



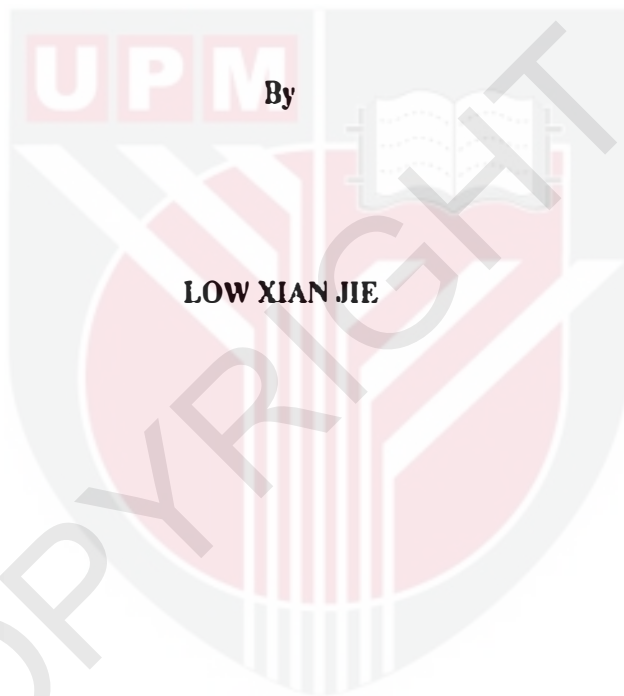
UNIVERSITI PUTRA MALAYSIA

***ANION EXCHANGE CAPACITY OF CHICKEN LITTER BIOCHAR IN
REGULATING PHOSPHATE RETENTION OF AN ACID SOIL***

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**ANION EXCHANGE CAPACITY OF CHICKEN LITTER BIOCHAR IN
REGULATING PHOSPHATE RETENTION OF AN ACID SOIL,**



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**A Project Report Submitted in Partial Fulfillment of the Requirement
for the Degree of Bachelor Bioindustrial of Science in the
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ABSTRACT

Management of Anion Exchange Capacity (AEC) and phosphate retention are important in sustainable agriculture management because both of them have direct effects on the availability of P for plants uptake that would directly affect soil productivity and fertility. An incubation study was carried out in the Research Centre of UPMKB for 40 days with different treatments. The objective of the study was to determine the anion exchange capacity of an acid soil and retention of phosphate ions of an acid soil after amended with chicken litter biochar. The available P of soil samples was determined using Bray II method whereas total P are determined using Aqua Regia method. For AEC, solution was extracted by centrifugation with ammonium chloride, NH_4Cl and potassium nitrate, KNO_3 then silver nitrate, AgNO_3 was added into the samples solution to determine the weight of silver chloride precipitates, AgCl . Acid soils amended with chicken litter biochar showed higher P content but did not show any significant effect on AEC. This happened due to organic anions and acids released by chicken litter biochar after decomposition binded with Al and Fe ions in the soils thus increased available P but it didn't have effect on AEC due to reduction in protonation by Al, Fe and H ions in the soil as a result of the binding of organic anions and acids with these ions. It can concluded that acid soils amended with chicken litter biochar can improve P availability and not AEC.

ABSTRAK

Urusan Kapasiti Pertukaran Anion (KPA) dan pengekalan fosfat adalah penting dalam pengurusan pertanian yang mampan kerana kedua-duanya mempunyai kesan langsung terhadap ketersediaan P untuk pengambilan tanaman yang secara langsung akan mempengaruhi produktiviti dan kesuburan tanah. Untuk ini, kajian inkubasi dijalankan di Pusat Penyelidikan UPMKB selama 40 hari dengan rawatan yang berbeza. Objektif untuk menjalankan inkubasi adalah untuk menentukan kapasiti pertukaran anion tanah berasid dan pengekalan ion fosfat dalam tanah berasid selepas diubah dengan biochar tahi ayam. Sampel tanah yang didapati P ditentukan menggunakan kaedah Bray II manakala jumlah P ditentukan menggunakan kaedah Aqua Regia. Untuk KPA, larutan sampel diekstrak dengan sentrifugasi dengan ammonium klorida, NH_4Cl dan kalium nitrat, KNO_3 maka nitrat perak, AgNO_3 ditambah ke dalam larutan sampel untuk menentukan berat perak klorida, AgCl . Keputusan menunjukkan bahawa tanah berasid yang diubah dengan biochar tahi ayam mempunyai kandungan P yang lebih tinggi tetapi tidak menunjukkan sebarang kesan yang signifikan terhadap KPA. Ini berlaku disebabkan oleh anion dan asid organik yang dikeluarkan oleh biochar sampah ayam selepas penguraian terikat dengan ion Al dan Fe di dalam tanah sehingga meningkatkan P yang didapati tetapi tidak berkuat kuasa pada KPA disebabkan pengurangan fotonasi oleh Al, Fe dan ion hidrogen di dalam tanah kerana pengikatan anion dan asid organik dengan ion-ion ini. Kita dapat membuat kesimpulan bahawa tanah asid yang dipinda dengan biochar sampah ayam dapat mempertingkatkan ketersediaan P dan tiada pada KPA.

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APPROVAL SHEET

I certify that this research project report entitled "Anion exchange capacity of chicken litter biochar in regulating phosphate retention of acid soil" has been examined and approved as a partial fulfillment of the requirement for the degree of Bachelor Science Bioindustry in the Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia Bintulu Sarawak Campus.

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LIST OF ABBREVIATIONS

AAR	Applied Agriculture Resource
AEC	Anion Exchange Capacity
ADP	Adenosine diphosphate
AgCl	Silver chloride
Al	Aluminium
AlOH	Aluminum hydroxide
ANOVA	Analysis of Variance
ATP	Adenosine triphosphate
C	Carbon
Ca	Calcium
CEC	Cation Exchange Capacity
CLB	Chicken Litter Biochar
cmol	Centimoles
CRD	Completely Randomized Design
Fe	Iron
g	Gram
H_2PO_4^-	Dihydrogen phosphate
HCl	Hydrochloric acid
HNO_3	Nitric acid
KCl	Potassium chloride
kg	Kilogram
KNO_3	Potassium nitrate
M	Molarity

MBC	Maximum Buffering Capacity
mL	Millilitre
mol	Moles
N	Nitrogen
NaOH	Sodium hydroxide
NH ₄ Cl	Ammonium chloride
OM	Organic Matter
P	Phosphorus
PO ₄ ³⁻	Phosphate
ppm	Particles per million
RCOO ⁻	Carboxylate group
R-C=O	Acyl group
R-C-OH	Alcohol
rpm	Rotation per minute
SAS	Statistical Analysis System
TOC	Total Organic Matter
TSP	Triple Superphosphate
UV	Ultra violet

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Phosphorus (P) is a basic component for plants. In corrosive soils such as oxisols and ultisols, P constitutes the most constraining supplement for generations and the cost of crop production. These soils lose supplements due to precipitation and high weathering. In these soils, abundance of aluminum (Al) and iron (Fe) tend to bind with dissolvable inorganic P (Adnan *et al.* 2003). Phosphorus is most accessible to crops around pH 6 – 7. In acid soils, P reacts with Al and Fe oxides whereas it reacts with Al and Fe hydroxides in alkaline soils (pH>7) to become less accessible. This prompts extensive utilization of P fertilizers to saturate Al and Fe so as guarantee P accessibility for plants uptake (Rahman *et al.* 2014). However, this method is neither economical nor environmental friendly. Excessive or unbalanced use of P fertilizer could cause water pollution such as eutrophication through leaching of P.

To improve the fertility of Ultisols and Oxisols, organic amendments are used to mitigate the high P-fixing capacity of soils. Soil chemical properties and nutrient bioavailability are also improved with the addition of organic amendments in the tropical soils especially P for reducing P sorption sites in the soils (Ohno and Amirbahma 2010; Ohno *et al.* 2007). To improve soil fertility and crop productivity, soil water retention, and carbon sequestration, organic amendments are used because they have the potential and capability (Galinato *et al.* 2011). Chicken litter biochar is one of them.

