

# Analysis of humic acid from compost of urban wastes and soil by fluorescence spectroscopy

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## SUMMARY

The aim of this work was to analyze humic acids (HAs) extracted from compost and soil from Cordoba, Argentina through the utilization of fluorescence emission spectra in order to find similarities and/or differences in the aromatic character and size and/or weight between soil and compost HA in comparison with HA from IHSS. These characteristics are directly related with the biodegradation process of HA and in this way with the physical and chemical soil fertility.

The HAs samples used in this work were: International Humic Substances Society (IHSS) soil HA standard (1S102), leonardite HA standard (1S104H); Soil HA from different origin from Province of Cordoba, Argentina and compost derived HA. Soil HAs studied in this work have the following molecular size and/or aromatic condensation order: Gramma < Pastizal < Desmonte < Algarrobo < Leonardite < Soil standard, in agreement with the fluorescence spectra, thus the HAs derived from soil of the province of Córdoba, Argentina, are more easily biodegradable in soil environment. The fluorescence behavior of compost HA (CHA) studied in this work suggest that this CHA have the lowest molecular size and aromatic condensation content because the short period of humification that takes place in the composting process. These compounds are HAs in neof ormation or have similar structure to fulvic acids.

**Key words:** soil humic acids, compost humic acids, fluorescence spectroscopy, organic matter.

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## RESUMEN

El objetivo de este trabajo fue analizar ácidos húmicos (AH) extraídos de compost y suelos de Córdoba, Argentina, utilizando espectroscopía de fluorescencia de emisión con el propósito de encontrar similitudes y diferencias en el carácter aromático, tamaño y/o peso entre los AH de suelo y compost en comparación con los AH de la IHSS. Estas características están relacionadas con los procesos de biodegradación de los AH y consecuentemente con la fertilidad física y química del suelo.

Las muestras de AH estudiadas fueron: AH soil standard (1S102), AH leonardite standard (1S104H) de la Sociedad Internacional de Sustancia Húmicas (IHSS), AH de suelos de diferentes orígenes, de la Provincia de Córdoba, Argentina y AH derivados de compost.

Los AH de suelo estudiados tienen, según los espectros de fluorescencia, el siguiente tamaño molecular y/o carácter aromático: Gramma < Pastizal < Desmonte < Algarrobo < Leonardite < Soil standard; con lo cual los AH de suelos de la provincia de Córdoba, Argentina, son más fácilmente biodegradables en el medioambiente del suelo. El comportamiento fluorescente de los AH de compost (CHA) estudiados en este trabajo sugiere que éstos tienen el menor tamaño molecular y condensación aromática debido al corto período de humificación que tiene lugar durante el proceso de compostaje. Estos compuestos son AH en neoformación o tienen estructura similar a los ácidos fúlvicos.

**Palabras clave:** ácidos húmicos de suelo, ácidos húmicos de compost, espectroscopía de fluorescencia, materia orgánica

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## INTRODUCTION

It is well known that intensive agronomic practices have a great influence on the characteristics of soil organic matter, generally causing substantial losses of organic carbon (Oades *et al.*, 1988; Capriel *et al.*, 1997). The addition of organic amendments and crop rotation systems are traditional farming practices employed to increase the soil content of organic carbon (Paustian *et al.*, 1992). The organic matter (OM) is known to play important roles in the interaction and transport of many toxic organic or inorganic chemicals and in nutrient cycling throughout the environment (Burgos, *et al.*, 2000; LeBoeuf & Weber, 2000 a,b). Humic acids (HA), the most resistant fraction to microbial degradation of the OM in soil, are complex polymeric organic acids with a wide range of molecular weights. They are heterogeneous mixtures of a variety of organic compounds, consisting of aromatic, aliphatic, phenolic, and quinolic functional groups with varying molecular sizes and properties (Aiken *et al.*, 1985; Gu *et al.*, 1995). In addition,

the size, chemical composition, structure, and functional groups of HAs may vary greatly, depending on the origin and age of the material (Chin *et al.*, 1998). They affect a variety of chemical, physical, and biological reactions.

