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Simple Method of Purifying Humic Acids Isolated from Tropical Hemists (Peat Soil)

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Abstract: The objectives of this study were: To investigate whether a purification period of HA isolated from Hemists peat soils could be reduced and to investigate whether distilled water could be used to purify HA isolated from Hemists peat soils. Standard procedures were used to extract and fractionate HA in a Hemists peat soil. The isolated HA was purified by suspending the HA 50 mL distilled water, centrifuged for 10 minutes, supernatant decanted and the liquor analyzed for K, Ca, Mg and Na by atomic absorption spectrophotometry. The entire procedure was repeated five times after which the purified HA samples were oven dried at 40ºC to a constant weight. Washing HA for 5 consecutive times (10 min for each washing) reduced the ash (mineral matter) content of the HA to an acceptance level of 2%. This finding was associated with significant decrease in K, Ca, Mg and Na contents with increased washing time. This observation also suggests that the distilled water used during the purification process served as Bronsted-Lowry acid thereby donating more H^+ which may have replaced some of K^+, Ca^{2+}, Mg^{2+} and Na^+ at the exchange sites of the HA. The C, carboxylic COOH, phenolic OH, total acidity and E4/E6 values of the purified HA were consistent with standard values, a further indication of the effectiveness of using distilled water in purifying HA from Hemists peat soil. Humic acids isolated from Hemists peat soil can be purified within one hour using distilled water without altering the true nature of HA.

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

INTRODUCTION

The processes involved in isolating Humic Acids (HA) from soils are extraction, fractionation and purification. Each of these processes is laborious and time consuming. The usually time of HA is 24 h[1-4]. In a recent research, extraction time in isolating HA from Hemists peat soil has been reduced to 4 h[5]. Fractionation time usually ranges between 12 and 24 h[4,6,7]. Recently, Susilawati *et al*.[5] managed to reduce fractionation time in isolating HA from Hemists peat soil to 2 h.

It takes between 1-7 days to purify HA[8,9]. One of our studies have shown that HA isolated from composted pineapple leaves could be purified within 6 h[10] but an information such as this is lacking for peat soil HA. The purification process of HA is characterized by the use of expensive chemicals such as hydrochloric acid or sulphuric acid as well as hydrogen fluoride. The purification process also employs an expensive process called dialysis. To date, no much attempt has been made to addressed the aforementioned problems associated with HA purification particularly for HA isolated from peat soils.

This study was conducted with the following objectives: (i) To investigate whether a purification period of HA isolated from Hemists peat soils could be reduced and (ii) To investigate whether distilled water could be used to purify HA isolated from Hemists peat soils.

MATERIALS AND METHODS

Peat soil (Hemists) samples were taken at 0-15 cm in a 50 m^2 plot at Kuala Tatau, Sarawak, Malaysia using a peat augur. The HA extraction was carried out by the methods of Stevenson[6] and Susilawati *et al*.[5] with some modifications. Five gram (dry-weight basis) peat

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soil samples were placed into polyethylene centrifuge bottles and 0.10 M KOH solution was added and the bottles stoppered tightly with rubber stoppers. The samples were equilibrated at room temperature (about 25°C) on a reciprocal mechanical shaker at 180 rpm. At the end of the extraction period, the samples were centrifuged at 21,000 g for 15 minutes. The dark-coloured supernatant liquors was decanted, the pH of the solutions adjusted to 1.0 with 6N HCl and the HA allowed to stand at room temperature (about 25ºC). The extraction (shaking) period used was 4 h[5].

The fractionation periods used immediately after acidification was 2 h. At the end of fractionation, the suspensions containing the HA were transferred to polyethylene bottles and centrifuged at 21,000 g for 10 min. The HA samples were purified following the procedures described by Susilawati *et al*.[5], Ahmed *et al*.[10] and Ahmed *et al*.[11]. This purification was done by washing the HA in 50 mL of distilled water through centrifugation at 21,000 g for 10 min to reduce mineral matter and HCl (during acidification) used during extraction and acidification, respectively. The entire procedure was repeated 5 times and afterwards, the washed HA samples were oven dried at 40ºC to a constant weight. The complete process of extraction and fractionation for this experiment was replicated 3 times.

Ash and organic C in HA were determined by combusting the HA at 750ºC[12]. The carboxylic COOH and phenolic OH functional groups and total acidity were determined by the method described by Inbar *et al*.[2]. Humification level of the HA was ascertained by analysing the E_4 and E_6 (HA) by the method outlined by Stevenson[6]. The concentrations of K, Ca, Mg and Na during purification were determined by atomic absorption spectrophotometry. The effects of purification periods on the concentrations of K, Ca, Mg and Na were analysed statistically by analysis of variance and Tukey's test using Statistical Analysis System (SAS) version 9.1.

RESULTS AND DISCUSSION

One of the ways of confirming the purity of HA following washing is to check the ash or mineral content. The ash content of the HA after five consecutive washing was found to be 2%, a value that compared well with the generally accepted value of ±1%[6], suggesting that the HA was relatively pure or well washed. This observation also demonstrates relatively low content of inorganic ions in the HA[6].