Chicken litter biochar is an organic amendment derived by pyrolyzing chicken litter. When chicken litter biochar decomposes, it releases humic and fulvic acids for adsorbing metal cations such as Al and Fe that reduced P fixation in soil colloids by competing for binding sites (Hariprasad and Niranjana 2008) and this increase P concentration in the soil solution (Wandruszka 2006).

1.2 Hypothesis

1. Chicken litter biochar will increase the availability of phosphate ions in an acid soil.
2. Chicken litter biochar will improve anion exchange capacity (AEC) and the retention of phosphate ions in an acid soil.

1.3 Objectives

1. To determine the anion exchange capacity of an acid soil amended chicken litter biochar.
2. To determine retention of phosphate ions in an acid soil amended with chicken litter biochar.

1.4 Significance of Study

Chicken litter biochar could be used to improve P availability in acid soils.

CHAPTER 2

LITERATURE REVIEW

2.1 Importance of Phosphorus to Plants

Phosphorus is a basic component for plant development since it influences biological processes. It plays role in biosynthesis of nucleotides, nucleic acids, coenzymes, phosphoproteins, phospholipids, and sugar phosphates (Brady and Weil 2002). During photosynthesis and digestion of starches, energy is produced in phosphate compounds. The nucleotides, adenosine diphosphate (ADP) and adenosine triphosphate (ATP) are critical for energy storing and move in plant biochemical processes. The reaction in which ATP is changed back to ADP or known as phosphorylation likewise required phosphorus (Havlin *et al.* 1999).

Sufficient P availability for plants is important for root development and improvement of plant reproductive parts such as seeds and organic products (Sanchez 2006). Lack of P in plants causes hindered protein synthesis that prompts to low yields and low quality as the vegetative and reproductive development is inhibited. Developments of plants are staggered with thin stems, lesser leaves and constrained root frameworks. Due to the aggregation of anthocyanin, more established leaves turn purple or blue green and cause hindered plant seedlings (Brady and Weil 2002). Chlorosis and rot happening on older leaves since P is mobile in plants and translocated from older to younger leaves. Less and short new shoot and contorted fruits of the plants is the effects of P inadequacy in soils (Sanchez 2006).

2.2 Phosphorus Form in Soil

Soil P exists in organic and inorganic structures (Brady and Weil 2002). The inorganic P forms start from build-ups of P compost with amendments and weathering of minerals. Natural P form is considered as recalcitrant in the soils which are humified materials that balanced out in the soil lattice. It starts from organic matters, plant build-ups, and soil microorganisms (Campbell and Edwards 2001). Add up to P fixation in soils ranges from 50 to 3000 mg/kg, of which 15 to 80% happens in the organic types of inositol phosphates, phospholipids and nucleic acids (Pierzynski *et al.* 2005; Sims *et al.* 2002). The inositol phosphates are the most predominant type of organic P in soils. Phospholipids which are formed from P is approximately represent around 1 to 10% of the organic P in soil. Nucleic acids that are created by plant, creature and microbial biomass including the decomposed products made up to 10% of soil organic P (Brady and Weil 2012).

2.3 Organic Matter

Organic matter portion in soil contribute to P fixation by forming complex compounds of organic matter, metal cations, and phosphates. Soil organic matter comprises of humic and fulvic acids containing useful functional groups such RCOO^- , R-C=O , R-C-OH and others. These groups adsorb metal cations and hence increasing sorption of P in soil (Bianchi *et al.* 2008). In addition, the low molecular weight organic acids decomposition of organic matter increases the P sorption sites on cations by suppressing polymerization and crystallization of metal cations. However, the most organic matters association with soil in a way that it mobilizes P in soil and thus it enormously increases P uptake by crops (Wandruszka 2006). Organic acids

decrease the adsorption of added phosphorus on soil colloids by competing for the binding sites (Hariprasad and Niranjana 2008). Organic acid anions are adsorbed faster on soil surface than P and this buildup P concentration in the soil solution (Wandruszka 2006). Solubilization of P compounds by organic acids also happens through complex development between organic acids and metal ions, particularly for Al, Fe, and Ca. Metal complexation and dissolution reactions decrease the amount of sorption sites and more P is freed for plant uptake (Wandruszka 2006; Geelhoed *et al.* 1999; Bolan *et al.* 1994) Organic acids are also a fast soluble carbon source for microorganisms and therefore affect the rhizosphere microbial population and therefore promote plant development (Bolan *et al.* 1994; Yang *et al.* 1994)

2.4 Soil pH and Exchangeable Al, Fe, and Ca concentration

The solubility of the compounds holding P is directly associated with soil pH. Phosphorus is most available in the pH range of 6.5 to 7 (Troeh and Thompson 2005). An alteration in soil pH to outside this range influences the P species on the surface of the adsorbing particles and in the solution. Increase in pH results in P adsorbing sites gradually being negatively charged. This makes the reactions with negatively charged phosphate ions become harder and this diminishes P adsorption as well as changes the fraction of P species in solution (Whitelaw 2000). In acidic soils more P binds with Al and Fe to form insoluble phosphate compounds. The solubility of these phosphates buildup while increase in soil pH. In alkaline and calcareous soils, P reacts with overabundance Ca forming insoluble compounds. The solubility of these phosphates increases while decreasing soil pH. Fixation of P is generally higher in the adsorption site for P in calcareous soils. Soluble P interacts with CaCO_3 to form low solubility

calcium phosphates which later may form to precipitated P compounds under alkaline soil condition. In strongly alkaline soil with presence of sodium, sodium phosphates are formed. Sodium phosphates are able to be soluble compared to calcium phosphates. Therefore Phosphorus availability is not the main issue at soil pH over 9. Plant development is the one that is influenced by other adverse conditions (Troch and Thompson 2005). For instance, a buildup in P availability at high pH reduces the concentration of micronutrients like Cu, Fe, and Zn in soil (Flocking 2001; Whitelaw 2000).

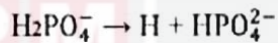
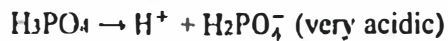
2.5 Phosphorus Retention in soils

The P cycle in soils is a dynamic system including soil, plants and microbes. In the closed natural system, basically P uptake by plants is released into the soil either as plant or animal remains. However, in horticulture systems some P is exited from soil system with harvested crops and only small portion of this P returns to the soil. Phosphorus changes in soils include mineralogical, chemical, physico-chemical and biological processes (Frossard *et al.* 2000). Chemical activities incorporate precipitation and dissolution, while physico-chemical processes incorporate adsorption and desorption, and biological processes include immobilization and mineralization.

2.6 Factor Affecting Phosphorus Sorption from Soil

2.6.1 Soil pH

Soil pH has significant effect on the amount and manner in which soluble phosphates become adsorbed. When soil is acidic, the dominant P ion species present in H_2PO_4^- and when soils become alkaline, the dominant ion become PO_4^{3-} (Tan 1998). This is represented by equation below:



2.6.2 Organic Matter

Decomposition of organic matter leads to buildup of P availability in soils. The formation of phosphor-humic complexes which are absorbed by plants easily, anion substitution of the phosphate by the humate particles, and the development of a protective coat for sesquioxide from humus help to reduce P retention capacity of soil (Tan, 1998). The reaction of Al and Fe with P is decreased when certain organic anions form stable complexes with Al and Fe and in where they block the adsorption sites for P (Hunt *et al.* 2007). It was further stated that those complex ions can free formerly retained P by same mechanism. At the beginning stage, P bonded to anion exchange sites on organic matter are consequently changed into less soluble Al and Fe phosphates (Chintala *et al.* (2013). In a related study, P adsorption capacity of soil and index of bonding energy of the soil particle for P diminishes due to increase of soil pH

along with organic matter and lime application (Lucesio and Duque 1999). The maximum buffering capacity (MBC) directly corresponded with soil organic matter, potential acidity, and contrarily related with based saturation (Guilherme *et al.* 2000).

