

Article

Extraction Kinetics and Molecular Size Fractionation of Humic Substances From Two Brazilian Soils

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No presente trabalho foi estudada sistematicamente a extração de substâncias húmicas (SH) de um Oxisol e de um Mollisol do Sul do Brasil, empregando-se soluções de NaOH 0.1 e 0.5 mol L⁻¹ e de Na₄P₂O₇ 0.15 mol L⁻¹ a pH 7. A cinética e o rendimento de extração foram monitorados através de espectroscopia UV/Vis. Os ácidos húmicos (AH) e os ácidos fúlvicos (AF) foram fracionados segundo o seu tamanho por ultrafiltração em multi-estágios (seis frações) no intervalo de peso molecular de 1 a 100 kDa. Os resultados obtidos mostraram que o rendimento de extração de SH dependeu não apenas do extrator, porém também do tipo de solo. Em 3 h, aproximadamente 90% de SH solúveis foram extraídas por ambos os métodos e pouca ou nenhuma modificação estrutural foi constatada conforme a constância do valor da razão E₃₅₀/E₅₅₀, ficando evidente porém uma cinética complexa de extração. No Mollisol, a extração com pirofosfato foi mais efetiva, sugerindo que grande parte das SH ocorreram como macromoléculas ligadas aos argilo-minerais e entre si através de pontes catiônicas. No Oxisol o maior rendimento foi obtido pelo método alcalino, provavelmente devido à fixação de SH nas superfícies dos óxidos via pontes de hidrogênio e/ou complexação de superfície. Em geral, as SH extraídas com solução de pirofosfato apresentaram peso molecular médio superior ao das SH extraídas pelo método alcalino.

In the present study, the extraction behaviour of humic substances (HS) from an Oxisol and a Mollisol from South Brazil, by using 0.1 and 0.5 mol L⁻¹ NaOH and 0.15 mol L⁻¹ neutral pyrophosphate solutions, respectively, was systematically studied. The kinetics and efficiency of HS extraction were evaluated by means of UV/Vis spectroscopy. The isolated humic acids (HA) and fulvic acids (FA) were size-classified by multistage ultrafiltration (six fractions) in the molecular weight range of 1 to 100 kDa. The obtained data show that the HS extraction yield depended not only on the extractant, but also on the soil type. Within 3 h approximately 90% of the soluble HS could be extracted following complex extraction kinetics by both methods and none or little structural modification was verified as observed from their stable extinction ratio E₃₅₀/E₅₅₀. In the Mollisol the pyrophosphate extraction was more effective, suggesting that a great part of HS occurred as macromolecules bonded to clay minerals and aggregated between themselves through cationic bridges. In the Oxisol a higher HS yield was verified with the alkaline method, presumably due to HS fixation onto the oxide surface by H-bonds and/or surface complexation reactions. In general, HS extracted by the pyrophosphate procedure showed higher molecular weights than those extracted by NaOH.

Keywords: *humic substances, oxisol, mollisol, molecular-weight fractionation, extraction kinetics*

Introduction

The main mass of refractory organic carbon in soils is concentrated in humic substances (HS), a polydisperse

mixture of related macromolecules formed by microbial degradation of plant remnants¹. HS in soils occur mainly: a) as insoluble macromolecular aggregates, b) as macromolecules bound together by di- and tri-valent cations (*e.g.*

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Ca²⁺, Al³⁺ and Fe³⁺), c) associated with clay minerals through cationic bridges, H-bonds and ligand exchange reactions, d) occluded in the interlayers of expanding-type clay minerals². The abundance of each form and the quality of HS depends greatly on the soil type and soil mineralogy.

