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Chemical Characterization and Functional Group Analysis of Humic Substances Extracted from Enriched Compost Prepared by using Various Organic

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A B S T R A C T

The present investigation on chemical characterization and functional group analysis of humic substances extracted from enriched compost prepared by using various organics was conducted during the year 2018-19 at research farm, department of soil science and agricultural chemistry. The enriched compost was prepared by pit method with sorghum stubbles, wheat straw and shredded cotton stalk and glyricidia leaf along with rock phosphate @ 12 per cent. The mixture containing sorghum stubble, wheat straw, shredded cotton stalk and glyricidia leaf along with rock phosphate @ 12 per cent and Trichderma viride @ 1 kg per tonne of crop residues were decomposed up to 90 days and thereafter kept for 30 days for curing. Turning was given at 10 days interval up to 90 days. Glyricidia leaves @ 10% (100 kg tonne^{-1}) were incorporated in the pit at 15 days interval in T7 and T8 treatment. The enriched compost sample were collected treatment wise at 15, 30, 60, 90 and 120 days during decomposition and analyzed for various chemical parameter. The organic carbon, total N and tota p content of enriched compost prepared by various crop residues was improved as compared to initial. The C:N ratio of prepared compost was decreased in the range of 20.94 to 26.45. the decomposed compost was subjected for extraction of of various humic substances (HS). The result indicated that, compost prepared from various combination of crop resided and different ingredient yielded quite high recovery of HS. The higher value of all the functional was observed in humic acid than fulvic acid. The total acidity, carboxylic and phenolic functional group range between 7.8 to 9.4, 2.9 to 3.6 and 4.2 to 6.1 meq/g respectively. The humic acid and fulvic acid extracted from compost prepared from 100% wheat straw (glyricidia leaves at 15 days interval) recorded higher values total acidity and phenolic(OH) group.

Keywords

Humic, Fulvic, Carboxylic, Phenolic, Compost, Straw and Leaves

Introduction

The term "humus" originates from the Romans, when it was familiarly used to signify the entire soil. Later the term was used to denominate soil organic matter and compost or for different parts of this organic matter, as well as for complexes created by chemical agent treatments to a wide palette of organic substances. The principal definition of humus, as decomposed organic matter, originates from 1761. His comprehensive study on the acidic nature of HAs is thought to be his most important benefit to humus chemistry. Research on the chemical properties of HS was extended by the Swedish researcher Berzelius, whose main contribution was the isolation of two light-yellow coloured HS from mineral water and slimy mud rich in iron oxides. Enormous advances have been made during the last decade thanks to modern physicochemical methods. Nevertheless, the structural chemistry of lignin and HS did not advance so fast as the chemistry of animal-originated biopolymers.

The humic substances are differentiated on the basis of their extractability-precipitation in alkali-acid solutions yielding three fractions (i) HA (Humic acid), fraction of SOM that coagulates when the alkaline extract is acidified; (ii) FA (Fulvic acid), both acid and alkaline soluble fraction; and (iii) Humin, insoluble in both acid and alkali. These materials originate from the decomposition and further subsequent polymerization of organic residues of plant and animal origin lead to heterogeneity in these fractions with distinct functional groups, elemental composition and properties from the type of OM and existing environment. In any ecosystem, status and composition of these humic substances were governed by most of the factors as importantly management > climate > biota > topography = parent material > time. Thus, better understanding of characteristics and functional groups of humic substances induced by land use management may serve as essential guide in the study of soil organic matter. Therefore, present study was carried out to know the elemental composition mainly C and N, total acidity and spectral characterization i.e., E4/E6 ratio of humic substances extracted from soils of major land use systems (LUS) viz., forests (natural and social forests), horticultural systems like coffee and coconut plantations; vegetable and potato fields, field crops (paddy) and mulberry fields of Hassan district of Karnataka (India).

Humic substances can be divided into components according to their solubility in different media. humic acids and fulvic acid represent alkali-soluble humus fragments; HAs are commonly extracted using diluted alkali and precipitated with an acid, and so are separated from the soluble fulvic acid. Humin represents the insoluble residue.

