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Characterisation of soil Humic and Fulvic acids from different land use patterns

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Abstract

Humic substances are the major fractions (nearly 80%) of soil organic matter, constituting humic acid (HA) and fulvic acid (FA) extracted from soils of different land use patterns viz., agriculture land, forest land, fallow land, pasture land, salt affected land, horticulture land and agroforestry land under varied agroclimatic zones of western Maharashtra (India) were evaluated for their individuality and properties through E4/E6 ratio and functional groups. The HA and FA were extracted from soils and further studied for their functional groups. It was observed that the total acidity (7.92 and 6.85 meq total acidity g⁻¹ HA and 9.35 and 8.58 meq total acidity g⁻¹ FA) and functional groups of humic acid and fulvic acid viz. CO₂H group (7.82 and 6.87 meq g⁻¹ HA and 9.82 and 8.86 meq g⁻¹ FA), Alcoholic group (7.88 and 6.95 meq g⁻¹ HA and 8.32 and 7.45 meq g⁻¹ FA) and Phenolic group (6.96 and 5.97 meq g⁻¹ HA and 7.96 and 6.98 meq g⁻¹ FA) were recorded higher in soil under Mahabaleswar forest as compared to other land use patterns i.e. conventionally cultivated and fallow land at depths 0-15 and 15-30 cm, respectively. In both humic fractions, carboxylic groups contributed a higher amount of total acidity than phenolic groups. E4/E6 ratio was recorded higher in FA than HA. Soils from fallow land showed lower total acidity than forest land.

Keywords: humic acid, fulvic acid, land use patterns, E4/E6 ratio and functional groups

Introduction

Humic substances are the colloidal substance having high specific surface area, surface charge, which control the mobility of heavy metals and pesticides in soil. It plays major role in chelation and complexation reaction with metal ions, nutrient cycling, improve the aggregate structure, improve the buffering capacity of soil and also interact with mineral, oxides, metal ions and organic compounds (Gautam *et al.* 2021) [6]. Soil is the largest pool of organic carbon storing more than three times carbon as compared to atmospheric and biotic pool. Humic substances represent the recalcitrant carbon pools SOM. (Galantini *et al.* 2014, Wiesmeier *et al.* 2019) [5, 22].

Functional group is specific group of atom or bond within the compound which is responsible for chemical reaction (Schnitzer, 1965). Functional group play important role in chelation and complexation reaction with metal ions and control mobility and availability of metal ions in soil and help to develop charges on organic colloid and increase the availability of metal ions for plant uptake by protecting the chelated ions from unwanted chemical reactions (Tewari, 2018) [20].

Total acidity indicates the presence of carboxylic group and phenolic group and contribute 1/3rd of total acidity. Carboxylic group is important component of amino acid, oxalic acid, acetic acid which play major role in cellular respiration. Phenolic group consist of aromatic ring having one or more OH group. It controls the soil organic matter decomposition and nutrient cycling. Alcoholic group consist of aliphatic carbon having one or more OH groups. E4/E6 ratio shows the absorbance at 465/665 nm. It shows degree of aliphaticity and aromaticity of humic substances. (Zalba *et al.*, 2016. Ukalska *et al.* 2021) [23, 21]. Our goal was studying the effect of different land use patterns on HS of the examined soils and characterise humic components of soil under seven land use patterns. Proposed technique is seen to be very helpful in understanding organic substances of soil, particularly when it becomes important to determine how changes in the environment have influenced the soil.

Materials and Methods

The study was conducted at department of Soil Science and Agricultural Chemistry, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra (India) in the year 2020-2021.

