UNIVERSITY FOR DEVELOPMENT STUDIES, TAMALE

MANAGEMENT OF SHEA NUT WASTE WITH INDIGENOUS SOIL BACTERIA

BY

FRANCIS MWIN-IR-ME DANIKUU (MSc. CLINICAL MICROBIOLOGY)

(UDS/BT/0001/2009)

THESIS SUBMITTED TO THE DEPARTMENT OF BIOTECHNOLOGY, FACULTY OF AGRICULTURE IN FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF PhD DEGREE IN BIOTECHNOLOGY

NOVEMBER, 2016

Declaration

Student

I hereby declare that this thesis is the result of my own original work and that no part of it has been presented for another degree in this university or elsewhere:

Candidate's Signature: ….....…… Date ……

Name: Francis Mwin-Ir-Me Danikuu

Supervisors'

I hereby declare that the preparation and presentation of the thesis was supervised in accordance with the guidelines on supervision of thesis laid down by the University for Development Studies.

Principal Supervisor's Signature: …… Date ……

Name: Prof. Elias N. K. Sowley

Co-Supervisor's Signature (if any): .... Date ……

Name: Prof. Albert K. Quainoo

Abstract

Shea nut cake contains high levels of tannins which make it toxic and recalcitrant to biodegradation. Soils receiving shea nut cake are polluted. Microbial removal of tannins from shea nut cake will permit easy degradation of the waste by soil microbes and its conversion to economically useful products. Three hundred and twenty four (324) soil samples were collected at three depths (0-20 cm, 21-40 cm and 41-60 cm) from shea nut cake polluted and unpolluted locations in Jisonayili, Gurugu and Kasalgu in the Northern Region of Ghana, from September, 2010 to July, 2011. A completely randomized multi-factorial design and single factor experiments were used to study the physico-chemical and microbiological properties of the soils and identify potential microbes for the degradation of tannins in shea nut cake. Dependent variables measured were pH, moisture, nitrogen, carbon, tannin contents, bacteria and fungi populations. ANOVA was used to analyze the results. Tannin degrading bacteria were isolated with mineral salt medium supplemented with 2% shea nut cake. GUR/09 and GUR/38 gave good growth in 5% shea nut cake with yeast extracts at room temperature and were identified biochemically as *Pseudomonas aeroginosa* and *Pseudomonas putida* respectively. The ability of GUR/09, which performed better than GUR/38, to degrade tannins in fresh and boiled shea nut cake was investigated by inoculating with GUR/09 and monitoring tannin concentration. Moisture, pH, carbon, nitrogen contents and microbial counts were significantly higher in shea nut cake polluted than unpolluted soils and highest (bacteria, 7.566 and fungi, 3.657 $\log_{10}$ cfu) in the 0-20 cm depth. Moisture and bacterial counts were higher in rainy season than dry season with peak in September. Fungal counts were highest in November. Shea nut cake added organic matter and nutrients to the experimented soil which probably increased the microbial populations. *Pseudomonas aeroginosa* (GUR/09) degraded 92 % tannin in fresh shea nut cake in 20 days and 95% in 20 days when shea nut cake was boiled. *Pseudomonas aeroginosa* GUR/09 can be used to

manage the waste. Polluted soils are reliable sources of bacteria to manage wastes. Government should encourage the use of bacteria to manage agricultural wastes.

Acknowledgements

I thank my supervisors, Prof. Elias N. K. Sowley and Prof. Albert K. Quainoo for their motivational support and patience throughout the project period. I am most grateful to the late Dr. Demuyakor Bawa for encouraging me to register for the programme, suggesting the topic to me and agreeing to supervise; and Prof. Elias N. K. Sowley for agreeing to assist Dr Demuyakor Bawa and especially helping me to come out of a traumatic shock after the death of Dr Demuyakor Bawa. I must say that Prof. Elias N. K. Sowley's patience and support saw me through this academic exercise. Sincere thanks go to Prof. Juventus B. Ziem of the School of Medicine for his motivation and advice.

I sincerely thank the staff of Tungteiya Women Association Shea Butter Extraction Centre, Jisonyilli; Tiehisuma, Shea Butter Processing Centre, Gurugu (located on Tamale-Kumbugu road); and Sekaf Shea Butter Village, Kasalgu for their cooperation during the field study.

I am also thankful to Mr Maxwell Budu and Mr. Edward B. Kuppo, Chief Laboratory Technicians for helping in the laboratory studies. Sincere thanks go to Mr. Fidelis B-Baguo, Senior Medical Laboratory Technologist of the School of Medicine and Health Sciences, for working with me throughout the exercise. I am very grateful to Mr. Emmanuel Zoletor of the Soil Science Department of Savannah Agricultural Research Institute (SARI), Nyankpala, for his assistance in the determination of some of the chemical properties of the soil samples. I am also grateful to Mr. Stephen Danuor and Mr. Joseph Laare of the Public Health Laboratory, Ghana Health Service, Tamale, for their co-operation, support and storage of the bacteria isolates. I am sincerely grateful to Dr. Bayor Hipolite of the Department of Horticulture, Faculty of Agriculture, for his assistance in the statistical analysis.

F. M. DANIKUU

Dedication

I dedicate this piece of work to my late parents and also to the late Dr. Demuyakor Bawa who encouraged me to pursue this study. May they rest in perfect peace.

TABLE OF CONTENTS

List of Tables

List of Figures

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

The shea tree (*Vitellaria paradoxa),* the source of raw material for the shea butter industry is mainly found in the Northern, Upper East, Upper West, part of Brong Ahafo and Volta Regions of Ghana (Iddrisu, 2013). The first three regions mentioned above are among the poorest areas in the country, depending solely on rain-fed subsistence agriculture with erratic and unreliable rain fall. A long period of the year (November to April) is wasted as a dry unproductive period which is characterized by mass exodus of the youth to the south in search of non-exiting jobs. Soil fertility is a problem partly due to overgrazing, bush fires and over exploitation of the soil resulting in low crop yield. The area is deprived of important natural resources that the rest of the country in the southern sector can boast of such as gold, diamond, manganese, cocoa and timber. Even though gold is prospected in the North in recent years, exploitation is not developed to benefit the area as in the southern sector of the country. However the area is blessed with the important oil plant, the shea tree the source of shea butter.

Investment in, and development of the shea butter industry, a major source of income, is a promising area that can guarantee jobs and economic empowerment of the people especially the youth and women, most of whom become victims of North-South migration with its associated problems, such as risky head porter business (Kayaye) and sexual exploitation. The shea butter industry has the potential to outgrow the cocoa industry in future with such revelations as shea butter being a good replacement of cocoa butter in the manufacture of margarine and extending the shelf-life of chocolate (Hall *et al*., 1996; De Muelenaere, 1997). Resourcing state institutions, including research institutions to expand the shea butter

industry and adding economic value to the large volumes of waste generated annually will make the sector a powerful source of employment and foreign exchange.

The tree is an important oil crop in West Africa, rated second to oil palm (Hall *et al.*, 1996). Shea kernel has become popular in the international market and thus played a significant role in the foreign exchange earnings of the countries in the shea belt (Harsch, 2001). Regions of developing countries where the economic tree abounds and shea butter extraction is an important economic activity, especially amongst women, are among the poorest and socially deprived. Poverty alleviation interventions in these areas of countries of the shea belt have not succeeded partly because exploitation of this important natural resource has been completely neglected.

The largest producer of shea nuts in the West African Sub-Region is Burkina Faso where about 80% of land is occupied by the plant (Harsch, 2001). In Burkina Faso, shea kernel and shea butter constituted the second largest export in the 1980`s, third in 2001 and the business engages over 300,000-400,000 women (Harsch, 2001). Ghana is the largest exporter of shea products in the West-African Sub-Region to the European countries (Harsch, 2001). About 70% of nuts are processed and consumed locally in Ghana (Hall *et al.*, 1996). The highest shea butter extraction rate of 83% available fat has been recorded from Dagomba women in the Northern Region of Ghana (Harsch, 2001). Attempts are being made by Ghana Cocoa Board, with a research station in Bole in the Northern Region of Ghana, to domesticate the plant and improve yield and nut quality (Fobil, 2002). The higher market potential and demand for shea butter than the kernel in the international market, is attracting big processing firms such as Olam Ghana Limited; Kassardjan Ghana Limited, Juapong Oil Mills Limited and Sekaf Ghana Limited among others into shea butter extraction and export (Dei *et al.*, 2008). Shea butter recorded the highest world price among both traditional and non-traditional export trades in Ghana in 2004 and 2005 (Table 1).

