PHOSPHORUS CHANGES IN INTENSIVE CROPPING PADDY SOIL UNDER NATURAL AND CONTROLLED CONDITIONS FROM MADA, KEDAH

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by

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LIST OF SYMBOLS AND ABBREVIATION

ANOVA	Analysis of Variance
g	Gram
mg	Milligram
µg	Microgram
kg	Kilogram
ha	Hectare
cm^2	Centimeter square
cm	Centimeter
mm	Millimeter
nm	Nanometer
mL	Milliliter
L	Liter
Min	Minute
Rpm	Revolutions per minute
M	Molar (Moles per liter)
µM	Micromolar / micro(moles per liter)
mM	Millimolar / milli(moles per liter)
^0C	Celsius
%	Percentage
s.e.	Standard error

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PERUBAHAN FOSFORUS DALAM TANAH TANAMAN PADI SECARA INTENSIF DALAM KEADAAN SEMULAJADI DAN TERKAWAL DARI KAWASAN MADA, KEDAH

ABSTRAK

Fosforus merupakan salah satu nutrien utama yang diperlukan oleh tumbuhan untuk menjalankan pelbagai aktiviti. Walau bagaimanapun, fosforus di dalam tanah mudah diikat. Dalam tanah pertanian, fosforus dibekalkan secara utama melalui pembajaan. Sebahagian besar petani akan cenderung untuk menambah baja fosforus secara berlebihan untuk meningkatkan hasil dan perbuatan ini akan menyebabkan perubahan kepekatan nutrien di dalam tanah terutamanya kolam fosforus. Oleh itu, kajian ini telah dijalankan untuk mengetahui perubahan kolam fosforus di dalam tanah padi dari kawasan MADA, Kedah dimana secara umumnya diketahui bahawa kawasan tersebut telah menjalankan penanaman padi secara intensif selama 50 tahun. Untuk mengetahui status dan perubahan fosforus di dalam tanah tanaman padi, dua eksperimen telah dijalankan di bawah dua keadaan persekitaran; semula jadi (penyampelan luar) dan terkawal (kajian rumah tumbuhan). Untuk persekitaran terkawal, terdapat tiga jenis rawatan yang telah diberikan iaitu baja kimia, vermikas serta rawatan terkawal untuk mengkaji perubahan kepekatan fosforus untuk dua kali tempoh penanaman. Parameter tanah yang diambil adalah tesktur tanah, pH tanah dan nutrien tanah (bahan organik, nitrogen dan fosforus) manakala parameter tumbuhan yang diambil adalah biomas dan nutrien tumbuhan. Untuk mengetahui kolam fosforus di dalam tanah, kaedah pecahan fosforus secara berturutan telah dilakukan. Dalam keadaan semula jadi, perubahan kolam fosforus untuk setiap jenis tanah adalah berbeza secara nyata kerana perbezaan pH tanah dan tesktur tanah

untuk setiap jenis tanah. Hasil kajian korelasi pula menunjukkan segilintir kolam fosforus terpilih untuk setiap jenis tanah sebahagian besarnya dipengaruhi oleh pH tanah. Dalam keadaan terkawal dimana padi ditanam dengan penambahan baja yang berbeza, perubahan kolam fosforus untuk setiap jenis rawatan dalam setiap jenis tanah adalah berbeza dalam dua tempoh penanaman. Perbezaan tekstur tanah dan jenis rawatan yang berbeza dipercayai mampu mempengaruhi perubahan kolam fosforus semasa dua kali tempoh penanaman. Pengambilan fosforus oleh padi dalam rawatan baja kimia adalah yang tertinggi manakala pengambilan fosforus oleh padi dalam rawatan terkawal adalah setanding dengan rawatan vermikas. Keputusan ini menunjukkan apabila keadaan tanah yang tidak ditambah dengan fosforus, pokok padi mampu mengambil dan menggunakan fosforus untuk pertumbuhan kerana kandungan fosforus dalam biomas padi adalah melebihi paras kritikal untuk kekurangan fosforus. Secara keseluruhan untuk kajian ini, perubahan kolam fosforus untuk setiap jenis tanah (tanpa mengira keadaan semula jadi dan keadaan terkawal) adalah berbeza secara nyata kerana perbezaan tekstur tanah, pH tanah dan jenis baja. Perubahan ketara pada kolam fosforus di dalam tanah dapat diperhatikan di dalam tanah P1 dan P3, masing-masing merupakan tanah lom dan tanah lom lempung. Tanah P1 mempunyai peratusan lempong yang paling sedikit (19.2%), kolam fosforus (sederhana labil, kurang labil dan tak labil) dipengaruhi oleh pH tanah secara signifikan. Sementara itu, tanah P3 yang mempunyai peratusan lempung tertinggi (35.8%) dan penurunan pH tanah secara signifikan telah mempengaruhi kolam labil dan sederhana labil secara signifikan. Terdapat perubahan yang nyata untuk perubahan fosforus dalam keadaan semula jadi dan keadaan terkawal dimana perbezaan itu yang mungkin disebabkan oleh penyampelan tanah yang berlainan untuk kajian ini (tanah pukal dan tanah rizosfera). Keputusan yang berbeza untuk

perubahan fosforus dalam tanah dibezakan keadaan semula jadi dan keadaan terkawal ini boleh diperhatikan dengan keputusan korelasi dimana hubungan di antara parameter tanah dan kolam fosforus dalam tanah adalah berbeza secara signifikan diantara kedua-dua keadaan. Rumusannya, tekstur tanah, pH tanah dan jenis baja mempunyai kesan yang signifikan terhadap perubahan kolam fosforus dalam tanah padi. Maka, pengetahuan yang lebih mendalam berkenaan kepekatan fosforus tanah dan perubahan fosforus di dalam tanah boleh difahami. Selain itu, hasil kajian ini juga mampu menyumbang kepada penambahbaikan dan pembangunan protokol piawai untuk penentuan fosforus dalam tanah pertanian.

