CHAPTER 2

LITERATURE REVIEW

2.1 Leonardite

Leonardite is an oxidized form of lignite with brown and coal-like appearance and often found at shallow depths overlying more compact coal in a coal mine. Leonardite, named after A. G. Leonard in recognition of his research contributions to the substance and humified organic matter, contains 30–80% humic acid (HA) and can be used as an organic fertilizer or a soil amendment (Ayuso *et al*., 1996). Abundant natural resources of leonardite are available in many places in the world and the deposits in USA were found in Texas, Wyoming, New Mexico, North Dakota, Idaho, and Florida. (Fernández *et al*., 1996). There are economic deposits of humate-rich material in Arkansas, Florida, Louisiana, New York, North Dakota, Michigan, Minnesota, Texas, and Wyoming, according to Burdick (1965). Humic substances can serve as nitrogen, phosphorus, and sulfur reservoirs; improve soil structure, aeration, and drainage; and increase buffering and exchange capacities. (Stevenson, 1982 and Stevenson. 1979) Leonardite has soil amendment potential and beneficial effects on bean growth. (Akinremi *et al*., 2000 and Ece *et al*., 2007). Applications of HA also showed improved plant growth, seed germination, and fruit quality. (Mackowiak *et al*., 2001) However, most of these investigations focused on studying plants grown with different sources or levels of humic substances, and the correlation between total amount of humic substances and plant growth. (He *et al*., 2009, Xu *et al*.,2009 and Liu *et al*., 2008). Only Serenella *et al*. (2002) investigated whether low-molecular size HA endowed with a high aromatic and carboxyl C content, and whether high-molecular size HA endowed with peptidic and carbohydratic carbons positively influence the metabolic parameters of plants. Low-molecular size humus was found to be capable of reaching the plasma membrane of root cells and was translocated, while a high-molecular size fraction is not absorbed and only interacts with the cell wall. Vaughan (1974) and Vaughan *et al*. (1985) attributed cell elongation by humic substances to the formation of strong Fe complexes with HAs, resulting in a reduction in a wall-bound hydroxyproline.

Using Leonardite directly or HAs produced from it as soil amendments or plant promoters is expected to improve the properties of soil and growth of plants. Humic and fulvic acids are usually used in agricultural production and are widely known as having agronomic potential (Ece *et al*., 2007). Humic substances (humic and fulvic acids), components of soil organic matter, are mostly used to eliminate adverse effects of chemical fertilizers such as soil pH (Chen and Aviad, 1990; Akıncı *et al*., 2000; Katkat *et al*., 2009). Humic substances (humics, HS) constitute an important fraction of soil organic matter, have a positive influence on soil fertility and the physical integrity of soil, and increase the availability of nutrients (Stevenson, 1979; Akinremi *et al*., 2000). Humic substances are refractory, dark-colored heterogeneous organic compounds produced in the decay of the total biota in the environment (Stevenson, 1994). Their unique structure makes them a versatile material with applications in industry, medicine, environmental protection, and agriculture. It is becoming clearer, that the presence of humic acid in soil is necessary for sustainable agriculture, due to their ability to improve the soil, enhance its stability and increase its resistance to erosion (Laker *et al*., 1993; Spaccini *et al.,* 2002), ensure enhanced biological activity (Canellas *et al.,* 2002; Canellas *et al.,* 2008; Nardi *et al*., 2000a; Zandonadi *et al.,* 2007) and obtain higher crop yields (Antošová *et al*., 2008; Brownell *et al*., 1987; Eyheraguibel *et al*., 2008), and may be used in soil remediation (Fava *et al*., 2004; Stehlíčková *et al*., 2009; von Wandruszka 2000).

Leonardite, a coal-like appearance material (Fig. 2.1), is often found associated with near surface lignite deposits. Liginite is used to generate electricity however leonardite cannot be used as fuel because of its low heating content. Therefore, in the past, leonardite is considered as waste product of mining in Thailand. Lignite is used as a feedstock in the production of electricity about 20 million tons per year, so leonardite wastes from mining is increasing. Recently, many researchers recognized the high value of leonardite because it contains high humic acid. (Kohanowski, 1970; Ayuso *et al*., 1996; Dudley *et al*., 2004). Leonardite is a great feature to agriculture in many respects such as high cation exchange capacity (CEC) (Pertuit *et al*., 2001; Kalaitzidis *et al*., 2003; Dudley *et al*., 2004) thus high water and nutrients retention, contains many kinds of nutrient elements (Simandl *et al*. 2001; Kalaitzidis *et al*., 2003) and high humic acid content and organic matter (Conxita *et al*., 2005).

