SOIL PHYSICO-CHEMICAL PROPERTIES AND FUNGAL DIVERSITY UNDER DIFFERENT AGRICULTURAL MANAGEMENT PRACTICES IN BHAKTAPUR, NEPAL

A Dissertation Submitted for the Partial Fulfilment of the

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DECLARATION

I, Sunita Sharma, student of M.Sc. Botany, Central Department of Botany, Tribhuvan University, Kirtipur, hereby declare that the research work entitled "SOIL PHYSICO-CHEMICAL PROPERTIES AND FUNGAL DIVERSITY UNDER DIFFERENT AGRICULTURAL MANAGEMENT PRACTICES IN BHAKTAPUR, NEPAL" submitted by myself at Institute of Science and Technology, Tribhuvan University for Partial Fulfilment of Master's Degree in Botany, is a record of bonded work carried out by me under the supervision of Dr. Chandra Prasad Pokhrel, Assoc. Professor, Central Department of Botany, Tribhuvan University, Kirtipur.

I further declare that the work reported in this research work has not been submitted and will not be submitted, either in partial or in full, for the award of any other degree in this or any other institute or university.

Sunita Sharma

February, 2021

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TABLE OF CONTENTS

RECOMMENDATION	ii
LETTER OF APPROVAL	iii
DECLARATION	iv
ACKNOWLEDGEMENT	V
TABLE OF CONTENTS	vi
LIST OF FIGURES	ix
LIST OF TABLES	X
CHAPTER ONE: INTRODUCTION	1
1.1 Background	1
1.2 Research Hypothesis	4
1.3 Objectives	4
1.4 Problem statement	5
1.5 Limitations	5
CHAPTER TWO: LITERATURE REVIEW	6
2.1 Overview of soil quality	6
2.2 Land management practices	6
2.3 Soil Moisture	7
2.4 Bulk density	7
2.5 Soil pH	
2.6 Soil organic matter	9
2.7 Soil electrical conductivity	10
2.8 Soil organic carbon and carbon sequestration	10
2.9 Total nitrogen	12
2.10 Carbon nitrogen ratio	
CHAPTER THREE: MATERIAL AND METHODS	

3.1 Description of the study area	15
3.2 Farming system	16
3.3 Site selection	16
3.4 Soil sample collection	17
3.5 Soil analysis	17
3.5.1 Soil sample preparation	
3.5.2 Determination of moisture	
3.5.3 Determination of bulk density	
3.5.4 Determination of soil pH	
3.5.5 Determination of EC	19
3.5.6 Determination of soil organic carbon	19
3.5.7 Determination of total Nitrogen	
3.5.8 Determination of carbon nitrogen ratio	21
3.6 Culture of fungi	21
3.6.1 Sterilization technique	21
3.6.2 Preparation of media	
3.6.3 Isolation of soil fungi	
3.6.4 Identification of fungi	23
3.6.5 Statistical analysis	23
CHAPTER FOUR: RESULTS	24
4.1 Physical and Chemical properties of soil at different manager	ment practices of
land	
4.1.1 Moisture content	24
4.1.2 Soil bulk density	
4.1.3 Soil pH	26
4.1.4 Electric conductivity (EC)	27
4.1.5 Soil organic carbon	

4.1.6 Soil organic matter	30
4.1.7. Soil organic carbon stock	31
4.1.8 Total nitrogen	
4.1.9 Carbon nitrogen (C/N) ratio	
4.2 Correlation between various physical and chemical parameters	34
4.2.1 Relationship between bulk density and soil organic carbon (%)	34
4.2.2 Relationship between pH and SOC %	
4.4 Soil fungi isolated from different management practices of land	
CHAPTER FIVE: DISCUSSION	39
5.1 Moisture Content	39
5.2 Bulk density	
5.3 Soil pH	40
5.4 Soil electric conductivity	40
5.4 Soil organic matter	41
5.5 Soil organic carbon and soil organic carbon stock	41
5.6 Total nitrogen	
5.7 Carbon Nitrogen ratio of soil	
5.8 Soil fungal diversity	
CHAPTER SIX: CONCLUSION AND RECOMMENDATIONS	44
Conclusion	44
Recommendations	44
REFERENCES	45
PHOTOPLATES	59

LIST OF FIGURES

Fig 1 Map of study area15
Fig 2 Mean monthly temperature and rainfall of Bhaktapur16
Fig 3 Moisture content in soil depth of 0-10 cm and 10-20 cm in different land
management practices
Fig 4 Bulk density in the soil depth of 0-10 cm and 10-20 cm in different land
management practices
Fig 5 Soil pH in the soil depth of 0-10 cm and 10-20 cm in different land management
practices
Fig 6 Soil electrical conductivity in the soil depth of 0-10 cm and 10-20 cm in different
land management practices
Fig 7 Soil organic carbon in the soil depth of 0-10 cm and 10-20 cm in different land
management practices
Fig 8 Soil organic matter in the soil depth of 0-10 cm and 10-20 cm in different land
management practices
Fig 9 Soil organic carbon stock in the soil depth of 0-10 cm and 10-20 cm in different
land management practices
Fig 10 Total nitrogen in the soil depth of 0-10 cm and 10-20 cm in different land
management practices
Fig 11 Carbon nitrogen ratio in the soil depth of 0-10 cm and 10-20 cm in different land
management practices
Fig 12 Correlation between bulk density and SOC % in soil depth (0-10) cm35
Fig 13 Correlation between bulk density and SOC % in soil depth (10-20) cm35
Fig 14 Correlation between pH and SOC % in (0-10) cm depth
Fig 15 Correlation between pH and SOC % in (10-20) cm depth

LIST OF TABLES

Table 1: List of fungi isolated from the	tudied land management practices

CHAPTER ONE: INTRODUCTION

1.1 Background

Soil is an important component of biosphere, its function not only production of food but also maintenance of local, regional and global environment quality (Glanz 1995). Soil is a thin layer covering the earth surface. Johnson *et al.* (1997), defined soil quality as a measure of the condition of soil relative to the requirement of one or more biological species and or to any human purpose. Soil quality has been degraded worldwide by the change in biological physical, chemical and contamination by organic and inorganic chemicals (Arshad & Martin 2002). According to soil science society of America the soil quality is defined as 'the fitness of specific kind of soil to function within natural or managed ecosystem boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality and support human health and habitation'. Soil quality is an indicator of environmental quality (National Research Council 1993) and food security (Lal 1999).

Soil quality refers to the status of soil as a result of management (Karlen *et al.* 2003). Soil quality parameters is divided into physical, chemical and biological parameters i.e., water holding capacity, relative field capacity to water saturation, macro porosity, bulk density, cation exchange capacity, contamination presence, pH, exchangeable sodium, etc. (Reynolds *et al.* 2009; Zaid *et al.* 2017). Degradation of soil in arid and semi-arid regions such as Egypt is due to lack of knowledge about soil condition in farmers and lack of proper equipment. Also, in such circumstances, soil was found to be with poor soil quality, high temperature, poor soil fertility, low available water holding capacity, soil organic carbon and high concentration of salt and pH (Zaid *et al.* 2017). The status of soil structure, porosity, water holding capacity greatly effects plant growth and health. Soil water relationship and soil plant relationship is affected by soil physical parameters. Crop yield response will be optimal when soil quality parameters are in optimal range (Reynolds *et al.* 2009).

Soil is the main resources of natural and agricultural ecosystem. Only 22% (3.26 billion ha) of the total area of planet is suitable for agriculture and only 3% (450 million ha) has high production capacity (Lal 1995). Soil productivity is affected by different physicochemical parameters (Imhoff *et al.* 2016).

Bulk density reflects the soil quality to function for structural support, water and solute movement and soil aeration. Bulk density is influenced by amount of organic matter in soil, texture of soil, constituent mineral and porosity. Bulk density is regularly checked soil porosity in agricultural system for distinguishing soil compactness, aeration and infiltration (Dalal & Moloney 2000; Reynolds *et al.* 2009).

Soil pH is most informative properties of the soil. It is a measure of the hydrogen ion concentration in soil and also determines the solubility and availability of most nutrients in soil. Soil pH not only determines the plant growth but also influences the availability of essential nutrients. Most horticultural crops grow satisfactorily in soil having pH between 6 (slightly acidic) and 7.5 (slightly alkaline). Soil texture and organic matter are the key components that determine soil water holding capacity. As Soil Organic Matter (SOM) increases, water holding capacity (WHC) increases. The higher osmotic pressure around the roots prevents an efficient water absorption by the plant. Each plant has specific electrical conductivity tolerating capacity. The factor that influences electrical conductivity (EC) is soil salinity, clay content and cation exchange capacity, soil pore size and distribution, soil moisture content and temperature (Mcneill 1992; Rhoades *et al.* 1999). Nitrogen is an essential component of atmosphere which helps in photosynthesis of plants.

Microbes include fungi, bacteria and viruses. Fungi colonize in upper parts of plants and provide many benefits, including drought tolerance heat tolerance resistance to insects and resistance to plant disease. The micro-organisms present in soil are soil algae, bacteria, Actinomycetes, Bacteriophages, Protozoa, Nematodes and fungi. Microbes are essential part of soil and play vital role in carbon and nitrogen cycling and ecosystem functioning (Doran 1987). Soil microbes enhance soil quality, soil health, growth, yield and quality of crops (Singh *et al.* 2011). Microbial community of soil contains population of micro-organisms including plant growth promoting rhizobacteria, Nitrogen fixing cyanobacteria, plant disease suppressive bacteria and fungi, soil toxicant degrading microbes, actinomycetes & other useful microbes (Singh *et al.* 2011). Microbes show direct relation with soil carbon in agricultural soil (Smith and Paul 1990) and microbial activity is directly related to soil carbon. It is proved that microbes are sensitive towards changes in carbon level through natural as well as

anthropogenic disturbances (Powlson *et al.* 1987; Ju *et al.* 2006). Agricultural tools also have adverse impact on soil properties (Rienzi *et al.* 2016).

Carbon is mainly stored in oceans, atmosphere and terrestrial system. Ocean is main reservoir of carbon (38,000 Pgc) followed by terrestrial system (2060 Pgc). In terrestrial plants, carbon is stored in soil and SOM is high in surface soil layer, so small influence on land use pattern greatly changes in atmospheric CO_2 concentration (Post *et al.* 1990; Raich & Schlesinger 1992; Schlesinger & Bernhardt 2013). SOM is made from residue of biological biomass such as plant animal and microbes. SOM plays vital role in functioning of soil ecosystem, enhance agricultural productivity and soil fertility. Because it maintains soil structure, boost WHC and release of plant and nutrients (Lefevre et al. 2017). Soil organic matter plays great role in productivity (Wendling et al. 2010). The various factors decomposing soil organic matter (SOM) are aeration, temperature and water content (Ashgrie et al. 2007). Intensive tillage practice and higher use of chemical fertilizer in conventional agriculture erode the soil C pools (Wen-jie et al. 2011). The factor such as land use type, soil type climate and vegetation effects soil organic carbon sequestration (Guo & Gifford 2002). The amount of carbon is significantly affected by land use change and climate change (John et al. 2005; Smith 2008; Don et al. 2011; Munoz-Rojas et al. 2013).

