

Preparation and characterization of natural and synthetic humic acids by thermogravimetry analysis

In this study, a polymer with characteristics analogous to that of the humic acid was obtained through oxidative polymerization in alkaline medium using para-benzoquinone as precursor and compared to natural humic acid (HA) extracted from a soil. Complexes of as-prepared synthetic humic acid (SHA) with the Cu²⁺, Fe³⁺ and Mn²⁺ ions were also prepared. Elemental analysis and thermogravimetry were used for comparing the SHA characteristics with those of HA and for evaluating the effect of the metal-type for complexing with SH and SHA. Element analysis showed that SHA presented almost the same atomic constitution of HA, except for or sulfur and nitrogen contents, fact that is expected due to the precursor compounds. TGA curves of HA and SHA are quite similar in spite the thermogravimetric index (TGI) indicates higher aromatic portion in SHA. SHA-metallic ions (SHA-Cu²⁺, SHA-Fe³⁺ and SHA-Mn²⁺) complexes presented different thermal stability as compared among them and with the precursor SHA. The data indicate that the complexation is dependent of metallic ion-type and provokes different rearrangements in the SHA matrix.

Keywords: Humic acid; Synthetic humic acid; Metal cation; Thermal stability by TGA curves.

Preparação e caracterização de ácidos húmicos naturais e sintéticos por análise termogravimétrica

Neste estudo, um polímero com características análogas às do ácido húmico foi obtido por polimerização oxidativa em meio alcalino, utilizando a para-benzoquinona como precursor, e comparado ao ácido húmico natural (AH) extraído de um solo. Também foram preparados complexos de ácido húmico sintético (SHA) preparado com os íons Cu²⁺, Fe³⁺ e Mn²⁺. A análise elementar e a termogravimetria foram utilizadas para comparar as características do SHA com as do HA e para avaliar o efeito do tipo de metal na complexação com SH e SHA. A análise dos elementos mostrou que o SHA apresentava quase a mesma constituição atômica do AH, exceto os teores de enxofre e nitrogênio, fato esperado devido aos compostos precursores. As curvas TGA de HA e SHA são bastante semelhantes, apesar do índice termogravimétrico (TGI) indicar maior porção aromática no SHA. Os complexos de íons SHA-metálicos (SHA-Cu²⁺, SHA-Fe³⁺ e SHA-Mn²⁺) apresentaram estabilidade térmica diferente quando comparados entre eles e com o precursor SHA. Os dados indicam que a complexação é dependente do tipo de íon metálico e provoca diferentes rearranjos na matriz SHA.

Palavras-chave: Ácido húmico; Ácido húmico sintético; Cátions metálicos; Estabilidade térmica por curvas de TGA.

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INTRODUCTION

Humic substances (HS) are close to be the most abundant of the organic components of the nature (Stevenson, 1994). They are present in all soils and in natural waters due to its organic matter fraction. Because of their ability to interact with various components of the environment, HS play important role in soil and aquatic chemistry and, beyond this, are involved in most of the chemical reactions in the soil. Humic substances, of which the humic acid (HA, insoluble at acidic pH) and fulvic acid (FA, water soluble at acidic to alkaline pH) are the major fractions, possess various chemically reactive functional groups, including carboxyls, phenolics and alcoholic hydroxyls, with pH-dependent properties (STEVENSON, 1982).

The analytical limitations and the complex and dynamic nature of the organic matter make hard its characterization, as well as difficult the understanding of processes that regulate their behavior in the soil. Such organic matter presents high chemical and structural complexity reason for why their molecular formula is still not well defined (MEURER, 2006). Synthetic compounds, such as phenolic polymers, have been used with the intent for obtaining further information regarding the chemical structure of humic substances (KOVALENKO et al., 2004; WUNDERWALD, 2000; HANNINEN, 1987). Zunino et al. (1979) studied the competitive behavior of Zn^{2+} and Mg^{2+} for complexing to humic and fulvic acids polymeric models. They concluded that polymers containing both carboxylic (-COOH) and phenolic (Ar-OH) groups presented higher complexing power for both Zn(II) and Mg(II) as compared to those containing only -OH groups, besides Zn(II) is preferred over Mg(II) in fulvic and humic acid-typed polymer having also -NH₂ and -SH groups.