Recently, the use of leonardite in agriculture at the commercial scale is expanding. However, information related to its properties is quite limited. High plant nutrients and humic acid content in leonardite samples indicates its possible use to improve organic matter, humic acid and some plant nutrient levels in soil. Robert (1997) reported that leonardite contains 48.60% carbon (C), 3.31% hydrogen (H), 1.03% nitrogen (N), 2.11% sulfur (S), 23.73% oxygen (O) and 21.22% ash. Conxita *et al.* (2005) reported that leonardite contains 79% humic acid (HA), 55.2% C, 3.4% H, 0.8% N, 2.4% S, 38.1% O, 2.87 meq/g of CEC, 3.12 meq/g of -COOH groups and 2.07 meq/g of -OH groups. Besides plant nutrients, leonardite also contained a certain amount of heavy metals. Alfredo *et al*. (2005) reported that leonardite contained 34.9 mg/kg arsenic (As), 0.83 mg/kg cadmium (Cd) and 22.0 mg/kg lead (Pb). Heavy metal limits for Thai organic fertilizers standards are as follows; 50.0 mg/kg As, 5.0 mg/kg Cd and 500 mg/kg Pb (Department of Agriculture, 2005).

Although leonardite contains valuable plant nutrients, organic matter and humic acid, that can improve soil quality and plant growth, it also contains potentially toxic elements that can cause soil contamination, phytotoxicity and undesirable residues in plant and animal products. Therefore, the use of leonardite for agricultural purposes should be done with care. Since there are multiple sources of leonardite around the world, the quality and nutrients composition in leonardite vary widely from place to place. So, in order to take advantage of leonardite in agricultural efficiency and does not impact negatively on the environment in the long term, the properties of the leonardite should be analyzed. Evaluation of its effects on soil nutrients and heavy metal levels, and plant growth should be done both in pot and field experiments prior to farmer field application.

Figure 2.1 The character with black color of leonardite

2.1.1 Sources of leonardite in Thailand

There are several sources of leonardite in Thailand occurring with lignite, which is mined in northern Thailand, particularly Mae Moh mine, Lampang province (Fig. 2.2). Mae Moh mine might be a major source of leonardite humate ore with an area of about 38 square kilometers of lignite mine. Lignite coal (Fig. 2.3) has a higher carbon content thus higher heating value than leonardite. The largest open-pit coal mine (lignite) in Thailand is located in Mae Moh district, Lampang province. Leonardite was found at bench outcrops of the mine, and has humic acid content of 20 – 40% or more and economically used for humic acids extraction. The amount of lignite coal in this reserve area are about 1,139 million tons which represents highest volumes of both lignite and leonardite in Thailand.

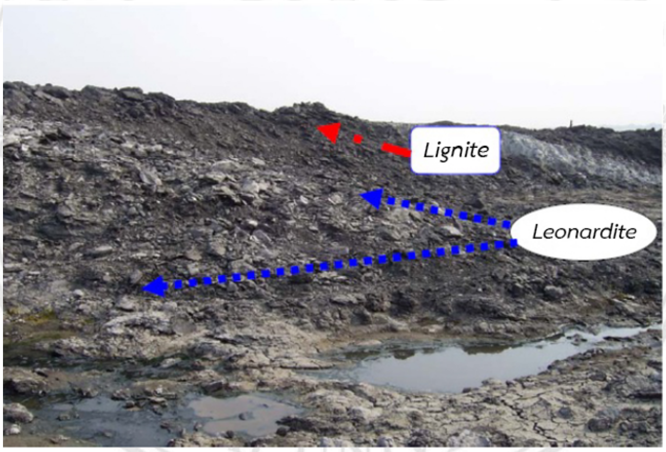

Figure 2.2 Leonardite deposit in Mae Moh mine, Lampang province

There are other sources of leonardite in the new mining areas such those in Lamphun, Phayao and Krabi provinces. Quality and property of leonardite found in Thailand might vary widely from deposit to deposit. For appropriate use and application of leonardite, evaluation of its chemical properties is of importance.

