

## Determination of Minimal Duration Essential for Isolation of Humic Acids From Soils in Forest Restoration Programmes

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**Abstract:** This study was conducted to investigate whether a simple and rapid method could be developed for extracting, fractionating and purifying soil HA in forest rehabilitation programmes. Humic acids from 10 g of soil were extracted with 100 mL of 0.10 M NaOH. Different extraction periods (4, 8, 12, 16, 20 and 24 h) were tested. Samples were centrifuged (16,211 G for 15 min) at the end of each extraction period. The dark-coloured supernatant liquor containing HA was decanted and the pH of the solution adjusted to 1.0 using 6 M HCl. After acidification, the fractionation periods evaluated were 4, 8, 12, 16, 20 and 24 h. After each fractionation period, the sample was transferred to a polyethylene bottle and centrifuged (16,211 G for 10 min). The HA were purified by suspending them in 100 mL distilled water, centrifuged (16,211 G for 10 min). After repeating this procedure three times, the supernatant was analyzed for Na, Mg and K. Standard procedures were used to characterize the HA (C, E<sub>4</sub>/E<sub>6</sub>, phenolic OH, carboxylic COOH, total acidity) and soil (pH, C, organic matter). Although there was significant effect of different extraction periods on yield of HA, there was no significant relationship between fractionation period and yield of HA. There was also no significant relationship between fractionation periods and yield of HA for different extraction periods studied. In terms of purification, the distilled water used in this study was able to effectively purify HA (e.g., reduction in mineral matter such as Na<sup>+</sup>) of the soil without altering the true nature of HA as C, E<sub>4</sub>/E<sub>6</sub>, phenolic OH, carboxylic COOH, total acidity values of the acids were consistent with those reported in the literature. The significance of this work is that it enables the isolation of HA from soil within 9 h (4 h extraction period, 4 h fractionation period and 1 h purification period) instead of the existing range of 2-7 days, hence helping in facilitating the idea of producing for instance ammonium and potassium-humates from soils, a practice that could have less undesirable environmental effects.

**Key words:** humic acids isolation, humic substances, forest soils, degraded soils, re-forestation

### INTRODUCTION

Tropical forests are decreasing at the rate of 16.9 million hectares per year due to clearance for agriculture. In Malaysia, tropical rainforest covers about 19.37 million ha (60%) of its total land area. Out of this, 8.71 million ha can be found in Sarawak, Malaysia<sup>[1]</sup>. Besides excessive logging and mining, shifting cultivation is the major cause of deforestation in Sarawak. About 2.25 million ha were under shifting cultivation in the 1960s and by 1985, the figure increased to 3.3 million ha. Even Permanent Forest Estate in Sarawak was not spared of this practice as 116,000 ha have been degraded. To reverse the

situation, restoration programmes were initiated. Among the on-going rehabilitation programmes is the joint project between Universiti Putra Malaysia, Universiti Malaysia Sarawak and Japanese Center for International Studies in Ecology at Bintulu, Sarawak.

Since the inception of the project in 1990, some successes in terms of establishment of selected indigenous tree species and tree planting techniques on the degraded soils have been reported<sup>[1]</sup>. Nonetheless, time demands that a rapid method is developed to assess the accumulation of humic substances such as Humic Acids (HA) which in a way indicates the stability of soil carbon and soil carbon stock for that matter. Furthermore, this aspect could serve as is one of

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the indicators of forest soil health (e.g., carbon sequestration). From economic point of view, a simple, rapid and efficient method of isolating HA from forest soils may facilitate the use of these acids in producing organic fertilizer such as ammonium-humate in particular to reduce ammonia volatilization from fertilizers such as urea in agriculture. As one of the major components of humic substances, humic acids play a major role in soil cation exchange capacity, complexation of heavy metal ions and pesticides<sup>[2,3]</sup>, soil conditioning, carbon and nitrogen cycles<sup>[2]</sup>, plant and growth development and reduction of ammonia volatilization from soil applied urea<sup>[4]</sup>.