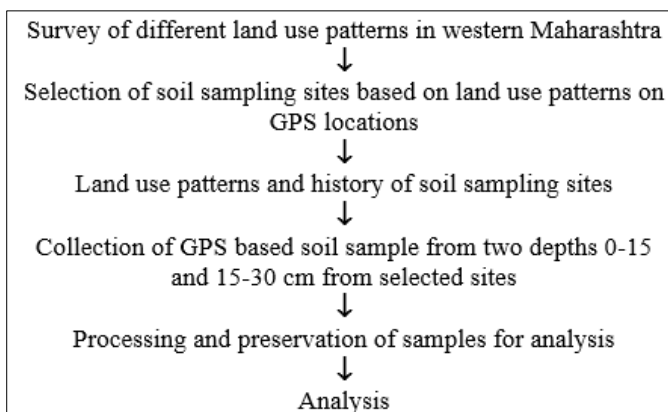


Fig 1: Experimental Details: (Steps involved in research project)

Location and Extent

Table 1: The study was conducted in eight districts of western Maharashtra with different land use patterns

| Sr. No. | Land use patterns | Locations |
|---------|-----------------------------------|---|
| 1. | Agriculture | Solapur, Borgaon (Satara), Igatpuri (Nashik), Rahuri |
| 2. | Forest land | Mahabaleshwar (Satara), Borgaon (Satara), Nandurbar, Radhanagri (Kolhapur). |
| 3. | Fallow land | Kolhapur, Solapur, Pune, Rahuri (Ahmednagar) |
| 4. | Pasture land | Shirval (Satara), Kolhapur, Rahuri, Igatpuri (Nashik) |
| 5. | Salt affected land (Saline/Sodic) | KasbeDigranj (Sangli), Padegaon (Satara), SavalivihirandRahuri (Ahmednagar) |
| 6. | Permanent Horticulture | Igatpuri (Nashik), Ganeshkhind (Pune), Kolhapur, Rahuri (Ahmednagar) |
| 7. | Agro-forestry | Kolhapur, Igatpuri (Nashik), Solapur, Rahuri |

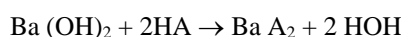
Characterization of humic and fulvic acid by chemical methods (Functional Group Analysis)

E₄/E₆ Ratio

The E₄/E₆ ratios were determined by dissolving 2 mg of HA and FA samples in 10 mL of 0.05(N) NaHCO₃ (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) [2].

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 0.2N Ba(OH) only. Displace air in each flask by N₂ stopper the flask carefully and shake for 24 hours at room temperature. Filter suspension wash the residue thoroughly with CO₂-free distilled water, and titrate filtrate plus washing potentiometrically with standard 0.5 N HCL solutions to pH 8.4.



The calculation is as follows

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

CO₂ H Group

Place between 50 to 100 mg of humic material in a 125-ml ground- glass stoppered Erlenmeyer flask, and 10 ml of 1N Ca (OAc), solution and 40 ml of CO₂ -free distilled water only. After shaking for 24 hours at room temperature, filter suspension, wash residue with CO₂ -free distilled water, and combine filtrate and washing and then titrate the potentiometrically with standard 0.1 N NaOH solution to pH 9.8. The calculation is as follows:

$$\text{meq CO}_2 \text{ H Groups/g of HA} = \frac{(\text{Volume blank} - \text{Volume sample}) \times \text{Normality} \times 1000}{\text{Weight of sample (mg)}}$$

Phenolic OH groups

Calculate the amount of Phenolic hydroxyls in the following manner:

$$\text{meq phenolic hydroxyl group/} = (\text{meq total acidity/g HA}) - (\text{meq carboxylic groups/g of HA})/\text{g of humic preparation.}$$

Characterisation of humic substances

Humic acid and Fulvic acids extracted from soil 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) [14].

E₄/E₆ Ratio of Humic Acid and Fulvic Acid

Humic and fulvic acids are characterised by their optical densities at 465 and 665 nm in relation to one another. As a potential humification index, the relationship E₄/E₆ ratio relates to the aromaticity and degree of condensation of the chain of aromatic carbons in humic substances. (Srilatha *et al.*, 2013, Stevenson, 1982 and Schnitzer and Khan, 1972) [15, 16, 14].

E₄/E₆ Ratio of Humic Acid

The data with respect to the E₄/E₆ ratio of humic acid are presented in Table and Fig. 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. 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 stabilisation and cycling of carbon in terrestrial ecosystems may be indicated by this property of humic acids. It was found that humic acid E4:E6 ratios were higher in surface soils (0-15 cm) than subsurface soils (15-30 cm) under all land use patterns. The E4/E6 ratio of FA are high as compared to HA and this might be due to presence of aliphatic structure, low molecular weight, low degree of polymerization, high oxygen containing functional groups and high degree of solubility (Stevenson, 1994) [17]. These findings are similar with Haddad *et al.*, (2015) [7], Zalba *et al.*, (2016) [23].

