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Department of Environmental Science
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**Determination of the relationship between organic carbon and
environmental factors in agricultural Saraburi soils
by comparative analysis of methods**


การหาความสัมพันธ์ระหว่างคาร์บอนอินทรีย์และปัจจัยทางสิ่งแวดล้อมในดินเกษตรจังหวัดสระบุรี
โดยการเปรียบเทียบวิธีการวิเคราะห์ที่แตกต่างกัน

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Project Title **Determination of the relationship between organic carbon and environmental factors in agricultural Saraburi soils by comparative analysis of methods**
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Accepted by the Department of Environmental Science, Faculty of Science, Chulalongkorn University in Partial Fulfilment of the Requirements for the Bachelor's Degree


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จังหวัดสระบุรี โดยการเปรียบเทียบวิธีการวิเคราะห์ที่แตกต่างกัน

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บทคัดย่อ

ปริมาณคาร์บอนอินทรีย์ในดินมีความสำคัญต่อคุณภาพและกระบวนการต่าง ๆ ในดินรวมถึงการสะสมคาร์บอนที่ส่งผลกระทบต่อ การเปลี่ยนแปลงสภาพภูมิอากาศ โดยทั่วไปการวัดปริมาณคาร์บอนอินทรีย์ในดินจะวัดโดยวิธีการเผาไหม้แบบแห้งด้วยเครื่องวิเคราะห์อัตโนมัติหรือวิธีการออกซิเดชันทางเคมีแบบเปียก เช่น วิธีของ Walkley & Black การวิเคราะห์ด้วยวิธีการเผาไหม้แบบแห้งเป็นวิธีที่มีความละเอียดและไม่ซับซ้อน แต่มีค่าใช้จ่ายสูง ในขณะที่วิธีของ Walkley & Black มีค่าใช้จ่ายต่ำกว่าวิธีการเผาไหม้แบบแห้ง แต่จำเป็นต้องมี Correction factor ในการปรับแก้ เนื่องจากวิธี Walkley & Black นั้นวิเคราะห์ปริมาณคาร์บอนอินทรีย์ได้ต่ำกว่าความเป็นจริง อย่างไรก็ตามค่า Correction Factor มีความหลากหลายตามพื้นที่ศึกษาขึ้นอยู่กับชนิดของดิน การจัดการดิน และปัจจัยทางสิ่งแวดล้อม งานวิจัยนี้จึงมีวัตถุประสงค์เพื่อ 1) ตรวจสอบปริมาณคาร์บอนอินทรีย์ในดินและหาความสัมพันธ์ระหว่างวิธีของ Walkley & Black และวิธีการเผาไหม้แบบแห้ง 2) ศึกษาความสัมพันธ์ระหว่างคาร์บอนอินทรีย์ที่ได้รับจากวิธี Walkley & Black และวิธีการเผาไหม้แบบแห้ง และปัจจัยทางสิ่งแวดล้อม ได้แก่ ค่าความเป็นกรดต่าง ค่าการนำไฟฟ้า ปริมาณไนโตรเจน และฟอสฟอรัสที่เป็นประโยชน์ในดินเกษตรจากจังหวัดสระบุรี การศึกษานี้ได้ทำการเก็บตัวอย่างดิน 30 จุดจากพื้นที่เกษตรกรรมในจังหวัดสระบุรี การวิเคราะห์คาร์บอนอินทรีย์ในดินด้วย 2 วิธี คือ วิธีของ Walkley & Black และวิธีการเผาไหม้แบบแห้ง ผลจากการศึกษาพบว่าปริมาณคาร์บอนอินทรีย์ที่วิเคราะห์โดยวิธีของ Walkley & Black มีค่าเฉลี่ย คือ $1.55 \pm 0.51\%$ ในขณะที่การวิเคราะห์โดยการเผาไหม้แบบแห้ง ให้ค่าเฉลี่ยคาร์บอนอินทรีย์ คือ $2.03 \pm 1.08\%$ อัตราการฟื้นฟูปริมาณคาร์บอนอินทรีย์ในวิธี Walkley & Black ต่อวิธีการเผาไหม้แบบแห้งมีค่าแตกต่างกันระหว่าง 44.4% ถึง 114.6% ดังนั้นค่า Correction Factor จึงมีค่าอยู่ในช่วง 0.87 ถึง 2.25 นอกจากนี้ยังพบว่าคาร์บอนอินทรีย์ที่ได้รับจากวิธี Walkley & Black และวิธีการเผาไหม้แบบแห้งมีความสัมพันธ์กันสูงอย่างมีนัยสำคัญ ซึ่งมีค่า r^2 เท่ากับ 0.675 ($p < 0.001$) ปริมาณคาร์บอนอินทรีย์ในดินมีความสัมพันธ์ทางบวกกับค่าความเป็นกรดต่างและค่าการนำไฟฟ้าอย่างมีนัยสำคัญ แต่มีความสัมพันธ์กับปริมาณฟอสฟอรัสที่เป็นประโยชน์ในดินในทางตรงกันข้าม การศึกษานี้ได้มีการเสนอสมการถดถอยเชิงเส้นหลายตัวแปรเพื่อทำนายปริมาณคาร์บอนอินทรีย์ที่ได้รับจากการวิเคราะห์ด้วยวิธี Walkley & Black และวิธีการเผาไหม้แบบแห้งจากค่าความเป็นกรดต่าง ค่าการนำไฟฟ้า และปริมาณฟอสฟอรัสที่เป็นประโยชน์ในดินเกษตรในจังหวัดสระบุรี ผลจากการศึกษานี้ช่วยเป็นข้อมูลสำหรับเลือกวิธีในการประเมินคาร์บอนอินทรีย์เพื่อหาปริมาณคาร์บอนอินทรีย์ที่แม่นยำ และหาค่า Correction factor ที่เหมาะสมและเฉพาะเจาะจงสำหรับดินจังหวัดสระบุรี นอกจากนี้สมการความสัมพันธ์ระหว่างปริมาณคาร์บอนอินทรีย์และค่าความเป็นกรดต่าง ค่าการนำไฟฟ้า และปริมาณฟอสฟอรัสที่เป็นประโยชน์ในดินเกษตรในจังหวัดสระบุรีสามารถเป็นทางเลือกในการทำนายค่าปริมาณคาร์บอนอินทรีย์ในดินได้

คำสำคัญ : คาร์บอนอินทรีย์ในดิน วิธีของ Walkley & Black วิธีการเผาไหม้แบบแห้ง Correction Factor อัตราการฟื้นฟูปัจจัยทางสิ่งแวดล้อม

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Abstract

Soil organic carbon content was important for soil properties, and processes include the accumulation of carbon affected by climate change. SOC was commonly measured by dry combustion with automated analyzers or a wet chemical oxidation method such as Walkley & Black method. The dry combustion technique was accurate and uncomplicated, but the cost was high. On the other hand, the Walkley & Black method was less expensive than dry combustion but needed a correction factor because of its underestimation of SOC content. However, correction factors had variability across study areas depend on soil type, management practices, and environmental parameters. This study aims to i) measure SOC stocks and investigate the associations between Walkley & Black and dry combustion methods ii) determine the relationship between SOC derived by both Walkley & Black and the dry combustion method and environmental factors such as pH, electrical conductivity (EC), nitrogen (N), and available phosphorus (P) in the agricultural soil of Saraburi. To compared different methods, we used 30 soil samples from the cropland area in Saraburi Province. The techniques used to measure SOC were the Walkley & Black and dry combustion method. The results showed mean \pm SD concentration values were 1.55 \pm 0.51% for the Walkley & Black method and 2.03 \pm 1.08% for the dry combustion technique. The percentage recovery (R) of SOC by the Walkley & Black method to the dry combustion technique varied between 44.4% to 114.6%. The correction factor (100/R) ranged between 0.87 and 2.25. In addition, there was a strong linear relationship for SOC content between the Walkley & Black method and dry combustion technique ($r^2 = 0.675$, $p < 0.001$). The SOC exerted a significantly positive effective effect on the pH and EC value, but negative relationship on available P content ($p < 0.01$). The multiple regression equations were presented to predict SOC analyzed by Walkley & Black method and dry combustion technique from pH, EC, and available P values in agricultural Saraburi soils. The results of this study indicated that the choice of assessment methodology is a critical decision for the suitable quantification of SOC content. This work has established equations between SOC content and pH, EC, and available P values in agricultural Saraburi soil for the SOC estimation.

Keywords: Soil Organic Carbon, Walkley & Black, Dry combustion, Correction factor, Recovery rate, Environmental factors

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CHAPTER 1

INTRODUCTION

1.1 Background and Significance

Of the soil carbon pool, soil organic carbon (SOC) stock constitutes the largest pool of terrestrial organic carbon. Over 60% of the soil carbon pool is SOC and 40% is soil inorganic carbon. SOC is usually sensitive to environmental conditions, which can be relatively resistant to environmental condition (Qin, 2013). SOC content and quality are key drivers of soil physical, chemical, and biological properties and processes (Fageria, 2012) as well as in predicting feedback of the terrestrial carbon cycle to climate change (Kirschbaum, 2000). Moreover, SOC also plays an important role in ecosystem services (Lal, 2011) and in agronomic yields (Tiessen et al., 1994). Agricultural soils attract great attention as a potential sink for atmospheric carbon dioxide (CO₂) by sequestering SOC (Smith et al., 2000; Pan et al., 2003). The loss of SOC has adverse effects on soil structure and soil fertility, contributing to warming the globe by further adding the atmospheric CO₂ concentration (Lal, 1999; Lützow and Kögel-Knabner, 2009). Agro-ecosystem is a highly sensitive part of the global carbon cycle, providing a large potential for carbon sequestration and presenting a feasible option for mitigating atmospheric CO₂ (Sun et al., 2010).

