

Original Research Article

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Characterization of Humic Substances by Functional Groups and Spectroscopic Methods

M. Eshwar^{1*}, M. Srilatha¹, K. Bhanu Rekha² and S. Harish Kumar Sharma¹

¹Department of Soil Science and Agricultural chemistry, College of Agriculture, PJTSAU, Rajendranagar, Hyderabad- 500 030, Telangana, India

²Department of Agronomy, Agriculture College, Palem, Nagarkurnool

*Corresponding author

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The present study was carried in the Department of Soil Science and Agricultural Chemistry, College of Agriculture, Rajendranagar. The humic and fulvic acids were extracted, isolated and purified from manures and were studied for their functional groups and spectroscopic methods. It has been found that the total acidity (9.4 me g⁻¹), carboxyl group (6.2 me g⁻¹) and phenolic-OH (3.2 me g⁻¹) groups were higher in case of fulvic acid compared to humic acid (7.2, 4.2 and 3.0 me g⁻¹ respectively). Among these functional groups, the percent contribution of carboxylic groups towards total acidity was higher than that of phenolic-OH groups in both humic fractions. The ratio of optical densities at 465 and 665 nm i.e. E₄/E₆ ratio was higher in case of fulvic acid (6.17) compared to humic acid (4.34). The UV spectra of humic and fulvic acid were similar in nature in spite of the differences in their composition and showed a decreasing optical density with increasing wavelength.

Introduction

Humic substances are often described as coiled, long chain molecules of two or three dimensional cross linked macromolecules which may vary in molecular weight depending on type of humic acid and method of measurement (Stevenson, 1994). They form the largest fraction of soil organic matter (OM) and play vital role in improving soil productivity. On account of their wide range of molecular sizes and properties, humic substances are usually fractionated to obtain materials with similar properties. The three fractions of humic substances are: i) fulvic acid (FA), (ii) humic acid (HA) and (iii)

humin. Among these humic fractions, FA is soluble in both acid and alkali. HA is the fraction which is soluble only in alkali. The most insoluble fraction of humus is humin, which is neither soluble in acid nor alkali.

Soil organic matter fractions are capable of forming complexes with metal ions. The ability of humic substances to form stable complexes with metal ions can be accounted for their high content of oxygen containing functional groups viz., carboxylic, phenolic aliphatic and alcoholic -OH groups. As these complex formation reactions between metal

ions and humic substances are helpful in understanding the problems of plant nutrition (Stevenson *et al.*, 1993). During complexation, numerous compounds including humic acid (HA) and fulvic acid (FA) are involved which control the distribution and supply of micronutrients to plants and interact with metal ions through their functional groups forming metal complexes of varying stabilities.

The spectroscopic studies of humic compounds have been the focus of researchers in the recent past. The humic-fulvic acids have been extensively characterized by UV-VIS spectroscopy. Schnitzer (1977) studied that HAs and FAs extracted from soils formed under different geographic and pedologic environments had similar analytical characteristics and chemical structures. The colour of humic substances is an important physical property, which is used to characterize humic fractions of soil (Tan and Van Schuylenborgh 1961; Kononova 1966; Schnitzer 1971; Flaig 1975). The colour ratio is used as an index for the rate of light absorption in the visible range. Absorption of humic acid in the ultraviolet range is based on the aromatic groups that contribute to the molecular structure, *i.e.* the sum of phenyl propane units, and on several chromophoric structural elements. The evidence for the complexation of micronutrient metal ions with humic acid (HA) and fulvic acid (FA) has been demonstrated by several workers employing chemical methods and IR spectroscopy (Sujana Reddy *et al.*, 1998; Stevenson *et al.*, 1993 and Schnitzer and Hansen, 1970). The studies on the interactions of metal ions with humic acids extracted from the soils of Hyderabad have been reported earlier (Sailaja, 1999 and Srilatha, 2001).