Table 1: World Prices for Ghanaian Exportable Commodities, 2001-2005 (United state Dollars)

	Commodity	2001	2002	2003	2004	2005
Traditional	Cocoa Beans(tonne)	1020.8	1260.5	1949.5	1586.9	1524.7
	Timber (m^3)	355.2	386.8	391.9	465.8	486.5
	Gold(oz)	271.6	309.5	364.5	410	445.3
	Diamond(carat)	23.3	20.7	25	28.7	35.2
	Bauxite(tonne)	24.1	23.2	22.3	21.3	21
	Manganese (tonne)	33	24.5	20.9	18.6	19.5
Non - Traditional	Pineapple	N/A	N/A	N/A	650.3	579.3
	Coffee	N/A	N/A	N/A	396.3	251.4
	Kola Nut	N/A	N/A	N/A	82.2	63.1
	Shea Butter	N/A	N/A	N/A	33,436.9	11,761.3
	Cashew Nut	N/A	N/A	N/A	267.2	279.1
	Tuna	N/A	N/A	N/A	713.9	613.8

Source: Bank of Ghana in Ghana Statistical service, (2007)

Key: N/A = **No data available**

1.2 Shea butter

Shea butter, among numerous uses, is a main cooking oil of the people in the shea belt. It is a rich source of vitamins A, D, E, F, fatty acids and glycerol (Orkorley *et al.*, 2005). Shea butter is extracted from the kernels of the nuts of the shea tree, (*Vitellaria paradoxa)*, traditionally and by agro-food transformation factories such as vegetable oil mills, soap and cosmetic factories, producing an important waste called the shea nut cake (Hall *et al*., 1996).

The oil-rich kernel (Fig. 1) is the most valuable of the shea tree and in a fresh state is about 15-20 % of the entire fruit weight (Hall *et al*., 1996). The kernel is reported to have a high amount of vitamin A (Vivien, 1990).

Figure 1 Shea Nut kernels

Hall *et al*. (1996) reported that some kernels yield solid fats while others yield oil. They also indicated that kernels of the subspecies *nilotica* produce oil of lower melting point with less unsaponifiable matter than subspecies *paradoxa.* An average of 41-60 % fat content per dry kernel has been reported (Adu-Ampomah *et al.*, 1995).

1.3 Processing of the shea fruits for the extraction of shea butter

The processing of shea fruits for the extraction of shea butter involves a number of stages outlined below and in Fig. 2. Picking of shea nuts is carried out mainly by women and children (Hall *et al.*, 1996), from May to September, when the fruits (Fig. 2A) which are ready for consumption (Busson, 1965) are plucked from tree or collected after dropping from the tree (Hall *et al*., 1996). Depulping, **t**he removal of fleshy part may be done by fermentation (through burying) or boiling to obtain the nuts (Fig. 2B). The depulped nuts are boiled to kill the embryo, coagulate latex in kernels and to cause kernel to shrink and detach from the shell, to facilitate dehusking. The nuts are dried prior to dehusking.

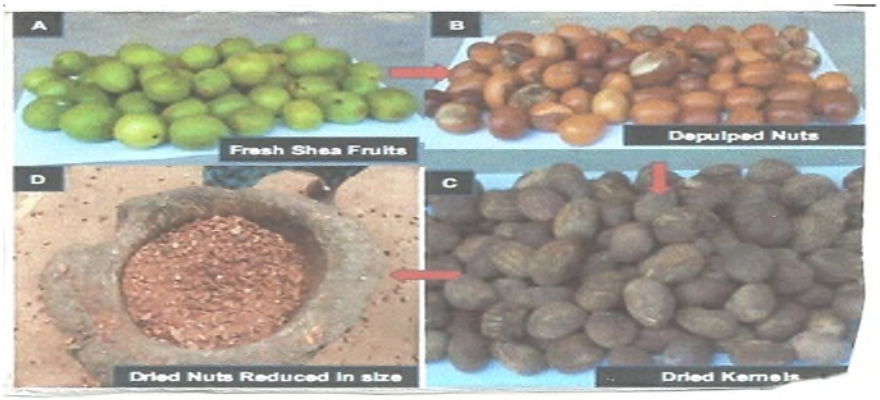


Figure 2Stages in the processing of shea fruit into shea butter

Source: Iddrisu (2013)

Drying of nuts may take up to 10 days in the sun or shorter when mechanical dryers at higher temperatures are used. Sun-drying reduces the moisture content to about 15-30% and 6-7% at 56^{O} C (Hall *et al.,* 1996). Dehusking is the removal of the hard shell covering the kernel (Fig. 2 C) and is done by pounding in a mortar with pestle or by crushing with stone, after which the kernels are dried to further reduce moisture content to prevent fungal growth. The nuts may then be crushed (Fig. 2D) and stored or used for shea butter extraction.

1.4 Methods of shea butter extraction

1. The traditional water-based extraction (Home-based) method is the commonly used method of extraction by the indigenous people in the Savannah zone of Northern Ghana.

2. Mechanical extraction also known as the press method (Cold/Wet press and Hot-press) uses screws and hydraulic instruments.

3. Chemical extraction method which uses solvents such as Ether is industrial-based extraction process, which depends on improved technology and inputs (Hall *et al.*, 1996).

Dagomba women have been reported to be among the first to introduce mechanisation into the traditional shea butter extraction process (Hall *et al.*, 1996). These innovations, aimed at

improving extraction efficiency include adapting a corn mill for crushing kernel and grinding crushed and roasted kernels into paste. These have improved extraction efficiency up to 80% (Salunkhe *et al.*, 1992). The introduction of mechanical processing technology, in the form of expellers and hydraulic pressers and chemical extraction techniques was aimed at eliminating the tedious and time consuming processes inherent in the traditional extraction process while increasing extraction efficiency and oil yields. High temperatures in traditional process reduce quality of Vitamin E, a natural anti-oxidant, causing rancidity (Hall *et al*., 1996). A major limitation in mechanical extraction is that the latex may agglutinate and block machinery, making mechanical extraction difficult and impedes solvent extraction (Hall *et al*., 1996).

1.4.1 The Traditional Extraction Method

The traditional processing of shea fruit and extraction of shea butter, reported by Dalziel in 1937, as labour-intensive, women dominated, time consuming and tedious, yielding only 25% of butter (Hall *et al*., 1994; Iddrisu, 2013) have still not changed. The traditional extraction method described here and shown in Fig. 3 is that used by the shea butter extraction centres studied for this thesis.

The dried kernel is crushed by pounding in large mortars, crushing with stones or mills (Fig. 3-1) and Fig. 3-2) and roasted to concentrate the oil (Fig. 3-3). Roasted and crushed kernels are dried (Fig. 3-4) to further reduce moisture content and ground into paste (Fig. 3-5). Kneading of paste vigorously with warm water (Fig. 3-6) is carried out until a white coagulated crude shea butter paste containing oil separates from the water suspension (Fig. 3-7). This paste is whisked out of the water (Fig. 3-8) into a pot and boiled in water until oil floats on top (clarification) (Fig. 3-9). The remaining brown suspension containing mainly nut deposits (Fig. 3-10) which solidifies to form the shea nut cake (Fig. 3-11) is discarded as waste. The oil, in liquid form, is scooped out into containers (Fig. 3-12), leaving behind in the

pot a black paste as waste (Fig. 3-13), being charred nut deposits that followed the crude shea butter from step 3-7. The oil is then allowed to cool and solidify at room temperature into shea butter. This process is aided by constant stirring with clean dry sticks. The solidified butter is then packaged either as balls or into large containers for market (Fig 3-14).

