

Current Journal of Applied Science and Technology



39(37): 12-22, 2020; Article no.CJAST.63371 ISSN: 2457-1024 (Past name: British Journal of Applied Science & Technology, Past ISSN: 2231-0843, NLM ID: 101664541)

FTIR Spectroscopy and Optical Density Characterization of Humic Substances Extracted from Reclaimed Alkali Soils under Different Tillage and Management Practices

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Authors' contributions

This work was carried out in collaboration among all authors. Author SNS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors NA, SCD, KMM, TKD and RK managed the analyses of the study. Authors V. Kumar, V. Kumari and RL managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/CJAST/2020/v39i3731083 <u>Editor(s):</u> (1) Dr. Orlando Manuel da Costa Gomes, Lisbon Accounting and Business School (ISCAL), Lisbon Polytechnic Institute, Portugal. <u>Reviewers:</u> (1) Caba Ioan Ladislau, The National Institute of Research –INMA and Politehnica University Timisoara, Romania. (2) António Benjamim Mapossa, University of Pretoria, South Africa. Complete Peer review History: <u>http://www.sdiarticle4.com/review-history/63371</u>

> Received 10 September 2020 Accepted 12 November 2020 Published 08 December 2020

Original Research Article

ABSTRACT

In the present investigation, different tillage and management practices interventions were assessed to note the changes in the composition of humic substances using Fourier transform infrared (FTIR) techniques, optical density (E_4/E_6) measurement and elemental composition of humic acid. One uncultivated and four reclaimed alkali surface (0-0.15 m) soil samples after adoption of four years different tillage and management practices were collected from Central Soil Salinity Research

Institute, Karnal. Optical density (E_4/E_6) of fulvic acid was higher than that of humic acid. This was due to the more condensed and more matured nature of humic acid than fulvic acid. The trend of E_4/E_6 ratio was farmers practices in rice-fallow-wheat cropping system with puddled rice and conventional tillage wheat without crop residue addition (FP in R-F-W) > integrated crop and resource management in rice-wheat-mungbean cropping system (ICRM in R-W-M)> futuristic and diversified (CA) based system of maize-wheat-mungbean cropping system (FDCA in M-W-M) >U_nK> Conservation agriculture (CA) based system in rice-wheat-mungbean cropping system (CA in R-W-M). The highest stability was recorded in CA in R-W-M and the lowest in FP in R-F-W. Elemental composition of extracted humic acid revealed that carbon content in humic acid varied from 47.53% in uncultivated soils to 51.32% in ICRM in R-W-M. The nitrogen content in humic acid varied from 4.38% in FDCA in M-W-M to 4.89% in CA in R-W-M. Soils having the lowest C/N value was observed 10.03 in CA in R-W-M and the highest C/N ratio of value 11.72 was recorded in FDCA in M-W-M. Wave no. (cm⁻¹) & proposed functional group of extracted humic acid revealed that FTIR peak at 1507-1508 cm⁻¹ in CA in R-W-M, aromatic -C=C- peak was absent in FDCA in M-W-M and in FP in R-F-W it was replaced by aliphatic C-H stretch.

Keywords: Fulvic acid; humic acid; FTIR; E_4/E_6 ; humus stability.

ABBREVIATION

Has HA FA E ₄ /E ₆	 Humic Substances Humic Acids Fulvic Acids The ratio of Absorbances at 465 and 665 nm
C/N CA FP in R-F-W	 Carbon and Nitrogen Ratio Conservation Agriculture Farmers practices in Rice- Fallow-Wheat Cropping System with Puddled Rice and Conventional Tillage Wheat without Crop Residue adDition
ICRM in R-W-M	: Integrated Crop and Resource Management in Rice-Wheat-Mungbean Cropping System
CA in R-W-M	: Conservation agriculture (CA) Based Rice-Wheat- Mungbean Cropping system
FDCA in M-W-M	: Futuristic and Diversified (CA) Based Maize-Wheat- Mungbean Cropping System

