

CHEMICAL AND SPECTROSCOPIC MEASUREMENTS ON THE HUMIC ACIDS EXTRACTED FROM SOME ORGANIC COMPOSTS

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ABSTRACT

Humic acids were extracted from different composts and have been characterized by chemical and spectroscopic methods. Meaningful differences in the composition were revealed by UV, Visible and IR spectroscopies. The obtained data showed that carbon, hydrogen and nitrogen of the studied humic acids were slightly affected by the source of the humic acids, indicating that these humic acids appeared to be closely related structurally. However, the C/H and O/H values for Yan-HA were higher than the other humic acids. This could indicate that this humic acid is more humified and in a more condensed state. The UV and Visible spectra of the isolated humic acids exhibited the same trend showing no minimum or maximum absorption. San-HA and Enz-HA have the lowest value of E_4/E_6 ratio indicating a higher degree of aromatic condensation and low aliphatic structure. The IR spectra of the studied humic acids indicated that, there was a broad similarity among the different humic acids studied. The intensities of the absorption band vary slightly from one to other. They differ mainly in the ratios of the number of functional groups and the degree of polymerization. On the other hand, humic acid extracted from sludge (Bos-HA) contains the highest percentage of aliphatic carbon, associated with polysaccharides structures.

Keywords: Compost, humic acids(HA), elemental analysis, UV& visible absorption, IR spectroscopy.

INTRODUCTION

Humic substances and its composition are important diagnostic criteria that reflect the nature and direction of the present soil formation process. These materials arise from the chemical and biological degradation of plant and animal residues and from synthetic activities of microorganisms (Schnitzer,1978). Humic substances are dark-coloured, acidic, predominantly aromatic, hydrophilic, chemically complex and polyelectrolyte-like materials that range in molecular weights from a few hundreds to several thousands (Stevenson,1982 and Soler Rovira *et al.*2002).The elementary analysis of humic acids provides information on the distribution of carbon, hydrogen, nitrogen and oxygen. Humic acid elemental composition is usually in the range: C, 43.8 to 58.7%; H, 3.2 to 6.2%; N, 0.8 to 4.3% and O, 32.8 to 38.3% (Schnitzer,1978). The O/H mole ratio for soil humic acids is usually about 0.5, while the C/H mole ratio is approximately 1.0 (Steelink, 1985). Taha (1991) and Abo El-fadl *et al.*(1992) reported that humic acids are chemically similar, but they different in molecular weight and ultimate analysis.

The spectroscopic investigations of humic acids in the UV region (200-400 nm) and visible region (400-800 nm) are important for both the

characterization and the determination of genetic differences as well as for transition between the types of humic acids (Flaig *et al.*, 1975). In contrast to the relatively few absorption bands observed in the UV and visible regions, the infrared (IR) spectra of humic acids contain a variety of bands that are diagnostic of specific molecular structures (Stevenson, 1982) who stated that IR spectroscopy is a valuable technique, hence it provides a key information regarding the nature, reactivity, structural arrangement of oxygen-containing functional groups and the occurrence of protein, carbohydrate constituents and inorganic impurities (metal ions, clay) in the extracted humic acids. Also, infrared (IR) analysis has contributed substantially to the knowledge of the chemistry of humic acids (Schnitzer and Khan, 1972 and Stevenson, 1982).

The main objective of this work is to determine and discuss comparatively the compositional, structural, and functional properties of the humic acids isolated from different types of organic fertilizers (composts).

MATERIALS AND METHODS

Source of humic acids:

Humic acids were isolated from different organic fertilizers (composts) used in the Kingdom of Saudi Arabia, namely: 1) Khaldia (animal wastes, Kha-HA); 2) Sanbest (plant & animal residues, San-HA); 3) Yanbost (plant residues, Yan-HA); 4) Al-Kharj (Cow wastes, Cow-HA); 5) Al-Enzy (animal & plant residues, Enz-HA) and 6) Bostan (sludge wastes, Bos-HA). Some chemical properties of these composts are given in Table (1).

