

Simple and Rapid Method of Isolating Humic Acids from Tropical Peat Soils (Saprists)

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Abstract: Problem Statement: The isolation (extraction, fractionation and purification) of humic acids (HA) from soils is laborious, time consuming and expensive. The extraction, fractionation and purification periods of these substances vary from 12 h-7 days. In order to facilitate production of HA at competitive cost, this study was conducted to investigate whether a simple and rapid procedure could be developed for isolation of HA from well decomposed tropical peat soils (Saprists). **Approach:** A 0.1 M KOH was used to isolate HA of air dry peat soil at 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and 24 h extraction periods after which samples (liquid obtained after centrifugation at 16,211 G for 15 min) were fractionated (using 6 M HCl) at 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and 24 h. Samples were purified by washing them five times using distilled water instead of using HCl, HF, and an expensive process called dialysis that requires 1 to 7 days to purify HA. Each washing time was 10 min. Standard procedures were used to ascertain the purity (Ash, C, E₄/E₆, carboxylic, phenolic, total acidity, and K, Ca, Mg, and Na) and quantity of HA yield. Statistical Analysis System (SAS) was used for statistical analysis. **Results:** Although there was a linear relationship between extraction period and HA yield, there was no relationship between fractionation period and yield of HA. Distilled water used in this study was effective in purifying HA of the Saprists within 1 h without altering the true chemical nature of HA as it significantly reduced the mineral content of HA. Besides, C, E₄/E₆, carboxylic, phenolic, and total acidity of the isolated HA were typical of standard ones. **Conclusion:** The isolation of HA from peat soils can be reduced to 9 h (4 h extraction period, 4 h fractionation period and 1 h purification period) instead of the existing range of 1-7 days.

Key words: Humic acids isolation, tropical peat soils, humic substances, saprists

INTRODUCTION

About 2.5 million ha of peatland can be found in Malaysia. Pineapples and oil palms are among the major crops grown on peat soils. However, the inherent nature of peat soils has been militating against sustainable cultivation of most crops on them as desired. For instance, mature oil palms tend to lean and in some cases fall off. In addition, lack of good anchorage leads to oil palm yield reduction on these soils. In the case of pineapple cultivation on peat soils, the yield decreases with time not withstanding high application of fertilizers even though the practice is not economically viable and environmentally unfriendly^{1,2}. Besides these impediments, frequent burning of peat soils with the accompanied undesirable economic losses cannot be overemphasized.

Being organic in nature, peat soils are rich in humic substances such as humic acids (HA). At the moment, Malaysia imports HA based fertilizers (mostly foliar). One of the reasons for the importation of HA is that the isolation of these acids from soils is laborious, time consuming and expensive. For example, the extraction, fractionation and purification periods of these substances vary from 12 h-7 days³⁻⁵. Extraction period ranges between 12 and 48 h even though 24 h is the usual practice⁶⁻⁸. Fractionation period ranges between 12 and 24 h^{4,6}, although most studies usually use 24 h⁶⁻⁸. It must be emphasized that where the state of the art technologies are not available or expensive to afford, particularly in developing countries, it usually takes between 2 to 7 days to purify (removal of mineral matter like ash) HA

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Our recent studies have shown that the purification period of HA from composted pineapple leaves could be reduced from 7 days to between 1 and 2 h^[9,10] but an information such as this is lacking for HA of well decomposed peat soils (Saprists). In another study, we managed to reduce extraction and fractionation periods of HA in compost from 48 to 17 h and 24 to 3 h, respectively^[9]. These relationships are important because they enable the calculation of the maximum and optimum yields of HA with extraction or fractionation or both procedures, an information lacking for HA of Saprists. This study was carried out to investigate whether a simple and rapid procedure could be developed for isolation of HA from Saprists to facilitate the idea of producing organic fertilizers such as ammonium and potassium humates from these soils.