1. INTRODUCTION

Humus and humic substances are very important soil constituents. Depending on climatic conditions and cultural practices, the humus content of soil often stabilizes at fairly definite amount depending on the chemical composition of the SOM are also very important [1-2]. The complex forming capacities of humic substances with ions depend on its molecular dimension and presence of functional groups. The optical density, E4:E6 ratio and coagulation thresholds of humic substances indicated more maturity due to intense humification process than that of humic substances [3]. In southern region of the United States with a prevailing subtropical climate, soil humus content seldom exceeds 3.5% and the carbon /nitrogen (C/N) ratio usually narrows down in a humification process from a value in excess of 20 for fresh material to 8 to 20 for humus [4]. The fate of natural organic matter and especially humic substances (HSs) has attracted increasing interests of scientists representing various disciplines over recent decades. Humic substances are a relatively stable fraction of soil organic matter pool and are able to partly resist microbial decomposition and hence they help to maintain the Soil organic matter level [5]. These substances are dark colored. acidic. predominantly aromatic. hvdrophilic. chemically complex and polyelectrolyte like materials that range in molecular weights from a few hundreds to several thousands [6]. The reactivity of humic substances is attributed to the high content of oxygen containing functional group -COOH, phenolic-OH, enolic-OH, alcoholic-OH, C=O, auinones, hydroxyl auinines etc. The content of oxygen containing functional group seem to be higher in fulvic acids than those in humic acids [7]. The acting mechanisms by which humic substances improve the soil physical, chemical and biological properties can be attributed to their main component-humic acids [8-9]. Humic acid data on humification is important to assess the rate and magnitude of soil carbon sequestration. Tivet et al. [10] observed selective preservation by aromaticity of SOM is the predominant mechanism in that environment and land use changes modified the arrangement of organic compounds necessitating the diversification of agro-ecosystems and conversion to NT farming with Fourier-transform infrared (FTIR) spectroscopy on whole soil samples to 1-m depth and three functional groups were selected based on FTIR: aliphatic- C_1 (1404 cm⁻¹), aromatic-C (1632 cm⁻¹), and aliphatic- C_2 (2852 and 2922 cm⁻¹).

Fourier-transform infrared (FTIR) spectroscopy has been widely used for the characterization of components into micro molecular and humic substances from 1980 onwards. The FTIR method adopted to characterize humic substances was described by Francioso et al. [11]. The FTIR spectroscopy worked as fast, efficient and precise techniques for analyzing the degree of SOC humification, functional C groups and hence the efficiency of NT cropping systems in promoting long-term carbon sequestration in soils was reported by Tivet et al. [10] Humic acids were divided into two groups according to the aromatic and aliphatic compounds in their molecules. The highest aromatic degree and stability was found in lignite HA and Haplic Chernozem HA. Humic acids isolated from grassland and hydric soils contained more aliphatic and newly formed compounds [12] Effect of five years application of different integrated nutrient management (INM) interventions was assessed to note the changes in the fulvic acid and humic acid composition fourier transform infrared (FTIR) usina techniques indicated that application of 8 t FYM ha⁻¹ in soybean and 16 t FYM ha⁻¹ in wheat in soil helped in the formation of long chain humic acid which can sequester more organic carbon and subsequently improve soil quality and health [13]. Long-term field trial under no-tillage for 30 years and tillage system showed that the soil organic matter in notillage system decreased slowly whereas in the tilled system it fell steeply to a relatively low level. The total C and C of humic substances and hot water soluble C are comparable in both the systems up to 0.3 m but they are lower in tilled system from 0.3 to 0.5 m. [14] Conservation agriculture plays a vital role in carbon sequestering carbon in soil-plant svstem through change in management cropping practices. use of improved systems, less disturbance of soil and hence less disruption of carbon rich soil aggregates and retention of crop residues in soil [15-16]. The more alkyl-aromatics and N-compounds, resistant to chemical oxidation were associated

with the A_1 and B_w fine clay fraction, whereas alkyl-aromatics as well as carbohydrate, phenols, lignin, lipids and N-compounds were found in C-horizon sample [17]. The Nheterocycles that were identified in many soils are mostly of pyrogenic origin. Other potential sources for N-heterocycles are the purines bases in nucleic acids, and secondary metabolites for various purposes [18]. The synthesized alleged inherent stability of natural Nheterocycles (eg. Purine bases etc.) against decomposition has never been conclusively demonstrated while several sources report on the biodegradability of non-hydrolyzable N. Pyrogenic heterocyclic N within charred organic matter will experience slow turnover due to specific properties of the black carbon (BC).

