

USE OF THE EPR TECHNIQUE TO DETERMINE THERMAL STABILITY OF SOME HUMIFIED ORGANIC SUBSTANCES FOUND IN SOIL ORGANIC-MINERAL FRACTIONS

Sérgio da Costa Saab*

Departamento de Física, Universidade Estadual de Ponta Grossa, Av. Carlos Cavalcanti, 4748, 84030-000 Ponta Grossa - PR

Ladislau Martin-Neto

Embrapa Instrumentação Agropecuária, CP 741, 13569-970 São Carlos - SP

Recebido em 25/7/02; aceito em 27/11/02

In this work, using the EPR spectroscopy, we analysed the thermal stability of some organic-mineral compounds found in a Gleysoil from Rio Janeiro. It was observed a complete disappearance of the EPR signal around 600 °C for the $\leq 2 \mu\text{m}$ fraction and a residual EPR signal of semiquinone free radical for the 2-20 μm and 20-53 μm fractions at the same temperature. Also, the experiments showed that the 2-20 μm fraction had a larger concentration of semiquinone free radical per g of carbon and a smaller line width indicated a larger humification of this fraction. This is an evidence that the soil organic matter of this fraction (2-20 μm) is more stable than the other ones.

Keywords: semiquinone free radical; stabilization; organic matter soil.

INTRODUCTION

An alternative procedure to study soil organic matter is the use of physical fractionation using the ultrasound technique. With this technique, some organo-mineral fractions with different sizes; above 150 μm , 50 - 150 μm , 50-20 μm , 20-2 μm and smaller than 2 μm ¹⁻³ can be obtained. When compared with the solubility method, one of the main advantages of the technique is the capability of obtain information from lighter fractions of soil organic matter (particles larger than 50 μm) and humified organic matter (particles smaller than 50 μm)^{1,4,5}.

Quiroga *et al.*⁶, studying some semi-arid soils from Pampa region of Argentina, showed that the amount of stable organic matter (particles with size lower than 50 μm) is significantly related to the clay content in the soil, and the light organic matter (larger than 100 μm) is strongly influenced by the soil management. Biodegradable and humified organic matter fractions of the soil are differently attacked by microorganisms and some studies indicate that it can be associated to soil intrinsic chemical complexity (chemical stability) and to the association degree of the mineral fraction of the soil (physical stability). According to Tate e Theng⁷, the organic matter resistance to the decomposition is more related to the physical protection of the mineral fraction than the chemical complexity of the organic matter of the soil. The stabilization of soil organic matter, due to interaction with minerals, is probably more important in tropical soils than in temperate soils because of favorable climatic conditions to the decomposition of organic matter. The protecting effect of minerals is more pronounced to constituent labels, as polysaccharides or proteins⁸.

Christensen⁹, applying the incubation and examining CO₂ methods to organo-minerals fractions, showed that the decomposition of the soil organic matter occurs in the following order: sand (larger 53 μm) > clay (smaller 2 μm) > silt (2 - 20 μm). Organic matter from silt fraction (2-20 μm) has a larger resistance to the microbial attack, suggesting that the carbon found in this fraction is more recalcitrant than the one found in clay fractions (0-2 μm).

Using Electron Paramagnetic Resonance (EPR) technique to

investigate litter samples, Tam *et al.*¹⁰ showed a small increase in spin concentration (semiquinone free radical) and a small decrease in line width with increasing sample depth. It suggested a more advanced humification of the litter with depth.

The main objective of this work was to evaluate the stability of the fraction 2 - 20 μm in temperatures above 100 °C and if the EPR spectroscopy was able to evaluate changes in stability.