The association of HS with Al- and Fe-oxide surfaces has been investigated by means of sorption studies^{3,4,5} and selective extractions^{6,7}. It was observed that pedogenic Al and Fe oxides exerted a stabilizing effect on the soil humic matter⁸. The interaction between HS and Fe-oxides surfaces occurs via ligand exchange of surface -OH or -OH₂ with organic anions and via H-bonds². On the other hand, the association of HS with clay minerals occurs mainly via cationic bridges². An enhancement of HS adsorption on montmorillonite, kaolinite and illite has been observed by the addition of Ca²⁺^{9,10} and of Al³⁺¹¹.

A differentiating analysis of HS in soils by a variety of chemical and spectroscopic methods available for this purpose, generally requires their preceding isolation¹. The extractant conventionally used for HS isolation from soil samples is NaOH solution, which is also the most efficient one¹². Since alkaline extraction may cause chemical alteration of the HS, the Na₄P₂O₇ solution, a milder solvent, is also frequently employed^{12,13,14,15}. The extractability of soil organic matter (SOM) with NaOH solution and with pH-unadjusted Na₄P₂O₇ solution from soils widely differing in pedological characteristics has been investigated¹³. In that study, variations in extraction yield and quality of HS for the different samples were related to the different mineralogy. In pyrophosphate extracts of SOM from Oxisols, suspended particles with the same Al- and Fe-mineralogy of the corresponding clays were observed⁶. On the contrary, pyrophosphate extracts of Alfisols contained Fe and Al (0.3 to 6.1 g Fe kg⁻¹ and 0.3 to 10.9 g Al kg⁻¹) mainly complexed to SOM.

Another important parameter considerably influencing the reaction of HS in soil is their molecular size and the functionalities contained in the different HS fractions². Therefore a number of recent studies have focused on the molecular size differentiation of HS in both soils and aquatic systems. Humic acids (HA) were fractionated from an Andosol and from a Cambisol by successive gel permeation chromatography (GPC) and it was verified that the fractions differed chemically¹⁶. Recently, a multistage ultrafiltration (MST-UF) device was adapted to fractionate aquatic HS and it was verified that HS fractions of higher molecular size exhibited a different composition from that of the smaller ones¹⁷. Moreover, it was observed that the HS molecular size distribution determined with MST-UF and with GPC were in good agreement in the range of 1-50 kDa¹⁸.

The present study on HS in two Brazilian soils of different mineralogy (Oxisol and Mollisol) was concentrated on the HS extraction behaviour operationally influ-

enced by the applied extractants (NaOH, Na₄P₂O₇). The main objectives were: (i) to investigate the extraction kinetics of HS from soils of different mineralogy; and (ii) to evaluate the influence of the extraction procedures used and the soil mineralogy on the yield and the molecular size distribution of the HS under study. For the latter purpose MST-UF already successfully applied for size classification of aquatic HS had to be adapted for the fractionation of dissolved soil HS¹⁷.

Materials and Methods

Soils

Humic substances were extracted from the A-horizon (0 to 10 cm) of an Oxisol and of a Mollisol from Rio Grande do Sul state, Brazil. The Oxisol, located in Santo Angelo, was under agricultural use (crop rotation oat/soja), contained 27 g kg⁻¹ soil of organic Carbon, and its clay mineralogy was dominated by Fe-oxides and kaolinite¹⁹. The Mollisol, located in Rio Pardo, was under native vegetation, contained 21 g kg⁻¹ soil of organic carbon, and its clay mineralogy consisted mainly of smectites and kaolinite²⁰.

Kinetic tests

Three extractant solutions were employed: a) 0.1 mol L⁻¹ NaOH; b) 0.5 mol L⁻¹ NaOH and c) 0.15 mol L⁻¹ Na₄P₂O₇ adjusted to pH 7. In a 10 mL centrifuge tube, 5 mL of extractant solution was added to ca. 240 mg soil. During 24 h, 3 mL of the supernatant solution were collected periodically (10 min., 30 min., 1 h, 2 h, 3 h, 4 h, 6 h and 24 h) for UV/Vis spectroscopy measurements and returned again to the tube. After 24 h the extract was separated from the soil by centrifugation at 2000 rpm and a second and a third 24 h-extraction were carried out. The pH level was measured in each extract after 24 h.

