



UNIVERSIDAD POLITÉCNICA DE CARTAGENA
DEPARTAMENTO DE CIENCIA Y TECNOLOGÍA AGRARIA

**MANAGEMENT OF SOIL ORGANIC CARBON USING
FARMYARD MANURE
IN MURCIA PROVINCE, SE SPAIN.**

Asuman YANARDAĞ

2015



Universidad Politécnica de
Cartagena
Departamento de Ciencia y
Tecnología Agraria



Grupo de Investigación Gestión,
Aprovechamiento y Recuperación de
Suelos y Aguas

Tesis Doctoral

Management of Soil Organic Carbon Using Farmyard
Manure in Murcia Province, SE Spain.

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FARMYARD MANURE IN MURCIA PROVINCE, SE SPAIN.**

Tesis Doctoral

Trabajo presentado por **ASUMAN YANARDAĞ** para aspirar al
grado de Doctora por la Universidad Politécnica de Cartagena

Dirigida por

**DR. ÁNGEL FAZ CANO
DR. AHMET MERMUT**

Cartagena, 2015.



Universidad
Politécnica
de Cartagena



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Reunido en el día de la fecha el Tribunal nombrado por el Sr. Rector Magnífico de la Universidad Politécnica de Cartagena, por resolución **R-224/15, de 10 de abril**:

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Para juzgar la Tesis Doctoral realizada por **Dña. ASUMAN YANARDAG**, con NIE nº **Y0074166M**, con el título "**Management of soil organic carbon using farmyard manure in Murcia Province, SE Spain**", en el Departamento de **Ciencia y Tecnología Agraria**, tras seguir el Programa de Doctorado: "**MINERIA, MEDIO AMBIENTE Y DESARROLLO SOSTENIBLE**", bienio **2007-2009**, dirigida por los doctores **D. Ángel Faz Cano y D. Ahmet Ruhi Mermut**.

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Once the defense of the doctoral thesis titled *"Management of soil organic carbon using farmyard manure in Murcia Province, SE Spain"*, directed by doctors Mr. Ángel Faz Cano and Mr. Ahmet Ruhi Mermut, has been carried out by MRS. ASUMAN YANARDAG on the 24th April 2015, and after counting the votes cast by the members of the board,

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THE PRESIDENT OF THE DOCTORAL COMMITTEE



Signed: Pablo Fernández Escámez



To my husband and son

To my grandparents and my dear mom

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ASUMAN BÜYÜKKILIÇ YANARDAĞ

SCIENTIFIC JOURNALS AND BOOK CHAPTER

M.Gomez-Garrido, S. Martinez-Martinez, A. Faz Cano, **A. Büyükkılıç Yanardağ** and J.M. Arocena. Soil Fertility Status and Nutrients provided to Spring Barley (*Hordeum distichon* L.) by Pig Slurry. Chilean Journal of Agricultural Research. 74(1): 73-82.

Asuman Büyükkılıç Yanardağ, Melisa Gomez Garrido, İbrahim Halil Yanardağ, Ángel Faz Cano, Ahmet Mermut. Pig Slurry Application to Improve Soil Quality and Sustainability in Semiarid Region of Spain. Communication in Soil Science and Plant Analyses (Under review).

Asuman Büyükkılıç Yanardağ, Ahmet Mermut, Ángel Faz Cano, İbrahim Halil Yanardağ and Melisa Gomez Garrido. Organic Carbon Fluxes in Undisturbed Soil Columns Treated with Pig Slurry in SE Spain. Soil Science (Under review).

Asuman Büyükkılıç Yanardağ, Ángel Faz Cano, Ahmet Mermut, İbrahim Halil Yanardağ and Melisa Gomez Garrido. Stable Carbon Isotope Composition of Soil and Plant under Pig Slurry Applications from SE, Spain. Geoderma (Under review).

İbrahim Halil Yanardağ, **Asuman Büyükkılıç Yanardağ**, Angel Faz Cano, Ahmet Ruhi Mermut. Chapter 24 - Effect of Pig Slurry Application on Soil Organic Carbon, Pages 689-706. Soil Remediation and Plants Book. Prospects and Challenges. Edited by: Khalid Hakeem, Muhammad Sabir, Munir Ozturk and Ahmet Mermut. ISBN: 978-0-12-799937-1

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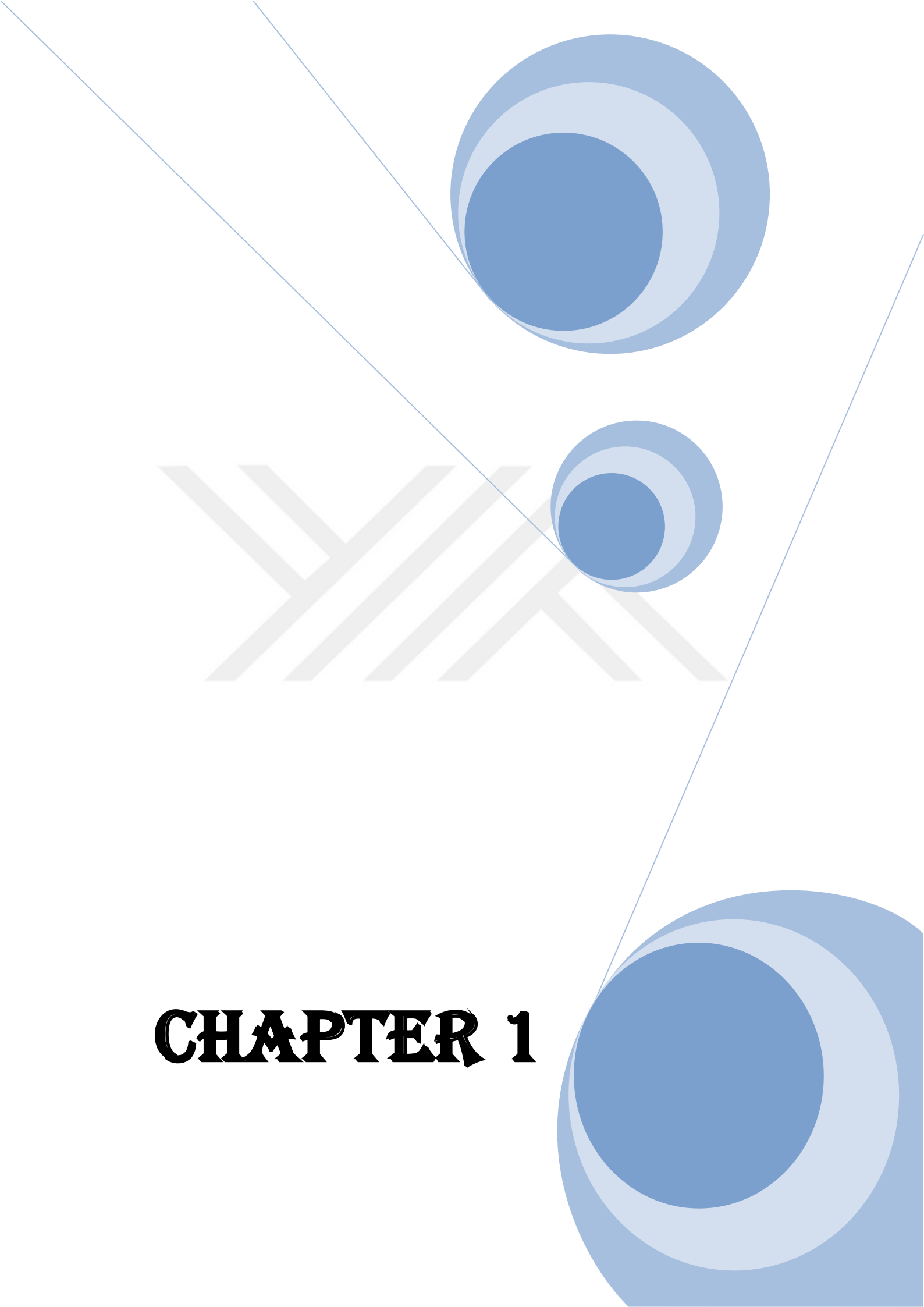
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CHAPTER 1

CHAPTER 1

INTRODUCTION

1.1. BACKGROUND INFORMATION

A better knowledge of the magnitude and dynamics of organic C and the C in carbonates is important for a more complete understanding of the global C cycle, and to evaluate the storage and fluxes between the atmosphere, the biosphere, and the pedosphere (Lal et al, 2000). Buyanovski and Wagner, (1998) suggest that soil and crop management can greatly improve the residence time and new C storage in the soil, which is worthy of consideration under the Kyoto Protocol. Different land uses and agronomic practices were evaluated with respect to their effect on carbon sequestration or release (Lal, 1999; Batjes, 1996). A distinction is made between practices causing a decrease of carbon loss, an increase in carbon input into the soil, or a combination of both.

The recycling of pig slurry in agricultural soils is an alternative and valuable practice in countries such as Spain with significant European pig production

(2.529 million sows in 1998). This is particularly the case since many regions are arid and are comprised of poor soils (<1% organic matter) that support intense mineralization. Recycling supplies agricultural soils with organic matter, nutrients, and micronutrients, but also adds unwanted substances such as food additives, pesticides, pharmacological products, along with their metabolic and degradation by-products, and fecal contamination. In Spain, nearly 2.5×10^6 Mg (dry wt.) of pig manure is collected annually (Bigeriego, 1995), and it has been acknowledged (MAPA, 1997) that approximately 50% of this manure is spread on agricultural land.

The great intensification of pig production in Murcia in recent years and the untying of agriculture are associated with a large quantity of manure that can be generated pollutant. Only in the Region of Murcia are a potential annually 4 million tones of slurry (amount calculated from the RD 324/2000). In Murcia region, the area of Guadalentin Valley has more than 50% of the total pig production. With proper management the slurry can be recovered on farmland as fertilizer. Under the Code of Good Agricultural Practices (CBPA) in establishing guidelines for such reuse surplus needed only 94 117 ha cultivated, less than 16% of the total cultivable area in the Murcia Region if applied in the soil up to 170 kg Nitrogen / hectare per year.

Current European Environmental Council Directives such as the Waste Directive (European Union, 1991) consider the need for integrated environmental assessment to ensure “the protection of the environment and human health,” through use of the best available methodologies. Therefore, to evaluate the use of pig slurry, and assess its toxicity, knowledge of the fate and bioavailability of its

components and estimate of potential routes of contamination and effects on organisms are required.

Management of soil organic carbon with different manure application is a very important research topic for southeast region. "Development of recycling pig slurries for agricultural practices in Murcia Region-Spain" research project financed by the Agriculture and Water Ministry and FECOAM (Federation of Agricultural Cooperatives in the Region of Murcia) for 3 years and supervised Research Group Sustainable Use, Management, and Reclamation of Soil and Water (GARSA) from Universidad Politecnica de Cartagena (UPCT). During this a research was carried out an exhaustive monitoring and analyses of physical-chemical properties of the soil – water – plant system, given the excellent results obtained, it is considered necessary to expand substantially parallel lines of research, focusing on a rigorous characterization of the carbon cycle as well.

1.2. HYPOTHESES

The hypotheses of this study were:

1. The amount of carbon will gradually be increase (sequestrated) with the application of pig slurry.
2. The physical-chemical soil properties will change, organic carbon content of the soils that treated with different doses of pig slurry application will increase.
3. Temporal changes in soil characteristics will take place with pig slurry.

1.3. OBJECTIVES

The objectives of this thesis are to:

1. determine the effects of different doses of manure applications on soil C cycle,
2. monitor the changes in soil properties soil-water-plant (cereal) system with manure application,
3. Identify sustainable amount of manure mix with the soil system, minimize the possibility of environmental pollution in Guadelentin Valley,
4. characterize physical-chemical properties of leachates after the application of pig slurry and determine carbon balance in undisturbed soil columns,
5. Study the stable isotope geochemistry and functional groups of carbon in the pig slurry and soil system using isotopes and NMR techniques.
6. Improve soil organic carbon picks for NMR analyses using removal sand method.

1.4. STRUCTURE OF THE THESIS

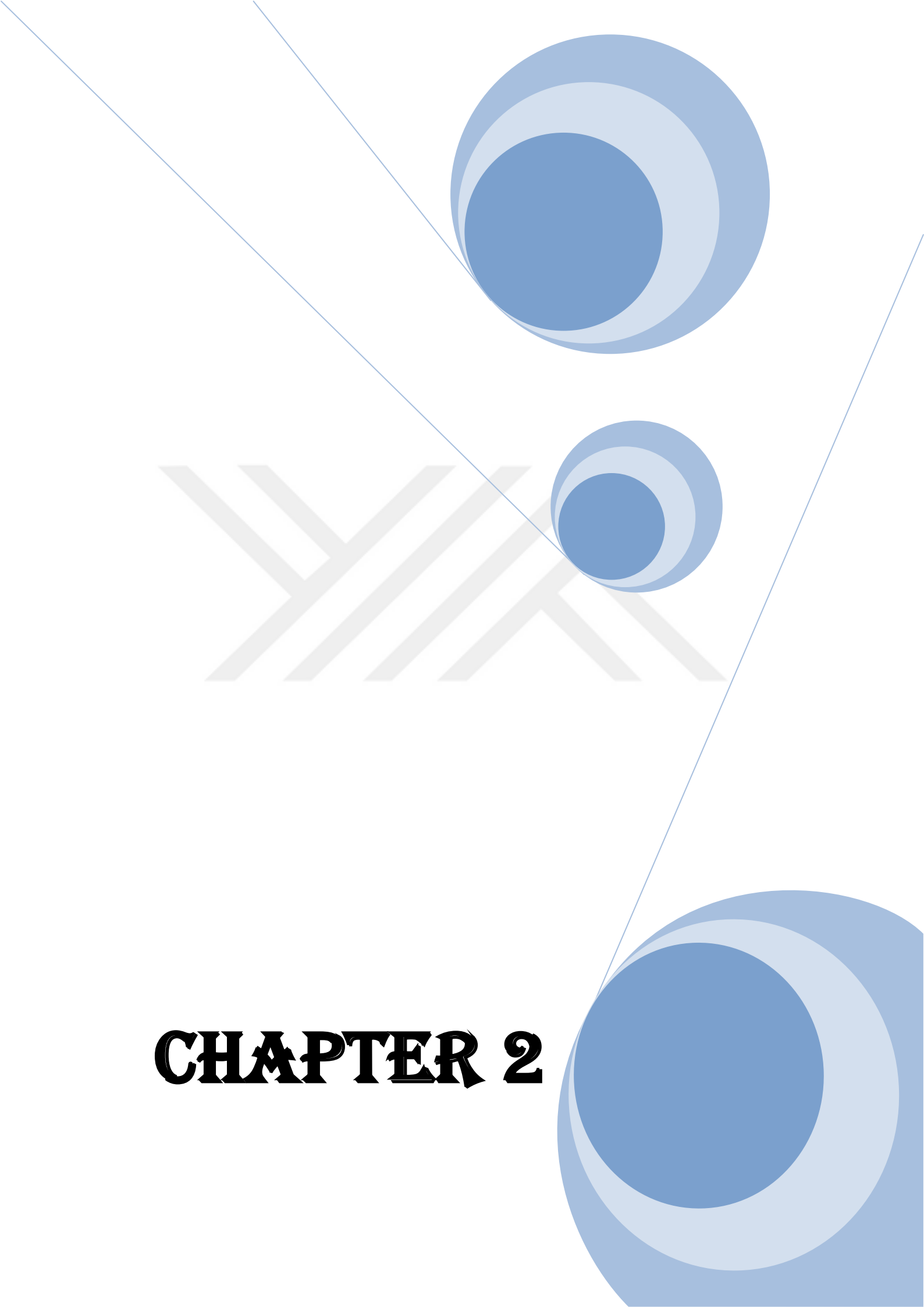
The relevant themes including; pig production of Spain, pig slurry application in the region, soil organic carbon content in the area, waste directives about pig slurry and management of soil organic carbon are described in the Introduction Section (Chapter 1) of the thesis.

The experimental results obtained during the development of the study and relevant realized studies mentioned above are organized in Chapters 3, 4, 5, and 6 of this document. Each chapter is written in the form of a scientific paper and

consists of the following sections: introduction, materials and methods, results and discussion. Chapter 7

Finally, Chapter 7 includes the main summary and conclusions of the work, Chapter 8 the list of references used in the study, Chapter 9 the list of appendices.



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CHAPTER 2

CHAPTER 2

LITERATURE REVIEW

2.1. IMPORTANCE OF SOIL ORGANIC CARBON IN THE SOIL SYSTEM

Soil organic matter comprises other important elements such as nitrogen, phosphorus, and sulphur. Soil organic matter is a fundamental component of healthy soil ecosystems and is a major contributor to the sustainability of productive output from cropping and grazing systems (Baldock and Skjemstad., 1999). Organic matter is important because it affects all the soil quality functions (Figure 2.1) (Fenton et al., 1999) including:

- sustaining biological activity, diversity, and productivity,
- regulating and partitioning water and solute transport,
- filtering, buffering, degrading, immobilizing, and detoxifying organic and inorganic materials, including industrial and municipal byproducts and atmospheric deposition,
- storing and cycling nutrients and other elements within the earth's biosphere,

- The impact of soil organic matter on the soil qualities listed above and soil functions can be summarized as follows :

Organic materials added to soils contain a wide range of C compounds that vary in their rate of decomposition. The biological breakdown of the organic material depends on the rate of degradation. Changes in environmental factors can cause changes in the rate of decomposition of organic materials in soils, such as soil moisture status, soil aeration, soil temperature, pH, and availability of minerals (Baldock and Skjemstad, 1996).

2.1.1. Global Carbon Pools

The major components of the natural global carbon pools are the oceans (38,000 Pg C; Pg C= 10^{15} g), the atmosphere (750 Pg C) and carbon stored in terrestrial ecosystems: 2300 Pg C in soils and 560 Pg C in plants (Figure 2.2). Dissolved organic carbon is moved from the land to the ocean through the rivers at a rate of 0.4 Pg C a⁻¹. A further 0.4 Pg C a⁻¹ flux of dissolved inorganic carbon (DIC) is assumed to be from the weathering of CaCO₃, taking up CO₂ from the atmosphere in a 1:1 ratio (Schlesinger., 1990, 1997).

An increase in the atmospheric from the soils, biota, or oceanic pools can lead to global warming and climate change (Lal et al., 1995a, Mermut., 2009). Important greenhouse gases in the atmosphere are water vapor, carbon dioxide (CO₂), methane (CH₄), nitrous oxide (N₂O), oxides of nitrogen (NO_x), tropospheric ozone (O₃), carbon monoxide (CO), and halocarbons (Lal et al., 1995b).

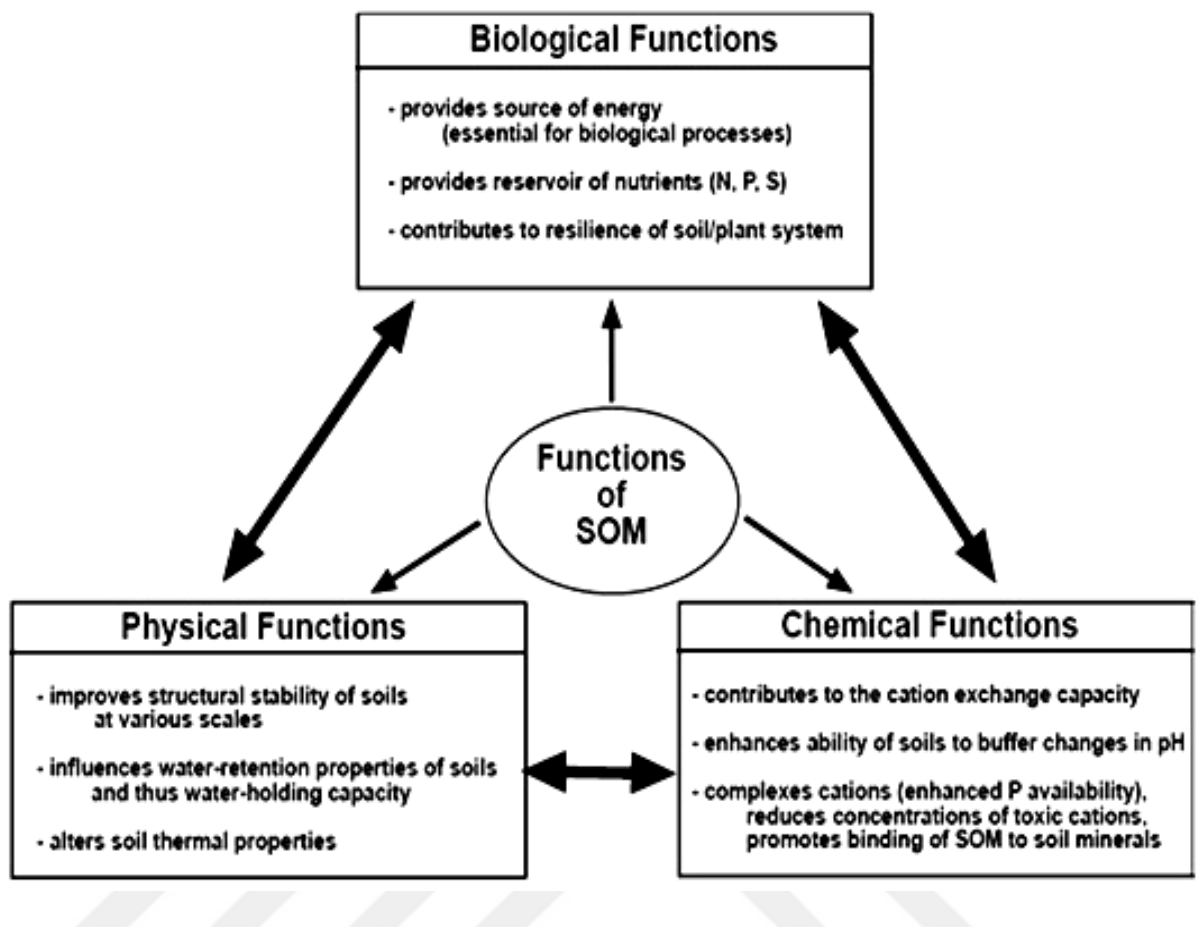


Figure 2.1. Functions of soil organic matter (from Baldock and Skjemstad, 1996)

The atmospheric concentration of CO₂ has increased from 280 ppm (in 1750) to 367 ppm (in 1999) (31%) (IPCC, 2001a). Atmospheric methane (CH₄) concentrations have increased by about 150% (1,060 ppb) since 1750. The atmospheric concentration of nitrous oxide (N₂O) has steadily increased during the Industrial Era and is now 16% (46 ppb) larger than it was in 1750. Major natural sources of these gases are terrestrial ecosystems, including soils, biota, wetlands, and volcanic eruptions (IPCC, 2001a). A doubling of preindustrial CO₂ concentrations by the end of the twenty-first century is expected to increase global mean surface temperature by about 2°C (Mosier, 1998).

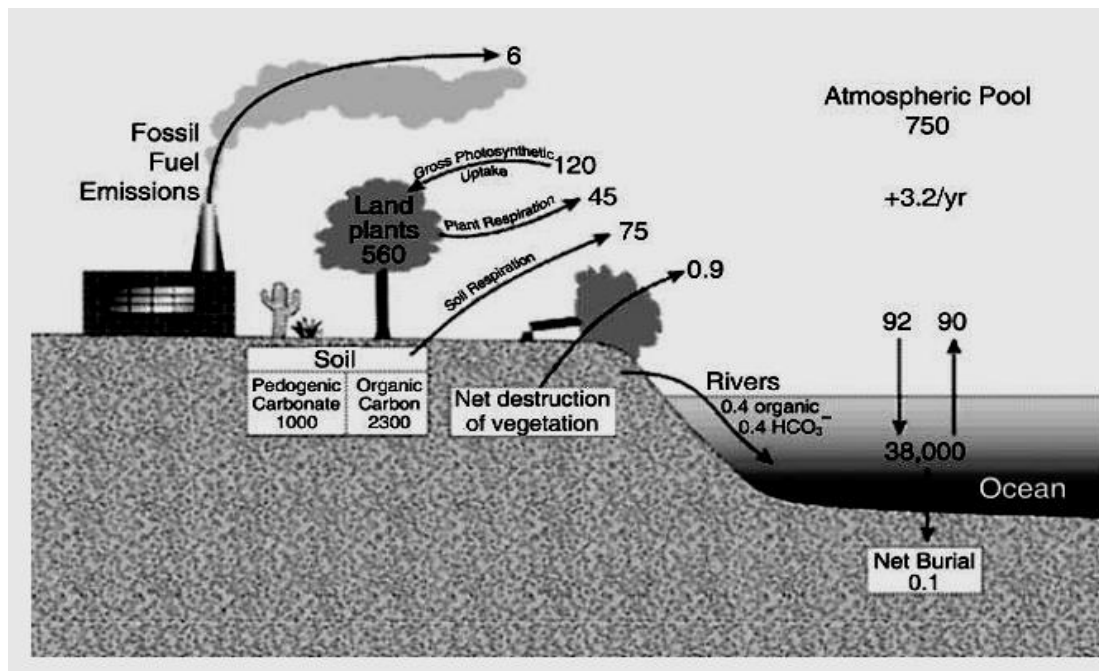


Figure 2.2. The global C cycle, showing in the size reservoirs (10^{15} GtC) and the annual flux between them (10^{15} gC/yr) (Modified from Schlesinger., 1997).

Soils contain the largest “active” terrestrial carbon pool on Earth, and through soil respiration, annual flux of CO_2 to the atmosphere is about 10 times greater than from fossil fuel combustion (Schlesinger, 1997). Unlike forests, agricultural lands, and other terrestrial ecosystems are source rather sink. One-half to two-thirds of the estimated historic C loss from cultivated soils could be recovered in the next 50 years, (IPCC, 2001b) with the potential soil C increases of about 0.4 to 0.6 Gt C a^{-1} from the better management of available agricultural soils. The current evaluation of the potential of biological decrease options is about 100 Gt C (cumulative) by 2050, equivalent to about 10% to 20% of expected fossil fuel emissions during the period. Increased soil carbon pools through the appropriate management of terrestrial ecosystems can only partly decrease fossil fuel emissions (Flessa et al., 1996).

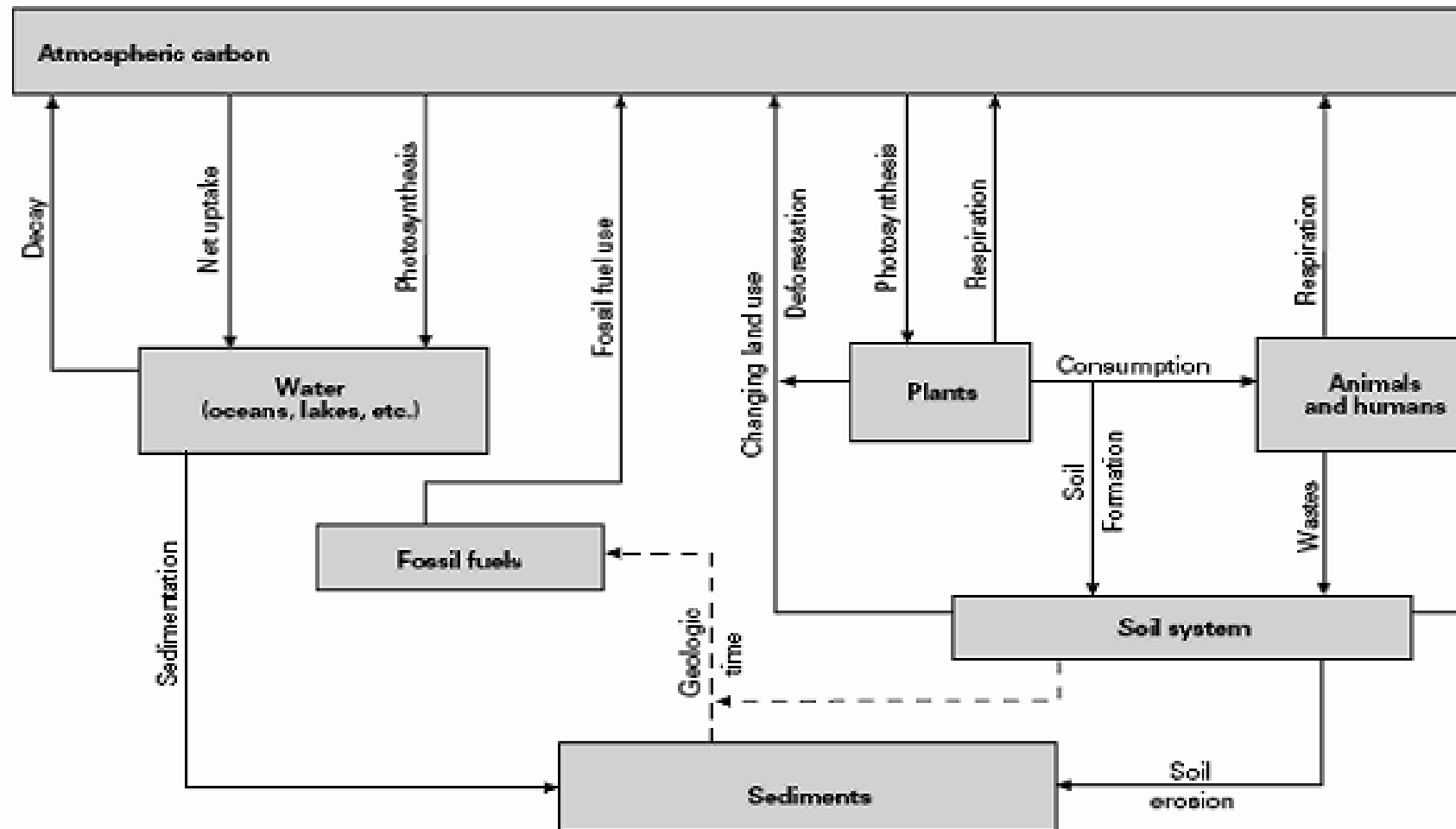


Figure 2.3. Carbon cycle aspects (modified from Paul and Clark, 1996).

Global soils contain approximately 1500 Pg carbon (Post et al., 1995). As can be seen in Figure 2.3, soils can be either net sources or sinks of the atmospheric carbon dioxide. Changes in land use and agricultural activities during the past 200 years have made soils act as net sources of atmospheric CO₂. Experimental studies suggest that carbon losses due to oxidation and erosion can be reversed with soil management practices that can minimize soil disturbance and increase plant yield, through fertilization. Improved land management can result in a significant amount of increasing rate of carbon sequestration. Because of the relatively long turnover time of some soil carbon fractions, this could result in storage of a large amount of carbon in the soil (Lal et al., 1998, 1999).

2.1.2. Forms of Soil Organic Carbon

Globally, carbon is continually flowing from one stock to another, such as the transformation of carbon dioxide to plant carbon, through to soil carbon and then back again to its gaseous form in atmosphere. Within the soil phase, carbon occurs in various chemical compositions and rate of decomposition. It can be divided into the four major pools of soil organic carbon: labile, humic, inert, and microbial pools (Table 2.1). They all have important and different roles for the continued health of soil systems. The labile pool may remain in soils for a relatively short time period (weeks to months) compared with the humic (10s to 100s of years) and inert (100s to 1000s of years) pools. The labile pool provides the critical functions of food and energy for the microbial biomass, and is associated with macro-aggregate stability. The more resistant forms also provide

other vital functions including developed nutrient availability and retention, and micro-aggregate stability (Buldock and Skjemstad., 1999).

Soil organic carbon exceeds atmospheric C by a factor of two (Schlesinger 1995). Among the components of soil organic C, labile organic C (LOC) is particularly important. This is due to of its closed association with soil microorganisms. Since soil microbes control heterotrophic respiration, they can accelerate decomposition of resistant soil organic matter by the priming effect of LOC (Kuzyakov 2002; Hamer and Marschner 2005).

Table 2.1. Forms of soil organic carbon

Fraction	Particle size	% of SOC	Functions	Decomposition Rate
Labile,active, fast, non living	Disolved OC < 0,5 µm Particulate OC > 53µm	Varies: 1-85 %	Food/energy, nutrients, macro-aggregate stability	Fast: week to months
Slow,humus	< 53 µm	~50	Charges, micro aggregate stability, water holding capacity	Slow: 10s-100s years
Inert,resitant, recalcitrant	< 2 µm (clay -size)	0-50	Thermal, charges, water holding capacity	Very slow: 100s-1000s of years
Living microbial biomass	µm-cm	1-50 % of SOM	Decomposers, nitrifiers, immobilisers	

2.1.3. Influence of Management System on Organic Carbon

Increasing organic matter inputs through the agricultural management systems is the key to increase in SOC content (Janzen et al., 1992). Similarly, qualities of organics (i.e. lignin, C–N ratio etc.) also influence the quantity of SOC development in the soil. In intensive cropping system, burning or removal of

crop residues is a common practice, where combine harvester is used. Thus, only the roots are recycled under intensive cultivation. In this case, there is a need to quantify the C inputs return back to soil.

The composition and losses of SOC was studied by several researchers, especially C and N, under cropping and continuous cultivation systems (Dalal and Mayer, 1986, Dawe et al., 2000 and Swarup et al., 2000). So the restoration and loss of SOC could be effected through land use management practices such as cropping frequency (Campbell et al., 1995), reduced tillage (Reicosky et al., 1995), fertilizer (Gregorich et al., 1996) and manure application (Sommerfeldt et al., 1988). Therefore, long-term sustainability and overall productivity of cropping systems are directly related to the maintenance of soil organic matter

The information on the long-term effect of manure and fertilizer on crop residue production and its following effects on SOC storage and soil quality is limited in sub-humid and semi-arid tropical cropping systems (Dawe et al., 2003). Quantity of SOC in relation to different crop management practices is importance for SOC dynamics in the soil in identifying sustainable management systems in a given area. This is extremely important to understand the effects of cropping practices and soil types on SOC storage, which could influence yield sustainability and soil quality. Major factors influencing the carbon turnover and sequestration in soils are the climate, soil relief, and geological properties of a site, soil texture, pH, and anthropogenic influences (Scheffer and Schachtschabel, 2002).

Table 2.2. Land use for C sequestration

Management Strategies		
Land use	Soil Management	Crop management
Cultivation	Tillage	Varieties
Rangeland	Residue management	Crop rotations
Forestry	Fertility	Cover crops
	Water management	
	Erosion	

(Adapted from Lal et al., 1998)

Agriculture in the 1800s and early 1900s relied upon the plowing the soil with low crop yields and crop residues. The agricultural practices resulted in reducing the replenishment of organic C to the soil. In recent decades, higher yields, return of crop residues, and development of conservation tillage practices have caused an increase of SOM. Table 2.2. depicts several practices affecting the soil's ability to sequester C (Landi et al., 2004; Lal et al., 2000).

2.2. MANAGEMENT SYSTEM OF ORGANIC CARBON

Buyanovski and Wagner, (1998) suggest that soil and crop management can greatly improve the residence time and new C storage in the soil, which is worthy of consideration under the Kyoto Protocol. Different land uses and agronomic practices were evaluated with respect to their effect on carbon sequestration or release (Lal, 1999; Batjes, 1996). A distinction is made between practices causing a decrease of carbon loss, an increase in carbon input into the soil, or a combination of both (Figure 2.4).

