ELECTRON PARAMAGNETIC RESONANCE AND THERMO-GRAVIMETRIC CHARACTERIZATION OF HUMIC ACIDS IN NUTRIENT-RICH SOILS FROM THE RAPOSA-SERRA DO SOL INDIAN RESERVE, RORAIMA, BRAZIL

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SUMMARY

Little is known about the organic matter status in nutrient-rich cultivated soil in Amazonia since most studies dealt with slash-and-burn nutrient-poor soil. In Roraima (north Amazonia) Macuxi Indians have cultivated food crops (maize, manihoc and beans) on nutrient-rich Alfisols for many years in the Flexal indian community. The attributes of humic acids fraction from Chernosols under different land uses in the Raposa Serra do Sol Indian reserve were characterized. The humic substances were extracted according to procedures recommended by the International Humic Substances Society and characterized by means of elemental analysis, thermogravimetry, and electron paramagnetic resonance (EPR). The cultivated Chernosol, under long-term burning practices, showed increasing production of oligomers of humic acids, indicated by the largest concentration of semiquinone-type free radicals. High fertility uncultivated soils in fallows showed less concentration of semiquinone-type free radicals compared with cultivated soils and less fertile soils from the same area. Positive correlations were obtained between the values of semiquinone-type free radicals concentration and the thermogravimetry indexes for temperature >350ºC. The most weathered soils and those under more intense land use showed an overall higher degree of humification.

Introduction

Soil management practices can affect the natural ecosystem equilibrium, modifying the organic compounds both in quality and amount, resulting in different chemical and physical dynamics from the original conditions, with positive or negative alterations (Stevenson, 1994). Changes in the natural ecosystems depend on anthropogenic actions such as burning and cultivation, and can affect the soil nutrient cycling and soil fertility (Cerri *et al*., 1996), even in the indigenous areas. These changes, in turn, will also affect the composition of soil organic matter, notably the humic substances (Melo, 2002; Melo *et al.,* 2010). The changes can further lead to significant modifications in pedoclimate, enhancing CO_2 emissions (Fearnside, 1997), depending on the magnitude of the impacts.

Humic substances (HS) are natural organic compounds that influence the physical, chemical and biological soil properties (Stevenson, 1994). They represent important carbon and nutrients stocks for plants (Mangrich, 2001) and are characterized by various structures and reactions. These HS are separated based on their solubility under different pH values, and can be classified as humin, humic and fulvic acids (Swift, 1985; Camargo *et al*., 1999). Quantitative and qualitative studies of HS are made through fractionation and purification, by physical and chemical processes, followed by spectroscopic characterization (Stevenson, 1994). Humic acids (HA) are organic compounds with prevalence of aromatic structures of high complexity that present various different forms in soils and waters (Jonas and Kozler, 1995).

The methods to evaluate the degree of humification of the soil are still up to debate, because there is not a clear definition of the HS structure (Piccolo, 2001). However, HS are usually studied through indirect methods that indicate the occurrence of structural changes during the humification process. Several techniques have been developed to characterize the degree of humification of soil organic matter, such as measurement of the E4/E6 ratio

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CARACTERIZACIÓN DE ÁCIDOS HÚMICOS DE SUELOS EUTRÓFICOS EN LA RESERVA INDÍGENA DEL FLECHAL, RORAIMA, BRASIL, POR RESONANCIA ELECTRÓNICA PARAMAGNÉTICA Y TERMOGRAVIMETRÍA

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RESUMEN

Poco es conocido sobre el estado de la materia orgánica en los suelos cultivados que presentan alta fertilidad de la Amazonia, considerando que la mayoría de los trabajos se han desarrollado en tierras de fertilidad baja en los sistemas de agricultura de tala y quema. En Roraima los indígenas Macuxi han desarrollado cultivos en Nitosoles, Chernosoles y Cambisoles de fertilidad alta durante muchos años, en la comunidad flexal. Esta práctica puede provocar alteraciones en las substancias húmicas de los horizontes Chernozêmicos en diferentes sistemas de uso del suelo en la Reserva Indígena Raposa Serra do Sol. Las sustancias húmicas se extrajeron de acuerdo con los procedimientos recomendados por la Sociedad Internacional de Sustancias Húmicas y fueron caracterizadas usando análisis elemental, termogravimetría y resonancia electrónica paramagnética. El Chernosuelo cultivado, con quemas de largo historial, mostró incremento de la polimerización de ácidos húmicos, indicada por una mayor concentración de radicales libre del tipo semiquinona. Los suelos no cultivados y de buena fertilidad mostraron menor concentración de radicales libre del tipo semiquinona comparado con los suelos de más baja fertilidad y los suelos cultivados. Correlaciones positivas fueron obtenidas entre la concentración de radicales libres y los índices termogravimétricos en temperaturas >350ºC. Los suelos más intemperizados y con mayor intensidad de uso presentaron mayor grado de humificación.