Soil organic carbon (SOC) is an indicator of soil quality and environmental sustainability. SOC has impact on crop production information and global climate change. Higher carbon sequestration enhances the productivity and sustainability of agricultural system (Lal 2004), reduces atmospheric CO₂ concentration (Sperow *et al.* 2003) and enhances soil fertility, minimizes nutrient runoff and improves water quality (Kurkalova *et al.* 2004; Lubowski *et al.* 2006; Feng *et al.* 2007).

Management practices results in soil erosion, atmospheric pollution, salinization and desertification (Oldeman 1994). Landuse change has great impact in soil quality. Management practices such as cropping pattern, tillage practice, fertilizer and pesticide use have great influences on soil quality (Doran & Zeiss 2000). Soil management practice is important either to consume or produce atmospheric gases such as CO₂, nitrous oxide and methane (Rolston *et al.* 1993; Mosier 1998). Landuse management is a serious concern as it influences on global climate change and ozone depletion through elevation level of greenhouse gases and altered hydrological cycles (Bengtsson 1998).

Tillage is the main cause of loss of organic matter which leads to decrease in soil biological activity, effect in soil physicological properties ultimately leading to low crop productivity (Du Preez *et al.* 2001). The soil management practice change from tillage to no tillage system is most effective factor for SOC sequestration (Parton *et al.* 1987; Lee *et al.* 1993; Jarecki & Lal 2003). Land management practices has direct influence on soil quality parameters such as soil organic matter, nutrient supply, soil vegetation and compactness of soil are major factors for soil degradation (Dunjo *et al* 2003).

In the developing country like Nepal, soil is deteriorating at an alarming rate due to landuse change lowering carbon sequestration (IPCC 2000). Population growth is associated with urbanization, agricultural expansion and industrialization which lead to land use change. Expanding drift of commercialization of all types of food and bioenergy will likely increase land requirements (Knickel 2012). Land use change (LUC) changes the biotic and abiotic characteristics of an area, consequently influence biodiversity. LUC causes change in organic matter ultimately to SOC. Agriculture requires sustainable use of soil resources as it can easily lose quality through change in practice (Kiflu & Beyene 2013).

1.2 Research Hypothesis

Physicochemical properties and fungal diversity of soil varies with the landuse type and management practices.

1.3 Objectives

General objective

• The main objective of this study is to determine the current situation of physicochemical properties and fungal diversity of soil under different land management practices in Bhakatpur, Nepal.

Specific objectives

- To analyze soil physico-chemical properties including soil organic matter and carbon stock under different land management practices.
- To isolate and identify the fungi from the soils of different land management practices.

1.4 Problem statement

Due to population increase and need for the increase of the living standard, farmers extensively use agricultural land. Expanding of commercialization of all types of food and bioenergy will likely increase land requirements (Knickel 2012). The excessive use of pesticides, insecticides, fertilizer etc. in the name of high yield, deteriorates natural soil quality. The quality of soil is highly correlated with its physicochemical parameters and organism present in it. However, crop yield response will be best when soil quality parameters are in optimal range (Reynolds *et al.* 2009). So, soil parameters are a major issue to be kept in mind for sustainable use of land and good production of crops.

Soil is the major reservoir of carbon. The increase in atmospheric carbon increases earth atmospheric temperature. Land management practices cause large alternation in soil quality and its carbon content. In the developing country like Nepal, soil is deteriorating at an alarming rate due to landuse change lowering carbon sequestration (IPCC 2000).

There is research gap in spatial and temporal variation in management practices and landuse impact in soil physicochemical properties. Studies related to management practice and land use impact in soil physicochemical parameters have been done in limited areas of Nepal. Such studies provide status of soil according to management practices and landuse practice. This study aims to analyze physicochemical properties and fungal diversity of soil in different management practices of land in Bhaktapur district. This study helps to find out if there is any anomaly in the soil physicochemical parameters and suggest ways of remedy if any found. These parameters should be regularly monitored so as to keep soil sustainable for agriculture.

1.5 Limitations

- The study was conducted in two municipalities in Bhaktapur.
- Only one media (Potato dextrose agar medium) was used for isolation of fungi.
- Identification of fungi was done up to genus level, few up to species level.
- The measured parameters may also vary with the season which was not considered for this study.

CHAPTER TWO: LITERATURE REVIEW

2.1 Overview of soil quality

Soil quality and soil health are defined as the ability of soil to act as vital living system within land use practices. It focuses on sustaining biological productivity of the soil and maintaining quality of surrounding environment and human health (Laishram *et al.* 2012). Soil quality is the capacity of a specific kind of soil to function within natural or managed ecosystem boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality and support human health and habitation according to soil science society of America (1995).

According to Bajracharya *et al.* (2007), the concept of soil quality is vague because it includes different factors of ecosystem such as water, air, flora and fauna; as well as these factors have great influence on human health and habitation. Different research done in developing countries like Nepal has shown that mitigating soil degradation and increasing productive capacity also helps in declining soil erosion. The soil quality depends on different factors like soil textural class, soil organic matter, pH and major nutrients available in the soil. There is a wide range of agro ecological parameters which are additional factors for soil quality.

The research carried on organic and conventional farms found that organic carbon and soil quality depends on quality and quantity of applied manure types. It has more effect on microbial biomass and its activities than that of Organic Carbon (Fließbach *et al.* 2007).

2.2 Land management practices

Land use change has direct influence on soil quality parameters. Soil organic matter, nutrient supply, soil vegetation cover and compactness of soil are major factor for soil degradation. In order to maintain quality of soil, these factors should be managed properly (Dunjo *et al.* 2003).

Gomiero (2013), states that soil conservation is a challenge because it is influenced by different factors one of which is rapid increase in population which causes increase in pressure for food production to them and intensive agricultural practices. Therefore, for soil conservation we should adopt alternative practices mainly based on organic farming

because it helps to preserve soil condition as well as to fulfill the demand of food for whole ecosystem.

According to Wen-jie *et al.* (2011), the practice of reduced tillage and increasing carbon content maintain good soil fertility, increase soil organic carbon and reduce atmospheric CO_2 rise. The combination of no tillage and organic input increased the level of Soil organic carbon by 140 % over conventional agriculture. No tillage practice on soil has also effect in microbial biomass level and reduces organic carbon.

2.3 Soil Moisture

Soil moisture is the amount of water present in the soil and expressed as percentage. It helps in chemical and biological activities in soil and growth of plants. Liang *et al.* (2002), describes the effect of soil moisture and nutrients on crop land productivity as well as the method through which crop productivity can be increased. This research is based on improving cropland ecosystem, effect of fertilizers on crop productivity under different patterns of rainfall and relationship between fertilizers and productivity.

Soil water acts as a solvent and medium for carrying the nutrients. It is also a necessary ingredient for photosynthesis. Identifying how soil moisture changes under different land management practices is necessary to maintain the viable use of water and soil resources. It also helps in the restoration of vegetation in arid and semiarid ecosystems (Tang *et al.* 2019).

Soil moisture helps in maintaining the soil temperature and microbes in soil also need moisture for their metabolic activities. The management practice of land acts a vital role in regulating the spatial and temporal variation of soil moisture by affecting infiltration rates, runoff and evapotranspiration, which is significant to crop growth and vegetation in semiarid environments (Niu *et al.* 2015). The change in the land management causes variability of soil moisture which results in soil deterioration, decrease in agricultural productivity and land degradation (Biro *et al.* 2013).

2.4 Bulk density

Bulk density of soil is defined as the ratio of oven-dried mass of soil to its bulk volume and depends on the densities of soil particle such as sand, slit, clay and organic matter and their packing arrangement (Aşkın & Özdemir 2003). Soil bulk density defines various characteristics of soil; explains the packing structure of soil and is also necessary for determination of soil carbon stock and nutrient assessment (Reidy *et al.* 2016). Bulk density generally increases with soil depth, due to changes in organic matter content, porosity and compaction (Chaudhari *et al.* 2013).

The research done by Bogunovic *et al.* (2017) in vineyards in (0 - 20 cm) soil depth show that use of tractor raises the soil compactness and erosion. The experiment done by Kapoor *et al.* (2015) in organic, inorganic and integrated management practices of Himanchal Pradesh shows high bulk density value in inorganic and lowest in organic management practices. Research states that bulk density and organic matter are negatively correlated (Nanganoa *et al.* 2019)

The research carried by Agbede (2010), in southwestern Nigeria found highest bulk density value in no tillage area when compared with the conventional tillage area which had significantly low bulk density. Similar research was done in Southwestern Nigeria by Jimmy *et al.* (2005), in four different types of tillage practices. It was found that bulk density was higher in no tillage field followed by manual tillage, plough-plough and plough-harrow tillage operations. Also, soil bulk density was found to increase as time after cultivation increased.

2.5 Soil pH

Soil pH acts as an important factor that regulates soil microbial activities, soil nutrient availability and crop growth and development (Zhang *et al.* 2019). Maintaining soil pH is one of the simplest and most significant factors in all plant-growing ecosystems. Optimum pH level for growth of most vegetable crops is between 6 and 7 (Brust 2019).

High value of pH (greater than 7.5) helps weathering of minerals, surge in the bacterial populations and increase in the release of cations; however, it suppresses the solubility of salts including carbonate and phosphates. If pH levels are low (less than 5.5), then the availability of nitrogen, phosphorous, potassium, calcium, magnesium, sulphur and molybdenum reduces. Availability of aluminium and manganese can surge to toxic levels at pH levels below 5. Low pH levels decline the activity of microbial decomposers which may reduce the biological conversion of organic material to useable nutrients for plant growth (Crozier *et al.* 2010).

Smith and Doran (1996), state that pH of soil provides acidity level and biological activities in soil influence pH level. The soil of manmade forest, natural forest and grassland was more acidic than arable land use type (Pham *et al.* 2018). According to Ghimire and Bista (2016), pH of forest soil was higher than agricultural soil in Nuwakot. The pH value of grassland was found higher followed by forest, Grazing and cultivated land (Tufa *et al.* 2019).

