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STUDIES ON THE STRUCTURE OF HUMIC SUBSTANCES EXTRACTED FROM DIFFERENT SOILS

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ABSTRACT

Humic and fulvic acids were extracted from normal (non-saline), saline, alkaline and newly reclaimed salt affected soils. These humic substances were characterized in terms of chemical composition, functional groups analysis, infrared spectrometry (IR) and solid-state ^{13}C cross-polarization magic angle spinning nuclear magnetic resonance ^{13}C(CPMAS)NMR spectrometry. Large variations in chemical , functional groups and spectroscopic measurements were observed among the extracted humic and fulvic acids. More humic substance were extracted from the normal soil than other soils, with the majority being humic acids. Small amounts of fulvic acids were recovered from all studied soils.

Data from elemental analysis and ^{13}C NMR spectroscopy indicated that humic and fulvic acids extracted from normal and saline were more aromatic than those of alkaline and newly reclaimed soils. Fulvic acids extracted from all soils contained mostly aliphatic, O-substituted alkyl, and carboxylic groups and small amounts of carbonyl groups. No difference were detected between the humic substances by IR spectroscopy . The humic acids extracted from the newly reclaimed soil is less humified than those of the other soils.

Keywords: humic acids, fulvic acids , elemental composition , functional groups, IR, ^{13}C(CPMAS)NMR

INTRODUCTION

Humic acids are complex orangic substances formed during biogeochemical degradation of plant debris and animal residues and condensation of the degraded segments. Because of their ubiquity in surface aquatic and groundwater system, humic acids often play important roles in environmental processes governing the fate and transport of organic and inorganic pollutants in natural system (Bartschat *et al.*, 1992 and Stevenson, 1994).

The chemical composition, structures and conformation of humic substances may vary greatly, depending on the origin of its source materials (Leinweber *et al.*, 1996). Chemical characteristics of humic substances are also affectel by agricultural practices (Ding *et al.*, 2002). Thus, identification of the overall chemical structure of humic substances is important to understand and elucidate its interactions with organic or inorganic contaminants.

The compositional differences of humic substances among soils were reported by Hatcher *et al.* (1981) with the application of ^{13}C CPMAS NMR. These studies indicate that ^{13}C NMR spectra of humic acids extracted from soils belonging to various soil orders are different.

Nuclear magnetic resonance studies by Saiz- Jimenez *et al.* (1986) showed wide differences in the aromatic character of humic acids extracted from Inceptisols and Mollisols of Australia and Spain, respectively. Lobartini and Tan (1988) provided ^{13}C NMR spectra of humic acids extracted from

Entisols, Inceptisols, Mollisols, Spodosols and Ultisols of Indonesia, the USA and Argentina and concluded that a distinctive spectrum was produced from each humic acid studied.

The objective of this study is to characterize and compare humic and fulvic acids extracted from the surface layer (0-30cm) of four different soils in Egypt, namely; a- normal; b- saline; c- alkaline and d- newly reclaimed salt affected soil. The humic substances (humic and fulvic acids) were examined by chemical, infrared (IR) and ^{13}C NMR (^{13}C CPMAS NMR) techniques.

MATERIALS AND METHODS

Four soil samples were collected from the surface layer (0-30 cm) to represent normal (non-saline), saline, alkaline and newly reclaimed salt affected soils from Egypt. The soil samples were air dried , ground and passed through a 2 mm sieve. The soil properties of the selected soils are shown in Table 1.

Table (1): Chemical and physical properties for the soils used in this investigation

Soil Samples	Organic carbon%	pH soil paste	ECe dS/m	ESP %	$CaCO_3$ %	Particle size distributions(%)			Soil texture
						Sand	Silt	Clay	
Normal soil	1.35	7.92	1.14	5.72	3.45	21.42	22.84	49.96	Clay
Saline soil	1.02	8.02	28.69	13.41	1.62	16.24	25.98	54.40	Clay
Alkaline soil	0.94	8.70	3.83	18.07	2.89	18.62	27.76	49.11	Clay
Newly reclaimed soil	0.51	8.12	2.17	13.18	2.26	23.09	25.83	47.94	Clay

Soil characterization:

Particle size distribution were determined using the pipette methods of Kilmer and Alexander (1949) and Soil Survey Staff (1972). Organic carbon (OC) contents were determined by dry combustion (950 ^{0}C) according to the methods of Nelson and Sommers (1982). Calcium carbonate ($CaCO_3$) content was estimated using Collin's calcimeter (Wright, 1939). Soil pH and ECe were measured in soil paste and soil paste Extract, respectively according to Jackson (1967).

