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Article

Studies of Semiquinone Free Radicals by ESR in the Whole Soil, HA, FA and Humin Substances

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Nesse trabalho foram realizadas análises espectroscópicas por ESR da matéria orgânica de solos (gleissolos), do Rio de Janeiro, incluindo avaliações nas macromoléculas de ácido humico, ácido fulvico e humina. O nível de radicais livres semiquinona e a largura de linha das frações humina correlacionaram melhor com o solo inteiro.

In this work it was studied soil organic matter from a gley soil, using ESR (electron spin resonance spectroscopy). The studied soil samples were collected in Rio de Janeiro, Brazil. Humic and fulvic acid, humin macromolecules, and whole soil samples were analyzed. The results showed that the amount and line width of semiquinone free radical from whole soil samples had good correlation with humin fraction and no correlation with humic and fulvic acids contents.

Keywords: semiquinone free radical, acid humic, fulvic acid, humin, whole soil

Introduction

Soil organic matter (SOM) is a mixture of simple and complex organic compounds including macromolecular structures usually classified as humic acid (HA), fulvic acid (FA) and humin (HU). They are basically classified in relation to their solubility in alkali and acid.[1] The use of conventional chemical methods and advanced physico-chemical techniques including capillary electrophoresis, fourier transform infrared (FTIR), fluorescence, electron spin resonance (ESR) and nuclear magnetic resonance (NMR) spectroscopies have allowed the elucidation of several aspects of the structures, functionalities and reactivity of HA.[2-6]

ESR can detect free radical compounds, determine its concentration and, sometimes, their origin. Humic substances contain very stable semiquinone free radicals that are supposed to be related with polymerization-depolymerization reactions and to interact with pesticides and toxic poluents.[7,8] The semiquinone free radicals (spins), which are believed to be stabilized by condensed aromatic structures,[9-11] have been associated with humification degree of soil humic substances.[5,6,12]

Tam *et al.*,[13] using ESR technique to investigate litter samples, showed a small increasing in spin concentrations (semiquinone free radical) and a small decreasing in line width to the depth of the sample. This fact suggested a more advanced humification of the litter in relation to the depth of the sample is found in soil profiles.

Martin-Neto *et al.*[5] showed that the spin concentration of the HA samples exhibits a highly significant positive correlation with mean annual rainfall in samples from the Argentine Pampa.

Generaly, studies of humic substances [(HA), (FA) and (HU)] concentrate in one of its fractions, usually HA. The FA(s) fraction, many times appear in small amounts in many soils, and are related to structural aspects.[14] In litter studies, it was found that is difficult to dissociate humin fraction from the mineral part of the soil and, consequently, very few information from this fraction can be available.

This work studied and compared the fractions of Humic Substances (HA, FA and HU), and Whole Soil (WS) samples through ESR spectroscopy.

Experimental

Samples

The analysed Gley soil, seven samples from horizon A, 0- 20 cm layer, were collected in Rio de Janeiro – Brazil, in a region named “Região dos Lagos”. The percentage of carbon of these samples is show in Table 1.

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Table 1. Percentage of organic carbon in the soils samples

Soil	1	2	3	4	5	6	7
C%	20.2	13.4	11.2	11.0	8.7	5.5	2.6

Extraction of humic substances

The extraction of humic substances from the soil samples was made followng the methodology suggested by International Humic Substances Society (IHSS),[15] using NaOH (0.1mol L^{-1}) as extraction agent. The extracted HA was dialyzed against water and silver nitrate was used to test for removal of excess chloride ions.[16] The resulting sample was stored as a homogenized freeze-dried powder. The ash content of HA was about 6.0 %. The fraction FA was passed in the resin XAD 8 in pH approximately 2 and washed with NaOH (0.1 mol L^{-1}). The obtained fractions were HA, FA and HU, all were dried in pH around 4.0. The fractions HU and FA were not purified so have high ash content. The elemental analysis (C) of this sample was done using Carlo Erba equipment.

