Determination of Minimal Duration Essential and Extractant Ratio for Humic Acid Isolation from Rice Straw Compost

By

Yeong Yee Yue

F14A0414

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DECLARATION

I hereby declare that the work embodied in this Report is the result of the original research and has not been submitted for a higher degree to any universities or institutions.

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Student

Name:

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I certify that the Report of this final year project entitled “Determination of Minimal Duration Essential and Extractant Ratio for Humic Acid Isolation from Rice Straw Compost” by Yeong Yee Yue, matric number F14A0414 has been examined and all the correction recommended by examiners have been done for the degree of Bachelor of Applied Science (Agrotechnology) with Honours, Faculty of Agro-Based Industry, Universiti Malaysia Kelantan.

Approved by:

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Supervisor

Name:

Date:

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ABSTRACT

The method for accumulate humic substances especially humic acid is laborious and time consuming. The extraction, fractionation and purification periods of humic acid vary from 12 hours to 7 days. The common extractant ratio used for isolation is 1:10 which is recommended by International Humic Substances Society. However, the duration and extractant ratio for isolation of humic acid from different sources vary significantly. The objective of this study is to determine then minimum duration essential and extractant ratio for humic acid isolation from rice straw compost. A ratio of 1: 5 and 1: 10 of compost sample and chemical used were performed in humic acid extraction. A 5 g of compost sample was mixed with 0.5 M sodium hydroxide and were extracted for different extraction period (1, 3, 5, 7, 9, 12 and 24 hours). After the extraction period, the samples were centrifuged at 10, 000 rpm for 15 minutes. Then, the samples were acidified to pH 1 using 6 M hydrochloric acid and fractionated for 24 hours. The samples were centrifuged for 15 minutes at 10, 000 rpm after 24 hours. The humic acid samples were purified using 50 ml of distilled water and centrifuged three times at 10,000 rpm for 10 minutes. There was significant effect of different extraction periods and extractant ratio on the yield of humic acid. This study indicated that approximately 12 hours are required to extract humic acid from rice straw compost optimally and can be purified within 1 hour using distilled water. The significance of the study is humic acid from rice straw compost can be isolated within 12 hour of extraction period, 24 hours of fractionation period and 1 hour of purification period. Thus, this may help in reducing time and costs needed to produce the humate product from this compost. The extractant ratio of 1:10 is more preferred because of the higher humic acid yield obtained.

Keywords: humic acid, extraction period, extractant ratio, rice straw compost, yield of humic acid

Penentuan Tempoh dan Nisbah Pengekstrut Minimal bagi Pengasingan Asid Humik dari Kompos Jerami Beras

ABSTRAK

Kaedah untuk mengumpul bahan-bahan humik terutama asid humik adalah sukar dan memakan masa. Pengekstrakan, pemeringkatan dan tempoh pembersihan asid humat berbeza dari 12 jam hingga 7 hari. Nisbah pengekstut yang digunakan untuk pengasingan biasanya ialah 1:10 yang dicadangkan oleh International Humic Substances Society. Walau bagaimanapun, tempoh dan nisbah ekstaksi untuk mengasingkan asid humik dari pelbagai sumber adalah berbeza. Objektif kajian ini adalah untuk menentukan tempoh dan nisbah pengekstrut minimal untuk pengasingan asid humat dari kompos jerami beras. Nisbah 1: 5 dan 1: 10 sampel kompos dan bahan kimia yang digunakan telah digunakan dalam pengasingan asid humik. 5 g sampel kompos telah bercampur dengan 0.5 M natrium hidroksida dan diekstrak untuk tempoh pengekstrakan yang berbeza (1, 3, 5, 7, 9, 12 dan 24 jam). Selepas tempoh pengekstrakan, sampel telah disentrifugasi pada 10, 000 rpm selama 15 minit. Kemudian, sampel telah diasidkan kepada pH 1 menggunakan asid hidroklorik 6 M dan dimeringkat selama 24 jam. Sampel telah disentrifugasi selama 15 minit pada 10, 000 rpm selepas 24 jam. Sampel asid humik dibersihkan menggunakan 50 ml air suling dan disentrifugasi tiga kali pada 10,000 rpm selama 10 minit. Kajian ini terdapat kesan yang kerata dari tempoh pengekstrakan dan nisbah ekstraksi yang berbeza terhadap hasil asid humik. Kajian ini menunjukkan kira-kira 12 jam diperlukan untuk mendakan asid humik dari kompos jerami beras secara optimum dan boleh dibershikan dalam masa 1 jam dengan air suling. Kepentingan kajian ini adalah asid humik dari kompos jerami boleh diasingkan dalam 12 jam tempoh pengekstrakan, 24 jam tempoh pemeringkatan dan 1 jam tempoh pembersihan. Oleh itu, ini boleh membantu dalam mengurangkan masa dan kos yang diperlukan untuk menghasilkan produk humate dari kompos ini. Nisbah ekstraksi 1:10 lebih dicadangkan dalam kajian ini kerana kuantiti hasil asid humik lebih tinggi.

Kata kunci: asid himik, tempoh pengekstrakan, nisbah ekstrak, kompos jerami, hasil asid humat

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LIST OF ABBREVIATIONS AND SYMBOLS

ATP	Adenosine Triphosphate
$Ba(OH)_2$	Barium Hydroxide
°C	Celcius
C	Carbon
CEC	Cation Exchange Capacity
Ca	Calcium
COOH	Carboxylic
Cu	Copper
E_4/E_6	Ratio 0f Absorbance Intensities at 465nm And 665nm
FA	Fulvic Acid
Fe	Iron
H	Hydrogen
HA	Humic Acid
HCl	Hydrochloric Acid
HF	Hydrofluoric Acid
HS	Humic Substances
K	Potassium
Mg	Magnesium
Mn	Manganese
N	Nitrogen
Na	Sodium
$Na_4P_2O_7$	Tetrasodium Pyrophosphate
Na_2CO_3	Sodium Bicarbonate
NaOH	Sodium Hydroxide
O	Oxygen
OH	Phenolic
OM	Organic Matter
rpm	Revolutions per Minute
P	Phosphorous
S	Sulphur
UV-VIS	Ultraviolet and Visible Spectroscopy
Zn	Zinc

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CHAPTER 1

INTRODUCTION

1.1 Research Background

Humic substances (HS) are derived from organic matter which are mainly formed through the microbial degradation of plant material and decayed organic matter that mainly found in manure, peat, lignite coal, and leonardite (Baldock & Nelson, 1999). There are three fractions of HS namely, humic acid (HA), fulvic acid (FA) and humin. They are categorised based on their solubility (Reshi & Tyub, 2007; White, 2003).

The information, structure and function of HS are not well understood, although many researcher put a large research effort over the times (Stevenson, 1994). It is very difficult to extract intact HS from soils because they are bound with mineral surfaces. Because of the expenditure and time involved with these extractions, many researchers are more preferred use commercial humic products for their studies. The yield of extractable HA and FA from HS and its chemical characteristics depend on the origin from which HA is extracted, temperature, grain size, frequency of extraction, the extracting agent and its strength and drying procedure (Schnitzer, 1978).

Because of its molecular structure, it provides abundant benefits to plant growth and soil physiochemical properties. Humic substances increase the cation exchange capacity (CEC), providing the soil a stronger buffer capacity to withstand sudden drastic chemical changes, that will indirectly affect the soil fertility and environmental quality (Reshi & Tyub, 2007; Kim H. Tan, 2014). Toxic materials that introduced into the soil will be interrupted and adsorbed by HA application. Humic substances are known to have indirect and direct effects on plant growth and crop production in terms of increasing root growth and nutrient uptake (Tan, 2014; Billingham, 2015; Nardi, Concheri, & Dell'agnola, 1996). Increasing soil aggregation, water holding capacity, and improving aeration are some of the beneficial indirect effects in soil physics.

The method for accumulate humic substances especially HA is laborious and time consuming. The extraction, fractionation and purification periods of HA vary from 12 hours to 7 days. Most of the studies using 24 hours of extraction period and 24 hours of fractionation for their researches. However, there are various studies reported that the isolation can be reduced to less than 2 days varies sources of organic matter by Ahmed, Husni, Anuar, & Hanafi (2005), Chen, Ahmed, Majid, and Jalloh (2009); Kasim, Ahmed, Majid, andYusop (2007), and Palanivell, Susilawati, Ahmed, and Muhamad (2012).

The extractant ratio is also one of the factors that affect HA yield production. The International Humic Substances Society (IHSS) recommends the extractant ratio of 1:10 which gives the highest yield of HA isolated from HS (Swift, 1996). However, there is a study by Asing, Wong, and Lau (2009) concluded that the extractant ratio of 1:5 and 1:10 had no significance effect on the HA yield isolated in compost.

1.2 Problem Statement

There is a dearth information on the relationship between HA yield and time of extraction time under different extractant ratio. Most of the studies focused on the isolation duration of HS but not extractant ratio. To date, there is also lack of information about the studies toward HA derived from rice straw compost.