Extraction of humic and fulvic acids:

Humic substances were extracted and purified using produres outlined and distributed by International Humic Substances Society (Aiken *et al.*, 1985). Briefly, 1 kg of 2- mm dry soil were suspended in 0.1M NaOH (soil/solution ratio of 1:10) under an N_2 atmosphere for 24 h at room temperature. The extract was acidified with 6M HCl to a pH of ~1. Humic and fulvic acids were then separated by centrifugation at 10000 rpm for 15 min. The humic acid fraction was purified with 0.1 M HCl -0.3 M HF mixture, and then dialyzed in deionized water. Fulvic acids were purified with repeated passages through Amberlite XAD-8 no.A-6525, 1-0.5 mm resin (Sigma Chemical Co, St. Louis, MO), followed by washings with 0.3 M HF and dialyzed in the same way as the humic acids. To ensure removal of salts, the fulvic acids were then passed four times in suecession through Bio-Rad Ag MP-50, .05- to 0.2- mm resin (Bio-Rad Laboratories, Richmond, CA) in the H from. Both fractions (humic and fulvic acids) were then freeze-dried and stored.

Characterization of humic and fulvic acids :

Carbon, hydrogen and nitrogen contents (oven-dry basis) were determined for humic and fulvic acid samples by dry combusion method as described by Mann and Saunder (1960). Oxygen was calculated by subtracting C% +H% +N% from 100. Total acidity, carboxyl groups, and phenolic-OH groups were measured on all humic acids samples using the method described by Schnitzer (1982). Sufficient quantities of fulvic acids were not available for these measurements.

Infrared absorption spectra for all humic and fulvic acid samples were obtained using the KBr pellet technique described by Schnitzer (1982). Spectra were collected on a Beckman model 4250 diffuse double-beam IR spectrophotometer.

Solid-State ^{13}C CPMAS NMR spectra for all humic acids were obtained on a Bruker CXP-100 spectrometer operating at 22.6 MHz. Samples were spun at the magic angle (54.7 ^{0}C) at approximately 3 to3.5 kHz. Spectra based on 1200 scans were obtained using a contact time of 1 ms and a recycle time of 1S. Solid-state ^{13}C CPMAS NMR spectra for all fulvic acids were obtained on a Nicolet NT-150 spectrometer operating at 15MHz. Samples were spun at the magic angle at 3.8 kHz. Spectra based on 9000 scans were obtained using a contact of 1 ms and a recycle time of 1 s. Chemical shifts in the humic and fulvic acids were reported downfield from tetramethylsilane. The spectra were evaluated in a manner similar to that of Hatcher *et al* (1983).

RESULTS AND DISCUSSION

The organic carbon (OC) of the soil samples selected for this study range from 0.51% to 1.35% (Table 1). The normal soil (non saline) has OC content that are 2.5 times greater than that in the newly reclaimed salt affected soil. The pH values of all the studied soils are alkaline, the alkali soil has the highest pH value as a result of the high ESP value (18.07%) (Table 1). The saline soil has the highest value of EC (28.69 dS/m) whereas the newly reclaimed salt affected soil has the lowest value (2.17 dS/m). The saline soil possess the lowest contents of $CaCO_3$ (1.62%) because the salts increase the solubility of calcium carbonate. The mineral fractions of the studied soils are dominated by clay (Table 1).

Chemical analyses of humic substances:

Yields of humic substances extracted from all four soils have been expressed as a percentage of the total organic carbon (TOC) content (Table 2). Thirty-nine to 52% of the TOC content of the soils was extracted. These values are similar to those reported by Lowe (1969). The normal and saline soils yielded approximately 2 times more humic acids than the newly reclaimed soil. In contrast, the yields of fulvic acids were much lower than those of humic acids in all soils. Approximately, fulvic acids were similar for the normal, saline and alkaline soils, but high in the newly reclaimed salt affected soil. This may be attributed to the high rate of decomposition of organic matter in this soil as a result of optimum soil conditions which increase the activity of soil microorganisms. Similar findings were found by Ruchko (1984) who indicated that fulvic acids were more readily mineralized by the microorganisms in soil than humic acids. Also, Sonbol and El-Arquan (1978) stated that fulvic acids are the first stage of the formation of humic acids, therefore the significant increase of fulvic acids and the decrease of humic acids in the newly reclaimed salt affected soil represent the intermediate stage between normal soil and the newly reclaimed salt affected soil.

