

Characterization by Electron Paramagnetic Resonance of Organic Matter in Whole Soil (Gleysoil) and Organic-Mineral Fractions

Sérgio Costa Saab^{*,a} and Ladislau Martin-Neto^b

^aDepartamento de Física, Universidade Estadual de Ponta Grossa, 84031-510 Ponta Grossa-PR, Brazil

^bEmbrapa Instrumentação Agropecuária, CP 741, 13560-970 São Carlos-SP, Brazil

A espectroscopia de Ressonância Paramagnética Eletrônica (EPR) foi aplicada em sete amostras de solos (Gleissolos), com o objetivo de quantificar o grau de humificação da matéria orgânica destes solos e comparar com as suas respectivas frações físicas. O sinal dos radicais livres do tipo semiquinona foi detectado e quantificado identificando o grau de humificação da matéria orgânica do solo inteiro que correlacionou com a humificação da matéria orgânica de suas frações.

Electron Paramagnetic Resonance (EPR) spectroscopy was applied in whole soil samples (Gleysoil). The objective of this work was to quantify the degree of humification of the organic matter of the soil, whole soil (Gleysoils) without any chemical treatment, and to compare with its organic-mineral fractions. The signal of semiquinone free radical was detected and quantified showing close relationship with its organic-mineral fractions.

Keywords: free radical, humification, organic-mineral fractions, whole soil

Introduction

The importance and need of studies of the dynamics of the organic matter of the soil are consent in different areas involving soil researchers and environmental sciences.¹ However, the complexity and difficulty for a good understanding of the organic matter in soils and other ecosystems are also accepted.²

The most used procedure for studies of organic matter of the soil is the chemical extraction based on the characteristic of solubility of the humic substances, humic acid, fulvic acid and humin.³ But it can have limitations in the product generated due to the drastic chemical treatment used in its isolation that can promote alterations in the fractions of their isolation matter of the soil.

An alternative, less aggressive than the chemical extraction, is the sorting out of the organic-mineral fractions using supersonic sound waves to disperse soil particles with different sizes, above 53 μm , between 53-20 μm , 2-20 μm

and smaller than 2 μm .⁴⁻⁶ An advantage of this procedure is the possibility of also obtaining information of the light fraction of the organic matter of the soil (particles with smaller size than 53 μm) as well as of the humification fraction of the organic matter (particles with smaller size than 53 μm).^{5,6}

The characterization of these samples was accomplished by the elemental analysis (CHN) and then spectroscopic methodologies such as Nuclear Magnetic Resonance (NMR),^{7,8} Electron Paramagnetic Resonance (EPR)⁹⁻¹¹ and Infrared with Fourier Transform (FTIR).¹² Recently Milori *et al.*¹³ showed that can estimates the degree by Laser- Induced Fluorescence in whole soil. However, the use of some of these spectroscopies in the organic-mineral fractions or whole soil might be impossible in soils with high content of the ion Fe^{3+} and other paramagnetic species, due to the interference in the data acquisition, mainly in NMR and EPR. In NMR the limitation is due to the reduction of the time of relaxation of the excited nuclei and in EPR it is due to the intense signal associated to the paramagnetic ions that overlap the signal of the semiquinone free radicals.^{10,11,14}

*e-mail: scsaab@uepg.br

Rex made the first investigation by EPR of soil organic matter in 1960.¹⁵ He showed that the single line spectrum (3400 G and 9.5 GHz) obtained from such material was due almost entirely to the base-soluble fraction, humic acid. An EPR spectrum obtained from a sample of whole soil was made by Steelink and Tollin, in 1962;¹⁶ they obtained a broad line due to paramagnetic metal ions.

It was demonstrated that the content of semiquinone free radicals is associated with the humification degree of humic substances,^{17,18} in litter samples¹⁹ and in soil organic-minerals.^{10,11}

The objective of this work was to quantify the degree of humification of the organic matter of a soil, whole soil (Gleysoils) and organic-mineral fractions, through the use of the EPR spectroscopy.

Experimental

Samples

The analyzed soils (Gleysoils) were collected in the area of the Lakes of the State of Rio de Janeiro, Brazil, in the amount of seven samples with content of carbon varying from 26 to 202 g kg⁻¹, in the depth of 0-0.20 m. The agricultural activity in this area became intense, however, in inadequate ways of handling of the soil that has caused alterations in the quality and amount of organic matter of the soil, resulting in an accentuated reduction of the productivity in a short length of time, abandonment of the area and consequent degradation of the organic matter of the soils.

The analysis of carbon was made using the CHNS-OCE-Instruments EAUGER 200. All the analyses were performed three times and the average value of those analyses are presented.

