

ELEMENTAL ANALYSIS, E4/E6 RATIO AND TOTAL ACIDITY OF SOIL HUMIC AND FULVIC ACIDS FROM DIFFERENT LAND USE SYSTEMS

SHARANBHOOPAL REDDY, M.S. NAGARAJA¹, T.S. PUNITH RAJ, A.S. POLICE PATIL² AND PRABHUDEV DHUMGOND

Department of Soil Science and Agricultural Chemistry, UAS, Bengaluru – 560 065, Karnataka

Received: March, 2014; Revised accepted: May, 2014

ABSTRACT

Humic substances, the major fractions (nearly 85%) of organic matter, constituting humic (HA) and fulvic acid (FA) extracted from soils of different land use systems viz., forests (both natural and manmade), cultivated systems (coffee plantations, paddy, potato, mulberry, coconut and vegetable) under varied climatic conditions in Hassan district of Karnataka (India) were evaluated for their individuality and properties through elemental analysis, E4/E6 ratio and total acidity. Humic acid fraction was composed of higher EI-C and EI-N (ranging from 48.4-54.7% and 5.1-5.8%, respectively) than FAs (38-47.8% and 2.6-3.2%). Higher degree of aromaticity of HA's was evidenced by lower E4/E6 ratio value (1.92-3.38) than FA's (6.77-11.41) in all the LUS. Fulvic acid in common observed with higher total acidity (6.2-11.0 meq g⁻¹ humic material) depicting more of functional groups. Soils from coconut plantations showed lower total acidity (4.7) than forest (7.0) and coffee systems (6.9). The distinctiveness of humic substances was influenced by land use management and climatic parameters (temperature and rainfall).

Keywords: Humic acid, fulvic acid, land use systems, elemental analysis, E4/E6 ratio, total acidity

INTRODUCTION

Natural organic matter (OM) is the prime attribute and centre for most of the chemical, physical and biological processes occurring in environment and soil. Hence, organic fraction can exert a profound influence on soil properties and ecosystem functioning (Tan, 2003). The term soil organic matter (SOM) has been used to encompass all organic materials found in soil (Stevenson, 1994), ranging from as low as < 0.5% carbon in Aridisols to 33% in Histosols (Sombroek *et al.*, 1993). Soil organic matter composed of both humic and non-humic fractions, humic substances being major fraction of SOM (85%) have a greater influence on soil fertility through involving and regulating most of the soil processes for example, solubility and availability of plant nutrients, serves as energy and carbon source for soil biological entity, thermal characteristics of soil, water retention capacity to restrict water loss through drainage and erosion etc (Huang, 2004). Hence, most of the time humus is synonymously termed as SOM. 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 en bloc. 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).

MATERIALS AND METHODS

Study area (Hassan district) is situated eastern side of the Western Ghats, in the southern part of Karnataka state and lies between 12° 13' and 13° 33' N; and 75° 33' and 76° 38' E. District is exposed for an equitable mm, but most of the hilly area receives higher rain whereas, dry regions receives rainfall in the range of 500 to 700 mm. The mean annual air temperature is 23.3 °C. April is generally the hottest and December is the coldest month. The soil temperature regime is categorized as iso-hyperthermic. Study area represents four distinct state

Corresponding author email: sbpatil90@gmail.com

1. Department of Soil Science and Agricultural Chemistry, UHS, Bagalkot - 587 102 (Karnataka)

2. Department of Agronomy, UAS, Bengaluru-560 065 (Karnataka)

agro-climatic zones (ACZ) and predominant LUS were selected from these ACZ's namely Central Dry Zone (ACZ - 4)- coconut (CNP), mulberry (MLF) and vegetable (VEG) systems; Southern Dry Zone (ACZ - 6)- coconut, mulberry, vegetable, potato (POT) systems; Southern Transition Zone (ACZ -7)- coffee (CFP), paddy fields (PDF), vegetable, potato, coconut, mulberry fields and forests (both natural-NTF and manmade-MNF); climate with average annual rainfall of 1031 and Part of Hilly Zone (ACZ - 9)- coffee plantations and natural forests. Each LUS was represented by 15 individual farms spreading across the entire study area with an exception to coffee as the crop was restricted only to the western region. The surface composite soil samples (0-0.15 m) were pooled from 3 soil sampling points, within a pre-identified representative site for the LUS during Feb-April (summer), 2009 to lessen the seasonal effects on soil properties.