Numerous methods have been developed to analyze SOC. The method of Walkley & Black (1934) is most frequently used because it is simple and relatively quick (Gillman et al., 1986). The major limitation is that the recalcitrant fraction of organic carbon is not determined, resulting in an underestimation of the total SOC content and consequently a correction factor is needed. The most frequently used correction factor is 1.33. More recently the dry combustion method has been adopted. This method is based on thermal oxidation of the sample at a temperature of ~1,200 °C and determining the CO₂ produced by gas chromatography or infrared analysis (LECO, 1996). The method is considered the most accurate method to determine total carbon. However, several studies have shown different correction factors depend on soil type, land use, management practices and soil depth. In the majority of the studies, correction factors were obtained from linear regressions (using a regression coefficient without intercept) between the SOC content obtained by the Walkley & Black and the dry combustion method. The correction number derived by two techniques in the agricultural soils within Thailand is still limited. This research gap may be due to high cost of laboratory analysis, limitation of samples, and time consumption. SOC variation is related to the combined action of physical, chemical and biological processes as well as anthropogenic land use patterns (Goovaerts, 1998). Saraburi is one of the major provinces of cropland which is a major land use. Rice paddy and corn are main crops in Saraburi. Considering the large landscape, paddy soils play an important role in mitigation of atmospheric CO₂. Changes in the size of cropland soil carbon pool could significantly impact carbon budget in a macroscale. However, the sequestration of SOC response to different types of crops is limited because of large variations in crop productivity, crop residue properties and the addition of organic amendments (Chen, 2016). Enrichment of soil with organic carbon is more important in tropical and subtropical regions where soil is inherently low in organic carbon content and production system is fragile (Sharma et al. 2005).

Cropland SOC is correlated with both environmental factors and management practices. Spatial variability of soil chemical properties such as pH, electrical conductivity (EC), SOC, available phosphorus (P) and nitrogen (N) have been studied by different researchers in different soils under different management systems across the world (Li et al., 2011). Soil pH is an important property of soils determining the availability and toxicity of nutrients. It contributes to improve soil properties and helps in enhancing exchangeable cations

in soil thereby reducing the adverse effect of soil acidity (Karlton et al., 2013). In addition, N and P are the most important nutrients for productivity; thus, chemical N and P fertilizers are commonly applied in the fields to achieve a high yield. In some areas, the application of N in excess of crop requirements has induced soil acidification of cropland. The soil environmental quality has been seriously damaged due to significant decrease in pH. Although studies have been conducted on N fertilization under different cropping systems, their impact on SOC and N is not clearly understood (Biederbeck et al., 1996; Jantalia and Halvorson, 2011).

The goal of this study is aimed to provide a correction factor obtained by correlating SOC quantification from the Walkley & Black method to the dry combustion method. In the similar ecological condition where some resources for SOC analysis may act as constraints, this correlation number is anticipated to easily be applied. Furthermore, to have a better understanding of which environmental factors influence on the SOC content, this research emphasizes the association between SOC content with environmental covariates i.e., pH, EC, N, and P. According to the results, we could plan to increase SOC content and promote carbon sink in Saraburi agricultural areas.

1.2 Objectives

1. To measure SOC stocks and investigate the associations between Walkley & Black and dry combustion methods.
2. To determine the relationship between SOC (derived by Walkley & Black and the dry combustion method) and environmental factors (i.e., pH, EC, N and P).

1.3 Outcome of research

1. Recommendation analysis method for SOC stock measurement for specific soil group.
2. This research can be used as a guide for identifying the areas of deficiency of SOC, N, and P content and their management through efficient fertilizer recommendations to make the crop more productive.

CHAPTER 2

LITERATURE REVIEWS

2.1 Soil organic carbon

SOC refers only to the carbon component of organic compounds. SOC is extremely important in all soil processes. Organic material in the soil is essentially derived from residual plant and animal material, synthesized by microbes and decomposed under the influence of temperature, moisture and ambient soil conditions. Higher SOC promotes soil structure or tilth meaning there is greater physical stability. This improves soil aeration (oxygen in the soil) and water drainage and retention and reduces the risk of erosion and nutrient leaching. SOC is also important to chemical composition and biological productivity, including fertility and nutrient holding capacity of a field. As carbon stores in the soil increase, carbon is sequestered and the risk of loss of other nutrients through erosion and leaching is reduced. An increase in SOC typically results in a more stable carbon cycle and enhanced overall agricultural productivity, while physical disturbances of soil can lead to a net loss of carbon into the surrounding environment due to formation of CO₂ (Corning et al., 2016). Sequestering carbon in SOC is seen as one way to mitigate climate change by reducing atmospheric CO₂. The argument is that small increases over very large areas in agricultural and pastoral lands will significantly reduce atmospheric CO₂. Although the overall impact of climate change on SOC stocks is very variable according to the region and soil type, rising temperatures and increased frequency of extreme events are likely to lead to increased SOC losses (Johns, 2017).

2.2 Soil properties

Soil consists of the mass of solid particles produced by the physical and chemical disintegration of bedrock found in various thickness mantling the ground surface (Johnson and DeGraff 1988; USBR 1998). It may or may not contain some proportion of organic material. For engineering geologic purposes, the soil should be considered as a mass consisting of the solid particles and the intervening spaces between particles containing either air or water. This perspective is important because the qualities needed to use soil or some fraction thereof as a building material, to support structures, or to excavate into it are controlled by the mineralogical and physical character of the solid particles in combination with the presence and proportion of air and water in the void spaces (Graff, 2017).

2.2.1 Soil pH

Soil pH or soil reaction is an indication of the acidity or alkalinity of soil and is measured in pH units. Soil pH is defined as the negative logarithm of the hydrogen ion concentration. The pH scale goes from 0 to 14 with pH 7 as the neutral point. As the amount of hydrogen ions in the soil increases the soil pH decreased thus becoming more acidic. From pH 7 to 0 the soil is increasingly more acidic and from pH 7 to 14 the soil is increasingly more alkaline or basic (Bickelhaupt, 1989). Soils tend to become acidic as a result of; 1) rainwater leaching away basic ions (calcium, magnesium, potassium and sodium); 2) CO₂ from decomposing organic matter and root respiration dissolving in soil water to form a weak organic acid; 3) formation of strong organic and inorganic acids, such as nitric and sulphuric acids (H₂SO₄), from decaying organic matter and oxidation of ammonium and sulfur fertilizers. Strongly acid soils are usually the result of the action of these strong organic and inorganic acids. The desirable pH range for optimum plant growth varies among crops. While some crops grow best in the 6.0 to 7.0 range, others grow well under slightly acidic conditions. Soil properties that influence the need for and response to lime vary by region. A knowledge of the soil and the crop is important in managing soil pH for best crop performance (SUNY, 2020).

2.2.2 Soil electrical conductivity

Soil EC is a measure of the number of salts in soil (salinity of soil). It is an excellent indicator of nutrient availability and loss, soil texture, and available water capacity. It affects crop yields, the suitability of the soil for certain crops, the amount of water and nutrients available for plant use, and the activity of soil micro-organisms, which influences key soil processes such as the emission of greenhouse gases, including nitrogen oxides (NO_x), methane, and CO_2 . Excessive salts hinder plant growth by affecting the soil and water balance. Soils containing excessive salts occur naturally in arid and semiarid climates. Salt levels can increase as a result of cropping, irrigation, and land management. Although EC does not provide a direct measurement of specific ions or salt compounds, it has been correlated to concentrations of NO_3^- , potassium, sodium, chloride, sulfate, and ammonia. For certain non-saline soils, determining EC can be an easy, economical way to estimate the amount of N available for plant use. Soil EC can also be an effective way to determine the texture of the surface layer because smaller clay particles conduct more electrical current than larger silt and sand particles (USDA, 2014).

2.3 Soil nitrogen

N is the most abundant element in the atmosphere and is usually the most limiting crop nutrient. N cycles through soil in various processes and forms. Some processes are necessary to convert N into forms which plants can use. Some processes can lead to N losses such as leaching or volatilization. N is added to soil naturally from N fixation by soil bacteria and legumes and through atmospheric deposition in rainfall. Additional N is typically supplied to the crop by fertilizers, manure, or other organic materials. Soil NO_3^- -N is an excellent indicator of N-cycling in soils, whether carryover N was used by the previous crop and whether additional N is needed (USDA, 2014).

In agricultural and natural ecosystems, nitrogen occurs in many forms covering a range of valence states from -3 (in ammonium ion (NH_4^+)) to +5 (in NO_3^-). The changes from one valence state to another depends primarily on environmental conditions and is mainly biologically mediated. N is readily distributed by hydrologic and atmospheric transport processes. The transformations and flows from one to another constitute the basis of the soil N cycle. (Hofman and Cleemput, 2004) The principal forms of N in soil are NH_4^+ , NO_3^- , and organic N-compounds. At any time, the inorganic N in the soil is only a small fraction of the total soil N. Most of the N in a surface soil is present as organic N. It consists of proteins (20 to 40 percent), amino sugars, and complex unidentified compounds formed by reaction of NH_4^+ with lignin, polymerization of quinones with N compounds and condensation of sugars and amines. These different N fractions are susceptible to various transformation processes.

Inherent factors such as soil drainage, soil texture, and slope steepness impact N-transport and N-transformation processes that limit availability to crops or lead to losses. Inherent factors such as rainfall and temperature; and site conditions such as moisture, soil aeration (oxygen levels), and salt content (electrical conductivity) affect rate of N mineralization from organic matter decomposition, N cycling, and N losses through leaching, runoff, or denitrification. Organic matter decomposes releasing N more quickly in warm humid climates and slower in cool dry climates. This N release is also quicker in well aerated soils and much slower on wet saturated soils.