PHOSPHORUS CHANGES IN INTENSIVE CROPPING PADDY SOIL UNDER NATRURAL AND CONTROLLED CONDITIONS FROM MADA, KEDAH

ABSTRACT

Phosphorus is one of the primary nutrients needed by plant to perform its activities. However, phosphorus in soil can easily being fixed. In agricultural soil, phosphorus was mainly supplied by fertilizer application. Majority of farmers tend to apply more phosphorus fertilizer to increase yield and this practice may lead to nutrient concentration changes in soil particularly phosphorus pools. Therefore, this study was carried out to determine the changes of phosphorus pool in paddy soil from MADA, Kedah which was known to practice intensive paddy cropping for almost 50 years. In order to determine phosphorus status and changes in paddy soil, experiments was conducted under two environmental conditions; natural (field sampling) and controlled (plant house study). In controlled condition, three treatments such as inorganic fertilizer, vermicast and control were applied to study the changes of phosphorus concentration in two cropping periods. Soil parameters measured were soil texture, soil pH and soil nutrients (organic matter, nitrogen and phosphorus) while the plant parameters measured were biomass and plant nutrients. To determine the phosphorus pool in soil, sequential phosphorus fractionation was carried out. Under natural condition, the changes in phosphorus pool in different soils were varied significantly due to different soil pH and soil texture. The correlation results showed that selected phosphorus pools in different soil was largely influenced by soil pH. Under controlled condition where paddy was grown with different amendment, the changes of phosphorus pools in different treatment in different soil showed different changes in both cropping periods. The different soil texture and different treatments were believed could influence the changes of phosphorus pools in both cropping periods. Phosphorus uptake by paddy in the inorganic fertilizer treatment was the greatest while the uptake by paddy in control treatment was as good as vermicast treatment. This result demonstrated that in condition of no addition phosphorus into the soil, paddy was able to utilize phosphorus for their growth since the phosphorus content of paddy biomass was above critical level for phosphorus deficiency. Overall, in this study, the changes in phosphorus pool in different soils (regardless of natural and controlled conditions) varied significantly owing to different soil texture, soil pH and type of fertilizers. The greater change of soil phosphorus pools was observed in P1 and P3 soil, which were loam and clay loam, respectively. P1 soil with the lowest clay percentage (19.2%), phosphorus pools (moderate labile, less labile and non labile) was significantly influenced by the decreasing soil pH. Meanwhile, in P3 soil with the highest clay percentage (35.8%) and significantly decreasing of soil pH was significantly influenced the labile and moderate labile pool. There were significant different changes of phosphorus in natural and controlled conditions which could be due to different soil sample for this study (bulk and rhizosphere soils). This different result of phosphorus changes in soil under natural and controlled conditions could be seen in this correlation result where the relationship of soil parameters and phosphorus pools in soil was significantly different between two conditions. In summary, soil texture, soil pH and types of fertilizer have significant effects on the changes of phosphorus pools in paddy soil. Hence, in depth knowledge on soil phosphorus concentration and its changes in paddy soil was understood. Besides, the

outcome of this study also can possibly contribute to improvising and developing a standard protocol for phosphorus determination in agricultural soil.

CHAPTER 1

INTRODUCTION

1.1 Background study

Rice is a staple food for half of the world's population and the demands on the rice production have been increased from time to time (Rajamoorthy *et al.*, 2015). Paddy is one of the most important crops in Malaysia besides oil palm, rubber and cocoa (Department of Agriculture, 2014). In 2007, about 87% lowland area had been used paddy cropping which occurred in granary area (Najim *et al.*, 2007). Paddy cropping can be found largely in northern region of Peninsular Malaysia especially in Kedah which reflected the highest land usage for paddy cultivation, comprising of 104362.3 hectares which covered 29% of paddy area in Malaysia (Department of Agriculture, 2014). In Southeast Asia, there are more than 100 types of paddy variety with the common variety being planted are Midon, Anak Daro, Jasmine, and Bonla Pdao. In Malaysia, common paddy varieties planted by the farmers were MR 219, MR 220 and MR 220 Clear Field 1 (CL1) (Department of Agriculture, 2014). With such increasing demand for paddy production, more proper and efficient ways in producing high yield and better quality of paddy are important.

Fertilization is much required for paddy cultivation as the main source of nutrient supply for paddy growth and development. Phosphorus (P) is one of the essential elements needed by plant for growth and contribute to the later amount of yield (Brady & Weil, 2002; Schachtman *et al.*, 1998). The concentration of available P in soil is commonly scarce for plant uptake. The low concentration of available P in soil could be attributed to the ability of P being easily fixed with soil matrices (Maathuis & Diatloff, 2013). In order to overcome this problem, farmers have resorted to applying excessive fertilizer to enrich P concentration in soil. Similar to nitrogen, soil containing high P concentration from excessive use of fertilizer can lead to eutrophication of nearby water bodies (Brady & Weil, 2002), through leaching or surface runoff (Sharpley *et al.*, 2014). Therefore, sufficient amount and types of fertilizer are needed to ensure paddy could take up P sufficiently and mitigate the environmental impact which could lead to this phenomenon. Previous study highlighted that, sole application of inorganic fertilizer can provide nutrient directly to the plant but in a long run this could also negatively affect the arable soil (Huang *et al.*, 2013; Simpson *et al.*, 2015). In certain cases, organic fertilizer was chosen to substitute the use of inorganic fertilizer to increase soil fertility (Bhattacharyya *et al.*, 2015; Zhou *et al.*, 2016). However, poor performance of organic fertiliser addition towards plant growth was largely due to the reaction of organic P with soil physical and chemical factors rendering P becoming unavailable (Bah *et al.*, 2006). Hence, some researches proposed the combination of fertilizers as the best way to maximise paddy yield (Bhattacharyya *et al.*, 2015) and improve the soil health (Huang *et al.*, 2013; Zhou *et al.*, 2016).

In Malaysia, most of the farmers used inorganic fertilizer and tend to mismanaged the application of inorganic fertilizer (Mohamed *et al.*, 2016) which is excessively used. In the year of 2012/2013, the average amount of P_2O_5 fertilizer being applied into paddy soil was 52.5 kg/ha (Department of Agriculture, 2014). On other hand, according to Malaysian Agricultural Research and Development Institute (MARDI), there were excessive use of P_2O_5 (~18%) in Malaysian paddy soil (Appendix A) compared to the report by the Department of Agriculture (2014). According to FAO (2002), the demand fertilizer was expected to increase in the future to sustain crop yield. Ismail (1994) stated that the excessive use of inorganic fertilizer and pesticide can lead to the increment of heavy metal accumulation into paddy soil. Thus, food safety in rice will become a bigger issue to be dealt with in future.