HAs are one of most active fractions of OM, they improve the absorption of nutrients by plants and soil microorganisms, have a positive effect on the dynamic of N and P in soil, stimulate plant respiration and the photosynthesis process, and favor the formation of soil aggregates, etc. (Hernandez *et al.*, 2001). The fluorescence of HAs is a general phenomenon (Schnitzer & Khan, 1972). The fluorophores in HAs are thought to contain substituted and condensed aromatic structures (Seitz, 1981). It has been demonstrated that the fluorescent intensity of HAs and fulvic acid (FA) increase with decreasing molecular size (Ewald *et al.*, 1988; Aoyama, 2001). Fluorescence and UV spectroscopy have recently been used to establish differences in dissolved OM composition (McKnight *et al.*, 1997).

The humification degree produce changes in the HAs fluorescent structures. The relative fluorescence intensity is lower when the humic acids are more ancient and another pronounced maximum in the spectra at a longer wavelength appears when the aromatic condensation degree and complexity of the humic acid molecule are greater (Miikki *et al.*, 1997). Miano *et al.* (1988) systematically studied the fluorescence spectra of HAs and FA of various origins. They found that broad emission spectra showed maximum intensity wavelengths that were lower for FA than for HAs, indicating a greater degree of condensed aromatic character in HA.

The sensitivity and nondestructive nature of fluorescence techniques are well suited for studies of the chemical and physical properties of HAs (Pullin & Cabaniss, 1995; McKnight *et al.*, 2001).

The aim of this work was to analyze HAs extracted from compost and soil from Cordoba Argentina through the utilization of fluorescence emission spectra in order to find similarities and/or differences in the aromatic character and size and/or weight between soil and compost HA in comparison with HA from IHSS. These characteristics are directly related with the biodegradation process of HA and, in this way, with the physical and chemical soil fertility.

## MATERIALS AND METHODS

### Humic acids

The HAs samples used in this work were: International Humic Substances Society (IHSS) soil HA standard (1S102), leonardite HA standard (1S104H); Soil HA from different origins from Cordoba, Argentina and compost HA.

The soil from which HAs were extracted and purified are: - Entic Haplustol with different vegetable coverages, the plots are: with Gramma Rhodes and with natural grass land. HA are labeled as HA Gramma, and HA Pastizal; - Typic Entisol with different vegetable coverages, the plots are: mount with Algarrobo (*prosopis sp*) and natural grass land without trees. HA are labeled as HA Algarrobo and HA Desmonte.

The compost material from which HAs were extracted and purified are: compost obtained with a mixture of horse manure 30 %, urban wastes 40 %, vegetal material 30% from a plant located in La Para (Province of Córdoba). The procedure of composting was carried out in open air piles. Once a week, the piles were turned and their humidity percentage (50-60%) was controlled. Sampling was made at the end

of the stabilization process after 270 days. These HAs are labeled as HA La Para.

### Extraction of the humic acids

Soil and compost derived humic acids (CHA) were extracted with a slightly HA modified procedure recommended and used by the IHSS to isolate standards of humic acid. The dried soil and compost samples were sieved through a 1 cm sieve. The sample was treated with 0.1 M HCl at room temperature at a final ratio solution-raw sample 10-1. The suspension was shaken for 4 h and the supernatant was separated from the residue by low speed centrifugation. The soil residue was neutralized to pH 7.0 with 1 M NaOH and 0.1 M NaOH added to result in a final extractant to soil ratio of 10:1. The suspension was extracted by shaking for a minimum of 8 h in N<sub>2</sub> atmosphere. The alkaline suspension was allowed to settle overnight and the supernatant collected by centrifugation. The supernatant was acidified with 6 M HCl with constant stirring to pH 1.0 and then allowed to stand for 24 h. It was then centrifuged to separate the humic acid (precipitate) fraction. The humic acid fraction was redissolved by adding a minimum volume of 0.1 M KOH. Solid KCl was added to obtain a concentration of 0.3 M [K<sup>+</sup>] and then centrifuged at high speed to remove the suspended solids. The humic acid was reprecipitated by adding 6 M HCl with constant stirring to pH 1.0 and allowed to stand again for 12 to 16 h. The sample was centrifuged and the supernatant discarded. The humic acid precipitate was suspended in a mixture of 0.1 M HCl – 0.3 M HF solution in a plastic container and shaken overnight at room temperature. After centrifuging, the HCl-HF treatment was repeated to obtain a sufficiently low ash content. The humic acid was washed with distilled water until the water gave a negative test for chloride with silver nitrate AgNO<sub>3</sub>. The humic acid preparative was then dried at room temperature until constant weight.