EXPERIMENTAL

The soil sample analyzed was collected in an area called Região dos Lagos, located in Rio de Janeiro, in the depth of 0-20 cm, horizon A. Sieving by 2 mm and sedimentation analyses followed the physical fractionation of the organo-mineral compounds. After sieved, the samples were submitted to ultrasound, to promote dispersion of the soil aggregates. The ultrasound tune-up was 240 W and the samples were dispersed for 6 min. Our samples were suspensions composed by 20 g of soil and 100 mL of distilled water. The dispersion process was conducted controlling the temperature of the samples using an ice bath. After the dispersion, the 53 μm soil particles were completely separated from the soil suspension (the process was finished when it was verified the absence of soil particles in the water collected through the sieving process). Particles larger than 53 μm were oven dried during 48 h at 50 °C. Particles smaller than 53 μm were placed in tubes for sedimentation analyses. 10 mL of a NaOH solution 0,5 mol L⁻¹ was added to each liter of sample, to promote dispersion of the aggregates of the sample. The Stokes law of sedimentation was used to obtain the particle size distribution of the investigated soil. In this analysis, it was considered $d = 2,65 \text{ g/cm}^3$ for the particle density of the soil² and the collection was made in plastic recipients of 1 L. To increase velocity of sedimentation 10 mL of calcium chloride were added (1 molL⁻¹) to the particles with size smaller than 2 μm after the collection. These samples were also oven dried during 48 h at 50 °C. Different groups of particles were obtained: 53-2000 μm , by sieving in soil suspension, and the fractions 20-53 μm , 2-20 μm and 0-2 μm by sedimentation process³.

In order to investigate temperature effects, the different soil fractions, were heated to 100, 200, 400, 600, 800 and 1000 °C for

*e-mail: scsaab@uepg.br

two hours. After that, the soil fraction stability was investigated using Electron Paramagnetic Resonance (EPR).

EPR spectra were recorded for freeze-dried physical fraction samples using a Bruker – EMX EPR spectrometer with a rectangular cavity operating at X-band frequency (9.4 GHz). Experimental conditions of the EPR experiments were: magnetic field centred at 0.34 T, 0.2 mW microwave power and 0.02 mT amplitude modulation. The relative area of EPR signals was obtained by conventional approximation, intensity \times (line width)^{4,11}. The absolute concentration for semiquinone free radical was obtained using a ruby crystal (as secondary standard) calibrated with strong pitch reference^{4,5}.

RESULTS AND DISCUSSION

The amount of the semiquinone free radical by g of C (Spin/gC) of each fraction and line width (at room temperature) were determined, and the results are shown in the Table 1.

Table 1. Line Width, ΔH , in mT, concentration of semiquinone free radical (SFR) (Spin/gC) $\times 10^{18}$ and Carbon percentage (%C) in each fraction of the soil (Gleysoil sample) at room temperature

Fractions (μm)	0 - 2	2 - 20	20 - 53	>53
ΔH (mT)	0,57	0,37	0,42	0,45
SFR (Spin/gC)	$1,6 \pm 0,1$	$1,9 \pm 0,1$	$1,5 \pm 0,1$	$0,9 \pm 0,1$
%C	11,9	12,5	7,2	10,2

As it can be seen, the fraction 2-20 μm had a larger concentration of semiquinone free radical per g of C and a smaller line width, indicating a larger humification¹⁰. This is an evidence that the soil organic matter in this fraction (2-20 μm) is more stable than the other ones^{1,10}.

The concentration of semiquinone free radical (Spin/g) as a function of the temperature for the fractions < 2 μm , 2-20 μm , 20-53 μm and > 53 μm of the soil are shown in the Figure 1. It is observed that the free radical signal increases in all the fractions for temperatures up to 200 °C. This result can be explained considering that the aliphatic C fraction of the organic matter is decomposed first, increasing the relative concentration of the aromatic C fraction associated to the semiquinone free radicals⁶. Around 400 °C it was found a decreasing of the semiquinone free radical content in all the fractions studied, being the signal associated to the fraction > 53 μm the lowest in this temperature. For the fraction 0-2 μm the complete disappearance of signal was observed at 600 °C. For the fractions 2-20 μm and 20-53 μm , a residual of semiquinone free radical content remained, even at this temperature (600 °C). These results can be explained considering that in the fractions 2-20 μm and 20-53 μm the humified organic matter can be more firmly recalcitrant or linked to the mineral (probably in aggregates or clay fractions).

Therefore, the formation of aggregate in this soil is the most likely reason of the stabilization of the organic matter¹².

