

COMPOSITION AND INFRARED SPECTRA OF HUMIC AND
FULVIC ACIDS IN SOIL ORGANIC MATTER

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ABSTRACT

Organic matter, total carbon and nitrogen contents were determined in the soil samples collected from three different districts of El-Fayoum Governorate. Fe, Mn, Cu and Zn were extracted from the investigated soils by DTPA and $\text{Na}_4\text{P}_2\text{O}_7$ -NaOH mixture.

Also, iron contents associated with humic and fulvic acids were determined. The investigated soils were characterized by low content of the organic matter and hence the total carbon. However, carbon contents and total Fe of humic acids predominated over fulvic acid in the samples under investigation.

Furthermore, fulvic acid had infrared characteristics rather similar to humic acid except for the following differences: a) Absorption band at 2850 cm^{-1} disappeared in humic acid. b) Strong absorption band at 1400 cm^{-1} was noticed for fulvic acid, while humic acid showed a weak band at the same region.

INTRODUCTION

For the achievement of evaluation of nutrients status in soil, it is necessary to determine the available amount of soil organic matter, and total Fe content of humic and fulvic acids.

The standard method of Kononva (1966) stated that the use of $\text{Na}_4\text{P}_2\text{O}_7$ -NaOH mixture at PH 13 could be considered as a rapid method for the maximum extraction of humus substances from the soil. Also, in testing soils for available Fe, Mn, Cu and Zn, several extracts are commonly used. The DTPA (diethylene triamine penta acetic acid) soil test method developed by Lindsay and Norvell (1978) proved to be one of the best in predicting Fe, Mn, Cu and Zn availability.

Although it is not possible to give any structural formula for the humic and fulvic acids, it is possible to identify the functional groups they contain, Tan and McGreery (1970).

Stevenson and Goh (1971) stated that humic and fulvic acids have somewhat similar spectra in the IR absorption, but the main difference being in the band at 1720 cm^{-1} . It is considerably stronger in fulvic acid than in humic acid due to the occurrence of more carboxylic groups in the former. Moreover, Schnitzer and Ghosh (1982) confirmed the major functional groups of fulvic and humic acids which involved with internal complexing were -COOH and phenolic OH groups.

Amer (1986) revealed that humic substances showed no minimum or maximum absorption at ultraviolet regions.

The aim of this investigation is to provide information about some micronutrients status as related to organic matter and the chemical characteristics of humic and fulvic acids as the main constituents of the soil organic matter extracted from two different layers to represent the alluvial soils in Fayoum Governorate.

MATERIAL AND METHODS

(1) Material:

Composite surface (0-20 cm) and sub-surface (20-50cm) soil samples were collected from three different districts (Abshway, Sunnoris and Tameya) to represent the alluvial soils in Fayoum Governorate.

The physical and chemical properties of the soil samples under investigation are recorded by Abdellah (1991).

(2) Methods of analysis:

- Organic matter contents: was determined according to the method described by Jackson (1967).
- Total organic carbon: The soil total carbon was determined using the method recommended by Kononova (1966)
- Total nitrogen in the samples was determined using Kjeldahl method.

- The available Fe, Mn, Cu and Zn were determined after extraction with DTPA according to Lindsay and Norvell (1978).

(3) Extraction and purification of humic substances:

The extraction of humic substances was carried out using mixture of 0.1 N Na₄ p₂ O₇ -0.1 N NaOH at PH 13 according to the method of Flaig et al (1975). Humic and fulvic acids were fractionated according to Flaig et al (1975). The purification of the isolated fractions was carried out by dialysis in cellophane bags due to the method of Chen et al. (1978).

- a) Humic and fulvic acids carbon contents were determined due to the procedure of kononova (1966).
- b) Iron content of humic and fulvic acids: were determined using 0.1 N Na₄ p₂ O₇-0.1 N NaOH mixture.

(4) Spectrophotometric analysis of humic and fulvic acids:

U.V. spectrum of the samples was determined at wavelength 200-400 nm using a spectronic 21, Baush and Lomb .

