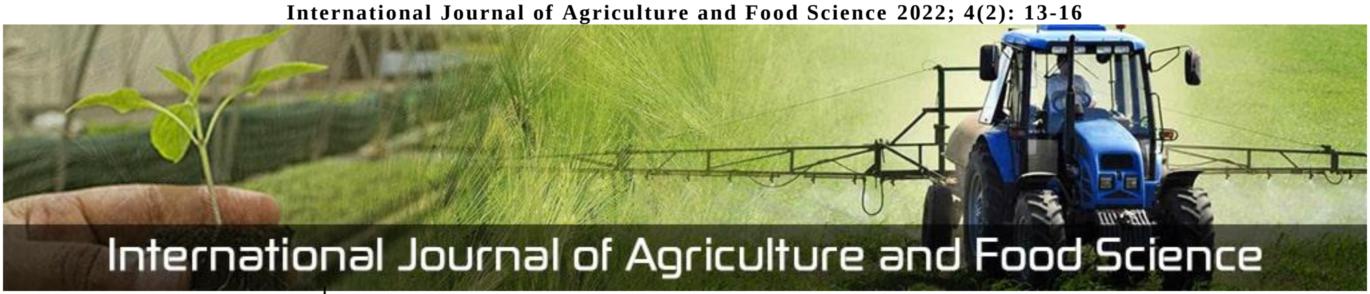

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SP Kale
Department of Soil Science and Agricultural Chrmistry, Mahatma Phule Krishi Vidyapeeth, Rahuri, Ahemdnagar, Maharashtra, India

BD Bhakare
Department of Soil Science and Agricultural Chrmistry, Mahatma Phule Krishi Vidyapeeth, Rahuri, Ahemdnagar, Maharashtra, India

Ritu S Thakare
Department of Soil Science and Agricultural Chrmistry, Mahatma Phule Krishi Vidyapeeth, Rahuri, Ahemdnagar, Maharashtra, India

VS Patil
Department of Soil Science and Agricultural Chrmistry, Mahatma Phule Krishi Vidyapeeth, Rahuri, Ahemdnagar, Maharashtra, India

AG Durgude
Department of Soil Science and Agricultural Chrmistry, Mahatma Phule Krishi Vidyapeeth, Rahuri, Ahemdnagar, Maharashtra, India

Corresponding Author:
SP Kale
Department of Soil Science and Agricultural Chrmistry, Mahatma Phule Krishi Vidyapeeth, Rahuri, Ahemdnagar, Maharashtra, India

E4/E6 ratio and total acidity of soil Humic and Fulvic acids from different land use patterns

SP Kale, BD Bhakare, Ritu S Thakare, VS Patil and AG Durgude

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 patterns *viz*., agriculture, forest, fallow, pasture, salt affected, horticulture and agroforestry land under varied agroclimatic zones of western Maharashtra (India) were evaluated for their individuality and properties through E4/E6 ratio and total acidity. Higher degree of aromaticity of HA was evidenced by lower E4/E6 ratio than FA in all the LUP. Soils from fallow land showed lower total acidity than forest. 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

Humic substances are often described as coiled, long chain molecules of two or three dimensional cross linked macromolecules which may vary in molecular weight depending on type of humic acid and method of measurement (Stevenson, 1994) [6]. They form the largest fraction of soil organic matter (OM) and play vital role in improving soil productivity. 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. Among these humic fractions, FA is soluble in both acid and alkali. HA is the fraction which is soluble only in alkali. The most insoluble fraction of humus is humin, which is neither soluble in acid nor alkali. Soil organic matter fractions are capable of forming complexes with metal ions. The ability of humic substances to form stable complexes with metal ions can be accounted for their high content of oxygen containing functional groups *viz*., carboxylic, phenolic aliphatic and alcoholic –OH groups. As these complex formation reactions between metal ions and humic substances are helpful in understanding the problems of plant nutrition (Stevenson *et al*., 1994) [6]. During complexation, numerous compounds including humic acid (HA) and fulvic acid (FA) are involved which control the distribution and supply of micronutrients to plants and interact with metal ions through their functional groups forming metal complexes of varying stabilities.

Carbon storage in soil is related to various drivers including climate, topography, parent material, soil properties, interaction with organisms (vegetation, animals, soil biota), and land use and management. Among the soil factors, soil aggregation and texture, mineralogy, and the related specific surface area are considered as key variables controlling the potential of carbon storage in soils (Wiesmeier *et al*., 2019) [7].