Extraction, fractionation and purification of humic substances: Soil residue after acid treatment (0.1N HCl) was extracted with 50 mL of 0.1 M NaOH in 0.1 M sodium pyrophosphate and repeated thrice 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 (Stevenson, 1994). Further purified by treating the extracted HA fraction with HCl - HF mixture for 24 hours, later acid mixture was separated by centrifugation. Finally, the residue was thoroughly washed with distilled water and freeze dried. The purification of FA was done by transferring suspension to 100 mwco (Molecular Weight Cut Off) dialysis bags and dialyzed against double distilled water for 24 hours (Wander and Traina, 1996). The dialyzed fraction was evaporated under low temperature and finally freeze dried. The dried sample were weighed and stored for further analysis.

Characterization of HA and FA: Humic acid (HA) and fulvic acids (FA) extracted from soils of different LUS were analyzed for EI-C and EI-N by adopting modified Walkely Blacks wet digestion method (Amato, 1983) and micro Kjeldahl distillation method (Jackson, 1973) using automatic Gerhardt - Vapodest 30 distillation, respectively. 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 was titrated potentiometrically with standard 0.05 N HCl solution to pH 8.4. Spectral characterization (E4/E6 ratio) was

done by measuring absorbances of humic substances at 465 and 665nm using UV-VIS spectrophotometer.

RESULTS AND DISCUSSIONS

Organic matter in soil is a key factor and known to regulate and improve the soil properties in chemical, biological and physical functions and it also get influenced by many factors. Tree based systems under high rainfall regions were observed with acidic pH (< 6.0) and greater soil organic carbon (> 10 g kg⁻¹) status (Table 1). Leaching of exchangeable bases to under depths due to high rainfall causing acidity in surface layers and frequent biomass additions to soil surface through leaf fall and other plant residues might have contributed to higher organic carbon status of these soils. Intensive monocropping of paddy, potato and vegetable fields with higher amount of acid forming fertilizers might have lead to acidity with pH of 5.74, 5.76 and 6.39, respectively. None of the soil samples showed higher salinity levels. Among LUS study, EC values were slightly higher in mulberry (0.21 dS m⁻¹), vegetable (0.16 dSm⁻¹) and paddy systems (0.17 dSm⁻¹) while, the other LUS recorded conductivity of about 0.1 dS m⁻¹. Continuous and excess application of fertilizer nutrients in mulberry might have added salts. Seasonal application of organic matter through FYM, organic manures etc and decomposition of applied organic matter due to greater microbial activity (mesophiles) at higher temperature (35-45⁰C) prevailing under these drier regions and anthropogenic interventions caused lower organic carbon status (Doran, 2002) of cultivated systems (< 6.0 gkg⁻¹) under study. Similar variations in organic carbon status were observed in six soil profiles from different altitudes (400-600 MSL) and places having reasonably wide rainfall (767-1190 mm) and vegetation cover (cultivated, barren and forests) of Maharashtra (Waikar *et al.*, 2004).

Table 1: Chemical properties of soils of different LUS

LUS	pH	EC (dS m ⁻¹)	Soil Organic carbon (g kg ⁻¹)
NTF	5.75 ± 0.23	0.12 ± 0.05	13.18 ± 2.20
MNF	5.76 ± 0.31	0.13 ± 0.07	10.62 ± 1.98
CFP	5.88 ± 0.25	0.12 ± 0.03	12.56 ± 2.03
MLF	7.80 ± 0.29	0.21 ± 0.07	6.57 ± 1.61
CNP	7.11 ± 0.37	0.12 ± 0.05	4.76 ± 1.13
POT	5.76 ± 0.79	0.12 ± 0.07	4.61 ± 1.45
PDF	5.74 ± 0.38	0.17 ± 0.06	5.98 ± 1.48
VEG	6.39 ± 0.56	0.16 ± 0.03	6.16 ± 1.95
S.Em ±	0.112	0.016	0.45
CD (at 0.05)	0.314	0.044	1.26

Note: Natural forests (NTF), Manmade forests (MNF), Coffee plantations (CFP), Mulberry fields (MLF), Coconut plantations (CNP), Potato plots (POT), Paddy fields (PDF), Vegetable fields (VEG)

