



Isolation and Characterization of Soil Fulvic Acid

Mir Munsif Ali Talpur^{1*}, Tajnees Pirzada¹, Waheed Ali¹, Mohammad Hussain Arain¹, Samina Rajper² and Mazhar Iqbal Khaskheli³

¹Institute of Chemistry, Shah Abdul Latif University, Khairpur, Sindh, Pakistan.

²Department of Computer Science, Shah Abdul Latif University, Khairpur, Sindh, Pakistan.

³Institute of Advanced Research Studies in Chemical Sciences, University of Sindh, Jamshoro, Pakistan.

*Corresponding Author Email: mirmunsif_salu@yahoo.com

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Abstract

Fulvic acid was isolated from the agriculture soil of District Naushahro Feroz, Sindh, Pakistan by International Humic Substances Society (IHSS) method. The nutrient contents of the soil like K, Ca, Na, Fe and Zn were determined by using the Atomic Absorption spectrophotometer (AAS). The spectroscopic analysis was carried out by studying the UV-Vis, FT-IR and NIR spectra of isolated compounds. The data has been compared with the literature and correlated. Moisture as well as texture shows good water holding capacity and silt-loam type of soil. pH and EC are indicators of the fertility of soil to be beneficial for plantation. The spectral data (UV-Visible, FTIR and NIR) supports the characteristic functional groups (-COOH, C=O, -OH, -NH₂, C=C, CH₂ and polysaccharides) present in fulvic acid. E₄/E₆ values depict its hydrophilic nature, having less aromatic and more aliphatic groups. The presence of metal ions indicates its chelating ability.

Keywords: Fulvic acid; Agriculture soil; AAS; UV-Visible; FTIR and NIR

Introduction

Soil Organic matter (SOM) is very essential to determine the fertility status of soil because it is a source of majority of micronutrients (about 70%) and essential elements like carbon, nitrogen, sulfur and phosphorous [1,2]. Organic matter also maintains the physico-chemical and biological properties of the soil along with microbial activities [2,3]. SOM also influences electrical properties therefore a rough indicator to determine the dissolved salts in soil available for plant growth [4].

SOM is most widely spread organic product used for biosynthetic purpose on globe and also plays vital role in natural environment to regulate ion concentration in solution [5]. Major portion of SOM (80%) are humic substances, these substances improve certain properties of soil like buffering capacity, moisture retention and

availability of micronutrients [6,7]. Phosphorous uptake of plants is also increased by humic substances [8]. SOM include Humins, Humic Acid (HA) and Fulvic Acid (FA) [5,9]. FAs occur in most soils and water at lower levels than HAs [10] and is one of the basic components of well known Ayurvedic medicine Shilajith [11]. FA is lower molecular weight macromolecule, soluble at all pH values, mixture of yellow to brown color. These molecules are known to be heterogeneous polyacids with polymeric structure formed by different types of functional groups like aliphatic and aromatic organic acids having many aromatic rings attached to (carboxyl, phenols, quinones & semiquinones) [5,8,9,12] and the number of oxygen containing functional groups is more than HA [13].

Since long intense research has been made on humic substances but the molecular structure of

HA and FA are not fully understood [1]. These studies has generated the idea that HA are not natural product but it is collection of different macromolecular species, formed by dynamic associations stabilized by hydrophobic interactions and hydrogen bonds [5, 6].

Pakistan is an agricultural country with high population. In order to resolve the food problems it is necessary to increase the crop production by solving the problems of agriculture, will be of national economical interest. Humic substances (HA, FA) play vital role in this regard; hence their study is much beneficial and important. The role of FA is also vital in improving the solubility, its effect on dissolution and permeation of drugs is also reported [14].

Materials and Methods

Sample collection

Soil samples were collected at depth of 10 cm from different agriculture lands of District Naushahro Feroze, Sindh, Pakistan. Air dried at room temperature till constant weight, powdered with pestle and mortar, passed through 2mm sieve and stored at suitable place in labeled plastic bags for further analysis.

Sampling stations

Three Talukas of Naushahro Feroze District were selected for sampling i.e Moro, Naushahro Feroze and Bhirya. Sampling stations are:

1. Phull
2. Moro
3. Sadhoja
4. Wada Dangraj
5. Darya Khan Mari
6. Abran
7. Khair Wah
8. Jatoi
9. Khahi Qasim
10. Mithiani
11. Tharu Shah
12. Bhorthi
13. Bhirya
14. Bhirya Road
15. Dalipota
16. Bela Wah

Fulvic acid extraction

The method used for separation and purification of FA was based on the procedure described by International Humic Substances Society (IHSS) [14, 15]. 100g of air dried and sieved samples of soil were washed by using acidic

solution (10% HCl), stirred for 1-2 hours for decalcination and then allowed to dissolve in 0.5M NaOH to maintain pH 12 using 1:10 ratio of sample/extractant, respectively. The same was shaken for 7-8 hours using mechanical shaker, the suspension was left over night at room temperature to settle down the residue and then centrifuged for 10 minutes at low speed (1500rpm) to separate the alkaline solution from the residue. The dark brown solution of HA and FA was obtained by the decantation of supernatant solution, which was acidified with 6M HCl to maintain pH 1, with constant stirring and allowed to stand for 12-16 hours. The supernatant material (FA extract) was saved, freeze-dried and preserved for analysis.