A comprehensive study on P concentration of paddy soil is needed to acquire in depth views and knowledge particularly on P pools. Unfortunately, data regarding P pools in paddy soil especially in Malaysia was limited probably due to lack of accessed publication. Although some of the data of P status in paddy soil are probably notarised, the data on P pools in paddy soil are poorly documented. Previously, a study on P transformation and dynamics in Malaysian soil have been done by Gikonyo *et al.* (2008) by growing setaria grass (*Setaria Anceps Stapf.* cv. “Kanzungula”) in acidic soil amended with different inorganic fertilizers and manure treatments. Another study by Ch’ng *et al.* (2014) was done by incubating the acidic soil with different organic amendments. Both studies determined the influence of various treatments towards P pools in soil. Although both studies were conducted using different types of Malaysian soil but paddy soil was not included. Thus, limited information on the soil P status in paddy soil is the main reason for this study to be carried out to elucidate P pools of Malaysian paddy soil.

Sequential P fractionation method is widely used to determine the changes in soil P pools. The pioneer of P fractionation method was developed by Chang and Jackson in 1957. However, the original method was modified extensively to suit different soil types and consistency of the results (Bowman & Cole, 1978; Hedley *et al.*, 1982; Tiessen & Moir, 1993). This method elucidates P concentration in different types of pools by extraction using different strength of extractants. Besides, P changes in each pool can be studied temporally and possible factors causing such changes and the ability of P to become available can also be deduced by this method.

1.2 Significance of contribution, aims and objectives

The aims for this study to be conducted are to provide relevant information on soil P pools in paddy soil as well as to develop an efficient method for determination of P pools in our tropical soil. The benefit from this study could also help to minimise the risk of environmental pollution associated with P losses from agriculture systems. In addition, the findings will also provide the baseline data of soil P pools thus helping the growers to utilise the soil P bank. Consequently, this could lead to reducing the reliance on P fertilisers and resulting in substantial economic benefits. This study will address the following objectives:

a) To improvise a standard protocol for P pools determination.
b) To study the changes of P pools in both natural and controlled environments.
c) To determine the relationship between P pools and soil parameters in both natural and controlled environments.

CHAPTER 2

LITERATURE REVIEW

2.1 Importance of Phosphorus and P uptake mechanisms

Phosphorus (P) is the second limiting macronutrient after nitrogen that needed by plants after nitrogen and it is important for plant activities such as; 1) plant growth, 2) cellular respiration, 3) photosynthesis, 4) reproduction, 5) protein regulation activity and 6) maturation (Brady & Weil, 2002; Hopkins & Hüner, 2009; Maathuis & Diatloff, 2013; Schachtman *et al.*, 1998). Plant takes up P via root by diffusion in soil (Hopkins & Hüner, 2009). Less than 1% of P in soil can be taken up by plant (Richardson *et al.*, 2009) in the form of available P. According to Maathuis and Diatloff (2013), P in soil is low within the range of 0.1-1 µM. The amount of P uptake by plant is varied due to P concentration in soil which is affected by physical, biological and environmental conditions (Rashid *et al.*, 2005). In addition, the availability of P in soil is also influenced and limited by ecological conditions (Turner, 2008), topography (Kitayama *et al.*, 2000) and chronosequence of soil (Huang *et al.*, 2014). Plant takes up P in the form of ion in soil solution; $H_2PO_4^-$ in acidic soil and HPO_4^{2-} in alkaline soil (Brady & Weil, 2002; Schachtman *et al.*, 1998) through the root membrane via Pi transporters availability which can regulate the uptake of P depending on the P concentration in soil (Raghothama, 2000). Furthermore, P in soil can be taken up by plant by removing P from adsorp clay soil surface or element particles (Barrow, 2015). According to Richardson *et al.* (2009), plant can take up the highest P concentration at the surface layer compared to soil depth. This is due to the slow mobility of P at low depth and the limitation of P concentration that strongly bounded with the elements (Amaizah *et al.*, 2012).

In plant, P contained about 0.2% from its biomass (Schachtman *et al.*, 1998), however, deficiencies of P in plant are largely occurred due to unavailable P in the soil solution (Maathuis & Diatloff, 2013). P is unavailable in soil for plant uptake because of P is bound with mineral elements and this process is called fixation (Brady & Weil, 2002). About 90% of P from the additional of P fertilizer into soil tended to be fixed by other soil matrix (Maathuis & Diatloff, 2013). The fixation process is occurred precipitation and specific adsorption at mineral surfaces which is influenced by soil pH and the concentration of ion (Iqbal, 2012). Soil pH is important because it can affect nutrient availability for plant (Huang *et al.*, 2013; McCauley *et al.*, 2009) particularly P and consequently affect plant yield (Cregan & Scott, 1998). High acidity of soil pH can make P become unavailable by the interference from several factors such as; 1) agricultural activities (acidification in soil due to excessive use of ammonia fertilizer (Kochian *et al.*, 2004)) and 2) environmental factors (acidic rain which commonly occurred in arable soil (Zhang *et al.*, 2014)).

Continuous of P accumulation in soil will cause P to undergo recalcitrant form when P adsorption site of moderate labile pool is reaching maximum capacity. Thus, it will impact P to become unavailable for plant uptake in the future (Darilek *et al.*, 2010). However, if P fixation is lower, plant can take up P in recalcitrant form and being able to supply P towards plant in the future (Sattari *et al.*, 2012) by mineralization and/ or mobilization to another pool (Saleque & Kirk, 1995; Saleque *et al.*, 2004) with the influenced of soil pH (McCauley *et al.*, 2009).

If most of P being fixed into soil matrix, plant will undergo P deficiency (Meng *et al.*, 2014). Deficiency of P in plant can be seen by several signs and symptoms such as; 1) older leaves has abnormal discoloration in which leaves turn to dark greenish with purple colour (Brady & Weil, 2002; Hopkins & Hüner, 2009), 2) stunted plant growth, 3) necrotic spots on leaves, 4) leaf malformation, 5) short stem and 6) reduced crop yield (Hopkins & Hüner, 2009).

In order to overcome P deficiency, plant modified its characteristics to improve the efficiency (Richardson *et al.*, 2009) and maximization of P uptake in P deficient soil (Bates & Lynch, 2000). The uptake of P in plant can be improved by; 1) mycorrhizal association (Schachtman *et al.*, 1998), 2) solubilisation of P by certain chemicals from plants exudation (Kirk *et al.*, 1998; Kochian *et al.*, 2004) or soil microbes (Acosta-Martinez *et al.*, 2007; Tarafdar & Claassen, 1988) and 4) structural root modification (Föhse *et al.*, 1988; Kirk *et al.*, 1998; Kochian *et al.*, 2004).