Table 2: **Yield of humic and fulvic acids expressed as a percentage of the total organic carbon**

Soil Samples	**Total Organic C (TOC %)**	**Yield of humic substances (%)**		
		Humic acids	**Fulvic acids**	**Total**
Normal soil	1.35	38.04	14.13	52.17
Saline soil	1.02	34.65	15.08	49.73
Alkaline soil	0.94	28.59	16.62	45.21
Newly reclaimed soil	0.51	18.48	20.61	39.09

The elemental composition of the humic acids extracted in this study ranged from 51.14 to 55.37% C, 3.75 to 4.68% H, 3.69 to 4.16% N and 36.81 to 40.02% O of oven dry humic acid (Table 3). The composition is similar to that of an ideal soil humic acid reported by Schnitzer (1977) and humic acids extracted from a Gray Solonetz and a Brown Solod reported by Lowe (1969). Some trends, however, are apparent. The carbon content of the humic acid extracted from the newly reclaimed salt affected soil was lower and the oxygen content was higher than that of humic acids extracted from the other soils as illustrated by the differences in the O/C ratios (Table 3). This suggests that humic acids of the newly reclaimed soil is less humified than humic acids of the other soils. The perceived differences in hummification may be indicative of differences in the length of time the sites have been cultivated. Some differences can also be noted between the extracted humic acids. The humic acid extracted from alkaline and newly reclaimed soils had wider H/C ratios and higher H and N cotents than the other two soils. Generally, H and N contents increased with decrease in organic carbon content of the soil (Visser, 1983). Wide H/C ratios are indicative of humic substances with less condensed or more open aromatic type structures and a relatively high content of aliphatic components (Visser, 1983). This implies that the humic acids extracted from the alkaline and newly reclaimed soils were not as structurally condensed as the humic acids extracted from normal and saline soils.

Elemental composition of the fulvic acids ranged from 40.57 to 43.96% C, 3.42 to 3.81% H, 2.24 to 2.94% N and 50.38 to 52.68% O of oven dry fulvic acids (Table 3). The composition is similar to that of an ideal fulvic acids reported by Schnitzer (1977) and Stevenson (1994). Steenlink (1985) reported that the H/C, O/C and N/C ratios for fulvic acids typically cluster around 1.0, 0.9 and 0.5, respectively The data generated in this study suggest some slight compositional differences between the fulvic acids extracted from the different soils.

Table 3: Elemental composition and ratios of humic and fulvic acids

Soil Samples	C%	H%	N%	O%	Elemental ratios		
					H/C	O/C	N/C
			Humic acids				
Normal soil	55.37	3.75	3.69	37.19	0.81	0.50	0.057
Saline Soil	54.32	3.92	3.81	37.95	0.87	0.52	0.060
Alkaline soil	54.94	4.23	4.02	36.81	0.92	0.50	0.063
Newly reclaimed	51.14	4.68	4.16	40.02	1.10	0.58	0.069
			Fulvic acids				
Normal soil	43.96	3.42	2.24	50.38	0.93	0.86	0.044
Saline Soil	42.45	3.54	2.69	51.32	1.00	0.91	.0054
Alkaline soil	41.68	3.66	2.81	51.85	1.05	0.93	0.058
Newly reclaimed	40.57	3.81	2.94	52.68	1.13	0.97	0.062

Total acidity values of the humic acids ranged from 6.12 to 7.29, carboxylic groups from 4.49 to 5.30 and phenolic OH groups from 1.61 to 1.99 meq/g HA (Table 4).

Functional groups analyses were not performed on fulvic acids because of insufficient sampler. Humic acid extracted from saline soil contained higher quantities of total acidity, carboxylic and phenoloic- OH groups than those extracted from the other soils. The high values for carboxylic groups point to the inherent reactivity of the humic acids. The values of the functional groups are similar to that of an ideal soil humic acid reported by Schnitzer (1977).

Table 4; Total acidity, carboxylic and phenolic OH groups of humic acids

Soils	meq/g. HA		
	Total acidity	Carboxylic groups	Phenolic-OH*
Normal	6.78	5.17	1.61
Saline	7.29	5.30	1.99
Alkaline	7.08	5.21	1.87
Newly reclaimed	6.12	4.49	1.63

*Determined by difference between total acidity and carboxylic groups.