Total N is measured by combusting the sample in an oxygen atmosphere, then measuring the nitrogen dioxide (NO_2) produced. This gives the total elemental N present in the sample in both organic and inorganic forms. Combustion analysis requires specialized and expensive laboratory instrumentation. Total N can be analyzed using persulphate/UV or persulphate oxidative digestion followed by analysis of the NO_3^- -N formed. These methods are

not suitable for samples with high organic loading or for some industrial organic N compounds. Total N may be calculated (approximately) by adding Total Kjeldahl Nitrogen (TKN) and NO_xN (Hill laboratories, 2016). TKN is defined by the test method used which determines nitrogen in the trivalent state. The method does not determine N present in azide, azine, azo, cyanide, hydrazone, nitroso, oxime, or semicarbazone forms, nor as NO₃⁻ or NO₂⁻, and also does not recover N from some industrial chemicals. TKN can be considered to comprise NH₄-N, protein N and non-protein N e.g., urea, DNA, benzalkonium salts. In addition to, investigation of the research showed that the amounts of NO₃⁻ and NO₂⁻ in the soils were too small to affect the results of Kjeldahl analysis and that pretreatment of the soils with water had no effect on their analysis by the Kjeldahl method (Bremner, 1959).

2.4 Soil phosphorus

P is often the most limiting nutrient for crop and forage production. The primary role of P in the plant is to store and transfer energy produced by photosynthesis for use in growth and reproductive processes. P in soils occurs almost exclusively in form of orthophosphate with total P concentrations usually in range of 500-800 mg/kg dry soil. Quite a substantial amount of this P is associated with organic matter and in mineral soils the proportion of organic P lies between 20 and 80% of the total P. Organic P solubility in the soil is low. There is equilibrium between solid phase P in soil and the P in the solution. Plants can only take up P dissolved in the soil solution, and since most of soil P exists in stable chemical compounds, only a small amount of P is available to the plant at any given time. When plant roots remove P from the soil solution, some of the P adsorbed to the solid phase is released into the soil solution in order to maintain equilibrium. The types of P compounds that exist in the soil are mostly determined by soil pH and by the type and amount of minerals in soil. The optimal pH range for maximum P availability is 6.0-7.0. In many soil decompositions of organic material and crop residue contributes to available P in the soil (SMART, 2020).

Inherent soil properties and climate affect crop growth and how crops respond to applied P fertilizer and regulate processes that limit P availability. Climate and site conditions, such as rainfall, temperature, moisture, soil aeration (oxygen levels) and salinity (salt content/EC) affect the rate of P mineralization from organic matter decomposition. Organic matter decomposed releasing P more quickly in warm humid climates and slower in cool dry climates. P is released faster when soil is well aerated (higher oxygen levels) and much slower on saturated wet soils (USDA, 2014).

2.5 Walkley & Black method

The indirect methods are a particular element is present in the organic matter in the soil to such a constant degree that the percentage of organic matter can be obtained from the amount of this element by use conversion factor. This method can give the organic matter content from the carbon content of the sample only if the carbon content of the organic matter is known. Much effort has been devoted to the determination of the carbon factor. The most usual value is 1.724, which is based on 58 percent of carbon content of humus (Sprengel, 1826). Estimates of total organic carbon are used to assess the amount of organic matter in soils. The method measures the amount of carbon in plant and animal remains, including soil humus but no charcoal or coal. Levels are commonly highest in surface soils but wide variations from almost zero to above 15% carbon are possible. Presence of chlorine will produce a positive interference in saline soils (>0.5% Cl). The bias resulting from the presence of Chlorine can be corrected if required.

The determination of SOC is based on the Walkley & Black method. Oxidizable matter in the soil is oxidized by 1 N Potassium dichromate (K₂Cr₂O₇) solution. The reaction is assisted by the heat generated when two volumes of H₂SO₄ are mixed with one volume of dichromate.

The remaining dichromate is titrated with ferrous sulphate. The titrate is inversely related to the amount of carbon present in the soil sample (Department of Sustainable Natural Resource, 2008).

2.6 Dry combustion method

For analysis of total organic carbon by dry combustion, an automatic chemical analyzer, is used. Advantages of using an autoanalyzer are increased accuracy and ability. An autoanalyzer can be used to quantify carbon, N and sulfur. Disadvantages of using an autoanalyzer are equipment initial cost, operating and maintenance costs and the lower number of labs using an autoanalyzer worldwide.

This method is based on the Dumas dry combustion principle (Dumas, 1831). The sample is burned at high temperature (between 900 and 1000°C or 1400 and 1600°C) in an atmosphere of pure oxygen. Under these conditions, all carbon-containing compounds are completely decomposed and converted into CO₂. The autoanalyzer measures and reports the total organic carbon value based on the concentration of CO₂ present using various procedures (Bertsch et al., 2019). The end product of the combustion is CO₂ which is quantitated by titrimetric, gravimetric, manometric, spectrophotometric, or gas chromatographic techniques (for example, a carbon gas detector and thermal differences between gas columns). Dry chemistry techniques are typically cited as total carbon techniques but, there are not in the absence or removal of inorganic carbonates, these techniques can be used to quantify total organic carbon (Schumacher, 2002).

2.7 Review of related literatures

Rodríguez Martín et al. (2015) reported assessment of the SOC stock in Spain. The researchers informed SOC stocks assessments at a national level are essential in the climate change mitigation role. Their study provides the first assessment of the SOC stored in entire Spanish surface soils obtained from one sampling protocol within a regular sampling framework in a short edaphic time. The objectives of this study were to quantify current SOC, and SOC stock in Spain separated by three different land uses including agriculture, forestlands, and grasslands, and to generate resolution estimates of surface soil SOC stocks. In this study, they measured SOC, soil bulk density, and stoniness to estimate SOC stock. Organic carbon was analyzed by oxidizing carbon with acidic dichromate (Walkley, 1935). This procedure has been the “reference” method followed to make comparisons with other methods in numerous studied (Schumacher, 2002), and is widely used as it is simple and rapid with minimal equipment requirements (Nelson and Sommers, 1982). The results showed that half the Spanish areas obtained a SOC value below 1%. The lowest SOC levels were associated with agricultural soils. However, no statistically significant differences were found between forestlands and grasslands. The mean SOC stock in the Spanish topsoil layer was 56.57 Mg C ha⁻¹. The total organic carbon stock stored in topsoil was 2.819 Pg C. There was inherently wide spatial variability in stocks in Spain. SOC stock tended to be higher in north-westerly areas of the Spanish peninsula, while the lowest contents were located in the south because climate conditions. In addition, certain changes in agricultural practices that adopt conservation tillage with cover crops can increase carbon sequestration in soils and may be converting some unproductive croplands into other land uses (forestlands and grasslands), which can multiply the SOC content in Spain.

Wang et al. (2010) described SOC changes under different fertilization schemes across China's croplands. The researchers search data from online database, China's Academic Magazine and Weipu Science and Technology Literature, for publications on long-term fertilization experiments. They classified six categories of fertilization schemes, including N (inorganic nitrogen fertilization only), NP (compound inorganic nitrogen and phosphorus

fertilizers), NPK (compound inorganic nitrogen, phosphorus and potassium fertilizers), O (organic fertilizers only), OF (combined inorganic/organic fertilization) and Others (other unbalanced fertilizations such as P only, K only, P plus K and N plus K). They compared the relative change in SOC content between rice paddies and dry croplands soils. As the result, there was an overall temporal increase in topsoil SOC content, and relative annual change (RAC, $\text{g kg}^{-1} \text{y}^{-1}$) ranged -0.14 to 0.60 (0.13 on average) for dry cropland soils and -0.12 to 0.70 (0.19 on average) for rice paddies. SOC content increase was higher in rice paddies than in dry croplands. SOC content was higher under organic fertilization and combined organic/inorganic fertilizations than chemical fertilizations. Moreover, there was a much higher SOC increase under balanced fertilization compared with unbalanced fertilizations. The higher increase in SOC under rational fertilization can be sustained in dry croplands soils for <20 years, but >20 years in rice paddies soils, although RAC values decreased generally when the experiment duration increased. These findings suggest that fertilization, as a critical factor in agro-management practices, may affect SOC sequestration. Therefore, the extension of rational fertilization in China's croplands may offer a strategy to enhance carbon sequestration potential and to sustain long-term crop productivity.

Meersmans et al. (2009) reported determining SOC for agricultural soils by a comparison between the Walkley & Black and the dry combustion methods in north Belgium. SOC change over time are important because of CO_2 fluxes between soil and atmosphere and cropland decrease in SOC. Generally, the analytical methods underestimate total SOC content, correction factors are needed to avoid methodological bias when comparing SOC data from sampling campaigns using different analytical procedures. The traditional method for SOC analysis used to be, and in most cases is wet oxidation in $\text{K}_2\text{Cr}_2\text{O}_7$, known as the Walkley & Black method. The aim of this study is to quantify the recovery of the organic carbon content obtained by the Walkley & Black method in order to determine land use and soil type specific correction factors. Two different variants of the Walkley & Black method were applied to agricultural soils in north Belgium: the classic Walkley and Black (1934) method as well as a modified version as used by the Belgian Soil Service. The sample organic carbon content was determined using three different methods, including 1) the classic dichromate oxidation method of Walkley & Black (1934), 2) a modified dichromate oxidation method, the Belgian Soil Service adds in a modified version external heat to the oxidation reaction based on the French standard X31-109 until the boiling point is reached (Baize, 2000) and 3) the dry combustion method by an elemental analyzer (Carlo Erba NA 1500 Milan, Italy). The results showed a mean recovery rate of 0.70 ± 0.14 for the classic Walkley & Black method was estimated and 0.84 ± 0.14 for the modified Walkley & Black method. Regression between SOC content obtained by the Walkley & Black method and the dry combustion method show that the intercept is not significant ($P < 0.05$). This study suggests the use of a factor of 1.47 for the correction of SOC values obtained by the classic Walkley & Black method for north Belgian agricultural soils. For the modified Walkley & Black method, an overall correction factor of 1.2 was determined. Most samples with high carbon values (>4-5%) are characterized by low recovery rates compared with samples with lower carbon content. There were considerable differences in recovery between the two texture classes for both the classic and modifies Walkley & Black method. Significantly higher correction factors were estimated for sand than for soil loam grassland soils and correction factors increase with soil wetness. Comparison of their results with others suggests that no general factor can be used to correct for incomplete recovery which is inherent in the Walkley & Black method, but where possible a soil type and land use specific factor should be applied.