In some cases where plant root is associated with mycorrhiza, this plant will have greater efficiency of obtaining P from P deficient soil. Mycorrhiza is important for P partitioning in plants (Turner, 2008) and about 90% of plant root is infected by mycorrhiza to help plant obtains P while in return, mycorrhiza obtains carbon from plant (Schachtman *et al.*, 1998). The mutualistic symbiosis of roots and mycorrhiza will increase P uptake efficiency together with the changing of root morphology such as branching, volume and root hairs (Kochian *et al.*, 2004; Richardson *et al.*, 2009). Hence, plant can exploit more P in larger soil volume (Richardson *et al.*, 2009). The hyphae of mycorrhiza help to transport inorganic P into root (Schachtman *et al.*, 1998). In addition, mycorrhiza also help to increase the ability of P solubilizing bacteria to convert unavailable P into available P for plant uptake (Cong *et al.*, 2011). Moreover, P uptake by plant through mycorrhiza is found to be more efficient rather than through the assistance of soil microbe (Schachtman *et al.*, 1998).

Other mechanisms that regulated plant P uptake in P deficient soil are by exudation of phosphatase (Craine & Jackson, 2010; Tarafdar & Claassen, 1988) and organic acid (Richardson *et al.*, 2009). Phosphatase and organic acid are two different chemicals produced by plant exudates. Phosphatase is a type of enzyme, while, organic acid is secondary metabolite of plant (Hu *et al.*, 2005). Both chemicals are exuded from root and their function is to solubilise P (Richardson *et al.*, 2009). In addition, soil microbes also can exude both chemicals to solubilise P (further explanation in sub chapter 2.3) (Zhu *et al.*, 2018).

Plant or soil microbe will release phosphatases to hydrolyse P in order to become available P (Tarafdar & Claassen, 1988) through acidification (Acosta-Martinez *et al.*, 2007; Kochian *et al.*, 2004; Wang *et al.*, 2014). Soil order and land management can influence the soil phosphatase activity such as organic matter content, parent material and type of clay (Acosta-Martinez *et al.*, 2007). In the soil with decreasing of available P concentration, soil phosphatase activity will increase in order to increase available P concentration (Zhang *et al.*, 2012). However, phosphatase activity can be reduced when carbon concentration in soil decreased (Acosta-Martinez *et al.*, 2007; Zhang *et al.*, 2012). Moreover, phosphatase activity is influenced by soil pH, while the soil pH is regulated by soil properties such as soil organic matter, soil texture or others (Acosta-Martinez *et al.*, 2007; Liu *et al.*, 2015; Zhang *et al.*, 2012).

Plant also exudes organic acids to solubilize P by the influence of soil acidification (Panhwar *et al.*, 2013; Wang *et al.*, 2014) which almost similar with phosphatase. Generally, the amount of organic acid exuded by plant was around 10-20 mM (Jones, 1998). However, different plant may exudes different types of organic acids, and different P pools are solubilized by different types of organic acids (Richardson *et al.*, 2009). The common organic acids that exuded by plants are citric acid (Kirk *et al.*, 1998), malic acid (Jones, 1998; Kirk *et al.*, 1998) and oxalic acid (Jones, 1998). However, the effectiveness of organic acids in converting unavailable P into available P is differ among plant for P uptake as it depends on the type of plant (Bhattacharyya *et al.*, 2003; Chen *et al.*, 2003). Organic acid exuded by plant also can trigger soil microbes to release their own organic acids (Chen *et al.*, 2003). Organic acid makes P become more available by decreasing the P adsorption sites of ferum (Fe) and aluminium (Al) as these sites are occupied by the organic acids (Jiao *et al.*, 2007). Organic acid also can mobilize adsorbed P from clay minerals (Hu *et al.*, 2005). The reasons of increased available P resulting from exudation of organic acid are; 1) P binding site in soil are reduced by chelation of Al and Fe by organic acids and/or 2) there are competition of P binding site in Al and Fe between organic acid and P solution ($H_2PO_4^-/HPO_4^{2-}$) at soil surface (Jiao *et al.*, 2007). Organic acids have the same affinity of P and can cause site exchange at mineral surfaces (Al and Fe) between P and organic acids. Hence, P become more freely and available (Guppy *et al.*, 2005; Lee *et al.*, 2004; Park *et al.*, 2004).

The other mechanism for plant to uptake P in P deficient soil is by morphological change for example increasing the number of root hairs (Bates & Lynch, 2000, 2001). Root hairs can take up about 90% of total P in low P soil (Föhse *et al.*, 1991). Increasing root length, root surface area and root volume are among of morphological traits exhibited by plant in accessing P from P deficient soil (Meng *et al.*, 2014). Moreover, the root shape also can increase the efficiency of nutrient uptake especially under evocative structure (Comte *et al.*, 2013). Due to the changes in root morphology, root to shoot ratio will increase (Guertal & Howe, 2013; Kirk *et al.*, 1998) as the biomass of shoot decreases (Guertal & Howe, 2013). In paddy plant, root biomass was increased even the total paddy biomass was decreased in low soil P (Kirk *et al.*, 1998) due to more focus was directed on the P uptake by the roots.

2.2 P in Soil

P pool is the category of P that was classified according to its availability for plant to uptake. The changes of P pools in soil are influenced by plant type, soil orders, soil properties (pH and soil texture), climatic condition and land management practices (application fertilizer) (Chen *et al.*, 2003; Lee *et al.*, 2007a; Tiessen *et al.*, 1984; Zhang & MacKenzie, 1997; Zubillaga & Giuffré, 1999). According to Parent *et al.* (2014), P cycle in soil is mainly influenced by soil pH. Soil pH is affected by hydroxide, silicate and carbonate in wetlands soil (Xu *et al.*, 2012) typically paddy soil.

Inorganic P (Pi) is fixed in soil by forming an aggregate or complex with organic matter, clays, sesquioxides and/or other elements (Richardson *et al.*, 2009). In alkaline soil, Pi tends to fix with calcium, Ca (apatite) while in acidic soil, Pi tend to fix with Al (variscite), Fe (strengite), clay and silicate (Lee *et al.*, 2007a; Lee *et al.*, 2007b; Park *et al.*, 2004). Pi in soil solution will increase when the organic P (Po) is mineralized by soil microbial activity and P cycling pool becomes active (Perrott & Mansell, 1989). The availability of Pi is depending on the rate of mineralization of Po from soil microbial activity (Kitayama *et al.*, 2000). According to Damon *et al.* (2014), the intensity of Po mineralization into Pi depends largely on soil microbial biomass and soil pH.

