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PREPARATION CONDITIONS OF HUMIC ACIDS FROM VILLAGE'S COMPOST FUEL (VCF) AND THEIR EFFECTS ON HUMIC ACIDS CHEMICAL CHARACTERISTIC ALTERNATION DURING ISOLATION

PART II. EFFECT OF THE EXTRACTION CONDITIONS ON THE FUNCTIONAL GROUPS CONTENTS OF HUMIC ACIDS AS REVEALED BY SPECTROSCOPIC ANALYSIS

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Abstract

IR spectra of humic acids (HAs) extracted by NaOH solution under different conditions showed that HAs extracted by 0.1 M after 6h and 16 h have identical finger print regions. These spectra indicate the presence of highest number of functional groups and highest aromatic COOH group content, in comparison with (HAs) extracted by 0.1M after 2h and 24 h. IR spectrum of humic acids HAs extracted by 0.2M after 2h was generally similar with the two latter spectra. The IR spectra of HAs extracted after 2h by 0.5M, 1M, and 2M not only show the disappearance of aromatic COOH bands but also indicate the hydrolysis of ester moieties and increase the OH groups content of the extracted HAs. The IR spectra of HAs extracted at the latter three concentrations showed attacked finger print regions. Uv-Vis spectra show two clearly observable peaks at 208 and 280 nm which are characteristic to these humic acids. The spectral change of the latter are in agreement with the results obtained by IR analysis According to results obtained, the extraction by 0.1M NaOH after 16h leads to production of HAs of highest condensation, highest functional group content.

Introduction

Humic substances (HSs) are the major organic constituent of soil, they are formed through plant and animal tissues breakdown by chemical and biological processes. They are widely distributed over earth's surface and occur in almost all terrestrial and aquatic environments (Tatjana *et al.*, 2001). HSs can be divided into three components : Fulvic acid (FAs), humic acids (HAs) and humin, as shown in Fig. (1). The important part of HSs is HAs. HAS are natural dark solid paramagnetic, macromolecular, heterogeneous substances with a high degree of polydispersity, extremely complex structure and high molecular weight of 10^3 - 10^5 Da (Da = Dalton(1 a.m.u)).They contain similar functional groups such as C=C, COOH, OH, OCH₃, CO, or NH₂, arrangements such as redox qunone, semiquinon-hydroquinone, charge transfer planar complexes... etc. Humic acids have remarkable properties and are used in many practical applications, Eladia (2005). When applied to soil they influence significantly the quality and productivity of the

soil in addition to the improvement of the soil physical properties and soil fertility Lotosh (1991), Zhang and He (2004). HAs have good effects on seed germination and stimulation of plant growth, Miguel et.al.(1996). Industrially humus and humus containing materials have been used in concrete, leather, wood working, ceramic, paper industry. Humic acids are used in production of plastics, electricity conducting paper and food processing Eladia(2005). Also, it enhance extraction of uranium from its ores, Duncan *et. al.*(1981). Humic substances are produced on a large commercial scale and are used in veterinary and human medicine. Another reason for increasing attention devoted to humic acids is their antiviral, profibrinolytic and anti-inflammatory and estrogenic activity Yamada *et. al.* (1998), antibacterial, Anorg (1978), Skliar *et. al.* (1998) and anticancer activities Van Rensburg *et al.* (2002).

Humic substances can be extracted from soil (1-5% in the highest 20-30 mm soil layer), Tatjana (2001), organic dungs (5-15%) Aziz et al. (2000), Natural Water, Merk Burian (2003), peat (10-40%), Cedric et al. (2000), Lignite (10-30%) and can be reach to 50-80% by oxidation under special conditions, Bituminous coal by exposure to oxygen in stirred pressure vessel at 40 bar and 180oC, Van de Venter et al., (1991), and Leonardite Nobili (1990).