CONCLUSION

This work showed that the fraction 2-20 μm of this gleysoil, was more stable than the other fractions. Also, formation of aggregate is the most probable reason of the stabilization of the organic matter of this gleysoil.

ACKNOWLEDGEMENTS

The first author would like to thanks Prof. Dr. F. A. M. Cássaro

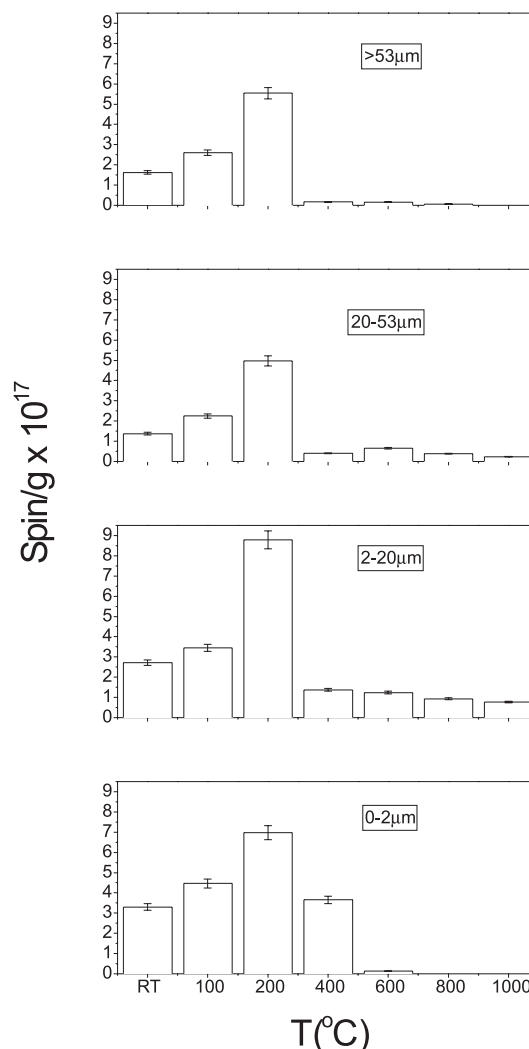


Figure 1. Concentration of semiquinone free radical as a function of the temperature (RT. is the room temperature in this case around 28 °C) in the physical fractions of the soil. The error bar of the measurements is shown

for constructive reviews of this paper. The authors thank M. Conceição from Embrapa-solos, Rio de Janeiro, for Gleysoils samples.

REFERENCES

- Bayer, C.; Martin-Neto, L.; Mielniczuk, J.; Ceretta, C. A.; *Soil and Tillage Research* **2000**, 53, 95.
- Christensen, B. T. In *Advances in Soil Science*; Christensen, B. T.; Springer-Verlag, eds.; New York, 1992, vol. 20.
- Feller, C.; Beare, M. H.; *Geoderma* **1997**, 79, 69.
- Martin-Neto, L.; Andriullo, A. H.; Tragheta, D. G.; *Soil Sci.* **1994**, 157, 365.
- Saab, S.C.; *Tese de Doutorado*, Universidade de São Paulo, Brasil, 1999.
- Quiroga, A. R.; Buschiazzo, D. E.; Peinemann, N.; *Soil Sci.* **1996**, 161, 104.
- Tate, K. R.; Theng, B. K. G. In *Soil with variable charge*; Theng, B. K. G., ed.; Palmerston north, New Zeland, Society of Soil Science, 1980, p. 225.
- Zech, W.; Senesi, N.; Guggenberger, G.; Kaiser, K.; Lehmann, J.; Miano, T. M.; Miltner, A.; Schroth, G.; *Geoderma* **1997**, 79, 117.
- Christensen, B. T.; *Soil Biol. Biochem.* **1987**, 19, 429.
- Tam, S. C.; Sposito, G.; Senesi, N.; *Soil Sci. Soc. Am. J.* **1991**, 55, 1320.
- Bayer, C.; Martin-Neto, L.; Mielniczuk, J.; Saab S. C.; Milori, M. P. D.; Bagnato, V. S.; *Geoderma* **2002**, 105, 81.
- Stevenson, F. J.; *Humus Chemistry*, 2nd ed., John Willy & Sons: New York, 1994.