

Characterisation of Organic Matter in Four Soils of Peninsular Malaysia. I. Extraction, Fractionation and Purification of Humic Substances

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The procedures for extraction, fractionation and purification of humic substances from four soils of Peninsular Malaysia are detailed. The problems involved in the experimental procedures and ways to overcome them are also discussed.

The clay minerals were observed to be linked quite strongly to the humic acid (HA) fraction. This was confirmed by infra-red and X-ray diffraction studies as well as by the chemical composition of the ash of unpurified and purified humic and fulvic acids (FA).

The complete organic matter fraction of a soil comprises live organisms as well as their undecomposed, partly decomposed and completely transformed remains. Although soil organic matter is a heterogenous mixture, it can be broadly classified into two main groups^{1,2}:

- The unaltered materials which include fresh debris and non-transformed components of older debris
- The transformed products or humus, which bear no morphological or chemical resemblance to the parent material from which they were derived.

Further, the transformed products can be separated into two major groups, the humic and non-humic substances. *Figure 1* illustrates the various components of soil organic matter.

In studying such a complex system as humic substances of soil organic matter,

it is essential first to extract and then to fractionate them into components which are reasonably homogenous in molecular size and properties. Several solvents have been used to extract humic substances from soils. However, the work reported in the literature, are focussed mainly on temperate soils which have properties and mineralogy different from those of tropical soils. A detailed study on the procedures involved in the extraction, fractionation and purification of humic substances of four soils of Peninsular Malaysia is accounted herein.

Previous work on organic studies of Malaysian soils^{3,4} did not include shaking the soil organic matter extracts with Na₂SO₄ and further purification of the reprecipitated HA. These two steps were found necessary in order to obtain relatively pure samples of HA with very low ash content.

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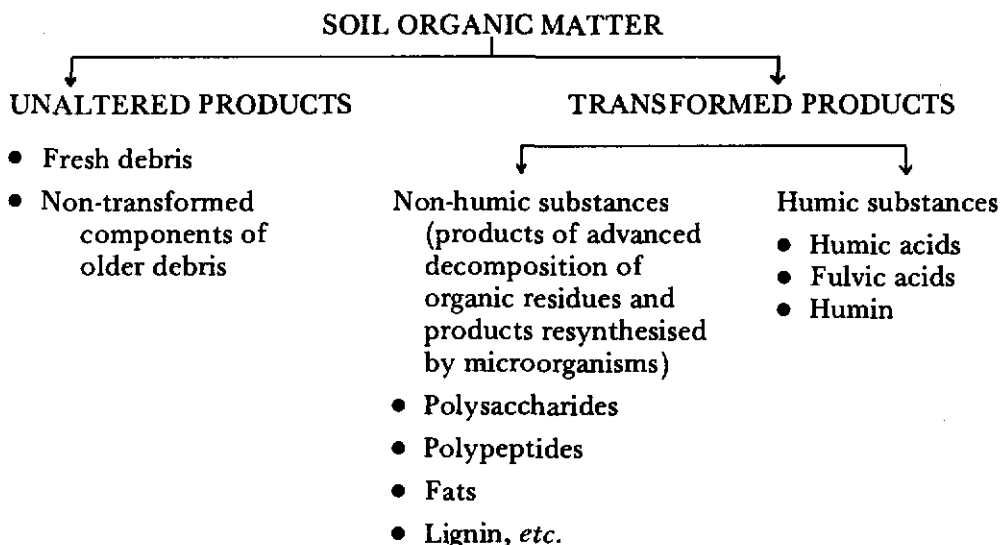


Figure 1. Schematic illustration of the different components of soil organic matter.

MATERIALS AND METHODS

Soils

Organic matter was extracted from four soils of igneous origin, namely Rengam, Jerangau 1, Jerangau 2 and Kuantan. These soils are commonly cultivated with rubber (*Hevea brasiliensis*).

Table 1 gives the type of parent material from which the soil is derived and the sampling location.