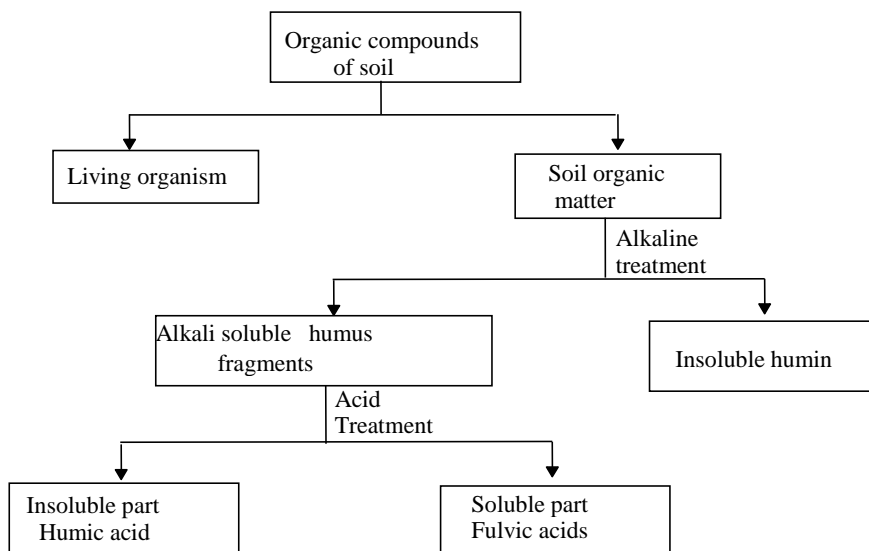


Fig. (1) Scheme of divisions of humic substances in dependence of their alkaline solubility (Eladia *et al.*, 2005).

Several extractants have been used for extraction of humic substance including for example, NaOH, KOH, Na₂CO₃, Na₂P₂O₇, EDTA, organic solvents, acetylacetone and formic acid. The problems found during alkaline extraction include autooxidation and alternation of the product during extraction, so the extraction carried out in absence of air, Alken, *et. al.*, (1985), Kim (1998). In spite of the importance of humic substances, their commercial production and marketing at many areas in the world, their structure remains largely unknown as a consequence of their heterogeneity and great complexity. Some formulae were suggested, e.g. Stevensen(1982), Donald (1995). The studying of different methods of extraction, analytical characteristics, their reaction with heavy metals, their biological and environmental effects are general trends in humic acids researches.

According to the available large number of literatures, there is little concern about studying the alternation of humic acids during extraction with NaOH. In Part I, Badr (2004), the effect of NaOH solutions of different strengths on some characteristics such as elemental analysis, acidity and stimulating abilities were studied. It was found that the stimulating abilities of the extracted HAs on germination of seeds were affected by the extraction conditions. The aim of this study includes the studying of the FT-IR and Uv- Vis spectra of HAs extracted by NaOH solutions of different strengths and after different extraction intervals. Also to determine the optimum extraction condition required to extract HAs of least alternation during extraction, and contain the highest content of functional groups, especially those responsible for their biological activity i.e. COOH and OH groups Cacco and Dell'Agnola (1984), Piccolo *et al.* (1992)

Material and methods

The VCF source (Village's Compost Fuel) is a type of fuel was used in Egyptian villages and made from cattle manure mixed with plant residues. This was grounded, the plant residues were removed as possible. The grounded fuel was used for extraction of humic acids as described before (Badr 2004) and outline as follows: Two sets of experiments were carried out. The first include the extraction of HAs by 0.1 M NaOH and the time was varied for 02, 06, 16m 24 hours. For the second set of experiments, the extraction time was 2 hours and different NaOH concentrations namely, 0.2, 0.50, 1.0 or 2.0M were used. The extracts were separated quickly by filtration through a suitable tissue and the pH was adjusted to 1-2 by adding HCl (2M) to precipitate humic acids. The solutions contained humic acids precipitates were left 24 hours for coagulation. The humic acids sludge were separated by decantation and filtration or centrifugation. The purification processes were carried out as usual, Badr (2003).The FT-IR spectra of pure and dried humic acids samples

were recorded over a wave number range 400-4000 cm^{-1} by using FT/IR-460, plus, Jasco, spectrophotometer using KBr disc method.. Ultraviolet and visible spectrophotometer measurements were carried out on humates solutions between 200-600 nm with a Unicon UV₂-300 UV/visible spectrophotometer using suitable blank. Humates solution were prepared by dissolving 0.02g of each HAs in minimum amount of 0.1M KOH under N₂ the volume was completed to 50 ml to 0.2 M KCl. pH was adjusted to 7, Theng *et. al.*, (1967).

Results and Discussion

FTIR Analysis

Effect Of Extraction Time

The FT-IR spectra of HAs extracted by 0.1M NaOH solution under different time intervals are shown in Fig. (2). Table (2) show the assignments of group wave number of HAs extracted from the given source by 0.1M NaOH and 2h. Table (3) shows the assignment of the additional bands appear or disappear by increasing the extraction time to 6,16 and 24hr. Table (4) shows the band width of 1655 cm^{-1} peak with some obtained results for HAs extracted under the conditions studied

The IR spectra of HAs extracted by 0.1 M NaOH after 2hr is shown in Fig. (2-c). These bands are in general agreement with those characteristics to humic acids e.g. Kim (1998), Tatjana *et.al.*(2001)and Soumaia *et.al.*(2003). By prolonging the time of extraction to 6h., HAs gave the same bands with five additional bands as shown in the Fig. (2-b) and assigned in Table (3). By increasing time to 16h, the above effect was observed but the band became more clear, Fig. (2-a) and Table (3). Generally it can be concluded that, the dissolution of a new humic acid component or components, which attached to one or more of these assignments increased with increase the time of extraction to 16 h. Fig (2-d) shows the IR spectra of HAs extracted by increasing the time of extraction to 24 h. In the latter spectrum, the five additional bands at 2645.2, 2548.9, 1677.4, 887.1, and 778.3 Cm^{-1} outlined and assigned in Table (3) are disappeared. This means the removal of these assignments by increasing the time of extraction to 24h.

