Hydrophobicity of an Entisol under loblolly pine (*Pinus taeda)* plantation

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Abstract - The understanding of soil carbon stabilization processes can be very useful in the development of mitigation techniques for CO_2 emissions and global warming. The greater the hydrophobicity of soil organic matter the more stabilized soil organic carbon. Therefore, hydrophobicity can be a sensitive index to characterize the 'quality' of soil organic matter. In this context, the present work aimed to characterize the chemical structures of humic acids collected at three different depths in a hydrophobic Entisol (Neossolo) under loblolly plantation. The results of spectroscopic and chemical analyses (UV-Vis, fluorescence, EPR and X-ray diffractometry) indicated that, as soil depth increased, so did the content of conjugated organic structures, aromatic groups, and free organic radicals, leading to higher humification indices. Aliphatic groups in these fractions were more concentrated in the surface layer than in deeper ones, which can be explained by the constant input of litter. The greater hydrophobicity of the surface soil sample was due to these non-humic components of the organic matter, as suberin and cutin.

Index terms: Humic acids, water repellency, forest soil, fluorescence, EPR, X-ray.

Hidrofobicidade em Neossolo litólico sob plantação de *Pinus taeda*

Resumo - Compreender os processos de estabilização do carbono no solo pode ser muito útil no desenvolvimento de técnicas de mitigação das emissões de CO_2 e do aquecimento global. Quanto maior a hidrofobicidade da matéria orgânica do solo, mais estabilizado é o carbono do solo. Portanto, a hidrofobicidade pode ser usada como indicador para caracterizar a qualidade da matéria orgânica do solo. O presente trabalho caracterizou as estruturas químicas de ácidos húmicos extraídos de solos coletados a três profundidades de um Neossolo sob plantação de *Pinus taeda*. Os resultados das análises espectroscópicas e químicas (ultra-violeta, fluorescência, ressonância paramagnética eletrônica e difração de raio-X) indicaram que a ocorrência de estruturas conjugadas, grupos aromáticos e radicais orgânicos livres e, portanto, maiores índices de humificação, aumentaram com a profundidade do solo. Grupos alifáticos na fração húmica estavam mais concentrados nas camadas superficiais, o que pode ser explicado pelo constante aporte de liteira na superfície. Testes da repelência à água apontaram maior hidrofobicidade na superfície do solo, provavelmente devido a componentes não húmicos da matéria orgânica, tais como suberina e cutina.

Termos para indexação: Solos florestais, repelência à água, RPE, fluorescência, raio-X.

Introduction

A mechanistic understanding of soil carbon stabilization can be very useful in developing CO_2 emission and global warming mitigation techniques. This biospheric fraction shelters the largest terrestrial carbon pool, which is estimated at 2,300 Gt C in the top 3 m of soil (Jobbágy & Jackson, 2000; Lorenz et al., 2007). According to Lützow et al. (2006), important processes that can reduce the accessibility of soil organic matter (SOM) to degradation are: occlusion of organic matter (OM) by aggregation; intercalation of OM within phyllosilicates and other inorganic lamellar structures; hydrophobicity of OM; and encapsulation in other organic macromolecules. Therefore, the hydrophobic nature of OM plays an important role in soil carbon protection, organic matter dynamics, microbial biomass production, aggregate stability, water infiltration,

