T.R.
KAHRAMANMARAŞ SÜTÇÜ İMAM UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

EFFECT OF CARBON TO NITROGEN RATIO ON SOME PROPERTIES OF ORGANIC SOILS

RAWAZ KHASRO QADER

MASTER'S THESIS
DEPARTMENT OF BIOENGINEERING AND SCIENCES

KAHRAMANMARAŞ - TURKEY 2015

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A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in
Bioengineering and Sciences

KAHRAMANMARAŞ - TURKEY 2015

M.Sc. thesis entitled “Effect of Carbon to Nitrogen Ratio on Some Properties of Organic Soils” and prepared by Rawaz Khasro QADER, who is a student at Department of Bioengineering and Sciences, Graduate School of Natural and Applied Sciences, Kahramanmaraş Sütçü İmam University, 28/05/2015 was certified by all the majority jury members, whose signatures are given below.

Prof. Dr. Hüseyin DİKİCİ (Supervisor) ……………………………….
Department of Soil Science and plant Nutrition
Kahramanmaraş Sütçü İmam University

Prof.Dr. Kadir YILMAZ (Member) ……………………………….
Department of Soil Science and plant Nutrition
Kahramanmaraş Sütçü İmam University

Prof.Dr. Adem KAMALAK (Member) ……………………………….
Department of Animal Science
Kahramanmaraş Sütçü İmam University

I approve that the above signatures related to the members.

Assoc. Prof.Dr. MUSTAFA ŞEKKELİ ……………………………….
Director of Institute of Science and Technology
Kahramanmaraş Sütçü İmam University

DECLARATION

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KARBON / AZOT ORANININ ORGANİK TOPRAKLARIN BAZI ÖZELLİKLERİ ÜZERİNE ETKİSİ

(YÜKSEK LİSANS TEZİ)

RAWAZ KHASRO QADER

ÖZET

Bu çalışma C/N oranının Kahramanmaraş İli, Sağlık Ovası organik topraklarının bazı toprak özellikleri üzerine olan etkisini belirlemek amacıyla yürütülmüştür. Bu amaç için alandan 50 adet toprak örneği alınmış, analizleri yapılmış, daha sonra veri seti hesaplanan C/N oranlarına göre üç farklı gruba ayrılmıştır. Grup 1 en düşük C/N oranlarına sahip iken grup 3 en yüksek C/N oranlarını içermiştir. Analizi yapılan toprak özelliklerinden pH, katyon değişim kapasitesi, kireç içeriği, organik madde, toplam azot, organik karbon ve bitkice alınabilir P, Mg, Na, Cu, Zn ve Mn konsantrasyonları üzerine C/N oranlarının etkisi istatistiksel olarak önemli bulunurken, bitkiye yarayışlı Ca ve K bu değişimlerden etkilenmemiştir. C/N oranı grup 1'den grup 3'e doğru arttıkça, organik madde, organik karbon, pH, KDK, MVR yöntemiyle hümik asit değerleri de artış göstermiştir. Bu sonuçlar C/N oranlarının organik topraklarda toprak kalitesinin bir göstergesi olarak kullanılabileceğini göstermektedir.

Anahtar Kelimeler: C/N oranı, Toprak Degradasyonu, Organik Toprak, Humik Asit

Kahramanmaraş Sütçü İmam Üniversitesi
Fen Bilimleri Enstitüsü
Biyomühendislik ve Bilimleri Anabilim Dalı Haziran / 2015

Danışman: Prof. Dr. Hüseyin DİKİCİ
Sayfa sayısı: 46

EFFECT OF CARBON TO NITROGEN RATIO ON SOME PROPERTIES OF ORGANIC SOILS

(M.Sc. THESIS)

RAWAZ KASRO QADER

ABSTRACT

This study was performed to determine the effect C/N ratio on some properties of organic soils in Sağlık Plain, Kahramanmaraş. To achieve this objective, 50 soil samples were collected from the area and analyzed for some soil attributes, and the data set were divided into three almost equally numbered groups based on the calculated C/N ratio values. The group 1 had the lowest C/N ratios, and the group 3 had the highest among the three. The measured soil attributes such as pH, electrical conductivity, cation exchange capacity, lime content, organic matter, total nitrogen, organic carbon and extractable P, Mg, Na, Cu, Zn, and Mn were significantly affected by C/N ratios except for plant available Ca and K. As C/N ratio increased from group 1 to 3, soil organic matter, organic C, soil pH, CEC, humic acid contents by MVR and CDFA methods also increased significantly. The results showed that C/N ratio can be used as an indicator of soil quality in organic soils.

Key words: C/N ratio, Soil degradation, Organic soil, Humic acids.

University of Kahramanmaras Sutcu Imam
Graduate School of Natural and Applied Sciences
Department of Bioengineering and Sciences, June / 2015

Supervisor: Prof. Dr. Hüseyin DİKİCİ

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ACKNOWLEDGEMENT

First of all, I would like to thank ALLAH, who gave me this chance to study and finish my Master degree.

I would like to express my gratitude to my whole family and especially my parents, who gave me the strength to overcome all obstacles, and I thank them for what they have done for me.

I would like to express my sincere gratitude to my advisor Prof. Dr. Hüseyin DİKİCİ for his academic support, supervision, and his generous advice during my Master study.

Besides my advisor, I would like to thank Research Assistant Ömer Faruk DEMİR, Ardalan Jalal MAJEED and Khadijah Muhammed ALI for their support and their help.

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SYMBOLS AND ABBREVIATIONS

°C	:Degree Celsius
ANOVA	:Analysis of Variance
C	:Carbon
C/N	:Carbon to Nitrogen Ratio
Ca	:Calcium
CDFA	:California Department of Food and Agriculture
CEC	:Cation Exchange Capacity
$\mathbf{CH_3COOH}$	:Glacial acetic acid
cm	:Centimeter
Cu	:Copper
D.W	:Distilled Water
DTPA	:Diethylene Triamine Pentaacetic Acid
EC	:Electric Conductivity
Fe	:Iron
g	:Gram
$\mathbf{H_3BO_3}$	:Boric Acid
$\mathbf{H_2SO_4}$	:Sulfuric Acid
H	:Hydrogen
H.A	:Humic Acid
HCl	:Hydrochloric Acid
$\mathbf{HNO_3}$	:Nitric Acid
hr	:Hour
ICP	:Inductively Coupled Plasma
K	:Potassium
kg	:Kilogram

M	:Molarity
Mg	:Magnesium
ml	:Milli Litters
µm	:Micro Meter
mm	:Milli Meter
Mn	:Manganese
MVR	: Mesa Verde Resources
N	:Nitrogen
N	:Normality
Na	:Sodium
NaOH	:Sodium Hydroxide
$\mathbf{NH_4OAc}$	:Ammonium Acetate
O	:Oxygen
OM	:Organic Matter
OC	:Organic Carbon
P	:Phosphors
RPM	:Rounds per Minute
SOM	:Soil Organic Matter
SOC	:Soil Organic Carbon
SPSS	:Statistical Packages for the Social Sciences
TEA	:Triethanolamine
TN	:Total Nitrogen
TSE	:Turkish Standard Institute
Zn	:Zinc

1. INTRODUCTION

The biogeochemical cycles of C and N have important effects in terrestrial ecosystems. Soil organic C and total N content of soils can play a crucial role in sustaining soil quality, crop production, and environmental quality (Bauer and Black, 1994; Doran and Parkin, 1994; Robinson et al., 1994).

Average of soil C/N ratio is different from soil to soil, but the value between "8-17" is considered as a typical value (Alistair, 1979). C/N ratio is a sensitive index for soil quality (Zhang et al., 2011), and this ratio have been vastly used as an indicator of the rate at which OM will decompose. The ratio of C/N also determines that how much N can be mineralized per units of C respired, and the amount of this N that will be immobilized by decomposers (Accoe et al., 2004). Moreover, this ratio can affect some other soil properties such as pH, C and N cycles, nutrient accumulations, humic substances (Yano et al., 2000). Therefore, understanding C/N ratio and its effects on soil properties is vitally important.

Soils are one of the most important C and N pools. Soils contain approximately 75% OC and 95% TN (Baldwin et al., 1938). Organic soil can be characterized by dark in color, and with their at least 20% OM content.

Another part of this research is to measure humic substances and their relations with C/N ratio. Humic substances are general categories of naturally occurring biogenic, and heterogeneous organic substances that can be easily characterized by yellow to black color, high molecular weight, and being resistant to degradation (Stevenson, 1994). Depending on its solubility, it consists of several groups of substances: humin is the humic substance which is insoluble in water at all pH values; humic acids which are insoluble under acidic conditions (pH<2), but it's soluble at higher pH values; and fulvic acid that is the fraction soluble in water at any pH value (Stevenson, 1994).

Humic substances form most of the organic compounds of the soils, peatlands, and natural water. It can influence on the process of the formation of fossil fuels, and play a major role in the global C geochemical cycles (Geel, 1978). Significant reserves of organic C are stored in wetland soils, and as much as 60‑85% of the OM in peat soils is humic substances (Garnier-Sillam et al., 1999). Soil C/N ratio is considered as an indicator of the process of humification (Brady, 1990; Miller and Gardiner, 1998). The ratios of C/N in humic compounds vary. Humic acid has much lower C/N ratio than fulvic acid.

Generally, C/N ratio of 10 – 15 is considered as a good ratio for well developed humic substances in soils. Therefore; humic and fulvic acids are considered as an active component for soil, and they are important to agriculture (MacCarthy et al., 1990). They can be commercially sold for agricultural and horticultural use as a fertilizer or soil amendments. There are many different methods to determine humic acids in organic soils, and three of them are Turkish Standard Institute (TSE) method (Anonymous, 2003), Mesa Verde Resources (MVR) method (Bruce, 1999), and California Department of Food and Agriculture (CDFA) method (Page, 1982).