It is known that C=O group of carboxylic acids is in the same region as C=O stretch of ketons, aldehydes, and esters. The group wave numbers required to confirm the assignment of carboxylic are as summarized in Table (1) (Brian 1998). By comparing the spectrum at 2h and 6h -spectra c, b in Fig. (1)- it is noted that the bands at 1677 cm^{-1} and 887.1 cm^{-1} appear simultaneously with strengthen of bands at 1422 cm^{-1} and at 1223.4 cm^{-1} which are more pronounced at the spectrum (a) of

HAs extracted at 16h in Fig (1). According to data shown in Table (1), the latter effects may be due to aromatic carboxylic moieties. So it can be said that the prolonging of the extraction time to 16 h during the extraction by 0.1 M NaOH may lead to extraction of HAs include components rich in carboxylic groups which are strongly H bonded through OH groups.

Table (1): The group wave number of carboxylic acid (all number in cm^{-1})

vibration	Wave number range
Saturated C=O stretch	1730-1700
Aromatic C=O stretch	1710-1680
C-O stretch	1320-1210
O-H stretch	3500-2500 (broad and intense)
O-H in-plane bend	1440-1395
O-H out-of-plane bend	960-900

By prolonging the time of extraction to 24h, the obtained spectrum was similar to that obtained for HAs extracted after 2hr with disappearance of the five additional bands and the effect of carboxylic groups discussed above is removed. This means that a component or components may lose their carboxylic groups. This decarboxylation effect is confirmed by the observed shifting of the related band at 3390.8 cm^{-1} of HAs extracted after 24h toward higher energy in comparison with the bands of HAs extracted after 16h, according to Hook's law (Barian 1998). The increase of carboxylic groups concentration with increasing time to 6 and 16 h, then decreasing with increasing time to 24h is confirmed by chemical analysis, as shown in Table (4).

Broad infrared bands are observed for samples of many chemical environments, Brian (1998). Humic acids show many broad bands. Among these is the band at 1655 cm^{-1} . It was found that there is a gradual increase in this band width with molecular weight of HAs fractions. Aziz *et al.* (2000). $Q_{4/6}$ (ratio of absorbance at 400nm to absorbance at 600nm of sodium humates solution) is another indication of molecular weight where it decreases with increasing of molecular weight. According to data available in Table (4) for HAs isolated after 2, 6, 16h, both $Q_{4/6}$ ratio and band width at 1655 cm^{-1} varied in opposite direction and a maximum band width observed for HAs of least $Q_{4/6}$ which correspond to highest molecular weight (i.e. highest condensation). The general coincidence of the finger print region of the spectra in fig (1) indicate that components of HAS extracted by 0.1 M NaOH under this set of experiments including change of time to 2,6,16 and 24hr have generally the same identities. This confirms that the latter conditions do not affect HAs basic structures but their action only were on the functional groups.

Table (2) The assignments of Group wave Numbers of VCF humic acids (Has) Extracted by 0.1 M NaOH solution at 2h

Region	$\bar{\nu}cm^{-1}$	Assignments
Hydrogen-stretching region 3700 cm^{-1}	3362.5	Broad and strong band of stretching of alcoholic and phenolic intermolecular H.bonding and probably including those of COOH and N-H group.
	3080.7	=CH ₂ asymmetric stretching
	2927.2	Aliphatic CH stretch of CH ₂ and CH ₃ .
	2830.7	
Triple bond stretching region	2387.7 2338.0	-NH ₂ , >NH, ≡N (2400-2200)
Double and triple stretching region 2000-1600	1655.0	C=O stretch amid I, quinones and aromatic C=C or/and 1-keto-2 hydroxy or amino aryl ketones
Finger print region	1450.3	aromatic C=C stretch, NH deformation in amide II.
	1513.2	
	1458.2	C-CH ₃
	1422.7	O-H in plane bending of COOH
	1362.9	in plane bend of phenolic OH
	1306.5	C-C-O stretch of aromatic ester, (1310-1282)
	1274.6	
	1226.0	O-C-C ester linkage of carboxylic and phenolic acid ,C-O stretch of COOH, CH ₂ twisting
	1124.9	O-C-C of ester,C-OH, C-C, typical of glucoside linkage polymeric substance and Si-O impurities of humic acid.
1030.7	orthosubstitution, C-C-O of primary alcohol	
Aromatic ring vibration region	832.2	out plane vibration of isolated C-H group found in bituminous brown coal also due to cis cyclic ether, epoxy compound
	734	O-H vibration of COOH