Total Acidity and Functional Group (CO₂H Group, Alcoholic Group and Phenolic Group) of Humic Acid

Total acidity of humic acid

The data with respect to total acidity of humic acid are presented in Fig 1. 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. This might be due to the presence of carboxylic group and phenolic group which contribute 1/3rd of total acidity (Reddy *et al.*, 2014) [11].

CO₂H group of humic acid

Maximum CO₂H group was observed in forest land with mean values of 7.61 and 6.51 meq g⁻¹ HA in surface and subsurface soils, respectively (Fig 2). However, it was followed by agroforestry land (6.49 and 5.36 meq g⁻¹ HA), pasture land (5.55 and 4.49 meq g⁻¹ HA), horticulture land (4.80 and 3.63 meq g⁻¹ HA), agriculture land (3.76 and 2.67 meq g⁻¹ HA), salt affected land (3.30 and 2.47 meq g⁻¹ HA) in surface and subsurface soils, respectively and minimum CO₂H group was recorded in fallow land (2.26 and 1.41 meq g⁻¹ HA) in surface and subsurface soils, respectively. This might be due to continues deposition of organic matter inputs and soil cover with vegetation throughout the year without any soil disturbance which reduces the loss of soil by erosion (Lopez *et al.*, 2020) [24].

Table 2: 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 | KasbeDigraj | 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 |

Alcoholic group of humic acid

The forest land recorded higher alcoholic group of humic acid with mean value of 7.67 and 6.81 meq g⁻¹ HA in surface and subsurface soils, respectively (Fig 3). However, it was followed by agroforestry land (6.60 and 5.70 meq g⁻¹ HA), pasture land (5.72 and 4.83 meq g⁻¹ HA), horticulture land (4.62 and 3.77 meq g⁻¹ HA), agriculture land (3.37 and 2.53 meq g⁻¹ HA), salt affected land (2.41 and 1.57 meq g⁻¹ HA) in surface and subsurface soils, respectively and lower alcoholic group of humic acid was recorded in fallow land (1.40 and 0.67 meq g⁻¹ HA) in surface and subsurface soils, respectively.

Phenolic group of humic acid

Maximum phenolic group of humic acid was observed in forest land with the mean value of 6.79 and 5.84 meq g⁻¹ HA in surface and subsurface soils, respectively (Fig 4). However, it was followed by agroforestry land (5.83 and 4.85 meq g⁻¹ HA), pasture land (4.49 and 3.60 meq g⁻¹ HA), horticulture land (3.82 and 2.92 meq g⁻¹ HA), agriculture land (2.72 and 1.84 meq g⁻¹ HA), salt affected land (2.30 and 1.43 meq g⁻¹ HA) in surface and subsurface soils, respectively and minimum phenolic group of humic acid was recorded in fallow land (1.29 and 0.55 meq g⁻¹ HA) in surface and subsurface soils, respectively. Humic substances and functional group were recorded high in forest soil might be due the continues deposition of above ground biomass inputs, high microbial diversity, minimum soil disturbance, high rainfall and low temperature condition which reduces the rate of decomposition of SOM and low in fallow land may be due to the lower turnover of OM and soils exposed to direct sunlight which increases oxidation of organic matter.

Reddy *et al.* (2014) [11] reported that the values of FA were higher than HA values, as FA have more aliphatic compounds than HA fractions.

Total acidity and functional group (CO₂H group, alcoholic group and phenolic group) of fulvic acid

Total acidity of fulvic acid

The similar trend was associated with the total acidity and functional groups of fulvic acid, the soil of forest land recorded the highest value of total acidity of fulvic acid with mean value of 9.24 and 8.39 meq g⁻¹ FA in surface and subsurface soils, respectively. However, it was followed by agroforestry land (8.51 and 7.61 meq g⁻¹ FA), pasture land (7.52 and 6.67 meq g⁻¹ FA), horticulture land (6.80 and 5.90 meq g⁻¹ FA), agriculture land (5.33 and 4.48 meq g⁻¹ FA), salt affected land (4.81 and 3.81 meq g⁻¹ FA) in surface and subsurface soils, respectively and minimum total acidity of fulvic acid was recorded in fallow land (2.35 and 1.49 meq g⁻¹ FA) in surface and subsurface soils, respectively (Fig 5). Similar result was found by Navnage (2022) [9] that functional groups in humic acid and fulvic acid varies with different land use system such as fallow, conventionally cultivated and mango orchard.