In addition, Po can be taken up by plant through manipulation of several plant mechanisms as being mentioned in previous literatures (Adams & Pate, 1992; Audette *et al.*, 2016; Tarafdar & Claassen, 1988). Total P in soil contains about 30-80% of Po (Richardson *et al.*, 2009) and in agricultural soil, Po is in labile and moderate labile pools accounted 5-52% of total P (Dodd & Sharpley, 2015). Po is important in soil but is less favoured by plant as compared to P availability (Guo *et al.*, 2000). Organic sources such as; 1) organic N fertilizer (Shafqat & Pierzynski, 2010), 2) plant residue, manure (Brady & Weil, 2002), 3) soil organic matter (McCauley *et al.*, 2009) and 4) clay surfaces interference (Condron *et al.*, 2005) contribute to the accumulation of Po in soil by soil microbial activity (Damon *et al.*, 2014). Moreover, Po can be immobilized by soil microbial activity and abiotic stabilize P (by fixing with Fe, Al, hydroxide, organic matter or precipitation with cations) (Dodd & Sharpley, 2015). Several types of Po that can be found in soil are phosphoinositide, nucleotides and recalcitrant humic substances decomposed from organic molecules (Hedley *et al.*, 1982; Reddy *et al.*, 1999; Tiessen & Moir, 1993). However, Po in agricultural soil usually in the form of orthophosphate monoesters and diesters (Condron *et al.*, 2005). In acidic soil, Po consists of DNA, inositol hexakisphosphate and phosphonates (Turner & Blackwell, 2013). According to Gerke (2015), the most dominant Po in soil is phytate which is slow decomposable Po. Nevertheless, based on the literature above, type of Po and amount of Po in soil are different due to different soil properties. The advantage of Po is reducing soil degradation through binding with micro-aggregate soil particles occluded with macro-aggregate soil particle (Dodd & Sharpley, 2015).

2.3 Soil Microbial P

Soil microbes are important as it can act as source or sink of nutrients for soil fertility (Aulakh & Pasricha, 1991; Damon *et al.*, 2014; Kouno *et al.*, 1995). Microbial P in soil is able to mineralize Po into available P for plant, accumulating P in P pools by immobilization and decomposition of organic matter (Damon *et al.*, 2014; Huang *et al.*, 2013). In a meantime, soil microbial P also can benefit from the immobilized P and utilized P for themselves, hence, reduce the availability of P towards plant (Dijkstra *et al.*, 2015). This is because soil microbial activity is depended on the soil nutrient condition (Dai *et al.*, 2017; He *et al.*, 2008; Seeling & Zasoski, 1993). Moreover, soil microbial P has similar mechanisms with plant such as solubilizing, mineralizing and mobilizing P by releasing phosphatase (Acosta-Martinez *et al.*, 2007; Liu *et al.*, 2015; Zhang *et al.*, 2012) and releasing organic acids (Panhwar *et al.*, 2013). Hence, the beneficial effect of soil microbial P for supplying P to the plant can be seen for a long-term (Ilstedt *et al.*, 2003) and small population size of soil microbes can affect the availability of P (Jonasson *et al.*, 1999). Soil microbial P also can act as biological indicator in determining the soil quality through its activities (Lima *et al.*, 2013) and their community (Ge *et al.*, 2012).

Microbial activities in soil is commonly influenced by; 1) soil pH, 2) amount of P in soil, 3) soil texture, 4) C:N ratio in soil, 5) elements in soil (He *et al.*, 2008; Kuramae *et al.*, 2012; Ma *et al.*, 2016), 6) soil organic carbon (He *et al.*, 2008; Liu *et al.*, 2012) and 5) total nitrogen concentration in soil (Bhattacharyya *et al.*, 2015; Birkhofer *et al.*, 2008; Ma *et al.*, 2016). However among these factors, P concentration in soil is the main factor influencing microbial community (He *et al.*, 2008; Kuramae *et al.*, 2012; Ma *et al.*, 2016). The concentration and forms of microbial P is influenced by P availability, soil microbial growth stage and microbial community (Bünemann *et al.*, 2011). Microorganisms in soil especially fungal are more responsive towards P in soil compared to bacteria (Kuramae *et al.*, 2012). In humid tropical forest, amount of P usually limits the microbial community due to highly weathered soils (Gnankambary *et al.*, 2008).

Soil microbial activity increased under application of inorganic and organic fertilizer due to the increased of N availability (Zhou *et al.*, 2010) and organic matter (Audette *et al.*, 2016). Moreover, available N and organic matter are the most important factor that increase the microbial biomass (Ma *et al.*, 2016).

Po is mineralized into Pi by soil microbial activity through decomposition of organic matter to obtain carbon (Spohn & Kuzyakov, 2013) due to the fact that carbon can influence the microbial activity (Guppy *et al.*, 2005).

2.4 Excessive P in Soil and its Disadvantages

Excessive P in soil is will lead to the environmental problem such as land degradation and eutrophication in water system (Brady & Weil, 2002). Eutrophication makes water becomes hazardous to organisms (Sharpley & Beegle, 2001) due to the toxicity of algal bloom and reduced the water quality (Lee *et al.*, 2007a). There are two factors contributing to eutrophication which are point inputs (directly) and non-point inputs (indirectly) (Carpenter, 2005). A non-point input is characterized as P losses from agricultural soil (Dodd & Sharpley, 2015; Liang *et al.*, 2016; Zhang *et al.*, 2003) especially in paddy field (Park *et al.*, 2016). The pathways for non-point inputs are 1) surface runoff (Zhang *et al.*, 2003) by soil erosion due to high water runoff in granular form (Quynh *et al.*, 2005) and/ or 2) through soil crack and/or biopores of percolating water (Huang *et al.*, 2013). In general, P distribution and loss are affected by weather conditions, history of land management, soil properties and slope position (Laio *et al.*, 2001; Negassa & Leinweber, 2009; Zhang *et al.*, 2003). In North America and Europe, the soil residual P can reduce water quality of the aquatic ecosystem by surface runoff and leaching to water bodies (Sharpley *et al.*, 2014). In order to reduce P leaching, proper management of fertilizer application such as the dosage reduction should be applied (Zhang *et al.*, 2003), for instance, fertilizer application onto agricultural soil was halted for several years (Bhattacharyya *et al.*, 2015; Wang *et al.*, 2014) to make unavailable P in soil to become available for the future crop (Sattari *et al.*, 2012).

