

Chemical and Instrumental Characterization of Humic Acid of Diverse Soil Orders under Paddy Cultivation

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ABSTRACT

Humic acid is a blend of aliphatic and aromatic molecules with a variety of composite structures that are found in the natural world. The fundamental role of humic acid is retaining metal ions and organic pollutant and also in carbon sequestration. The purpose of this study was to characterize humic acids isolated from several soil orders such as Entisol, Inceptisol, Vertisol, and Alfisol in a continuously paddy growing area using chemical and instrumental methods.

According to elemental analysis, all humic acids extracted from different soil orders has higher carbon and lower oxygen content representing typical value of humic acid. The Vertisol recorded higher total acidity (6.90 meq g⁻¹) while lower in the Entisol (3.80 meq g⁻¹) and the E4/E6 ratio was higher in the Entisol (4.56) indicating that humic acid was less condensed and less matured than humic acids in other soil orders. The FTIR spectra of Vertisol showed broad and sharp peaks of aromatic C-C, strongly H-bonded C-O of conjugated ketones and similar spectra were displayed for Inceptisol 1 and Inceptisol 2 and weak and reduced IR spectra was appeared for Alfisol and Entisol. The dominances of smectite and smectite rich minerals in Vertisol showed by X-ray diffractograms has higher affinity towards soil organic matter which lead to greater humification of organic matter in these soil.

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INTRODUCTION

Soil organic matter (SOM) is made up of 58 % carbon, is both a sink and a source of carbon in soil. Soil organic matter is a mixture of plant and animal residues and most of the soil organic matter originates from plant residue. Plant leftovers decompose in the soil via a biological process that involves the physical breakdown and biochemical transformation of complex organic molecules into simpler organic

and inorganic molecules. Humic compounds are the most chemically resistant component of soil organic carbon. Humic substances are defined as amorphous, brown to black, acidic and polydisperse, with molecular mass ranging from few hundred to several thousand. Based on their solubility in water at various pH values, the International Humic Substances Society categorized soil organic matter into three fractions: Fulvic acids soluble at all pH values, humic acids soluble in alkaline media but insoluble at pH 1.0, and humin insoluble at all pH values.

Soil organic carbon content of agricultural soil should range from 1-1.5% but it has come down to 0.3-0.4 % in the country. It is well below the acceptable limit and is a cause for concern. This rapid decrease is due to increased atmospheric temperature, depletion of resources, intensive mining activities, soil deterioration, poor soil and crop management practices, indiscriminate use of fertilizers and accelerated soil erosion.

Organic matter (OM), especially humic compounds, plays a critical role in soil formation and development. OM is also a key measure of soil fertility and it has a significant impact on agricultural efficiency, environmental quality and global climate change. Humic substance increase soil buffering capacity, provide accessible micronutrients to plants and immobilize organic pollutants and metals. Humic substances also determine the soil production potential and play an environmental role by participating in global carbon cycling. The quantity and quality of humic substances in the soil organic matter may vary with soil types, climate, altitude, soil pH, vegetation type and soil management practices.

So far, the majority of study has focused on SOM from upland soils. The properties of SOM from paddy soils are poorly understood. India has the world's largest rice-growing area and second-largest producer and exporter of rice. In comparison to the uplands, lowland cultural practices and fertilizer use activities in rice may have a significant impact on the composition of humic compounds. As a result, the primary goal of this research is to report on the chemical composition, functional groups and nature of humic acid isolated from various soil orders under

Table 1. Initial soil properties.

Soil orders	pH	CEC (Cmol(p+)/kg)	Aail. OC (%)	TOC (%)
Entisol	6.673	12.81	0.34	0.45
Vertisol	7.783	22.44	1.39	1.57
Inceptisol 1	7.732	19.25	0.48	0.40
Inceptisol 2	7.763	18.21	0.58	0.67
Alfisol	6.83	20.11	1.10	1.25

paddy cultivation.

MATERIALS AND METHODS

Location and soil sampling

Soil samples were collected from different paddy growing states of India having diverse soil orders like Entisol from Nadia, West Bengal, Vertisol from Belgaum, Karnataka and Inceptisol 1 from Nadia, West Bengal, Inceptisol 2 from Sonipat, Haryana and Alfisol from Davanagere, Karnataka. The composite soil samples were collected at a depth of 0-30cm in replications whereas the Inceptisol 2 soil order was the normal agriculture soil. The initial soil properties of the soil are listed in Table 1.