Ramalakshmi (2011) [10] and Banik and Sanyal (2006) [11] reported that total acidity was observed high in FA than HA. Sujana Reddy and Rao (2000) and Sanyal (2002) [12] found that increase in total acidity with decrease in degree of polymerization and molecular weight of HS.

CO₂H group of Fulvic acid

Maximum CO₂H group of fulvic acid was observed in forest land with mean value of 9.58 and 8.70 meq g⁻¹ FA in surface and subsurface soils, respectively. However, it was followed by agroforestry land (8.42 and 7.53 meq g⁻¹ FA), pasture land (7.60 and 6.74 meq g⁻¹ FA), horticulture land (6.41 and 5.56 meq g⁻¹ FA), agriculture land (5.45 and 4.61 meq g⁻¹ FA), salt affected land (4.32 and 3.46 meq g⁻¹FA) in surface and subsurface soils, respectively and minimum CO₂H group of fulvic acid was recorded in fallow land (2.46 and 1.60 meq g⁻¹ FA) in surface and subsurface soils, respectively (Fig 6).

Fulvic acid have higher carboxyl group content due to its low particle weight, which indicates that humic acid has high degree polymerization due to its molecular weight. (Lal and Mishra, 2000. Srilatha *et al.*, 2013) [8, 15].

Alcoholic group of fulvic acid

The forest land recorded maximum alcoholic group of fulvic acid with mean value of 8.17 and 7.28 meq g⁻¹ FA in surface and subsurface soils, respectively. However, it was followed by agroforestry land (7.34 and 6.44 meq g⁻¹ FA), pasture land (6.51 and 5.60 meq g⁻¹ FA), horticulture land (5.27 and 4.38 meq g⁻¹ FA), agriculture land (4.665 and 3.78 meq g⁻¹ FA), salt affected land (4.32 and 3.48 meq g⁻¹FA) in surface and subsurface soils, respectively and minimum alcoholic group of fulvic acid was recorded in fallow land (2.33 and 1.44 meq g⁻¹ FA) in surface and subsurface soils, respectively (Fig 7).

Phenolic group of fulvic acid

Maximum phenolic group of fulvic acid was observed in forest land with mean values of 7.84 and 6.87 meq g⁻¹ FA in surface and subsurface soils, respectively. However, it was followed by agroforestry land (6.27 and 5.38 meq g⁻¹ FA), pasture land (5.42 and 4.55 meq g⁻¹ FA), horticulture land 4.22 and 3.36 (meq g⁻¹ FA), agriculture land (3.77 and 2.79 meq g⁻¹ FA), salt affected land (3.30 and 2.39 meq g⁻¹FA) in surface and subsurface soils, respectively and minimum phenolic group of fulvic acid was recorded in fallow land (2.16 and 1.30 meq g⁻¹ FA) in surface and subsurface soils, respectively (Fig 8).

It was observed that higher content of carboxyl groups in both humic acid and fulvic acid than phenolic-OH groups indicate that carbohydrates and phenolic compounds produced were easily degradable and readily converted to carboxyl groups on subsequent oxidation. These results are in accordance with the findings of Erdogan *et al.* (2007) [3], Satisha and Devarajan (2011) [13], Banik and Sanyal (2006) [1], Eshwar *et al.* (2017) [4].

