

Physico-chemical Attributes of Humic Acid Extracted from Tropical Peat

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ABSTRAK

Kajian asas mengenai asid humik pada gambut tropika adalah terhad. Satu kajian mengenai ciri-ciri asid humik yang diekstrak dari gambut tropika telah dijalankan dengan menganalisis elemen dan kumpulan berfungsi, spektrum inframerah dan ujian terus pada asid humik dengan menggunakan mikroskop elektron imbasan. Berdasarkan kepada analisis elemen, jumlah C yang didapati adalah 40.26 - 53.26%, H 4.84 - 6.36%, O 38.39 - 51.62%, N 1.51 - 2.64%, dan P 0.02 - 0.06%. Kajian dalam kumpulan berfungsi menunjukkan kumpulan karboksil yang wujud dalam asid humik di gambut tropika adalah dua kali ganda lebih banyak daripada kumpulan hidroksil fenolik. Daripada spektrum inframerah, asid humik didapati mempunyai ciri-ciri aromatik. Analisis inframerah juga menunjukkan spektrum yang sama dan selaras tanpa mengira perbezaan tempat asalnya, kompaun humik yang diekstrak mempunyai struktur dan komposisi kumpulan berfungsi yang sama. Pemeriksaan menerusi mikroskop elektron pada asid humik kering menunjukkan bentuk yang berblok dan separa berliku dengan saiz yang berbeza. Semua asid humik yang diekstrak menunjukkan bentuk yang sama.

ABSTRACT

Basic research conducted on humic acid (HA) in tropical soils is limited. A study was conducted to characterize HA extracted from tropical peat by elemental and functional group analyses, infrared (IR) spectra and by direct examination on HA using scanning electron microscopy (SEM). From the elemental analysis it was found that C ranged from 40.26 - 53.26%, H 4.84 - 6.36%, O 38.39 - 51.62%, N 1.51 - 2.64%, and P 0.02 - 0.06%. Studies of functional groups indicated that the carboxyl group present in HA of tropical peat was about twice as high as that of phenolic hydroxyl groups. From IR spectra, the HA had an aromatic character. Results of IR analysis also exhibited consistently similar spectra, demonstrating that regardless of place of origin, the extracted humic compound had a similar structure and was composed of the same functional groups. Electron microscopic examination of dried HA showed a sub-angular blocky shape which varied in size. All extracted HA exhibited similar form.

INTRODUCTION

Humic acid (HA), defined as the fraction that is soluble in dilute base and coagulated upon acidification of the alkaline extract, is the main component of humic fraction in both temperate and tropical peat (Norhayati 1989; Garcia *et al.* 1991). HA is

comprised of highly reactive polymeric compounds of varying molecular weight, containing C, H, O, and N, and its reactivity is influenced by the nature and amount of oxygen-containing functional groups (Stevenson and Goh 1972).

According to Cheshire *et al.* (1967),

peat humic acids have 52% C, 5.1% H, 2.2% N and 40.3% O content. A study by Visser (1987) on humic acids extracted from decomposing plant and peat core samples showed content of 34-55% C, 5-11% H and 40-55% O, different from the values of mineral soils 55-60% C, 3-5% H, 2-6% N and 30-40% O (Kononova 1961). HA extracted from Sphagnum and Carex peat showed 49.7 and 55.4% C, 6.0 and 5.0% H, 42.0 and 35.5% O, 2.3 and 3.5% N content, respectively (Garcia *et al.* 1991).

IR spectra of HA extracted from temperate peat showed aromatic character and great similarity among different samples of HA (Visser 1987; Garcia *et al.* 1991). Studies by Norhayati (1989) also indicated that the HA extracted from tropical peat exhibited very similar IR spectra.

SEM provides a non-destructive method for direct recording of the shape of tiny humic particles (Bohr and Hughes 1971; Orlov and Glebova 1972). Ghosh and Schnitzer (1982) found under SEM, humic acids to consist of small discrete sphenoid, flattened aggregates, linear chain assemblies of these aggregates and flattened filaments.

There is a dearth of information on the study of elemental composition and reactivity of HA, and its structure under SEM. This study was therefore carried out to characterize the HA extracted from tropical peat by elemental and functional group analyses, its IR spectra and its structure by SEM.

MATERIALS AND METHODS

Fourteen samples from major peat areas of Malaysia were used in this experiment, twelve from the west coast of Peninsular Malaysia and two from Sarawak (peat samples 5 and 6). Percentage of organic matter was determined as loss on ignition from sample maintained at 500°C for 4 h (Storer 1984).

