Humic Substances and Organic Matter in Soil and Water Environments: Characterization, Transformations and Interactions
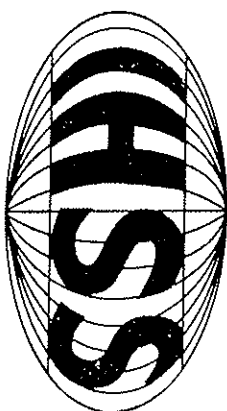
52 Chemical Characteristics of Humic Substances Extracted from Wheat Straw Compost

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ABSTRACT

Humic acids (HAs) and fulvic acids (FAs) extracted from composing wheat (*Triticum aestivum* L.) straw with and without rock phosphate enrichment were characterized by functional group analysis and by infrared spectroscopy. The results showed that 16.2 and 10.5% of organic matter of the straw was extracted as HAs and FAs, respectively, at 120 days of composing. The enrichment of straw with both Mussoorie Phos (MP) and Hyper Phos (HP) retarded HA production and enhanced FA production. The HAs required a higher content of hydrogen (H) and oxygen (O) while the carbon (C), nitrogen (N) and sulfur (S) decreased with composing time. The production of FAs decreased with composing time and contained lower amounts of C, H, N, and S, but had higher contents of O throughout the composing period. The HAs were less acidic and retained lower amounts of carboxyl, phenolic, and alcohol hydroxyl groups. The addition of both MP and HP to decomposing straw did not affect the chemical nature of HAs and FAs, except by increasing their carboxyl groups. The infrared spectra of both HAs and FAs confirmed the presence of all the above mentioned functional groups, including the presence of sharp bands for methoxyl, amino, C-H, and C-O stretching of polysaccharides.

INTRODUCTION

Humic substances (HS) are the major end product of organic matter decomposition. They differ widely in their chemical nature or reactivity due to the composite effect of several factors involved at the various stages of decomposition and humification of different kinds of wastes. In natural conditions, the humification of ligno-cellulosic waste is very slow, but it can be increased by enhancing the microbial activity through optimization of environmental factors and nutritional requirements

(Martin & Haider, 1971; Singh, 1987). Organic wastes containing aromatic amino acids, lignin, and tannin-like substances rapidly acquire the property of HS (Kononova, 1966). Schnitzer and Khan (1972) demonstrated that the reactivity of HS was mainly due to oxygen-containing functional groups through which they can react with minerals, metals, pesticides, and a variety of organic compounds. The total acidity or exchange capacity depends mainly on the occurrence of ionizable hydrogen in carboxyl (COOH) and phenolic hydroxyl (OH) groups.

The burning or dumping of organic wastes which are increasingly available in large quantities either from agricultural farms or from cities has passed a serious consequence onto our environment. Composting is the cheapest and best way to utilize all kinds of organic wastes. Little information, however, on humification and on the chemical nature of products of organic matter decomposition, particularly during composting, is available in the literature. Adequate knowledge about the chemical properties of organic matter at different stages of composting is desirable, for consideration of the disposal of the organic waste/refuse that is available in large quantities. Composting also helps to save foreign currency used in importing raw materials for the preparation of chemical fertilizers. The enrichment of composting wastes with single superphosphate, rock phosphate (Singh et al., 1987), nitrogen (Singh, 1987), micro-nutrients, and the use of inoculants (Singh et al., 1980) reduces the time of composting. Molasses incorporation enhances the inherent capacity of HS to solubilize insoluble inorganic P during composting (Singh & Amberger, 1991) and it also increases the crop-utilizing efficiency of chemical fertilizers through their chelating properties, if applied together.

In this paper we report the chemical characteristics of humic acids (HAs) and fulvic acids (FAs) extracted during different stages of wheat straw composting where the medium was enriched with two different types of low grade rock phosphate. This study was necessary to discover at which stage of composting HS acquired the highest solubilizing or chelating properties.