Clay mineralogy study

Clay separated from soil using Jackson's technique (1985) and clay mineralogy was studied by differentially treated clays X-ray diffractograms with a Philips diffractometer using Ni-filtered Cu-K radiation at a scanning speed of 1.5°2 θ min⁻¹.

Extraction and characterization of humic acid (HA)

The HA was extracted by the procedure of International Humic Substance Society (Swift 1996). The extracted HA was stored in a freezer at -20°C before drying in a lyophiliser. The CHNS analyzer was used to identify the elemental composition of HA while the difference factor was used to determine oxygen content. E4/E6 was determined by the method outlined by Stevenson (1994). Total acidity and carboxylic acid functional group was also determined by the method of Schnitzer and Gupta (1965). The difference between total acidity and carboxylic group acidity was

used to compute the phenolic-OH groups.

FTIR spectroscopy of humic acid

For IR analysis, the isolated samples were vacuum dried at room temperature and ground to a fine powder with an agate mortar and pestle. The Bruker (Alpha) instrument was used to characterize HA, clay and clay-humus complex samples. The FTIR spectra of all the three samples were examined using a spectral range of 600-4000 cm^{-1} . The spectra were recorded on pressed tablets made by combining samples with KBr (1:100, w/v).

Statistical analysis

Data were analyzed using standard Analysis of Variance (ANOVA) technique and means were compared using Duncan's Multiple Range Test (DMRT) using Windows-based SPSS version 10.0.

RESULTS AND DISCUSSION

Clay mineralogy of soils

X-ray diffractograms of clay isolated from different soil orders (Fig. 1) revealed that clay in Vertisol of Belgaum were mainly dominated by smectite at peak positions ranging from 4.5 to 5.1 $^{\circ}2\theta$ and peaks ranging from 5.2 to 6.2 $^{\circ}2\theta$ adjacent to smectite were due to presence of chlorite, hydroxyl-interlayered minerals (HIMs), vermiculite or smectite-rich mixed layer minerals interstratified with illite or chlorite or

other hydroxyl-interlayered minerals. In Inceptisol 1 and 2 soil clays contains illite rich minerals with peak positions spread between 8.3 and 8.8 $^{\circ}2\theta$ is characterized as illite. There were a few more peaks near the illite peak ranging from 9.2 to 10.3 $^{\circ}2\theta$ which could be due to the presence of illite rich illite/smectite or illite/chlorite interstratified minerals. Alfisol clay contains reflection of kaolinite mineral and sharper and stronger with peak positions at 12.3 to 12.4 $^{\circ}2\theta$ and peaks adjacent to that of kaolinite ranging from 10.5 to 12.3 $^{\circ}2\theta$ were considered as interstratified kaolinite with illite or smectite. Alfisol clays showed weak diffuse reflections, attributed to poor crystallinity and the presence of iron oxide coatings. Entisol clays showed major reflection peaks at 4.5, 7.2 and 12.5 $^{\circ}2\theta$ due to the presence of smectite, illite and kaolinite. The results were in accordance with the previous findings of Moore and Reynolds (1997), Datta *et al.* (2015) and Das *et al.* (2019).

Elemental composition, acidic functional groups and spectral characterization of humic acids of different soil orders

The elemental composition of humic acids extracted from different soil orders is listed in the Table 2. Humic acids of all soil orders have higher carbon and lower oxygen content representing typical value of HA. The C content ranged from 53.66 to 57.39 % with significantly highest value in Vertisol. Due to intensive humification, greater degree of condensation of aromatic rings in Vertisol lead to greater elemental carbon, nitrogen and sulfur in humic materials than

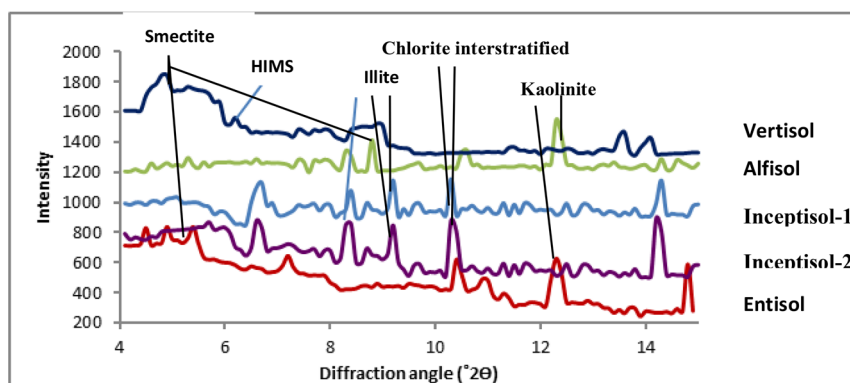


Fig. 1. X-ray diffractograms of clay isolated from different soil orders.