I.R. absorption spectra of humic and fulvic acids were determined in KBr pellets using Pye Unicam SP-1100 Spectrophotometer.

RESULTS AND DISCUSSION

Organic matter content, total nitrogen and C/N ratio:

The total carbon and hence the organic matter contents of all investigated soil samples were low, ranging from 0.5 to 1.45% as shown in Table (1). Such low values is mainly due to the arid conditions as well as the relative high temperatures. However, it can be noticed that the total carbon and the organic matter tends to accumulate in relatively higher levels in surface layers due to the relatively higher supplemental of organic residues to the surface soil layers, Stevenson (1982).

The nitrogen content in the samples under investigation, Table (1) showed that a noticeable correlation to a great extent, with soil contents of the organic matter, since the

Table (1): Total carbon (T.C.), organic matter (O.M.), total nitrogen (T.N.) and C/N ratio of the investigated soils.

District	Village	Vegetation	Depth (cm)	T.C. (%)	O.M. (%)	T.N. (%)	C/N
	Aboxa	Orchards	0 - 20	0.73	1.26	0.130	5.60
			20 - 50	0.53	0.91	0.070	7.57
		Crops	0 - 20	0.68	1.18	0.140	4.90
			20 - 50	0.43	0.75	0.086	5.00
Abshway							
	El-Nassaria	Orchards	0 - 20	0.81	1.40	0.120	6.80
			20 - 50	0.30	0.66	0.089	9.20
		Crops	0 - 20	0.77	1.40	0.099	7.70
			20 - 50	0.51	0.62	0.066	9.40
Sunnoris							
	Shakshuk	Crops	0 - 20	0.56	0.95	0.078	6.60
			20 - 50	0.54	0.94	0.064	8.50
	Manshate-	Crops	0 - 20	0.53	0.91	0.064	8.30
		Tantawy	20 - 50	0.52	0.90	0.062	8.40
	Komoshem	Crops	0 - 20	0.84	1.45	0.120	7.00
			20 - 50	0.29	0.50	0.036	8.05
	Kasser-	Crops	0 - 20	0.84	1.45	0.113	7.40
		Rashwan	20 - 50	0.64	1.10	0.079	8.10

organic matter is considered as the main source of nitrogen in soils. This correlation explains why the total nitrogen content tended to decrease with depth.

Data in Table (1) show that the C/N ratios are very narrow and fluctuates between 4.9 and 9.4. Such low values reveals that the decomposition of the organic matter in the soils under study is very rapid. these results are in agreement with that obtained by Amer (1986).

Extractable Fe, Mn, Cu and Zn with DTPA:

The obtained data of the DTPA-extractable (available) Fe, Mn, Cu and Zn are shown in Table (2). Data indicate that Fe content of the samples fluctuated between 6.0 and 9.0 ppm. Mn contents vary from 6.4 to 11.0 ppm. The corresponding values of Cu and Zn ranged from 2.8 to 5.4 ppm and 0.8 to 2.8 ppm respectively. According to Lindsay and Norvell (1978) all samples contain sufficient amounts of available Fe, Mn, Cu and Zn.

Extractable Fe, Mn, Cu and Zn with Na₄ P₂ O₇-NaOH mixture:

Sodium pyrophosphate-Sod. hydroxide mixture was used as an extractant for the active humic substances (humic and fulvic acids) and hence the metal ions associated with them were able to be determined. data in (Table 2) revealed that the soil contents of Fe, Mn, Cu and Zn were fluctuating between (3.5 to 8.5 ppm Fe), (0.5 to 2.5 ppm Mn), (2.0 to 12.5 ppm Cu) and (traces to 0.5 ppm Zn). Generally, in most cases, the studied elements tended to accumulate in the surface layers in relatively high amounts than that in the sub-surface layers. Such results are in accordance with the data obtained by Amer (1986).