Sample preparation for the analysis was as follows: 1 mg of HA was dissolved in 1 ml 0.036 M NaOH. For fluorescence analysis the stock solution was diluted 100 times. All HAs work solutions were analyzed after 10 days of preparation, because it was found that after this time solution stability was reached.

### Analysis

Elemental analyses of HAs were carried out with a Carlo Erba Model 1108 Elemental Analyzer. The analyses were made in triplicate. The total content of carbon, nitrogen and hydrogen in soil HAs from IHSS were determined on a Perkin Elmer 2400

CHNS/O analyzer and the ash content was obtained by burning a certain amount of HA in an oven at 900°C for 6 h. Optical densities of HAs solution (3 mg of HA in 10 ml 0.05N NaHCO<sub>3</sub>) were measured at 465 (E<sub>465</sub>) and 665 nm (E<sub>665</sub>) wavelengths on a Spectronic 20 Genesys and these values were used for the calculation of the E4/E6 coefficient value (Chen *et al.*, 1977).

Fluorescence spectra were obtained for the aqueous solution of HAs at a concentration of 10 ppm (pH=8) using an Aminco Bowman Serie 2 Luminiscence Spectrometer Thermospectronic equipped with a 1 cm quartz cell. Emission spectra were recorded over a scan range of 400 – 700 nm by measuring the fluorescence intensity at a fixed excitation wavelength of 355 nm. The pH values were measured using a glass G202C electrode, a standard calomel electrode K401 of Radiometer and a Precision Digital pH meter OP-208/1 of Radelkis while standard buffer solutions of Radiometer and/or Radelkis 5 were used for the calibration.

## RESULTS AND DISCUSSION

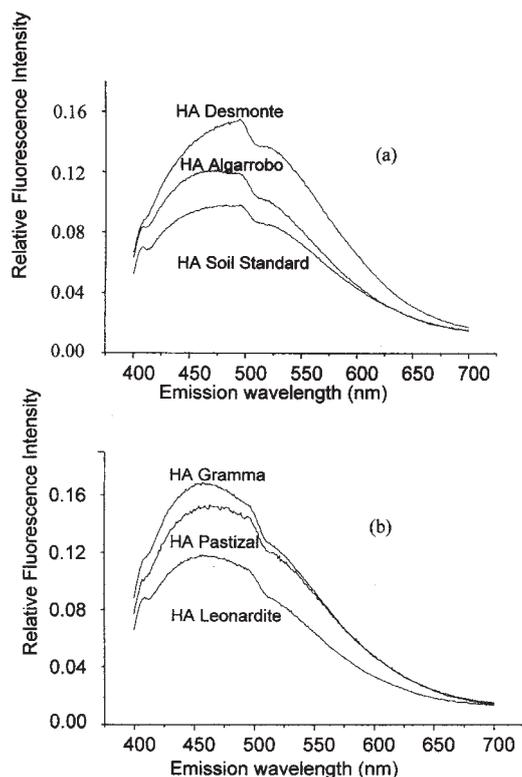
Elemental analysis for HA was carried out and data are given in Table 1. Level of ash contents in compost derived HA is higher than in soil standard HA, however, leonardite standard HA presents the highest content. Carbon to nitrogen (C/N) ratios vary in a wide range (13.2 to 71.9) for soil HA while C/N ratio for compost HA samples was the lowest value probably due to the raw material used in the composting process such as horse manure and vegetal materials have a high nitrogen content and because of the short and incomplete period of humification that takes place during the composting process.