Extraction

To extract the humic substances from the soil, 250 g of air-dry, <2 mm size surface soil was weighed into several erlenmeyer flasks in batches of 25 g each; 250 ml of 0.2 N sodium hydroxide was added to each flask. The flasks were then shaken for 16 h on a mechanical shaker.

The suspension was filtered through glass wool to remove any suspended material, centrifuged and the supernatant decanted. The supernatant was shaken

TABLE 1. PARENT MATERIAL AND SAMPLING LOCATION OF SOILS STUDIED

Soil	Parent material	Sampling location
Rengam	Granite	Sungei Buluh, Field 65
Jerangau 1 ^a	Granodiorite	Amber Estate
Jerangau 2	Granodiorite	Kulai
Kuantan	Basalt	Jeram Kuantan Estate Field 1965 R.P.

^aJerangau 1 is an Ultisol and Jerangau 2 an Oxisol. Jerangau 1 may have been renamed.

overnight with about 20 g of anhydrous sodium sulphate. This salted out most of the clay particles and a clear suspension was obtained after centrifugation. All of the filtrate was collected into a large beaker.

Fractionation

The collected filtrate was then acidified to pH 1 with concentrated sulphuric acid and allowed to equilibrate overnight. HA was precipitated out while FA remained in the filtrate. HA was separated from FA by centrifuging and decanting off the FA.

Purification of Humic Acid

The precipitate of HA was purified by dissolving it in 0.2 N sodium hydroxide. The pH of the solution was again adjusted to 1 with sulphuric acid and allowed to equilibrate overnight.

Reprecipitation and redissolution of the HA was thus carried out in the same manner three times.

After the final precipitation, the HA was placed in cellulose tubing and dialysed against distilled water for one week, with daily change of the distilled water. The mixture was then centrifuged. The residue of HA was subsequently shaken for 48 h with 100 ml of HCl-HF mixture to desorb the HA completely from the silicate impurities.

The acid mixture was removed by centrifuging for half an hour and decanting the supernatant. The residue of HA was washed with distilled water until free of Cl^- (as shown by tests with silver nitrate solution) and freeze-dried.

Purification of Fulvic Acid

To purify FA, it was first placed in cellulose tubing and dialysed against distilled water for one week* with daily change of the distilled water. The dialysed FA was then allowed to pass through an ion-exchange resin, Amberlite IR-120 and the leachate was freeze-dried. The FA was not preconcentrated prior to freeze drying, as preconcentration was seen to cause some changes to it.

Spectral Analysis

Infra-red spectrophotometry. The infra-red spectra of the acids were recorded on a Beckman spectrophotometer using the KBr pellet technique. The HA and FA together with the KBr were stored for at least 48 h in a dessicator prior to analysis, to prevent absorption of atmospheric moisture.

X-ray Diffraction

X-ray diffractograms were obtained using the Phillips X-ray Diffractometer (PW 1050/25) and CoK_α radiation.

Chemical Analysis

The ash contents of the acids were obtained by igniting the acids at 700°C for 4 h. The ash content indicates the purity of the acid obtained. The ash was also analysed for its elemental composition. Treatment with HF was first carried out to volatilise any silica present. The amount of silica present in the ash was determined gravimetrically. The residue was then dissolved completely by digesting it in 2 ml of 4 N $\text{HCl-H}_2\text{SO}_4$ mixture, evaporated until 1 ml of the solution was left, cooled and made up to 50 ml with distilled water. The solution was analysed for Fe, Cu, Zn, Mn, Ca and

*On exhaustive dialysis of FA, the outer solution was observed to turn yellowish in colour. This was believed to be due to dialysis of the low molecule weight component of FA. On prolonged dialysis, a brown flocculant precipitate similar in appearance to HA was observed.

Mg by AAS; Na and K by flame photometer and Al and P by colorimetry using the auto-analyser⁵.