MATERIALS AND METHODS

Preparation of Rock Phosphate-Enriched Compost

Wheat (*Triticum aestivum* L.) straw was collected from the Durnast Farm of the Institute and ground to pass a 2-mm sieve. The chemical composition of the wheat straw, Mussoorie Phos (MP) and Hyper Phos (HP) is given in Table 1. Both rock phosphates are from sedimentary rock pertaining to soft grade. The MP contains a lower percentage of P_2O_5 than HP, but the presence of organic carbon (C) in significant amounts shows the loose configuration of apatite structure. The value of 2% formic acid-soluble P_2O_5 in MP is very low due to its high free $CaCO_3$ content.

Twenty grams of wheat straw were weighed into 100-mL plastic bottles and both rock phosphates were added separately at the rate of 5 g per 20 g straw on a freeze-dried basis. This ratio had given maximum solubilization of added rock phosphate in the earlier experiment (Singh, 1987). Molasses (Mol) at the rate of 2.5 g per bottle was added to enhance microbial activity during composting (Alexander, 1977). The C to N ratio of the mixture was adjusted to 30 in each case by adding cattle urine for quick decomposition. The mixture was then inoculated with 5 mL of aqueous extract of natural inoculation, containing 5% each of soil, cattle dung, and old straw compost.

Table 1. Physico-chemical characteristics of raw material used for composting.

Characteristics	Wheat straw	MP	HP
Origin	Germany	Sedimentary	Sedimentary
pH (1:5, soil:water)	7.35	7.95	8.15
EC (dS m⁻¹)(1:10, soil:water)	0.15	0.93	1.43
Organic C (%)	42.15	1.61	0.27
Total N (%)	0.511	ND†	ND
Total P$_2$O$_5$	0.061	19.51	30.48
Formic acid P$_2$O$_5$ (% of total P$_2$O$_5$)	ND	7.07	8.15
Total Ca (%)	0.58	22.80	37.00
CaCO$_3$ (%)	ND	20.30	16.70
Substitution (%) PO$_4$ by CO$_3$	ND	9.67	18.33
Length 'a' axis (Å)	ND	9.352	9.328

† ND = Not determined.

The contents of the bottles were mixed after 7, 20, and 60 days of incubation and distilled water was added to return the moisture to the original level. Duplicate bottles for each treatment were removed at 30, 60, and 120 days of incubation. After freeze-drying, the samples were analyzed for organic matter loss, N (micro-Kjeldahl method) and C (Morris & Schnitzer, 1967).

Finally, the moisture was maintained at 200% of the freeze-dried weight of the mixture. After thorough mixing, 6 bottles each for straw alone, straw + MP, straw + HP, straw + HP + straw + MP + Mol, straw + HP + Mol were loosely-stopped and incubated at 30 °C.

Extraction of Humic and Fulvic Acids

A manure sample of 20 g was shaken with 300 mL of 0.1M NaOH solution under N_2 in a 500-mL plastic bottle for 24 h. After centrifugation at 500 rpm for 10 min, the supernatant solution was filtered through glass wool and adjusted to pH 2 with 2M HCl. The residue was washed again with 200 mL of 0.1M NaOH for 1 h and the procedure repeated. Finally, the residues were washed with distilled water and added to the previous supernatant solution. The pH of whole solution was readjusted to 2 with 2M HCl again, and allowed to stand overnight. The coagulated HAs were separated from soluble FAs by centrifugation, redissolved in 0.1M NaOH, reprecipitated with 2M HCl and centrifuged as before.

The HAs obtained from different compost samples contained more than 9% ash, but the value was reduced to less than 5% by shaking with dilute HCl-HF solution (Schnitzer, 1982). The FAs were purified by passing repeatedly through DOWEX-50 cation exchange resin until their ash contents were reduced to 5%. Both HAs and FAs

were dried on a rotatory evaporator, and kept in glass vials for chemical and functional group analyses.