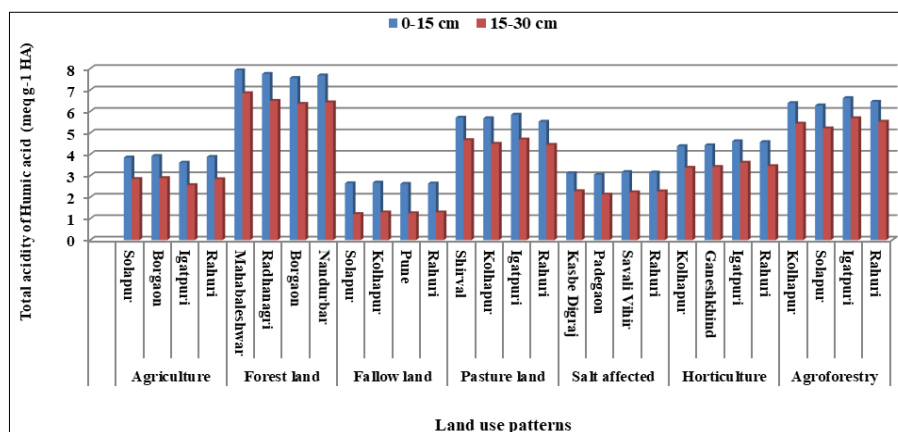


Fig 1: Effect of different land use patterns on depth wise soil total acidity of humic acid

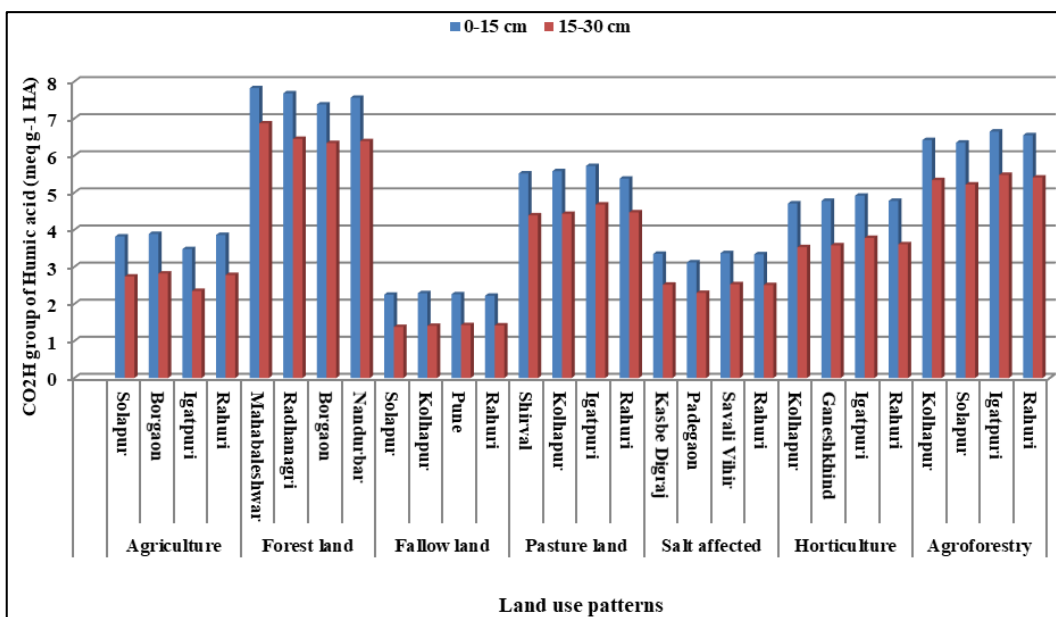


Fig 2: Effect of different land use patterns on depth wise soil CO₂H group of humic acid