Infrared Spectroscopy (IR).

Infrared spectra of both the humic and fulvic acids (Fig. 1 and 2) show a strong-OH absorption band at 3430 to 3250 cm^{-1} , a C-H absorption band at 2960 to 2900 cm^{-1}, a C=O stretch of COOH absorption band at 1730 to 1710 cm^{-1}, and an aromatic C=C and an aromatic C-C absorption band at 1640 to 1610 cm^{-1}. All humic acid spectra exhibit a weak band indicative of C=O stretching of amides and quinones at 1650 cm^{-1}. Other bands common to both the humic and fulvic acid spectra are attributable to weak -OH deformation and C-O stretching of phenolic-OH or COO-antisymmetric stretching at 1420 to 1390 cm^{-1} , a C-O stretch and –OH deformation of COOH groups at 1230 to 1200 cm^{-1} and a C-O stretching of polysaccharide-like substances or Si-O of silicate impurities at 1110 to 1080 cm^{-1}. All humic acids exhibit a =C-H out- of- plane bending of aromatic and alkene structures at 900 cm^{-1} (Pavia *et al.*, 1979).

It is interesting to note that the spectra of fulvic acids exhibit a weaker band at 1640 to 1610 cm^{-1} than those of the humic acids, which indicates that there was less aromatic character in the fulvic acids than the humic acids. The IR spectral patterns of humic acids and fulvic acids in this study are similar to those reported by Taha and Modaihsh (2003).

No differences are evident by IR spectroscopy among the humic and fulvic acids extracted from the different four soils in this study. Infrared analyses did reveal that humic and fulvic acids were composed of aromatic, aliphatic, carbonyl (as carboxylic acids, ketones and phenolic- OH) groups and polysaccharide-like components and that the fulvic acids were not as aromatic as the humic acids.

^{3}C CPMAS NMR spectra:

The ^{13}CNMR spectra for the extracted humic and fulvic acids are presented in Fig. 3 and 4, respectively. Each spectrum was divided into four regions of resonance frequencies for major organic structures. The most significant peaks in each resonance frequency region will be identified and the significance of each region discussed.

In the aliphatic regions (0-50 ppm), spectra of humic acids from alkaline and newly reclaimed soils exhibit a broad peak (18-32 ppm), whereas those of normal and saline soils show a relatively well-defined peak at 32 to 34 ppm (Fig. 3). This suggests that, although humic acids from all soils contained methyl-C in long chains and alkyl-C bonded to aromatic ring structures (Taha et al, 2000), the humic acids extracted from alkaline and newly reclaimed soils may have contained a more heterogeneous mixture of aliphatic structures than the normal and saline humic acids.

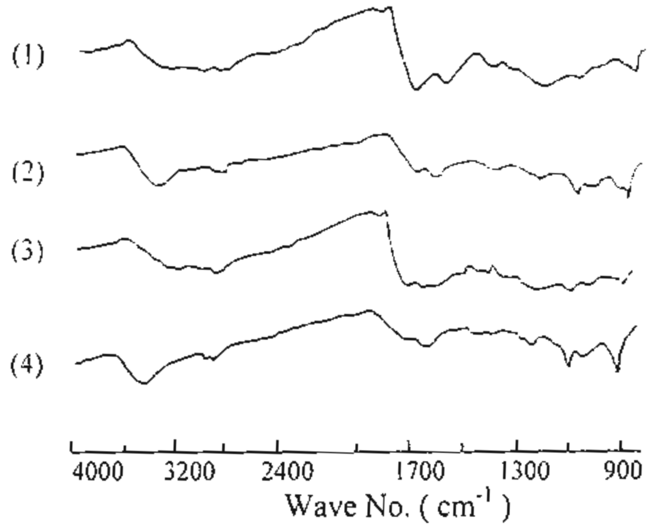


Fig.(1): IR spectra for (1) Alkaline, (2) Normal, (3) Newly reclaimed and (4) Saline humic acids.

