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Extraction and Characterization of Humic and Fulvic Acids from Latosols under Tea Cultivation in South India

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Abstract: Humic substances are considered as the most important constituents of soils. They form the largest fraction of soil organic matter and play a dominant role in improving soil productivity. Humic substances promote plant growth and improve the organic matter status of soil and soil fertility. The common method of extracting of humic substances is by treatment with alkali; dilute sodium hydroxide is generally used. Initially, the soil should be washed with 1 N HCl solution. Humic acid showed broad absorption centered around at 3360, 1406, 1233 and 1060 cm^{-1} regions. The phenolic (OH), amide, methyl, free-NH- bond, carboxylic and carbonyl group are present in the humic and fulvic acids. The absorption around 1715 cm^{-1} region was identified to be due to C=O stretching of carbonyl or ketonic groups. The organic matter content of humic and fulvic acids are highly influenced by physical parameters of soil. The organic matter content in humic acid was higher (61%) in Munnar soils, followed by Gudalur and Anamallais soils 56 and 55%, respectively. The organic matter content in fulvic acids is lower when compared to humic acid. The organic matter of the soil is positively correlated with organic matter of humic acid. The available magnesium content in fulvic acid is negatively correlated with potassium content of fulvic acid. The organic matter of the soil is negatively correlated with exchangeable K of humic acid and the same trend observed between available K and organic matter of humic acid.

Key words: Humic acid, fulvic acid, organic matter, extraction, nutrients, organic matter

INTRODUCTION

Humic substances are considered as the most important constituents of soils. They form the largest fraction of soil organic matter and play the dominant role in improving soil productivity. Humic substances are formed by the decomposition of plant and animal residues by micro organisms. Organic minerals are intrinsic and essential components of all soil. It functions as storehouse of nutrients like nitrogen, phosphorus, sulphur, potassium, calcium, magnesium, zinc, copper, iron, etc. and hence treated as important parameter for productivity and quality of tea. The humic substances play an important role in increasing productivity and soil fertility. The organic part of the soil consists of a complex system of substances, the dynamics of which is determined by the continuous admission of organic residues of plant and animal origin into the soil and their continuous transformation under the action of chiefly biological factors, but also to certain extent by chemical and physical

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factors. In developed soils, the second group forms a large part of the total reserve of organic matter up to 80-90% (Stevenson, 1994; Saikh and Chandra, 1999). The nitrogen dose may be adjusted based on organic matter status of tea fields, which is an integrated approach for sustainable productivity of tea in South India. Humic substances are reported to promote plant growth and improve the organic matter status of soil and soil fertility (Szajdak and Osterberg, 1996; Szajdak *et al*., 2003; Madrid *et al*., 2004; Verma and Palani, 1997). The main aims of the present study were (1) develop methodology for extraction of humic substances from tea soil, (2) characterise humic and fulvic acid fractions, (3) find out the nutrients present in humic and fulvic acids and (4) conduct correlation study between soil properties nutrient contents/organic matter of extracted acid fractions.

MATERIALS AND METHODS

Study Site

The study was conducted in three regions viz., Anamallais, Vandiperiyar and Munnar. The Anamallais is located on the western face of Western Ghats at elevation ranging from 1000 to 1400 m above Mean Sea Level (MSL) with 3000 to 3500 mm of annual rainfall. Munnar is at an elevation of 1500- 2000 m above MSL with 3952 mm annual rainfall. The other study site, Vandiperiyar, is in Kerala state at an elevation of 1000 m above MSL with 1921 mm annual rainfall. The mean minimum temperatures of the study site varied between 4 and 10°C, while, the maximum temperatures ranged from 16 to 32°C. The experiment was conducted at UPASI Tea Research Institute, Valparai during 2007-2009.

Soil Sampling and Analysis

From each region 15 to 20 soil samples were taken, using sampling auger (Eijkelkamp, ME 52C09). The individual soil samples collected from each region were mixed together by hand on a polythene sheet. The samples were air dried and passed through 2 mm sieve.