CARACTERIZAÇÃO DE ÁCIDOS HÚMICOS DE SOLOS EUTRÓFICOS DA ÁREA INDÍGENA DO FLECHAL, RORAIMA, BRASIL, POR RESSONÂNCIA PARAMAGNÉTICA DE ELÉTRONS E TERMOGRAVIMETRIA

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RESUMO

Pouco é conhecido sobre o comportamento da matéria orgânica nos solos cultivados da Amazônia que apresentam alta fertilidade, considerando que na sua maioria, os trabalhos têm sido desenvolvidos em solos de baixa fertilidade em sistemas de agricultura de derruba e queima. Em Roraima os índios Macuxi desenvolvem seus cultivos (milho, mandioca, feijão) em Nitossolos, Chernossolos e Cambissolos de alta fertilidade por muitos anos, na comunidade indígena do Flexal. Esta prática pode provocar alterações nas frações húmicas de horizontes chernozêmicos em diferentes sistemas de uso do solo na área indígena Raposa Serra do Sol. As substâncias húmicas foram extraídas de acordo com procedimentos recomendados pela Sociedade Internacional de Substâncias Húmicas e caracterizadas usando análise elementar, termogravimetria e ressonância paramagnética de elétrons. O Chernossolo cultivado, com queimar de restos culturais, mostrou incremento da polimerização de ácidos húmicos, indicada pelos maiores valores na concentração de radicais livres do tipo semiquinona. Os solos não cultivados e de melhor fertilidade mostraram menor concentração de radicais livres do tipo semiquinona comparado com solos de mais baixa fertilidade e solos cultivados. Foram obtidas correlações positivas entre os valores de concentração de radicais livres e os índices thermogravimétricos em temperatura >350ºC. Os solos mais intemperizados e com maior intensidade de uso mostraram maior grau de humificação da fração ácido húmico.

(the ratio of optical absorbance at 465nm and 665nm in aqueous extract; Chen, *et al.*, 1977; Stevenson, 1994), aromatic C content by CP-MAS 13C nuclear magnetic resonance (Preston, 1996; Ykeya *et al.*, 2004), condensed or substituted aromatic rings with a large π-electronic system by UV and visible fluorescence (Milori *et al.*, 2002), and C:H, C:O e C:N ratios (Rosa *et al.*, 2005), amongst others.

Thermogravimetry is based on the identification of the degree of complex formation of humic substances as a function of temperature changes up to combustion (Benites, 2002). This method has been extensively used in the characterization of organic material because of its simplicity. It precludes any special sample pre-treatment, providing information on the thermal behavior and structural properties of HS. Studies regarding the effect of temperature on thermal stability of HS are of particular interest, since thermal pre-treatment has a strong impact on HS water-content, structure, stability, microbial degradability, and solubility (Kolokassidou *et al.*, 2007).

Electron paramagnetic resonance (EPR), a technique of molecular spectroscopy, estimates the degree of humification of organic matter as a function of the concentration of stable semiquinone-type free radicals (Martin-Neto, *et al.*, 1998; Bayer *et al.*, 2002). Complex aromatic structures are believed to stabilize semiquinone free radicals in humic substances (Riffaldi and Schnitzer, 1972; Senesi, 1990; Stevenson, 1994) in coexistence with carbon-centered “aromatic” radicals (Paul *et al.*, 2006), although contributions from methoxybenzene and nitrogen-associated radicals cannot be excluded (Senesi, 1990).

The present study aimed to characterize the soil humic acid fraction properties of nutrient-rich soils under different land use systems by aborigine inhabitants of Roraima, Amazonia, by means of combined studies of thermogravimetry, EPR and elemental analysis.