The goal of this study was to determine the relationship between carbon and nitrogen, and its effect on some properties of organic soil. In addition, the determination of humic acids in organic soil, and their relations with C/N ratio will be studied.

2. LITERATURE REVIEW

2.1 Gavur Lake Study Area

Wetland areas are known to be important ecosystems, and they are the most convenient areas which produce the highest number of organic substances. They have a special economic value, and they are accepted to be important resources for scientific research. Gavur Lake is one of the most important wetland area in Turkey because of its bio-geographical and ecological situation, and especially for its current agricultural important. Kinds of soils that present in this place includes; Entisol, inseptisol, vertisol, hydromorphic, and organic soils (Yılmaz, 2006).

Gavur Lake is in the Mediterranean climatic zone. Because it is located at the cross section of 3 geographical regions that are Mediterranean, East Anatolian, and Southeast Anatolian regions, the climate of the area is called “Modified Mediterranean Climate”. The average temperature ranges between 15.5^{o}C to 18.0^{o}C in the region. The average precipitation is 691.4 mm in a year (Gürbüz et al., 2003; Yilmaz, 2006).

2.2 Organic Soil

2.2.1 Background of organic soil

Soil represents one of the most complex and dynamic natural systems, which is studied by the scientists (Chesworth, 2008). Soil is the primary link between components such as bedrock, air, water, and biota which produce our environment. Ingredients may interact with each other to provide many of goods (e.g., food, fuel and fiber) to support organisms. The word "soil" means different things to different people, but basically it can be defined as solids on the surface of the earth that results from weathering reactions and biological activities on the soil parent material or underlying hard rock (Dawson, 2012).

There are organic and non-organic soils that differ from each other because they are composed of different types of materials (Baldwin et al., 1938). Organic soil can be made up of plant and animal wastes. It can be found in wet areas because the remains of plants degrade slowly in wet environments. Organic soils contain at least 20% organic matter, they are black in color, light in weigh, porous, and their water and nutrient holding capacities are very high. They are sometimes referred as "peat" or "muck". Organic soil can be divided according to the thickness of the organic material to shallow (30 – 80 cm), moderate (80 – 130 cm), and deep (more than 130 cm) soils (Okruszko, 1994).

Organic soils are developed in the lower topographical positions characterized by higher temperature, higher frequency of frost, and higher relative humidity of the air. Organic soils may be colder than mineral soils during the summer months and hotter during the winter (Ilnicki, 1965).

Organic soil can change its basic functions from natural carbon and water reservoir sinks to the source of greenhouse gas emissions and water deficiency after drainage (Okruszko, 1993).

2.2.2 Physical properties of organic soils

Soil is made up of solid, liquid, and gas phases. The solid phases of organic soil is made up of plant fibers, humus, and mineral matters like sand, silt, and clay fractions as well as amorphous substances in the form of carbonates, phosphates, and hydroxides. The rate at which organic material can degrade depends on many factors such as pH, temperature, humidity, and chemical composition.

Substrates contain carboxyl and phenol groups that have the ability to absorb cations (Puustjarvi, 1982). Cation exchange capacity is expressed in "$\mathrm{cmol_c\ kg^{-1}}$" or on volume basis. Cation exchange capacity depends on pH value, because carboxyl and phenol groups can accept protons under acidic conditions. High CEC value can reduce the risk of unexpected changes in salt concentrations in the liquid phases.

Organic soils usually have very high water contents, the water content will decrease with the degree of humification process, and it can increase the decomposition process of organic materials (Jarrett, 1982).

2.2.3 Carbon and nitrogen cycles

Damman (1988) suggested that the critical C/N ratio for N mineralization must be an exponential function of the microbial N requirements. The critical C/N ratio of 14 to 15 should meet 100% of the microbial N requirements in a particular ecosystem.

One of the major problems related to the drainage of organic soils is subsidence. There are several factors that play important roles in subsidence of organic soils that are biological oxidation, depth to the water table, leaching of soluble organic materials, compaction, burning, wind erosion, and water erosion. The depth to the water table is certainly one of the major factors involved in subsidence. Subsidence of organic soils occurs in two stages; primary and secondary. Primary subsidence occurs relatively fast (4 to 10 years) and involves the loss of force and compaction of the organic soils due to drainage. Secondary subsidence, which is much slower, includes biological oxidation, wind and water erosion, and leaching of organic materials (Everett, 1983). A study in Indiana (Jongedyk et al., 1950) showed annual subsidence rates of 1.1, 1.8, and 3.0 cm where water tables were maintained at depths of 42, 68, and 98 cm, respectively. This rate of subsidence is considerably less than that reported by Stephens (1956), and is likely related to the colder and freezing winter temperatures that occur in Indiana. In a Minnesota study over a 5 year period, subsidence of 15 cm and 60 cm was noted with water table levels of 30 and 135 cm, respectively.

Burning is another factor that can increase or decrease subsidence of organic soils. Before 1950, fires accounted for the majority of the loss of organic materials in the U.S. Some fires were the result of deliberate burning to increase soil pH and soil nutrient levels; others were natural fires caused by lightning (Lucas, 1982). Extremely hot fires, which often occur after periods of severe drought, can burn deeply into the organic layers and destroy the forest stand. Severe fires were likely the cause of the formation of the many lakes in the Okefenokee Swamp (Cypert, 1961), and the deep, treeless pools in some of the cypress swamps (Ewell and Mitsch, 1978).

Wind erosion occurs as the surface of the organic soil dries and decomposes. Even under the most careful management, the resulting loose, powdery surface is easily removed by wind. This type of erosion can be a serious problem during severe windstorms. Constructing and maintaining shrub windbreaks can reduce wind erosion, as can maintaining a cover on the soil surface at all times.

Proper management techniques can reduce, although not totally stop, the subsidence. Management of organic soils involves protection from development followed by an active management program to counteract the effects of the lowering of the water tables. Constructing shrub windbreaks that can reduce wind erosion. Maintaining a high water table is one of the primary means of reducing biological oxidation and subsidence (Collins and Kuehl, 2001).

2.3 Carbon to Nitrogen Ratio

There are two chemical elements in OM which are extremely important. Especially, in their relationship or proportions to each other, that they are carbon and nitrogen. All OM made up of essential amounts of C which combined with lower amounts of N (Miller, 2000). The path of decomposition of OM may affect by the presence of each C and N. The balance of these two elements is called carbon to nitrogen ratio.

The C/N ratio is a ratio of the mass of C to the mass of N in a matter. C/N ratios are an indicator for N limitations of plants or other organisms which can be identified whether molecules found in the sediments under study that came from land based or algae plant. Therefore, C/N ratio could serve like a tool to understand the sources of OM that can drive to information on ecologies, climates, and ocean circulations at different times of the Earth’s history (Ishiwatari and Uzaki, 1987).

C/N ratio is a sensitive index for soil quality (Zhang et al., 2011). It is even considered as a mark for capacity of soil N mineralization. High soil C/N ratio could slow down the decomposition rate of OM by limiting the soil microbial activity abilities. While low soil C/N ratio could accelerate the processes of microbial decompositions of OM, which is not conducive for C sequestrations (Wu et al., 2001). Carbon is important because of its energy content in the form of species like carbohydrates, while, N is essential for plant growth. Average C/N ratios differ from country to country depends on the dominant soil types, but the value between 8 and 17 is considered as a typical value. Table 2.1 shows C/N ratio of some materials (Alistair, 1979).

C/N ratio determines how much N may be mineralized per unit of C which respires and affects the amount of this N that would be immobilized by the decomposers (Accoe et al., 2004). This ratio considered as a critical index for biogeochemical cycles that used to predict the impacts of agriculture and other disturbance on OM decompositions and N cycles in the terrestrial ecosystem (Chapin et al., 2002).

According to Miller (2000) any fertilizers that add to soils for regulating C/N ratio, can be considered. When O.M is added to soils the crush of the molecules by microbes may cause changes in C/N ratio. It will be important that all fertilizers which add to soils must have sufficient levels of N or the fertilizer would have negative impact. Berg and McClaugherty (2003) suggested that in the humus layers C/N ratio positively could affect by the stance volume, the larger the volume, the higher the C/N ratio.

C/N ratio of the soils in surface horizons could be found only by getting the information about total C and N. Then, this information could be used as an indicator which can provide relatively credible information about biological activities and equilibriums of these two nutrients, which have been subjected to the antagonistic process of mineralization or immobilizations of N content. In a region with moderate climates, the C/N ratio would be approximately 10 – 12 for uncultivated soil and mainly this ratio will decrease with increasing the depth of soil. In conformed soils N could be significantly occluded in clay soils, especially in deep horizons (Pansu et al., 1998). In the forest soils, peat horizon and podzols, C/N ratios could be reached to 20 – 30 or even higher, because of the formation of slightly biodegradation complexes that have low N content (e.g. Spodic horizons). At a threshold C/N ratio which below or near 20:1 generally positive N mineralization would be observed. In cultivated soils, farming residues which recycle in the field has a C/N ratio that ranges between 15 and 60 due to the present of lignin celluloses that are compounds with a slow rate of degradation level. Under forest situation, the C/N ratios could reach to the ratio of 150 or even higher (Pansu et al., 2001).

C/N ratio could be influenced by land use system, this ratio shows narrower in a soil that is cultivated as compared to other soil uses indicating that mineralization and oxidation of OM will be higher in a cultivated soil. This is also agreed by Seeber and Seeber (2005) which reported cultivated land could alter humus contents or even can narrow the C/N ratio. Native soils that usually has high C and N contents than abandoned soils or cultivated soils, because cultivation could lead to the loss of C and N contents in soils. However, the losses of N content is much lower than the losses of C content due to cultivation, then the C/N ratio will be narrower (Sebber and Seeber, 2005). Some differences in C/N ratio between the systems of land uses might be reflect variations in quality of an organic residue which enters the soil organic matter pools and can be attributed to the contrast vegetation covers. Caravaca et al. (2002) discovered lower C/N ratios in cultivated areas than for uncultivated areas and suggested higher C/N ratio to the input of the recent matters of plant and microbial origins in uncultivated areas. John et al. (2005) determined higher C/N ratios in forest lands than agricultural lands. Same suggestion with Puget and Lal (2005) who noted higher C/N ratios in forest lands as compared to cultivated lands.