RESULTS AND DISCUSSION

Of the various possible extractants, sodium hydroxide was chosen because of its known ability to extract appreciable amounts of organic matter^{6,7}. However, several problems were encountered during the extraction. The soils studied have very high percentages of clay. Sodium hydroxide at the concentration used was found to disperse the clay. Therefore, on centrifuging the suspension, not all of the fine colloidal clay particles were sedimented. The clay particles were observed to be linked to the organic matter as organo-clay-metal complexes. This was revealed in the spectral studies (Figures 2 and 3).

From the literature, it is known that high concentrations of Na^+ with SO_4^{2-} as the counter ions will coagulate clay particles. Thus, anhydrous sodium sulphate was added to the supernatant with this objective in mind. The clay was then

observed to be salted out and a clear alkali extract obtained.

To fractionate the HA and FA, the alkali extract was acidified to pH 1 with concentrated sulphuric acid. Yuan⁸ has shown that the pH of precipitation had a considerable effect on the yield. His studies showed that the amounts of HA precipitated increased as pH decreased. Sequi *et al.*⁹ have also observed that incomplete separation of HA was likely if the extracted impure organic matter extract was not acidified to pH 1.

Hydrochloric acid was not used to precipitate the HA because an earlier trial showed that it was difficult to obtain Cl^- -free HA even after several washings. These washings resulted in an appreciable loss of the HA due to dissolution. Moreover, HCl tends to enhance the probability of trace-metal-chloride complexation.

The precipitate of HA was redissolved and reprecipitated twice followed by exhaustive dialysis against distilled water

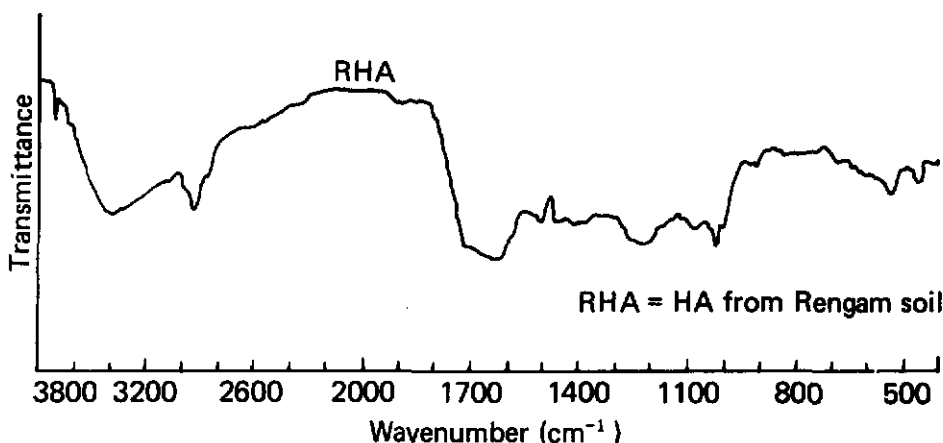


Figure 2. Infra-red spectrum of humic acid after three reprecipitations and redissolutions followed by exhaustive dialysis.

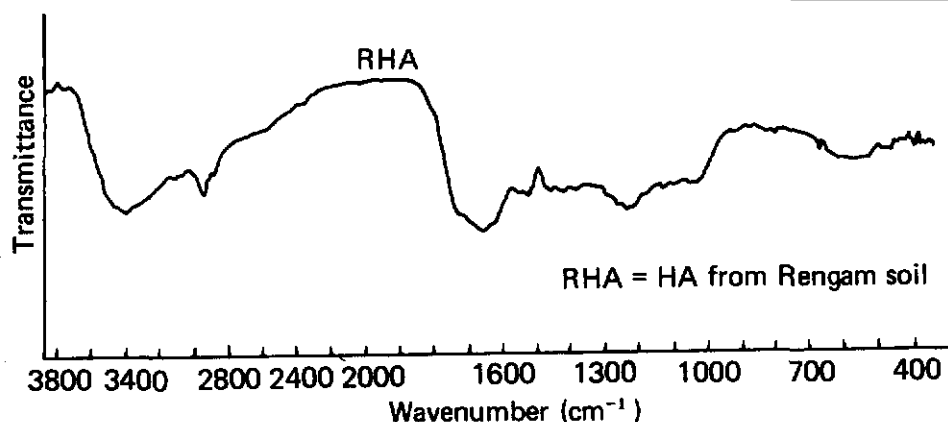


Figure 3. Infra-red spectrum of relatively pure humic acid.