1.3 Hypothesis

H0: There is no significance different in extraction duration under different extractant ratio for isolation of HA from rice straw compost

Ha: There is a significance different in extraction duration under different extractant ratio for isolation of HA from rice straw compost.

1.4 Research Question

Can rice straw compost's HA extraction duration and extractant ratio be reduced from conventional period and ratio?

1.5 Objectives

1. To extract, fractionate and purify the HA from rice straw compost
2. To determine the minimum duration to extract HA from rice straw compost
3. To determine the minimum extractant ratio to extract HA from rice straw compost

1.6 Scope of study

This study focuses on determination of minimal duration and extractant ratio essential for HA isolation from rice straw compost.

1.7 Significance of Study

This study help to determine the minimal duration and extractant ratio of HA isolation from standard isolation method that is costly, time and labour consuming. This study also may provide an alternative way to handle the agricultural waste by adding value to them. The information gained from this study enables the quantification of the maximum and optimum HA yields with extraction and fractionation or both procedures.

CHAPTER 2

LITERATURE REVIEW

2.1 The Nature and Distribution of HS

Humic substances are complex organic compounds that occur naturally in terrestrial, geological and water. Humic products are basically manufactured from soil, water, brown coals, brown-black coal, peats, composts and other organic materials. Many product found in market mostly are derived from peats and brown coal. Humic substances defined by Stevenson (1994), are a general category of naturally occurring, biogenic, heterogeneous organic substances that can be generally characterized as yellow to black in colour, of high molecular weight, and refractory to degradation.

They are tremendously complex super-molecules with random structures (Billingham, 2015; Reshi & Tyub, 2007). They are pervasive in nature, being formed naturally by humification from organic matter. Humic substances are both highly chemical reactive and resist to microbial decay. They are closely related with soil fertility and quality, nutrient dynamics, pollutants and contaminants, and the global carbon sequestration in soil. They have high potential in complexation and are precursors of many carcinogenic compounds, causing their elimination or immobilization in the environment (Reshi & Tyub, 2007).

They consist of three groups of substances, depending on their solubility. Humin is the HS fraction that is insoluble in water at any pH; humic acid (HA) is the fraction insoluble in water under acidic conditions with pH level less than 2, but is soluble at greater pH; and fulvic acid is the fraction soluble in water at all conditions at any pH values (Stevenson, 1994).

Many investigators now believe that all dark coloured HS are part of a system of strongly related, but not completely similar, high molecular weight polymers. According to this concept, differences between HA and FA can be explained by variation in molecular weight, numbers of functional groups (carboxyl, phenolic OH) and extent of polymerisation as described by Stevenson (1982) in Figure 2.1.

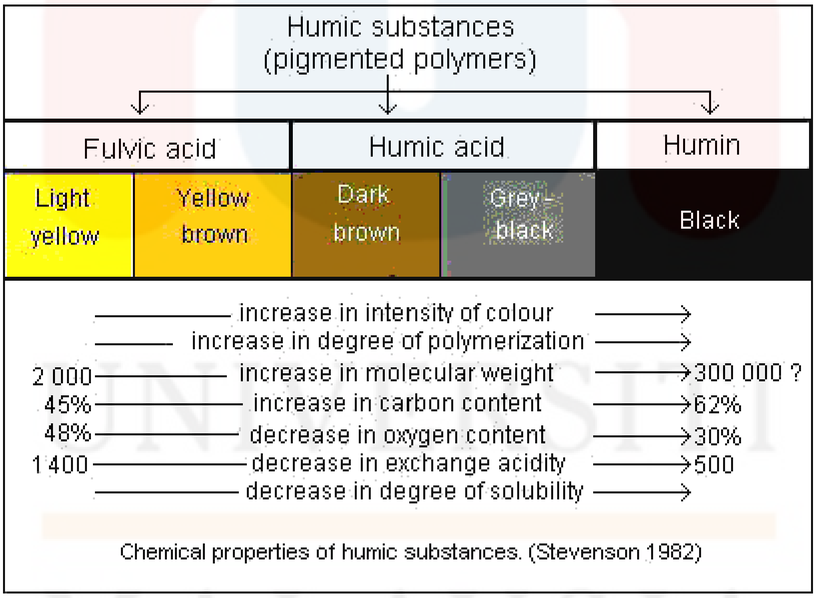


Figure 2.1: Chemical properties of humic substance (Stevenson, 1982)

Carbon and oxygen contents, acidity, and degree of polymerisation, all change systematically with increasing molecular weight. The low molecular weight FA have higher oxygen but lower C content than the high molecular weight HA because of FA contains more functional groups of an acidic nature, mainly COOH. The total acidities of FA (900-1400 meq/100g) are also considerably higher than HA (400-870 meq/100g) (Reshi & Tyub, 2007).

2.1.1 Humic Acid

Humic acid is the HS that is insoluble in aqueous solution and precipitate when the pH value is lower than 2, but soluble at higher pH values. Humic acid are dark brown to black colour with large molecular size, ranging from 10,000 to 100,000 g/mol (Stott & Martin, 1990).

Humic acid consists of a mixture of weak aliphatic (C chains) and aromatic (C rings) organic acids. They are termed polydisperse due to their flexible chemical features and are thought to be complex aromatic macromolecules with amino acids, amino sugars, peptides and aliphatic compounds involved in linkages between the aromatic groups.

Humic acid functions as important ion-exchange and metal-complexing (chelating) systems because they are readily bind with clay minerals to form stable organic-clay complexes and form salts with inorganic trace mineral elements that can be readily utilized by various living organisms (Reshi & Tyub, 2007). Therefore, HA products generally used as plant growth amenders and as an ingredient in fertilizer.

Humic acid contains many functional chemical groups that help to physically modify and improve the soil's chemical properties and biologically stimulate plant growth (Arancon, Edwards, Lee, & Byrne, 2006; Munawar & Mindari, 2015; Wright & Lenssen, 2013). The oxygen containing functional groups such as carboxyl, phenol, hydroxyl and ketone, tend to increase the soil CEC. These functional groups including the aromatic backbone and amines are also cause HA to be biologically active.

2.1.2 Fulvic Acid

Fulvic acid is the fraction of HS with a mixture of weak aliphatic and aromatic organic acids that is soluble under all pH conditions. They change colour depending on pH (Stevenson, 1994). They appear as straw yellow colour at very low pH values. When pH rises to 3, FA turns to orange colour and then change to wine red colour at higher pH. Fulvic acid have molecular weight range from approximately 1,000 to 10,000 g/mol which are generally considered to be smaller molecules with lower molecular weights than HA (Billingham, 2015).

Their composition and shape is quite variable due to FA have many carboxyl (-COOH) and hydroxyl (-COH) groups which make them extremely chemically reactive. Due to the large total number of carboxyl groups in FA, the exchange capacity of FA is more than twice over HA. FA are low in phenols, and less aromatic compared to HA (Nardi et al., 1996). Fulvic acid molecules can carry trace minerals and are readily enter plant roots, stems and leaves due to their relatively small size which is an essential role for plant growth. Fulvic acid are also the key ingredients in high quality foliar fertilizers (Reshi & Tyub, 2007).

2.1.3 Humin

Humin is insoluble in water at any pH conditions. It is the oldest among the HS fractions and is most resistant to decomposition in soil (Rice, 2001). Humin are considered macro-organic substances because their tremendously large molecular weights range approximately from 100,000 to 10,000,000 g/mol. The chemical and physical properties of this fraction are only partially understood largely because of the difficulties in extraction process (Baldock & Nelson, 1999).

2.2 Chemical Composition of HS

Humification is the process of the plant parts being physically and chemically transformed to a black, amorphous material called humus. The plant carbohydrates and proteins are decomposed and their microbial analogues are synthesised during the process. Polymerisation reactions that involving aromatic compounds mainly carboxyl and phenolic groups occur and contributed to the CEC. When amino acids bind with the phenols before polymerization, N is also incorporated into the polymers (Nardi et al., 1996; White, 2003).

The main structure of the HA and FA fractions is a combination of aliphatic and aromatic groups such as carboxyl, hydroxyl, carbonyl and alkyl group covalently bonded to form high molecular weight polymers, with abundant branching and folding. The aromatic and alkyl group are bound together principally by C-C bonds and ether linkages to form the backbone of the humic molecules which is random and is not characterized by a regular sequence of aromatic and alkyl groups. Polysaccharides and protein materials do not contribute more than 20% of the total mass although they occupy large spaces in the folded and branched macromolecules (Baldock & Nelson, 1999). This is because these molecules may be covalently bonded or held by electrostatic attraction or hydrogen-bonding.

Approximately 35-45% of the HA fraction is aromatic, consisting mainly of single ring structures that are highly substituted, mostly with carboxyl and phenolic groups of differing acid strength. HA also contain long aliphatic chains that either with carboxyl groups, link the aromatic structures or exist as separate side chains attached to the rings. Meanwhile, approximately 25% of FA is aromatic. FA have more highly charged, and more polar, although FA molecules are smaller than HA molecules. Repulsion of negative charges cause the FA molecules to be more linear than the randomly coiled HA molecules (Schnitzer, 1978; White, 2003).