Table 2.1 C/N ratio contents of some materials (Alistair, 1979)

Sources	C/N ratio content
Sandy loam (fine)	7:1
Humus	10:1
Food scraps	15:1
Alfalfa hay	18:1
Rotted manure	20:1
Vegetable trimmings	25:1
Leaves	35:1 to 85:1
Corn stalks	60:1
Straw	80:1
Pine needles	60:1 to 110:1
Alder sawdust	134:1
Newspaper	170:1
Douglas fir bark	491:1

2.3.1 Total nitrogen

Nitrogen is the seventh most abundant element in the universe. It’s the single most common element in the earth’s atmosphere, comprising about 78% of the gas that makes up our atmosphere. N is found in all soils, and is required by all living creatures. In plants, N is the nutrient required in the largest amounts. It is a key constituent of critical organic molecules such as amino acids, nucleic acids, and proteins. N is found in marine and freshwater and is present in some minerals. In short, N is found in every ecosystem and in every part of the global environment (Walworth, 2013).

Most soils contain inorganic N in the form of NH_4 and NO_3. The NO_2 also may be present, but the amount is usually too small to warrant its determination, except in cases where NH_4 or NH_4-forming fertilizers are applied to neutral or alkaline soils (Mulvaney, 1996). Until the 1950s, inorganic N was believed to account for <2% of total soil N, on the assumption that NH_4 and NO_3 are completely recovered by extracting soil with a neutral salt solution. The validity of this assumption was challenged by the finding that some soils contain NH_4 in a form that is not extracted by exchange with other cations (Rodrigues, 1954; Dhariwal and Stevenson, 1958; Stevenson and Dhariwal, 1959; Bremner and Harada, 1959; Schachtschabel, 1960, 1961; Young, 1962), and by estimates that the proportion of soil N in this form can exceed 50% for some subsurface soils (Stevenson and Dhariwal, 1959; Young, 1962).

Nitrogen is an important element for soil formation and development. It is considered as a unique element among nutrients. In the sense; that it has its origin in the atmosphere. It’s closely tied to OM, but rarely accumulates to a significant degree on soil exchange complexes (Johnson and Ball, 1996). With knowing that OM mineralization process plays an important role in the N cycle, it is responsible for the transformation of organic N present in plant tissues into simple inorganic forms (Franzluebbers et al., 1994). N availability along with low temperatures can control the productivity of ecosystems (Côté et al., 2000; Mäkipää et al., 1998). N in soil is an essential part of the OM or humus. Thus, the N content of a soil is indicative of the humus content and vice versa. N constitutes about 5 to 6% of the SOM by weight. The N in soil results from biological fixation and from accumulation of plant residues over a long period of time (Wells et al., 1997).

2.3.2 Soil organic carbon

Soil organic carbon is the C stored in SOM. Organic carbon enters the soil through the decomposition of plant and animal residues, root exudates, living and dead microorganisms, and soil biota. SOC as defined by (IPCC, 2006) comprises “organic carbon in soils to a specific depth chosen, also including live and dead fine roots within the soil”. SOC is one of the most important constituents of the soil due to its capacity to affect plant growth as both a source of energy and a trigger for nutrient availability through mineralization. SOC fractions in the active pool are the main source of energy and nutrients for soil microorganisms (Edwards et al., 1999). It improves the physical properties of soil, increases CEC and water holding capacity of sandy soils, and it contributes to the structural stability of clay soils by helping to bind particles into aggregates (Leeper and Uren, 1993).

Soil organic carbon is very interesting to study, as the soil is estimated to store twice as much C as the atmosphere, and three times that contained in the aboveground biomass (IPCC, 2001). Carbon in the soil is an important factor when studying global C budgets (Lehtonen et al., 2004; Berg and McClaugherty, 2003; Wilding et al., 2001; Liski et al., 2000). Soil organic carbon can act as a source or as a sink for CO_2 in the atmosphere (Fröberg, 2004; Högberg et al., 2002; Liski et al., 2000; Fisher and Binkley, 2000), and can be considered as the biogeochemical linkage between the other major C reservoirs: biosphere, atmosphere and hydrosphere (Wilding et al., 2001). The stock of SOC results from the balance between litter input and decomposition over time (Liski et al., 2002); while stored in the soil as humus and related stable organic compounds, the C is not circulating through the atmosphere (Berg and McClaugherty, 2003). As a part of the dynamic C cycle, SOC is linked to the development of vegetation (Liski et al., 2002; Nabuurs et al., 1997). Estimating its stock in reliable values is necessary for understanding the global C cycle, and estimating its spatial variability is also important when developing C budgets, explaining climate change and characterizing ecosystems (Davis et al., 2004).

2.3.3 Soil organic matter and its characteristics

The quantity of SOM can affect engineering and physicochemical properties of soil, which include specific gravities, water contents, liquid limits, plastic limits, bulk densities, the CEC, the hydraulic conductivities, and strengths.

Soil organic matter is defined as "it's an organic fraction of soils, which include plants, animals, and residues of microbes" (SSSA, 1979). As suggested by Stevenson (1994) SOM may include total organic matters in soil. By given a complexity of a SOM, it could be generally classified into two main categories: non living OM and living OM (Figure 2.1). Living OMs are the minor portions of SOM which consisted of soil biota like bacteria, fungi, algae, or undecomposed plant and animal residues. Non-living OM is the major fraction of a total organic component in a soil, which is a plant or animal residue at different stages of decompositions and transformations. Non living OMs are normally can be divided to humic substances and non-humic substances (Hayes and Swift, 1978). Non-humic substance refers to every recognizing plant and animal residues with each of the resemble classes of organic compounds in their original or transform phases. The organic compounds can be considered as amino acids "with including polypeptides" carbohydrates "which includes monosaccharides, oligosaccharides, and polysaccharides", and lipids "that include fat, wax, and resin" (Schnitzer and Kahn, 1972). Humic substance could refers to general categories of naturally occurring, biogenetic, and heterogeneous organic matters which can be easily characterized by black to yellow in colour, high molecular weight, and also can be refractory for degradations (Sparks, 2003). These substances can't be identified by belongs to the establish groups of organic compounds. They are classified into fulvic acids, humic acids, or humins.

Non humic matters can be easily offensive by micro-organisms in soils and also existed in soils for short periods of time (Schnitzer and Kahn, 1972, Sparks, 2003). Therefore, the term "soil organic matter" is generally used as an index for synonym of humus. Also, SOMs can refer to only these organic matters which follow soil particles that they are smaller than "2 mm" in size (SSSA, 1979).

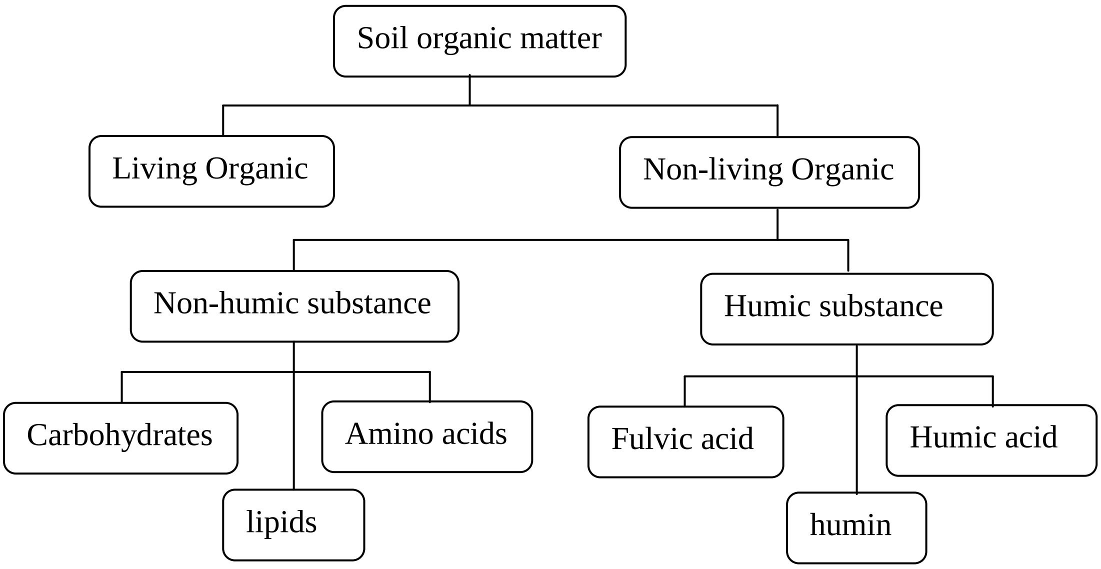

Figure 2.1 Classification of soil organic matter (Hayes and Swift, 1978).