The relatively low ash content of the purified HA may be ascribed to washing during the purification process because washing of the HA with distilled water consistently reduced the contents of K, Ca, Mg and Na (Fig. 1-4). The concentrations of K, Ca, Mg and Na at first washing were generally high but the concentrations of these elements consistently decreased remarkably with increasing time of washing. In a related study, Ahmed *et al*.[8] reported significant decreased in the contents K, Ca, Mg and Na following purification (using distilled water) of HA isolated from composted pineapple leaves. Aside from being capable of removing the cations in solution, the excess distilled water had the ability to serve as Bronsted-Lowry acid thereby donating more H^+ which may have replaced some of K^+, Ca^{2+}, Mg^{2+} and Na^+ at the exchange sites of the HA which were perhaps not replaced by H^+ (during acidification) during the fractionation process.

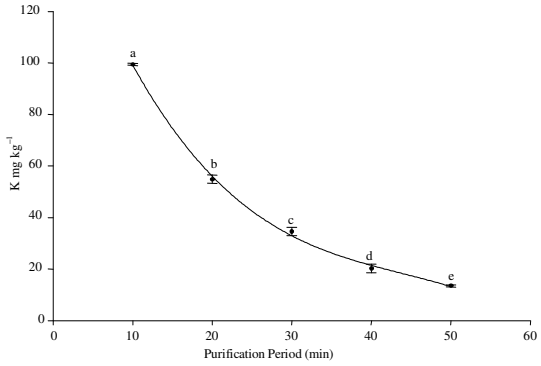

Fig. 1: Effect of washing HA with distilled water on removal of K. Different letters indicate significant difference between means using Tukey's test p = 0.01. Bars indicate standard error of menans

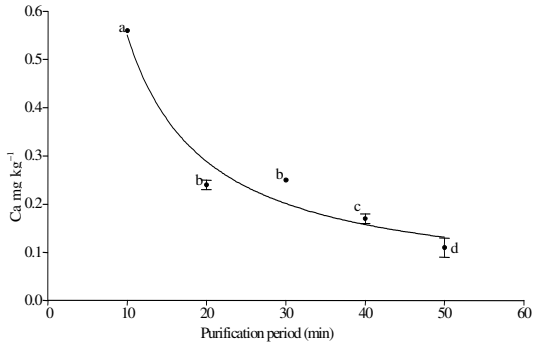

Fig. 2: Effect of washing HA with distilled water on removal of Ca, Different letters indicate significant difference between means using Tukey's test p = 0.01. Bars indicate standard error of menans

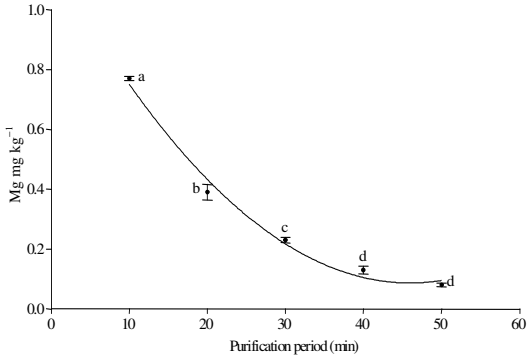

Fig. 3: Effect of washing HA with distilled water on removal of Mg. Different letters indicate significant difference between means using Tukey's test p = 0.01. Bars indicate standard error of menans

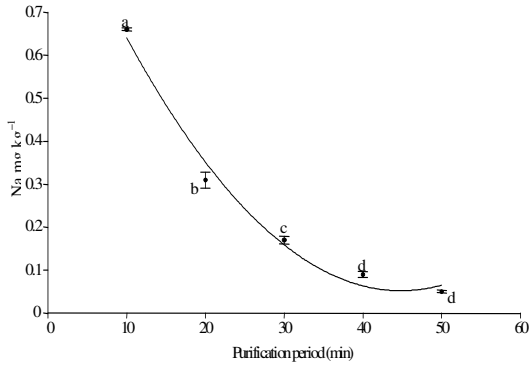

Fig. 4: Effect of washing HA with distilled water on removal of Na, Different letters indicate significant difference between means using Tukey's test p = 0.01. Bars indicate standard error of menans

Another indication of the purity of the washed HA is the similarity of the E_4/E_6 value (HA.) to those reported by Tan[13] (Table 1). The relatively high E4/E6 value of the HA indicates. The consistency of the C value (Table 1) with that reported given by Stevenson[6] is another attestation that a typical HA being dealt with.

The tendency of HA to condition soil, chelate heavy metals, adsorb pesticides and other toxic pollutants depends partly on the presence of oxygen containing functional groups such as carboxylic COOH and phenolic OH. The carboxylic COOH and phenolic OH values (Table 1) were found to be 300 and 200 cmol kg^{-1}, respectively. These values were within the ranges reported for carboxylic COOH and phenolic OH[6,13,14] The cation exchange capacity of HA is represented by the total acidity (summation of carboxylic COOH and Phenolic OH values) of the HA. The total acidity value (Table 1) of the HA was found to be 500 cmol kg^{-1}, a value that was found to be within the range reported by other workers[6,13,14]. The fact the carboxylic COOH, phenolic OH and total acidity values were comparable with those of other workers also buttress the purity of the washed HA besides demonstrating the successfulness of the purification process using distilled water.

Table 1: Effect of purification on selected chemical characteristics of humic acids (HA)

Variable	Range obtained	Typical standard range
E_4/E_6	7.08	7-8
Carbon (C%)	56.84	54-59
Carboxylic (cmol kg^{-1})	300.00	240-540
Phenolic (cmol kg^{-1})	200.00	150-440
Total acidity	500.00	390-980

Source: Stevenson[6], Tan[13] and Schnitzer[14]

CONCLUSION

Humic acids isolated from Hemists peat soil can be purified within one hour using distilled without altering the true nature of HA.

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