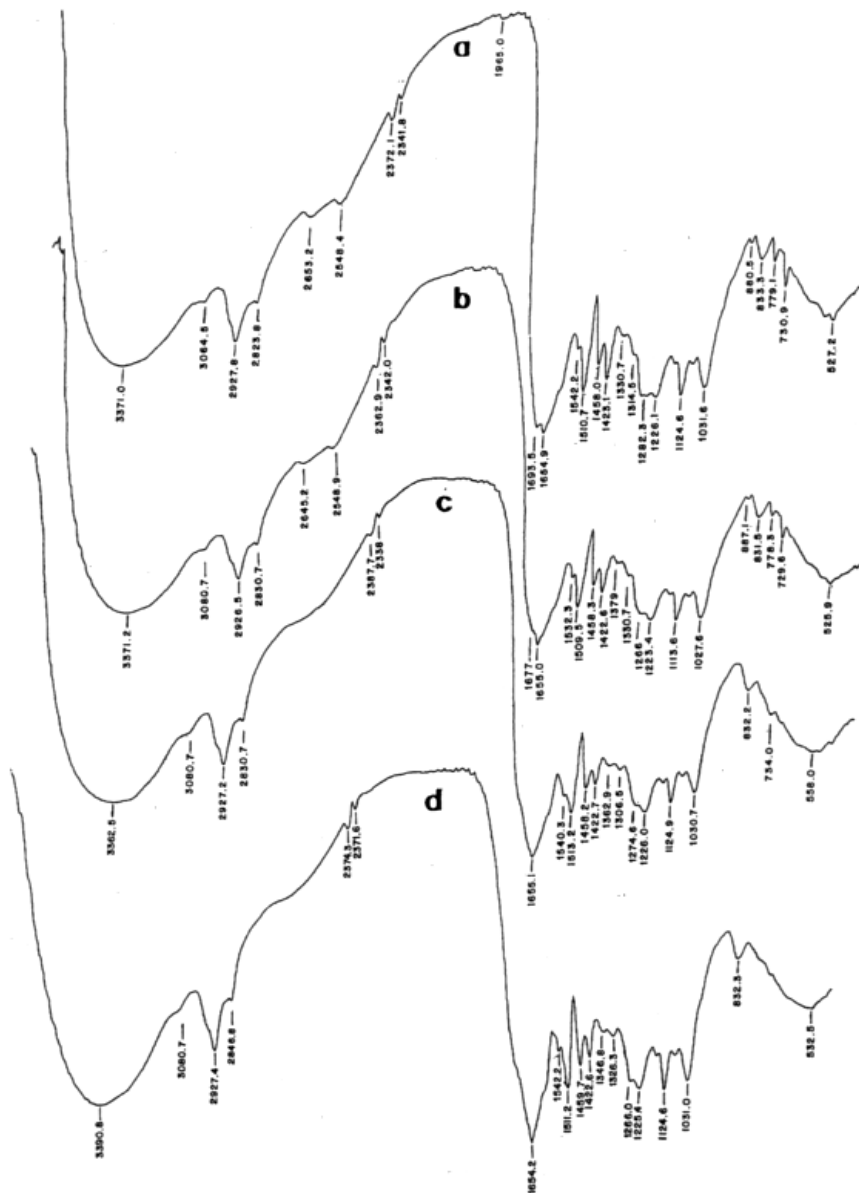


Fig. (2): FT-IR spectra of HAs isolated by 0.1 M NaOH after :
 (a) 16h, (b) 6h, (c) 2h and (d) 24h

Table (3): Effect of increasing time more than 2 hours, during the extraction of humic acids from VCF source by 0.1 M NaOH solution on IR bands (all wave length in cm⁻¹).