Determination of soil texture

Soil texture was determined by Hydrometer method. 50g of fine powdered soil was taken into 250 ml of distilled water. 5g of calgon dispersing agent was taken in a separate beaker, then added 50ml distilled water and stirred the calgon get dissolved. Calgon as well as soil solution was put into a dispersion cup. Repeat it until the entire sample was transferred. Dispersion cup was stirred for 15 minutes on stirrer. Soil suspension was transferred to a sedimentation cylinder and water was added to adjust the volume to 1L. A drop of amyl alcohol was added to remove foam on the liquid surface. Temperature reading of the water was taken (approx room temperature). Gently stir with plunger for 2 minutes, through the cylinder (25-30 plunges), quickly then dip hydrometer into the suspension. Hydrometer reading was noted at 40 seconds and finally after 2 hours. Rinse it well between readings of the different samples.

pH and conductivity of soil

The pH of soil samples (suspension with water 1:1) was measured after collection using a Eutech Instruments Bench pH meter Cyber Scan pH 500 Ptc. Ltd Singapore. The conductivity of all the soil samples 1:1 with water was measured by using a Eutech, Cyber scan Con 11 conductivity/TDS/C Meter, made in Singapore using Dahnke and Whitney 1988 process.

Detection of metals in fulvic acid

Concentration of all the elements was estimated from FA samples digested in 0.1M nitric acid by using Perkin Elmer Atomic Absorption Spectrophotometer Analyst 800. Instrumental zero was adjusted with distilled water as blank. Addition of chemicals and other reagents for experiment were set according to the specifications given in the instrument's working manual. The concentration of each element was estimated with reference to standard solutions of the element.

UV/Visible spectroscopy

Ultraviolet and visible spectroscopic measurements of FA were made between 200 to 700 nm by using Birchrom Libra 522 spectrophotometer using quartz cell (1cm x 1cm). The spectral absorbance at 254, 436, 465 and 650 nm were measured after equilibrating the prepared solution at room temperature (25°C). The ratio of the measured spectral absorbance i.e. A_{254}/A_{436} , D_4/D_6 and E_4/E_6 were calculated.

Fourier transform infrared spectroscopy (FTIR)

The FTIR spectra of FA samples were recorded on Bruker vector: 22 spectrophotometer using the potassium bromide (KBr) disc technique. The samples (1 mg) were mixed with KBr (about 200 mg) in a clean glass pestle and mortar and compressed to obtain a pellet. The base line was corrected and scanning was performed from 4000–400 cm^{-1} .

Near infrared spectroscopy (NIR)

The NIR spectra of FA samples were recorded on spectrophotometer Mpa 1590 opus version 6.5 build 6597.

Results and Discussion

Moisture content

Table 1 shows moisture contents. At room temperature it varies from 1.75 to 14.15%, indicating the good water holding capacity of the soil and excellent for irrigation purpose [1,16]. At

105°C its percentage varies from 2.9 to 21.5% close to the data reported in literature 1.00 to 15.57% [17] and it is the maximum temperature to eliminate free water, without significant loss of organic matter [17]. Loss on ignition (LOI) at 550°C is used to analyze the organic matter content which ranges from 4.97 to 24.2% whereas 2.63 to 47.96% values are also reported [18].

Table 1. Moisture content in soil at different temperatures.

Sample No.	Moisture % at 25°C	Moisture at 105°C %	LOI at 550°C%
1	7.5	12.05	16.1
2	7.5	12.05	15.1
3	8.0	11.91	14.5
4	9.0	13.38	15.6
5	1.94	4.11	5.63
6	3.85	4.97	6.8
7	4.26	5.68	7.37
8	1.75	3.15	4.97
9	11.73	16.54	19.04
10	10.26	13.39	15.15
11	9.33	12.05	13.69
12	7.74	10.35	11.54
13	14.15	16.25	19.25
14	13.9	15.5	18.1
15	7.5	2.9	16.65
16	8.0	9.5	16.55

Soil texture

The data of soil texture has been presented in Table 2, which is the relative measurement of sand, silt and clay particles, having size less than 2mm [19, 20]. It is very important characteristic indicating strong effect on water and nutrient retention, infiltration, drainage, aeration, pH buffering and porosity. Movement of water through the soil is influenced by the texture. All soil samples were categorized as silt clay, silt loam, loam and silt clay loam. Sample no: 2,3,4,5,8,10,12,14 and 15 are slit loam, sample no: 6,11,13,16 are loam while sample no: 7 and 9 are slit clay loam. All samples were found to be non-saline in nature [21].