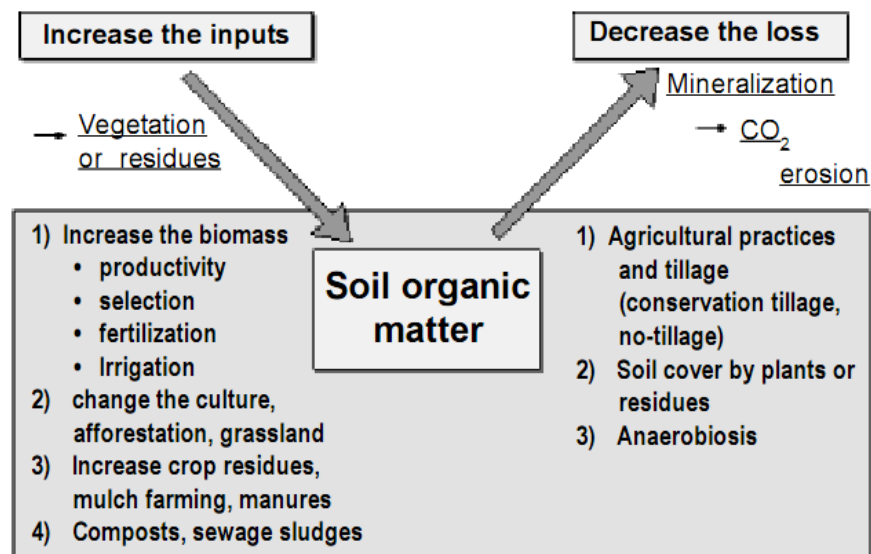


Figure 2.4. Management of soil organic matter in agriculture (Lal, 1999; Batjes, 1996).

The basic principles of soil management to improve soil quality are already well known. For agricultural soil management, they can be categorized as

- 1) Increase in soil organic matter content;
- 2) Improvement in soil structure;
- 3) Increase in soil biodiversity;
- 4) Reduction of erosion rates; and
- 5) Increase in nutrient capital and recycling mechanisms.

The rapid increase of agricultural production in recent years has been achieved by the intensification of land use, the use of motorized farm equipment, and the application of agrochemicals. These technological inputs frequently warn the loss of soil flexibility and environmental pollution in many agricultural areas (Dalal and Chan, 2001).

2.2.1. Tillage

Agricultural management practices influence organic matter in the soil (Reicosky and Lindstrom, 1995), micro- and macro aggregates distribution, rate of soil organic matter (SOM) turnover, saturation limit and SOM stability and their steady state level (Stewart et al., 2007). Intensive tillage have caused a decrease in soil C of between approximately 30 and 50 per cent within the last 100 years (Schlesinger, 1986).

Soil aggregation itself and consequently the C stock, soil organic carbon (SOC) dynamics and organic matter quality is strongly influenced by agricultural management intensity (Pulleman and Marinissen, 2004, Bono et al., 2008; Alvaro-Fuentes et al., 2009; Simon et al., 2009). Soil structure and organic matter storage are also affected by the quality and quantity of organic inputs and the use of pesticides, fertilizers and manure (Droogers and Bouma, 1997). Soil organic matter and structure are mutually related: SOM binds with mineral particles to form soil aggregates and, in turn, stable aggregation can provide physical protection of otherwise mineralisable SOM (Tisdall and Oades, 1982; Gupta and Germida, 1988; Beare et al., 1994b).

In the 20th century, agricultural practices have caused the loss of SOM from cultivated soils (Lal and Kimble, 1997; Smith et al., 2000). The loss of SOM effects the soil structure and soil quality (Reeves, 1997; Six et al., 1998), consequently the released organic C contributes to global warming (IPCC, 2001, 2007). An important objective of sustainable use of soil resources is to increase the soil organic C pools (Paul et al., 2001).

The hierarchical model of aggregate formation by Tisdall and Oades (1982) illustrates the role of various organic binding agents in the organization of stable soil aggregates. Generally, microaggregates are relatively stable and bound by persistent polysaccharide-based glues produced by roots and microbes and by calcium bridges. On the other hand, microaggregates are bound into macroaggregates by a network of roots and hyphae. Therefore, macroaggregate stability is thought to respond more rapidly to changes in soil management such as tillage and organic inputs (Elliott, 1986).

Several researchers have found that aggregate stability and SOM contents of stable macro aggregates were higher in native grassland or reduced tillage compared to conventionally tilled and cultivated systems (Cambardella and Elliott, 1994; Beare et al., 1994a,b; Jastrow et al., 1996; Six et al., 1998). Others reported higher mean residence times of SOM for less-tilled soils (Balesdent et al., 1990; Six et al., 1998; Collins et al., 2000; Dalal et al., 2007; Metay et al., 2007) that were attributed to a better physical protection of soil aggregates in the absence of conventional tillage (CT) practices.

The simulated total soil carbon changes for a depth of 0-20 cm are presented in Figure 2.5 (Smith, 1999). Adverse effects of continuous cropping systems, involving conventional tillage, on soil organic carbon dynamics and crop productivity are widely recognised (Lal 2002; Nyamadzawo et al. 2007).

Soil organic carbon (SOC) is closely linked to soil structural stability, soil fertility and hydraulic properties, and hence crop productivity (Lal 2002). Large amounts of literature are available on tillage effects on soil properties and crop productivity (e.g. Chivenge et al. 2007; Nyamadzawo et al. 2007).

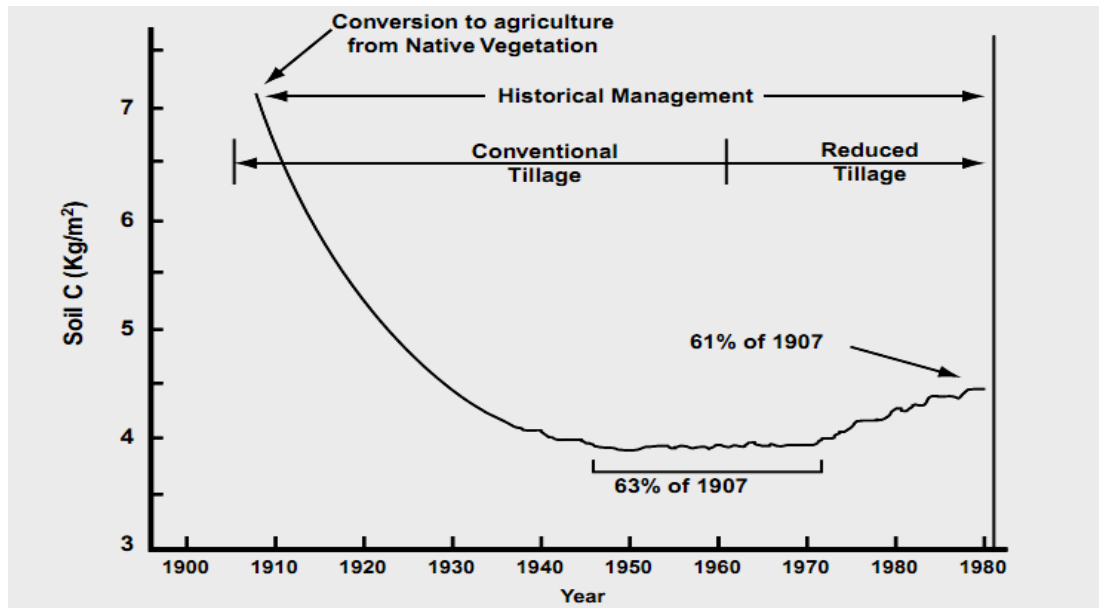


Figure 2.5. Simulated total organic carbon changes (0 to 20 cm. depth) from 1907 to 1990 for the central U.S. A. corn belt (from Smith, 1999).

Conservation tillage has been reported to reduce soil erosion and improve the soil moisture and crop yields compared to conventional tillage (Nyakatawa et al. 1996; Nyamudeza 1998; Nyagumbo 1999). Yield increases under conservational tillage were linked to improved soil moisture. However, yields were fluctuated considerably, depending on seasonal rainfall patterns. By comparison, studies involving retention of crop residues on the soil surface, such as no-till and minimum tillage systems are relatively limited.

2.2.2. Commercial Fertilizers

There are five major types of fertilizers: complete inorganic, special-purpose, liquid, slow-release and fertilizer with insecticide or herbicide. Complete

inorganic fertilizers provide some amounts of each inorganic nutrient (N:P:K) (Nuttall., 2009)

The primary advantage of using packaged commercial fertilizer is that nutrients are immediately available to the plants. The exact amounts of a given element can be calculated for a given plant (Stewart et al., 2005). Disadvantages of using commercial fertilizer, especially nitrogen, is easily washed below the level of the plant's root system, through the leaching of rain or irrigation. An application which is too heavy or too close to the roots of the plants may cause "burning" (actually a process of desiccation by the chemical salts in the fertilizer). As well, overly heavy applications of commercial fertilizers can build up toxic concentrations of salts in the soil and consequently creating chemical imbalances. If organic materials are readily available and cheap, the expense of the commercial fertilizer should also be considered (Stewart et al., 2005).

2.2.3. Crop

Benefits to crop production can be realized when organic fertilizers are used as a nutrient source. Owing to their organic nature, these materials can build soil organic matter reserves, thus improving the soil quality. The beneficial functions of soil organic matter include its capacity to: (1) serve as a nutrient reserve; (2) increase soil cation exchange capacity; (3) provide energy substrates for microbial transformations; (4) increase soil water-holding capacity; (5) stabilize soil structure; (6) reduce soil crusting; (7) increase water infiltration into soil; and, (8) buffer against rapid changes in acidity, alkalinity, and salinity (Tisdale et al., 1985).

Generally, most soils of dry-land ecosystems are characterized by low SOC contents. In the semiarid environment, conservation soil management techniques allow the increase of C only in the surface soil (Alvaro-Fuentes et al., 2008; Bell et al., 2003). The contribution to SOM concentration is determined by the incorporation of new organic matter in the coarse fraction and the reduction of mineralization processes in the fine ones (Oue'draogo et al., 2005).

Soil organic carbon has also been identified as a possible C sink for sequestering atmospheric carbon dioxide (Jenkinson et al., 1991; Cox et al., 2000; Jones et al., 2005; Farage et al., 2007). Under cropping, it is generally agreed that the no-tillage (NT)/reduced tillage and conservation tillage (CT) favors soil carbon sequestration. From the information available, the scope of soil carbon sequestration under conservation tillage is more limited in Australia than elsewhere but the reasons are not clear (Chan et al., 2003).

Kern and Johnson (1993) reported that soil organic carbon under conservation tillage and conventional tillage in 18 field trials across Australia and found lower amounts of soil carbon than those reported in the soils of USA. The effects of tillage and stubble management are often not separated. The effects of the two practices on soil carbon dynamics are different and can be opposite: no-tillage reduces the rate of organic carbon breakdown (compared to conventional tillage) and potentially can reduce soil carbon losses, while stubble retention/burning directly affects the rate of organic input. In practice, it is desirable to quantify the effect of each of the practices individually so that their contributions can be evaluated separately.

2.3. INORGANIC CARBON

In recent years more works were carried out on the role of soil organic carbon (SOC), (Lal et al., 2005; 2007) than soil inorganic carbon (SIC)(Lal, 2000; Batjes, 1996) in the global carbon cycle. Because, there is difficulty differentiating lithogenic from pedo- genic carbonates because most chemical methods determine the total quantity of carbonates in the soil without regard to origin. The amount of pedogenic carbonate formed in soils is typically estimated from visible segregations in the field (Soil Survey Staff, 1999) or from stable carbon isotope analysis to differentiate the pedogenic carbonate (Nordt,1998). Gile and Grossman (1979) and Landi et al. (2003), consider that much of the calcium in carbonates results from carbonate dusts or from calcium in rainwater. Chadwick and Capo (1993) believe that at least 95% of the Ca in carbonates of arid and semiarid regions is derived from dusts. Many soils also have lithogenic carbonate inherited from alluvial, till, and bedrock parent materials. It is possible that as little as 10% of the total SIC reported in soils is formed from the authigenic weathering of calcium-bearing minerals.

The flux rates of inorganic carbon stocks require knowledge of soil chronology coupled with mass balance reconstruction analyses to determine the magnitude of gains or losses (Chadwick et al., 1994; Nordt et al., 2000; Drees et al., 2001).

Table 2.3 presents SIC and TC stocks according to global soil moisture and temperature conditions. It is interesting to note that in Mediterranean climates with winter rain, SIC contributes 56% of the TC stock. Because of the semiarid condition the SOC pool is less important than SIC. From the above comments, it

is not surprising that a little over 38% of the TC stock (2472 PgC) is SIC (946 PgC), with arid (30%) and semiarid (6%) regions serving as major contributors (Table 2.3).

Table 2.3. Soil inorganic carbon (SIC) and total carbon (TC) stocks according to moisture conditions (from Eswaran et al., 2000)

Moisture Condition	TC		SIC		SIC/TC	SIC/TC
	Pg	% Global	Pg	% Global	% Region	% Global
Permafrost	405	16.4	18	1.9	4.4	0.7
Arid	877	35.5	732	77.8	83.5	29.7
Mediterranean	90	3.6	50	5.4	55.6	2.0
Semiarid	471	19.1	134	14.2	58.5	5.5
Humid	539	21.9	4	0.5	0.7	0.2
Perhumid	85	3.4	2	0.2	2.4	0.1
Total	2467	99.9	940	100	205.1	38.2

Global pool of SIC is quite large, its formation, quantity, and dynamics are still uncertain. The major problems in determining SIC stocks are the incomplete global database and the difficulty in separating pedogenic from lithogenic carbonate sources. To provide better estimates of SIC stocks, more sampling and analysis are needed that involve good soil chronology, stable carbon isotopes to differentiate pedogenic from lithogenic carbonates, and techniques to quantify the SIC stocks (Wilding et al., 2006).

2.4. INFLUENCE OF MANURE APPLICATION ON SOIL ORGANIC CARBON

The application of organic materials to the soil increases organic matter and nutrient supply. The external sources of organic matter in the soil include crop residues and animal manure. Mixing animal excrete with straw, which often serves as bedding material, makes the resulting material more suitable for land

application, because the straw lowers the excessive water content of the animal excreta. The management of organic materials requires knowledge about their quality and fate in the soil (Saviozzi et al. 1997).

The use of manure can increase the soil organic matter (SOM) and this effect has been recognized and used for nearly 4000 years in China, Japan, and Korea in order to restore soil fertility and obtain a satisfactory yield (Dormaar et al., 1988), because SOM contributes to nutrient supply, improvement of soil physical properties, protection from erosion, and promotion of biological activity (Jimenez et al., 2002). Fertilization is often recommended to increase SOM level and carbon (C) sequestration in soils of highly managed multiple cropping systems (Yang et al., 2005; Denef et al., 2007; Gulde et al., 2008). Many studies have shown that application of organic manure, either alone or in combination with mineral fertilizers, is more effective in increasing SOM and its fractions than mineral fertilizers alone (Wu et al., 2003; Yang et al., 2005; Blair et al., 2006; Rudrappa et al., 2006; Purakayastha et al., 2008). Fertilization could promote aggregate formation (Sleutel et al., 2006) and stabilization (Blair et al., 2006), then enhance the spatial inaccessibility for decomposing organisms (Kögel-Knabner et al., 2008).

Manure contains most elements required for plant growth including N, P, K, and micronutrients. Soil organic matter is considered nature's signature of a productive soil. Organic carbon from manure provides the energy source for the active, healthy soil microbial environment that both stabilizes nutrient sources and makes those nutrients available to crops (Bigeriego, 1995).

2.4.1. Organic Residues

The level of soil organic matter in a particular system is a function of three major factors: climate, soil type and land management. These three factors are closely related, and the level of influence of each of these factors will vary according to each individual system. Apart from the addition of organic amendments, the primary method for increasing carbon in the soil is through the transformation of gaseous carbon into plant matter from photosynthesis, many losses from erosion, decomposition and product removal (Dalal and Chan., 2001).

Plants can contribute through both shoot and roots to soils, and the level of plant matter addition is determined by the amount and type of plant grown. The amount of plant is primarily a function of climate and soil type. Therefore, wetter regions will have a greater potential to grow more plant than drier regions of similar soil type. More fertile soils will encourage greater plant growth and, therefore, more soil organic matter accumulation when compared to lower fertility soils from a similar climate. The type of plant can also influence how much carbon is added to the soil. For example, pasture plants with greater root/shoot ratios can add more organic matter to the soil compared with cropping plants with lower root/shoot ratios. Pasture life cycles are also generally longer and therefore can contribute organic residues to soils over longer periods compared to shorter term cropping species (Dalal and Chan., 2001).

Organic residue decay, nutrient release, and humus formation are controlled by environmental factors and by the quality of organic materials. Along with high C/N ratios, high contents of lignin and polyphenols slow the decomposition process, causing organic matter to accumulate while decreasing the availability of

nutrients. The carbon-to-nitrogen (C/N) ratio of most soils is variable, generally near 12:1. Soil management for increasing organic matter levels should include some supplying nitrogen (Skeffington, 1990).

2.4.2. Farmyard Manure

Manure (animal waste) is generated in beef cattle feedlots, swine operations, dairy barns, poultry houses, and other livestock operations. The number of animals and the number of large production facilities in the U.S.A. have significantly increased in the past 10 years (Anonymous., 2000). Manure, as well as composts and biosolids, is a renewable resource and an excellent source of macro- (N, P, K, Ca, Mg, and S) and micronutrients (Zn, Cu, Fe, Mn, etc.) that are essential for growing plants. For centuries manure was used throughout the world for improving soil fertility and enhancing crop productivity. However, with the advent of synthetic fertilizers after World War II, manure was considered more a liability than a nutrient resource for crop production (Eghball and Barbarick., 2006).

When animals are grazing on pastures and range lands, manure is dispersed across a large area and little management is needed, because the material is not concentrated and decomposes rapidly. However when animals are concentrated in small feeding areas the quantity of manure increases greatly. Significant amounts of manure are generated each year in the U.S.A. and elsewhere in the world from the confined feedlots of livestock.

The amount of N, P, and K present in the manure from these species would replace 25%, 25%, and 45% of the purchased N, P, and K fertilizers, respectively, in U.S.A. If utilized agronomically they can replace or reduce the use of commercial fertilizer. Crop producers are generally reluctant to use manure, because of factors such as hauling and spreading costs, potential introduction of weed seeds, nonavailability of manure where needed, uncertainty about availability of manure nutrients to plants, and problems of smell and application uniformity. The global numbers of major livestock species are given in Table 2.4.

Table 2.4. Global numbers of major livestock species in 1997 (from FAO., 2001).

Animal Species	Animals (million)
Cattle	133
Chickens	14156
Sheep and Goats	1754
Swine	837

2.4.3. Recycling of Pig Slurry

The recycling of pig slurry in agricultural soils is an alternative and valuable practice in countries such as Spain with significant European pig production (2.529 million sows in 1998). Spain is the second pig producer in the Europe with 19% production power, the meaning of this ratio is 3.7 billion € (Hansson, 2003) (Table 5). This is particularly the case since many regions are arid and are comprised of poor soils (<1% organic matter). Recycling supplies agricultural soils with organic matter, nutrients, and micronutrients, but also adds unwanted substances such as food additives, pesticides, pharmaceutical products, along with their metabolic and degradation by-products.

Table 2.5. Pig Production in Europe between 2005-2007

EUROPE	2005	2006	2007
Germany	44833	45402	44800
Spain	38993	40100	39780
Benelux	32800	32850	33100
Denmark	26857	26000	26900
Poland	25500	26000	25500

In Spain, nearly 2.5×10^6 Mg (dry wt.) of pig manure is collected annually (Bigeriego, 1995), and it has been acknowledged (MAPA, 1997) that approximately 50% of this manure is spread on agricultural land. In Murcia region, the area of Guadalentin Valley has more than 50% of the total pig production (Figure 2.6).

Current European Environmental Council Directives such as the Waste Directive (European Union, 1991a) consider the need for integrated environmental assessment to ensure “the protection of the environment and human health,” through use of the best available methodologies. Therefore, to evaluate the use of pig slurry, and assess its toxicity, knowledge of the fate and bioavailability of its components and estimate of potential routes of contamination and effects on organisms are required.

The N content of the slurry needs to be determined to accepting ways of decreasing its environmental impact from an agricultural perspective (CD 91/676/EEC; Diez et al., 1998). A further essential is to establish the available soil N including potentially mineralizable N (Sanchez et al., 1998). In addition, irrigation quantity needs to be regulable to satisfy for evapotranspiration losses and thus minimize water losses owing to drainage (Diez et al., 1997).

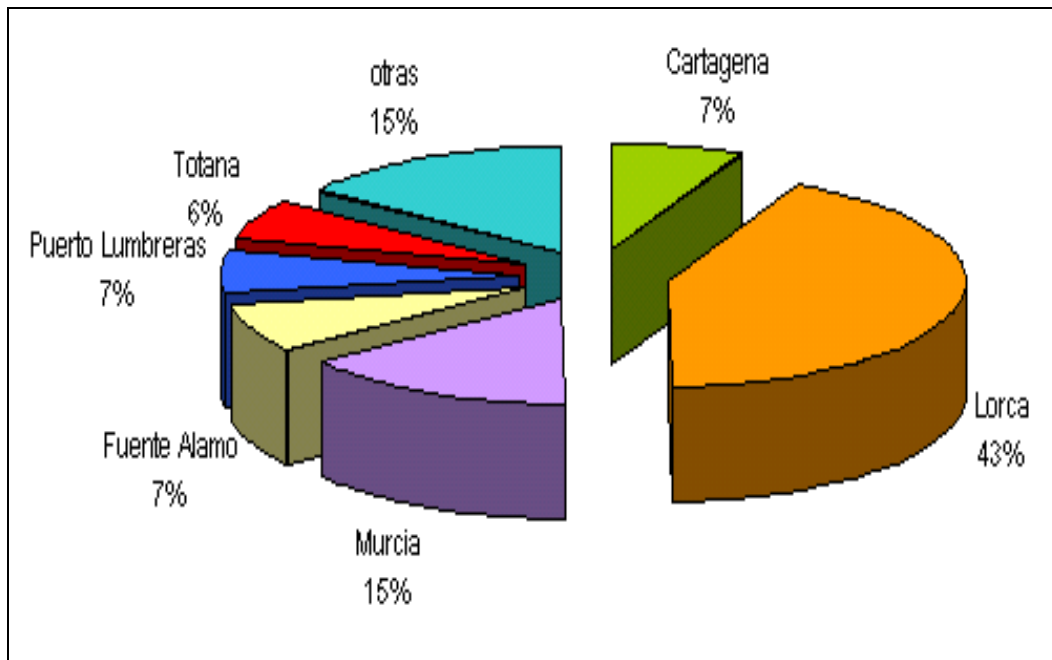


Figure 2.6. Percentage of pig production in Murcia Region.

2.4.4. Efficiency of Farmyard and Other Organic Residues

The SOM is a biological buffer, which plays an important role in maintaining the balanced supply of available nutrients for plant growth. The higher SOM content reflects higher soil productivity (Karama et al., 1990). Therefore, application and management of biosolids as fertilizers are viable options to improve plant growth. Soils with low SOM content lose their buffering capacity and productivity, and have low fertilizer use efficiency. In areas of intensive cropping an increased level and better quality of SOM can contribute to high productivity significantly (Finck, 1998). Organic fertilizers vary widely in terms of their nutrient contents (Table 2.6) depending on the biosolids and their constituents (Anonymous, 1961).

The chemical composition of decomposing materials was considered a critical factor in determining rates of decay (Saviozzi et al. 1993). The pool of available C has an extremely high influence on the rate of decomposition of organic materials (Knapp et al. 1983), however there are only little experimental work to determine the pattern of decomposition of organic materials in the soil in the presence or absence of their labile C pool. Reinertsen et al. (1984) determined the relationship between soluble C and rate of wheat straw decomposition, after leaching water-soluble C from the residue, concluded that the decomposition rate in the early stages is dictated by the size of the soluble C pool and an intermediately available C fraction that is not water-soluble. Christensen (1985), studying the influence of leaching on decomposability of barley straw, confirmed that microbial respiration rate was reduced following soluble C losses.

It is difficult to estimate general changes in total C input into the soil by condensation of crop production: Mineral fertilization changes the amounts of C separated under the ground as well as C soil pools (Kuzyakov and Domanski, 2000). In studies of wheat (Liljeroth et al., 1990), maize (Anderson, 1988) and horticultural plants (Kuzyakov et al., 2002) it was observed that the relative amount of belowground simulated C decreased due to N fertilization. This shows that measures to optimize above-ground plant growth and total fixed C (total dry mass production) result in a decrease of below-ground changed portion of simulated C, although the amount of simulated C increases.

Nitrogen provides N delivery to plants and minimizing nitrate leaching and optimal management depends on an understanding of the dynamics of N

transformation in soils. The addition of inorganic amendments on a regular basis may lead to an increase in SOM and N (Gregorich et al., 1996).

Table 2.6. Nutrient analysis of some common biofertilizers (Anonymous., 1961)

Manures	Nutrient content (percent)		
	N	P₂O₅	K₂O
Fresh dung			
Cattle	0,3-0,4	0,1-0,2	0,1-0,3
Horse	0,3-0,4	0,3-0,4	0,3-0,4
Sheep	0,5-0,6	0,4-0,6	0,3-1,0
Poultry manure (fresh)	1,0-1,8	1,4-1,8	0,8-0,9
Farmyard manure (dry)	0,4-1,5	4,0-5,0	0,3-1,9
Crop residues			
Rice	0,36	0,08	0,71
Corn	0,42	1,57	1,65
Wheat	0,53	0,1	1,1
Green manure			
Sesbania aculeate	0,62	-	-
Cymposis tetragonolaba	0,34	-	-
Crotolaria juncea	0,75	0,12	0,61

As micronutrients have been showed in sewage sludge, the potential negative effects of heavy metals added to pig diets (Chaudri et al., 1993; McGrath et al., 1999). Therefore, maximum permitted heavy metal limits have been established for agricultural soils treated with slurry (European Union, 1986). Other compound, such as phenols and indols (Hammond et al., 1989; Lo et al., 1994), encourage the bacterial decomposition of proteins in the pig intestine (Jensen et al., 1995), surfactants (Warren-Hicks and Parkhurst, 1992), PCBs (ECME, 1999), and pathogenic microorganisms (Dewi et al., 1994) have been determined, while their environmental relation, in terms of toxicological and

ecotoxicological results, are only just beginning to be appreciated (de la Torre et al., 2000).

It is well known that for many organic compounds, toxicity can often be directly related to organic matter content (Van Gestel and Ma, 1990) and their bioavailability in the soil pore water will depend on soil properties and other factors, such as their sorption capacity (Van Straalen and Van Gestel, 1998).

2.4.5. Oxidation and Turnover Rate

Soil organic matter (SOM) plays a major role in sustaining ecosystems by regulating nutrient cycling and impacting physical properties. The quantity of SOM is dependent on the balance between primary productivity and the rate of decomposition. The presence of silt and clay generally protects some C from primary production and increases SOM under certain environments. The highest accumulation of C is found in marshes (723 t ha^{-1}) where NPP is high but decomposition is inhibited by a lack of O_2 (Table 2.7). Decomposition also is high inhibited by cold in tundra soils and C tends to accumulate there primarily as litter and POM.

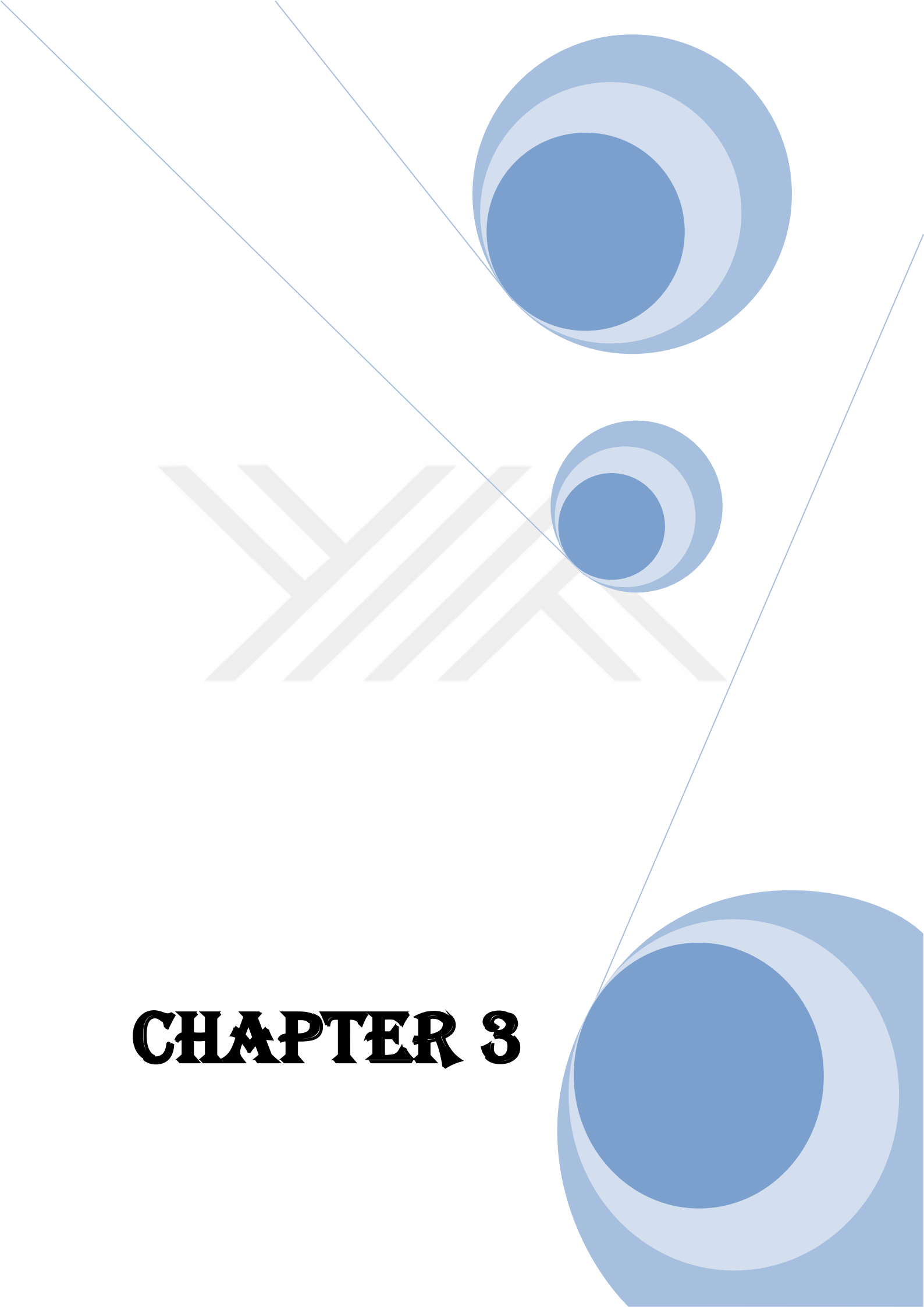
Soil organic matter includes a number of fractions with different turnover rates, rather than the one value given for total SOM. The overall turnover time mirrors the rapid turnover of the recent plant residues. The majority of the SOM turns over much more slowly, with mean residence times, often thousands of years for the humic fractions protected by clay minerals (Paul et al., 2001).

Table 2.7. The area, stock of C, NPP, and turnover of various biomes ^a

Biomes	Global C stock				NPP (PgC/year)	Turnover (years) ^b
	World Area (10 ⁹ ha)	Plant	Soil	Total		
Tropical forests	1.76	340	213	553	17.8	38
Temperate forests	1.04	139	153	292	7.3	29
Boreal forests	1.37	57	338	395	2.9	91
Tropical savannas and grasslands	2.51	79	247	326	16.3	10
Temperate grasslands and scrublands	1.52	23	176	199	6.15	61
Deserts and semideserts	3.66	10	159	169	2.45	37
Tundra	0.76	2	115	117	0.75	490
Croplands	1.48	4	165	169	5.45	21
Wetlands	0.35	15	225	240	4.3	520
Total	15.0	669	1791	2460	63.4	

^a Adapted from Houghton et al. (2001).^b Adapted from Raich and Schlesinger (1992). Turnover of soil C assumes 30% of soil respiration is derived from root respiration.

Consequently, carbon is very important in terms of sustainable agriculture, organic carbon is highly related to the soil type and management systems. Research on the impact of tillage practices and crop rotation has demonstrated that no-till and permanent vegetation are more effective in storing carbon in the soil. The use of crop rotation and conservation tillage, in addition to effective farmyard manure and carbon management systems, contributed significantly to improving soil carbon status. The value of carbon storing through conservation practices can be very significant in improving soil quality and productivity.

The background features a light grey 'X' shape formed by two thin lines crossing. Three blue circles of varying sizes are positioned at the top, middle, and bottom right. The circles are composed of concentric rings of different shades of blue. The text 'CHAPTER 3' is located in the lower-left quadrant.

CHAPTER 3

CHAPTER 3

PIG SLURRY APPLICATION TO IMPROVE SOIL QUALITY AND SUSTAINABILITY IN SEMIARID REGION OF SPAIN.

Abstract: Pig slurry (PS) application effects on soil properties, quality, and productivity were studied. Three different doses on a silty loam soil were applied: Single (D_1), Double (D_2), Triple (D_3) and unfertilized plots (C) served as controls. Additions of pig slurry to soils at agronomic rates (D_1) to match crop nutrient requirements showed that the soil quality improved with the applications of PS, depending on the soil characteristics in the semiarid regions. Dose 1 (D_1) is the agronomic rate of N-requirement (170 kg N/ha/yr) (European Directive 91/676/CEE) and appears to be very appropriate in term of sustainable agriculture. The yield decrease in D_2 and D_3 in comparison to D_1 were due to accumulation of salt existing in the PS applied. Soil OC was increased with the amount of PS applied. The level of benefits from the use of PS depends on the management in terms of the soil quality and productivity.

Keywords: Organic C, Crop Yield, Barley, Pig Slurry.

3.1. INTRODUCTION

For centuries manure was used throughout the world for improving the soil fertility and enhancing crop productivity. Pig slurry (PS) is not a waste product: it is a valuable plant nutrient resource of macro- (N, P, K, Ca, Mg, and S) and micronutrients (Zn, Cu, Fe, Mn, etc.) that are essential for plant growth, with the added benefit of improving soil quality (Torres, 1993; Yanardag et al., 2014). Despite the availability of published data there are still a lack of information about the agronomic benefits and disadvantages of the organic matter from anaerobically digested pig slurries, although they are now widely used in agricultural soils. This is of particular interest since soil organic matter is decreasing in the cultivated soils, which is a matter currently receiving much attention in Europe

Pig manures may increase soil fertility, and thus the crop production potential possibly by changes in soils physical and chemical properties including nutrient bioavailability, soil structure, water holding capacity, cation exchange capacity, soil pH, microbial community and activity (Walker et al., 2004; Agbede et al., 2008; Muhammad and Khattak, 2009).

The recycling of PS in agricultural soils is a quiet common practice, especially in southeastern Spain, which is arid and are comprised of poor soils with organic matter (OM) < 1%. A healthy soil in properly-functioning ecosystem is often characterized by a good soil quality and sufficient OM content in which essential nutrients are readily available to support plants and organisms (Sollins et al., 2006; Ottenhof et al., 2007). In Spain, nearly 2.5×10^6 Mg (dry wt.) of pig manure is collected annually (Bigeriego, 1995), and it has been acknowledged (MAPA, 1997) that approximately 50% of this manure is spread on agricultural land.

Effects of manure addition on increasing soil organic matter content are more pronounced on soils of low organic matter content and fertility. It well known that the application of manure can increase the SOM and contributes to nutrient supply, improving soil physical and chemical properties (Loro et al., 1997).

The objectives of this chapter were to: 1) find the best rate of PS applications in terms of soil characteristics, quality, and productivity, 2) determine plant uptake of macro and micronutrients.

3.2. MATERIAL AND METHODS

3.2.1. Description of the Study Area and Samplings

The study area is located southeast of Lorca, Murcia region, Southeast of Spain (Guadalentin Valley-37° 55' 27, 87" N -1° 49' 37, 84" W), which has 18 m with 3.4% slope. Climate is semiarid, with mean annual average rainfall of 300 mm and mean annual temperature of 18°C and potential evapotranspiration is >900 mm yr⁻¹ (López-Bermúdez et al., 2002).