Methods of Analysis

Carbon and H were analyzed by dry combustion, N by the micro-Kjeldahl method, S by the method of Soep and Demoent (1960) and O by difference [100% - (C+H+N+S)]. Total acidity was determined by Ba(OH)$_2$ titration under N$_2$ and COOH by the calcium acetate method (Wright & Schnitzer, 1959). Phenolic-OH content was obtained by subtracting COOH groups from total acidity. Similarly, the alcoholic-OH group was obtained by difference between phenolic-OH and total-OH groups. The carbonyl groups were determined by the method of Fritz et al. (1959). All the values were expressed on dry ash-free basis. The infrared spectra were recorded on a Perkin Elmer 983 IR-Spectrophotometer for both HAs and FAs extracted only from straw alone, straw + Mol + MP, and straw + Mol + HP treatments after 120 days of composting. The KBr pellets were prepared by the procedure of Stevenson and Goh (1974).

RESULTS AND DISCUSSION

Maturity of compost was evaluated by measuring the rate of organic matter mineralization and C:N ratios of decomposing waste at different time intervals (Table 2). Mineralization, as determined from loss of organic matter, increased significantly with composting time in all the treatments. Mineralization was very slow, and reached 29.2% at 120 days when wheat straw decomposed alone, but it was increased drastically and reached 50.4 and 49.6% on the addition of MP + Mol and HP + Mol, respectively. This showed that P was the limiting factor for microbial growth because straw initially contained very small amounts of P (Table 1). In the presence of MP, the loss of organic matter was observed to be comparatively higher than with HP, most probably due to its higher CaCO$_3$ content which induced microbial growth during composting. The C:N ratio of decomposing waste narrowed in all the treatments due to loss of organic matter with time. It came down to 17.3, 9.4 and 10.0 at 120 days of composting, respectively, in straw alone, in the straw + Mol + MP and in the straw + Mol + HP treatments. The loss of organic matter of more than 46.9% and a C:N ratio of less than 10 in both rock phosphate- and molasses-enriched composts suggested that both these composts had matured within 120 days of composting, whereas more time was needed to compost straw alone. Alexander (1977) reported that humus, an end product of organic matter decomposition, generally contained a C:N ratio from 5 to 15, with an average value of 10. Microbial cells also contained similar values for the C:N ratio, and these continued to decompose organic matter for their N requirement until the C:N ratio of the wastes reached an average value of 10 (should other essential nutrients not be the limiting factors).

Table 2. Loss of organic matter and the C:N ratios of decomposing wheat straw enriched with rock phosphates.

Treatments	Organic matter loss			C:N ratio		
	Composting period (days)					
	30	60	120	30	60	120
	------%------					
Wheat straw alone†	14.2	24.8	29.2	22.5	19.6	17.3
+ MP	21.5	31.6	42.3	20.7	17.3	14.0
+ MP + Mol	24.6	39.2	50.4	18.3	12.7	9.4
+ HP	17.3	26.8	37.8	21.3	18.5	15.3
+ HP + Mol	21.9	34.4	46.9	18.8	13.9	10.0
LSD (0.05)	Treat. 1.38; Days 1.08; Treat. x Days 2.40					

† Initial C:N ratio of wheat straw maintained at 30.

Humic and Fulvic Acids

The data on relative percentage of HAs and FAs in total organic matter of decomposing waste (Table 3) showed that the production of HAs increased continuously, whereas FAs decreased after 30 days of composting in all treatments. This suggested that FAs were the initial product of humification during straw composting (Kononova, 1966). Initially high quantities of FAs were produced and these were gradually converted into HAs as the compost matured (Singh & Amberger, 1990). Both rock phosphates retarded HA formation and enhanced the formation of FAs. In the presence of MP, the production of HAs was higher than in the presence of HP. This trend was reversed in the case of FAs. The maximum conversion of organic matter into HAs + FAs was 27 to 35% at 30 days, and then decreased gradually, reaching between 24 and 27% at 120 days in different treatments. However, on purification, the percentage recovery decreased to 17 to 21% at 30 days and thereafter increased gradually, reaching 20 to 30% at 120 days of incubation.