2.6 Soil organic matter

Soil organic matter (SOM) is the organic matter portion of soil, consisting of plant and animal detritus at various stages of decomposition, soil microbial cells and tissues, and soil microbial substances. SOM provides a wide range of benefits to the physical and chemical properties of soil and regulatory ecosystem services (Brady & Neil 1999). SOM also affects soil properties like bulk density, aggregate stability, cation exchange capacity and biological activity. It also protects and neutralizes soil pH, promotes the absorption of air and water into the soil, and improves soil water retention. SOM acts as a slow-release reservoir for plant macronutrients (particularly nitrogen) and micronutrients (Doran *et al.*, 1996).

Soil organic matter performs vital roles in improving soil health (Burst 2019). Carter (2002) studied wide range of management in Canada over the last two decades. The study conclude that SOM and aggregate stability are the main factors to access sustainable land use. Organic matter fraction like macro-organic matter, light fraction, microbial biomass and mineralizable carbon explains the quality of SOM. They have biological significance for various soil functions and soil processes and it also changes the amount of total SOM. They have biological significance for various soil functions and soil process and it also changes the amount of total SOM. They have biological significance on soil compatibility, friability and soil water holding capacity. Similarly, aggregated SOM has great effect on functioning of the soil in regulating air and water infiltration, conserving nutrients soil permeability and erodibility overall organic input, total SOM and soil aggregation process are the key factors for maintaining and regulating quality of soil. Guimaraes *et al.* (2013) states that land use change plays vital role in SOM status.

2.7 Soil electrical conductivity

Soil electrical conductivity (EC) is the measure of salinity of soil (amount of salt present in soil) and important indicator of soil health. High concentration of salt in soil suppresses plant growth. Measure of soil electrical conductivity is necessary to understand the soil salinity levels. Salinity above threshold levels in soil results in decreased crop yield and agricultural productivity. Soil salinity is most far-flung land degradation problem and thus currently a global environmental problem (Touch *et al.* 2015).

Land management practices, irrigation, application of fertilizer and cropping pattern also plays role in increasing salt concentration of soil. 20% of cultivated and 33% of irrigated soils in the world is salt-affected and degraded. This can be boosted by climate change, excessive use of groundwater (mainly if near to sea) or low-quality water in irrigating and high use of irrigation in intensive farming (Machado & Serralheiro 2017).

Electrical Conductivity of soil provides information about crop yields, nutrient condition and biological activities of soil. Nitrification and denitrification processes that are accomplished by bacteria can be greatly influenced by soil EC at levels that are well below the commonly used salinity thresholds. Most soils are considered slightly saline if the EC of a saturated paste extract exceeds 2 dS/m (Smith & Doran 1996).

Naturally high concentration of salt present in arid and semi-arid climates. Patel (2015), conducted experiment in Gujarat and states that EC is an important soil quality indicator. The result showed the 99.77% of soil samples are salt free and concluded that study area was good for agriculture practice.

2.8 Soil organic carbon and carbon sequestration

Post & Kwon (2000), explained about land-use change effects on soil organic carbon sequestration. When agricultural land is allowed to grow its own natural vegetation or planted to perennial vegetation, SOC accumulation process increases while changing from natural vegetation to agricultural land SOC accumulation decreases.

Yadav *et al.* (2002), described about input of sewage water for irrigation adds soil organic carbon, major micro-nutrients in the soil. They also compared distribution of N, P, K and other micronutrient to the soil. It was found that sewage used for irrigation

improves organic matter to 1.24 - 1.78% and fertility status of soil up to distance 1km along the disposal channel. They observed that total nitrogen content in sewage irrigation land was 2908 kgh⁻¹ as well as available total phosphorus and potassium was 2115 kgh⁻¹ and 412 kgh⁻¹ respectively in surface 0.15 m soil. It indicates that domestic sewage can effectively increase water resources for irrigation but continuous monitoring of the concentration of toxic metals like heavy metals in soil plants and ground water is still unknown.

According to Scharlemann *et al.* (2014), the amount of carbon present in soil is more than carbon present in phytomass and atmosphere due to land use and management practices. Although huge quantity of carbon is stored as soil organic carbon, consensus is lacking on the size of global SOC stock. This research mainly focused on impacts on SOC stock due to management practices. In order to mitigate soil organic carbon losses and enhance soil carbon stock, proper study of geographical distribution and better management policies are needed.

The research done by Kalita *et al.* (2016), in Barak valley India explains about managing soil under higher age tea plant providing environment services coupled with economic gain. The spread of SOC stock was evaluated in different layers of soil depth up to 1m depth under an age sequence of tea agro forestry system. SOC in the system was found to be ranging from 65.14 to 141.4 Mg C/ha with a mean 101.39 Mg C/ha. About 46% of estimated SOC was found in 0-30 cm layer, 65% occurred in 0-50 cm layer and 36% in 50-100 cm layer. It was found that SOC decreased with depth and negatively related with bulk density. Proper management practices like managed tillage, proper shade, surface mulching, compost input, proper maintenance of floor litter mass was considered as positive factor for preservation of soil C reserve under tea agro forestry system.

The experiment was done by Mulat *et al.* (2018), in Eastern Ethiopia in three land management practices: Grazing, cultivated and fallow lands in which soil sample was collected from (0-20) cm, (20-40) cm and (40-60) cm depth. It was found in results that grazing land had high soil organic carbon stock (42.9 t/ha and 32.9 t/ha) followed by cultivated land use type (32.6 t/ha and 26.3 t/ha) and fallow land use type (23 t/ha and 12.5 t/ha) in surface and sub surface layer respectively. Also, SOC stock was found reducing with soil depth and negatively correlated with bulk density.

The rising concentration of carbondioxide in atmosphere can be reduced by a good strategy of soil carbon sequestration by enhanced land use (Ghimire *et al.* 2019).

2.9 Total nitrogen

Total Nitrogen along with soil organic carbon plays a vital role in sustaining soil quality, crop production and environmental quality (Bauer & Black 1994). Knowing about the storage of carbon (C) and nitrogen (N) in soil allows us to understand how ecosystems respond to natural and anthropogenic disruptions under various land management ways (Zhang *et al.* 2013).

Wang *et al.* (2016), explains the influence of land use in TN. Conversion of natural forest into agricultural land cause extreme soil degradation. In comparison to cropland TN was higher in shrub land by 10.8% and forest by 39.8%. The degradation of soil quality is due to inappropriate tillage practices and anthropogenic activities. It was found that, bulk density and pH value had significant and negative effect on SOC and TN concentration. The use of organic fertilizer along with chemical fertilizer and farming practices like corn-wheat rotation to rice-wheat rotation increase soil TN (Huang *et al.* 2007).

The research done by Havlin *et al.* (1990), in eastern Kansas soils showed higher nitrogen and carbon value in no tillage soil treatment. This was related to the crop systems with rotations that included high residue producing crops. Soil carbon and nitrogen was only slightly increased by Nitrogen fertilizer.

Zhang *et al.* (2013), reported higher nitrogen value in grassland which was found to be gradually decreasing with depth. They also pointed out that SOC and TN content of soil can be improved by the reconversion of the sloppy croplands to forestlands and grasslands. Albera & Belachew (2011), reported higher TN value in natural forest in (0-5) cm depth than in cultivated land in Bale, South Eastern Ethiopia.

2.10 Carbon nitrogen ratio

Carbon to Nitrogen ratio (C:N) is a proportion of the mass of carbon to the mass of nitrogen in a substance. Such as a C: N of 10:1 implies for each unit of nitrogen in a substance, there are ten units of carbon (USDA 2011). Swangjang (2015), explains Carbon Nitrogen ratio as a significant aspect for soil capability and carbon storage.

Ratio of C: N demonstrates the rate of decomposition of organic matter and outcome of this is the mineralization or immobilization of soil nitrogen. C: N ratio of less than 20 means that mineral N is released in beginning phases of biodegradation process.

The mass of carbon is higher in organic matter than that of nitrogen. The carbon to nitrogen ratio is represented as C:N and usually a single number (Flavel and Murphy 2006). The lower C:N ratio means the quick release of nitrogen into soil for instant crop uptake (Watson *et al.* 2002). C:N ratio greater than 35 outcomes in bacterial immobilization. Soil C: N ratio was reported as strongest predictor of fungal richness in the research by Yang *et al.* (2017), on soil fungal diversity on Tibetian Plateau.

2.11 Soil microbes

Research explains role of soil microbes in productivity and factors that influence growth of micro-organism. Microorganisms undertake important biochemical activities such as fixing atmospheric nitrogen, decomposing organic matter, detoxifying toxic substance, transforming nitrogen, phosphorous, potassium and other secondary micro-nutrients. The population of microbes found in soil is affected by various factors such as soil depth, organic matter, porosity, oxygen, carbondioxide, pH etc. Less number of microbes is found in compact soil and soil with low organic matter percentage (Bhattarai *et al.* 2015). Fungi can be present in almost any environment and can survive in a wide variety of pH and temperatures. (Frac *et al.* 2015).

Study done by Asadu *et al.* (2015), shows significant correlation between some soil chemical properties and microbial densities, which points that microorganisms have vital role in building up of soil nutrients. It was also found that abandoned land had significantly lower pH value and higher bacterial population than in cultivated land.

Though the effect of soil microbes on ecosystem is not fully understood, they are major regulators of plant diversity and productivity in terrestrial ecosystem. For instance, mycorrhizal fungi and nitrogen fixing bacteria are found to provide 5-20% (grassland and savannah) to 80% (temperate and Boreal forest) of all nitrogen and up to 75% of phosphorous that is taken up by plants yearly. Similarly, free living microbes are responsible for regulating plant productivity and microbial pathogens are also important regulators of plant community dynamics and plant diversity as well as determining plant abundance (van der Hejiden *et al.* 2008).

Microbial population exhibit spatial scale patterns that are influenced by geographical separation and environmental diversity. Anthropogenic management play a vital role in microbial distribution in nearby and regional scale. Mycorrhizal fungi are essential part of terrestrial biological system creating symbiotic relation with plant roots. This in turn has significant effect on the ecology and fertility of soil (van der Gast *et al.* 2011).

Difference in soil microbial community composition has been strongly related with total PLFA (Phospholipid ester-linked fatty acid) which is the measure of soil microbial biomass which hints that unstable soil organic matter has affect on microbe composition. Moreover, inputs such as compost, herbicide and water system are related with particular microbial community composition of the distinct cultivated land management types (Steenwerth *et al.* 2002).

