

Effects of Extractants on the Yields and Selected Chemical Characteristics of Humic Acids Isolated from Tropical Saprists Peat

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Abstract: Problem statement: Humic acids are beneficial to soil aggregation, binding of heavy metal, plant growth and many more. However, the isolation of Humic Acids (HA) from its origin is not only time-consuming, but the isolation is also affected by factors such as temperature and the types of extractants and their concentrations. Different concentrations of extractant are said to alter the chemical characteristics of HA. Although this kind of information is important in HA studies, it is lacking for tropical peats. **Approach:** This study was conducted to investigate the yields and selected chemical element contents of HA isolated from tropical saprists peat as affected by NaOH and KOH with different concentrations. Humic acids were isolated from tropical saprists peat taken from Sarawak, Malaysia. Yields of HA and selected chemical properties were determined using standard procedures. **Results:** Yields of HA isolated using different concentrations of NaOH and KOH showed significant difference at each level of concentrations. For the chemical characteristics tested, only total acidity showed no significant difference. For TOC and ash, the KOH used exhibited inconsistent results compared to that of NaOH. As for E_4/E_6 values, the high values obtained suggests that HA in Sarawak peats contain relatively lower molecular weight. **Conclusion:** For the purpose of studying chemical characteristics, 0.2, 0.3 and 0.4 M of both NaOH and KOH were good enough to be used in isolating HA. This is because the results of study showed that these 3 levels of concentrations yielded HA with more homogenous chemical characteristics. On the other hand, extractants with higher concentrations are preferred when the yield of HA is of concern.

Key words: Humic substances, extractants, humic acids, tropical peats

INTRODUCTION

Generally, humic matter, or humic material, refers to the humified organic material fraction of humus. Based on solubility, humic matter can be further divided into three groups, namely Fulvic Acids (FA), Humic Acids (HA) and humin (Brady and Weil, 2002). A collective term for these groups is humic substances. Humic substances are mixture of amorphous, polydispersed substances with yellow, brown to black colour. Other common characteristics including hydrophilic, acidic and high in molecular weight (Hayes, 2006), ranging from several hundreds to thousands of atomic units or Daltons are well known. Humic substances can be found in all terrestrial and aquatic environments. Isolation of humic substances can be accomplished according to a fractionation scheme based on their water solubility under acidic or alkaline conditions (Zaccone *et al.*, 2007).

In agriculture, humic matter has drawn the attention of many scientists as the performance of crops

have always been better when they are grown in soils rich in humic matter. Studies have shown that HA is in general beneficial to plant, such as growth promoting, tolerance to soil contaminant or utilization of nitrogenous fertilizer (Atiyeh *et al.*, 2002; Tan and Binger, 1986; Tan and Nopamornbodi, 1979). However, isolation of humic substances such as HA is laborious and time consuming. Factors that affect the quality and quantity of HA yield isolated from soils include extraction, fractionation and purification periods, types of extractants (Zaccone *et al.*, 2007) and others. Common extractants include neutral pyrophosphate, mixture of pyrophosphate, sodium hydroxide and potassium hydroxide (Hayes, 2006). However, some reagents are said to induce auto-oxidation of humic substances. This alters the chemical composition of HA. Besides the nature of reagents, concentration of the reagents play important role too. For instance, stronger NaOH solution extracts more HA but it alters the chemical characteristics of HA.

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Previous investigations on the influence of various extractants on the yields or structural properties of HA have been focused mainly on mineral soil HA. In contrast, relatively little attention has been devoted to HA isolated from peat (Zaccone *et al.*, 2009), especially tropical peat. Thus, the objective of this study was to investigate the yields and selected chemical element contents of HA isolated from tropical saprist peat as affected by 2 different extractants (NaOH and KOH) with different concentrations.

MATERIALS AND METHODS

Peat soil (saprist) samples were taken at 0-15 cm of secondary forest adjacent to an oil palm plantation at Kuala Tatau, Sarawak, Malaysia using peat auger. Humic acids isolation was carried out by the method of Stevenson (1994) and Ahmed *et al.* (2005) with some modifications. Ten gram (dry-weight basis) peat soil samples were placed into polyethylene centrifuge bottles and 100 mL extractant at different concentrations was added. The bottles were stoppered tightly with rubber stoppers, followed by equilibrating at room temperature on a reciprocal mechanical shaker (24 h, 180 rpm). At the end of the shaking period, samples were centrifuged at 21,000G for 15 min (Susilawati *et al.*, 2008). The dark colored supernatant liquors were decanted while the pH of the solutions was adjusted to one (Zaccone *et al.*, 2007). Afterwards, the solutions were allowed to equilibrate at room temperature.