Table 2. Elemental composition of humic acids isolated from diverse soil orders.

Soil orders	C (%)	H (%)	N (%)	S (%)	O (%)
Entisol	53.66 ^e	3.50 ^e	3.25 ^e	0.19 ^e	39.90 ^a
Vertisol	57.39 ^a	4.55 ^a	4.64 ^a	0.45 ^a	33.07 ^e
Inceptisol 1	54.49 ^{cb}	4.05 ^b	4.01 ^b	0.37 ^b	36.93 ^d
Inceptisol 2	54.89 ^b	3.90 ^{cb}	3.92 ^{cb}	0.28 ^d	37.11 ^c
Alfisol	53.77 ^d	3.85 ^{dc}	3.67 ^d	0.30 ^c	38.41 ^b

other soil orders. Nature of OM (leaf litter, plant residues, FYM, organic manures) added to the soils and its decomposition under varied climates (dry and transitional heavy rainfall zones) will influence the elemental composition of humic substances. The C content in the soil orders follows the order as Vertisol > Inceptisol 2 > Inceptisol 1 > Alfisol > Entisol (Srinivasarao *et al.* 2009). Hydrogen, nitrogen and sulphur significantly higher in Vertisol followed by Inceptisol 1 while lowest in the Entisol.

The functional groups data of the HAs shown in the Table 3 revealed that total acidity (meq. g⁻¹) ranged from 3.80 in Entisol to 6.90 in Vertisol whereas, Vertisol recorded significantly highest total acidity (6.90 meq g⁻¹) followed by Inceptisol 1 (6.33 meq g⁻¹), Inceptisol 2 (6.26 meq g⁻¹) and Alfisol (5.96 meq g⁻¹), while significantly lowest in Entisol (3.80 meq g⁻¹). The variations in total acidity may be attributed to the inherent differences in chemical composition and molecular weight of HAs. Similar trend was observed in case of carboxyl and phenolic groups which were significantly highest in Vertisol and lowest values were featured by Entisol.

The degree of aliphaticity or aromaticity of humic substances i.e., E4/E6 ratio was significantly lower in Vertisol followed by Inceptisol 2 and Inceptisol 1 whereas higher in Entisol followed by Alfisol. Generally, the HAs with ratio of < 5 serves as an index of humification and ratio narrows with increasing molecular weight and condensation. Greater values of E4/E6 ratios indicates low molecular weight, low degree of aromatization and presence of relatively large proportion of aliphatic structures (Stevenson 1994), this is true in case of Entisol due to time as a limiting factor (Satisha and Devarajan (2011), Srilatha *et al.* 2013 and Tahiri *et al.* 2016).

Table 3. Acidic functional groups and E4/E6 ratios of humic acids.

Soil orders	Total acidity (meq. g ⁻¹)	Carboxyl group (meq. g ⁻¹)	Phenolic-OH (meq. g ⁻¹)	E4/E6 ratio
Entisol	3.80 ^e	2.40 ^e	1.40 ^e	4.56 ^a
Vertisol	6.90 ^a	3.85 ^a	3.05 ^a	3.13 ^e
Inceptisol-1	6.33 ^b	3.74 ^a	2.26 ^c	3.59 ^c
Inceptisol-2	6.26 ^{cb}	3.60 ^a	2.96 ^{ba}	3.40 ^{dc}
Alfisol	5.96 ^d	3.49 ^b	2.03 ^d	3.96 ^b

FTIR spectra of humic acids (HAs)