Extraction of Humic and Fulvic Acid

Humic substances were extracted by treatment with alkali; dilute NaOH is used. Initially, the soil was washed with 1 N HCl solution (Mukherjee and Ghosh, 1984). Soil sample was taken in the conical flask and tio NaOH solution was added at 1:20. All conical flasks were shaken for 24 h using rotatory shaker (Model: Pelican equipments-ROTEKLES). The temperature was maintained at 25°C at 150 rpm. After 24 h, the solution was centrifuged at 6000 rpm and the supernatant was adjusted to the pH between 1 and 2. The solution was settled for another 24 h, the insoluble humic substances were precipitated. The precipitate was collected and the supernatant was again adjusted to pH between 4.5 and 5 using NaOH solution. The fulvic acid fraction got was settled after 24 h. Both the humic substances and fulvic acid fractions were washed with distilled water to remove impurities. Finally, black coloured fine crystals of humic acid and brown coloured crystals of fulvic acid fractions were obtained.

Infrared spectra of the humic and fulvic acid substances were recorded at room temperature on a Shimadzu double beam IR spectrometer within a scanning range of 4000-400 cm^{-1} using pellets containing KBr as matrix.

Soil pH was measured in 1:2 soil: water mixture using Orion pH meter (Orion, 950), electrical conductivity was measured using conductivity meter (Systronics, 304), organic matter by Wakley and Black (1934) procedure, available phosphorus (P) by Bray and Kurtz (1945) method, potassium (K) by Hanway and Heidal (1952) procedure and soil particle distribution (sand, silt and clay) by international pipette method.

Statistical analysis were carried out by the standard method (Gomez and Gomez, 1984). The correlation coefficients were worked out using SPSS software.

RESULTS AND DISCUSSION

The physico-chemical properties of the Anamallais, Munnar and Gudalur soils were analysed and the results are furnished in Table 1. The organic matter content was higher (97 g kg^{-1}) in Munnar soils, while the phosphorus content was higher (125 mg kg^{-1}) in Anamallais and potassium content was higher (460 mg kg^{-1}) at Munnar. Such difference existed in available magnesium and calcium also (Table 1). The analysis of mechanical property indicated that clay fraction dominated in the soils of Anamallais while the sand portion was predominant in the soils of Munnar.

Spectral Analysis

Infrared spectra of humic and fulvic acid are shown in Fig. 1 and 2. In humic acid show broad absorption centered around at 3360, 1406, 1233 and 1060 cm^{-1} regions (Garcia *et al*., 1992; Ayuso *et al*., 1996). The Si-O bending, metal-oxygen stretching absorption is occurred at 590 cm^{-1}. The phenolic (OH), amide, methyl, free-NH- bond, carboxylic and carbonyl group are presented in the humic and fulvic acids (Sharmah and Bordoloi, 1993). The absorption are around at 1715 cm^{-1} region is due to the C=O stretching of carbonyl or ketonic groups (Haworth, 1971). The absorption are around at 1630 cm^{-1} region is due to the C=C skeletal

Table 1: Physico-chemical properties of tea soils

Properties	Anamallais	Gudalur	Munnar
pH	4.50	4.80	4.60
EC (dS m^{-1})	0.08	0.11	0.23
OM (g kg^{-1})	68.00	60.00	97.00
Available P (mg kg^{-1})	125.00	110.00	108.00
Available Mg (mg kg^{-1})	53.00	60.00	72.00
Exchangeable potassium (mg kg^{-1})	405.00	410.00	460.00
Available Ca (mg kg^{-1})	350.00	290.00	290.00
Clay (g kg^{-1})	190.00	185.00	170.00
Silt (g kg^{-1})	100.00	95.00	90.00
Sand (g kg^{-1})	710.00	720.00	740.00

