

APPLICATION OF ¹H-NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY TO STUDY THE STRUCTURE OF SOME HUMIC ACIDS

Taha ,A.A.; A.S. Modaihsh and M.O. Mahjoub
Soil Sci. Dept., College of Agric., King Saud University, Saudi Arabia

ABSTRACT

A study has been made using the ¹H-n.m.r. spectroscopic technique to investigate the structure of some humic acids extracted from different composts.

The results gave useful information about the nature of the extracted humic acids, which differed slightly from one to another and quite similar with a little difference in the intensity of the resonances. The ¹H-NMR spectra of the extracted humic acids identified polymethylene compounds and terminal methyl groups of polymethylene chains. In addition, protons attached to carbon β to carboxylic groups or aromatic rings have also identified. ¹H-NMR spectra of the studied humic acids showed that protons of aromatic rings are presented in humic acids.

The humic acid extracted from Bostan compost (Bos-HA) contained the highest aliphatic hydrogen content. On the other hand, humic acids from Sanbest (San-HA) and Al-Enzy (Enz-HA) composts exhibited the highest aromatic hydrogen content.

Keywords: compost, humic acids (HA), ¹H-NMR spectroscopy.

INTRODUCTION

Nuclear Magnetic Resonance spectroscopy (NMR) has been an invaluable technique in elucidating the structure of organic compounds.

Application of nuclear magnetic resonance to organic molecules is concerned largely with proton resonance. The resonance frequency varies slightly for hydrogens in different molecules, and for hydrogens in different environments in the molecule, so that different types of hydrogens in an unknown structure can be distinguished in a NMR spectrum (Stevenson, 1982).

¹H-NMR spectra have been obtained for soil humic acids (Lentz *et al.*, 1977 and Ruggiero *et al.*, 1980_b) but the results have provided little information on the chemical structure of humic substances. Spectra obtained for humic substances often show broad and poorly resolved resonances, possibly caused by their extreme molecular complexities. Ogner and Gronneberg (1977) have recorded the spectra of degraded humic materials. In all the ¹H-NMR spectra, the general features were the same. The signals at 0.90, 1.22 and 3.40 ppm were found. They concluded that the aromatic entities in humic substances are highly substituted and the aliphatic contributions of straight chains of 6-8 units are significant. Taha (1992) and Taha *et al.* (2000) investigated ¹H-NMR spectra for humic acids extracted from different soils. They reported that the signal from 6.0-8.3 ppm arises from aromatic protons including quinones, phenols and oxygen-containing heteroaromatic aromatics. Also, polycyclic aromatics can be characterized by a resonance at 8.1 ppm due to sterically hindered periprotons.

Taha, A. A. et al.

Recently, Fourier Transform NMR techniques (F.T.) have greatly improved spectral quality, and hitherto unobtainable spectra of complex mixtures have been recorded (Deiana, *et al.* 1990).

The objective of this study is to elucidate the structure of some humic acids extracted from different composts by means of ¹H-nuclear magnetic resonance.

MATERIALS AND METHODS

Humic acids were extracted and 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 wests; Bos-HA). Some chemical properties of these composts are given in Table (1).

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.

The humic acids were extracted from the previous composts with 0.1M NaOH solution according to the method of Kononova (1966) and Taha *et al.* (1993) using a sample : extract ratio of 1 : 5. The extracted humic acids were isolated and purified according to the method of Chen *et al.* (1978). Some chemical characteristics of the isolated humic acids are shown in Table (2).

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

Source of HA	%				Atomic ratio		
	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
Sanbest	50.55	4.45	4.90	40.10	12.04	0.95	0.56
Yanbost	46.91	4.16	6.34	42.59	8.63	0.94	0.64
Al-Kharj	44.16	4.41	7.49	43.94	6.88	0.83	0.62
Al-Enzy	44.09	5.42	4.01	46.48	12.83	0.68	0.54
Bostan	51.23	6.38	7.63	34.76	7.83	0.67	0.34

¹H-NMR :

Nuclear magnetic resonance protonic spectra were obtained by dissolving 50 mg of the purified humic acid in 2 ml of deuterated dimethyl

sulphoxide solution (DMSO-d₆) according to the method of Wilson *et al.* (1983). DMSO-d₆ was preferred to other organic solvents, since it is a good solvent for humic substances.