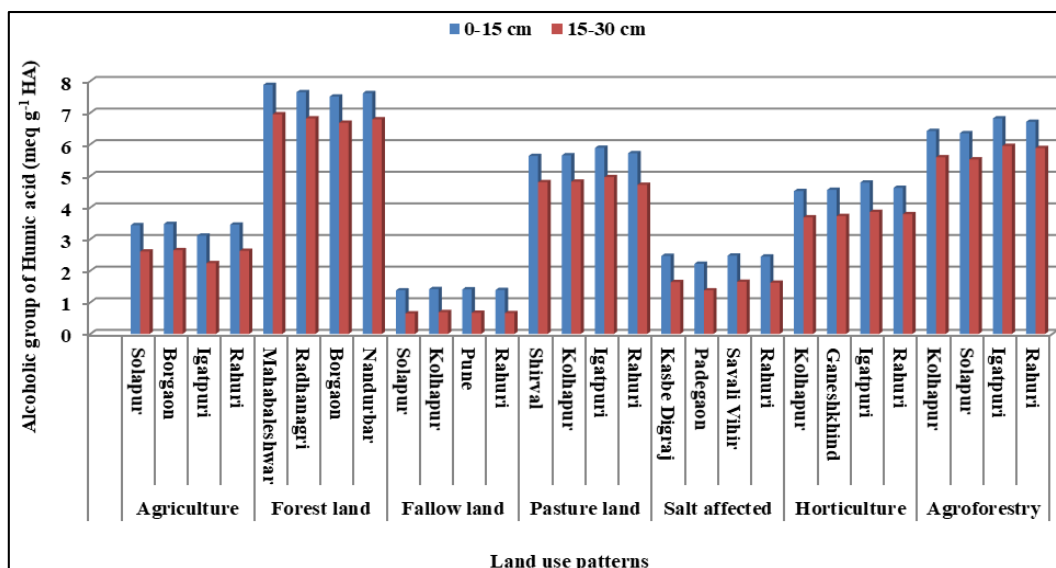


Fig 3: Effect of different land use patterns on depth wise soil alcoholic group of humic acid

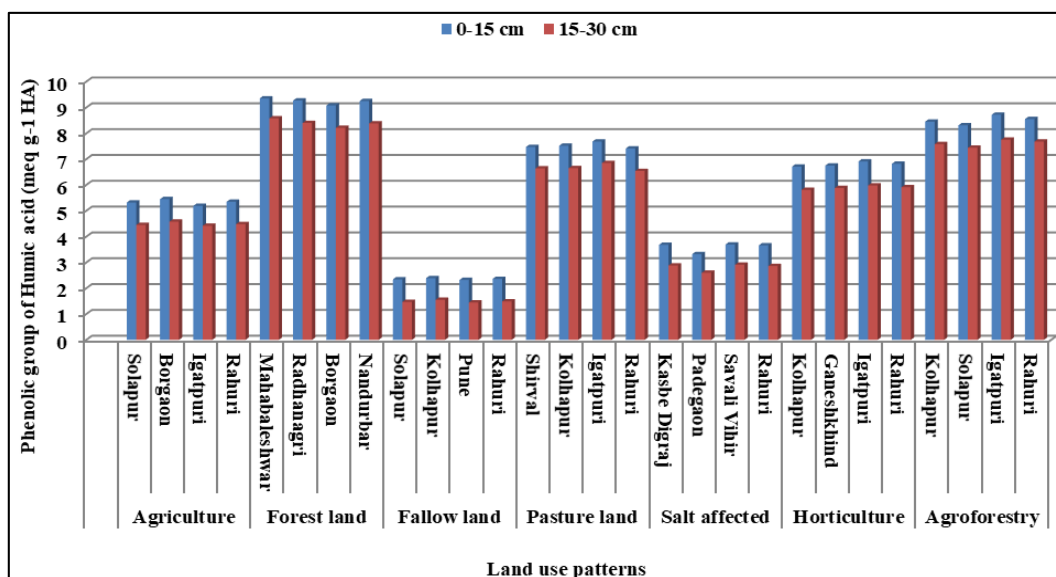


Fig 4: Effect of different land use patterns on depth wise soil phenolic group of humic acid