2.7 Acid soil

In Malaysia, formation of an acid soil such as Ultisols involved clay mineral weathering, leaching of basic cations from soil profile and, accumulation of clay matters in an argillic or kandic horizon. Generally, Ultisols are formed on old land surfaces and usually under forest vegetation savanna or swap vegetation. Ultisols are characterized by a relatively acidic B horizon that has less than 35% of the exchange capacity satisfied with base acidity, a kandic horizon that often have an ochric or umbric epipedon. Subsoils of Ultisols are quite acidic and low in plant nutrients even consists of both an epipedon. (Brady and Weil 2002)

Judging on weathering and acidity of soils, Ultisols are more highly weathered and acidic than Alfisols, yet less acidic than Spodosols and less weathered than Oxisols. Because of the buildup of iron oxides in the subsurface, the subsurface horizons are generally red or yellow in color. Plinthite is a horizon with iron-rich mottled material that formed under fluctuating of humidity in certain. Providing the materials remain moist, this material is soft and can dig out effortlessly. Plinthite is unusable for cultivation when air dried and solidifies irreversibly into a sort of ironstone (Brady and Weil 2002).

2.8 Biochar

Biochar is produced through pyrolysis. This biomass-derived dark C is generally used as organic soil amendment. It is like black C which is normally found in fire-ecosystem and charcoal produced through traditional or modern pyrolysis technique. Biochar is normally utilized as a soil amendment to enhance soil nutrient status, C stockpiling, and filtration of permeating soil water (Lehmann and Joseph 2009). It comprises of similar key characteristics incorporating long residence period in soil and a soil conditioning effect (Glaser *et al.* 2002).

In spite of various studies that have demonstrated enhanced P uptake with the presence of biochar, directly or indirectly affect biochar but whether it could decrease P fixation in soil and its effectiveness still not well-known for the types of mechanism. In a study about natural oxidation of black C in soil by Cheng *et al.* (2008), there was plenty Anion Exchange Capacity (AEC) in the acid pH range for fresh biochar which can originally be in abundance of the total CEC of the biochar. It is possible that these positive exchange sites contend with Al and Fe oxides for sorption of soluble P. This presumption is similar to observation on humic and fulvic acids in the study by Hunt *et al.* (2007). For P sorption and availability utilizing biochar and soil-biochar mixture, it demonstrated that assimilation of biochar to an acid soil at 40g/kg increased the equilibrium solution P concentration (decreased sorption) and increased available adsorbed P (Chintala *et al.* 2013).

To this date, there is lack of studies assessing the impacts short-term anion exchange capacity on P cycling and availability. The positive exchange sites on biochar surfaces decay while negative charge sites increase as biochar age (Cheng *et al.* 2008).

Phosphorus availability and recycling might be affected by biochar CEC over long period and in soils that have low exchange capacity. Biochar may promote the formation and recycling of labile P part by decreasing the free Al and Fe close to root surfaces. A study by Morales *et al.* (2013) about sorption and desorption of P on biochar and soil-biochar mixture had found that the 600 Celsius slow pyrolysis biochar had a lessened capacity to sorb P (4 – 10 times less) in respect to biochar formed at 400 to 500 Celsius. This study demonstrates the diversity in P chemistry that can be expected when biochar is a soil amendment and proposes the potential to create biochar with properties to meets particular objectives.

2.9 Potential of Biochar in Phosphate Retention

2.9.1 Mechanisms of Biochar in Reducing Soil P Fixation

Further decomposition take place when biochar is added into the soil producing a whole range of organic compounds are synthesized by the decomposer microorganisms. These compounds can bind strongly with Al and Fe in the soil through complexation of Al and Fe with (i) well-defined compounds such as organic acids, phenols, and carboxyls, phenolic acids, or (ii) functional group such as carboxyls and phenols in humic substances (humic acids, fulvic acids and humin) known to negatively charged in alkaline condition (Haynes and Mokolobate 2011).

2.9.2 Increase of Soil pH upon application of Biochar

Increasing soil pH to near neutral (pH 6 -7) reduces P fixation in soils by reversing the precipitation process and anion replacing process in chelation. Thus, allowing the free phosphates in soil solution to become available for plants uptake. The increase in soil pH following biochar application to soils can be explained by different mechanisms. This mechanism includes oxidation of organic anions present in the decomposing co-composts, ammonification of biochar and compost organic N, and specific absorption of organic molecules produced during biochar decomposition (Yan *et al.* 1996). For example, biochar increase pH by specific adsorption of humic substances or organic acids onto Al and Fe hydrous oxide with the consequent release of OH⁻ ions (Iyamuremye and Dick 1996).

2.9.3 Direct phosphate source from Biochar

Application of biochar either in short term nor long term will cause accumulation of humic substances in the soil. This helps to increase soil CEC and reducing Al and Fe in soil. The solubility of Al and Fe may decrease when the humic substance form coating on the surfaces of Al and Fe. For instance, concentration of organic acids in soil solution is likely to increase during the initial stage of decomposition due to rapid microbial degradation in soils. But after certain period, the humic substances become more stable and less susceptible to degradation and this may provide a more permanent solution to reduce P fixation (Iyamuremye and Dick 1996). The presence of P from decomposition of biochar will be adsorbed onto oxide surfaces, thus increasing the proportion of adsorption sites occupied by P. As a result, less subsequently added P is adsorbed and P availability is therefore increased.

2.10 Anion Exchange Capacity

Positive charges in the soil originate either from rupture of planes of the structural units and the resulting edge charges or of iron and aluminium oxides that cover some crystalline clays or occupy an interlayer position in lattice layers. These charges induce adsorption of anions (Zelazny *et al.* 1996).

Before 1975, anion exchange capacity (AEC) was rarely studied mainly because in the main soils of the temperate zones, the influence of anions is weaker than cations which are linked with negatively charged surfaces. This is particularly true for 2:1 clays whose zero point charge is badly defined, as the permanent negative charge is too great to be balanced by the less significant positive charges which appear at the edge of the rupture zones of the structural units, giving very high ratios of CEC to AEC (Bingham *et al.* 1965).

As the CEC is relatively low in 1:1 clays, the effects of the edge charges give low ratios of CEC to AEC, and the influence of AEC is consequently more significant, especially if the particles are small and display a significant level of disorder. These phenomena are significant in certain acid tropical soils including metallic oxides and organic anions (Theng 1979; Tate and Theng 1980; Eick *et al.* 1999). In Spodosols, Ultisols, Oxisols and Andosols, the values can vary from 1 to 10 mmol kg⁻¹ with ratios of CEC to AEC. In Andosols, allophane-imogolite and organic matter content can drastically modify exchange properties. The development of positive charges at the soil pH is high in these soils which have a ratio of silica to alumina of near one (Wada and Okamura 1977 1980; Okamura and Wada 1978; Cruz Huerta and Kientz 2000).

On the other hand, if these soils contain aluminium-humus complexes, the positive charges are weak, and this makes it possible to differentiate Al-OH complexes in humus and in the mineral fraction (organic matter anions has a low p*H*0 which lowers the soil p*H*0 when it fixes large organic). There is significant adsorption of P in andosols that involves exchange mechanisms as well as structural displacements (Rajan 1975; Parfitt and Henmi 1980). The polymers of iron oxides and aluminium hydroxides induce a strong AEC (Schwertmann and Taylor 1989). In strongly weathered soils, the AEC values can be higher than the CEC values; for example when the method of Gillmann (1979) is used, acrohumox can have a CEC of 0.6 cmol (+)kg⁻¹ and an AEC of 3.7 cmol (-)kg⁻¹.