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leaching of organic and inorganic pollutants, and in the chemical composition and dynamics of dissolved organic matter (Capriel, 1997). A higher hydrophobicity of SOM appears to stabilize the soil's organic C, caused either by a specific reduced biodegradability of OM or indirectly by increased aggregate stability (Bachmann et al., 2008). Thus, hydrophobicity can be a sensitive index to characterize the 'quality' of SOM (Capriel, 1997). On the other hand, hydrophobicity or soil water repellency can also lead to the reduced water affinity of soils, causing them to resist wetting for periods ranging from a few seconds to hours, days or weeks (Doerr et al., 2000). Soil water repellency is a common phenomenon in forest soils and is usually attributed to the hydrophobic nature of OM (Doerr et al., 2000; Buczko et al., 2005). In addition to fungal and other microbiological metabolites, many plant species, especially coniferous and resinous trees, produce hydrophobic compounds, resulting in a hydrophobic compounds-rich litter. The compounds identified in water-repellent soils can be divided into two main groups: the long-chain aliphatic hydrocarbons and the amphiphilic hydrocarbons, with both hydrophilic and hydrophobic (structure) ends, which are frequently found in suberin and cutin (Doerr, et al., 2000; Hansel et al., 2008). Recently, Hansel et al. (2008) suggested that large amounts of preserved cutin and suberin in soil could be the principal constituents of the hydrophobic organic layer that prevents the penetration of water in an Entisol repellent soil.

It is well known that SOM is a complex mixture composed mainly of plant residues and excretions, whose chemical composition varies from biopolymers (such as lignin, cellulose, protein, suberin, and cutin) to small molecules (saccharides, organic acids, amino acids, and terpenes), in different phases of decomposition or humification. Considering that OM solubility-based fractionation (humic fractions at different pHs, as in Swift, 1996) does not distinguish between humic and non-humic structures due to operational reasons, any humic acid (HA) fraction shall contain some hydrophobic components originating from plant metabolism without undergoing any chemical transformation. Therefore, studies about hydrophobicity of humic substances are not always associated, to compounds which cause soil water repellency.

Many spectroscopic techniques can be used to chemically characterize SOM. The 465 nm (E_4) and 665 nm (E_6) absorptions, for example, are used to estimate the E_4/E_6 ratio, which is associated with the degree of condensation and conjugation (Chen et al., 1977). The 270 nm (A_2) and 407 nm (A_4) wavelengths have been used to estimate the A_2/A_4 ratio, which is employed to distinguish the marine and terrestrial origin of humic and fulvic acids (Fooken & Liebezeit, 2000). Fluorescence spectroscopy by UV-visible light provides information about the origin, genesis and nature of SOM (Senesi et al., 1991). This technique is also used to identify molecular structures and their functionalities, and the presence of heavy metal and organic contaminants (Senesi, 1990). With its high sensitivity and selectivity, this technique highlights only fluorescent chemical groups. Fluorescence spectra of humic substances result from the sum of the individual spectra of different fluorophore groups that are present in these complex and heterogeneous samples (Milori et al., 2002). Electronic Paramagnetic Resonance provides information about free organic radicals associated with conjugated structures and phenols (Stevenson, 1994), usually from aromatic compounds responsible in part for the hydrophobicity of SOM.

This work aims to characterize the chemical structures of humic acids collected at three different depths in a hydrophobic Entisol under loblolly pine plantation and to correlate the hydrophobic indices determined by different spectroscopic techniques.

Materials and Methods

Soils samples were collected in a 16 year-old loblolly (*Pinus taeda*) plantation at Piraí do Sul, Paraná, in southern Brazil. The soil was classified as a litholic dystrophic Neossolo, according to the Brazilian soil classification system (Sistema…, 1999), as a Lithosol, according to the FAO soil classification system, or Entisol, according with US Soil Taxonomy, medium texture, wavy relief (Sistema…, 1999). Samples were collected at depths of 0 to 5, 5 to 10 and 10 to 30 cm during the autumn of 2005.

Hydrophobicity was measured using the Water Drop Penetration Time (WDPT) test, an easy, quick and widespread method which involves placing a drop of water on a soil surface and recording the time elapsed for its complete penetration (Letey, 1969, cited by Doerr, 1998; Hansel et al., 2008). This test was standardized by placing each soil sample on a Petri dish. Then, using a Pasteur pipette, 4 drops (~ 40μL each) of distilled water

were applied on the soil and the penetration times were measured. The hydrophobicity classification criteria used here were those proposed by Bisdom et al. (1993).

