Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.5454

ISSN: 2320 – 7051

Int. J. Pure App. Biosci. **5 (6):** 1254-1259 (2017)

Research Article

International Journal of Pure & Applied Bioscience

OPEN ACCESS

Functional and Spectral Characterization of Humic Fractions Obtained from Organic Manures

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Received: 16.08.2017 | Revised: 22.09.2017 | Accepted: 28.09.2017

ABSTRACT

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 characterise the physico – chemical properties of humic fractions. It has been found that the total acidity in humic acids of farmyard manure and vermicompost was 8.3 and 7.3 respectively, carboxylic groups 5.08 and 3.44 and phenolic- OH groups 3.05 and 3.86 while that of fulvic acids were 9.61 and 8.7, 6.4 and 5.1 and 3.21 and 3.6 me g^{-1} respectively. Among the humic fractions fulvic acid had higher total acidity, carboxylic and phenolic-OH groups than the corresponding humic acid obtained from farmyard manure and vermicompost. The ratio of optical densities at 465 and 665 nm i.e. E_4/E_6 ratio was higher in case of fulvic acids are ranging 8.4 and 6.2 and humic acids were narrow ranging 4.9 and 4.4, respectively.

Key words: *Farmyard manure (FYM), Vermicompost (VC), Humic acid (HA), Fulvic acid (FA), total acidity, carboxyl groups, E_4/E_6 ratio and UV-Spectra.*

INTRODUCTION

Humic substances are considered as the most important constituents of soil. They form the largest fraction of soil organic matter (SOM) and play pivotal role in improving soil productivity. They occur in soils, sediments and water as a product of the chemical and biological transformation of animal and plant residues. They are colloid-sized, polymeric substances having dark colours. 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. Humic acids (HAs) and fulvic acids (FAs) are the most natural widespread complexing agents.

Humic acid (HA) and fulvic acid (FA) components of humus are extracted from organic manures using the classical fractionation procedures based on their solubility characteristics[6].

Cite this article: Rajashekhar, D., Srilatha, M., Rao, P.C.S., Sharma, S.H.K. And Rekha, K. B., Functional and Spectral Characterization of Humic Fractions Obtained From Organic Manures, *Int. J. Pure App. Biosci.* **5(6):** 1254-1259 (2017). doi: http://dx.doi.org/10.18782/2320-7051.5454

They differ in quantity and chemical composition depending on many variables such as climate, parent material, altitude and vegetation type. Fulvic acid plays an important role in influencing fertility and productivity of soil. The reactivity of HA and FA is attributed to their molecular weights, the quantity and quality of the functional groups present in the molecules and the proportions of aliphatic and aromatic rings[3]. Organic manures obtained from department of entomology, college of agriculture, rajendranagar, have long has been recognised as potential source of humic materials[9].

In this work humic acids and fulvic acids were extracted from farmyard manure and vermicompost. After purification, their basic characteristics are evaluated. The total acidity, contents of COOH and phenolic OH as weakly acidic function groups were determined by baryta and direct potentiometric titration methods.

MATERIAL AND METHODS

The FYM and VC used for isolation of humic substances and were extracted and fractioned using standard procedures[6]. The general properties of FYM and VC have been furnished in the Table 1. Farmyard manure and vermicompost after acid treatment (0.1 *N* HCl) was extracted with 50 mL of 0.1 *N* NaOH repeated several times for complete extraction of humic fractions. The soluble FA was separated from coagulate (HA fraction) by centrifugation. The process of precipitation and centrifugation were repeated to attain partial purification of HA fraction[22]. Further purified by treating the extracted HA fraction with HCl - HF mixture, transferring suspension to 100 mwco (Molecular Weight Cut Off) dialysis bags and dialyzed against double distilled water for 24 hours. The dialyzed fraction was evaporated under low temperature and finally dried. The dried sample were weighed and stored for further analysis.

The purification of FA was adsorbed on a charcoal column of activated charcoal was repeatedly washed with 1 *N* H_2SO_4, was then eluted from the column by 1 *N* NH_4OH. Thus obtained fulvic acid was dried at 40^0c and used for further analysis.

Characterization of HA and FA: Humic acid (HA) and fulvic acids (FA) extracted from farmyard manure and vermicompost were analyzed for total acidity was determined by preparing suspension of humic substances (HAs and FAs) and $Ba(OH)_2$, was filtered and the residue was washed thoroughly with double distilled water further filtrate and washings were titrated potentiometrically with standard 0.05 *N* HCl solution to pH 8.4[15]. Carboxylic groups was determined by preparing suspension of humic substances (HAs and FAs) and $CH_3COO)_2Ca$, was filtered and the residue was washed thoroughly with double distilled water further filtrate and washings were titrated potentiometrically with standard 0.1 *N* NaOH solution to pH 9.8[16].