Fractionation starts right after acidification. The period used in this study was 24 h. At the end of fractionation period, the solutions were transferred into polyethylene bottles and centrifuged at 21,000G for 10 min. The supernatant part of the samples (FA) was decanted. The remainder parts which contained HA were purified following a modification of the method of Ahmed *et al.* (2005) by washing them in 100 mL of distilled water through centrifugation at 21,000 G for 10 min with the purpose of reducing mineral matter and HCl. This procedure was repeated five times. 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 samples used. The whole isolation procedure was replicated four times.

The elemental composition of HA obtained was analyzed to determine the influence of extractant on HA. Prior to analysis, the HA samples were ground into fine powder to ensure homogeneity. Total Organic Carbon (TOC) was determined by dry combustion

method (Cheftetz *et al.*, 1996). Ash content was determined by combusting HA at 750°C (Inbar *et al.*, 1990). Analysis of functional groups (carboxylic, phenolic and total acidity) of HA was conducted according to the method described by (Inbar *et al.*, 1990). Humification level of HA was ascertained by E₄/E₆ ratio followed the method described by Stevenson (1994).

Analysis Of Variance (ANOVA) was performed to detect treatments effect using Statistical Analysis Software (SAS) version 9.1. Tukey's test at p = 0.05 was conducted for separation of means.

RESULTS

Data obtained (Fig. 1) shows that different concentrations of extractant affected the yields of HA significantly, regardless of the type of extractant. Comparison between the 2 extractants showed that only the yields of HA using 0.1 and 0.3 M were significantly different yields of HA. In the case of the other 4 concentrations tested, yields of HA isolated by using NaOH and KOH did not differ significantly.

As shown Table 1, TOC did not show significant difference across different levels of NaOH concentrations except for 0.6 M. As for KOH, the condition was getting more complicated starting from 0.3 M. Similar to NaOH, 0.6 M of KOH also exhibited significantly different values of TOC compared to other concentrations except for 0.5 M. Comparison between the 2 extractants with same concentrations showed no significant difference except for 0.5 M. The data on ash (Table 2) showed similar pattern as that of TOC.

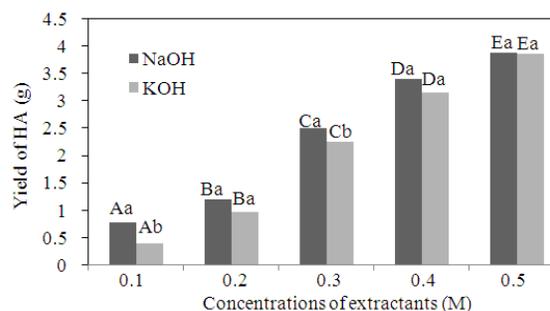


Fig. 1: Yield (g) of HA isolated using different concentrations of NaOH and KOH; Means with the same capital letter within the same row are not significantly different at p = 0.05 (Tukey's test). Means with the same letter within the same column are not significantly different at p = 0.05 (Tukey's test)

Table 1: Total organic carbon (%) of HA isolated as affected by different concentrations of NaOH and KOH

Extractants	0.1 M	0.2 M	0.3 M	0.4 M	0.5 M
NaOH	57.227 Aa	57.517 Aa	56.550 Aa	55.873 Aa	55.497 Aa
KOH	57.517 Aa	57.420 Aa	56.357 ABa	54.907 ABa	53.650 Bb

Note: Means with the same capital letter within the same row are not significantly different at $p = 0.05$ (Tukey's test). Means with the same letter within the same column are not significantly different at $p = 0.05$ (Tukey's test)

Table 2: Ash (%) of HA isolated by different concentrations of NaOH and KOH

Extractants	0.1 M	0.2 M	0.3 M	0.4 M	0.5 M
NaOH	1.334 Aa	0.834 Aa	2.500 Aa	3.667 Aa	4.334 Aa
KOH	0.834 Aa	1.000 Aa	2.833 ABa	5.333 ABa	7.500 Bb

Note: Means with the same capital letter within the same row are not significantly different at $p = 0.05$ (Tukey's test). Means with the same letter within the same column are not significantly different at $p = 0.05$ (Tukey's test)

Table 3: Total acidity (cmol/kg⁻¹) of HA isolated by different concentrations of NaOH and KOH

Extractants	0.1 M	0.2 M	0.3 M	0.4 M	0.5 M
NaOH	868.75 Aa	925.00 Aa	875.00 Aa	912.50 Aa	856.25 Aa
KOH	862.50 Aa	856.25 Aa	875.00 Aa	831.25 Aa	856.25 Aa

Note: Means with the same capital letter within the same row are not significantly different at $p = 0.05$ (Tukey's test). Means with the same letter within the same column are not significantly different at $p = 0.05$ (Tukey's test)

Table 4: E₄/E₆ value of HA isolated by different concentrations of NaOH and KOH

Extractants	0.1 M	0.2 M	0.3 M	0.4 M	0.5 M
NaOH	7.0490 Ba	8.6837 Aa	8.5339 Aa	8.2683 Aa	7.8202 ABa
KOH	8.5801 Ab	8.4909 Aa	8.4716 Aa	8.4058 Aa	7.8841 Ba

Note: Means with the same capital letter within the same row are not significantly different at $p = 0.05$ (Tukey's test). Means with the same letter within the same column are not significantly different at $p = 0.05$ (Tukey's test)

In both comparison between 2 extractants and comparison among different concentrations of 1 extractant, the value of total acidity did not exhibit any significant difference (Table 3).