Aula et al. (2016) described effect of fertilizer nitrogen on SOC, total N, and soil pH in long-term continuous winter wheat. Soil samples (0-15 cm) were analyzed and compared to soil test results from 1993 to 2014 to determine the effect of long-term N application on SOC,

total N, and soil pH and to evaluate differences in SOC, total N, and soil pH over time in continuous winter wheat. The researchers used three long-term continuous winter wheat fertility experiments: Experiment 222 (E222), Experiment 502 (E502) and the Magruder Plots in this study. They found soil pH decreased with increasing N fertilization. N application significantly increased total N experiment 502, located in Lahoma and classified as Grant Silt Loam, from 1993 to 2014. Total N tended to be high at high N rates. SOC followed a linear pattern where an increase in rates of N fertilization. Rates that exceeded 67 kg N ha^{-1} resulted in increased sequestration of atmospheric CO_2 . Fertilizer N significantly increased SOC with time from 1993 to 2014, especially when N rates exceeded 90 kg ha^{-1} where it increased by 1.37 g kg^{-1} relative to the unfertilized check point in E222. In E502, SOC was consistently higher at an application rate of 112 kg N ha^{-1} over the 22-year period (1993 to 2013) considered in this study. Manure played an important role in improving SOC, which is important soil property and improve soil health. In conclusion, long-term application of fertilizer N was cause acidification of surface soils. Manure played an important role in improving SOC, which is an important soil property because manure application raises soil pH and retain grain yields at acceptable levels. Accumulation of SOC and total N in the surface soil was significantly when at least 90 kg N ha^{-1} was annually applied. This may improve the physical, chemical, and biological properties of the surface soil.

CHAPTER 3

MATERIALS AND METHODS

3.1 Sampling site

Saraburi is one of the central provinces of Thailand. Saraburi has tropical savanna climate. The climate is arid with little rain in winter, relative high temperature in summer, and rain from May to October. Saraburi is an agricultural area in which rice is dominant cropping. The main bodies in Saraburi are those soils developed on the lower terraces of the Pasak River, one of the tributaries of the Chao Phraya River, which enters the Central Plain from the eastern highlands (Fukui and Takahashi, 1969). According to the soil series reports of the Land Development Department in Thailand (2010), there are 24 soil groups in Saraburi province. This study focused on the agricultural areas. Of 24, five soil groups were included in the sampling locations namely soil group_1, 4, 16, 28, and 62. Soil group_1 comprised poorly drained, fine-textured, high fertility status, and neutral to moderately alkaline. Soil group_4 consisted of poorly drained, fine-textured (silty clay loam to silty clay), moderately fertility, and neutral to slightly alkaline. Soil group_16 contained poorly drained, medium-textured (silt loam to silty clay loam), low fertility, and strong to very strong acid. Soil group_28 was well-drained, moderately fertility, and neutral to moderately alkaline. Soil group_62 included steep lands and vary soil qualities. A total of 30 distributed samples were taken from the top 0-15 cm of agricultural soils in Saraburi. The locations within Saraburi province in Thailand are indicated in Figure 1.

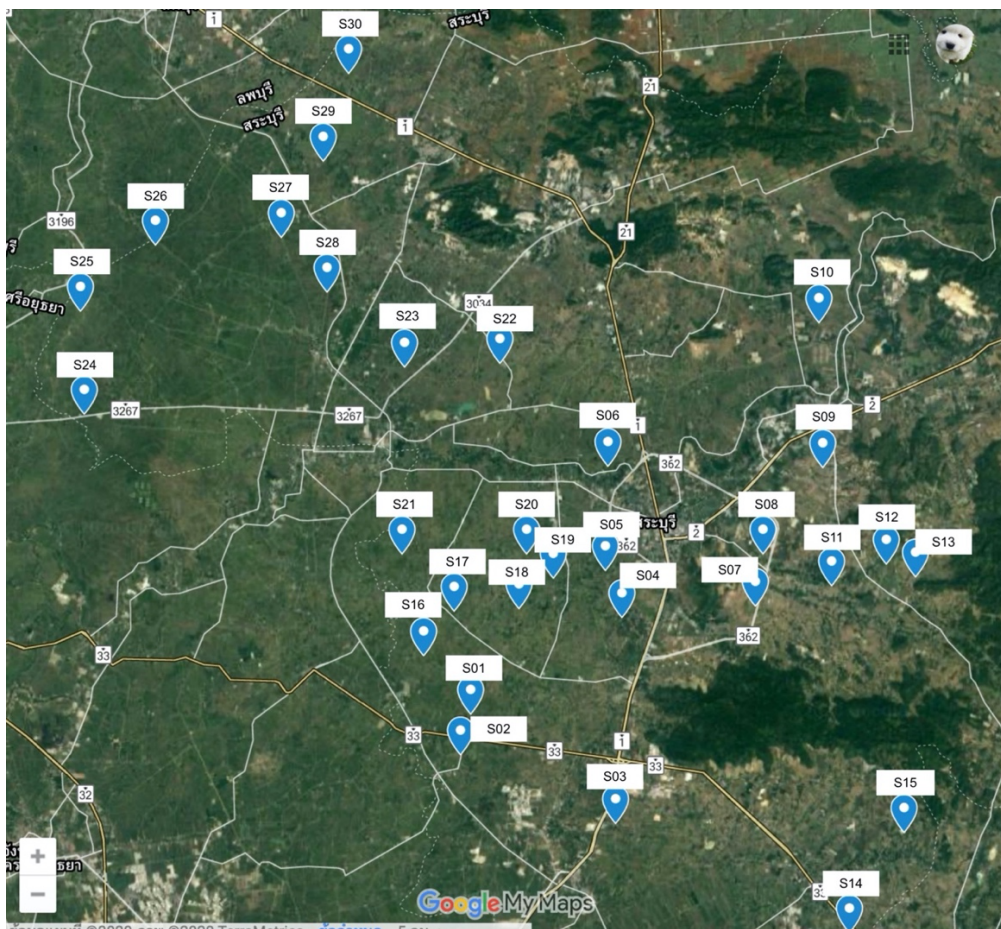


Figure 1. Map of the study area showing sampling sites of agricultural soil in Saraburi province (n=30) Satellite source: Google maps (November 15, 2020).

3.2 Soil sampling

A total of 30 sites was sampled at the same depths (0-15 cm) in the August 2020 rainy season. Each site represents soil Tambon. We mixed 3 sub-samples within the triangle $2 \times 2 \times 2$ m plots to represent one soil sample. Each soil sample was placed in a ziplock bag until it was transported to the laboratory. We recorded the latitude, longitude for sampling point using a handheld Global Position System (GPS).

3.3 Sample preparation

Soil samples were air-dried at room temperature for at least a week. The soil aggregates were sieved using 2-mm and 0.5-mm sieves. After removing the visible organic materials, stoned and fine roots by hand, the samples were divided into two parts (0.5-2 mm and <0.5 mm). After that, we stored the samples in a ziplock bag.

3.4 Analysis of total organic carbon

Organic carbon analysis was first determined SOC in each soil sample by the standard Walkley-Black method (Walkley, 1947). First, transfer 1 g air dried soil (0.5 mm) into a 250 ml conical flask then add 10 ml 1 N $K_2Cr_2O_7$ and 20 ml concentrated H_2SO_4 . To avoid underestimation due to incomplete oxidation specific to the Walkley & Black method. Next, swirl the content of the flask 2 or 3 times and allow the flask to stand for 30 minutes for the reaction to complete. After that, add 50 ml distilled water to the flask to dilute the suspension and stand for a cool down. Then, add 5 drops of O-phenanthroline. A green color will appear. Afterwards, Titrate it with 0.5 N Ferrous ammonium sulphate will the color changes from green to red brown. Note the volume of the ferrous ammonium sulphate used in titration and carry out a blank titration (without soil) in the same way.

$$\% \text{ Organic carbon, OC} = \frac{10 \times (B-S) \times 100 \times 3 \times 100 \times N}{B \times 77 \times 1000 \times W} \quad (1)$$

$$\text{Or } \% \text{OM} = \% \text{OC} \times 1.724 \quad (2)$$

Where B is volume of ferrous ammonium sulphate used titration with blank, S is volume of ferrous ammonium sulphate used titration with samples, W is weight of soil used and N is concentration of $K_2Cr_2O_7$ (1N).

We further determined SOC by dry combustion with the aj-Analyzer multi N/c 3100 total analyzer. Dry combustion total analyzer method is completely oxidizing the organic molecules in soil samples to CO_2 , measuring the resultant CO_2 concentration, and expressing this response as carbon concentration. First, all the samples were pre-treated with HCl before putting the samples into the oven for 2 hours in order to remove inorganic carbon. After that, weigh 250 mg of soil samples in a boat then put the samples into aj-Analyzer multi N/C 3100 for 1,199 °C to analyze total organic carbon which appears as %TOC.

The percentage of recovery (R) of SOC content was determined for the Walkley & Black method and was calculated as follows:

$$R (\%) = (SOC_{WB}(\%)/SOC_{DC}(\%)) \times 100 \quad (3)$$

Where R is percent recovery, SOC_{WB} is SOC content determined by the Walkley & Black method and SOC_{DC} is SOC content determined by the dry combustion method.

The correction factors (CF) between the Walkley & Black determination when compared to the dry combustion methods was obtained by the following equation:

$$CF = 100/R(\%) \quad (4)$$

3.5 Analysis of pH and EC

Soil pH was determined in suspensions with a 1:1 ratio of air-dried soil to water in the laboratory. Take 20 g soil in 100 ml beaker and add 20 ml of distilled water into it. The suspension was stirred at a regular interval for 30 minutes. Determined the pH value by pH meter with temperature compensation (United States Environmental Protection Agency).

EC was measured with 1:5 of soil/water ratio. Take 4 g of soil in a 100 ml beaker, add 20 ml of distilled water and shake intermittently for 30 minutes. Determine the conductivity of the supernatant liquid by an electrical conductivity meter. Measure the electrical conductivity of the 0.01M KCl at the same temperature as the soil suspensions. Record the value indicated on the conductivity meter with temperature compensation (Visconti and De Paz, 2016).