Elemental composition provides worthwhile information on the reactivity of humic substances in soil. The EI-C content (%) in HA fraction ranged from 48.4 in paddy soils to 54.7 in coconut plantations (Table 2). Whereas, the EI-N content (%) ranged from 5.1 in natural forests and coffee plantations while, it was 5.8 in vegetables and potato fields. The C:N ratios of HA fractions ranged from 8.8 in vegetable gardens to 10.5 in coffee plantations. The HA of forests and coffee plantations recorded wider C:N ratios than agricultural soils and may be due to N supplementation and its complexation with SOM in agricultural systems (Martin *et al.*, 1998). Intensive humification due to greater degree of condensation of aromatic rings in semi arid tropical climates lead to greater EI-N of humic materials than transitional high rainfall

climates. In case of FAs, EI-C and EI-N content ranged from 38.0 and 2.6 in potato soils to 47.8 and 3.2 in coffee plantations, respectively. Thus, C:N ratio was found in the range of 14.2- 16.2 with a slightly wider ratios in forest related systems to narrower ratios in agriculture LUS. From elemental composition, it is evident that HAs contained more C than FAs. This could be explained by the fact that the formation of FA was accompanied by loss of carbon and nitrogen and gain in oxygen (Schnitzer, 2000). Nature of OM (leaf litter, plant residues, FYM, organic manures etc.) added to the soils and its decomposition under varied climates (dry and transitional heavy rainfall zones) and land use management (forests, cultivated, barren etc) will influence the elemental composition of humic substances.

Table 2: Elemental composition of humic and fulvic acid fractions of soils from different LUS

LUS	Humic acid			Fulvic acid		
	EI-C (%) ^a	EI-N (%) ^b	C:N ratio	EI-C (%) ^a	EI-N (%) ^b	C:N ratio
NTF	53.3	5.1	10.5	45.4	2.8	16.2
MNF	51.2	5.3	9.7	42.3	2.7	15.7
CFP	53.3	5.1	10.5	47.8	3.2	14.9
MLF	54.3	5.5	9.9	42.6	3.0	14.2
CNP	54.7	5.3	10.3	40.2	2.7	14.9
POT	52.3	5.8	9.0	38.0	2.6	14.6
PDF	48.4	5.3	9.1	39.3	2.6	15.1
VEG	51.2	5.8	8.8	41.2	2.8	14.7

Note: ^a EI-C = Elemental Carbon (%), ^b EI-N = Elemental Nitrogen (%)

The degree of aliphaticity or aromaticity of humic substances i.e., E4/E6 ratio and index of functional groups (such as carboxylic and phenolic-OH groups) expressed as total acidity are depicted in Table 3. The E4/E6 ratios of HA fraction in soils ranged from 1.92 to 3.38. The values of FA were higher than that of HA values, suggesting fact that the FAs have more aliphatic compounds than HA fractions. Generally, the HAs with ratio of < 0.5; while, FAs > 0.5, serves as an index of humification and ratio narrows with increasing molecular weight and condensation. The E4/E6 ratios of fulvic acid are generally higher than the HA indicating that the former ones are with low molecular weights and are less polymerized. Relatively wider ratios of FAs than those of HAs reflect a low degree of aromatization and presence of relatively large proportion of aliphatic structures in FAs (Stevenson, 1994). Higher total acidity (meq g⁻¹ HA) values were observed in natural forests (7.0) and coffee plantations (6.9) whereas, lower values for cultivated systems i.e., from 4.7 to 6.6. Similar to HA, FA total acidity (meq

g⁻¹ FA) was higher in tree based forests and coffee systems (9.6 to 11.0) and lower in agricultural systems (6.2 – 7.5). The variations in total acidity may be attributed to the inherent differences in chemical composition and molecular weights of HA and FA.

Table 3: E4/E6 ratio and total acidity of humic and fulvic acids in soils of different LUS

LUS	Humic Acid		Fulvic Acid	
	E4/E6 Ratio ^a	Total acidity (meq g ⁻¹)	E4/E6 Ratio ^a	Total acidity (meq g ⁻¹)
NTF	3.38	7.0	11.41	9.6
MNF	2.86	6.1	9.50	10.1
CFP	2.79	6.9	9.97	11.0
MLF	2.78	6.6	9.01	10.3
CNP	2.19	4.7	6.77	6.2
POT	2.17	5.4	8.49	7.5
PDF	2.03	5.7	7.70	7.2
VEG	1.92	6.1	7.96	7.0

Note: ^a E4/E6 ratio => ratio of absorbance's at 465 and 665 nm, Total acidity (meq g⁻¹ of humic material)

Present study revealed that agriculture systems applied with almost decomposed OM may better and immediately involved in soil fertility improvement due to greater CEC and simultaneously serving as a carbon source for soil biological community. While, forest and coffee systems with frequent biomass additions were observed with higher OC status but under varied state of humification in

surface layers i.e., initial stages of decomposition; as evidenced by wider E4/E6 ratio and higher total acidity values. Agriculture systems with high humified materials may be due to prevalence of these systems under dry regions with higher temperature than transition climates causing greater microbial activity on applied OM (FYM) and simultaneously causing rapid decomposition.

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