Organic-mineral fractions

Sieving by 2 µm and sedimentation analyses followed the physical fractionation of the organo-mineral compounds. After sieving, 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. They 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^{4,5} 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 soil and the collection was made in plastic recipients of 1 L. To increase velocity of sedimentation 10 mL of calcium chloride were added (1 mol L⁻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 process.³

EPR

The measures of free radicals from the type semiquinone were accomplished using the spectrometer of EPR of Bruker-EMX rectangular cavity, band X (9.0 GHz). The absolute concentration for semiquinone free radical was obtained using ruby as a secondary standard, calibrated with strong pitch reference of known free radical content obtained by Bruker.²¹ Samples of around 20×10^{-6} kg were placed in quartz tubes and the amounts of free radicals were normalized for the mass of the samples.^{11,18} The potency of microwaves used to obtain the signal of the semiquinone free radicals was 0.2 mW and the width of modulation 0.02 mT,²¹ and the measurements accomplished at room temperature (298 K).

Results and Discussion

Table 1 shows results of the content of carbon (C) and humification degree, Spin (g C)⁻¹, of the samples of Gleysoils. The content of C varied from 26 to 202 g kg⁻¹

Table 1. Content of organic carbon and humification degree of organic matter, Spin (g C)⁻¹, of whole soil (gleysoils)

Soil	1	2	3	4	5	6	7
C / (g kg ⁻¹)	202	134	112	110	87	55	26
Spin (g C) ⁻¹ × 10 ¹⁸	1.81	1.74	1.46	1.30	1.38	1.29	1.15

and the degree of humification of organic matter varied from 1.15 a 1.81×10^{18} Spin (g C)⁻¹.

In Figure 1 the spectrum of EPR associated to semiquinone free radicals of the sample 3 is shown. All the other samples, with different contents of carbon, had the spectra of the semiquinone radical similar to the one observed in Figure 1. It is verified that there is no influence of paramagnetic signals in *g ca.* 2, mainly Fe³⁺, being Figure 1 very similar to the signal of the free radical in purified samples of humic and fulvic acids.^{17,18} Figure 2 shows the signal of resonance of Fe³⁺ together with the one of the semiquinone free radical in the whole soil, sweeping the magnetic field from 0 to 500 mT. This illustration shows that there is little amount of paramagnetic metals (being just observed Fe³⁺) in these soils interfering very little in the signal of the free radical.^{5,13}

The signals of Fe³⁺ were observed in *g* = 9, *g* = 5, *g* = 4.3, *g* = 3.3 and *g* = 2. The signals in *g* = 9 and 4.3 are

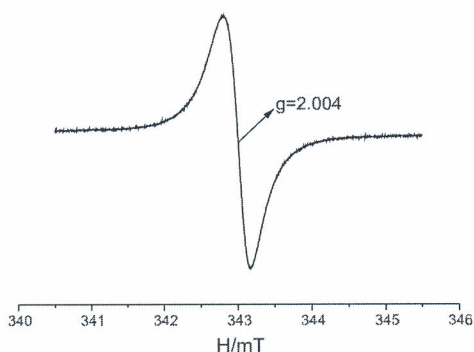


Figure 1. EPR spectrum of the semiquinone free radicals in sample 3 whole soil (Gleysoil).

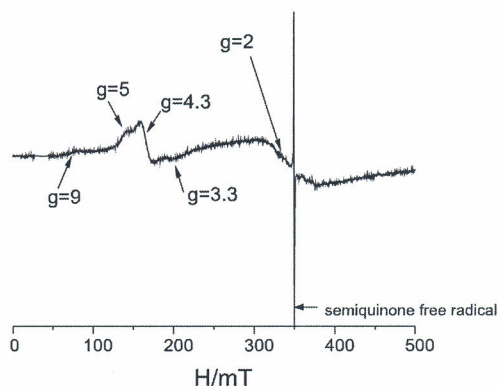


Figure 2. EPR spectrum of the whole soil in microwaves frequency of 9.7 GHz.

due to the ion Fe³⁺ in rhombic symmetry. The values in *g* = 5 and 3.3 are due to the ion Fe³⁺ in higher symmetry²² those signals are due to the mineral material.²³ The signal in *g* = 2 is probably due to the ion Fe³⁺ in the oxide form, that is in the surface of the mineral or organic matter.²²

The possibility of the use of the EPR spectroscopy to detect the semiquinone free radical in whole samples of gleysoils was due to combination of the high content of carbon with the reduced amount of paramagnetic ions (especially Fe³⁺) in these samples as shown in Figures 1 and 2. Also, the appropriate use of experimental parameters as potency of microwaves and modulation width are certainly the additional factors that allowed the measurements in whole samples of gleysoils.^{6,20}