The AEC was long considered to be a less important measurement than the CEC because the different anions involved in these exchanges are seldom retained by simple electrostatic bonds, but only by more complex strengths. Without selective fixation, only the exchange of Cl⁻, NO₃⁻ and ClO₄⁻ anions is possible: the exchange of one anion with another anion of similar ionic force does not modify the electrophoretic mobility of the particles. There is no change in specificity related to the size of the anion and probably not to phenomena of steric impossibility (Schwertmann and Taylor 1989). Other anions can be more strongly retained on the oxide surfaces by coordination bonds or chemisorption (e.g. different silicates, molybdates, arsenates, selenates, organic anions). Acetate is adsorbed by coulombic interaction, whereas citrate, which forms ligands with aluminium, is strongly retained. As reported by Bowden *et al.* (1980) adsorption model, it able to defined behaviour of the anions.

CHAPTER 3

MATERIALS AND METHODS

3.1 Characteristics and Physiography of the Study Area

The soil samples used in incubation study are collected from an uncultivated area of Universiti Putra Malaysia Bintulu Campus Sarawak (UPMKB) as the Figure 3.1 below with the coordinate of $3^{\circ}12'11''\text{N}$, $113^{\circ}04'25''\text{E}$ with the depth of 0-25cm. The incubation study is carried out at the Research Centre of UPMKB and the treatments are arranged in Completely Randomized Design (CRD) with three replications. The selected physical and chemical properties of the soils were determined before and after the study.

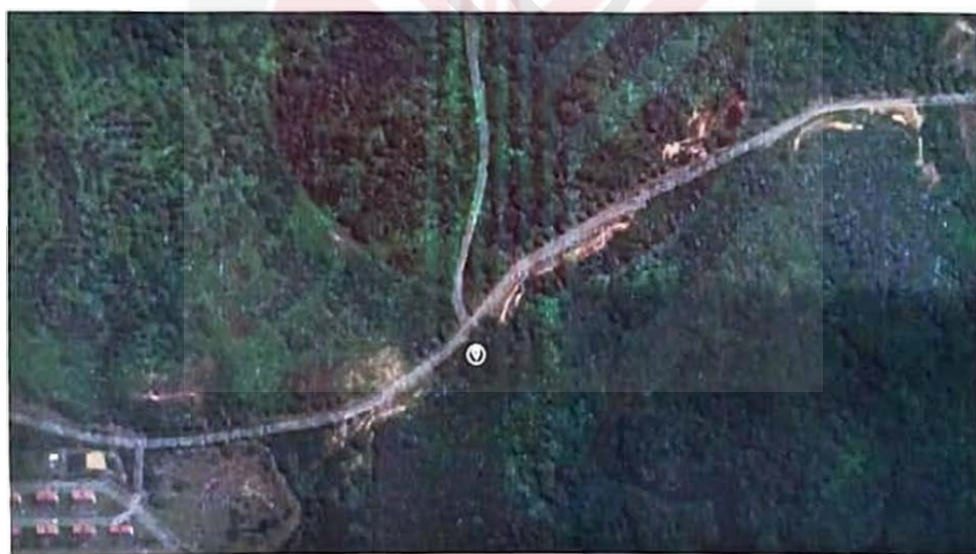


Figure 3.1 Soil sampling location in Universiti Putra Malaysia Bintulu Campus

3.2 Incubation Study

The incubation study is carried out for 40 days with 4 batches which are 10 days, 20 days, 30 days, and 40 days of incubation. Table below is the summary of the treatments used in the incubation study.

Table 3.1 Treatments with organic amendments and fertilizer application

Treatments	Soil (g)	CLB (g)	TSP (g)
T1	300		
T2	300		4.8
T3	300	180	
T4	300	90	2.4

*CLB = Chicken Litter Biochar

*TSP = Triple Superphosphate

3.3 Chemical Analysis

3.3.1 Soil pH Determination in Water and KCl

The air-dried soil samples were oven dried at 60 °C for 24 hours. A 10g of soil samples was weighed into plastic vial and 25 mL of distilled water or 1M KCl are added. The lids were close and the suspension was shaken at 180 rpm for 15 minutes (Peech, 1965) using an orbital shaker. The mixture with distilled water was left overnight and a digital pH meter was used to determine the pH of the soil while the mixture with KCl was determined after they are shaken at 180 rpm for 15 minutes.

3.3.2 Anion Exchange Capacity (AEC) Analysis

The moisture content of the sample was measured to bring back the results to soil dried at 105°C. 10 mL of the 1 mol NH₄Cl solution was added to the samples (the pH of this solution should be around 6.0). The samples were shaken for 30 min and centrifuged at 5000 g and discard the supernatant. The centrifugation pellet was suspended five times in 10 mL of the 0.1 mol NH₄Cl solution centrifuging between each treatment. The Cl⁻ ion was moved by five additions of 10 mL of 1 mol KNO₃ solution centrifuging between each treatment; mix the extracts and brought to 50 ml. 5 ml of AgNO₃ were added to 20 mL of samples and the samples are constantly shaken to ensure precipitates could form properly. 5 mL of AgNO₃ are added into the samples until no precipitates are formed. The samples are filtered with filter paper to obtain the mass of precipitates. To obtain the mass of precipitates, the formulas are as below:

Weight of Precipitates (g) = Weight of dry filter paper + Precipitates (g) – Weight of dry filter paper (g)

3.3.3 Determination of Available Phosphorus in Soil – Double Acid Method

Available phosphorus was measured using UV spectrophotometer. A 5 g of soil sample was weighed into a 250 mL conical flask and 20 mL extract solution was added (0.05M HCl + 0.025M H₂SO₄) and shaken for 30 rpm for 15 minutes. The solution was filtered with filter paper No.2 into a plastic vial and the solution was used to identify the P concentration using UV spectrophotometer. The formula for Available P is as below:

$$\text{Available P} = \text{UV-VIS} \times \frac{\text{Extract Solution (mL)}}{\text{Weight of Soil Used (g)}} \times \frac{\text{Marked up Volume (ml)}}{\text{Volume of Sample Used (ml)}}$$

3.3.4 Determination of Total Phosphorus in Soil – Aqua Regia Method

Total P was measured using UV spectrophotometer. A 2 g of soil sample was weighed into a 250 mL conical flask and 20 mL aqua regia solution (3HCl : 1HNO₃) was added. The sample was heated until the volume of aqua regia solution is halved. The samples were filtered with filter paper into 50 mL volumetric flask and the solution is marked up to 50 mL using distilled water. The solution was used to identify the P concentration using UV spectrophotometer. The formula for Total P is:

$$\text{Total P} = \text{UV-VIS} \times \frac{\text{Aqua Regia Solution (mL)}}{\text{Weight of Soil Used (g)}} \times \frac{\text{Marked up Volume (mL)}}{\text{Volume of Sample Used (mL)}}$$

3.3.5 Determination of Total Organic Carbon and Organic Matter by Dry Combustion Method (Loss on Ignition Method)

Air-dry sample was placed in an oven and dry it at 60°C for 24 hours and cool it in a desiccators. The initial weight was taken of the silica or porcelain dish (crucible) plus the sample. The sample was placed in a muffle furnace and initially ash at 300°C for 1 hour. The temperature was raised to 550°C and continues with the ashing for 8 hours and the sample is allowed to cool down before inspection. The weight of the samples and crucibles are taken after ashing. The OM and TOC were calculated using the formula below:

$$\text{OM} = \frac{\text{Initial weight of sample (g)} - \text{Final Weight of Sample (g)}}{\text{Initial weight of sample (g)}} \times 100$$

$$\text{TOC} = \frac{\text{Initial weight of sample (g)} - \text{Final Weight of Sample (g)}}{\text{Initial weight of sample (g)}} \times 100 \times 0.58$$