Extraction and isolation of HA and FA

HS were extracted separately with 0.5 mol L⁻¹ NaOH and with 0.15 mol L⁻¹ Na₄P₂O₇ (pH 7) solutions (250 mL /11 g soil) during 3 h under continuous shaking, based on Swift (1996). The HS extract separated by centrifugation was immediately acidified to pH 2 with 0.1 mol L⁻¹ HCl. After 24 h, the precipitated HA was separated by centrifugation from the remaining FA solution, washed twice with distilled water and dried at room temperature in a desiccator over P₄O₁₀.

The obtained FA solution (pH 2) was run over a XAD-8 column and then eluted by a 0.1 mol L⁻¹ NaOH solution¹⁷. After neutralization by diluted HCl this FA concentrate was dried by rotatory-evaporation at 70 °C.

Fractionation of HA and FA by multistage-UF (MST-UF)

HA and FA were size-fractionated (6 fractions; F1: > 100, F2: 50-100, F3: 10-50, F4: 5-10, F5: 1-5, and

F6: < 1 kDa) by using the multistage-UF technique of Burba *et al.*¹⁷. 10 mL of the humic substance solution under study (*ca.* 0.5 mg mL⁻¹, pH 7) were pumped through the MST-UF device (Pall Filtron, OMEGA membranes based on polyethersulfone, 25mm, nominal molecular weight cut-off: 100, 50, 10, 5 and 1 kDalton) and then washed with 10 mL of high purity water (Millipore MilliQ system)¹⁸.

The collected HS fractions (1 mL, each) were washed out quantitatively with 2 mL water, and kept for UV/Vis spectroscopy. Their relative concentration (%) of each fraction was calculated by relating its absorbance at 450 nm to the absorbance in the same wavelength of the HS solution to be fractionated (100%)¹⁷.

UV/Vis spectroscopy

UV/Vis spectra of the extracted HS (pH 13 and pH 7), of the fulvic acids (FA), of the humic acids (HA) and of their respective MST-UF-fractions were recorded in the range of 200-700 nm (Scanning double-beam spectrometer Varian Cary 1/3). For the kinetic tests, the absorbances of the first pyrophosphate-extracts were measured at pH 7 within 24 h. The values were then corrected for pH 13, employing a calibration curve calculated from the measurements at pH 7 and at pH 13, obtained in a parallel experiment.

The spectroscopic method for the determination of HS distribution in the UF-MS tests had been calibrated by Burba *et al.*¹⁷, and thus it was also employed in the kinetics tests. The relative amount of extracted HS was estimated from the subtraction $[E_{450}-E_{700}]$, where E_{450} and E_{700} are the absorbances at 450 nm and 700 nm, respectively. The determination of total organic carbon via oxidative methods, could not be employed for the calculation of HS concentration, because this chemical method is destructive and hence would render impossible the monitoring of the kinetics of the extraction in the same extract.

The E_{450} was chosen because absorbance in this region is concentration dependent²³ and less influenced by HS structural and chemical characteristics than at the shorter wavelengths²⁸, since the different chromophores absorb in the UV- region^{1,21}. Simultaneously, the E_{450} absorbances were generally above the error limit of the experimental measurements, in contrast to the absorbances at larger wavelengths in some samples. The E_{700} is preferably caused by small amounts of remaining particles in the sample solution, and its subtraction was employed as a correction of the E_{450} values.

From the UV/Vis data, the ratio E_{350}/E_{550} (absorbance at 350 and 550 nm, respectively) was calculated. These wavelengths were chosen instead of the usually employed E_{465}/E_{665} , because the absorbance at 665 nm was sometimes too low, that the values could not be considered. In the present work, it is proposed that the shift of the λ_{max} of

the chromophores to longer wavelengths due to molecular condensation²¹ can also be qualitatively estimated from the ratio E_{350}/E_{550} . Like the ratio E_{465}/E_{665} , the proposed index should be adequate for comparison among the studied samples.