ESR spectroscopy

ESR spectra were recorded for freeze-dried WS, AH, AF, and HU samples using a Bruker – EMX EPR spectrometer with a rectangular cavity operating at X-band (9.4 GHz). Experimental conditions for the ESR experiments were: magnetic field centred at 0.34 T, 0.2 mW microwave power, and 0.02 mT amplitude modulation. 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.[6]

Results and Discussion

The spectra of semiquinone free radical sign to all investigated fractions are shown in Figure 1. Higher amount of soil organic carbon and the reduced quantity of paramagnetic ions permitted us to obtain very good spectra to whole soil samples, as well as to all humic fractions.[17] The signs were similar to all the samples and, the obtained g value, approximately 2.004 (see Figure 1), shows that the paramagnetic species (whole soil and HA, FA, HU fractions) have the same origin.[18] In other words, they were originated from semiquinone free radical.[11]

In Table 2, values of line width and the amount of free radical per mass are shown. It can be seen that the largest value of the line width was observed to FA and the smallest value was observed to HU. This result can be explained by knowing that the semiquinone free radical in FA is less protected, permiting it to interact with its neighbors what decreases its relaxing time, and increases its line width. This conclusion was also confirmed using potency saturation measurements (the intensity of the sign is proportional $P^{1/2}$ until the limit of the saturation of the sign[19]), as show in Figure 2. Potency saturation is smaller for HU, indicating a larger time of relaxation and a smaller value associated to the line width.[20]

The line width value, ΔH, for HU was smaller in all the samples when compared with the other humic fractions, and also close to value related to the whole soil (Table 2).

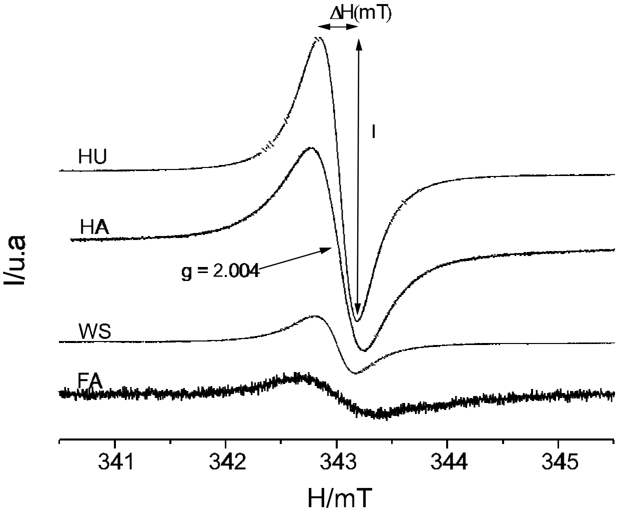

Figure 1. EPR spectra of semiquinone free radicals in HA, FA and HU fractions and, Whole Soil (WS). H indicating line width and I the intensity of the signal.

Table 2. Line Width (ΔH) in Gauss and the amount of free radical (FR) in 10^{17} (Spin (S) / g) to the whole soil (WS), HA, FA, and HU fractions

Samples	1	2	3	4	5	6	7
WS							
ΔH	3.7	4.13	3.93	3.98	4.16	3.95	3.79
FR	3.62	1.69	1.63	1.43	1.21	0.71	0.23
HU							
ΔH	3.38	3.93	3.78	3.71	4.12	3.87	3.38
FR	10.1	3.71	1.79	2.04	4.1	0.66	0.49
HA							
ΔH	4.66	4.68	4.22	4.57	4.37	4.48	4.62
FR	4.40	6.05	9.47	5.61	9.32	4.92	3.92
FA							
ΔH	6.54	5.56	6.41	5.50	5.42	6.36	5.42
FR	0.37	0.09	0.21	0.07	0.08	0.23	0.13