In case the conductivity meter did not have temperature compensation, we used the temperature of the sample. The EC measurement needed to be expressed at a standard temperature of 25°C. This is because the EC of all materials depends on temperature. Specifically, the EC of soils increases as temperature increases. Thus, unless the temperature of all soils is adjusted to 25°C by equilibration in thermostatic baths, all measurements must be corrected to 25°C. This is done by measurement of the temperature of the under test, and subsequent application of an adequate equation to convert from EC at T to EC at 25°C, carry out the follow calculations based on the EC evolution of KCl 0.01 M solutions with temperature.

$$EC_{25} = \frac{EC_T}{1+0.0191(T-25)} \quad (5)$$

3.6 Analysis of Nitrogen

Total N is estimated by the Kjeldahl method as per procedure suggested by AOAC (1995). The Kjeldahl method are more frequently used because of its performance and simplicity, utilization of ordinary equipment, and low-cost analyses (Pereira et al., 2006). The Kjeldahl method consists of three steps are digestion, distillation and titration.

First step is digestion. Weigh 1 g of soil samples and transfer it to the digestion tube. Add 7 g of catalyst mixture (K_2SO_4 : $CuSO_4 \cdot 5H_2O$:Se with 100:10:1 ratio) and 20 ml of concentrated H_2SO_4 to the sample. Load the digestion tubes into the digester and heat the digestion block then switch on the digestion unit and set initial temperature 100°C till frothing is over. After that, block temperature us raised to 400°C. The effective digestion starts only at 360°C and beyond 410°C. The sample turns light green color or colorless at the end of the digestion process.

Second step is distillation. After cooling the digestion tube, load the tube in distillation unit and other side of hose keep 60 ml of distilled water in the tubes. 80 ml NaOH (32%) is automatically added by distillation unit program. The digested sample is heated by passing steam at a steady rate and the liberated ammonia absorbed in 65 ml of 2% boric acid containing mixed indicator solution kept in the tubes. With the absorption of ammonia, the pinkish color turn to green. Nearly 60 ml of distillate is collected in about 8 minutes. Simultaneously, blank sample is to be run.

Third step is titration. The green color distillate is titrating with 0.1 N H_2SO_4 and the color changes to original shade (pinkish color). Note the blank & sample titer reading (ml) and calculate the total nitrogen content present in soil samples.

$$\% \text{ TKN content in soil} = \frac{(S-B) \times \text{Normality of acid} \times \text{Atomic weight of N} \times 100}{\text{Sample weight (g)} \times 1000} \quad (6)$$

$$\text{Or } \% \text{ TKN content in soil} = \frac{(S-B) \times \text{Normality of acid}}{\text{Sample weight (g)}} \times 1.4 \quad (7)$$

Where S is volume of H₂SO₄ used titration with samples and B is volume of H₂SO₄ used titration with blank.

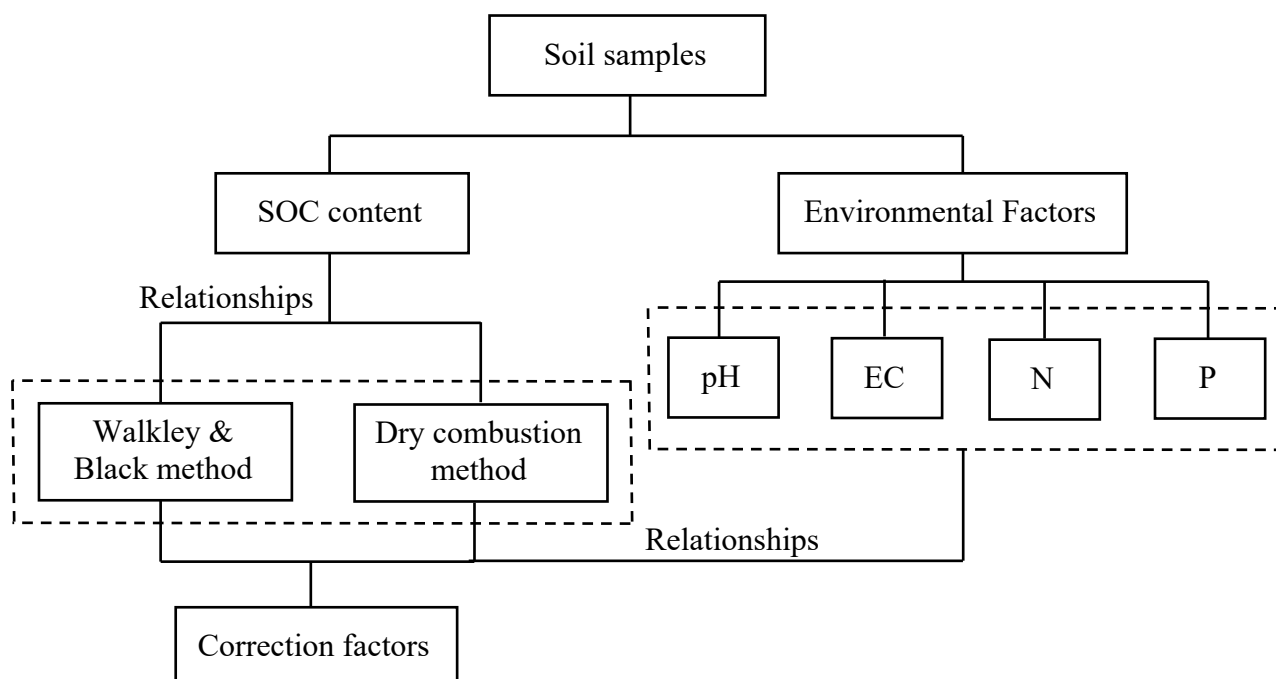
3.7 Analysis of Phosphorus

A colorimetric measurement was performed to determine the amount of P in agricultural soils. We added 5 g of soil sample in 150 ml conical flask and 200 mg of Darco G-60 activated charcoal. Added 20 ml of extracting solution (0.5N NaHCO₃) and shook the solution for 10 minutes in an orbital shaker. A similar process was run for a blank without soil. We filtered the suspension through the Whatman no. 42 paper in 50ml volumetric flask before adding distilled water till mark. We pipetted 5 ml aliquot of extract in a 50ml volumetric flask and added 10ml ammonium molybdate solution contains H₂SO₄. The volumetric flask was then shaken to mix the solution. We added 4 ml of mixed reagent consist of antimony-ascorbic acid and then adjusted volume with distilled water. The intensity of blue color was read on spectrophotometer at 840 nm wavelengths after 10 minutes. A standard curve was done to compare the P content in soil.

3.8 Statistical analysis

Descriptive statistics were used to describe the characteristics of soil properties. Linear regression was performed to identify relationship of SOC content between Walkley & Black and dry combustion method. Spearman's correlation and linear regression would be used to determine the relationship between the correction factors derived from the calculation and the environmental factors including pH, EC, N and available P. The analyses were analyzed by R for R 4.0.2 GUI 1.72 Catalina build (7847) © R foundation for Statistical Computing, 2020 for Mac OS.

3.9 Conceptual framework



CHAPTER IV

RESULTS AND DISCUSSION

4.1 Descriptive parameters for Saraburi soils

4.1.1 Soil group

Soil group_4 represented the major soil group (n=15) in this study area. It contained high amount of SOC and EC, and low pH value. The soil group_16 (n=7) was higher in N and P concentration when compared to the other soil groups. The soil group_28 hold the lowest amount of N and available P content. The soil group_62 and 16 were weakly acidic soils. The mean SOC, EC, N, and available P values and range of pH were presented in Table 1.

4.1.2 Soil pH

The pH of the soil in agricultural areas ranged between 4.9 and 7.8, indicating moderately acidic to slightly alkaline soils. Soil pH value of soil group_16 and 62 was slightly acidic more than other soil groups. Soil group_16 and 62, located at west of Saraburi province, were surrounded by residential area and cultivation land use. The reasons for low soil pH in this region included replacement of the layer of accumulated litter by a large humus layer, containing humic and fulvic acids, lowering the soil pH (Cleveland et al., 2006). Moreover, acid conditions occur in soil having parent material high in elements such as silica, high levels of sand with low buffering capacities, and in regions with high amounts of precipitation. An increase in precipitation enhanced leaching of base cations and lowest the soil pH (McCauley, 2009). In contrast, the researcher found most agricultural soils have basic conditions with average pH values ranging from 7 to 8 (Jacobsen, 2003; Belden, 1994). For those cases, it is primarily due to the presence of base cations associated with carbonates and bicarbonates found naturally in soils and irrigation waters (Yang et al., 2006).

4.1.3 Electrical Conductivity

The EC analyzed at 25°C in all samples ranged between 0.028 and 0.408 dS/m with a mean±SD of 0.092±0.076 dS/m (Table 1). The EC of a solution is related to the total cations or anions in the solution. EC has generally been associated with determining soil salinity; however, EC also can serve as a measure of soluble nutrients. Thus, within a specific range, the EC would indicate better nutrient availability for plants, with the low value indicating nutrient-poor soil that is structurally unstable and disperses readily and the high-value salinity problems. Our results indicated that the EC values were insufficient for plant growth. The samples had low EC value attribute to generally flat and very well-drained, little surface runoff occurs on this field. The salt tolerance of crops varies considerably and ranges from soil EC_{1:1} of 0.6 to 2.0 dS/m for salt-sensitive species and 1.7 to 5.1 dS/m for salt tolerant species (Smith and Doran, 1996). The soils with high EC attribute to greater slope, higher precipitation, and finer textured soil (Kitchen, 2003).