The C, N and S contents in HAs decreased while H and O increased with the rise in composting time in all the treatments (Table 4). In the case of FAs, the trend was reversed. The gradual increase in C and decrease in O with time in the FAs confirmed their conversion into HAs as the compost matured. The addition of both rock phosphates decreased C, N and S and increased the H and O contents in both the HAs and FAs extracted at all time intervals. The HAs retained comparatively higher amounts of C, N, and S, but lower amounts of O than the FAs (Challa et al., 1985). The relatively low N and S contents of the latter might be due to sorption of nitrogenous and sulphur compounds during passage over the H-resin.

Table 3. Relative percentage of humic and fulvic acids produced during the preparation of rock phosphate-enriched straw compost.

Treatment	Composting period (days)		
	30	60	120
	---------------% of organic matter ---------------		
	Humic acids		
Wheat straw alone	8.4 (5.2)[†]	11.6 (8.7)	15.7 (13.2)
MP	7.5 (5.6)	10.0 (7.9)	13.7 (12.8)
MP + Mol	7.6 (4.7)	10.3 (7.2)	13.8 (12.5)
HP	6.2 (4.6)	7.8 (6.8)	11.9 (11.7)
HP + Mol	6.8 (4.0)	8.2 (6.3)	12.5 (11.4)
LSD (0.05)	Treat. 0.36; Days 0.36; Treat. x Days 0.73		
	Fulvic acids		
Wheat straw alone	19.4 (12.2)[†]	13.8 (9.8)	9.2 (7.2)
MP	21.6 (13.3)	15.6 (10.5)	11.6 (8.5)
MP + Mol	26.8 (16.0)	20.5 (14.3)	13.9 (10.5)
HP	23.5 (14.5)	17.0 (11.1)	12.8 (9.3)
HP + Mol	27.6 (16.3)	21.7 (14.8)	14.3 (10.9)
LSD (0.05)	Treat. 0.40; Days 0.42; Treat x Days 0.84		

[†] Values in parenthesis indicate relative percentage of humic and fulvic acids after their purification.

The functional group analysis of HAs and FAs (Table 5) indicated that FAs were more acidic than HAs, and this acidity was mainly due to presence of COOH groups (Chen et al., 1977). All O-containing functional groups (except the phenolic-OH groups) increased gradually in the HAs, but these decreased in FAs, mainly in the straw compost. This indicated that FAs produced during composting of ligno-cellulosic waste are less stable than HAs. The distribution of OH groups in the HAs appeared to have higher abundance than the COOH groups, whereas FAs had the higher values of COOH groups. The incorporation of MP and HP helped in acquiring all the O-containing groups except the phenolic-OH groups in both HAs and FAs. The ratios of phenolic-OH to COOH in HAs ranged from 1.3 to 2.5 and then decreased with composting time, as well as with rock phosphate additions. In the case of FAs the ratios were considerably lower, ranging from 0.3 to 0.6. The amounts of ketonic groups per unit weight were found to be lower than the other O-containing groups in both HAs and FAs.

Table 4. Chemical composition of humic and fulvic acids extracted from straw compost enriched with rock phosphate.