3.3.6 Determination of Soil Exchangeable Acidity, Aluminium, and Hydrogen Ions

Soil exchangeable acidity and Al^{3+} were determined using acid-base titration method (Rowell, 1994). The solution was extracted by shaking the soil samples with 1 M potassium chloride (KCl) at a ratio of 1:3 for 15 minutes using a reciprocal shaker. Afterwards, the suspension was filtered into 100 ml volumetric flask using filter paper No. 2 after which it was marked up with 1M KCl. The exchangeable acidity was determined by titrating 50 ml aliquot with 0.01M of NaOH after adding 5 drops of phenolphthalein indicator. The titre value was recorded after the first permanent pink endpoint after which drops of 0.01M HCl were added until the permanent pink endpoint turn into colourless. Afterwards, exchangeable Al^{3+} was determined by adding 10 mL of sodium fluoride (NaF) to the colourless solution and titrated with 0.01M HCl until the pink coloured turns into colourless. To calculate exchangeable acidity and aluminium, the formulas are as below. Exchangeable hydrogen is calculated using exchangeable acidity subtracted exchangeable aluminium:

$$\text{Exchangeable Acidity} = \frac{\text{Volume of NaOH used} \times \text{Concentration of NaOH} \times 100}{\text{Weight of soil sample used}}$$

$$\text{Exchangeable Aluminium} = \frac{\text{Volume of HCl used} \times \text{Concentration of HCl} \times 100}{\text{Weight of soil sample used}}$$

3.4 Experimental Design and Statistical Analysis

Data were statistically analyzed using analysis of variance (ANOVA) to detect treatment effects and means was compared using Tukey's test at $P \leq 0.05$. Statistical Analysis System (SAS Version 9.4) was used for the statistical analysis.



CHAPTER 4

RESULT

4.1 Chemical Analysis of soil sample *Nyalau* series

The selected chemical properties of soil samples *Nyalau* series used in this study are consistent with those reported by Applied Agriculture Resources (AAR) in Table 4.1.

Table 4.1 Selected chemical properties of soil samples *Nyalau* series

Chemical Analysis	Data Obtained (0-15 cm)	AAR (0-15cm)
pH (water)	4.10	4.7
pH (KCl)	3.68	4.0
%OM	3.8	Nd
%TOC	2.20	1.30
Available P (ppm)	4.40	Nd
Total P (ppm)	20.11	Nd
Exchangeable Acidity (cmol/kg)	21.57	2.20
Exchangeable Al (cmol/kg)	18.1	2.14
Exchangeable hydrogen (cmol/kg)	4.13	0.06
AEC (ppm)	4373.602	Nd

*Nd – Not determined

4.2 pH in KCl

Based on Figure 4.1, T3 showed the highest pH_{KCl} value and followed by T4 with 2nd highest pH_{KCl} value. T1 and T2 had lower pH_{KCl} value compared to T3 and T4 and they showed similar pH_{KCl} value. This trend was consistent throughout the period of incubation study.

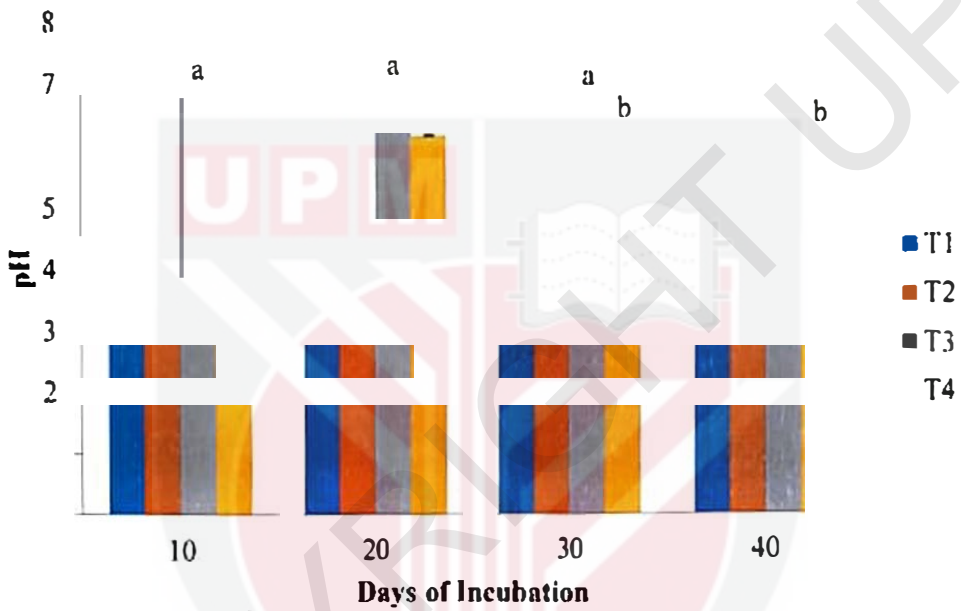


Figure 4.1 pH_{KCl} of soil samples from 10 days to 40 days of incubation

4.3 pH in Water

In Figure 4.2, the pH_w values were similar with pH_{KCl} where T3 had the highest pH_w value and T4 is the 2nd highest. T1 and T2 had lower pH_w value compared to T3 and T4 and they had similar pH_w value. This trend was consistent throughout the period of incubation study.

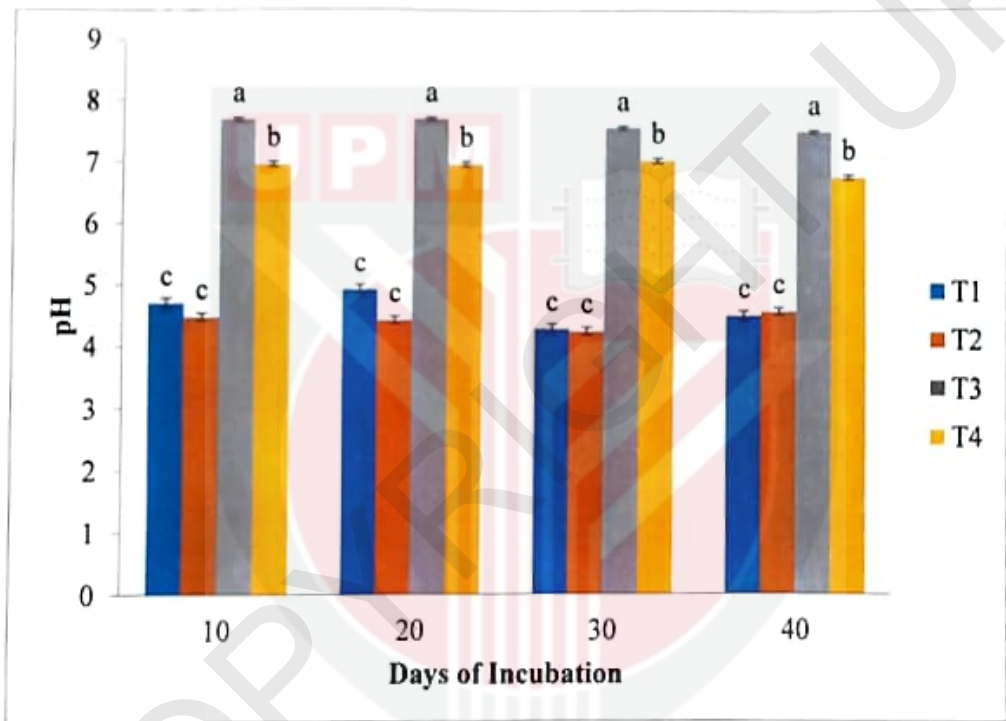


Figure 4.2 pH_w of soil samples from 10 days to 40 days of incubation

4.4 Exchangeable Acidity

Based on Figure 4.3, T1 had the highest exchangeable acidity. T2 had slightly higher exchangeable acidity compared to T3 and T4. T3 and T4 had lower exchangeable acidity. This trend was consistent throughout the period of incubation study.