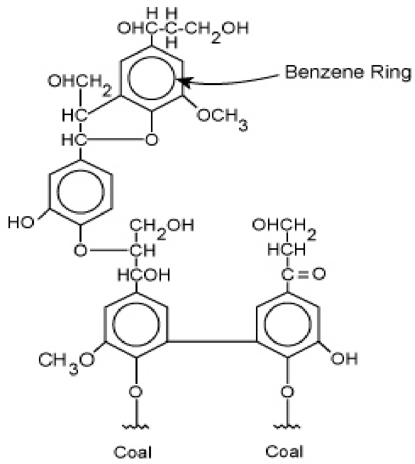

Figure 2.3 A structural model of lignite

2.1.2 The benefits of Leonardite

As mentioned aboved, leonardite contains high organic matter and humic acid thus make it a versatile material for various application both in agriculture and environment (Table 2.1). Leonardite can be used as soil amendments, organic fertilizer, filter material for treatments of waste water, organic compounds and heavy metals etc. The benefit of organic materials in soils is well known and application of organic materials on agricultural soils positively affects physical, chemical and biological properties of soils (Flaig *et al*., 1977). The effects of organic material (leonardite) and humic acids which extracted from organic materials are more apparent in soils with low organic matter than in soils with high organic matter.

Leonardite acts as conditioner for the soil and as bio-catalyst and bio-stimulant for the plant. Compared to other organic products, leonardite enhances plant growth particularly (biomass production) and fertility of the soil. Another advantage of leonardite is its long-term effectiveness, as it does not consume up so quickly as animal manure, compost or peat. As leonardite is completely decomposed, it does not enter into nutritional competition with plants for nutrients such as for nitrogen. This is not the case with incompletely decomposed compost, whereby the organic substances in soil are rapidly consumed up by micro-organisms and mineralized entirely without humus formation.

Tabel 2.1 The benefits of Leonardite

Type	The benefits
Agricultural	Soil amendments
waste water	Organic and metal filter
Oil drilling	drilling mud
the foundry	Additives green sand

2.1.3 Humic substances

The sources of humus (humic substances and non-humic substances) are various kinds of organic matters such as organic fertilizers and compost. One of the significant component of organic fertilizers such as compost is the humic acid. Besides natural persistence in soil and organic fertilizers, humic acid has been extracted from various sources such as lignite, leonardite, peat, coal, farmyard manure and compost. Among organic matters, leonardite contains very high humic and fulvic acids (40-85%) compare to that of peat (10-20%) and compost (2-5%) (Table 2.2).

Tabel 2.2 Comparison on contents of humic and fulvic acid from various sources.

Natural source	Content of humic and Fulvic acid (%)
Leonardite	40-85
Black peat	10-40
Sapropel peat	10-20
Brown coal	10-30
Dung	5-15
Compost	2-5
Soil	1-5
Sludge	1-5
Hard Coal	0-1

Humic acid is a long chain molecule, which is high in molecular weight, dark brown and is soluble in an alkaline solution (Stevenson, 1982). Humic acid is one of the major components of humic substances. Humic substances is formed through the chemical and biological humification of plant and animal matter through the biological activities of microorganisms (Anonymous, 2010). The effects of humic substances on plant growth depend on the source and concentration, as well as on the molecular fraction weight of humus. Lower molecular size fraction easily reaches the plasma lemma of plant cells, determining a positive effect on plant growth, as well as a later effect at the level of plasma membrane, that is, the nutrient uptake, especially nitrate. The effects seen on the intermediary metabolism are less understood, but it seems that humic substances may influence both respiration and photosynthesis (Nardi *et al*., 2002).

Humus contained in organic matter is the key factor to promote soil fertility, crop yield and microbial activities. Humic substances are a good source of energy for beneficial soil organisms. Humic substances and non-humic (organic) compounds provides the energy and many of the mineral requirements for soil microorganisms and soil animals. Beneficial soil organisms lack the photo synthetic apparatus to capture energy from the sun thus must survive on residual carbon containing substances on or in the soil. Energy stored within the carbon bonds function to provide energy for various metabolic reactions within these organisms. Giannouli *et al.* (2009) evaluated the use of Greek peat and coal samples for applications in the agricultural/horticultural sector and assesses the suitability of a certain peat/coal either as soil conditioner or as raw material for manufacturing organic fertilizers. Twenty-six samples of different rank ranging from peat to subbituminous coal obtained from several Greek peat/coal deposits, were studied. The majority of the samples reveal moderate to high ash yields (16–80 wt.%), a slightly acidic to neutral character and electrical conductivity ranging from 100–2500 μS/cm. Concerning the environmental impact of the sensitive trace elements, which might be leached, As, Mn, Ni and Sr show relatively strong mobilization in some samples, although severe impacts are not expected. The soil's CEC is generally improved, although it remains at moderate levels. The most interesting aspect is the humic acids content, which ranges between 9.6 and 52.2 wt.% on a dry basis.