Humic acid was extracted with 0.2 M NaOH using a soil to extractant ratio of 1:10. The mixture was shaken continuously on a mechanical shaker for 24 h and the soluble organic matter was separated from the peat soil by centrifugation at 10,000 rpm for 15 min. The residue was washed with distilled water in a ratio of 1:5 and washing water separated from the residue by centrifugation. The combined supernatants were acidified with concentrated H₂SO₄ to pH 1.0 and allowed to stand for 24 h. The coagulated humic acid (HA) precipitate was purified by re-precipitation and re-dissolution using H₂SO₄ and NaOH, respectively. The alkaline extract was then shaken for 48 h with excess HCl-HF mixture (0.5%, v/v). The acid mixture was removed by centrifugation and the residue thoroughly washed with distilled water until the filtrate became free of chloride. The humic acid was then dialyzed against distilled water until there was no colour change in the outer solution (distilled water) and finally frozen and dried at ambient temperature (Schnitzer 1982; Norhayati and Verloo 1984).

Carbon, H and N were determined by dry combustion using a CHN analyser (VARIO-EL), O was calculated by difference and P by dry ashing at 500°C and determined colorimetrically at 882 nm (Murphy and Riley 1962). Carboxyl groups were measured by Ca(OAc)₂ method (Schnitzer and Gupta 1965) and the phenolic hydroxyl groups by the colorimetric method using Folin Ciocalteu's reagent (Tsutsuki and Kuwatsuka 1978). These determinations were represented as the average result of two duplicate samples.

IR spectra were recorded on a Beckman Fourier transfer IR spectrophotometer using the KBr pellet technique. Prior to the analyses, the HA with the KBr was stored for at least 48 h in a desiccator to prevent absorption of atmospheric moisture.

For SEM examination, the dried HA obtained directly after extraction was attached to Al-stubs with the aid of colloidal silver paint. To make the specimens surface conducting, they were coated with a layer of gold-palladium. The prepared specimen were examined on a SEM (JOEL 35C) operated at 25kV.

RESULTS AND DISCUSSION

The organic matter contents per 100 g peat of the 14 peat samples studied ranged from 44 - 97% (Table 1). Percentage of HA obtained ranged from 8.30 - 39.52/100 g peat.

The data on the elemental composition of HA in terms of percentage weight are presented in Table 2. Carbon content ranged from 40.26 - 53.26%, H 4.84 - 6.3%, O 38.39 - 51.62%, N 1.51 - 2.64% and P 0.02 - 0.06% with means of 49.34% C, 5.64% H, 43.05% O, 1.99% N and 0.03% P. Comparing these with the elementary composition of HA reported

by Visser (1987) and Garcia *et al.* (1991) indicated that our results fell within the reported range for temperate peat.

The analysis of the major oxygen-containing functional groups is shown in Table 3. The carboxyl group of HA extracted from tropical peat ranges from 2.50 - 3.10 meq/g of HA while phenolic hydroxyl groups range from 0.99 - 1.75 meq/g of HA. Mean values of functional groups showed 2.84 meq COOH and 1.36 meq phenolic hydroxyl/g of HA. The carboxyl groups present in HA are more than twice as numerous as in the phenolic hydroxyl groups, implying that the pH-dependent charge in peat is controlled by carboxyl groups.

IR spectra and the absorption bands of HA extracted from peat are shown in *Fig. 1* and Table 4, respectively. The broad absorption band at 3550-3200 cm^{-1} region can be attributed to H-bonded OH groups, while peaks between 2920-2860 cm^{-1} are due to aliphatic C-H stretch. According to

TABLE 1
Classification and yield of HA from peat

Peat Sample No.	¹ Classification	Depth (cm) ² (%)	O.M. ³ (%)	HA
1	A	0-15	44	21.23
2	A	0-15	82	26.88
3	A	0-15	86	18.30
4	B	15-30	88	25.78
5	A	0-15	90	23.78
6	B	15-30	94	8.40
7	A	0-15	97	35.67
8	A	0-15	97	20.68
9	B	0-15	76	23.45
10	A	0-20	94	36.52
11	A	20-40	97	12.38
12	B	0-15	89	19.82
13	B	20-40	97	8.30
14	A	0-15	96	26.92