Materials and Methods

The present study was carried out at the laboratory for characterization of humic

substances by functional groups and UV spectra at department of Soil Science and Agricultural Chemistry, College of Agriculture, Rajendranagar in the year 2016-17.

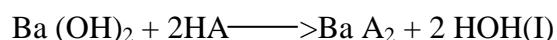
Characterisation of humic substances

Humic acid and Fulvic acids extracted from manures were used for this study and characterized for their functional groups and spectral analysis.

Determination of functional groups

Total acidity

Total acidity in HA/FA was determined by Ba (OH)₂ method. Wright and Schnitzer (1961) applied this method for humic substances. In the modified procedure of Schnitzer and Gupta (1965), the sample is allowed to react with an excess of Ba (OH)₂, the unreacted Ba (OH)₂ could be determined by back titration with standard acid.



Fifty milligrams of HA/FA along with blank was taken in separate stoppered flasks and 20 ml of 0.2N Ba (OH)₂ was added. The flasks were shaken for 24 h at room temperature. The suspension was filtered and the residue was washed with CO₂ free distilled water. The filtrate and washings were titrated against 0.5 N HCl to pH 8.4 potentiometrically. Identical blanks were maintained simultaneously.

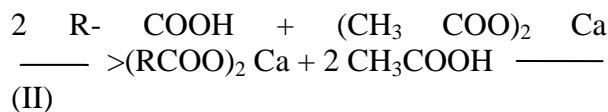
$$\text{Total acidity} = \frac{(\text{TV for blank} - \text{TV for sample}) \times \text{Normality of acid}}{(\text{me g}^{-1}) \text{ Weight of sample (mg)}} \times 1000$$

Where

TV - Titre Value

Carboxylgroups

HA / FA were treated with calcium acetate, acetic acid liberated is titrated with standard 0.1 N NaOH (Schnitzer and Khan, 1972).



For this, fifty milligrams of HA/FA was taken in a stoppered flask to which 10 ml of 1 N(CH_3COO)₂Ca and 40 ml of CO_2 free distilled water were added. A blank was also set up simultaneously. After shaking at room temperature for 24 h the suspension was filtered and the residue was washed with CO_2 free distilled water. The filtrate and washings were titrated potentiometrically with standard 0.1 N NaOH to pH 9.8.

$$\text{COOH groups} = \frac{(\text{TV for sample}-\text{TV for blank}) \times \text{Normality of base (0.1 N)} \times 1000}{(\text{me g}^{-1}) \text{ Weight of sample (mg)}}$$

Phenolic-OH groups

The phenolic-OH groups were calculated as the difference between total acidity and carboxylic group acidity.

$$\text{Phenolic-OH groups} = (\text{Total acidity}) - (-\text{COOH acidity}) \quad (\text{me g}^{-1})$$

Spectral Characteristics

E₄/E₆ ratios

Fifteen milligrams of HA/FA were dissolved in 200 ml of Std. 0.1 N NaOH (Kononova, 1966) and the optical densities were measured at 465 and 665 nm on a UV-visible spectrophotometer. The ratios of optical densities at 465 and 665 nm were calculated and were expressed as E₄/E₆ ratios.

UV-spectra of humic acid/fulvic acids

Spectral characteristics of HA/FA were evaluated in UV- range in the wavelength range of 200 to 300 nm.

For this purpose, the solution containing 10 mg HA/FA ml⁻¹ of distilled water was prepared and the absorbance was recorded on a UV-visible spectrophotometer.

Results and Discussion

Total acidity

The total acidity of humic acid was 7.2 me g⁻¹ that of fulvic acid 9.4 me g⁻¹ indicating that fulvic acid has high total acidity than humic acid (Table 1).

Functional groups obtained from humic and fulvic acids are similar to those obtained by Ramalakshmi (2011).

Banik and Sanyal (2006) also reported high total acidity in case of fulvic acid over humic acid. Srilatha (2014), Sanyal (2002) and Sujana Reddy and Rao (2000) observed that increase in total acidity with decreasing molecular weight was in consistence with increasing degree of oxidation of low molecular weight fractions.