Thermal analysis methods have been extensively used for characterizing organic material (e.g., humic substances) because beyond providing information on the thermal behavior and structural properties of humic substances such methods are simple and further special sample pre-treatments are not needed (KOLOKASSIDOU et al., 2007).

In this study humic acid was synthesized from oxidative polymerization and labeled as SHA. Complexes of SHA with the Cu^{2+} , Fe^{3+} and Mn^{2+} ions were prepared and characterized through FTIR spectroscopy, elemental analysis and thermogravimetry. So, the studies were devoted for comparing the characteristics of the SHA with a natural humic acid (HA) extracted from a soil and for evaluating the effect of the metal ions in SHA.

METHODOLOGY

Experimental

Extraction of humic acids (HA) from soil

Humic acid (HA) was extracted from Brazilian subtropical Paleudult soil, following the methodology proposed by the International Humic Substances Society (IHSS). The soil sample used for the extraction of humic acid (HA) was collected from an area of red Argisol under nine (9) years of management, involving the direct cultivation of oats with common vetch/corn with cowpea.

Preparation of synthetic humic acids (SHA)

The polymer (SHA) was prepared with 5.4g of *para*-benzoquinone dissolved in 60 mL of water containing 2.8 g of KOH. The solution was placed in flasks and a stream of purified air was bubbled at a rate of 1 L min⁻¹. The air was purified by first passing it through solid NaOH pellets and after through a solution of 0.8 mol L⁻¹ NaOH. After 22 h of air bubbling, the resulting black viscous solution was treated with 6 mL of concentrated hydrochloric acid under mechanical stirring until appearing as precipitated as a brownish black mass. Such polymeric product was collected by vacuum filtration, washed repeatedly with deionized water, and dried in air for one day and subsequently maintained in a oven at 60°C for one day.

Preparation of synthetic humic acids (SHA) - metal complexes

Complexes of the polymeric substance SHA with the Cu²⁺, Fe³⁺ and Mn²⁺ ions were prepared by adding 0.1 g of polymer to 40 mL of a 0.10 mol L⁻¹ solution of each the metal sulphate. The suspensions were shaken for 16 h. After, the samples were centrifuged at 2,200 rpm for 20 min and the supernatant discarded. Deionized water (10 mL) was added to the solid and shaken for more 2 min and again centrifuged at 2,200 rpm for 20 min and the supernatant discarded. This washing procedure was repeated further twice and the purified products (subsequently referred to as SHA-Cu, SHA-Fe and SHA-Mn, respectively) were collected, freeze-dried and lyophilized.

Elemental Analysis and Determination of the Ash Contents

The elemental composition of the as-prepared humic acids was characterized based on nitrogen, carbon, hydrogen and sulfur quantifications using a CHNS-O analyzer (CE-Instruments EAGER 200). The oxygen percentage was obtained by mass balance, by applying: %Oxygen = 100 – (C+H+N+S %).

The ash content was determined gravimetrically by weighing and calcinations (approximately 10 mg) of synthetic or natural humic acids (HA and SHA) in a platinum crucible at a temperature of 600°C until reaching constant mass.

Infrared spectroscopy

The infrared spectra of SHA, SHA-Cu²⁺, SHA-Fe³⁺ and SHA-Mn²⁺ samples were obtained from KBr pellets using a FTIR spectrophotometer Perkin-Elmer model Paragon 1000 in wave number range from 4000 to 400cm⁻¹ at a resolution of 4 cm⁻¹, by accumulating 64 scans for acquiring each spectrum.