The fact that N-heterocycles tend to be enriched in smaller size fraction of soils where they appear to be rather resilient over time suggests that their preservation in soil environment is likely a matter of protection in mineral-organic interaction [19]. The literature available shows most of the studies on carbon sequestration but the area on soil-carbon bonding has been less explored. So, there is a need to study the strength with which C is held along with soil so as to understand its stability as well as retention. Against this backdrop, an attempt was made in the present investigation with the objective to characterize the humic substances especially humic acid in reclaimed alkali soil under effect of different tillage and management practices on stability of clay-humus in different soils by elucidating the typical absorption bands FTIR using spectroscopy.

2. MATERIALS AND METHODS

The soil samples varying in depth of 0-15, 15-30, 30-45 and 45-60 cm were collected from all three replications of four different treatments and uncultivated sites of conservation agriculture field of Cereal System Initiative for South Asia (CSISA) project, CSSRI, Karnal, India (29°70'N latitude 76° 96'E longitude). The experimental soil is a reclaimed alkali loam with alkaline pH, average semi-arid climate with annual rainfall of 700 mm and minimum temperature of 0-4°C in January, maximum temperature of 41-44°C in June and relative humidity of 50-90 % throughout the year. The design of the experimental field is randomized completely block design (RCBD).

2.1 Experimental	Details	and
Management		

Four cropping system treatments (scenarios) varying in crop sequence, tillage, establishment

method, residue management and other management practices were replicated thrice in a randomized complete block design. Plot size was 2000 m^2 (20 m x100 m). The details of treatments are briefly summarised in Table 1.

Table 1. Summary of the different tillage and management practices in reclaimed alkali soils at CSSRI, Karnal

	FP in R-F-W	ICRM in R-W-M	CA in R-W-M	FDCA in M-W-M
	Farmer`s practice	Integrated crop and resource management	Conservation agriculture based system	Futuristic and diversified system based on principles of CA
Drivers of change	None	The need to increase cereal production and farmers income	The need to increase cereal production and farmers income in the face of increasing scarcity of water, labour and energy and soil degradation	The need to diversify and increase cereal production and farmers income in the face of increasing scarcity of labour and energy and even greater water scarcity and food demand
Approach	Current farmers practices	Intensification and best management practices	Intensification and best management practices including zero tillage and residue retention	Intensification and best management practices including zero tillage and residue retention and diversification by replacing rice with maize
Crop rotation	Rice-wheat	Rice-wheat- mungbean	Rice-wheat- mungbean	Maize-wheat- mungbean
Tillage	Conventional tillage Rice: Puddling Wheat: conventional tillage	Conventional /zero tillage Rice: Puddling Wheat: zero till Mungbean: zero tillage	Zero tillage Rice: zero tillage Wheat: zero tillage Mungbean: zero tillage	Zero tillage Maize: zero tillage Wheat: zero tillage Mungbean: zero tillage
Crop establishment method	Rice: transplanting Wheat : broad casting	Rice: transplanting Wheat :drill seeding Mungbean: drill /relay broadcasting	Rice: drill seeding Wheat :drill seeding Mungbean: drill /relay broadcasting	Maize: drill seeding Wheat :drill seeding Mungbean: drill /relay broadcasting
Crop residue management	All residues removed	Wheat : partially (anchored) incorporated Mungbean: fully incorporated during puddling of rice Rice: partially(anchored) incorporated	Full (100%) rice and mungbean ; partial (anchored) wheat residues retained on the soil surface	Full (100%) maize and mungbean ; partial (anchored) wheat residues retained on the soil surface

Treatment	% C	% N	C/N
FP in R-F-W	50.42	4.63	10.89
ICRM in R-W-M	51.32	4.68	10.97
CA in R-W-M	49.04	4.89	10.03
FDCA in M-W-M	51.30	4.38	11.72
US	47.53	4.64	10.24

 Table 2. Elemental composition of humic acid extracted from reclaimed alkali surface soils (0-15 cm) after four of conservation agriculture at CSSRI, Karnal

FP in R-F-W= Farmers practices in rice-fallow-wheat cropping system with puddled rice and conventional tillage wheat without crop residue addition