CHAPTER THREE: MATERIAL AND METHODS

3.1 Description of the study area

The study was carried in Bhaktapur district which is located in Bagmati Province and has area of 119 km². From geographical point of view, Bhaktapur covers the region between the northern latitude of 27°36' - 27°44' and the eastern longitude of 85°21' - 85°32'. The altitude ranges from 1,331 meters to 2,191 meters above the sea level. The vegetation type is subtropical and temperate with three distinct seasons: hot and dry summer (Feb-May), hot and moist season (Jun-Sep) and cold dry winter (Oct-Jun).

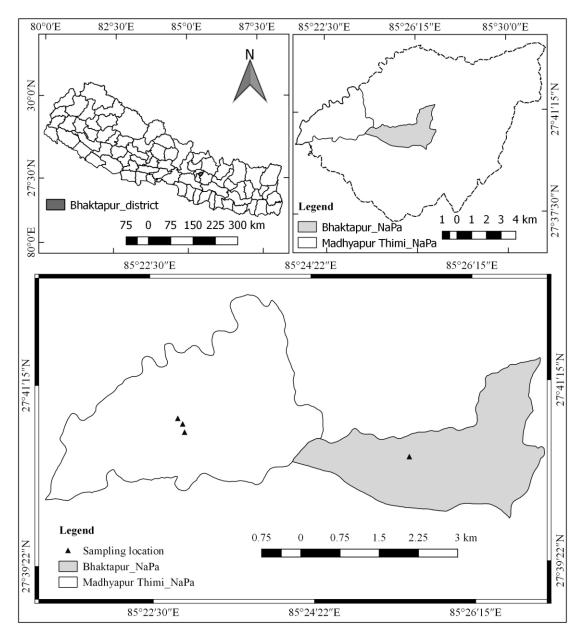
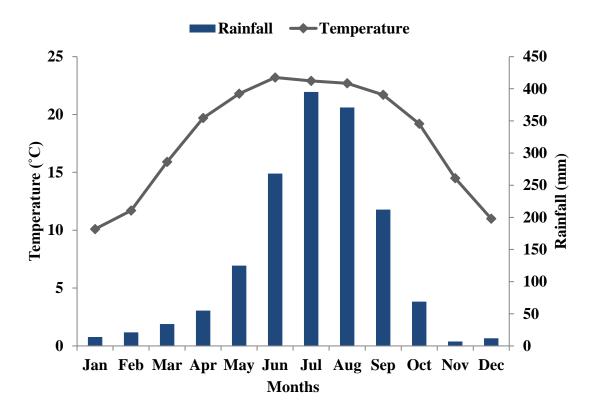
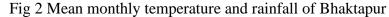


Fig 1 Map of study area





Source: climate-data.org

3.2 Farming system

Two types of farming system were found in our study sites i.e. commercial vegetable farming and cereal farming. Farmers grow seasonal as well as non-seasonal vegetables for commercial farming and cereals such as paddy, wheat, lentil etc. according to season. Farmers use compost fertilizer, animal dung, chicken manure and in some place sewage for irrigation and manure

3.3 Site selection

The sampling site was selected on the basis of land management practices and management practices and other parameters which affect in soil quality.

The study constituted four land management practices:

• Rice field: Rice is cultivated in rainy season. Wheat, lentils are grown in winter. This site lies in Bhaktapur Municipality.

- Vegetable farm treated with fertilizer: Seasonal and Non-seasonal vegetables are grown. Animal dung, chicken manure, fertilizer (Urea, DAP and Potash) is used in farm. This site lies in Bhaktapur Municipality.
- Vegetable farm treated with fertilizer and sewage: Seasonal and non-seasonal vegetables are grown. Farm is irrigated with sewage and animal dung, chicken manure, fertilizer (Urea, DAP and Potash) is used in farm. This site lies in Madhyapur Thimi Municipality.
- Abandoned land: Land left uncultivated for several years. This site lies in Bhaktapur Municipality.

3.4 Soil sample collection

The soil sampling was done in December 2017. Soil samples were collected from four sites (abandoned land, rice field, vegetable farm treated with fertilizer and vegetable farm treated with fertilizer and sewage) from the depth of (0-10) cm and (10-20) cm. Random sampling method was applied. The soil was collected from 5 quadrates of $1 \times 1m^2$ from each landuse type. The distance between two quadrates was 10m (systematic random sampling). From each land use type and management practice, 10 composite samples were taken (five from 0-10 cm and five from 10-20 cm soil depth). Soil samples were cored from four corners and centre of each quadrate and mixed to make a composite sample and kept in an air tight plastic bag then tagged properly. For bulk density, 10 samples were separately collected from (0-10) cm depth and (10-20) cm depth from undisturbed site within a quadrate, then kept in air tight plastic bag and bags were tagged properly.

These samples were brought to the laboratory of Central Department of Botany, Tribhuvan University for analysis. About 2 g of soil sample from each composite sample was separated, tagged and stored in refrigerator at 4 °C for fungal isolation to avoid interspecies microbial interaction. Hence, microbe population will be more or less same at the time of soil sampling.

3.5 Soil analysis

In laboratory analysis of different physical properties such as moisture, bulk density and chemical properties such as EC, SOC, pH, and TN content of soil was done.

3.5.1 Soil sample preparation

Each of the composite samples collected from study area was dried for 15 days, crushed and passed through 2 mm mesh size. Fined soil sample was used for other physicochemical analysis except moisture.

3.5.2 Determination of moisture

Soil moisture content was calculated by the following procedure suggested by Buckman and Brady (1997). 10 g of soil sample was kept inside hot air oven at a constant temperature of 180 °C for 24 hrs. Then dry weight of the soil sample was measured.

Percent of moisture was calculated by using following formula:

$$moisture \ content \ (\%) = \frac{wt \ of \ fresh \ soil \ - \ wt \ of \ oven \ dried \ soil}{wt \ of \ oven \ dried \ soil} \times 100$$

3.5.3 Determination of bulk density

Core method (Blake 1965), was used to determine bulk density of the soil. The surface materials of the soil were removed. Then the core tube was pressed into the soil to collect the soil sample. The samples were brought to lab, oven dried at 105 °C for 24 hrs and weighted to calculate bulk density.

Following relation was used to calculate the bulk density:

Bulk density
$$(g/cm^3) = \frac{Mass of oven dried soil}{volume of soil}$$

3.5.4 Determination of soil pH

Fischer's digital pH meter was used on soil water mixture in the ratio 1:2 for finding out the pH of soil (ASTM G51-95 2012). For preparing the mixture, 10 g of soil was taken in a beaker and 20 ml of distilled water was added to it. The soil water suspension was stirred with a glass rod thoroughly for about 5 minutes and then left for half an hour. The pH meter was turned on and allowed to warm up for 15 minutes. Before measuring the soil pH, the pH meter was calibrated using the buffer solution of pH (pH 4 and pH 7). For measurement electrode was dipped into the beaker containing soil water suspension and the pH value was noted after waiting for 30 seconds.

3.5.5 Determination of EC

EC of soil was measured by using digital EC meter on soil water mixture mixed in ratio of 1:5. For the mixture, 3 g of sieved soil was taken in a beaker and 15 ml of distilled water was added to it and the soil water suspension was stirred with a glass rod for about 3 minutes. The EC meter was turned and allowed to warm up. The EC meter was washed with distilled water before measuring the EC of soil. For measurement, electrode of the EC meter was dipped into the beaker containing soil water suspension and value of EC was noted.

3.5.6 Determination of soil organic carbon

Soil Organic carbon was determined by Walkey and Black method (Gupta 2000). 0.5 g soil sample which was passed through fine sieve (0.5mm) was taken in 500ml conical flask and added 5ml of 1N K₂Cr₂O₇ and 10 ml of conc. H₂SO₄ and reagents were mixed together by gentle swirling. As the reaction was exothermic, the flask was left for about 30 minutes to cool down to room temperature. To that mixture 100ml of distilled water, 5ml Orthophosporic acid and 1ml Diphenylamine indicator solution were added successively and shaken until violet colour appeared. The sample was titrated with 0.5N Ferrous Ammonium sulphate till there was change of colour from violet to brilliant green. The volume of ferrous ammonium sulphate used for titration was noted.

Similar process was applied for blank sample (without soil).

Formula used to calculate SOC,

Percentage Organic Carbon (R) =
$$\frac{(B-T) \times S \times 0.003 \times 100}{W}$$

where,

B = volume of Ferrous Ammonium Sulphate solution used for blank titration

- T = volume of Ferrous Ammonium Sulphate solution consumed with soil
- S = strength of Ferrous Ammonium Sulphate
- W = Amount of soil sample taken in gm

Total Organic Carbon (%) = $R \times 1.3$ Organic Matter (%) = $R \times 1.3 \times 1.724$ SOC Stock = $SOC \times BD \times Soil depth$ (Poeplau et al. 2017)

3.5.7 Determination of total Nitrogen

Nitrogen content of the soil was determined by using micro-Kjeldahl's method (Jackson 1967). This method involves the conversion of organic nitrogen into ammonia by boiling with conc. H₂SO₄: the ammonia was subsequently liberated from its sulphate by distillation in presence of an alkali, which is titrated against HCl.

Digestion

1gm of oven dried soil screened through 0.2mm sieve was taken in a Kjeldahl digestion flask along with 3.5gm K₂SO₄ and 0.4gm CuSO₄ and 6ml of conc. H₂SO₄ was added to the mixture with gentle shaking. The mixture was heated on the preheated heating mantle at low heat until bubbles disappeared from the black mixture. The heat was raised until the content of the flask changed to grey or greenish in colour for complete digestion. The digestion sample was cooled down to room temperature and about 50ml of distilled water was added to the mixture with gentle shaking.

Distillation

The digested materials in the kjeldahls distillation flask were assembled in distillation chamber and warmed up for 15 minutes of adjusting the heating mantle adjuster at 30. In the kjeldahl distillation flask, 30ml sodium hydroxide (40% NaOH) was added through the funnel connected to tube of distillation flask and the cork was set. In cleaned dry 100ml beaker, 10ml boric acid indicator was pipette and placed below the nozzle of the condenser in such a way that the end of nozzle dips into the indicator. The heating mantle's temperature adjuster was set at 70. When the distillate began to condense, the colour of boric acid indicator changed from pink to green. Distillation was continued until the volume of distillate in beaker reached about 450ml.

Titration

50ml distillate containing in beaker was titrated with hydrochloric acid (0.1N) in burette. The volume of volume of HCl consumed by distillate to change the green colour into pink was recorded. The same procedure was followed for other samples. For every 10 samples, 1 blank sample (without soil) was taken.