The steric arrangement of different functional groups such as carboxyl, phenolic-OH, and carbonyl C=O facilities the complexing of metal cations. The non-extractable humins are believed to be HA-type compounds that are strongly adsorbed, or precipitated, on mineral surfaces as metal salts or chelates (Koopal, Riemsdijk, & Kinniburgh, 2001; White, 2003).

2.3 Isolation of HS

A proper analytical procedure is required for accomplishing the purpose of extracting the real HS. Isolation and purification are considered as integral parts of HS extraction to get the real humic compounds isolated from each other, and free from co-extracted materials or contaminants (Tan, 2005). The ideal of the extraction procedure contingent on the correct use of extraction agent. Humic substances are usually gained from the soil or other organic materials by alkaline extractant to separate HA and FA. The ideal extractant for humic substances is NaOH and usually used in research from earlier times (Stevenson, 1994).

Many techniques have been employed, depending upon the nature of the material to be examined. Therefore, nonpolar compounds (fats, waxes, resins, etc.) can be extracted with such organic solvents as hexane, ether, carbon tetrachloride, alcohol-benzene mixtures, and others. Hydrolysis procedures have been used for isolating individual monomers, such as amino acids and sugars (Stevenson, 1994).

Standard method for HS isolation from diverse sources have been developed to compare the obtained results. The ideal extraction method should meet the following criteria that discussed by Stevenson (1994):

1 The method should lead to the isolation of unaltered material.

2 The extracted humic materials must be free of inorganic contaminants, such as clay and polyvalent cations.

3 The complete extraction should be insuring representation of fractions from the entire molecular weight range.

4 The method is universally applicable to all soils at any condition.

2.3.1 Extraction of HS

The basic extraction procedure most agreed are to a soil: solution ratio of 1:5 by the researchers (Stevenson, 1994; Kim H. Tan, 2005). A study from Asing et al. (2009) indicated that the ideal sample to extractant solution ratio for HA extraction is either 1:10 or 1:5. However, 1:10 ratio was preferred and recommended by IHSS because of easy handling during the process and able to obtain the highest yield percentage from HS (Swift, 1996). Yet, in commonly, the ratio to be used depends on the organic content of the soil sample. The higher ratio is preferred for use with soil samples rich in organic matter. A flow sheet of the basic extraction procedure is outlined in Figure 2.2, whereas the analytical details are provided as follows.

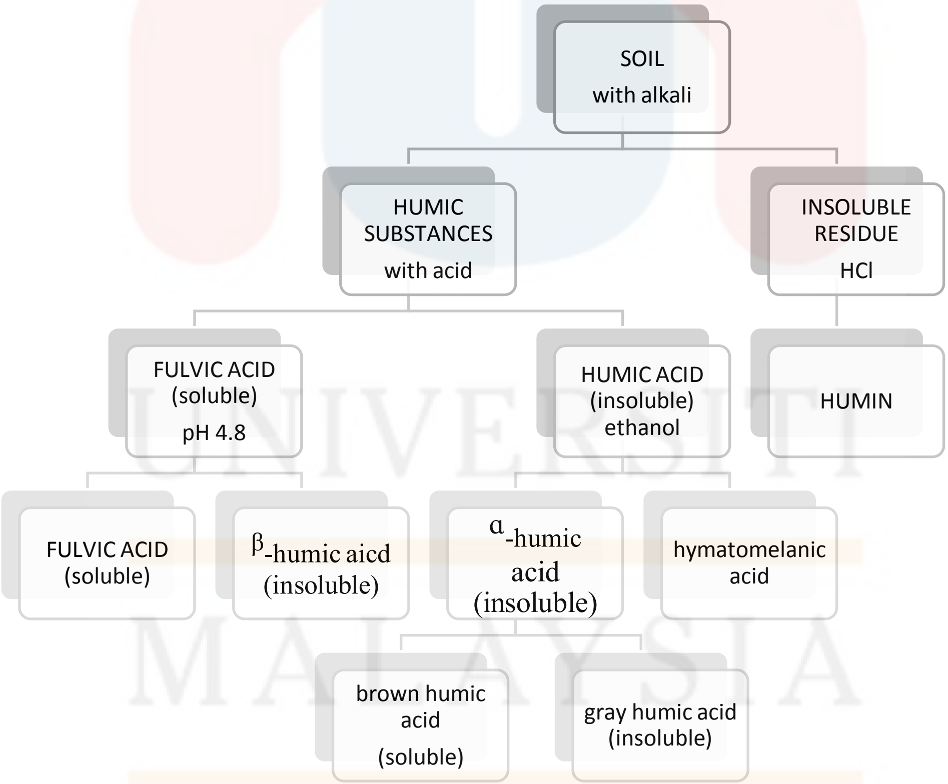


Figure 2.2: Flow sheet for extraction of humic matter from soils, peat, lignite and other terrestrial deposits (Tan, 2005).

Sodium hydroxide and Na_2CO_3 solution of 0.1 to 0.5 N in water and a soil to extractant ratio of from 1:2 to 1:5 (g/mL) have been widely used for recovering organic matter. The solubility of HS in alkali is thought to be affected by disruption of bonds holding organic material to inorganic soil components and conversion of acidic components to their soluble salt form. Diluted HCl used to leach the soil not only to remove Ca and other polyvalent cations but also to increase the efficiency of extraction of organic matter with alkaline reagents. In general, extraction of soil with 0.1 or 0.5 N NaOH able to recover approximately 2/3 of soil organic matter (Stevenson, 1994; Tan, 2005). Table 2.1 shows the suggested extractants of HS. The alkaline extractants are generally being used as it shows the highest effectiveness.

Table 2.1: The suggested extractants of HS commonly used in research. (Stevenson, 1994)

Extractant	**OM Yield (%)**
Strong alkaline extractants	
NaOH	80
Na_2CO_3	30
Ethylenediamine 2.5M	~63
Organic solvents	
Formic acid (HCOOH)	55
Ethlenediamine (anhydrous)	~5
Sulfur-containing (dimethylsulfoxide, etc)	~22
Pyridine	~36
Mild extractants	
$Na_4P_2O_7$ and other	30
Organic chelants such as hydroxyquinoline, acetylacetone, cupferron	30

The optimum extraction period varies by the origin or type of the organic matter, temperature, frequency of extraction and the types of extractant agent used and its concentration. The extraction period usually require 12 to 24 hours to recover HA from organic matter. Many of the study commonly used 24 hours to extract HA from their samples which is time consuming. A study from Ahmed, Husni, Anuar, and Hanafi, (2005) and Chen, Ahmed, Majid, and Jalloh, (2009) found that, extraction period can be completed within 24 hours from different kind of organic matters and can complete the whole isolation less than 7 days.

2.3.2 Fractionation of HS

Fractionation is a necessary step in the characterization of soil organic matter. The primary objective of fractionation is to help the application of analytical techniques by reducing heterogeneity of isolated material. There are some factors that affect the separations found on the basis of solubility characteristics. The amount of organic matter precipitated by acid is affected by metals ions in the soil organic matter extracts. The concentration of NaOH used for extraction may also affect this ratio by improved the disruption of FA to HA binding by intermolecular forces (Stevenson, 1994).

2.3.3 Purification of HS

Purification is extraordinarily important process in humic matter isolation in order to remove or reduce unwanted substances or containments such as humin, clay particles, plant residue before biological studies can be commenced. As Stevenson (1982) suggested that high speed centrifugation may remove inorganic impurities for clay and treatment with diluted HCl/HF to diminish ash content. The separation of organic impurities is rarely completely successful because they are strictly bound to HS (Nardi et al., 1996).

2.4 Characterization of Humic Matter

2.4.1 Chemical Characterization

2.4.1.1 E_4/E_6 Ratios

The ratio of optical density or absorbance of dilute aqueous HA and FA solutions at 465 and 665 nm is widely used by soil scientists for the characterization of these materials. This ratio, usually referred to as E_4/E_6 has been reported to be independent of concentration of humic materials but differ for humic materials extracted from different type of HS (Kononova, 1966; Schnitzer, 1978)

The light absorption of HS appears to increase with increase in: 1) the degree of condensation of aromatic rings in these substances; 2) the ratio of C in aromatic “nuclei to C in aliphatic side chains; 3) total C content; 4) molecular weight (Y. Chen, Senesi, & Schnitzer, 1977).

In general, E_4/E_6 ratio in HA is 2-5 while FA is 8-10. Usually, a high E_4/E_6 ratio reflects a low degree of aromatic condensation and deduces the presence of the relatively large proportions of aliphatic molecules. Analytically, the determination of E_4/E_6 ratio of HS is a rapid and convenient procedure that does not need a complex equipment or advanced technical skill, nonetheless can provide potentially valuable information (Baldock & Nelson, 1999; White, 2003).

According to Y. Chen, Senesi, and Schnitzer (1977), the E_4/E_6 ratio of HA's and FA's is: 1) mainly governed by the particle size (or particle or molecular weight); 2) influenced by pH; 3) associated with the free radical concentration, contents of O, C, CO_2H and total acidity in as far these parameters are also functions of the particle size or particle or molecular weight; 4) apparently indirectly correlated to the relative concentration of condensed aromatic rings; 5) independent of HA and FA concentration at least in the 100-500 rpm range (Schnitzer, 1978).