Thermogravimetric analysis (TGA)

The TGA curves were obtained in TA Q 500 thermoanalyzer for samples: HA, SHA, SHA-Cu²⁺, SHA-Fe³⁺ and SHA-Mn²⁺. Mass of approximately 2 mg of sample was used in each run. The sample was heated from 25°C to 800°C at heating rate of 10°C min⁻¹ and under synthetic air flowing 20 mL min⁻¹.

RESULTS AND DISCUSSION

Obtainment of natural (HA) and synthetic (SHA) humic acids

It is known that polyhydroxy phenols, as well as hydroquinones and quinones such as *para*-benzoquinone, are highly sensitive to the action of aqueous alkali, yielding polymeric products known as synthetic humic acids (SHA) (CATALDO, 1998). The reason for synthesizing these polymers was that the information obtained from these synthetic precursors could be used to shed light on the complex structure of natural humic acids.

The SHA polymer formed from the *para*-benzoquinone reaction provides a compound constituted mostly by polyhydroxy phenols, in which the main groups responsible for the adsorbing capability are -OH and -COOH (CATALDO, 1998). The SHA structure can be described by a sequence of phenyl rings having three hydroxyl groups per each ring, intercalated by monohydroxylated quinone units (Figure 1) (CATALDO, 1998). According to Cataldo (1998), the presence of the carboxylic group is due to the partially degradation of the quinone aromatic ring during polymerization. This structure is similar to lignin, one of the major constituents of the organic matter of soil (CATALDO, 1998).

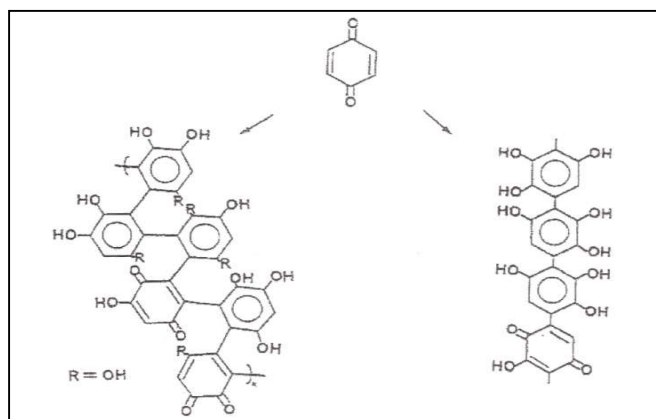


Figure 1: SHA obtained by oxidative polymerization in alkali. **Source:** Cataldo (1998).

The extraction of humic acid from the red Ultisols yielded 0.246 %, i. e., 246 mg of humic acid were obtained per kilogram of soil. On the other hand, the SHA synthesis presented an excellent performance as compared to the extraction yielding, yielding 76.36% in weight of polymerized *para*-benzoquinone.

Elemental Analysis and Determination of Ash Content

Table 1 lists the elemental analysis and ash content results from HA and SHA. The SHA contained a large amount of oxygen, 40.7%. However; this is lower than the amount of oxygen found in natural humic acid, which also contains 46.0 %. According to Schnitzer et al. (1978), the elemental analysis of humic acids indicated the following composition (in average): 53.8-58.7% of carbon, 3.2-6.2% of hydrogen, 32.8-38.3% of oxygen, 0.8-4.3% of nitrogen and 0.1-1.5% of sulfur. These values are quite similar to the ones obtained for HA obtained by extraction process (Table 1). The synthesized polymer (SHA) differed significantly from the values described by Schnitzer et al. (1978) only for sulfur and nitrogen contents, fact that is expected due to the precursor compounds.

Table 1: Physical and chemical characteristics of the natural humic acid (HA), extracted from soil and synthetic humic acid (SHA).