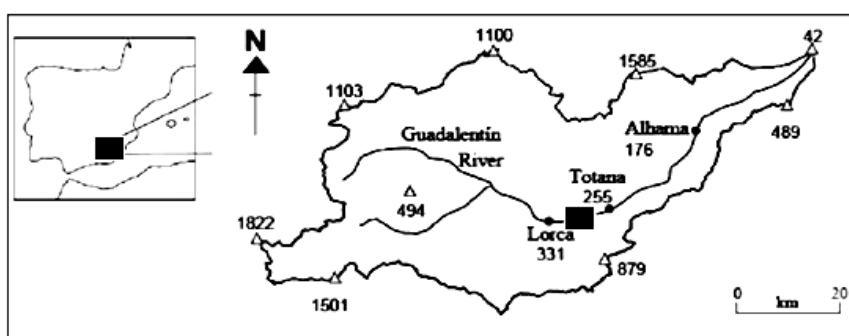


Figure 3.1. Location of the study area (adapted from Mateus, 2006).

The soil studied could be classified as Typic Haplocalcid according to Soil Survey Staff (2010). Texture classification is silty loam. pH is slightly alkaline (between 7.2

and 7.8) with no salinity. Organic carbon content ranged between 13.08 and 14.31 g kg⁻¹. Soil has very low contents of total nitrogen (TN) (app.0.5 g kg⁻¹) and cation exchangeable capacity (CEC) (8.7-18.3 cmol⁽⁺⁾ kg⁻¹, high contents of lime (CaCO₃) (app. 55%) and phosphorus (P) (6.3-62.0 mg kg⁻¹) in the profile.

The area of each plot was 25 m² (5x5 m). Single doses (D₁), double doses (D₂), and triple doses (D₃) of PS were applied to the each plot (Figure 3.2.). They were calculated the agronomic rate of N-requirement as 170 kg N ha⁻¹ yr⁻¹ (European Directive 91/676/CEE), 340 kg N ha⁻¹ yr⁻¹ and 510 kg N ha⁻¹ yr⁻¹ respectively. Soil samples were collected from surface and subsurface horizons (0-30 cm and 30-60 cm. Figure 3.3 and Figure 3.4). Total seven soil sampling and two PS application results were evaluated. The experiment was conducted between 2009 and 2011. Pig slurry was applied once per a year mostly in August. Plants samples were harvested once per a year during three year.

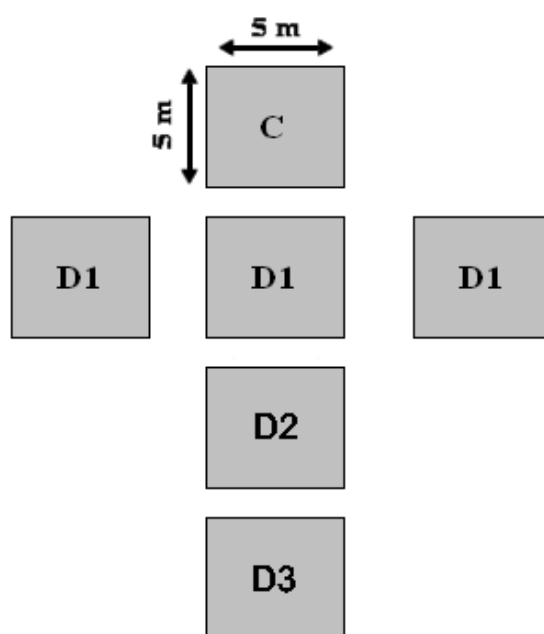


Figure 3.2. Experimental design of the plots from study area (C: Control Plot, D₁: Single Dose, D₂: Double Dose; D₃: Triple Dose)



Figure 3.3. Equipment used and method of pig slurry application in study area



Figure 3.4. Collected soil from two depths (Surface and Subsurface).

3.2.2. Analytical Methods and Statistical Analysis

The collected soil samples were air-dried in the lab, passed through a 2-mm sieve, homogenized, and stored in plastic bags at room temperature prior to laboratory analyses. We analyzed pH (1:2.5 w/v), EC (1/5 w/v), organic carbon, nitrogen, cation exchange capacity according to Peech's method (1965), Bower and Wilcox (1965), Duchaufour (1970), Chapman (1965), respectively.

Barley plants were taken in each plot from 1 m² at harvest time. Samples were separated and dried in an oven at 60 ° C for 48 hours. The grain samples were ground and stored in the special glass containers to determine chemical characteristics of plants.

Plant yield were measured and calculated on the basis ton/ha for the year 2010 and 2011.

In order to determine nutrients and phosphorus content in plant; 0.7 g samples were weighted and ashed in a muffle furnace at 480°C for 24 h. The ash residue was digested in 6 N nitric acid and the solution used to determine nutrients (Na, K, Ca, Mg, Zn, Cu, Fe and Mn) by an atomic absorption spectrophotometer and phosphorus by a spectrophotometer. Plant available phosphorus (P_2O_5) was measured according to Watanabe and Olsen (1965), based on the extraction of phosphorus $NaHCO_3$ 0.5 M solution (Olsen and Dean, 1965) and photo colorimetric determination of molybdenum blue (Murphy and Riley, 1962), by UVmini-1240 Spectrophotometer Shimatzu. Total N was measured by Kjeldahl method (KjelFlex Büchi K-360), as described by Duchaufour (1970).

pH (Peech, 1965), electrical conductivity (EC) (Bower and Wilcox, 1965), Total Nitrogen (TN) with Kjeldahl method (Duchaufour, 1970), Total phosphorus (Olsen and Dean., 1965) were analyzed in PS applied. Raw PS was diluted (1/10) in a 100 ml flask, manually shaken 1 minute, then filtered with Albet paper No 242. This extract was kept to measure trace elements (Cu, Zn, Fe and Mn) by atomic absorption flame (AAnalyst 800, Atomic Absorption Spectrometer by Perkin Elmer). All data were analyzed by parametric analysis of variance (ANOVAs) using the software package “SPSS Version 19.0” for selected subsets of data.

3.3. RESULTS

3.3.1. Selected Properties of Pig Slurries and Soil

Pig slurry applied characteristics were given in Table 3.1. Density was approximately 1 g mL^{-1} . Humidity was changed between 95% and 96.3% in each year. The dry matter content of the PS of first year application was higher than the second year, electrical conductivity (EC) of applied PS in the second year was similar with first year. Total nitrogen of PS was changed between 1.0 and 1.4 g L^{-1} in first year, which was higher than second year.

Table 3.1. Characteristics of applied pig slurry from the study area (n:3)

Parameter	1st year		2nd year	
	Content	SD	Content	SD
Density (g mL^{-1})	1.0 $\pm$	0.0	0.9 $\pm$	0.1
pH	7.2 $\pm$	0.0	7.6 $\pm$	0.1
EC(dS m^{-1})	16.1 $\pm$	0.1	16.7 $\pm$	2.0
Humidity (%)	95.0 $\pm$	1.3	96.3 $\pm$	1.5
Dry Matter (g L^{-1})	9.5 $\pm$	2.6	36.9 $\pm$	5.1
Cu (mg L^{-1})	0.7 $\pm$	0.0	0.5 $\pm$	0.3
Zn (mg L^{-1})	3.4 $\pm$	0.3	3.9 $\pm$	2.0
Fe (mg L^{-1})	3.8 $\pm$	0.7	6.5 $\pm$	2.6
Mn (mg L^{-1})	0.4 $\pm$	0.1	0.7 $\pm$	0.2
Mg (mg L^{-1})	71.1 $\pm$	2.5	144.9 $\pm$	15.4
Ca (mg L^{-1})	113.1 $\pm$	5.5	140.7 $\pm$	13.5
K (mg L^{-1})	836.0 $\pm$	11.6	1020.3 $\pm$	95.1
Na (mg L^{-1})	868.4 $\pm$	18.8	1194.3 $\pm$	29.6
TN (g L^{-1})	1.0 $\pm$	0.1	1.4 $\pm$	0.0
AN (g L^{-1})	0.8 $\pm$	0.1	1.2 $\pm$	0.0
PO ₄ (mg L^{-1})	360.0 $\pm$	26.5	580.0 $\pm$	27.7
P (mg L^{-1})	117.7 $\pm$	8.6	184.3 $\pm$	24.8

(TN: Total Nitrogen, AN: Amoniacal Nitrogen, PO₄: Phosphate, P: Phosphorus)

Changes in selected soil properties can be seen in Table 3.2 and 3.3 for the surface and subsurface horizons. Soil pH was between 7.44 and 7.69 in the surface, 7.44 and 7.72 in the subsurface horizons. A slightly decline was observed with D₂, D₃ and

approximately stable with D_1 in the surface horizon. pH was more affected by PS application in D_2 and D_3 . The effect of PS on salinity was insignificant ($P > 0.05$) in the surface horizon (Table 3.2). It was slightly decreased with D_1 and D_2 , an increment was observed with D_3 in the subsurface horizon (Table 3.3). Due to the high amount, PS was leached from surface and accumulated in the deeper horizons and increased soil salinity. Carbonate contents were slightly increased which were statistically insignificant ($P > 0.05$) (except of D_3).

The different doses of PS application were not significantly affected CEC in surface and subsurface horizons. Plant available P were affected by PS application and increased with increasing doses ($D_3 > D_2 > D_1 > C$). The results of SOC are given in Figure 3.5. They were changed between 10 and 20 g kg⁻¹, which was increased with all doses application and remarkable increased were observed with D_2 and D_3 application in the study area. Total N contents were only slightly increased with D_1 .

Soil macro-micro nutrient contents were given for the surface in Table 3.4 and for the subsurface horizons in Table 3.5. Sodium and K contents of the soil were not significantly effected ($P > 0.05$) by PS application in the area studied. Calcium contents were remarkable increased especially with D_3 . Magnesium was decreased with D_2 and D_3 in the surface horizon, whereas, it was slightly increased in the subsurface with D_1 and D_2 .

Copper, Mn, Zn, and Fe contents were given in Table 3.4 and 3.5 for the surface and subsurface horizons. Copper and Mn contents were not affected by PS application. The increased in Zn contents were statistically insignificant in the surface horizon. However, D_3 application was significant in the subsurface soil. A slight decreased in Fe content was observed with D_1 and D_2 , but D_3 were not.

Table 3.2. Selected soil properties in the surface horizons (n:3, S: Year of sampling, D: Doses).

Soil Properties									
S	D	pH	EC (dS m ⁻¹)	CaCO ₃ (%)	CEC (cmol ⁽⁺⁾ kg ⁻¹)	SOC (g kg ⁻¹)	TN (g kg ⁻¹)	C/N (%)	P (mg kg ⁻¹)
1	C	7,44ab(0,18)	0,12a(0,04)	57,21ab(1,32)	16,55b(0,77)	10,69ab(0,84)	0,62a(0,08)	17,32a(0,98)	62,09a(0,81)
2		7,69b(0,04)	0,17a(0,02)	54,37ab(1,05)	9,32a(0,75)	10,58ab(0,85)	0,56a(0,01)	19,06a(0,90)	62,65a(0,95)
3		7,55ab(0,08)	0,14a(0,03)	57,09ab(0,87)	9,01a(0,03)	15,81abcd(0,75)	0,43a(0,05)	37,53b(0,76)	61,58a(0,81)
4		7,48ab(0,05)	0,20ab(0,04)	56,98ab(0,95)	8,50a(0,11)	13,14abc(0,47)	0,53a(0,01)	24,80a(0,45)	60,65a(0,54)
5		7,61ab(0,00)	0,16a(0,00)	60,53abc(0,00)	8,57a(0,00)	10,06a(0,81)	0,08a(0,00)	33,88ab(0,73)	62,02a(0,68)
6		7,44ab(0,00)	0,17a(0,00)	59,57abc(0,00)	8,74a(0,00)	10,48a(0,25)	0,55a(0,00)	19,20a(0,70)	60,87a(0,88)
7		7,53ab(0,00)	0,16a(0,00)	60,05abc(0,00)	8,65a(0,01)	10,27a(0,15)	0,31a(0,00)	33,07ab(0,68)	61,31a(0,19)
1	D ₁	7,45ab(0,03)	0,33ab(0,01)	56,05ab(0,39)	10,88a(0,73)	10,91ab(0,84)	0,66a(0,10)	19,00a(0,48)	56,87a(0,92)
2		7,64b(0,05)	0,19ab(0,02)	53,48ab(0,59)	9,74a(0,27)	12,20ab(0,85)	0,66a(0,02)	19,66a(0,26)	70,29a(0,52)
3		7,58ab(0,04)	0,20ab(0,03)	59,39abc(0,15)	8,84a(0,72)	14,50abc(0,75)	0,65a(0,05)	23,88a(0,55)	88,98a(0,98)
4		7,49ab(0,05)	0,21ab(0,01)	52,50a(0,78)	8,15a(0,46)	15,64abcd(0,47)	0,66a(0,06)	24,20a(0,97)	81,26a(0,65)
5		7,45ab(0,01)	0,29ab(0,04)	61,31abc(0,88)	10,61a(0,29)	11,97ab(0,77)	0,74a(0,02)	17,62a(0,43)	98,03ab(0,61)
6		7,45ab(0,04)	0,19ab(0,02)	61,55abc(0,64)	8,88a(0,66)	13,09abc(0,45)	0,67a(0,09)	19,60a(0,80)	97,05ab(0,46)
7		7,45ab(0,01)	0,24ab(0,03)	61,43abc(1,02)	9,74a(0,96)	12,53abc(0,15)	0,70a(0,04)	18,38a(0,78)	105,90ab(0,40)
1	D ₂	7,37a(0,07)	0,31ab(0,01)	56,22ab(0,66)	9,56a(1,39)	17,21bcd(0,90)	1,66ab(0,08)	14,49a(0,99)	74,18a(0,90)
2		7,62b(0,03)	0,26ab(0,04)	59,38abc(0,53)	10,74a(0,79)	18,92abc(0,51)	1,04a(0,05)	20,50a(0,84)	90,10a(0,49)
3		7,59ab(0,03)	0,20ab(0,03)	54,72ab(0,83)	8,15a(0,38)	14,28cd(0,98)	0,63a(0,08)	23,13a(0,71)	126,67ab(0,34)
4		7,54ab(0,03)	0,24ab(0,05)	57,15ab(0,33)	8,00a(0,79)	12,20ab(0,88)	0,55a(0,07)	22,67a(0,53)	100,41ab(0,84)
5		7,43ab(0,10)	0,40ab(0,05)	61,50abc(0,66)	9,30a(0,39)	15,67abcd(0,64)	0,90a(0,05)	19,41a(0,74)	201,38bc(0,68)
6		7,46ab(0,04)	0,34ab(0,08)	63,49bc(0,60)	8,08a(0,16)	16,29abcd(0,82)	0,75a(0,14)	21,44a(3,24)	144,49ab(0,80)
7		7,44ab(0,03)	0,37ab(0,06)	62,49abc(0,56)	8,69a(0,12)	15,98abcd(0,98)	0,82a(0,11)	19,96a(0,73)	132,56ab(0,61)
1	D ₃	7,40a(0,05)	0,48abc(0,04)	57,10ab(0,64)	10,59a(0,59)	12,54abc(0,83)	1,22ab(0,14)	10,34a(0,99)	234,57bc(0,93)
2		7,72c(0,07)	0,49bc(0,04)	68,08c(1,06)	8,45a(1,08)	15,17abcd(0,68)	0,88a(0,06)	34,14ab(0,73)	246,03c(0,79)
3		7,55ab(0,08)	0,65bc(0,03)	50,52a(0,47)	11,29a(0,99)	18,27cd(0,46)	2,71b(0,09)	8,64a(0,49)	255,75c(0,79)
4		7,53ab(0,07)	0,78c(0,02)	56,73ab(0,30)	8,97a(0,73)	16,39bcd(0,60)	2,41b(0,07)	9,89a(0,80)	203,53bc(0,66)
5		7,63b(0,00)	0,22ab(0,00)	64,62bc(0,00)	7,34a(0,13)	17,83bcd(0,56)	0,72a(0,00)	24,76a(0,20)	227,67bc(0,29)
6		7,51ab(0,00)	0,35ab(0,00)	63,78bc(0,00)	6,80a(1,08)	20,33d(0,73)	0,85a(0,00)	23,87a(0,43)	180,66ab(0,86)
7		7,57ab(0,00)	0,28ab(0,00)	64,20bc(0,00)	7,07a(0,47)	19,08cd(0,68)	0,79a(0,00)	24,28a(0,29)	209,46bc(0,42)
f		5,72***	6,58***	5,30***	4,00***	1,70*	7,85***	5,02***	2,73***

Table 3.3. Selected soil properties in the subsurface horizons (n:3, S: Year of sampling, D: Doses).

Soil Properties									
S	D	pH	EC (dS m ⁻¹)	CaCO ₃ (%)	CEC (cmol ⁽⁺⁾ kg ⁻¹)	SOC (g kg ⁻¹)	TN (g kg ⁻¹)	C/N (%)	P (mg kg ⁻¹)
1	C	7.52ab(0,06)	0,14a(0,04)	53,59ab(0,52)	12,05b(0,38)	7,62a(0,45)	0,30ab(0,04)	25,86a(0,55)	34,37a(0,36)
2		7.66bc(0,09)	0,16a(0,01)	51,94a(0,51)	7,61ab(0,47)	8,21ab(0,37)	0,37ab(0,01)	22,37a(0,50)	46,97ab(0,57)
3		7.59abc(0,04)	0,14a(0,03)	63,29bc(0,42)	6,94a(0,91)	10,03ab(0,64)	0,22a(0,02)	46,32ab(0,62)	56,61ab(0,56)
4		7.47a(0,02)	0,12a(0,01)	61,95abc(0,44)	8,59ab(0,43)	7,44a(0,20)	0,36ab(0,04)	21,51a(0,44)	61,14ab(0,51)
5		7.60abc(0,00)	0,14a(0,00)	67,59c(0,00)	8,44ab(0,00)	8,33ab(0,24)	0,15a(0,00)	55,56ab(0,00)	60,77ab(1,35)
6		7.46a(0,00)	0,15a(0,00)	66,92bc(0,00)	6,63a(0,00)	15,89c(0,22)	0,32ab(0,00)	48,93ab(0,66)	58,06ab(0,44)
7		7.53ab(0,00)	0,14a(0,00)	67,26bc(0,00)	7,54a(0,01)	12,11abc(0,79)	0,24a(0,00)	51,03ab(0,85)	63,16b(0,52)
1	D ₁	7.55ab(0,03)	0,16a(0,03)	59,85abc(0,34)	10,06ab(0,73)	6,82a(0,73)	0,31ab(0,01)	22,29a(0,38)	30,39a(1,20)
2		7.64bc(0,13)	0,18a(0,03)	53,93ab(0,40)	8,82ab(0,22)	7,82ab(0,14)	0,36ab(0,04)	22,45a(0,41)	36,79a(1,23)
3		7.61bc(0,01)	0,12a(0,04)	61,50abc(0,66)	8,08ab(0,54)	14,47bc(0,73)	0,37ab(0,10)	40,37ab(1,26)	58,94ab(1,56)
4		7.49ab(0,03)	0,18a(0,06)	53,05a(0,31)	8,10ab(0,35)	14,94bc(0,70)	0,44ab(0,08)	38,86ab(0,68)	65,64b(0,80)
5		7.55ab(0,02)	0,19a(0,05)	63,53bc(0,92)	9,94ab(0,76)	11,88abc(0,54)	0,38ab(0,09)	34,01ab(0,59)	64,28b(0,81)
6		7.44a(0,10)	0,17a(0,05)	64,70bc(0,43)	9,93ab(0,28)	12,97abc(0,20)	0,43ab(0,01)	39,94ab(0,68)	70,28b(0,89)
7		7,50ab(0,05)	0,18a(0,03)	64,12bc(0,46)	9,94ab(0,78)	12,43abc(0,55)	0,41ab(0,05)	34,28ab(1,09)	81,75b(0,41)
1	D ₂	7.57abc(0,04)	0,20a(0,05)	62,49abc(0,33)	8,58ab(0,39)	12,22abc(0,24)	0,3ab(0,06)	41,26ab(1,12)	37,53a(1,11)
2		7.70c(0,01)	0,22a(0,06)	68,09c(0,31)	8,84ab(0,15)	12,78abc(0,26)	0,24a(0,04)	55,59ab(0,37)	48,75ab(1,40)
3		7.61abc(0,02)	0,21a(0,05)	57,18ab(0,41)	7,05a(0,54)	15,94bc(0,71)	0,22a(0,04)	74,26b(1,95)	62,76b(1,43)
4		7.51ab(0,04)	0,17a(0,08)	58,16abc(0,96)	8,42ab(0,53)	15,15bc(0,51)	0,24a(0,01)	64,01ab(1,27)	60,72ab(0,46)
5		7.59abc(0,03)	0,27a(0,02)	64,73bc(0,75)	7,97ab(34)	13,00abc(0,47)	0,2a(0,06)	66,28b(1,63)	71,36b(1,16)
6		7.47a(0,05)	1,76b(0,03)	67,45bc(0,25)	7,06a(0,97)	13,78bc(0,26)	0,28ab(0,07)	53,63ab(1,17)	70,75b(0,98)
7		7.53ab(0,01)	1,01ab(0,08)	66,09bc(0,81)	7,51a(0,43)	13,39abc(0,67)	0,24a(0,05)	58,25ab(0,86)	124,38c(3,40)
1	D ₃	7.47a(0,03)	0,52a(0,07)	62,42abc(0,65)	10,44ab(0,98)	10,07ab(0,27)	0,56b(0,03)	19,73a(1,03)	58,38ab(1,21)
2		7.72c(0,04)	0,47a(0,06)	68,95c(0,73)	8,12ab(0,63)	10,56ab(0,21)	0,42ab(0,04)	27,32ab(0,66)	71,30b(0,69)
3		7.71c(0,10)	0,43a(0,03)	61,03abc(0,03)	7,49a(0,36)	17,07c(0,48)	0,37ab(0,06)	46,72ab(0,69)	62,37b(1,18)
4		7.58abc(0,06)	0,47a(0,06)	65,67bc(0,93)	6,19a(0,48)	16,60c(0,69)	0,49ab(0,03)	42,49ab(0,96)	63,17b(0,58)
5		7.56abc(0,00)	0,45a(0,00)	66,90bc(0,00)	7,02a(0,24)	12,35abc(0,51)	0,30ab(0,00)	40,72ab(1,23)	68,26b(0,87)
6		7.58abc(0,00)	0,81ab(0,00)	68,81c(0,00)	6,34a(0,61)	19,01c(1,08)	0,36ab(0,00)	52,54ab(0,92)	79,36b(0,72)
7		7.57abc(0,00)	0,63a(0,00)	67,86c(0,00)	6,68a(0,89)	15,68bc(0,83)	0,33ab(0,00)	47,15ab(0,97)	168,31c(2,82)
f		9,85***	2,84***	9,14***	3,30***	1,33ns	10,42***	2,61***	4,11***

Table 3.4. Selected soil nutrients in the surface horizons (n:3, S: Year of sampling, D: Doses).

Soil Nutrient Contents									
S	D	Na (cmol ⁽⁺⁾ kg ⁻¹)	K (cmol ⁽⁺⁾ kg ⁻¹)	Ca (cmol ⁽⁺⁾ kg ⁻¹)	Mg (cmol ⁽⁺⁾ kg ⁻¹)	Cu (mg kg ⁻¹)	Mn (mg kg ⁻¹)	Zn (mg kg ⁻¹)	Fe (mg kg ⁻¹)
1	C	0,89a(0,04)	1,65ab(0,12)	23,98a(1,30)	0,90a(0,08)	2,43a(0,17)	23,34a(0,71)	3,98a(0,50)	3,56a(0,29)
2		1,06b(0,02)	0,38a(0,13)	62,31c(1,00)	0,70a(0,04)	2,04a(0,37)	22,22ab(0,63)	0,32a(0,27)	6,05abc(0,67)
3		0,16a(0,01)	0,36a(0,09)	16,59a(1,06)	0,69a(0,02)	2,02a(0,15)	15,40a(0,66)	0,51a(0,04)	3,54a(0,60)
4		0,39a(0,04)	0,42a(0,04)	23,52a(1,15)	0,71a(0,03)	2,65a(0,25)	16,73a(0,79)	0,46a(0,18)	3,6ab(0,05)
5		0,65a(0,09)	0,24a(0,00)	70,27c(0,00)	0,91a(0,00)	1,81a(0,00)	22,47a(0,47)	0,42a(0,00)	4,46ab(0,00)
6		1,3a(0,08)	0,28a(0,00)	15,88a(0,00)	0,65a(0,00)	1,12a(0,00)	11,59a(0,24)	0,32a(0,00)	1,70a(0,00)
7		0,98a(0,03)	0,26a(0,05)	23,07b(0,87)	0,78a(0,05)	1,47a(0,00)	17,03a(0,35)	0,21a(0,00)	3,08a(0,00)
1	D ₁	1,26a(0,04)	1,05a(0,05)	24,71a(0,26)	1,01a(0,06)	2,04a(0,12)	28,53a(0,44)	3,69a(0,06)	3,99ab(0,21)
2		1,61a(0,05)	0,83a(0,06)	64,69c(0,78)	0,86a(0,01)	2,56a(0,06)	68,15b(0,97)	4,85a(0,51)	7,16abc(0,40)
3		0,18a(0,01)	1,10a(0,11)	16,16a(0,38)	0,81a(0,06)	2,42a(0,05)	20,85a(0,76)	3,29a(0,47)	3,82ab(0,32)
4		1,22a(0,09)	1,07a(0,11)	21,67a(0,58)	0,85a(0,07)	2,53a(0,24)	20,80a(0,37)	2,91a(0,06)	3,75ab(0,07)
5		0,95a(0,06)	1,00a(0,11)	58,91bc(0,86)	1,17a(0,05)	2,29a(0,16)	27,63a(0,25)	4,48a(0,31)	4,56ab(0,36)
6		1,47a(0,09)	0,84a(0,03)	15,85a(0,92)	0,79a(0,03)	1,17a(0,14)	14,75a(0,91)	2,20a(0,02)	1,76a(0,26)
7		1,21a(0,04)	0,92a(0,04)	37,38ab(0,69)	0,98a(0,01)	1,73a(0,15)	21,19a(0,80)	3,34a(0,17)	3,16a(0,31)
1	D ₂	3,08a(0,09)	1,67ab(0,09)	22,12a(0,29)	3,15b(0,05)	3,95a(0,17)	49,66(0,70)	14,96a(0,13)	5,91abc(0,45)
2		0,85a(0,05)	0,62a(0,21)	48,19bc(0,88)	1,76a(0,05)	4,82a(0,23)	106,37ab(0,70)	18,26a(0,74)	14,34ab(0,82)
3		1,28a(0,03)	0,79a(0,08)	15,17a(0,42)	1,47a(0,03)	2,38a(0,34)	25,03b(0,66)	9,40a(0,97)	5,01ab(0,90)
4		0,63a(0,04)	0,77a(0,23)	19,63a(0,77)	1,33a(0,15)	2,14a(0,22)	19,31a(0,79)	7,31a(0,66)	4,40ab(0,41)
5		1,34a(0,08)	1,30ab(0,31)	53,16bc(1,01)	1,78a(0,30)	2,78a(0,30)	33,07a(0,47)	16,29a(0,21)	6,80abc(0,27)
6		0,78a(0,04)	1,47ab(0,17)	12,54a(0,55)	1,24a(0,01)	1,34a(0,35)	16,13a(0,24)	7,04a(0,79)	2,81a(0,87)
7		1,06a(0,07)	1,38ab(0,13)	32,85ab(1,10)	1,51a(0,14)	2,06a(0,16)	24,60a(0,35)	11,67a(0,43)	4,80ab(0,13)
1	D ₃	2,55a(0,18)	1,48ab(0,35)	26,21a(0,29)	2,05ab(0,37)	4,23a(0,15)	39,96a(0,44)	11,82a(0,01)	3,98ab(0,22)
2		1,86a(0,24)	1,00a(0,31)	55,10bc(0,67)	1,87a(0,46)	4,10a(0,15)	50,61ab(0,97)	17,71a(0,54)	8,29bc(0,48)
3		0,86a(0,08)	2,73b(0,42)	12,55a(0,62)	4,26a(0,53)	10,86b(0,14)	38,44ab(0,76)	46,30b(0,60)	10,20c(0,36)
4		1,12a(0,09)	2,79b(0,34)	15,87a(0,67)	3,40b(0,44)	9,97b(0,16)	43,07ab(0,37)	35,26ab(0,85)	8,45bc(0,91)
5		1,34a(0,09)	0,40a(0,00)	60,94c(0,00)	1,06b(0,00)	2,85a(0,00)	23,56a(0,25)	13,99a(0,00)	6,46abc(0,00)
6		0,38a(0,02)	1,41ab(0,00)	14,22a(0,00)	1,69a(0,00)	2,35a(0,00)	17,14a(0,91)	13,25a(0,00)	4,20ab(0,00)
7		0,86a(0,01)	1,51ab(0,13)	37,58ab(0,00)	1,38a(0,08)	2,60a(0,00)	20,35a(0,89)	13,62a(0,00)	5,33ab(0,00)
f		5,96***	1,70*	4,55***	48,21***	13,83***	5,96***	12,81***	7,55***

Table 3.5. Selected soil nutrients in the subsurface horizons (n:3, S: Year of sampling, D: Doses).

Soil Nutrient Contents									
S	D	Na (cmol ⁽⁺⁾ kg ⁻¹)	K (cmol ⁽⁺⁾ kg ⁻¹)	Ca (cmol ⁽⁺⁾ kg ⁻¹)	Mg (cmol ⁽⁺⁾ kg ⁻¹)	Cu (mg kg ⁻¹)	Mn (mg kg ⁻¹)	Zn (mg kg ⁻¹)	Fe (mg kg ⁻¹)
1	C	0,89(0,14)	0,60ab(0,03)	23,97a(0,32)	1,24ab(014)	1,17abc(0,07)	15,03abc(0,42)	0,53a(0,09)	2,96ab(0,30)
2		3,82(0,27)	0,18a(0,04)	64,20bc(0,61)	0,75a(0,08)	1,91bc(0,05)	39,02c(0,80)	0,16a(0,01)	6,21bc(0,24)
3		2,46(0,37)	0,21a(0,01)	13,79a(0,78)	0,68a(0,03)	1,24abc(0,10)	13,76abc(0,62)	0,42a(0,10)	3,46abc(0,28)
4		2,33(0,36)	0,30a(0,05)	23,83a(0,31)	0,73a(0,10)	1,99bc(0,03)	14,15abc(0,71)	0,21a(0,07)	3,82abc(0,27)
5		0,93(0,00)	0,17a(0,00)	66,56c(0,00)	0,95ab(0,00)	1,58abc(0,00)	21,80c(0,00)	0,43a(0,00)	5,02abc(0,00)
6		2,36(0,00)	0,10a(0,00)	14,85a(0,00)	0,64a(0,00)	0,58a(0,00)	9,62a(0,00)	0,30a(0,00)	1,10a(0,00)
7		1,65(0,10)	0,13a(0,08)	40,70ab(0,50)	0,80a(0,16)	1,08ab(0,00)	15,71abc(0,00)	0,21a(0,00)	3,06ab(0,00)
1	D ₁	0,99(0,24)	0,42ab(0,02)	23,24a(0,21)	0,82a(0,08)	1,10ab(0,02)	13,97abc(0,36)	0,13a(0,01)	3,26ab(0,45)
2		1,66(0,20)	0,19a(0,02)	55,07bc(0,31)	0,81a(0,03)	1,45abc(0,02)	38,08c(0,98)	0,27a(0,09)	5,46bc(0,38)
3		1,21(0,20)	0,43ab(0,06)	16,47a(0,38)	0,78a(0,04)	1,73abc(0,09)	16,41abc(0,33)	0,73a(0,09)	4,06abc(0,25)
4		1,57(0,09)	0,38ab(0,20)	21,21a(0,27)	0,89ab(0,06)	1,76abc(0,11)	17,05abc(0,42)	0,28a(0,02)	3,44abc(0,41)
5		1,79(0,30)	0,31a(0,05)	64,03bc(0,47)	1,33ab(0,05)	1,51abc(0,09)	20,38bc(0,51)	0,73a(0,09)	4,28abc(0,37)
6		0,44(0,09)	0,18a(0,02)	15,39a(0,31)	0,81a(0,07)	0,86ab(0,17)	13,44ab(0,30)	0,11a(0,04)	2,06a(0,33)
7		1,11(0,14)	0,25a(0,03)	39,71ab(0,52)	1,07ab(0,11)	1,18abc(0,05)	16,91ab(0,24)c	0,42a(0,11)	3,17ab(0,04)
1	D ₂	1,66(0,23)	0,57ab(0,15)	27,12a(0,31)	1,19ab(0,13)	1,13ab(0,09)	14,42abc(0,86)	0,39a(0,10)	3,13ab(0,51)
2		1,48(0,23)	0,64ab(0,19)	54,46bc(0,26)	1,12ab(0,07)	1,16abc(0,06)	42,94c(0,63)	0,33a(0,07)	5,85bc(0,41)
3		0,47(0,11)	0,28a(0,05)	16,53a(0,39)	1,18ab(0,31)	1,05ab(0,06)	18,07bc(0,71)	0,89a(0,06)	3,55abc(0,46)
4		0,80(0,06)	0,24a(0,03)	18,39a(0,42)	1,14ab(0,13)	1,02ab(0,07)	15,44abc(0,83)	0,19a(0,02)	3,32abc(0,23)
5		0,72(0,19)	0,74ab(0,53)	50,98bc(0,58)	1,92b(0,40)	0,94ab(0,11)	17,89bc(0,44)	0,88a(0,05)	3,75abc(0,27)
6		0,80(0,13)	0,24a(0,16)	13,45a(0,69)	1,09ab(0,11)	0,58a(0,12)	11,77abc(0,61)	0,49a(0,07)	1,39a(0,36)
7		0,76(0,20)	0,49ab(0,24)	32,22ab(0,85)	1,51b(0,25)	0,76a(0,12)	14,83abc(0,50)	0,68a(0,06)	2,57ab(0,32)
1	D ₃	3,37(0,21)	0,44ab(0,10)	26,55a(0,85)	1,02ab(0,13)	1,26abc(0,11)	14,76abc(0,75)	0,69a(0,08)	2,87ab(0,40)
2		2,23(0,55)	0,27a(0,06)	60,16bc(0,65)	1,21ab(0,13)	1,85abc(0,14)	41,46c(0,92)	4,82b(0,85)	6,51c(0,59)
3		0,73(0,24)	1,00b(0,25)	17,04a(0,39)	1,25ab(0,16)	1,39abc(0,11)	17,76abc(0,98)	1,51ab(0,20)	3,21ab(0,29)
4		1,86(0,45)	0,68ab(0,29)	19,32a(0,34)	1,31ab(0,12)	2,28c(0,18)	16,89abc(0,45)	5,09b(0,92)	8,45c(0,67)
5		0,59(0,00)	0,25a(0,00)	81,86c(0,00)	0,84ab(0,00)	1,13ab(0,15)	16,75abc(0,00)	1,43ab(0,00)	3,46abc(0,00)
6		4,76(0,00)	0,43ab(0,00)	16,00a(0,00)	1,07ab(0,00)	0,59a(0,20)	8,86a(0,00)	1,54ab(0,00)	0,68a(0,00)
7		2,68(0,55)	0,34ab(0,20)	48,93b(0,25)	0,96ab(0,25)	0,86ab(0,12)	12,80ab(0,39)	1,48ab(0,00)	2,07a(0,00)
f		4,95***	1,33ns	2,79***	46,27***	5,62***	4,95***	43,88***	2,65***

Table 3.6. Selected plant nutrient contents (n:3, S: Year of sampling, D: Doses).