Spectral characterization

E_4/E_6 ratio was done by measuring absorbances of humic substances at 465 and 665nm using UV-VIS spectrophotometer For this purpose, the solution prepare thirty milligrams of HA/FA was dissolved in 100 ml of 0.1 *N* NaOH[6].Spectral characteristics were evaluated in UV- range for HA/FA 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 DISCUSSIONS

Functional characterization of humic fractions

The humic and fulvic acids isolated and extracted from farmyard manure and vermicompost were analysed for their functional groups. HA and FA's were highly relative natural polymers. Functional group analysis provides information about the occurrence of major functional groups in HA and FA and are thus an index of their activity.

The contents of oxygen containing functional groups, obtained from humic acid and fulvic acid are presented in Table 2. Close perusal of data revealed that the oxygen containing

functional groups viz., carboxyl, phenolic and total acidity were high in FA compared to HA. These results are in conformity with those Srilatha *et al*.[20] and Ramalakshmi[11]. The contents of total acidity, carboxylic and phenolic-OH groups of both humic acid samples obtained from farmyard manure and vermicompost were 8.3 and 7.3, 5.08 and 3.44 and 3.05 and 3.86 while that of fulvic acid were 9.61 and 8.7, 6.4 and 5.1 and 3.21 and 3.6 me g^{-1} respectively. Fulvic acid had higher total acidity, carboxylic and phenolic-OH groups than the corresponding humic acid obtained from farmyard manure and vermicompost. Banik and Sanyal[1] reported fulvic acids had higher total acidity than the corresponding humic acid. Xiaowei *et al*.[28] observed that increase in total acidity with decreasing molecular weight was in consistence with increasing degree of oxidation of low molecular weight fractions.

The total acidity and carboxylic groups of fulvic acids were higher than those humic acids may be due to their low particle weight because decarboxylation did not occur before polymerisation or condensation to high molecular weight humic acids, thus indicating degradation of humic acid, which may also result in high content of carboxyl groups[8,20] . Carboxylic and phenolic – OH contents were higher in humic acid extracted from farmyard manure than humic acid obtained from vermicompost[4].

Table 2: Total acidity, carboxyl groups and phenolic- OH groups of humic acid and fulvic acids obtained from Farmyard manure and vermicompost

Source	Humic acid (me g^{-1})			Fulvic acid (me g^{-1})		
	Total acidity	Carboxyl groups	Phenolic-OH	Total acidity	Carboxyl groups	Phenolic-OH
FYM	8.13	5.08	3.05	9.61	6.4	3.21
VC	7.3	3.44	3.86	8.7	5.1	3.6

The percent contribution of –COOH groups in humic acids obtained from farmyard manure and vermicompost was 62.48 and 52.88 % and that of fulvic acids obtained from farmyard manure and vermicompost was 66.59 and 47.12%, respectively whereas the phenolic-OH groups towards total acidity was 37.52 (FYM) and 52.88 % (VC) in humic acid and 33.41 (FYM) and 41.37% (VC) in fulvic acid (table 3) indicating that higher percent contribution of carboxylic group (–COOH group) towards total acidity than that of phenolic-OH groups, irrespective of humic fractions and sources. Similar trend was reported by Lal and Mishra[8] and Srilatha *et al*.[20]. The acidity of humic acid and fulvic acid was predominantly due to carboxyl groups whereas phenolic-OH groups contributed nearly one third of the total acidity[10].

Table 3: Contents of carboxyl and phenolic-OH groups (expressed as % of Total acidity) of humic acid and fulvic acids obtained from farmyard manure and vermicompost

Source	Humic acid		Fulvic acid	
	Content (% of total acidity)		Content (% of total acidity)	
	Carboxyl groups	phenolic-OH	Carboxyl groups	phenolic-OH
FYM	62.48	37.52	66.59	33.41
VC	52.88	47.12	58.62	41.37

Characterization of humic fractions for Spectral properties

UV spectra:

The absorbance of light in UV range is due to the presence of multiple bonds and due to unshared pair in the organic molecule. These group which confer colour to the humic substances, are called chromophores. The typical chromophores known to occur in humic acid are C=C and C=O groups. For description of molecular properties of humic acids (HA) and fulvic acids (FA) UV-Vis

spectroscopy is widely used as simple and informative method[19].