Carboxyl groups

The carboxyl group contents of humic acid was 4.2 me g⁻¹ and fulvic acid was 6.2 me g⁻¹ respectively. Higher content of carboxyl groups in fulvic acid may be due to their low particle weight because decarboxylation did not scan before polymerization or condensation due to high molecular weight thus indicating degradation of humic acid which may also result in high content of carboxyl groups (Srilatha *et al.*, 2013 and Lal and Mishra, 2000).

Phenolic-OH groups

The phenolic-OH group contents of fulvic acid was 3.2 me g^{-1} while it was 3.0 me g^{-1} in humic acid. From the close perusal of data on functional groups it was observed that higher content of carboxyl groups in both humic acid and fulvic acid than phenolic-OH groups suggesting that the carbohydrates and phenolic compounds produced were easily degradable and thus readily converted to carboxyl groups on subsequent oxidation. These results are in accordance with the findings of Satisha and Devarajan (2011), Erdogan *et al.*, (2007) and Banik and Sanyal (2006).

From the data, percent contribution of carboxylic groups and phenolic -OH groups were also calculated (Table 2). The data indicated higher percent contribution of (carboxylic group) -COOH group towards total acidity than that of phenolic-OH groups. The percent contribution of -COOH groups in humic acid was lower (58.3%) than fulvic acid (65.9%). The percent contribution of phenolic-OH groups towards total acidity was (41.6%) and (34.0%) in humic acid and fulvic acid respectively. The acidity of fulvic acid was predominantly due to carboxyl groups whereas phenolic-OH groups contributed to nearly one third of the total acidity (Pandeya and Singh, 2000).

Fig.1 UV spectra of humic acid

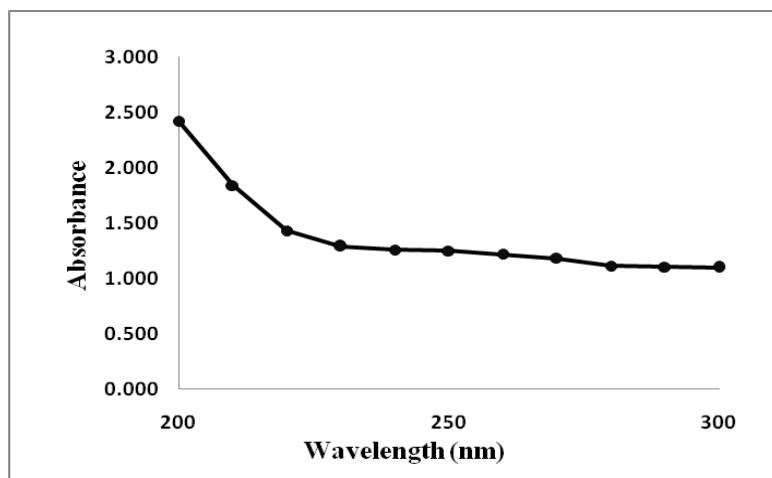


Fig.2 UV spectra of fulvic acid

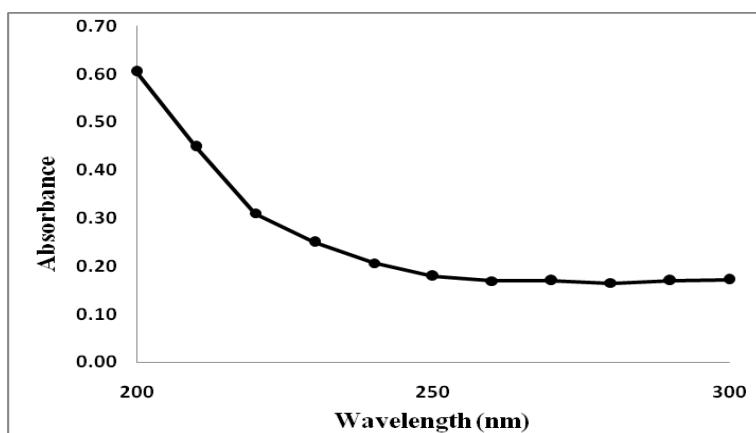


Table.1 Functional group (total acidity, carboxyl, phenolic-OH and E₄/E₆) contents of Humic and fulvic acids