2.4 Humic Substances

Humic substance consists of humic acids, fulvic acids, and humins. Fulvic acids are the coloured soil organic matter which can be soluble in any solutions either alkali or acid. Humic acids are the dark coloured of OM that they can only soluble in alkaline solutions. Humins are the SOM fractions which are insoluble in alkaline and will be remained after the extractions of the humic or fulvic acids with diluted alkaline. The distributions of those three fractions of SOM vary with the type and depth of soil. However, the elemental compositions may very similarly in those three fractions of SOM. Usually, the elemental composition includes C, H, N, O, and S. The typical rages of humic acid's elemental composition will be C (54-59%), H (3-6%), N (1-6%), O (33-38%), and S (0.1-1.5%). For fulvic acids, the typical rages will be C (41-51%), H (4-7%), N (1-3%), O (40-50%), and S (0.1-3.5%). For humins, this range could be much closed to ranges of humic acids. Table 2.2 shows the ranges of elemental compositions for humic acids in different soils (Schnitzer and Kahn, 1972). Besides the element compositions, group compositions are also used for characterizing humic substances because it's giving information about the chemistral and structural properties of humic substances (Purdue

1998, Tan et al. 2002). Fulvic acid contains more functional groups comparing with humic acids. The total acidity of fulvic acid is 900-1400 cmol $100g^{-1}$ which extremely higher than the acidity of humic acid which is ranged between 400-870 cmol $100g^{-1}$.

Humic and fulvic acids from different resources and from the same resource could be varied frequently in structures "e.g., the degrees of aromaticities per aliphalicities" (Wilson 1987). Indeed, humic acids can be produced from sequential extractions from the same sources that have been showed to have significant differences chemically and structurally (Kang and Xing, 2005). Humic and fulvic acids can be extracted in great quantities from humates, which includes an assortment of naturally occurring organic lithology with large content of humic substances (Simandl et al., 2001). Those substances including leonardites "oxidized lignite from a particular geologic deposit in North Dakota", weathered lignites, subbituminous coals, and a varieties of carbonaceous rocks like mudstone, shale, and claystone (Kohanowski 1957, 1970; Hoffman et al. 1993). Humates as raw ores and their extracts can be marketed and sold to the purpose of agricultural and horticultural community as a soil amendment or as a fertilizer. Figure 2.2 shows the fraction of humic substances and properties in soils which vary with the fractions (Swift, 1996). These properties may consist of molecular weights, C contents, O contents, acidities, CEC contents, N contents, or resemblances to lignins.

Table 2.2 Elemental composition of humic acids extracted from soils from widely different Climates (Schnitzer and Kahn, 1972)

Element (%)	Arctic	Cool, temperate		Subtropical	Tropical
		Acid soils	Neutral soils		
C	56.2	53.8-58.7	55.7-56.7	53.6-55.0	54.4-54.9
H	6.2	3.2-5.8	4.4-5.5	4.4-5.0	4.8-5.6
N	4.3	0.8-2.4	4.5-5.0	3.3-4.6	4.1-5.5
S	0.5	0.1-0.5	0.6-0.9	0.8-1.5	0.6-0.8
O	32.8	35.4-38.3	32.7-34.7	34.8-36.3	34.1-35.2

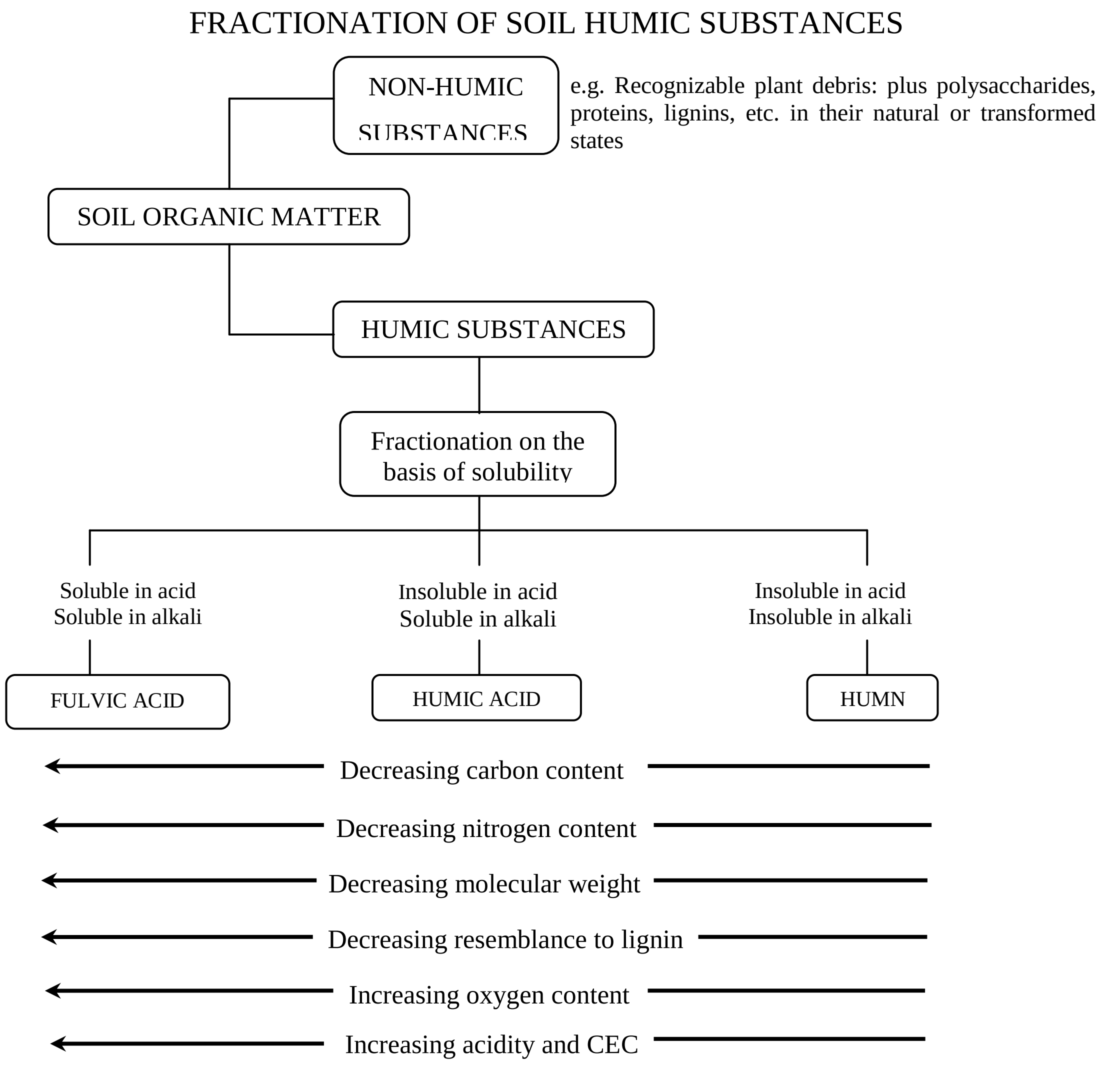

Figure 2.2 Property variations for three fractions of humic substance (Swift, 1996)

2.4.1 History of humic substances

The term "humus" comes from the Romans, when it was usually have been used for signifying the entire soil. Then the term have been used for denominating SOM and composts or to different parts of this OM in soils, as well as for complex made by the treatments of chemical agents for wide palettes of organic matters. The major definition of humus as a decomposed OM came from 1761 (Stevenson, 1982).

The first pertinent studies of the origins and chemical natures of humic substances were searched out by Sprengel (1839). His overall study on the acidic natures of humic acid was thought to be his most important benefits to the chemistry of humus. Researches on the chemical properties of humic substances were stretched by the Swedish researcher Berzelius, who major contributions were the isolations of two light yellow coloured humic matters from mineral water and slimy mud which rich in iron oxide (Berzelius 1839). Enormous progresses have been making during the last decades for modern physicochemical methods. Nevertheless, the structural chemistries of lignins or humic matters didn't advance very fast as the chemistry of animals who originated biopolymers.

The processes of the formations of humic substances have been studied carefully and for a long time period. Their formations are still a thing of long standing and continued research. Some theory has lasted for years, for example the "sugar amine condensations" theories, the "lignin" theories and the "polyphenol" theories. A demo of such theories could be established in monographs of Davies and Ghabbour (1999). Nowadays, the most implementers supposed that humic substances are formed from lignin (Oglesby et al., 1967).

Polyphenols came essentially from the lignin during its biodegradations. Possibly, it can play as a key role in the composition processes. Also, polyphenol is regarded as a main agent in the formations of humic substances from some plants which doesn't contain very high lignin and from non lignin consisting plants. Polyphenols could be accounted as a humic acid precursor. They themselves possess enough reactive sites to permit over and above transformations like some reactions of condensation.

The humic substance systems are made by the associations of various components which present in the humification processes, like amino acid, lignin, pectin and carbohydrate, during inter molecular forces. It's very clear that any mechanism of the formation of humic matters could be a bit different, depending on the geographical, climatic, physical or biological state of affairs. Those compounds could be composed by several ways, and the roles of lignin are important in the majority of those processes (Burdon, 2001; Davies et al., 2001). Burdon (2001) submitted that the humic of OM would consist generally of a mixture of plant and microbial constituents with the same constituents in various phases of decompositions.

2.4.2 Humic acid

A normal humic acid consists of polymeric brown to black organic acids which are ubiquitous in nature. Nearly, they can be found in all soils or water surfaces (Aiken, 1985; Berzelius, 1893; Davies, 1996; Hoppe-Seyler, 1889; Kononova, 1966; Stevenson, 1982). The chemical characteristics and physical properties of humic acids vary depending on the sources from which they have been extracted. Humic acid presents in soils are acknowledged to be important for the fertility of soil (Vaughan and Malcolm, 1985; Visser, 1986), and refractive to chemical and biological decompositions (Hedges and Oades, 1997), leading to their dominance at the OM found in soils. Humic acids are also precursors to or inclusions in many of the abundant of natural resources such as peats, bitumens, coals or petroleums (Janecek and Chalupa, 1969, Priegnitz, 1986; Reichert, 1966; Visser, 1973; Ziechmann, 1996).

The term "humic acid" was mainly used for describing the brown to black, polymer, alkali soluble organic acid fractions of humus, which can be found in geological sediment, soil, wetland, surface or underground water, and that more recently have been identified in assure living fungi or plant matters (Kuhnert et al., 1982).