Sample	Elemental composition (%)					Ash Content (m/m) %
	N	C	H	S	O*	
HA	3.9	44.5	4.9	0.7	46.0	7.0
SHA	0.3	55.6	3.4	0	40.7	0.8
	H / C (atomic ratio)		C / N (atomic ratio)		C / O (atomic ratio)	
HA	0.11		11.4		1.0	
SHA	0.06		185		1.4	

*Calculated by difference: % O = 100 – % (C + H + N + S).

Evaluation of SHA-metallic ion complexation through Infrared Spectroscopy

The infrared spectra obtained for SHA-Cu²⁺, SHA-Fe³⁺ and SHA-Mn²⁺ samples are presented in Figure 2. Differences in the spectra are observable mainly in the region between 1750 and 1550 cm⁻¹. According to Piccolo et al. (1982), the conversion of COOH to COO⁻ (ionization of carboxyl groups) leads the loss absorption at close to 1,700 cm⁻¹ (due to C=O groups of COOH) and at 1,200 cm⁻¹ (due to -C-OH stretching and -O-H deformation, both of carboxyl group). The complexes' spectra showed absorption decrease close to 1,700 cm⁻¹ (stretching of C=O of COOH) and to 1,209 cm⁻¹ and an intensity rising of band at 1,618 cm⁻¹ (absorption of COO⁻). Such changes are evidences of -COOH conversion to COO⁻ due to interaction of SHA with Cu²⁺, Fe³⁺ and Mn²⁺ ions. Also, such results are quite similar to those reported for humic acid extracted from soil. For instance, Prado et al. (2006) performed study of interactions between humic acid (HA), extracted from Brazilian peat soil, and copper (II) and zinc (II) ions. Interactions between the metal ions and COOH groups were evidenced in such study. Such evidences are based on: i) reduction of intensity of the band at 1,250 cm⁻¹, which was associated to COOH of HA-Cu and HA-Zn complexes; and ii) on increasing of intensity of the peak at 1,660 cm⁻¹, that was attributed to asymmetric stretching in COO⁻ groups.

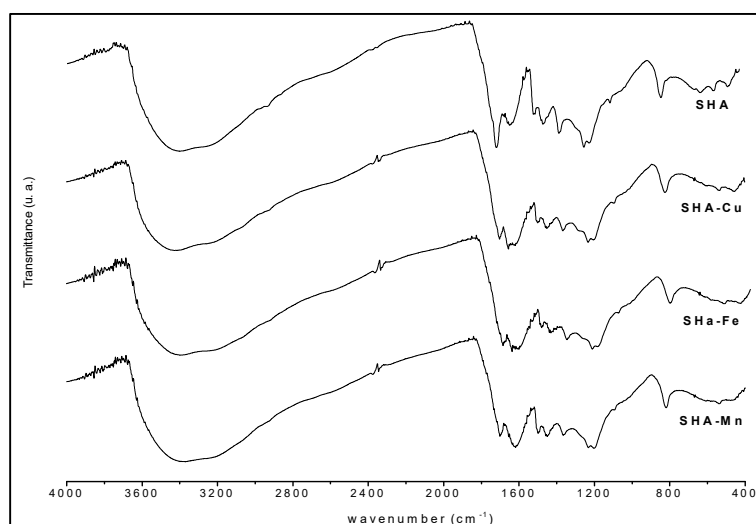


Figure 2: Infrared spectra of SHA, SHA-Cu²⁺, SHA-Fe³⁺ and SHA-Mn²⁺ samples.

Thermal analyses of HA, SHA and SHA-metallic ion complexes

Thermogravimetric curves (TG) and respective first derivatives (DTG) of HA, SHA and of complexes SHA-Fe²⁺, SHA-Fe³⁺ and SHA-Mn²⁺ are presented in Figure 3. The following parameters, characteristics of such materials, were determinate based on such curves: i) the amount of adsorbed water (in the range 25-105

°C);ii) the weight loss due to thermal degradation (in 105-350 °C and 350-650 °C ranges);the amount of ash (at 800 °C); and the thermogravimetric index (TGI). According to Benites, 2005, the values of TGI can be calculated by the following ratio: $WL_{105-350\text{ °C}} / WL_{350-650\text{ °C}}$ being the WL_{i-j} the weight loss occurred in the temperature range i to j, in °C. The data relative to thermal evaluation are presented in Table 2.