Analytical method :

¹H-NMR spectra were recorded using the Fourier- Transform technique (F.T.) as reported by Vila *et al.* (1976) and Deiana *et al.* (1990). ¹H-NMR spectra were recorded at 100 MHz on a JEOL – Eclipse*400 spectrometer by using an expansion scale of 2Hz mm⁻¹ and a sweep rate of 2 Hz sec⁻¹. The calibration of the spectra was done using a Hewlett-Packard 5521-A frequency counter. A small amount of tetramethylsilane (TMS) was added to generate the reference and lock signal.

RESULTS AND DISCUSSION

The most significant ¹H-n.m.r. of the extracted humic acids are shown in Figs. 1-6. It must be emphasized, however, that ¹H-n.m.r. spectra of all the studied humic acids are quite similar to each other, where each spectrum is consisting of a series of broad bands with a few difference in the resonances and its intensities. The ¹H-n.m.r. data and assignments recorded to date are summarized in Table 3.

As shown in Figs. 1-6, the ¹H-n.m.r. spectra of the studied humic acids are divided into three main ranges of chemical shifts : aliphatic hydrogen (0.0-3.0 ppm), aromatic hydrogen (6.0-8.5 ppm), and hydrogen atoms adjacent to strong electron-donating centers such as oxygen and to nitrogen-containing groups (3.0-5.0 ppm). In addition, four sharp resonances are present at 1.24, 2.15, 3.25 and 8.41 ppm. The region around 5 ppm is dominated by the exchangeable protons (Deiana,*et al.* 1990).

The sharp signal at 1.24 ppm appears to be a singlet and thus may arise from methyl groups α - to unsaturated groups. On the other hand, the sharp resonances at 2.15 and 3.25 ppm may arise from protons of methyl groups α - to carbon attached to oxygen groups, respectively.

As Fig. 6 shows, the humic acid which extracted from Bostan compost (Bos-HA) exhibited the striking differences because this humic acid has the highest aliphatic hydrogen content, which is mainly distributed between methylene (1.24 ppm) and terminal methyl (0.82 ppm) groups in long aliphatic chains (Preston, 1987).

As indicated from Figs. 1-6, the resonance at 0.82 ppm indicates methyl groups of alkyl chains at least three carbons removed from deshielding groups. Ogner and Gronneberg (1977) have recorded ¹H-n.m.r. spectra for different humic materials. In their studies it was indicated that in all the ¹H-n.m.r. spectra, the general features were the same. Signals at 0.9, 1.22 and 3.40 ppm were found. They concluded that the aromatic entities in humic substances are highly substituted and the aliphatic contributions of straight chains are significant.

On the other hand, Bos-HA contains higher content of protons in carbohydrates, peptidic and methoxyl functionalities as indicated by the large peaks at 2.54 and 3.25 ppm in Fig.6 and the data in Table 3.

Taha, A.-A. et al.

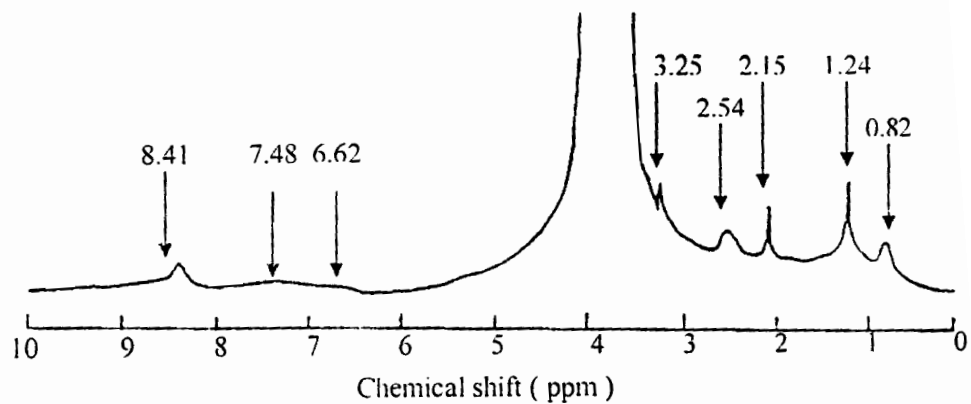


Fig. 1. ^1H - FT-n.m.r. spectrum of humic acid from Khalidia compost.