Humic acids (HA) were extracted following the method recommended by the International Humic Substances Society (Swift, 1996), and were characterized spectroscopically by electronic paramagnetic resonance (EPR), fourier transform infra red (FTIR), diffuse reflectance ultraviolet-visible light absorption (DRUV-VIS) and fluorescence, and X-ray diffraction techniques. The DRUV-VIS analyses of solid samples were performed with a Shimadzu UV-2401PC spectrometer, equipped with an integration sphere 240-52454-01 and a solid sample cell holder. FTIR spectra were recorded using a Bomen FTIR MB 100 Spectrophotometer. Pellets were obtained by mixing 1 mg of HA and 99 mg of spectroscopic grade dried KBr. X-ray diffraction analyses were performed using a Shimadzu XRD-6000 diffractometer with Cu radiation, CuKα (α=1,5418 Å), at 40 kV and 40 mA. The range of diffraction angles (10 to 60°, 2θ) was arrayed for 30 minutes. EPR experiments were carried out at room temperature (~300 K) on a Bruker ESP 300E spectrometer operating at a 9.5 GHz frequency (X-band), with a 100 kHz modulation frequency, 202.4 T modulation amplitude and 2 mW microwave power for the HA free radical study. HA free radicals were detected and quantified using the approximation intensity = $(\Delta H_{pp})^2$ at a peak-to-peak line width of ΔH_{pp} (Gonçalves et al., 2000). The areas of the EPR peaks, obtained by integration of the derivative EPR spectra of 5 mT EPR peaks, were calibrated with the peak corresponding to the EPR signal of a reference ''weak pitch'' of known g-value and free radical content, obtained from Bruker. For the fluorescence analyses, the HAs were reduced to a concentration of 20 mg L^{-1} and brought to pH 8 by diluting them in a solution of 0.05 mol L^{-1} $NaHCO_3$.

Fluorescence spectra – emission and synchronous-scan excitation modes – were acquired with a Perkin Elmer LS-50B luminescence spectrophotometer. The emission and excitation slits were adjusted to a bandwidth of 10 nm and a scan speed of 500 nm min^{-1} was selected for both monochromators. To obtain the A_4/A_1 index (Zsolnay et al., 1999), the area of the last quarter of the spectrum (570–641 nm), acquired in the emission mode with excitation at 240 nm, was divided by the area of the first quarter (356–432 nm). The basic proposal of this method is that humification leads to condensed aromatic rings which would shift the fluorescence emission to red. Therefore, the higher A_4/A_1 index the more humified the sample. The A_{465} index (Milori et al., 2002) corresponded to the area of the spectrum acquired in the emission mode with excitation at 465 nm. Spectra in the synchronous-scan excitation mode, acquired with a $\Delta\lambda$ of 55 nm, were used to calculate the I_{454}/I_{399} index (Kalbitz et al., 1999), which was obtained from the ratio of the signal intensity at 454 nm to the signal intensity at 399 nm.

Results and Discussion

The repellency test showed a significant difference between the upper soil layer (0-5 and 5-10 cm) and the 10-30 cm layer (Table 1). The surface layer (0-5 cm), which showed the highest WDPT, was classified as strongly hydrophobic, as was the next layer (5-10 cm). These results suggest that surface layers are strongly influenced by the deposition of hydrophobic compounds, which can be originate from litter or from microbiological ativity.

Table 1. Repellency class indicated by water drop penetration time (WDPT) at different depths of an Entisol.

Depth (cm)	WDPT (s)	Repellency class*
0-5	430	Strongly hydrophobic
5-10	66	Strongly hydrophobic
10-30	1	Hydrophilic

*According to classes proposed by Bisdom et al. (1993), cited by Doerr (1998)

The A_2/A_4, ratio increased with soil depth (Table 2), indicating a higher lignin derivative content (absorption at 270 nm) in the deeper sample. The E_4/E_6 ratio for HAs is usually less than 5.0 and decreases with increasing condensation or organic conjugated structures (Stevenson, 1994; Chen et al., 1977). The HA samples showed a small decrease with soil depth, suggesting a slight increase of their degree of aromaticity.