Organic carbon and iron contents of humic and fulvic acids:

Data illustrated in Table (3) show that carbon contents of humic acids exceeded than that of fulvic acids in all the investigated samples except the surface layer of Komoshem. It is obvious also that carbon contents of both humic and fulvic acids were relatively higher values in the upper layers than the lower ones. Such data may be attributed to the higher rate of decomposition of soil organic matter as a

Table (2): Extractable Fe, Mn, Cu and Zn with DTPA and $\text{Na}_4\text{P}_2\text{O}_7$ -NaOH mixture from the investigated soils.

District	Village	Vegetation	Depth (cm)	DTPA extractable				$\text{Na}_4\text{P}_2\text{O}_7$ -NaOH mixture extractable				
				Fe	Mn	Cu	Zn	Fe	Mn	Cu	Zn	
				mg/kg soil (ppm)				mg/kg soil (ppm)				
Abshway	Aboxa	Orchards	0 - 20	8.0	11.0	3.6	0.9	5.5	2.0	9.5	0.0	
			20 - 50	8.0	9.5	3.2	0.8	6.5	0.5	9.5	0.0	
	Crops	0 - 20	7.8	8.8	2.8	1.8	7.0	1.5	12.5	0.5		
		20 - 50	7.6	8.4	3.4	1.8	6.0	0.5	12.5	0.0		
Sunnoris	El-Nassaria	Orchards	0 - 20	9.0	10.0	4.2	1.2	8.0	2.5	3.5	0.0	
			20 - 50	9.0	9.8	4.8	1.6	4.5	1.0	2.0	0.5	
		Crops	0 - 20	7.5	7.8	5.4	2.8	5.5	2.5	3.0	0.5	
			20 - 50	7.4	7.6	5.2	2.8	4.5	1.0	3.5	0.5	
	Shakshuk	Crops	0 - 20	8.7	6.4	3.6	2.8	5.0	1.5	11.0	0.5	
			20 - 50	8.5	6.4	3.4	2.3	4.5	1.0	8.0	0.5	
		Manshate-	Crops	0 - 20	9.0	8.0	3.2	1.3	8.5	1.0	5.5	0.0
				20 - 50	7.8	7.8	3.8	1.4	8.5	1.0	4.0	0.0
Tameyu	Komoshem	Crops	0 - 20	6.0	9.2	4.8	1.4	7.5	2.0	2.5	0.0	
			20 - 50	6.0	9.2	4.6	1.6	8.5	0.5	2.5	0.5	
	Kasser-	Crops	0 - 20	8.0	7.2	4.0	1.8	5.5	1.5	2.0	0.0	
			20 - 50	6.5	7.8	5.0	1.4	3.5	1.5	3.5	0.0	

Table (3): Organic carbon and iron contents of extracted humic, fulvic acids and humin.

District	Village	Vegetation	Depth (cm)	Total carbon %	Percent of total carbon			CHA	CHA+CFA	Fe (HA+FA) ppm	Fe (HA) ppm	Fe (FA) ppm
					CHA	CFA	CHum					
Abshway	Orchards		0 - 20	0.73	19.5	17.0	63.5	1.15	0.57	5.5	4.0	1.5
			20 - 50	0.53	18.5	16.5	65.0	1.12	0.69	6.5	4.5	2.0
	Crops		0 - 20	0.68	23.3	17.7	59.0	1.30	0.69	7.0	3.0	4.0
			20 - 50	0.43	18.5	17.2	64.3	1.07	0.58	6.0	4.5	1.5
El-Nassaria	Orchards		0 - 20	0.81	20.8	18.0	61.2	1.15	0.63	8.0	3.5	4.5
			20 - 50	0.36	19.1	17.9	63.0	1.06	0.59	4.5	3.5	1.0
	Crops		0 - 20	0.77	22.1	18.3	59.6	1.21	0.68	5.5	4.0	1.5
			20 - 50	0.51	18.7	17.1	64.2	1.09	0.56	4.5	3.0	1.5
Sunnoris	Shakshuk	Crops	0 - 20	0.52	23.2	16.1	63.7	1.44	0.57	5.0	4.0	1.0
			20 - 50	0.54	19.4	17.2	63.4	1.13	0.58	4.5	4.0	0.5
	Manshate-	Crops	0 - 20	0.53	20.8	18.2	61.0	1.14	0.64	8.5	6.5	2.0
			20 - 50	0.52	19.2	17.8	63.0	1.13	0.59	8.5	6.0	2.5
Tameya	Komoshem	Crops	0 - 20	0.84	17.8	18.1	62.1	1.09	0.61	7.5	4.5	3.0
			20 - 50	0.29	19.3	18.1	62.6	1.07	0.60	8.5	4.0	4.5
	Kasser-	Crops	0 - 20	0.84	20.2	17.4	62.4	1.16	0.60	5.5	2.5	3.0
			20 - 50	0.64	18.6	17.1	64.3	1.09	0.56	3.5	2.0	1.5