4.1.4 Soil organic carbon

SOC levels at the surface 0-15 cm depth varied between 0.68 and 2.68% for the Walkley & Black method, between 0.69 and 5.27% for the dry combustion method. The mean±SD SOC concentration values were 1.55±0.51% and 2.03±1.08% for the Walkley & Black and dry combustion methods, respectively (Table 1). For every sample, the SOC values measured by dry combustion technique were higher than determined by the Walkley & Black method. This difference was most likely due to the incomplete oxidation of organic matter by the wet-combustion method. Rodriguez Martin et al. (2015) reported the SOC concentration across

Spain ranged from 0.006 to 18.40%. The mean SOC concentration values were 1.70%, which have generally lower SOC concentration levels than critical levels for a temperate area (Loveland and Webb, 2003) had suggested that 1% is a threshold value. The SOC average in forestlands (3.70%) and grasslands (3.27%) was more than in croplands (1.05%). Our results indicated that had higher SOC stocks in agricultural soils were attributed to different cultivation, management practices, and climate zone.

The organic C pool capacity of the world soils has been variously estimated for principal each area. Despite the value of SOC is not measured solely by its global warming potential. Unlike more inert forms of carbon such as coal, soil organic matter (of which about 40% is SOC) performs numerous functions that promote plant productivity, agricultural efficiency, and environmental quality. The value of this service should be considered and added to the value of atmospheric carbon abatement when evaluating programs to encourage sequestration. Important soil organic matter mediated processes include mineralization and nutrient supply, enhancement of water relations and water supply, reductions in energy required for tillage, enhanced soil tilth, pH buffering and, disease suppression (Wander and Nissen, 2003).

4.1.5 Nitrogen

TKN values have the lowest value of TKN obtained was 0.004% and the highest 0.121%. Mean TKN content in all soil samples at Saraburi province was $0.038 \pm 0.037\%$ (Table 1). The result indicated the amount of N in Saraburi soil samples was lower than the requirement, which was 3.55% for rice fields (Chai Tai, 2020). N deficiency often results from chemical solubility in water, made it easily to be washed out of soil. Most of N lost by soil erosion is in organic forms and does not represent a loss of readily available N. It is a large loss of potentially available N, however, that eventually is deposited in streams and oceans with little opportunity to be recycled into agricultural systems (Legg and Meisinger, 1982). There are various transformation processes involved in N losses. Most of the native N that is potentially available to plants is associated with organic matter. Organic N can transform into inorganic N through the mineralization process. Moreover, there are denitrification processes that reduce NO_3^- or nitrites NO_2^- commonly by bacteria in soil that usually results in the escape of N into the air. Consequently, these reasons provide a low level in the amount of N accumulation in the soil. There were many studies interested in the amount of N. Marsh total N content in North Carolina soil ranged from 0% to 1.63%, which separated in ten marsh including North Carolina Phosphate (0.01 and 1.63%), Oregon inlet (0.10 and 0.18%), Pine Knoll Shores (0.02 and 0.03%), Snow's cut (0.08 and 0.37%), and Texasgulf (0.07 and 1.32%) (Craft et al., 1991). Difference of N content in soil due to microbial activity led to N mineralization process and soil organic matter runoff by topography (Rumpel and Kögel-Knabner, 2011; Doetterl et al., 2016).

4.1.6 Phosphorus

The available P values varied from 0.004 to 0.100 mg/kg. The mean content of available soil P in all samples was 0.030 ± 0.022 mg/kg (Table 1). The result showed relatively low available P in the soil samples. Soil P was quickly removed from the dissolved (plant-available) form by sorption, precipitation, and microbial immobilization (Roberts and Johnston, 2015) or was lost through surface runoff (in particulate and dissolved forms), subsurface flow (leaching and throughflow via the soil matrix and macropores), drain flow and groundwater which may accelerate the eutrophication of P sensitive waters (Heathwaite and Dils, 2000; Hively et al., 2006; Holman et al., 2008; Sims et al., 1998). Furthermore, soil available P is affected by agricultural management practices, such as crop rotation (Karasawa and Takebe, 2012), fertilization, and tillage (Cade-Menun et al., 2010; Messiga et al., 2012). This result suggests

that the large proportion of soil P occluded with Al and Fe oxides may be an important factor in the low P availability and P limitation on the growth of plants and microbes in this study area (Hou et al. 2012; Liu et al. 2012). Due to P is a major limiting nutrient for crop growth in many agroecosystems. Therefore, soil P needs to be replenished to satisfy plant demand. Organic matter applied to soil as an amendment is also reported to increase the availability of existing soil P (Zhu et al., 2017).

Table 1 Mean and SD of SOC, EC, N, and available P and range of pH on agricultural soil sample each soil group.

Soil group	n	SOC _{WB} (%)	SOC _{DC} (%)	pH	Electrical Conductivity (dS/m)	Nitrogen (%)	Available P (mg/kg)
1	4	2.09±0.43	3.35±1.35	6.0-7.4	0.187±0.163	0.043±0.047	0.017±0.013
4	15	1.62±0.44	1.81±0.58	5.3-7.6	0.096±0.046	0.035±0.035	0.022±0.012
16	7	1.01±0.31	1.10±0.33	4.9-6.5	0.043±0.013	0.053±0.044	0.058±0.021
28	3	1.86±0.21	3.65±0.81	6.7-7.8	0.080±0.023	0.012±0.006	0.010±0.006
62	1	1.189	1.56	5.5	0.030	0.049	0.052
All	30	1.55±0.51	2.03±1.08	4.9-7.8	0.092±0.076	0.038±0.037	0.030±0.022

4.2 Relationship between Walkley & Black carbon (SOC_{WB}) and the dry combustion carbon (SOC_{DC})

4.2.1 Overall recovery rate and correction factors

The percentage of C recovery by Walkley & Black for the C content measured by the dry combustion method ranged from 44.4% to 114.6%. The average recovery was least for the soil group_28 (51.9%), and the greatest recovery was found for the soil group_16 (93.1%) (Table 2). Samples with >2% C tend to have lower recovery values for the Walkley & Black method than samples with <2% C. The correction factor (100/R) varied between 0.87 and 2.25 of all samples (n=30). The correction factors were 1.96 for soil group_28, 1.58 for soil group_1, 1.31 for soil group_62, 1.12 for soil group_4, and 1.09 for soil group_16 (Table 2). Walkley & Black (1934) suggested 1.33 of correction factor without using external heating for whole soil. The soil group_28 and 1 were highly the correction factor than a reference value (1.33) and attributed the difference to the more stable organic compounds in the former fraction. The differences in correction factors between soil samples indicate the need for the inclusion of specific environmental factors to correct the Walkley & Black method for incomplete oxidation. La Manna et al. (2007) verified the effects of vegetation type on soil organic carbon contents, and Lettens et al. (2007) noted that recovery factor can be influenced by land use, soil texture, and depth. As the SOC content increased, the size of the active and slow pool also increases, which affects the recovery efficiency of Walkley & Black. Maybe, for this reason, Konen et al. (2002) suggested the inexistence of a universal factor applied to different soils, and De Vos et al. (2007) recommended a specific recovery factor for each laboratory and soil type to standardize the results of soil C organic estimation.

In most of the samples, SOC content determined through Walkley & Black method was lower than that obtained through dry combustion, possibly because of incomplete oxidation of the total organic carbon in the Walkley & Black procedure (McGeehan & Naylor, 1988; Nelson & Sommers, 1996). Lettens (2007) explains this result by the presence of phenolic and lignin compounds under graminaceous perennial pastures (such as corn, paddy, and cane) that resist oxidation at the temperatures obtained with H₂SO₄ in the Walkley & Black analytical

procedure. Baker (1936) describes that Walkley & Black recovery of starches, hemicelluloses, and cellulose (easily decomposable non-lignin fractions) is almost 100% and therefore the lignin and lignin-like materials must account for the average SOC recovery of 76%. The recovery means of all soils for the Walkley & Black method without heating was higher than some reported values. Considering the average percent recovery obtained for all samples (n=30), the efficiency of these measurements was high, 83.4% for all soils (Table 2). These results indicate SOC in Saraburi soil samples high efficiency can be oxidized. Hence, a more appropriate conversion factor for all soil samples would be 1.26. The correction factor of 1.33 over-estimates the organic carbon contents of these soils by recovery determination. On the other hand, from the soil group_28 and 1 were percentages of recovery, which were 52 and 66%, respectively, lower than the reference value (76%). As the SOC recovery was less than the assumed recovery with the Walkley & Black method, the conversion factor of 1.33 would underestimate the SOC contents of the soil group_1 and 28. It can be explained by the characteristically high heterogeneity of the soil, which contains a wide range of carbonaceous compounds whose relative amounts can vary considerably according to their geographic origin (Jiménez & García, 1991). Moré et al. (1987) presented that the oxidation degree depends on the chemical nature of the organic compounds, especially the number of aromatic molecules, nitrogen-heterocyclic groups, and polymerization degree. In addition, chloride, ferrous iron, and higher oxides of manganese, which possible constituents of the soil samples can interfere with the dichromate-based Walkley & Black method. In some cases, there was SOC content obtained by Walkley & Black method higher than the dry combustion technique. The higher SOC values by the Walkley & Black method can be attributed to oxidation of inorganic elements in the soil matrix, such as chloride, ferrous, and manganese compounds (Walkley, 1947), which can consume $K_2Cr_2O_7$ leading to higher apparent SOC values (Vitti, 2015). The former two can lead to high levels of organic carbon and latterly can result in low values (Nelson and Sommers, 1996). The soils were thoroughly air-dried before analysis, thus reducing the possibility of the presence of ferrous iron, and there was no significant effect of silver sulfate on the level of organic carbon determined (Kamara et al., 2007).