Malaysia imports HA based fertilizers (mostly foliar) from China and Australia, instead of producing them locally using available resources such as composts and soils. One of the reasons could be due to the laborious and time consuming processes involved in the isolation of these substances. For example, the extraction, fractionation and purification periods of HA vary from 12 h to 7 days<sup>[2,3,5,6]</sup> (but, an extraction time of 24 h<sup>[7-9]</sup> is the usual practice. Fractionation period ranges between 12 to 24 h<sup>[2,3,10]</sup> but most studies usually use 24 h<sup>[2,3,8,11,12]</sup>. Where latest technologies are not available or expensive to afford, particularly in developing countries, it usually takes between 2-7 days<sup>[5]</sup> to purify these acids. Even though purification time for HA from composted pineapple leaves has been reduced from 7-1 day<sup>[11]</sup>, information of this kind is lacking for rehabilitated forest soils HA. A recent study has managed to reduce extraction of HA in compost from 48-17 h<sup>[13]</sup> and has established relationship between HA yield and period of extraction and fractionation period and the relationships between these two variables and compost HA yield but not for rehabilitated soils. These relationships are important because they enable the estimation of the maximum and optimum yield of HA yield with extraction or fractionation or both procedures. This study was conducted to investigate whether a simple and rapid method could be developed to isolate (extracting, fractionating and purifying) soil HA in forest rehabilitation programmes.

## MATERIALS AND METHODS

The soil (Tipik Tualemkuts, Nyalau Series) used in this study was taken from a 16 year old rehabilitated forest at Universiti Putra Malaysia Bintulu Campus, Bintulu, Sarawak, Malaysia. The size of the experimental plot was 40×40 m. Fifteen soil samples were randomly taken at 0-5 cm soil depth using an augur. Three samples were taken at each sampling point and bulked, after which they were air dried to pass a 2 mm sieve. Afterwards, they kept in air tight plastic vials

for analysis. The pH of the soil was determined in a 1:2.5 soil: distilled water suspension and/or 0.1 M KCl using a glass electrode<sup>[14]</sup>. Total C and organic matter were determined by the combustion method<sup>[2]</sup>.

The HA extraction was conducted using the procedures described by some workers<sup>[3,11,13,15]</sup> with some modifications. Ten gram (dry-weight basis) soil samples at natural moisture content were placed into 250 mL polyethylene centrifuge bottles, 100 mL 0.10 M NaOH solution added and the bottles stoppered tightly with rubber stoppers. The samples were equilibrated at room temperature (25°C) on a reciprocal mechanical shaker. The extraction periods used were 4 (EP4), 8 (EP8), 12 (EP12), 16 (EP16), 20 (EP20) and 24 (EP24) h. At the end of each extraction period, the samples were centrifuged at 16,211 G (16,211 G) for 15 min (SIGMA, Model 6 K 15, Osterode am Harz, Germany). The dark-coloured supernatant liquors were decanted, the pH of the solutions adjusted to 1.0 with 6 M HCl and the HA allowed to stand at room temperature. The fractionation periods used immediately after acidification were 4 (FP4), 8 (FP8), 12 (FP12), 16 (FP16), 20 (FP20) and 24 (FP24), 21 (FP21) h. After each fractionation period, the samples were transferred to a polyethylene bottle and centrifuged at 16,211 G for 10 min. The HA samples were purified by washing them in 100 mL of distilled water through centrifugation at 16,211 rpm for 10 min to reduce mineral matter (e.g., Na<sup>+</sup>) and HCl (used during acidification). This procedure was repeated three times after which the washed HA samples were oven dried at 40°C to a constant weight, weighed and yields expressed as percentage by weight of HA in the soil used. Carbon in HA was determined by combusting the HA at 750°C<sup>[16]</sup> while the carboxylic, phenolic functional-groups and total acidity were determined by the method described by<sup>[8]</sup>. E4/E6 of the HA was determined by the method described by Stevenson<sup>[3]</sup>. It is worth noting that the complete process (extraction, fractionation and fractionation) for this study was replicated 3 times, hence values used in this work were the means of these replications. Analysis of variance was used to test for treatment effects and means of treatments were compared using Tukey's test using the Statistical Analysis System (SAS) version 9.1.