CHA : Carbon in humic acid.

CFA : Carbon in fulvic acid.

CHum : Carbon in humin.

result of optimum soil conditions which increase the activity of soil microorganisms in the surface layers. The total carbon contents of humic and fulvic acids ranged from 19.5 to 23.3% and from 16.1 to 18.3% respectively in the surface layers. Such values ranged from 18.5 to 19.4% for the former and from 16.5 to 18.1% for the latter in the lower layers.

Such results could be explained according to Schnitzer (1978) who reported that humic acids are able to form water insoluble complexes with metal ions, whereas fulvic acid form water soluble complexes. Thus, the fulvic acid is exposed to be leaching from the soil surface in relatively higher amounts than humic acid. Consequently the latter tend to accumulate in the surface layers in higher amounts than fulvic acid.

Data in Table (3) show that the greater the amounts of humic acids in humus, the less are humines and vice versa.

Iron contents of humic and fulvic acids:

The total extracted iron associated with humic and fulvic acids were determined and the obtained results are presented in Table (3).

In general, the total Fe contents of the samples under investigation ranged from 3.5 to 8.5 ppm. Also, most samples of humic acid contained higher values of total Fe than that of fulvic acid. These values ranged from 2.0 to 6.5 ppm for the former, and from 0.5 to 4.5 ppm for the latter.

The obtained results are in accordance with those reported by Bohn et al (1985). They proposed that various low molecular weight components of organic matter e.g. fulvic acid form stable complexes (chelates) with Fe and other polyvalent cations. these shield the cations from hydrolysis and precipitation reactions.

Spectroscopic analysis of humic and fulvic acids:

The isolated humic and fulvic acids after fractionation and purification were examined through UV and IR spectroscopic analysis.

Ultraviolet absorption spectra of the samples showing neither maximum nor minimum absorption bands. These data means to the uncharacteristic spectra in the U.V regions. Such results might be attributed to the heterogeneity of molecular constituents in the structure of humic and fulvic acids (Flaig *et al*, 1975).

Infrared spectroscopic analysis was used to identify the functional groups of the extracted humic and fulvic acids. IR spectra of such compounds might be considered as a fingerprint with a few exceptions since the intensities of the absorption bands vary slightly.

Major absorption bands in the IR spectra of humic acids are illustrated in Figs. (1 to 4) were in the following regions : 3300-3520 cm^{-1} (H-bonded OH), 2900 cm^{-1} (aliphatic C-H stretching), 1720 cm^{-1} (C=O of CO_2H and C=O stretch of Carbonyl), 1625 cm^{-1} (Conjugated C=C, H-bonded C=O of carbonyl, COO^-), 1400 cm^{-1} (aliphatic C-H), 1230 cm^{-1} (OH deformation of CO_2H). 1200 cm^{-1} (phenolic OH stretching) and 1100 cm^{-2} (C-O stretching or Si-O of silicate).