1.4.2 Mechanical Extraction Method

Despite the introduction of some technological innovations in the traditional extraction processes such as mechanical crushers, mills and kneading machines, shea butter yields and extraction efficiency by this method are still woefully low (Iddrisu, 2013). The mechanical extraction method involves the use of expellers and hydraulic pressers; this method is mostly used by big shea butter extraction firms such as the Ghana Nut Company (GNL) in Techiman in the Brong-Ahafo Region of Ghana. The mechanical extraction technique involves heating the nuts to 15-20^{o}C, crushing and then pressing the crushed nuts to release the oil and a first extraction cake, (Fig. 4) (Iddrisu, 2013). The first extraction cake is further pressed in a second expeller to release more oil and a second extraction cake. As much as 25-80% of shea butter is produced from shea nuts with the mechanical process as against about 25% from the traditional water-based method (Iddrisu, 2013).

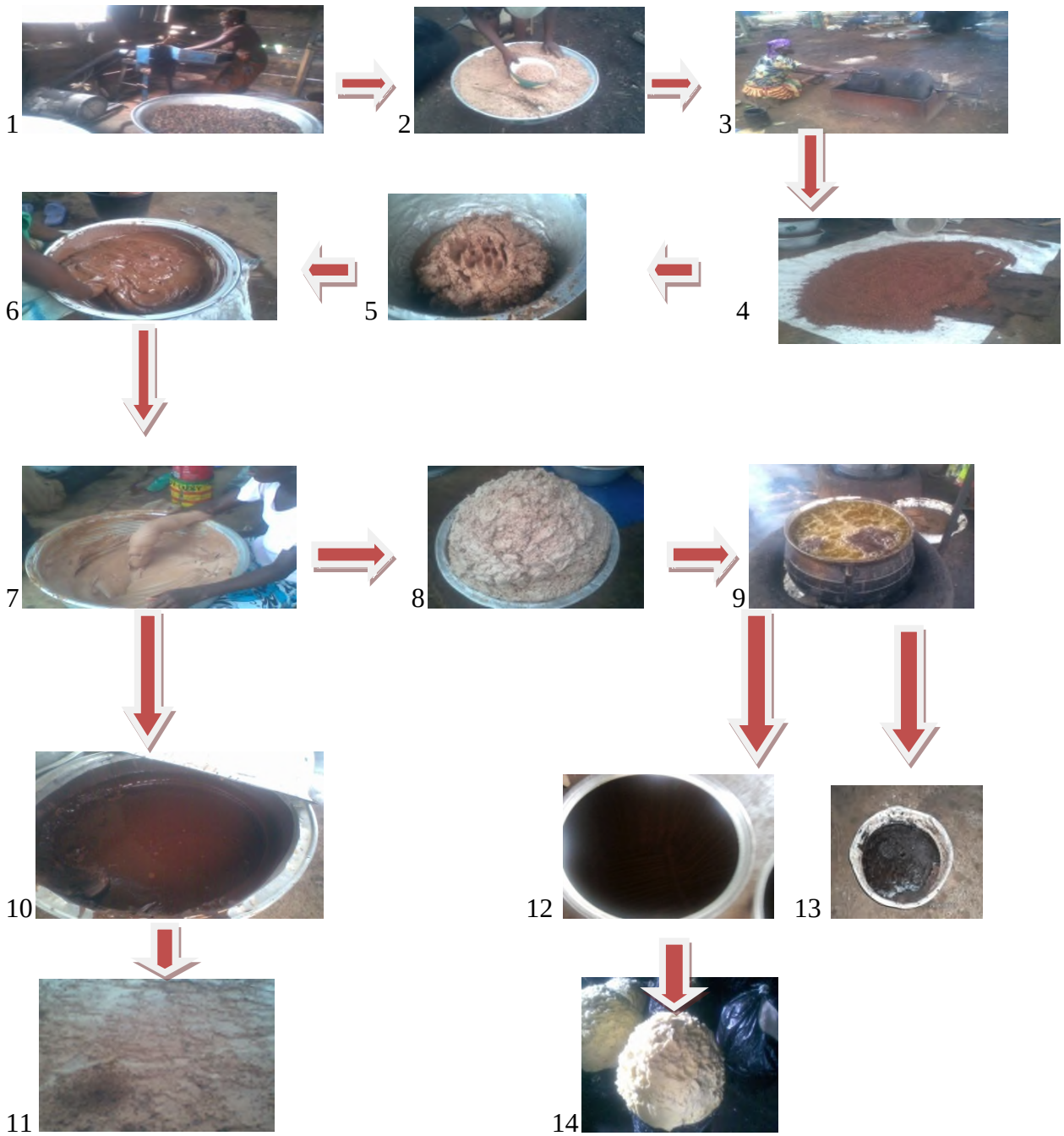


Figure 3 Stages of traditional shea butter processing from kernel

KEY:3-1=Crushing of kernels; 3-**2**= Crushed kernels; 3-**3**= Roasting of crushed kernels; 3-**4**= Drying of roasted kernels; 3-**5**= Kernel paste; 3-**6**= Kneading of paste; 3-**7**= Concentration of crude shea butter;3-**8**= Crude shea butter; 3-**9**= Clarification;3-**10**= Suspension containing nut deposits; 3-**11**= Shea nut cake 1st extraction; 3-**12**= Shea butter (Freshly scooped out in liquid form); 3-**13**= Shea nut cake 2nd extraction, 3-14 = Shea butter (Solidified).

1.4.3 Chemical Extraction Method

This method employs a solvent such as hexane or ether and extraction efficiency is highest as compared to techniques described in 1.4.1 and 1.4.2. The solvent is mixed with the crushed shea nut and after the butter is extracted in the solvent the solvent is subsequently separated from the solvent-oil mixture by distillation. The method can be applied alone or in combination with the mechanical press method as is done in the GNL (Iddrisu, 2013). In this integrated method the second extraction cake is directed into a chemical plant where a suitable solvent, such as hexane is added at a ratio of 5 L to a tonne of shea nut (Iddrisu, 2013). The solvent is subsequently separated in a distillation plant. The process yields 98% extraction efficiency (Iddrisu, 2013). Extraction efficiency is higher in mechanical than traditional and highest in chemical method of extraction, especially when mechanical and chemical are combined (Iddrisu, 2013).

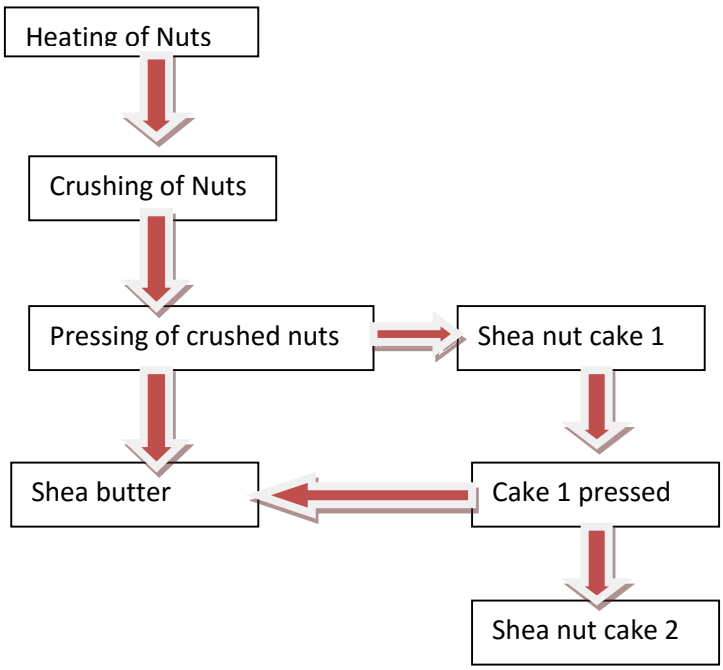


Figure 4 Flow chart for mechanical extraction of shea butter from kernel

(Source: Iddrisu, 2013)