for one week with daily change of the distilled water, to remove the inorganic contaminants. Infra-red studies showed that a large amount of the inorganic contaminants was still present in the HA.

Figure 2 shows the infra-red spectrum of HA extracted from Rengam soil, that had been reprecipitated and redissolved thrice followed by exhaustive dialysis. The principal features in this spectrum are similar to those reported in the literature¹⁰⁻¹³. The spectrum was in general rather complex for definite interpretation of the functional groups present in the acids. Nevertheless, some idea of the functional groups present can be deduced.

Broad adsorption bands at 3400 cm^{-1} which can be attributed to H-bonded OH were seen in the spectra of all four HA extracted from the four soils. Other features observed were strong intensity peaks between 300 cm^{-1} and 2920 cm^{-1} due to C-H stretching of CH_2 and CH_3 groups; a shoulder at 1700 cm^{-1} indicative of C=O or COOH groups and broad absorption bands around 1650 cm^{-1} and 1220 cm^{-1} . The former may be due to

aromatic C=C and/or H-bonded C=O as in quinones, while the latter may be due to C=O of phenolic OH groups. Infra-red spectra of HA extracted from the other three soil series were similar in character to that of Figure 2, hence, they are not illustrated here.

In all the spectra of HA of the four soils the peak at 1030 cm^{-1} was present. This peak can be ascribed to the presence of silicate impurities in the HA samples, in spite of the repeated precipitation, dissolution and exhaustive dialysis treatments. Dialysis removed most of the non-polymeric organic materials but could not separate the complexed or strongly adsorbed metals or metal hydroxides from the humic materials. The peak at 1030 cm^{-1} was observed to be absent in the spectrum of Figure 3 where the HA was desorbed from the silicates, free silicic acid and clay minerals which were subsequently dissolved in the acid mixture of HCl-HF and removed. This indicated the successful removal of the silicate impurities from the HA by the acid treatment. This was true for all the HA extracted from the four soils.

Figure 4 shows the IR spectra of FA extracted from Rengam and Kuantan soils which was dialysed and freeze-dried, while Figure 5 shows the IR spectrum of FA that was dialysed, passed through Amberlite IR-120 resin and freeze dried. The spectrum in Figure 5 was rather featureless showing absorption bands in the region of 3400, 2920, 1650, 1150 and 1050 cm^{-1} . The absorption band at 3400 cm^{-1} as described earlier, is associated with H-bonded OH groups; 2920 cm^{-1} of aliphatic stretching; that of 1650 cm^{-1} and 1400 cm^{-1} due to carboxyl groups in the carboxylate forms, COO^- .

The shoulder between 1200 cm^{-1} and 1100 cm^{-1} can be assigned to C-O stretching of phenols and/or alcohols. The band between 1100 cm^{-1} and 1000 cm^{-1} cannot be assigned to the presence of silicate impurities as in HA because all silica present would have been precipitated out together with the HA since they are insoluble in an acid medium. This band can be assigned to C-C, C-OH and C-O-C vibrations similar to that exhibited by soil polysaccharides¹³.