Results and Discussion

Extraction kinetics and method yield

The plot of $[E_{450}-E_{700}]$, which expresses the amount of solubilized HS, as a function of time, shows that the HS extraction proceeded rather fast within the first 10 min (Fig. 1). The pH constancy and the high ionic force (0.15 mol L⁻¹ in pyrophosphate, 0.1 and 0.5 mol L⁻¹ in the alkaline solutions) in each extract during the 24 h, assure that the absorbance results are related to the amount of extracted HS rather than to HS with different macromolecular shape.

In both samples the 0.1 mol L⁻¹ NaOH extractant solution reached its maximum extraction yield, MEY, after 2 h. The MEY corresponds to 100% of the extraction amount of HS after 24 h. Within 3 h, the pyrophosphate solution extracted *ca.* 80% (Oxisol) and 95% (Mollisol) of its respective MEY's, and 0.5 mol L⁻¹ NaOH extractant reached 90% (Oxisol) and 80% (Mollisol) of its MEY's (Fig. 1).

In the Oxisol the 0.5 mol L⁻¹ NaOH solution extracted a higher amount of HS than pyrophosphate within 3 h. On the contrary, a higher HS yield by pyrophosphate was obtained in the Mollisol. This last result agrees with those verified in a Mollisol¹⁴ and in a Gleysol¹⁵ by sequential HS extractions with pyrophosphate solutions at different pH levels.

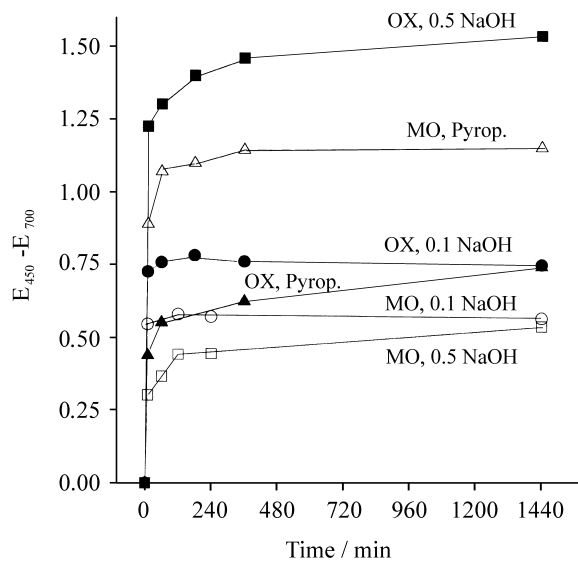


Figure 1. Variation of $E_{450}-E_{700}$ with time, of HS extracted from the Oxisol (OX) and from the Mollisol (MO) with 0.1 mol L⁻¹ NaOH (0.1 NaOH), with 0.5 mol L⁻¹ NaOH (0.5 NaOH) and with 0.15 mol L⁻¹ Pyrophosphate (Pyrop.) solutions.

Due to the complex and heterogeneous chemical nature of humic substances, different sorption/retainment mechanisms in the environment are likely to act simultaneously²². Our findings suggest that the extent of each mechanism is strongly influenced by the soil mineralogy, which affects the extraction efficiency of a given method.

The $\text{Na}_4\text{P}_2\text{O}_7$ extraction solubilizes HS mainly by disrupting cationic bridges of polyvalent cations which bind organic molecules between themselves or link them to the negative charged surface of minerals². The pyrophosphate anion forms with such cations either insoluble precipitates (e.g. Ca^{2+}) or soluble coordination compounds (e.g. Al^{3+} and Fe^{3+}), leaving the negative sites on the organic molecules free for the (abundant) Na^+ ions. In the alkaline extraction, the pH-dependent charge of the mineral surface as well of the HS, is converted to its negative form, and HS solubilization occurs due to repulsion between equally negative charged surfaces and formation of soluble HS salts. By this extraction, mainly H-bonding and surface complexation interactions between HS and mineral surfaces are broken up².