Humic acids are firstly extracted in peat bogs in Germany by Achard (1786). Then, it was extracted in plant materials by Vauquelin (1797), later in soils by many investigators the first comprehensive study was published by Sprengel (1826). Sprengel extracted humic acids in alkali soils, the same method that Achard used for peats, and this have been the distinguished method ever since for extractions of humic acids. Oden (1919) redefined humic acids as yellow to brown to black to brown substance of unknown constitutions, which formed from nature by the decompositions of OM under an atmospheric affect or in the laboratories by chemical actions.

2.4.3 Fulvic acids

Fulvic acid is a part of humic substance that can soluble in water under all pH values, which is light yellow to yellow to brown in colour. It can remain in solutions after removing humic acids by acidification processes (Aiken et al., 1985). Fulvic acids may contain many reactive functional groups, which includes carbonyl, hydroxyl, phenol, quinon and semiquinone. Those functional groups can make fulvic acids a candidate to form both metal chelates and antioxidant activities (Murray and Linder, 1983). Fulvic acid is an active principle in humic substances that can absorb mineral ions from soil to be fixed in vegetation tissues at large proportions (Navarrete et al., 2004; Navarrete et al., 2005).

Biologically, fulvic acids are more active than humic acids. It contains more O, less C and its noteworthy more acidic than humic acid. Fulvic acids have much lower molecular weights, provides more potential for mobility within the plants, which in turn eases a greater affect on metabolic process. It's far more multilateral than humic acids, and biologically they are the most valuable input in agriculture (HGS, 2011).

2.4.4 Humification process

Deactivated vegetations represent the major sources of OM that transforms to humic substance in the environments. Humification is a continuous historical process, which decomposes remains and makes humus. Humus is a dynamic system of each of chemically active and passive components (Gonzalez et al. 2003). Chemically, humification is a microbiological process of transformation of dead residue to humus or humic substance (Hedges and Oades, 1997). Each of degradations and synthetic processes through decaying of living OMs can be described as humification process. In general, it means transformation of many groups of substances such as protein, carbohydrate, and lipid, and individual molecule present in living OMs into the groups of substances with similar properties, and, finally, into the compounds of mineral carbon (Francioso et al., 2003).

2.4.5 Benefits and effects of humic substances on soil

The OM of soil generally consists of humic and fulvic acids that called humic substances (Schnitzer, 1982; Andriesse, 1988). It was recorded that these organic complexes can affect on chemical, physical and biological properties of any soil (Vaughan and Linehan, 1976; Boyle et al., 1989; Schnitzer, 1992; Khattak, and Muhammad, 2008).

Humic substance could modify soil properties by making them more fragile, by buffering pH, by raising soil water holding capacity, by convention with trace metals and making them more available to plants, and by releasing the bounds of nutrients such as phosphates from clay, by keep sandy soils from the process of leaching (Stevenson, 1982; Aiken et al., 1985; Jardine et al., 1989; Spark et al., 1997a). Because of the tenacity nature of humic substance, it also can control the release rates of C into the atmosphere. Also humic substances can affect on the growth of plants and crop yields. In the additions, the stimulation of ion uptakes with applications of humic substances leads many investigators to propose that these substances can affect membrane permeability (Zientara, 1983).

2.4.6 Relation between C/N ratio and humic substances

Carbon and nitrogen can be used as a source to form humic substances, and the relation between them is considered as an index for humification processes (Brady, 1990; Miller and Gardiner, 1998), because of the specific activities of microbes in an aerobic and acidic environment, and also enrichment of the mass of organic soils with N compounds of bacterial origins (Borgmark, 2005). This ratio can be used as a measure of the degradation levels of organic soils. Decreasing in this ratio could denote increases in the decomposition process of organic soil "due to microbial activity" and vice versa. It is interesting to note, that soil fulvic acid has a higher C/N ratio in comparison to humic acids that ranged between 18.4 and 37.8 (Anderson and Hepburn, 1986). The N content is approximately 2 to 3 times higher in humic acids than in fulvic acids, which might indicate that the N fixation could increase with increasing the process of humification from fulvic acids to humic acids (Kim, 2003).

Generally, C/N ratio of humic substances might fall to nearly stable value. Carbon content of 50 to 57% and N content of 4 to 5% can give C/N ratio between 10 and 14. This range is considered as a good range for well developing humic acids in soil. The stored N in the humic molecules will be released again after the decomposition and mineralization process of the humic substances (Kim, 2003).

3. MATERIALS AND METHODS

3.1 Study Area

To study the effects of C/N ratio on organic soil properties, numerous samples were taken at a depth of approximately 0 to 20 cm from four fields from Sağlık Plain, Kahramanmaraş. The fields, in which the soil samples were collected are located in "37,330,612 – 37,323,186 N" and "36,844,764 – 36,841,320 E" (Table 3.1). The soils samples, then, were air-dried and sieved through 2 mm prior to laboratory analysis. The physical and chemical soil analyses run on soil samples are given below.

Table 3.1 GPS coordinates of the point where the soil sample taken Sağlık Plain.

Sample No.	Coordinates		Sample No.	Coordinates	
	E	N		E	N
1	37,330,612	36,844,764	26	37,326,935	36,843,568
2	37,330,476	36,844,764	27	37,326,833	36,843,509
3	37,330,309	36,844,801	28	37,326,701	36,843,514
4	37,330,126	36,844,828	29	37,326,577	36,843,530
5	37,329,934	36,844,850	30	37,326,411	36,843,552
6	37,329,815	36,844,860	31	37,326,231	36,843,578
7	37,329,631	36,844,877	32	37,326,022	36,843,605
8	37,329,452	36,844,898	33	37,325,860	36,843,611
9	37,329,307	36,844,914	34	37,325,681	36,843,621
10	37,329,183	36,844,925	35	37,325,532	36,843,643
11	37,329,064	36,844,941	36	37,325,400	36,843,653
12	37,328,940	36,844,952	37	37,325,263	36,843,680
13	37,328,799	36,844,946	38	37,325,144	36,843,696
14	37,328,706	36,843,342	39	37,324,896	36,841,282
15	37,328,578	36,843,364	40	37,324,756	36,841,288
16	37,328,420	36,843,385	41	37,324,585	36,841,277
17	37,328,236	36,843,412	42	37,324,427	36,841,277
18	37,328,087	36,843,428	43	37,324,273	36,841,245
19	37,327,925	36,843,444	44	37,324,120	36,841,245
20	37,327,801	36,843,471	45	37,323,962	36,841,245
21	37,327,639	36,843,482	46	37,323,783	36,841,256
22	37,327,477	36,843,493	47	37,323,634	36,841,272
23	37,327,332	36,843,509	48	37,323,476	36,841,293
24	37,327,196	36,843,535	49	37,323,341	36,841,293
25	37,327,076	36,843,557	50	37,323,186	36,841,320

3.2 Soil pH and EC

100 g of soil sample was weighed into a plastic container. Distilled water was added to the soil sample by using burette until the soil became saturated by using a spatula. Saturation pastes were allowed to stand overnight. Then, pH was measured by pH-meter

by glass electrode, and EC was measured by adding the soil paste into the conductance meter YSI model 32 (Black, 1965).

3.3 Total Lime

Approximately, 0.5 g of soil sample was placed in a reaction bottle. A plastic vial was inserted into the bottle with the aid of a pipette, and 5-10 ml of diluted HCl in the pipette was added into the vial. The monometer of the calcimeter was adjusted to set zero, and the rubber cap at the end of the plastic tube which is connected to the monometer was tightly fastened to the reaction bottle in a way that it formed a seal. Then, the sample in the reaction bottle and acid in the vial were mixed and shaken vigorously to allow the reaction between acid and soil particles. Shaking continued until the gas release had stopped. The volume of CO_2 gas released at the monometer was recorded (Vt). The temperature and atmospheric pressure in the laboratory were also recorded. The real gas volume (Vo, at 0°C and 760 mmHg) was calculated by using Boyle-Mariotto formula (Gülçur, 1974).

$$Vo = \frac{Vt * (b - e) * 273}{760 * (273+T)}$$

$$CaCO_3\ \% = \frac{V_0 * 0.4464}{A} * 100$$

Vo = Gas volume converted at normal conditions (cm3).

Vt = Gas volume read on monometer (cm^3).

b = Recovered Barometer pressure (mmHg).

e = Vapor pressure of water at ‘t’ ºC (mmHg).

t = Temperature (ºC).

A = Weigh of soil sample (g).

3.4 Cation Exchange Capacity (CEC)

Sodium acetate was used to determine CEC of soil samples (Chapman, 1965). Soil samples were shaken with $C_2H_3NaO_2$ solution resulting in replacement of cations on the colloidal surfaces with added Na. Subsequently, samples were washed with ethyl alcohol. Ammonium acetate was then added, which replaces the adsorbed Na with NH_4^+. The concentration of displaced Na was determined by Inductively Coupled Plasma (ICP).

- **Extraction reagents**

- Sodium acetate 1 *N* (NaOAc): 136 g of $NaC_2H_2O_2.3H_2O$ is dissolved by D.W. in a 1 L conical flask. pH was adjusted to 8.2 by using diluted acetic acid or NaOH solutions.
- Ethyl alcohol (%99).
- Ammonium acetate 1 *N* (NH_4OAc): 57 ml of glacial acetic acid (%99.5) is diluted with D.W. to a volume of approximately 500 ml. Then, 69 ml of concentrated ammonium hydroxide (NH_4OH) is added. pH was adjusted by using NH_4OH or acetic acid, and the volume was filled to 1 L with D.W.