Formula for the calculation of % of Nitrogen:

Nitrogen content of soil (%) =
$$\frac{14 \times N \times (C - B)}{W} \times 100$$

where,

N=Normality of HCl used for titration

C=Volume of HCl consumed during titration (ml)

B=Volume of HCl consumed during blank titration (ml)

W=Amount of soil used for the test (gm)

3.5.8 Determination of carbon nitrogen ratio

Carbon nitrogen ratio was determined by dividing SOC concentration by TN concentration. So, the ratio we have calculated can be called as organic carbon to Nitrogen ratio.

3.6 Culture of fungi

3.6.1 Sterilization technique

Sterilization is the destruction or removal of micro-organisms including bacteria and their endospores, virus and fungi. This can be accomplished by physical method such as heat, radiation and filtration or chemical methods. The wide application of sterilization processes makes it necessary to impose strict control measure to validate the results. The methods of sterilization were used in laboratory.

Dry sterilization: Glass ware such as petridishes, conical flask, beaker, test tube etc. were wrapped in newspaper and kept in hot air oven at 15 °C for 2 hrs.

Wet sterilization: PDA media was kept in autoclave at 121 °C under the pressure of 15 lb for 30 minutes.

The laminar air flow is sterilized by using sprit before starting culture process. Hand was sterilized by using sprit and inoculating needles were sterilized by using sprit and heating over flame for few seconds.

3.6.2 Preparation of media

Potato dextrose agar (PDA) was used for the isolation and growth of fungi. Antibacterial antibiotics (Amoxicillian 30mg/L) were used to inhibit the bacterial growth in culture media.

Composition of PDA for 1000 ml media:

Distilled water:	1000 ml
Potato:	200 g
Dextrose:	20 g
Agar:	20 g

3.6.3 Isolation of soil fungi

Serial dilution plate method (Walkman 1972): 1 gm of soil sample from each was added in 9 ml of distilled water. Then 1ml solution was taken and transfer into nest tube containing 9 ml of distilled water. Similarly, solution of 10⁻³, 10⁻⁴ and 10⁻⁵ solution was prepared in order to avoid fungal colonies.

Then spread plate method was done for this 1ml of the dilution was poured in petridish over which 15ml molten media was poured. Then the Petridish was rotated by hand in 8-shape for uniform dispersion of solution on the solidified medium. Then those petridish were kept in incubator in inverted position at 25 ± 5 °C for 15 days.

Several fungal colonies were sporulate in petriplate. These colonies were re-isolated separately by the help of sterilized needle into freshly prepared PDA medium. It is difficult to obtain pure culture so spread method is applied.

3.6.4 Identification of fungi

Fungi were identified based on the morphological character, colony structure through microscopical examination. Fungi were stained by using lactophenol and cotton blue. Cotton blue was used for staining cytoplasm resulting light blue background and lactophenol was used for cleaning agent. Fungal morphology was studied macroscopically by observing colony features colour, texture and microscopically by staining with lactophenol cotton blue and observed under microscope for the conidia, conidiophores and arrangement of spores. The fungi were identified with the help of standard literature and book (Webster & Weber 2007; Gilman 1975).

3.6.5 Statistical analysis

One way analysis of variance (ANOVA) was used to analyze moisture, bulk density, pH, EC, SOC, SOM, SOC stock and total nitrogen among the soil of different management practice. Tukey test was performed to compare differences in means of the parameters at significance level of p < 0.05. Comparison of soil parameters between the soil depth of (0-10) cm and (10-20) cm was analyzed using independent sample T-test. All the statistical analysis was performed using SPSS software (version 20).

CHAPTER FOUR: RESULTS

4.1 Physical and Chemical properties of soil at different management practices of land

4.1.1 Moisture content

In the soil depth (0-10) cm, the moisture content varied among different land management practices and it was found to be highly significant (p < 0.001). The average value of moisture content was the highest in vegetable farm with chemical fertilizer and sewage (20.11%) followed by rice field (12.24%), fertilizer applied vegetable farm (11.61%) and least in abandoned land (10.34%) (fig 3).

Similarly, in soil depth 10-20 cm, highly significant difference was found in moisture content among different land management practices (p < 0.001). The average value of moisture content was the highest in vegetable farm with sewage and fertilizer (20.89%) followed by rice field (12.84%), vegetable farm treated with fertilizer (10.92%) and the least in abandoned land (9.016%) (fig 3).

No significant difference between two depths was found in all four sites: rice field (p=0.064), vegetable farm with fertilizer field (p=0.563), vegetable farm with sewage and fertilizer (p=0.620) and in abandoned land (p=0.029) (fig 3).

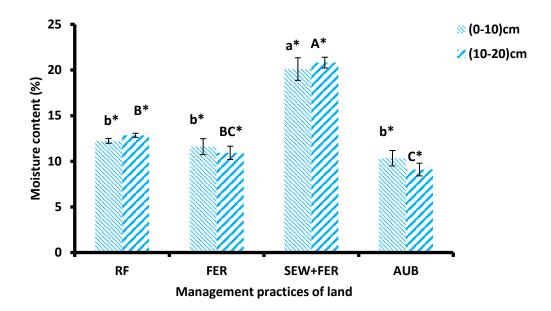


Fig 3 Moisture content in soil depth of 0-10 cm and 10-20 cm in different land management practices

(RF=Rice field, FER= vegetable farm with fertilizer, SEW+FER = vegetable farm treated with sewage and fertilizer, AUB = Abandoned land; Letters above the error bar indicate differences in the mean value among the groups and stars indicate differences between two depths)

4.1.2 Soil bulk density

In the soil depth of 0-10 cm, there was no significant difference in the bulk density of soil among different land management practices (p = 0.341). The average value of bulk density was the highest in Vegetable farm treated with fertilizer (1.26 g/cm³) followed by rice field (1.12g/cm³), abandoned land (1.16g/cm³) and the lowest in the soil of vegetable farm treated with sewage and fertilizer (0.94 g/cm³) (fig 4).

Similarly, in the soil depth of 10-20 cm, significant difference was not found in the bulk density of soil among different land management practices (p=0.099). The average value of bulk density was the highest in the soil of vegetable farm treated with fertilizer (1.43 g/cm³) followed by abandoned land (1.38 g/cm³), rice field (1.23 g/cm³) and the lowest in cultivated land irrigated with sewage (1.05 g/cm³) (fig 4).

The bulk density of soil increased with the depth of the soil in all land management practices and the difference in bulk density between the two depth was not significant in all land management practices: RF (p=0.885), FER (p=0.053), SEW+FER (p=0.173) and AUB (p=0.117) (fig 4).

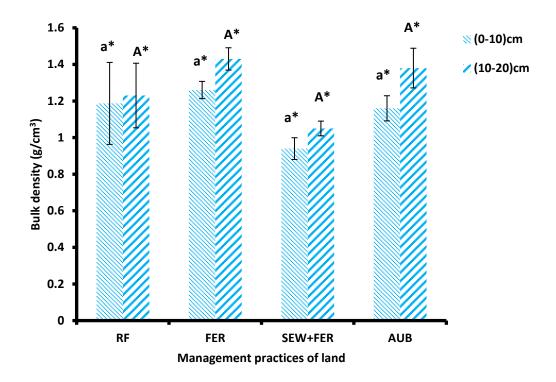


Fig 4 Bulk density in the soil depth of 0-10 cm and 10-20 cm in different land management practices

(RF=Rice field, FER= vegetable farm with fertilizer, SEW+FER = vegetable farm treated with sewage and fertilizer, AUB = Abandoned land; Letters above the error bar indicate differences in the mean value among the groups and stars indicate differences between two depths)

4.1.3 Soil pH

In the soil depth of 0-10 cm, there was no significant difference in soil pH among different land management practices (p = 0.101). The average value of soil pH was the highest in vegetable farm treated with sewage and fertilizer (6.776) followed by abandoned land (6.54), vegetable farm treated with fertilizer (6.21) and the least in rice field (6.1668) (fig 5).

Similarly, in soil depth of 10-20 cm, there was significant difference in pH among different land management practices (p<0.001). The average value of the soil pH was the highest in vegetable farm treated with sewage and fertilizer (6.93) followed by fertilizer applied vegetable farm (6.47), rice field (6.25) and the lowest in abandoned land (6.042) (fig 5).

The pH value increases with depth in all land management practices. The difference in soil pH between the depth of 0-10 cm and 10-20 cm was significantly different only in the abandoned land (p < 0.02) (fig 5).

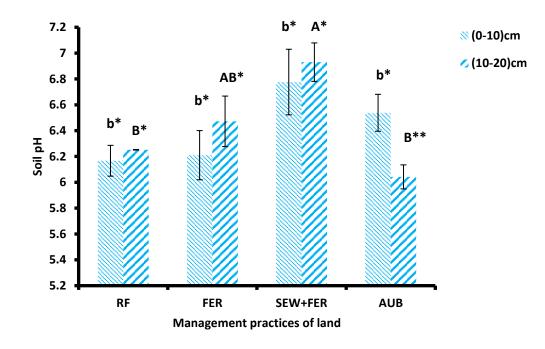


Fig 5 Soil pH in the soil depth of 0-10 cm and 10-20 cm in different land management practices

(RF=Rice field, FER= vegetable farm with fertilizer, SEW+FER = vegetable farm treated with sewage and fertilizer, AUB = Abandoned land; Letters above the error bar indicate differences in the mean value among the groups and stars indicate differences between two depths)

4.1.4 Electric conductivity (EC)

In the soil depth of 0-10 cm, the EC of the soil was highly significant among different land management practices (p<0.001). The EC was the highest in vegetable farm treated with fertilizer (0.256 dS/m) followed by rice field (0.06 dS/m), the abandoned land

(0.054 dS/m) and the least in the fertilizer and sewage applied vegetable farm (0.5dS/m) (fig 6).

Similarly, in the soil depth of 10-20 cm, it was highly significant in EC of soil among different land management practices (p < 0.001). The EC was the highest in vegetable farm treated with sewage and fertilizer (0.048 dS/m) followed by the vegetable farm treated with fertilizer (0.11 dS/m), the abandoned land (0.04 dS/m) and the lowest in the abandoned land (0.04 dS/m) (fig 6).

Though the EC of soil decreased with soil depth in all land management practices the difference in EC of soil between the soil depths was significant only in abandoned land (p = 0.008) (fig 6).