Table 2. UV-Vis light absorption ratios at 270 and 407 nm (A_2/A_4), and 465 and 665 nm wavelengths (E_4/E_6) of HA, free radical concentration (in spin g^{-1} x 10^{16}), and corrected g-values of HAs samples extracted from three different depths of an Entisol.

Sampling depth (cm)	A_2/A_4	E_4/E_6	Free radical (Spin g^{-1}) X 10^{16}	g-factor
0-5	0.71	1.11	2.96	2.0026
5-10	0.86	1.10	8.11	2.0025
10-30	0.83	1.08	8.57	2.0027

The FTIR spectra of HAs (Figure 1) are typical for this type of material (Senesi, 2003). A strong absorption broad band in the region of 3400 cm^{-1} was attributed mainly to the OH stretching of phenol and alcohol groups. The broadening of these bands is due to the different degrees of hydrogen bonding among the OH groups and oxygen and/or nitrogen atoms of the present chemical structure. Bands at 2918 and 2850 cm^{-1} correspond to aliphatic C-H stretching (-CH2 and -CH_3), and are less evident in the sample from the greatest depth (10-30 cm). This result can be explained by the higher concentrations of compounds originated from cutin and suberin, such as alkanoic, alkanedioic and hydroxyalkanoic acids and alkanols, according to findings obtained previously, which indicated the preservation of suberin and cutin biopolyesters (Hansel et al., 2008). Alkyl C from polymethylenic compounds is among the most biologically stable forms of soil organic carbon (Lützow et al., 2006).

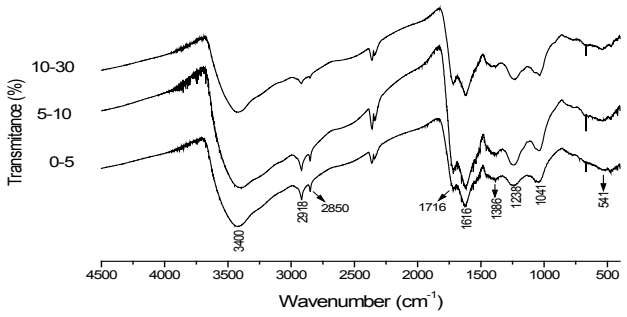


Figure 1. FTIR spectra of humic acid samples from different depths of an Entisol.

The broad bands at around 2,500 and 2,000 cm^{-1} are due the first overtones of 1,238 cm^{-1} (C-O of carboxylic group) and 1,041 cm^{-1} (C-O of carbohydrate), respectively (Janik et al., 2007). The absorption bands at 1,716 (C=O stretching of ketonic and carboxylic groups) and at 1,616 cm^{-1} (aromatic C=C and H-bonded C=O groups) are also present. From 1,400 to 1,370 cm^{-1}, the absorption bands are due to O-H deformation, C-H deformation (-CH_2; -CH_3) and COO^- symmetric stretching. The bands between 1,170 – 950 cm^{-1} correspond to absorptions of C-O stretching from alcohols, and/or phenols and/or carbohydrates and Si-O of silicate impurities (Rosa et al., 2000; Stevenson, 1994). Lastly, the absorptions at 541 cm^{-1} are commonly attributed to H-N bending.

The X-ray diffractograms of HA (Figure 2) of humic materials are typical of very amorphous material but can be used to indicate their stability, aromatic origin, and chemical reactivity. The diffractograms can be divided in two main areas: aromatic structure band at around ~ 2 θ of 25,5° and aliphatic structure band at around 2 θ of 20,5° (Naidja, 2002). The HA 10-30 cm sample has an evidently more intense aromaticity than that of surface samples.