The elemental analysis of all HA studied is in the same range as that obtained by other authors (Stevenson 1994).

Kononova (1966) believes that the magnitude of E4/E6 is related to the degree of condensation of the aromatic C network, with a low ratio indicative of a relatively high degree of condensation of aromatic humic constituents. Conversely, a high E4/E6 ratio reflects a low degree of aromatic condensation and suggests the presence of relatively large portions of aliphatic structures. Chen *et al.* (1977) suggested that values of E4/E6 ratio depend on the average molecular weight or particle size (negative correlation). Soil and coal derived HAs, Gramma, Pastizal, Desmonte, Algarrobo, Soil Standard and Leonardite have the lowest values of E4/E6 ratio that indicates a high degree of condensation of aromatic constituents and/or significantly high molecular size and weight. Compost derived HA, La Para has a high E4/E6 ratio indicating the presence of relatively large proportions of aliphatic structures, low degree of aromatic C network content and low average molecular weight, probably due to a short period of humification that takes place in the composting process. This E4/E6 value suggests that during the composting process the HAs produced are similar to fulvic fraction and in this way they can undergo more microbial degradation.

### Fluorescence of Soil HAs

The soil HAs and compost derived HAs studied, show fluorescence in the wavelength range of 400 - 700 nm ( $C_{HA} 10 \text{ mg l}^{-1}$ ;  $\lambda_{exc} 355 \text{ nm}$ ). Figures 1a and b, show the fluorescence spectra of soil derived and standard HA. Those HAs have two shoulders, the first one at a shorter wavelength has a higher relative fluorescence intensity and is more defined than the second shoulder. In agreement with the fluorescence spectra, the soil HA studied in this work can be classified into two groups: the first group -Soil Standard, Algarrobo and Desmonte- (Figure 1a) and



**Figure 1:** Fluorescence emission spectra of soil and coal derived HA. The concentration of all HA was: 10 mg l<sup>-1</sup>;  $\lambda_{exc} = 355 \text{ nm}$ . a) A comparison of HA extracted from Entisol with soil standard AH of IHSS b) A comparison of HA extracted from Haplustol with coal derived HA leonardite standard (IHSS).

**Table 1:** Review of elemental analysis and ash content concerning HA studied.

Humic Acid	Type	C (%)	H (%)	N (%)	O (%)	C/N	E4/E6	Ash (%)
Soil IHSS Standard	soil	58.13	3.68	4.14	34.08	16.4	4.68	0.88
Leonardite	coal	63.81	3.70	1.23	31.27	60.5	4.76	2.58
Gamma	soil	50.94	5.24	2.16	41.66	27.6	5.45	1.56
Pastizal	soil	49.81	4.77	2.30	41.59	25.3	5.30	1.65
Algarrobo	soil	51.11	5.17	1.66	40.67	36.0	4.46	1.79
Desmonte	soil	53.53	4.28	0.88	40.69	71.9	4.88	2.03
La Para	compost	51.80	4.90	4.60	38.70	13.2	6.18	1.27

Content of C, H, and O is related to free humic acids.

C, H, N, O are the elemental composition in % (w/w) of the dry, ash free samples.

Ash is the % (w/w) of inorganic residues in a dry sample.

the second group -Gamma, Pastizal and Leonardite- (Figure 1 b), which have similar profiles in each groups. Among both groups there are some differences. In the first group, (Soil Standard, Algarrobo and Desmonte) separation between both shoulders is clear and the second shoulders have less intensity than the first one. In Figure 1a the maxima of the first shoulder are about 490 nm and this wavelength is greater than the first maxima of the HAs in the second group (460nm) (Figure 1b). This behavior can indicate that the HAs of the first group (Soil Standard, Algarrobo and Desmonte) have a greater degree of aromatic condensation than the HAs of the second group (Gamma, Pastizal and Leonardite), in agreement with the E4/E6 values (Table 1). The second group of HAs (Gamma, Pastizal and Leonardite) (Figure 1 b) presents similar fluorescence spectra, the first shoulder with a maxima at 460 nm and the second one less pronounced. In Figures 1 (a, b) it can be seen that Argentinean soil HAs (Algarrobo, Desmonte, Pastizal y Gamma) have more fluorescence intensity than IHSS Soil Standard and Leonardite HAs.