Table 2. Texture of soil.

Sample No.	Sand%	Clay %	Silt %	Texture Class
1	18.4	31	50.4	silt clay
2	22.4	23.6	53	silt loam
3	22.4	24	53.6	silt loam
4	26.4	20	53.6	Silt loam
5	20.4	20	59.6	Silt loam
6	26.4	24	49.6	Loam
7	20.4	28	51.6	Silt clay loam
8	26.4	22	51.6	Silt loam
9	20.4	27	52.6	Silt clay loam
10	24.4	24	51.6	Silt loam
11	28.4	26	45.6	Loam
12	20.4	22	57.6	Slit loam
13	38.4	19	42.6	Loam
14	24.4	18	57.6	Silt loam
15	30.4	14	55.6	Silt loam
16	37.4	20	42.6	Loam

pH of soil

The obtained pH values have been shown in Table 3. In soil management pH is a key parameter because it impacts on nutrient availability and solubility of materials [2,22]. Sample no: 1,2,6 and 14 have 7.7 pH, depicting slightly alkaline nature. However all others show 7-7.2 pH indicating their neutrality, pH data point out that soil is very good from agriculture point of view as solubility of the most essential nutrients in soil solution increases in the range 6.5 to 7.5 pH. N, K, Ca, Mg, S and P are more available at 6.5 to 8 pH range, however in basic or alkaline medium Zn, Al, Mn, Cu, Co, Fe are very rare for the plants uptake [23].

Electrical conductivity (EC)

Electrical conductivity measures the total dissolved salts in the soil; it determines the nature of soil as saline or non-saline [24]. Various fertility factors of the soil like salinity level, texture, clay contents etc. are determined by its EC, so it acts as rapid indicator for crop management [25]. EC values are depicted in (Table 3) ranges from 7.3 to 30.1 mS/cm, with the highest value of sample 1

and lowest of 16. These values suggest that all soil samples belong non-saline, showing good fertility status [1, 2, 21].

Yield of fulvic acid

Table 3 shows FA yield. FA was precipitated as lead fulvate by using concentrated lead chloride solution [26]. The obtained (%) yield ranges from 0.504 to 1.121 which is close to the data reported in literature 1-4% [27] 2.4 to 5mg/g [28].

Table 3. pH, electrical conductivity and yield of soil samples.

Sample No	pH	EC (mS/cm)	Yield (%)
1	7.7	30.1	0.610
2	7.7	13.8	1.121
3	7.1	15.64	0.504
4	7.0	11.47	0.568
5	7.1	16.93	1.052
6	7.7	13.1	0.615
7	7.0	7.43	0.689
8	7.1	9.29	0.986
9	7.2	7.62	0.661
10	7.1	8.25	0.572
11	7.0	14.6	0.714
12	7.1	8.28	0.89
13	7.2	7.97	0.787
14	7.7	7.5	1.092
15	7.0	10.45	0.746
16	7.1	7.3	0.581

Co-relation coefficient

The 'r' co-efficient data has been shown in Table 4. The 'r' co-efficient data shows moderate relation between EC and pH, poor relation between pH, moisture content and quantity of FA, while no relation between moisture content, EC and quantity of FA.

Table 4. Co-relation coefficient between EC, pH, Moisture content and Quantity of FA.

	EC	pH	Moisture (%)	Yield (%) of FA
EC	1.00			
pH	0.39	1.00		
Moisture (%)	-0.25	0.12	1.00	
Yield (%) of FA	-0.12	0.30	-0.12	1.00

Elemental analysis

The deficiency of Na, K, Ca, Fe and Zn reduces the crop production and nutritional quality of grains [29-32]. Availability of essential nutrients depends upon the presence of SOM [33]. Therefore isolated FA was analyzed for some major and trace elements and presented in Table 5. FA has capability of mineral chelating and plant growth [34], probably due to smaller structure and higher solubility.

Table 5. Major and trace elements concentration ($\mu\text{g/g}$) in fulvic acid from soil.

