

Physicochemical Characteristics of Humic and Fulvic Acids Extracted from Different Feedstocks

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ABSTRACT

There is a lack of knowledge regarding the variation physicochemical characteristics of humic substances (HS) extracted from various feedstock. For this purpose, humic acid (HA) and fulvic acid (FA) were extracted from soil, compost and clover straw to present several types of feedstock. The physicochemical properties of these humic substances were characterized for organic elements (C and N), total acidity and functional groups as well as IR spectra analysis. Data showed the feedstock recorded a significant effect on physicochemical properties of HS. Carbon and nitrogen concentrations varied widely among the extracted HS. The carbon and nitrogen concentrations in the extracted HA were more than the isolated fulvic acid. The C/N value of humic acid extracted from the soil was less than the other humic acids extracted from other sources. This trend was the same with FA indicating that the HA and FA extracted from the soil are more humified than other feedstock. Total acidity of FA was higher than HA with the superiority to those extracted from soils. The total content of active functional groups (COOH, total -OH, Phenolic -OH and Alcoholic-OH groups) was higher in FA as compared with HA, and the soil type was the most abundance source in most cases. Fourier transform infrared (FTIR) spectra confirm the presence of hydroxyl, aromatic and aliphatic fractions including quinone groups in humic and fulvic acids but in different values based on the feedstock.

Keywords: Humic acid, Fulvic acid, C/N ratio, Functional groups, IR spectra.

INTRODUCTION

Humic substances (humic acids, fulvic acids and humin) are the active part of organic substances, which reached the final degradation stage and exhibited a very long carbon chain with abundance of active functional groups (Stevenson, 1994). HS are formed through biological, chemical and geological decomposition of various feedstock. HS are existed in naturally in the ecosphere (particularly lithosphere and hydrosphere) either in solid state (70% from the soil organic matter) or in aqueous state (25% of dissolved organic carbon). They are intimately associated with soil sustainable productivity given their high ability to maximize water and nutrient supply potentials (Plaza *et al.*, 2006). In addition, HS can act as biostimulants to encourage seeds germination, root growth and elongation as well as the potentiality to deliver bioactive molecules and free radical scavenging (Mosa *et al.*, 2018). HS can be classified to humin, humic acid, and fulvic acid depending on their solubility/extractability at different pH values of aqueous suspension (Lee *et al.*, 2004). Humins are macromolecular species, which are insoluble in solutions at any pH-value and resistant to decomposition. Humic acids are the species of higher molecular weight, which are soluble in alkaline solutions. Fulvic acids, however, are classified with lower molecular weight and higher solubility state at a wide range of pH values as compared with humic acids (Stevenson, 1994). Comparing with other HS, fulvic acids exhibited higher carboxylic (-COOH) and hydroxyl (-OH) groups, which make them chemically reactive (Pettit 2004). Several field investigation have proved the capability of HS to maximize nutrients availability/retention, water holding capacity, soil aggregates stability and soil buffering capacity (Selim and Mosa 2018; Qin and Leskovar 2018). However, there is a lack of knowledge regarding the beneficial effect of HS on soil microorganisms, which are the main pool for improving soil fertility. In this regard, there are recent reports suggesting the probability of low molecular weight HS to act as nutritive substance for soil microorganisms (Lipczynska-Kochany 2018). To deal with this recent

hypothesis, studying the physicochemical characteristics of HS in relation of the feedstock is of great interest.

Humic substance can be derived from several feedstock (e.g. soil, crop residues and humified materials). There are two main scientific theories describing the origination of HS: (i) the oxidation of organic biopolymers (e.g. lignin), thereby transformation into humin, which may further oxidized to humic and fulvic acids, and (ii) abiotic condensation of organic molecules with low molecular weight following the microbial decay of plant residuals by soil microorganisms (e.g. bacteria and fungi) into fulvic acid, humic acid, and finally humin as the most recalcitrant material (Van Trump *et al.*, 2006; Leenheer, 2007). The objectives of this work are to extract, purify and isolate of humic and fulvic acids from different sources and studying their chemical properties.

MATERIALS AND METHODS

Three samples were collected from different sources to extract and isolate humic and fulvic acids and studying their chemical characteristics as follows: (1) soil sample was taken from a fertile clay soil at the Agricultural Experiment Station of the University of Mansoura, (2) compost sample was obtained from Mansoura, Dakahlia Governorate, and (3) clover straw sample was collected from a private Farm in Dakahlia Governorate, Egypt.