The IR spectra of the isolated fulvic acids, Figs. (5 to 8) resembles with those of humic acids except for the following differences : a) the absorption band noticed at 2850 cm^{-1} is assigned to the carboxylate ion. Such band disappeared in the spectra of humic acid, b) strong absorption band was observed at 1400 cm^{-1} reflecting large proportions of aliphatic C-H structure. On the other hand, humic acids spectra showed a weak band at the same region. The obtained data are in agreement with those of Tan and Giddens (1972) and Schnitzer and Ghosh (1982).

Consequently, it could be postulated that the IR absorption spectra of humic and fulvic acids isolated from Fayoum governorate soils supports the interpretation that the structure of such acids are rather similar, at least in the constructure of the internal "nucleus" however, they differ mainly in the ratios of the number of functional groups.

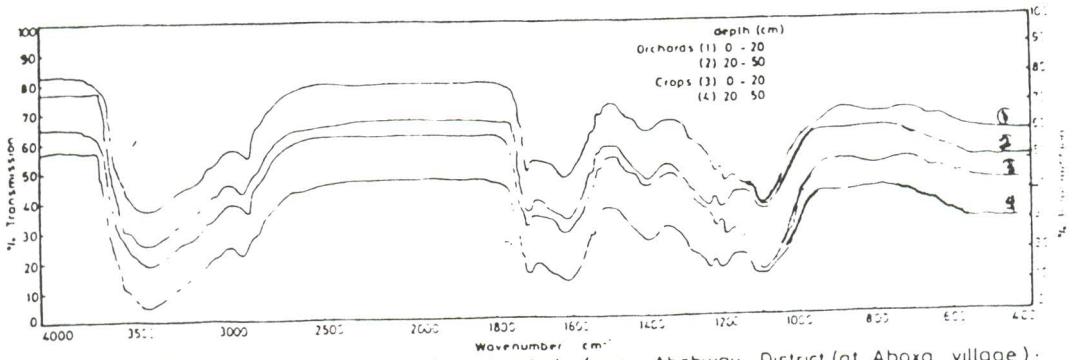


Fig. (1) : IR spectra of humic acids extracted from Abshway District (at Aboxa village).

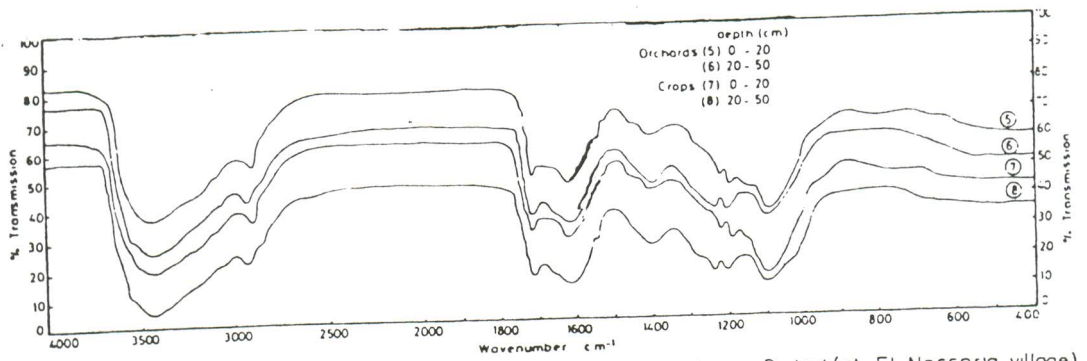


Fig. (2) : IR spectra of humic acids extracted from Abshway District (at El-Nassaria village)