P leaching is affected by soil microbial activity (Dodd & Sharpley, 2015) and soil texture (Li *et al.*, 2016). The abundance of microbial communities in soil enhance Po mineralization which lead to the P leaching (Dodd & Sharpley, 2015). A research study by Li *et al.* (2016) had showed that soil with high texture of small soil aggregate have potential to runoff and cause soil erosion. Similarly, a research conducted by Maguire *et al.* (2002) had reported that small soil aggregate can runoff easily compared to larger soil aggregates, and small soil aggregate has high P buffering capacity to bind P. In addition, larger soil aggregates had low risk of runoff due to its physical properties such as low water holding capacity, deeper infiltration level and reduced loss by evaporation (Austin *et al.*, 2004).

Different P pools are one of the factors that lead to the P losses. Less or non-labile pool is rarely contributed to eutrophication in water bodies but it can be mobilized into labile pool (Irshad *et al.*, 2008). The losses of P concentration from moderate labile pool might be due to the run off of colloidal matter through pass flows (Negassa & Leinweber, 2009) or deposited in sediments (Darilek *et al.*, 2010). In paddy field, the dominant P loss in paddy soil is caused by runoff of particulate P (Zhang *et al.*, 2003).

2.5 P Pools in Soil

Soil P pools and the accumulation of P are influenced by nutrients availability (Malhi *et al.*, 2011), soil physico-chemical properties and climatic condition (Magid & Nielsen, 1992). Forms of P in soil is affected by parent materials, soil pH, pedogenesis and vegetation cover (Spohn & Kuzyakov, 2013).

Since P has many forms and able to fix with several elements, a proper method is needed to precisely determine P pools in soil. To date, sequential P fractionation method is the most reliable method to elucidate P status and changes (Gikonyo *et al.*, 2008). According to Roy *et al.* (2016), P fractionation was used to elucidate long term of P fixation in agricultural tropical soil that related to sustainable P management (Roy *et al.*, 2016).

Several methods are developed to determine various fractions of P as proposed by Chang and Jackson (1957), Bowman and Cole (1978), Hedley *et al.* (1982) and Tiessen and Moir (1993). The sequential P fractionation was proposed by Hedley *et al.* (1982) in order to determine P pools in soil based on its lability (Levy & Schlesinger, 1999; Tiessen & Moir, 1993). According to Saleque *et al.* (2004) and Quintero *et al.* (2007), this method is the proper method for evaluating P pools in paddy soil. According to Tiessen and Moir (1993), Hedley P fractionation method underestimated the organic P concentration because the persulfate digestion in various extractions was not analyzed.

P fractionation method can access the concentration of Pi and Po in soil and their lability. However, those methods are developed differently due to different soil P conditions and different extractants being used. In this study, sequential fractionation by Tiessen and Moir (1993) was employed by using different extractants (from mild to strong extractants) based on assumption on the lability degree of each P pool (Hedley *et al.*, 1982). Sodium bicarbonate ($NaHCO_3$) extractant is used to extract P as the most labile P for plant uptake (Xu *et al.*, 2012). $NaHCO_3$ extractable Pi is known as the labile P which can be directly taken up by plant (Hedley *et al.*, 1982; Tiessen & Moir, 1993) and Po of $NaHCO_3$ is also known as labile Po that can easily mineralized into available P (Bowman & Cole, 1978;

Seeling & Zasoski, 1993). Sodium hydroxide (NaOH) extractable P is regarded as moderately labile pool which Pi is fixed with Al and Fe while Po is fixed with fulvic and humic acid (Tiessen & Moir, 1993). Hydrochloric acid (HCl) extractable P is known as less labile pool and P in this pool is commonly fixed with Ca (Tiessen & Moir, 1993). The remaining P determined after the extraction of the above extractants is considered as recalcitrant P after acid digestion (Bowman & Cole, 1978) and they are usually dominant in Po form (Levy & Schlesinger, 1999). The smallest P pool is contributed by microbial pool which generally has the lowest percentage of P fractions (Guo *et al.*, 2000).

The determination of available P in different soil types varies according to different methods (Quintero *et al.*, 2007). The most common methods used for available P method are Mehlich-1, Bray-1 (Raij *et al.*, 2009), Mehlich-3, Bray-2, Resin, Olsen, $CaCl_2$, Fe-oxide Pi, calcium acetate lactate (CAL), Dithionite, 0.5M HCl, Oxalate, LiCl and water (Wünscher *et al.*, 2013). However, most of the extractant used for each method in determining available P coincidently extracts moderate or less labile P (Raij *et al.*, 2009). In this study, Resin-P method was chosen to determine available P. This method was proposed by Kouno *et al.* (1995) and according to Raij *et al.* (2009), this method is the most reliable method to determine available P. This method does not change the chemical formation in soil solution (Xu *et al.*, 2012). However, according to Wünscher *et al.* (2013), proper method to determine available P was Mehlich-3 but Resin-P was the best predictor to determine the amount of P uptake in their research. The factors that might differentiate their findings might be due to soil pH, soil minerals, soil texture and soil P adsorption capacity.

2.6 Paddy: Plant and Soil

2.6.1 Morphology and Characteristics of Paddy Plant

Oryza sativa. L or locally known as paddy is a tribe of Oryzae in grass family (Poaceae/Gramineae) which has 24 chromosomes and diploid species (Bardenas & Chang, 1965). Paddy is an annual grass (Moldenhauer & Slaton, 2001) with many varieties based on the plant characteristics and traits (Bardenas & Chang, 1965). According to Yang *et al.* (2012), the variety of paddy is depending on the number of organelles like mitochondria, golgi apparatus and amyloplasts. Paddy variety is varied among the other countries because of the environmental growth conditions such as; 1) soil fertility, 2) climate, 3) planting method, 4) topography, 5) meteorological condition and 6) paddy life cycle (Bardenas & Chang, 1965).

The common morphological characteristics of paddy plant are the culm is round, hollow and jointed while the leaf blade is narrow, flat and sessile, which connected to leaf sheath with collars. In addition, paddy plant was characterized by sickled-shaped, hairy auricles and terminal panicles (Moldenhauer & Slaton, 2001). The shape of the seed is polyhedral and densely packed (Chungcharoen *et al.*, 2015). Paddy plant has three different stages to complete its life cycle; 1) vegetative stage, 2) reproduction stage and 3) grain filling with maturation stage, however, the life cycle of paddy largely depends to the environmental factor and paddy varieties (Moldenhauer & Slaton, 2001).