Extraction and purification of humic and fulvic acids:

Extraction of humic substances was run according to the method described by Sanchez-Monedero *et al.* (2002). 10 kg of soil or compost or clover straw were suspended in 0.1 M KOH (soil/solution ratio of 1:5) for 24 h at room temperature. Next morning, the dark coloured supernatant solution was siphoned off. Then potassium sulphate saturated solution was added to coagulate fine mineral particles and then filtered. The filtrate was acidified with H₂SO₄ drop by drop to pH of 2-3. Humic acid gel was coagulated, filtrated and washed with distilled water to remove the excess of H₂SO₄. The precipitated humic acid was transferred into a large jar and dried at 40 °C in an oven, while fulvic acid was siphoned off and passed through activated charcoal followed by elution of

the charcoal. The fulvic acid solution was concentrated to small value by placing it inside an oven with a fan and left at room temperature. The concentrated solution was transferred to membrane filter and electro dialysed until the dialysate was free from Cl^- and SO_4^{2-} .

Carbon and nitrogen contents of the purified humic acids were determined by the dry combustion method by using NC analyzer (Thermo Scientific, flash 2000) as described by Mann and Saunders (1960). Whereas carbon of fulvic acids was measured by using Walkely's & Black method (Kononova, 1966) and nitrogen was determined by the conventional method of Kjeldahl that reported by Bremner and Mulvaney (1982).

Total acidity and carboxylic groups of humic acids and fulvic acids were measured by BaCl_2 and $\text{Ca}(\text{CH}_3\text{COOH})_2$ methods, respectively (Dragunova, 1958) and (Schnitzer and Gupta, 1965). Total hydroxyl groups were estimated by acetylation method as described by Brooks *et al* (1958). Phenolic were determined by subtracting COOH groups content from total acidity according to (Kononova, 1966). Alcoholic hydroxyl groups were estimated by subtracting phenolic hydroxyl groups from total hydroxyl groups (Kononova, 1966).

Fourier transform infrared spectroscopy (FTIR) analysis was identified by a Thermo Scientific-Nicolet iS10 to obtain surface functional existed in humic and

fulvic acids. KBr at a rate of 100:1 (w/w), compressed into pellets, and analyzed within the observable absorption spectra 4000 and 400 cm^{-1} .

RESULTS AND DISCUSSION

Carbon and nitrogen concentration in humic and fulvic acids:

Data presented in Table 1 show that carbon and nitrogen contents were slightly affected by the source of the humic substances, indicating that these humic acids and fulvic acids appeared to be closely related structurally (Schnitzer, 1978). Contents of carbon in humic acids varied from 55.10% to 53.40% with an average of 54.46% while carbon contents in fulvic acids ranged from 46.2% to 44.5% with an average of 45.5%. It means that carbon range of humic acid is higher than of fulvic acid. These data are in agreement with those reported by Gomah *et al.* (1978), Taha (1989) and Allam (2005). Nitrogen contents in humic acids were found to vary between 3.90% and 4.20% with an average of 4.07%. However, nitrogen contents in the fulvic acids were found to vary between 3.4% and 3.8% with an average of 3.63%. This means that humic nitrogen concentration was higher in humic acid as compared with fulvic acid. These results are in harmony with that reported by El-Ghozoloi (1998).

Table 1. Carbon and nitrogen concentrations in humic and fulvic acids:

Source of humic substances	Humic acid			Fulvic acid		
	C%	N%	C/N	C%	N%	C/N
Soil	53.40	4.20	12.7:1	45.7	3.8	12.0:1
Compost	54.90	4.11	13.4:1	46.2	3.7	12.5:1
Clover straw	55.10	3.90	14.1:1	44.5	3.4	13.0:1
Average	54.46	4.07	13.4:1	45.5	3.63	12.5:1

Data presented in Table 1 also show that C/N ratios of humic acids ranged between 12.7:1 and 14.1:1. This narrow ratio could indicate that isolated humic acids from soil were more humified than the other humic acids, while the C/N ratios of fulvic acids are ranged between 12.0:1 and 13.0:1. These narrow ratios are also similar to that of fulvic acid extracted from the soil. These results are in agree with that reported by Sweed, (2005).

Total acidity and functional groups:

Total acidity of extracted humic acids is located between 4.97 and 6.7 mmole/g. HA (Table 2). COOH

groups varied from 3.8 to 4.1 mmole/g. HA. Phenolic -OH groups varied from 1.17 to 2.60 mmole/g. HA. and alcoholic- OH groups ranged from 2.1 to 3.6 mmole/g. HA. It is noticeable that the soil humic acid contains more COOH, phenolic -OH and less alcoholic- OH groups than that of the other humic acids, which extracted from compost and clover straw. This would be explained by the fact of the humic acid isolated from soil was more mature than the others. Generally, these results are in harmony with those of Stevenson, (1994) and Allam (2005)

Table 2. Oxygen-containing functional groups in the humic acids (mmole/g. HA)

Source of HA	Total acidity	COOH groups	Total -OH groups	Phenolic -OH groups	Alcoholic-OH groups
Soil	6.7	4.1	4.7	2.60	2.1
Compost	5.2	3.9	4.9	1.30	3.6
Clover straw	4.97	3.8	3.7	1.17	2.53

Whereas total acidity of the isolated fulvic acids ranged from 11.5 and 12.7 mmole/g. FA (Table 3). COOH groups in fulvic acids varied from 7.3 to 8.4 mmole/g. FA. Phenolic -OH groups in fulvic acids is located between 4.2 and 4.4 mmole/g. FA, and alcoholic- OH groups is located between 3.5 and 5.0 mmole/g. FA. It is noticeable that the fulvic acids extracted from soil contain more COOH groups, alcoholic-OH groups and less phenolic-OH than

that of the other fulvic acids isolated from compost and clover straw. This would be explained by the fact that the fulvic acids isolated from soil was more mature than the others. These results are in agreement with those of Stevenson, (1994) and Allam (2005).

In general, it was noticed from Tables 2&3 that the fulvic acids content of total acidity, COOH groups and total-OH groups are higher than of humic acids.

Table 3. Oxygen-containing functional groups in the fulvic acids (mmole/g.FA)

Source of FA	Total acidity	COOH groups	Total -OH groups	Phenolic -OH groups	Alcoholic-OH groups
Soil	12.7	8.4	9.3	4.3	5.0
Compost	11.5	7.3	8.1	4.2	3.9
Clover straw	11.8	7.4	7.9	4.4	3.5

IR spectra of the humic substances

Infrared (IR) spectra of humic substances showed a number of bands that provide information about the nature, reactivity, and structural arrangement of oxygen-containing functional groups (Stevenson, 1994). IR spectra of the fulvic acid extracted from soil is shown in Fig. 1. The broad

and strong band at 3421cm^{-1} showed the presence of H-bonding OH. A band at 2922cm^{-1} due to aliphatic CH stretching, Quinone C=O groups at the strong and narrow band (1638cm^{-1}). The weak band at 1109cm^{-1} is due to C-O stretching of polysaccharides and Si-O of silicate impurity.

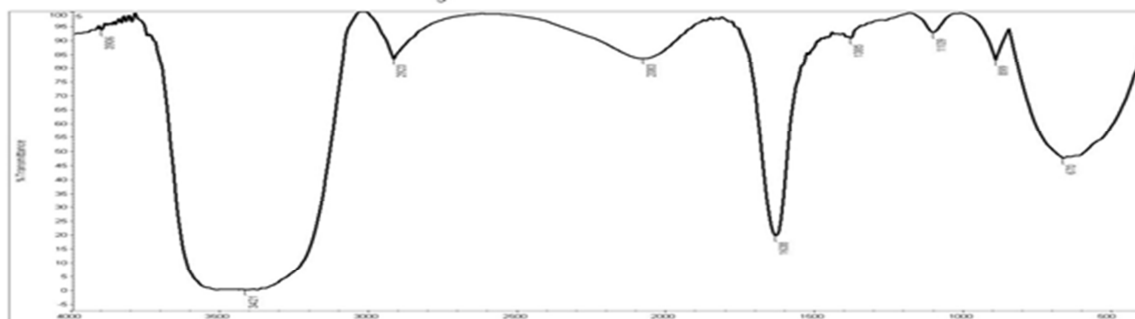


Fig. 1. IR spectra of fulvic acid extracted from soil.

IR spectra of the humic acid extracted from compost is shown in Fig. 2. The strong band at 3422cm^{-1} was indicate to H-bonded OH groups. The band at (1703cm^{-1}) indicates C=O of ketones, quinone C=O groups indicated by the weak band (1653cm^{-1}). The weak bands of

(1545 and 1514cm^{-1}) indicate ionized carboxyl groups, the strong band at (1190cm^{-1}) refers to C-O stretching and OH deformation of COOH, there was narrow band at 1066cm^{-1} due to C-O stretching of polysaccharides and Si-O of silicate impurity.

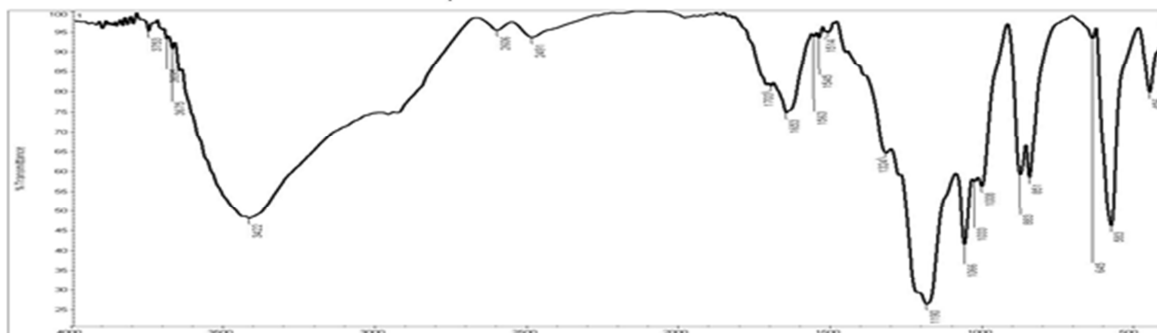


Fig. 2. IR spectra of humic acid isolated from compost.

While IR spectra of fulvic acid isolated from compost in Fig. 3 shows the strong and broad bands at (3550 , 3432 and 3358cm^{-1}) refer to H-bonding OH, The weak band existed at (2835cm^{-1}) is due to presence of

carboxylate ion. Quinone C=O groups at the strong band (1638cm^{-1}). The weak band at 1107cm^{-1} is due to C-O stretching of polysaccharides and Si-O of silicate impurity.

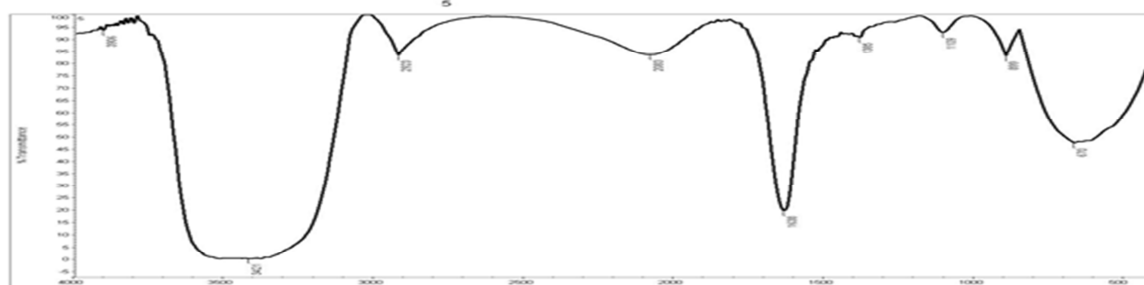


Fig. 3. IR spectra of fulvic acid extracted from compost.

IR spectra of humic acid extracted from clover straw is shown in Fig. 4. The strong band at 3424cm^{-1} was indicate to H-bonded OH groups. The advantage of this spectrum was the broad band at range of 3600cm^{-1} and 3100cm^{-1} indicating hydroxyl groups. This rang included many types of OH reported by (Taha , 1985; Sanchez-Monedero *et al.*, 2002). A band at 2922cm^{-1} refers to aliphatic CH stretching , the weak band of aliphatic CH

ranged between 2970cm^{-1} and 2840cm^{-1} were previously reported by Gonzalez Perez *et al.*, (2004), Quinone C=O groups indicated by the weak band (1653cm^{-1}) was reported by Canellas *et al.*, (2001). The weak bands between (1545 and 1522cm^{-1}) indicated ionized carboxyl groups, Also the narrow band at 1106cm^{-1} is due to C-O stretching of polysaccharides and Si-O of silicate impurity.

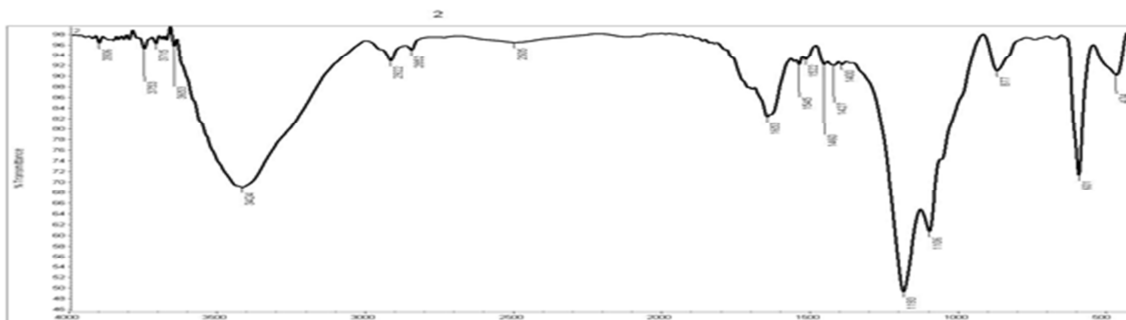


Fig. 4. IR spectra of humic acid isolated from clover straw.