Humic substances	Total acidity (me g ⁻¹)	Carboxylic groups (me g ⁻¹)	Phenolic groups (me g ⁻¹)	E ₄ /E ₆ ratio
Humic acid	7.2	4.2	3.0	4.34
Fulvic acid	9.4	6.2	3.2	6.17

Table.2 Content of carboxyl and phenolic-OH groups (expressed as % Total acidity) of Humic and fulvic acids

Functional groups	Humic acid (% Total acidity)	Fulvic acid (% Total acidity)
Carboxyl groups	58.33	65.95
Phenolic-OH groups	41.66	34.04

E₄/E₆ ratio

The ratio of optical densities at 465 and 665 nm is used for characterization of humic and fulvic acids. The relationship E₄/E₆ is related to aromacity and degree of condensation of the chain of aromatic carbons of humic substances, and could be used as a humification index (Srilatha *et al.*, 2013, Stevenson, 1982 and Schnitzer and Khan, 1972). This ratio is referred to as E₄/E₆ ratio (Lal and Mishra, 1999) is independent of concentration of humic and fulvic acids but varies with humic material extracted from different soil types (Tahiri *et al.*, 2016 and Sailaja and Rao, 2000).

The E₄/E₆ ratio of humic acid was 4.34 that of fulvic acid was 6.17 (Table 1) and it could be observed from the data that the fulvic acid had higher E₄/E₆ ratio in comparison to the humic acid. It could be due to the higher degree of aromaticity in carbon atoms of humic acid. Tahiri *et al.*, (2016), Srilatha *et al.*, (2013) and Satisha and Devarajan (2011) also reported slightly higher E₄/E₆ ratio of fulvic acid than humic acid.

A low ratio of E₄/E₆<5.0 indicates high degree of condensation of aromatic humic

components, high molecular weight and low acidity which are characteristics of humic acid fraction where as high E₄/E₆ ratio of fulvic acid reflects a low degree of aromatic condensation and presence of a relatively large proportion of aliphatic structure. These results are in line with those of Haddad *et al.*, (2015), Petrus *et al.*, (2009) and Banik and Sanyal (2006).

UV Spectra

The UV spectra of humic and fulvic acid was in the range of 200 to 300 nm with a decreasing optical density and increasing wavelength (Figs. 1 and 2). The UV spectra of humic and fulvic acid were similar in nature in spite of the differences in their composition. The absorbance of light in UV range was due to the presence of multiple bonds and due to unshared electronic pair in the organic molecule. These groups which confer colour to the humic substances are called chromophores. The typical chromophores that are known to occur in humic acid are C=C and C=O groups (Stevenson, 1982). Lack of absorbance in UV range could be due to the fact that the humic substances are considered to be an intermediate stage of development between

lignin and coal (Srilatha, 2014; Ramalakshmi et al., 2013; Sailaja and Rao, 2000 and Stevenson, 1982).

From the data it is evident that total acidity, carboxyl group and phenolic-OH group were higher in case of fulvic acid compared to humic acid. The increase in total acidity with decreasing molecular weight was in consistence with increasing degree of oxidation of low molecular weight humic fractions. Higher content of carboxyl groups in fulvic acid may be due to their low particle weight because decarboxylation did not scan before polymerization or condensation. The acidity of fulvic acid was predominantly due to carboxyl groups whereas phenolic-OH groups contributed to nearly one third of the total acidity. The UV spectra of humic and fulvic acid were similar in nature in spite of the differences in their composition. The humification index (E_4/E_6 ratio) of fulvic acid is higher than humic acid.

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