The indigenous areas. These cycling and soil fertility and can affect the soil nutritive or negative alterations in quality and amount, result- ing in different chemical and physical dynamics from the original conditions, with postive 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 af- fect 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 CO2 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 dif- ferent 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 pro- cesses, followed by spectroscopic characterization (Ste- venson, 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 tech- niques have been developed to characterize the degree of humification of soil organic matter, such as measurement of the E4/E6 ratio.

KEYWORDS / Amazon / Humic Substances / Slash and Burn Agriculture / Soil /

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Valdinar Ferreira Melo. Agro- nomist. D.Sc. in Soil and Plant Nutrition, Universidade Federal de Viçosa (UFV), Brazil. Professor, Universi- dade Federal de Roraima (UFRR), Brazil. Address: Depart- ment of Soil and Agricul- tural Engineering, UFRR, 69310-250, Boa Vista, Rorai- ma, Brazil. e-mail: valdinar@yahoo.com.br

Carlos Ernesto G. R. Schaefer. Agronomist. Ph.D. in Soil Science, Reading University, UK. Professor, UFV, Brazil.

Sandra Cátia Pereira Uchoa. Agronomist. D.Sc. in Soil and Plant Nutrition, UFV, Brazil. Professor, UFRR, Brazil.


José Frutuoso do Vale Júnior. Agronomist. D.Sc. in Soil and Plant Nutrition, UFV, Brazil. Professor, UFRR, Brazil.
CARACTERIZACIÓN DE ÁCIDOS HÚMICOS DE SUELOS EUTRÓFICOS EN LA RESERVA INDÍGENA DEL FLECHAL, RORAIMA, BRASIL, POR RESONANCIA ELECTRÓNICA PARAMAGNÉTICA Y TERMogravimetrÍA

Valdir Ferreira Melo, Carlos Ernesto G.R. Schaefer, Sandra Cátia Pereira Uchôa, Marcelo Luiz Simões, Ladislau Martin-Neto y José Frutuoso do Vale Júnior

RESUMEN

Poco es conocido sobre el comportamiento de la materia orgánica en los suelos cultivados que presentan alta fertilidad de la Amazonía, considerando que la mayoría de los trabajos se han desarrollado en tierras de baja fertilidad en los sistemas de agricultura de derruba y quema. En Roraima los indígenas Macuxí han desarrollado cultivos en Nitosoles, Chernossoles y Cambissóis de alta fertilidad en diferentes años, en la comunidad Flexal. Esta práctica puede provocar alteraciones en las sustancias húmicas de los horizontes Chernozémicos 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 queemas 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.

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

Valdir Ferreira Melo, Carlos Ernesto G.R. Schaefer, Sandra Cátia Pereira Uchôa, Marcelo Luiz Simões, Ladislau Martin-Neto y José Frutuoso do Vale Júnior

RESUMEN

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 Macuxí desenvolvem seus cultivos (milho, mandioca, feijão) em Nitossóis, Chernossóis e Cambissóis de alta fertilidade por muitos anos, na comunidade indígena do Flexal. Esta prática pode provocar alterações nas frações húmicas de horizontes Chernozémicos 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 elemental, termogravimetría e resonância 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 termogravimétricos em temperatura >350ºC. Os solos mais intemperizados e com maior intensidade de uso mostraram maior grau de humificação da fração ácido húmico.

(continued from previous page)
Materials and Methods

Study area and soil sampling

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

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 indigenous 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); Orthic 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 mid-slope (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).

Table I.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Soila</th>
<th>pH H2O</th>
<th>CEC</th>
<th>Ca2+</th>
<th>Mg2+</th>
<th>K+</th>
<th>P</th>
<th>C</th>
<th>Texture (%&lt;2mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NVe</td>
<td>5.7</td>
<td>12.21</td>
<td>2.63</td>
<td>2.48</td>
<td>0.10</td>
<td>0.96</td>
<td>2.5</td>
<td>19  28 52</td>
</tr>
<tr>
<td>2</td>
<td>MEo</td>
<td>6.1</td>
<td>14.84</td>
<td>6.87</td>
<td>3.16</td>
<td>0.15</td>
<td>2.50</td>
<td>2.4</td>
<td>49  23 28</td>
</tr>
<tr>
<td>3</td>
<td>CXbe</td>
<td>6.8</td>
<td>14.39</td>
<td>8.69</td>
<td>2.79</td>
<td>0.05</td>
<td>1.33</td>
<td>1.5</td>
<td>34  34 32</td>
</tr>
<tr>
<td>4</td>
<td>MEov</td>
<td>7.0</td>
<td>11.88</td>
<td>9.31</td>
<td>0.04</td>
<td>0.05</td>
<td>1.16</td>
<td>2.1</td>
<td>42  29 29</td>
</tr>
<tr>
<td>5</td>
<td>CXbe</td>
<td>6.5</td>
<td>10.63</td>
<td>6.75</td>
<td>0.37</td>
<td>0.04</td>
<td>1.22</td>
<td>1.9</td>
<td>37  28 35</td>
</tr>
</tbody>
</table>