Similar profiles are observed in TG and DTG curves of HA and SHA (Figure 3), suggesting chemical similarity in HA and SHA structures (MELO *et al*, 2009). The first event of weight loss observed in such curves in the range of 25 to 105 °C corresponds to loss of adsorbed water. The thermal degradation of HA and SHA occurred in temperature range from 105 to 650 °C. According to Benites (2005) and Cunha (2007), the weight loss occurred in the range 105 to 350 °C corresponds to the decomposition of functional groups of aliphatic, while the thermal degradation of groups bonded to aromatic rings occurs at superior temperatures. Table 2 shows that the values of TGI for HA and SHA are quite different the TGI for HA is 1.8 while for SHA the value of TGI is 4.2. This large difference is a strong indicative that such materials are different, in chemical composition, as expected. The lower value of TGI for HA (TGI = 1.8) is due to its weight loss (WL = 29.9%) in first event of thermal degradation (in 105-350°C range) and the WL (= 53.9%) in second event of thermal degradation (at 350-650 °C range). Compared, the SHA sample presented lower weight loss WL = 17.1% in the first event of thermal degradation and higher WL (= 71.9%) in the second event of thermal degradation. The higher value of TGI for SHA indicates higher fraction of aromatic groups as compared to HA. These data match to the H/C and C/N ratios, calculated from elemental analysis, and presented in Table 1. According to Benites (2005), a correlation between the value of TGI and the values of H/C and C/N ratios exist and can be due to the combination of lower H/C atomic ratio and higher TGI values. This is an indicative of higher proportion of aromatic groups relative to aliphatic groups on the sample structure. Oppositely, higher C/N atomic ratio and lower TGI values indicate predominance of aliphatic groups.

Table 2: Parameters obtained from TG/DTG curves of HA, SHA, SHA-Cu²⁺, SHA-Fe³⁺ and SHA-Mn²⁺ samples: moisture content, weight losses (at the ranges: 105-350 °C and 350-650°C), thermogravimetric index (TGI) and mass residue (ash content) at 800 °C.

Sample	Moisture (%)	Weight loss (%)		TGI	
	25-105 °C	105-350 °C	350-650 °C	800 °C	
HA	9.2	29.9	53.9	6.9	1.8
SHA	11.0	17.1	71.9	0.6	4.2
SHA-Cu ²⁺	5.7	49.8	36.2	7.9	0.7
SHA-Fe ³⁺	9.9	35.9	51.3	2.7	1.4
SHA-Mn ²⁺	10.8	17.4	69.6	2.7	3.9

Other important difference between HA and SHA given by the experimental data collected in this work is the ash content: the HA possesses 6.9% in ash, while the SHA presented only 0.6% of ash, in weight. This evidences that the HA contains ten folds more content of inorganic moieties than the SHA.

After the complexation of SHA with metallic ions (Cu²⁺, Fe³⁺ and Mn²⁺), the respective TGA curves profiles changed substantially. The changes can be easily observed in the respective DTG curves and by comparison to the DTG curve of precursor SHA. For instance, the first event of thermal degradation in the SHA-Fe³⁺ complex shifted to lower temperatures, ascribing a decrease in thermal stability. Despite this shifting, it remains as only one event because only one peak was observed in DTG curve for this complex (see

Fig. 3). Differently, the DTG curves for SHA-Cu²⁺ and SHA-Mn²⁺ complexes presented two peaks in the 105-350 °C range, instead only one for SHA-Fe³⁺ or SHA. For the SHA-Cu²⁺ complex, both peaks appeared on DTG curve at lower temperatures, as compared to SHA. For the SHA-Mn²⁺ complex, only one of these peaks appeared at lower temperature while the temperature where the other peak appeared does not changed as compared to not complexed SHA. This means that thermal properties of SHA-metallic ions (Cu²⁺, Fe³⁺ and Mn²⁺) are dependent of the metallic ion type evolved in the complex forming.