In view of the mineralogy of the two studied soil samples, it is not surprising that the pyrophosphate method showed a higher yield in the Mollisol, where minerals with permanent negative charge predominate. From the Oxisol, where the mineralogy consists mainly of Fe-oxides and kaolinite, the alkaline method extracted much more HS than the pyrophosphate one. Those results are indicative for the specificity of the two methods in solubilizing soil HS by different mechanisms.

The absorbance ratio E_{465}/E_{665} of HS is a traditional parameter to estimate their humification degree and/or their molecular size^{21,23}. Though, other ratios like E_{350}/E_{450} ¹⁷ or E_{350}/E_{550} can be used with the same purpose. In the present study, the variation range of the absorbance ratios E_{350}/E_{550} from 10 min to 24 h, of the extracted HS are shown in Table 1.

For all extracts, the values increased with time, and this fact is probably more related to a gradual enrichment in structures of lower molecular sizes in the two alkaline and in the pyrophosphate extracts, and less to a strong chemical modification. It has been reported that absorbance ratios vary inversely with the molecular size^{17,21}. Furthermore, in an investigation of the influence of the oxidative atmosphere in the NaOH treatment, an increase on the oxidation

of HA and FA extracted under O_2 was not observed when compared to those extracted from the same sample under an inert atmosphere²⁴.

In both samples, within the first 3 h (data not shown here), the E_{350}/E_{550} ratios of the two alkaline and of the pyrophosphate HS extracts increased no more than one unit, and because of that the 3 h long extraction was employed for the separation and isolation of HS that were further fractionated by MST-UF.

For each soil sample, the 0.5 mol L⁻¹ NaOH extract showed the highest E_{350}/E_{550} ratio (Table 1), suggesting that this extracting agent solubilized HS with a smaller average molecular size. Probably, the rupture of the inter/intra-molecular hydrogen bonding of the HS in the NaOH solution accounts for the higher observed E_{350}/E_{550} values. The same trend with E_{400}/E_{600} ratio values has been observed by other authors¹³.

Another parameter hardly considered with respect to HS isolation from soil samples is its extraction kinetics which was assessed in the present study by means of $[\ln(E_{\max}-E_t)/E_{\max}]$ as a function of the time, where E_{\max} (450 nm) corresponds to the absorbance of the HS extract at 24 h and E_t to the absorbance at the time t . The shape of the extraction curves in Fig. 2 indicates that the HS solubilization from both soil samples by the two tested extractants is much more complex than a first order reaction. The same conclusion was reached by the investigation of the alkaline extraction of HS at different temperatures from brown coals, and in that work it was supposed that the opening of aromatic rings due to an alkaline oxidation, was responsible for the complex extraction kinetics²⁵. Since in the present work, the alkaline as well as the neutral extraction data showed a similar behaviour, other factors might have influ-

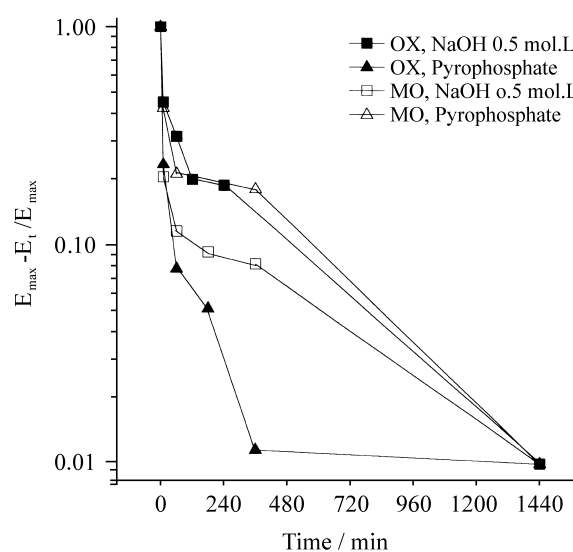


Figure 2. Variation of $\ln(E_{\max} - E_t) / E_{\max}$ with time of HS extracted from the Oxisol (OX) and from the Mollisol (MO) with 0.5 mol L⁻¹ NaOH and with 0.15 mol L⁻¹ Pyrophosphate solutions.