ICRM in R-W-M= integrated crop and resource management in rice-wheat-mungbean cropping system CA in R-W-M= Conservation agriculture (CA) based rice-wheat-mungbean cropping system FDCA in M-W-M=futuristic and diversified (CA) based maize-wheat-mungbean cropping system US=Uncultivated soil

Table 3. E_4/E_6 of fulvic and humic acid extracted from reclaimed alkali surface soils (0-15 cm) after four of Conservation Agriculture at CSSRI, Karnal

Treatment	E ₄ /E ₆ of fulvic acid	E₄/E ₆ of humic acid	
FP in R-F-W	6.55	4.35	
ICRM in R-W-M	7.14	4.23	
CA in R-W-M	6.54	3.56	
FDCA in M-W-M	6.79	4.11	
US	7.82	3.74	

FP in R-F-W= Farmers practices in rice-fallow-wheat cropping system with puddled rice and conventional tillage wheat without crop residue addition

ICRM in R-W-M= integrated crop and resource management in rice-wheat-mungbean cropping system CA in R-W-M= Conservation agriculture (CA) based rice-wheat-mungbean cropping system FDCA in M-W-M=futuristic and diversified (CA) based maize-wheat-mungbean cropping system US=Uncultivated soils

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2.2 Extraction and Characterization Humic Acid

Humic acid was extracted by the procedure of International Humic Substance Society. The soil samples were first equilibrated to a pH value of 1-2 with 1M HCl at room temperature and adjusted solution volume with 0.1M HCl to provide a final concentration that had ratio of 10:1 :: Liquid: dry sample. The suspension was shaken for 1 hour and the supernatant was separated from the residue by decantation after allowing solution to settle (by low speed centrifugation). Neutralized the soil residue with 1M NaOH to pH= 7.0 and then added 0.1N NaOH under an atmosphere of nitrogen to give a final extractant to soil ratio of 10:1. Extracted the suspension under nitrogen atmosphere with intermittent shaking for a minimum of 4 hours and allowed the alkaline suspension to settle overnight and collected the supernatant by means of centrifugation. The supernatant was acidified with 6M HCl with constant stirring to pH =1.0 and then allowed the suspension to stand for 12-16

hours. The humic acid (precipitate) and fulvic acid(supernatant) fractions were separated by centrifugation. The extracted humic acids were purified for removing the impurities from it. This was done by first dissolving the humic acid fraction by adding a minimum volume of 0.1M KOH under nitrogen atmosphere. The solution was treated with solid KCI to attain 0.3 M (K^+) and then centrifuged at high speed to remove suspended solids. Acidification of the supernatant was carried out with 6M HCI by continuous stirring to pH 1.0 till the precipitation of the humic acid. The supernatant separated from the humic was acid precipitate by centrifugation. The humic acid precipitate was suspended in 0.1M HCI/ 0.3M HF solution in a plastic container and was shaken overnight at room temperature. Centrifugation and HCI/ HF treatment was repeated, if necessary, until the ash content was below 1 percent. The precipitate was transferred to a visking dialysis water, which gave a negative chloride test with the AgNO₃. The extract of humic acid stored in a freezer at -20°C before drying in a lyophiliser.

2.3 Elemental Composition of Humic Acid

The C and N contents of humic acid was done by dry combustion method in a CHNS analyser (EuroVector make, Euro EA3000 model) N, C:N ratio of humic acid was also computed.

2.4 IR of Humic Acid

The identification of functional groups in humic acid was done by FTIR spectroscopy (Bruker, model Alpha) with the help of Opus Wizard software (Cheng et al., 2006). The tablet of humic acid was prepared by mixing 5 mg (0.1mm sieved) with 1 g analytical grade KBr powder by applying pressure in a hydraulic press. The spectra were recorded from 4400 to 400 cm^{-1} by averaging 200 scans at 2cm^{-1} resolution.

2.5 Optical Densities of Humic Acid Solution

Optical densities of HAs solution (3 mg of HA in 10 mL of 0.05 N NaHCO₃) were measured at 465 (E_{465}) and 665(E_{665}) wavelength and these values were used for the calculation of E_4/E_6 coefficient value [20].