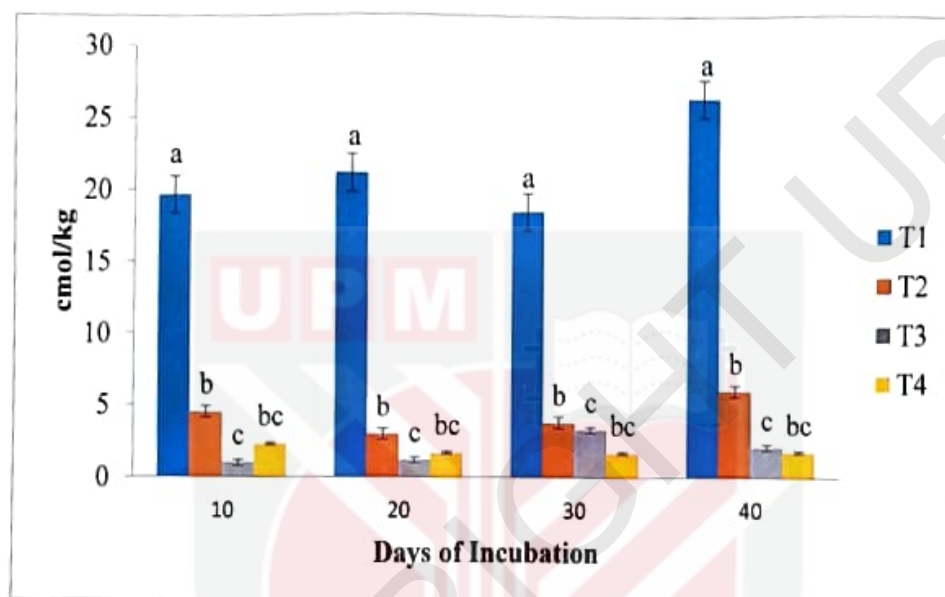


Figure 4.3 Exchangeable Acidity of soil samples from 10 days to 40 days of incubation

4.5 Exchangeable Aluminium

Based on Figure 4.4, T1 showed the highest exchangeable aluminium. Exchangeable aluminium for T2, T3 and T4 were not able to detect. This trend was consistent throughout the period of incubation study.

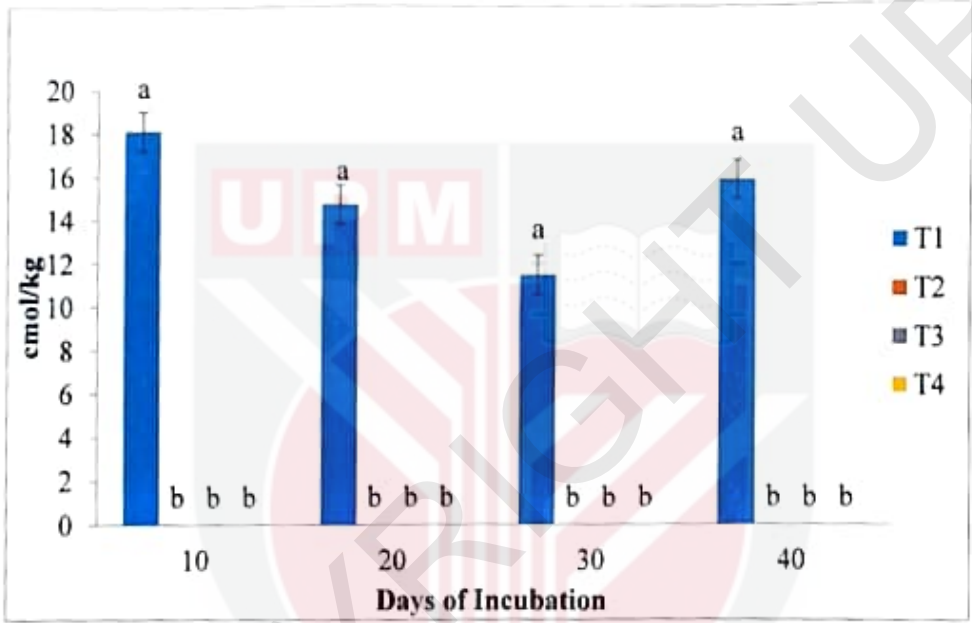


Figure 4.4 Exchangeable Aluminium of soil samples from 10 days to 40 days of incubation

4.6 Exchangeable Hydrogen

Based on Figure 4.5, T2 showed the highest exchangeable hydrogen at 10 days of incubation. T1 had the highest exchangeable hydrogen from 20 days to 40 days incubation. T4 had the least exchangeable hydrogen among the treatment. T3 had lesser hydrogen compared to T4 at 20 days of incubation and the exchangeable hydrogen are higher than T4 at 30 days of incubation but decreased to similar that of exchangeable hydrogen of T4 at 40 days of incubation.

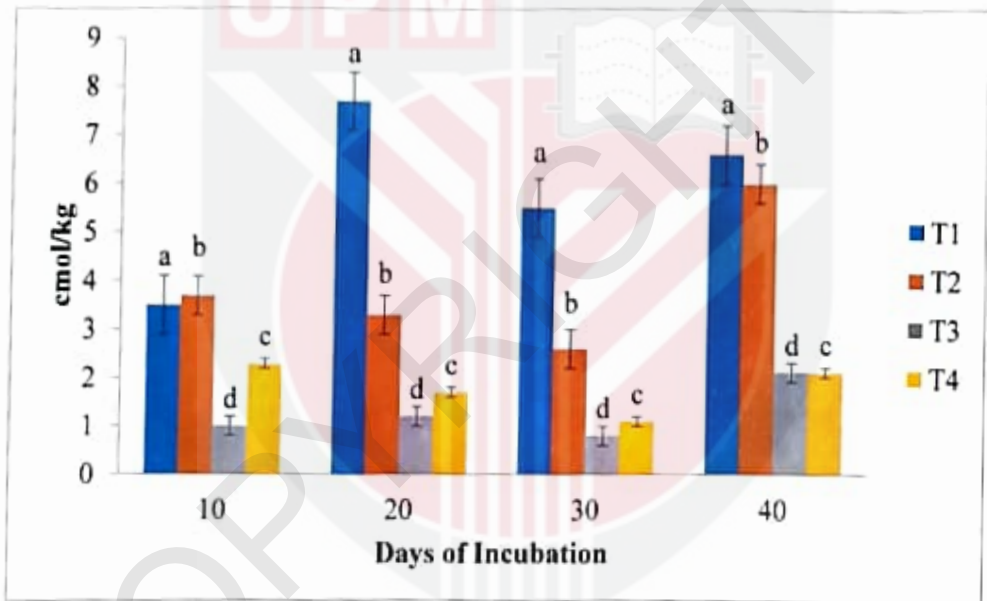


Figure 4.5 Exchangeable Hydrogen of soil samples from 10 days to 40 days of incubation

4.7 Organic Matter

In Figure 4.6, T3 had the highest organic matter and T4 was the 2nd highest. T1 and T2 had similar percentage of organic matter but they had lower organic matter compared to T3 and T4. This trend was consistent throughout the period of incubation study.