Extraction, purification and isolation of humic acids:

Humic acids were extracted from the different composts with 0.1M NaOH solution (Kononova, 1966 and Taha, *et al.*, 1993) using a sample: extractant ratio of 1: 5. For each extraction step, the compost was shaken mechanically with 0.1M NaOH solution for 3 h., and the suspension was allowed to settle overnight, then the supernatant liquid was siphoned off, followed by centrifugation at 10000 rpm for 15 min. to remove the suspended materials. The combined alkaline extract was then acidified with HCl to pH= 1, left standing for 24 h to allow the complete precipitation of humic acid. The acid soluble fraction, which represent fulvic acid, was discarded. The humic acid precipitate was purified by dissolution in 0.1M NaOH. The suspension was centrifuged, the residues were discarded, and the alkaline supernatant was acidified with HCl to pH=1. The suspension was left standing for 24 h. at room temperature. The precipitated humic acid was then recovered with distilled water, dialyzed until free of Cl⁻ ions, and freeze dried.

Elemental analysis of the isolated humic acids:

The elemental composition (C, H, N) were performed on a Perkin Elmer C, H, N, S/ O analyzer, series II model 2400. Oxygen was calculated by subtracting of C% + H% + N% from 100 (Schnitzer, 1978).

Ultraviolet (UV) and Visible spectra:

Ultraviolet (UV) (200-400 nm) and visible light (400- 800 nm) spectra of the studied humic acids were carried out using a MILTON ROY

Spectronic 1001 plus spectrophotometer. Solutions of sodium humates were prepared by dissolving 2 mg of the purified humic acid in 10 ml of 0.05N NaHCO₃ solution according to the method of Chen *et al.*(1977). The ratio between the absorbance at wavelengths of 465 and 665 nm, which is conventionally indicated as E₄/E₆, was measured on the same solutions of sodium humates.

Infrared spectra (IR):

The infrared spectra (4000- 600 cm⁻¹) were performed on KBr pellets using a Perkin Elmer 883 IR spectrophotometer. Pellets were obtained by accurately weighing 1 mg of humic acid and 300 mg of dry KBr. The mixture was finely milled, transferred into a 13 mm die, and finally pressed under vacuum at 10 tons for 1 min.

The resulting pellets were weighed again and dried under vacuum at 70°C (Schnitzer, 1978).

Table 1: Chemical analysis of the organic fertilizers (composts).

Source	% moisture	pH*	% O.M.	% N	% P	% K	ppm							
							Fe	Cu	Zn	Mn	Pb	Cd	Ni	Co
Khaldia	43.75	8.70	28.87	1.68	0.63	1.89	2800	14	172	168	34	2	12	4
Sanbest	12.50	7.38	14.58	0.78	0.35	0.58	9680	28	732	232	64	2	16	12
Yanbost	29.63	8.10	19.18	1.28	0.46	0.66	11340	286	606	226	78	2	30	4
Al-Kharj	10.20	8.29	14.83	1.28	0.67	0.66	6980	150	458	110	82	3	36	2
Al-Enzy	2.73	8.44	11.94	0.20	0.14	0.72	3320	8	64	44	40	4	16	4
Bostan	35.42	7.44	30.77	1.68	0.68	0.58	7060	156	118	68	112	6	32	2

* pH was measured in 1:5 suspension.

RESULTS AND DISCUSSION

Elemental composition:

The elemental analysis of humic acids provides an useful inventory of the distribution of the major elements in the humic substances. Also, the elemental analysis provides C/N, C/H and O/H ratios which give an indication about the aromaticity, the aliphatic compounds contents and the degree of oxidation (Drozd and Licznar,1983).

The elemental composition data of the isolated humic acids are given in Table (2), showing the percentage of carbon, hydrogen, nitrogen and oxygen, in addition to the elemental ratios (C/N, C/H and O/H ratios). Carbon content ranged from 44.09% to 53.68%. The low carbon content in Enz-HA and Yan-HA are very similar to typical values reported in the literature for marine or lacustrine humic acids (Ishiwatari,1985),whereas the carbon percentages obtained for Kha -HA, San-HA and Bos-HA fall within the range typical of soil humic acids (50.3-54.8%) (Schnitzer,1977) obtained from widely differing environments. Hydrogen contents of these humic acids varied between 4.16% and 6.38%. It is of interest to notice that Yan-HA had the lowest percentage (4.16%) of hydrogen indicating more humification than the other humic acids. On the other hand, all of the humic acids show a very high nitrogen content (range from 4.01% to 7.63%) that is closed to the highest

limit of the range usually found for humic acids(2-7%) (Schnitzer,1985). This particularly true for the humic acid obtained from Bostan fertilizer, which showed the highest percentage(7.63%). Oxygen contents of the studied humic acids varied between 34.76% and 46.48%.

Table (2) also show that the C/N ratios of the isolated humic acids varied between 6.88 and 12.83. The ratios are much lower for the humic acids extracted from Al-Kharj and Bostan fertilizers, respectively because of their humic acids contain the highest percentages of nitrogen. These narrow ratios would indicate that Kha -HA and Bos-HA may be in a mature state. However, Visser (1964) stated that no constant change could be detected in C/N ratio with the degree of humification, possibly owing to the nitrogen content which depends on more factors than only the state of decomposition or humification.

The C/H ratios of the isolated humic acids ranged from 0.67 to 0.95 where that of the San-HA and Yan-HA have the highest values (0.95 and 0.94, respectively), indicating that these humic acids in a high degree of humification. Gomah, *et al.*(1978) and Kaloosh (1984) reported that C/H ratio is usually considered to be parallel to the degree of condensation of aromatic rings in the humic substances or to indicate the degree of humification.

The O/H ratios of the studied humic acids were found to vary from 0.34 to 0.64, with the highest value for Yan-HA (0.64). This could indicate that the process of oxidation and dehydrogenation appeared to proceed more in this humic acid than the others. Kononova (1966) stated that the increase in O/H ratio for the humic substances could indicate more oxidation and humification of the molecule of humic acids. These data are in harmony with those reported by Taha (1989 and 1991).

Table 2: Elemental composition and atomic and E_d/E_s ratios of the humic acids

Source of HA	%				Atomic ratio			E_d/E_s
	C	H	N	O	C/N	C/H	O/H	
Khaldia	53.68	5.40	5.65	35.27	11.08	0.83	0.41	6.50
Sanbest	50.55	4.45	4.90	40.10	12.04	0.95	0.56	7.90
Yanbost	46.91	4.16	6.34	42.59	8.63	0.94	0.64	5.70
Al-Kharj	44.16	4.41	7.49	43.94	6.88	0.83	0.62	6.40
Al-Enzy	44.09	5.42	4.01	46.48	12.83	0.68	0.54	5.30
Bostan	51.23	6.38	7.63	34.76	7.83	0.67	0.34	4.60

Absorption in the UV(200-400nm) and Visible(400-800nm) regions:

Absorption in the UV and visible regions of the isolated humic acids is due to presence of multiple bonds and to unshared electron pairs in organic molecules (Schnitzer and Khan, 1972). As shown in Figs. (1&2), humic acids, like many relatively high molecular weight materials, yield generally uncharacteristic spectra. The absorption spectra of the studied humic acids in the UV and visible regions are characterized by a monotonous decrease of absorbance with increasing wavelength, showing no minimum or maximum absorption. This could be due to the heterogeneity of molecular

constituents found in the structure of humic acids such as phenolic compounds and their oxidation products, amino acids and their condensation products with phenols in oxidizing medium (Flaig, *et al.*, 1975; Chen *et al.*, 1977; Schnitzer, 1978; Lichtszeld, 1963 and Taha, 1991). As indicated in Figs (1&2), the absorbance decreases as the wavelength increases. Higher absorbance at shorter wavelengths has been attributed to increased mobilities of π electrons over aromatic carbon "nuclei" and over unsaturated structures conjugated with these "nuclei" (Schnitzer and Khan, 1972).

As shown in Fig. (1), it was observed that the spectra of the extracted humic acids in the UV region exhibited the same trend. However, there is a slight maximum absorption is indicated in the 250 to 280 nm regions for Kha - HA and Enz-HA, respectively and this may be related to the differences in degree of humification (Stevenson, 1982).