Aoyama (2001) and Miano & Alberts (2001), among others, found an inverse correlation between molecular size, aromatic condensation degree and/or molecular weigh with the relative fluorescence intensity and a direct correlation with wavelength of the fluorescence spectra in HA. Our results can indicate that Argentinean soil HAs are characterized by a lower molecular size and lower aromatic condensation degree than standards HAs from IHSS. This result can indicate a greater degradation possibility of Argentinean HAs and in this way less contribution to the chemical and physical fertility of the soil from which they were extracted in comparison with AHs from IHSS.

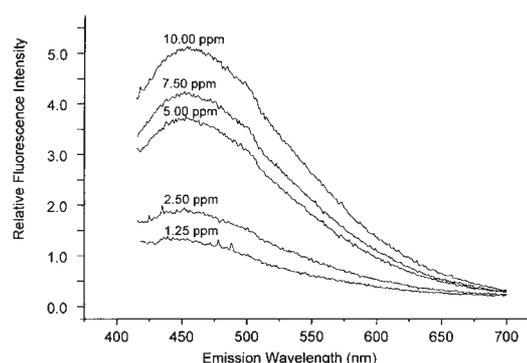
In agreement with the fluorescence spectra, the soil HA studied can have the following molecular size and/or aromatic degree condensation order:

Gamma < Pastizal < Desmonte < Algarrobo < Leonardite < Soil Standard. This order is in agreement with those obtained through E4/E6 value.

### Effect of the HAs concentration on the fluorescence spectra

Figure 2 shows the variation of relative fluorescence intensity with the HAs concentration for HAs extracted from urban waste compost (La Para HA). It shows an increase of relative fluorescence intensity with an increase of HA concentration from 1.25 to 10 ppm.

A similar experiment, relative fluorescence intensity vs. concentration, was carried out for Soil HAs (Gamma HA) and the results are shown in Figure 3, which shows a relative fluorescence intensity increase with an increase of HA concentration from 2.5 to 20 ppm. In both cases the profiles of the fluorescence spectra were similar for the different concentration studied.



**Figure 2:** Fluorescence emission spectra of compost derived HA "La Para". Variation of relative fluorescence intensity with the concentration. Range of concentration: 1.25 ppm to 10.00 ppm.  $\lambda_{exc.} = 355$  nm.

Soil HAs fluorescence spectra show two shoulders for all concentrations studied. The first shoulder with a higher intensity than the second, at 490 nm and 520 nm respectively. Compost HAs fluorescence spectra (Figure 2) show only one broad shoulder in the region 400 – 700 nm unlike those observed in the corresponding spectra for soil HA. In this case the maximum has a higher intensity than the soil HAs and this maximum is observed at 470 nm. This can indicate that compost HAs (La Para) have a lower molecular size, a lower aromatic condensation degree and a high heterogeneity of fluorescent groups present in more simple structural unities in comparison with soil HAs (Gamma), probably due to the short and incomplete period of humification that takes place in the composting process. The fluorescence behavior of compost HA studied in this work is in agreement with the fact that a decrease in the age of the HAs shows an increase in the relative intensity of fluorescence (Miikki *et al.*, 1997).