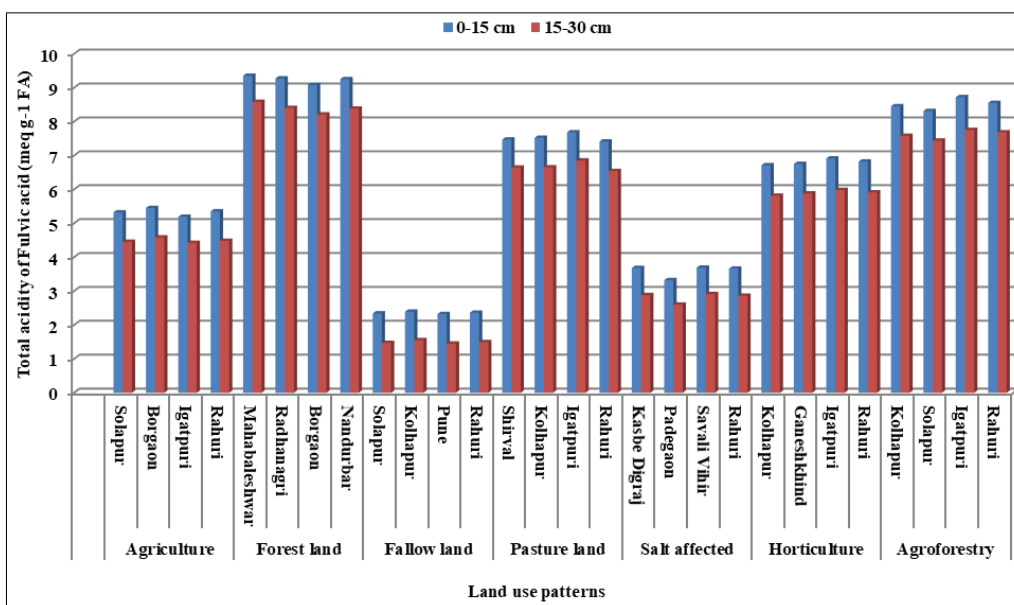


Fig 5: Effect of different land use patterns on depth wise soil total acidity of fulvic acid

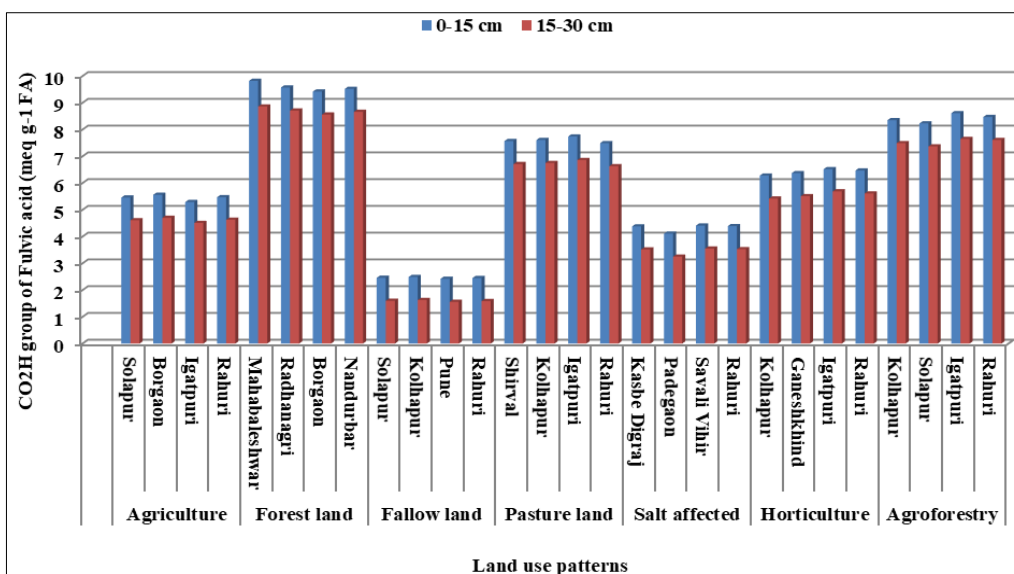


Fig 6: Effect of different land use patterns on depth wise soil CO₂H group in fulvic acid