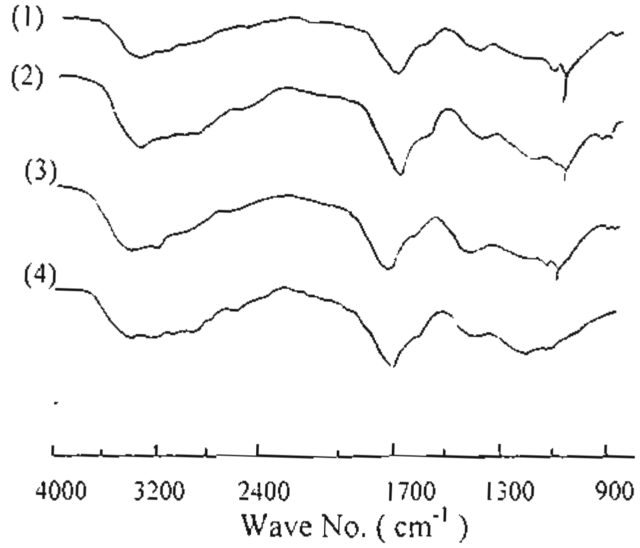


Fig (2): IR spectra for (1) Alkaline, (2) Normal, (3) Newly reclaimed and (4) Saline fulvic acids.

The most intense peak in the humic acids spectra of this study occurred at 129 to 134 ppm, which Schnitzer and Preston (1986) have attributed to aromatic structures substituted with alkyl groups. Minor peaks, indicative of phenolic-OH groups, also occurred at 153 to 154 ppm in all humic acids.

A distinct peak appears in the 160 to 190 ppm range in all the studied humic acids, which is commonly assigned as carboxylic carbons including free carboxylic acids and esters with possible contributions from peptides and quinones (Amalfitano *et al.*, 1995). Minor peaks in the carbonyl region (190-240 ppm) of the spectra were evident for all four humic acids (Fig. 3)

The ^{13}C NMR spectra of the extracted fulvic acids are shown in Fig. 4. In the aliphatic region, spectra for all fulvic acids showed a broad, noisy peak between 30 and 41 ppm. The fulvic acids extracted from alkaline and newly reclaimed soils exhibited a shoulder peak at 17 to 18 ppm which indicated an aliphatic-side- chain C and C from terminal methyl groups (Li *et al.*, 2003), this suggests that there are some differences between the fulvic acids extracted from the normal, saline and the alkaline and newly reclaimed soils.

All fulvic acids spectra exhibited a weak signal at 56 ppm that provided little evidence for lignin and lignin-like products. Strong absorptions peaks at 70 to 72 ppm suggested that carbohydrate and carbohydrate-like components are major constituents. Hatcher *et al.*, (1983) have reported that soil fulvic acids are dominated by carbohydrate and carbohydrate-like components. Unlike for the humic acids, the peaks indicative of aromaticity occurring between 130 and 137 ppm were not the most intense signals in the fulvic acids spectra. Saleh et al (1983) suggested that this indicates that, although fulvic acids have some aromatic character, they are mainly alkyl substituted. The most prominent peak in the fulvic acid spectra occurs between 172 and 174 ppm and is due to carboxylic acids, amides, and esters. A dominance of carboxylic acid adsorption peaks has been noted previously by Saiz-Jimenez *et al* .(1986) in spectra of soil fulvic acids. All fulvic acids spectra exhibited some minor peaks in the carbonyl region, but the signals were weak..

In the O-alkyl region (50-110 ppm), peaks are evident in the spectra of all humic acids at 57 to 58 and 73 to 74 ppm. The peak at 58 ppm has been attributed to methoxy associated with lignin and lignin-like products (Piotrowski *et al.*, 1984), whereas the 74 ppm peak has been attributed to carbohydrates (Preston *et al.*, 1997).

CONCLUSIONS

A combination of the elemental analysis, functional groups, IR and solid-state ^{13}CNMR spectroscopy was used to examine the compositional and structural differences among the humic and fulvic acids extracted from four different soils. Data from elemental analysis and ^{13}C NMR spectroscopy indicated that humic and fulvic acids of normal and saline soils were more aromatic than those of the alkaline and newly reclaimed soils. Also, the data of the functional groups analysis and ^{13}C NMR spectroscopy indicated that humic acids extracted from saline soil contained more carboxylic groups than those of the other soils. No differences were detected between humic substances of the studied soils by IR spectroscopy. The humic acid extracted from the newly reclaimed soil is less humified than those of other soils.