Plant Nutrient Contents									
D	S	Na (g kg ⁻¹)	K (g kg ⁻¹)	Ca (g kg ⁻¹)	Mg (g kg ⁻¹)	Cu (g kg ⁻¹)	Zn (g kg ⁻¹)	Fe (g kg ⁻¹)	Mn (g kg ⁻¹)
C	2009	0,32 a (0,05)	4,61 a (0,41)	0,56 a (0,04)	0,59 a (0,07)	2,04 (2,09)	12,14 a (3,54)	30,71 ab (1,91)	14,19 a (1,47)
	2010	0,50 ab (0,04)	4,19 a (0,33)	0,5 a (0,03)	0,94 bc (0,06)	3,06 (2,49)	19,86 ab (2,89)	21,94 a (1,56)	17,20 a (1,2)
	2011	0,42 ab (0,04)	4,97 ab (0,33)	1,03 c (0,03)	1,21 c (0,06)	5,46 (2,49)	17,23 a (2,89)	39,04 bc (1,56)	18,95 bc (1,2)
D ₁	2009	0,45 ab (0,03)	5,83 ab (0,24)	0,51 a (0,02)	0,68 a (0,04)	4,04 (1,49)	17,68 a (2,04)	28,02 ab (1,1)	16,67 a (0,85)
	2010	0,24 a (0,03)	3,95 a (0,24)	0,58 ab (0,02)	0,83 ab (0,04)	2,41 (1,49)	17,99 ab (2,04)	21,7 a (1,1)	15,89 a (0,85)
	2011	0,72 ab (0,03)	6,52 bc (0,19)	0,82 b (0,02)	1,05 bc (0,03)	5,70 (1,83)	28,1 ab (1,67)	40,91 c (0,9)	23,00 c (0,69)
D ₂	2009	0,96 c (0,05)	7,63 c (0,41)	0,6 ab (0,04)	0,88 ab (0,07)	8,50 (2,09)	32,15 b (3,54)	34,8 bc (1,91)	27,18 bc (1,47)
	2010	0,41 ab (0,04)	3,83 a (0,33)	0,65 ab (0,03)	0,8 ab (0,06)	2,70 (2,49)	22,95 ab (2,89)	25,38 a (1,56)	16,78 a (1,2)
	2011	0,95 c (0,04)	6,92 bc (0,33)	1,14 c (0,03)	1,35 c (0,06)	7,33 (2,49)	57,54 c (2,89)	45,52 c (1,56)	31,6 c (1,2)
D ₃	2009	0,28 a (0,05)	6,44 bc (0,41)	0,46 a (0,04)	0,57 a (0,07)	2,75 (2,09)	22,99 ab (3,54)	32,21 ab (1,91)	14,8 ab (1,47)
	2010	0,4 a (0,04)	4,66 a (0,33)	0,73 ab (0,03)	1 bc (0,06)	3,12 (2,49)	28,36 ab (2,89)	31,99 ab (1,56)	26,85 ab (1,2)
	2011	0,62 ab (0,04)	6,84 bc (0,33)	0,99 c (0,03)	1,14 bc (0,06)	8,20 (2,49)	41,14 b (2,89)	43,57 c (1,56)	24,08 c (1,2)
f		33,28***	17,70***	64,32***	18,68***	0,54 ^{ns}	19,52***	36,93***	23,38***

3.3.2. Nutrient Uptakes by Plants and Crop Yield

Some selected nutrients contents of plants were depicted in Table 3.6. All parameters were significantly affected by PS application except of Cu contents. Sodium uptake of barley was increased with D₁ and D₂ applications. However, D₃ doses on Na uptake were insignificant. Potassium contents were increased with all different doses of PS application, whereas, Ca and Mg uptakes were not statistically significant.

Plant available P contents, between 7.93 and 11.18 g kg⁻¹, 7.95 and 10.83 g kg⁻¹, 9.36 and 12.47 g kg⁻¹ in the first, second and third year, respectively, which were slightly increased with D₂ and D₃ applications. The amount of total N were changed between 16.14 and 27.10 g kg⁻¹, 12.30 and 19.89 g kg⁻¹, 15.66 and 20.86 g kg⁻¹ in 2009, 2010, and 2011, respectively. Also, it was decreased with all doses, especially with D₂. Zinc, Fe and Mn uptakes of plants were increased with all doses, whereas, increases for Cu uptakes were ($P > 0.05$).

Crop yield for the year 2010 was 1.50 tons/ha and for 2011 it was 0.85 tons/ha for control and for PS application it was 1.87, 1.67, and 1.64 tons/ha for D₁, D₂, and D₃, respectively for the year of 2011 they were 0.98, 0.92, and 0.93 tons/ha for D₁, D₂, and D₃, respectively.

3.4. DISCUSSION

3.4.1. Effects of Pig Slurry on Soil Characteristics

Despite the fact that slightly lower of pH of the PS than the soil studied, the changes of this characteristics in the soil was statistically not significant. Another reason for stability of soil pH could be attributed the high content of carbonate.

Similar conclusion was mentioned in the literature by other scientists (Ukrainetz et al., 1996; Chang et al. 1990). High pH value of the PS used in our study appears to be related with high EC and humidity. Nonetheless, soil pH was greatly influenced by the nature and amounts of organic amendments used (Whalen et al., 2000; Walker et al., 2004). Chang et al. (1990) observed a decrease in soil pH with time and suggested that some soils might eventually become acidic with continuous application of farm manure in Canadian prairies.

Our results showed that salinity was increased with increasing doses of PS applied owing to the high amount of salts specifically K together with Na in animal feeding diets. Dose 3 applications were caused a slight increase of EC in the subsoil and this was attributed to leaching of salt in the medium textured soil of the area (Table 3.4). Animal manures could contain appreciable amounts of salts and excessive application of solid or liquid phases could naturally cause an accumulation of salt and consequently could be harmful to crops and soil structure. Chang et al. (1990) observed a linear increase in electrical conductivity over time with increasing rates of cattle manure application. Soil salinity was one of the most important factors reducing soil quality and productivity (Shortall and Liebhardt 1975). Diez et al. (2001) observed that salinization usually occurred over the first few days following slurry application, but it decreased because of rainfall and irrigation.

The soil studied was excessively carbonatic and additions of organic substances to the soil were not expected to change it at significant level. Our results confirms that there were not significantly influenced ($P > 0.05$) on carbonate contents by the application of PS with all doses; due to slightly alkaline pH of the soil and PS. The work by Doner and Lynn (1989) support our findings.

The soil studied had low CEC, due to low clay and high carbonate contents. Cation exchange capacity was slightly higher than the subsoil and this is attributed to the slightly higher content of organic matter in the surface soil. According to Porta (1999), the CEC increased with increasing doses of PS, in the soil with low clay content, giving rise to a positive correlation between the values of organic carbon and exchange capacity.

Magnesium, Cu, Mn, Zn, and Fe contents were decreased in the surface soil. However, increase in Na, Cu, Fe contents were not significant ($P > 0.05$) with D_1 , on the contrary they were decreased with D_2 and D_3 in the subsurface horizon due to leaching out from the soil to the deeper layer to contaminate the groundwater (Tables 3.4, and 3.5). The impact of PS application on metal content in the soil is a soil quality issue that is of both agronomic and environmental interest. Pig slurry contained essential nutrient for plants and could increase the bioavailable concentrations of metals (Chang et al., 1991; Qian et al.2003).

The assessment of available phosphorus was interest to establish the characteristics of soil fertility, and that together with potassium and calcium, is the most extracted by plants (Chesworth et al., 2008, Sanchez, 2007). Plant available P contents were statistically significantly ($P < 0.05$) increased with increasing doses of PS. This is due to high amount of plant available P in the PS (Table 3.2 and 3.3), which could be used as a source of P fertilizers especially when organic agriculture considered. Similar results were found by other workers. Furthermore, plant available P in the fields that received high rates of P inputs across the sites ranged from 28 to 106 mg P kg⁻¹ compared with 10-35 mg P kg⁻¹ in the fields with low or no P inputs (Hooda et al.,

2001). As suggested by Barberis et al., (1996) inorganic forms of P in agricultural soils varied widely, depending on the soil type, management history and climatic condition.

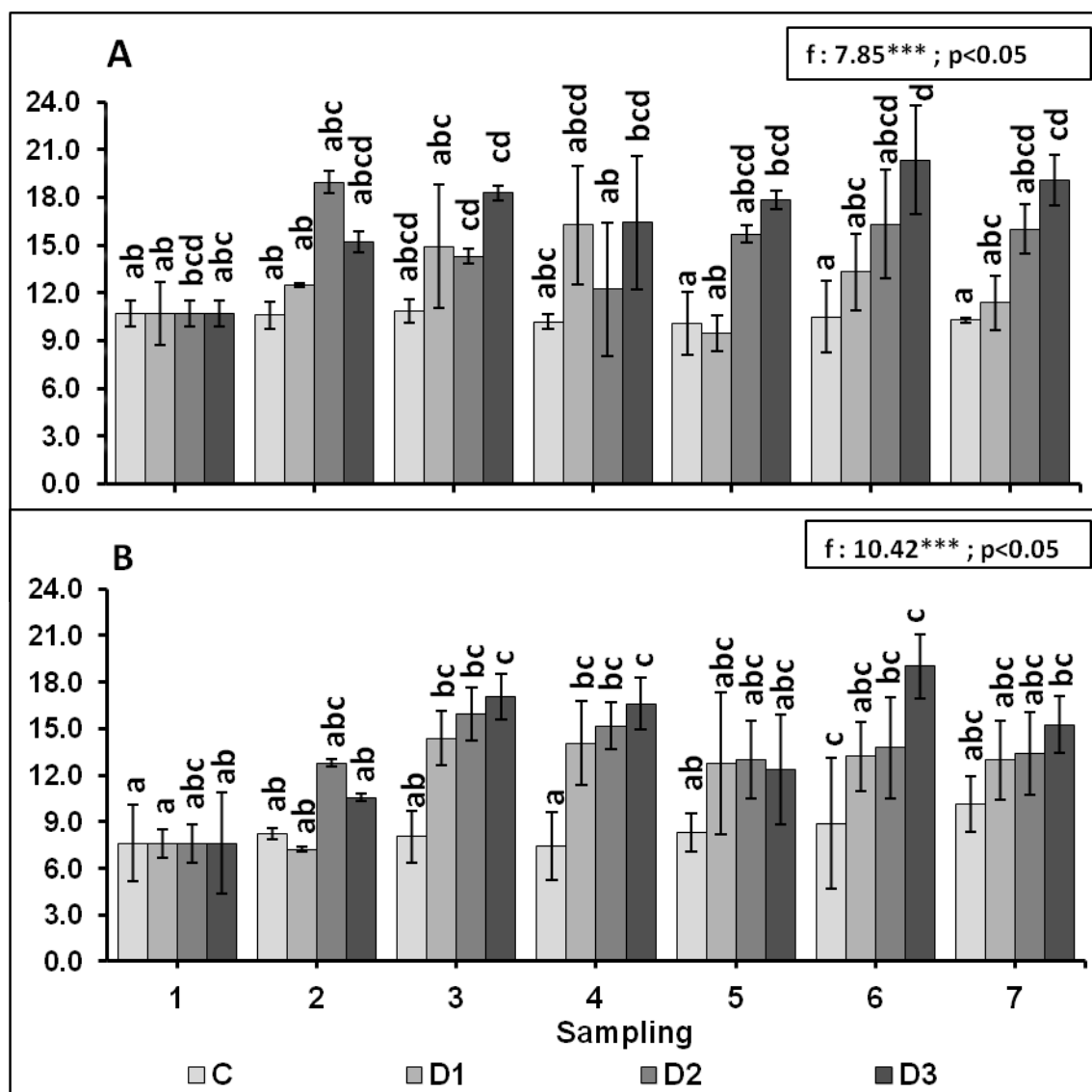


Figure 3.5. Evolution of soil organic carbon (g kg^{-1}) **A**-Surface, **B**-Subsurface Horizon (n:3)

The effect of different doses of PS on SOC contents were statistically significant ($P < 0.05$) (Figure 3.5). Soil OC was increased with the amount of PS applied, due to high OC content of PS. Our results showed that SOC was increased with increasing doses of PS applied owing to the high amount of OC in PS in both top and subsoil. However, increase in OC in the surface horizon was more visible. Increase of organic

matter in the surface horizon is very essential for sustainable crop production especially in soils which are poor in organic matter.

Soils in arid region, including area studied, are poor in organic matter content and need to be improved properly for better use and management of land resources in such regions. The role of manures in maintaining and increasing levels of SOC in agricultural soils has been well documented (Rudrappa et al. 2006). Qualities of organics (i.e., lignin, C/N, etc.) also influence the quantity of SOC development in the soil (Yanardag et al., 2014). Our results clearly confirmed; PS could directly influence the soil properties and quality, also some researcher supported our findings (McLaughlin et al., 1994; Weggler-Beaton et al. 2000).

3.4.2. Nutrient Uptakes and Crop Yield

The nutrient contents (Na, K, Zn, Cu, Mn and Fe) of plants were significantly increased ($P < 0.05$) with PS application both in the year of 2010 and 2011. This was due to the amount and availability of the nutrients present in PS. Our result clearly supports the view that organic amendments such as PS could be used not only the source of macro but micronutrients for soil management practices especially in organic agriculture (Charles, 1999; Schoenau et al., 2004).

Kornegay et al. (1976) reported increased corn leaf Cu, Zn, P, and K with pig manure (pigs feeding high Cu diets; 250 to 370 mg Cu kg⁻¹). In other studies, Zhu et al. (1991) reported increased plant growth and Cu uptake from Cu-amended pig manure compared with control (Bailey and Buckley, 1998).

The PS applications were caused increase in P concentration of barley crop, and the magnitude was greater for P than N (Data not shown). Similarly, Grant et al., (2001)

reported that additions of PS were significantly increased P concentration than N concentration (Qian et al., 2003).

Diez et al. (2001) indicated that application of high rates of slurry appeared to increase plant N uptake without increasing the grain production. Some researchers reported that liquid hog manure applications were increased leaf or seed N and P concentrations (Sutton et al. 1982; Faz et al., 2004). They concluded that nutrients in excess of quantities removed by crops were potential pollutants in surface and ground waters, or soil.

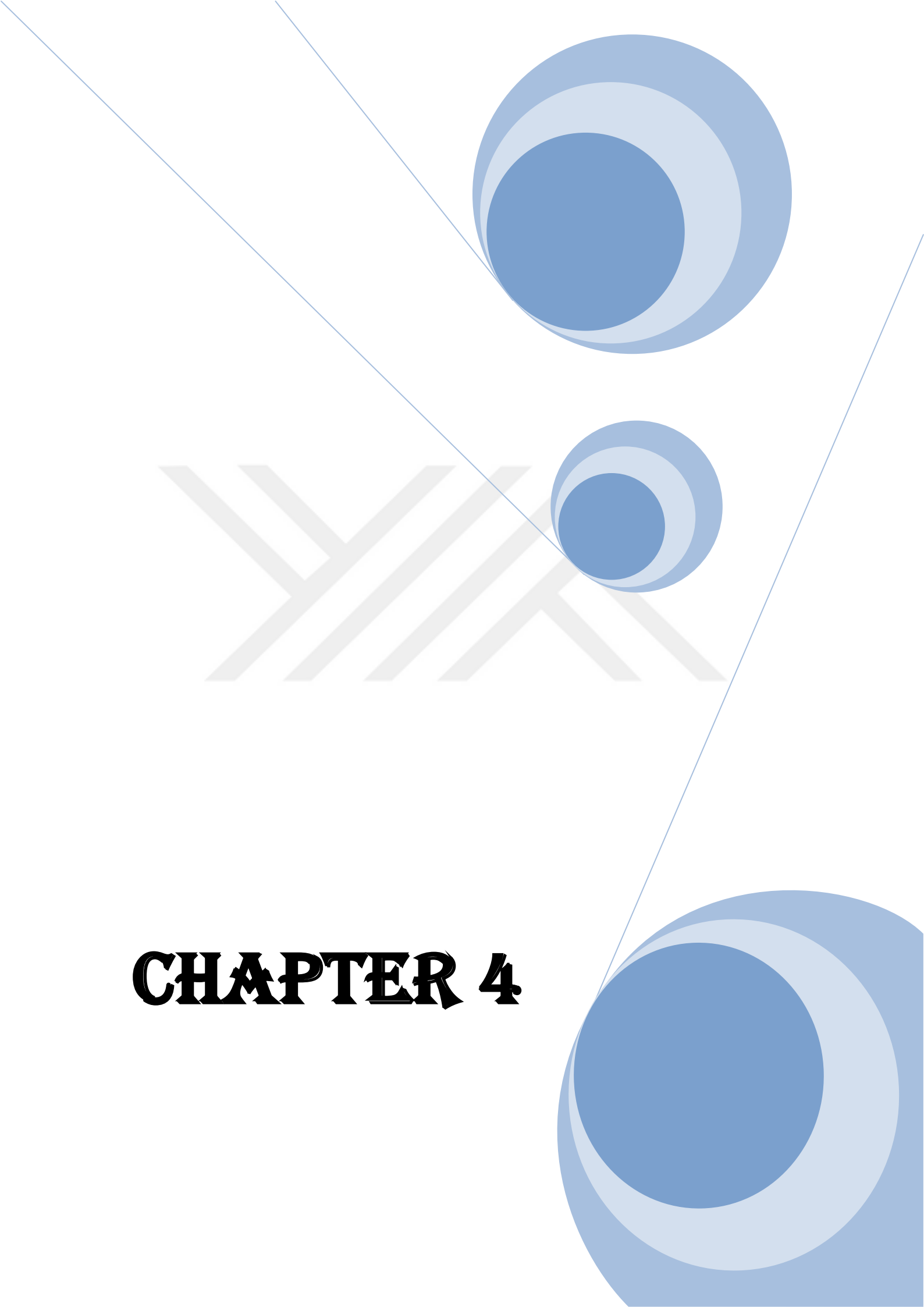
Pig slurry applications had positive effect on barley yield both in the year of 2010 and 2011. This finding was supported by Lal and Marthur (1989) in cereals in India. The yield increase in D₁ was significantly higher (~25%) whereas, D₂ and D₃ applications resulted in somewhat lower yield than D₁ in both years. It is a surprise to notice the yield decrease in D₂ and D₃ in comparison to D₁. As it can be seen in Table 3.2 and 3.3, PS causes increased amount of salinity in the soil. This is likely the reason why the increased amount of PS application causes reduction in yield. From our experiment, the D₁ seems to be an appropriate dose, as also suggested by legislated doses by the European Community to be applied for barley production in the area.

3.5. CONCLUSIONS

Our study clearly supports the view that organic amendments such as pig slurry (PS) could be used as an organic manure to provide required nutrients for plants. This is also an important consideration in terms of organic agriculture and environmental protection. The yield increase in D₁ was significantly higher (~25%) than control. Dose 2 and D₃ applications resulted in slightly lower yield than D₁ in both years. We found

this a surprise to notice the yield decrease in D_2 and D_3 in comparison to D_1 . This could be attributed to amount of salt accumulation in the profile that could cause reduction in production. Dose 1 is the agronomic rate of N-requirement (170 kg N/ha/yr) (European Directive 91/676/CEE) and appears to be very appropriate in term of sustainable agriculture.



The background features a light grey 'X' shape formed by two intersecting diagonal lines. Three blue circles of varying sizes are positioned around the page: a large one in the top right, a medium one in the center right, and a large one in the bottom right. Each circle is composed of three concentric layers of blue, with the innermost being the darkest and the outermost being the lightest. The text 'CHAPTER 4' is located in the lower-left quadrant.

CHAPTER 4

CHAPTER 4

ORGANIC CARBON FLUXES IN UNDISTURBED SOIL COLUMNS TREATED WITH PIG SLURRY IN SE SPAIN.

Abstract: Large amount of organic residues, including pig slurry (PS), are added to agricultural lands every year which have significant effects on the C flux in the soil. The objectives of this study were to: 1) determine the amount of dissolved organic carbon (DOC) in leachate and 2) quantify the CO₂ released from the soil to establish the C fluxes with time. Total of 9 undisturbed soil columns were used in the study. They were amended with low dose (LD) (129.7 mL) and high dose (HD) (389.2 mL) of PS with three replications, considering the N content by Spanish legislations. Irrigation was carried out on weekly bases by simulating the monthly rainfall events of the area.

Pig slurry applications have a greater effect on increasing organic carbon (OC) levels in the terrestrial ecosystem. The topsoils (0-10 cm) have sequestered 15.84 and 24.68% (1.97 and 3.15 tonnes OC ha⁻¹) with LD and HD, respectively. Carbon leaching in the control column showed 0.68% (0.09 tonnes OC ha⁻¹) loss of OC in one year. A large portion of the added OC was accumulated in the subsoil owing to the higher leaching in the medium textured soil studied. Thus, the benefits from the use of PS application depend on the soil management practices and environmental factors. We suggested that the amount of application doses should be calculated detailed measurement of C fluxes in the soil.

Keywords: Carbon, Column, Fluxes, Leachate, Undisturbed.

4.1. INTRODUCTION

Storage of soil organic carbon (SOC) in agricultural soils is mainly a function of carbon additions from organic amendments and the carbon losses through organic matter decomposition and a lesser extent as dissolved organic carbon (DOC) (Christ and David 1996). The release of additional organic substrates might not only affect the fluxes of DOC but also the quality. Natural annual carbon fluxes are huge, so that even small forced changes in the natural balance would be very significant at global level (Mermut and Eswaran 2001). Reliable data shows that agriculture is a source of atmospheric CO₂. About 30 to 50% of soil organic matter has been lost since the cultivation of native prairies in Canada (Mermut 2002).

Agricultural management practices such as irrigation, fertilization (organic and inorganic), tillage and residue management, crop rotation or their different combinations can influence the amount of carbon in the soil (Baggs et al. 2006). Farmyard manure together with organic residues and wastes is used increasingly in agriculture (Fearnside 2000). In Southeastern Spain, there are many swine farms, and their waste is often used as an agricultural fertilizer. Nearly 2.5×10^6 Mg (dry wt.) of pig manure is collected annually, approximately 50% of this manure is utilized in agricultural land. This practice is an easy, inexpensive solution for the disposal of swine manure on agricultural property.

The addition of pig slurry to the soil can favor the carbon contents in the soil (Mondini et al. 2007). Southeast of Spain is arid and has poor soils with <1% organic matter. Recycling of pig slurry in agricultural soils provide nutrients (macro and micronutrients), in addition to organic carbon (Faz et al. 2005). Objectives of our study were to: 1) determine the total amount of dissolved organic carbon (DOC) in leachate and 2) quantify the CO₂ released from the soil to establish the carbon fluxes with time.

4.2. MATERIAL AND METHODS

4.2.1. Site Description

The study area was located in La Hoya near the Lorca city of Murcia Province, southeast of Spain. Application of pig slurry to the soil was a common practice in this area which was semiarid, with mean annual rainfall of 300 mm, mean annual temperature of 18°C and potential evapotranspiration was >900 mm yr⁻¹ (Faz et al. 2005). The typical Mediterranean summer drought meant that there was almost no rain (3 mm in July), whereas autumn and spring were the rainy seasons. Most agriculture in the area was rain-fed. Cereals were the main crops in the area. Soil in the area studied could be classified as Typic Haplocalcid (Soil Survey Staff 2010). For details see Chapter 3.

4.2.2. Characteristics of the Soil and Pig Slurry

Selected soil properties of the area studied were given in Table 4.1. Calcium carbonate contents were changed between 55 and 60 % and soil organic carbon was between 13.08 and 14.31 g kg⁻¹. Organic carbon contents were similar to Mediterranean soils under arid-semiarid climate, which was less than 20 g kg⁻¹. Soils had alkaline reaction, low EC, low organic carbon, very low total nitrogen (Villalbi and Vidal 1988), and high phosphorus contents. Cation exchangeable capacity of the soil was also low and this was due to the silty loam texture.

Solid and liquid part of pig slurry was measured and created Table 4.2, which was given the characteristics of pig slurry used in the study. Both liquid and solid part together was applied to the columns. Ions including phosphate and dry matter contents were high in the pig slurry. Although Na and K concentrations were very high,

however, bioavailable microelement concentrations, including Cu, Zn, Fe, and Mn were not at elevated levels.

Table 4.1. Selected soil properties of the area studied.

Soil Profile	pH (1:2.5)	EC (dS m ⁻¹)	SOC (g kg ⁻¹)	TN (g kg ⁻¹)
A1 (0-20 cm.)	8.2±0.1	0.27±0.0	13.55±0.2	0.6±0.0
A2 (20-40 cm.)	8.6±0.0	0.15±0.0	14.31±0.1	0.5±0.1
CK1 (40-65 cm.)	8.7±0.2	0.11±0.1	13.08±0.2	0.5±0.1
CK2 (≥65 cm.)	8.8±0.1	0.20±0.0	13.44±0.3	0.4±0.0
Soil Profile	P _{Olsen} (mg kg ⁻¹)	CEC (cmol ⁽⁺⁾ kg ⁻¹)	CaCO ₃ (%)	Texture
A1 (0-20 cm.)	62.0±0.4	9.0±0.3	55.31±0.9	SL
A2 (20-40 cm.)	40.2±0.6	18.3±0.4	55.52±1.2	SL
CK1 (40-65 cm.)	14.3±0.5	8.7±0.6	57.93±0.8	SL
CK2 (≥65 cm.)	6.3±0.8	10.0±0.5	60.51±0.6	SL

(EC: Electrical Conductivity, SOC: Soil Organic Carbon, TN: Total Nitrogen, P: Phosphorus, CEC: Cation Exchange Capacity, CaCO₃: Lime contents, SL: Silty Loam)

Table 4.2. Characteristics of the pig slurry applied to undisturbed soil columns (n: 3).

Parameter	Content	SD	Parameter	Content	SD
Density (g cm ⁻³)	1.04	± 0.05	Cu (mg L ⁻¹)	0.22	± 0.06
pH	7.34	± 0.01	Zn (mg L ⁻¹)	1.12	± 0.03
EC(dS m ⁻¹)	21.20	± 0.20	Fe (mg L ⁻¹)	1.86	± 0.09
Humidity (%)	82.49	± 0.07	Mn (mg L ⁻¹)	0.22	± 0.03
Dry Matter (g L ⁻¹)	44.50	± 0.05	Mg (mg L ⁻¹)	88.13	± 4.13
DOC (g L ⁻¹)	1.28	± 0.02	Ca (mg L ⁻¹)	45.66	± 2.51
DIC (g L ⁻¹)	0.97	± 0.00	K (mg L ⁻¹)	1102.66	± 10.29
TC (g kg ⁻¹)	366.22	± 10.25	Na (mg L ⁻¹)	762.72	± 10.81
TOC (g kg ⁻¹)	317.88	± 9.52	TN (g L ⁻¹)	2.32	± 0.10
TN (g kg ⁻¹)	19.65	± 0.94	AN (g L ⁻¹)	2.06	± 0.09
TON (g kg ⁻¹)	19.45	± 1.25	DN (g L ⁻¹)	1.04	± 0.05
P _{Olsen} (mg L ⁻¹)	67.33	± 1.53	PO ₄ ⁻ (mg L ⁻¹)	230.00	± 10.00

(EC: Electrical Conductivity, DOC: Dissolved Organic Carbon, DIC: Dissolved Inorganic Carbon, TC: Total Carbon, TOC: Total Organic Carbon, TN: Total Nitrogen, TON: Total Organic Nitrogen, AN: Ammoniacal Nitrogen, DN: Dissolved Nitrogen, P: Phosphorus, PO₄: Phosphate)

4.2.3. Columns Sampling and Experimental Design

Nine undisturbed soil columns were taken from silty loam soils of the area. Soils were extracted using transparent metacrylate cylinder, 60 cm long, 25 cm inside diameter, and 45 cm thick with bulk density of about 1.18 g cm⁻³ and three replicates. Each column weight was about 11.25 kg. Undisturbed soil columns were extracted

using equipment designed based on Mihaljevic et al. (2004), Ashworth and Alloway (2004), and recent modifications by Carmona and Faz (2004). Experimental design of column is given in Figure 4.1. Sea sand, washed with distilled water, was used as supporting material below the columns. They were saturated with approximately 1000 mL distilled water by capillarity placing the columns in a tray with water by superficial addition, before the application of the pig slurry. Simulated rainwater was given to columns by peristaltic pump.

Soil columns were amended with low (129.7 mL) and high (389.2 mL) doses of pig slurry with three replications, applied and mixed in the surface layer of 10 cm. Experiment was carried out for 12 weeks; equaling 170 kg N/ha/yr as low dose (LD), and 510 kg N/ha/yr as high dose (HD). Three untreated columns as control (CT) were added to the experiment. The applied doses were calculated from the agronomic rate of the European legal N-requirement of 170 kg N/ha/yr, for vulnerable zones to nitrates pollution, which corresponds to the LD used in our experiment. Soil samples were taken from 0-5, 5-15, 15-25, 25-35, and 35-45 cm along the column profile from field at the initial of the study. At the end of the experiment we dismantled the columns and soil samples were again taken from 0-5, 5-15, 15-25, 25-35 and 35-45cm depth to compare the initial and final stages of carbon contents (Table 4.4). This enabled us to determine the C fluxes (leached and released to the atmosphere), at the end of the experiment.

Irrigation was carried out on weekly bases using distilled water at a rate of 80 mL h⁻¹ to simulate the monthly rainfall events in the area studied. Leachates and the surface soil of 5 cm deep were collected, and analyzed in each week to determine the amount of carbon in the columns. Leachates were filtered with a Whatman n° 42 paper, and refrigerated at 5 °C until chemical analysis.

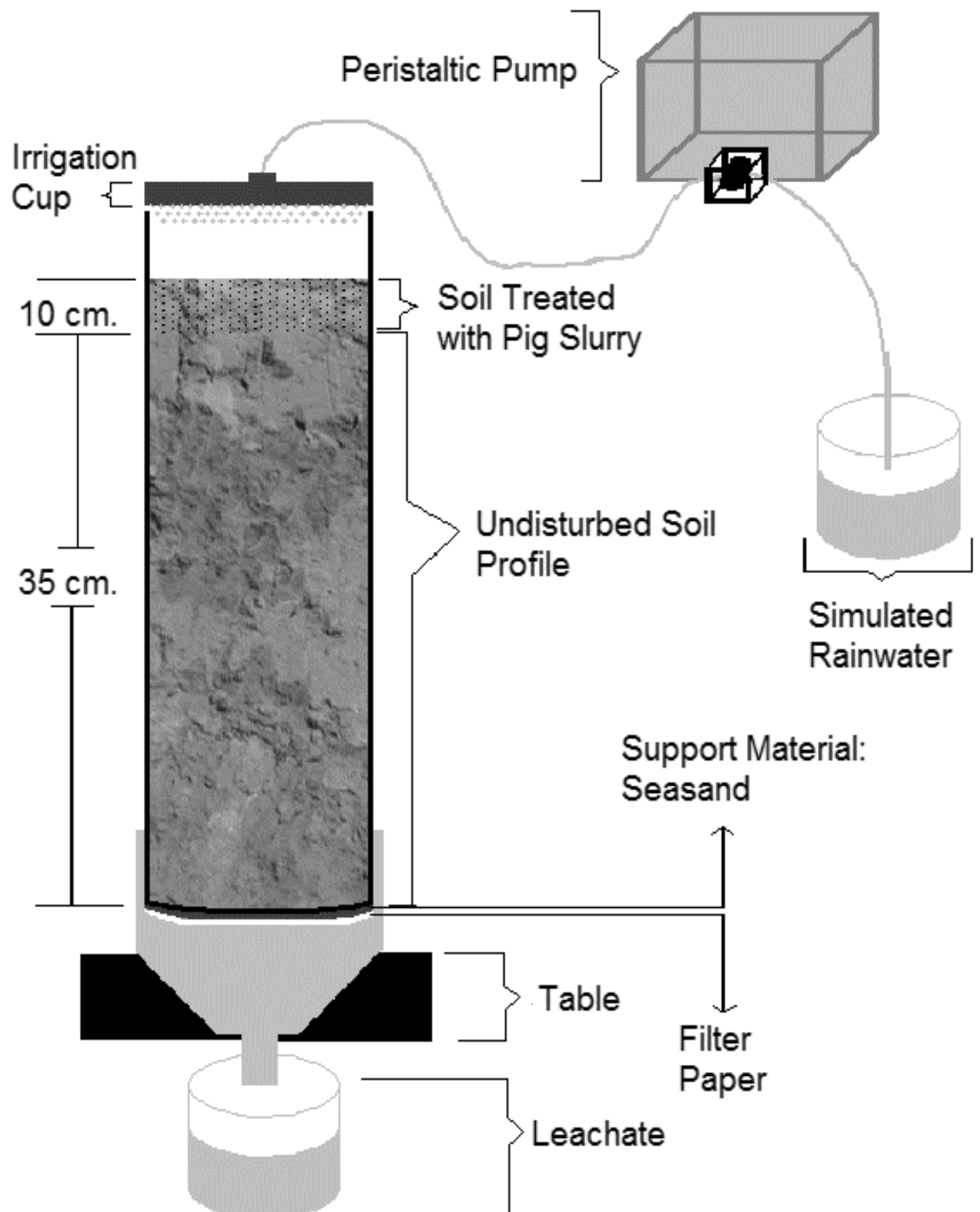


Figure 4.1. Experimental design of the undisturbed soil column.

Table 4.3. Quantity of rainfall simulated, temperature and pressure values of simulated month (n: 3).

Codes	Simulated Months	Temperature (°C)			Pressure (kPa)			Simulated Rainwater (mL.)
Sp	September	25	±	0.3	99.6	±	0.2	1010.02^a
Oc	October	24	±	1.5	100.9	±	0.2	383.09
Nv	November	24	±	0.6	99.9	±	0.5	462.95
Dc	December	26	±	0.0	100.0	±	0.0	351.28
Ja	January	26	±	1.3	99.7	±	0.2	530.81
Fb	February	23	±	1.1	100.0	±	0.1	312.41
Mr	March	26	±	2.1	100.0	±	0.2	541.41
Ap	April	27	±	0.5	100.3	±	0.2	515.26
My	May	26	±	1.5	100.0	±	0.7	650.96
Ju	June	25	±	0.0	100.0	±	0.0	185.18
Jl	July	26	±	2.5	99.9	±	0.2	31.10^b
Au	August	25	±	2.4	100.0	±	0.1	301.10

a: Maximum and **b:** Minimum value of simulated rainwater.

The experiment was continued 3 months (12 weeks) as simulated one year, each week rains was simulated as one real month. The amount of leachates, simulated months, temperature, pressure and codes in each week are given in Table 4.3. There were no leachates in Week ₁₁, which was coincided the month of July, and this was due to the lower amount of simulated rainwater applied. Temperature and pressure were between 23.4 and 26.7 °C and 99.6 and 102.1 kPa, respectively.

4.2.4. Analytical and Statistical Methods

Selected chemical properties of PS, leachate and soil were determined using the following routine methods: soil pH, Eh and electrical conductivity (EC) were measured using deionised water (1:2.5, 1:2.5 and 1:5 w/v, according to Peech (1965) and Bower and Wilcox (1965). Soil organic carbon (SOC) and total nitrogen (TN) were determined according to FAO-ISRIC system (1990). Total phosphorus (P) was measured according to Olsen and Dean (1965). Bulk density was measured according to Culley (1993). Texture analysis carried out by using the FAO-ISRIC system (1990) using a

combination of pipette. The carbonate content of the samples was determined by a calcimeter. Bioavailable metals such as Copper (Cu), Zinc (Zn), Iron (Fe) and Manganese (Mn) were extracted with DTPA in the ratio of 1:2 soil-extractant (Lindsay and Norwell 1978). Cation exchange capacity (CEC) was determined according to Chapman (1965). Metals and cations (Na, K, Ca, Mg) were analysed, using atomic absorption spectrophotometer (AAAnalyst 800, Perkin Elmer).