The biological activity of humic substances has been recognized in 1917 (Bottomley, 1917), while their hormone–like nature was reported later (Canellas *et al*. 2008; Nardi *et al.* 2002). Aguirre *et al.* (2009) noted that the biological activity of humic substances is based on their ability to promote the expression of selected genes encoding enzymes like FeIII chelate reductase, plasma membrane H^+ATPase, and FeII high affinity transporter. Mora *et al*. (2010) questioned this “hormone-like” idea, since no cytokinins, gibberellins and indolacetic acid were found in humic acid samples.

Instead, they hypothesized that humics enhance the activation of root plasma membrane H+ATPase, since this may cause significant changes in the root-to-shoot distribution of NO_3^- and therefore of cytokinins and polyamines. Piccolo *et al.* (1992) showed that the humic fraction with the highest acid functionality and the smallest molecular size had the greatest effect on plant nitrate uptake and hormone-like activity. In contrast, neither the aliphatic nor the aromatic content of the extracts appeared to play a role in the biological activity (Nardi *et al.* 2000a, b, and 2002) confirmed those observations and concluded that the smaller molecular size fractions can be partially taken up by the plasmolemma of higher plant cells, whereas the larger fractions (> 3.5 kDa) interacted only with the cell walls. In contrast, Canellas *et al*. (2010) showed that the size fractions of vermicompost humics obtained by preparative HPSEC (high performance size exclusion chromatography) had similar biological activities. In the work of Vlčková *et al.* (2009), the highest biological activity of humic substances toward *Zea mays* was observed for 35–175 kDa molecular weight fraction

Benefits of Humic Acids

Current scientific studies show that the fertility of soil is determined to a very large extent by the content of humic acids. Their high cation-exchange capacity (CEC), the oxygen content as well as the above average water holding capacity are the reasons for the high value of using humic acids for improving soil fertility and plant growth. The most important feature of humic acids lies in their ability to bind insoluble metal ions, oxides and hydroxides, and to release them slowly and continually to plants when required. Due to these properties, humic acids are known to produce three types of effects: physical, chemical and biological as summarized in the followings;

Physical Benefits: Humic acids physically modify the structure of the soil.

- Improve soil structure: Humic acids physically bind soil particles together, creating stable aggregates that help air and water flow, prevent high water and nutrient losses in light, sandy soils.
- Increase water holding capacity of soil and thus help resist drought.

Chemical Benefits: Humic acids chemically altered the properties of the soil.

- Improve and optimize the uptake of nutrients and water by plants.
- Increase CEC thus the buffering properties of soil.
- Provide a natural chelator for metal ions.

- Contain organic and mineral substances essential to plant growth.
- Increase plant nutrients availability
- Reduce the availability of toxic substances in soils.

Biological Benefits: Humic acids biologically stimulate microbial activities thus plant growth.

- Stimulate plant hormone production by microbes thus the root growth and nutrients uptake
- Enhance plant immune system thus better cope with disease and pest.
- Increase germination and viability of seeds.
- Improve yield quality

Ecological Benefits of Humic Acids

The ecological benefits of humic acids are diverse and present profitable and effective solutions for environmental problems and the preservation of the environment. Soils with a high content of humic acids can reduce nitrate leaching thus lower the risk of potential pollution to groundwater. As a high CEC substance, humic acid has a high potential to counteracts and detoxifies soil contaminants such as heavy metals, pesticides and toxic chemicals. Humic acids acts as a cementing within the soil structural units and stabilizes soil aggregates thus are an effective means to fight against soil erosion.