1.5 Fatty acid composition of shea butter

Although shea butter has about 16 saturated and unsaturated fatty acids the five most dominant ones with values greater than 0.01% are oleic acid, stearic acid (28.65-30.95 %), palmitic acid (6.52-8.12%), linoleic acid (6.18-7.79%) and arachidic acid (0.65-0.90%) for Uganda shea butter, with oleic acid and stearic acid reaching values between 45-57% and 30 - 34% for both West and Central Africa shea butter respectively (Okullo *et al.,* 2010)

Shea nut oil can be divided into fatty acids, glycerides and unsaponifiable matter (Fig. 5). Natural vegetable oils are in high demand because of their role in human nutrition as important sources of dietary energy. About 80% of the world fat and oil supply comes from vegetables (FAO, 2007). Shea butter is a vegetable oil and a raw material for important products in the pharmaceutical, soap and confectionary industries (Hall *et al.,* 1996). It is a good cocoa butter replacement in the manufacture of margarine and has been reported to prolong the shelf-life of chocolate (Hall *et al.,* 1996). Cocoa butter is made from oil high in mono-unsaturated symmetrical triglycerides SOS, distearin (StOSt), stearopalmitine (StOP) and dipalmitin (POP) and can only be replaced with oils high in SOS triglycerides, which is lacking in cultivated vegetable oil except palm oil. Palm oil is high in only POP and has to be blended with StOSt for compatibility. Shea butter on the other hand has high levels of StOSt triglycerides (29.6%) and 6.4% StOP, which permit shea butter to conveniently replace cocoa butter without loss in product quality (Hall *et al*., 1996).

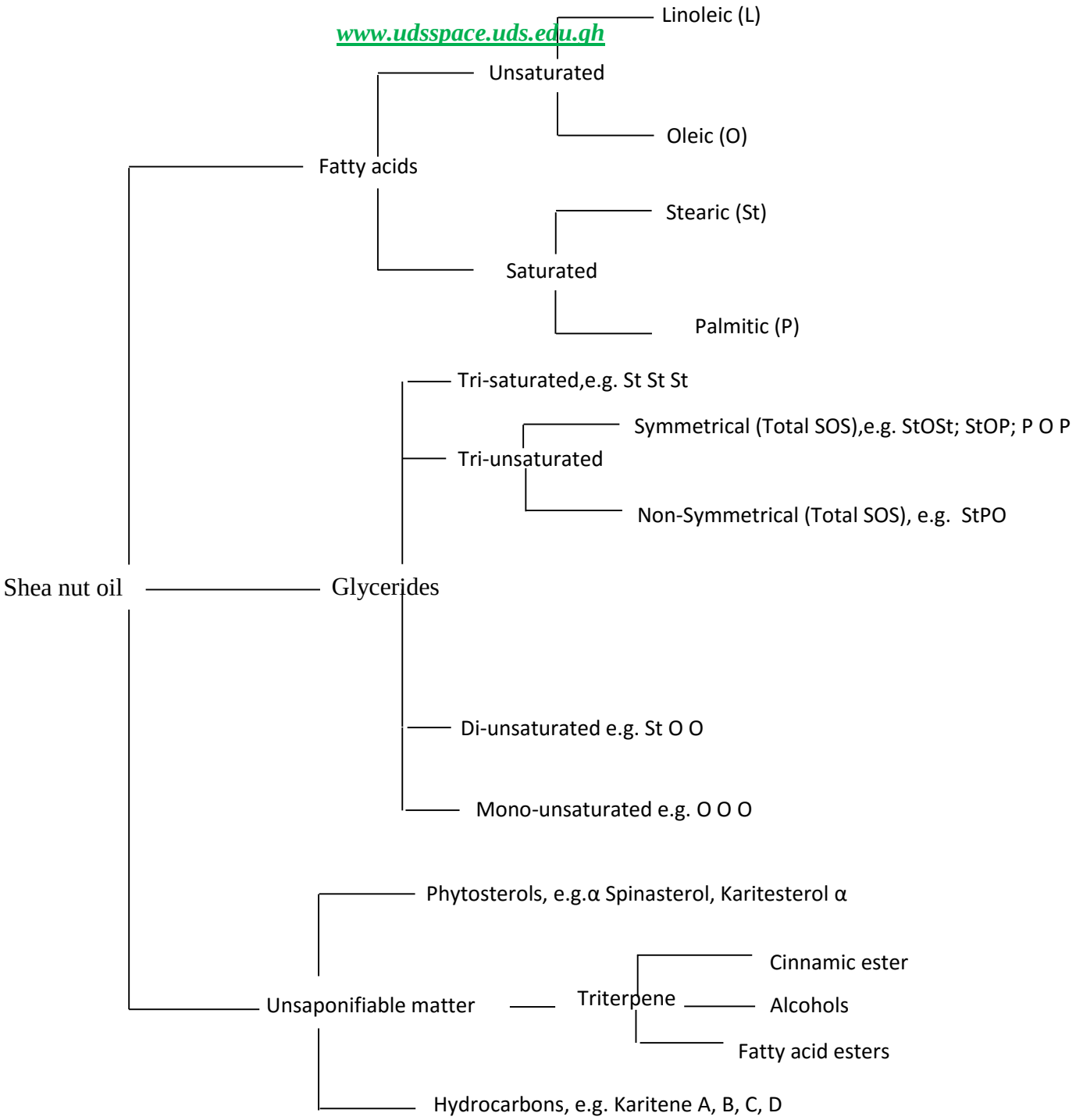


Figure 5 Chemical constituents of shea nut oil (Source: Hall *et al.*, 1996)

1.6 Some traditional uses of shea butter

The numerous beneficial traditional uses of shea butter described by Roger Caillié in 1930, have seen little change (Hall *et al.*, 1996). Shea butter is a source of fat for soap-making and a reliable source of income. The rancid butter is not discarded but also suitable for soap (Schreckenberg, 1992). Shea butter is used as a cosmetic, a moisturizer and a hair relaxer

(Hall *et al*., 1996). It is also used to relieve pains, to heal wounds, correct dislocations. Shea butter is used to treat swellings, bruises, skin infections and ulcers (Hall *et al.*, 1996). Shea butter is used to massage pregnant women and children (Marchant, 1988). It is used for protection against the weather and sun and to treat skin diseases (Hall *et al.*, 1996). Shea butter is used as a medium of administration of most traditional herbal preparations due to its high melting point (32-45^{o}C) close to body temperature (Hall *et al*., 1996). Shea butter is used traditionally for storage of cowpeas (*Vigna* spp.). It protects the beans from insect damage, particularly *Callosobruchus maculatus* spp. (Hall *et al.,* 1996). Poor quality butter is used as water proofing on doors and windows. Refuse or waste water from shea butter extraction has been reported to keep insects (termites) away from places it is disposed of.

1.7 The Shea nut waste

Three types of waste identified in the shea butter extraction process are seed husks, brown suspension containing mainly nut deposits and the black deposit obtained after clarification of crude shea butter. The black residue is used for filling cracks on local home walls and the brown shea nut cake is used to plaster walls of mud buildings as waterproofing material to protect them from rain and wind action (Wallace–Bruce, 1995). The brown shea nut cake is also reported as feed for animals with latest recommendation of the addition of low percentage in animal feed. However research is still on-going to make shea nut cake acceptable to animals as feed. Shea nut cake is used in Europe as non-nutritional bulk for compound cakes, marketed by UNILEVER (Hall *et al*., 1996). The seed husks are useful in removing heavy metals such as lead (Pb II) ions from aqueous solutions and mulching of soil (Hall *et al.,* 1996). Waste water from shea butter extraction has been reported to have insecticidal properties, keeping termites away from places it is disposed (Hall *et al*., 1996). The potential of shea nut cake as an energy source has also been reported. Shea nut cake is

biodegradable and if co-fermented with cow dung in an anaerobic digester could generate up to 61.4% methane (Ofosu, 2009).

The potential of shea nut cake in plant nutrition though variously reported, has not been given serious attention. Its use as fertilizer has always been mentioned in passing. Shea nut cake is applied to the soil as fertilizer though too bulky and recalcitrant to biodegradation by most soil microbes. There is a wide information gap on the use of soil microbes to transform the tannin-rich shea nut cake into an easily degradable fertilizer by the numerous otherwise beneficial soil microbes inhibited by the product.