Table 1. Range of E_{350}/E_{550} data from 10 min to 24 h of the HS extracted from the Oxisol (OX) and the Mollisol (MO).

Treatment	E_{350}/E_{550} -OX	E_{350}/E_{550} -MO
NaOH 0.1 mol L ⁻¹	5.7 - 5.9	5.3 - 6.8
NaOH 0.5 mol L ⁻¹	8.1 - 9.6	8.1 - 8.6
Pyrophosphate 0.15 mol L ⁻¹	7.8 - 8.8	5.6 - 6.2

enced the extraction kinetics of HS in the Oxisol and Molisol.

Consecutive extractions

The extraction efficiency of the chosen HS extractants was studied by means of consecutive extractions, each consisting of a 24 h-long period. As shown in Fig. 3, the greater part of the HS was extracted in the first stage (in the Molisol by pyrophosphate and in Oxisol by 0.5 mol L⁻¹ NaOH solutions) and continuously decreased after that. Furthermore, the different yields of the two methods in the first extraction are not compensated by the subsequent ones, and supports the considerations made previously about the selectivity of the two extractants tested.

Moreover, from the E₃₅₀/E₅₅₀ ratio of the HS extracts decreasing continuously in the consecutive extractions (Fig. 3), it can be suggested that the solubilization of chemically/structurally different HS fractions occurred in each extraction stage.

Molecular-size fractionation by MST-UF

The fractionation of HA and FA by MST-UF into six molecular weight fractions was performed at pH value 7 and a constant electrolyte concentration of 0.05 mol L⁻¹ NaCl based on experimental conditions optimized in Burba *et al.*¹⁷. Afterwards, the HS molecular distribution (in %) was determined according to Aster *et al.*¹⁸. The molecular size distributions of the studied HS are given in Fig. 4.

In general, the investigated FA were mainly found in the smaller fractions (F6 to F4) in contrast to the corre-

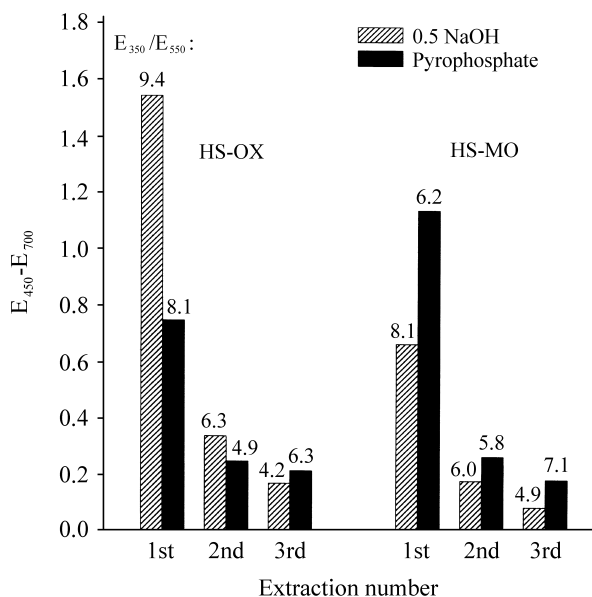


Figure 3. E₄₅₀-E₇₀₀ values for each of the three 24 h-extractions of HS extracted from the Oxisol (OX) and from Molisol (MO) with 0.5 mol L⁻¹ NaOH and with 0.15 mol L⁻¹ Pyrophosphate solutions. E₃₅₀/E₅₅₀ ratio values for each extraction are given above the column.