Table 2. Means and SD of SOC levels, recovery rate, and correction factor in Saraburi soils each soil group determination by Walkley & Black (WB) and dry combustion (DC) methods

Soil group	n	SOC _{WB} (%)	SOC _{DC} (%)	R (%)	CF
1	4	2.09±0.43	3.35±1.35	65.83±0.13	1.58±0.38
4	15	1.62±0.44	1.81±0.58	90.35±0.10	1.12±0.11
16	7	1.01±0.31	1.10±0.33	93.09±0.12	1.09±0.16
28	3	1.86±0.21	3.65±0.81	51.87±0.08	1.96±0.30
62	1	1.19	1.56	76.22	1.31
All	30	1.55±0.51	2.03±1.08	83.40±0.17	1.26±0.34

Recovery rate, $R = (SOC_{WB} / SOC_{DC}) \times 100$

Correction factor, $CF = 100 / R$

4.2.2 Relationship between Walkley & Black and the dry combustion method

There was a linear relationship between SOC derived by Walkley & Black and the dry combustion method with r^2 (coefficient of determination) values of 0.675. The analysis for linear showed that Walkley & Black method was linearly positive related to the dry combustion method ($p < 0.001$). The regression relationships between SOC values determined by the Walkley & Black method and dry combustion techniques (Figure 2) also demonstrated that the Walkley & Black method tends to underestimate SOC, particularly SOC content was lower than 2%. The model was effective when SOC content was lower than 2%, whereas SOC content

was higher than 2% this model had begun to vary. The model could be improved by selecting SOC content was <2% and removing SOC content was >2% to increase the suitability model. By taking of the linear regression in Figure 2, a correction equation is thus proposed to be applied to the SOC content results of Walkley & Black. The relationship between SOC contents of the dry combustion method and Walkley & Black method described by a regression equation:

$$\text{SOC}_{\text{DC}} = 1.7464\text{SOC}_{\text{WB}} - 0.6748 \quad (8)$$

In this relationship, the intercept was negative and significantly different from zero ($p < 0.1$). Although, Kamara, Rhodes, and Sawyer (2007) reported a very high coefficient of determination between Walkley & Black C and dry combustion carbon and observed a significant positive intercept. In addition, the intercepts are significantly different from zero ($p = 0.0737$) for all samples, which means that more accuracy can be obtained using a single factor but considering also the intercept. Some studies forced the intercept to zero to find a single factor, but this was avoided in this study (Fernandes et al., 2015). The significant intercept agreed with certain published results (Grewal, Buchan, and Sherlock, 1991; Wang, Smethurst, and Herbert 1996; Konen et al., 2002) and disagreed with others (Spain et al., 1982; Lowther et al., 1990). It indicated that the removal of structural water, the loss of H₂O from hydroxyl groups in sesquioxides, and the destruction of element carbon may be affecting the SOC content derived by the dry combustion method (Jha et al., 2014).

The using of dry combustion analysis for SOC appears more accurate, however, it is only valid in soils with no carbonates (Mikhailova, 2003). The results have reliable for SOC when the error caused by free lime was obtained (Sippola, 1982). This technique has been recognized as a standard procedure to measure the SOC content in acidic soils (Navarro et al., 1993). Moreover, the dry combustion method gives reproducible results, and the operation of the instrument is fast. However, expensive equipment provides problems for many laboratories (Chacón et al., 2002). On the other side, Walkley & Black presented drawbacks such as a long analytical procedure as well as the use of potentially harmful chemicals (H₂SO₄ and K₂Cr₂O₇) for the operator and the environment. Even if these disadvantages, there are utilizing this method throughout in laboratories. Nevertheless, the measurement of SOC content should be had a standard recovery reference for each method to calculation accurate total SOC concentration in the soil. Most studies compare the dichromate oxidation method (Walkley & Black) with the dry combustion method. In this study, the linear regression analysis indicated that Walkley & Black performed quite well in the agricultural soils at Saraburi province. Therefore, in the absence of dry combustion equipment, the Walkley & Black procedure would be adequate for SOC determination in these soils.

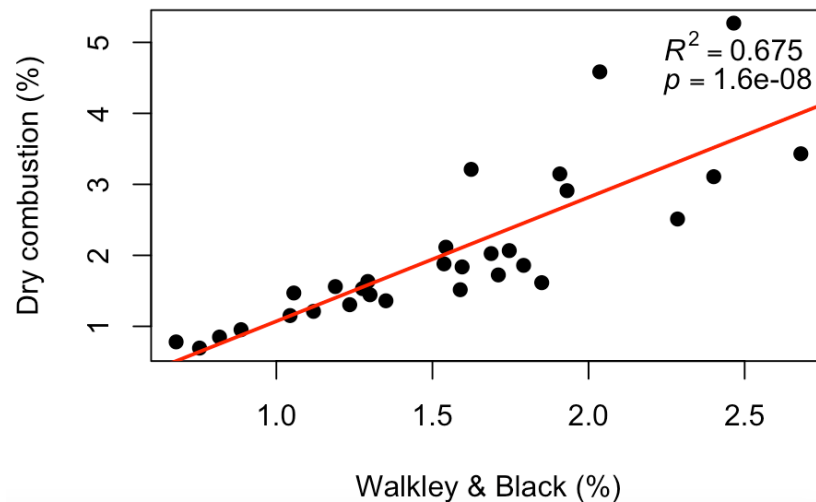


Figure 2 Relationship between SOC concentration obtained by the Walkley & Black method and a dry combustion method in all soil samples (n = 30) at Saraburi province.

4.3 Relationships of SOC derived by Walkley & Black and the dry combustion method versus environmental factors

4.3.1 Correlation of SOC and soil properties

The Spearman's correlation indicated that the results were significant, positive associations between the SOC derived by Walkley & Black method and available EC ($p < 0.001$), pH values ($p < 0.05$), while that was negatively correlated with available phosphorus ($p < 0.001$). Compared to SOC concentration derived by dry combustion method, that had a significant positive correlation with pH ($p < 0.001$) and EC ($p < 0.05$) and negative correlation with available P ($p < 0.001$). On the other hand, SOC derived by both Walkley & Black and dry combustion methods were not significantly related to the amount of TKN in this study.

The soil pH was similarly correlated with SOC derived by both Walkley & Black and dry combustion methods (Table 3). Soil pH gradient caused considerable changes in the concentrations of SOC ($r = 0.53$ in Walkley & Black method and 0.58 in dry combustion method). In this case, the apparent SOC concentrations were maximal at the 6 to 7 pH range. There was another research suggest both differ and agree with the result from this study. For example, Purnomo et al. (2000) reported a positive correlation between soil pH and SOC ($r = 0.59$) in surface Australian Spodosols. In contrast, Weigand et al. (1995) did not found a correlation between SOC and soil pH ($r = 0.12$, $p > 0.05$) when they analyzed these properties in 32 different surface soils derived from different parent materials under different climatic conditions in Bavaria, Germany. SOC concentrations in these soils were between 0.55% and 1.90%. Most of the previous studies involved soils from different locations then some of them originated from the different parent material. Consequently, when using soils with different origins, other factors, such as clay content, mineral composition, and climate conditions could affect the results (Aciego Pietri and Brookes, 2008).

The EC was highly correlated with SOC derived by Walkley & Black ($r = 0.61$) and the dry combustion method ($r = 0.57$) showed in Table 3. The EC variability across the site was somewhat related to historical erosion, with higher EC found in areas of exposed subsoils with higher clay content. Terra et al. (2004) reported EC values were relatively low compared with other studies because of the sandy nature of these soil surfaces, low organic carbon and soil water content, and low ionic strength of the soil solution. Since EC variability was related

to soil-terrain characteristics that largely control soil properties (Mueller et al., 2003). Results for correlation between SOC and EC are similar in the study; some studies have found positive correlation (Kitchen et al. 2003; Damian et al. 2011), while others have found negative correlation (Johnson et al. 2001; Terra et al., 2004). Due to EC has been shown to have significant temporal variability, primarily as the result of changes in soil profile moisture amount and distribution (Sudduth et al., 2001). Therefore, comparing EC across various sites, whether the sites are similar or dissimilar in soil type, may be misleading. A few contrasts of the other soil parameters are notable.

We found a significantly negative correlation between available P content and SOC concentration in both Walkley & Black and dry combustion methods ($r = -0.79$ and -0.77 , respectively, Table 3). Schlesinger et al. (1998) and Huang et al. (2012) reported the continuous accumulation of soil organic C could essentially lead to binding of the P pool, thus reducing soil P availability and leading to P limitation on plant growth. Moreover, the work of Thenard and Schutzenber (1861) showed that union may happen between various forms of artificial humus and phosphates under certain conditions and indicated that a combination may occur in the soil between organic carbon and inorganic P. In contrast, this result seems inconsistent with the study by Shah et al. (2019) found a significantly positive correlation between SOC and available P ($r = 0.095$, $p < 0.05$). Wang et al. (2006) found that SOC concentration positively related to the concentrations of soil soluble organic P fractions, whereas it was negatively related to the concentrations of soil soluble inorganic P fractions in depressional and riparian fresh wetlands in North-east China. Different studies have reported that soil containing high organic carbon content showed less P sorption and retention capacities for P (Guppy et al., 2005), which indicates the high P availability for uptake. However, Hou et al. (2012) revealed the increase in SOC content can enhance soil P sorption capacity. The relationships between organic carbon and phosphate ion in soil solution could also be partly explained through microbial processes, which occurred before soil sampling. Immobilization of phosphate ions in micro-organisms and mineralization of organic P due to microbial and enzymatic activities could affect the concentration of phosphate ions in the soil solution (Oehl et al. 2001; Achat et al. 2010), and microbial and enzymatic activities are generally correlated to organic C (Bünemann, 2015). This process leads to increasing organic C in soil when soil reactivity to phosphate ions was decreased. Enzyme activities play a vital role in the biochemical reactions of C, N and P cycling (Acosta-Martinez et al. 2007; Böhme et al. 2005; Fliebach, 2007). Nayak et al. (2007) suggested that phosphatase activities increased by increasing soil organic matter. The phosphatase enzyme was hydrolyse organic P compounds to inorganic polyphosphates which occur in soils, which is essential for P cycling for soil deficient in P (Kumar et al., 2011). In another study, Ohm et al. (2017) also found positive relationship between soil carbon and phosphatase activities. Therefore, studying phosphatase activities could be helpful to understand the lability of P.