1.8 Proximate composition of shea nut cake

Earlier studies on the composition of shea nut cake revealed that the waste product is high in carbohydrates, proteins, fats (Table 2) and minerals (Table 3), suggesting a high potential of shea nut cake in animal nutrition, soil fertility and nutrients for soil microbial growth.

Table 2: Proximate composition of first and second extractions of shea nut cake

Composition	First extraction cake (%)	Second extraction cake (%)
Total ash	3.17	4.17
Moisture	6.17	5.00
Crude protein	13.32	12.87
Crude fat	17.42	25.36
Fibre	10.57	8.09
Carbohydrate	66.13	57.13

Source: Iddrisu (2013)

Table 3: Mineral composition of Shea nut cake

Mineral	Concentration (% dry matter in mg/Kg)
Nitrogen	2.370-3.425
Phosphorus	0.155-0.260
Potassium	2.915-4.710
Sodium	0.345 -0.468
Calcium	0.420 – 0.665
Magnesium	0.618 – 2.253
Copper	0.038–0.183
Mercury	0.049 – 0.196
Lead	0.010 – 0.200

Source: Iddrisu (2013)

1.9 Shea nut cake and animal nutrition

Intake of large amounts of Shea nut cake predisposes animals to severe gastric disturbances such as digestive mucosal injury, blood haemolysis and vomiting (Hall *et al*., 1996). High tannins in shea nut cake have been reported to decrease nitrogen utilization in animals and poultry, cause loss of appetite and low weight gain (Hall *et al*., 1996). Pigs have been reported to vomit on consumption of shea nut cake though the whole shea fruit is a good meal (Hall *et al*., 1996). Chicken are reported to decline in growth when fed on diet containing more than 5% shea nut cake (Osei Amaning, 1993). Konlan (2010) recommended the inclusion of only limited amounts of up to 23% in the feed of Djallonke sheep above which he noted decreased feed intake.

1.10 Challenges Associated with the Shea Industry

Governments of countries in the shea belt are increasingly becoming aware of the potential of the industry as a source of foreign exchange, employment and economic empowerment of the rural families, hence attempts to develop the sector by way of establishment of some organizations such as Savannah Accelerated Development Authority (SADA) by the Ghana Government, with development of the shea industry as one of its core mandates. However, the industry is not without problems, which are as old as the industry itself with very little attention from Government and stakeholders in countries of the shea belt.

The shea tree grows in the wild, with associated poor nut yield and quality. Shea butter extraction process is labour intensive with low extraction efficiency. There is lack of political commitment to invest in and develop the industry as in the cocoa sector. The industry has no regulatory body on production of quality nuts, butter and marketing of end products. Waste disposal is a big challenge in the industry in the presence of tannin, an environmental pollutant which is toxic to soil microbes, makes the waste recalcitrant to biodegradation and unacceptable to animals as feed.

Value addition such as removal of anti-nutritional factors from shea nut cake and converting it to economic products such as integrating with animal feed and easily degradable organic fertilizer for soil fertility among others can create more jobs, increase income, improve agriculture and free the environment of toxic pollutants such as tannins (constituents of the shea nut waste). There is lack of serious committed research into all sectors of the industry funded by central government, stakeholders and non-governmental organizations to unearth and exploit the full benefits of the sector. Training in the sector geared towards improving the sector which still depends on very old extraction methods is lacking.

Apart from domesticating the plant and improving nut yield and quality, a mandate of Ghana Cocoa Board (Fobil, 2002), the low shea butter extraction efficiency of 25% for the traditional shea butter extraction (Iddrisu, 2013), the predominant method in the industry suggests that quite a quantity of butter is lost with the wastes in the extraction process. The inability to make use of the wastes generated from the extraction process especially shea nut cake and consequently the disposal of shea nut cake are some important challenges. Ofosu (2009) estimated that about 450-600 kg of shea nut cake is produced from every metric tonne of nut processed into butter. This huge quantity of tannin-rich product is discarded into the soil where it is also destroying useful but vulnerable soil microorganisms instead of being converted to useful products such as animal feed and easily degradable fertilizer for soil fertility (Hall *et al.*, 1996).

While so much research has gone into the shea tree, uses of its numerous parts and products, including shea butter (Hall *et al.*,1996; Yidana, 2004), not much has been done about the waste, shea nut cake which at best has been described as a product of no economic value (Hall *et al.*, 1996). However, some researchers in the West African Sub-Region say otherwise, especially the potential of the waste in animal husbandry as feed and biogas generation among others (Osei-Amaning, 1993; Hall *et al.*, 1996; Aluyor, 2009; Ofosu, 2009;

Konlan, 2010). The high content of polyphenolic compounds (anti-nutritional agents) especially tannins which make shea nut cake unacceptable to animals as feed, the consequences of ill-health, reduced growth associated with consumption of high amounts of shea nut cake and its unacceptable mode of disposal into the environment (Hall *et al.*, 1996; Nitiema *et al.*, 2010) are research challenges that need attention.

1.11 The study area and people

Sagnarigu District is located in the Northern Region of Ghana. Sagnarigu District (Fig. 6), one of the newly created Districts in 2012 by Legislative Instrument (L.I.) 2066, was originally part of the Tamale North Sub-Metropolitan area of Ghana.

Geographically it falls between latitudes 9^{o} 16”and 9^{o} 3” N. and longitudes 0^{o} 36” and 0^{o} 57”W. The district has an estimated land size of 200.4 square kilometres, representing 26% of total land mass of the region. Sagnarigu is 180 metres above sea level. It shares boundaries to the north with Saveligu-Nanton Municipality, to the south and east with Tamale Metropolitan Area, to the west with Tolon and to the north-west with Kumbungu (Fig. 6). Temperatures are generally high throughout the year with a minimum of 23^{o} C at night and a maximum of 42^{o} C during the day, with a mean ranging between 21^{o} C and 32^{o} C. The weather is characterized by two seasons: raining season (May to October) and dry season (November to April). Main soil types are sandstone, gravel, mudstone and shale which give rise to sand, clay and laterite ochrosols. Vegetation is the Guinea Savannah type consisting of tall grasses with scattered fire-resistant trees such as shea, dawadawa, nim and baobab (SDAPU, 2013). The Shea tree has been recognized to be of economic value to the people, the picking and processing of the nuts engaging thousands of households, increasing household incomes and has the potential to eradicate poverty among the people (SDAPU, 2013).