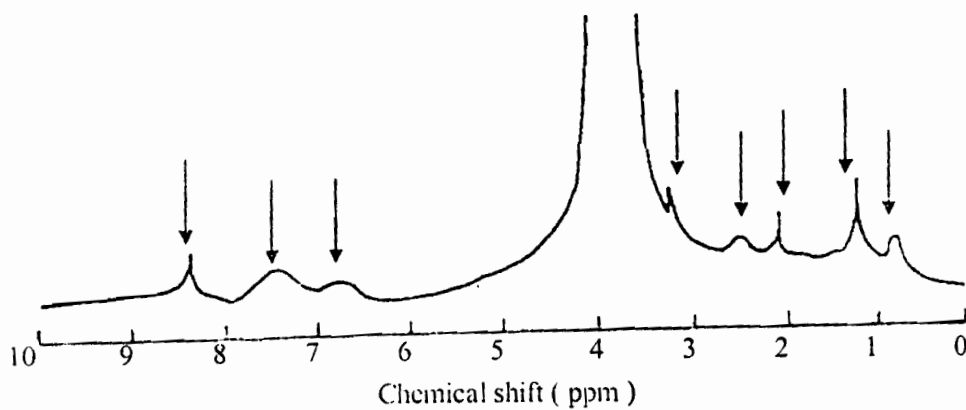


Fig. 2. ^1H - FT-n.m.r. spectrum of humic acid from Sanbest compost.

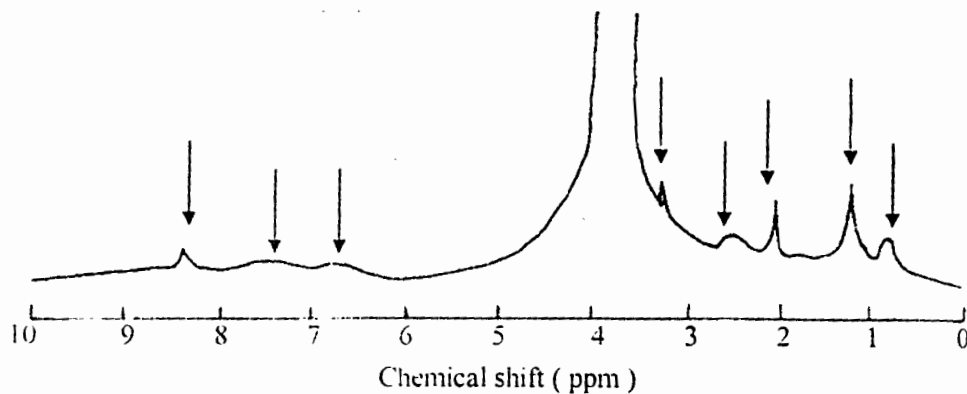


Fig. 3. ^1H - FT-n.m.r. spectrum of humic acid from Yanbost compost.