- **Procedure**

4.0 g of soil sample and 33 ml of 1 *N* NaOAc were added into a 50 ml plastic tube. The tube was, then, shaken for 5 minutes on a mechanical shaker at normal speed (RPM=175). The samples were centrifuged for 5 minutes, and the supernatant was discarded. The processes were repeated two more times. Then, very same processes were repeated using 33 ml of 99% ethyl alcohol instead of 1 *N* NaOAc. Finally, the procedure was run with 33 ml of 1 *N* NH_4OAc but this time the supernatants were decanted into a 100 ml volumetric flask, and the solution was filled up to the volume by ammonium acetate solution. Cation exchange capacity of soil was determined measuring Na in the filtrate by using Inductively Coupled Plasma (ICP) and converting it to centi mole charge per kg of soil.

3.5 Phosphorus in Soil

0.5 N $NaHCO_3$ method employing an UV-VIS spectrophotometer was used to determine the concentration of phosphorus in organic soil (Olsen et al., 1954).

- **Ascorbic acid reagents**

- 14 ml of concentrated sulfuric acid (H_2SO_4, 2.5 *M*) is diluted into 100 ml volumetric flask with D.W.
- 2 g of ammonium molybdate tetrahydrate $[(NH_4)_6Mo_7O_{24}.4H_2O]$ dissolved in 50 ml D.W.
- 0.1314 g of antimony potassium tartrate $[K(SbO).C_4H_4O_6.\frac{1}{2}H_2O]$ is dissolved in 50 ml D.W.
- 1.76 g of ascorbic acid $C_6H_8O_6$ (0.1 *M*) is dissolved in 100 ml D.W.

- Mixed reagent: 100 ml of sulfuric acid, 30 ml ammonium molybdate tetrahydrate solution, 60 ml of ascorbic acid, and 10 ml of antimony potassium tartrate solutions were mixed thoroughly

- **Extraction reagent 0.5 *N* $NaHCO_3$**

42.0 g of sodium bicarbonate ($NaHCO_3$) is weighed and dissolved in 1 L D.W. pH was adjusted to 8.5 by using either 50% NaOH or 0.5 *N* HCl.

- **Procedure**

2.0 g of soil sample and 40 ml of extraction reagent were added into 50 ml plastic tube. The mixture was shaken for 30 min (RPM= 165), and filtered through Whatman no.42 filter paper. Then 3 ml of filtrate solution and 5 ml of ascorbic acid solution were added into 25 ml volumetric flask and filled to volume with D.W., the mixture was measured by Optima SP-3000 spectrophotometer at 880 nm.

3.6 Organic Matter of Soil

The Walkley-Black method was used for determining OM in soil samples (Nelson and Sommers, 1996).

- **Reagents**

- 49.04 g of potassium dichromate $K_2Cr_2O_7$ (0.167 *M*, 1 *N*, dried at 105^oC) was weighed into 1000 ml volumetric flask, dissolved and brought the volume to 1000 ml with D.W.
- Concentrated of sulfuric acid H_2SO_4 (not less than 96%).
- 0.16 g of barium diphenylamine sulfonate ($C_{24}H_{20}BaN_2O_6S_2$) is dissolved in 100 ml D.W. as an indicator.
- 140 g of ferrous sulfate hepta-hydrate ($FeSO_4.7H_2O$, 0.5 *M*) was dissolved with D.W. in 1000 ml volumetric flask, 15 ml of concentrated sulfuric acid H_2SO_4 was added, and filled to volume with D.W.

- **Procedure**

0.5 g of soil sample, which was sieved to pass 100 μm and 10 ml of potassium dichromate were added into a 500 ml conical flask. The flask was gently swirled. 20 ml of concentrated H_2SO_4 was added. The mixture was heated for 1 minute. Then, the flask was allowed to cool for 10 minutes. 200 ml of D.W. and 10-15 drops of diphenylamine indicator were added to the flask. Finally, the solution was titrated with 0.5 M ferrous sulfate. During the titration, the color of solution was changed from brown to dark green color. The blank sample was also determined in the same manner to standardize potassium dichromate.

meq OC= 10 * (1-A/B)

% O.M= (meq OC/W)*0.003*2.238*100

A= Volume of $FeSO_4.7H_2O$ that added to soil samples

B= Volume of $FeSO_4.7H_2O$ that added to blank

W= Weight of soil samples

3.7 Total Nitrogen

The Kjeldahl procedure was used for determining total N in organic soil (Bremner, 1996). Nitrogen in the organic nitrogenous compounds is converted into ammonium during digestion stage. The NH_4^+ ions in the distillate are volatilized by distilling with NaOH. The liberated NH_4^+ is absorbed in boric acid and back titrated with a standard H_2SO_4. Potassium sulphate is added to raise the boiling point of the mixture during digestion and copper sulphate and selenium powder mixture is added as a catalyst. The procedure determines all soil N with the exceptions of nitrate and nitrite-N.

- **Extraction reagents**

- Concentrated H_2SO_4 (96%)
- Diluted H_2SO_4: 2.77 ml of H_2SO_4 is diluted with D.W. in 1000 ml volumetric flask
- Kjeldahl tablets: Consisted of 100 parts of Na_2SO_4 or K_2SO_4, 10 parts of Copper Sulphate ($CuSO_4.H_2O$), and 1 part selenium powder.
- Sodium Hydroxide (%40): 400 g of NaOH is dissolved with D.W. in 1000 ml volumetric flask (for distillation procedure).
- Sodium Hydroxide (%20): 200 g of NaOH is dissolved with D.W. in 1000 ml volumetric flask (for digestion procedure).
- Mixed indicator: 0.099 g of brome-cresol green and 0.066 g of methyl red is dissolved in 100 ml of 95% ethanol.

- Boric acid solution (%2): 20 g of boric acid is weighed into 1000 ml volumetric flask, and D.W. is added, and shaken until it dissolves. Then, 20 ml indicator is added to solution and filled to 1000 ml volume with D.W.

❖ **Procedure**

0.5 g of soil sample was added into the digestion tubes. One Kjeldahl tablet (2 g of catalyst mixture), and 15 ml of concentrated H_2SO_4 were placed into the each tube. Digestion tubes were placed into the preheated digestion blocks, and the samples were digested for 3 hours at 375^oC. The samples, then, were allowed to cool, and 20 ml of D.W. was added to the each tube. For distillation of the samples, 25 ml of boric acid was poured into a 250 ml Erlenmeyer flask and placed into the condensation end of the unit to capture volatile NH_3. A digestion tube was placed to steam-distillation unit, and the machine was set to pour adequate amounts of NaOH and water for volatilization of ammonium in the tube, and, then, distillation started. Finally, in the third stage of the N analysis, captured ammonia in boric acid was titrated with 0.1 N of H_2SO_4. The end color was from green to pink.

$$\%N = \frac{(S - B) * N * meq}{W} * 100$$

S = ml of H_2SO_4 required for titration of sample

B = of H_2SO_4 required for titration of sample

N = Normality of H_2SO_4 (0.1 *N*)

Meq = Meq. Weight of N (0.014)

W = Weight of soil sample

3.8 Plant available Macronutrients (K, Ca, Mg and Na)

1 *N* of ammonium acetate NH_4OAc (pH 7.0) is used to determine K, Mg, Ca, and Na (Helmke and Sparks, 1996) using Inductively Coupled Plasma (ICP).

❖ **Reagents**

57 ml of glacial acetic acid CH_3COOH (99.5%) and 69 ml of concentrated ammonium hydroxide (NH_4OH) are mixed in 1L volumetric flask which contained at least 0,5 L distilled water. (or: 77.1 g of NH_4OAc is weighed and dissolved in 1L D.W.). The pH of the extraction solution was adjusted to 7.0 with using either 3 *N* acetic acid

(CHCOOH) or 3 *N* ammonium hydroxide (NH_4OH). Then the mixture was filled to the volume with D.W.

- **Procedure**

4.0 g of soil sample was weighed into 50 ml plastic tube, and 40 ml of extraction solution was added. The solution was shaken for 1 hour on a mechanical shaker at a medium speed (RPM = 175) and, then, filtered through Whatman No. 42 filter paper. Finally, the levels of extractable K, Ca, Mg and Na in the filtrate were determined by Inductively Coupled Plasma (ICP).

3.9 DTPA-TEA Extractable Micronutrients (Cu, Mn, and Zn)

The DTPA-TEA extraction method that was developed by Lindsay and Norvell (1978) for extracting metal micronutrients in neutral and calcareous soils was used for measuring plant available Mn, Cu, and Zn by using Inductively Coupled Plasma (ICP).

- **Reagents**

The DTPA (Diethylene Triamine Pentaacetic Acid $C_{14}H_{23}N_3O_{10}$) solution consists of 0.005 *M* DTPA, 0.01 *M* $CaCl_2$, and 0.1 M TEA (Triethanol amine). 14.92 g of TEA, 1.967 g of DTPA, and 1.47 g of $CaCl_2.2H_2O$ were dissolved in 1000 ml D.W. The pH of the solution was adjusted to 7.3 with 1.0 *M* HCl.

- **Procedure**

20 g of soil sample was weighed into 100 ml Erlenmeyer flask, and 40 ml of the DTPA-TEA extraction solution was added to the flask. All flasks were covered by stretch parafilm and shaken on a mechanical shaker for 2 hrs at a medium speed (RPM= 175). The mixture was filtered through Whatman No. 42 filter paper. The amounts of Cu, Mn, and Zn were determined by Inductively Coupled Plasma (ICP).

3.10 Humic Acids

Three different methods were used to determine humic acids, Turkish Standard Institute (TSE) method (Anonymous, 2003), Mesa Verde Resources (MVR) method (Bruce, 1999), and California Department of Food and Agriculture (CDFA) method (Page, 1982).

3.10.1 Turkish Standard Institute (TSE) method

This method shares the same methodology with the Walkley Black method that is used to determine organic matter in a soil (Anonymous, 2003).