Sample No.	Na	K	Ca	Fe	Zn
1	13.87	7.1	5.58	0.307	0.013
2	13.90	6.98	3.19	0.720	0.013
3	11.19	8.45	4.66	0.402	0.013
4	7.88	8.80	5.12	0.179	0.021
5	9.1	8.4	2.91	0.246	0.034
6	6.88	7.12	3.88	0.212	0.051
7	7.55	6.99	6.4	0.280	0.050
8	8.81	8.10	4.88	0.189	0.047
9	10.6	5.69	5.32	0.268	0.047
10	6.93	7.63	3.12	0.121	0.011
11	5.58	4.88	2.98	ND	0.014
12	4.60	8.23	5.16	ND	0.013
13	8.22	6.20	4.28	0.329	0.019
14	9.28	7.12	3.69	0.105	0.023
15	7.69	8.69	5.61	0.107	0.029
16	7.80	6.83	3.32	ND	0.010

Sodium can be found as HCO_3^{1-} , CO_3^{2-} and Cl^- in the earth crust. It has direct effect on fertilization rates of agriculture soil and decrease the crop production by the base exchange reaction, replacing Ca and Mg, resulting in alkalinity of the soil. The Na concentration of isolated FA is between 13.60-13.97 $\mu\text{g/g}$ which is comparable with the data (7.30 to 24.30 $\mu\text{g/g}$) reported elsewhere [35].

Potassium stimulates the early growth of plant by increasing protein production in plant cell and improves resistance against diseases and insects [2,36]. It is the third most abundantly found element in plant cell. It regulates osmosis [1], activates dozens of enzymes acting in metabolism Potassium concentration lies between 4.88-8.80 $\mu\text{g/g}$, which is higher than reported data (0.01 to 1.76 $\mu\text{g/g}$) [37].

Calcium is a vital nutritional mineral for all body functions. Its ionic form when absorbed play role for enzymatic and starch metabolic activity. Ca ranges between 2.91-6.4 $\mu\text{g/g}$, which is within the limits of reported data (0.01-11.63 $\mu\text{g/g}$) [37].

Iron is very important micronutrient for many organisms, animals and plants, where it behaves as catalyst. In agriculture, iron is the main ingredient in manufacturing plant food due to its vital role in formation of chlorophyll [38]. Iron concentration ranges between 0.105-0.720 $\mu\text{g/g}$, which is comparable with the data 0.01-3.67 $\mu\text{g/g}$ [37,39] and 806-1197 $\mu\text{g/g}$ reported in literature [27].

In soil Zn being a part of enzyme system plays important role for the plant growth and carbohydrate transformation. It is absorbed by plants as divalent ion through diffusion. It is very essential for synthesis of protein and metabolism in plants. Zinc deficiency cause delay in maturity, photosynthesis, decrease in leaf size, hadda disease in rice, leaf mottling and interveinal chlorosis and short internodes in plants [40,41,42]. Highest quantity of zinc was found in sample no.6 as 0.051 while lowest in sample no. 16 as 0.010 $\mu\text{g/g}$, which is less than reported data 66.1 mg/g and 10-23 $\mu\text{g/g}$ [27,43].

The correlation- coefficient data has been placed in Table 6. The 'r' co-efficient data shows a very good relation between iron and sodium, where as a minus values depict no relation and other imply very weak or poor relation.

Statistical parameters have been placed in Table 7. The average value is highest for Na and lowest for Zn. Mode is 7.12 for K and 0.013 for Zn. median, max, min and St. dev. is maximum for Na and minimum for Zn.

Table 6. Co-relation coefficient data of element concentration in soil fulvic acid.

	Na	K	Ca	Fe	Zn
Na	1.00				
K	-0.007	1.00			
Ca	0.0626	0.241	1.00		
Fe	0.717	-0.275	-0.175	1.00	
Zn	-0.100	-0.014	0.376	-0.289	1.00

Table 7. Statistics of elements concentration in soil fulvic acid.

	Na	K	Ca	Fe	Zn
Average	8.74	7.32	4.38	0.27	0.06
Mode	#N/A	7.12	#N/A	#N/A	0.03
Median	8.05	7.12	4.47	0.26	0.02
Max	13.90	8.80	6.40	0.72	0.05
Min	4.60	4.88	2.91	0.15	0.01
Std div	2.51	1.07	1.07	0.16	0.04

UV-Visible measurements

The UV-Visible spectra of FA samples are depicted in Fig.1, indicating monotonous decrease in absorption with increasing wavelength. UV-visible spectra indicate the presence of chromophores. The FA samples have shown sharp maxima at 235nm. Absorption in UV region is normally due to the $\pi-\pi^*$ transition in unsaturated and conjugated aromatic rings [44, 45].