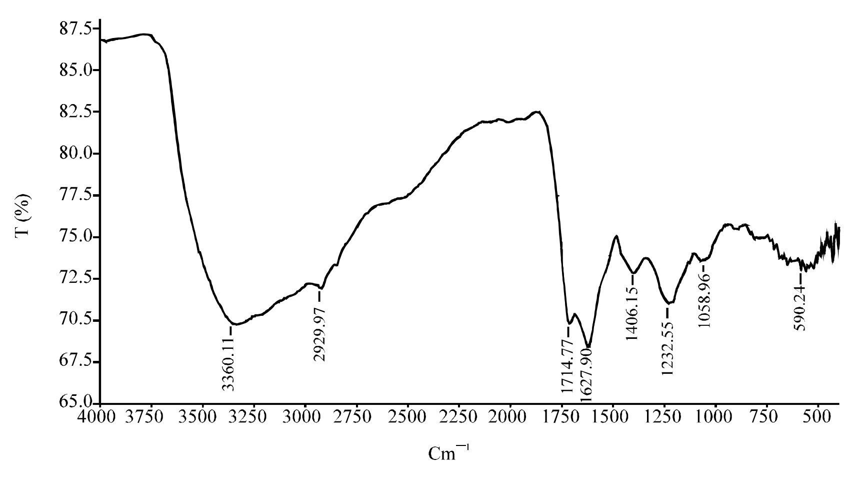

Fig. 1: Infrared spectrum of humic acid fractions

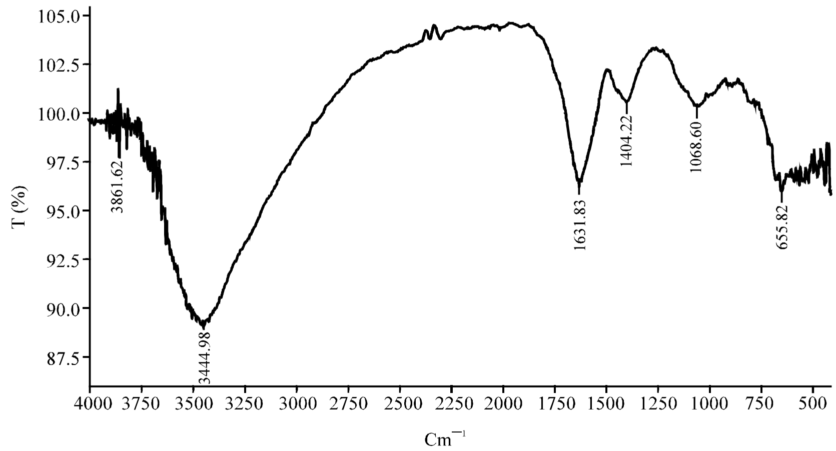

Fig. 2: Infrared spectrum of fulvic acid fractions

Table 2: Relative intensity of major IR absorption bands of humic and fulvic acids soils

Frequency (cm^{-1})	Assignment	Bond nature
Humic acids		
3360	H-bonded OH, Free OH, Intermolecular bonded OH.	Broad
2930	Aliphatic C-H, C-H_2 C-H_3 stretching	Weak
1715	C=O stretching of carboxylic acids, cyclic and acyclic aldehydes and ketones, quinones	Weak
1630	C=C stretching vibration of double bonds of cyclic and acyclic compounds, benzene ring substitution	Shoulder
1406	Aliphatic C-H deformation	Medium
1233	C-O stretching of phenols, carboxylic acids, ethers and COOH groups	Medium
1059	Si-O stretching	Weak
590	Si-O bending, metal oxygen stretching	Shoulder
Fulvic acids		
3445	H-bonded OH, Free OH, Intermolecular bonded OH.	Broad
1631	C=C stretching vibration of double bonds of cyclic and acyclic compounds, benzene ring substitution	Strong
1404	Aliphatic C-H deformation	Weak
1068	Si-O stretching	Medium
56	Si-O bending, metal oxygen stretching	Shoulder

Table 3: Chemical properties of humic and fulvic acids

	Humic acids			Fulvic acids		
Parameters	Anamallais	Gudalur	Munnar	Anamallais	Gudalur	Munnar
pH	1.80	1.82	1.89	5.27	4.49	3.95
EC (dS m^{-1})	6.80	5.90	5.40	3.60	7.40	8.60
OM (%)	55.00	56.00	61.00	24.00	20.00	19.00
Available P (mg kg^{-1})	0.21	0.22	0.14	0.81	0.72	0.29
Available Mg (%)	0.111	0.129	0.124	0.094	0.047	0.087
Exchangeable potassium (%)	0.513	0.504	0.465	0.402	0.383	0.259
Available Ca (%)	0.348	0.407	0.334	0.459	0.227	0.376
Ammoniacal nitrogen (%)	1.522	2.011	1.718	1.190	1.047	0.839

vibrations, H-boned quinines and COO^- groups bonded to metal ions (Dorado *et al*., 2003). The 1200 cm^{-1} band is assigned to C-O stretching of phenols, carboxylic acids, ethers and OH- deformation of –COOH (Stevenson, 1994). The relative intensity of major IR absorption bands of humic and fulvic acids of soil are shown in Table 2.