FTIR spectra of the representative HAs isolated from diverse soil orders are illustrated in the Fig. 2. Assignments of various absorption peaks are tabulated in the Table 4. Most important features were 3850-3620 cm⁻¹ (H-bond OH stretching), 2940-2900 cm⁻¹ (aliphatic C-H stretching), 2398-2310 cm⁻¹ (triple bond C≡N and C≡C), 1698-1691 cm⁻¹ (COOH vibrations), 1607-1623 cm⁻¹ (aromatic C=C and C=O in amide, ketone, and quinone group stretching), 1517-1506 cm⁻¹ (COO⁻ symmetric stretching, N-H bending, C=N stretching), 1220-1205 cm⁻¹ (C-O stretching and OH deformation of COOH, C-O stretching of aryl ethers), 1030-950 cm⁻¹ (C-O stretching of polysaccharide and Si-O of silicates impurities) and 750-790 cm⁻¹ (aliphatic CH₂ chains).

The FTIR spectra of HAs isolated from different soil orders has a number of common major absorption peaks with smaller differences in their relative intensity. The major difference observed that, broad and sharp absorption peaks of aromatic C-C and strong H-bonded C-O of conjugated ketones stretching (1620-1615 cm⁻¹) in Vertisol, Inceptisol 1 and Inceptisol 2 and strong and sharp COOH vibrations (1698-1691 cm⁻¹) was detected in Entisol and Alfisol spectra. The higher percentage of aromatic C in Vertisol represents a more advanced stage of humification with a relative increase in the concentration of recalcitrant organic compounds this is due to predominant of smectite and smectite rich clay minerals in Vertisol and these minerals have higher affinity towards the organic matter, as they are having greater surface area for adsorption and also contains high layer charge than any other clay minerals like illite, kaolinite and chlorite. The greater the amount of active sites on the clay mineral surface, the higher

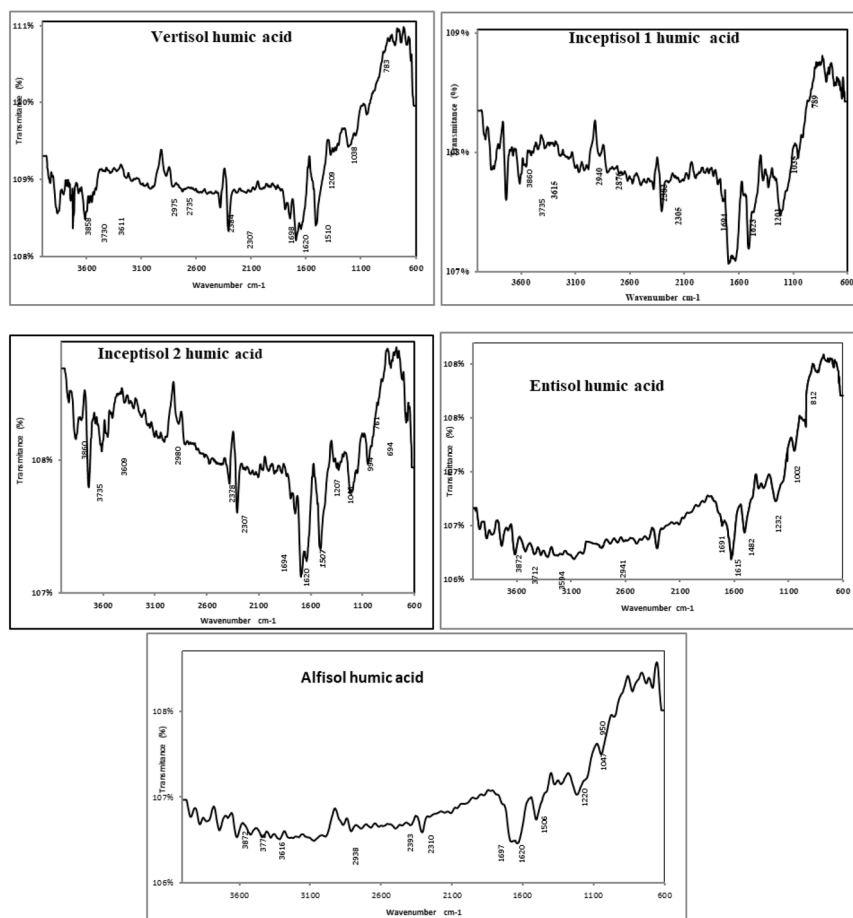


Fig. 2. FTIR spectra of humic acids isolated from diverse soil orders.