2.1.4 Chemical composition of Leonardite

Leonardite is a highly oxidized coal normally found in lignite mine. With its high humic acids content, there has been much interest in understanding the structure and composition of leonardite because of the potential role in soil quality improvement, plant growth and yield enhancemen,t and heavy metals complexation and detoxification. The organic matter in leonardite contains significant amounts of oxygen, sulfur and nitrogen heteroatoms incorporated in various functionalities (e.g. carboxylic group), which vary in abundance depending on the specific conditions of the coal-beds. Analysis of leonardite indicated that it contains 48.60% carbon (C), 3.31% hydrogen (H), 1.03% nitrogen (N), 2.11% sulfur (S), 23.73% oxygen (O) and 21.22% ash (Robert, 1997). The use of XRD analysis indicated various mineral composition in leonardite such as gypsum, calcite and kaolinite (Table 2.3). Characterization of leonardite would be useful for obtaining information about the structure and reactivity of the macromolecular organic matter.

Table 2.3 Characterized composition of the mineral found in leonardite with the XRD

No	Mineral composition
1	Gypsum ($CaSO_4$•$2H_2O$)
2	Calcite ($CaCO_3$)
3	Kaolinite (Al_2O_3•$2SiO_2$•$2H_2O$)
4	Muscovite ($KAl_2(AlSi_3O_{10})(F,OH)_2$)
5	Quartz (SiO_2)
6	Iron sulfide (FeS_2)

2.2 The Role of Microorganisms

Microorganisms such as bacteria, cyanobacteria, fungi, and actinomycetes, are able to decompose almost any existing natural organic materials. Microorganisms transform organic matter into plant nutrients that are assimilated by plants and resynthesized the decomposed materials into the end valuable products called humic substances.

Most microorganisms exist in topsoil, where organic matters are plentiful, than in subsoil. They are especially abundant in the rhizosphere soil, where sloughed-off cells and root exudates secreted by plant roots provide excellent food sources. Plants can promote changes in the soil microbial community through the exudation of different combinations of organic compounds by the roots depending on environmental conditions (Bais *et al.,* 2006). Plants cultivated under optimal conditions or in low P or N availability tend to exhibit significant differences in the composition of root exudates (Carvalhais *et al.,* 2011), which in turn cause changes in the population density of microbial groups in the soil (Eilers *et al*., 2010). Soil microbes are primary decomposers of organic matter. In addition, they fix dinitrogen (N_2) and provide available N for plants, detoxify toxic substances, suppress plant diseases, and produce plant hormones that stimulate plant growth and development. Soil microorganisms are also a good source of most antibiotic medicines we use to fight diseases.

2.2.1 Influence of Soil Microorganisms on Plant Growth

Soil microorganisms play an important role in determining the composition of plant communities. They responsible for nutrients transformation (e.g. nitrogen, phosphorus, and sulfur) thus re-cycling organic and inorganic material and alter the physico-chemical characteristics of the environment. The microbes also form mutualistic associations with plants, these activities resulting in greater plant growth (Sylvia *et al.,* 2005). The quality of root exudates can promote a diverse group of microorganisms present in the rhizosphere soil (Wolfe and Klironomos, 2005). The rhizosphere soil microbes usually interact intensively with the plant and can have a positive, neutral, or negative interference on plant growth and, thus, potentially determining the composition of plant communities (Klironomos, 2002).

Mycorrhiza Fungi

Different symbiotic associations between plants and fungi occur, almost ubiquitously, in a wide range of terrestrial ecosystems and involve a broad range of plant hosts (Brundrett, 2009). It is believed that at least 85% of plant species are able to establish symbiotic associations with fungi, of which 70% are associated with the arbuscular mycorrhizas of the phylum Glomeromycota (Wang and Qiu, 2006). The studies on interactions between plants, soil microorganisms and the dynamics of plant communities is interesting because plant-microorganism interactions can be decisive for the establishment of a species in a given environment (Klironomos, 2002; Callaway *et al.,* 2004; van Grunsven *et al*., 2009).

Mycorrhizal associations can influence plant community composition and diversity, since plants that establish this type of association can obtain competitive advantages (O'connor *et al.,* 2002; Shah *et al.,* 2008) or facilitate the establishment of other species (Chen *et al.,* 2004). Plant inoculated with arbuscular mycorrhizal fungi (AMF) grew, on average, 11.8 times more than those uninoculated, and that the distribution of nitrogen and phosphorus between the species varied depending on the AMF present. Thus, arbuscular mycorrhizal fungi can redistribute resources among plants of different species, allowing their coexistence (van der Heijden *et al*., 2003). Mummey and Rillig (2006) found that in areas dominated by the invasive species *Centaurea maculosa* Lam., the diversity of AMF was much smaller than in areas dominated by native species. Thus, the composition of plant communities and

AMFs are influenced by feedback interactions in each community (Bever, 2003; Hart *et al.,* 2003).