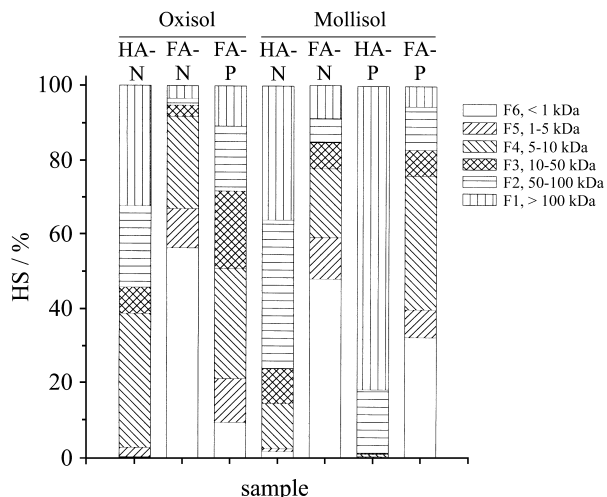


Figure 4. Molecular size distribution of the HA and FA extracted with 0.5 mol L⁻¹ NaOH (N) and with 0.15 mol L⁻¹ Pyrophosphate (P) solutions.

sponding HA, whose main fractions were found between 50 and 100 kDa and > 100 kDa (F2 and F1, respectively). In both samples, the fraction F6 was more abundant in the FA extracted with NaOH when compared to the FA-P, indicating that the alkaline solution produced smaller FA molecules. Furthermore, in the Molisol, HA-P was richer in fraction F1 than the corresponding HA-N (Fig. 4).

From the plot in Fig. 4 the nominal average molecular weight $\langle M_w \rangle$ presented by at least 50% of the HS can be estimated. For example, in the sample OX-HA-N, if the contents of HS were added from fraction F6 up to the bigger fractions, the value of 50% HS is already reached by the addition of fraction F2. In other words, in this sample, at least 50% of HS presented a molecular weight ≤ 100 kDa. The values obtained for these nominal $\langle M_w \rangle$ are given in Table 2 excepted for MO-HA-P where 77% HS is concentrated in the fraction F1, and the $\langle M_w \rangle$ was classified as greater than 100 kDa.

Table 2. pH value (pH₀) and E₃₅₀/E₅₅₀ ratio of the initial solution and average molecular weight $\langle M_w \rangle$ of the humic acids (HA) and fulvic acids (FA) from the Oxisol (OX) and the Molisol (MO).

Sample	pH ₀	E ₃₅₀ /E ₅₅₀	$\langle M_w \rangle$ (kDa)
OX-HA-N	7.2	8.2	≤ 100
OX-FA-N	7.2	15.4	≤ 1
OX-FA-P	7.0	15.1	≤ 10
MO-HA-N	7.3	8.4	≤ 100
MO-FA-N	7.1	15.1	≤ 5
MO-HA-P	6.8	4.0	> 100
MO-FA-P	7.1	8.5	≤ 10

Legend: -N: first 3 h-long NaOH extract; P: first 3 h-long pyrophosphate extract.

The pyrophosphate-extracted HA and FA generally exhibited higher $\langle M_w \rangle$ and lower E350/E550 values than the corresponding NaOH extracted HS. (Table 2). This result might be a hint that cationic bonds (broken by pyrophosphate) are formed preferably by larger HS molecules, while smaller moieties interact mainly through H-bonds and ligand exchange reactions. It has been verified that pyrophosphate extracted a higher molecular weight organic matter than NaOH solution¹³. Some authors²⁶ proposed that Al^{3+} and Fe^{3+} are preferably associated with larger humic molecules, since pyrophosphate, which extracted HS of higher molecular weight, also has an affinity for those metals. Furthermore, it was verified that HS molecules > 50 kDa formed stronger complexes with Mn(II), Zn(II), Al(III) and Fe(III) than the smaller ones¹⁸. On the other hand, the possibility that in the present work, the alkaline solution disrupted further inter-molecular H-bonds which were resistant to pyrophosphate treatment, cannot be excluded.