2.4.1.2 Functional Groups

The analysis of functional group in HS is carried out by standard organic chemistry methods as follows (Swift, 1996). Total acidity is usually found by titration of the HS up to pH 7 (which is an arbitrary end point). The HS are treated with a known excess of barium hydroxide, $Ba(OH)_2$, the precipitate is filtered and the remaining base is titrated with HCl to pH level of 8.4. The amount of carboxylic groups is obtained by treatment with calcium acetate, and the released protons are titrated with NaOH. The difference between the total acidity and carboxylic contents is considered to be phenolic contents (Molina, 2013).

Higher acidity of FA is clearly observed compared with HA. The carbonyl contents are found to vary between different samples (Schnitzer, 1978). In some cases, ketonic and quinoidic carbonyl are not differentiated, thus total carbonyl is reported (Molina, 2013). The total acidity also shows some variations because of the origin of the HS. These values are ascribed to the sum of the carboxyl and phenolic-OH group content, and point out the cation exchange and complexing capacities of HS. A higher total acidity value is indicative of a higher CEC and complexing power.

2.5 Agronomic Importance of HS

2.5.1 Importance in Soil

2.5.1.1 Physically Soil Properties

Humic substances and non-humic (organic) compounds provide soil organisms' energy and mineral requirements. Soil organisms that lack photosynthetic system survive on residual carbon containing substances on or in the soil. These organisms, in turn, simplified the mineral complexes that able to absorb by plants for growth development and influence soil fertility (Billingham, 2015; Tan, 2014).

Some of the studies have shown that the soil quality and crop yield were improved when organic matter applied to eroded soil (Delfine, Tognetti, Desiderio, & Alvino, 2005; Hopkins & Stark, n.d.; Wright & Lenssen, 2013). Humus enhance water holding capacity of soils due to their large surface area and high CEC, function as water sponges and have the ability to hold water seven times their volume. A greater water holding capacity of HS facilitates water infiltration and hold water within the root zone (Nardi, Pizzeghello, Muscolo, & Vianello, 2002). That is the main reason why the growers prefer to apply humate-based fertilizers and integrate production practices to preserve HS, which can frequently harvest a crop during drying seasons.

Aggregate stability in most agricultural soils is climate dependent. It has been found that the application of a mixture of HA and FA to soil positively influence seed germination and the plant roots' and shoots' growth and development when soil aggregation increased (Tonder, 2008). Humic substances bind the primary particles in the aggregates, physically and chemically, and thus in turn increase the stability of the aggregates and limit their breakdown during the wetting process (Nardi et al., 1996).

2.5.1.2 Chemically Soil Properties

Humic acid plays an important role as pH buffer due to its large buffer capacity in a wide pH range, which arises fundamentally from the separation of acidic functional groups. Although HA did not have a strong buffer capacity to a strong acid, they are an excellent buffer to base additions that resisted pH change in between pH 5.5 and 8 (Campitelli, Velasco, & Ceppi, 2006; Pertusatti & Prado, 2007). Humic acid contains chemical reactive functional groups such as carboxyl, phenolic and alcoholic hydroxyls that have pH dependent charge properties. The cation exchange sites on a HA or FA will be saturated with H^+ ions and when HS are added to the soil, these H^+ ions will detach from their functional groups, lowering the pH of the soil solution (Mikkelson, 2005).

Humic substances really important and useful in an agricultural system because they able to complex metal ions. Humic acid cans form aqueous solutions with trace elements, and yet not to the same extend as many synthetic chelating agents (Koopal et al., 2001). A generally accepted explanation is that HS form complexes with clays through polyvalent cations that act as a bridge or chelate. The carboxylic and phenolic-OH functional groups dissociate leaving multiple negative charges on the humic molecule (Imbufe, Patti, Surapaneni, Jackson, & Webb, 2004). These humic-metal-clay complexes protects from the action of water, by amassed into micelles and films with hydrophilic and hydrophobic parts (Piccolo & Mbagwu, 1999). The hydrophilic end, which is negatively charged functional groups strongly adsorb to the metal-clay complex forming the interior of soil micro-aggregates which will not easily leach out of the soil.

2.5.1.3 Biological Soil Properties

Humic substances can affect microorganisms indirectly by their CEC which can supply essential cations such as chelated Fe or can chelate toxic concentrations of Cu, and this assists microbial growth in soil. Humic substances may also indirectly affect the microbial metabolism when their molecular size is suitable for uptake by soil microorganisms (Charest, Beauchamp, & Antoun, 2004).

In a soil nutrient cycle, one of the most critical aspects is litter decomposition in which microbes are directly responsible for the process. These soil animals can enhance decomposition through litter fragmentation and defecating into the soil, and modifying the microbial activity and components (Ayres, Dromph, & Bardgett, 2005). Humic acid could increase and promote the bacterial number and modification of cellular activity and growth in bacteria through their influence on cell membrane permeability or on nutrient absorption (Gümüş & ŞEKER, 2013; Schroeder, 2014).

2.5.2 Importance in Plant Growth

2.5.2.1 Root Growth

Application of HS to soils and crops has a significant effect toward crop yield and soil physiochemical. Various studies have found that humate products induced a significant effect in root growth and great in root mass and length (Delfine et al., 2005; Mohajerani, Mojtaba, Hamid, Lak, & Modhej, 2016; Schroeder, 2014; Waqas et al., 2014).

2.5.2.2 Nutrient Uptake

Humic substances influence the uptake and transport of nutrients. Humic substance concentration and the pH of the medium that related to influence on ion uptake are more or less selective and variable (Fahramand, Moradi, Noori, & Sobhkhizi, 2014). Because of the presence of HS such as HA and FA, the yield production increase significantly in quantity and quality by increasing nutrient uptake. The stimulatory effect of HS have been directly associated with enhance macronutrients uptake such as N, P, K and S (Bakry, Taha, & Abdelgawad, 2014; Danyaei, Hassanpour, Baghaee, & Dabbagh, 2017; Tonder, 2008; Zimmer, 2004) and micronutrient uptake such as Fe, Zn, Cu and Mn was also found. Humic substances improve nutrient uptake through the microbial activity stimulation (Zimmer, 2004).

2.5.2.3 Plant Biology and Physiology

Several researches have shown that HS can have a positive effect on plant growth (Arancon et al., 2006; El-bassiouny, Bakry, Attia, & Abd Allah, 2014; Lodhi et al., 2013). They have a direct effect through HS absorption by the plant which influencing the enzyme activities and membrane permeability (Nardi et al., 2002). The HS can also indirectly influence on the plant by changing the soil structure, increase of soil CEC, and stimulation of microbial activity and has the ability to solubilize or complex certain soil ions.

The biological activity of HS mostly are regardless of their stimulatory or inhibitory, incorporates activities in amending plant biochemical and physiological processes. Various of known affected mechanisms are formation of ATP, amino acids, carbohydrates and proteins, membrane permeability, selective effects on enzyme activities, activation of Kreb’s cycle and respiration, ion protein carriers, synthesis of nucleic acid and photosynthesis (Charest et al., 2004; Nardi et al., 1996).

CHAPTER 3

METHODOLOGY

3.1 Composting

The rice straw was shredded and air-dried before the composting process. The compost was produced by mixing 80% shredded RS + 5% chicken feed + 10% goat manure slurry + 5% molasses. The rice straw was served as substrate (bulking material) and the goat manure slurry were used as source of moisture, microbes, and nutrients. The chicken feed was included as source of energy for the microbes. Molasses was added to provide carbohydrate for the microbes. Mixing of the compost was done manually prior to composting. The chicken feed and molasses were added gradually while mixing the rice straw and goat manure slurry so as to obtain a uniform mixture. The composting material was turned when necessary. The ambient temperature and compost temperature will be monitored daily (7 a.m., 1 p.m., and 7 p.m.) using a digital thermometer. The composting was completed at 60th day.

3.2 Compost Characterization

3.2.1 Soil pH and Soil Electrical Conductivity (EC)

Potentiometric method was used to determine the soil pH and EC (Peech, 1965). A ratio 1:5 of compost: distilled water was performed in compost pH determination since its ratio is closer to natural conditions compared to other wider ratios. A 5 g of air-dried soil with 25 ml of distilled water was added into a conical flask and shaken for 15 minutes at 180 rpm. Then, the samples were left for 24 hours before using Beckman pH meter and EC meter for pH and EC determination respectively.

3.2.2 Total N Determination

The total N content of compost 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 instrument was prepared by following procedures outline in the operator's instruction manual, that is perform maintenance, check gas supplies, and perform leak checks. Next, the blanks was analysed until the instrument is stable. After that, a 0.25 g of compost sample that had passed 250 mesh sieve was weighed into a 528-203 Combustion Boat and analysed.