Comparison of E₄/E₆ values among different concentrations of NaOH showed that 0.1 M was significantly different from others (Table 4). This observation was different from what was observed in KOH. Apart from 0.5 and 0.6 M KOH, the E₄/E₆ values as affected by other concentrations were not significantly different. As for comparison between extractants, only 0.1 M showed significant different result.

DISCUSSION

It is generally agreed that the higher the concentration of extractant, the higher the yield of HA (Stevenson, 1994). Hence, it can be concluded from Table 1 that isolation of HA from tropical saprists peat by using both NaOH and KOH, agreed with the above

mentioned statement. This observation could be associated with the exchange ability of extractant. With more Na⁺ and K⁺, the exchange process happened at the hydroxyl and carboxylic functional groups of peat was more complete for the extractant with higher concentration compared to isolation by using low concentration of extractants. Apart from this, Stevenson (1994) also pointed out that the organic matter extracted from soil with increasing concentration of alkali might be due to slow depolymerization of high molecular weight complexes. In the comparison between 2 extractants, only 0.1 and 0.3 M showed significant difference. This might due to stronger reactivity of Na⁺ compared to K⁺ in binding soil organic matter. However, when the concentration increased, the effect of the reactivity was not so obvious.

Values of TOC from HA isolated in this study were within the range reported by Li *et al.* (2003) and Stevenson (1994). Humic acids isolated by different concentrations of NaOH did not show significant difference in TOC. The trend for KOH was inconsistent as there was no single KOH concentration that was significantly different from other concentrations. However, 0.5 M KOH did show significant difference in TOC value compared to those of 0.1 and 0.2 M. For comparison between the 2 extractants, significant difference was only detected in the concentration level of 0.5 M. According to Krosshavn *et al.* (1992), there was significant loss of carbon in the extraction of humic substances by using 0.5 M NaOH as extracting solution. Though the TOC value of HA isolated by NaOH was higher than the one isolated by KOH, the above mentioned statement suggests the possible cause of this difference.

The value of ash was in general agreement with what was reported by (Zacccone *et al.*, 2007). The overall trend was similar to that of TOC (no significant difference among the 5 concentrations of NaOH as compared to those of KOH). Though there was no significant difference compared to others, the TOC values of 0.1 and 0.2 M (using both NaOH and KOH) were considered very low. This suggests that lower concentrations of extractant tend to isolate HA with less alteration on chemical characteristics. However, in the comparison between 2 extractants, only 0.5 M showed significant difference. Generally K⁺ is weaker than Na⁺ in exchange ability. The isolation of HA using 0.5 M KOH might not be as effective as using 0.5 M NaOH. Hence, HA isolated by 0.5 M KOH might contain more foreign materials than the one isolated by 0.5 M NaOH.

Total acidity obtained in this study was consistent with those reported by Campitelli *et al.* (2006) and also Stevenson (1994). The value of total acidity, either

compared within the same column or compared within the same row, showed no significant difference. Hence, it could be concluded that both extractants and concentration had no effect on the values of carboxylic and phenolic groups of HA.

The E_4/E_6 values reported in this study were slightly higher compared to the one reported by Sim and Mohamed (2007). According to Stevenson (1994), E_4/E_6 value has an inverse relationship with molecular weight of humic substances. The relatively high value reported in this study indicated that HA isolated is lower in molecular weight. The above mentioned statement is supported by the study of Sim and Mohamed (2007) as their study showed that Sarawak humic substances possess relatively lower molecular weight. Comparison between the 2 extractants showed no significant difference in all the concentrations tested, except for 0.1 M. Both Na and K are in the same group of periodic table. This might be the possible reason why their chemical reaction is similar to each other.

CONCLUSION

Humic substances have been extracted from different origins for different purposes. For the purpose of maintain HA chemical characteristics, 0.2, 0.3 and 0.4 M of both NaOH and KOH could be a good choice as the results of study showed that these concentrations yielded HA with less difference in the chemical characteristics of the isolated HA. However, if yield of HA is of concern, extractants with higher concentration should be used.

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