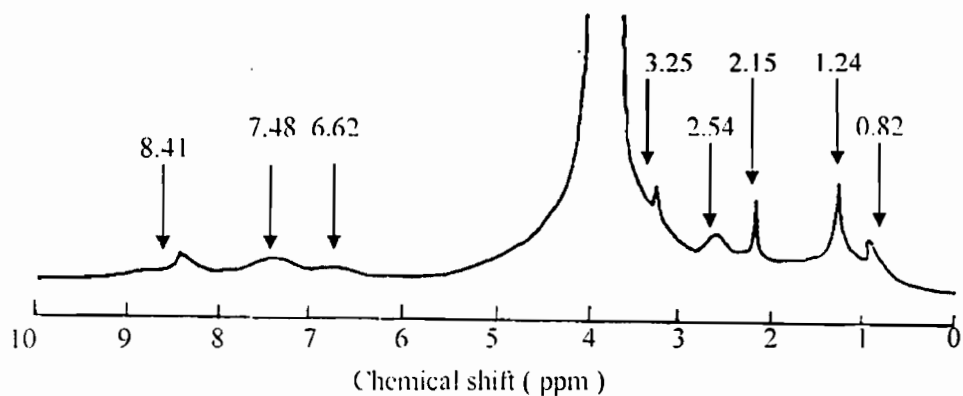


Fig. 4. ^1H - FT-n.m.r. spectrum of humic acid from Al-Kharj compost.

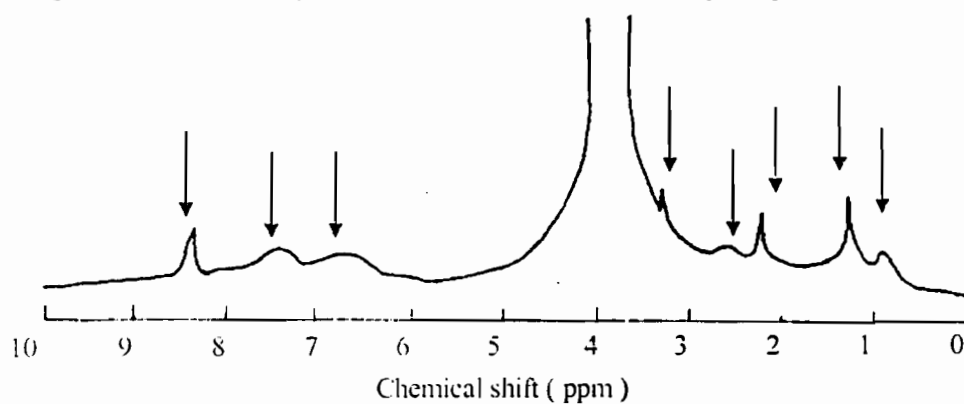


Fig. 5. ^1H - FT-n.m.r. spectrum of humic acid from Al-Enzy compost.

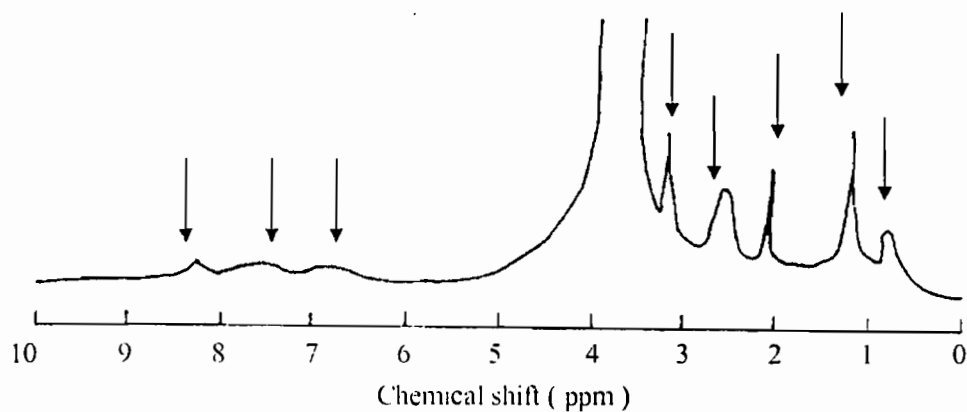


Fig. 6. ^1H - FT-n.m.r. spectrum of humic acid from Bostan compost.