MATERIALS AND METHODS

The peat soil used in this study was a well decomposed peat from Craun Research, Mukah, Sarawak, Malaysia. The HA extraction was conducted using the procedures described by other authors^[9,10,11] with some modifications. Five gram (dry weight basis) peat soil samples at natural moisture content were placed into polyethylene centrifuge bottles (250 mL) and 50 mL 0.1 M NaOH solution was 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 for 15 min (SiGMA, Model 6K 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. At the end of each fractionation period, the excess supernatant liquors (fulvic acid) were siphoned off from the acidified extracts. The remainder of the suspensions containing the HA were transferred to polyethylene bottles and centrifuged at 16,211 G for 10 min. The HA samples were purified by washing them in 50 mL of distilled water through centrifugation at 16,211 G for 10 min to reduce mineral matter (e.g., Na⁺) and HCl (used during acidification). This procedure was repeated 5 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 peat soil used. Carbon and ash

contents in HA were determined by combusting the HA at 750°C^[12] while the carboxylic, phenolic functional groups and total acidity were determined by the method described by^[8]. E₄/E₆ of the HA was determined by spectroscopy.

It must be noted that 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. The relationship between extraction period, fractionation period and the yield of HA, as well the relationship between both variables (extraction period and fractionation period) on the yields of HA were evaluated using the statistical analysis system (SAS) version 9.1.

RESULTS

The pH in water and 1 M KCl of the peat soil were 3.77 and 2.45, respectively. These values were low but they were typical of tropical peat soils. The organic matter and total carbon contents of the soil were 97 and 56.21%, respectively. These values were also typical of Saprists of Sarawak, Malaysia.

There was a linear relationship between extraction period and yield of HA, (Fig. 1) from the tropical peat soil studied. Contrary to period of extraction, no significant relationship was found between the period of fractionation with yield of HA (Fig. 2). There was no significant interaction between extraction period and fractionation period (Fig. 3).

When HA are extracted they are normally not free from mineral matter. As a result, the purity of HA is usually ascertained from ash content (mineral matter), elemental composition, functional groups, spectral characteristics and so forth. The ash content should not be more than 1%. The ash content of the HA was found to range from 0.3-0.83%, a range less than the generally accepted one.

As shown in Table 1, the contents of Na, K, Ca and Mg at first washing were generally high but their contents consistently decreased considerably at the fifth washing (Table 1).

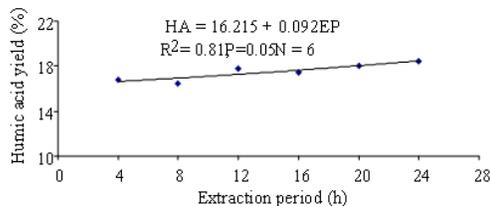


Fig. 1: Relationship between extraction period and humic acids yield

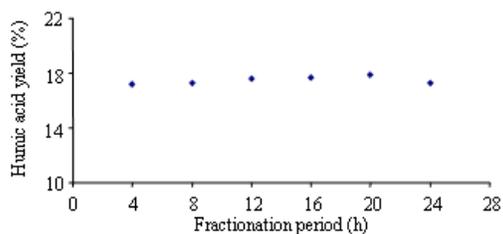


Fig. 2: Relationship between fractionation period and humic acids yield

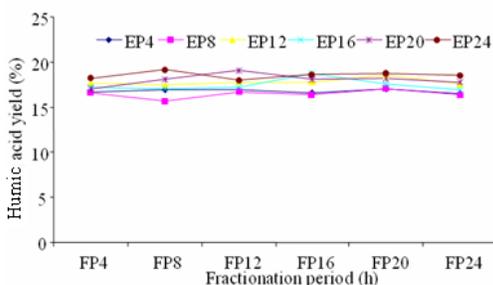


Fig. 3: Performances of fractionation periods under different Extraction Periods (EP). Note, no significant relationship $p = 0.05$ between different fractionation periods and yield of humic acid for different extraction periods studied