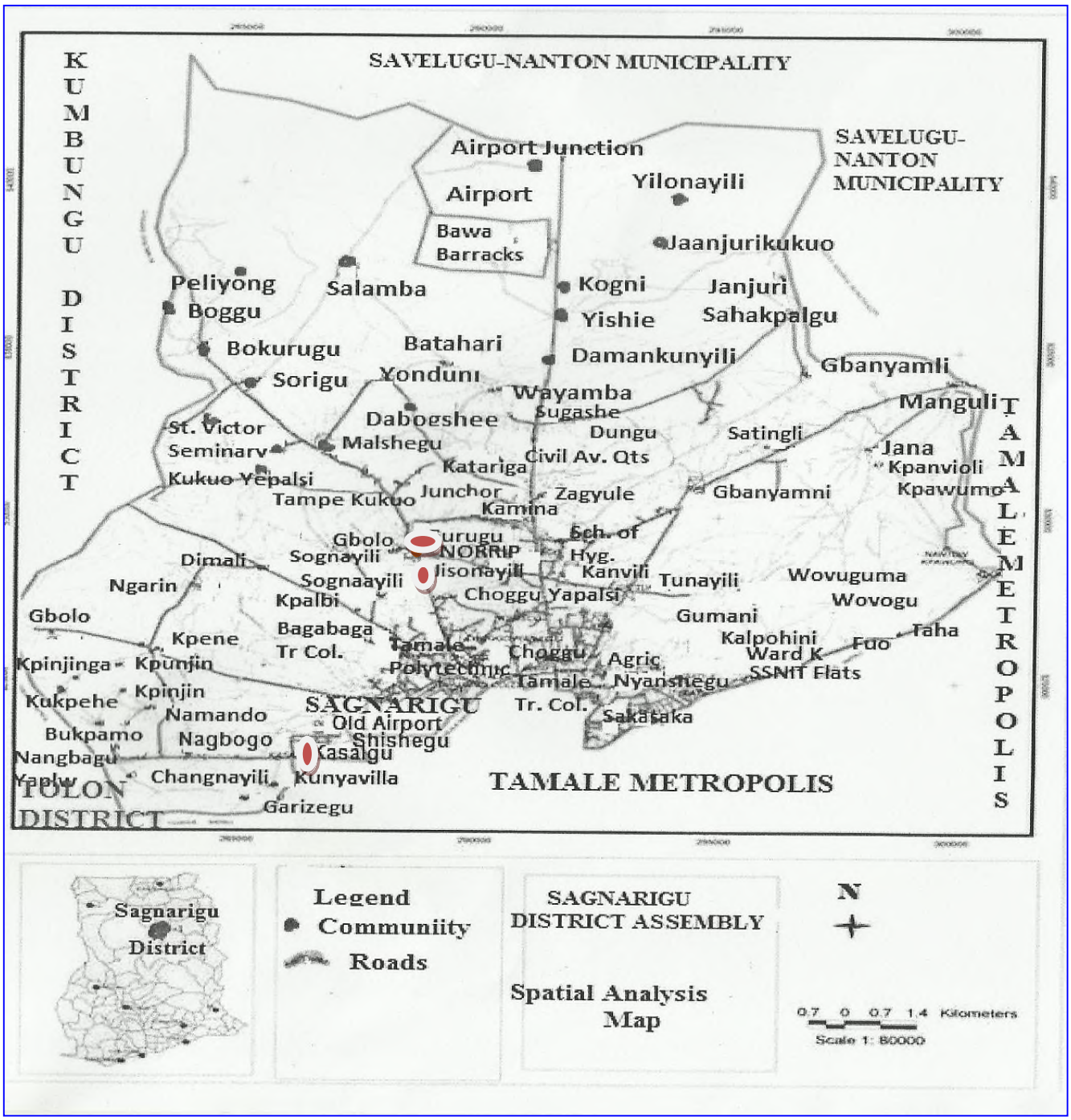


Fig. 6 Sagnarigu District Map

Source: Sagnarigu District Assembly.

Sagnarigu has a human population of 148,099 comprising 74,886 males (50.50%) and 73,213 (49.5%) females, predominantly Dagomba with other tribes from all over the country. Religion is predominantly Islam with other faiths (Ghana Statistical Service, 2010 Population and Housing Census). Major economic activity is subsistence agriculture. Shea butter extraction is an important women-dominated business in the district with the shea butter extraction centres in Sagnarigu employing the traditional water-based technique.

1.12 Problem Statement

1.12.1 Shea Nut cake Disposal

The common waste generated in the shea butter extraction process of major concern to this research is the brown amorphous suspension containing nut deposits after the crude shea butter has been removed for clarification. It is often described as shea nut cake (Hall *et al*., 1996; Dei *et al.*, 2008; Nitiema *et al.*, 2010) as it rapidly forms a dry mass on exposure to air (Fig.7b). Shea nut cake is of no economic value (Hall *et al.*, 1996) and soil is the ultimate recipient. Shea nut cake contains high levels of tannins (Nitiema *et al.*, 2010). Tannins are a group of polyphenolic compounds, described as environmental pollutants, being recalcitrant to biodegradation and toxic to microorganisms (Nitiema *et al.*, 2010). Tannins form indigestible complexes with proteins, precipitating them out of solutions (Hall *et al*., 1996; Nitiema *et al.,* 2010). They exert their antimicrobial activity by inhibiting the absorption of iron and their nonspecific interaction with all forms of enzymes (including metabolic enzymes) and bacteria cell membrane phospholipids (Hall *et al*., 1996; Nitiema *et al.,* 2010). The disposal and management of shea nut cake remains a serious challenge to the shea butter extraction industry and in most cases poses a problem to the environment (Nitiema *et al.*, 2010).

The disposal of toxic industrial, agricultural and agro-based industrial wastes high in polyphenolic compounds has become a global concern (Mazzafera, 2002). Around the world microbes are being investigated as agents that can turn vast agricultural and agro-based industrial wastes into animal feed and toxic pollutants into harmless substances without producing new toxic substances (Ray, 1994; Mazzafera, 2002). This innovation has worked for some, such as converting tannin-rich coffee husks and pulp to animal feed (Mazzafera, 2002) and clearing oil spills from hydrocarbon polluted soils and water (Ray, 1994). Very often, a polluted environment had been the source of the microorganism to do the job (Ray, 1994; Mazzafera, 2002), hence the need to try the technology on the management of shea nut cake. Microbial biodegradation is cost-effective and more environmentally friendly than physical and chemical methods of organic waste disposal, especially that the waste is biodegradable. The technology which is gradually gaining ground in the scientific world is the use of microorganisms to break down complex substances into simple ones (Biodegradation).

Common methods of disposal practised by home-based extractors, which are widely used by all in the industry including the locations selected for this study, include open disposal onto the soil surface (7a and 7b), ploughing of the waste into deeper soil depth 7c) and the pit system (7d), all of which need attention. The open disposal method involves discarding the brown suspension openly onto the surface of land (Fig. 7a). A visit to the selected shea butter extraction centres revealed patches of freshly thrown suspension of nut deposits onto the soil, which upon evaporation of the water forms a cake over the soil surface (Fig. 7b). This method is practiced by small scale home-based extractors. The commercial centres require large tracts of land space. The waste, eventually cakes, and covers the soil surface for a long period of time, reducing oxygen, water and nutrient supply to soil microbes. Plant life is inhibited in the area during the period the soil is covered with the cake. The scene on large

tracts of land is very nasty, especially during the raining season. Shea nut cake may be ploughed into the soil (7c), where it can form a cake obstructing percolation of water, air and nutrients in addition to directly interacting negatively with soil microorganisms. Fresh shea nut cake may also be disposed into a pit (Fig. 7d). The suspension is allowed to settle over days, the supernatant is drained off and the deposit removed and discarded onto the soil somewhere else. If the cake in the pit is not evacuated the pit eventually gets full and overflows causing an environmental nuisance, especially in residential areas (Fig. 7e).

a: Fresh shea nut cake on land surface

b: Solidified shea nut cake on land surface

c: Shea nut cake ploughed into soil

d: Pit disposal of shea nut cake in Kasalgu

e: Overflow of shea nut cake from Pit in Tungteiya women Assoc Centre; Jisonayilli

Figure 7 (a-e): Shea nut cake disposal

The potentials of shea nut cake as animal and poultry feed, fertilizer and for biogas production have been recognized and still at research level (Hall *et al*., 1996; Ofosu, 2009). The use of the product to improve animal nutrition in particular has not been favourable. Shea nut cake is thus described as a waste product of no economic value resulting in the indiscriminate dumping of the waste in soil in an environmentally unfriendly manner (Nitiema *et al*., 2010). The potential in bacteria to remove anti-nutritional factors such as tannins in the waste to make shea nut cake acceptable to animals as feed is also beginning to unfold. Earlier studies on shea nut cake have indicated that it is biodegradable (Ofosu, 2009; Nitiema *et al*., 2010). The potential of biodegradation as a better option to render shea nut cake environmentally friendly and economically useful is coming into focus.

Microbial, (bacterial and fungal) population dynamics of organic wastes polluted soils, such as sewage, some agricultural wastes, some agro-based industrial wastes and petroleum hydrocarbon polluted soils have been extensively researched and well documented. Earlier research observed that soils polluted with organic wastes have higher bacterial and fungal populations than unpolluted soils. Bacterial and fungal populations at different soil depths, locations, seasons, in relation to the various physical and chemical properties of polluted soil and pollutant contamination levels have been reported (Dkhar, 1983; Zwolinski *et al.*, 1988; Shukla *et al.*, 1989; Smith *et al.*, 1994; Arunachalam *et al.*, 1997; Laukova *et al.*, 2002; Ebuehi *et al.*, 2005; Kennedy *et al.*, 2005; Truong, 2005; Adesemoye *et al.*, 2006; Bahig *et al.*, 2008; Tangjang *et al.*, 2009; Adeduntan, 2009; Hamzah *et al.*, 2010). The findings of such research have been extensively used to improve crop yield and in bioremediation applications. However, the microbial population dynamics of shea nut cake polluted soil have not been investigated.