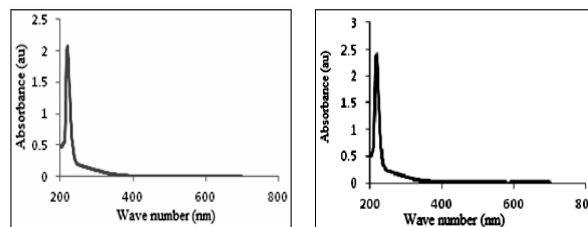


Figure 1. UV-Visible spectrum of soil fulvic acid

E_4/E_6 ratio and optical density ratio (D_4/D_6) have been placed in Table 8. E_4/E_6 ratio ranges from 6.0 to 8.0 for FA [45] and is used to determine the degree of humification. Higher ratio indicates more degree of humification, low molecular size and weight, concentration of chromophores and low aromaticity whereas lesser ratio is vice versa. [46]. It is inversely proportional to degree of condensation and molecular weight but directly proportional to aliphatic structures [47]. Optical density ratio (D_4/D_6) is independent of concentration of FA but depends upon the soil type. It varies from 6.2 to 8.4 [48]. More is the ratio; mature is the FA, lesser value shows its young nature [49]. Yellow color of all isolated samples imply about the presence of conjugated double bonds in FA [50].

Table 8. The specific spectral absorbance and their ratios at particular wavelengths by UV-Visible spectroscopy of fulvic acid.

Sample No.	A ₂₅₄	A ₄₃₆	A ₄₆₅	A ₆₆₅	E ₄ /E ₆	D ₄ /D ₆
1	0.576	0.144	0.150	0.052	2.884	2.552
2	0.630	0.137	0.142	0.008	17.75	5.534
3	0.449	0.024	0.017	0.005	3.4	2.833
4	0.177	0.002	0.001	0.001	1.0	2.137
5	0.232	0.002	0.001	0.001	1.0	1.929
6	0.092	0.001	0.001	0.001	1.0	3.1
7	0.094	0.001	0.001	0.001	1.0	0.666
8	0.148	0.000	0.001	0.001	1.0	0.214
9	0.048	0.002	0.002	0.001	2.0	4.344
10	0.013	0.003	0.003	0.001	3.0	3.914
11	0.254	0.009	0.005	0.001	5.1	4.685
12	0.845	0.036	0.023	0.003	7.666	6.212
13	0.219	0.003	0.002	0.001	2.0	1.664
14	0.085	0.001	0.001	0.001	1.0	7.055
15	0.055	0.002	0.002	0.003	0.666	0.697
16	0.180	0.003	0.002	0.001	2.0	1.832

E_4/E_6 ratios of sample no: 2, 11 and 12 are 17.75, 5.1 and 7.66 is greater than 5 indicating the good quality of FA, more humified, presence of more aliphatic and less aromatic fraction, low molecular weight and low degree of condensation [44, 46]. While all other samples have less than 5 value, showing the poor quality of FA, with high molecular weight, high degree of condensation, less aliphatic but more aromatic fraction [47,49].

FTIR analysis

The FTIR spectra of FA are shown in Fig. 2, the main absorption bands with

corresponding assignments are present in Table 9. Absorption bands in the region between 3450–3050 cm^{-1} phenolic groups [47]. The FA spectrum has strong absorption band at 3400 cm^{-1} followed by strong band at 1650 cm^{-1} and 1000 cm^{-1} . These bands are attributed to vibrations of OH, aliphatic C-H, carry (C=O) followed by carboxylate COO form & ethyl, vinyl $-\text{CH}_2=\text{CH}_2-$, aromatic, aldehyde, amine and SH groups, respectively [16,50]. In case of FA, a sharp peak occurred at 1056 cm^{-1} as shown in fig. for C-O stretch of polysaccharide, Si-O stretching, which does not occur in other humic fractions [44,45,49,51].

