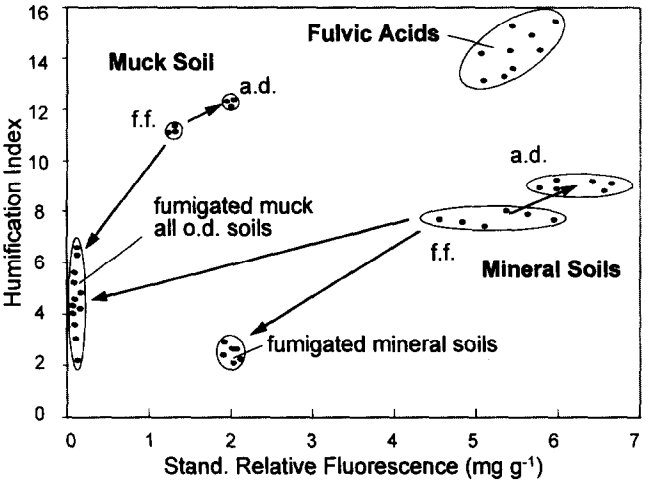

Fig. 3. Plots of the fluorescence attributes of the investigated samples direct from the field (f.f.) and their alteration due to air drying (a.d.), oven drying (o.d.) and fumigation. The transitions are shown by the arrows. Standard relative fluorescence is given in quinine sulphate dihydrate equivalents. See text for details.

Acknowledgements-

This research was partially supported by a grant from the European Commission (EV5V-CT94-0470).

References

1. Á. Zsolnay, Dissolved humus in soil waters. In: *Humic Substances in Terrestrial Ecosystems* (Edited by A. Piccolo). Chap. 4. Elsevier, Amsterdam (1996).
2. Á. Zsolnay and H. Görlitz, Water extractable organic matter in arable soils: Effects of drought and long-term fertilization. *Soil Biol. Biochem.* **26**, 1257-1261 (1994).
3. S.A. Green and N.V. Blough, Optical absorption and fluorescence properties of chromophoric dissolved organic matter in natural waters. *Limnol. Oceanogr.* **39**, 1903-1916 (1994).

4. M.M.D. Sierra, O.F.X. Donard, M. Lamotte, C. Belin and M. Ewald, Fluorescence spectroscopy of coastal and marine waters. *Mar. Chem.* **47**, 127-144. (1994).

5. N. Senesi, T.M. Miano, M.R. Provenzano and G. Brunetti, Characterization, differentiation, and classification of humic substances by fluorescence spectroscopy. *Soil Sci.* **152**, 259-271 (1991).

6. M.J. Pullin and S.E. Cabaniss, Rank analysis of the pH-dependent synchronous fluorescence spectra of standard humic substances. *Environ. Sci. Technol.* **29**, 1460-1467 (1995).

7. R.F. Chen and J.L. Bada, The fluorescence of dissolved organic matter in pore waters of marine sediments. *Mar. Chem.* **45**, 31-42 (1994).

8. R.W.P.M. Laane, Influence of pH on the fluorescence of dissolved organic matter. *Mar. Chem.* **11**, 395-401. (1982).

9. Y.H. Yang and D.H. Zhang, Concentration effect on the fluorescence spectra of humic substances. *Commun. Soil Sci. Plant Anal.* **26**, 2333-2349 (1995).

10. R.A. Velapoldi and K.D. Mielenz, *Standard reference material 936: Quinine sulfate dihydrate*. National Bureau of Standards, Washington, D.C (1979).

11. E.D. Vance, P.C. Brookes and D.S. Jenkinson, An extraction method for measuring soil microbial biomass C. *Soil Biol. Biochem.* **19**, 703-707 (1987).

12. F.J. Stevenson. *Humus Chemistry*. Wiley, New York (1982).

13. H. Haken and H.C. Wolf, *Molekülphysik und Quantenchemie*. Springer, Heidelberg (1992).

14. M. Ewald, P. Berger and S.A. Visser, UV-visible absorption and fluorescence properties of fulvic acids of microbial origin as function of their molecular weights. *Geoderma* **43**, 11-20 (1988).

15. C. Belin, C. Quellec, M. Lamotte, M. Ewald and P. Simon, Characterization by fluorescence of the dissolved organic matter in natural water application to fractions obtained by tangential ultrafiltration and XAD resin isolation. *Environ. Technol.* **14**, 1131-1144 (1993).

16. M.U. Kumke, H.-G. Löhmannsröben and Th. Roch, Fluorescence spectroscopy of polyaromatic compounds in environmental monitoring. *J. Fluor.* **5**, 139-153 (1995).

17. G. Lüttig, Plants to peat: the process of humification. In: *Peat and Water* (Edited by C.H. Fuchsman). Chap 1. Elsevier, Amsterdam (1986).

18. S.-C. Tam and G. Sposito, Fluorescence spectroscopy of aqueous pine litter extracts: effects of humification and aluminium complexation. *J. Soil Sci.* **44**, 513-524 (1993).