Humic acids, one of the most important components of HS, help break up clay and compacted soils, assist in transferring micronutrients from soil to plants, enhance water retention, increase seed germination rates, and stimulate the development of micro flora populations in soils. Humic acids also slow down water evaporation from soils. This is especially important in soils where clay is present at low concentration or not at all, in arid areas, and in sandy soils without the capability to hold water. Humic acids provide also sites for microflora to colonize. Bacteria secrete enzymes which act as catalysts, liberating calcium and phosphorous from insoluble calcium phosphate, and iron and phosphorous from insoluble iron phosphate. The chemical structure of HAs is very complicated and depends on their source. The elemental composition of different FAs and HAs shows that the major elements in their composition are C, H, O, N, and S. These major elements are always present regardless their origin and country or continent. Moreover, besides elemental composition, group composition is used to characterize HS as it gives information about the chemistry and structural properties of HS. Fulvic acids contain more functional groups of an acidic nature, particularly COOH. The total acidities of fulvic acids (900. 1400mmol/100g) are considerably higher than for humic acids (400.870mmol/100g). Another important difference is that while the oxygen in fulvic acids is largely in known functional groups. (COOH, OH, C=O), with a high oxygen content, the acidity and degree of polymerisation all change systematically with increasing molecular weight. The proportion of oxygen in humic acids seems to occur as a structural component of the nucleus.

Materials and Methods

Preparation of compost

The enriched compost was prepared by decomposing various crop residues (wheat straw, shredded cotton stalk, glyricidia leaves and sorghum stubbles), cow dung slurry with rock phosphate, urea, elemental sulphur and PDKV, decomposer. The enriched compost was prepared by pit method. Turnings are given at 10 days interval up to 120 days of decomposing. After 90 days of decomposing, heaps of enriched compost ware collected at one place as treatment wise and allowed to cure for another 30 days. Compost was watered regularly at 10–15 days of interval so as to maintain moisture content up to 60-70%.

Extraction of humic Subtances

50 grams of air dried compost samples were taken in 500 ml conical flask to which 300 ml of 0.5 *N* NaOH was added under stirring for half an hour. After 24 hour the content were centrifuged for 15 minutes at 5000 rpm. This operation was repeated until the supernatant was obtained clear. This combined fractions comprised humic and fulvic acids. To separate the humic acids, the pooled supernatants were then treated with concentrated HCl (pH 2). The acidified extracts allowed to stand overnight and centrifuged to separate humic acids from fulvic acids. The precipitated portion (crude humic acid) was collected by centrifugation. The supernatant (crude fulvic acid fraction) was filtered through Whatman No. 1 filter paper.

Qualitative analysis of extracted Humic acid and Humin

E4/E6 ratio recorded at 90 and 120 days after decomposition of enriched compost measure the absorbance at 465 and 665 nm wavelength in spectrophotometer as described by Schnitzer (1982).

Functional Group analysisd of Humic acid and Humin

Carboxylic

The method is based on the liberation of acetic acid when acids are treated with calcium acetate and its titration with standard 0.1 *N* NaOH (Schnitzer and Khan, 1972).

2 R- COOH + (CH3 COO)2 Ca ------> (R COO)2 Ca + 2 CH3COOH----- (II)

To fifty milligrams of HA/FA in a stopper flask, 10 ml of 1 *N* (CH3COO)2Ca and 40 ml of CO2 free distilled water were added, a blank was also set up simultaneously. After shaking at room temperature for 24 hr the suspension was filtered and the residue was washed with CO2 free distilled water. The filtrate and washings were titrated potentiometrically with standard 0.1 *N* NaOH to pH 9.8.

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

Total acidity

Total acidity in HA/FA was determined by Ba(OH)2 method (Wright and Schnitzer

1961). 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.

Ba(OH)2 + 2HA ----> Ba A2 + 2 HOH--- (I)

Fifty milligrams of HA/FA along with blank was taken in separate stopper flasks and 20 ml of 0.2 *N* Ba(OH)2 was added. The flasks were shaken for 24 hr 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 potentiometricaily. Identical blanks were maintained simultaneously.

$$\text{Total acidity (me g}^{-1}) = \frac{(\text{TV for blank - TV for sample}) \times 0.5\,N \times 1000}{\text{Wt. of sample (mg)}}$$

Phenolic

The Phenolic-OH groups were calculated as the difference between total acidity and COOH acidity.

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

Results and Discussion

Extraction of humic substances from enriched compost.

The humic substances were extracted from various compost, the recovery of humic acid, fulvic acid and humin are presented in Table 8. The recovery fulvic acid varied from 20.36 to 24.92 % in compost prepared from various crop residues. The significantly highest value of fulvic acid (24.92%) was recorded in compost prepared from 25% wheat straw + 25% shredded cotton stalk + 25% glyricidia leaves + 25 % sorghum stubbles followed by 30% wheat straw + 30% shredded cotton stalk +20% glyricidia leaves and 20% sorghum stubbles. Significantly lower fulvic acid content (20.36%) was observed in compost prepared from 100% shredded cotton stalk followed by 50% wheat straw + 50 % shredded cotton stalk.