There are several factors in determining the yield quality of paddy (Datta & Datta, 2006) such as water content in soil, salinity (Batlang *et al.*, 2013), climate (Cong *et al.*, 2011) and temperature (Moldenhauer & Slaton, 2001). Drought and salinity can reduce paddy yield due to paddy physiological and biochemical were affected (Batlang *et al.*, 2013). In addition, climatic changes between two seasons of paddy can affect the yield as well. For example, a study by Cong *et al.* (2011) showed that the production of paddy grain was different despite the amount of fertilizer applied was similar for both seasons due to the climatic changed. Similarly research conducted by Yu *et al.* (2001) had found a significant correlation between climatic factors and yield variance. Besides, temperature is among the important factors needed for paddy growth (Moldenhauer & Slaton, 2001). However, each stage of paddy development has specific requirement of different optimum temperature (Moldenhauer & Slaton, 2001). Paddy usually obtains nutrient in a dissolved forms which the nutrient availability is affected by water management and fertilizer input (Anda & Subardja, 2013).

2.6.2 Characteristics of Paddy Soil

Paddy ecosystem is the biggest artificial wetland ecosystem and management (Guo *et al.*, 2015). However, the properties of paddy soil changed from time to time by soil formation due to the flooded condition (Kögel-Knabner *et al.*, 2010). According to Kyuma (2004), paddy soil defined as ‘a soil used or potentially usable for growing aquatic rice’. Paddy soil is classified as artificial soil due to modification and proper management to suit with paddy cultivation condition (Prakongkep *et al.*, 2008). Paddy soil had undergoes an array of mechanical activities for soil formation such as flooding, leaching, oxides formation with redistribution and accumulation of organic matter at topsoil (Luster *et al.*, 2014). However, biogeochemistry of paddy soil is influenced by soil properties such as pH, redox potential, organic matter solubility and organic matter degradation (Kögel-Knabner *et al.*, 2010). Paddy soil contains high organic matter due to slow rate of organic matter decomposition and most of the time it is in anaerobic/flooded condition (Kögel-Knabner *et al.*, 2010). Besides, high soil microbial activity in paddy soil indicated that the soil is fertile

(Liu *et al.*, 2015). In order to create new paddy field in a specific area, topography and soil conditions are the important criteria to be evaluated for better paddy soil management (Anda & Subardja, 2013).

The properties of paddy soil are depending on the soil genesis and land management practices. In soil genesis, paddy soils are varied based on the disparity of the soils' morphological, chemical and thermal reaction status that can give specific information about paddy cultivation (Tan, 1968). Research done by Prakongkep *et al.* (2008) on Thailand paddy soil found that parent material had shown an influence effect on the texture of paddy soil. It was shown that the clay concentration in paddy soil had increased corresponding to the soil depth (Prakongkep *et al.*, 2008).

Furthermore, water and fertilizer are important factors for land management practices in paddy soil. Paddy soil has strong relationship with hydrological activity because water can change paddy soil characteristics in term of soil physical properties and soil microbial activity in long run (Luster *et al.*, 2014). Moreover, water management is important for paddy growth and physiology but it is also depends on the paddy variety (Chu *et al.*, 2014). According to Kato *et al.* (2016), water management has more influence on the nutrients in paddy soil than fertilizer application. In addition, high soil water content had assisted for an efficient P diffusion mechanisms to occurs rapidly in rhizosphere soil (Huang *et al.*, 2013). Therefore, it is important for maintaining the root activity and confers a positive growth to paddy (Yang *et al.*, 2012). However, continuous flood condition had alleviated the capacity of paddy to uptake nutrient due to unfavourable physico-chemical environment and Fe toxicity towards paddy root (Yang *et al.*, 2004).

Hence, the evaluation of paddy soil properties is needed from time to time to ensure the status of soil fertility and toxicity is in balanced condition.

2.6.3 Role of P in Paddy

Malaysian soil is largely categorized under the order of Ultisol and Oxisols in which the soil texture in most places are in the form of fine clay and clay loam (Chee & Peng, 2006). It is expected that the source of labile P in soil comes from Po and soil P transformation resulting from land management activities, different soil type and climate changes (Tiessen *et al.*, 1984). The changes of labile and moderate labile into less or non-labile P pools is fast in Ultisol and Oxisols because of high temperature, acidity and variability of charge in pedogenic oxides (Negassa & Leinweber, 2009) occurred within the regions. Furthermore, in terrestrial land especially paddy soil, soil P is strongly influenced by pedogenesis and weathering of parent material (Huang *et al.*, 2013).

The solubility of P in flooded soil is influenced by pH, organic matter, time and temperature (Quintero *et al.*, 2007; Scalenghe *et al.*, 2002). Land management practices is generally a decisive factor in controlling the fluctuation changes of soil P in paddy soil (Huang *et al.*, 2013). For example, Darilek *et al.* (2011) suggested that the changes in P pools are significant in flooded condition compared to aerobic condition. However, two soil orders; Ultisols and Oxisols which contain high Al^{3+} and Fe^{3+} with strong acidic reaction are frequently reported as P deficient soil (Saleque *et al.*, 2004). Fe-P is the most dominant P pools in wetland soil (Irshad *et al.*, 2008) especially in paddy soil. Most of P depleted in paddy soil is due to improper land management such as the cropping intensity, amount of fertilizer application and the employment of high yielding paddy varieties (Ali *et al.*, 1997). However, an improper management of fertilizer application also resulted in soil P accumulation in which over the time become unavailable for plant (Irshad *et al.*, 2008) due to high P fixation rate has occured (Maathuis & Diatloff, 2013). Fertilization of paddy soil only increase Pi rather than Po (Wang *et al.*, 2015) and these P being fixed simultaneously after the fertilizer application. In a worst case, the residue from the fertilizer can lead to the pollution in paddy soil due to the improper fertilizer management (Aishah *et al.*, 2010), particularly if the paddy soil is initially low in P adsorption which high P solution (Jalali & Matin, 2013). In addition, in long term older paddy soil has low capacity to receive P absorption due to reducing of P sorbent (Huang *et al.*, 2014) as well as low clay content (Jalali & Matin, 2013) due to particulate P runoff (Zhang *et al.*, 2003) or leaching (Li *et al.*, 2015b).