- **Extraction reagents**

- 15 g of sodium pyrophosphate and 7 g of sodium hydroxide were dissolved with D.W. in 1000 ml volumetric flask.
- Concentrated sulphuric acid (H_2SO_4 96%)
- 2 g of potassium dichromate $K_2Cr_2O_7$ is dissolved in 100 ml flask with D.W.
- Indicator: 0.75 g of phenanthroline, and 0.5 g of ammonium ferrous sulphate were dissolved in 50 ml flask with D.W.
- $(NH_4)_2Fe(SO_4)_2.6H2O$: 40 g of ammonium ferrous sulphate is dissolved in 1000 ml volumetric flask with D.W.

- **Procedure**

0.5 g of soil sample and 150 ml sodium pyrophosphate were placed into a 250 ml erlenmeyer flask. The flask was placed into a water bath for 2 hours at 80^oC (RPM=100). After bathing, the samples were filtered through Whatman No. 42 filter paper. 5 ml of filtrate was transferred into 250 ml Erlenmeyer flasks. 5 ml of $K_2Cr_2O_7$ and 15 ml concentrated H_2SO_4 were also poured into the flask. The flasks were put into the bath water again for 30 minutes at the same temperature 80^oC (RPM=100). Samples were allowed to cool for 5 minutes. Then, 100 ml of D.W, and 15 drops of indicator were added. Finally, solutions were titrated with ammonium ferrous sulphate. The color change was from green to red.

$$\%H.A = \frac{(V_0 - V_1) * meq * N}{0.59 * W} * \frac{V_e}{V_a} *100$$

V_0 = ml of $(NH_4)_2Fe(SO_4)_2 .6H_2O$ required for titration of blank

V_1 = ml of $(NH_4)_2Fe(SO_4)_2.6H_2O$ required for titration of sample

meq = meq. weight of C (0.003 g)

N = Normality of pyrophosphate (0.1)

Ve = volume of the extraction solution that was added to the soil (150 ml)

W = Weight of sample

Va = volume of filtrate (5 ml)

3.10.2 Mesa Verde Resources (MVR) method

Similar to Olsen et al. (1954), colorimetric method was used to determine humic acids (Bruce, 1999).

- ❖ **Extraction reagents**

- In a 2 liter volumetric flask with 500 ml of D.W H_2O, 80g of NaOH with 8ml ethanol added and brought to volume.
- Weigh out 0.1075 g of Humic Acid and diluted to 100 ml with extraction solution. Shaken for 1 hour (standard stock solution).

- ❖ **Procedure**

2 g of sample is weighed and transferred to a 50 ml centrifuge tube. A sufficient amount of the extraction solution was added and thoroughly mixed on a mechanical shaker for one hour, and centrifuged at 2000 nm for 30 minutes. The supernatant was taken (diluting with distilled water if necessary). Aldrich humic acid prepared from a standard stock solution (1000 ppm), in 100 ml conical flask 5, 10, and 20 ml were taken from standard stock solution to get 50 ppm, 100 ppm, and 200 ppm, respectively. Set spectrophotometer at 450 nm. Standard solutions and samples were read (Used D.W as blank).

3.10.3 California Department of Food and Agriculture (CDFA) method

This method is to be used for analyzing humic acid of solid samples containing a minimum of 0.5%, and liquid samples containing a minimum of 0.5% of humic acids (Page, 1982).

- ❖ **Extraction reagents**

- Concentrated Hydrochloric Acid HCl
- 1% Sodium Hydroxide NaOH
- 0.5 *N* Sodium Hydroxide NaOH

- ❖ **Procedure**

Weighed 0.5 g of soil sample into a centrifuge bottle, and 50 ml of 0.5 *N* NaOH is added. Shaken for 1.5 hours, and centrifuged for 20 minutes at 2000 rpm. The supernatant is decanted into second centrifuge bottle (previously weighed). To first centrifuge bottle, 10 ml of 1% NaOH was added. Stirred well and centrifuged for 20 minutes at 2000 rpm. Combine second supernatant with first by decanting into second centrifuge bottle. To the combined extracts in the second bottle, added approximately 10 ml of concentrated HCl (to lower pH ≤ 1). Samples were centrifuged again for 20 minutes, and carefully discarded the liquid. 25 ml of D.W (previously adjusted the pH ≤ 1 with HCl) is added to sample. Shaken and centrifuged again, and carefully discarded the liquid. The bottles were dried overnight at 100–110 ^oC. Samples were cooled in desiccators for 2-3 hours, and weighed.

$$\% \text{ Humic acid} = \frac{\text{Wt. of dried precipitate}}{\text{Wt. of sample}} * 100$$

3.10. Statistics

In order to measure the effect of C/N ratio on soil properties, data set were divided into three almost equally numbered groups based on the calculated C/N ratio values that were sorted from lowest to highest and, then, data set were divided into the groups (Table 3.2). The group 1 had the lowest C/N ratios, and the group 3 had the highest among the three. One way analysis of variance and Duncan’s multiple range test were used to test the differences for the measured properties of the soils among the different C/N ratio groups. The differences were accepted as significant if the p value was smaller than 0.05 ($p < 0.05$). All statistics were performed using SPSS (1998) software.

Table 3.2 The grouping of the data set based on C/N ratios

C/N Groups	C/N Ranges	Number of soils in each group
Group 1	8.99-10.29	17
Group 2	10.30-11.70	17
Group 3	11.71-13.20	16

4. RESULTS AND DISCUSSION

The summary statistics of the measured soil attributes are given in Table 4.1. The organic matter levels ranged between 21.50 and 52.70%, and the mean value was 39.32%. The numbers show a medium range of variability in organic soils. Organic carbon, of course, followed similar trends and changed between 12.50 and 30.60% with a mean value of 22.81%. The variations in organic matter and carbon can be an indicator of degradations in organic soils. Leeper and Uren (1993) reported organic matter values between 27 and 33% for peat soils they studied. Total nitrogen values were between 1.39 and 2.55%, and the mean value was 2.05%. Kaila (1958) and Scheffer (1976) reported that organic soils they studied contained between 0.5 to 2.6% nitrogen in the top 20 cm. soil. Soil C/N ratio which is one of the best indicator of organic matter degradation ranged between 8.99 and 13.20, and the mean value was 11.00.

Table 4.1 The summary statistics of the some properties of organic soil

	Minimum	Maximum	Mean	Std. Deviation
O.M (%)	21.50	52.70	39.32	9.16
C (%)	12.50	30.60	22.81	5.31
N (%)	1.39	2.55	2.05	0.33
C/N	8.99	13.20	11.00	1.18
H.A/TSE (%)	6.00	15.20	10.30	2.40
H.A/MVR (%)	1.67	5.23	3.33	1.27
H.A/CDFA (%)	2.58	30.57	16.56	7.74
pH	6.80	7.53	7.22	0.25
EC (dS m^{-1})	1.78	3.70	3.19	0.42
CEC (cmolc kg^{-1})	35.20	90.10	68.37	10.93
Lime (%)	2.10	4.80	3.77	0.67

Soil C/N ratio is a sensitive indicator of soil quality (Zhang et al., 2011), and C/N ratio also serves as a tool for understanding the sources of organic matter which can lead to information about the ecology, climate, and ocean circulation at different times in the Earth’s history (Ishiwatari and Uzaki, 1987). Average C/N ratios vary from soil to soil depending on the predominant soil type, and Alistair (1979) reported that C/N ratio between 8 and 17 is typical for soils. High C/N ratio can immobilize and low C/N ratio can mineralize nitrogen in soils. Tate (1995) and Bengtston et al. (2003) considered C/N ratio of 20 to be a threshold point where either net nitrogen mineralization or net nitrogen immobilization occurs. Pansu et al. (1998) reported C/N ratios from 10 to 12 for the uncultivated soils in regions with a temperate climate. Caravaca et al. (2002) found that in forest soils, peat horizons, or podzols, C/N ratios can reach 20–30 or even higher because of the formation of only slightly biodegradable complexes which are low in nitrogen. Berg and McClaugherty (2003) reported that the C/N ratio in the humus layer is positively influenced by the stand volume; the higher the volume, the larger ratio in the humus. Also, in cultivated soils, farming residues recycled in the field have C/N ratios ranging between 15 and 60 due to the presence of lignin-cellulose compounds with a slow rate of degradation. Under forest with acidifying litter, the C/N ratios can reach 150 or even higher (Pansu et al., 2001).

Humic acid contents of the organic soils were measured using three different methods (TSE, MVR, and CDFA methods). These different methods measured different humic acid contents. Turkish Standard Institute (TSE) method is based on the determination of organic carbon by wet oxidation in the extracted humic and fulvic acids (Anonymous, 2003). This method extracted humic acid content between 6.00 and 15.20%, and these values are in agreement with the findings of Bozkurt (2005) who reported 5 to 15% humic acids content for the peat soils he studied. Çelik (2003), however, found higher humic acid levels (15-30%) in his study.

The second method, Mesa Verde Resources (MVR), is a colorimetric method. This method measured humic acids between 1.67 and 5.23%. This method generally gives lower humic acid contents compared with the other two methods because this method only measures humic acid content not both humic and fulvic acids together.

The third method was California Department of Food and Agriculture (CDFA) method. The method originally measures humic acid in organic matter. In our study it was expressed as percent humic acid in soil. The values ranged between 2.58 and 30.57%, and the mean value was 16.56%. Similar and higher humic acid contents for peat soils (15-42%) reported elsewhere (Anonymous, 1996; Lobartini et al., 1997). Humic acid contributes to the formation and stabilization of soil aggregates, as well as adding to the nutritional value of the soil. They increase water holding capacity, decrease soil bulk density, control retention and release of micro and macro-nutrients and are involved in carbon, nitrogen, and phosphorus cycles (Kononova, 1966). They act as buffers that regulate pH and bind many metal ions (Guminski et al., 1983; Vaughan and McDonald, 1971) in such a way that they are easily available for uptake by the roots of plants.