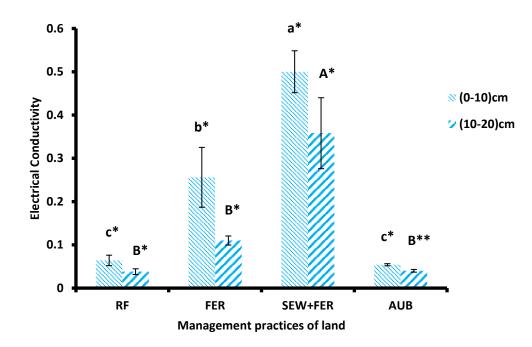


Fig 6 Soil electrical conductivity in the soil depth of 0-10 cm and 10-20 cm in different land management practices

(RF=Rice field, FER= vegetable farm with fertilizer, SEW+FER = vegetable farm treated with sewage and fertilizer, AUB = Abandoned land; Letters above the error bar indicate differences in the mean value among the groups and stars indicate differences between two depths)

4.1.5 Soil organic carbon

Soil organic Carbon varied among different land management practices. In the soil depth of (0-10) cm, highly significant difference was found among land management practices (p<0.001). Soil organic was found the highest in abandoned land (2.568%) followed by sewage and fertilizer applied vegetable farm (2.09%), fertilizer applied vegetable farm (1.3%) and the lowest in rice field (1.214%) (fig 7).

Also, in soil depth (10-20) cm highly significant difference was found among different land management practices (p<0.001). SOC was found the highest in sewage and fertilizer applied vegetable farm (1.98%) followed by abandoned land (1.6%), fertilizer applied vegetable farm (1.03%) and the lowest in rice field (0.90%) (fig 7).

However, SOC decreases with depth in all management practices of land, the significant difference was found only in abandoned land (p<0.01) (fig 7).

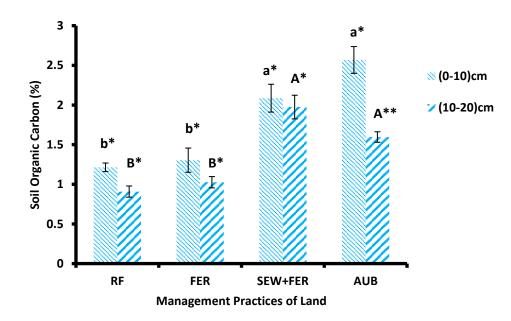


Fig 7 Soil organic carbon in the soil depth of 0-10 cm and 10-20 cm in different land management practices

(RF = Rice field, FER = vegetable farm with fertilizer, SEW+FER = vegetable farm treated with sewage and fertilizer, AUB = Abandoned land; Letters above the error bar indicate differences in the mean value among the groups and stars indicate differences between two depths).

4.1.6 Soil organic matter

In soil depth (0-10) cm, it was highly significant difference among different land management practice (P<0.001). The SOM was highest in the abandoned land (5.75%) followed by sewage and fertilizer applied vegetable farm (4.68%), vegetable farm treated with fertilizer (2.92%) and lowest in rice field (2.72%) (fig 8).

In soil depth (10-20) cm also it was found highly significant difference among different land management practices of SOM (P<0.001). The SOM value was the highest in sewage and fertilizer treated vegetable farm (4.42%) followed by abandoned land (3.57%), vegetable farm treated with fertilizer (2.29%) and lowest in rice field (2.04%) (fig 8).

SOM decreases with depth in all management practices of land. No significant difference was found between two depth in all management practices of land. (fig 8).

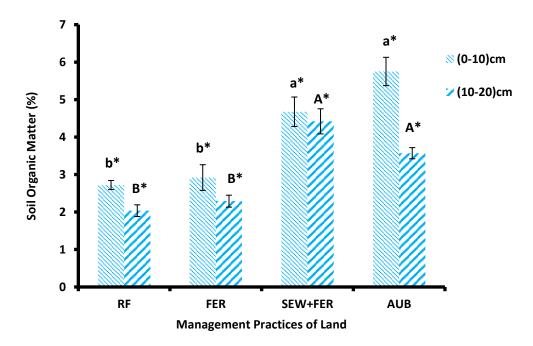


Fig 8 Soil organic matter in the soil depth of 0-10 cm and 10-20 cm in different land management practices

(RF= Rice field, FER= vegetable farm with fertilizer, SEW+FER = vegetable farm treated with sewage and fertilizer, AUB = Abandoned land; Letters above the error bar indicate differences in the mean value among the groups and stars indicate differences between two depths)

4.1.7. Soil organic carbon stock

There is a variation in SOC Stock among different management practices of land. In soil depth (0-10) cm, significant difference was found in SOC Stock in different land management practices (P=0.004). The SOC content was high in abandoned land (30.07 Mg C/ha) followed by vegetable farm treated with sewage and fertilizer (19.6 Mg C/ha), vegetable farm treated with fertilizer (16.43 Mg C/ha) and the lowest in rice field (14.824 Mg C/ha) (fig 9).

In soil depth (10-20), highly significant difference was found between SOC stock of different land management practices (P<0.001). The SOC content was highest in the abandoned land (21.134 Mg C/ha) followed by vegetable farm treated with sewage and fertilizer (41.43 M C/ha), vegetable farm treated with fertilizer (29.04 M C/ha) and the lowest in rice field (11.109 Mg C/ha) (fig 9)

SOC stock decreases with depth in all management practices of land. The significant difference between the two soil depths was found only in abandoned land (p=0.046) (fig 9).

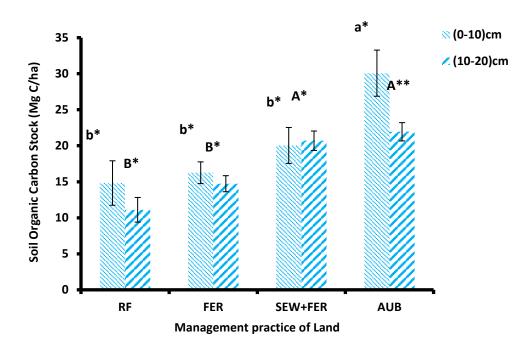


Fig 9 Soil organic carbon stock in the soil depth of 0-10 cm and 10-20 cm in different land management practices

(RF=Rice field, FER= vegetable farm with fertilizer, SEW+FER = vegetable farm treated with sewage and fertilizer, AUB = Abandoned land; Letters above the error bar indicate differences in the mean value among the groups and stars indicate differences between two depths)

4.1.8 Total nitrogen

Total nitrogen of soil decreases with depth. Total nitrogen was highest in vegetable farm treated with fertilizer and sewage and lowest in rice field in both soil depths. In soil depth (0-10) cm, highly significant difference was found in total nitrogen of soil in different management practices of land (P<0.001). Total nitrogen was the highest in vegetable farm with fertilizer and sewage (2.924 mg/g) followed by abandoned land (2.69 mg/g), vegetable farm treated with fertilizer (2.06 mg/g) and the lowest in rice field (2.742 mg/g) respectively (fig 10).

In soil depth (10-20) cm, significant difference was found in total nitrogen of soil in different management practices of land (P<0.02). Total nitrogen was highest in the vegetable farm treated with fertilizer and sewage (2.742 mg/g) followed by abandoned land (1.8 mg/g), vegetable farm treated with fertilizer (1.6 mg/g) and the lowest in rice field (1.468 mg/g) (fig 10).

Total nitrogen decreases with depth and significant difference between two depth was found in RF (P<0.001) and FER (P<0.05) only (fig 10).

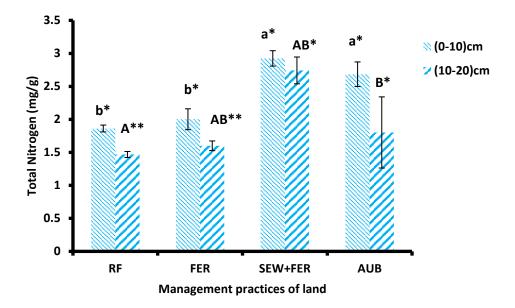


Fig 10 Total nitrogen in the soil depth of 0-10 cm and 10-20 cm in different land management practices

(RF=Rice field, FER= vegetable farm with fertilizer, SEW+FER = vegetable farm treated with sewage and fertilizer, AUB = Abandoned land; Letters above the error bar indicate differences in the mean value among the groups and stars indicate differences between two depths)

4.1.9 Carbon nitrogen (C/N) ratio

Carbon nitrogen ratio decreases according to depth except in sewage and fertilizer treated field. It was found highly significant difference in (0-10) cm in different management practices of land (P<0.001). The highest C/N was observed in abandoned land (1.02) followed by vegetable farm treated with fertilizer (0.67), rice field (0.65) and the lowest in the vegetable farm treated with sewage and fertilizer (0.54) (fig 11).

In soil depth (10-20) cm, significant difference was observed among different land management practices (p=0.005). The highest C/N ratio was observed in abandoned land (1.07) followed by vegetable farm treated with sewage and fertilizer (0.72), followed by vegetable farm treated with fertilizer (0.64) and rice field (0.62). (fig 11). No significant difference was found between two depths (fig 11).

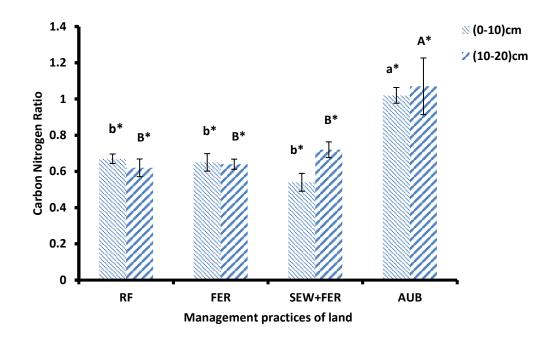


Fig 11 Carbon nitrogen ratio in the soil depth of 0-10 cm and 10-20 cm in different land management practices

(RF=Rice field, FER= vegetable farm with fertilizer, SEW+FER = vegetable farm treated with sewage and fertilizer, AUB = Abandoned land; Letters above the error bar indicate differences in the mean value among the groups and stars indicate differences between two depths)

4.2 Correlation between various physical and chemical parameters

4.2.1 Relationship between bulk density and soil organic carbon (%)

In soil depth of (0-10) cm, bulk density showed negative correlation with SOC percentage (r = -0.084, p=0.725) (fig 12)

Again, bulk density exhibited negative correlation with SOC percentage in 10-20 cm soil depth (r = -0.370, p=0.108) (fig 13).