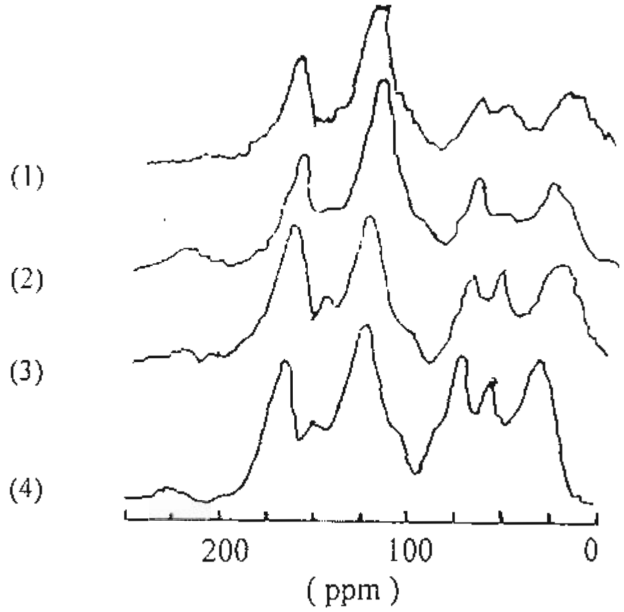
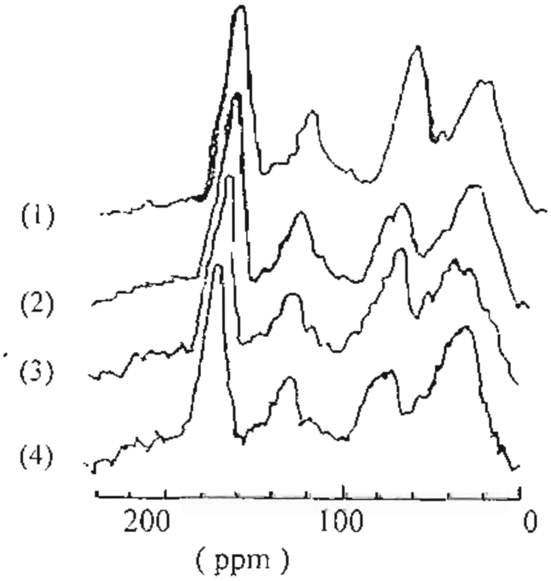


Fig (3): Solid – state ^{13}C NMR of (1) Alkaline, (2) Normal, (3) Newly reclaimed and (4) Saline humic acids.



Fig(4): Solid – state ^{13}C NMR of (1) Alkaline, (2) Normal, (3) Newly reclaimed and (4) Saline fulvic acids.

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دراسات على تركيب الأحماض الدبالية المستخلصة من أراضى مختلفة

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تم إستخلاص أحماض الهيوميك (Humic acids) وأحماض الفلفيك (Fulvic acids) من أربعة أراضى مختلفة: أرض عادية (غير ملحية) ، أرض ملحية ، أرض قلوية و أرض متأثرة بالأملاح ومستصلحة حديثا. وقد تم دراسة تركيب هذه المواد الدبالية بالطرق الكيميائية والطرق الضوئية مثل Infrared (IR) وإستخدام خاصية التردد النووى المغناطيسى الكربونى(^{13}C CPMAS NMR).

أوضحت النتائج أن هناك إختلافات واضحة فى كل من التركيب الكيميائى والمجاميع الفعالة لأحماض الهيوميك وأحماض الفلفيك المستخلصة من هذه الأراضى.

هذا وقد تميزت الأرض الغير ملحية بسيادة كمية المواد الدبالية المستخلصة عن بقية الأراضى حيث كان حمض الهيوميك هو المكون السائد فى هذه التربة، بينما تم إستخلاص كميات قليلة جدا من حمض الفلفيك من جميع الأراضى تحت الدراسة.

أوضحت نتائج التحليل العنصرى و^{13}C NMR أن أحماض الهيوميك المعزولة من كل من الأرض الغير ملحية والملحية تحتوى فى تركيبها على مركبات حلقية أكثر من كل من الأرض القلوية والأرض المستصلحة حديثا بينما إحتوت أحماض الفلفيك على مركبات أليفاتية كما تميزت هذه الأحماض بمحتواها المرتفع من مجاميع الكربوكسيل.

لم تظهر إختلافات واضحة عند دراسة التركيب الكيميائى للأحماض الدبالية بواسطة الأشعة تحت الحمراء(IR) .

حمض الهيوميك المعزول من الأرض المستصلحة حديثا كان أقل نضجا وتدبلا من بقية الأحماض الدبالية الأخرى.