Materials and Methods

Study area and soil sampling

The studied area is located at 'Maloca do Flechal,' one of the main indian communities of the Raposa Serra do Sol indigenous reserve, Northeastern Roraima, Brazil, at 4°35'10"N and 60°11'25"W (Figure 1).

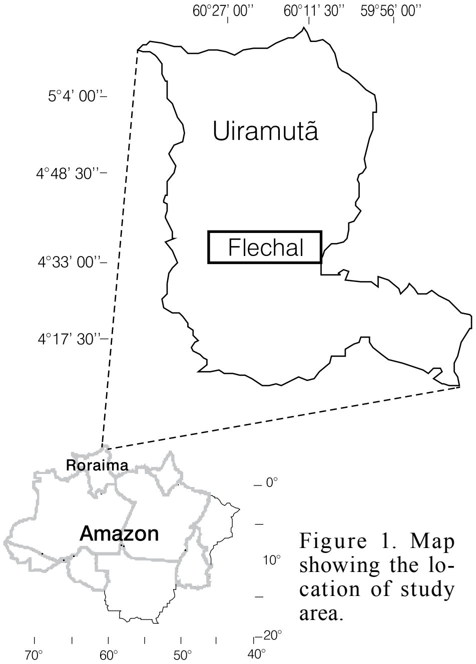

Figure 1. Map showing the location of study area.

Five surface soil horizons were selected, all belonging to soils developed from nutrient-rich mafic rocks (Diabase, Pedra Preta Formation) long cultivated by the local indian community, having shifting agriculture, pastureland and fallows. The climate is characterized by well defined wet and dry seasons, a five month dry season and mean rainfall of 1300mm per year (Pinheiro, 1990).

Soils were collected along a toposequence across the main soils developed from mafic rocks. We observed pedological variations according to topography, land use and management applied, and eventually areas with pasture, slash-and-burn agriculture for more than 20 years by local indians, and a 20 year old fallow, were selected. The five soils were classified, according to the Brazilian system of soil classification (soil taxonomy orders in brackets), as: Eutrophic Red Nitosol (Alfisol); Ortic Ebanic Chernosol (Mollisol); Eutrophic Haplic Cambisol (Mollisol), in lowland; Vertic Orthic Ebanic Chernosol (Mollisol); and Eutrophic Haplic Cambisol (Mollisol), in slope relief.

In each type of different land use, soil pits were dug for morphological description, sampling and classification. Fifteen composite surface samples were collected at 0-10cm depth at each profile, from which we obtained a single sample at each site, used to carry out detailed studies of humic substances.

Soil classification

Soils were classified according to the Brazilian Soil Classification System (Embrapa, 2006). Five soil classes were identified, varying mainly in function of relief, as indicated above and shown in Table I.

In the plain and lowest area, Orthic Ebanic Chernosol occurs, with deeper mollic A horizon richer in organic carbon, being cultivated for >20 years and with annual burning of crop remnants. In the midslope (8% slope) Eutrophic Red Nitosol occurs, being under pasture and with a greater weathering degree. In the upslopes, Eutrophic Tb Haplic Cambisols (Mollisol) and vertic Orthic Ebanic Chernosols (Mollisol) occur on steeper slopes under pasture; there, a reference Eutrophic Tb Haplic Cambisol was covered by a 20 year old fallow, formed by secondary forest. All soils studied were eutrophic, with pH >5.7, high base saturation, and had a comparable mollic A horizon (Chernozemic epipedon).

Extraction and purification of HA

The humic acids (HA) were extracted and purified following the recommendations of the International Humic Substances Society (Swift, 1996). HA were extracted with NaOH 0.1mol·l^{-1} under N_2 atmosphere. After shaking for 24h, the material was centrifuged at 10000g for 30min. The solution was collected and the pH was immediately adjusted to 2.0 with HCl 6mol·l^{-1}. After 18h the fulvic acid fraction was siphoned and discarded. The remaining material was centrifuged at 5000g for 10min and the supernatant was discarded. The precipitate was redissolved in 200ml of NaOH 0.1mol·l^{-1} under N_2 atmosphere and centrifuged at 10000g for 30min. The solution was collected, and pH was immediately adjusted to 2 with HCl 6mol·l^{-1}. The acidified solution was centrifuged at 5000g for 10min. Precipitated HA was treated twice with 0.5% HF+HCl solution for 24h and again centrifuged at 5000g for 10min. The purified samples were washed with 200ml of HCl 0.01mol·l^{-1}, centrifuged at 5000g for 10min and transferred to 100ml cellophane bags, dialyzed and lyophilized.