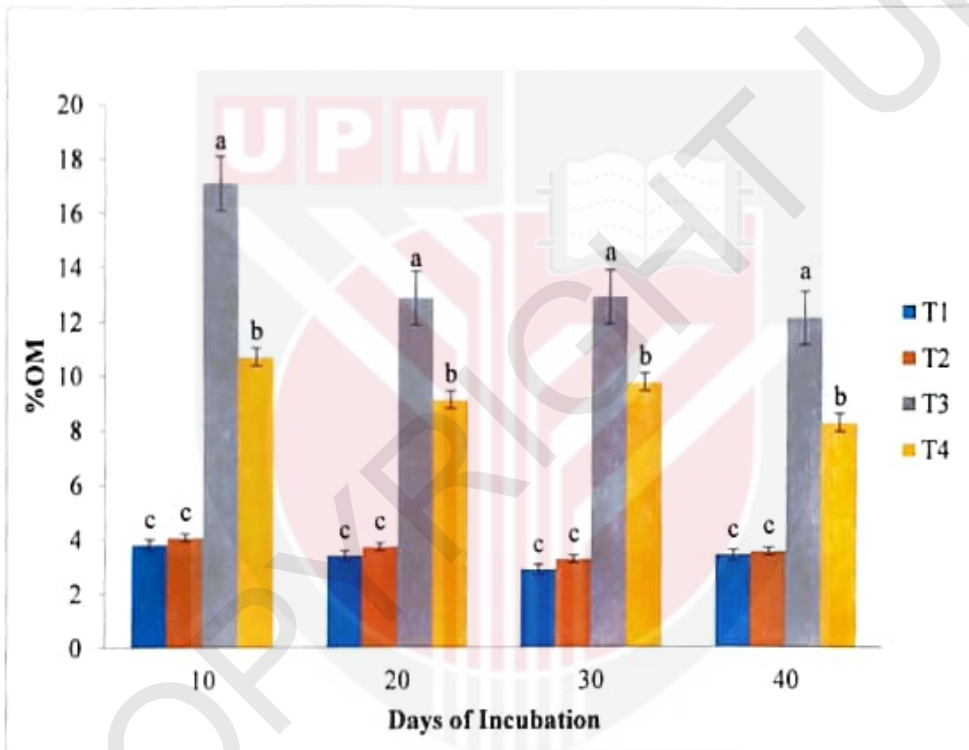


Figure 4.6 Organic matter of soil samples from 10 days to 40 days of incubation

4.8 Total Organic Carbon

Based on Figure 4.7, total organic carbon was similar to organic matter of T3 which showed the highest % TOC and T4 is the 2nd highest. For T1 and T2, both of their %TOC was similar and they were lower compared to T3 and T4. This trend was consistent throughout the period of incubation study.

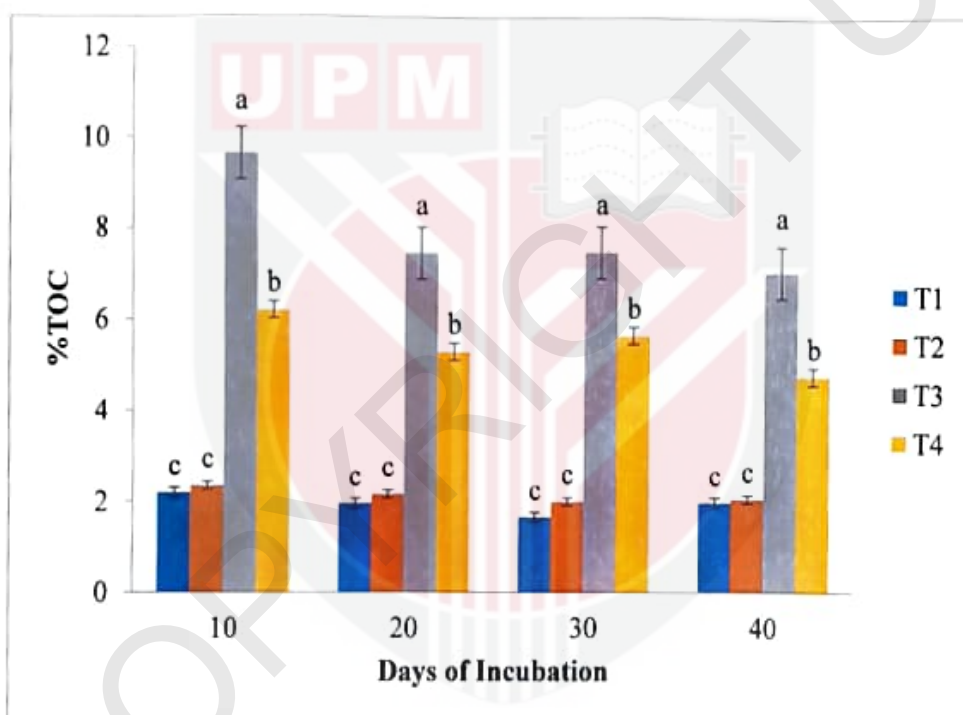


Figure 4.7 Total organic carbon of soil samples from 10 days to 40 days of incubation

4.9 Available Phosphorus

Based on Figure 4.8, T3 and T4 showed higher available P compared to T2. T2 had higher available P compared to T1 but it was slightly lower than T3 and T4. T1 had the least available P. This trend was consistent throughout the period of incubation study.

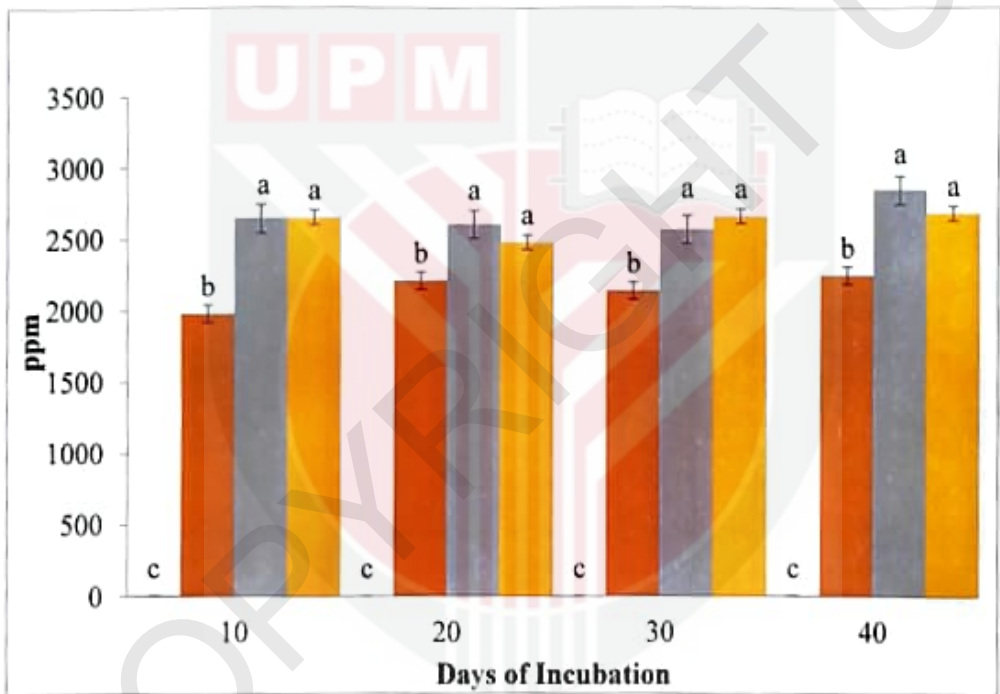


Figure 4.8 Available P of soil samples from 10 days to 40 days of incubation

4.10 Total Phosphorus

Based on Figure 4.9, total P had similar trend compared to available P where T3 and T4 had higher total P. T2 had higher total P compared to T1 but it was slightly lesser than T3 and T4. T1 had the least total P. This trend was consistent throughout the whole incubation study.

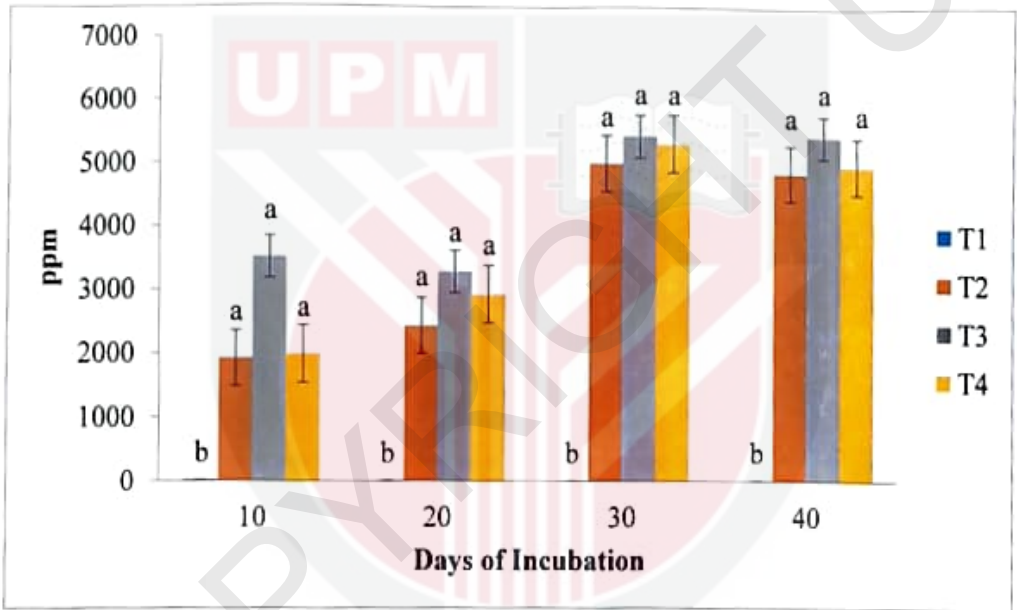


Figure 4.9 Total P of soil samples from 10 days to 40 days of incubation

4.11 Anion Exchange Capacity

Based on Figure 4.11, all of the treatments were quite similar among each other. T1 had the highest AEC at 10, 20, and 40 days of incubation. T4 had the highest AEC at 30 days of incubation.