Another important group of soil microorganisms are the dark septate endophytes (DSEs), which can associate with the roots of several plant species (Grünig *et al.*, 2002; Weishampel and Bedford, 2006). Sometimes, DSEs are found colonizing roots containing AMF (Rains *et al.,* 2003; Weishampel and Bedford, 2006). The importance of DSE appears to increase with the increasing severity of environmental conditions, since in high-stress environments the occurrence of plant associations with DSE is much more frequent than the associations with AMFs (Barrow, 2003; Postma *et al.,* 2007).

Nitrogen Fixing Microorganisms

Various groups of soil bacteria are important as plant growth promoting microorganisms apart from fungi. Symbiotic association between diazotrophic bacteria (N_2 fixing bacteria) and plants is well documented (Franche *et al.,* 2009). Phosphate-solubilizing microorganisms are also very important since they are able to solubilize precipitated P and provide available phosphorus to plants (Gyaneshwar *et al.*, 2002). Besides N_2 fixing and P solubilizing bacteria, there is a group of bacteria called PGPR (plant growth promoting rhizobacteria) also plays an important role, since they can promote plant growth by different mechanisms (Saharan and Nehra, 2011).

Nitrogen-fixing bacteria may associate around the plant rhizosphere or enter the root cells, leading to the formation of structures called nodules responsible for N_2 fixation (symbiotic nitrogen fixation) (Bhattacharjee *et al.*, 2008; Franche *et al.,* 2009; Masson-Boivin *et al.,* 2009). Nitrogen supply is increased when plants associated with these bacteria, and in the case of symbiotic associations, over 90% of nitrogen contained in the plant can be fixed by bacteria (Franche *et al.*, 2009). Thus, in the soil with low level of nitrogen, N-fixing bacteria can improve plant growth, as well as promote increased nitrogen content in the soil (Walker *et al.*, 2003).

Phosphate Solubilizing Microorganisms

Phosphate solubilizing microorganisms (PSMs) are naturally present in soils associated or not with plant roots (Rodrýìguez and Fraga, 1999; Gyaneshwar *et al.,* 2002). PSMs can solubilize insoluble P (precipitated and/or adsorbed phosphorus) by means of various mechanisms and have great potential to promote plant growth

(Gyaneshwar *et al.,* 2002). Higher P uptake and growth of various plant species were obtained with PSM inoculation (Pal, 1998; Kumar and Narula, 1999; Peix *et al.*, 2001). Since available P is in the soil solution, not all of the solubilized P can be transported to the plant (Rodrýìguez and Fraga, 1999). In this case plants able to recruit greater populations of these microorganisms can gain a competitive advantage over others, especially in soils where phosphorus is scarce.

Plant Growth Promoting Microorganisms

Plant growth promoting rhizobacteria (PGPR) are able to enhance plant performance by means of a wide variety of mechanisms (Saharan and Nehra, 2011). Studies have shown that inoculation of plants with PGPR can increase nutrient content (Orhan *et al*., 2006; Karthikeyan *et al.,* 2010) and resistance to pathogens (Saravanakumar *et al.*, 2007; Maksimov *et al.,* 2011). Moreover, some PGPR are able to produce phytohormones, increase the population of other beneficial microorganisms and control the population of harmful ones in the rhizosphere (Saharan and Nehra, 2011). Thus, plants able to recruit greater populations of these microorganisms into their rhizospheres present greater survival, growth, and reproduction (Gholami *et al.,* 2009), and consequently higher competitive ability.

2.2.2 Microorganisms and Soil Organic Matter Decomposition

Soil microorganisms gain their carbon and energy for the formation of new cells from soil organic matter (SOM) decomposition. The organic matter of soils includes the remains of plant, animals and microorganisms in all stages of decomposition. The level of organic matter in soils influences a number of soil chemical and physical properties. Soil organic matter is chosen as the most important factor of soil quality and agronomic sustainability, due to its impact on physical, chemical and biological properties indicators of soil quality (Limtong, 2012). Soil organic matter has two components called the active (35%) and the passive (65%) SOM. Active SOM is composed of the "living" and "dead" fresh plant or animal material which is food for microbes and is composed of easily digested sugars and proteins. The passive SOM is resistant to decomposition by microbes and is higher in lignin.