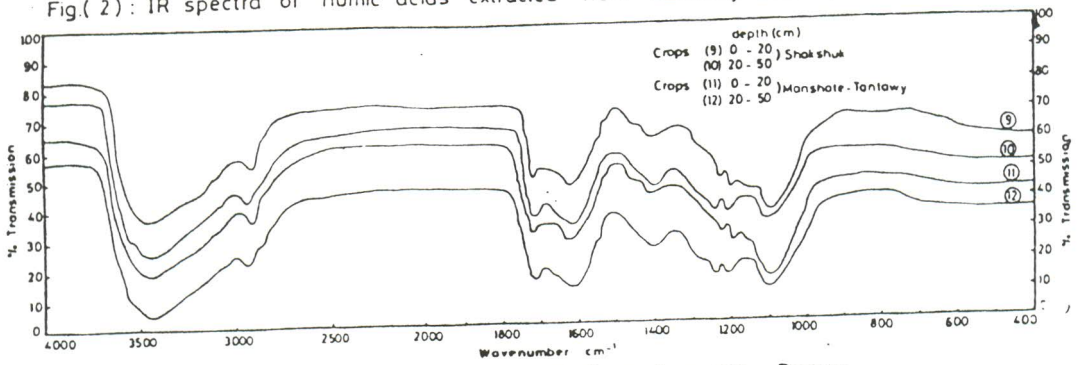


Fig. (3) : IR spectra of humic acids extracted from Sannoris District (at Shakshuk and Manshate- Tantawy villages)

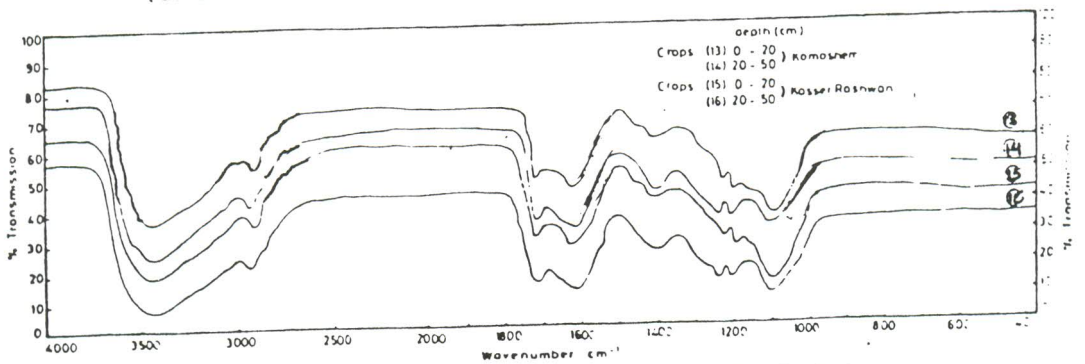


Fig. (4) : IR spectra of humic acids extracted from Tameya District (at Komoshem and Kasser - Rashwan villages)

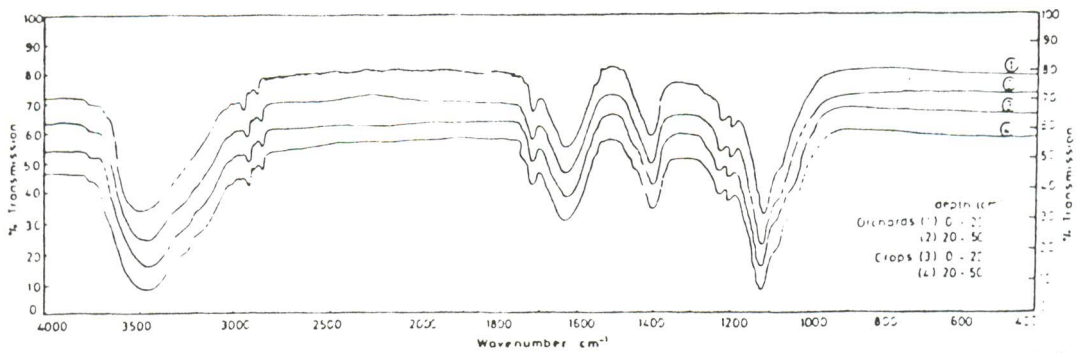


Fig (5) IR spectra of fulvic acids extracted from Abshway District (at Aboxa village)