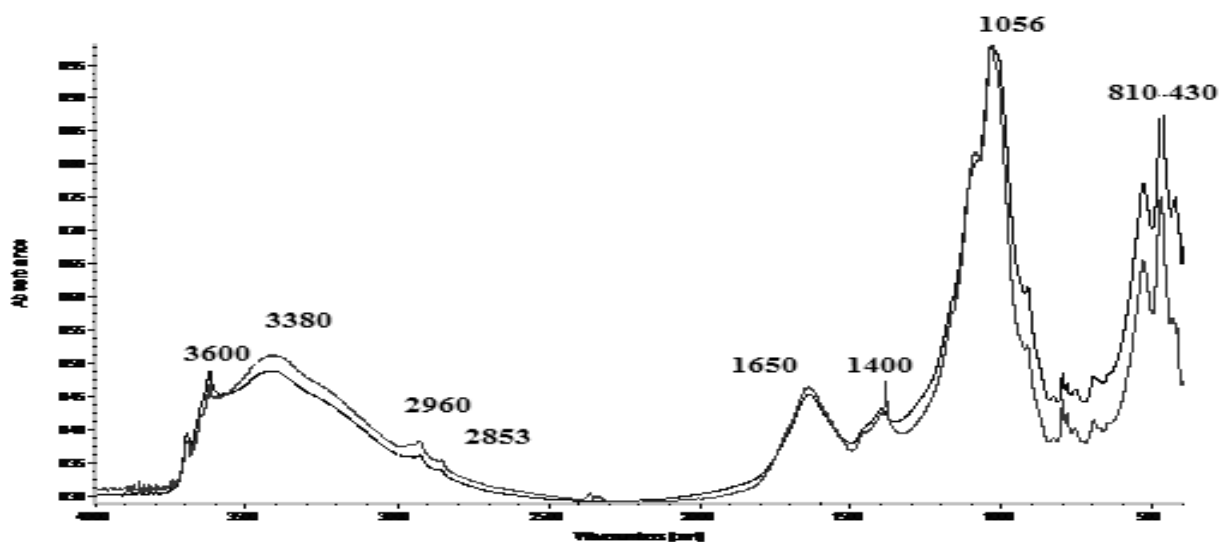


Figure 2. FTIR spectrum of soil fulvic acid

Table 9. FTIR assignments.

Range cm^{-1}	Assignment	Fulvic Acid
3600-3500	O-H stretching of kaolinite and sincalite	3600
3380	O-H or N-H stretch	3380
2960-2900	Aliphatic C-H stretching in CH_3 and CH_2 group	2960
2856-53	Aliphatic C-H stretching in CH_3 and CH_2 groups	2850
1650	C=O amide groups (amide I band), aromatic C=C carboxylate ion C=O of quinine and/or H-bonded conjugated ketones	1650
1400	COO^- antisymmetric stretching O-H deformation and C-O stretching of phenolic groups	1400
1160-1100	C-C, C-OH, C-O-C typical of glucosidic linkages, Si-O impurities, C-O stretch of polysaccharides	1100
1050	C-OH stretching of aliphatic O-H and polysacchrides	1050
775-870	Out-of-plane bending of aromatic C-H	870

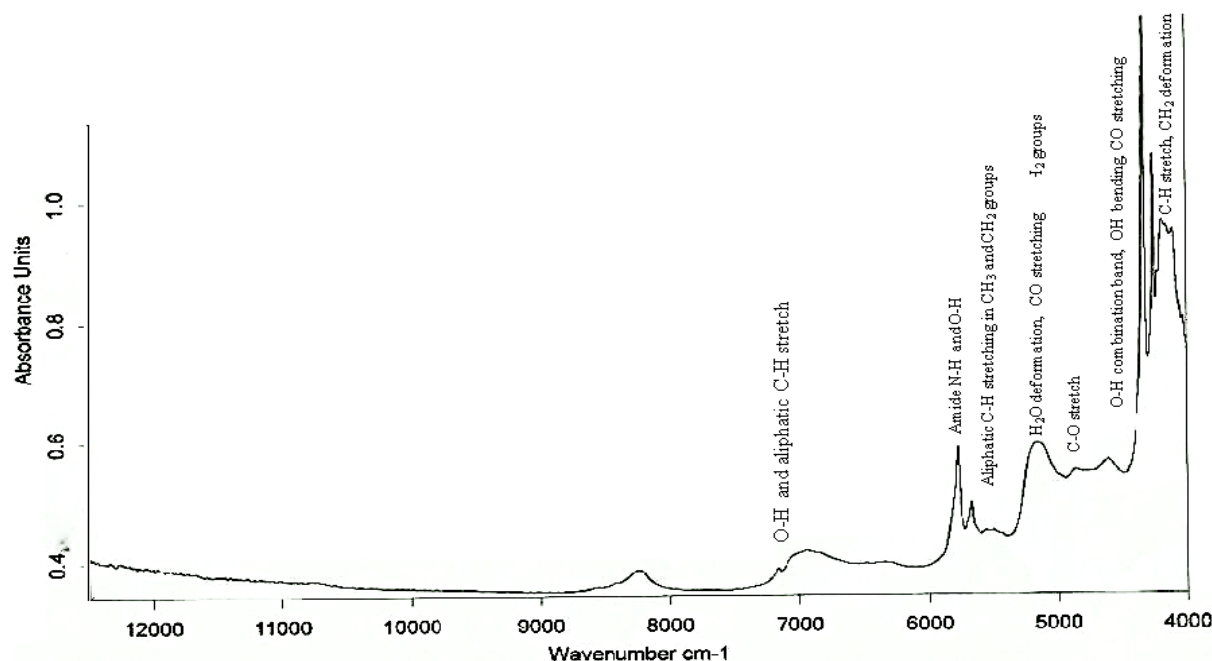


Figure 3. NIR spectrum of soil fulvic acid with assignments

NIR analysis

NIR is considered reflection part of organic compounds. NIR spectroscopy (4000-12000 cm^{-1}) is successful analytical technique for investigation of agriculture product.