More importantly information on suitable microorganisms capable of degrading tannin complexes in shea nut cake is scanty. The few attempts centred on soil from rice fields

(Quattara *et al*., 1992) and gut contents of animals fed on tannin-rich plant products (Nitiema *et al*., 2010) as bacteria sources, which yielded anaerobic *Streptococci* from anaerobic digester capable of degrading hydrolysable tannin. From available literature indigenous soil constantly receiving shea nut cake has not been investigated for indigenous aerobic bacteria capable of degrading tannin in shea nut cake. The physico-chemical characteristics and microbial population dynamics of shea nut cake polluted soil have also not been investigated. The potential of bioremediation in the management of shea nut cake and shea nut cake polluted soils is exploited in this study. The choice of the bioremediation strategy to manage the shea nut cake disposal problem will require information on whether or not bacteria are present in the shea nut cake polluted soil, if bacteria are present the type of bacteria and the growth conditions to increase the bacteria population and enhance biodegradation of the recalcitrant and toxic tannins in shea nut cake by the bacteria.

1.13 Study objectives

1.13.1 Major objective

To use indigenous soil bacteria for the degradation of tannins in shea nut cake as a major objective, with the following specific objectives:

1.13.2 Specific objectives

1. To determine the physico-chemical characteristics of soil polluted with shea nut cake.
2. To determine the bacterial and fungal populations in soil polluted with shea nut cake.
3. To isolate and identify aerobic soil bacteria with high potential for the degradation of tannin in shea nut cake.
4. To identify the optimum growth conditions to increase population of the bacteria and hasten biodegradation of tannins in shea nut cake.

1.14 Significance of study

Shea butter extraction, the only business that directly benefits the poor women in rural areas (Harsch, 2001), is on the increase in the Savannah zone of West Africa, especially Northern Ghana. With increasing financial support from Non-Governmental Organizations, participation of more women in shea butter extraction and introduction of technological innovations, the increased shea butter extraction will be accompanied by increased production of the waste (shea nut cake). This is expected to rise further with the implementation of the Savannah Accelerated Development Authority (SADA) programme by the Ghana Government. One of SADA's main objectives is to promote the Shea butter industry as a means of creating employment and poverty alleviation in the Northern sector. With an estimated annual consumption level 60,000 metric tonnes of kernels producing about 30,300,000 Kg of shea nut cake by traditional extractors alone it is necessary to find an effective way of making the waste disposal environmentally friendly or adding value to the waste by making it possible to convert shea nut cake into economically useful products.

The study is significant because it is aimed at isolating bacteria inherent in the shea nut cake polluted soil capable of degrading tannin complexes. If such bacteria are isolated and the conditions for the optimum degradation of tannin-complexes in the shea nut cake are identified, the bacteria can be used to degrade the tannins in shea nut cake before they are disposed into the environment or further processed into economically useful products.

The research is critical to the sustainability of the shea nut industry which is a major socio-economic activity of the shea nut belt in Ghana and elsewhere. In view of the staggering quantities of shea nut cake indiscriminately dumped into the environment and the inability of the shea nut cake polluted soil to support plant life, it is obvious that the shea nut industry is unlikely to be sustainable unless effective measures are taken not only to prevent further indiscriminate dumping of shea nut cake but also reclaim shea nut cake polluted fields.

2.0 CHAPTER TWO

LITERATURE REVIEW

2.1 The shea tree

The shea plant (*Vitellaria paradoxa),* belongs to the family Sapotaceae. The species *paradoxa*consists of two sub-species *paradoxa* and *nilotica* (Hall *et al.,* 1996). *It* is widely distributed in the Savannah zone of West and Central Africa respectively, from Senegal to Uganda (Fig. 8).

Figure 8The Shea Belt in Africa (Source: Harsch, 2001)

Countries covered by subspecies *paradoxa* include Ghana, Benin, Burkina Faso, Central Africa Republic, Tchad, Cộtre d'Ivoire, Guinea, Guinea Bissau, Mali, Niger, Senegal and Togo. Subspecies *Nilotica* covers Ethiopia, Sudan, Uganda and Zaire (Hall *et al*., 1996).

The shea tree is so socially and economically important in Africa that it has been captured in the priority list of the African Genetic Resources by the Food and Agriculture Organization (Hall *et al.*, 1996). The tree, which has a spreading canopy (Fig. 9) grows in the wild within the Savannah zone and is much cherished and protected by the communities for its social and economic values.

Figure 9The Shea tree

The plant is deciduous, fire resistant, surviving the frequent threats of bush fire, a common feature of its natural habitat and can reach heights of 10-15 metres and up to 25 metres in protected areas (Hall *et al.*, 1996). Different names such as 'Tama' within the Mole-Dagbani group, are ascribed to the tree by the numerous communities within which the tree exists over a land space of nearly 5,000 kilometres (Hall *et al.*, 1996).

The leaves are mostly borne at the end of branches and often along branches (Hall *et al.*, 1996). The fruit, round to oval-shaped mostly contain a single seed, but occasionally two and rarely three seeds (Hall *et al*., 1996). Fruit size and shape depend on the number of seeds in the fruit (Hall *et al.*, 1996). Fruit yield per tree per year has been estimated at 15-20 kg or more depending on soil fertility (Hall *et al.*, 1996) and 5 kg of dried kernels per tree (Schreckenberg, 1996). Researchers are however divided in opinion as to the maturing time for fruiting of the plant with some suggesting 20-25 years (Greenwood, 1929). According to Hall *et al*. (1996) the fruit pulp is mostly eaten fresh, and is a rich source of important mineral salts, such as (mg $100g^{-1}$) Mg (26.3), Ca (36.4), Zn (0.47), Mn (0.24), Cu (0.11), Fe (1.93), P (18), ascorbic acid **(**196.1 for ripe fruit)) and sugars (2.9-5.6).

2.2 Some traditional uses of parts of the shea tree

Various parts of the shea tree have many uses. For instance, the wood is used for making domestic utensils (mortar and pestle, bowl, ladle, kitchen stirring rod), handles of hoes, guns, axes and cutlasses. The wood is used as fuel wood, for charcoal production and for construction (home frames, roofing timber, road culverts and fences), furniture (e.g. stools). The leaves (foliage) have medicinal applications, for the treatment of stomach ache in children (Millee, 1984), vapour bath for headaches in Ghana and eye bath (Hall *et al*., 1996). The leaves, which lather well in water, are used for washing (Abbiw, 1990). Masks are also produced from the wood by people of Burkina Faso (Millee, 1984). The bark infusion has antimicrobial properties against some bacteria and used in combination with other herbs and salt as a dewormer (Malcolm and Sofowora, 1969). The glue property of the latex is exploited when mixed and heated with palm oil (Hall *et al.*, 1996), and made into a ball for children to play with (Hall *et al*., 1996).

2.3 Tannins and microorganisms

Tannins interact with enzymes of bacterial origin and cell membrane phospholipids, interfering with their functions and bacterial survival. Tannins are a threat to aquatic life (Mazzafera, 2002; Nitiema *et al.*, 2010). However, some soil bacteria and fungi are able to produce enzymes to degrade tannin-containing compounds for carbon and energy to support their growth. *Pseudomonas* spp. and *Bacillus* spp. are among such bacteria with *Pseudomonas* well known to produce inducible enzymes for the degradation of most complex substances including cyclic compounds (Bhatia and Ichhpujani, 2005). *Aspergillus* spp. and *Penicillium* spp. are among the best known fungal degraders of tannins (Bennet *et al*., 2002).