Table 3 : ¹H-Resonance of the studied humic acids

Chemical shift (ppm)	Assignment
0.8---1.0	terminal methyl groups of methylene chains.
1.0---1.4	methylene of methylene chains; CH ₂ ,CH at least two carbons or further from aromatic rings, or polar functional groups.
1.4---1.7	Methylene of alicyclic compounds.
1.7---2.0	Protons of methyl and methylene groups \downarrow to aromatic rings; δ protons of indanes and tetralins.
2.0---3.3	Protons of methyl groups and methylene groups \downarrow to aromatic rings; protons \downarrow to carboxylic acid groups; \downarrow protons of indans and tetralins.
3.3---5.0	protons \downarrow to carbon attached to oxygen groups; sugars of carbohydrates.
5.0---6.5	Olefins
6.5---8.1	aromatic protons including quinones, phenols, oxygen containing heteroaromatics aromatics.
8.1---9.0	sterically hindered protons of aromatics; nitrogen heteroaromatics; formate.

It was observed from Figs. 1-6 and data in Table 3, that the signal from 3.0-5.0 ppm arises from aliphatic protons including protons \downarrow to carboxylic groups; protons of methyl groups and methylene groups; protons \downarrow to carbon attached to oxygen groups; and sugars of carbohydrates. No aromatic hydrogens were identified in this signal.

From the previous data, it is certain, therefore, that polymethylene chain are important contributors to humic substances (Ogner and Gronneberg,1977; Ruggiero *et al.*,1980_b; Wilson,1981 and Taha *et al.*,2000). The signal from 6.0-8.5 ppm arises from aromatic protons including quinones, phenols and oxygen-containing heteroaromatics aromatics. As indicated from Figs. 1-6, San-HA and Enz-HA exhibited the highest aromatic hydrogen content as indicated by the peaks at 6.62 and 7.48 ppm, respectively, indicating that these humic acids are in a higher degree of aromatic condensation than the other humic acids. This finding has been confirmed from a recently study of Taha and Modaihsh (2003).

The sharp resonance at 8.41 ppm in all the studied humic acids as shown in Figs. 1-6 may arise from formate ion which is a decomposition product of the humic acid in sodium hydroxide.

Wilson *et al.* (1978) detected only small amounts of aromatic protons in some potable and swamp water humic materials. The fact that aromatic rings extends as low as 6.0 ppm suggests that phenols are present (Collin *et al.*, 1980). Also, Hatcher *et al.* (1980_a) found the aromatic protons of humic acids isolated from marine sediments to much less than those from terrestrial environments.

The spectra given here are very similar to the spectra reported for humic acids extracted from different organic substances (Wilson,1981)

which indicated that protons of aromatic rings are usually present in humic substances.

In conclusion, the study of $^1\text{H-NMR}$ may throw more light on the nature of the chemical structure of the isolated humic acids since it points out that the spectra of all humic acids are similar with a little difference in the intensity of resonances. The chemical shifts of protons which used to deduce the structure of humic acids samples identified polymethylene terminal methyl groups of polymethylene chains. It is certain therefore, that polymethylene chains are important contributors to humic acids. It is also evident that protons of aromatic rings are usually present in humic substances.