3.2.3 Total Organic Matter (OM) and Total Carbon (C) Determination

The total OM and total C of compost sample were determined by gravimetrical dry combustion method (Allison, Bollen, & Moodie, 1965). The weight of empty porcelain dishes were weighed before fill in 5 g of compost samples. The samples were placed into muffle furnace at 300 °C for an hour and temperature will be increased to 550 °C for another 8 hours. Lastly, the soil sample were allowed to cool before inspection. The final weight of the porcelain dish with soil sample were weighed. The total OM and total C were calculated using following calculation:

$$total\ OM = \frac{initial\ weight\ of\ sample\ (g) - final\ weight\ of\ sample\ (g)}{initial\ weight\ of\ sample\ (g)} \times 100\% \tag{3.1}$$

$$total\ C = \frac{initial\ weight\ of\ sample\ (g) - final\ weight\ of\ sample\ (g)}{initial\ weight\ of\ sample\ (g)} \times \frac{58}{100}\% \tag{3.2}$$

3.2.4 Total P, K, Mg, Ca, Na, Fe, Cu and Zn Determination

According to Miller (1998), the total P, K, Mg, Ca, Na, Fe, Cu and Zn determination were analysed using Single Dry Ashing Method. A 1 g of compost samples were weighed and placed into porcelain crucible. The samples were then be placed in a muffle furnace to be ash at 300 °C for an hour. After that, the temperature of muffle furnace was raised to 520 °C and continue ashing for 5 hours. The samples were cooled in desiccator after the added with a few drops of distilled water and 2 ml concentrated HCl. The samples were evaporated to dryness in the fume chamber by using hot plate. Next, 10 mL of 20% HNO_3 was added to the sample and allow to heat for one hour. Later, the samples were filtered into 100 mL volumetric flask and were made up to volume. Then, the samples were aspirated into AAS and the absorbance reading was recorded to determine total K, Mg, Ca, Na, Fe, Cu and Zn. The molybdenum blue method (Murphy & Riley., 1962) was used to determine the total P of the sample. The blue colour of the samples were analysed in UV-VIS spectroscopy at 882 nm wavelength.

3.3 Isolation of HA

The isolation of HA was done by using the method referred to Stevenson (1994). A ratio of 1: 5 and 1: 10 of compost sample and chemical used was performed in HA extraction. A 5 g of compost sample and 50 mL of 0.5 M sodium hydroxide (NaOH) were added in the centrifuge bottle and closed tightly (Gracia, Cegarra, Bernal, & Navarro, 1993). The samples were equilibrated at room temperature on a mechanical shaker at 180 rpm for 1, 3, 5, 7, 9, 12 and 24 hours. After the extraction period, the side of the bottles were washed by using distilled water and the mixture were centrifuged at 10, 000 rpm for 15 minutes. The dark colour of supernatant liquors were decanted and filtered. The pH of the solution was adjusted to 1.0 by using 6.0 M HCl. Then, the HA were allowed to equilibrate for 24 hours. After that, the supernatant which is FA was siphoned off from the acidified extracts (Aiken, Mcknight, Wershaw, & MacCarthy, 1985). The remains of the suspensions were transferred to centrifuge bottles and centrifuged for 15 minutes at 10, 000 rpm.

Purification was conducted by using method that described by Ahmed, Husni, Anuar, Hanifi, & Angela (2004). The HA was purified by suspended HA into 50 ml of distilled water and centrifuged at 10,000 rpm for 10 minutes till the supernatant decanted and repeated three times. Then, the HA was air dried at 40 °C until constant weight. The yield of HA was expressed as the percentage of weight of compost used.

3.4 Determination of Humification Level and Functional Groups of HA

Humification level was determined using E_4/E_6 ratio (465 and 665 nm) by spectroscopy (Stevenson, 1994). A 0.003 g of HA was dissolved in 10 mL of 0.05 M sodium bicarbonate ($NaHCO_3$). 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 stated by Inbar, Chen, and Hadar (1990). A 0.02 g sample of HA was dissolved in 4 mL of 0.08 M NaOH and equilibrated at room temperature and in 180 rpm on a reciprocal shaker for 30 minutes. The initial pH was recorded before titrated. The solution was titrated with 0.10 M HCl to pH 2.5 within 15 minutes. Phenol content was calculated by assuming 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 of the phenols and carboxyls. Total acidity is the summation of the carboxyl and phenolic. The phenolic, carboxyl and total acidity of humic acid were calculated by equation 3.3, 3.4 and 3.5.

$$phenolic = \frac{volume\ of\ HCl\ (ml) \times 2 \times 10}{weight\ of\ sample\ (g)} \tag{3.3}$$

$$carboxyl = \frac{volume\ of\ HCl\ (ml) \times 10}{weight\ of\ sample\ (g)} \tag{3.4}$$

$$total\ acidity = phenolic + carboxyl \tag{3.5}$$

3.5 Data Analysis

The data obtained from the laboratory test was analysed by using the Statistical Package for the Social Science (SPSS) Version 21. Analysis of Variance (ANOVA) was used to detect the significance difference between HA yields under different extractant ratio and extraction duration. Tukey's test ($p \leq 0.05$) was used to separate the means between HA yields and extraction duration.

CHAPTER 4

RESULT AND DISCUSSION

4.1 Selected Physico-Chemical Properties of Rice Straw Compost

Table 4.1 shows the results for the selected physico-chemical properties of rice straw compost.

Table 4.1: selected physico-chemical properties of rice straw compost

Property	**Value obtained**
pH (water)	7.53
EC	1.53 dS/m
Organic matter (%)	72.53%
Total carbon (%)	42.65%
Total N (%)	2.75%
Total P (ppm)	2351.1
Total K (ppm)	150.767
Total Ca (ppm)	43.663
Total Mg (ppm)	230.067
Total Fe (ppm)	18.207
Total Cu (ppm)	0.977
Total Zn (ppm)	2.243
Total Na (ppm)	13.417

4.1.1 pH and EC of Compost

The pH of the rice straw compost in the experiment was 7.53. The value was around the range reported by studies of Jusoh, Manaf, and Latiff (2013) and Mahmoud, Ibrahim, Robin, Akkal-Corfini, and El-Saka (2009) for rice straw compost. The EC value in the experiment was 1.53 dS/m. There is a big different between EC value when compare to a study from Mahmoud et al. (2009). This might due to the ingredient of the rice straw compost added during composting in current study and the origin of the rice straw are different from the study.

4.1.2 Total Organic Matter and Total Carbon of Compost

The compost samples contained 72.53% of OM and 42.65% of total C. The values of OM and total C were relatively similar to that of study by Enev, Pospíšilová, Klučáková, Liptaj, and Doskočil (2014). The values of compost samples were higher than rice straw compost without goat manure, chicken feed and molasses in the study of Enev et al. (2014) because goat manure, chicken feed and molasses are high in OM and total C.

4.1.3 Total N in Compost

The percentage of the total N in compost sample was 2.75%. The value was much higher than other studies by Devi, Sharma, and Singh (2012) and Jusoh et al. (2013). Goat manure and chicken feed are high in N compared to rice straw. Therefore, when both animal and plant based organic matter are mixed together, total N became higher.

4.1.4 Total P in Compost

The value of total phosphorous obtained from this study was 2351.1 ppm or 0.235% which is within the range reported by (Jusoh et al., 2013).

4.1.5 Total Cation in Compost

Table 4.1 shows the total cation (K, Ca, Mg, Na, Fe, Cu and Zn) of rice straw compost. The exchangeable cation K, Ca, Mg, Na, Fe, Cu and Zn were 150.767, 43.663, 230.067, 18.207, 0.977, 2.243, and 13.417 ppm respectively, which overall are lower than the studies by Ayinla, Olayinka, and Etejere (2016); Cuevas (1997); Jusoh et al. (2013) and Mahmoud et al. (2009) . The values obtained were different is because of the feedstock used in composting and their origin were different.

4.2 Isolation of HA

Table 4.2.1 shows the percentage of HA yield obtained for different extraction period and extractant ratio from rice straw compost. The means comparison of effect of different extraction periods on the yield of HA under different extractant ratios were consistent with the studies by Ahmed et al. (2005), Kasim et al. (2007) and Ahmed, (2012).

The observation from the result obtained in this study also revealed that: (1) yield of HA extracted under 1 hour in different extractant ratio were both significantly lower compared to other extraction period; (2) HA yield extracted under 12 and 24 hours with extractant ratio 1:5 and 1:10 were not statistically different in this study. This indicated that the HA yield was significantly low under 1 hour because the time was not sufficient for the H ions to displace Na ions and other cations. Furthermore, the difficulty of HA extracting at initial shorter period at 1 hour, might due to difficulty of wetting the compost, since it have been air-dried. The reduction of high molecular-weight complexes deploymerization, the wetting and solubilization of the rice straw compost increased with increasing extraction period (Ahmed et al., 2005). The exchange process between K from the extractant and the exchange sites mainly hydroxylic, phenolic and carboxylic functional groups in the compost progressed with extraction period until a maximum period is achieved when the maximum number of exchange sites might have been saturated with K ions (Palanivell et al., 2012). Besides, this extraction process might have made the compost highly soluble. From the Table 4.2.1, extraction period under 12 and 24 hours with both extractant ratio of 1:5 and 1:10 produced the highest HA yields. These result can be implied that the solubility and the maximum exchange sites were saturated with K ions.