In Fig. 3 NIR spectra of FA labeled with characteristics group peaks are presented. The peaks at 4000 cm^{-1} are attributed to C-H stretching and CH_2 deformation while the region between 4025-4295 cm^{-1} indicates O-H combination band, OH bending, CO stretching. The peak at 4770 cm^{-1} is due to CO stretching. The bend appeared at 5198 cm^{-1} is due to H_2O deformation, CO stretching. Peak at 5856 cm^{-1} indicates aliphatic C-H stretching in CH_3 and CH_2 groups. The band at 6000 cm^{-1} is due to N-H, whereas a small bend at 7048 cm^{-1} predicts O-H stretching and aliphatic C-H stretching [52,53].

Conclusion

Moisture as well as texture shows good water holding capacity and silt- loam type of soil. pH and EC values indicate the fertile nature of soil

beneficial for plantation. The spectral data (UV-Visible, FT-IR and NIR) supports the characteristic functional groups ($-\text{COOH}$, $\text{C}=\text{O}$, $-\text{OH}$, $-\text{NH}_2$, $\text{C}=\text{C}$, CH_2 and polysaccharides) present in FA. E_4/E_6 values depict its hydrophilic nature, having less aromatic and more aliphatic groups. Concentration of metal ions present in FA $\text{Na} > \text{K} > \text{Ca} > \text{Fe} > \text{Zn}$ indicates its chelating ability.

References

1. P. Ravikumar and R. K. Somashekar. *Proceedings of the Int. Acad. Ecol. Environ. Sci.*, 3 (2013) 330.
2. Mu Li, Yinguang Chen, Yinglong Su, Rui Wan and Xiong Zheng. *RSC Adv.*, 6 (2016) 14993.
3. B. Sannappa and K. G. Manjunath, *Scholars Acad. J. Biosci.*, 1 (2013) 200.
4. M. A. Malicki and R. T. Walczak, *Eur. J. Soil Sci.*, 50 (1999) 505.
5. A. K. Kiprop, Marie-Camille J-Coumon, E. Pourtier, S. Kimutai and S. Kirui, *Int. J. App. Sci. Tech.*, 3 (2013) 28.
6. D. V. Guimarães, M. I. S. Gonzaga, T. O. da Silva, T. L. da Silva, V. da Silva and M. I. S.