The humc acid varied from 10.09 to 13.45 % in compost prepared from various crop residues. The significantly highest value of humic acid (13.45 %) was recorded in compost prepared from 40% wheat straw + 40% shredded cotton stalk + 20% glyricidia leaves followed by 25% wheat straw + 25% shredded cotton stalk + 25% glyricidia leaves + 25% sorghum stubbles. Significantly lower humic acid content (10.09 %) was observed in 100% shredded cotton stalk followed by 50% wheat straw + 50 % shredded cotton stalk.

The humin varied from 59.12 to 69.55 % in compost prepared from various crop residues. The significantly highest value of humic acid (69.55 %) was observed in compost prepared from 100% shredded cotton stalk followed by 50% wheat straw + 50% shredded cotton stalk. Significantly lower humin content (59.12 %) was observed in 25% wheat straw + 25% shredded cotton stalk + 25% glyricidia leaves + 25% sorghum stubbles followed by 30% wheat straw + 30% shredded cotton stalk + 20% glyricidia leaves and 20% sorghum stubbles.

The fulvic and humic acid contents obtained from farmyard manure were 0.62 and 0.92% respectively, where in vermicompost 0.86 and 1.26% respectively. Among the manures, humic acid and fulvic acid contents were more in vermicompost than farmyard manure. The data shows that humic acid content was more than fulvic acid content in both the organic manures. Similar results were reported by several workers (Shailaja, 2006; Ramalakshmi, 2011; Kar *et al.,* 2012; Revathi, 2012; Srilatha *et al.,* 2013 and Metwally *et al.,* 2014).

E4/E6 ratio in humic substances

The E4/E6 ratio in humic acid, fulvic acid and humin varied from 2.50 to 3.67, 7.30 to 8.04 and 3.17 to 3.84. Significantly highest value of E4/E6 ratio in humic acid (3.67) of compost prepared from 25% wheat straw + 25% shredded cotton stalk + 25% glyricidia leaves + 25 % sorghum stubbles followed by 30% wheat straw + 30% shredded cotton stalk + 20% glyricidia leaves + 20% sorghum stubbles. Significantly lower E4/E6 ratio in humic acid acid (2.50) was observed in compost prepared from 100% shredded cotton stalk followed by 25% wheat straw + 25% shredded cotton stalk + 25% glyricidia leaves + 25 % sorghum stubbles.

The significantly highest value of E4/E6 ratio in fulvic acid (8.07) of compost prepared from 25% wheat straw + 25% shredded cotton stalk + 25% glyricidia leaves + 25 % sorghum stubbles followed by 40% wheat straw + 40% shredded cotton stalk + 20% glyricidia leaves. Lower value of E4/E6 ratio in fulvic acid (7.30) was observed in 100% shredded cotton stalk followed by 100% wheat straw.

The ratio of optical densities at 465 nm (E4) and 665 nm (E6) is used for characterization of humic and fulvic acids. The relationship of E4/E6 is related to the aromacity and to the degree of condensation of the chain of aromatic carbons of humic acids, and could be used as a humification index (Kononova, 1966, Schnitzer and Khan, 1972 and Stevenson, 1982). This ratio, referred to as E4/E6 ratio, is independent of concentration of humic and fulvic acids but varies with humic material extracted from different soil types (Sailaja and Rao, 2000, Pospisilova and Fasurova, 2009 and Tahiri *et al.,* 2016).

Functional group of extracted humic substances

The total acidity in humic acid and humin varied from 8.10 to 9.73 m eq g^{-1} and 5.08 to 6.18 m eq g^{-1} respectively. The significantly highest value of total acidity in humic acid i.e. 9.73 m eq g^{-1} extracted from compost 25% wheat straw + 25% shredded cotton stalk + 25% glyricidia leaves + 25% sorghum stubbles followed by 100% wheat straw. The lower value of total acidity in humic acid (8.10 m eq g^{-1}) was observed in 50% wheat straw + 50% shredded cotton stalk. Significantly highest value of total acidity in humin (6.18 m eq g^{-1}) was recorded in 25% wheat straw + 25% shredded cotton stalk + 25% glyricidia leaves + 25 % sorghum stubbles followed by 50% wheat straw + 50% shredded cotton stalk. Significantly lower total acidity in humin (5.08 meq g^{-1}) was recorded extracted from compost of 40% wheat straw + 40% shredded cotton stalk + 20% glyricidia leaves.

The carboxylic OH in humic acid and humin varied from 2.90 to 4.10 meq g^{-1} and 1.90 to 3.90 meq g^{-1} respectively. The significantly highest value of carboxylic OH in humic acid (4.10 meq g^{-1}) was found in 30% wheat straw + 30% shredded cotton stalk + 20% glyricidia leaves and 20% sorghum stubbles followed by 50% wheat straw + 50% shredded cotton stalk. Significantly lower carboxylic OH in humic acid (2.90 m eq g^{-1}) was observed in 100 % shredded cotton stalk.