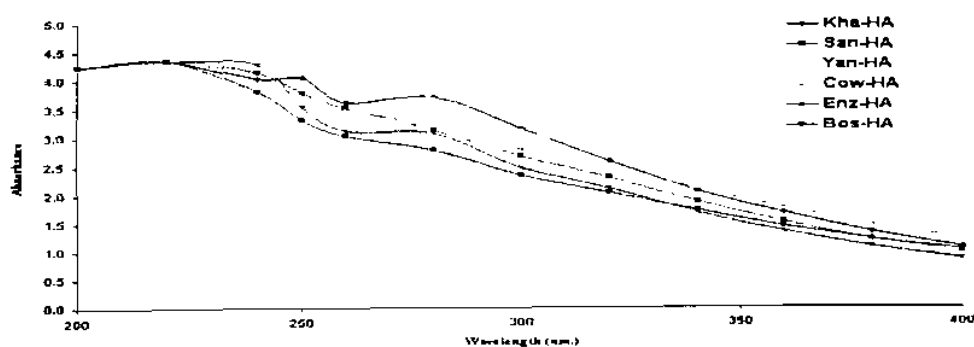


Fig.(1) UV spectra of the isolated humic acids

It is also indicated from Fig.(2) that the absorbance of the studied humic acids in the visible region were slightly affected by the source of the humic acids and they exhibited the same trend showing no minimum or maximum absorption. It was concluded that the similarity of humic acids spectra, regardless of origin, method of extraction, and purification suggests

that these humic acids had similar basic structures. These findings are in harmony with those reported by Kononova (1966) and Taha *et al.* (1993). In this respect, Hayes and Swift (1978) assumed that humic materials contain numerous chromophors whose absorption bands overlap throughout this spectral region and to absorb with growing intensity as the wavelength decreases. Thus it is not possible to observe or measure one particular chromophore or derive definitive information with regard to chemical composition or structure. It is, however, possible to utilize the general features of the spectrum, namely the intensity of absorption and the gradient of the spectrum.

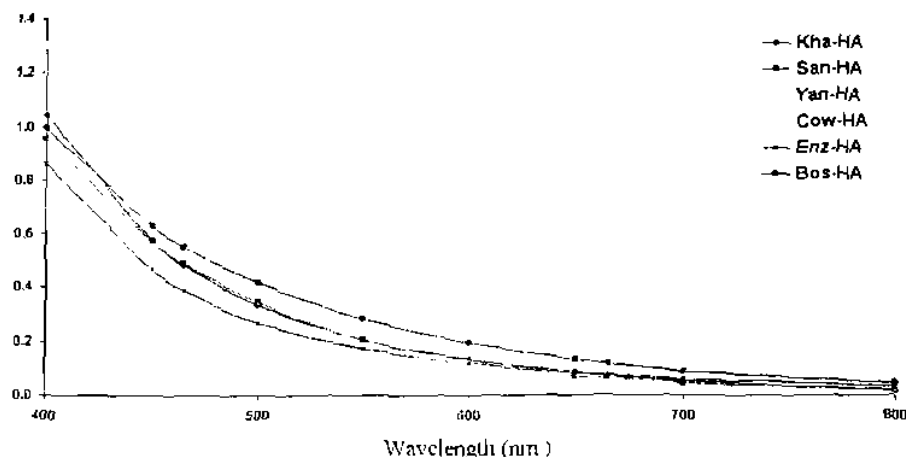


Fig.(2) Visible spectra of the isolated humic acids

The E_4/E_6 ratio:

The ratio of absorbance at 465 and 665 nm, referred to as the E_4/E_6 ratio, has been widely used for characterization of humic acids. As indicated in Table (2), the E_4/E_6 ratio for the extracted humic acids ranges from 4.6 to 7.9. This result agreed with the data reported by Kononova(1966). In general, progressive humification and increased condensation are indicated by a decrease in the E_4/E_6 ratio (Kononova, 1966). Thus a low ratio may be indicative of a relatively high degree of condensation of aromatic constituents; a high ratio reflects a low degree of aromatic condensation and infers the presence of more aliphatic structures (Chen *et al.* 1977). The results here could indicate a lower molecular weight of San-HA as well as a higher content of carboxylic functionalities in this humic acid than the others. The E_4/E_6 values obtained for the other humic acids are close to those normally reported in the literature for soil humic acids (Chen *et al.*, 1977 and Deiana *et al.*, 1990). However, an inverse

relationship has been observed between the E_4/E_6 ratio and the mean residence time of humic materials; specifically, the humic material with the highest E_4/E_6 ratio had the lowest mean residence time, indicating that the more humified and highly condensed (aromatic) substances were of more ancient origin (Stevenson, 1982).