Element	Straw compost			MP-Mol compost			HP-Mol compost		
	30	60	90	30	60	90	30	60	90
	Composting period (days)								
	- % -								
	Humic acids								
C	59.91	58.95	57.31	57.62	56.12	53.81	57.84	56.62	34.42
H	4.85	5.00	5.32	5.21	5.43	5.92	5.13	5.31	5.71
N	3.75	3.56	3.24	3.50	3.42	3.11	3.60	3.31	3.00
S	0.65	0.50	0.47	0.50	0.48	0.45	0.55	0.52	0.46
O	30.84	31.99	33.66	33.17	34.55	37.71	32.78	34.24	36.41
	Fulvic acids								
C	45.61	46.33	47.04	42.12	43.61	43.92	41.72	42.93	43.90
H	4.82	4.75	4.72	4.64	4.58	4.21	4.60	4.55	4.18
N	1.31	2.10	2.42	1.98	2.11	2.39	1.91	2.10	2.35
S	0.10	0.16	0.15	0.10	0.12	0.12	0.10	0.12	0.12
O	48.06	46.16	45.57	51.16	50.58	50.36	51.67	51.30	50.75

Infrared Spectra

Infrared spectra for HAs and FAs extracted after 120 days of composting are shown in Fig. 1. The spectra of HAs obtained from straw compost enriched with, and without rock phosphate indicated similar chemical structures, differing mainly in their intensities of absorption bands and in the absence of a band at 1380 cm⁻¹ in both MP- and HP-enriched compost. The spectra of FAs resembled those of HAs, but in general they had higher intensities for all the bands, and had especially high intensities for the COOH band at 1720 cm⁻¹. The strong absorption at 3425 cm⁻¹, with the addition of a shoulder at 3325 cm⁻¹ in the FAs, appeared because of the OH stretching vibration due to free amino and H-bonded OH groups, suggesting the retention of applied N through cattle urine during composting (Dyer, 1978). The additional development of a weak band at 3100 cm⁻¹ in both HAs and FAs extracted from rock phosphate-enriched compost indicated that enrichment increased the retention of total OH groups. The HAs showed sharp and strong bands at 2920 and 2860 cm⁻¹ due to the stretching vibration of aliphatic C-CH, -CH₂, and -CH₃ groups. These bands were less sharp for the FAs. A strong band at 1720 cm⁻¹ for the FAs indicated higher numbers of COOH groups than for the HAs, as already indicated in Table 3.

Table 5. Functional group analysis of humic and fulvic acids extracted from straw compost enriched with rock phosphate.

	Straw compost			MP-Mol compost			HP-Mol compost		
Element				Composting period (days)					
	30	60	120	30	60	120	30	60	120
					--- me g⁻¹ ---				
Humic acids									
Total acidity	8.0	8.6	8.9	8.4	9.2	10.1	8.2	9.1	9.6
-COOH	3.3	3.0	3.4	3.0	4.0	5.5	2.8	3.8	4.2
Total -OH	7.1	7.6	7.9	7.3	7.8	9.1	7.4	7.7	8.5
Phenolic -OH	5.7	5.6	5.5	5.4	5.2	5.6	5.4	5.3	5.4
Alcoholic -OH	1.4	2.0	2.4	1.9	2.6	3.5	2.0	2.4	3.1
Ketonic -C = O	1.8	1.6	1.3	1.5	1.4	1.2	1.4	1.3	1.2
Fulvic acids									
Total acidity	14.7	12.5	11.8	16.2	12.9	12.1	16.6	13.4	12.6
-COOH	9.4	8.3	7.9	11.2	9.5	9.1	11.5	9.8	9.4
Total -OH	9.3	8.2	7.1	11.0	8.7	7.4	10.9	8.5	7.3
Phenolic -OH	5.3	4.2	3.9	5.0	3.4	3.0	5.1	3.6	3.2
Alcoholic -OH	4.0	4.0	3.2	6.0	5.8	4.4	5.8	4.9	4.1
Ketonic -C = O	2.0	1.8	1.5	1.8	1.5	1.1	1.7	1.5	1.1