The ratio between the optical density (absorbance) of diluted solutions of HA and FA (E4/E6) determined at 465 and 665 nm has been used for years by soil scientists to characterize organic matter (Chen *et al*, 1977) [1]. Although this ratio has been related to the degree of condensation of the aromatic carbon network, to the carbon content, and to the molecular weight of the HS, there is little evidence in the literature to confirm this hypothesis. There are even less reports in the literature on the determinations of the E4/E6 ratio in FA.

Our aim was to characterization of humic substances of soil under seven land use patterns and establishes structural differences in the HS of the compared soils. The proposed methodology is considered to be highly useful for the study of organic substances in soil, particularly in those cases calling for an evaluation of the impact of change on soil.

Materials and Methods

The present study was carried out at the laboratory for characterization of humic substances by functional groups at department of Soil Science and Agricultural Chemistry, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, RahurI in the year 2020-2021.

E_4/E_6 Ratio

The E_4/E_6 ratios were determined by dissolving 2 mg of HA and FA samples in 10 mL of 0.05(N) $NaHCO_3$ (pH~8.0) and measuring optical densities at 465 and 665 nm with a spectrophotometer according to the method described by Chen *et al.* (1977) [1].

Total Acidity

Place between 50-100 mg of humic material in a 125 ml ground-glass stopper stoppered Erlenmeyer flask, and add 20 ml of 0.2*N* Ba(OH) only. Displace the air in each flask by N_2 stopper the flask carefully and shake for 24 hours at room temperature. Filter the suspension wash the residue thoroughly with CO_2 - free distilled water, and titrate the filtrate plus washing potentiometrically with standard 0.5 *N* HCL solutions to pH 8.4.

$$Ba(OH)_2 + 2HA \rightarrow BaA_2 + 2\,HOH$$

The calculation is as follows

$$\text{meq total acidity/g of HA} = \frac{\text{(Volume blank – Volume sample) x Normality x 1000}}{\text{Weight of sample (mg)}}$$

3.5.2 CO_2H Groups

Place between 50 to 100 mg of humic material in a 125-ml ground- glass stoppered Erlenmeyer flask, and 10 ml of 1*N* Ca (OAc), solution and 40 ml of CO_2 - free distilled water only. After shaking for 24 hours at room temperature, filter the suspension, wash the residue with CO_2 - free distilled water, and combine the filtrate and the washing, and titrate the potentiometrically with standard 0.1 *N* NaOH solution to pH 9.8. The calculation is as follows:

$$\text{meq } CO_2\text{ H Groups/g of HA} = \frac{\text{(Volume blank – Volume sample) x Normality x 1000}}{\text{Weight of sample (mg)}}$$

Characterisation of humic substances

Humic acid and Fulvic acids extracted from manures were used for this study and characterized for their functional groups

Characterization of Humic and Fulvic acid by Chemical Methods (Functional Group Analysis)

Functional group analysis of Humic acid was given by Schnitzer and Khan (1972) [5].

E4/E6 Ratio of Humic Acid and Fulvic Acid

The ratio of optical densities at 465 and 665 nm is used for characterization of humic and fulvic acids. The relationship E4/E6 is related to aromacity and degree of condensation of the chain of aromatic carbons of humic substances, and could be used as a humification index (Srilatha *et al*., 2013, Stevenson, 1982 and Schnitzer and Khan, 1972) [6, 5]. This ratio is referred to as E4/E6 ratio (Lal and Mishra, 2000) is independent of concentration of humic and fulvic acids but varies with humic material extracted from different soil types (Tahiri *et al*., 2016).

E4/E6 Ratio of Humic Acid

The data with respect to the E4/E6 ratio of humic acid are presented in Table 1 and Fig 40. The results revealed that E4/E6 ratio of humic acid was recorded highest in forest land with mean value of 4.81 and 4.70 in surface and subsurface soils, respectively. However, it was followed by agroforestry (4.35 and 4.26), pasture (3.87 and 3.77 and) and horticulture land (3.43 and 3.29) in surface and subsurface soils, respectively. Whereas, relatively low content of E4/E6 ratio of humic acid was associated with soil under agriculture (2.68 and 2.60) and salt affected land (2.36 and 2.26) and lower E4/E6 ratio of humic Acid was recorded in soil under fallow land with mean value of 1.89 and 1.78 in surface and subsurface soils, respectively.