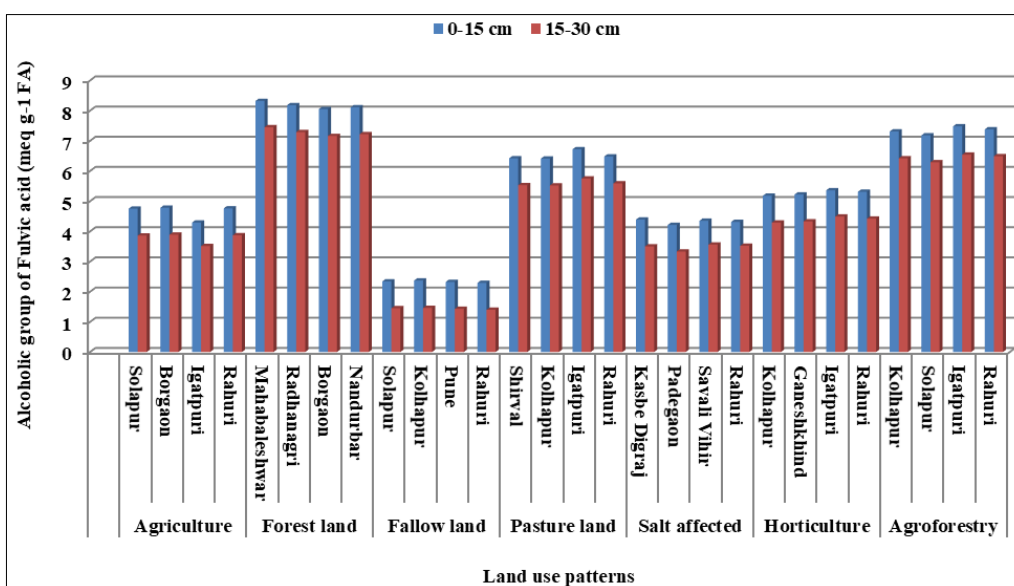


Fig 7: Effect of different land use patterns on depth wise soil alcoholic group in fulvic acid

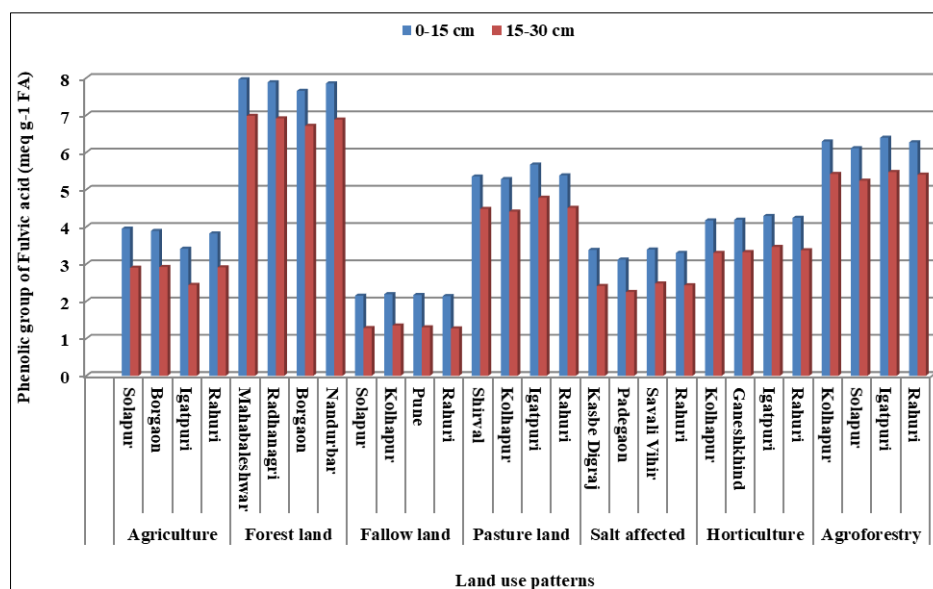


Fig 8: Effect of different land use patterns on depth wise soil phenolic group in fulvic acid

Conclusions

It can be concluded that the forest-based land use pattern under varied agroclimatic zones was found superior for E4/E6 ratio and functional groups (total acidity, carboxyl group and phenolic-OH). However, it was followed in order of agroforestry, pasture, horticulture, agriculture, salt affected and fallow land. It is clear from the results that fulvic acid has higher overall acidity, carboxyl group, and phenolic-OH group as compared to humic acid was due to presence of aliphatic structure, low molecular weight, low degree of polymerization, high oxygen containing functional groups and high degree of solubility. Soil under the forest land improved the soil nutrient status through the addition of litter fall recycling of SOM at deeper layer.

This is a very helpful way for figuring out molecular changes in humic substances as a result of changes in land use patterns since distinct variations were found between the E4/E6 ratios and different functional groups.

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