Thermal stability of humic acid and its complexes with different metallic ions (Fe³⁺, Mn²⁺, Ca²⁺ and Na⁺) was investigated by Lu et al. (2007) through FTIR emission spectroscopy, at different temperatures. For the HA, they pointed out that as the temperature was increased the amount of aromatic portion of HA increased and the amount of aliphatic decreased. It was proposed that aromatic C-H bonds were formed at the expense of aliphatic C-H ones through dehydrogenation of hydro-aromatics, or via dehydrogenative cyclisation (VASSALLO et al., 1992). Beyond this, Lu et al. (2007) observed that during heating of metal ion-free HA gradual decreases in the intensities of the bands at 1720 and 1600 cm⁻¹ occurred at temperatures below 300 °C and were due to slow-rate decarboxylation process. However, above 300 °C such decomposition is accelerated and provokes rapid decrease in the intensity of the COOH band that completely disappeared at temperatures above 600 °C. According to Lu et al. (2007), in HA-metallic ion complexes the decarboxylation occurs at lower temperature and it is accompanied by a monoxide carbon formation. The lowering in thermal stability observed by Lu et al. (2007) on HA-metallic ion complexation leads to the following thermal stability sequence: HA-Fe³⁺ < HA-Mn²⁺ < HA-Ca²⁺ < HA-Na⁺. So, as observed in Figure 3, the complexing HA with the Mn²⁺ changed the thermal stability, splitting the only one peak at DTG of HA on two peaks, being that both peaks appeared at higher than 420-430 °C. Buurman et al. (2002) also observed changes in thermal stability of humic material after be complexed with mono, di and trivalent metallic cations.

Possibly, the changes in thermal stability are related to matrix-cation interaction intensity as bind energy. Some plant micronutrients (Zn, Cu, Fe, Mn) bind to the functional groups without the interposition of the water molecules, thus forming inner-sphere complexes with a tendency to irreversibility, and the particle surface becomes more positive. Probably, conformational reorganization associated to the metal ions-SHA complexation resulted in changes in the thermal stability. This can be also observed by comparison of the values of TGI of SHA and complexes (SHA-Cu²⁺, SHA-Fe³⁺ and SHA-Mn²⁺). The complex SHA-Mn²⁺ showed values close to SHA, while SHA-Fe³⁺ and SHA-Cu²⁺ presented lower TGI values. An explanation for this behavior is that metal cations bind favorably with different parts (with predominance of aliphatic on SHA), possibly provoking reorganization in the matrix (VASSALLO et al., 1992). According to Lu et al. (2007), stronger bonding of the transition metal adducts to humic substances gives metal forms of humic substances with lower thermal stability because of the effect of strain due to the formation of a metal chelate. It was also observed that the presence of metal ions increased the residue content in relation to the observed value for SHA, as expected.