Elemental analysis

The elemental composition of the HA was determined in two replicates using a Perkin Elmer 2400 CHN analyzer. The C, H and N values were corrected for dry ash free weight, using the amount of moisture and ashes obtained by the thermogravimetric analysis. Oxygen was determined from the difference in the corrected data. The H:C, C:N and O:C atomic ratios were then calculated, using the following equations: NS

$$C:H = (\%C/12) / (\%H/1)$$

$$C:N = (\%C/12) / (\%N/14)$$

$$O:C = (\%O/16) / (\%C/12)$$

Thermogravimetry

The thermal decomposition of HA was studied by means of a TGA-50 SHIMADZU thermogravimetric analyzer using 3.3 ±0.1mg of sample mass. The initial weight was stabilized at 30°C and the heating curve was obtained from 5°C·min^{-1} to 105°C, with a holding time of 10min, followed by heating at 5°C·min^{-1} up to 650°C. The thermal decomposition values were acquired by a microcomputer using the TA-50 WSI standardized according to Benites (2002).

Electron paramagnetic resonance (EPR)

The EPR spectra were obtained in a Bruker EMX spectrometer operating in the X-band (~9GHz) at room temperature. For quantitative analysis, quartz tubes were filled with freeze-dried HA samples, registering their respective masses for posterior data normalization. To obtain

TABLE I
CHEMICAL AND PHYSICAL PROPERTIES IN SURFACE SAMPLES OF STUDIED SOILS

Sample	Soil[a]	pH	CEC	Ca^{2+}	Mg^{2+}	K^+	P	C	Texture (%<2mm)		
		H_2O	..........cmol.kg^{-1}..........				mg·kg^{-1}	%	Sand	Silt	Clay
1	NVe	5.7	12.21	2.63	2.48	0.10	0.96	2.5	19	28	52
2	MEo	6.1	14.84	6.87	3.16	0.15	2.50	2.4	49	23	28
3	CXbe	6.8	14.39	8.69	2.79	0.05	1.33	1.5	34	34	32
4	MEov	7.0	11.88	9.31	0.04	0.05	1.16	2.1	42	29	29
5	CXbe	6.5	10.63	6.75	0.37	0.04	1.22	1.9	37	28	35

[a] NVe: Eutrophic Red Nitosol, MEo: Orthic Ebanic Chernosol, CXbe: Eutrophic Haplic Tb Cambisol, MEov: Vertic Orthic Ebanic Chernosol, CEC: cation exchange capacity.

the areas of the signals, the I×$(\Delta H_{PP})^2$ approximation was used (Poole, 1967), where I is the derivative signal intensity and ΔH_{PP} is the peak-to-peak line width. To determine the relative concentration of semiquinone-type free radicals (in spin/g of C) the signal area of the HA samples was compared with a standard ('strong pitch') of known spin concentration supplied by the manufacturer. A secondary standard, according to Singer's method (Singer, 1959; Martin-Neto *et al.*, 1998) was also used to detect possible alterations in the Q-values of the EPR cavity. The experimental parameters were 0.2mW microwave power, 100kHz modulation frequency and 1 Gauss modulation amplitude.

TABLE II
ELEMENTAL COMPOSITION OF HUMIC ACIDS, ATOMIC RATIO AND OF SPIN/G OF C IN THE SURFACE SAMPLES OF STUDIED SOIL

Samples	Soils	C	H	N	O	C:N	C:H	C:O	Mass of C (mg)	Spin/g of C (×10^{16})
		%								
1	NVe	29.96	2.85	1.92	65.27	18.21	0.88	0.61	7.891	3.73
2	MEo	52.28	3.90	2.82	41.00	21.63	1.12	1.74	15.041	12.10
3	CXbe	29.99	3.49	2.98	63.54	11.74	0.72	0.63	7.286	0.98
4	MEov	33.32	5.30	4.37	57.01	8.89	0.52	0.78	4.118	0.36
5	CXbe	44.33	4.35	3.49	47.83	14.82	0.85	1.99	9.136	1.12