## RESULTS AND DISCUSSION

The pH in water and 1 M KCl of the soil were 4.30 and 3.33, respectively. These values are typical of Ultisols<sup>[17]</sup>. The organic matter and total carbon contents of the soil were 7.56 and 4.38%, respectively. These values are relatively high probably due to unavoidable very fine roots in the soil but the values are typical of forest Ultisols.

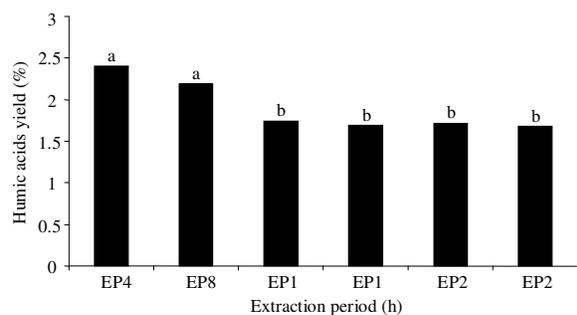


Fig. 1: Effect of Extraction Period (EP) on the yield of humic acids. Note: Bars with different letters indicate significant difference between means using Tukey's test at  $p = 0.05$

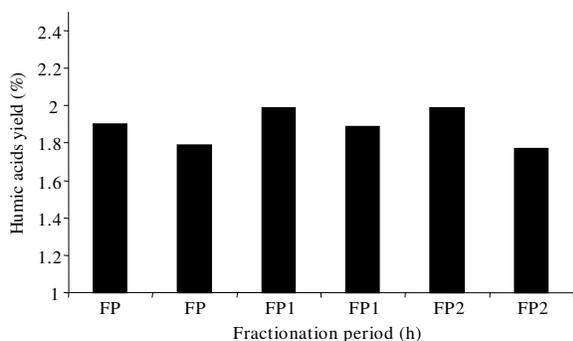


Fig. 2: Effect of Fractionation Period (FP) on the yield of humic acids. Note: No significant relationship  $p = 0.05$  between different fractionation periods and yield humic acids

The HA yields of EP4 and EP8 were statistically similar but they were significantly different from those of EP12, EP16, EP20 and EP24 (Fig. 1), indicating that the yield of HA decreased after extraction period EP8. This observation could be partly attributed to the fact exchange sites (carboxylic, phenolic functional groups and so forth) of the HA need to be at some time saturated with Na ions<sup>[18]</sup>. Additionally, the lower values with longer extraction periods are because prolonged extraction period causes significant chemical changes in HA<sup>[3]</sup>. Since the HA yields of EP4 and EP8 were not statistically different, extraction period of 4 h corresponding to HA yield of 2.41% could be considered optimum. This is because beyond 4 h, the yield of HA may not be time wise economically justifiable.

Contrary to extraction period, the yield of HA was not significantly affected by duration of fractionation immediately after acidification (Fig. 2). In view of this observation, because the yield of FP4 was not

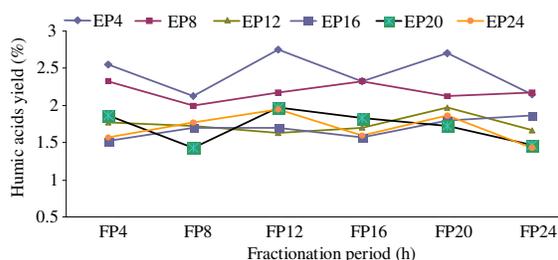


Fig. 3: Performances of fractionation periods under different Extraction Periods (EP). Note: No significant relationship ( $p = 0.05$ ) between different fractionation periods and yield of humic acid for different extraction periods studied

Table 1: Ranges of the elemental concentrations in the supernatant solutions at first and third washing of rehabilitated forest soil Humic Acids (HA) during purification