Microbes need regular supplies of active SOM in the soil to survive in the soil. Long-term no-tilled soils have significantly greater levels of microbes, more active carbon, more SOM, and more stored carbon than conventional tilled soils. A majority of the microbes in the soil exist under starvation conditions and thus they tend to be in a dormant state, especially in tilled soils. Dead plant residues and plant nutrients become food for the microbes in the soil. Soil organic matter (SOM) is basically all the organic substances (anything with carbon) in the soil, both living and dead. SOM includes plants, blue green algae, microorganisms (bacteria, fungi, protozoa, nematodes, beetles, springtails, etc.) and the fresh and decomposing organic matter from plants, animals, and microorganisms.

Soil organic matter can be broken down into its component parts. One hundred grams (g) or 100 pounds (lbs) of dead plant material yields about 60–80 g (lbs) of carbon dioxide, which is released into the atmosphere. The remaining 20–40 g (lbs) of energy and nutrients is decomposed and turned into about 3–8 g (lbs) of microorganisms (the living), 3–8 g (lbs) of non-humic compounds (the dead), and 10–30 g (lbs) of humus (the very dead matter, resistant to decomposition). The molecular structure of SOM is mainly carbon and oxygen with some hydrogen and nitrogen and small amounts of phosphorus and sulfur. Soil organic matter is a by-product of the carbon and nitrogen cycles.

2.3 Soil Fertility Degradation

Soil fertility degradation in agriculture land of South East Asia and Africa has led to the declining per capita food production (Sanchez *et al*., 1997). Soil fertility decline includes plant nutrients depletion, SOM decline/loss, soil compaction, acidification, and an increase in toxic elements such as aluminum (Hartemink, 2003). Recently, Soil degradation in most agricultural land including paddy fields, has become an important issue in Thailand and are challenging the concept of sustainability of current land management system. After the Green Revolution, modern agriculture (increased use of hybrids, increased chemical use, mechanization, and a trend toward monocropping) has become a major cause of land degradation. In most cases, inappropriate use of machinery has led to soil compaction; poor vegetation management has exposed soils to erosion; and substitution of organic inputs with chemical fertilizers has led to declining organic matter and acidification of vulnerable soils. There are indications that the highly productive fertilizer and seed technologies introduced over the past three decades may be reaching a point of diminishing returns (Bouis 1993; Cassman *et al.* 1995; Flinn and De Datta 1984). Recently, after several decades of inappropriate soil managements under the green revolution, the adverse cumulative effects of this management become apparent. For the above reasons, organic materials should be applied to cultivated land to improve soil fertility and crop yield.