TABLE III
THERMOGRAVIMETRY OF HUMIC ACIDS FROM SOIL SAMPLES OF STUDIED AREA

Samples	Soil	Humidity %	Ash	PPI %		QM ºC
				105-350ºC	350-650ºC	
1	NVe	4.30	0.50	24.43	75.07	627
2	MEo	6.95	0.35	33.24	66.41	647
3	CXbe	4.30	3.40	12.35	84.34	623
4	Meov	2.80	4.40	50.27	49.72	590
5	CXbe	5.10	3.00	40.37	59.63	597

Median values. PPI: loss of weight by burning, QM: maximal temperature for total combustion.

Results and Discussion

Electronic paramagnetic resonance

The quantification of semiquinone-type free radicals, as spin/g of C (Table II), provided information about the qualitative evaluation of the HA of the studied soils. The obtained values of spin concentration varied from 0.36 to 12.10×10^{16} spin/g of C, indicating substantial differences among soil types and land management systems. Larger spin concentration in Chernosol and Nitosol, both under cultivation, corroborate the effect of the agricultural practices and soil fertility on the HA quality. These values are lower than those obtained by Rosa *et al.* (2005) in low-fertility Amazon soils of the Rio Negro basin, demonstrating the influence of the soil chemical attributes in the quality of humic substances, in Amazonia. No EPR data on nutrient-rich soils in the Amazon was found in the literature to allow comparison.

The increasing concentration of semiquinone-type free radicals in the Chernosol under cultivation is consistent with the frequent burning performed just before planting. Long-term cultivation, therefore, systematically increases the HA aromaticity, with greater concentration of semiquinone-type free radicals. This is probably the result of oxidation of the H_2 from OH in phenol groups, according to Riffaldi and Schnitzer (1972), following burning of plant residues. This, in turn, increases the aromaticity of HA, with a decrease of carboxilic groups from aliphatic structures (Almendros *et al.*, 1992). It is postulated that the formation of such "chernozemic" (mollic) A horizon on nutrient-rich soils is the direct result of burning and cultivation, similarly to the process described for Indian Black Earth from Amazonia (Neves *et al.*, 2003; Cunha *et al.*, 2010).

Aromatic structures are more resistant to mineralization processes and can indicate the presence of "black carbon" in areas with high frequency of fires and high oxidation rates (Haumaier and Zech, 1995). This information allows inferences about the organic matter quality in different environmental conditions and its implications to soil genesis. The uncultivated soils (soils 3, 4 and 5), with higher fertility, showed less spin/g of C, resulting in greater nutrient cycling.

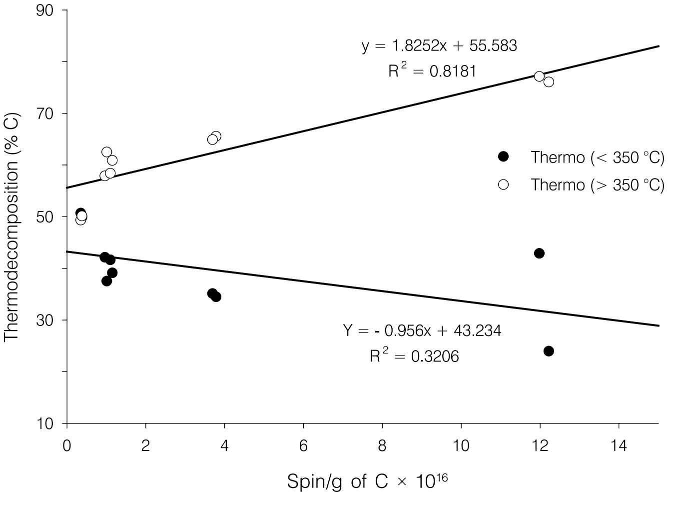

Figure 2. Correlation between the concentrations of semiquinone-type free radicals (in spin/g of C), obtained by EPR, and carbon percentage determined in the combustion by thermogravimetry.

The thermal resistance of HA of surface horizons (Table III) shows different events of decomposition between materials, with well defined phases. The mass losses at temperatures below 350ºC correspond to the decomposition of functional groups of aliphatic structure, while at temperatures >350ºC thermal decomposition of functional aromatic groups occurrs (Mangrich, 2001 and Benites, 2002).