Purification stage	NA (mg L <sup>-1</sup> )	K (mg L <sup>-1</sup> )	Mg (mg L <sup>-1</sup> )
First purification	114.67-48.47	2.56-0.02	0.47-0.06
Third purification	9.48-1.83	0.75-0.00	0.16-0.05

statistically different from other periods, it could be implied that it takes about 4 h for the exchange sites of HA of soil to be saturated with hydrogen ions after acidification. Furthermore, the fact that there was no significant interaction between extraction period and fractionation period (Fig. 3) suggests that the performances of the different fractionation periods with any of the extraction periods is the same, therefore it is reasonable to assume that 4 h of equilibration after acidification is sufficient to displace the sodium ions with hydrogen ions at the exchange sites of the HA. This could also mean that the effectiveness of fractionation was dependent on the duration of extraction.

When HA are extracted they are usually not free from for instance mineral matter. As a result, they are purified before chemical characterization. As summarized in Table 1, the contents of Na, K and Mg at first washing (using distilled water) were generally high but their contents consistently decreased considerably at third washing (using distilled water). Apart from removing cations in solution, the excess water used during purification may have served as Bronsted-Lowry acid thereby donating more hydrogen ions which may have effectively replaced most of the remaining Na<sup>+</sup>, K<sup>+</sup> and Mg<sup>2+</sup> at the exchange sites of the HA which were perhaps not replaced by hydrogen ions (during acidification with 6 M HCl) during fractionation (precipitation). Similar findings have been reported<sup>[10, 18]</sup>.

Table 2: Comparison of ranges of carbon, phenolic OH, carboxylic COOH, total acidity and E<sub>4</sub>/E<sub>6</sub> of rehabilitated forest soil Humic Acids (HA) with those in the literature

Variable	HA, present study	HA, literature
Phenolic OH (cmol kg <sup>-1</sup> )	100-200	30-220 <sup>a</sup>
Carboxylic COOH (cmol kg <sup>-1</sup> )	183-275	150-570 <sup>a</sup> 380-450 <sup>a</sup>
Total acidity (cmol kg <sup>-1</sup> )	300-450	430 <sup>b</sup>
E <sub>4</sub> /E <sub>6</sub>	5.63-7.42	4.32-5.5.49 <sup>c</sup> 7-8 <sup>c</sup>

<sup>a</sup>: Stevenson<sup>[3]</sup>, <sup>b</sup>: Senesi *et al.*<sup>[19]</sup>, <sup>c</sup>: Tan<sup>[20]</sup>, <sup>c</sup>: Tan<sup>[20]</sup>

Humic acids are noted for having the ability to reduce ammonia volatilization from urea, chelate heavy metals, adsorb pesticides, maintain soil structure and so on partly because of the presence of functional groups such as phenolic OH and carboxylic COOH in them. The phenolic OH, carboxylic COOH and total acidity ranges of the HA in this study were found to be within the ranges reported in the literature (Table 2), a further indication of the purity of the HA and the effectiveness of the washing process. Additionally, the E<sub>4</sub>/E<sub>6</sub> values of the HA which indicate level of humification were comparable with those reported in literature (Table 2). The relatively high E<sub>4</sub>/E<sub>6</sub> values indicate prominence of aliphatic components or the HA in this study are of relatively low molecular weights<sup>[3]</sup>. The relatively low content of C (28-42 %) of the HA is reflection of the relatively low content of the HA in the soil studied.

In summary, it could said that the adoption of extraction period of 4 h, fractionation period of 4 h and purification period of 1 h the isolation of HA of rehabilitated forest soils can be done in less than 10 h.

## CONCLUSIONS

The optimum yield of HA from rehabilitated forest soil could be obtained at extraction of period of 4 h. Fractionation period immediately after acidification does not significantly affect the yield of HA from rehabilitated forest soil but approximately 4 h is required to precipitate the HA of this soil. There is no significant interaction between extraction period and fractionation period. The HA can be purified within 1 h using distilled water. The significance of this study is that HA of reforested soils can be isolated within 9 h (i.e., 4 h extraction period, 4 h fractionation period and 1 h purification period) or less instead of the existing range of 2 to 7 days, hence helping in facilitating the idea of producing ammonium and potassium-humates from soils.

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