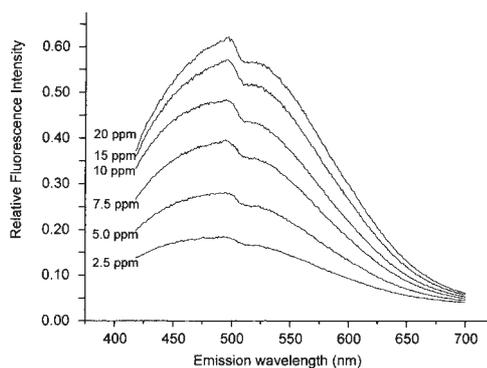
All the fluorescence spectra show numerous peaks in the spectra indicating that the resultant is the overlapping of a set of spectra of different substances that have this fluorescent properties in the HA to very close wavelengths. This characteristic is more evident in compost HAs which have, probably, a lower molecular size and a relative high content of low molecular weight polyphenolic compounds that give higher intense fluorescence emission as compared with those of soil HAs (Chen *et al.*, 2003). Thus, the HAs extracted from compost are compounds with a limited oxidation and humification process and in this way these HAs are HAs in neoforation or have a structure similar to fulvic acids.

### Effect of pH on the HAs Fluorescence spectra

The variation of the fluorescence intensity with pH was studied for compost HAs (La Para) and the results are shown in the Figure 4. It shows that the relative fluorescence intensity increase faster with the increase of pH from pH 1.5 to pH 6. For higher pH values, the fluorescence intensity was approximately constant (from pH 6 to 11) (Fig.4).

According to Laane 1982, the change of fluorescent intensity with pH is probably due to the alteration of the molecular orbital of excitable electrons. This can be the consequence of ionization of the fluorescent molecules after modification pH.

Another possible explanation can be related to the macromolecular configuration of humic substances: more rigid structures that give better fluorescent yields. Ghosh & Schnitzer (1980) observed that the structure of humic substances varied with the modification in their environment, such as pH

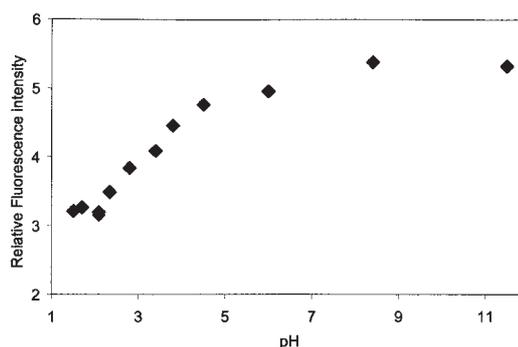


**Figure 3:** Fluorescence emission spectra of soil derived HA "Gamma". Variation of relative fluorescence intensity with the concentration. Range of concentration: 2.5 ppm to 20 ppm.  $\lambda_{exc.} = 355$  nm.

changes. Their conclusion is that humic substances have linear structures at high pH and they coil when pH decreases. This result can also explain that the fluorescent intensity increase with increasing pH. A spherocolloidal configuration could mask some fluorophores inside their structure. At higher pH, the configuration becomes linear and some fluorophores, which are not anymore masked, can fluoresce, increasing the fluorescent intensity.

As fluorescence properties depend on the environmental conditions, such as the ionization state, a molecule whose structure has acid function near a fluorophore, can be a model of the fluorescence intensity dependency. Fluorophores depend on the acid functions on the structure, but unfortunately, constant acidity measurements on the sample do not give so extreme pKa, because the fluorescence is not directly related to the acid-base functions.

This pH effect (decrease of fluorescence emis-



**Figure 4:** Variation of fluorescence intensity with pH. Relative fluorescence intensity at  $\lambda_{em.} = 455$

sion upon acidification from pH 6 to pH 1.5) suggests that the fluorescence process is strongly dependent on the ionization state of macromolecules or of fluorophores.

## CONCLUSION

The soil HAs studied in this work have the following molecular size and/or aromatic condensation order: Gramma < Pastizal < Desmonte < Algarrobo < Leonardite < Soil standard, in agreement with the fluorescence spectra, thus the Argentinean HA are more easily biodegradable in the soil environment.

The fluorescence behavior of compost HAs studied in this work indicates that this CHA have the lowest molecular size and aromatic condensation content because of the short period of humification that takes place in the composting process. These compounds are HAs in neoformation. In this way, CHA can be more easily biodegradable than soil HAs, but when composts are used as a soil organic amendment, their HAs in neoformation can be incorporated in the soil HAs structure and contribute to chemical and physical fertility and they increase the soil organic matter content.

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