- Matias, *Soil Till Res.*, 126 (2013) 177.
7. S. Yang, Z. Zhang, L. Cong, X. Wang and S. Shi, *J. Soil Sci. Plant Nutr.*, 13 (2013) 526.
 8. S. Şahin, M. R. Karaman and N. Gebologlu, *Sci. Res. Essays*, 9 (2014) 586.
 9. D. H. Goenadi, R. A. Pasaribu, H. H. Isroi, and R. Misman, *Menara Perkebunan*, 67 (1999) 40.
 10. E. A. Ghabbour and G. Davies, *Annals of Environ. Sci.*, 3 (2009) 131.
 11. Ayurpharm, *Int. J. Ayur. Alli. Sci.*, 2 (2013) 327.
 12. J. Bruneton, *Pharmacognosy, Phytochemistry, Medicinal Plants*, 2 (1995) 330.
 13. L. Bravo, *Nutrition Reviews*, 56 (1998) 317.
 14. Shamama Javed, Kanchan Kohli and Waquar Ahsan. *Int. J. Drug. Dev. Res.*, 8 (2016) 08.
 15. F. J. Stevenson, *Humus Chemistry, Genesis, Composition, Reactions*, John Wiley and Sons (1994).
 16. R. B. Grisso, M. Alley, W. G. Wysor, D. Holshouser and W. Thomason, *Precision Farming Tools: Soil Electrical Conductivity, Virginia Cooperation Extension publication*, (2009) 442.
 17. M. Pansu and Gautheyrou, *Handbook of Soil Analysis: Mineralogical, Organic and Inorganic Methods* (2006) 209.
 18. W. C. Dahnke and D. A. Whitney, *Measurement of Soil Salinity*, In W. C. Dahnke Ed, (1988) 32.
 19. A. E. Hartemink, M. R. Balks, Z. S. Chen, P. Drohan, D. J. Field, P. Krasilnikov and C. Walter, *Geoderma*, 217 (2014) 1.
 20. Khaled Mohamed Mossa Bushnaf., *Ph.D Thesis*, Environmental Engineering group School of Civil Engineering and Geosciences (2013).
 21. P. R. Chaudhari, D. V. Ahire, V. D. Ahire, M. Chkravarty and S. Maity. *Int. J. Sci. Res. Pub.*, 3 (2013) 1.
 22. M. Schirrmann, R. Gebbers, E. Kramer and J. Seidel. *Sensors*, 11 (2011) 573.
 23. N. C. Brady and R. R. Weil. The nature and properties of soil, 13th ed. *Prentice Hall, Upper Saddle River, NJ* (2007).
 24. D. L. Corwin and S. M. Lesch, *Agronomy J.*, 95 (2003) 455.
 25. J. P. Molin and G. D. C. Faulin, *Scientia Agricola*, 70 (2013) 1.
 26. M. Schnitzer and S. U. Khan, *Humic Substances in the environment*, (1972).
 27. J. O. Nriagu and R. D. Coker, *J. Am. Chem. Soc.*, 14 (1980) 443
 28. N. Fausurova and L. Pospisilova, *Soil and Water Research-UZEI* (2011).
 29. N. C. Brady and R. R. Weil, *Nitrogen and Sulfur Economy of Soils*, (2002) 524.
 30. M. Welch and R. D. Graham, *Plant and Soil*, (2002) 205.
 31. J. Alloway, *Micronutrients and crop production: An introduction. In. Micronutrient deficiencies in global crop production. Springer, Netherlands* (2008) 1.
 32. S. A. Shahid, A. A. Qidwai, F. Anwar, I. Ullah and U. Rashid, *Molecules*, 17 (2012) 12587.
 33. F. J. Stevenson, *Soil Science Society of America: Madison, WI. Second Ed.* (1991) 145.
 34. N. Senesi, *Special Publications of the Royal Society of Chemistry.*, 135 (1993) 73.
 35. K. Hussein, and Hassan A. Faway, *Soil and Water Res.*, 6 (2011) 21.
 36. R. George and S. Micheal, *Potassium for crop production, University of Minnesota/Extension. Germany*, 2 (1997) 1063.
 37. S. E. Calvert, S. Mukherjee and R. J. Morris, *Oceanologica Acta*, 8 (1985) 167.
 38. M. K. Bennett, J. Garcia-Arraras, I. A. Elferink, K. Peterson, A. M. Fleming, C. D. Hazuka and R. H. Scheller, *Cell*, 74 (1993) 863.
 39. B. S. Rauthan and M. Schnitzer, *Plant and Soil*, 63 (1981) 491.
 40. T. Kösesakal, M. Ünal and G. C. Öz Fresenius, *Env. Bulletin*, 18 (2009) 2402.
 41. F. M. Chaudhry and A. Wallace, *Plant and Soil*, 45 (1976) 697.
 42. T. Kim, H. A. Mills and H. Y. Wetzstein, *J. Plant Nutr.*, 25 (2002) 1987.
 43. L. Borůvka and O. Drabek, *Plant. Soil. Env.*, 50 (2004) 339.
 44. A. Naidja, P. M. Huang, D. W. Anderson and C. Van Kessel, *App. Spectroscopy*, 56 (2002) 318.
 45. T. J. F. Cunha, E. H. Novotny, B. E. Madari,, L. Martin- Neto, M O de O

- Rezende, L. P. Canelas, and V. de M. Benites, Spectroscopy characterization of humic acids isolated from Amazonian Dark Earth Soils (terra preta de Índio). Amazonian Dark Earths: Wim Sombroek's Vision. Springer Sciences + Business Media B.V. (2009) 363.
46. Renuka, J. V. Shukla, P. K. Prajapati, *Ayurpharm Int. J. Ayur. Alli Sci.*, 2 (2013) 327.
47. M. Srilatha, P. C. Rao, S. H. K. Sharma and G. Padmaja, *Int. J. Adv. Res.*, 1 (2013) 343.
48. G. F Huang, Q. T. Wu, J. W. C. Wong and B. B. Nagar, *Bioresour Technol.*, 97 (2006) 1834.
49. O. Francioso, E. Ferrari, M. Saladini, D. Montecchio, P. Gioacchini and C. Ciavatta. *J. Haz. Mat.*, 149 (2007) 408.
50. K. H. Tan, *Environmental Soil Science*, 2nd ed. Marcel Dekker, New York (2000).
51. H. Li, Y. Li and C. Li, *Asian J. Chem.*, 25 (2013) 10087.
52. A. Vergnoux, N. Dupuy, M. Guiliano, M. Vennetier, F. Théraulaz, P. Doumenq. *Talanta*, 80 (2009) 39.
53. M. Chodak. *Pol. J. Environ. Stud.*, 17 (2008) 631.