The HA resistance to thermal decomposition indicates composition variations which may occur both in aliphatic and aromatic nuclei. Soil samples 3 and 4, with greater similarity of HA composition, were both nutrient-rich Chernosols with higher CEC and more stable organic matter, with humic substances with a lower degree of polymerization.

The presence of organic structures of high aromaticity was observed in the correlation between the thermogravimetry data and EPR, where a significant positive correlation between the concentration of the semiquinone-type free radicals (spin/g of C) and the thermal decomposition values at >350ºC were detected. A positive correlation between spin/g of C and the percentage of thermal decomposition of HA >350ºC was also obtained (Figure 2), indicating a relation between the resistance to decomposition and complexity of the organic structures in these nutrient-rich soils.

Elemental composition

The analysis of the relative contents of C, H, N and O in the elementary composition of

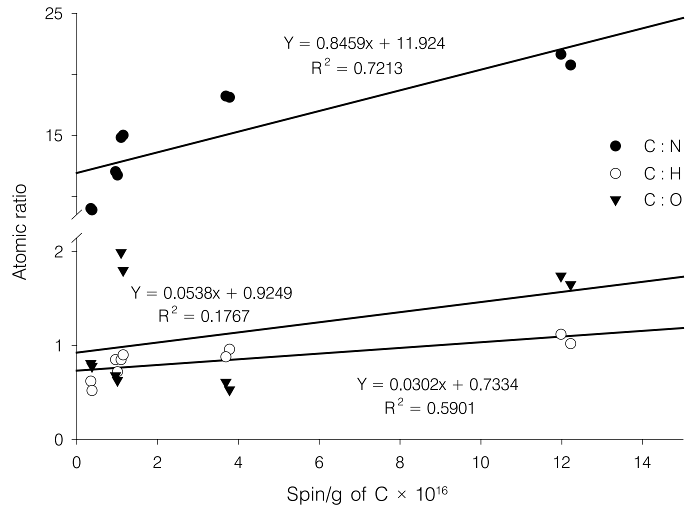

Figure 3. Correlation between the concentrations of semiquinone-type free radicals, given in spin/g of C, and the atomic ratios.

HA revealed its chemical composition and quantity. The atomic ratio has been used to indicate the aromaticity and the mineralization degree of soil organic matter. According to Stevenson (1994), larger values of C:H, C:O and C:N atomic ratios are associated with a higher humification degree and content of carbohydrate, amino acids and protein. Carbon values were lower than the mean values reported by Steelink (1985), and only sample 2 was within the range, with greater carbon and nitrogen contents, compared to the other samples. It indicates an increasing humification degree, as postulated by Canellas *et al.* (1999).

The results indicate that there was a tendency of greater carbon cycling in these Eutrophic soils, retarding humification. This also suggests that in the Chernosols under long-term cultivation and burning, formation of HA with a high polymerization degree was favored (Santos *et al.*, 2001), increasing the stable carbon pool and leading to the formation of mollic (chernozemic) surface horizon by an anthropic effect. Figure 3 reveals a significant correlation between the EPR signal and the C:N ratio. However, there was no correlation between C:H and C:O values and semiquinone-type free radicals concentration. These data are consistent with Rosa *et al.* (2005), who showed a lack of correlation between semiquinone-type free radicals concentration and such ratios; these authors pointed out the need for complimentary studies with high temperature combustion of humic substances to induce the formation of alkaline carbonates ($NaCO_3$) that are stable to temperatures up to 950ºC (Rosa *et al.*, 2005).

Conclusions

The formation of a mollic (chernozemic) A horizon on eutrophic soils from this part of Northern Amazonia has a strong anthropic influence.

Chernosols under cultivation, with burning of crop remnants, showed increasing polimerization of the humic acid fraction, accompanied by consistent high values of semiquinone-type free radicals, obtained by EPR.

The soils of higher fertility, in fallows, with less anthropogenic pressure and not cultivated at present, showed a smaller concentration of semiquinone-type free radicals.

Positive correlations were obtained between the values of semiquinone-type free radicals concentration and the thermogravimetry indexes in temperatures above 350ºC.

The most weathered soils, and with greater land use intensity, showed a higher degree of humification, observed by EPR and confirmed by thermogravimetry.

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