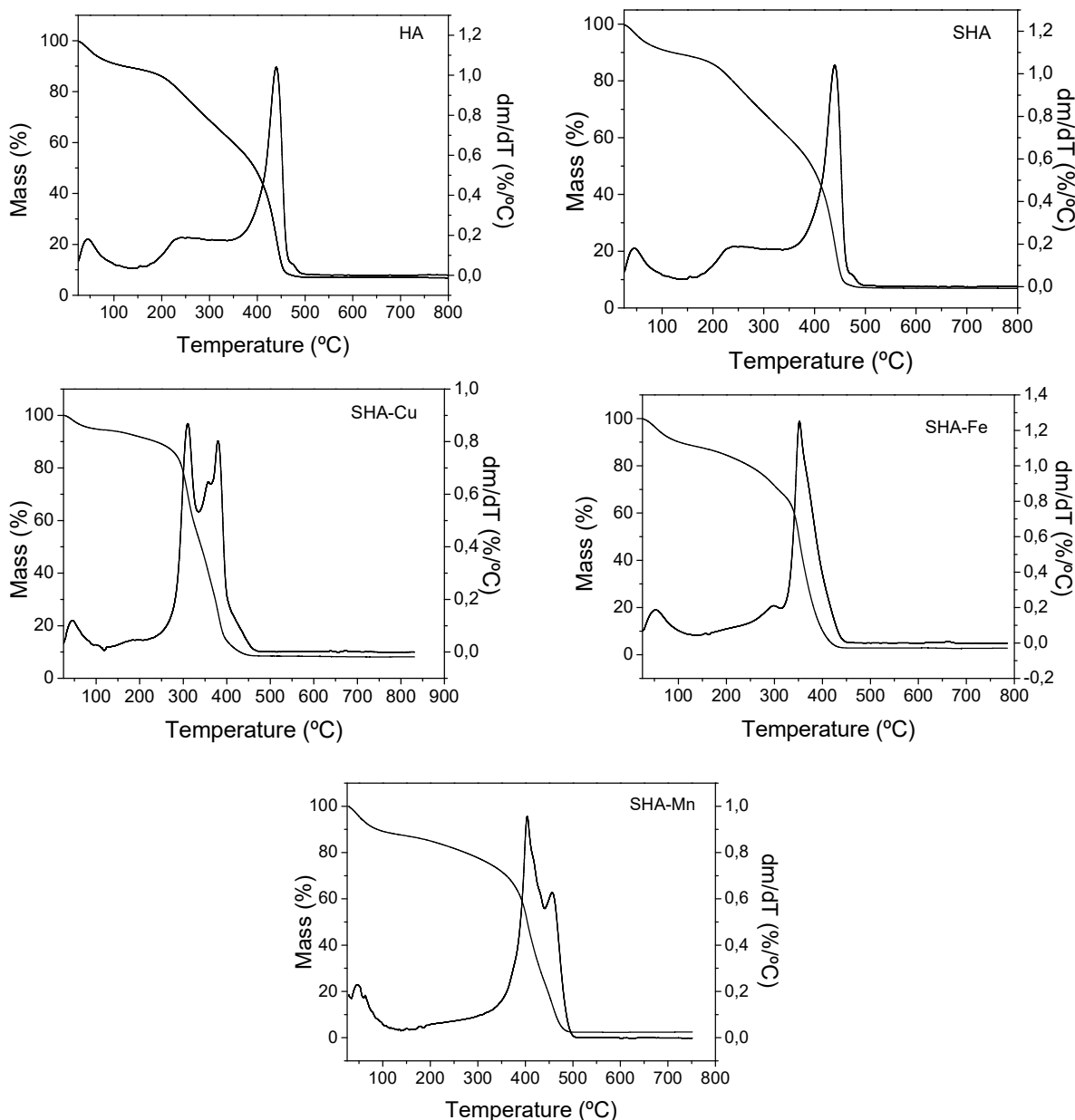


Figure 3: TG and DTG curves obtained for HA, SHA, SHA-Cu²⁺, SHA-Fe³⁺ and SHA-Mn²⁺ samples.

CONCLUSIONS

Interactions between SHA and the metallic ions Cu²⁺, Fe³⁺ and Mn²⁺ were confirmed by infrared spectroscopy in which the intensity of the peak due to the stretching frequency of C=O in COOH groups decreased following complex formation. Elemental analysis showed that SHA presented atomic constitution similar of the HA, except for sulfur and nitrogen contents, fact that is expected due to the precursor compounds. The TGA curves of HA and SHA are quite similar. However, the complex of SHA-metallic ions (SHA-Cu²⁺, SHA-Fe³⁺ and SHA-Mn²⁺) presented different thermal stability as compared among them and also to the precursor SHA. The data indicate that the complexation is dependent of metallic ion-type and provokes different rearrangements in the SHA matrix.