The organic matter content in humic acid was higher (61%) in Munnar soils, followed by Gudalur and Anamallais soils 56 and 55%, respectively (Table 3). The organic matter

Table 4: Correlation coefficients worked out between nutrient content in humic and fulvic acid and physical properties of soil

	Clay	FA-Ca	FA-K	FA-Mg	FA-N	FA-OM	HA-Ca	HA-K	HA-Mg	HA-N	HA-OM	Sand	Silt
FA-Ca	0.003												
FA-K	0.002	1.000**											
FA-Mg	0.138	-0.990**	-0.990**										
FA-N	0.994**	0.107	0.108	0.032									
FA-OM	0.945**	-0.327	-0.326	0.4546	0.905**								
HA-Ca	0.181	0.984**	0.984**	-0.949**	0.284	-0.151							
HA-K	0.882**	0.472	0.473	-0.346	0.927**	0.679	0.624						
HA-Mg	-0.700	0.714	0.714	-0.804	-0.620	-0.895**	0.576	-0.279					
HA-N	-0.403	0.915**	0.915**	-0.962**	-0.303	-0.680	0.828*	0.077	0.936**				
HA-OM	-0.933**	-0.360	-0.360	0.227	-0.966**	-0.764	-0.522	-0.993**	0.396	0.047			
Sand	-1.000**	-0.003	-0.002	-0.138	-0.994**	-0.945**	-0.181	-0.882**	0.700	0.403	0.933**		
Silt	1.000**	0.003	0.002	0.138	0.994**	0.945**	0.181	0.882**	-0.700	-0.403	-0.933**	-1.000**	
Soil OM	-0.904**	-0.427	-0.429	0.298	-0.945**	-0.715	-0.584	-0.999**	0.327	-0.0273	0.997**	0.904**	-0.904**

HA: Humic acid; FA: Fulvic acid; OM: Organic matter; *Significant at 5% level; **:Significant at 1% level

content in fulvic acids was lower when compared to humic acid. The organic matter in humic acid was the lowest in the Anamallais (55%) while fulvic acid it was the highest in Anamallais (24%). Ammoniacal nitrogen content was varied between 0.839 and 2.011% in both humic and fulvic acids. The soils of Gudalur had higher quantum of ammoniacal nitrogen. The available magnesium content in humic acid was higher when compared to fulvic acid. The available magnesium content in humic acid was higher (0.129%) in Gudalur soils, followed by Munnar and Anamallais soils 0.124 and 0.111%, respectively.

The organic matter of the soil is positively correlated with organic matter of humic acid (r = 0.9973; p = 0.01), it revealed that when the soil is having high organic matter it increased high organic carbon content in humic acid (Basak and Ghosh, 1999). The organic matter content of humic and fulvic acids are highly influenced by physical parameters of soil (Table 4). The proportion of sand is positively correlated (r = 0.933; p=0.01) whereas silt and clay are negatively correlated (r = -0.933; p=0.01 and r = -0.933; p=0.01) with organic matter of humic acid. A similar trend was observed in fulvic acid (Saikh and Chandra, 1999). The organic matter of the soil is negatively correlated with exchangeable K of humic acid (r = -0.9987 at 1% level), the same trend observed between available K and organic matter of humic acid. The correlation coefficient was positive in between the humic and fulvic acid in the case of calcium (r=0.984). The available calcium content in humic and fulvic acids are almost same, it varies between 0.227 to 0.459%. The available magnesium content in fulvic acid is negatively correlated with potassium content of fulvic acid (Dorado *et al*., 2003). The higher potassium input in tea soils enhances the antagonism with magnesium, (Venkatesan *et al*., 2006; Jayaganesh and Venkatesan, 2006) the similar kind of trend is observed in the case of fulvic acids.

CONCLUSION

Humic substances are formed by the decomposition of plant and animal residues by micro organisms. The content of humic acid was higher in Munnar soils, followed by Gudalur and Anamallais while fulvic acid constituents lower in comparison to humic acid. The study confirmed the importance of soil organic matter in improving soil health due to the presence of constituents such as humic and fulvic acids.

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