Figure 6 shows the spectrum of FA of Rengam soil which was treated in the

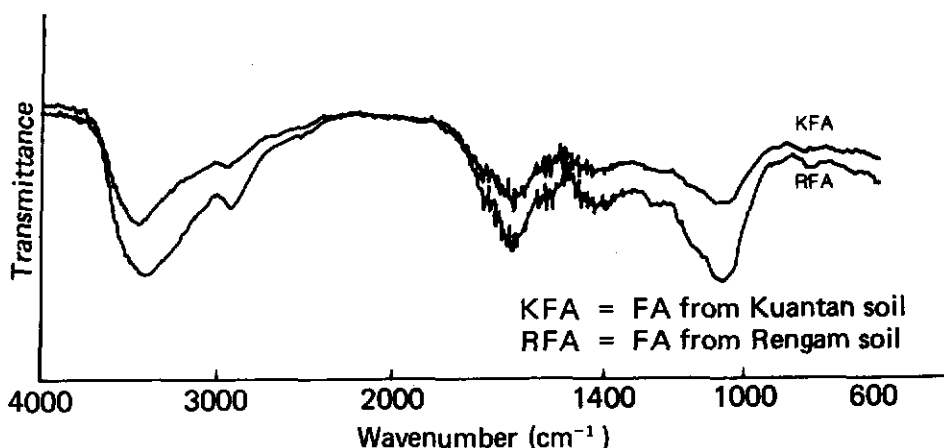


Figure 4. Infra-red spectra of unpurified fulvic acids.

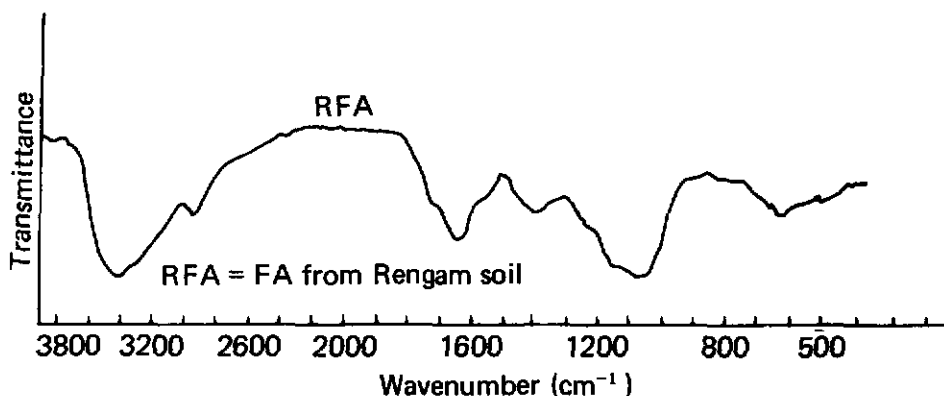


Figure 5. Infra-red spectrum of purified FA by passing through Amberlite IR-120.

same manner as the sample in *Figure 5*, but prior to freeze drying it was pre-concentrated. Some differences were seen between the two spectra. The band at 1725 cm^{-1} was more marked in *Figure 6* while in *Figure 5* it was only a shoulder. The shoulder between 1200 cm^{-1} and 1100 cm^{-1} and the band between 1100 cm^{-1} and 1000 cm^{-1} were changed in character. A strong broad absorption band between 1200 cm^{-1} and 1050 cm^{-1} was seen instead of the two absorption bands. The peaks at 2920 cm^{-1} and 1400 cm^{-1} were also seen to be decreased in their intensities. This clearly showed that preconcentration resulted in appreciable structural changes of the FA.

The IR spectra of the HA and FA extracted from the four soils were basically similar in character. This suggests that they possess similar basic structures and functional groups in their building blocks.

X-ray Diffraction

X-ray diffractions of HA and FA using the Debye Sherrer camera showed

diffused diffraction peaks. It has been shown by several workers^{14,15} that more accurate diffraction peaks could be obtained by using the counter technique. This was not attempted in this study.

Nevertheless some interesting information was obtained from the diffractograms of the unpurified and purified HA and FA. The presence of inorganic contaminants such as silicates and goethite in the unpurified extracts were confirmed as deduced from their diffraction peaks at 3.5 and 4.2 and 2.69Å respectively.