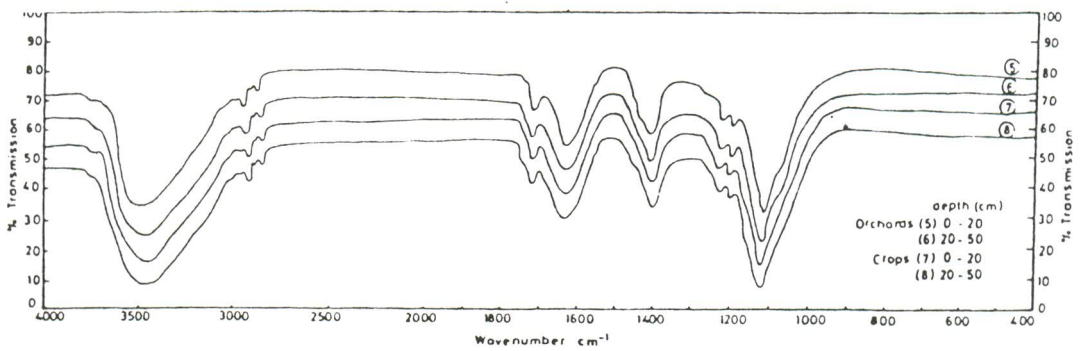


Fig (6) IR spectra of fulvic acids extracted from Abshway District (at El-Nassarka village)

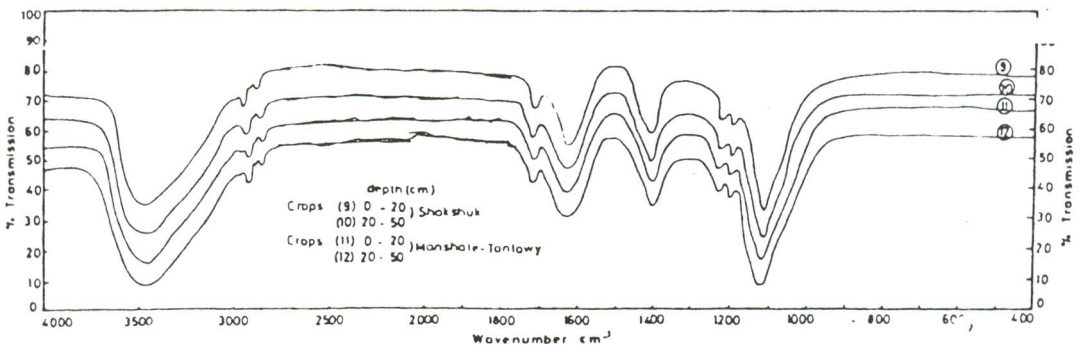


Fig (7) IR spectra of fulvic acids extracted from Sannoris District (at Shakshuk and Manshate- Tantawy villages)

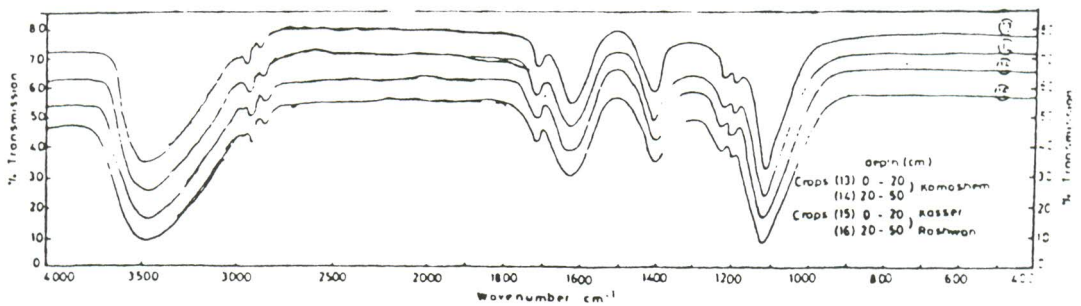


Fig (8) IR spectra of fulvic acids extracted from Tameya District (at Komoshem and Kasser - Rashwan villages)