Since both extractant ratios obtained the same optimum extraction period, the ratio was analysed between the extractant ratio under 12 and 24 hours to detect any significant different by Tukey's test at $p \leqslant 0.05$. Table 4.2.2 shows that the HA yield extracted using 1:10 extractant ratio was significantly higher than 1:5 extractant ratio in both 12 and 24 hours extraction period. The result is consistent with the IHSS recommended data (Swift, 1996).

Table 4.2.1: Effect of extraction period on the yield of humic acids with different extractant ratio.

Treatment **Extraction Period**	Humic Acid Yield (%) **Extractant Ratio** **1:5**	 **1:10**
1hr	5.20 ± 0.2^{BC}	6.60 ± 0.12^{D}
3hr	5.27 ± 0.18^{BC}	9.14 ± 0.13^{C}
5hr	4.70 ± 0.30^{C}	9.53 ± 0.58^{C}
7hr	6.06 ± 0.24^{B}	9.80 ± 0.12^{C}
9hr	6.10 ± 0.50^{B}	13.20 ± 0.12^{B}
12hr	8.90 ± 0.50^{A}	14.46 ± 0.29^{A}
24hr	9.06 ± 0.27^{A}	13.40 ± 0.31^{AB}

Table 4.2.2: Effect of extractant ratio on the humic acid yield under extraction period of 12 and 24 hours.

Treatment **Extractant Ratio**	Humic Acid Yield (%) **Extraction Period** 12hr	 24hr
1:5	8.90 ± 0.50^{B}	9.06 ± 0.27^{B}
1:10	14.46 ± 0.29^{A}	13.40 ± 0.31^{A}

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

4.3 Functional Groups and Humification Level of HA

Generally, the ranges of phenolic OH, carboxylic COOH, total acidity under 1:5 extractant ratio were within the ranges reported in previous study by K.H. Tan (2003). The carboxylic groups were found to be between 483 to 950 cmol kg^{-1} under extractant ratio of 1:10. This study were relatively higher compared to study by Tan (2003). This might due to the unstable HA and caused the high value range of total acidity in that particular ratio. The high E_4/E_6 ratio of HA obtained mainly in compost samples which indicates that the higher rate aliphatic structures have in compost than the aromatic constituents which suggest the early phase of humus formation (Velasco, 2004). Even though the E_4/E_6 ratio of both extractant ratio HA were relatively lower than previous study, the values were still within the common range of E_4/E_6 ratio in HA which is 2-5 (Schnitzer, 1978; Stevenson, 1994). The low E_4/E_6 ratio obtained in HA samples could be due to intensive microbial activity. Usually, a low E_4/E_6 ratio reflects a high degree of aromatic condensation and high molecular weight.

Table 4.3 comparison of ranges of phenolic OH, carboxylic COOH, total acidity, and E_4/E_6 of rice straw compost humic acid with those in literature.

Variable	Extraction period (hours)	HA, present study Extactant ratio		HA, literature (Tan, 2003)
		1: 5	1:10	
Phenolic OH (cmol kg^{-1})	1	233.33	166.67	240 – 540
	3	200	266.67	
	5	200	266.67	
	7	300	200	
	9	200	266.67	
	12	266.67	233.33	
	24	233.33	133.33	
Carboxylic COOH (cmol kg^{-1})	1	250	483.33	150 – 440
	3	316.67	483.33	
	5	383.33	833.33	
	7	383.33	833.33	
	9	400	900	
	12	300	883.33	
	24	483.33	950	
Total acidity	1	483.33	650	500 – 700
	3	516.67	750	
	5	583.33	1100	
	7	683.33	1033.33	
	9	600	1166.67	
	12	566.67	1116.66	
	24	716.66	1083.33	
E_4/E_6	1	5.163	5.479	7 – 8
	3	5.069	3.968	
	5	4.872	3.158	
	7	3.401	3.520	
	9	4.175	2.49	
	12	2.53	2.521	
	24	5.787	3.058	

CHAPTER 5

CONCLUSION AND RECOMMENDATION

The optimum yield of HA from rice straw compost can be obtained at extraction period of 12 hours in both extractant ratio of 1:5 and 1:10. The process required approximately 12 hours to extract HA from rice straw compost and one hour of purification process using distilled water. The significance of the study is HA from rice straw compost can be isolated within 12 hours of extraction period, 24 hours of fractionation period and 1 hour of purification period or less instead of existing range of 2 to 7 days. Thus, this may help in reducing time and costs needed to produce the humate product from this compost. The extractant ratio of 1:10 is more significant in terms of higher HA yield obtained.

This study can be further improved by including different fractionation periods with different ratio of extractant to HS samples to determine whether there is any interaction between fractionation period with different extractant ratio on the HA yield. The determination of K, Ca and Mg contents of isolated HA after each washing should also be determined to assess the purify level of HA being isolated.

REFERENCES

Ahmed, O. H., Husni, M. H. A., Anuar, A. R., & Hanafi, M. M. (2005). Effects of Extraction and Fractionation Time on The Yield of Compost Humic Acids. *New Zealand Journal of Crop and Horticultural Science*, *33*, 107–110. https://doi.org/10.1080/01140671.2005.9514338

Ahmed, O. H., Husni, M. H. A., Anuar, A. R., Hanifi, M. M., & Angela, E. D. . (2004). A Modified Way of Producing Humic Acids from Pineapple Leaves. *J. Susy. AGric.*, *25*, 129–139.

Aiken, G. R. ., Mcknight, D. M., Wershaw, R. L., & MacCarthy, P. (1985). An Introduction to Humic Substances in Soil, Sediment and Water. In G. R. Aiken (Ed.), *Humic Substances in Soil, Sediment and Water.: Geochemistry, Isolation, and Characterization* (pp. 1–9). New York: John Wiley and Sons.

Allison, L. E., Bollen, W. B., & Moodie, C. . (1965). Total carbon. In C. A. Black, D. D. Evans, J. L. White, L. E. Ensminger, & F. E. Clark (Eds.), *Methods of Soil Analysis, Part 2- Chemical and Microbiological Properties* (pp. 1346–1366). Madison: American Society of agronomy.

Arancon, N. Q., Edwards, C. A., Lee, S., & Byrne, R. (2006). Effects of humic acids from vermicomposts on plant growth. *European Journal of Soil Biology*, *42*, 65–69. https://doi.org/10.1016/j.ejsobi.2006.06.004

Asing, J., Wong, N. C., & Lau, S. (2009). Optimization of Extraction Method and Characterization of Humic Acid Derived from Coals and Composts. *J. Trop. Agric. and Fd. Sc.*, *37*(2), 211–223.

Ayinla, A. A., Olayinka, B. U., & Etejere, E. O. (2016). Rice straw : A Valuable Organic Manure for Soil Amendment in The Cultivation of Groundnut (Arachis hypogaea). *Environmental and Experimental Biology*, *14*, 205–211.

Ayres, E., Dromph, K. M., & Bardgett, R. D. (2005). Do Plant Species Encourage Soil Biota that Specialise in The Rapid Decomposition of Their Litter? *Soil Biol. Biochem.*, *38*, 183–186.

Bakry, B. A., Taha, M. H., & Abdelgawad, Z. A. (2014). The Role of Humic Acid and Proline on Growth , Chemical Constituents and Yield Quantity and Quality of Three Flax Cultivars Grown under Saline Soil Conditions. *Agricultural Sciences*, *5*, 1566–1575.

Baldock, J. A., & Nelson, P. N. (1999). Soil Organic Matter. In M. E. Sumner (Ed.), *Handbook of Soil Science* (pp. 62–65). CRC Press.

Billingham, K. (2015). *Humic Products, Potential or Presumption for Agriculture*. NSW Agriculture.

Campitelli, P. A., Velasco, M. I., & Ceppi, S. B. (2006). Chemical and Physicochemical Characteristics of Humic Acids Extracted from Compost, Soil and Amended Soil. *Talanta*, *69*(5), 1234–1239.

Charest, M. H., Beauchamp, C. ., & Antoun, H. (2004). Effects of the humic substances of de-inking paper sludge on the antagonism between two compost bacteria and Pythium ultimum. *FEMS*, *52*, 219–227.

Chen, C. H. H., Ahmed, O. H., Majid, N. M. A., & Jalloh, M. B. (2009). Reduction of Isolation Period of Coal Humic Acids. *American Journal of Applied Sciences 6*, *6*(7), 1327–1330. https://doi.org/10.3844/ajassp.2009.1327.1330

Chen, Y., Senesi, N., & Schnitzer, M. (1977). Information Provided on Humic Substances by E4/E6 Ratios. *Soil Science Society of America Journal*, *41*, 352–358.