Time	6		16		24		bands assignments
Effect	app.	disapp.	app.	disapp.	app.	disapp.	
Wave number $\bar{\nu}$ in cm ⁻¹	2645.2 2548.9	--	2653.2 2548.4	--	--	2645.2 (2548.4)	absorption between 2700-2500 is indicative only of a strongly H bonded OH groups also 2800-2500 and combination of bands
	1677.4	--	1693.5	--	--	1677.4 (1693.5)	Aromatic C=O RCOOH where R is a conjugated
	887.1	--	880.5	--	--	887.1 (880.5)	isolated aromatic CH resulting from condensation, trans cyclic ether (1) epoxy also NH ₂ wag (882), (-N stretch of H-CONH ₂). and O-H out of plane bend of COOH
	778.3		779.1			778.3 (779.1)	1,2,4 trisubstituted (diagnostic band of two adjacent H)

Table (4): Band width at 1655 cm⁻¹ peak in comparison with some results*

Condition	(COOH)	(OH)	Q _{4/6}	1655 cm ⁻¹ band width	**GI%
2h by 0.1M	1.70	5.30	8.53	143.7	152
6	2.43	3.18	8.01	155.4	190
16	4.56	4.48	6.60	158.7	390
24	1.88	3.73	9.10	146.1	331
0.2 M at 2h	1.58	4.98	9.33	155.0	129
0.5	1.41	4.44	9.18	153.1	412
1.0	1.06	4.84	8.2	151.0	187
2.0	1.06	5.16	9.03	142.03	467

*Badr 2004, ** GI% the germination index is a quantity which combined the effect of HAs on percentage of germination and root growth (Miquel *et al.*, 1996).

Effect of NaOH concentration

This part of discussion is concerned with the effect of using NaOH solutions of different strengths after 2h extraction interval on the functional groups contents of HAs. Fig. (3) Shows the FT-IR spectra of HAS extracted under these conditions. By comparing the spectra (a) and (b) in Fig. (3), it is observed that the spectra of HAS extracted at 0.2 M NaOH, (b), is free from the five bands showed and assigned in Table (3). This means that extraction by 0.2 M NaOH after 2h has the effect caused by the extraction with 0.1 M NaOH after 24 h. The increasing of NaOH concentration to 0.5M, 1 M and 2M has the latter effect, also as shown by the spectra (c), (d) and (e) in Fig. (3) respectively. Moreover, it is clear from comparison of these spectra with that of HAs extract after 16h, fig (3 -a) that the finger print region began to be attacked with appearance and disappearance of many bands. The most observable changes occurred in, this case include, diminishing then disappearance of bands at 1330.7 cm^{-1} , 1314 cm^{-1} and 1282 cm^{-1} with simultaneous strengthen of the bands at 1030 cm^{-1} which overlapped with 1124 cm^{-1} band. According to Brain (1998) aromatic ester moieties may contribute to the bands at wave number: 1655 cm^{-1} (C=O stretch), 1314 cm^{-1} , 1282 , (C-C-O stretch), 1124 cm^{-1} (O-C-C stretch). The "O-C-C Stretch" Vibration involves the right hand side of the ester functional group and is very similar to C-C-O stretch of alcohol. So it can be concluded that the increase of NaOH concentration cause replacement of "O-C-C stretch" of ester by the very similar C-C-O stretch of alcohol and cause its strengthen. Accordingly, it can be said that during extraction of humic acid by NaOH solutions ester moieties of HAs undergo hydrolysis and more of these moieties hydrolyzed as concentration NaOH increased. This means that more OH groups were produced on HAs by increasing NaOH concentration which is accompanied by degradation of humic acids molecules. The starting of appearance of C-C-O stretch of alcohol at 976.6 cm^{-1} by using 0.5 M NaOH as shown in Fig. (3-c) and its strengthen by using 1M, 2M NaOH confirm the hydrolysis process and indicates that it begin to occur by using 0.5 M NaOH. The increase of HAs content of the OH groups, by increasing NaOH was confirmed by chemical analysis as shown in Table (4).

Generally, it can be concluded that, the isolation of humic acids from VCF source by 0.1M NaOH after 24 h or by 0.2M NaOH after 2h lead to isolation of humic acids of low carboxylic group's content. The extraction of humic acids by 0.5M, 1M or 2M NaOH after 2h lead to isolation of hydrolyzed HAs. But the extraction by 0.1M NaOH after 16h leads to isolation of HAs of highest COOH group's content, highest condensation.