The highest E4/E6 ratio of humic acid (4.91) was observed at 0-15 cm soil depth under Mahabaleswar forest and lowest E4/E6 ratio of humic acid (1.76) was noticed at 15-30 cm soil depth under fallow land of Rahuri location.

E4/E6 Ratio of Fulvic Acid

Land use patterns showed variation on E4/E6 ratio of fulvic acid in soil is presented in Table 2 and Fig 41. The results revealed that maximum E4/E6 ratio of fulvic acid was recorded in forest land and it shows decreasing trends with successive increase in soil depth of 0-15 cm with mean value of 8.74 and 15-30 cm with mean value 8.67. However, it was followed by agroforestry (7.80 and 7.74), pasture (7.38 and 7.26) and horticulture land (6.63 and 6.54) in surface and subsurface soils, respectively. Whereas, relatively low content of E4/E6 ratio of fulvic acid was associated with soil under agriculture (5.78 and 5.69) and salt affected land (5.24 and 5.15) in surface and subsurface soils, respectively and minimum E4/E6 ratio of fulvic acid was recorded in soil under fallow land with mean value of 4.46 and 4.34 in surface and subsurface soils, respectively.

The highest E4/E6 ratio of fulvic acid was observed in Mahabaleswar forest (8.92) at 0-15 cm soil depth and lowest E4/E6 ratio of fulvic acid (4.31) was recorded at 15-30 cm soil depth under fallow land of Rahuri location.

The E4/E6 ratio 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) [6]. These results are in line with those of Haddad *et al*., (2015) [2].

Table 1: Effect of different land use patterns on depth wise soil E4/E6 ratio of humic acid and fulvic acid

Land use patterns	Locations	E4/E6 HA		E4/E6 FA	
		0-15 cm	15-30 cm	0-15 cm	15-30 cm
Agriculture	Solapur	2.71	2.61	5.81	5.72
	Borgaon	2.78	2.69	5.88	5.77
	Igatpuri	2.52	2.47	5.61	5.53
	Rahuri	2.72	2.63	5.82	5.74
	Mean	2.68	2.60	5.78	5.69
	SD±	0.10	0.08	0.10	0.09
Forest	Mahabaleswar	4.91	4.82	8.92	8.93
	Radhanagri	4.86	4.76	8.77	8.68
	Borgaon, Satara	4.62	4.48	8.51	8.42
	Nandurbar	4.85	4.73	8.75	8.63
	Mean	4.81	4.70	8.74	8.67
	SD±	0.11	0.13	0.15	0.18
Fallow	Solapur	1.86	1.77	4.45	4.35
	Kolhapur	1.91	1.79	4.48	4.37
	Pune	1.89	1.78	4.46	4.34
	Rahuri	1.88	1.76	4.43	4.31
	Mean	1.89	1.78	4.46	4.34
	SD±	0.02	0.01	0.02	0.02
Pasture	Shirval	3.88	3.78	7.38	7.25
	Kolhapur	3.81	3.72	7.35	7.23
	Igatpuri	3.92	3.81	7.48	7.33
	Rahuri	3.85	3.77	7.32	7.21
	Mean	3.87	3.77	7.38	7.26
	SD±	0.04	0.03	0.06	0.05
Salt affected	Kasbe Digraj	2.38	2.26	5.21	5.13
	Padegaon	2.39	2.29	5.27	5.17
	Savalivihir	2.33	2.22	5.24	5.15
	Rahuri	2.34	2.25	5.22	5.14
	Mean	2.36	2.26	5.24	5.15
	SD±	0.03	0.02	0.02	0.01
Horticulture	Kolhapur	3.38	3.23	6.52	6.43
	Ganeshkhind	3.37	3.21	6.56	6.46
	Igatpuri	3.62	3.51	6.81	6.73
	Rahuri	3.36	3.22	6.62	6.55
	Mean	3.43	3.29	6.63	6.54
	SD±	0.11	0.13	0.11	0.12
Agroforestry	Kolhapur	4.29	4.18	7.86	7.78
	Solapur	4.23	4.14	7.55	7.54
	Igatpuri	4.51	4.42	7.91	7.84
	Rahuri	4.35	4.29	7.87	7.81
	Mean	4.35	4.26	7.80	7.74
	SD±	0.10	0.11	0.14	0.12