The results showed the percentage of N content appears to be independent of SOC obtained by both Walkley & Black and dry combustion method in this study. Contrast analysis showed that the amount of N was weak negatively related to the SOC concentration derived by Walkley & Black method with r of 0.096, while hardly related to the SOC concentration derived by the dry combustion method with r of 0.002 (Table 3). Therefore, this study did not consider N content in multiple regression model. Pagnoul (1900) found no fixed relation between the C and N of the soil, but the C, N, and humus varied in the same direction although irregularly. Moreover, Fulmer (1896) determine the results have the three classes of different variation between carbon and N contents provided good evidence that no one given relation would hold for all soils. Nevertheless, Müller (1861) thought he detected a tendency for the nitrogen to vary inversely as the carbon. Berthelot and André (1893) revealed the organic matter of the soil as of great importance since it prevented the loss of nitrogen thru drainage

because the nitrogen was held in insoluble combination in the organic matter. Since the nitrogen was always found to be associated with carbon it was regarded as being of organic origin and as being derived in part from the organic matter present in the clay (Hall and Miller, 1908). In addition, slight differences in SOC and N concentrations among assessment methods could translate into large differences in SOC and N content when expressed on a mass per area basis. Therefore, it is important to investigate the impact that assessment methods have on SOC and N to determine whether the choice of soil carbon, N, or C and N assessment method affects interpretations regarding the decomposition of soil organic matter (Brye and Slaton, 2003).

Table 3 Spearman's correlation coefficient (r) between SOC and environment factors in agricultural soil at Saraburi province

Methods analyzed SOC content	pH	EC	N	P
Walkley & Black	0.5305895**	0.6133005***	-0.09588432	-0.7877642***
Dry combustion	0.5835373***	0.5669951**	0.002002225	-0.7730812***

*** Highly, highly significant correlation ($p < 0.001$)

** Highly significant correlation ($p < 0.01$)

4.3.2 Multivariate relationship of SOC versus environmental factors

Multiple linear regression (MLR) was conducted to identify the impact of environmental factors on SOC concentration derived by the Walkley & Black and dry combustion method. Equation 9 and 10 present the relationship between the environmental factors and SOC concentration derived by Walkley & Black and dry combustion method, respectively, in all samples. Significant relationships ($p < 0.05$) were obtained.

The SOC content obtained by the Walkley & Black method was associated with pH, EC values, and the content of available P, as indicated by the equations:

$$\text{SOC}_{\text{WB}} = 0.07593\text{pH} + 2.42509\text{EC} - 11.85997\text{P} + 1.20497 \quad (9)$$

P** ($p < 0.001$) ; Adjusted $R^2 = 0.547$; $p = 3.963e^{-05}$

Where SOC_{WB} = % of organic C extracted by the Walkley & Black method, EC = dS/m of Electrical conductivity and P = mg/kg of available P.

The regression of SOC derived by the dry combustion method on pH, EC, and available P as indicated by the equations:

$$\text{SOC}_{\text{DC}} = 0.4461\text{pH} + 2.4608\text{EC} - 14.3503\text{P} - 0.6257 \quad (10)$$

pH* ($p < 0.01$) and P ($p < 0.1$) ; Adjusted $R^2 = 0.4999$; $p = 0.000132$

Where SOC_{DC} = % of organic C received by the dry combustion method, EC = dS/m of Electrical conductivity and P = mg/kg of available P.

The equations show the coefficients of pH and EC was positive whereas that of available P is negative. It suggests that as the values of pH and EC would increase the SOC will also increase. However, the reverse was the case for the amount of available P. In a relationship between Walkley & Black C and environmental factors, the intercept was positive (1.20497) and was found to be not significant ($p = 0.1167$) while the intercept of dry combustion carbon equation was negative (0.6257) and was found to be not significant ($p = 0.6674$). Although the intercept was not significant, we included intercept in our model as it improves the prediction efficiency of the model.

The multiple linear regression analysis (equation 9) showed that available P content was the major environmental factor influencing the Walkley & Black C ($p < 0.001$), indicating that SOC storage derived by Walkley & Black method may decrease in response to future

increases in available P content. In addition, P content in the soil had stronger influences on SOC derived by the Walkley & Black method than dry combustion method, which can be explained by increases in the activity of enzymes participating in soil organic P mineralization in line with influences in SOC content have been reported by many studies (Allison and Vitousek 2005; Singsabaugh et al. 2008). Increases in SOC content can provide more energy and C-structure materials for microbes, leading to elevated production of phosphate enzymes involved in the mineralization of soil organic P (Allison and Vitousek 2005; Singsabaugh and Moorhead 1994). According to an equation 10, it was possible to develop factors for calculating SOC content obtained by the dry combustion technique from pH value in surface soil. Soil pH is an important chemical factor that directly affects the existing forms, bioavailability, and migration of various elements (Yu et al., 2002). Bacterial diversity, richness, and activity were highly inhibited in acid or alkaline environments (Huang, 1994; Li et al., 2001; Fierer and Jackson, 2006). Hence, when microorganisms decompose less litter, lower levels of SOC. The EC was also related to SOC, but their contributions to both regression models were less.

Multiple linear regression equations explained large proportions of variation in EC and N. The variation represented the among-site variability that had not been captured by the selected environmental factors. The unexplained variance may be due to various other factors we did not measure, such as micro-environment, geologic parent material, and topography (Jenny, 1941; Xu et al., 2013). In addition, Stewart (1910) revealed the variations in the various samples considered in any single type are too great to permit the utilization of the factors in isolated cases. These multiple linear regressions were not significant all factors due to other components might be associated with SOC content obtained by both the Walkley & Black and the dry combustion methods. The factors related the SOC content provide a context for considering mechanisms and process that determine soil carbon (Conant et al., 1998), multiple mechanisms by which organic matter is stabilized by clays (Hassink et al., 1993; Plante et al., 2006), and relation of different soil organic fractions to climate and particle-size fractions (Amelung, Flach, and Zech, 1997; Franzluebbers et al., 2001). Previous case studies suggest that organic P mineralization depends on soil properties including organic carbon content, Al and Fe oxides, and phosphatase activity (Achat et al. 2013; Bünemann 2015). Organic P mineralization may also be linked to carbon and N mineralization (Achat et al. 2010; Spohn and Kuzyakov 2013; Bünemann 2015). For instance, Achat et al. (2010) found similar organic P mineralization rates and organic carbon and N mineralization rates (i.e., C: N: P stoichiometry in mineralization fluxes) under the basic condition of P in dead soil organic matter can be driven by a microbial need for carbon (Spohn and Kuzyakov, 2013). However, the generality of the coupling of carbon, N, and P in mineralization fluxes and their relationships with soil properties will have to be assessed before they can be incorporated into the model (Achat et al., 2015). Furthermore, Domagala (2013) conceded soil organic matter and nutrient availability are directly affected by fertilizer management. Nevertheless, it is assumed that these relationships between SOC and other soil properties might be assessed in different land used for different climatic scenarios in further studies to complete the recorded results of this investigation (Damian et al., 2011). Therefore, the study of environmental conditions in the sample area was necessary.

CHAPTER V

CONCLUSIONS AND RECOMMENDATION

5.1 Conclusions

In this research, a total of 30 soil samples of the agricultural soils at Saraburi province were studied. The mean SOC content derived by Walkley & Black method was $1.55 \pm 0.51\%$, while measured by dry combustion technique was $2.03 \pm 1.08\%$. This study found that Walkley & Black method tends to underestimate SOC contents compared to the dry combustion techniques. Thus, a correction factor should be determined to analyze total organic carbon in Saraburi soil samples. On average, for all soil samples, the recovery value ($83.40 \pm 0.17\%$) was obtained. Therefore, the correction factor was $1.26 \pm 0.34\%$, which lower than the currently used value. The regression analysis indicated a strong relationship between Walkley & Black and dry combustion methods ($p < 0.001$). Therefore, average Walkley & Black correction factors and regression equations of dry combustion C on Walkley & Black C as determined in this study can be effectively utilized in assessing C storage in this study area. However, the recover factor and regression equations must be generated considering local and regional condition. This clearly confirmed that generation of universal correlation factor for conversion of Walkley & Black C to dry combustion C is an unrealistic proposition. It was recommended to use experimentally derived correction factors related to characteristics of the samples when using Walkley & Black measurements for assessment of SOC stocks. Despite the dry combustion method gives reproducible results, and the operation of the instrument is fast. When the error caused by free lime is taken care of, reliable results for soil organic carbon are obtained. However, the dry combustion method was rather expensive and required more complex equipment than Walkley & Black method. Besides these limitations, researchers could easily use this relationship for determining the dry combustion C from Walkley & Black C would help in determining soil C content with greater accuracy with a suitable correction factor. Correlations of SOC accumulation in soil with pH, EC, and P available content were generally high across fields, whereas correlation with the amount of N was lower and more variable for the fields used in the study. As measured by a coefficient of determination, the SOC content and environmental parameters gave the models that could explain the relationship for them. Therefore, the multiple linear regressions could be used for prediction SOC content derived by the Walkley & Black and dry combustion method when there were pH, EC, and available P data. Regardless of the analytical procedure used available P and pH were important parameters in accounting for SOC content. While EC value are generally required to interpret the relationship for these equations. However, regression equations may vary under different soils due to the interference of specific soil constituents. Such a relationship could be modified by taking into account additional input other variables.

5.2 Recommendation

Until a reliable method of measuring SOC by different analytical methods is developed, comparisons of SOC among different analytical methods and different soils should be made with great caution. Moreover, there need for further research involving enough number of soil group to determine correction factors and regression equations appropriate for specific groups of soils in the country. There are needs for more intensive sampling and multiple regressions of physical, chemical and mineralogical properties of soils for more reliable information on soil properties on prediction of SOC. More research is needed to calibrate the observed association between SOC content and the environmental parameters with different situations, crops and topography.

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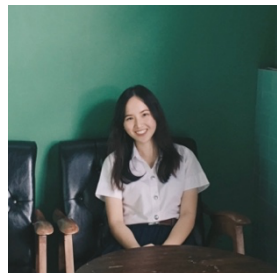
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