These peaks were absent in the diffractograms of the relatively pure HA and FA. The absence of the diffraction peaks at 2.8Å and 3.92Å in the purified FA extract indicated the removal of the sodium sulphate which was used to salt out the clay minerals.

Figure 7 shows the diffractograms of the unpurified and purified HA and FA extracted from Rengam soil. The HA and FA from the other three soils exhibited similar diffractograms. The X-ray dif-

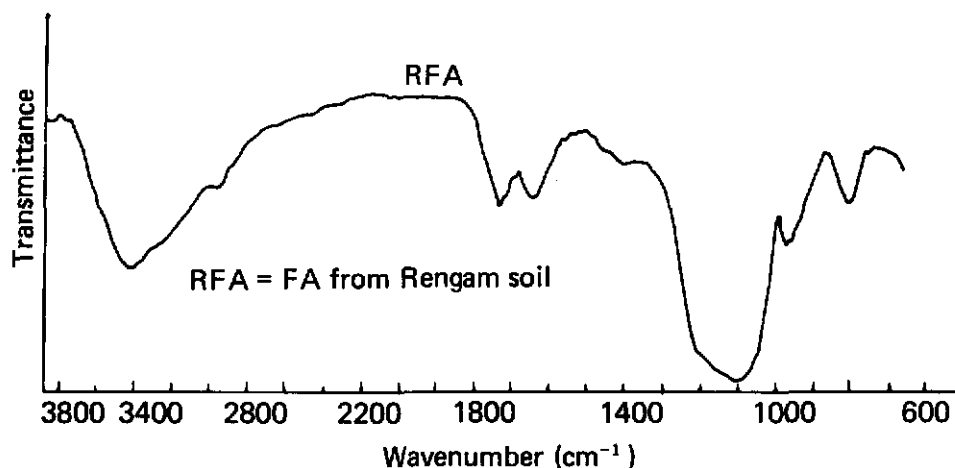


Figure 6. Infra-red spectrum of fulvic acid, purified by passing through Amberlite IR-120 resin and preconcentrated prior to freeze drying.

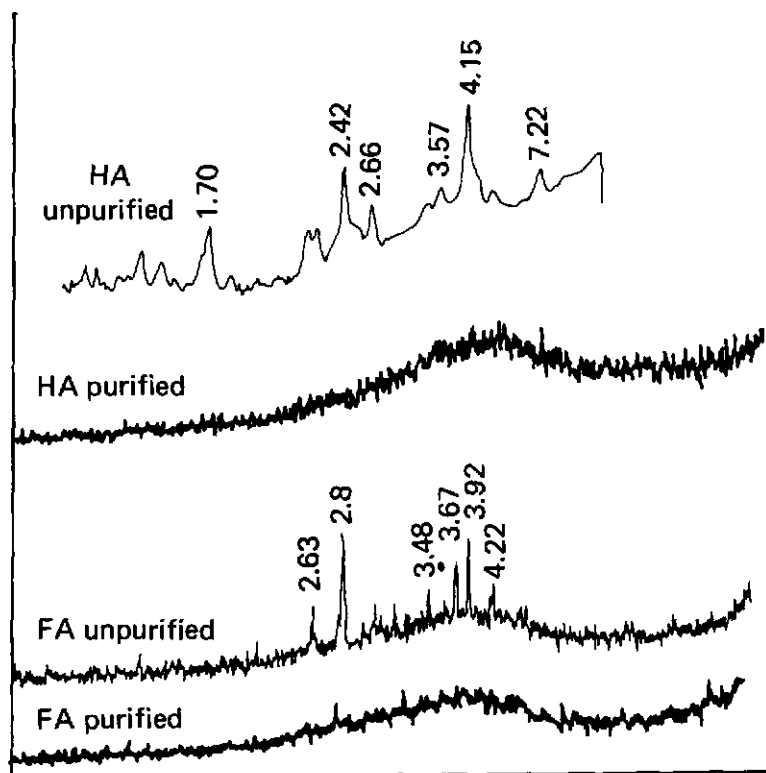


Figure 7. X-ray diffractograms of unpurified and purified humic and fulvic acids.

fractograms also failed to show any evidence of crystallinity in the structure of the acids.