Table 2: Effect of different land use patterns on depth wise soil total acidity and in humic acid and fulvic acid

Land use patterns	Locations	Total acidity (meq total acidity g^{-1} HA)		Total acidity (meq total acidity g^{-1} FA)	
		0-15 cm	15-30 cm	0-15 cm	15-30 cm
Agriculture	Solapur	3.85	2.85	5.32	4.45
	Borgaon	3.92	2.89	5.45	4.58
	Igatpuri	3.61	2.56	5.19	4.42
	Rahuri	3.88	2.84	5.35	4.48
	Mean	3.82	2.79	5.33	4.48
	SD±	0.12	0.13	0.09	0.06
Forest	Mahabaleswar	7.92	6.85	9.35	8.58
	Radhanagri	7.75	6.49	9.27	8.4
	Borgaon	7.56	6.35	9.08	8.21
	Nandurbar	7.68	6.42	9.25	8.38
	Mean	7.73	6.53	9.24	8.39
	SD±	0.13	0.19	0.10	0.13
Fallow	Solapur	2.65	1.21	2.34	1.47
	Kolhapur	2.68	1.29	2.39	1.55
	Pune	2.62	1.25	2.32	1.45
	Rahuri	2.64	1.29	2.36	1.49
	Mean	2.65	1.26	2.35	1.49

	SD±	0.02	0.03	0.03	0.04
Pasture	Shirval	5.71	4.66	7.47	6.64
	Kolhapur	5.68	4.49	7.52	6.65
	Igatpuri	5.85	4.69	7.68	6.85
	Rahuri	5.52	4.44	7.41	6.54
	Mean	5.69	4.57	7.52	6.67
	SD±	0.12	0.11	0.10	0.11
Salt affected	Kasbe Digraj	3.12	2.28	3.68	2.88
	Padegaon	3.05	2.12	3.32	2.60
	Savalivihir	3.17	2.23	3.69	2.91
	Rahuri	3.15	2.27	3.66	2.86
	Mean	3.13	2.23	3.51	2.81
	SD±	0.11	0.08	0.11	0.10
Horticulture	Kolhapur	4.38	3.37	6.71	5.81
	Ganeshkhind	4.42	3.41	6.75	5.88
	Igatpuri	4.61	3.61	6.91	5.98
	Rahuri	4.57	3.45	6.82	5.91
	Mean	4.50	3.46	6.80	5.90
	SD±	0.10	0.09	0.08	0.06
Agroforestry	Kolhapur	6.39	5.43	8.45	7.58
	Solapur	6.28	5.21	8.31	7.44
	Igatpuri	6.62	5.68	8.72	7.75
	Rahuri	6.45	5.52	8.55	7.68
	Mean	6.44	5.46	8.51	7.61
	SD±	0.12	0.17	0.15	0.12

Total acidity of humic acid

The data with respect to total acidity of humic acid are presented in Table 4.20 and Fig 42. The forest land recorded maximum total acidity of humic acid with mean value of 7.73 and 6.53 meq g^{-1} HA in surface and subsurface soils, respectively. However, it was followed by agroforestry land (6.44 and 5.46 meq g^{-1} HA), pasture land (5.69 and 4.57 meq g^{-1} HA), horticulture land (4.50 and 3.46 meq g^{-1} HA), agriculture land (3.82 and 2.79 meq g^{-1} HA), salt affected land (3.13 and 2.23 meq g^{-1} HA) in surface and subsurface soils, respectively and minimum total acidity was recorded in fallow land with mean value of (2.65 and 1.26 meq g^{-1} HA) in surface and subsurface soils, respectively.

The variations in total acidity may be attributed to the inherent differences in chemical composition and molecular weights of HA and FA (Reddy *et al*., 2014) [4].

Conclusions

- It can be concluded that the forest based land use pattern under varied agro climatic zones was found superior for different soil attributes studied. However, it was followed in order of agro forestry, pasture, horticulture, agriculture, salt affected and fallow land.
- The potential of agro forestry and fruit trees to sequester carbon may be equal to that of forest trees and the fact that these crops also provide food and income for farmers.

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