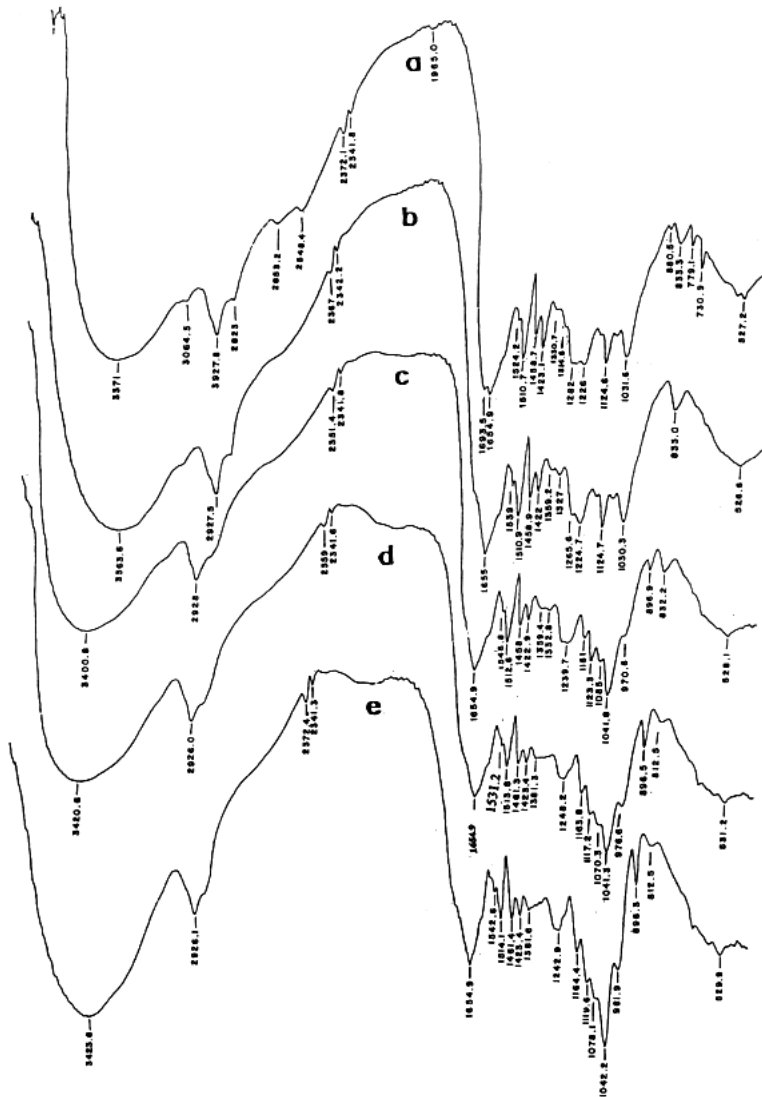


Fig. (3): FT-IR spectra of HAs isolated after 2h by (b) (0.2), (c) 0.5, (d) 1.0 and (e) 2.0 M NaOH in comparison with that of HAs excreted by 0.1 M after 16h (a)

Uv-Vis spectra under different extraction conditions

Generally, the UV-visible spectra of all HAs samples studied exhibit a continuous decrease in absorption with wavelength increasing as a typical with humic acids. Theng *et al.* (1967), Geoger (1985) Kim (1998), Soumia *et al.* (2003) Ludmila *et al.*, (2003). As seen from Fig (9) and Fig. (10), all spectra are similar,

this confirm the general resemblance of the basic structure of HAs obtained at all extraction condition studied. All humic acids obtained also gave two clear bands at 208 nm and 280 nm which are characteristic to these HAs and are attributed to $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ of CO groups, Theng (1967) and Gary (1980) which is confirmed by ir analysis. The change in the extraction conditions resulted in very clear spectral change in the absorbances intensities. As observed from Fig (9)and Fig (11 a,c), the increasing of the extraction time till 16h lead hyperchromism effect (increase in absorption intensities) at $\lambda_{max} = 208$ and 280 nm. Further increasing of the extraction time to 24h show hypochromism (decrease in absorption intensities) at $\lambda_{max} = 208$ and 280 nm. Accordingly it can be concluded that the increasing of the extraction time lead to increase of carbonyl groups content till 16h and further increase of time to 24h result in their decrease. after 2 h extraction time, NaOH solutions of different concentrations used lead to hypochromism spectral change at $\lambda_{max} = 208$ and 280 nm, Fig. (10), this leads to conclude that decrease in CO group resulted by increasing NaOH concentration during HAs extraction. These findings are in agreement with results obtained by both chemical and FT-TR analysis.

So HAs extracted by 0.1M NaOH after 16 h. have the highest number and highest concentration of functional groups and they are the least degraded humic acids can be obtained from this source by NaOH solution.

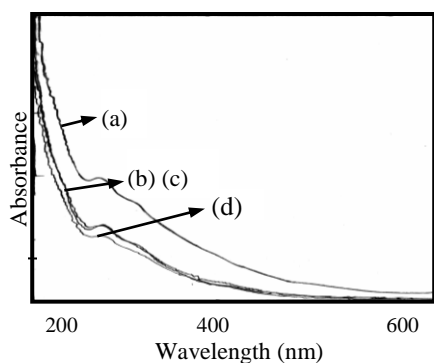


Fig. (4) UV-Vis spectra of humate solution (PH = 7) prepared from HAS extracted from VCF source by 0.1M NaOH after different extraction time(a) 16h, (b) 24h, (c) 6h, (d) 2h.

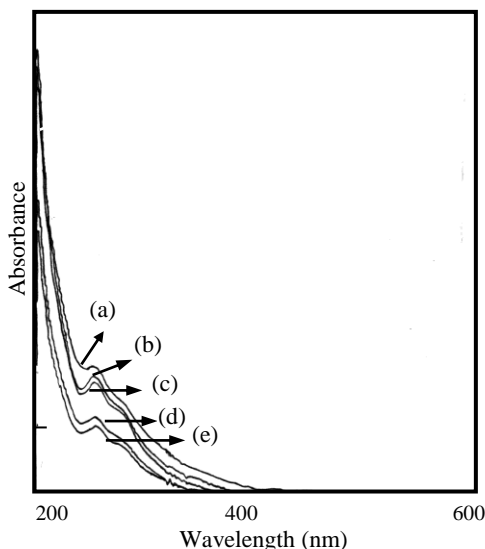


Fig. (4) UV-Vis spectra of humate solution (PH = 7) prepared from HAS extracted from VCF source by 0.1M NaOH after different extraction time (a) 16h, (b) 24h (c) 6h, (d) 2h

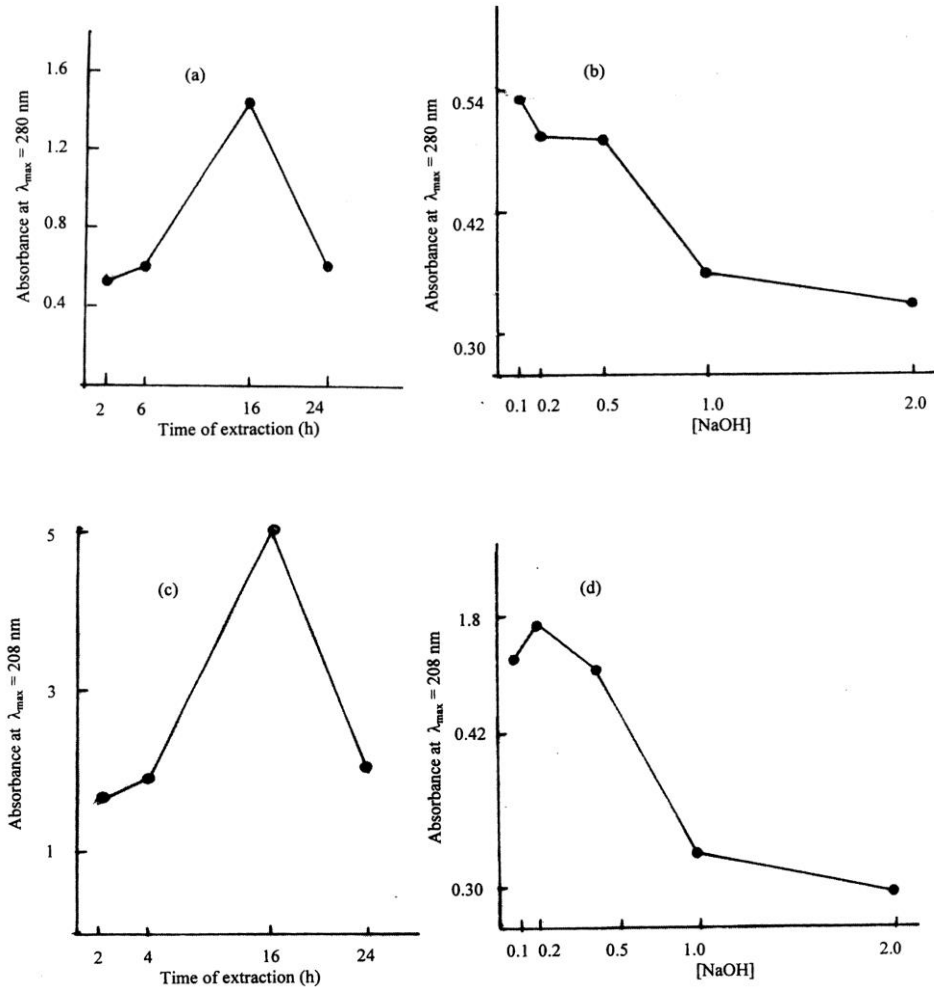


Fig. (6): Spectral change in absorption intensities at $\lambda_{\max} = 208$ nm and at $\lambda_{\max} = 280$ nm under the effect of change in the extraction conditions. Extraction of HAs by 0.1M NaOH and change in extraction time(a,c). extraction at 2h and change in [NaOH]. (b,d)

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تأثير ظروف التحضير علي تغير الخواص الكيميائية لأحماض الهيوميك أثناء استخلاصها من وقود الروث الريفي .