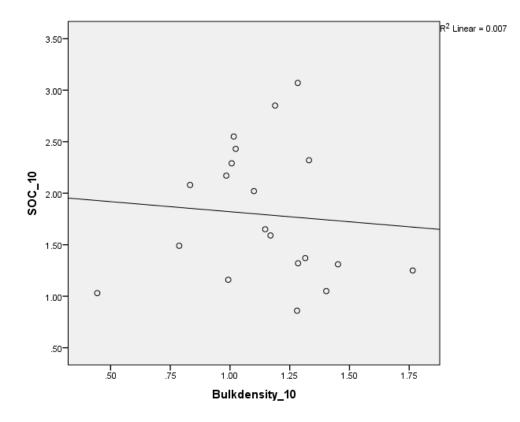


Fig 12 Correlation between bulk density and SOC % in soil depth (0-10) cm

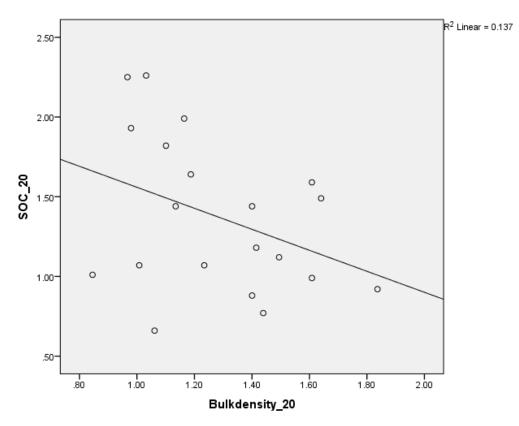


Fig 13 Correlation between bulk density and SOC % in soil depth (10-20) cm

4.2.2 Relationship between pH and SOC %

Soil pH showed positive correlation with SOC (r=0.299, p=0.2) in (0-10) cm soil depth (fig 14). Also, pH showed positive correlation with SOC (r=0.374, p=0.105) in (10-20) cm soil depth (fig 15).

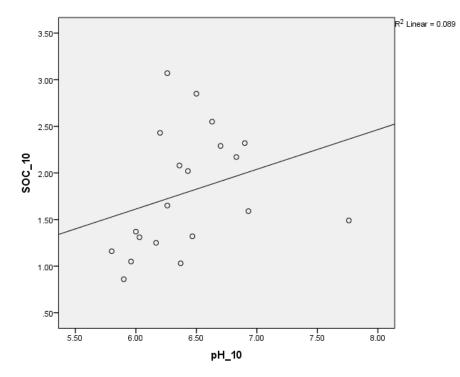


Fig 14 Correlation between pH and SOC % in (0-10) cm depth

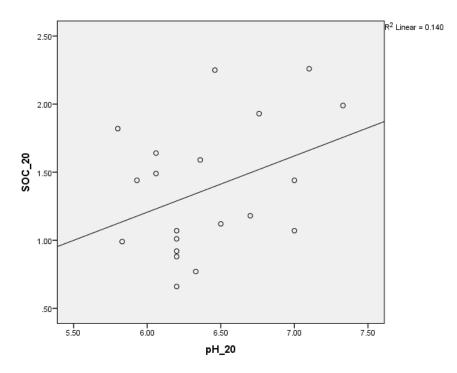


Fig 15 Correlation between pH and SOC % in (10-20) cm depth

4.4 Soil fungi isolated from different management practices of land

SEW + FER SEW + FER S.N. Fungi Class RF RF FER FER AUB AUB (10-20)(0-10)(0-10)(10-20)(0-10)(10-20)(0-10)(10-20)Aspergillus niger Eurotiomycetes 1 ++++++ +_ Mucoromycetes Mucor sp. 1 2 ++ ++++ +_ 3 Rhizopus sp. Mucoromycetes +++_ ++_ _ Sordariomycetes 4 Trichoderma sp. ++++_ _ _ -5 Geotrichum sp. Saccharomycetes +_ _ _ _ _ _ _ 6 Rhizomucor sp. Zygomycetes _ _ ++_ _ _ _ Sordariomycetes 7 Fusarium sp. 1 + _ _ _ +_ _ _ Fusarium sp. 2 Sordariomycetes 8 ++++_ _ _ _ 9 Alternaria sp. Dothiodeomycetes +_ _ _ _ _ _ _ Penicillium sp. Eurotiomycetes 10 +_ _ _ _ _ _ _ 11 Aspergillus Eurotiomycetes +_ _ _ _ _ -_ flavus 12 Mucor sp. 2 Mucoromycetes +++_ _ _ _ _

Table 1: List of fungi isolated from the studied land management practices

+ = presence of species

- = absence of species

Total 12 species of soil fungi were isolated. Number of fungal species is seen to be gradually decreasing with depth. In rice field, nine species were noted in (0-10) cm depth and one species in (10-20) cm depth. In vegetable farm treated with fertilizer, six species were found in (0-10) cm depth and similarly, four species were noted in (10-20) cm depth.

In vegetable farm treated with sewage and fertilizer, seven species were found in (0-10) cm depth and four species were found in (10-20) cm depth. In abandoned land, five species were found in (0-10) cm depth and three species were found in (10-20) cm depth.

CHAPTER FIVE: DISCUSSION

5.1 Moisture Content

High value of moisture content was in sewage treated vegetable farm in (0-10) cm depth and (10-20) cm depth (fig 3). This high value might be due to the regular irrigation in the field from the roadside sewage. Low was in abandoned land in surface and in subsurface of soil. Low value of moisture could be attributed to no any artificial irrigation on the abandoned land.

The difference in moisture content across depth was found insignificant across the studied land management practices. Gao *et al.* (2011), explains that the distribution of soil moisture according to depth differed in different seasons and soil texture plays a vital role in influencing the surface soil moisture. Soil moisture content was found to be significantly different among the land management practices. Niu *et al.* (2015), found that land use type affected soil moisture condition in semi-arid sandy soils.

5.2 Bulk density

Bulk density refers to the compactness of soil. Bulk density shows variation according to land use type. The highest bulk density value was recorded in vegetable farm treated with fertilizer and lowest value recorded in sewage treated vegetable farm in (0-10) cm (fig 4). Whereas highest value was recorded in vegetable farm with fertilizer and lowest value in sewage treated field in (10-20) cm depth (fig 4). Soil with a bulk density value higher than 1.6 g/cm³ tends to restrict the root growth.

Bulk density can be changed by management practices that affect soil cover, organic matter, soil structure, compaction and porosity. Excessive tillage destroys soil organic carbon matter and weakness the natural stability of soil aggregates making them susceptible to erosion caused by water and wind (Agbede 2010). According to Materechera (2018), the bulk density decreases in the surface horizon of the soil. Ghimire *et al.* (2019), also reported decreasing bulk density on all land management practices in their research done in four land management practices in Makawanpur district. Similar to them, our results show higher bulk density on 10-20 cm soil depth than 0-10 cm soil depth on all land management practices. This could be due to lower organic matter contents, less aggregation, fewer roots and other soil dwelling organisms and soil compaction (Liefeld *et al.*, 2005). Organic matter content in soil decreases with

increasing depth which ultimately reduces porosity and soil compactness (Chaudhari *et al.*,2013). Also, my result show lowest bulk value in sewage treated field corresponding to the results of Mojiri (2011).

5.3 Soil pH

Soil pH was affected by different land management practices. The amount of fertilizer and nutrients applied to the soil make differentiation in pH level.

Kenya Soil Survey 1987, classification of soil reaction rates soil with pH <4.5, 4.5-5.0, 5.1-5.5, 5.6-6.0, 6.1-6.5, 6.6-7.3, 7.4-8.4, 8.5-9.0, and >9.0 are classified as extremely acidic, very strongly acidic, strongly acidic, medium acidic, slightly acidic, neutral, mildly alkaline, strongly alkaline and very strongly alkaline. Based on this rating, our soils vary from slightly acidic to neutral. The soil pH values on rice field and vegetable farm treated with fertilizer are slightly acidic while the soil treated with sewage was neutral. It was found on both soil depths that the soil pH had positive correlation with the SOC concentration of soil.

The findings showed high pH value in sewage treated field on subsurface soil and lowest in abandoned land on subsurface soil (fig 5). This is similar to findings of long-term application of sewage water in farm increases pH level (Rusan *et al.* 2007; Yadav *et al.* 2002; Narwal *et al.* 1993). But, Singh and Verloo (1996) also reported lower pH in sewage water applied area.

5.4 Soil electric conductivity

The highest value of EC was recorded in sewage treated field and lowest in abandoned land (fig 6).

Electrical conductivity refers to the saline nature of soil. According to Kenya soil survey (KSS) 1987, key to salinity classes, the soil EC values (in dS/m) of <4, 4-8, 8-15, 15-30 and >30 are ranked as non-saline, slightly saline, moderately saline, strongly saline and excessively saline. Based on the ratings of EC of soil, less than 4 dS/m were classified as nonsaline.

The findings of our research showed EC values less than 4 so there was no risk of salinity in our study area. Highest value of EC was recorded in sewage treated vegetable farm which was similar to the results of Rusan *et al.* (2007), Narwal *et al.* (1993).

According to Mohammad and Mazahreh (2003), presence of TDS in waste water leads to high value of EC.

5.4 Soil organic matter

Soil Organic Matter is the reservoir of nutrients (such as N, P and K) also important for plant growth (Baldock & Nelson 2000; Powlson *et al.* 2013; Sparks 2003). Soil organic matter was high in rice field and lowest in abandoned land in (0-10) cm depth and in (10-20) cm soil SOM content was high in rice field and lowest in sewage treated field (fig 8).

According to Yadav *et al.* (2002) application of waste water in farm increases organic matter to 1.24 - 1.78% and fertility status of soil up to distance 1 km from disposal channel. But Rusan *et al.* (2007) reported there was no positive relation of waste water application in field with SOM. According to depth of soil, organic matter present in soil and aeration plays role in determining soil microflora and fauna. The organic matter and soil aeration plays positive relation for the formation of microflora and fauna whereas increasing depth decreases microflora and fauna (Bhattarai *et al.* 2015).

5.5 Soil organic carbon and soil organic carbon stock

Soil organic carbon is the chemical parameters to measure the soil quality. Soil plays major role in carbon sequestration. Soil organic carbon is measurable component of soil organic matter. Presence of high soil organic carbon was seen in abandoned land among different management practices types. This abandoned land was farm land a long time ago. The accumulation of carbon in land show higher SOC (Liu *et al.* 2014).

SOC value was found to decline with increasing depth in all soil use types. Higher value of SOC on surface layer can be attributed to higher organic matter content and less effect of the parent materials also SOC shows variation according to landuse types Ghimire *et al.* (2019) and Zhao *et al.* (2015) reported similar findings.

Soil organic carbon stock decline with depth in all management practices except in sewage treated vegetable farm (fig 9). SOC Stock was reported higher in abandoned land in soil surface and subsurface of soil whereas rice field contain lowest values.

SOC concentration of soil was reported to have negative correlation with bulk density in our research at both soil depths (fig 12,13). Also, Sakin *et al.* (2011), state that along with increasing depth SOC decreases on BD.