REFERENCES

- Abdellah, H.A. (1991)*. Status of iron in relation to organic matter of alluvial soils M.Sc. Thesis, Fac. of Agric. Móshtohor, Zagazig univ.
- Amer, A.A.(1986)*. Distribution and composition of humic substances as related for pedological features of some alluvial soils at Kafr-El Sheikh Governorate. Ph.D. Thesis, Fac. of Agric., Alex. Univ.
- Bohn, H.L., MCNeal, B.L. and O' Connor, G.A. (1985)*. soil chemistry 2nd Ed., A Wiley Interscience Publication. John Wiley and sons, New York, Toranto.
- Chen, Y.; Senesi, N. and Schnitzer, M. (1978)*. Chemical and physical characteristics of humic and fulvic acids extracted from soils of the Mediterranean region. *Geoderma*, 20: 87-104.
- Flaig, W.; Beutespacher, H. and Rietz, E. (1975)*. Composition and physical properties of humic substances. In: J.E. Gieseking (ed), *soil Components*, Springer-Verlag, New York, N.Y., pp. 1-211.
- Jackson, M.L. (1967)*. Soil chemistry analysis. Constable Co. Ltd., London.
- Kononova, M.M.(1967)*. "Soil organic matter" Its nature, its role in soil formation and in soil fertility. 2nd Pergamon Press.
- Lindsay, W.L. and Norvell, W.A. (1978)*. Development of a DTPA micronutrient soil test. *Soil Sci. Soc. Am. J.* 42: 421-428.
- Schnitzer, M. (1978)*. Humic substances: chemistry and reactions. In *soil organic matter*, Schnitzer and Khan (eds), Elsevier Scientific Publishing Co., Amsterdam, Oxford, New York: 1-59.
- Schnitzer, M. and Ghosh, K. (1982)*. Characteristics of water soluble fulvic acid-copper and fulvic acid-iron complexes. *soil sci.* 134, (6):354-363.
- Stevenson, F.J. (1982)*. Humus Chemistry. John Wiley and Sons, New York.

Stevenson, F.J. and Goh, K.M. (1971). Comparison on infrared spectra of Synthetic and natural humic and fulvic acids. Soil Sci., 112:392-400.

Tan, K.H. and Giddens, J.E. (1972). Molecular weights and spectra characteristics of humic and fulvic acids. Geoderma, 8: 221-229.

Tan, K.H. and Mcgreery, R.A. (1970). The infrared identification of humopolysaccharide esters in soil humic acid. Soil Sci., Plant analysis (U.S.A.), 1(2): 75-84.

مكونات وطيف الأشعة تحت الحمراء لحمض الهيوميك
والفولفيك للمادة العضوية في التربة

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في محاولة لتقييم تيسر بعض العناصر المغذية الصغرى في أراضى محافظة الفيوم أخذت عينات تربة سطحه وتحت سطحه من أبشواى وسنورس وطاميه وقدرت محتوياتها من المادة العضوية وكل من الكربون الكلى والنيتروجين الكلى وكذلك من الحديد - المنجنيز والزنك والنحاس المستخلصين بواسطة ثنائى الايثيلين ترائى أمين بنتا حمض الخليك ومخلوط البيروفوسفات مع هيدروكسيد الصوديوم.

ولتحديد مدى ارتباط تيسر الحديد كمثال للعناصر الصغرى بالمادة العضوية وخاصة المواد الديالية النشطة قدر الحديد المصاحب لكل من حامض الهيوميك، وحامض الفولفيك بعض استخلاصهما من المادة العضوية وتنقيتهما وقد أظهرت النتائج أن الارضى المختبرة قد تميزت بانخفاض محتواها من المادة العضوية ومن ثم من الكربون الكلى، إلا أن حامض الهيوميك قد تميز بارتفاع محتواه من الكربون الكلى وكذلك محتواه من الحديد عن حامض الفولفيك .

وأوضحت الدراسة باستخدام الأشعة تحت الحمراء التماثل النسبى بين خواص حامض الفولفيك والهيوميك إلا أنه عند ٢٨٥٠ سم^{-١} اختلفت منحنى الامتصاص الضامص بحامض الفولفيك . وكذلك عند ١٤٠٠ سم^{-١} أظهر حامض الفولفيك امتصاص قوى فى حين كان الامتصاص ضعيفا عند هذه المنطقة لحامض الهيوميك.