The band at 1650 cm⁻¹ due to C=O stretching of amide, quinone and ketone groups appeared sharp and larger in HAs extracted from straw compost than in HAs extracted from rock phosphate-enriched compost. However, a band at 1595 cm⁻¹, due to symmetrical stretching of COOH or N-H deformation emerged stronger and larger in HAs extracted from compost enriched with both rock phosphates. The sharp band at 1500 cm⁻¹ for aromatic C=C, at 1455 cm⁻¹ for aliphatic C-H, and at 1420 cm⁻¹ for OH deformation also appeared stronger in FAs than in HAs. The band at 1380 cm⁻¹ for nitro-HAs disappeared in HAs when extracted from rock phosphate-enriched compost. The other band at 1160 cm⁻¹ for C=O phenolic linkages, 1225 cm⁻¹ for OH deformation of COOH, 1120 cm⁻¹ for polysaccharides, along with a shoulder at 1080 and 1030 cm⁻¹ for amino compounds, appeared in HAs extracted from all three types of composts. In

the case of FAs the bands at 1355, 1260, 1120, 1080 and 1030 cm⁻¹ disappeared, and bands at 1325, 1225 and 1150 cm⁻¹, respectively, shifted to 1300, 1190 and 1140 cm⁻¹. It was interesting to observe the several weak bands between 600 and 900 cm⁻¹ in FAs and moderate to strong bands between 700 to 900 cm⁻¹ in FAs might have appeared due to possible interaction of inorganic materials, mainly SO₄, HPO₄, and various other functional groups (Singhal & Sharma, 1983).

SUMMARY

From the above results, it appears that rock phosphate enrichment did not affect the nature of both HAs and FAs produced during composting of wheat straw, although it enhanced the production of FAs and inhibited the production of HAs. The HAs were produced in higher quantities at 120 days of composting in all the treatments, but contained lower contents of COOH groups than did the FAs. The higher production of FAs in the initial days of composting was important with regard to the solubilization of added insoluble nutrients, as well as for chelating properties, because these were more highly acidic and had the highest contents of COOH groups.
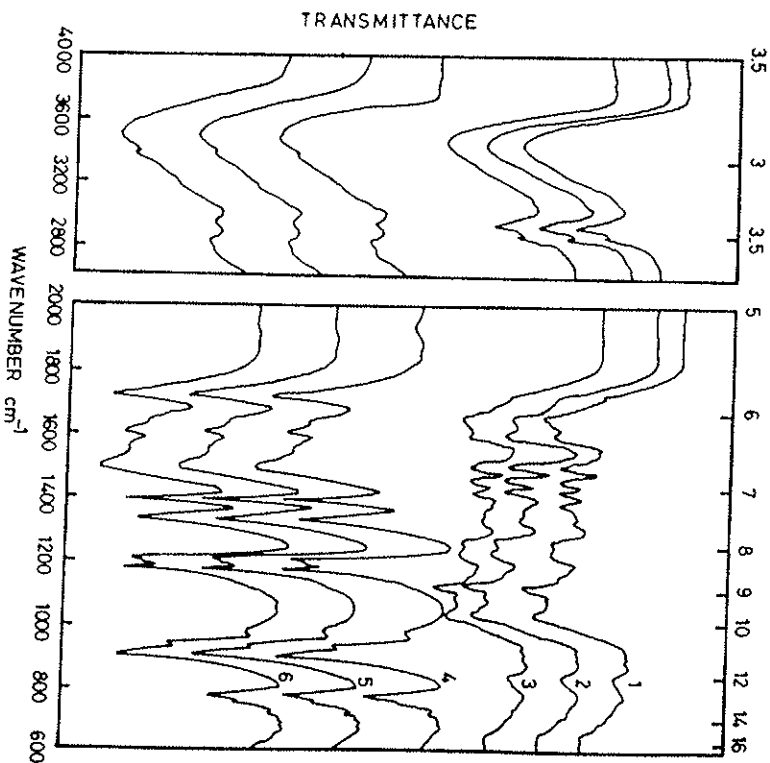

Fig. 1. Infrared spectra of: (1) HA from compost; (2) HA from MP-Mol compost; (3) HA from HP-Mol compost; (4) FA from compost; (5) FA from MP-Mol compost; (6) FA from HP-Mol compost.

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