Characterization of the PS was carried out according to the Standard Methods for the examination of water and wastewater (APWA, 2005). Raw PS was diluted (1/10) in a 100 mL flask, manually shaken 1 minute, then filtered with the filter paper (Albet paper No 242.). This extract was kept in refrigerator until used for analyses. For further analyses of leachates and PS, following analytical techniques suggested by APHA (1998) were used: Dissolved organic carbon (DOC) and Dissolved Nitrogen (DN) (on weekly bases) by TOC – V-CSH Shimadzu (Kyoto-Japan). CO₂ fluxes from the columns were measured according to Anderson (1982).

Soil organic carbon and total nitrogen were measured using CHNS-O Elemental Analyzer (model EA1108) from the surface soil in each week, in addition to initial and final stages. Organic carbon content was calculated through the following formula (1).

$$\text{Organic carbon} = (\text{TOC} + \text{OC of organic fertilizer}) - (\text{DOC} + \text{CO}_2) \quad (1)$$

Statistical analyses were performed with the software SPSS for Windows (Version 19.0) and used for soils and leachates, some data were analyzed by parametric analyses of variance. The data was submitted to one-way ANOVA to assess the differences among treatments and time. The fitting of the data to a normal distribution for all properties measured was checked with the Kolmogorov-Smirnov test. In comparison “T-paired Test was developed, one way Anova to compare treatments within each times and T-paired Test to compare evolution within each treatment.

4.3. RESULTS AND DISCUSSION

4.3.1. Changes of Soil Properties and Characteristics of Leachates

Selected soil properties of the columns with pig slurry application at the initial and final stage were given in Table 4.4. There were no significant differences between pH, EC, CEC, cations and bioavailable metals except of Zn contents at the initial and final stage of experiment. Total nitrogen and Zn contents of soil were significantly increased with all doses due to nitrogen and Zn concentration of pig slurry.

Distributions of SOC with depth at initial and final stages of the study were given in Table 4.5. Carbon contents in CT columns was not homogenous and the subsoil contains more organic carbon than surface, confirming that soils used in this study already received pig slurry and organic residues that moved down quickly with rainwater, in addition to initial distilled water added to the column to make it moist before the start of the experiment. They were changed between 6.81 and 15.50 tonnes OC ha⁻¹ at the initial, which were changed between 11.39 and 26.26 tonnes OC ha⁻¹ at the final stage in the entire profile, and they were increased with the increase doses of PS, specifically in the subsurface horizons. Portion of SOC with depth at initial and final stage of the study showed that; high amount of SOC was accumulated in the subsoil, especially with the HDs.

Volume of leachate was changed depending on simulated rainwater amount used. Correlation coefficient analyses of leachates in each simulated rainfall showed that direct effects of pig slurry on physical and chemical properties were observed in the leachate, which were consistent with other studies (Chao et al., 1996).

Table 4.4. Selected soil properties of the columns with pig slurry application at initial and final stages (n: 3).

Doses	pH			EC (mS cm ⁻¹)			CEC (cmol ⁽⁺⁾ kg ⁻¹)			Ca (cmol ⁽⁺⁾ kg ⁻¹)		
	Initial	Final	t	Initial	Final	t	Initial	Final	t	Initial	Final	T
LD	8.85b(0,00)	8,18(0,17)	***	0,13(0,05)	0,11(0,03)	***	9,54(1,25)	8,37(0,82)	**	20,00(2,86)	25,63(2,03)	***
HD	8.81b(0,00)	8,13(0,15)	***	0,13(0,05)	0,08(0,04)	***	9,48(0,95)	7,65(1,35)	***	21,53(1,68)	26,8(2,13)	***
CT	8.69a(0,00)	8,11(0,08)	***	0,11(0,03)	0,08(0,04)	***	9,21(0,49)	9,05(1,65)	ns	19,91(2,20)	25,44(3,57)	***
F	9.39***	0.87ns		1.47ns	1.67ns		0.52ns	2.59ns		2.36ns	1.17ns	
Doses	Mg (cmol ⁽⁺⁾ kg ⁻¹)			Na (cmol ⁽⁺⁾ kg ⁻¹)			K (cmol ⁽⁺⁾ kg ⁻¹)			Zn (mg kg ⁻¹)		
	Initial	Final	t	Initial	Final	t	Initial	Final	t	Initial	Final	T
LD	0,81b(0,11)	0,76a(0,11)	ns	0,28(0,31)	0,95(1,36)	ns	0,42a(0,08)	0,39ab(0,14)	ns	0,15a(0,08)	0,48b(0,33)	***
HD	0,70a(0,05)	0,88b(0,15)	***	0,36(0,16)	1,91(3,64)	ns	0,55b(0,15)	0,37a(0,16)	**	0,17a(0,10)	0,36ab(0,34)	*
CT	0,63a(0,07)	0,78ab(0,11)	***	0,37(0,31)	0,42(0,09)	ns	0,55b(0,08)	0,49b(0,10)	*	0,39b(0,17)	0,21a(0,06)	***
F	18.10***	4.37*		0.34ns	1.68ns		5,37***	2.65*		19.56***	3.89*	
Doses	Cu (mg kg ⁻¹)			Fe (mg kg ⁻¹)			Mn (mg kg ⁻¹)			TN (g kg ⁻¹)		
	Initial	Final	t	Initial	Final	t	Initial	Final	t	Initial	Final	T
LD	1,95a(0,75)	2,58b(2,05)	ns	3,74b(0,48)	2,01a(0,78)	***	14,15(3,24)	11,65(2,59)	*	0,10b(0,00)	0,61(0,19)	***
HD	2,91b(0,66)	1,21ab(0,48)	***	3,78b(0,63)	3,34a(2,76)	ns	16,51(3,52)	11,31(2,77)	***	0,10b(0,00)	0,51(0,25)	***
CT	3,14b(0,27)	1,63a(0,23)	***	2,95a(0,46)	1,89a(0,59)	***	14,54(1,42)	12,13(2,08)	***	0,07a(0,05)	0,56(0,11)	***
F	17.58***	4.91**		12.01***	3.49*		2.91ns	0.42ns		13.25***	0.98ns	

(**EC**: Electrical Conductivity, **CEC**: Cation Exchange Capacity, **Ca**: Calcium, **Mg**: Magnesium, **Na**: Sodium, **K**: Potassium, **Zn**: Zinc, **Cu**: Copper, **Fe**: Iron, **Mn**: Manganese, **TN**: Total Nitrogen)

Some properties of leachate such as pH ($r^2=0.334$, $p<0.01$), macro-micro nutrient (Mg; $r^2=0.275$, $p<0.01$; Na; $r^2=0.486$, $p<0.01$; Ca; $r^2=0.478$, $p<0.01$; K; $r^2=0.244$, $p<0.01$; Cu; $r^2=0.267$, $p<0.01$; Zn; $r^2=0.218$, $p<0.05$), and carbon ($r^2=0.449$, $p<0.01$) were significantly correlated with nitrogen ($r^2=0.227$, $p<0.05$), which are essential for plant growth (data not shown). It is well known that the pig slurry addition would be more pronounced in soils with low organic matter content and fertility (Loro et al., 1997).

Table 4.5. Distributions of SOC in the columns at initial and final stages of the study.

Depth (cm.)	LDI	HDI	CTI	LDF	HDF	CTF
g OC						
0-5	18.28	31.55	7.15	10.42	10.40	7.07
5-15	73.10	126.18	28.62	38.88	38.82	28.32
15-25	45.93	87.58	31.69	31.77	36.29	31.36
25-35	45.93	87.58	31.69	28.98	33.53	31.63
35-45	60.64	102.29	33.09	28.98	35.94	32.74
Total OC	243.87	435.18	132.25	139.03	154.98	131.11

(**LDI**: Low dose of initial stage at the end of at the end of September; **HDI**: High dose of initial stage at the end of September; **CTI**: Control of initial stage at the end of September; **LDF**: Low dose of final; **HDF**: High dose of final; **CTF**: Control of final)

Dissolved organic carbon (DOC) in cumulative manner were given in Figure 4.2. The results showed that DOC contents were changed between 0.00 and 3.42 kg ha⁻¹ carbon with the LD, 0.00 and 4.71 kg ha⁻¹ carbon with the HD. Dissolved organic carbon contents in two doses applied were closely associated with the amount of water applied, and it was tended to increase with time. Cumulative content of DOC were in the following order: HD > CT > LD. As expected, the DOC was increased in the leachate especially with HD application. Cumulative concentration of the total nitrogen were in the following order: HD > LD > CT (data not shown). The reason for the lower nitrogen content in leachate with HD was likely due to high microbial activity in increased organic matter.

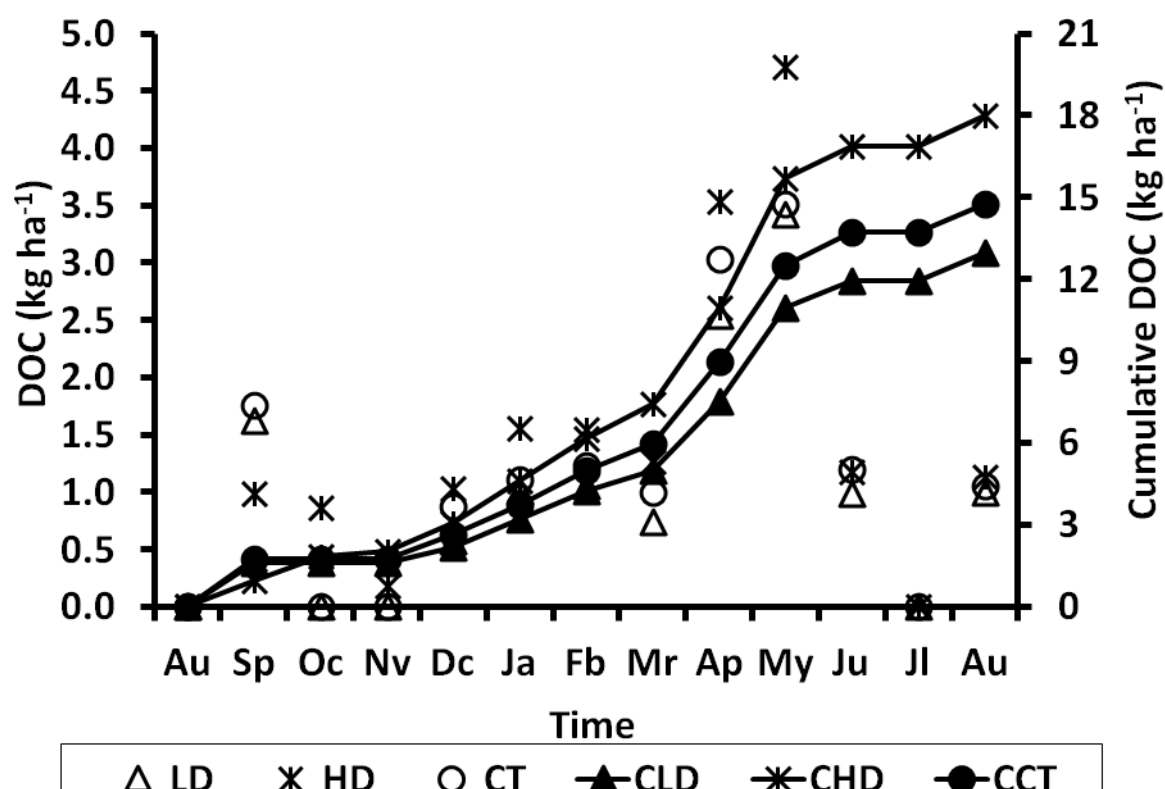


Figure 4.2. Changes in DOC on monthly bases and total losses in cumulative mode measured from the leachates (LD: Low dose, HD: High dose, CT: Control, CLD: Cumulative low dose, CHD: Cumulative high dose, CCT: Cumulative control; n:3).

The production and leaching of dissolved organic carbon (DOC) was affected by biological activity (Christ and David 1996) and chemical factors such as total nitrogen ($r^2=0.476$, $p<0.01$) and soil organic carbon ($r^2=0.572$, $p<0.01$). According to Qualls and Haines (1992) initially, pH was a more important factor for DOC leaching than temperature. Our results showed that pig slurry application were increased the amount of DOC in leachates with HD as also reported previously by Carmona and Faz (2004). DOC content in leachates was lower with LD, this is the important point for the legislated doses (LD), which was not contribute to losses of C by leaching in the system.

4.3.2. CO₂ Release

Soil respiration results in cumulative mode were given in Figure 4.3. The results showed that, without pig slurry application (CT) soil respiration was approximately 0.01 tonnes C-CO₂ month⁻¹ ha⁻¹, LD and HD were varied between 0.01 and 2.30 tonnes C-CO₂ month⁻¹ ha⁻¹; 0.01 and 7.16 tonnes C-CO₂ month⁻¹ ha⁻¹ respectively (Figure 4.3). They were in the following order: HD> LD> CT. The soil respiration was increased with the dose of pig slurry application. Recent studies on pig slurry application to silty loam soils also showed that soil CO₂ release was increased with amount of slurry applied (Bernal et al. 1998).

Cumulative respiration was higher in HD application and it was much lower in the control. We found a negative correlation between the CO₂ emission and temperature ($r^2=-0.223$, $p<0.05$), also humidity was positively correlated with respiration ($r^2=0.429$, $p<0.01$). Positive correlations were found in Russian Taiga zone (Kudeyarov and Kurganova 1998) and Canadian grassland ecosystem (Flanagan and Johnson 2005). Chodak et al. (2001) reported a negative effect of temperature on the DOC to CO₂ ratio, indicating that temperature has a weaker effect on DOC release than on CO₂ production. Lou et al. (2004) reported the maximum CO₂ fluxes during the summer and minimum during the winter and intermediate in autumn and spring.

The correlations between the soil humidity (%) and weekly CO₂ fluxes were statistically significant ($r^2=0.429$, $p<0.01$). The values of CO₂ rates were higher the first days of the application owing to higher soil humidity and temperature. Significant correlation were found between CO₂ in all treatments and temperature ($r^2=-0.223$, $p<0.05$), confirming the importance of the amount of water available in the soil, particularly in semi-arid eco-systems (Sainju 2008).

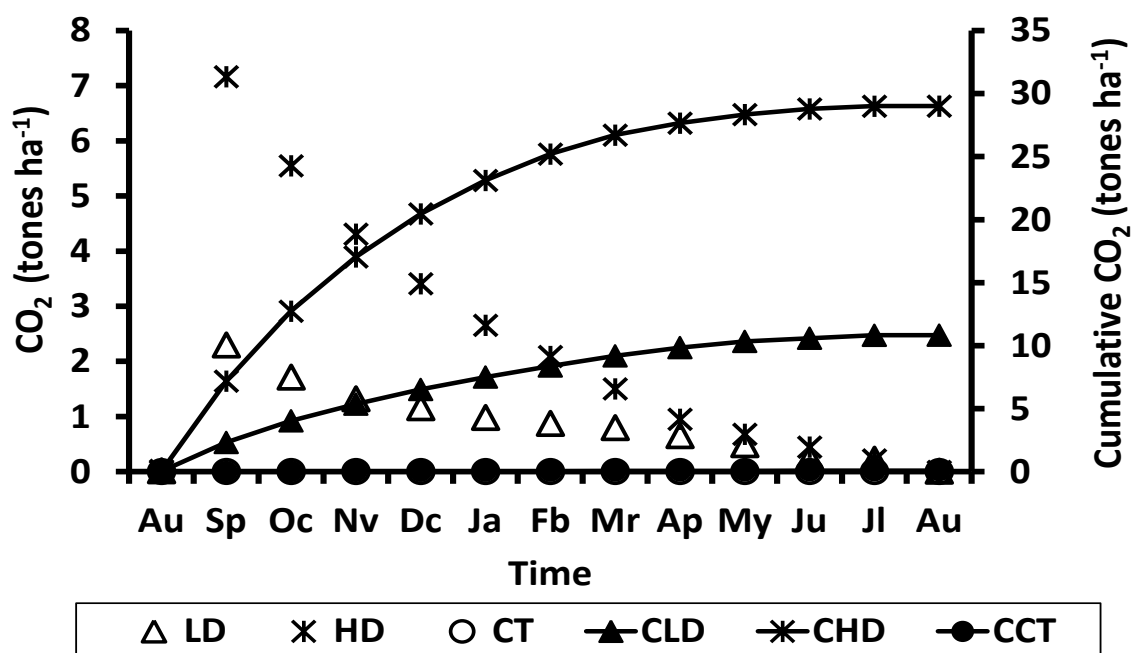


Figure 4.3. Monthly CO₂ release from the soil surface and cumulative expressions of the undisturbed columns used in the study (LD: Low dose, HD: High dose, CT: Control, CLD: Cumulative low dose, CHD: Cumulative high dose, CCT: Cumulative control; n:3).

Zhao et al. (2009) stated that soil moisture played the most significant role in CO₂ emission in the area under the shrub and interplant zone, rather than the soil surface temperature. This was attributed to the increased of microbial activity with respect to increased water in the soil. Same researcher also reported that CO₂ emissions were significantly correlated with soil organic carbon and total nitrogen reserves under desert conditions. Researchers have reported that the highest variations in CO₂ emission from the soil (65-85%) were derived from the difference in parent materials and were explained by soil temperatures (Lou et al. 2004).

According to Rochette et al. (2000), the majority of carbon loss occurred during the first week, following the application of pig slurry. The release of CO₂ immediately after pig slurry application was attributed to the metabolism of sugars and amino acids,

which were readily available to soil microorganisms. However, the increase in the CO₂ flux following the pig slurry application is not only due to microbial activity, but also C-CO₂ liberated from the dissociation of carbonates and to the rapid release of C-CO₂ when alkaline slurry (pH, 7.5) is applied to an acidic soil (pH, 5.5) (Sommer and Sherlock 1996). pH was 8, however this was not happening in our study.

4.3.3. Organic Carbon Fluxes

Organic C fluxes were statistically significantly related with SOC ($r^2=0.995$, $p<0.01$), CO₂ ($r^2=0.983$, $p<0.01$), DOC ($r^2=0.570$, $p<0.01$) and TN ($r^2=0.440$, $p<0.01$). The results of organic carbon loss as DOC+CO₂ have shown that; the losses of DOC from the columns were: 0.15 and 0.07 and 16.30 % with LD, HD, and the CT, respectively. CO₂ released from the pig slurry additions were 99.88; 99.94; and 83.70 % with LD, HD, and CT, respectively.

Total organic carbons from pig slurry additions remained were 18.2 and 10.9 % with LD and HD, respectively. The amounts in the surface layers (0-10 cm) in one year were between 15.85 and 24.65 % (1.97 and 3.15 tonnes OC ha⁻¹) with LD and HD, respectively, per cent of their organic carbon content in the soil. A result from CT column was 0.8% (0.11 tonnes OC ha⁻¹) loss of organic carbon in one year (Figure 4.4).

These results were consistent with a previous report by Wu et al. (2003), which estimated an SOC loss of 10%–40% in the cultivated soils relative to their non-cultivated counterparts in China. Guo and Gifford (2002) found that soil carbon stocks, at a global scale, declined by 59 % after the conversion of pasture to cropland. There is generally more SOC in grassland soil than in cropland soil (Cole et al. 1993). Lal (2002) reported a carbon pool loss of 30–50 % in the midwestern USA due to land use changes

from natural to agricultural ecosystems. Large amount of addition of crop residues or manure can offset the apparent losses. The loss is often attenuated by depth.

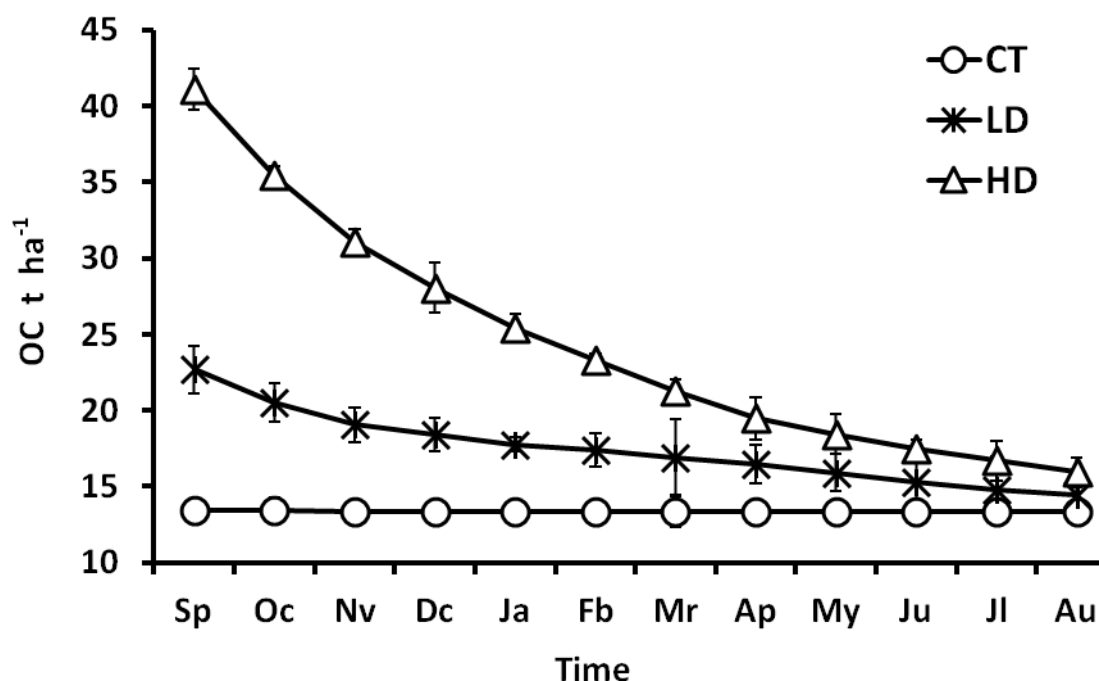


Figure 4.4. Evaluation of total organic carbon on monthly basis in undisturbed soil columns (LD: Low dose, HD: High dose, CT: Control, n:3).

Repeated measures analyses of variance of organic between doses and time were given in Table 4.6. Our results showed that; TOC, and CO₂ were statistically significant, in terms of dose and time, but DOC was significant only with time. Our analytical data in Table 4.7 showed that at the initial of the study, the soil organic carbon content was about 13.42 tones OC ha⁻¹ in the area studied. At the final of the experiment, the amounts of organic carbon remained in the soil were 14.4 and 15.93 tones OC ha⁻¹ with LD and HD, respectively. The change in control column (CT) was about -0.11 tones OC ha⁻¹ corresponding to 0.8 % of OC loss. About 81.64 and 91.21 % of added slurry were oxidized from the application of LD and HD. What remained in the soil corresponds to only 1.97 and 3.15 tones OC ha⁻¹ was increased annually in the carbon contents respectively.

Table 4.6. Repeated measures analysis of variance for organic carbon.

	TOC	DOC	CO ₂
<i>Between Subjects</i>			
Dose	366.9***	2.9ns	1250***
<i>Within Subjects</i>			
Time	105.0***	45.5***	233.2***
Dose x Time	48.8***	1.0ns	112.9***

*P<0.05, **P<0.01, ***P<0.001, ns; No significant

Table 4.7. Changes in organic carbon contents with time in entire soil columns treated with different amounts of pig slurry, expressed in OC ha⁻¹.

Treatment	Initial (tones OC ha ⁻¹)	Added (tones OC ha ⁻¹)	Total (tones OC ha ⁻¹)	Final (tones OC ha ⁻¹)
LD	12.43	10.73	23.16	14.4
HD	12.78	32.18	44.96	15.93
CT	13.42	-	-	13.32
Treatment	Changes of soil OC in %	Changes of soil OC in tones ha ⁻¹	Losses of Slurry %	
LD	15.85	1.97	81.64	
HD	24.65	3.15	91.21	
CT	-0.80	-0.11	-	

(LD: Low dose, HD: High dose, CT: Control)

Groundwater losses of DOC and inorganic carbon were less than 0.3 % of the total CO₂ flux to the atmosphere and this was due to decomposition at high temperature in deciduous forest in Tennessee (Edwards and Harris 1977). According to Giacomini (2008), working in laboratory conditions, observed about 45% of C mineralization within 25 days, when the slurry was applied to the soil surface. The substantial amount of slurry applied could remain and accumulate in the soil.

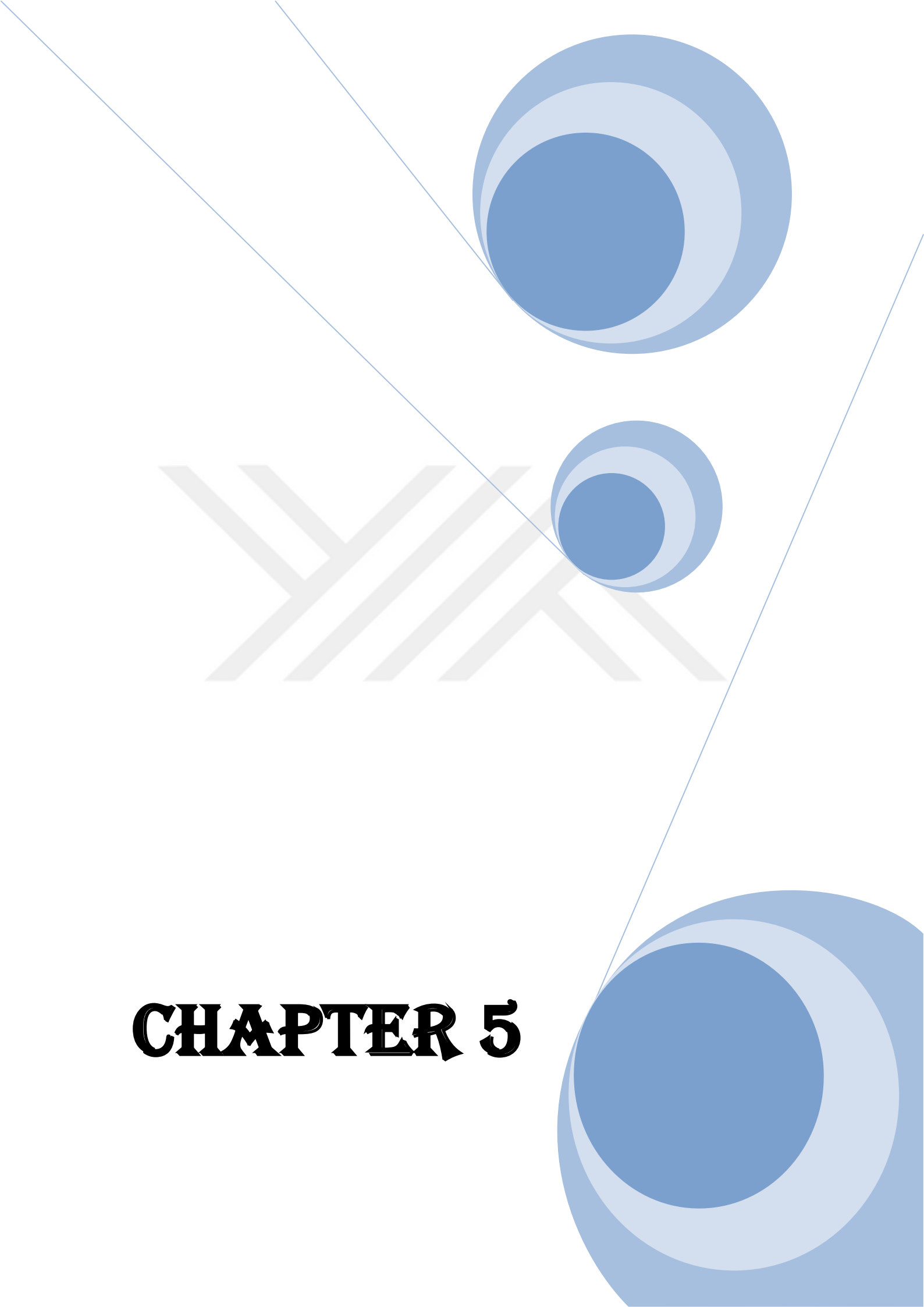
4.4. CONCLUSIONS

Pig slurry applications have a greater effect on increasing OC levels in the terrestrial ecosystem. It appears that the amount of legislated doses (LD) suggested were based on N content only. From our study we suggest that the C fluxes including the

movement of DOC in the soil need to be measured and calculated in details to decide on more realistic doses for farmers.

The technique used provides detail information about C fluxes in the soil system. The CO₂ emissions increase with increasing PS applications. The amount and losses of DOC is insignificant. Concentration of OC in the subsoil seems to be a surprise but this is related to the texture of the soil. Despite the fact that the area is semiarid, rainfall is <300 mm and potential evapotranspiration is >900 mm yr⁻¹. The rate of decomposition of PS appears to be higher than heavy soil, as the soil used in the study is loamy.

A large portion of the total and organic C is located in the subsoil. The topsoil (0-10 cm) have sequestered 15.84 and 24.68% (1.97 and 3.15 tonnes OC ha⁻¹) with LD and HD, respectively. Carbon leaching in the control column showed 0.68 % (0.09 tonnes OC ha⁻¹) loss of OC in one year. The benefits from the use of PS depend on management, amount added and soil characteristics.

The background features a light grey 'X' shape formed by two intersecting diagonal lines. Three blue circles of varying sizes are positioned around the page: a large one in the top right, a medium one in the center right, and a large one in the bottom right. Each circle is composed of three concentric layers of blue, with the innermost being the darkest and the outermost being the lightest. The text 'CHAPTER 5' is located in the lower-left quadrant.

CHAPTER 5

CHAPTER 5

STABLE CARBON ISOTOPE COMPOSITION OF SOIL AND PLANT UNDER PIG SLURRY APPLICATIONS IN SILTY LOAM SOIL OF THE MURCIA REGION, SPAIN.

Abstract: Pig slurry application on the soil has been a common practice in Spain in last decades. However, there is limited information, pig slurry impact on soil and plant carbon dynamics. Information about the use of the natural $\delta^{13}\text{C}$ abundance technique to study the dynamics of soil organic matter turnover is limited. The main source of organic carbon in the soil vegetative cover and other source could be recent application organic residues disposals such as pig slurry. The ^{13}C compositions of both sources are significantly different. We investigated the long-term effects of pig slurry on the distribution of stable carbon isotope ratio in a silty loam soil with cereal cultivation in the Southeast of the Spain, in order to elucidate the mechanism by which SOC was recycled in the silty loam soil ecosystem.

Three different doses of PS: $170 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, $340 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ and, $510 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, were compared with a control in a non-irrigated Mediterranean environment. Our conclusions are (i) soils were considerably enriched in $\delta^{13}\text{C}$ values with D1 application; (ii) $\delta^{13}\text{C}$ values were higher in second years application in the soil and plant; (iii) $\delta^{13}\text{C}$ values of organic C was lower at depth, suggesting that the leached

organic carbon was mainly soil carbon. Significant correlation exists between carbon isotopic values of organic carbon and pig slurry. This means that the stable isotope composition of soil and plant are affected by pig slurry application.

Keywords: $\delta^{13}\text{C}$, Soil, Plant, Pig Slurry

5.1. INTRODUCTION

Soil organic matter is an important pool of carbon (C) in the global C budget and a major component of the more active fractions involved in the earth's C cycle in terms of both the total amount and annual flux (Swift, 1996). It has been commonly estimated that soil organic matter contains approximately 1500 Pg C (Schlesinger, 1984). Also it plays an important role in soil biological (provision of substrate and nutrients for microbes), chemical (buffering and pH changes) and physical (stabilization of soil structure) properties. These properties, along with SOC, N and P, are considered critical indicators of the health and quality of the soil (Krull et al., 2004).

Recycling organic wastes as soil organic amendment in the form of compost is important for both environmental safety and sustainable agriculture (Caricasole et al., 2010). Application of compost to soil, besides improving its fertility functions, also enhances the role of soil as a sink of organic carbon (Piccolo et al., 2004). However, sequestration and losses of C from slurries applied to agricultural soils had been poorly understood (Chantigny et al., 2001; Fangueiro et al., 2007).

The ^{13}C natural abundance technique was first used to study the dynamics of SOC, and has been widely used in the field since (Nissenbaum and Shallinger, 1974; Balesdent et al., 1993). Natural abundance of ^{13}C (stable C isotope ratio, $\delta^{13}\text{C}$) in soil organic matter depends on the migration and decomposition dynamics of organic

matter itself and on plant type, and $\delta^{13}\text{C}$ can be used in ascertaining the rate of turnover of total SOC and that of movement or decomposition of some of its fractions (Haefelea et al., 2004; Ma et al., 2011).

The use of ^{13}C isotopic tracers for SOM studies derives from the fact that all plants discriminate against ^{13}C during photosynthesis but to different degrees depending on their photosynthetic pathway (Farquhar et al., 1989). Terrestrial plants with the C3 (Calvin cycle) pathway have $\delta^{13}\text{C}$ mean values of -27‰. Plants with the C4 (Hatch–Slack) pathway have higher $\delta^{13}\text{C}$ mean values of -12‰ (Stevenson et al., 2005). The isotopic composition of SOM closely resembles the isotopic composition of the vegetation from which it was derived because the fractionation during decomposition is small relative to the original fractionation during C fixation (Nadelhoffer and Fry, 1988). When one type of vegetation is replaced with another, $\delta^{13}\text{C}$ values can be used to identify SOM derived from residues from the original and new vegetation (Bernoux et al., 1998).

The objective of this chapter was to investigate the long-term effects of pig slurry on the distribution of stable carbon isotope ratio in a silty loam soil, and cereals, in order to trace the decomposition of added organic substances in the silty loam soil ecosystem of Southeast Spain.

5.2. MATERIAL AND METHODS

5.2.1. Experimental Design and Sampling

Field experiments were carried out for 3 years in an experimental field in the Murcia region, Guadelentin Valley (near the Lorca city, southeastern of Spain) (latitude:

37° 55' 27, 87" N; longitude: 1° 49' 37, 84" W) at a mean altitude of 18 m above sea level with 3, 4 % slope (Figure 5.1). The climate is semiarid Mediterranean with an average annual rainfall of 300 mm, occurring mostly in autumn and spring and a mean annual temperature of 18°C and potential evapotranspiration is >900 mm yr⁻¹ (López-Bermúdez et al., 2002). For details see Chapter 3.

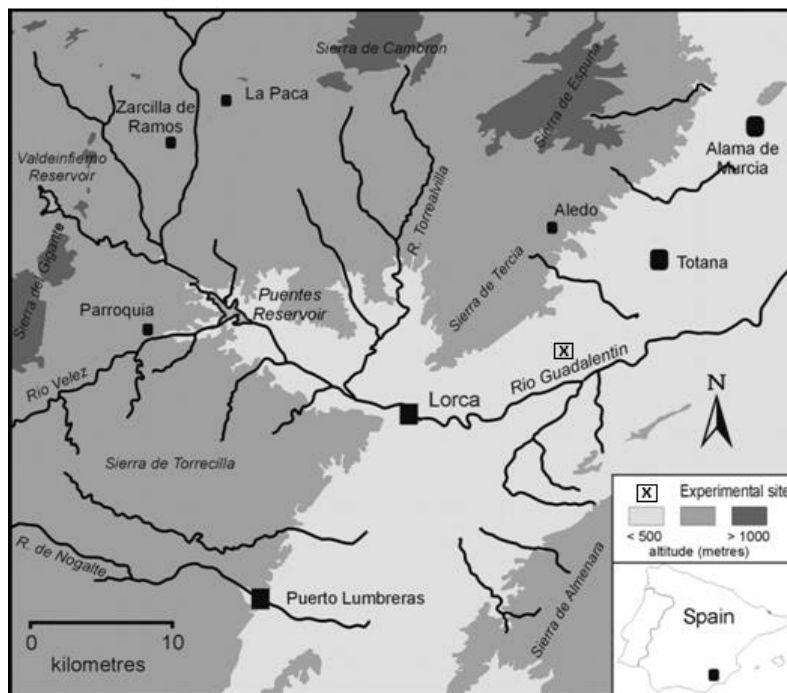


Figure 5.1. Location of the study area (Adapted from Mateus (2006)).