Cuevas, V. C. (1997). *Rapid Composting Technology in the Philippines: Its Role in Producing Good-Quality Organic Fertilizers*. *LibreroandTidon1997*. Retrieved from http://www.fftc.agnet.org/library.php?func=view&id=20110804171753

Danyaei, A., Hassanpour, S., Baghaee, M. A., & Dabbagh, M. (2017). The Effect of Sulfur-Containing Humic Acid on Yield and Nutrient Uptake in Olive Fruit. *Open Journal of Ecology*, *7*, 279–288. https://doi.org/10.4236/oje.2017.74019

Delfine, S., Tognetti, R., Desiderio, E., & Alvino, A. (2005). Effect of Foliar Application of N and Humic Acids on Growth and Yield of Durum Wheat. *Agronomy for Sustainable Development*, *25*(2), 183–191. https://doi.org/10.1051/agro

Devi, S., Sharma, C. R., & Singh, K. (2012). Microbiological biodiversity in poultry and paddy straw wastes in composting systems. *Brazilian Journal of Microbiology*, *43*(1), 288–296. https://doi.org/10.1590/S1517-83822012000100034

El-bassiouny, H. S. M., Bakry, B. A., Attia, A. A. E., & Abd Allah, M. M. (2014). Physiological Role of Humic Acid and Nicotinamide on Improving Plant Growth , Yield , and Mineral Nutrient of Wheat (Triticum durum) Grown under Newly Reclaimed Sandy Soil. *Agricultural Sciences*, *5*, 687–700.

Enev, V., Pospíšilová, L., Klučáková, M., Liptaj, T., & Doskočil, L. (2014). Spectral Characterization of Selected Humic Substances. *Soil and Water Research*, *9*(1), 9–17.

Fahramand, M., Moradi, H., Noori, M., & Sobhkhizi, A. (2014). Influence of humic acid on increase yield of plants and soil properties. *International Journal of Farming and Allied Sciences*, (1999), 2006–2008. Retrieved from www.ijfas.com

Gracia, D., Cegarra, J., Bernal, M. P., & Navarro, A. (1993). Comparative Evaluation of Methods Employing Akali and Sodium Pyrophosphate to Extract Humic Substances from Peat. *Communiation in Soil Science and Plant Analysis*, *24*(13–14), 1481–1494.

Gümüş, İ., & ŞEKER, C. (2013). Influence of humic acid applications on soil physicochemical properties, 1–11.

Hopkins, B., & Stark, J. (n.d.). Humic Acid Effects on Potato Response to Phosphorus. In *Idoho Potato Conference* (pp. 87–91).

Imbufe, A. U., Patti, A. F., Surapaneni, A., Jackson, R., & Webb, J. A. (2004). Effects of Brown Coal Derived Materials on pH and Electrical Conductivity of An Acid Vineyard Soil. In *SuperSoil: 3rd Australian New Zealand Soils Conference* (pp. 5–9). Sydney, Australia.

Inbar, Y., Chen, Y., & Hadar, Y. (1990). Humic Substances formed During The Composting of Organic Matter. *Soil Science Society of America Journal1*, *54*, 1316–1323.

Jusoh, M. L. C., Manaf, L. A., & Latiff, P. A. (2013). Composting of rice straw with effective microorganisms (EM) and its influence on compost quality. *Iranian Journal of Environmental Health Science & Engineering*, *10*(1), 17. https://doi.org/10.1186/1735-2746-10-17

Kasim, S., Ahmed, O. H., Majid, N. M. A., & Yusop, K. (2007). Effects of Extraction and Fractionation Period on The Yield of a Tropical Peat Soil (Hemists) Humic Acids. *American Journal of Agricultural and Biological Science*, *2*(3), 202–205. https://doi.org/10.3844/ajabssp.2007.202.205

Kononova, M. M. (1966). *Soil Organic Matter*. New York: Pergamon.

Koopal, L. K., Riemsdijk, W. H. van, & Kinniburgh, D. G. (2001). Humic Matter and Contaminants. General Aspects and Modeling Metal Ion Binding. *Pure Appl. Chem.*, *73*(12), 2005–2016.

Lodhi, A., Tahir, S., Iqbal, Z., Mahmood, A., Akhtar, M., Qureshi, T. M., … Naeem, A. (2013). Characterization of commercial humic acid samples and their impact on growth of fungi and plants. *Soil Environ.*, *32*(1), 63–70.

Mahmoud, E., Ibrahim, M., Robin, P., Akkal-Corfini, N., & El-Saka, M. (2009). Rice Straw Composting and Its Effect on Soil Properties. *Compost Science & Utilization*, *17*(3), 146–150. https://doi.org/10.13140/RG.2.1.4089.3283

Mikkelson, R. L. (2005). Humic Materials for Agriculture. *Better Crops*, *89*, 6–10.

Miller, R. O. (1998). High-Temperature Oxidation: Dry Ashing. In Yash P. Kalra (Ed.), *Handbook of Reference Methods for Plant Analysis* (pp. 53–61). New York: CRC Press.

Mohajerani, S., Mojtaba, A., Hamid, M., Lak, S., & Modhej, A. (2016). Effect of The Foliar Application of Humic Acid on Red Bean Cultivars (Phaseolus vulgaris L .). *Journal of Experimental Biology and Agricultural Sciences*, *4*(5), 519–524.

Molina, F. V. (2013). Functional Groups and Acid-Base Properties. In F. V. Molina (Ed.), *Soil Colloids: Properties and Ion Binding* (pp. 359–360). CRC Press.

Munawar, A., & Mindari, W. (2015). Effect of Humic Acid on Soil Chemical and Physical Charateristics of Embankment. *MATEC Web of Conferences 58*, *58*(1028).

Murphy, J., & Riley., J. P. (1962). A modified single solution method for the determination of phosphate in natural waters. *Anal. Chim. Acta.*, *27*, 31–36.

Nardi, S., Concheri, G., & Dell'agnola, G. (1996). Biological Activity of Humus. In A. Piccolo (Ed.), *Humic Substances in Terrestrial Ecosystems* (p. 363). Elsevier.

Nardi, S., Pizzeghello, D., Muscolo, A., & Vianello, A. (2002). Physiological effects of humic substances on higher plants. *Soil Biol. Biochem.*, *34*, 1527–1536.

Palanivell, P., Susilawati, K., Ahmed, O. H., & Muhamad, A. M. N. (2012). Effects of extraction period on yield of rice straw compost humic acids. *African Journal of Biotechnology*, *11*(20), 4530–4536. https://doi.org/10.5897/AJB11.3817

Peech, M. (1965). Hydrogen-ion activity. In Black, C. A., D. D. Evans, J. L. White, L. E. Ensminger, & F. E. Clark (Eds.), *Methods of soil analysis. Part 2. Chemical and microbial properties* (pp. 914–926). Madison: American Society of Agronomy.

Pertusatti, J., & Prado, A. G. S. (2007). Buffer Capacity of Humic Acid: Thermodynamic Approach. *J. Colloid Interface Sci.*, *314*(4), 84–489.

Piccolo, A., & Mbagwu, H. S. C. (1999). Role of Hydrophobic Components of Soil Organic Matter in Soil Aggregate Stability. *Soil Science Society of America Journal*, *63*, 1808–1810.

Reshi, Z., & Tyub, S. (2007). Humus Biosynthesis. In *Detritus and Decomposition in Ecosystems* (pp. 153–176). New India Publishing.

Rice, J. A. (2001). Humin. *Soil Sicence*, *166*, 848–857.

Schnitzer, M. (1978). Humic substances: Chemistry and Reactions. In M. Schnitzer & S. U. Khan (Eds.), *Soil Organic Matter* (pp. 11–12). Elsevier Scientific Pub. Co.

Schroeder, A. (2014). Plant Health and Soil Changes with Humic Substance Applications.

Stevenson, F. J. (1982). *Humus Chemistry, Genesis, Composition, Reactions*. New York:

John Wiley and Sons.

Stevenson, F. J. (1994). *Humus Chemistry : Genesis, Composition, Reactions*. Wiley.

Stott, D. E., & Martin, J. P. (1990). Synthesis and Degradation of Natural and Synthetic Humic Material in Soil. In P. MacCarthy (Ed.), *Humic substances in soil and crop sciences: selected readings.* (pp. 37–63). Madison, WI: Soil Science Society of America, Inc.

Swift, R. S. (1996). Organic Matter Characterization. In D. L. Sparks, A. L. Page, P. A. Helmke, R. H. Loeppert, P. N. Soltanpour, M. A. Tabatabai, … M. E. Sumner (Eds.), *Method of Soil Analysis: Chemical Methods. Part 3* (pp. 1018–1020). Madison, Wisconsin, USA: Soil Science Society of America, .

Tan, K. H. (2003). *Humic Matter in Soil and the Environment: Principles and Contoversies* (1st ed.). New York: Marcel Dekker Inc.

Tan, K. H. (2005). *Soil Sampling, Preparation, and Analysis* (Second Edi). CRC Press.

Tan, K. H. (2014). Agronomic Importance of Humic Matter. In *Humic Matter in Soil and the Environment: Principles and Controversies* (pp. 333–370). CRC Press.

Tonder, J. T. Van. (2008). *Effect of Potassium Humate on Soil Properties and Growth of Wheat*. University of the Free State BLOEMFONTEIN.