Chemical Analysis of Humic Acid and Fulvic Acid

Ash content and composition. The ash contents of HA and FA give an idea of their purity. The lower the ash content the purer is the acid. After several purification steps, HA of sufficiently low ash content (1.4 %) was obtained. However, the ash content of FA (19%) was not as easily reduced. The ash of FA is believed to be due to non-humic organic polymers which are not easily removed¹⁶⁻¹⁸.

The elemental compositions of the ash of HA and FA obtained after repre-

cipitation and redissolution three times followed by exhaustive dialysis are given in Table 2.

High amounts of Si, Al and Fe were present in the ash of HA and FA of the four soils. In the ash of the FA, the amounts of Si, Al and Fe were generally much less.

The ash contents of HA and FA extracted from Rengam soil which were further purified by shaking with HCl-HF mixture and passing through Amberlite IR-120, respectively, are shown in Table 3. This confirmed the earlier IR studies which showed that most of the inorganic contaminants had been successfully removed by the extra purification steps.

TABLE 2. ELEMENTAL COMPOSITION OF ASH OF HUMIC ACID AND FULVIC ACID

Soils series	Humic Substances	Element present (%)										
		Si	Al	P	Fe	Mg	Ca	Na	K	Zn	Cu	Mn
Rengam	HA	1.95	1.59	0.18	4.80	0.06	0.28	0.06	0.08	0.063	0.008	nd
	FA	0.57	1.33	0.09	0.06	0.02	0.10	0.65	0.07	0.022	0.004	nd
Jerangau 1	HA	3.11	3.17	0.30	8.91	0.07	0.15	0.40	0.14	0.032	0.012	nd
	FA	0.66	1.80	0.14	0.11	0.07	0.09	1.35	0.10	0.030	0.003	nd
Jerangau 2	HA	3.52	3.09	0.26	8.49	0.05	0.08	0.14	0.06	0.048	0.014	0.01
	FA	0.94	1.84	0.16	0.05	0.01	0.04	0.62	0.06	0.054	0.006	nd
Kuantan	HA	1.86	1.78	0.32	5.94	0.02	0.09	0.03	0.04	0.043	0.021	0.001
	FA	1.11	1.39	0.27	0.03	0.01	0.06	0.58	0.08	0.016	0.064	nd

nd = not detected

TABLE 3. ELEMENTAL COMPOSITION OF ASH OF PURE HUMIC ACID AND FULVIC ACID OF RENGAM SOIL

Element (%)	HA	FA
Si	nd	nd
Al	nd	nd
Fe	nd	nd
P	0.08	0.02

nd = not detected

CONCLUSION

Rigorous purification steps were necessary to obtain relatively pure samples of humic and fulvic acids from the four soils of Peninsular Malaysia. Additional treatments were incorporated into the extraction procedures. These include shaking the raw supernatant of humic substances with anhydrous sodium sulphate to salt out most of the clay particles; dialysing the acids exhaustively to remove most of the inorganic contaminants; treating the humic acid with HCl-HF mixture to desorb it from the silicate impurities and passing the fulvic acid through ion exchange resin Amberlite IR-120 to

remove inorganic contaminants that are still present in it.

Unlike soils of the temperate region, the clay minerals in the soils studied were observed to be bound strongly to the humic acid fraction. The main contaminants of the humic acid of the four soils are mainly sesquioxides, silicates and decomposed clay minerals.

Fulvic acids of the four soils studied have relatively high ash content. Non-humic organic polymers complexed chemically to the fulvic acid or associated strongly to it by other mechanisms are believed to be the main contaminants of the fulvic acid.

Relatively pure humic acid of 1.4 % ash content could be obtained after the various purification steps. However, the ash content of fulvic acid was not as easily reduced, even after passing the acid through the ion exchange resin, Amberlite IR-1200.

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