Infrared spectra (IR):

Infrared spectra of humic substances exhibit a number of bands that provides information about the nature, reactivity, structural arrangement of oxygen-containing functional groups (Stevenson, 1982). The IR spectra of the isolated humic acids are shown in Fig. (3). The assignment of the main recognizable absorption bands is given in Table (3). As indicated from the Fig. (3), the isolated humic acids exhibit similar infrared absorption patterns, with a few exceptions. The primary features of these spectra are the following: (1) a common intense, broad band at about 3400cm^{-1} (ranged between 3318 and 3403cm^{-1}), attributed to H-bonded OH, secondarily, to NH stretching of various functional groups (Taha, 1991). The centers of this band are seen to be different according to the differences in the humic acids samples investigated. Also, it may be attributed to different amounts of phenolic and/ or alcoholic OH groups (Soler Rovira *et al.*, 2002). (2) a peak at about 2925cm^{-1} (ranged between 2925 and 2937cm^{-1}), caused by the stretching of aliphatic C-H groups, whose intensity is much higher in the Bos-HA. (3) a peak at about 1710cm^{-1} (ranged from 1710 to 1713cm^{-1}), caused by C=O stretching of various carbonyl groups, including COOH groups, which is more pronounced in San-HA; Yan-HA and Cow-HA and absent in the other studied humic acids. (4) a common strong band in the region 1641 - 1660cm^{-1} , which is generally considered an envelope of a number of unresolved peaks primarily because of C=C aromatic vibrations, C=O stretching of amide groups (amide I band), quinines and/or C=O of H-bonded conjugated of ketones, and COO⁻ symmetric stretching, which is pronounced in all the studied humic acids. (5) a peak around 1540cm^{-1} (ranged between 1510cm^{-1} and 1540cm^{-1}), preferentially ascribed to N-H deformation and C=N stretching of amide (amide II band), which is very strong band in Kha HA and Enz-HA and faint or absent shoulder in the other humic acids. (6) a peak around 1460cm^{-1} , (ranged between 1450cm^{-1} and 1461cm^{-1}), attributed to aliphatic C-H, which is very intense band in Kha-HA and Enz-HA but faint or absent shoulder in the other humic acids. (7) a peak in the region 1412 - 1422cm^{-1} , preferentially assigned to OH deformation and C-O stretching of phenolic groups, which is more pronounced in Kha-HA, San-HA and Enz-HA and faint or absent in the other humic acids. (8) a band in the region 1220cm^{-1} - 1267cm^{-1} , generally ascribed to C-O stretching and OH deformation of COOH groups and C-O stretching of ethers and esters, which is more indicated in all the studied humic acids except for Cow-HA. (9) a peak in the range between 1124cm^{-1} and 1127cm^{-1} , possibly attributed to C-O of various alcoholic and ether groups which is apparent in all the isolated humic acids except for Yan-HA. (10) a peak in the region 1030 - 1034cm^{-1} evident in all the isolated humic acids, attributed to C-O stretching of polysaccharides-like components and/or to Si-O of silicate impurities.

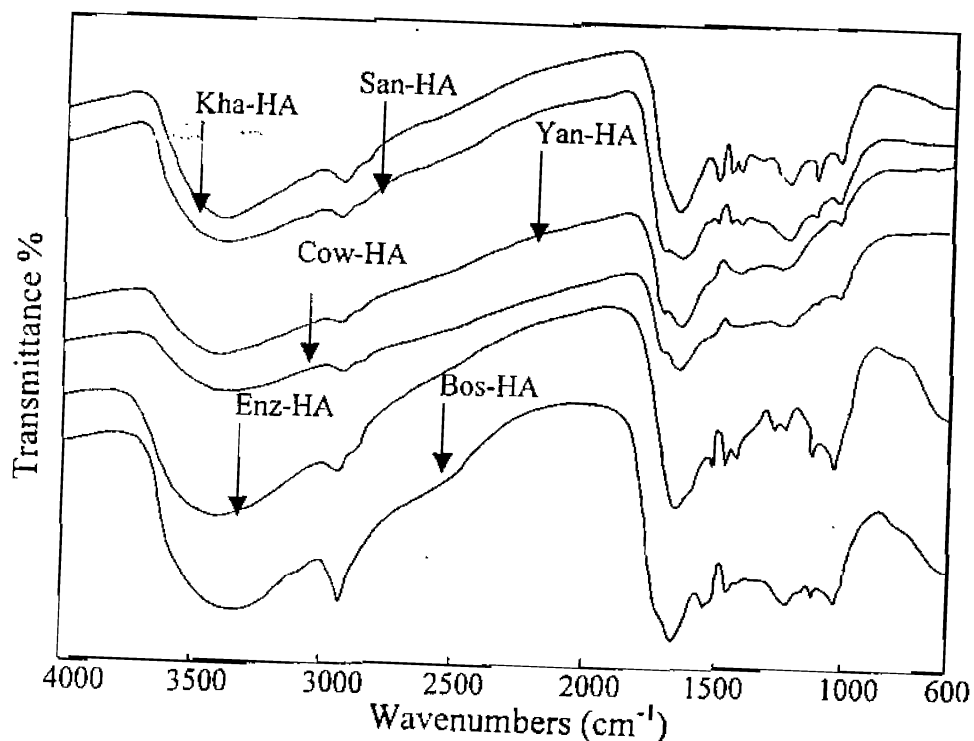


Fig. (3). IR spectra of the isolated humic acids.