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REFERENCES

BENITES, V. M.; KUCERÍK, J.; MADARI, B. E.. Thermal Analysis of humic substances extracted from contrasting pedoenvironments. **Chem. Listy**, v.99, p.s49-s652, 2005.

BUURMAN, P.; VAN LAGEN, B.; PICCOLO, A.. Increase in Stability Against Thermal Oxidation of Soil Humic Substances as a Result of Self Association. **Organic Geochemistry**, v.33, p.367-381, 2002.

CATALDO, F.. On the structure of macromolecules obtained by oxidative polymerization of polyhydroxyphenols and quinones. **Polymer International**, v.46, n.4, p.263-268, 1998.

HANNINEN, K. I.; KLÖCKING R.; HELBIG, B.. Synthesis and characterization of humic acid-like polymers. **The Science of the Total Environment**, v.62, p.201-210, 1987.

KOLOKASSIDOU, C.; PASHALIDIS, I.; COSTA, C. N.; EFSATHIOU, A. M.; BUCKAU, G.. Thermal stability of solid and aqueous solutions of humic acid. **Thermochimica Acta**, v.454, p.78-83, 2007.

KOVALENKO, A.; YUODOV, M.; PERMINOVA, I.; PETROSYAN, V.. Synthesis and characterization of humic derivatives enriched with hydroquinone and catecholic moieties. In: INTERNATIONAL MEETING OF IHSS, 12. **Anais**. São Pedro, 2004. p.472-473.

LU, X. Q.; VASSALLO, A.M.; JOHNSON, W. D.. Thermal stability of humic substances and their metal forms: an investigation using FTIR emission spectroscopy. **Journal of Analytical and Applied Pyrolysis**, v.43, p.103-114, 1997.

MELO, V. F.; SCHAEFER, C. E. G. R.. Matéria Orgânica em Solos Desenvolvidos de Rochas Máficas no Nordeste de Roraima, **Acta Amazônica**, v.39, n.1, p.53-60, 2009.

MEURER, E. J.. **Fundamentos de química do solo**. Porto Alegre: Evangraf, 2006.

PICCOLO, A.; STEVENSON, F. J.. Infrared Spectra of Cu²⁺, Pb²⁺, and Ca²⁺ Complexes of Soil Humic Substances. **Geoderma**, v.27, p.195-208, 1982.

PRADO, A. G. S.; TORRES, J. D.; MARTINS, P. C.; PERTUSATTI, J.; BOLZON, L. B.; FARIA, E. A.. Studies on copper(II)- and zinc(II)-mixed ligand complexes of humic acid. **Journal of Hazardous Materials B**, v.136, p.585-588, 2006.

SCHNITZER, M.; KHAN, S. U.. **Soil organic matter**. Amsterdam: Elsevier Scientific, 1978.

STEVENSON, F. J.. **Humus Chemistry: Genesis, Composition, Reactions**. 2 ed. New York: Wiley, 1994.

STEVENSON, F. J.. **Humus chemistry: genesis, composition, reactions**. New York: Wiley, 1982.

VASSALLO, A. M.; ATTALLA, M. I.. **J. Anal. Appl. Pyrol.** 1992.

WUNDERWALD, U.; KREISEL, G.; BRAUN, M.; SCHULZ, M.. Formation and degradation of a synthetic humic acid derived from 3-Fluorocatechol. **Appl Microbiol Biotechnol**, v.53, p.441-446, 2000.

ZUNINO, H.; AGUILERA, M.; CAIOZZI, M.; PEIRANO, P.; BORIE, F.; MARTIN, J. P.. Metal-binding organic macromolecules in Soil: 3. competition of Mg (II) and Zn (II) for binding sites in humic acid and fulvic-type model polymers. **Soil Sci.**, v.128, p.257-266, 1979.

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