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

Purification stage	Na	K (mg L^{-1})	Ca	Mg
First purification	91.8-143	0.4-1	6.7-10.8	1-1.4
Fifth purification	22.8-66	0.01-0.4	1.9-3.2	0.2-0.5

Table 2: Comparison of ranges of carbon, phenolic OH, carboxylic COOH, total acidity and E4/E6 of Saprists peat Humic Acids (HA) with related reports

Variable	Present study	Reference
Carbon (%)	57.05-57.90	48.90-58.50 54.00-59.00
Phenolic OH (cmol kg^{-1})	200-433	150-440
Carboxylic COOH (cmol kg^{-1})	433-533	240-540
Total acidity (cmol kg^{-1})	667-950	570-890; 390-980
E4/E6	6.46-7.24	7-8 or higher

Schnitzer^[6], Tan^[5]

The C content, phenolic OH, carboxylic COOH and total acidity ranges of the HA in this study were found to be within the ranges reported by other authors (Table 2). Additionally, the E₄/E₆ values of the HA which indicate level of humification were comparable with those reported elsewhere

DISCUSSION

The linear relationship between extraction period and yield of HA was consistent with the observations of Goh and Reid^[3] that the yield of HA increases with increasing extraction period. This finding for Saprists can be explained based on the fact that besides slowing down depolymerisation of high molecular weight complexes, the solubilisation of the peat soil increased with increasing period because the exchange process of Na ions at the exchange sites (carboxylic, phenolic functional groups) of peat progressed with the extraction period until 24 h, a period when most of these sites may have been saturated with Na ion, making the peat soil highly soluble and hence the maximum yield at this extraction period. Another reason could be the difficulty of extracting the humic substances in the initial shorter period (e.g., 4 h) because of the difficulty of wetting the peat soil due to irreversible drying associated with peat soils upon drying. It must, however, be pointed out that where the characterization of the HA is of primary importance instead of the amount of HA isolated, an optimum yield (16.54%) of HA equivalent to optimum extraction period of 4 h or less could be considered, as the longer the extraction period, the greater will be the chemical changes. Optimum yield was calculated as 90% of the maximum yield of HA corresponding to maximum extraction period of 24 h beyond which this yield will not be time wise economical^[9,10].

No significant relationship between the period of fractionation with yield of HA suggests that the yield of HA was not affected by fractionation period. Therefore, it is assumed that the minimum fractionation period in this study is 4 h. This is because the fractionation period was sufficient for the H ions (after acidification with 6 M HCl) to displace Na ions and other cations at the exchange complexes of the HA.

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.

The low ash content suggests that the HA in this study contained a relatively low content of inorganic ions. The relatively low ash content of the HA indicates that the purification process was effective in reducing mineral matter.

Apart from removing cations in solution, the excess water used during purification served as

Bronsted-Lowry acid thereby donating more hydrogen ions which effectively replaced some of the remaining Na, K, Ca and Mg at the exchange complexes of the HA which were not replaced by hydrogen ions (during acidification with 6 M HCl) during fractionation (precipitation). The consistency of the range of carbon content of the HA (Table 2) with those reported by^[13,15] also confirms the purity of the HA, a further indication of the purity of the HA.

The relatively high E_4/E_6 values indicate prominence of aliphatic components or the HA in this study are of relatively low molecular weights^[6,13].

CONCLUSION

The nature of the relationship between period of extraction and yield of HA from Saprists peat is linear. The optimum yield of HA can be obtained at extraction period of 4 h. There is no relationship between period of fractionation and yield of HA, so from an economic point of view 4 h is sufficient to equilibrate HA immediately after acidification for tropical peat soils. The HA can be purified within 1 h using distilled water. The significance of this study is that HA of tropical peat 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 1 to 7 days, hence helping in facilitating the idea of producing ammonium and potassium humates from tropical peat soils, a practice that could have less undesirable environmental effects.

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