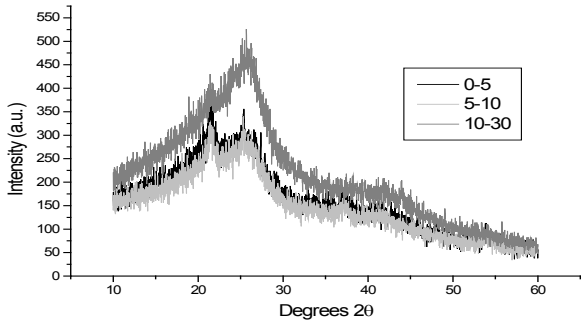


Figure 2. X-Ray diffractograms of humic acids at depths of 0-5, 5-10 and 10-30 (cm) of an Entisol.

The EPR spectra of all the samples in the magnetic field of 505 mT (Figure 3) presented two types of Fe^{3+} ion domains. The first was a diluted domain attributed to isolated Fe^{3+} ions occupying distorted sites of the HA structure, which showed a sharp EPR line (g ~4.3) in a magnetic field lower than 250 mT (Guimarães et al., 2001). The second domain was dominated by

nonhomogeneously broadened lines due to magnetic dipole–dipole interactions among Fe^{3+} ion centers ($\Delta H_{pp} > 50$ mT and $g \geq 2$), which is referred to as a concentrated domain. Such domains can be attributed to spin–spin interactions among ions in the oxy-hydroxides adsorbed in the HA structure.

The organic free radical signal in the EPR spectra (not shown) of HA samples showed g-values ranging from 2.0025 to 2.0027, which are typical of free radicals of aromatic organic structures. The concentration of free radicals per gram of HA (Table 2) increased with soil depth. This result is consistent with the X-ray and DRUV-VIS findings.

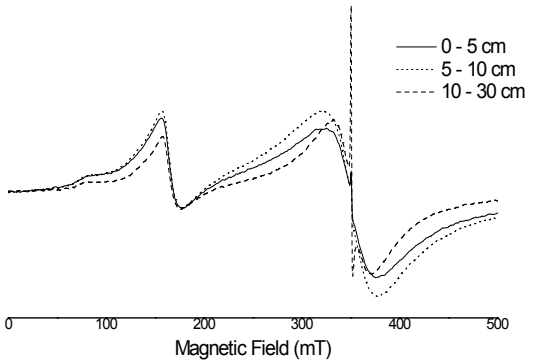


Figure 3. EPR spectra of humic acids extracted from different depths (cm) of an Entisol.

The humification index obtained by fluorescence and calculated by three different methods is accordant (Figure 4). These results showed an increase in the degree of humification down to a depth of 30 cm. Fresh OM from the surface layer contributes to a low degree of humification as a function of the addition of simple structures to the surface soil HA. The degradation and humification processes of organic matter in the first layer, promoted by microorganisms, create more complex structures which possibly migrate to deeper layers. These processes produce an accumulation of these types of structures at greater depths, which increase the molecular complexity of HA.

As soil lipids in surface soil was not removed before humic fractionation, it is reasonable to believe that water repellency in this layer is a complex property, arising from the interaction between different soil organizational components (mainly free lipid, 'fixed' lipid, macroscopic particulate organic matter and the concentration and maturity of humic substances (de Blas et al., 2010; Hansel et al., 2008).

A comparison was made of the fluorescence and EPR results by variation analysis (Figure 5), which showed a high correlation coefficient (R= 0.92, P= 0.26). Both techniques showed an increase of the humification index with increasing soil depth.