The most dominant soil types in the Guadalentín Valley are Regosols, Fluvisols, Calcisols, Gypsisols and Leptosols (FAO, 2006). Leptosols dominate on the steeper slopes of the limestone outcrops, whereas Calcisols, Gypsisols and Regosols are mostly found on the flatter terrain. Calcic and petrocalcic horizons are common in these soils. The main valley of the Guadalentín is dominated by Fluvisols formed in sediments rich in gypsum and calcium carbonate and often with high levels of salinity. The soil was classified as Haplic Calcicol (FAO, 2006) with a composition of 21.2% sand, 51.6% silt and 27.2% clay. The pig slurry was collected from Lorca city.

The study area represents one of the semi-arid (driest) regions of continental Europe. It is facing many problem associated with agriculture and the environment, including scarcity of water, salinisation, soil erosion, and desertification. The explosion of greenhouse cultivation within the last two decades in the region, using advanced plantation technologies, has created new challenges regarding the soil use and management, which has responded to external demand rather than taking into account the suitability of the soil to produce appropriate crops (Faz et al., 2005).

The study area was consisted of four experimental plots, 25 m²(5x5). Plots consisted of control and raw pig slurry (PS), applied at single (D1), double (D2), triple (D3) doses. Unamended soil used as a control (C) plot. D1 were the agronomic rate of N-requirement as 170 kg N ha⁻¹ yr⁻¹ (European Directive 91/676/CEE), D2 were 340 kg N ha⁻¹ yr⁻¹ and D3 were 540 kg N ha⁻¹ yr⁻¹. Soil samples were collected from two different depths, 0-30 cm and 30-60 cm. before and after applications. There were three soil and plant (*Barley-Hordeum Distichum L.*) sampling with two pig slurry applications in the study. The first sampling was before the application of PS in 2009, the second and third applications in 2010, 2011 were after applications.

5.2.2. Analytical Methods and Statistical Analysis

The collected soil samples were air-dried in the lab for 7 days, passed through a 2-mm sieve, homogenized, and stored in plastic bags at room temperature prior to laboratory analyses. We analyzed pH, EC, nitrogen, cation exchange capacity according to Peech (1965), Bower and Wilcox (1965), Duchaufour (1970), Chapman (1965), respectively. Plant available phosphorus (P₂O₅) was measured according to Watanabe

and Olsen (1965) by spectrophotometer (UVmini-1240 Spectrophotometer Shimatzu). Soil samples were analyzed for total organic carbon content on a LECO C 144 carbon analyzer as described by Merry and Spouncer (1988). Prior to analysis, each soil sample was tested with HCl for the presence of carbonate (Krull et al. 2005). The Bernard Calcimeter method with Na_2CO_3 was used to determine carbonate contents.

Barley (C3 plant) plants (*Hordeum Distichum L.*) were taken from 1 m² in each plot at harvest time. Plant samples were separated, washed with deionized water, and dried in an oven at 60 ° C for 48 hours, then ground to a fine powder by using a ball mill. The grain samples were stored in the special glass containers to determine chemical characteristics of plants.

In order to determine nutrients and phosphorus content in plant samples; 0.7 g. plant samples were weighted and ashed in a muffle furnace at 480°C for 24 h. The ash residue was digested in 6N nitric acid. The solution used to determine nutrients (Na, K, Ca, Mg, Zn, Cu, Fe and Mn) by an atomic absorption spectrophotometer and phosphorus by a spectrophotometer. Total phosphorus (P) was measured according to Olsen and Dean (1965). Total Nitrogen was measured by Kjeldahl method, as described in Duchaufour (1970) and analyzed by automatic titration machine (Metrohm 702 SM Titrino).

Selected chemical properties of applied PS were analyzed following the routine methods. We determined pH (Peech, 1965), electrical conductivity (EC) (Bower and Wilcox, 1965), Total Nitrogen (TN) with Kjeldahl method (Duchaufour, 1970), Total phosphorus (Olsen and Dean., 1965). Raw PS was diluted (1/10) in a 100 mL flask, manually shaken 1 minute, then filtered with Albet paper No 242. This extract was kept

to measure trace element (Cu, Zn, Fe and Mn) by Atomic Absorption Spectrometer (AAnalyst 800, Perkin Elmer Precisely).

For each property data were submitted to parametric multifactor analysis of variance (ANOVA's). The variance was related to the main treatments (year and amendment type) and to the interaction between them. If need nonparametric test with using the software package "SPSS Version 19.0" since the interaction between factors and processes was significant between soils/plants for selected subsets of data. A correlation analysis was also calculated, to evaluate the extent of association and its significance.

5.2.3. Samples Preparation and Measurement of Stable Isotope Composition of Organic C

The $\delta^{13}\text{C}$ value of organic C was measured for soil and plant samples. Horizons containing both organic C and carbonates were treated with 3 M HCl to remove carbonates, and then washed with deionized water using 0.22 μm Millipore filters to remove excess HCl and dried. All samples were ground to a fine powder using a ball mill. Soil samples were weighed 27.0 ± 6.0 mg; plant (C3) and pig slurry were weighed 2.0 ± 0.4 mg according to carbon contents. Acetanilide was the reference material used in the study, and weighed 1.5 ± 0.4 mg. microbalance (accurate to 0.01 mg).

The isotopic composition ($\delta^{13}\text{C}$ value) of soil organic matter was determined using a Europe Scientific Instruments elemental analyzer coupled to a Europe Instruments 20/20 mass spectrometer in continuous-flow mode. In this method, samples are loaded into tin capsules and combusted in a furnace at 1000 °C. The gas is passed

through traps to trap water and other unwanted gases. The gas stream passes into a gas chromatograph where components of interest are separated and is then bled into a mass-spectrometer where the carbon isotopes are ionized and then separated in a magnetic field. The isotopic species are detected separately and from their ratios (Landi, 2002).

The results of the isotope analyses are expressed in term of δ notation units of per mil (‰):

$$\delta^{13}\text{C} = \left(\frac{R_s}{R_{st}} - 1 \right) \times 1000 \quad [1]$$

where $R_s = {}^{13}\text{C}/{}^{12}\text{C}$ in sample, and $R_{st} = {}^{13}\text{C}/{}^{12}\text{C}$ in the reference standard ($R_{\text{PDB}}=0.0112372$) (Eq. (1)). The δ values for the $\delta^{13}\text{C}$ are reported relative to Vienna Pee Dee Belemnite (VPDB). The reproducibility of this method for C isotope ratios is $\pm 0.2\text{‰}$ (Mermut et al., 2000; Landi, 2002).

5.3. RESULTS AND DISCUSSION

5.3.1. Characterization of Soil, Plant and Applied Pig Slurries

The main soil properties were given in Table 5.1. Calcium carbonate and organic carbon contents were changed between 55 and 60%, 13.08 and 14.31 g kg⁻¹, respectively. Organic carbon contents were similar to Mediterranean soils under arid-semiarid climate condition, which were less than 20 g kg⁻¹ (Stevenson, 1994).

Soil bulk density in the area studied was about 1.18 gr cm⁻³. Soils were slightly alkaline reaction (Porta al, 1999), low EC, low organic carbon, very low total nitrogen with C/N ratio of > 20 (Villalbí and Vidal, 1988), and high bioavailable phosphorus contents (Dressing, 1993). Cation exchangeable capacity was also low (Porta, 1999) and this was due to the soil texture with a Haplic Calcisols nature according to FAO (2006).

Table 5.1. General soil properties of the study area.

Soil Profile	pH (1:2.5)	EC (mS m ⁻¹)	SOC (g kg ⁻¹)	TN (g kg ⁻¹)
A ₁ (0-20 cm.)	8.2±0.1	2.7±0.0	13.55±0.2	0.6±0.0
A ₂ (20-40 cm.)	8.6±0.0	1.5±0.2	14.31±0.1	0.5±0.1
CK ₁ (40-65 cm.)	8.7±0.2	1.1±0.1	13.08±0.2	0.5±0.1
CK ₂ (≥65 cm.)	8.8±0.1	2.0±0.0	13.44±0.3	0.4±0.0
Soil Profile	P _{Olsen} (mg kg ⁻¹)	CEC (cmol ⁽⁺⁾ kg ⁻¹)	CaCO ₃ (%)	Texture
A ₁ (0-20 cm.)	62.0±0.4	9.0±0.3	55.31±0.9	SL
A ₂ (20-40 cm.)	40.2±0.6	18.3±0.4	55.52±1.2	SL
CK ₁ (40-65 cm.)	14.3±0.5	8.7±0.6	57.93±0.8	SL
CK ₂ (≥65 cm.)	6.3±0.8	10.0±0.5	60.51±0.6	SL

EC: Electrical Conductivity, SOC: Soil Organic Carbon, TN: Total Nitrogen,

P: Phosphorus, CEC: Cation Exchange Capacity, CaCO₃: Lime contents, SL: Silty Loam.

Characteristics of PS showed that; cations, phosphorus and dry matter contents were high in the pig slurry. Although Na and K concentration were very high, however, bioavailable metal concentrations (Cu, Zn, Fe, and Mn) were not that high (Karaca, 2004).

According to Büyükkılıç Yanardağ et al. (2013), all chemical plant parameters were significantly affected by PS application except of Cu contents, which were increased with PS doses ($P < 0.05$). Na uptakes were increased with D1 and D2 applications. D3 applications were not significantly effected on plant Na uptake. Cereal K uptakes were increased with all doses of PS application. Ca and Mg uptakes were not significant with all PS applications. Phosphorus (P) uptakes of plants were slightly increased with D2 and D3 applications. Total Nitrogen (TN) was decreased with application of PS (especially with D2) owing to the microbial activity. Zn, Fe and Mn uptakes of plants were increased with all doses of PS applications whereas; Cu uptakes of plants were not statistically significant. The results showed that the optimum

application rate were D₁ application under field conditions in terms of sustainable plant production. For details see Chapter 3.

5.3.2. Organic Carbon Evolution with Pig Slurry Application

Soil organic carbon evolution in the surface and subsurface horizons were given in Figure 5.2 and 5.3. The effect of different doses application on soil organic carbon was found statistically significant ($P < 0.05$).

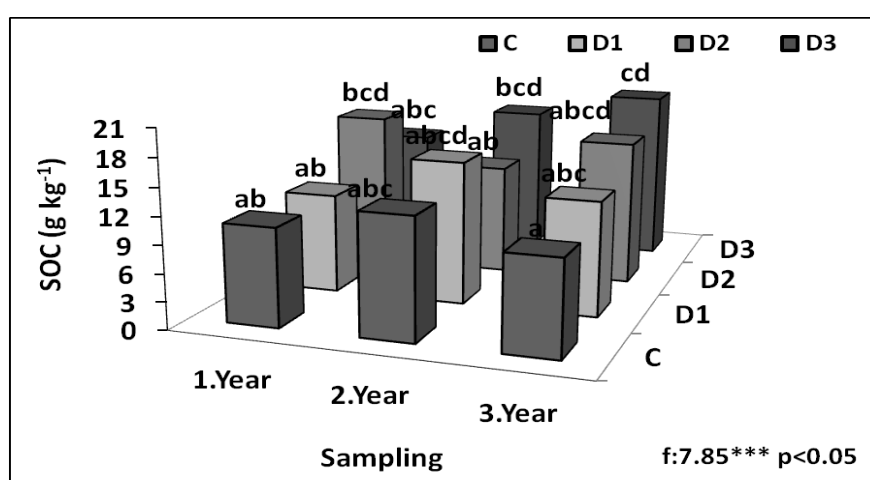


Figure 5.2. Soil organic carbon evolution in the surface horizon.

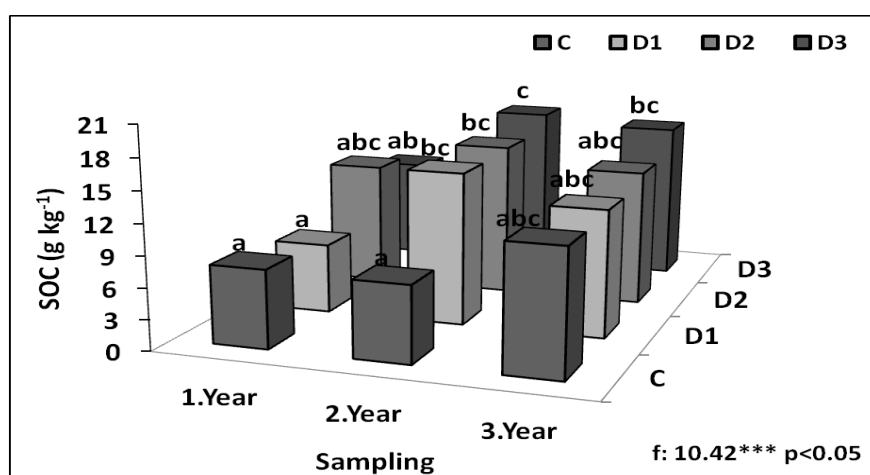


Figure 5.3. Soil organic carbon evolution in the subsurface horizon

Soil organic carbon was increased with the application PS, especially in the surface horizon. Organic carbon contents in the plants and applied pig slurry were given in Table 5.2. The results showed that; organic carbon contents of plants were directly increased with D3 application, in comparison with control. Organic carbon contents of pig slurry in the second year were higher than the first year. Therefore, they were increased with D1 and D2 application especially after second year application.

Table 5.2. Organic carbon contents (%) in the plant and applied pig slurry.

Samples	Treatment	1.Year	2.Year	3.Year
		% C		
Plant	C	23.54 ± 0.07	19.77 ± 0.06	14.72 ± 0.01
	D1	18.10 ± 0.05	17.69 ± 0.04	25.05 ± 0.06
	D2	30.92 ± 0.08	24.24 ± 0.01	28.12 ± 0.08
	D3	25.90 ± 0.06	26.89 ± 0.08	30.16 ± 0.09
Pig Slurry		NA	28.31 ± 0.07	34.76 ± 0.05

NA: No application in this year (n:3).

Effects of manure addition on increasing soil organic matter content would be more pronounced on soils of lower organic matter content and low fertility (Loro et al., 1997). Many researchers have observed such as our study increases in soil organic matter/carbon following repeated applications of manure (Campbell et al., 1997; Sommerfeldt et al., 1988; Larney et al., 2000).

Our results showed that; soil organic carbon content significant effected by the cumulative application of slurry over a 3 year period. It has been demonstrated that the annual addition of organic amendments, improved fertility of degraded soils of Mediterranean climate zones, due to positive effects on soil properties (Garcia et al., 2000). Isotopic composition of PS should be discussed before the isotopic composition of soil organic carbon.

5.3.3. Isotopic Composition of Soil Organic Carbon

Distributions $\delta^{13}\text{C}$ contents in the surface and subsurface soil were given in Table 5.3. The $\delta^{13}\text{C}$ values after 3 years, D2 were depleted. The $\delta^{13}\text{C}$ valued more depleted in D3 than D1, D2 and C in the subsurface horizon. The $\delta^{13}\text{C}$ value of the surface and subsurface soil ranged from -23.63‰ to -25.44‰, and -23.30‰ to -25.10‰ during the experiment, respectively.

Wang and Anderson (1998) have reported the average $\delta^{13}\text{C}$ value of the soil organic matter as -25.5‰ for the whole profile. An analysis of $\delta^{13}\text{C}$ value of the soil organic matter of the Amulet transect indicates that a lower $\delta^{13}\text{C}$ (-27.5‰) occurs in the surface horizon in the slough area, and becomes slightly enriched (-25.5‰) downward to the depth of 75 cm. (Nadelhoffer and Fry, 1988; Gregorich et al., 1995).

The C enrichment with depth is thought to be the result of overall discrimination against ^{13}C during organic matter decomposition or due to the preferential preservation of litter components or organic matter which is enriched in ^{13}C (Nadelhoffer and Fry, 1988). However, in the upper slope soil, the $\delta^{13}\text{C}$ value of soil organic matter is enriched (-22.5‰) in the surface horizon, and become more negative (-25.5‰) at 75 cm. The lower $\delta^{13}\text{C}$ values for the upper slope soils appear to be a likely consequence of a recent colonization of this site by blue grama grass, with the change to C plants showing up mainly in the surface horizons where organic additions are greater (Desjardins et al., 1994).

The $\delta^{13}\text{C}$ values of soil CO_2 for the Black soil should be about -21 to -22‰ based on the $\delta^{13}\text{C}$ value of organic matter which varies from -25‰ to -26‰, whereas, the CO_2 in the Dark Brown soil should have a $\delta^{13}\text{C}$ value of about -18.4‰, based on the average $\delta^{13}\text{C}$ value of -22.8‰ for the organic matter (Wang and Anderson., 1998).

Table 5.3. $\delta^{13}\text{C}$ contents in the surface soil, subsurface soil, and plant samples.

Treatment	1.Year	2.Year	3.Year	1.Year	2.Year	3.Year	1.Year	2.Year	3.Year
	$\delta^{13}\text{C}$								
	Surface (0-20 cm.)			Subsurface (20-40 cm.)			Plant		
C	-23.63	-24.32	-24.12	-24.35	-23.30	-23.71	-23.88	-23.85	-25.07
	-23.60	-24.31	-24.10	-24.36	-23.33	-23.71	-23.89	-23.86	-25.09
	-23.65	-24.32	-24.13	-24.35	-23.28	-23.70	-23.88	-23.85	-25.05
MA	-23.63	-24.32	-24.12	-24.35	-23.30	-23.71	-23.88	-23.85	-25.07
D1	-23.61	-24.38	-24.69	-24.23	-24.19	-24.26	-23.73	-25.55	-23.85
	-24.24	-24.47	-24.23	-23.66	-23.87	-23.66	-24.03	-24.94	-23.74
	-24.60	-24.71	-25.42	-23.38	-23.70	-24.61	-24.09	-24.30	-24.87
MA	-24.15	-24.52	-24.78	-23.75	-23.92	-24.17	-23.95	-24.93	-24.15
D2	-25.13	-25.18	-25.44	-23.76	-23.45	-24.80	-24.19	-23.64	-23.90
	-25.10	-25.19	-25.42	-23.76	-23.47	-24.80	-24.18	-23.64	-23.91
	-25.15	-25.18	-25.45	-23.77	-23.44	-24.81	-24.19	-23.65	-23.90
MA	-25.13	-25.18	-25.44	-23.76	-23.45	-24.80	-24.19	-23.64	-23.90
D3	-24.11	-25.21	-25.44	-23.82	-23.38	-25.10	-23.64	-23.72	-23.84
	-24.11	-25.20	-25.46	-23.81	-23.39	-25.10	-23.66	-23.69	-23.82
	-24.10	-25.21	-25.42	-23.82	-23.37	-25.11	-23.63	-23.75	-23.86
MA	-24.11	-25.21	-25.44	-23.82	-23.38	-25.10	-23.64	-23.72	-23.84

MA: Mean average of each treatment (n:3).

In a field experiment which was frequently cultivated with C3 crops that are ^{13}C depleted, resulting in an enriched $\delta^{13}\text{C}$ values of SOC, -22.68‰ to -19.21‰ from the surface to the subsurface horizons (Cheng et al., 2007). $\delta^{13}\text{C}$ values were affected by the PS application, which was observed in the third year. Slight enrichment of ^{13}C with depth corresponding to an increase in decomposition and humification has been observed in our study.

5.3.4. Isotopic Composition of Applied Pig Slurries and Plants

The $\delta^{13}\text{C}$ contents in the applied pig slurries and barley plants were given in Table 5.4. The results showed that $\delta^{13}\text{C}$ contents were higher in the second year of application. However, $\delta^{13}\text{C}$ contents were decreased with PS application in the plant samples. D2 and D3 are more negative than D1 and C plots. The $\delta^{13}\text{C}$ value of plants ranged from -23.64‰ to -25.07‰ during the experiment.

Table 5.4. $\delta^{13}\text{C}$ contents in the applied pig slurry samples.

	Applied Pig Slurry		
	1.Year	2.Year	3.Year
$\delta^{13}\text{C}$	NA.	-23.61	-25.05
		-23.60	-25.06
		-23.61	-25.04
MA	NA.	-23.61	-25.05

MA: Mean average of each treatment, NA: No application in this year (n:3).

Barley (C3) plants are slower to take in ^{13}C , so their total biomass ranges from -22‰ to -35‰, with a mean value of -26.5‰ (Anonymous, 2012). The application of carbon isotopes is based on the different ^{13}C composition of C3 plants and its preservation in SOM. $\delta^{13}\text{C}$ values of C3 plants range from approximately -32‰ to -

20‰ , with a mean of -27‰. C3 plants have distinct $\delta^{13}\text{C}$ ranges from -23‰ to -34‰ (Ogrinc et al., 2005).

Where vegetation has been compositionally stable, the $\delta^{13}\text{C}$ value of soil organic carbon in the upper soil profile (0–20 cm.) is similar to that of the plant community (Balesdent et al., 1993). This concordance between the isotopic composition of the soil organic matter in the upper profile and that of the plant community occurs because isotope fractionation is negligible during the early stages of organic matter decomposition in well-drained mineral soils (Balesdent and Mariotti, 1996; Boutton, 1996). However, older organic matter located deeper in the soil profile (below 20 cm.) has $\delta^{13}\text{C}$ values that are generally 1–3‰ greater than those for organic carbon in the upper profile (Balesdent et al., 1993).

Changes in quantities of organic carbon $\delta^{13}\text{C}$ values with depth are likely to reflect changes in vegetation over time. The $\delta^{13}\text{C}$ of pig slurry would indicate that increased plant production was also evident in our study. Other studies, for example, Huggins et al. (1998), and Collins et al. (1999) observed similar shifts in the $\delta^{13}\text{C}$ signature of soil C in the C3 system.

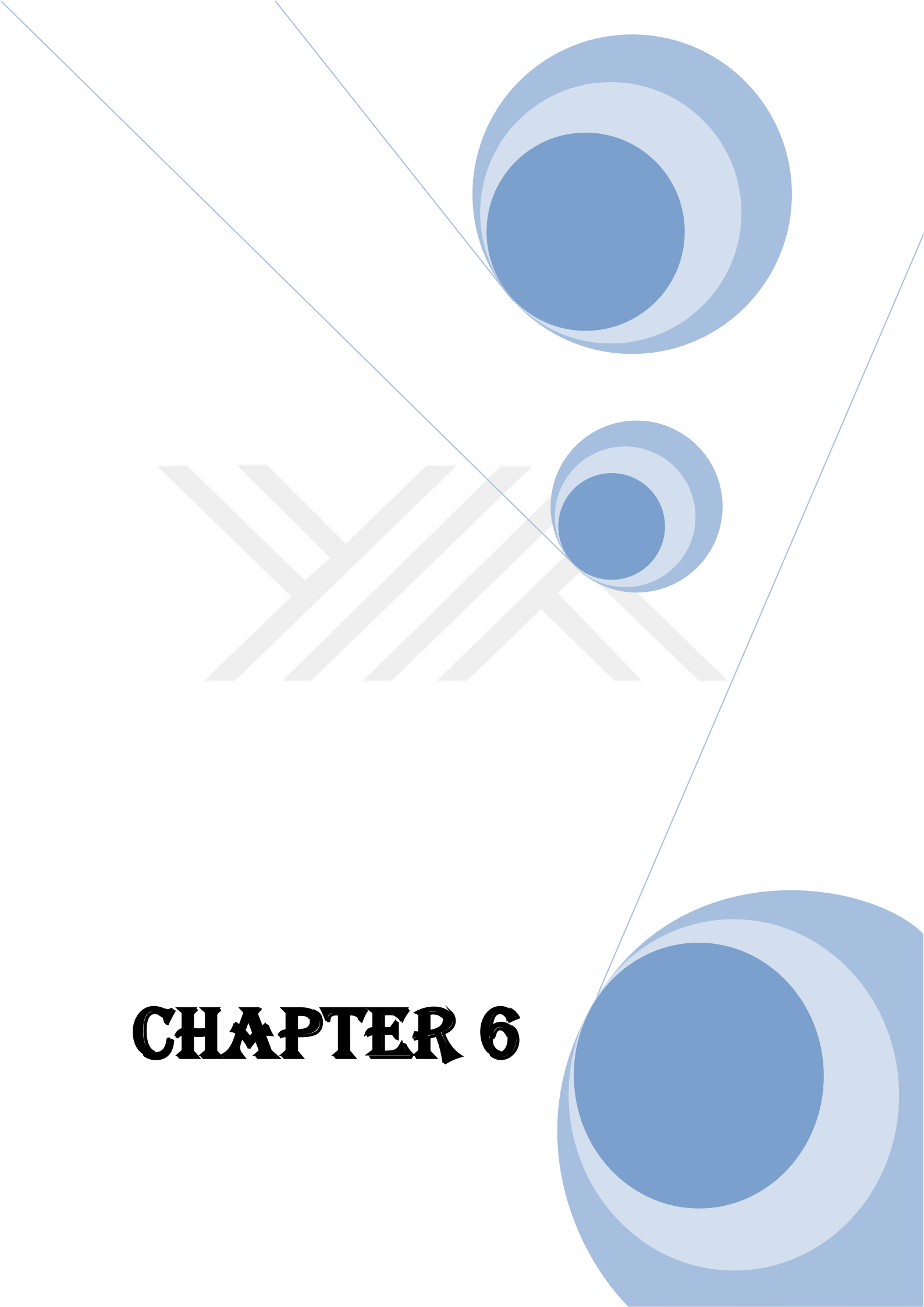
5.4. CONCLUSIONS

Isotopic measurements of soil organic C have been utilized to document the effects of land use practices on ecosystems, to quantify rates and patterns of vegetation dynamics in natural ecosystem, and to refine our understanding of vegetation-climate interactions. The C cycle of the soil is not only an important part of the global C cycle;

also it is a critical part of ecosystem energy and nutrient cycles. C isotopes provide a useful tool that can help us understand the dynamics of the soil C cycle.

Pig slurry applications increased the soil organic carbon in the terrestrial system, however D₃ applications has high micronutrient contents, therefore the benefits from the use of application depend on management of pig slurry, carbon and environmental factors. The application of manure directly influenced the SOC pools, plant productivity, and SOC turnover compared to control and treatments. D₁ application to silty loam soils has positive effect on carbon to the atmosphere and ground water in the Mediterranean ecosystem. This dose seems to be very suitable in term of sustainable agriculture and also can improve physical, chemical and biological soil properties. However, the application of $\delta^{13}\text{C}$ techniques showed that the soil exhibits a distinct shift in its isotopic signature, which is directly related to the type of residue input. There are distinct $\delta^{13}\text{C}$ values of organic C: (i) soils were considerably enriched in $\delta^{13}\text{C}$ values with D₁ application; (ii) $\delta^{13}\text{C}$ values were higher in second year application in the soil and plant samples; (iii) $\delta^{13}\text{C}$ values of organic C generally became enriched with depth.

Our stable isotope data have shown that the source of C in PS and plants is derived from the soil organic matter. There is a high correlation between carbon isotopic values of soil and pig slurry. This means that stable isotope composition of soil and plant are affected by pig slurry application, it is recommended that further silty loam soil studies should implement various management practices such as the type of cultivation, the rate of addition of manure and crop residues. Knowledge of these factors will promote our understanding of the dynamics of the SOC pools, their turnover rates, residue stabilization and the potential role of these systems to sequester C in the soil and promote long-term nutrient storage.

The background features a light grey background with several thin, light blue diagonal lines. Three concentric blue circles are positioned in the upper right and lower right areas. A series of parallel grey lines, resembling a stylized 'X' or a series of intersecting lines, are located in the center of the page.

CHAPTER 6

CHAPTER 6

IMPROVEMENT OF ^{13}C CP/MAS-NMR SPECTRA OF ORGANIC MATTER BY REMOVAL SAND CONTENT, SE SPAIN.

Abstract: Nuclear magnetic resonance (NMR) is a valuable tool for the characterization of organic matter and decomposition processes in soils. One of the advantages of NMR spectroscopy is that it can be used as non-invasive method for solid soil samples or any added organic sources. Artificial additives, such as pig slurry can also be detected in soils or individual soil fractions. Under semiarid climate and light textured. Low organic contents of soil effects quality of spectra and quantitative interpretation of such spectra becomes difficult and sometimes impossible. The aim of this chapter is to compare the effects of soil sand on ^{13}C CP-MAS NMR spectra of soil organic matter in Murcia region, South-East Spain.

The result showed that Alkyl, Metoxyl, diO-alkyl and phenolic were not changed significantly. But Aromatics and Carboxyl group in functional groups of organic matter were improved by the removal of sand fraction.

Keywords: Sand removal, NMR, Carbon, Quality

6.1. INTRODUCTION

Soil organic matter (SOM) influences the physical, chemical, and biological properties of soil, and it also acts as a source and reservoir of nutrients for bioata (Lal, 2000). SOM is composed of partially decayed plant residues and microorganisms, as well as products of OM decomposition and transformation that are no longer recognizable as parent material, i.e. humus (Paul and Clarck, 1996).

The composition of the organic substances in SOM and its precursors is very complex. Plant litter materials provide the primary resources for OM formation in soil (Kögel-Knabner, 2002; Gleixner et al., 2002). The amount of plant litter, its composition and its properties are essential controlling factors for the formation of SOM and humification processes in terrestrial ecosystems. The microbial biomass also represents a significant compartment of the terrestrial biomass and microbial residues in soil are an important parent material for humus formation (Haider, 1992). The major organic materials of plants are easily degradable and thus provide carbon and energy sources for microorganisms. Important components of maize leaves are carbohydrates, lignin monomers, n-fatty acids, n-alkanes, and sterols (Gregorich et al., 1996). Fungi consist mainly of homo- and herteropolysaccharides, lipids and melanin are quantitatively minor components of fungal cell walls. Bacterial cell walls are composed of a peptidoglucan (murein), which contains carbohydrate as well as amino acid elements, additionally bacteria produce structural components as lipopolysaccharides and aliphatic biomacromolecules which might serve as precursors for aliphatic components of the soil organic matter (Kögel-Knabner, 2002).

Fisher and Binkley (2000) reported that soil organic matter (SOM) constitutes a small fraction of forest soil (1-12%); however, forest quality change is strongly related to the changes in SOM content. Changes in SOM quality can be assessed non-destructively on whole soil using ^{13}C cross-polar-ization/magic angle spinning nuclear magnetic resonance (CP/MAS-NMR) spectroscopy (Preston, 1996). This technique has been used for about 25 years to study SOM composition.

Modern analytical techniques to characterize the chemical composition of SOC and its precursors include, among others, solid-state ^{13}C nuclear magnetic resonance (NMR) spectroscopy (Kögel-Knabner, 2002), direct pyrolysis-mass spectrometry (Py-MS; Schulten, 1996), Curie-point pyrolysis coupled on-line with a gas chromatography/mass spectrometry (Py-GC/MS) and GC-C-IRMS (gas chromatography-combustion-isotope ratio mass spectrometry), Fourier-transform infrared spectroscopy (FTIR), and UV/VIS (Schulten, 1996).

Several reviews have been published on ^{13}C CP/MAS-NMR of soil (Preston, 1996; Skjemstad et al., 1997; Mathers et al., 2000). Individual studies include, effects of fire on the change of molecular composition of soil organic C (Skjemstad et al., 1996, 1999; Golchin et al., 1997a,b; Schmidt et al., 1999; Guinto et al., 1999; Smernik et al., 2000; Czimczik et al., 2003) or effects of vegetation, parent material and different residue managements on SOM composition (Mathers and Xu, 2003; Faz et al., 2002), have used ^{13}C -NMR techniques. But nobody has been studied improve techniques of this method. The aim of this chapter is to evaluate differences of with/without sand soil samples to improve peak quality of ^{13}C -NMR techniques in two different soil characteristics.

6.2. MATERIAL AND METHODS

6.2.1. Study Area and Soil Sampling

The study area is located Lorca from agricultural fields in the Guadalentín Valley of Murcia province, SE Spain ($37^{\circ} 55' 27,87''$ N $-1^{\circ} 49' 37,84''$ W), which is 18 m. with 3,4 % slope. Climate is semiarid, with mean annual average rainfall of 300 mm and mean annual temperature of 18°C . and potential evapotranspiration is $>900\text{ mm yr}^{-1}$ (López-Bermúdez et al., 2002).

Under semiarid climate conditions, most agriculture in the region is rain-fed and generally cropping pattern is cereal. Therefore, study areas crops were identified cereal. Soil serie is Typic Haplocalcid according to Soil Survey Staff (2010) and Faz et al (2005). The lithology is varied, with carbonate materials predominating (hard limestones, calcareous conglomerates, calco-schists, marly limestones, and limestone-dolomites), and more rarely sandstones, marls, schists, slates, and gypsums.

Study area was consisted 4 horizons. Totally seven soils samples are evaluated. Soil samples were taken from the soil profiles. The collected soil samples were taken to the lab, air-dried for 7 days, passed through a 2-mm sieved, ground, homogenized, and stored in plastic bags at room temperature prior to laboratory analyses.

6.2.2. Laboratory Analysis

The soil sample analyses of pH, electrical conductivity (EC), total nitrogen (TN), phosphorus (P), cation exchangeable capacity (CEC), lime contents (CaCO_3) and texture of this study were determined as explained in previous chapter. Soil organic

carbon was analyzed using the dichromate oxidation technique. Method of Anne (1945), described by Duchaufour (1970), based on oxidation with H_2SO_4 and $\text{K}_2\text{Cr}_2\text{O}_7$, measured with Automatic Titrater Metrohm 702 SM Titrino with Salt Mohr. Soil samples for solid-state ^{13}C /MAS-NMR measurements were washed, and *sand* was separated from clay and silt with a 50. μm sieve. Samples were dried at oven in 40 $^\circ\text{C}$.

Dry soils were packed into cylindrical zirconia rotors (7 mm outside diameter, 5.6 mm internal diameter, and 17 mm height) and sealed with Kel-F caps, detailing the relevant Varian Unity 300. Solid-state ^{13}C /MAS-NMR measurements were performed at magic angle spinning (MAS) rates of 4 kHz, employing a Varian Unity 300, operating at 75 420 MHz for ^{13}C spectra acquired using 1.5 ms contact time, 4 s recycle time, 90 $^\circ\text{C}$ pulse of 6.7 μs , spectral width of 50 000 Hz and 60 000 scans, and processed using 100 Hz line-broasening and base line correction.

^{13}C /MAS-NMR spectra (background) were obtained using an empty rotor with and without different types of end-caps (Kel-F and boron nitrate) and also with no rotor. Spectra were obtained under the same conditions as the soil samples. All sample spectra were corrected by substacting the background before integration.

The ^{13}C -NMR spectra were divided into the 7 chemical shifts regions: alkyl-C (0-50 ppm); Methoxyl (50-60 ppm); O-alkyl C (60-98 ppm); di-O-Alkyl (98-112 ppm); Aromatic C (112-145 ppm); Phenolic C (145-163 ppm) and Carbonyl C (163-190 ppm). Areas of the chemical shift regions were measured by integration and were described as percentage of the total area (relative intensity). Sidebands contribution generated by the carbonyl region was corrected by multiplying the area under the peak to the low field sideband (215-230 ppm) by a factor of 2 and adding it to the carboxyl area (163-190

ppm). An area equivalent to the low field sideband was deducted from the 112-145 ppm area to correct for the high field sideband contribution in this spectra range (Faz et al., 2002).