Table.1 Recovery of humic substances

Treatment		Recovery of Humic Substances (%)			HI	HA/FA
		FA	HA	H		
T_1	100% WS	23.34	12.45	64.21	0.56	0.53
T_2	100% SCS	20.36	10.09	69.55	0.44	0.50
T_3	50% WS + 50% SCS	22.22	11.67	66.11	0.51	0.53
T_4	40% WS + 40% SCS +20% GL	23.90	13.45	62.65	0.60	0.56
T_5	30% WS+30% SCS +20% GL + 20% SS	24.15	11.90	63.90	0.56	0.49
T_6	25 % WS + 25% SCS +25 % GL +25% SS	24.92	12.67	59.12	0.60	0.51
SE (m) ±		**0.56**	**0.37**	**1.60**	_	_
CD at 5%		**1.66**	**1.09**	**4.77**	_	_

Table.2 Functional group of extracted humic substances

Treatments		Humic Acid			Humin		
		Functional Group (meq g^{-1} of HS)					
		Total Acidity	Carboxylic -OH	Phenolic-OH	Total Acidity	Carboxylic -OH	Phenolic-OH
T_1	100% WS	8.55	3.60	4.95	4.88	2.80	1.95
T_2	100% SCS	7.85	2.90	4.95	4.65	2.30	1.75
T_3	50% WS + 50% SCS	8.10	3.90	4.20	5.48	1.90	3.85
T_4	40% WS + 40% SCS + 20% glyricidia leaves	8.25	3.80	4.45	5.08	2.95	2.15
T_5	30% WS + 30% SCS + 20% glyricidia leaves + 20% sorghum stubbles	8.40	4.10	4.30	4.80	2.88	2.20
T_6	25% WS + 25% SCS + 25% glyricidia leaves + 25% sorghum stubbles	9.73	3.70	6.03	6.18	3.90	2.15
SE(m)±		**0.23**	**0.19**	**0.21**	**0.27**	**0.19**	**0.26**
CD at 5%		**0.67**	**0.58**	**0.62**	**0.81**	**0.56**	**0.78**

Significantly highest value of carboxylic OH in Humin (3.90 m eq g^{-1}) was observed in 25% wheat straw + 25% shredded cotton stalk + 25% glyricidia leaves + 25% sorghum stubbles followed 30% wheat straw + 30% shredded cotton stalk + 20% glyricidia leaves and 20% sorghum stubbles. Significantly lower carboxylic OH in humin (1.90 meq g^{-1})

extracted from compost of 50% wheat straw + 50% shredded cotton stalk.

The phenolic OH of humic acid and humin extracted from compost varied from 4.20 to 6.03 meq g^{-1} and 1.75 to 3.85 meq g^{-1}. The significantly highest value of phenolic OH in humic acid (6.03 meq g^{-1}) extracted from compost of 25% wheat straw + 25% shredded cotton stalk + 25% glyricidia leaves and 25% sorghum stubbles followed by 100% wheat straw. Significantly lower phenolic OH in humic acid (4.20 meq g^{-1}) was observed in 50% wheat straw + 50% shredded cotton stalk. Significantly highest value of phenolic OH in Humin (3.85 meq g^{-1}) was found in 50% wheat straw + 50% shredded cotton stalk. followed 30% wheat straw + 30% shredded cotton stalk + 20% glyricidia leaves and 20% sorghum stubbles. Significantly lower phenolic OH in humin (1.75 meq g^{-1}) was observed in 100% shredded cotton stalk.

High molecular weight humic acids which may also result in high content of carboxyl groups (Lal and Mishra, 2000 and Srilatha *et al.*, 2013). Total acidity and carboxyl group contents were higher in humic acid and fulvic acid extracted from compost than humic acid obtained from vermicompost. These results are in accordance with the results of Kar *et al.*, (2012).

From, the above the study, 25% wheat straw + 25% shredded cotton stalk + 25% glyricidia leaves and 25% sorghum stubbles found beneficial to enriched to compost in term to total N, P, K, S and micronutrient. The recovery of humic substances viz; humic acid, fulvic acid and humin are higher in compost prepared from 40% wheat straw + 40% shredded cotton stalk + 20% glyricidia leaves. The functional group viz; carboxylic OH, phenolic OH and total acidity was improve where humic acid, fulvic acid and humin were prepared from 25% wheat straw + 25% shredded cotton stalk + 25% glyricidia leaves and 25% sorghum stubbles. The elemental composition i.e. total nitrogen and sulphur content in humic acid, fulvic acid and humin was improved where compost prepared from 25% wheat straw + 25% shredded cotton stalk + 25% glyricidia leaves and 25% sorghum stubbles.

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