IR spectra of the fulvic acid extracted from clover straw is shown in Fig. 5. The strong band at 3412cm^{-1} was indicate to H-bonded OH groups , The weak band occurred at (2835cm^{-1}) is due to the presence of carboxylate ion.

Quinone C=O groups at the strong band (1638cm^{-1}). The weak band at 1115cm^{-1} is due to C-O stretching of polysaccharides and Si-O of silicate impurity.

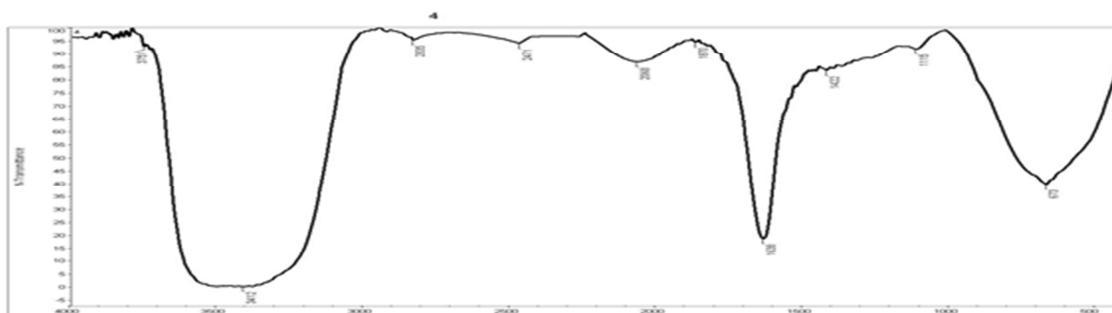


Fig. 5. IR spectra of fulvic acid isolated from clover straw.

Generally, the IR spectra for the various humic substances (humic and fulvic acids) showed that humic and fulvic acids isolated from different sources contain many amounts of different IR reactive groups. As well as , IR spectra of the studied humic and fulvic acids supports the interpretation that structure of these acids are rather similar, at least in the constructure of the internal. Mainly they are different in ratios of the number of functional groups and polymerization degree. These results are in harmony with those reported by Taha (1991); Canellas and Santos (2005).

CONCLUSION

The analysis of carbon and nitrogen contents of the studied humic and fulvic acids proved that these elements affected by the source of humic substances. Humic and fulvic acids isolated from soil is more mature state than compost and clover straw. Total acidity , COOH groups and phenolic OH groups content were differed with the sources of humic and fulvic acids. The IR spectra of the studied humic and fulvic acid showed that there are a big similarity among the different sources. IR spectra indicated that humic and fulvic acids had aromatic rings besides aliphatic compounds and include COOH, COO⁻, OH, C=O and C-O groups in their structures.

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الخصائص الفيزيائية والكيميائية لأحماض الهيوميك والفليك المستخلصة من المواد الخام الأولية

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تم استخلاص الأحماض الدبالية " من ثلاث مصادر مختلفة هي : تربة , كمبوست , تبن البرسيم . وتم دراسة هذه الأحماض Humic anf fulvic acids المستخلصة بالطرق الكيميائية وقد أوضحت النتائج تأثير الأحماض الدبالية علي محتوى كل من الكربون والنيتروجين حيث أن محتوى الكربون والنيتروجين المستخلص من حمض الهيوميك أعلى من المستخلص من حمض الفليك. وهذا يدل علي أن الهيوميك والفليك المستخلص من التربة أكثر نضجا من الآخرين. وتم تأكيد ان المجاميع الفعاله جزء من التركيب الكيميائي للأحماض الدباليه وذلك عند الاستعانة بالطرق (IR). الكيمياء وايضا باستعمال منحنيات الأشعة تحت الحمراء ولوحظ أن حمض الفليك المستخلص من مصادر مختلفة وهي التربة والكمبوست وتبن البرسيم يحتوي علي مجموعة الحموضة الكليه ومجموعة الكربوكسيل والهيدروكسيل بنسبه عاليه عن حمض الهيوميك. وتم تأكيد وجود مجموعات الهيدروكسيل ومجموعات الأروماتيك والأليفاتيك الفعاله والتي تتضمن مجموعات الكينون في كل من الهيوميك والفليك بواسطة الأشعة تحت الحمراء ولكن بكميات مختلفه.