Hatcher et al. (1982) reported aromaticity index indicating that the alkyl-C spectral region increases with decreases in the O-alkyl spectral region (Eq. (1)). Aromaticity index has been used to characterize the extent of humification of SOM during this process (Dai et al., 2001).

$$\text{Aromaticity} = [(Aromatic\ C\ (100-160\ ppm)) / (Aromatic\ C + Alkyl\ C + O-alkyl\ C\ (0-160\ ppm))] \times 100\ (1)$$

6.3. RESULTS AND DISCUSSION

6.3.1. Soil Characteristics of the Study Area

The characteristic of soil is given in previous chapters. Studied area had basic soil reaction (Porta al, 1999), no salty, low contents of total carbon and nitrogen (Villalbí and Vidal, 1988), high contents of phosphorus (Dressing, 1993), low cation exchangeable capacity (Porta, 1999), silty loam texture (FAO-UNESCO-ISRIC, 2010).

Soil results showed that, total organic carbon content were similar with Mediterranean soils under arid-semiarid climate, which were less than 20 g kg⁻¹ (Stevensen, 1994). Silty loam textures were predominant in the area studied (FAO-UNESCO-ISRIC). Silt contents were changed between 40 and 50%. For details see Chapter 3. As we know study area was applied pig manure for many years. The decomposition rates of organic manure are generally slow in the clay and rapid in

sandy soil (Pinamonti and Siche, 2001). Because of these reasons, texture is very important factor in terms of application organic residues.

6.3.2. ^{13}C -NMR spectra results of soil and compare with removal of sand

This improvement is important to increase the quantity NMR spectrum in the soil, sand fraction do have a limited affinity organic particles, due to its low surface area.

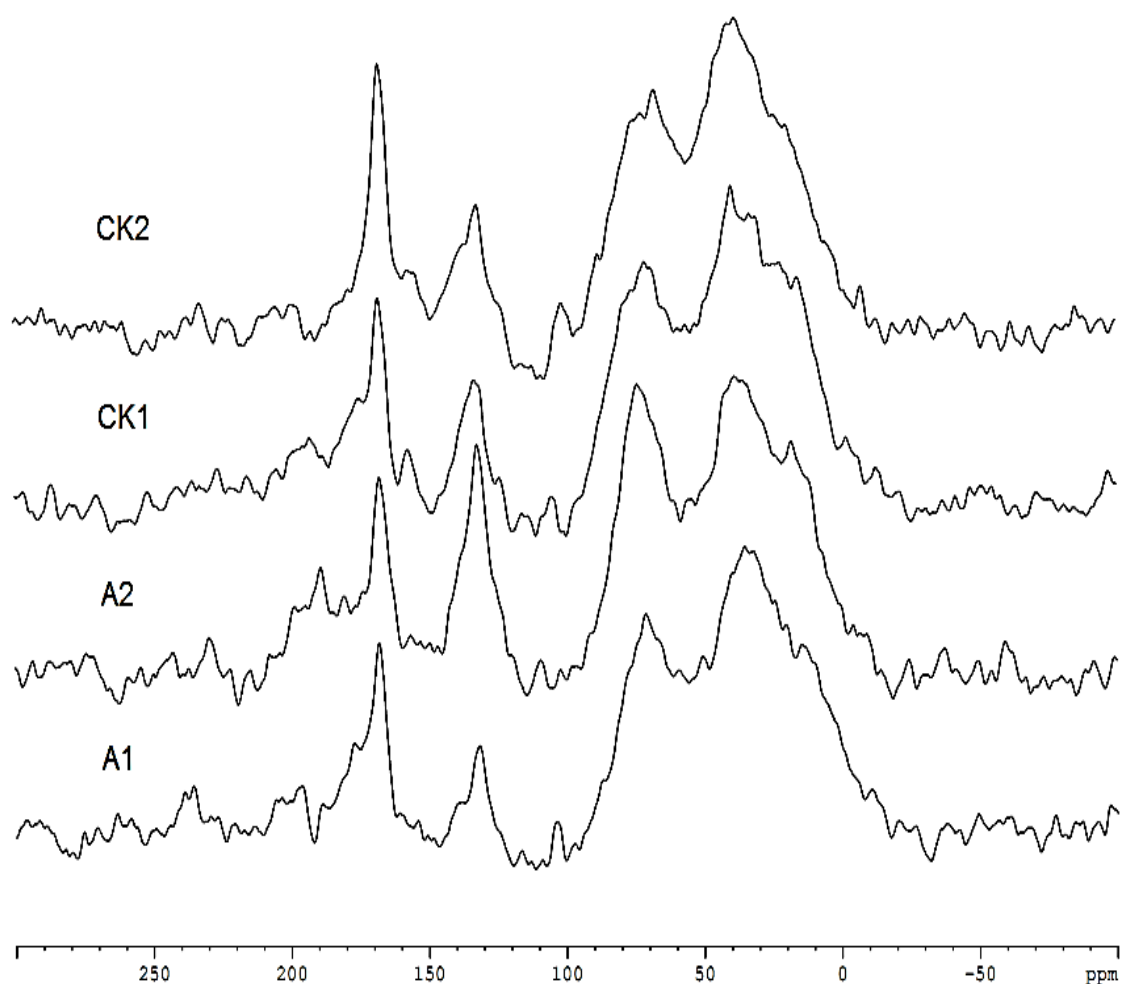


Figure 6.1. Carbon 13-CPMAS-NMR spectra of soils sampled from the area studied.

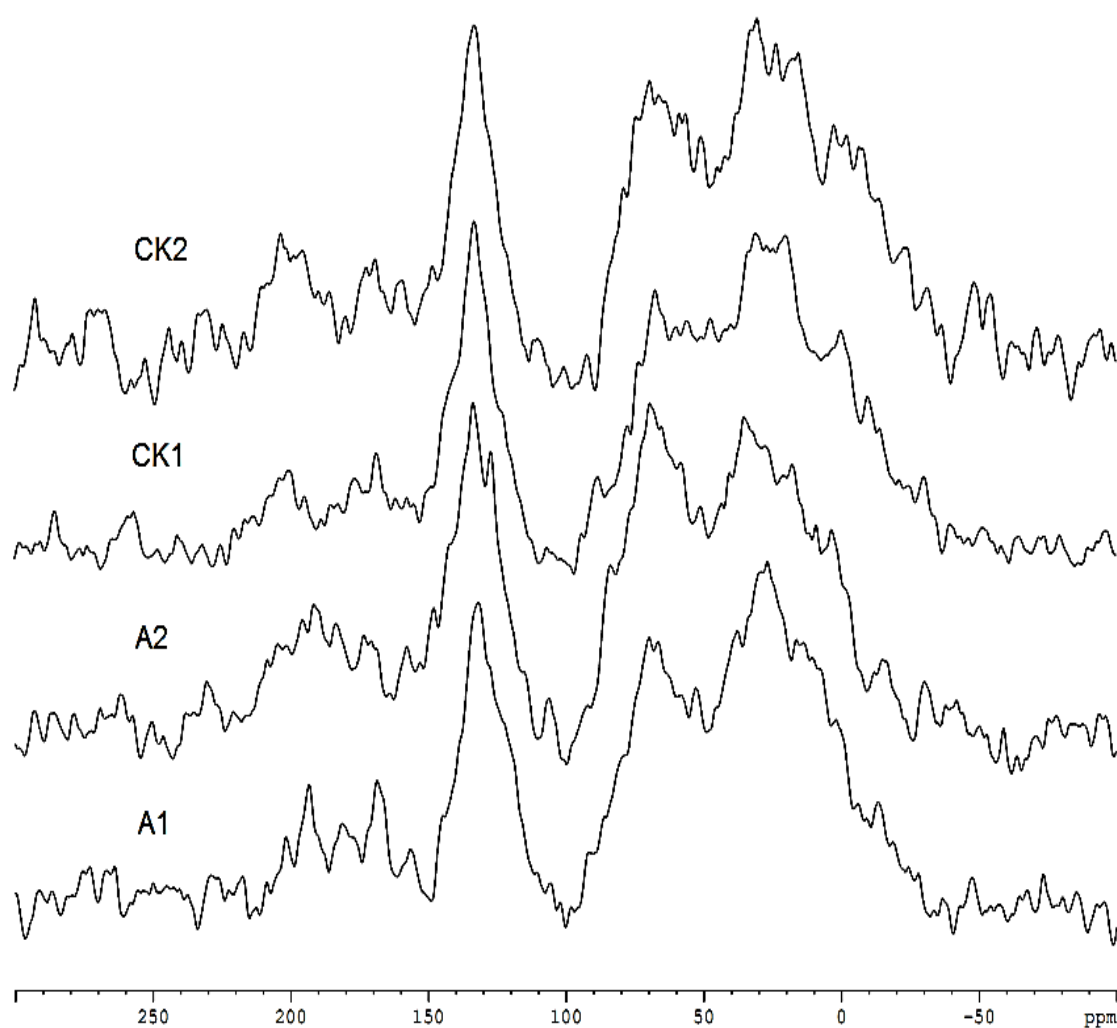


Figure 6.2. Carbon 13-CPMAS-NMR spectra of soils sampled from study area after removal of sand.

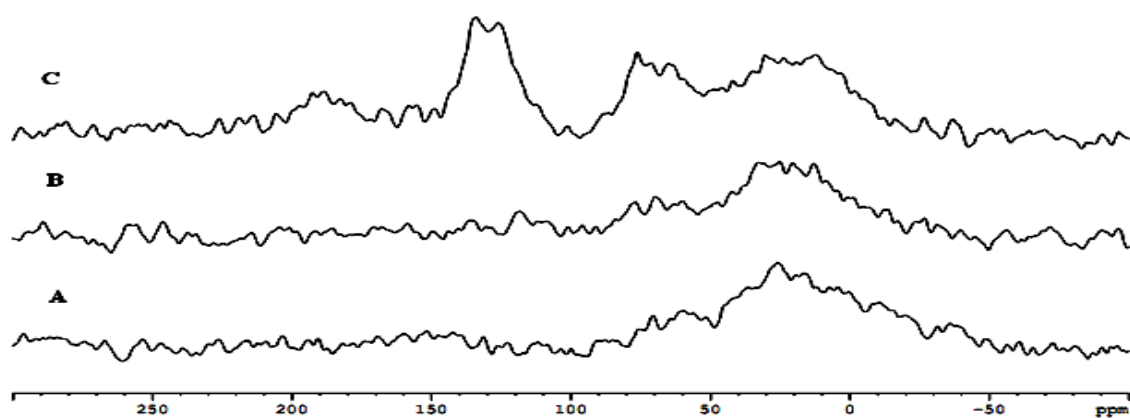


Figure 6.3. Carbon 13-CPMAS-NMR spectra of background **A**- No Rotor or Cap, **B**- Empty Rotor No Caps, **C**- Empty Rotor Kel-F Cap.

As shown in Figure 6.1, 6.2 and 6.3, there is a significantly differences between samples before and after the removal of sand in study area. Alkyl, Metoxyl, diO-alkyl and phenolic were not changed significantly. But Aromatic, Aromatics and Carboxyl group were improved by the removal sand method in soil used in the study.

The distribution of functional groups were in the following order Alkyl>O-alkyl>Aromatics>Aromatic>Carboxyl>Metoxyl>Phenolic>di-O-alkyl. The chemical shift assignments of the ^{13}C -NMR spectra and their relative abundance are presented in Table 6.1. The ^{13}C -NMR spectra in study area with and removal sand samples, and background were given in Figure 6.1, 6.2 and 6.3, respectively. Aromaticity indexes of sites studied varied from 1.44% to 2.22% in samples with the removal of sand fraction (Table 6.1). The most noticeable functional groups were at 100–150 ppm region i.e. di-O-alkyl, Aromatic and Phenolic. Another important difference was observed in carbohydrate C (50-112 ppm) content. Soils had also highest carbohydrate content (32%) compared to after the removal sand(27%).

A high amount of O-alkyl-C would indicate the soil C with a high potential for decomposition. Spectra for undecomposed plant litter gives ^{13}C -NMR spectra high O-alkyl-C signals, representing mainly plant polysaccharides, and comparatively small alkyl-C resonances (Almendros et al., 2000; Knicker and Lüdemann, 1995). Preston et al. (2000) analyzed 36 different forest litters and interpreted them using ^{13}C CP/MAS-NMR spectroscopy. Spectra of tree litters showed similar structures but in varying proportions. All litters had higher O-Alkyl C than Alkyl-C.

Table 6.1. Composition of organic matter (functional groups) with and without sand soil samples as determined by solid-state ^{13}C CP/MAS-NMR spectroscopy.

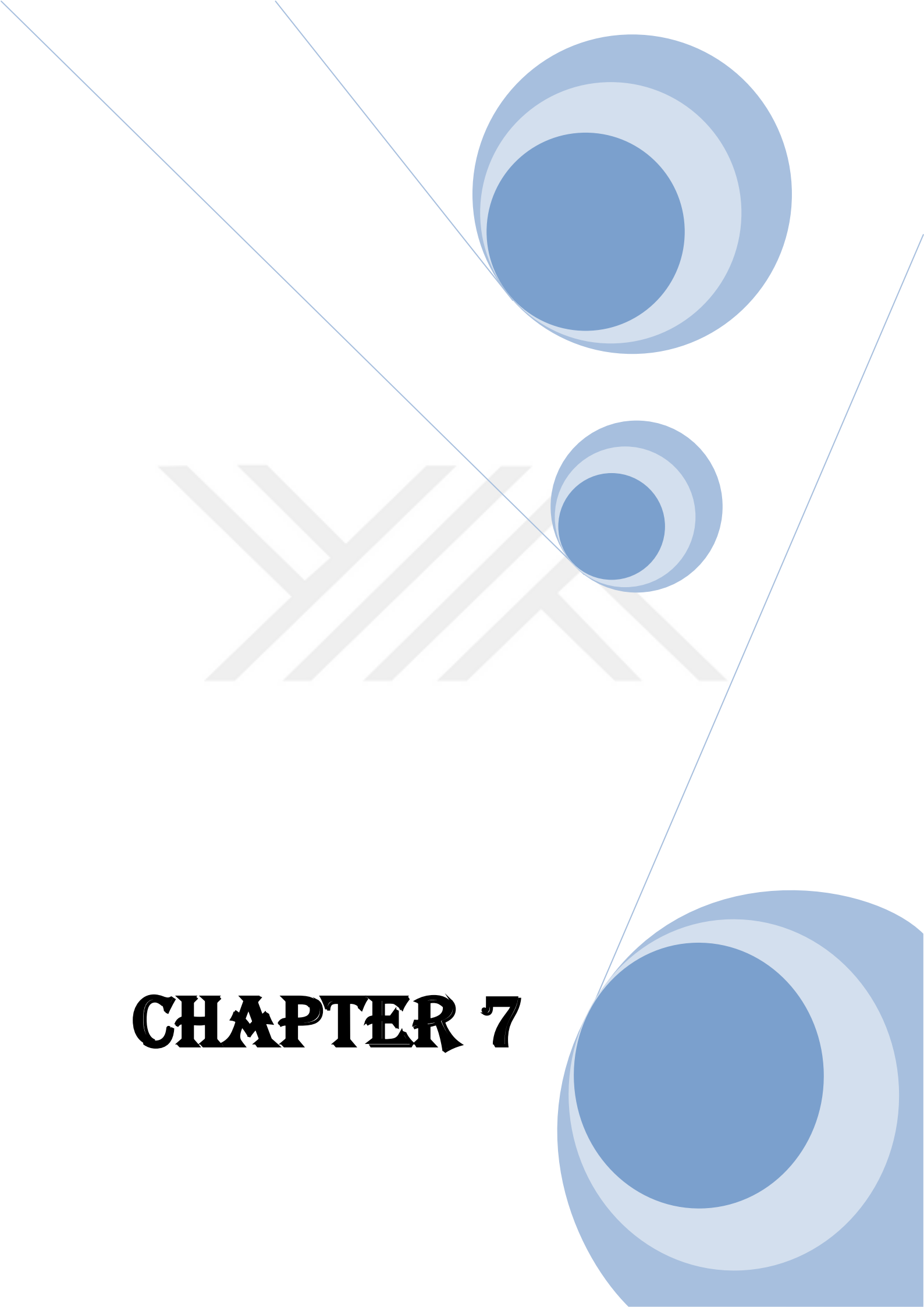
Sand	Horizon	Chemical shift range (ppm)									
		0-50	50-60	60-98	98-112	50-112	112-145	145-163	112-163	163-190	
		Alkyl	Methoxyl	O-alkyl	di-O-alkyl	Carbohydrate	Aromatic	Phenolic	Aromatics	Carboxyl	A/O-A
Relatively intensity of C region (%)											
Wi	A1	46.23	7.60	22.24	1.35	31.20	6.93	3.18	10.12	12.46	2.08
	A2	39.92	6.65	23.75	1.55	31.95	12.58	4.00	16.58	11.55	1.68
	CK1	44.05	7.42	23.85	0.87	32.14	8.20	3.36	11.56	12.24	1.85
	CK2	41.46	7.44	23.12	1.57	32.13	9.29	5.38	14.66	11.75	1.79
Wo	A1	42.39	6.89	19.49	1.20	27.58	19.61	2.94	22.55	7.48	2.17
	A2	36.78	7.18	21.65	1.17	29.99	19.59	5.44	25.03	8.20	1.70
	CK1	43.21	7.73	17.36	0.73	25.82	19.91	4.38	24.29	6.68	2.49
	CK2	41.33	7.37	18.58	1.14	27.10	19.18	5.16	24.34	7.23	2.22

Wi: Samples with sand, **Wo:** Samples without sand.

Alkyl carbon is expected to increase when stage of decomposition increases in the soil (Preston, 1996). Faz et al., (2002) reported that influences of soil order, parent material, vegetation, and climatic characteristics on the abundance of C functional groups of SOM were distinct.

6.4. CONCLUSIONS

There were no significant differences between soil samples with and without sand content. Alkyl, Methoxyl, diO-alkyl and phenolic were not changed in the studied area. But Aromatic, Aromatics and Carboxyl group in functional groups of organic matter were improved by removal sand method. Consequently the peaks of NMR spectra became more pronounced with removal of sand from the soil in Aromatic, Phenolic, Aromatics and Carboxyl groups of soil organic matter in the study area. This improvement method was a very important to determine very clear peaks of NMR spectra in terms of carbon quality.

The background features a light grey 'X' shape formed by two intersecting diagonal lines. Three blue circles of varying sizes are positioned at the top, middle, and bottom right. Each circle is composed of three concentric layers: a dark blue center, a medium blue middle ring, and a light blue outer ring. The circles are partially cut off by the edges of the page.

CHAPTER 7

CHAPTER 7

SUMMARY AND CONCLUSIONS

Understanding of the global carbon cycle is fundamental to evaluate the storage and fluxes between the atmosphere, the biosphere, and the pedosphere. Soils contain the largest “active” terrestrial carbon pool on Earth, and through soil respiration, annual flux of CO₂ to the atmosphere. Changes in land use and agricultural activities during the past 200 years have made soils act as net sources of atmospheric CO₂. Experimental studies suggest that carbon losses due to oxidation and erosion can be reversed with soil management practices that can minimize the soil disturbance and increase plant yield, through commercial and organic fertilization. Improved land management can result in a significant amount of increasing rate of carbon sequestration. Application of pig slurry (PS) may increase soil fertility and thus enhance the crop production potential.

The objectives of this thesis are to:

- determine the effects of different doses of manure applications on soil C cycle,
- monitor the changes in soil properties, soil-water-plant (cereal) system with manure application,

- identify sustainable amount of manure to mix with the soil system to minimize the possibility of environmental pollution in Guadalentin Valley,
- characterize physical-chemical properties of leachates after the application of pig slurry and determine carbon balance in undisturbed soil columns,
- monitor quantitative and qualitative carbon in the soil system,
- determine functional group of soil organic matter using ^{13}C NMR spectra.

Large amount of organic residues, including pig slurry (PS), are added to agricultural lands every year which have significant effects on the C flux in the soil. Three different doses of PS on a silty loam soil were applied: Single (D_1), Double (D_2), Triple (D_3) and unfertilized plots (C) served as controls. Additions of pig slurry to soils at agronomic rates (D_1) to match crop nutrient requirements showed that the soil quality improved with the applications of PS, depending on the soil characteristics in the semiarid regions. Dose 1 (D_1) is the agronomic rate of N-requirement (170 kg N/ha/yr) (European Directive 91/676/CEE) and appears to be very appropriate in term of sustainable crop production. The yield decrease in D_2 and D_3 in comparison to D_1 were attributed to accumulation of salt existing in the PS applied. This appears to be a limiting factor for the amount of PS that can be applied. Soil OC was increased with increased amount of PS applied.

Total of 9 undisturbed soil columns were used in a separate study. They were amended with low dose (LD) (129.7 mL) and high dose (HD) (389.2 mL) of PS with three replications, considering the N content by Spanish legislations. Irrigation was carried out on weekly bases by simulating the monthly rainfall events of the area. Pig slurry application on the soil has been a common practice in Spain in last decades. However, there is limited information, pig slurry impact on soil and plant carbon

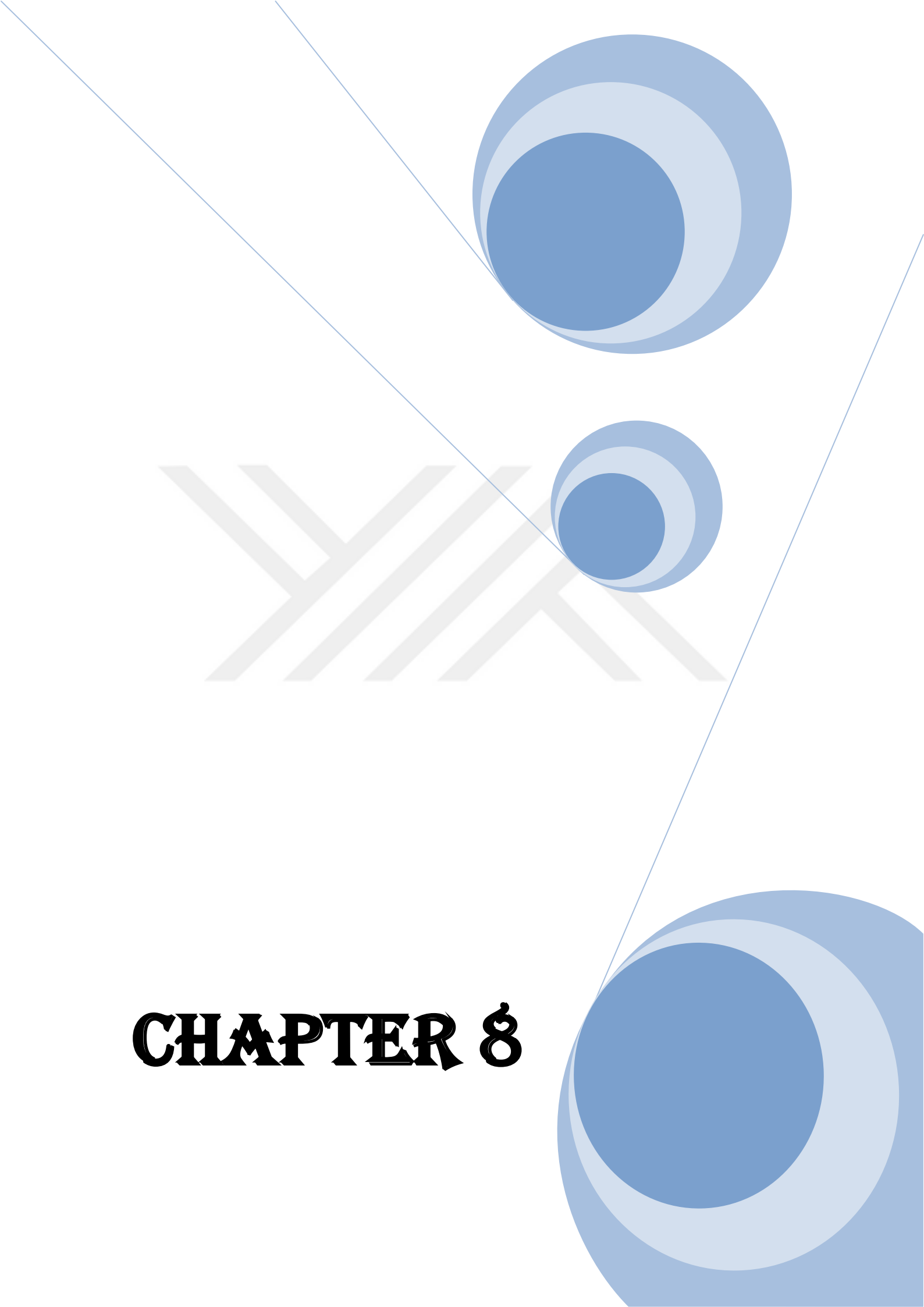
dynamics. From the column studies we found that PS applications have a greater effect on increasing organic carbon (OC) levels in the terrestrial ecosystem, but rapid oxidation do not allow significant accumulation in the soil. The topsoils (0-10 cm) have sequestered 15.84 and 24.68% (1.97 and 3.15 tonnes OC ha⁻¹) with LD and HD, respectively. Carbon leaching in the control column showed 0.68% (0.09 tonnes OC ha⁻¹) loss of OC in one year, which was insignificant. A large portion of the added OC was accumulated in the subsoil owing to the higher leaching in the medium textured soil studied. We concluded, the benefits from the use of PS application depend on the soil management practices and environmental factors. We suggested that the amount of application doses should be calculated on the basis of detailed measurement of C dynamics in the soil.

Information about the use of the natural $\delta^{13}\text{C}$ abundance technique to study the dynamics of soil organic matter turnover is rather limited. The main source of organic carbon in the soil derived from the vegetation cover and other source could be recent application organic residue disposals such as pig slurry. The isotopic ^{13}C compositions of both sources are significantly different. This may help to investigate the fate of added organic substances to the soil. We investigated the long-term effects of pig slurry on the distribution of stable carbon isotope ratio in a silty loam soil with cereal cultivation in the Southeast of the Spain, in order to elucidate the mechanism by which SOC was recycled in the silty loam soil ecosystem.

Three different doses of PS: 170 kg N ha⁻¹ yr⁻¹, 340 kg N ha⁻¹ yr⁻¹ and, 510 kg N ha⁻¹ yr⁻¹, were compared with a control in a non-irrigated Mediterranean environment. Our conclusions are: (i) soils were considerably enriched in $\delta^{13}\text{C}$ values with D1 application; (ii) $\delta^{13}\text{C}$ values were higher in second years application in the soil and

plant; (iii) $\delta^{13}\text{C}$ values of organic C was lower at depth, suggesting that the leached organic carbon was mainly added carbon. Significant correlation exists between carbon isotopic values of organic carbon and pig slurry. This means that the stable isotope composition of the soil and plant are all affected by pig slurry application

Nuclear magnetic resonance (NMR) is a valuable tool for the characterization of organic matter and decomposition processes in the soil. One of the advantages of NMR spectroscopy is that it can be used as a non-invasive method for solid soil samples or any added organic sources. Artificial additives, such as pig slurry, can also be detected in soils or individual soil fractions. Under semiarid climate condition soils are poor in organic matter contentment and light textured. Low organic matter contents of soil effects the quality of spectra and quantitative interpretation of such spectra becomes difficult and sometimes impossible. The aim of this chapter was to determine and interpret the ^{13}C CP-MAS NMR spectra of soil organic matter treated with pig slurry. The result showed that Alkyl, Metoxyl, diO-alkyl and phenolic were not changed significantly. But Aromatics and Carboxyl group in functional groups of organic matter were improved by the removal of sand fraction. This technique appeared to be very valuable in terms of determining which functional groups are more resistant to decomposition and fate of added organic constituents (amendments) to the soil.



CHAPTER 8

CHAPTER 8

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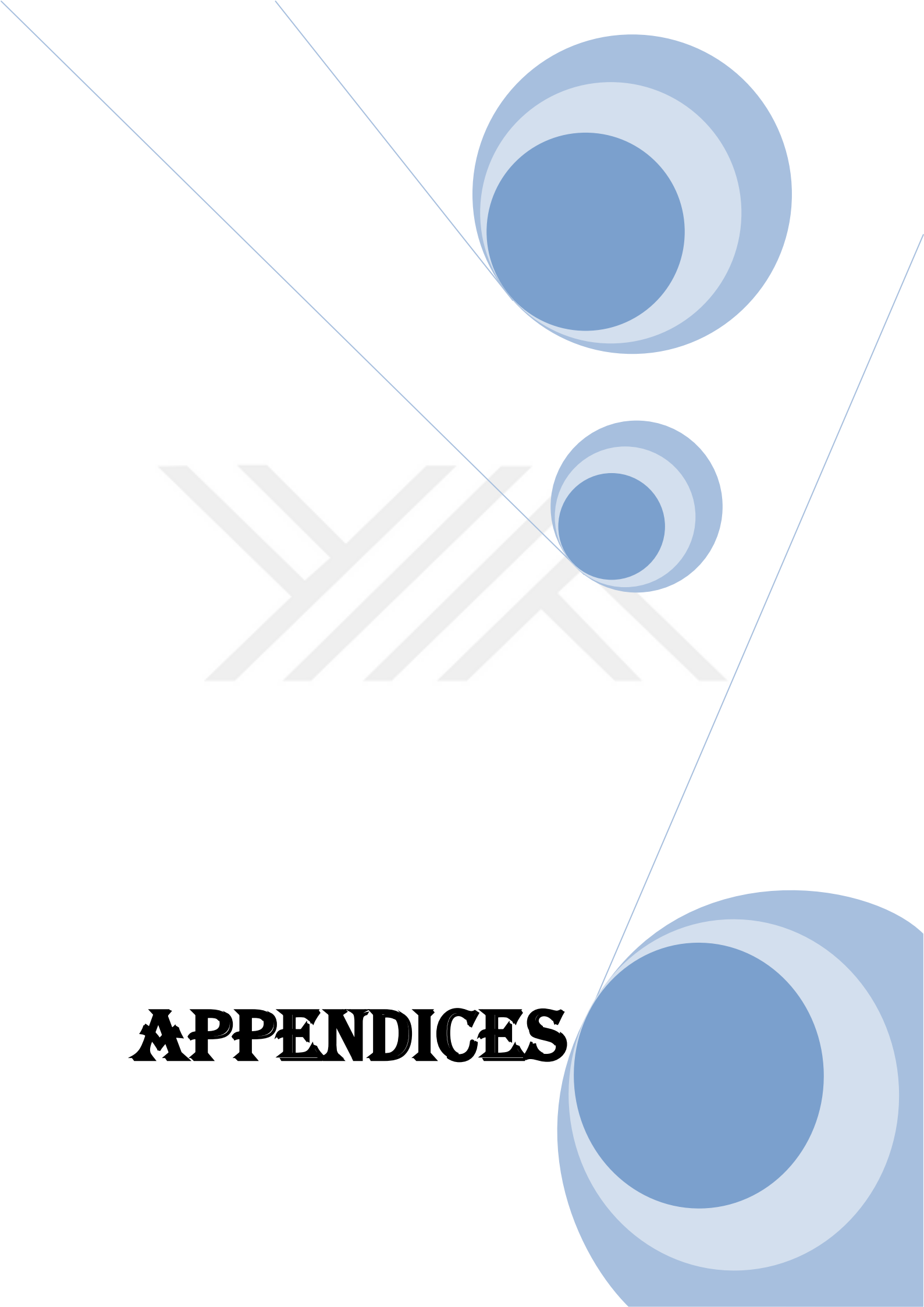
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The background features a light grey 'X' shape formed by two thin diagonal lines. Three blue circles of varying sizes are positioned at the top, middle, and bottom right. The circles are composed of concentric rings of different shades of blue. In the center, there is a faint, grey, stylized graphic that resembles a series of overlapping 'X' or 'Z' shapes.

APPENDICES

Appendix A

Physical and chemical analyses of the studied area as affected by pig slurry application



Table A1. pH values of studied area as affected by pig slurry application

Sampling	pH																	
Surface	C			D1			D1			D1			D2			D3		
1	8.39	±	0.03	8.35	±	0.08	8.41	±	0.12	8.37	±	0.46	8.02	±	0.16	8.32	±	0.11
2	8.41	±	0.22	8.17	±	0.01	8.23	±	0.34	8.24	±	0.15	8.35	±	0.10	8.35	±	0.24
3	8.59	±	0.14	8.42	±	0.06	8.52	±	0.06	8.43	±	0.17	8.41	±	0.22	8.05	±	0.25
4	8.41	±	0.25	8.55	±	0.06	8.41	±	0.10	8.51	±	0.28	8.53	±	0.15	7.95	±	0.20
5	8.41	±	0.00	7.75	±	0.06	7.81	±	0.06	8.04	±	0.13	7.43	±	0.66	8.21	±	0.00
6	8.16	±	0.00	8.02	±	0.11	8.02	±	0.07	7.91	±	0.08	7.81	±	0.07	7.86	±	0.00
7	8.29	±	0.00	7.89	±	0.07	7.92	±	0.07	7.97	±	0.08	7.62	±	0.34	8.04	±	0.00
Subsurface	C			D1			D1			D1			D2			D3		
1	8.51	±	0.18	8.55	±	0.10	8.61	±	0.10	8.49	±	0.13	8.27	±	0.26	8.50	±	0.04
2	8.58	±	0.13	8.55	±	0.31	8.42	±	0.24	8.51	±	0.15	8.69	±	0.07	8.16	±	0.05
3	8.92	±	0.05	8.61	±	0.15	8.79	±	0.10	8.97	±	0.18	8.77	±	0.26	8.11	±	0.15
4	8.76	±	0.02	8.72	±	0.06	8.68	±	0.11	8.97	±	0.14	8.95	±	0.03	8.36	±	0.26
5	8.64	±	0.00	8.08	±	0.10	8.23	±	0.10	8.56	±	0.14	8.20	±	0.14	8.09	±	0.00
6	7.95	±	0.00	8.16	±	0.04	8.23	±	0.08	8.36	±	0.11	7.78	±	0.14	7.70	±	0.00
7	8.30	±	0.00	8.12	±	0.03	8.23	±	0.04	8.46	±	0.12	7.99	±	0.04	7.90	±	0.00
pH (KCl)																		
Surface	C			D1			D1			D1			D2			D3		
1	7.44	±	0.18	7.44	±	0.01	7.44	±	0.03	7.44	±	0.10	7.44	±	0.17	7.44	±	0.05
2	7.69	±	0.04	7.59	±	0.10	7.63	±	0.05	7.67	±	0.01	7.70	±	0.03	7.76	±	0.07
3	7.55	±	0.08	7.55	±	0.06	7.59	±	0.04	7.58	±	0.03	7.67	±	0.03	7.59	±	0.28
4	7.48	±	0.05	7.43	±	0.01	7.48	±	0.05	7.54	±	0.05	7.61	±	0.03	7.57	±	0.17
5	7.61	±	0.00	7.41	±	0.04	7.45	±	0.01	7.45	±	0.10	7.50	±	0.10	7.67	±	0.00
6	7.44	±	0.00	7.43	±	0.06	7.40	±	0.04	7.50	±	0.03	7.53	±	0.04	7.55	±	0.00
7	7.53	±	0.00	7.42	±	0.02	7.42	±	0.01	7.48	±	0.04	7.52	±	0.03	7.61	±	0.00
Subsurface	C			D1			D1			D1			D2			D3		
1	7.52	±	0.06	7.52	±	0.03	7.52	±	0.03	7.52	±	0.03	7.52	±	0.04	7.52	±	0.03
2	7.66	±	0.09	7.53	±	0.13	7.59	±	0.01	7.69	±	0.02	7.65	±	0.01	7.76	±	0.04
3	7.59	±	0.04	7.56	±	0.01	7.53	±	0.02	7.65	±	0.03	7.56	±	0.02	7.76	±	0.10
4	7.47	±	0.02	7.43	±	0.03	7.38	±	0.05	7.55	±	0.04	7.46	±	0.04	7.63	±	0.06
5	7.60	±	0.00	7.50	±	0.02	7.46	±	0.03	7.59	±	0.03	7.54	±	0.03	7.61	±	0.00
6	7.46	±	0.00	7.36	±	0.10	7.38	±	0.06	7.48	±	0.02	7.42	±	0.05	7.63	±	0.00
7	7.53	±	0.00	7.43	±	0.05	7.42	±	0.02	7.54	±	0.03	7.48	±	0.01	7.62	±	0.00

Table A2. EC and CaCO₃ contents of studied area as affected by pig slurry application.