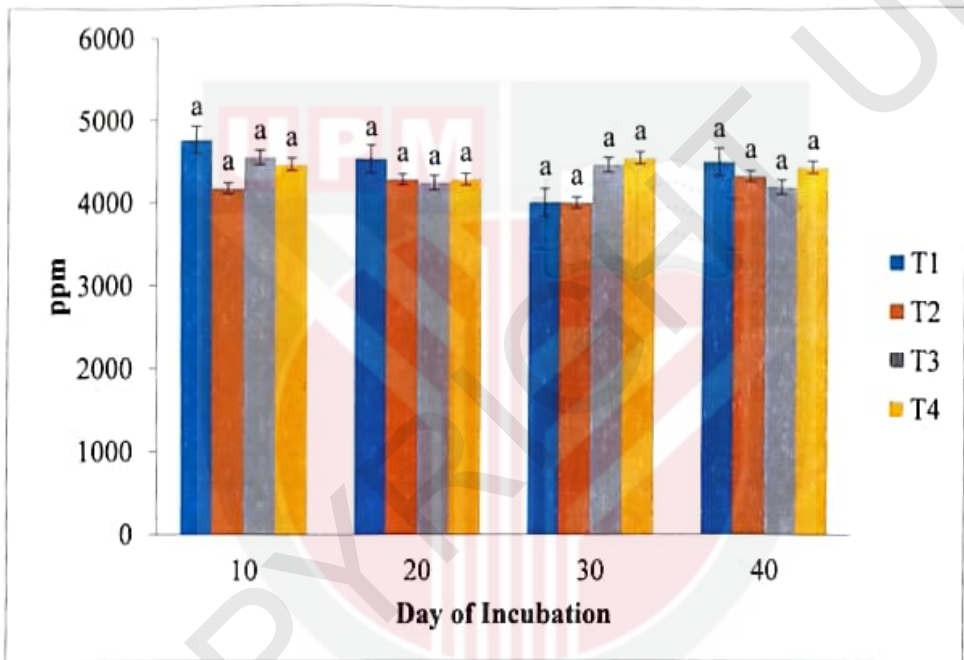


Figure 4.10 Anion Exchange Capacity of soil samples from 10 days to 40 days of incubation

CHAPTER 5

DISCUSSION

5.1 Fertilizer Treatments on pH of an Acid Soil

As shown in Figures 4.1 and 4.2, T3 and T4 which represent soil samples amended with chicken litter biochar had significant higher pH in both water and KCl compared to T1 and T2 that did not amend with chicken litter biochar. By applying biochar to soils, it increases soil pH by different mechanism such as specific absorption of organic molecules produced during biochar decomposition (Yan *et al.* 1996). As reported by Iyamuremye and Dick (1996), humic substances or organic acids bind onto Al and Fe hydrous oxide and they release of OH^- ions into the soil.

5.2 Fertilizer Treatments on Exchangeable Acidity, Aluminium and H

In the Figures 4.3, 4.4, and 4.5, T1 showed the highest exchangeable acidity, Al and hydrogen value. However, T2, T3, and T4 exchangeable Al was not detected in the soils. This is because of the organic matter in T2, T3, and T4 enabled reduction of Al and Fe in soils. As stated by Tan (1998), application of biochar caused accumulation of humic substances in soils by decreasing the solubility of Al and Fe to form a coating on the surfaces of Al and Fe. Other studies supported this claim as Haynes and Mokolobate (2011) reported that decomposition of organic matter releases functional groups to bind strongly with Al. Besides, exchangeable acidity is directly linked with pH of soils. This observation is further explained in Figures 4.2 and 4.3 as well as Figures 4.3, 4.4, and 4.5, where pH of T3 and T4 had higher pH compared to

T1 and T2 due to decomposition of biochar mechanism such as specific adsorption of organic molecules where organic acids reacts with Al and Fe hydrous oxide and released OH^- ions (Iyamuremye and Dick 1996).

5. Fertilizer Treatments on OM and TOC

In Figures 4.6 and 4.7, T3 and T4 showed higher organic matter and total organic carbon compared to T1 and T2. This phenomenon could be explained because chicken litter biochar is an organic amendment produced through pyrolysis and it is also a biomass derived black carbon. Biochar also improves C storage and soil nutrient status of soils (Lehmann and Joseph 2009). Furthermore, presence of biochar in soils increases soil pH where organic compounds released by biochar can bind strongly with Al and Fe in the soil (Haynes and Mokolobate 2011), to increase the availability of P to plants as showed that incorporation of biochar to an acid soil at 40g/kg increased the equilibrium solution P concentration and increased available adsorbed P (Chintala *et al.* 2013).

5.4 Fertilizer Treatments on Available Phosphorus and Total Phosphorus

Total and available phosphorus are directly related with exchangeable acidity and pH because these 2 factors affect fixation and retention of P. Based on Figures 4.8 and 4.9. Moreover, T3 and T4 showed higher available and total P which had organic amendment compared with T2 that had triple superphosphate and this could be due to P release when biochar undergo decomposition compete the binding sites of metal cations with P (Hariprasad and Niranjana 2008). Biochar releases organic anions

when it decomposes and to stable complexes with Al and block P adsorption sites (Hunt *et al.* 2007). Hence, this mobilizes P and releases organic anions adsorbed by soil colloids (Wandruszka 2006). Eventually, this greatly decreased the amount of P adsorption sites (Geelhoed *et al.* 1999; Bolan *et al.* 1994). Another important role played by biochar is increased the soil pH, as reported by Haynes and Mokolobate (2011), organic compounds released by biochar when decomposed can bind strongly with Al and Fe in the soil as well as undergo mechanism such as specific adsorption by reacts with Al and Fe hydrous oxide and released OH^- ions (Iyamuremye and Dick 1996).

5.5 Fertilizer Treatments on Anion Exchange Capacity

Based on Figure 4.10, it can be seen that there are no significant difference among the treatments with the addition of fertilizer and chicken litter biochar. All of the treatments showed similar AEC. AEC can be linked with the availability of cations such as Al and Fe because these cations enabled in promoting protonation of organic matter in soils and allowing them to adsorb anion such as phosphate.

CHAPTER 6

CONCLUSION

Amending acid soil with chicken litter biochar increases soil pH as shown by T3 and T4. These treatments also increased the OM and TOC in the soil.

Chicken litter biochar also reduced P fixation by releasing organic acids and anions that bind with Al and Fe and reduced the adsorption sites for P. Hence, it increased available P and total P as shown in T3 and T4 had higher P contents compared T2. This finding also suggests that chicken litter biochar had similar or better potential in reducing exchangeable acidity and chicken litter biochar compared to chemical fertilizer. For Anion Exchange Capacity, chicken litter biochar does not improve this soil properly.

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PUBLICATION OF THE PROJECT UNDERTAKING

This is to certify that I have no objection to publish the project entitled "Anion Exchange Capacity of Chicken Litter Biochar in Regulating Phosphate Retention of Acid Soil" by the supervisor in a joint authorship. However, it has to be evaluated by the Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia Bintulu Sarawak Campus and published in the form approved by the Faculty.



Low Xian Jie

Date: