International Journal of Applied Research

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Minimal duration of humic acid isolation from secondary forest soil of Kelantan, Malaysia

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Abstract

The extraction, fractionation, and purification periods of humic acid are very time-consuming which may vary from 12 hours to 7 days. However, the duration for isolation of humic acid from different sources vary considerably. The objective of this study was to determine the minimal duration essential to isolate humic acid from the secondary forest soil of Universiti Malaysia Kelantan Jeli Campus. A 5 g of soil were mixed with 50 ml 0.5 M sodium hydroxide. An extraction period of 24 hours was used in this study. Samples were centrifuged at 10,000 G for 15 minutes after 24 hours. The dark-coloured supernatant liquor containing both humic acid and fulvic acid was decanted and the supernatant was acidified to pH 1 using 6 M hydrochloric acid. Different fractionation periods (2, 4, 6, 8, 10, 12, and 24 hours) were tested in this study. Samples were centrifuged at 10,000 G for 15 minutes at the end of each fractionation period. The humic acid was purified using 50 ml of distilled water and centrifuged at 10,000 G for 10 min for three times. There was significant effect of different fractionation periods on the yield of humic acid. Fractionation period immediately after acidification (2 hours) did not significantly affect the yield of humic acid but 4 hours is required to precipitate the humic acid. The humic acid can be purified within 1 hour using distilled water. The significance of this study is that humic acid of Universiti Malaysia Kelantan Jeli Campus secondary forest soils can be isolated within 24 hours of extraction period, 4 hours of fractionation period, and 1 hour of purification period instead of the existing range, hence this may help to facilitate reduction of time and costs required to isolate humic acid from this soil.

Keywords: Humic acid, fractionation period, secondary forest soil, yield

1. Introduction

Humic acid (HA) are substances that occur naturally from complicated complex microbial degradation of animal debris and plants [1]. Humic acids are black or dark brown in colour, which are insoluble in acidic solution, but soluble in alkaline solution [2]. Humic acids are ample in natural environment and verified to group alkyl units and large polycyclic aromatic hydrocarbons as framework which attached with phenolic hydroxyl, carboxylic acid, alcoholic hydroxyl, ether and amino functional groups [3, 4]. Humic acid is part of the natural organic oligomer that can efficiently bypass the problem of matrix interference in low-mass site [5]. Humic acid also contains laser energy absorbing-transferring aromatic structure, inherently highly functionalized and rich in labile protons. Moreover, the structure and properties of solid HA is more stable than aquatic fulvic acid [6, 7].

The extraction, fractionation and purification periods of HAs are very time-consuming which may vary from 12 hours till 7 days. The general practices for extraction the HA is 24 hours. Meanwhile, the fractionation period can be between 12 to 24 hours even though most studies usually done for 24 hours. Purification period can vary between 2 to 7 days without unaffordable modern technologies. However, the duration for isolation of HAs from different sources vary considerably. According to Nur Hanisah *et al* [8], the optimum yield of HA from rehabilitated forest in Bintulu, Sarawak can be obtained within 4 hours of extraction period. There was no significant interaction between extraction period and fractionation period in the study. This is because fractionation after acidification did not significantly affect the yield of HA from the soils. However, approximately 4 hours were required to precipitate the HA from the rehabilitated forest soils. Purification was done by using distilled water for one hour. The objective of this study was to determine the minimal duration essential to isolate HA from the secondary forest soil of Universiti Malaysia Kelantan Jeli Campus.

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2. Materials and Methods

(a) Soil sampling and determination of selected physico-chemical properties of soil

Soil samples were collected from Universiti Malaysia Kelantan Jeli Campus's secondary forest. Ten soil samples were randomly taken and bulked together. After that, the soil samples were air-dried and grounded manually to pass through 2-mm sieve. In order to determine the characteristics of the soil, selected soil physico-chemical analyses were performed. The bulk densities at soil depth of 0-25 cm were determined by coring method [9]. The pH of the soil sample was determined by potentiometric method [10]. The soil texture was determined by using hydrometer method [11]. The total C and N content of soil samples was analysed using CHNS analyser (TruSpec Micro Elemental Analyser (NCHS), LECO, USA) in the Department of Land Management, Universiti Putra Malaysia Serdang Campus. The percentage of SOM was determined by taking the total C value divided by a conversion of factor of 0.58 [12]. The soil available P was extracted using Mehlich No.1 Double Acid Method [13]. The solution was analysed by the molybdenum blue method [14]. The developed blue colour was analyzed by UV-VIS spectrophotometer (Thermo Scientific Genesys 20) at 882 nm wavelength. The soil exchangeable cations (K, Ca, Mg, Na, Fe, Cu, and Zn) were extracted using Mehlich No.1 Double Acid Method [13]. An atomic absorption spectrophotometer (AAS) (Perkin Elmer AAnalyst 800) was calibrated and the extract was aspirated into the AAS and the absorbance reading was recorded.

(b) Extraction of humic acid

The extraction of HA was done by using the method described by Stevenson [15]. A 5 g of soil sample was placed in the centrifuge bottle, added with 50 mL of 0.5 M NaOH and the bottle was closed tightly. The samples were equilibrated at room temperature on a mechanical shaker at 180 rpm for 24 hours. After the extraction period, the side of the bottles was washed by using distilled water and the mixture was centrifuged at 10, 000 G for 15 minutes. The dark color of supernatant liquors containing HA was decanted. The pH of the solution was adjusted to 1.0 by using 6 M HCl. Then, the HA was allowed to equilibrate for 2, 4, 6, 8, 10, 12 and 24 hours. After that, the supernatant which is FA was siphoned off from the acidified extracts [16]. The remainder of the suspension was transferred to centrifuge bottles and the HA was centrifuged. Purification was conducted to purify the HA. It was done by using the method described by Ahmed et al. [17]. The HA was purified by suspended the HA into 50 mL of distilled water and centrifuged at 10,000 G for 10 minutes till the supernatant decanted. The procedures were repeated three times. The washed HA was oven-dried at 40 °C until it reached constant weight. The yield of HA was expressed as the percentage of the weight of soil used.

(c) Determination of humification level and functional groups of humic acid

Humification level was determined using E_4/E_6 ratio (465 and 665 nm) by spectroscopy [15]. A 0.003 g of the HA was dissolved in 10 mL of 0.05 M NaHCO3. The model of spectrometer used to determine E_4/E_6 ratio was UV-VIS spectrophotometer (Thermo Scientific Genesys 20). The carboxylic (-COOH), phenolic (-OH) functional groups and total acidity were determined by the method described by Inbar et al. [18]. A 0.02 g sample of HA was dissolved in 4 mL of 0.08 M NaOH and equilibrated at room temperature on a reciprocal shaker for 30 minutes. The initial pH was recorded. The solution was titrated with 0.10 M HCl to pH 2.5 within 15 minutes. Phenol content was calculated by assuming that 50% of the phenols were dissociated at pH 10. The acid consumption between pH 8 and 10 should represent half of the phenol. Carboxyl content was calculated based on the amount the acid required to titrate the suspension between pH 8 and the end point (pH 2.5). Total acidity was calculated by summation of the phenols and carboxyls.

3. Results and Discussion

(a) Selected physico-chemical properties of soil

The soil bulk density of the secondary forest was 1.11 g cm^{-3} (Table 1). The value was found to be within the range reported by Ch'ng et al. [19] and Leng et al. [20], which is between 0.9 to 1.6 g cm^{-3}. The low soil bulk density of this study suggests high soil porosity and relative low soil compaction. This is because the soil texture was found to be loamy sand. Besides, the lower value of soil bulk density in this study was also probably due to the accumulation of SOM content which will consequently decrease the soil bulk density value of the soil [19]. The riverine alluvium in the loamy sand texture also contributed to the low value of soil bulk density [21].

Table 1: Selected physico-chemical properties of soil in secondary forest of Universiti Malaysia Kelantan Jeli Campus.

Property	Value Obtained
Bulk Density (g cm^{-3})	1.11 ± 0.05
Soil Texture	Loamy Sand
pH (Water)	5.04 ± 0.03
Soil organic matter (%)	4.15 ± 0.22
Soil total carbon (%)	2.39 ± 0.12
Total N (%)	0.14± 0
Available P (ppm)	2.41 ± 0.04
Exchangeable K (ppm)	411.67 ± 17.86
Exchangeable Ca (ppm)	14.02 ± 1.29
Exchangeable Mg (ppm)	22.5 ± 1.61
Exchangeable Na (ppm)	123.67 ± 10.40
Exchangeable Fe (ppm)	375 ± 24.76
Exchangeable Cu (ppm)	0.14 ± 0.03
Exchangeable Zn (ppm)	1.77 ± 0.04

The soil pH of this study was 5.04 (Table 1). The value was relatively higher compared to the studies by Ch'ng et al. [19] and Leng et al. [20] which was also conducted the study for secondary forest soil. The difference between the soil pH in the current study and the soil pH in other studies proposes that different ages of secondary forests at different locations had significant effect on the soil pH. This was due to the rate of litter production at different ages and distribution of SOM of the ages of secondary forests and soil depths could be different [22]. The soil texture of the soil samples collected from the secondary forest was loamy sand (Table 1). The selected physico-chemical and field observations indicate that the soil is *Typic Dystropept* (Nangka series). The soil type was compared with the data recorded by Shamshuddin and Tessens [23]. The soil is characterised as a riverine alluvium. Structures of soil are weak, medium, and the consistence is friable Soil Survey Staff [21].

The secondary forest soil contained 4.15% of SOM and 2.39% of soil total C (Table 1). The values of SOM and soil total C reported in this study were relatively lower compared to the data reported by Ch'ng et al. [19], Ahmed et al. [22], Akbar et al. [24], and Leng et al. [20]. This is because the SOM recorded by Ch'ng et al. [19], Ahmed et al. [22], Akbar et al. [24], and Leng et al. [20] were from a 25-year-old secondary forest and 16-year-old rehabilitated forest. General increase in SOM with soil age could be due to decomposition and production of litter, which leads to the formation of humus. The lower value of SOM and soil total

carbon in this study also further suggests that the age of the secondary forest in UMK Jeli Campus is younger. It was also because of low N for efficient conversion of biomass C to humus C which is required for humification of biomass returned to the soil. Besides, the soil pH also has association with soil total C as well as SOM. This is because the acidic nature of the soil (as reported in this study) may have limited the abundance of microbes and their ability to decompose biomass to release more soil total C. Hence, the soil with low pH tends to have low SOM [25].

The percentage of total N of secondary forest soil was 0.14% and was typical of Ultisols (Table 1). The value was consistent with the studies by Ch'ng et al. [19], Akbar et al. [24], and Leng et al. [20]. The secondary forest soil available P in this study was 2.41 ppm (Table 1). The availability of P in the soil correlated with SOM [25]. Even though soil aeration also affects C/P ratio, in this study this factor may have played a significant role because the loamy sand texture indirectly affects the soil aeration and for that matter C/P ratio. Since the role of P is essential for the general health and vigour of all plants, the adequate supply of P to the plant would induce better plant growth. The exchangeable K, Ca, Mg, Na, Fe, Cu, and Zn are 411.67, 14.02, 22.5, 123.67, 375, 0.14 and 1.77 ppm, respectively (Table 1). The values were consistent with that reported by Soil Survey Staff [21].

(b) Isolation of humic acid

Table 2: Effect of fractionation period on the yield of humic acids.

Fractionation Period	Humic Acid Yield (%)
2 Hours	0.93 ± 0.33^D
4 Hours	1.45 ± 2.97^{AB}
6 Hours	1.80 ± 2.67^A
8 Hours	1.30 ± 1.16^{BC}
10 Hours	1.30 ± 1.16^{BC}
12 Hours	1.30 ± 1.53^{BC}
24 Hours	1.70 ± 2.40^{AB}

Note: Means within column with different letter(s) indicate significant difference between means by Tukey's test at $p \leq 0.05$.

Table 2 shows the value obtained for the HA yield for different fractionation period. The following means comparison of effect of different fractionation periods on the yield of HA revealed that: (1) yield of HA fractionated under 2 hours was significantly lower compared to those fractionated under 4, 6, 8, 10, 12, and 24 hours; (2) yield of HA fractionated under 4, 6, and 24 hours were not statistically different; (3) yield of HA fractionated under 4, 8, 10, 12, and 24 hours were not statistically different (Table 2). This indicates that the yield of HA was fractionated significantly low when fractionated under 2 hours. This observation could be partly attributed to the fact that exchange sites (carboxylic, phenolic functional groups and so forth) of the HA needed to be at some time saturated with Na ions [25].

Since the HA yields fractionated under 4, 6, and 24 hours were not statistically different, fractionation period of 4 hours corresponding to HA yield of 1.45% could be considered optimum. This is because beyond 4 hours, the yield of HA may not be time wise economically justifiable. In view of this observation, because the yield of HA fractionated under 4 hours was not statistically different from 6 and 24 hours, it could be implied that it takes about 4 hours for the exchange sites of HA of soil to be saturated

with H^+ ions after acidification. Even though, the HA yields show fluctuation which the yield increases with the increase of fractionation period from 2 till 6 hours. Starting from 8 till 12 hours of fractionation period, the HA decreased and remained constant. After 24 hours of fractionation period, the HA yield increased again. The previous study recorded by Nur Hanisah et al. [8] also showed fluctuation of HA yield with different fractionation periods. The lower values obtained with longer fractionation periods may be caused by the prolonged fractionation periods causing significant chemical changes in HA [15].

(c) Functional groups and humification level of humic acid

Phenolic, -OH, carboxylic, -COOH and total acidity (Table 3) were within range of the previous studies. The phenolic group of different fractionation period ranges from 150 to 200 cmol kg^{-1} which is relatively high value within previous studies reported by Nur Hanisah et al. [8] and Stevenson [15]. The carboxylic groups with range of 350 to 467 cmol kg^{-1} of this study also indicate that they were in the range of the recent study by Nur Hanisah et al. [8]. The relative high values of phenolic and carboxylic group may be because of the less stable HA. The high values of the functional groups

cause relatively high value of total acidity of the experimented HA. Even though the values of E_4/E_6 ratio is relatively high, the values are still within the range of the study reported by Nur Hanisah *et al*. [8]. The relatively high E_4/E_6 values in the secondary forest in this study may be due to the low molecular weights [2, 26].

Table 3: Comparison of ranges of phenolic -OH, carboxylic -COOH, total acidity, and E_4/E_6 of rehabilitated forest soil humic acids with those in the literature.

Variable	Fractionation Duration (hours)	HA, present study	HA, literature
Phenolic OH (cmol kg^{-1})	2	200	100-200[a] 30-220[b]
	4	200	
	6	200	
	8	200	
	10	150	
	12	150	
	24	200	
Carboxylic COOH (cmol kg^{-1})	2	200	183-275[a] 150-570[b] 380-450[b]
	4	233	
	6	267	
	8	233	
	10	250	
	12	200	
	24	250	
Total Acidity	2	400	300-450[a] 430[c]
	4	433	
	6	467	
	8	433	
	10	400	
	12	350	
	24	450	
E_4/E_6	2	6.00	5.63-7.42[a] 4.32-5.49[d] 7-8[d]
	4	5.84	
	6	6.35	
	8	6.14	
	10	6.60	
	12	6.11	
	24	6.33	

Note: [a]Nur Hanisah *et al*. [8]; [b]Stevenson (1994); [c]Senesi *et al*. [27]; [d]Tan [2]

4. Conclusion
The optimum yield of HA from secondary forest soil of Universiti Malaysia Kelantan Jeli Campus could be achieved at extraction period of 24 hours. Fractionation periods of 4 hours is required to precipitate the HA of this secondary forest soil. The HA can be purified within 1 hour by distilled water.

5. Acknowledgement

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