¹ A Typic Tropohemists, B Typic Tropofibrists

² Percentage of organic matter

³ % Humic acid (HA) per 100 g peat

TABLE 2
Elemental analysis of HA extracted from tropical peat

Peat Sample No.	Elements (%)				
	C	H	O	N	P
1	51.93	5.14	40.52	2.37	0.06
2	52.01	5.62	39.88	2.45	0.04
3	45.75	6.36	45.26	2.59	0.05
4	51.25	5.23	41.33	2.16	0.04
5	53.39	5.59	38.34	2.64	0.05
6	44.39	6.23	47.72	1.63	0.03
7	51.04	5.22	41.98	1.74	0.02
8	47.80	5.77	44.68	1.73	0.02
9	49.76	4.84	43.88	1.51	0.02
10	50.52	5.14	42.14	2.18	0.03
11	49.07	5.78	43.48	1.65	0.02
12	50.00	5.67	42.16	1.71	0.02
13	53.56	5.91	38.88	1.64	0.02
14	40.26	6.32	51.60	1.84	0.02
Range	40.26- 53.56	4.84- 6.36	38.39- 51.62	1.51- 2.64	0.02- 0.06
Mean	49.34	5.64	43.05	1.99	0.03

TABLE 3
Analysis of oxygen-containing functional groups of HA extracted
from tropical peat

Peat Sample No.	Functional groups (meq/g of HA)	
	COOH	Phenol OH
1	2.90	1.36
2	2.75	1.07
3	2.95	1.12
4	2.75	1.39
5	2.80	0.99
6	3.10	1.56
7	2.50	1.22
8	2.80	1.44
9	2.90	1.40
10	3.00	1.75
11	3.00	1.43
12	2.85	1.54
13	2.95	1.59
14	2.50	1.22
Range	2.50-3.10	0.99-1.75
Mean	2.84	1.36

TABLE 4
Absorption bands of infrared spectra of HA from tropical peat

Bands (cm ⁻¹)*	3550- 3200	2920- 2860	1725- 1705	1690- 1600	1550- 1500	1470- 1430	1280- 1200
Peat Sample No.	Relative intensity						
1#	s ⁺	m	m	s	a	w	w
2	s	m	m	s	a	w	w
3	s	m	w	s	a	w	w
4	s	m	m	s	a	w	w
5	s	m	m	s	a	w	w
6	s	m	m	s	a	w	w
7	s	m	w	s	w	w	w
8	s	m	m	s	w	w	w
9	s	m	m	s	w	w	w
10	s	m	w	s	w	w	w
11	s	m	m	s	w	w	w
12	s	m	m	s	w	w	w
13	s	m	m	s	w	w	w
13	s	m	m	s	w	w	w
14	s	m	m	s	w	w	w

* 3550-3200 (hydrogen-bonded OH); 2920-2860 (aliphatic C-H stretch); 1725-1705 (C=O of COOH and ketones); 1690-1600 (C=O stretch of quinones, C=O and/or C=O of H conjugated ketones); 1550-1500 (aromatic ring stretch, P- and O- distributed); 1470-1430 (aromatic ring stretch, O-distributed); 1280-1200 (C-O stretch, and deformation of COOH, C-O stretch of aryl ethers)

⁺ (s) strong; (m) medium; (w) weak; (a) absent

Sample number for HA extracted from respective peat soil

Bellamy (1969), bands in the region of 3000-2500 cm⁻¹ were produced by most carboxylic acids and arose from vibration of intermolecular OH...O band. Therefore, Schnitzer and Griffith (1975) indicated that the O-H stretch (<2800 cm⁻¹) was a test for the occurrence of H-bonded COOH groups. However, in this study only a medium peak occurred at this region similar to the results of Norhayati (1989).

Norhayati (1989) showed that higher amounts of carboxyl groups were present in HA molecules. Similar results were obtained in this study, where most of the HA spectrum exhibited a medium peak in the 1725-1705 cm⁻¹ region (band for carbonyl

group) with strong peaks in the 1690-1600 cm⁻¹ region (band for carbonyl group).

The spectra also showed weak bands at 1550-1500 cm⁻¹ and 1470-1430 cm⁻¹. Similar results were obtained by Garcia *et al.* (1991), where the above bands meant for HA extracted with alkali disappeared for HA extracted with pyrophosphate reagents in both Sphagnum and Carex peat. This indicated that HA extracted with alkali extractant exhibited stronger aromatic characteristics than that using pyrophosphate reagent.

A weak band obtained for all samples in the 1289-1200 cm⁻¹ region is attributed to OH, from COOH deformation. Other