Table 4. Wave no. (cm ⁻¹) & proposed functional gr	roup of humic acid extracted from reclaimed
alkali surface soils (0-15 cm) after four of cor	nservation agriculture at CSSRI, Karnal

Treatment	Wave no. (cm ⁻¹) & Proposed functional group
FP in R-F-W	2906- Aliphatic C-H stretch
	1698- COOH vibrations
	1647- C=O stretch(amide I)
	1558- N-H deformation and C=N stretch(amide II)
	1541-COO- anti-symmetric stretch
	1507- Aromatic C=C
	1457- CC-H₃
	1217- C-O ester linkages and phenolic C-OH
	1125-C-C, C-OH, C-O-C typical of glucosidic linkages, polymeric substances
	and Si-O impurities in humic compounds
	1029- OCH3 vibrations
ICRM in R-W-M	2919-C-H stretch of CH ₃
	1615- C=C and COO-
	1540- N-H deformation and C=N stretch(amide II)
	1228- C-O ester linkages and phenolic C-OH
	1031 -OCH3 vibrations
CA in R-W-M	3116- NH₃ stretch
	1698- COOH vibrations
	1541- N-H deformation and C=N stretch(amide II)
	1396- COO- anti-symmetric stretch
	1220- C-O ester linkages and phenolic C-OH
FDCA in M-W-M	1716- C=O stretch of carbonyl groups
	1698- COOH vibrations
	1615-C=C and COO-
	1558- N-H deformation and C=N stretch(amide II)
	1456- CC-H ₃
	1224- C-O ester linkages and phenolic C-OH
	1032- OCH3 vibrations
US	1715- C=O stretch of carbonyl groups
	1615- C=C and COO-
	1233- C-O ester linkages and phenolic C-OH
	1035- OCH3 vibrations
FP in R-F-W= Farme	ers practices in rice-fallow-wheat cropping system with puddled rice and conventional tillage
	wheat without crop residue addition
	= integrated crop and resource management in rice-wheat-mungbean cropping system

CA in R-W-M= Conservation agriculture (CA) based rice-wheat-mungbean cropping system FDCA in M-W-M=futuristic and diversified (CA) based maize-wheat-mungbean cropping system US=Uncultivated soils

3. RESULTS AND DISCUSSION

 E_4/E_6 of fulvic acid was higher than that of humic acid. This was due to the more condensed and more matured nature of humic acid than fulvic acid. Similar result was reported by Vennila, [21]. The trend of E_4/E_6 ratio was farmers practices in rice-fallow-wheat cropping system with puddled rice and conventional tillage wheat without crop residue addition (FP in R-F-W) > integrated crop and resource management of rice-wheatmungbean (ICRM in R-W-M)> futuristic and diversified (CA) based system of maize-wheatmungbean (FDCA in M-W-M) >US> Conservation agriculture (CA) based system of rice-wheat-mungbean (CA in R-W-M). The highest stability was recorded in CA in R-W-M and the lowest in FP in R-F-W. Higher the value E₄/E₆, lower is the humification and lees condensed will be aromatic network. Lower E_4/E_6 ratio indicates small particle size and weight. An inverse relationship has been observed between the E_4/E_6 ratio and mean residence time (stability) of humus, specifically the humic substances with the lowest E_4/E_6 value had the highest mean residence time (stability) indicating the more humified and highly condensed aromatic network. Elemental composition of extracted humic acid revealed that carbon content in humic acid varied from 47.53% in uncultivated soils (US) to 51.32% in ICRM in R-