2.4 Soil Organic Matter and Soil Fertility

Soil organic matter (SOM) has been directly and positively related to soil fertility and agricultural productivity potential. SOM generate favorable soil structure by binding soil particle into soil aggregates. Soil aggregates provide favorable conditions for soil microbial and faunal activity and plant growth. Certain components of SOM such as humic substances, polysaccharides, root material and fungal hyphae have an important role in soil aggregate stabilization. SOM acts as a significant reserve of plant nutrients and improves soil structure and water holding capacity. For this reason, organic matter depletion has led to a decrease in crop productivity. It is generally accepted that SOM has beneficial effects on soil biological, chemical and physical properties, which in turn influences the productive capacity of soils. It is also accepted that SOM is a major contributor of N, P and S as well as other nutrients to plants. Soil microbial activity is also dependent on SOM as a carbon source for metabolic activity which in turn influences nutrient fluxes and soil structure. Decline in soil fertility has also been related to the decline of soil nutrients and organic matter (Noble *et al*., 2000; Funakawa *et al*., 2006). Very recent work at the International Rice Research Institute has implicated organic matter quality as a key factor in nitrogen availability which appears to contribute to yield stagnation and decline in irrigated lowland rice (Cassman *et al.* 1995). There is almost no correlation between soil organic carbon content and the effective soil nitrogen supply capacity of lowland rice soils. In a survey of farmer's fields in Central Luzon (Philippines) there was a large range in the effective soil nitrogen supply capacity but no relationship between nitrogen uptake and soil organic carbon or total soil nitrogen (Cassman *et al.* 1996). Soil organic carbon and total nitrogen can increase without an increase in soil nitrogen supply capacity. Intercropping of maize with legumes has led better protection against soil erosion and higher grain yields than the conventional continuous monocropping of maize (Suwanarit *et al.* 1999). In contrast, monocropping of sugarcane for 30 years compared with undisturbed forest has resulted in a 3-fold reduction of available water content and a considerable reduction of wet aggregate stability (Caron *et al.*, 1996). Yasothon soil fertility was improved by organic compost. The more the amount of organic compost added to the soil, the more increasing in the percentages of soil nitrogen, soil available P and exchangeable K (Chuasavathi and Trelo-ges 2001). They also concluded that organic matter percentage in soil was increase with the levels of compost. However, the amount of organic matter was decreased with time and became similar at the final sampling period. These results implied that there was relatively high decomposition rate of the compost and it must be frequently applied to the soil in order to maintain high percentage of organic matter (Chuasavathi and Treloges., 2001). The problem cause by low soil fertility are usually addressed with a number of agronomic and farm management intervention that include zero tillage, mulching and the application of manure and compost. While these applications do improve the long term soil health and yield, they may not normally have an immediate effect on farm income (Saleth *et al*., 2009). Many studies have shown that crop yield and nutritional quality were improved by organic fertilizers and/or beneficial microorganisms. Organic fertilizers usually promote growth and activities of microorganisms and vice versa the value of organic fertilizer (humus) is created mainly by microorganisms. For these reasons, the major factors enhancing yield and nutritional quality might be the effect and interaction of both humus (humic substances) and beneficial microorganisms.

2.5 Rice Production and Fertilization

Rice is extremely important to the country of Thailand and its citizen. It remains the primary food in all parts of the country, particularly in rural areas. Thailand produces rice in excess of the country consumption and is a major exporter of milled rice. Rainfed lowland is the predominant rice ecology in Thailand. In the 1977/78 crop year, rainfed lowland accounted for 78.1 percent of the wet season rice area and 68.0 percent of production or about 60 percent of annual production. Average yield in the rainfed lowland of Thailand was extremely low, at about 1.30 t/ha during the 1977/78 crop year and 1.87 t/ha in the 1997/98 crop year, which needs to be improved. Higher average yield of 2.6 t/ha was achieved with higher production areas of modern rice varieties. Modern rice varieties became widely adopted because of their high yield performance, high response to chemical fertilizer and early maturity. The latter characteristics has allowed farmers in irrigated areas to cultivate two to three crops a year, increasing the application of agrochemicals as a consequence of higher cropping intensity. Major increases in aggregate agricultural production in this period have been associated with different kinds of soil degradation. The intense and increased pressure on land leads to its degradation and pollution, which may result in a partial loss of its productive capacity. The continuous production of rice has resulted in a decline in soil fertility, with an associated loss of productivity. The cultivated site has undergone a significant decline in soil pH as a result of high agrochemical input. Application of alternative technology such as crop residue management, compost and natural organic materials application (leonardite) have shown to improve soil fertility and crops yield. Larijani and Hoseini (2012) found that the rice grain yield obtained from organic fertilizer was higher than chemical fertilizer and there was no significant difference between chemical fertilizer and organic fertilizer alone. However, highest yield with 4772.4 kg/ha was belong to combination of organic fertilizer BIOL555 (1 ton/ha) + Urea application 50 kg/ha (25% as basal and 25% at PI). Cooper *et al*. (1998) found that humic acid extracted from soil, peat or leonardite increased 3-5% of phosphorus content in turf grass. Humic acid from leonardite significantly increased root weight of turf grass over the control. Studies have shown that humic substance could enhance seed germination of maize, wheat and barley. Fernandez-escobar *et al.* (1996) reported that foliar application of leonardite extracts to young olive plants stimulated shoot growth when they were growing without the addition of mineral elements to the irrigation water. Under field conditions, application of leonardite extracts stimulated shoot growth and promoted the accumulation of K, B, Mg, Ca and Fe in leaves. However, when leaf N and leaf K values were below the threshold limit for the sufficiency range, foliar application of humic substances was ineffective to promote accumulation of these nutrients in leaves.