The UV spectra of humic acids and fulvic acids obtained in this study are presented in Fig 1 and 2, respectively. The UV spectra of humic acids and fulvic acids were featureless and monotonously decrease with increasing wavelength, but there are UV regions and absorbances of spectra that can be used to analyse HA and FA[25]. It is interesting to observed that the humic acids of diverse origin were similar in spite of differences in their composition. The lack of absorbance in UV range could be due to the fact that the humic substances are considered to be an intermediate state of development between lignin and coal[11,14,16,18,21,23].

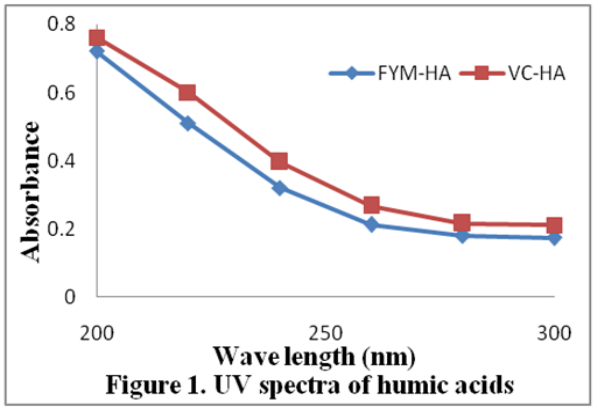
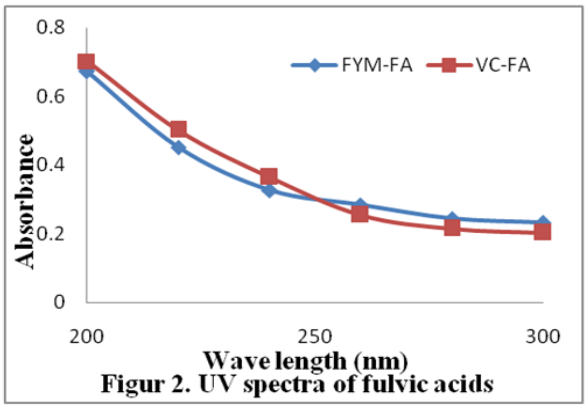


Figure 1. UV spectra of humic acids



Figur 2. UV spectra of fulvic acids

E_4/E_6 ratio:

Measurement of absorption in different regions of electromagnetic spectrum has been used for qualitative and quantitative investigations on HA's and FA's[23]. The ratio of optical densities at 465 and 665 nm is often used for characterisation of humic and fulvic acids. The relationship E_4/E_6 (the ratio absorbances at 465 nm and 665 nm) is related to the aromaticity and to the degree of condensation of the chain of aromatic carbons of the humic acids, and could be used as a humification index[6,23]. This E_4/E_6 ratio, is independent of concentration of humic and fulvic acid but varies with humic material extracted from different manures and soil types[11], Kar *et al.*[4], Srilatha *et al.*[26] and Reddy *et al.*[13].

Low E_4/E_6 ratio reflect a high degree of condensation of these structures while high ratios mean presence of large quantities of aliphatic structures and low quantities of condensed aromatic structures[2]. This ratio also is inversely related to the degree of aromaticity, particle size, molecular weight, and acidity[26]. Typically E_4/E_6 is larger for non-humified material by presence of proteins and carbohydrates, which increase the absorbtivity at the UV region of the spectrum[27].

The optical densities of humic acids have lower values 4.9, 4.4 and fulvic acids 8.4, 6.2 (Table .4). It could be due to the higher degree of aromaticity in carbon atom of HA. Lal and Mishra[8] and Kaddali also reported slightly higher E_4/E_6 ratios of FA than HA.

Table 4: E_4/E_6 ratio of humic and fulvic acids obtained from Farmyard manure and vermicompost

Source	Humic Acid	Fulvic acid
FYM	4.9	8.4
VC	4.4	6.2

Table 1: General properties of organic manures

Organic manure	OC (%)	N (%)	P (%)	K (%)
FYM	6.87	0.81	0.34	0.52
VC	9.82	1.52	0.97	1.58

CONCLUSIONS

Humic and fulvic acids extracted from organic manures showed that among humic fractions, humic acid content was more than fulvic acid. Analysis for oxygen containing groups (total acidity, carboxyl groups and phenolic – OH groups content) were high in fulvic acid than

humic acids and higher percent contribution of carboxylic group (–COOH group) towards total acidity than that of phenolic-OH groups. Spectral characteristics like E_4/E_6 ratios were also studied and found narrow ratios in HA than FA's.

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