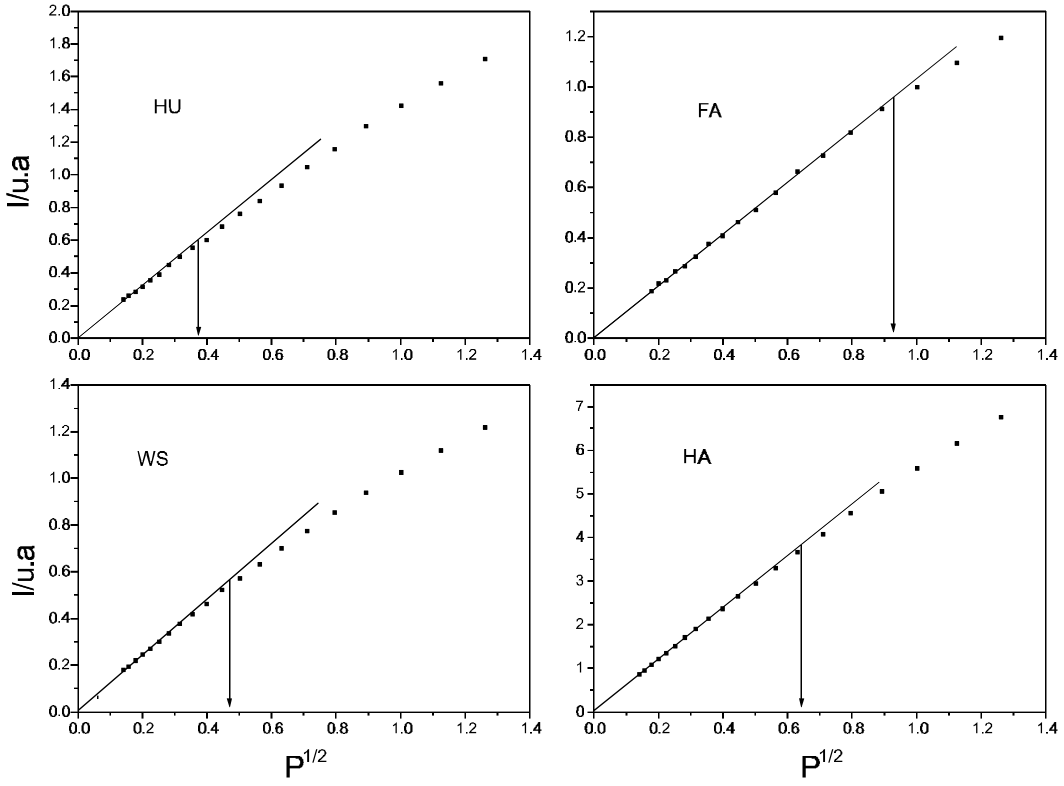

Figure 2. Potency saturation of the sample 3 (whole soil (WS), HA, FA, and HU).

It suggests that the humin is the largest fraction for gleysoil, and produces the largest contribution for the sign of free radical in the whole soil sample.

It can be observed in Table 2 that the fraction AH is the fraction associated to the largest amount of free radicals. It is because the fractions HU and AF were not purified and therefore, possess as the smallest amount of C. Table 3 shows the amount of free radical per gram of C in the whole soil and in the HU, AH and AF fractions. Table 3, shows the normalized amount of C of each fraction. It can be seen that AF possesses $10^{16} – 10^{17}$ spin/gC while AH and HU possesses about $10^{17} – 10^{18}$ spin/gC, (it was found that HU exhibit the largest amount of all the investigated fractions). It can be explained because the fraction HU has a larger molecular mass and high condensation degree. It means that HU has a larger amount of free radicals.[1,11] In Figure 3, the large value of r (r=0.91) shows a good correlation between semiquinone free radical and humin quantities. The amount of semiquinone free radical of the whole soil samples had direct correlation to humin fraction but no correlation to humic and fulvic acids (Figure 3).

Tabela 3. Free radicals for gram of C. (x 10^{18}), in the whole soil (WS) and in its fractions (HU, HA and FA)

Samples	1	2	3	4	5	6	7
WS	1.81	1.74	1.46	1.30	1.38	1.29	1.15
Fractions							
HU	7.30	3.30	2.50	2.85	5.70	2.35	2.90
HA	0.94	2.05	2.05	0.97	1.10	1.30	0.81
FA	0.33	0.089	0.20	0.044	0.088	0.20	0.13

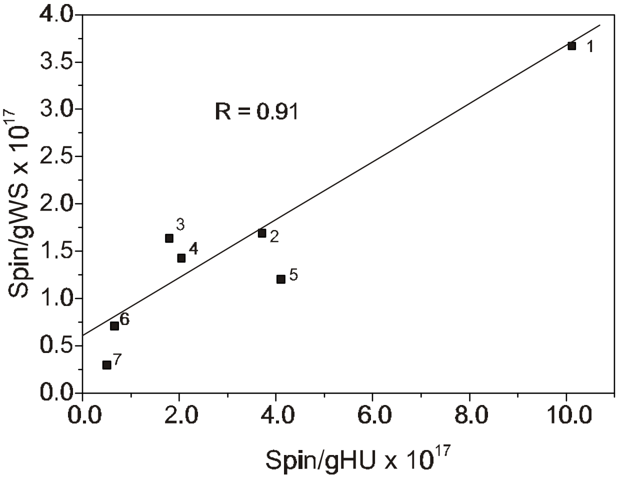

Figure 3. Graph showing the correlation among amount of semiquinone free radical among Whole Soil (WS) and Humin (HU). The numbers identify the samples.

Conclusion

ESR measurements from whole soils, and macromolecule humic acids, fulvic acids and humin (obtained by chemical fraction (for solubility) of the soil) shown that humin is the fraction with higher amount of free radicals in the investigated soil (Gley soil). This result could be confirmed based on Figure 3, that show a good correlation (r=0.91) between free radical contents to the whole soil and humin fractions. The free radical line width to the whole soil and HU, had a very similar behavior, indicating that HU is the predominant fraction in to these soils.

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