Sampling	EC (dS m ⁻¹)																	
Surface	C			D1			D1			D1			D2			D3		
1	0.32	±	0.04	0.32	±	0.41	0.32	±	0.31	0.32	±	0.17	0.32	±	0.10	0.32	±	0.14
2	0.17	±	0.02	0.25	±	0.03	0.16	±	0.01	0.16	±	0.02	0.26	±	0.04	0.49	±	0.08
3	0.14	±	0.03	0.17	±	0.01	0.17	±	0.03	0.26	±	0.05	0.20	±	0.03	0.65	±	0.24
4	0.20	±	0.06	0.13	±	0.01	0.17	±	0.04	0.32	±	0.11	0.24	±	0.05	0.78	±	0.23
5	0.16	±	0.00	0.36	±	0.08	0.27	±	0.04	0.24	±	0.04	0.40	±	0.05	0.22	±	0.00
6	0.17	±	0.00	0.18	±	0.06	0.18	±	0.07	0.21	±	0.06	0.34	±	0.08	0.35	±	0.00
7	0.16	±	0.00	0.27	±	0.03	0.23	±	0.05	0.23	±	0.05	0.37	±	0.06	0.28	±	0.00
Subsurface	C			D1			D1			D1			D2			D3		
1	0.17	±	0.04	0.17	±	0.03	0.17	±	0.03	0.17	±	0.04	0.17	±	0.05	0.17	±	0.17
2	0.16	±	0.01	0.18	±	0.03	0.16	±	0.02	0.18	±	0.07	0.22	±	0.06	0.47	±	0.16
3	0.14	±	0.03	0.11	±	0.04	0.13	±	0.05	0.13	±	0.05	0.21	±	0.05	0.43	±	0.13
4	0.12	±	0.01	0.16	±	0.06	0.23	±	0.10	0.16	±	0.01	0.17	±	0.08	0.47	±	0.16
5	0.14	±	0.00	0.21	±	0.05	0.17	±	0.03	0.18	±	0.03	0.27	±	0.02	0.45	±	0.00
6	0.15	±	0.00	0.17	±	0.05	0.15	±	0.06	0.18	±	0.08	1.76	±	0.37	0.81	±	0.00
7	0.14	±	0.00	0.19	±	0.03	0.16	±	0.04	0.18	±	0.03	1.01	±	0.18	0.63	±	0.00
CaCO ₃ (%)																		
Surface	C			D1			D1			D1			D2			D3		
1	55.25	±	2.32	55.25	±	3.69	55.25	±	2.49	55.25	±	5.70	55.25	±	5.66	55.25	±	2.64
2	54.37	±	6.05	50.80	±	5.59	56.84	±	3.17	52.81	±	3.36	59.38	±	5.53	68.08	±	10.06
3	57.09	±	0.87	61.51	±	0.15	62.00	±	0.36	54.66	±	4.94	54.72	±	0.83	50.52	±	7.47
4	56.98	±	0.95	48.26	±	0.78	52.93	±	2.03	56.32	±	3.88	57.15	±	4.33	56.73	±	4.30
5	60.53	±	0.00	59.78	±	0.88	62.84	±	4.53	61.31	±	0.75	61.50	±	2.66	64.62	±	0.00
6	59.57	±	0.00	61.22	±	1.64	61.27	±	1.56	62.17	±	0.23	63.49	±	2.60	63.78	±	0.00
7	60.05	±	0.00	60.50	±	1.02	62.05	±	2.84	61.74	±	0.49	62.49	±	2.56	64.20	±	0.00
Subsurface	C			D1			D1			D1			D2			D3		
1	62.27	±	8.52	62.27	±	3.14	62.27	±	4.78	62.27	±	2.62	62.27	±	2.33	62.27	±	1.65
2	61.94	±	5.11	63.77	±	4.04	52.92	±	9.78	55.10	±	5.68	68.09	±	2.31	68.95	±	2.73
3	63.29	±	2.02	63.61	±	1.66	64.87	±	1.75	56.02	±	7.04	57.18	±	1.04	61.03	±	0.03
4	61.95	±	2.44	49.75	±	3.01	53.78	±	0.68	55.63	±	3.66	58.16	±	4.96	65.67	±	0.93
5	67.59	±	0.00	65.72	±	3.92	62.54	±	2.82	62.34	±	0.32	64.73	±	0.75	66.90	±	0.00
6	66.92	±	0.00	63.24	±	3.43	62.98	±	1.37	67.88	±	0.96	67.45	±	2.25	68.81	±	0.00
7	67.26	±	0.00	64.48	±	1.46	62.76	±	1.18	65.11	±	0.50	66.09	±	0.81	67.86	±	0.00

Table A3. CEC and Na contents of studied area as affected by pig slurry application.

Sampling	CEC (cmol ⁽⁺⁾ kg ⁻¹)																	
Surface	C			D1			D1			D1			D2			D3		
1	16.55	±	7.77	16.55	±	3.12	16.55	±	1.92	16.55	±	0.93	16.55	±	1.39	16.55	±	3.59
2	9.32	±	0.75	14.21	±	0.81	15.10	±	0.35	16.92	±	0.27	17.74	±	0.79	14.41	±	2.08
3	9.01	±	0.03	13.98	±	0.19	14.38	±	0.14	15.21	±	0.72	15.14	±	0.38	14.61	±	0.99
4	8.50	±	0.11	12.91	±	0.68	12.82	±	0.10	15.74	±	0.46	15.00	±	1.79	14.74	±	1.73
5	8.57	±	0.00	15.11	±	0.58	16.88	±	1.33	16.88	±	1.29	16.30	±	0.39	14.19	±	0.13
6	8.74	±	0.00	13.93	±	0.97	14.94	±	0.58	14.78	±	0.66	15.08	±	0.16	16.43	±	1.08
7	8.65	±	0.01	14.52	±	0.73	15.91	±	0.94	15.83	±	0.96	15.69	±	0.12	15.31	±	0.47
Subsurface	C			D1			D1			D1			D2			D3		
1	12.05	±	5.38	12.05	±	0.73	12.05	±	1.32	12.05	±	0.80	12.05	±	1.39	12.05	±	1.98
2	7.61	±	2.47	9.13	±	1.10	10.62	±	0.95	12.69	±	0.75	12.32	±	0.15	9.73	±	0.63
3	6.94	±	0.91	9.27	±	0.54	9.86	±	0.30	11.07	±	1.10	10.52	±	0.54	9.66	±	0.36
4	8.59	±	0.43	8.50	±	1.35	10.14	±	0.79	11.62	±	0.47	11.89	±	1.53	8.56	±	0.48
5	8.44	±	0.00	11.60	±	0.76	12.09	±	1.21	12.10	±	0.73	11.44	±	1.34	9.92	±	1.14
6	6.63	±	0.00	10.58	±	1.18	11.57	±	0.77	13.62	±	5.66	10.53	±	0.97	10.51	±	1.61
7	7.54	±	0.01	11.09	±	0.78	11.83	±	0.24	12.86	±	3.19	10.99	±	0.43	10.21	±	0.89
Na (cmol(+) kg-1)																		
Surface	C			D1			D1			D1			D2			D3		
1	0.89	±	0.12	0.89	±	0.58	0.89	±	0.11	0.89	±	1.32	0.89	±	0.04	0.89	±	0.06
2	1.06	±	0.07	1.97	±	1.16	2.11	±	1.73	1.02	±	0.07	0.85	±	0.63	1.86	±	0.39
3	0.16	±	0.04	0.15	±	0.03	0.59	±	0.00	0.27	±	0.06	1.28	±	1.22	0.86	±	0.10
4	0.39	±	0.10	0.15	±	0.01	3.10	±	2.95	0.68	±	0.20	0.63	±	0.06	1.12	±	0.45
5	0.65	±	0.00	0.57	±	0.15	1.81	±	1.29	0.76	±	0.18	1.34	±	0.68	1.34	±	0.00
6	1.30	±	0.00	0.39	±	0.48	0.74	±	0.12	2.19	±	1.71	0.78	±	0.41	0.38	±	0.00
7	0.33	±	0.56	0.48	±	0.31	1.28	±	0.70	0.79	±	0.93	1.06	±	0.40	0.29	±	0.50
Subsurface	C			D1			D1			D1			D2			D3		
1	0.89	±	0.14	0.89	±	0.24	0.89	±	0.46	0.89	±	0.26	0.89	±	0.23	0.89	±	0.71
2	1.48	±	0.47	1.91	±	0.20	2.05	±	1.93	0.71	±	0.08	2.82	±	0.23	2.23	±	0.55
3	0.47	±	0.37	0.74	±	0.20	0.18	±	0.14	2.42	±	0.02	2.46	±	0.11	0.73	±	0.24
4	0.80	±	0.76	0.89	±	0.09	0.74	±	0.41	2.78	±	0.00	2.33	±	0.06	1.86	±	0.92
5	0.72	±	0.00	0.98	±	0.30	3.33	±	0.53	0.76	±	0.16	0.93	±	0.19	0.59	±	0.00
6	0.80	±	0.00	0.69	±	0.09	0.34	±	0.24	0.54	±	0.10	2.36	±	0.63	4.76	±	0.00
7	0.76	±	0.10	0.84	±	0.14	1.84	±	0.15	0.37	±	0.05	0.76	±	0.40	0.89	±	0.55

Table A4. K and Ca contents of studied area as affected by pig slurry application.

Sampling	K (cmol(+) kg-1)																	
Surface	C			D1			D1			D1			D2			D3		
1	0.62	±	0.52	0.62	±	0.13	0.62	±	0.05	0.62	±	0.38	0.62	±	0.29	0.62	±	0.35
2	0.38	±	0.13	1.59	±	0.24	0.46	±	0.06	0.44	±	0.06	0.62	±	0.51	1.00	±	0.81
3	0.36	±	0.09	0.48	±	0.06	0.60	±	0.11	2.23	±	0.39	0.79	±	0.08	2.73	±	0.71
4	0.42	±	0.04	0.51	±	0.28	0.88	±	0.11	1.83	±	0.26	0.77	±	0.23	2.79	±	0.34
5	0.24	±	0.00	0.86	±	0.17	0.70	±	0.11	1.44	±	0.44	1.30	±	0.91	0.40	±	0.00
6	0.28	±	0.00	0.80	±	0.47	0.40	±	0.03	1.31	±	0.05	1.47	±	0.57	1.41	±	0.00
7	0.09	±	0.05	0.83	±	0.30	0.55	±	0.04	1.38	±	0.24	1.38	±	0.67	1.04	±	0.53
Subsurface	C			D1			D1			D1			D2			D3		
1	0.47	±	0.03	0.47	±	0.24	0.47	±	0.02	0.47	±	0.01	0.47	±	0.15	0.47	±	0.10
2	0.18	±	0.04	0.18	±	0.05	0.17	±	0.02	0.21	±	0.01	0.64	±	0.39	0.27	±	0.06
3	0.21	±	0.01	0.32	±	0.06	0.25	±	0.06	0.73	±	0.31	0.28	±	0.05	1.00	±	0.75
4	0.30	±	0.05	0.30	±	0.05	0.52	±	0.20	0.32	±	0.03	0.24	±	0.03	0.68	±	0.59
5	0.27	±	0.00	0.33	±	0.10	0.25	±	0.05	0.34	±	0.08	0.74	±	0.53	0.25	±	0.00
6	0.24	±	0.00	0.24	±	0.06	0.14	±	0.02	0.18	±	0.06	0.24	±	0.16	0.43	±	0.00
7	0.26	±	0.08	0.29	±	0.06	0.19	±	0.03	0.26	±	0.02	0.49	±	0.44	0.22	±	0.20
Ca (cmol(+) kg-1)																		
Surface	C			D1			D1			D1			D2			D3		
1	26.21	±	1.30	26.21	±	8.99	26.21	±	2.26	26.21	±	9.65	26.21	±	1.29	26.21	±	1.29
2	62.31	±	5.00	66.52	±	17.47	61.17	±	4.78	66.38	±	4.89	48.19	±	4.88	55.15	±	3.67
3	16.59	±	1.06	16.80	±	0.74	16.57	±	0.38	15.11	±	0.60	15.17	±	0.42	12.55	±	1.62
4	23.52	±	1.15	22.95	±	1.82	22.77	±	1.58	19.29	±	1.62	19.63	±	2.77	15.87	±	2.67
5	70.27	±	0.00	57.83	±	8.34	59.85	±	13.86	59.04	±	12.87	53.16	±	17.01	60.94	±	0.00
6	15.88	±	0.00	17.61	±	0.60	15.09	±	0.92	14.86	±	0.49	12.54	±	1.55	14.22	±	0.00
7	34.36	±	24.87	37.72	±	4.30	37.47	±	6.69	36.95	±	6.52	32.85	±	9.10	32.53	±	21.69
Subsurface	C			D1			D1			D1			D2			D3		
1	24.20	±	3.12	24.20	±	12.90	24.20	±	1.78	24.20	±	2.11	24.20	±	3.11	24.20	±	2.85
2	64.20	±	6.10	54.93	±	8.40	53.75	±	7.66	56.53	±	3.21	54.46	±	8.26	60.16	±	12.65
3	13.79	±	3.78	16.54	±	1.53	17.19	±	0.95	15.70	±	1.38	16.53	±	1.39	17.04	±	2.39
4	23.83	±	2.31	23.78	±	3.26	19.85	±	4.43	19.99	±	2.07	18.39	±	0.82	19.32	±	3.04
5	66.56	±	0.00	74.64	±	8.78	50.34	±	15.17	67.10	±	9.74	50.98	±	5.08	81.86	±	0.00
6	14.85	±	0.00	16.54	±	1.73	15.75	±	1.25	13.87	±	1.31	13.45	±	0.69	16.00	±	0.00
7	33.57	±	23.50	45.59	±	4.55	33.04	±	7.05	40.49	±	5.52	32.22	±	2.85	36.31	±	2.25

Table A5. Mg and Cu contents of studied area as affected by pig slurry application.

Sampling	Mg (cmol(+) kg ⁻¹)														
Surface	C			D1			D1			D1			D2		
1	0.92	±	0.08	0.92	±	0.28	0.92	±	0.06	0.92	±	0.22	0.92	±	0.37
2	0.70	±	0.04	0.95	±	0.09	0.85	±	0.01	0.78	±	0.06	1.76	±	0.46
3	0.69	±	0.02	0.73	±	0.03	0.75	±	0.06	0.97	±	0.06	1.47	±	1.53
4	0.71	±	0.03	0.76	±	0.03	0.81	±	0.07	0.98	±	0.08	1.33	±	1.44
5	0.91	±	0.00	1.14	±	0.06	1.05	±	0.05	1.31	±	0.11	1.78	±	0.00
6	0.65	±	0.00	0.74	±	0.04	0.74	±	0.03	0.89	±	0.04	1.24	±	0.00
7	0.26	±	0.15	0.94	±	0.04	0.90	±	0.01	1.10	±	0.07	1.51	±	0.80
Subsurface	C			D1			D1			D1			D2		
1	0.70	±	0.14	0.70	±	0.37	0.70	±	0.09	0.70	±	0.08	0.70	±	0.13
2	0.75	±	0.08	0.99	±	0.10	0.74	±	0.03	0.71	±	0.03	1.12	±	0.13
3	0.68	±	0.03	0.70	±	0.04	0.73	±	0.02	0.91	±	0.24	1.18	±	0.36
4	0.73	±	0.10	0.75	±	0.05	0.82	±	0.04	1.11	±	0.06	1.14	±	0.49
5	0.95	±	0.00	1.07	±	0.20	1.07	±	0.20	1.85	±	0.35	1.92	±	0.00
6	0.64	±	0.00	0.72	±	0.04	0.68	±	0.03	1.03	±	0.17	1.09	±	0.00
7	0.27	±	0.16	0.89	±	0.09	0.87	±	0.10	1.44	±	0.11	1.51	±	0.55
Cu (mg kg ⁻¹)															
Surface	C			D1			D1			D1			D2		
1	1.82	±	0.17	1.82	±	0.12	1.82	±	0.05	1.82	±	0.75	1.82	±	0.95
2	2.04	±	0.37	2.94	±	0.61	2.27	±	0.20	2.46	±	0.10	4.82	±	1.15
3	2.02	±	0.15	2.06	±	0.05	2.39	±	0.39	2.80	±	0.43	2.38	±	1.94
4	2.65	±	0.25	2.46	±	0.24	2.24	±	0.13	2.88	±	0.29	2.14	±	1.86
5	1.81	±	0.00	2.46	±	0.16	2.01	±	0.25	2.41	±	0.54	2.78	±	0.00
6	1.12	±	0.00	1.20	±	0.14	0.90	±	0.07	1.40	±	0.32	1.34	±	0.00
7	1.47	±	0.00	1.83	±	0.15	1.46	±	0.11	1.91	±	0.42	2.06	±	0.00
Subsurface	C			D1			D1			D1			D2		
1	1.17	±	0.07	1.17	±	0.02	1.17	±	0.10	1.17	±	0.18	1.17	±	0.31
2	1.91	±	0.53	1.03	±	0.22	1.42	±	0.35	1.91	±	0.23	1.16	±	0.64
3	1.24	±	0.10	1.77	±	0.29	1.59	±	0.15	1.84	±	0.29	1.05	±	0.31
4	1.99	±	0.33	2.31	±	0.31	1.78	±	0.14	1.21	±	0.16	1.02	±	1.48
5	1.58	±	0.00	2.02	±	0.09	1.53	±	0.28	0.97	±	0.04	0.94	±	0.15
6	0.58	±	0.00	1.21	±	0.17	0.75	±	0.23	0.61	±	0.04	0.58	±	0.20
7	1.08	±	0.00	1.62	±	0.05	1.14	±	0.16	0.79	±	0.03	0.76	±	0.32

Table A6. Mn and Zn contents of studied area as affected by pig slurry application.

Sampling	Mn (mg kg-1)																	
Surface	C			D1			D1			D1			D2			D3		
1	21.27	±	4.21	21.27	±	3.40	21.27	±	1.30	21.27	±	13.40	21.27	±	3.89	21.27	±	7.54
2	42.22	±	4.32	98.69	±	32.37	56.75	±	4.61	49.02	±	1.34	106.37	±	14.22	50.61	±	19.37
3	15.40	±	0.97	17.10	±	0.05	20.16	±	2.38	25.28	±	6.99	25.03	±	3.70	38.44	±	7.59
4	16.73	±	0.67	17.62	±	2.39	21.90	±	2.72	22.88	±	0.75	19.31	±	2.63	43.07	±	23.83
5	22.47	±	0.00	31.52	±	1.92	24.40	±	1.95	26.97	±	6.66	33.07	±	2.00	23.56	±	0.00
6	11.59	±	0.00	14.41	±	0.58	13.43	±	1.09	16.42	±	2.63	16.13	±	3.35	18.63	±	0.00
7	17.03	±	0.00	22.97	±	0.86	18.91	±	0.97	21.69	±	4.65	24.60	±	0.72	18.84	±	11.75
Subsurface	C			D1			D1			D1			D2			D3		
1	14.42	±	3.42	14.42	±	1.26	14.42	±	1.34	14.42	±	1.49	14.42	±	2.86	14.42	±	1.65
2	39.02	±	5.80	37.12	±	4.98	37.29	±	3.82	39.83	±	4.98	42.94	±	4.63	41.46	±	2.12
3	16.03	±	1.62	16.08	±	1.11	15.29	±	1.80	17.85	±	3.87	18.07	±	2.71	20.74	±	1.98
4	18.18	±	4.31	16.54	±	2.20	20.52	±	1.41	14.10	±	0.54	15.44	±	0.83	15.49	±	4.45
5	19.70	±	0.00	23.58	±	1.51	21.67	±	4.32	15.90	±	1.08	17.89	±	1.34	19.43	±	0.00
6	11.68	±	0.00	14.56	±	1.20	13.41	±	1.61	12.36	±	1.92	11.77	±	2.41	12.59	±	0.00
7	15.69	±	0.00	19.07	±	0.24	17.54	±	1.37	14.13	±	0.97	14.83	±	1.40	16.01	±	7.39
Zn (mg kg-1)																		
Surface	C			D1			D1			D1			D2			D3		
1	0.22	±	1.50	0.22	±	0.08	0.22	±	0.06	0.22	±	0.19	0.22	±	0.13	0.22	±	0.01
2	0.32	±	0.27	12.56	±	8.50	1.24	±	0.51	0.75	±	0.15	18.26	±	5.74	17.71	±	5.54
3	0.51	±	0.04	0.62	±	0.11	2.87	±	3.47	6.37	±	3.19	9.40	±	3.97	46.30	±	13.60
4	0.46	±	0.18	0.56	±	0.51	1.00	±	0.06	7.17	±	2.32	7.31	±	1.66	35.26	±	9.85
5	0.42	±	0.00	3.58	±	1.25	2.28	±	0.31	7.58	±	3.76	16.29	±	3.21	13.99	±	0.00
6	0.00	±	0.00	0.78	±	0.53	0.83	±	0.02	4.99	±	0.96	7.04	±	1.79	13.25	±	0.00
7	0.21	±	0.00	2.18	±	0.85	1.56	±	0.17	6.28	±	2.35	11.67	±	1.43	13.62	±	0.00
Subsurface	C			D1			D1			D1			D2			D3		
1	0.22	±	0.19	0.22	±	0.01	0.22	±	0.03	0.22	±	0.07	0.22	±	0.31	0.22	±	0.83
2	0.16	±	0.01	0.44	±	0.09	0.18	±	0.05	0.17	±	0.03	0.33	±	0.17	4.82	±	2.85
3	0.42	±	0.12	0.37	±	0.06	0.42	±	0.08	1.41	±	0.65	0.89	±	0.46	1.51	±	1.02
4	0.21	±	0.07	0.16	±	0.02	0.35	±	0.23	0.32	±	0.15	0.19	±	0.02	5.09	±	2.92
5	0.43	±	0.00	0.68	±	0.19	0.59	±	0.08	0.93	±	0.32	0.88	±	0.25	1.88	±	0.00
6	0.00	±	0.00	0.16	±	0.13	0.04	±	0.04	0.14	±	0.09	0.49	±	0.37	1.54	±	0.00
7	0.21	±	0.00	0.42	±	0.11	0.31	±	0.02	0.53	±	0.14	0.68	±	0.26	1.48	±	0.00

Table A7. Zn and P assimilable contents of studied area as affected by pig slurry application.

Sampling	Fe (mg kg ⁻¹)																	
Surface	C			D1			D1			D1			D2			D3		
1	3.56	±	0.29	3.56	±	0.21	3.56	±	0.20	3.56	±	1.71	3.56	±	0.95	3.56	±	0.22
2	6.05	±	0.67	9.54	±	2.40	6.44	±	0.48	5.49	±	0.29	14.34	±	5.82	8.29	±	4.48
3	3.54	±	0.60	3.70	±	0.32	3.59	±	0.90	4.17	±	0.81	5.01	±	1.10	10.20	±	4.36
4	3.69	±	0.05	3.71	±	0.07	3.70	±	0.22	3.84	±	0.34	4.40	±	0.41	8.45	±	3.91
5	4.46	±	0.00	5.19	±	0.36	3.96	±	0.48	4.51	±	0.31	6.80	±	1.27	6.46	±	0.00
6	1.70	±	0.00	1.62	±	0.26	1.19	±	0.05	2.46	±	0.80	2.81	±	1.07	4.20	±	0.00
7	3.08	±	0.00	3.41	±	0.31	2.58	±	0.24	3.49	±	0.54	4.80	±	0.13	5.33	±	0.00
Subsurface	C			D1			D1			D1			D2			D3		
1	3.38	±	0.30	3.38	±	0.45	3.38	±	0.36	3.38	±	0.32	3.38	±	0.51	3.38	±	0.40
2	6.21	±	0.84	5.27	±	0.78	5.72	±	0.46	5.40	±	0.41	5.85	±	0.61	6.51	±	1.69
3	3.46	±	0.78	4.10	±	0.25	4.13	±	0.47	3.96	±	0.22	3.55	±	0.46	4.38	±	0.29
4	3.82	±	0.47	3.57	±	0.41	3.48	±	0.18	3.26	±	0.29	3.32	±	0.23	8.45	±	6.87
5	3.47	±	0.00	4.44	±	0.47	4.59	±	1.30	3.81	±	0.21	3.75	±	0.27	3.46	±	0.00
6	1.10	±	0.00	2.34	±	0.53	1.38	±	0.49	2.46	±	1.47	1.39	±	0.66	1.24	±	0.00
7	3.06	±	0.00	3.39	±	0.04	2.98	±	0.54	3.13	±	0.84	2.57	±	0.32	2.97	±	0.00
P (mg kg ⁻¹)																		
Surface	C			D1			D1			D1			D2			D3		
1	62.09	±	1.81	62.09	±	0.87	62.09	±	0.92	62.09	±	0.53	62.09	±	1.10	62.09	±	3.03
2	62.65	±	1.95	57.56	±	1.15	52.61	±	0.52	100.69	±	0.51	90.10	±	0.49	246.03	±	5.29
3	61.58	±	0.81	70.41	±	0.93	70.62	±	1.08	125.89	±	1.27	126.67	±	5.34	255.75	±	5.29
4	60.65	±	0.54	62.93	±	0.36	67.99	±	0.65	112.86	±	1.56	100.41	±	1.14	203.53	±	7.66
5	62.02	±	0.68	101.36	±	3.52	85.53	±	0.61	107.21	±	1.74	201.38	±	5.38	227.67	±	3.19
6	60.87	±	0.88	85.74	±	0.71	83.81	±	0.46	121.58	±	14.09	144.49	±	0.80	180.66	±	0.86
7	61.31	±	0.19	85.56	±	0.52	82.83	±	0.40	149.30	±	4.48	132.56	±	2.31	209.46	±	6.42
Subsurface	C			D1			D1			D1			D2			D3		
1	34.37	±	0.36	34.37	±	1.20	34.37	±	1.80	34.37	±	1.06	34.37	±	1.11	34.37	±	1.21
2	46.97	±	0.57	31.30	±	1.23	33.67	±	3.51	45.40	±	0.86	48.75	±	1.40	71.30	±	0.69
3	56.61	±	0.56	55.19	±	1.56	56.63	±	1.58	65.00	±	0.39	62.76	±	1.43	62.37	±	1.18
4	61.14	±	0.51	63.32	±	0.80	68.39	±	0.81	65.20	±	0.03	60.72	±	0.46	63.17	±	0.58
5	60.77	±	1.35	64.92	±	0.81	59.73	±	3.08	68.19	±	0.34	71.36	±	1.16	68.26	±	0.87
6	58.06	±	0.44	66.77	±	0.89	69.40	±	0.82	74.66	±	0.49	70.75	±	0.98	79.36	±	0.72
7	63.16	±	0.52	67.79	±	0.41	62.17	±	0.39	115.30	±	24.13	124.38	±	3.40	168.31	±	2.82

Table A8. SOC and Total N contents of studied area as affected by pig slurry application.

Sampling	SOC (g kg ⁻¹)																	
Surface	C			D1			D1			D1			D2			D3		
1	10.69	±	0.84	10.69	±	2.12	10.69	±	1.79	10.69	±	2.00	10.69	±	2.10	10.69	±	0.83
2	10.58	±	0.85	10.85	±	0.54	13.29	±	0.27	12.47	±	0.12	18.92	±	1.51	15.17	±	0.68
3	15.81	±	0.75	14.31	±	1.77	14.29	±	0.79	14.89	±	3.88	14.28	±	1.98	18.27	±	0.46
4	13.14	±	0.47	14.80	±	2.55	15.88	±	2.96	16.23	±	3.74	12.20	±	1.08	16.39	±	4.20
5	10.06	±	1.97	12.99	±	2.08	13.46	±	2.10	9.46	±	1.14	15.67	±	4.24	17.83	±	0.56
6	10.48	±	2.25	13.25	±	3.09	12.75	±	1.75	13.28	±	2.40	16.29	±	4.42	20.33	±	3.43
7	10.27	±	0.15	13.12	±	1.65	13.10	±	1.09	11.37	±	1.70	15.98	±	3.95	19.08	±	1.58
Subsurface	C			D1			D1			D1			D2			D3		
1	7.62	±	2.45	7.62	±	0.93	7.62	±	1.23	7.62	±	1.28	7.62	±	1.24	7.62	±	3.27
2	8.21	±	0.37	7.23	±	0.14	6.71	±	1.21	9.53	±	0.29	12.78	±	0.26	10.56	±	0.21
3	10.03	±	1.64	14.37	±	1.73	14.74	±	3.92	14.29	±	2.21	15.94	±	1.71	17.07	±	1.48
4	7.44	±	2.20	14.06	±	2.70	16.17	±	1.53	14.58	±	4.35	15.15	±	1.51	16.60	±	1.69
5	8.33	±	1.24	12.76	±	4.54	10.45	±	3.41	12.44	±	3.27	13.00	±	2.47	12.35	±	3.51
6	15.89	±	4.22	13.20	±	2.20	11.17	±	1.62	14.54	±	1.90	13.78	±	3.26	19.01	±	2.08
7	12.11	±	1.79	12.98	±	2.55	10.81	±	0.92	13.49	±	2.21	13.39	±	2.67	15.23	±	1.83
Total N (g kg ⁻¹)																		
Surface	C			D1			D1			D1			D2			D3		
1	0.62	±	0.08	0.62	±	0.02	0.62	±	0.10	0.62	±	0.47	0.62	±	0.38	0.62	±	0.14
2	0.56	±	0.01	0.87	±	0.21	0.57	±	0.02	0.55	±	0.04	1.04	±	0.45	0.88	±	0.66
3	0.43	±	0.05	0.50	±	0.02	0.55	±	0.05	0.90	±	0.13	0.63	±	0.08	2.71	±	1.91
4	0.53	±	0.01	0.52	±	0.04	0.66	±	0.06	0.82	±	0.06	0.55	±	0.07	2.41	±	1.77
5	0.08	±	0.00	0.63	±	0.11	0.70	±	0.02	0.88	±	0.34	0.90	±	0.25	0.72	±	0.00
6	0.55	±	0.00	0.64	±	0.04	0.64	±	0.09	0.74	±	0.05	0.75	±	0.14	0.85	±	0.00
7	0.31	±	0.00	0.64	±	0.07	0.67	±	0.04	0.81	±	0.14	0.82	±	0.11	0.79	±	0.00
Subsurface	C			D1			D1			D1			D2			D3		
1	0.30	±	0.04	0.30	±	0.01	0.30	±	0.02	0.30	±	0.03	0.30	±	0.06	0.30	±	0.30
2	0.37	±	0.01	0.28	±	0.04	0.35	±	0.05	0.43	±	0.04	0.24	±	0.04	0.42	±	0.14
3	0.22	±	0.02	0.38	±	0.10	0.32	±	0.04	0.41	±	0.10	0.22	±	0.04	0.37	±	0.06
4	0.36	±	0.04	0.50	±	0.08	0.54	±	0.10	0.27	±	0.05	0.24	±	0.01	0.49	±	0.33
5	0.15	±	0.00	0.44	±	0.09	0.44	±	0.06	0.26	±	0.06	0.20	±	0.06	0.30	±	0.00
6	0.32	±	0.00	0.55	±	0.01	0.41	±	0.08	0.33	±	0.28	0.28	±	0.07	0.36	±	0.00
7	0.24	±	0.00	0.50	±	0.05	0.43	±	0.07	0.29	±	0.11	0.24	±	0.05	0.33	±	0.00

Table A9. C/N ratio of studied area as affected by pig slurry application.

Sampling	C/N (%)																	
Surface	C			D1			D1			D1			D2			D3		
1	17.24	±	2.28	17.24	±	5.03	17.24	±	6.48	17.24	±	6.72	17.24	±	11.19	17.24	±	0.99
2	19.06	±	1.90	12.91	±	2.78	23.14	±	0.26	22.93	±	1.65	20.50	±	8.24	34.14	±	3.73
3	10.72	±	6.76	28.80	±	2.20	26.17	±	3.55	16.67	±	4.65	23.13	±	6.71	8.64	±	4.09
4	16.77	±	5.45	28.26	±	2.76	24.25	±	4.17	20.09	±	5.68	22.67	±	5.30	9.89	±	6.80
5	16.42	±	1.73	21.21	±	5.51	19.20	±	2.43	12.45	±	6.74	19.41	±	10.74	24.76	±	2.00
6	27.71	±	6.70	20.86	±	6.24	19.98	±	1.30	17.95	±	3.02	21.44	±	3.24	23.87	±	4.29
7	20.20	±	1.68	21.00	±	4.80	19.61	±	1.78	14.53	±	4.27	19.96	±	7.35	24.28	±	1.29
Subsurface	C			D1			D1			D1			D2			D3		
1	25.40	±	11.55	25.40	±	3.18	25.40	±	5.02	25.40	±	2.38	25.40	±	6.12	25.40	±	8.03
2	22.37	±	1.50	26.13	±	4.10	19.12	±	3.01	22.10	±	1.38	55.59	±	10.37	27.32	±	9.66
3	29.63	±	10.62	39.96	±	11.26	45.35	±	7.18	35.79	±	6.31	74.26	±	14.95	46.72	±	6.19
4	24.90	±	9.44	28.73	±	8.68	30.43	±	3.21	57.42	±	25.17	64.01	±	9.27	42.49	±	19.16
5	72.94	±	0.00	28.92	±	8.59	24.19	±	8.63	48.91	±	10.30	66.28	±	11.63	40.72	±	2.30
6	32.01	±	6.16	23.75	±	3.39	27.77	±	5.92	68.31	±	42.83	53.63	±	22.17	52.54	±	1.92
7	44.20	±	8.15	25.98	±	3.09	25.86	±	4.83	51.00	±	20.83	58.25	±	17.86	47.15	±	1.97

Table A10. Soil texture of studied area.

	SAND (%)			SILT(%)			CLAY(%)		
C	27.33	±	3.00	48.98	±	1.21	23.69	±	3.92
D1	26.19	±	1.94	50.71	±	2.73	23.10	±	1.01
D1	24.89	±	2.20	49.71	±	2.58	25.40	±	1.08
D1	27.54	±	4.34	48.63	±	6.07	23.82	±	3.57
D2	33.97	±	3.63	41.62	±	1.53	24.41	±	3.42
D3	25.22	±	2.09	46.55	±	3.06	28.23	±	0.98
	SAND (%)			SILT(%)			CLAY(%)		
C	29.76	±	3.11	47.90	±	3.50	22.34	±	3.58
D1	35.37	±	4.59	46.24	±	2.94	18.39	±	1.69
D1	34.64	±	9.00	43.70	±	9.42	21.66	±	0.56
D1	30.34	±	2.00	43.43	±	5.88	26.22	±	5.38
D2	24.90	±	3.23	47.32	±	2.99	27.78	±	0.24
D3	27.48	±	4.97	45.76	±	5.10	26.76	±	0.14

Table A11. Bulk density of studied area

	Bulk Density (g cm ⁻³)
As	1.18
Ap	1.25

Appendix B

Some photos of the studied area





Photo B1a. Soil sampling from the studied area



Photo B1b. Soil sampling from the studied area



Photo B2. Pig slurry application from the studied area.



Photo B3. Soil and plant sampling from the studied area.



Photo B4. Horizons of the studied area.



Photo B5. Column experiment in the field.



Photo B5. Pig slurry samples from the studied area.

