Molecular Proxies for Soil Carbon Storage Patterns in Humic and Fulvic Acids from Oxisols in Minas Gerais State (Brazil)

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1. Introduction

In tropical soils, there is the need for basic research on the structural features of the soil organic matter (MOS) in particular on the complementary pathways conforming the humification processes, which represent a keystone mainly in the terrestrial C cycle. Humic Oxisols are highly weathered soils, found mainly in the Southeast and South regions of Brazil, which present a thick A horizon (up to 200 cm), rich in organic matter. The occurrence of these soils is often related to dystrophic environments with undulated slopes and high altitudes.

Humic acids (HA) and fulvic acids (FA) from six humic Oxisols from Minas Gerais under different vegetation were isolated to describe structural features informing on the carbon sequestration performance in Oxisols showing well-developed A humic horizon.

2. Materials and Methods

Superficial (0–10 cm) and sub-superficial (60–100 cm) samples were collected in the A humic horizon. The HA and FAs were extracted with alkaline reagents and purified before its characterization.

To separate the HA and FA, the whole supernatant solution was acidified at pH 1–1.5 with 12 mol L⁻¹ HCl. The obtained FAs after centrifugation and filtering were saved for further purification. The precipitated HA were redissolved adding 0.1 mol L⁻¹ KOH under N₂ and, adding solid KCl until the concentration of 0.3 mol L⁻¹ followed by centrifugation for 20 min at 25,000 g. The process was repeated three times. The precipitated HA was re-suspended in 0.1 mol L⁻¹ HCl : HF (1:1) mixture and shaken during 2 h at 25 °C and centrifuged. The HA was recovered and transferred to dialysis tubes D-0405 (Sigma-Aldrich) and dialyzed against distilled water, until negative AgNO₃ chloride test. The samples were frozen and freeze-dried.
For purification of the FA fraction a glass column (30 mm i.d., 300 mm height) filled with Amberlite® XAD-8 (Rohm & Hass, Co) resin was used. The resin was previously washed with methanol, 0.1 mol L⁻¹NaOH, 0.1 mol L⁻¹ HCl and distilled water, respectively. The FAs (100 mL) were applied in the top of the column. The non adsorbed fraction (FA-1) was recovered. Then, the column was eluted sequentially with 100 mL of 0.1 mol L⁻¹ HCl and 100 mL of distilled water. Finally, the adsorbed FAs (FA-2) were eluted with 100 mL of 0.1 mol L⁻¹ NaOH and the eluate was immediately collected in a beaker containing a cationic exchange resin, Dowex®-50 (Dow Chemical Co.) in enough quantity to keep the pH balanced between 1 and 2. The fraction of FA-1 was distilled in cellophane bags against distilled water until negative AgNO₃ test. The of FA-1 and FA-2 fractions were concentrated in a rotary evaporator, frozen and freeze-dried.

The HAs and FAs were characterized by elementary analysis, visible spectrometry, Fourier Transform Infrared Spectroscopy (FTIR) and cross-polarization (CP) magic angle spinning (MAS) ¹³C-NMR.

3. Results and Discussion

The Van Krevelen diagram obtained from the H/C and O/C atomic ratios showed differentiation between the fractions according to their degree of carboxylation and alkylation (Figure 1).

![Figure 1: van Krevelen diagram obtained from the elementary composition of the humic acids (HA), FA-1 (FA fraction not adsorbed in XAD-8 resin) and FA-2 (adsorbed FAs)](image-url)
Figure 2: Second derivative of the visible spectra of humic acids extracted from different depths of 
Humic Oxisols (concentration 0.2 mg C mL⁻¹). OX1= soil under coffee; OX2= soil under pasture; 
OX3= soil under forest in Sericita-MG; OX4= soil under fern vegetations; OX5= soil under forest in 
Mutum-MG; OX6= soil from Cerrado ecosystem

The FA fraction not adsorbed in XAD-8 resin (FA-1) comparatively presented higher levels 
of H and O and, greater E₄/E₆ ratio than the FA fraction retained in this resin (FA-2).

In general the visible spectra of humic substances are monotonous. In order to detect any 
discrete humic fraction with repeating units, the second derivative spectrum was obtained, 
showing well defined valleys at circa 458, 570 and 620 nm. In particular, the HAs presented 
different concentration of polycyclic chromophors of fungal origin mainly in the 60–100 cm 
horizon (Figure 2).

Concerning E₄, the lower absorption intensity in the HAs, extracted from soils at the region of 
Mutum and Araçuaí, indicated significant differences as regards to samples from the region of 
Sericita.

The samples of HA showed E₄/E₆ ratios (data not shown) inside the interval of 3.6–5.2 
showing some tendency to increase with depth of the soil profile, which could be interpreted 
as the selective leaching of HAs of comparatively lower molecular size (e.g., FA-type) 
Average values of 17.6 and 9.9 were found for FA-1 and FA-2, respectively.

The FTIR spectra (Figure 3) reveal that FA-1 is composed mainly of carbohydrates but 
includes a component of lignin.
4. Conclusions

The results suggest the possibility to classify the performance for C sequestration of different Oxisols in terms of surrogate indicators consisting of molecular features of humic substances. In particular the extent of the carbohydrate domain in FAs could inform on the extent to which this soil fraction would consist of recent microbial metabolites or to secondary products from biodegradation of higher molecular weight organic matter forms in soils where tropicality is leading to increased levels of organic matter mineralization. This information is complemented with that from the HAs fractions, showing variable concentration of fungal biomarkers, indicative of moderate biological activity where positive balance for accumulation is in part due to a newly-formed humic matter which contrasted with Oxisols where a more marked lignin signature betrayed by the FTIR spectra indicates preservation of comparatively raw organic matter forms in ecosystems where long-term retention of C would depend more in variable climatic constraints than on the intrinsic resilience of the humic substances.