Velasco, M. I. (2004). Analysis of humic acid from compost of urban wastes and soil by fluorescence spectroscopy. *Agriscientia*, 8.

Waqas, M., Ahmad, B., Arif, M., Munsif, F., Khan, A. L., Amin, M., … Lee, I. J. (2014). Evaluation of humic acid application methods for yield and yield components of mungbean. *American Journal of Plant Sciences*, *5*(July), 2269–2276. https://doi.org/10.4236/ajps.2014.515241

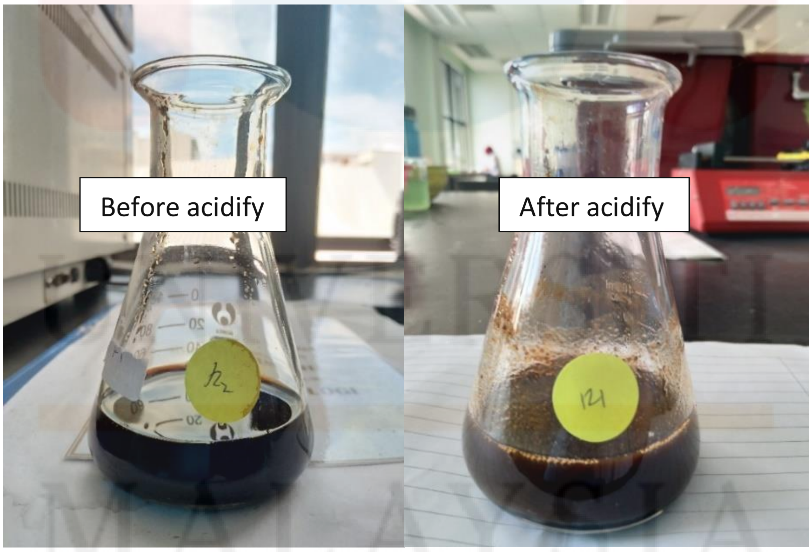
White, R. E. (2003). The Makeup of Soil. In *Soils for Fine Wines* (pp. 48–49). Oxford University Press.

Wright, D., & Lenssen, A. W. (2013). Humic and Fulvic Acids and Their Potential in Crop Production. Agriculture and Environment Extension Publications.

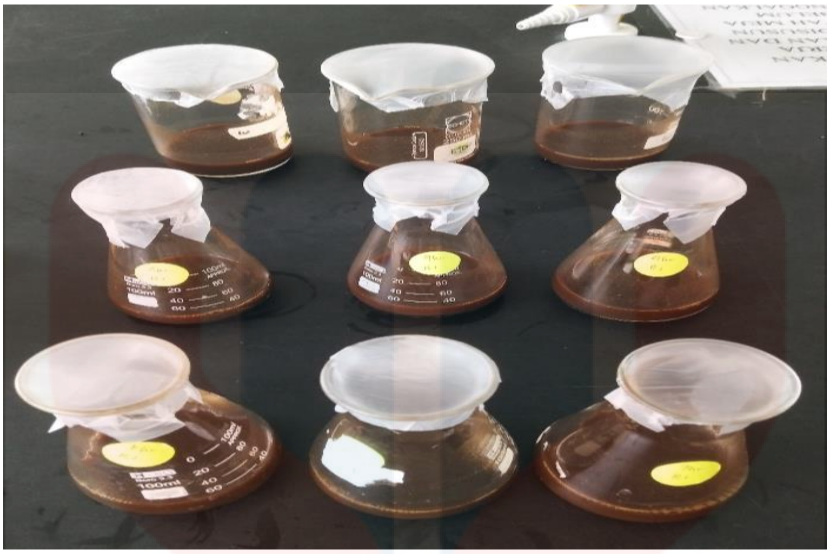
Zimmer, G. (2004). Humic Substances in Biological Agricultural Systems. *Acres USA Magazine*.

APPENDICES

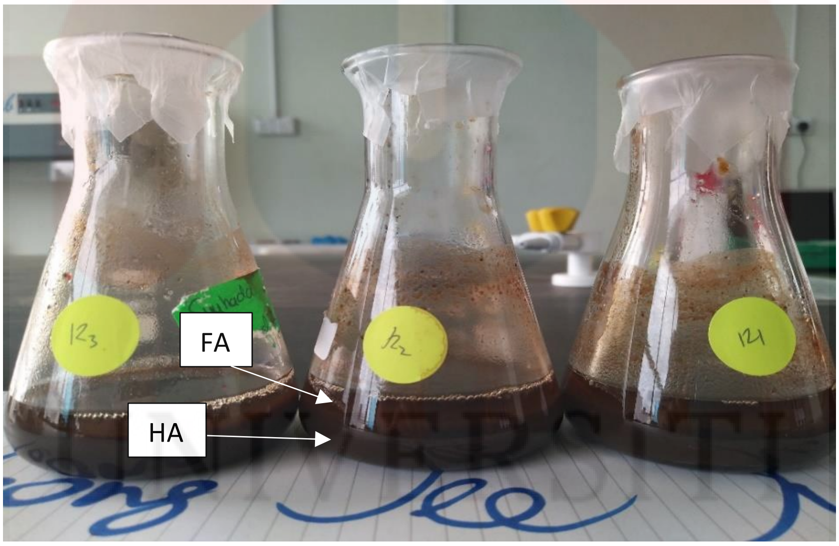
Appendix A: The extracted compost samples that ready to centrifuge.



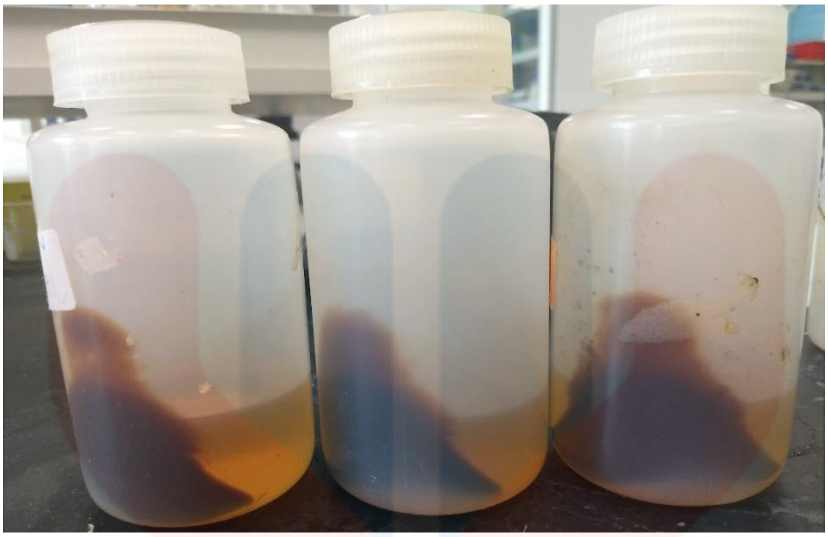
Appendix B: The dark colour supernatant liquors before and after acidified by 6M HCl.



Appendix C: The fractionate HA during fractionation period for 24 hours.



Appendix D: The fractionate HA that formed two level of FA and precipitate HA.

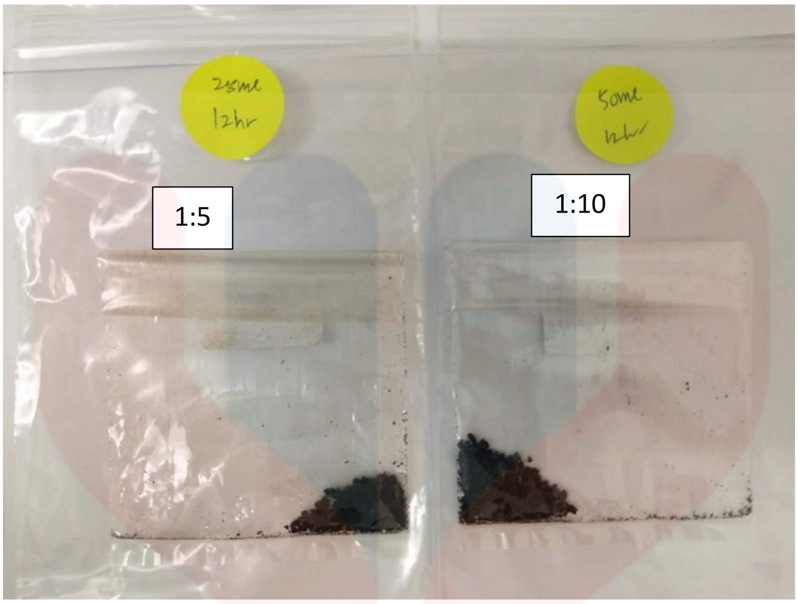


Appendix E: The HA samples that after centrifuged for 15 minutes at 10, 000 rpm.

Appendix F: The HA samples that after purified by distilled water.

Appendix G: The HA sample that ready for oven dried at 40 °C.

Appendix H: The HA samples that were fully dried.



Appendix I: The HA yield of different extractant ratio under 12 hours extraction period.