The IR results of the isolated humic acids confirm the compositional analysis results by showing the existence of marked structural and functional differences between these humic acids.

In general, it could be said that the IR spectra of the studied humic acids supports the interpretation that structure of these humic acids are rather similar, at least in the constructure of the number of the internal "nucleus". They differ mainly in the ratios of the number of functional groups and the degree of polymerization. These data are in good agreement with those reported by Stevenson(1982); Amer(1986); AboEl-Fadi *et al.*,(1992); Taha *et al.* (1993) and Soler Rovira *et al.*(2002).

Table 3: Absorption bands in the IR spectra of humic acids.

Wave number (cm ⁻¹)	Functional group
3318 - 3403	H-bonded OH and N-H stretching
2925 - 2937	aliphatic C-H stretching
1710 - 1713	C=O stretching
1641 - 1660	amides and aromatic C=C stretching
1510 - 1540	N-H and C=N stretching of amide
1450 - 1461	aliphatic C-H bending
1412 - 1422	OH deformation and C-O stretching
1220 - 1267	C-O stretching and C-O stretching of ethers
1124 - 1127	C-O of alcoholic and ethers
1030- 1034	C-O stretching of polysaccharides

REFERENCES

- Abo El-Fadl, M.; N.A. Bayoumi and S.M.A. El-Babary (1992). Characteristics of humic acids extracted from some soils of the Nile Delta in Egypt. *Egypt J. Soil Sci.*, 32(1): 119-129.
- Amer, A.A. (1986). Distribution and composition of humic substances as related to pedological feature of some alluvial soils at Kafr El-Sheikh Governorate. Ph.D. These, Fac. Agric. Alex. Univ., Egypt.
- Chen, Y.; N. Senesi and M. Schnitzer (1977). Information provided on humic substances by E_4/E_6 ratios. *Soil Sci. Soc. Am. J.*, 41: 352-358.
- Deiana, S.; C. Gessa; B. Manunza; R. Rausa and R. Seeber (1990). Analytical and spectroscopic characterization of humic acids extracted from sewage sludge, manure and worm compost. *Soil Sci.*, 150 (1): 419-424.
- Drozd, J. and M. Licznar (1983). Some properties of humic acids of the recent and fossil soils from eroded areas. In: *Studies about humus. International symposium, Prague- CSSR, August 1983.*
- Flaig, W.; H. Beutelspacher and E. Rietz (1975). Chemical composition and physical properties of humic substances. In: *Soil Components. Vol. I. Organic Components* (Ed. J.E. Giesking). pp. 1-219. Springer-Verlag, New York.
- Gomah, A.M.; M.H. El-Halafawi and T.M. El-Essawi (1978). Some chemical characteristics of humic acids isolated from different sources. *Alex. J. Agric. Rec.*, 26: 747-754.
- Hayes, M.H.B. and R.S. Swift (1978). The chemistry of soil organic colloids. In: *The chemistry of soil constituents. D.J. Greenland and M.H.B. Hayes (Eds.)*. pp. 179-320. Wiley-Interscience publication. New York.
- Ishiwatari, R.I. (1985). Geochemistry of humic substances in lake sediments. In: *Humic substances in soil, sediment and water. G.R. Aiken, D. M. McKnight, R.L. Wershaw, and P. MacCarthy (eds.)* Wiley-Interscience, New York, pp. 147-180.
- Kaloosh, A.A. (1984). Chemical composition and physical properties of humus extracted from some Egyptian soils. Ph.D. These, Fac. Agric., Alex. Univ., Egypt.
- Kononova, M.M. (1966). "Soil Organic Matter". 2nd Ed. Pergamon Press. Oxford.
- Lichszteid, K. (1983). The application of the absorption spectrophotometry in the UV region to the functional groups in humic acids. In: *Studies about humus. International symposium, Prague- CSSR, August 1983.*
- Schnitzer, M. (1977). Recent findings on the characterization of humic substances extracted from soils from widely differing climatic zones. In: *Soil organic matter studies. Vol. II. I.A.E.A., Vienna*, 117-132.
- Schnitzer, M. (1978). Humic Substances: Chemistry and reactions. In: "Soil Organic Matter". Schnitzer, M. and Khan, S.U. (Eds.). Elsevier, Amsterdam, Oxford, New York, 1-64.