Quantification of the free radical (Figure 3), Spin (g C)⁻¹, of each one of the organic-mineral fraction of each soil, showed that the smaller fractions than 53 μm

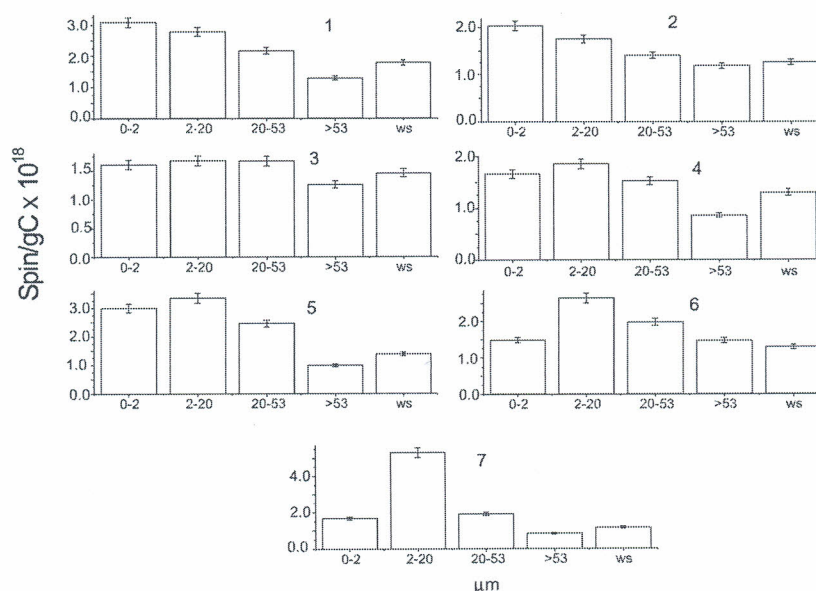


Figure 3. Comparison of the amount of free radical in the fractions and in the whole soil, free radical for mass of carbon of the samples. The fractions are in μm and ws is the whole soil of the respective fractions. The errors bars of the measures are shown.

Table 2. Percentage of the organic-mineral fraction (g per 100g) in each soil

Soil	1	2	3	4	5	6	7
Fractions / μm	Percentage						
0-2	30.5	30.2	56.7	38.0	61.5	63.4	54.9
2-20	33.9	38.6	26.2	24.3	28.5	21.1	17.7
20-53	9.5	7.1	4.9	7.4	3.0	6.2	14.3
>53	25.9	24.1	12.3	30.2	7.0	9.3	13.1

always contains larger amounts of free radicals. Similar result were obtained by Martin-Neto *et al.*¹⁰ studying the cultivation effects in a soil of mollisol type, attributing that result to the enhanced humification in the smaller fractions than 53 μm . Bayer *et al.*,⁵ studying organic-mineral aggregates, also obtained a larger amount of free radicals in the smaller fractions than 53 μm and they suggested that the aromaticity of the organic matter (due to the larger amount of free radicals) can be one of the decisive factors of its stability, not discarding however the contribution of the physical stabilization (organic-mineral) in those fractions.

The content of free radical in the whole soil is approximately the average of the free radical content from the fractions. That can be evidenced observing Table 2, where the percentage of each fraction is shown for each soil sample. For instance, sample 7 contains a greater amount of free radical in the 2-20 μm fraction (Figure 3), however the respective whole soil contains smaller amount and that difference is probably attributed to the fact that this fraction corresponds to only 17.7% of the whole soil (Table 2) and being like this more than 82% of the soil are in the other fractions that contribute less to the signal of the free radical in the whole soil. Sample 3 contains about 83% in the fractions smaller than 20 μm where are located the largest amount of free radicals, and therefore that the sample of whole soil has close values closer to the fractions smaller than 20 μm .

Figure 3 shows that there is a direct correlation between amount of semiquinone free radical in the whole soil and in the organic-mineral fraction of each soil.

The relationship between semiquinone free radicals and the degree of humification of the humic substances has been dealt with in the literature by several researchers.^{9,17-19} In this experiment it was possible to determine the degree of humification of the organic matter in whole soils, without any chemical treatment. Considering that the information is presented in Spins (g C)⁻¹, the values of the order of 10¹⁸ (Table 1) are indicative of the high humification of these soils, because these values are similar to the ones found in

samples of humic substances^{17,18} and higher than observed in litter samples,¹⁹ of the order of 10¹⁷ Spins (g C)⁻¹.

Conclusions

The combination of the high content of carbon with the reduced amount of paramagnetic ions (mainly Fe³⁺) allowed the detection and quantification of the semiquinone free radicals, through Electron Paramagnetic Resonance (EPR) spectroscopy in whole samples of gleysoils and its organic-mineral fraction.

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