Table 4. Summary of stretching vibrations of various functional groups present in humic acids.

Assignment	Entisol	Vertisol	Inceptisol 1 (Wave number cm ⁻¹)	Inceptisol 2	Alfisol
Free OH vibration of residual clay mineral or amine (N-H)	3872 3594	3858,3730	3860, 3735, 3615	3860, 3609	3778, 3616
Aliphatic C-H stretching	2941	2975,2735	2940,2874	2980	2938
Triple bond C≡N and C=C		2384,2307	2383	2307	2310
COOH vibrations	1691	1698	1694	1694	1697
Aromatic C-C, strongly H-bonded C-O of conjugated ketones	1615	1620	1623	1620	1620
COO ⁻ symmetric stretching, N-H bending, C=N stretching (amide II band)	1482	1510	1509	1507	1506
C-O stretching and OH deformation of COOH, C-O stretching of aryl ethers	1232	1208	1201	1207	1220
C-O stretching of polysaccharide and Si-O of silicates impurities	1002	1038	1035	1046	1047
Aliphatic CH ₂ chains	812	783	789	761	950

is the tendency to adsorb soil organic carbon (Sarkar *et al.* 2018 and Das *et al.* 2019). The 3850 to 3420 cm^{-1} region of Vertisol, Inceptisol 1 and Inceptisol 2 showed broad absorption peaks and this attributed to H-bonded OH group which is reduced and weak in case of Entisol and Alfisol. Absorption caused by aliphatic C-H stretching ($2940\text{-}2900\text{ cm}^{-1}$) varied considerably, being strong in Vertisol, Inceptisol 1 and Inceptisol 2 but not in Entisol and Alfisol. The reduced and weak peaks in Entisol because of less polymerization of humic substances and lower organic matter content due to time as limiting factor in this soil order. Alfisol has also recorded reduced and weak peaks as the Alfisol soils are rich in Kaolinite type of 1:1 clay minerals. The total surface area of these minerals is much lower than the expanding 2:1 clay minerals and its interlayer are not accessible by foreign molecules. This might be reason for lower humification of organic materials which also showed higher E4/E6 ratio in Alfisol. Other common absorption peaks in the region $1517\text{-}1506\text{ cm}^{-1}$ (COO^- symmetric stretching, N-H bending, C=N stretching), $1220\text{-}1205\text{ cm}^{-1}$ (C-O stretching and OH deformation of COOH), $1030\text{-}950\text{ cm}^{-1}$ (C-O stretching of polysaccharide and Si-O of silicates impurities) and $750\text{-}790\text{ cm}^{-1}$ (aliphatic CH_2 chains) were also shown in the FTIR spectra of Vertisol, Inceptisol 1, Inceptisol 2, Entisol and Alfisol. FTIR spectra interpretations were based on the descriptions given by authors Stevenson (1982), Rivero *et al.* (1998) and Suman *et al.* (2020).

CONCLUSION

X-ray diffractogram of Entisol contains illite and smectite clay minerals while Vertisol displayed predominance of smectite, followed by chlorite, vermiculite or smectite rich interstratified with illite minerals. Inceptisol 1 and 2 were rich in illite/smectite or illite/chlorite interstratified minerals similarly, Alfisol exhibited the dominance of kaolinite followed by vermiculite and illite clay minerals. The results obtained from elemental analysis, acidic functional groups and FTIR spectroscopy of HA isolated from different soil orders are in consistent. The dominance of expanding 2:1 type of clay minerals like smectite and smectite rich minerals which has larger layer charge and subsequently creates more active sites on the clay mineral's surface and has higher tendency to

adsorb soil organic carbon which lead to significantly higher carbon, total acidity and lowest E4/E6 ratio in Vertisol followed by Inceptisol 1, Inceptisol 2 and Alfisol, and lowest values featured by Entisol. Even though Entisol soils are rich in illite and smectite type of clay minerals showed lowest elemental composition and total acidity and highest E4/E6 ratio of HA due to time as a limiting factor has reduced the degree of condensation and humification of organic matter in these soils. The FTIR spectra of Vertisol and Inceptisol were similar and showed prominences of aromatic C-C, highly H-bonded C-O of conjugated ketones functional groups with broad and sharp peaks and also Alfisol showed weak and reduced peaks while least absorption peaks showed by Entisol humic acid.

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