- Schnitzer, M. (1985). Nature of nitrogen in humic substances. In: Humic substances in soil, sediment and water. G.R. Aiken, D.M. McKnight, R.L. Wershaw and P. MacCarthy (eds.). Wiley - Interscience, New York, 303-325.
- Schnitzer, M. and S.U. Khan (1972). "Humic Substances In The Environment". Marcel Dekker, Inc., New York.
- Soler Rovira, P.A.; G. Brunetti; A. Polo and N. Senesi (2002). Comparative chemical and spectroscopic characterization of humic acids from sewage sludges and sludge- amended soils. Soil Sci., 167 (4): 235-245.
- Steelink, C. (1985). Implications of elemental characteristics of humic substances. p.457-476. In: G.R. Aiken *et al.*(Ed.) humic substances in soil, sediment and water. John Wiley & Sons, New York.
- Stevenson, F. J. (1982). "Humus Chemistry ". John Wiley and Sons, New York.
- Taha, A.A. (1989). Chemical characteristics of humic acids isolated from different soils. J. Agric.Sci. Mansoura Univ., 14: 835-843.
- Taha, A.A. (1991). Some properties of humic acids extracted from some different soils. Egypt. J. Appl. Sci., 6 (10): 346-358.
- Taha, A.A.; A.O. Abd El-Naby and M.Y. El-Arquan (1993). Study on structure of some humic acids isolated from different sources. J. Agric. Sci. Mansoura Univ., 18(7): 2169- 2179.
- Visser, S.A. (1964). A physico- chemical study of the properties of humic acids and their changes during humification. J. Soil Sci., 15: 202-219.

دراسات كيميائية وضوئية على أحماض الهيوميك المستخلصة من بعض الأسمدة العضوية (الكومبوست).

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تم إستخلاص أحماض الهيوميك (Humic acids) من بعض الأسمدة العضوية الصناعية (الكومبوست) ولقد تم دراسة هذه الأحماض بالطرق الكيميائية و الضوئية. ولوضحت النتائج أن الأحماض الذبالية المستخلصة تحتوي على عناصر الكربون والهيدروجين والنيتروجين بالإضافة إلى الأكسجين، وأن كمية هذه العناصر لم تختلف كثيراً تبعاً لإختلاف نوع السماد العضوي وهذا يدل على أن هذه الأحماض تتشابه كثيراً من ناحية التركيب الكيميائي. ولكن وجد أن نسبة ك / يد ، أ / يد في الحامض الذبالي المعزول من سماد ينبت (Yan-HA) كانت أعلى من بقية الأحماض الذبالية الأخرى وهذا يدل على أن هذا الحامض في حالة أكثر نضجاً وتنبلاً (mature). وقد أظهرت منحنيات الإمتصاص في الضوء المرئي (Visible) وفي منطقة الأشعة فوق البنفسجية (UV) لهذه الأحماض الذبالية أنها متشابهة لحد كبير وأنها لم تظهر حد أدنى أو أعلى للإمتصاص. ووجد أن قيمة نسبة الـ E₄/E₆ لكل من حمض San-HA ، وحمض Enz-HA كانت قليلة جداً عن بقية الأحماض الذبالية الأخرى مما يدل على أن هذين الحمضين في درجة عالية من التركيب الحثلي ومحتوى أقل من المركبات الأليفاتية. أما دراسة الأحماض الذبالية باستخدام الأشعة تحت الحمراء (IR) فقد أظهرت النتائج أن هذه الأحماض متشابهة فيما بينها من حيث التركيب الكيميائي وأن الإختلافات فيها كانت بسيطة جداً وتمثلت في الإختلاف في عدد المجاميع الفعالة وفي درجة بلورة هذه الأحماض. ومن جهة أخرى فقد وجد أن حمض Bos-HA يحتوي على كربون أليفاتي (aliphatic carbon) أكثر من الأحماض الذبالية الأخرى.