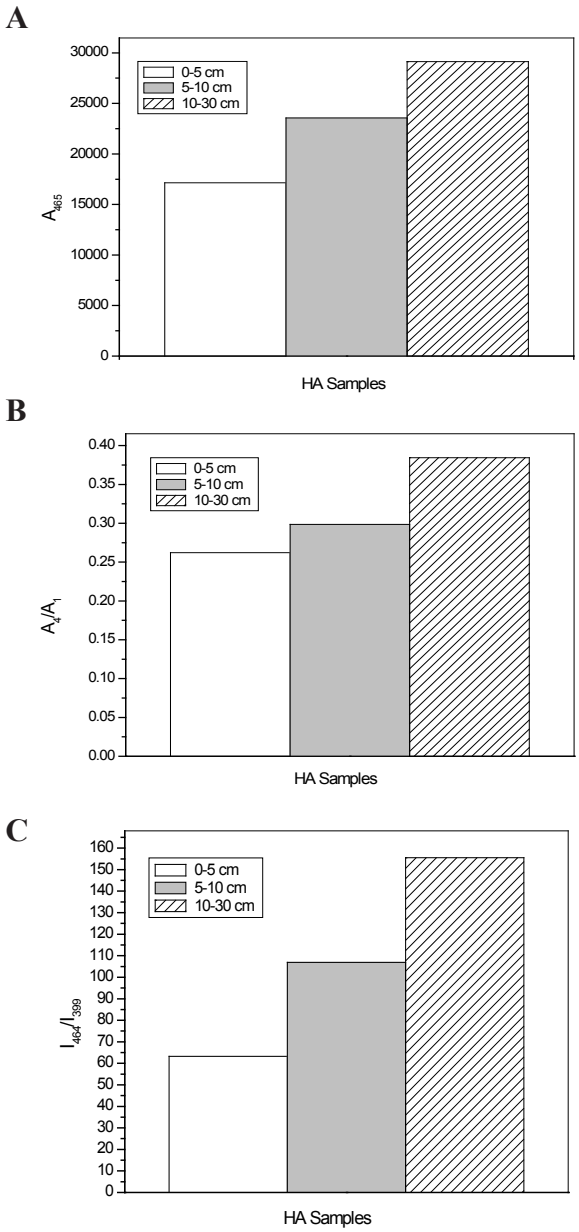
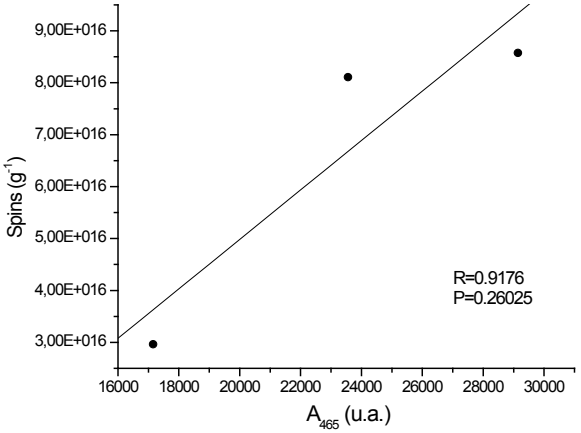


Figure 4. Humification index obtained by different fluorescence methodologies: A) A_{465} index (Milori et al., 2002), B) A_4/A_1 index (Zsolnay et al., 1999), C) I_{454}/I_{399} index (Kalbitz et al. 1999).

Figure 5. Correlation between EPR (Spins g^{-1}) and Fluorescence (A_{465}) results (according to Milori, et al., 2002 methodology).



Conclusions

Spectroscopic characterization of humic acids extracted from an Entisol under pine plantation showed an increasing content of conjugated organic structures and aromatic groups as soil depth increases, indicating higher humification indices, both by fluorescence spectroscopy and EPR.

Aliphatic groups in these fractions are more concentrated in the surface soil layer than in deeper ones, which can be explained by the constant input through litter deposition from loblolly pine plantation. The higher hydrophobicity of the surface soil sample was attributed especially to non-humic components of organic matter, such as those originating from cutin and suberin (alkanoic, alkanedioic and hydroxyalkanoic acids, aromatic compounds, and alkanols), indicating the preservation of suberin and cutin biopolyesters mainly in the topsoil of this pine plantation. Further studies are needed to understand the C stability in this fraction when compared with humic substances.

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