 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 N2 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 collected, registering the ions Mg2+, Ca2+ and centrifuged at 10000g for 10min. The purified samples were washed with 200ml of HCl 0.1mol·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 replications 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
Results and Discussion

Electronic paramagnetic resonance

The quantification of semiquinone-type free radicals, as spin/g of C (Table II), provided information about the quality of humic substances, with the frequent burning per-

the areas of the signals, the $I_1\Delta H_{pp}$ approximation was used (Poole, 1967), where $I_1$ is the derivative signal intensity and $\Delta H_{pp}$ 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 of the studied soils 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.

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 $>350^\circ\text{C}$ were detected. A positive correlation between spin/g of C and the percentage of thermal decomposition of HA $>350^\circ\text{C}$ 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

**Table II**

<table>
<thead>
<tr>
<th>Samples</th>
<th>Soils</th>
<th>C</th>
<th>H</th>
<th>N</th>
<th>O</th>
<th>C:N</th>
<th>C:H</th>
<th>C:O</th>
<th>Spin/g of C (mg)</th>
<th>QM %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NVe</td>
<td>2.85</td>
<td>1.92</td>
<td>0.52</td>
<td>0.78</td>
<td>1.12</td>
<td>1.74</td>
<td>9.136</td>
<td>0.98</td>
<td>612.0</td>
</tr>
<tr>
<td>2</td>
<td>MEO</td>
<td>3.02</td>
<td>2.12</td>
<td>0.52</td>
<td>0.78</td>
<td>1.12</td>
<td>1.74</td>
<td>9.136</td>
<td>0.98</td>
<td>612.0</td>
</tr>
<tr>
<td>3</td>
<td>CXBE</td>
<td>3.49</td>
<td>2.98</td>
<td>0.52</td>
<td>0.78</td>
<td>1.12</td>
<td>1.74</td>
<td>9.136</td>
<td>0.98</td>
<td>612.0</td>
</tr>
<tr>
<td>4</td>
<td>MEOV</td>
<td>3.32</td>
<td>3.03</td>
<td>0.52</td>
<td>0.78</td>
<td>1.12</td>
<td>1.74</td>
<td>9.136</td>
<td>0.98</td>
<td>612.0</td>
</tr>
<tr>
<td>5</td>
<td>CXBE</td>
<td>4.43</td>
<td>3.45</td>
<td>0.52</td>
<td>0.78</td>
<td>1.12</td>
<td>1.74</td>
<td>9.136</td>
<td>0.98</td>
<td>612.0</td>
</tr>
</tbody>
</table>

**Table III**

<table>
<thead>
<tr>
<th>Samples</th>
<th>Soil</th>
<th>Humidity</th>
<th>Ash</th>
<th>PPI %</th>
<th>QM %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NVe</td>
<td>4.30</td>
<td>0.50</td>
<td>24.43</td>
<td>75.07</td>
</tr>
<tr>
<td>2</td>
<td>MEO</td>
<td>6.95</td>
<td>0.35</td>
<td>33.24</td>
<td>66.41</td>
</tr>
<tr>
<td>3</td>
<td>CXBE</td>
<td>4.30</td>
<td>3.40</td>
<td>12.35</td>
<td>84.34</td>
</tr>
<tr>
<td>4</td>
<td>MEOV</td>
<td>2.80</td>
<td>4.40</td>
<td>50.27</td>
<td>49.72</td>
</tr>
<tr>
<td>5</td>
<td>CXBE</td>
<td>5.10</td>
<td>3.00</td>
<td>40.37</td>
<td>59.63</td>
</tr>
</tbody>
</table>

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

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.
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 stability 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 complementary studies with high temperature combustion of humic substances to induce the formation of alkaline carbonates (NaCO₃) that are stable to temperatures up to 950°C (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 polymerization 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 350°C.

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

REFERENCES