P is important to increase the yield of rice (Bhattacharyya *et al.*, 2015). Shen *et al.* (2004) suggested that P is the most second limiting factor after N for paddy growth and yield. The significant role of proper land management practices, optimum fertilizer application and soil fertility management are needed to obtain high yield and quality of rice with sustainable crop production (Shen *et al.*, 2004).

Usually, paddy takes up P in flooded condition at rhizosphere soil by root acidification to solubilize P and diffuse it into the root (Kirk *et al.*, 1998; Saleque & Kirk, 1995). In addition, acidity of soil pH is slightly increased due to P solubilisation at weak acid extractable P pool (Audette *et al.*, 2016). However, according to Kirk *et al.* (1998) the flooded condition was not the main factor that influence the availability of P as different soil types has different capacity in P adsorption. Under P deficiency paddy soil, P will be allocated economically in each plant parts due to P limitation (Amanullah, 2016). While, in response to the soil P deficiency in flooded condition, plant had undergo root and shoot modification by increasing root to shoot ratio and increasing shoot to total P ratio (Saleque & Kirk, 1995). The root also exuded H^+ from roots for acidification (Kirk *et al.*, 1998; Saleque & Kirk, 1995) and maximized root growth (Shao *et al.*, 2006). However, different genotypes of paddy exhibited different mechanisms (Kato *et al.*, 2016) and efficiency (Shen *et al.*, 2004) on P uptake. The association of mycorrhizal in flooded paddy cultivation has not been fully elucidated since the literature and research that had been conducted showed different interpretation and results (Huguenin-Elie *et al.*, 2003; Kirk *et al.*, 1998; Vallino *et al.*, 2014; Zhang *et al.*, 2016).

Flooded condition makes P becomes available (Elzenga & van Veen, 2010; Lee *et al.*, 2013) by iron oxides reduction dissolution (Fe^{3+} to Fe^{2+}) (Kirk *et al.*, 1998; Quintero *et al.*, 2007; Zhang *et al.*, 2006) and hence increased P diffusion (Zhang *et al.*, 2006) to the plant. Furthermore, according to Kato *et al.* (2016), flooded condition also caused P become available due to the mobilization in moderately labile and/or less or non-labile pool to labile pool.

2.7 Role of Fertilizer and the Influence of Fertilizer towards Soil and Plant

In arable or plantation soil, addition of substances such as fertilizer is important to supply adequate P for plant. Many studies have shown that additional of fertilizer can change P dynamic and total P in soil throughout time (Bhattacharyya *et al.*, 2015; Lee *et al.*, 2004; Perrott & Mansell, 1989; Zhang *et al.*, 2006). The addition of P fertilizer give various impacts to the elements in soil (Dang *et al.*, 2016) and the availability of P affecting the chemistry and mineralogy of the soil P sorption capacity (Damon *et al.*, 2014). P sorption capacity in soil is important if the soil has high P sorption capacity as the P in soil will bound to soil matrix, hence, P is unavailable for plant uptake (Huang *et al.*, 2014). High rate of fertilizer application will contribute to P losses by runoff especially soil containing high clay and organic matter (Zhang *et al.*, 2003).

The application of inorganic fertilizer gives deleterious impacts towards physical and chemical characters of soil. For example, the efficiency of P fertilizer applied into soil was decreasing over the time as P sorption site in soil matrix decreased (Barrow, 2015; Huang *et al.*, 2013; Simpson *et al.*, 2015). This was due to the physical and chemical damage occur at soil materials (Huang *et al.*, 2013). Moreover, soil stability also decreased in prolong application of inorganic fertilizer (Zhou *et al.*, 2016). In addition, inorganic fertilizer only makes soil pH to become more acidic (Ann, 2012) due to acidification by minerals fertilizer (Birkhofer *et al.*, 2008) containing organic N and ammonia (Iqbal, 2012). Hence, prolong application of inorganic fertilizer is not preferred in order to maintain the level of P pools (Mitran *et al.*, 2016).

An alternative solution to overcome the problem arising from inorganic fertilizer is by using an organic source. According to Bhattacharyya *et al.* (2015), P in soil can sustainably supply P for paddy consumption as the organic matter decomposition rate are low and organic sources can supply carbon and other nutrients. The accumulation of P from organic source is derived from mineralization of C (Damon *et al.*, 2014). Soil physical quality can be improved by using organic source (Zhou *et al.*, 2016). The organic source like manure can affect the nutrient stoichiometry in soil (Ma *et al.*, 2016), thus, making P more available and reduce in P adsorption (Bhattacharyya *et al.*, 2015; Guppy *et al.*, 2005; Li *et al.*, 2015a; Shafqat & Pierzynski, 2010) due to increasing of soil pH in acidic soil (Lee *et al.*, 2007a). In addition, according to Guppy *et al.* (2005), P sorption was delayed at the surface at minerals. Organic source application can increase negative charge of soil surface as compared to inorganic fertilizer or combination fertilizer application (Jiao *et al.*, 2007) which consequently increased labile P in paddy soil. Saleque *et al.*

(2004) also suggested that organic manure could substitute chemical fertilizer due to the P concentration in labile pool was higher than chemical fertilizer. Moreover, organic source can increase organic matter (Park *et al.*, 2004) and maintain the amount of organic matter through the time (Ann, 2012). In plant structural changes, the additional of organic source especially organic matter can alter root morphology and characteristics and consequently improving root activity (Yang *et al.*, 2004). However, the application of organic source only could not increase P availability for plant uptake directly (Bah *et al.*, 2006) and not enough for obtaining high crop yield because the availability of nutrient in organic source is low and need to be mineralize (Ann, 2012). In addition, excessive organic source (manure amendment) can increase the accumulation of P by immobilization in soil considerably in soil with high clay concentration (Ma *et al.*, 2016).

Hence, the integrated fertilizer management is needed to assist plant to obtain sufficient nutrient and sustain environmental condition. The combination of organic source and inorganic fertilizer can improve soil fertility (Bhattacharyya *et al.*, 2015; Huang *et al.*, 2014; Zhou *et al.*, 2016) and increased paddy yield (Bhattacharyya *et al.*, 2015). Plus, the combination of fertilizers can decrease P sorption and can supply high P to paddy crops (Bhattacharyya *et al.*, 2015). For example, total P in soil is highly increased with the combination of fertilizer application as compared to sole application of inorganic fertilizer (Lee *et al.*, 2004; Li *et al.*, 2015a). Pi concentration also increased due to decomposition of organic substance and mineralization of Po (Lee *et al.*, 2004).