الجزء الثاني دراسة تأثير ظروف التفاعل على محتويات أحماض الهيوميك من المجموعات الفعالة باستخدام التحليل الطيفي

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أحماض الهيوميك هي مخلوط طبيعي من عدة أحماض هيوميك متشابهة تتواجد معاً وتستخلص معاً وتختلف حسب المصدر. وهي ذات أوزان جزئية عالية وتركيب كيميائي غير معروف وتحتوي على عدد كبير من المجموعات الفعالة . هذه الأحماض هي جزء من مادة الهيوموس الناتج الطبيعي والنهائي لتحلل المواد النباتية والحيوانية لذلك فهي تتواجد في الفحم ، والبتروول ، التربة الزراعية ، المياه الطبيعية ، المخلفات النباتية المتحللة . و تلعب المجموعات الفعالة خاصة مجموعات الكربوكسيل و الهيدروكسيل دورا هاما في النشاط البيولوجي لها ، وأحماض الهيوميك موضع اهتمام كبير في الوقت الحالي نظراً للإقبال علي استخدامها في مجالات متعددة هامة،مثل استخلاص اليورانيوم. هذا إلي جانب دورها الهام في منع التلوث أو أحداث تلوث خطير في المياه عند تواجدها مع الكلور . ويهدف هذا البحث إلي دراسة تأثير ظروف التفاعل على محتوى أحماض الهيوميك من المجموعات الفعالة باستخدام التحليل الطيفي بهدف استخلاص أحماض الهيوميك من مخلفات المزارع الحيوانية ، ودراسة استخدامها في تحضير محاليل منشطة لنمو النبات بغرض زيادة الإنتاج الزراعي.

ونظراً لاختلاف تركيز محلول هيدروكسيد الصوديوم المستخدم بكثرة في استخلاص الأحماض من باحث لآخر ، كذلك وقت الاستخلاص ،فقد هدفت هذه الدراسة إلي استخدام كل الظروف المشار إليها و المستخدمة من قبل الباحثين لتطبيقها على استخلاص أحماض الهيوميك من هذا المصدر ثم دراسة التحليل الطيفي للنواتج لتحديد تأثير الظروف المختلفة علي محتوى الأحماض من المجموعات الفعالة ،ولتحديد أنسب الطرق لاستخلاص الأحماض من المصدر المختار . والمصدر المستخدم في هذه الدراسة هو نوع من الوقود المصنوع من روث الماشية والذي يمكن الحصول منه علي طاقة احتراق عالية، وقد أوضحت الدراسة أن أحماض الهيوميك المستخلصة بواسطة محلول 0,1 ، 0 عياري لفترة 6 ساعات أو 16 ساعة لها نفس طيف امتصاص الأشعة تحت الحمراء والمميز لأحماض الهيوميك ، وأنها تحتوي علي وجود أكبر عدد من المجموعات الفعالة ، كذلك تحتوي علي تركيز عالي من مجموعات الكربوكسيل خاصة المستخلصة لمدة 16 ساعة بالمقارنة بالأحماض المستخلصة بنفس المحلول لمدة 2 ساعة أو 24 ساعة . أما طيف الأشعة تحت الحمراء للأحماض المستخلصة لمدة 2 ساعة بواسطة محلول 0,2 ، 0 عياري فهو متشابه مع الطيفين في الحالتين الأخيرتين مع حدوث إزاحة لحزم الامتصاص .

أما طيف الأشعة تحت الحمراء للأحماض المستخلصة لمدة 2 ساعة بواسطة محلول 0,5 ، 1,0 ، 0,2 عياري فلم توضح الأطياف اختفاء مجموعات الكربوكسيل فقط ولكن أوضحت أيضا حدوث تمويؤ لمجموعات أستر أروماتية نتج عنه زيادة محتوى أحماض الهيوميك من مجموعات الهيدروكسيل ، كما أن الأطياف في الحالات الثلاثة الأخيرة غير متطابقة تماماً مع الأطياف الأخرى في منطقة بصمة الإصبع مما يشير إلي حدوث تغير في البنية الأساسية لهذه الأحماض. و بناء على ما سبق فإن انسب الظروف لاستخلاص الحمض من هذا المصدر هو محلول 0.1 ع هيدروكسيد الصوديوم وزمن استخلاص قدره 16 ساعة. ان الأحماض المستخلصة تحت هذه الظروف تحتوي تركيز أعلى من مجموعات الكربوكسيل والهيدروكسيل، و قد تأكدت هذه النتيجة بدراسة طيف الأشعة المرئية وفوق البنفسجية، ومسبقا بالتحاليل الكيميائية.