W-M. The nitrogen content in humic acid varied from 4.38% in FDCA in M-W-M to 4.89% in CA in R-W-M. Soils having the lowest C/N value was observed 10.03 in CA in R-W-M and the highest C/N ratio of value 11.72 was recorded in FDCA in M-W-M. The elemental analysis of all HA studied was in the same range as that obtained by other authors [22-23]. The fourier transform infrared (FTIR) spectra of humic acid (HA) extracted from reclaimed alkali soils under different tillage and management practices are shown in Figs. 1-5. The main absorption bands were: 2906 cm⁻¹- Aliphatic C-H stretch.1698 cm⁻¹ ¹- COOH vibrations, 1647 cm⁻¹- C=O stretch(amide I),1558 cm⁻¹- N-H deformation and C=N stretch(amide II), 1541 cm⁻¹-COO- antisymmetric stretch, 1507 cm⁻¹- Aromatic C=C, 1457 cm⁻¹ - CC-H₃ 1217 cm⁻¹ - C-O ester linkages and phenolic C-OH, 1125 cm⁻¹ -C-C, C-OH , C-O-C typical of glucosidic linkages, polymeric substances and Si-O impurities in humic compounds, 1029 cm⁻¹- OCH3 vibrations under Farmers practices in rice-fallow-wheat cropping system with puddled rice and conventional tillage wheat without crop residue addition (FP in R-F-W). Under integrated crop and resource management of rice-wheatmungbean (ICRM in R-W-M) absorption bands were: 2919 cm⁻¹-C-H stretch of CH₃ 1615 cm⁻¹-C=C and COO-, 1540 cm⁻¹- N-H deformation and C=N stretch(amide II), 1228 cm⁻¹- C-O ester

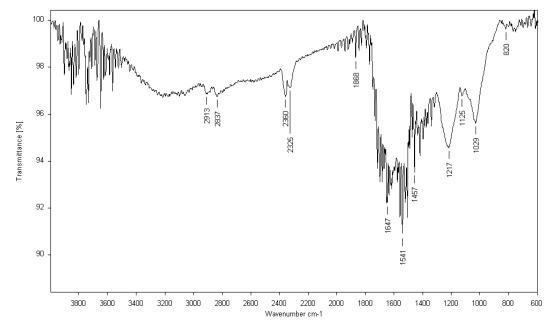


Fig. 1. FTIR spectra of humic acid extracted from reclaimed alkali soils after four years of farmers practices under rice-fallow-wheat cropping system

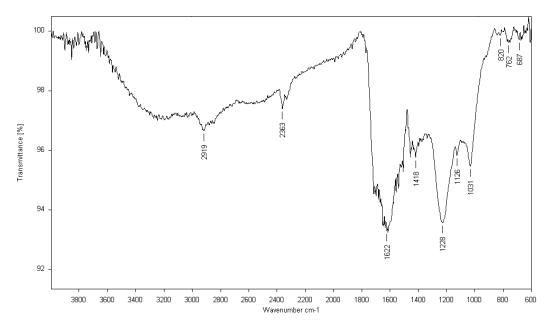


Fig. 2. FTIR spectra of humic acid extracted from reclaimed alkali soils after four years of integrated crop and resource management in rice-wheat-mungbean cropping system

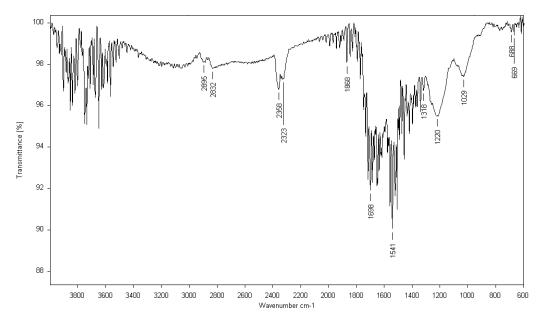


Fig. 3. FTIR spectra of humic acid extracted from reclaimed alkali soils after four years of Conservation agriculture (CA) based rice-wheat-mungbean cropping system

linkages and phenolic C-OH, 1031 cm⁻¹ -OCH₃ vibrations. Under Conservation agriculture (CA) based system of rice-wheat-mungbean (CA in R-W-M) absorption bands were: 3116 cm⁻¹- NH₃ stretch, 1698 cm⁻¹- COOH vibrations, 1541 cm⁻¹- N-H deformation and C=N stretch(amide II), 1396 cm⁻¹- COO- anti-symmetric stretch, 1220 cm⁻¹- C-O ester linkages and phenolic C-OH.