Synthetic fertilizers that are rich in nitrogen and phosphorus are commonly used but public pressure is growing to use organic fertilizers such as humic acids (Faust, 1996). Humic acids are known to be important for soil fertility (Vaughan and Malcolm, 1985; Visser, 1986), and refractive to chemical and biological decomposition (Hedges and Oades, 1997) leading to their dominance at the organic matter found in soils. Also, according to MacCarthy et al. (1990) humic acids are an active component of soil and aquatic organic matter and they are important for agriculture.

The pH values of the organic soils ranged between 6.80 and 7.53, and the pH value was 7.22. Similar (Bascomb, 1964), lower (Ponnamperuma, 1972; Collins et al., 1997), and higher pH values (Bridgham and Richardson, 1993) were reported for organic soils. Histosols in Alaska ranges from alkaline (pH >7.4) on the arctic coastal plain to slightly acid (pH < 6.6) on the arctic foothills, and ranges from slightly acid to strongly acid (pH 6.5 to 5.1) in the boreal zone (Riger et al. 1979).

High electrical conductivity measurements indicate that salinity is a problem in the organic soils. The CEC values ranged between 35.20 and 90.10 cmolc kg^{-1}, which are typical for organic soils. The lime content of the soils was low and less than 5%.

Table 4.2 Summary statistics of macro and micro-nutrients in organic soil

	Minimum	Maximum	Mean	Std. Deviation
P (mg kg^{-1})	6.01	11.27	8.41	1.30
Ca (mg kg^{-1})	6634.87	10904.50	8941.64	1046.09
Mg (mg kg^{-1})	2994.80	5426.08	4183.30	554.57
K (mg kg^{-1})	32.27	120.88	63.96	19.84
Na (mg kg^{-1})	36.30	60.09	47.43	5.56
Cu (mg kg^{-1})	0.42	1.27	0.91	0.15
Mn (mg kg^{-1})	2.88	11.48	7.61	2.08
Zn (mg kg^{-1})	1.58	3.38	2.54	0.44

Table 4.2 reveals that the values of analytical data of nutrients are within the normal ranges encountered in organic soils except for magnesium that was high in soil samples. The mean concentrations of this metal in soil samples was 4183 mg kg^{-1} Mg, while its concentration level normally encountered in remote or recently settled area soils is reported as 480-1500 mg kg^{-1} (Bridgham and Richardson, 1993). This is an indication of enrichment of this metal in organic soils of the study area. Most soil phosphorus including more than 95% in organic soils is in the organic form with cycling among P-forms

controlled by biological forces (Bridgham and Richardson, 1993). The organic soil of the study area was low in available phosphorus content, even lower than the results reported by Pote et al. (1996) who measured a mean value of 44 mg kg^{-1} extractable phosphorus in Wisconsin soil. The calcium content of the organic soils was higher than the results of Graham (1959) who found 4900 mg kg^{-1} of calcium in New Jersey soils. On the other hand, the mean concentration of potassium was lower than the results of Bolt et al. (1963) who found 94 mg kg^{-1} K in their soil samples. Soils contain low amount of sodium. This range was also lower than that reported by Rengasamy and Churchman (1999) who found 63 mg kg^{-1} Na in an organic soil. Soils are the reservoir for many harmful constituents, elemental and biological, including heavy metals and trace metals, henceforth referred to as metals (Cottenie and Verloo, 1984). Total metal content of soils is useful for many geochemical applications but often the bioavailability of these metals is more of a concern agriculturally in terms of what is biologically extractable (Cottenie et al., 1982). For this study, the DTPA procedure was examined to determine availability of micro-nutrients. The mean results of DTPA method for Mn, Zn, and Cu were 7.61, 2.54, and 0.91 mg kg^{-1}, respectively. The values were lower than that reported by Singh et al. (1997) who measured 12.36 mg kg^{-1} Mn, 5.8 mg kg^{-1} Zn, and 2.7 mg kg^{-1} Cu, respectively. Jackson (1973) found similar results of micronutrients in India, except for zinc, that was measured 5.5 mg kg^{-1}.

Table 4.3a The effect of C/N ratio on some soil properties

C/N Groups	%O.M	%C	%N	pH	EC dS m^{-1}	CEC $cmol_c$ kg^{-1}	Lime%	%H.A TSE	%H.A MVR	%H.A CDFA
1	31.02c*	18.00c	1.85b	7.01c	2.93b	59.15c	3.43b	8.70b	2.27c	9.76c
2	38.79b	22.49b	2.05b	7.22b	3.17b	69.03b	3.58b	10.02b	3.07b	15.38b
3	48.72a	28.26a	2.28a	7.43a	3.47a	77.46a	4.32a	12.28a	4.75a	25.05a

*Values not sharing the same letter are statistically different at $p < 0.05$.

Table 4.3b The effect of C/N ratio on some soil properties (Continued)

C/N Groups	P mg kg^{-1}	Mg mg kg^{-1}	Ca mg kg^{-1}	K mg kg^{-1}	Na mg kg^{-1}	Mn mg kg^{-1}	Zn mg kg^{-1}	Cu mg kg^{-1}
1	7.79b*	4006.05b	8708.78	57.14	45.52b	6.43b	2.21b	0.83b
2	8.08b	4065.07b	8710.25	63.97	46.13b	7.45b	2.60a	0.91ab
3	9.44a	4497.25a	9434.90	71.20	50.85a	9.04a	2.84a	1.00a

*Values not sharing the same letter are statistically different at $p < 0.05$.

There was a trend for the plant available nutrients to increase from C/N ratio group 1 to 3, these increases were statistically significant between group 3 and the other two groups for extractable P, Mg, Na, and Mn (Table 4.4). The differences were significant for group 1 and the groups 2 and 3 for DTPA extractable Zn, while the differences between groups 1 and 3 were significant for DTPA extractable Cu. There were no significant differences among the groups for plant available Ca and K.

Hammond (1968) studied two types of Ireland peat soils (Bogs, and fen soils). His experiments showed significant differences between soil C/N ratio and exchangeable K, Ca, Na, Cu, Mn, and Fe, and no significant differences with CEC, total lime, soil available Mg, P, and Zn with some other properties. Borgmark (2005a) studied humification process, and he used three different methods to analyze humic acids, which included IHSS, MVR, and CDFA Methods. He detected that C/N ratio has significant differences with all methodologies. The results of Rodhe and Seibert (1999) showed same significant differences among C/N ratio and the value of pH, OM content, CEC, and organic carbon, except for N that he found no significant differences with C/N ratio in organic soils.

As we expected, C/N ratio can influence some properties and nutrient availabilities of organic soils. Increases in this ratio significantly increase organic C and organic matter content in soils, which is consistent with the report of higher organic matter in peaty soils by Nelson and Sommers (1982). In addition, this increment could affect on the decomposition of organic matter by limiting the soil microbial activity abilities, higher C/N ratio lower decomposition process. C/N ratio could serve as an indicator for nitrogen limitations of plants or other organisms, and serves like a tool to understand the sources of organic matter that can drive to information on ecologies, climates, and ocean circulations at different times of the Earth’s history (Ishiwatari and Uzaki, 1987).

C/N ratio can affect pH and CEC values in the soil, increasing this ratio could change the soil to alkali, while decreasing make soil more acidic. Tate (1979) reported positive relationship between C/N ratio and pH value, higher the C/N ratio higher the soil pH. Cation exchange capacity depends on pH value, because carboxyl and phenol groups could accept protons under acidic conditions. C/N ratio increased pH value as well as CEC values in soil samples. The results of CEC were in line with Leeper and Uren (1993) who noted that C/N ratio can raise CEC value.

It has been shown that C/N ratio is an index for humification process (Brady, 1990; Miller and Gardiner, 1998). The value of 10-14 of C/N ratio is considered as a good range for well developing humic acids in soil. As C/N ratio increased from group 1 to group 3, the determination of humic acids by each of MVR and CDFA methods also increased, while C/N ratio could only affected at higher values on humic acids by TSE method. Brady (1990) used 2 different methods (MVR and IHSS) to determine humic acids; he also reported same increment between MVR humic acids and C/N ratio. Inverse determinations were found by Anonymous (2003) who found higher humic acids with lower values of C/N ratio. Results were in line with Anonymous (1996) who reported that C/N ratio can influence the humification process and increase humic acids in their soil samples.

5. CONCLUSION

Our objectives were to evaluate the effect of C/N ratio on some properties of organic soils. The values of C/N ratio were divided into three groups. It has been detected that increasing C/N ratio from group 1 to group 3 could significantly increase organic matter, carbon, pH, cation exchange capacity, and humic acids (MVR and CDFA method).

During the experiment we observed that C/N ratio had the greatest effect on pH and humic acids, which can be easily controlled by the pH value of the soil. Also, C/N ratio can improve the soil, making it more fertile, increase water and nutrient holding capacities, increase microbial activity, and make better root zones.

In this research, it has been detected that C/N ratio can be used as a good index for degradation levels and humification processes in organic soils, higher the organic matter content lower the degradation rate.

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CURRICULUM VITAE

Personal Identity

Name, Surname : Rawaz Khasro QADER
Nationality : Iraqi
Date and place of birth : 1 March, 1991 Erbil, Iraq
Marital Status : Single
Telephone : (+90) 534 636 87 96 - (+964)750 428 90 55
E-mail : rawazzkh@gmail.com

Educational Background

Degree	Place of Education	Date of Graduate
Master Degree	KSU, Graduate School of Natural and Applied Science Department of Bioengineering	2015
Bachelor's Degree	Salahaddin University College of Agriculture Department of Soil and Water Sci.	2012

Language Skills

Kurdish, English, Turkish, Arabic