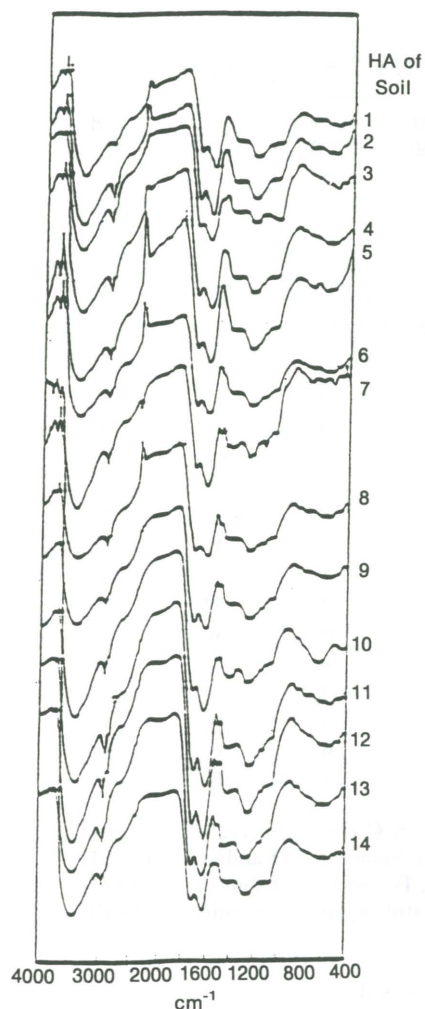


Fig. 1. Infrared spectra of HA extracted from tropical peat

bands, such as 1440, 1125 and 1034-1080 cm^{-1} were also observed. However, all these bands were of weak intensity, and were absent in some samples (Fig. 1). Bands occurring at 1440 cm^{-1} indicated the presence of the carboxyl anion (Schnitzer and Griffin 1975); 1125 and 1034-1080 cm^{-1} indicated aromatic ring P- and O- disubstituted, C-O-O stretch of aryl ethers and C-O stretch, aromatic ether, hydrated polyols and carbohydrates, respectively (Garcia *et al.* 1991). Thus, this study demonstrates that HA of tropical peat has a greater amount of carboxyl groups.

The IR spectra of the HA extracted from peat of diverse origin displayed similar bands and indicated the presence of similar structures, differing mainly in the number of functional groups. The results corresponded with those of Norhayati (1989).

The shape and particle arrangement of HA under SEM are shown in Plate 1. The HA materials are sub-angular blocky, vary in size, and are irregular in shape (Plate 1a). Plate 1b shows the plate-like structure of HA surface upon higher magnification (1 μm).

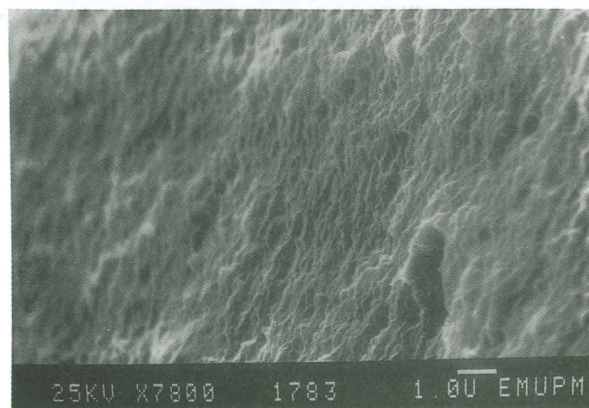
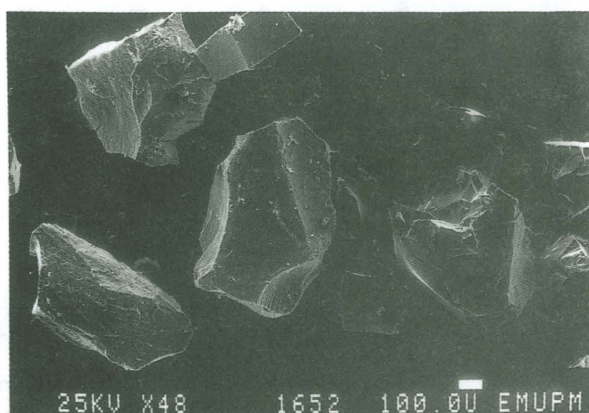


Plate 1. Scanning electron micrographs of shape and particle size humic acid; a) top: sub-angular blocky which vary in size and shape, b) bottom: plate like structure of HA surface.

CONCLUSION

The elemental analysis of HA showed C ranged from 40.26 - 53.26%, H 4.84 - 6.36%, O 38.39 - 51.62%, N 1.51 - 2.64% and P 0.02 - 0.06%. Studies of the functional groups indicated that carboxyl groups present in HA of tropical peat are about two times more numerous than phenolic hydroxyl groups. From IR spectra, the HA obtained is of aromatic character. The IR analyses also exhibited consistently similar spectra, demonstrating the possibility that regardless of sample site, the extracted HA has a similar structure and is composed of the same functional groups. SEM examination of dried HA showed sub-angular blocky shape of varying size was similar for all the HA extracted. These results support those obtained by Norhayati (1989) for Malaysian peat, and demonstrate the similarity between tropical and temperate peats regardless of plant origin or climate.

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