REFERENCES

- Chen, Y.; N. Senesi and M. Schnitzer (1978). Chemical and physical characteristics of humic and fulvic acids extracted from soils of Mediterranean region. *Geoderma*, 20; 87-104.
- Collin, P.J.; R. J. Tyler and M.A. Wilson (1980). $^1\text{H-NMR}$ study of tars from flash pyrolysis of three Australian coals. *Fuel* 59, 479-486.
- 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.
- Hatcher, P.G.; R. Rowan and M.A. Mattingly (1980_a). ^1H and $^{13}\text{C-NMR}$ of marine humic acids. *Organic Geochemistry* 2, 77-85.
- Kononova, M.M. (1966). "Soil Organic Matter". 2nd Ed. Pergamon Press. Oxford.
- Lentz, H.; H.D. Ludemann and W. Ziechmann (1977). Proton resonance spectra of humic acids from solum of a podzol. *Geoderma* 18, 325-328.
- Ogner, G. and T. Gronneberg (1977). Permanganate oxidation of methylated fulvic and humic acids in chloroform. *Geoderma* 19, 237-245.
- Preston, C.M. (1987). Review of solution NMR of humic substances. In *NMR of humic substances and Coal: Techniques, problems and solutions*. R. L. Wershaw and M.A. Mikita (eds.) Lewis Publishers, Chelsea, Wisc., pp. 3-92.
- Ruggiero, P.; F.S. Interesse and O. Sciacovelli (1980_b). $^1\text{H-NMR}$ evidence of exchangeable protons in fulvic and humic acids. *Soil Biology and Biochemistry* 12: 297-299.
- Stevenson, F.J. (1982). "Humus Chemistry". John Wiley and Sons, New York.
- Taha, A.A. (1992). A study on $^1\text{H-nuclear}$ magnetic resonance of a soil humic acid. *J. Agric. Sci. Mansoura Univ.* 17 (9): 3101-3106.
- Taha, A.A.; A.O. AbdEl-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.
- Taha, A.A. and A.S. Modaihsh (2003). Chemical and spectroscopic measurements on the humic acids extracted from some organic composts. *J. Agric. Sci. Mansoura Univ.*, 28 (6) : 5073-5082.

Taha, A. A. et al.

- Taha, A.A.; M.W.M. El-Agrodi; H.A. Sonbol and H.Z. Abdel-Salam (2000). Information provided on humic acids structure by ^1H - and ^{13}C -NMR spectrometry. Egypt. J. Soil.Sci. 40 No. 1-2, pp. 141-151.
- Vila, F.J.; H. Lentz and H.D. Ludemann (1976). F.T. C-13 Nuclear magnetic resonance spectra of natural humic substances. Biochemical and Biophysical Research Communications. 72: 1063-1069.
- Wilson, M.A. (1981). Applications of nuclear magnetic resonance spectroscopy to study of the structure of soil organic matter. J. Soil Sci. 32 : 167-186.
- Wilson, M.A.; A.J. Jones and B. Williamson (1978). NMR spectroscopy of humic materials. Nature, London 276, 487-489.
- Wilson, M.A.; P. J. Collin and K.R. Tate (1983). ^1H -nuclear magnetic resonance study of a soil humic acid. J. Soil Sci. 34 : 297-304.

استخدام خاصية التردد النووي المغناطيسي الهيدروجيني لدراسة تركيب بعض الأحماض الدبالية أحمد

عبد القادر طه ، عبد الله سعد المديهي و محمد عثمان محجوب
قسم علوم التربة- كلية الزراعة- جامعة الملك سعود- المملكة العربية السعودية

استخدمت خاصية التردد النووي المغناطيسي الهيدروجيني (^1H -NMR) لدراسة تركيب بعض الأحماض الدبالية المستخلصة من بعض الأسمدة العضوية (الكومبوست). أعطت النتائج معلومات مفيدة عن طبيعة تركيب الأحماض الدبالية المستخلصة حيث وجد أن هذه الأحماض متشابهة التركيب لحد ما ولم تختلف إلا في شدة التردد (الذبذبة). وباستخدام هذه الخاصية تم التعرف على وجود مركبات البولي ميثيلين ومجاميع الميثيل الطرفية في جميع الأحماض الدبالية المستخلصة ؛ كما تم التعرف على البروتونات المرتبطة بذرات الكربون في الوضع ألفا بالنسبة لمجاميع الكربوكسيل أو الحلقات العطرية. وقد تم التأكد من وجود بروتونات الحلقات العطرية في الأحماض الدبالية تحت الدراسة.

وقد أظهرت نتائج الدراسة أن حمض الهيوميك المستخلص من سماد البستان Bost- (HA) يحتوي على البروتونات التي في صورة أليفاتية أكثر من بقية الأحماض الدبالية الأخرى. ومن جهة أخرى فقد وجد أن الأحماض الدبالية المستخلصة من كل من سماد سنبيست (San-HA) وسماد العنزى (Enz-HA) تحتوي على البروتونات في صورة حلقيه أكثر من بقية الأحماض الدبالية الأخرى.