5.6 Total nitrogen

Nitrogen is one of the constraints for development of plant on soil. It exists in organic as well as inorganic forms. But on most soils, over 90 % of N is in structure of organic matter so that it is saved from being lost (Subbian *et al.* 2000). Management practices of land play vital role in the total nitrogen content of soil. Total nitrogen content showed high value in sewage treated vegetable farm (2.83mg/gm) and whereas lowest value in rice field (1.66mg/gm) in study site similar to this study Total nitrogen decreases with increasing depth was observed by Pham *et al.* (2018).

The total nitrogen values of <0.05, 0.05-0.12, 0.12-0.25 and >0.25 % are ranked as very low, low, moderate and high respectively (Tadesse 1991). Based on this ranking, the status of N of rice field and vegetable farm treated with fertilizer was moderate, farm treated with sewage and fertilizer was high whereas abandoned land has both high value on surface and moderate value on subsurface (fig 10). High value of Nitrogen on surface of abandoned land is due to biological production of N and releasing plant available nitrogen (Liu *et al.* 2014).

5.7 Carbon Nitrogen ratio of soil

Carbon nitrogen showed high value in abandoned land and low in rice field. The study carried by John *et al.* (2005) recorded greater C/N ratio in forest soil than agricultural soil. Lal and Puget (2005) found higher C/N ratio in forest soil than cultivated soil and pasture land. In this study we found that Carbon Nitrogen ratio was almost same at both depths (fig 11) of soil that was corresponding to the results of Yang *et al.* (2010).

5.8 Soil fungal diversity

In four different management practices of land 12 species of fungi were isolated among them one was unidentified. Fungal diversity was found more in sewage treated vegetable farm and lowest in abandoned land. There was a drop in number of fungi from surface to subsurface soil in all land management practices. Fierer *et al.* (2013) showed declining population of microorganism in increasing soil depth in their similar research.

Fungal diversity and activities of fungi are controlled by various biotic elements (plants and other organisms) and abiotic elements (soil pH, moisture, salinity structure and temperature) (López-Bucio *et al.* 2015; Rouphael *et al.* 2015). As microbial organisms are significant to the soil ecosystems, the effect of fertilization on soil microbial communities is a growing concern (Sun *et al.* 2016).

Greater number of fungal species was recorded in sewage treated vegetable farm and with compared to other studied land management practices (Table 1). This may be due to neutral nature (pH = 6.77) and organic matter content of soil treated with sewage. Sun *et al.* (2016) reported that addition of organic matter had a greater impact on fungal composition as compared to when solely treated with chemical fertilizer. Moreover, soil treated with different type of organic materials (wheat straw, cow manure and pig manure) had presence of significantly different fungal communities. They point that difference in the carbon composition of the different organic material may be a possible reason for this. According to Moll *et al.* (2015) resource type and availability controls the fungal communities of soil.

The common soil fungi are organic matter decomposers such as *Aspergillus* spp, *Alternaria* spp, *Fusarium* spp, *Mortirella* spp, and *Penicillium* spp. Arable soil contains higher fungal diversity than non-arable soil (Živanov *et al.* 2017). Similar to our study showed the major fungi found were organic matter decomposer type. Phytopathogenic fungi *Fusarium* spp. was common to all studied lands and few species like *Penicillium* spp. and *Trichoderma* spp. known as adversary to phytopathogenic species were also reported in this study. These fungi have role in protecting plant against pathogenic microorganisms as natural specialists which impact soil fertility (Frac *et al.* 2017). Species of *Trichoderma* (*T. asperellum*, *T. atroviride*, *T. virens* and *T. viride*) are widely used in biocontrol and also known as biostimulants for horticultural crops (López-Bucio *et al.* 2015).

CHAPTER SIX: CONCLUSION AND RECOMMENDATIONS

Conclusion

The findings of research showed variation in soil physico-chemical parameters among different land management practices. Soil total nitrogen and soil organic was high in vegetable farm treated with sewage and fertilizer with mildly alkaline, non-saline soil character having bulk density (0.94 g/cm³). Eight fungal species were isolated in this field.

Whereas rice field has low soil organic carbon and nitrogen content with non-saline slightly acidic soil character with bulk density (1.055 g/cm³) and eight fungi species. From this result we can conclude that management practice influence greatly on soil physicochemical parameters. Sewage and fertilizer treated field shows high nutrients than other management practices of land. Control use of sewage for irrigation increases nutrient of soil.

Recommendations

Soil physicochemical properties should be regularly monitored in different management practices so that effect of certain management practice on land can be understood. Farmers should compost crop residue and apply in the soils for increased sustainable crop production. Use of sewage of the field should be in controlled manner such that the soil physicochemical and fungal properties are not disturbed in long term.

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PHOTOPLATES



Site 1: Vegetable farm treated with sewage and fertilizer



Site 2: Vegetable farm treated with fertilizer



Group Photograph of fieldwork team on Site 3: Abandoned land



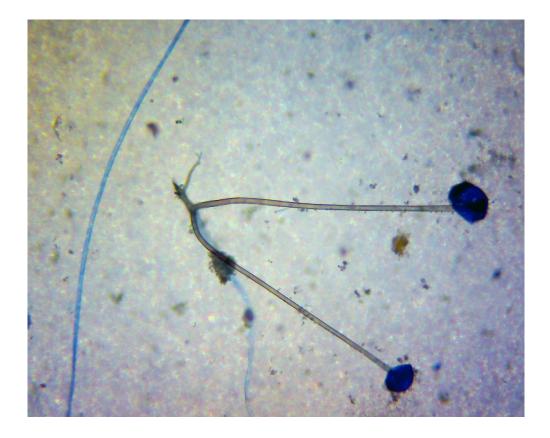
Laboratory work



Culture of fungus



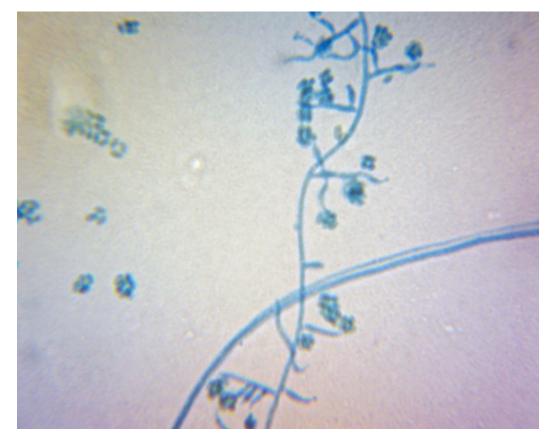
Culture of Fungus 2



Rhizopus sp.



Aspergillus flavus



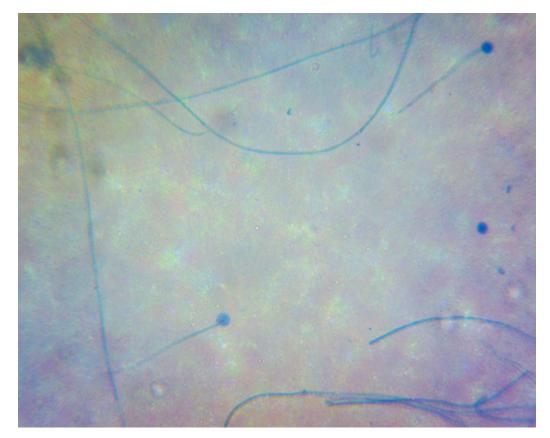
Trichoderma sp.



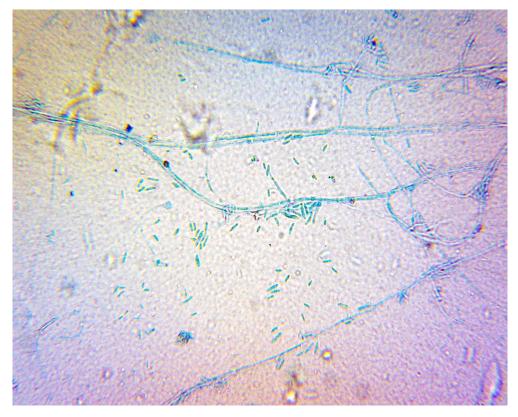
Aspergillus niger



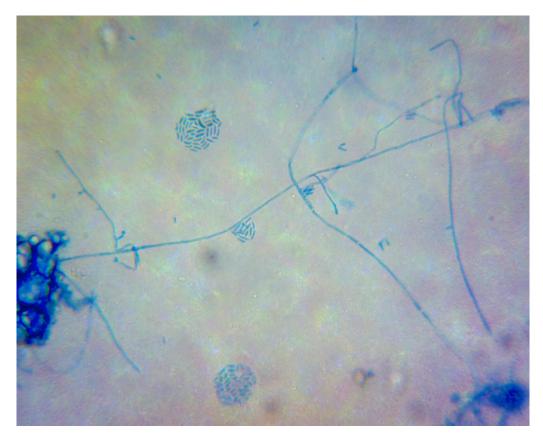
Mucor sp. 1



Mucor sp. 2



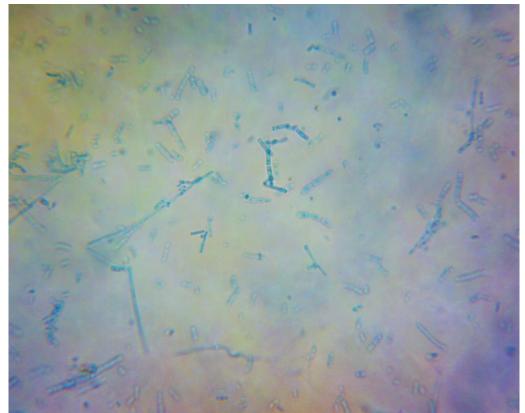
Fusarium sp. 1



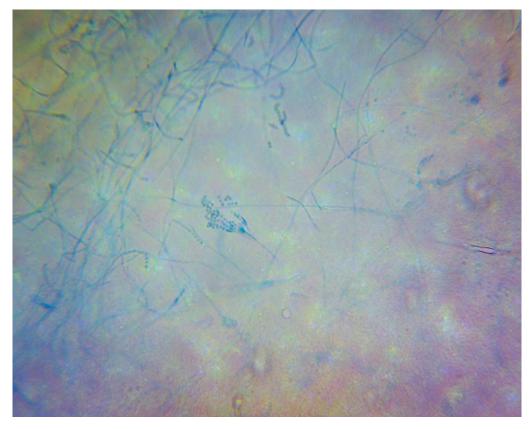
Fusarium sp. 2



Alternaria sp.



Geotrichum sp.



Penicillium sp.



Rhizomucor sp.