Practically no HA was extracted with pyrophosphate in the Oxisol, suggesting that in this Fe-oxide rich sample, the interaction between humic acid matter and mineral surfaces was mainly based on strong ligand-exchange and/or H-bonds. The HA extracted with NaOH in the first 3h-extraction (HA-N) showed similar $\langle M_w \rangle$ values in both soils.

For each studied HS sample, the E350/E550 ratio significantly decreases with the increasing molecular weight of the MST-UF fractions, but the values observed in comparable molecular weight fractions strongly differ among the samples (Table 3), indicating that structural/chemical characteristics also influence the obtained E350/E550 ratios. A similar inverse relationship between E350/E550 ratio and molecular size of fractionated aquatic HS has already been verified^{16,17}. It was also proposed the E_{460}/E_{660} ratio as a qualitative indicator of HS molecular size of different materials, because of the strong dependence of the UV/Vis spectra inclination on other parameters²⁷.

In the Oxisol (excluding the fraction 5 - 10 kDa) the FA's showed generally a higher value for E350/E550 ratio than the HA's (Table 3), as is usually observed for the classical E_{460}/E_{660} ratio. In the Mollisol, the pyrophosphate extracted HS (either FA or HA) showed the smallest E350/E550 ratio in the corresponding fractions.

Conclusions

From our study comparing systematically the extraction behaviour of HS in two Brazilian soils of different mineralogy, the following conclusions can be drawn:

(1) The extraction yield of HS not only depended on the extractant chosen but also strongly on the soil type. According to their solubilization mechanisms, the extractants solubilized different HS fractions but the yields of the tested procedures (alkaline and neutral pyrophosphate extraction, respectively) were partially equivalent.

(2) Approximately 90% or more of the soluble soil HS studied could be extracted within 3 h following complex extraction kinetics. No structural modification of HS was observed during this extraction period, as indicated by the ratio E350/E550 data.

(3) The absorbance ratio E350/E550 of the extracted HS differed considerably, depending on the chosen extractant.

(4) Considering the extraction mechanism of the two employed methods, it can be concluded that in the Mollisol, the greater part of HS occurred as macromolecules aggregated between themselves and bonded to clay minerals through cationic bridges (pyrophosphate extractable HS), while in the Oxisol the HS were presumably associated with oxide surfaces preferentially through H-bonds and/or ligand exchange reactions (NaOH extractable HS).

(5) Pyrophosphate-extractable HS exhibited higher molecular weights than those extractable by NaOH.

(6) Molecular-size classification of isolated soil HS by online MST-UF (standardized experimental conditions presupposed) can be considered as a simple and efficient procedure for their fractionation.

Table 3. Values of E350/E550 ratio in the six molecular weight fractions, separated by MST-UF.

Fraction: Sample	< 1kDa	1-5 kDa	5-10 kDa	10-50 kDa	50-100 kDa	> 100 kDa
OX-HA-N	n m	n m	11.7	7.2	6.8	5.14
OX-FA-N	19.1	11.7	9.8	12.9	8.7	8.4
OX-FA-P	n.m	17.2	16.1	11.1	10.4	10.2
MO-HA-N	n.m	n.m	11.3	10.0	9.2	7.4
MO-FA-N	15.8	11.7	11.6	8.3	8.6	5.4
MO-HA-P	n m	n.m	n m	n.m	5.4	4.2
MO-FA-P	15.1	8.2	7.9	7.5	5.9	5.6

Legend: see Table 2. n.m.: not measurable (no HS was separated in the given fraction).

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