Under futuristic and diversified (CA) based system of maize-wheat-mungbean (FDCA in M-W-M) absorption bands were:1716 cm⁻¹- C=O stretch of carbonyl groups, 1698 cm⁻¹- COOH vibrations, 1615 cm⁻¹-C=C and COO-, 1558 cm⁻¹-N-H deformation and C=N stretch(amide II),1456 cm⁻¹- CC-H₃,1224 cm⁻¹- C-O ester linkages and phenolic C-OH,1032 cm⁻¹- OCH₃ vibrations. Under uncultivated soils absorption bands were: 1715 cm⁻¹- C=O stretch of carbonyl groups, 1615 cm⁻¹- C=C and COO-, 1233 cm⁻¹-C-O ester linkages and phenolic C-OH, 1035 cm⁻¹ - OCH₃ vibrations. Thus, Wave no. (cm⁻¹) & proposed functional group of extracted humic acid revealed that FTIR peak at 1507-1508 cm⁻¹ in CA in R-W-M, aromatic -C=C- peak was absent in FDCA in M-W-M and in FP in R-F-W it was replaced by aliphatic C-H stretch. The presence of aromatic nature may be due to high humification and more maturity of humus and aliphatic nature may be due to less humification and less maturity of humus. Interpretations of IR spectra are based on the descriptions by authors [24-26].

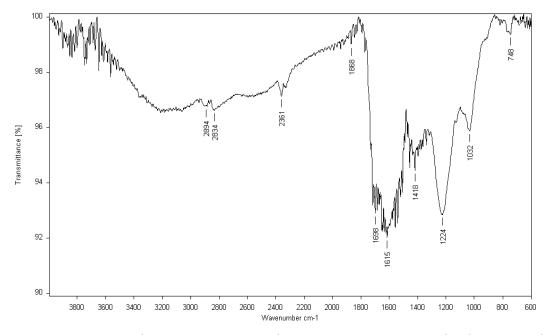


Fig. 4. FTIR spectra of humic acid extracted from reclaimed alkali soils after four years of futuristic and diversified (CA) based maize-wheat-mungbean cropping system

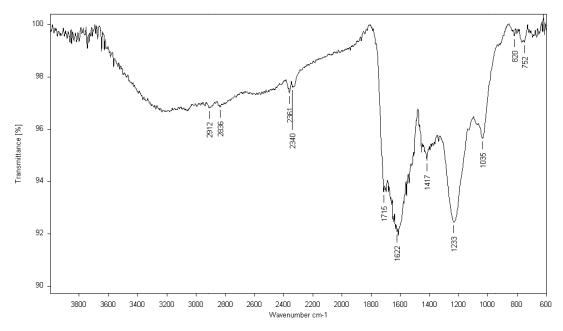


Fig. 5. FTIR spectra of humic acid extracted from reclaimed uncultivated alkali soils

4. CONCLUSION

 E_4/E_6 of fulvic acid was higher than that of humic acid. The trend of E_4/E_6 ratio was farmers practices in rice-fallow-wheat cropping system with puddled rice and conventional tillage wheat without crop residue addition (FP in R-F-W) > integrated crop and resource management of rice-wheat-mungbean (ICRM in R-W-M)> futuristic and diversified (CA) based system of maize-wheat-mungbean (FDCA in M-W-M) >US> Conservation agriculture (CA) based system of rice-wheat-mungbean (CA in R-W-M). The highest stability was recorded in CA in R-W-M and the lowest in FP in R-F-W. Elemental composition of extracted humic acid revealed that carbon content in humic acid varied from 47.53% in uncultivated soils to 51.32% in ICRM in R-W-M. The nitrogen content in humic acid varied from 4.38% in FDCA in M-W-M to 4.89% in CA in R-W-M. Soils having the lowest C/N value was observed 10.03 in CA in R-W-M and the highest C/N ratio of value 11.72 was recorded in FDCA in M-W-M.

ACKNOWLEDGEMENT

First author is highly thankful to RPCAU, Pusa (Bihar) and ICAR-IARI, New Delhi for financial assistance and academic facility, respectively during Ph.D. programme at Division of Soil Science and Agricultural Chemistry, PG school, IARI, New Delhi. He is also highly grateful to Dr., Y.S. Saharawat. Ex-Senior Scientist & Ex-Advisorv committee member. for his co-operation in Outline of Research Work (ORW) preparation and Soil sampling from two sites of Cereal System Initiative for South Asia (CSISA) project. Permission from In-charge of Cereal System Initiative for South Asia, CSSRI. Karnal conservation agriculture site for soil sampling is being gratefully acknowledged.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history: The peer review history for this paper can be accessed here: http://www.sdiarticle4.com/review-history/63371