2.4 Tannins and Human health

Favourable health benefits, such as antioxidants and anti-carcinogenic properties, have been associated with moderate intake of tannins by humans, however, excessive intake of tannins has been reported to have serious negative impact on human health, causing cheek and oesophageal cancer (Hagerman, 2002). Tannins and tannic acid have been listed as category 1 carcinogens by the Occupational Safety and Health Administration (OSHA) of the United States of America (Mellifera, 2009).Tannic acid has been reported to cause hepatic necrosis in humans, inhibit incorporation of amino acids into hepatic proteins, binds and precipitates epithelial proteins, causes gastroenteritis, damages gut mucosal lining and decreases digestibility and bioavailability of vitamin B12 (Hall *et al.*, 1996).

2.5 Physico-chemical properties of soil

Soil is a complex ecosystem delimited by physical and chemical properties which determine the type of nutrients (macro- and micronutrients) and prevailing environmental conditions (Tangjangg *et al*., 2009) which influence the diversity and population of microorganisms in a particular soil. The physical conditions include colour, texture and porosity, water holding capacity, moisture content and temperature. Chemical properties of soil include pH, cation

exchange capacity, organic carbon, total nitrogen and available phosphorus. Many soil properties influenced by texture include drainage, water holding capacity, aeration, susceptibility to erosion (erodability), organic matter content, cation exchange capacity (CEC), pH buffering capacity and soil drainage. Nutrients required for microbial growth in soil are inherent in the soil physico-chemical parameters, with soil organic matter having significant influence. All microbes require carbon, nitrogen and phosphorus for growth, while the combinations of other nutrients vary with different organisms.

2.6 **Soil microorganisms**

Many different types of microorganisms are in soil carrying out nutrient recycling from plants and animal remains entering soil. These include bacteria, fungi, protozoa and plant associated viruses. The important role soil insects, worms and rodents in the forefront of breaking down bulky material, initiating and facilitating microbial biodegradation cannot be ignored. Fungi and bacteria are the predominant players in converting these raw organic materials entering the soil to humus under favourable conditions inherent in the physical and chemical characteristics of soil. The nutritional and environmental needs of soil microorganisms are inherently linked to the physical and chemical properties of soil.

Soil bacteria have been classified by Winogadsky into autochnotus (indigenous species) and zymogenous organisms, on the basis of characteristics (Schlegel, 1995). Autochnotus bacteria are of uniform and stable population in the soil as their nutrition is derived from native soil organic matter. Zymogenous bacteria such as *Pseudomonas* and *Bacillus* species are fermentative in their metabolism and are of lower population since they require an external energy source (Schlegel, 1995). Their populations increase gradually when a specific substrate is added to the soil. This group includes cellulose degraders, nitrogen utilizers and ammonifiers (Schlegel, 1995). Most bacteria encountered in soil are members of *Pseudomondales*, *Eubacteriales* and *Actinomyctes* (Schlegel, 1995).

On the basis of nutrition, especially how they obtain their carbon, nitrogen, energy and other nutrient requirements, soil microorganisms may be classified into autotrophs and heterotrophs. Autotrophs synthesize their food from simple inorganic substances, using atmospheric carbon dioxide as carbon source and either derive energy from sunlight (photoautotrophs) or oxidation of simple inorganic substances (chemoautotrophs). Heterotrophs depend on pre-formed food, deriving their carbon source from complex organic matter and nitrogen from nitrates and ammonia compounds **(**Schlegel, 1995**)**. Most soil bacteria belong to this group. Some bacteria require amino acids, vitamins and other growth promoting substances **(**Schlegel, 1995**).**

The important role of bacteria and fungi in nutrient recycle, soil fertility and plant nutrition cannot be over emphasized (Diaz, 2004). Plants and animal remains entering the soil which otherwise would have accumulated till date are decomposed by bacteria and fungi, thus returning the locked up nutrients in them to the soil, thus contributing to soil fertility and plant nutrition and clearing the environment of these remains (Kummerer, 2004). They play very important role in geochemical transformations and in natural cycles such as those of carbon, phosphorus, nitrogen and sulphur resulting in cellulose, carbohydrate and protein degradation, ammonification, nitrification, denitrification, nitrogen fixation, oxidation and reduction of sulphur, iron and magnesium compounds. Soil microorganisms decompose plant and animal remains entering the soil into organic matter which impacts directly on the physical, chemical and biological properties of soil (Kummerer, 2004).

2.6.1 Soil microorganisms and biodegradation

Biodegradation is the use of a biological agent to break down complex substances. Microbial biodegradation therefore is the use of microorganisms or their products to break down complex materials into simpler forms. This is the mechanism by which soil microorganisms obtain their nutrients. The natural metabolic activities of microbes result in the breakdown of

nutrients. The use of microorganisms to break down agricultural wastes in composting is purely microbial biodegradation in action which is therefore not a new concept to humans but its application had been very limited (Schlegel, 1995). Some bacteria and fungi are able to use nutrients to synthesize enzymes to degrade complex compounds through metabolic pathways. Soil contains a myriad of microorganisms capable of degrading any form of organic material, including toxic pollutants. These microbes tend to increase in population with pollution (Ray, 1994; Schlegel, 1995).

Shea nut cake, like other organic wastes such as coffee husks and petroleum hydrocarbon can exert effect on the physical and chemical properties of soil and thereby influence microbial population and diversity in the soil. Bacteria and fungi survival in polluted soils depends on inherent biochemical, structural and physiological adaptations including the ability to use nutrients to synthesize the required metabolic enzymes to degrade the pollutant. These adapted bacteria often dominate the consortium of bacteria in the area with their population being significantly higher in polluted soil than unpolluted soil (Bahig *et al.*, 2008).

Microbial biodegradation can proceed to the end by one organism or may require other organisms in co-metabolism or synergism (Ray, 1994; Schlegel, 1995), when an intermediary substance is produced in the degradation process which is not utilized by the degrading organism. One other limitation to biodegradation is when the bi-product or intermediate product is toxic to the degrading microbe, which if not removed by another organism or by other means, may kill the degrading organism and stop biodegradation (Ray, 1994). High concentration of substrate, in the presence of adequate supply of other factors enhances microbial multiplication, selecting for microbes capable of utilizing that product as source of energy and carbon (Ray, 1994) and hastens biodegradation. However, high concentration of shea nut cake in the soil may result in paste or cake formation reducing percolation of water,

air and nutrient availability to the microbes, resulting in stress, death and reduction in biodegradation.

The success of biodegradation, a complex process involving different metabolic pathways and enzymes, depends on the substrate and the microorganisms involved. The application of the process requires knowledge in identification of particular microbes capable of degrading the substrate and the nutritional and environmental requirements of these microorganisms. These requirements are inherent in the physico-chemical properties of soil (Berry *et al.*, 2007).

2.6.1.1 Fungi and biodegradation

Fungi are known to be very good bioaccumulators i.e. absorbing products and not actually making use of them in body building or for energy (Bennet *et al.*, 2002). Fungi are known to produce many potent biodegradation enzymes which break down toxic pollutants and may not use the degradation products for growth (Bennet *et al.*, 2002). Fungi are efficient at degrading major plant polymers and many other organic molecules, which account for their ability to spoil our foods, buildings, crops through powerful degradation enzymes. However the use of fungi in bioremediation is limited compared to the use of bacteria (Bennet *et al.*, 2002).

Many of the oldest biotechnological practices such as baking, brewing, wine fermentation, pharmaceuticals are based on the catalytic power of fungi (Bennet *et al.*, 2002). Numerous beneficial fungal hydrolytic enzymes for proteins and carbohydrates have been purified, characterized and utilized in industries since the 20th century (Bennet *et al.*, 2002). He noted that the ability of fungi to grow on petroleum hydrocarbon has been well documented since the discovery of the growth of *Cladosporium resinae* in fuel pipes of jet aircrafts leading to

blockage of fuel lines and jet crushes in World War II and association of fungi with marine oil spills.

Fungi have been used in biodegradation of pollutants and toxic wastes, composting, to treat pesticides, munitions of wastes and bioaccumulation of heavy metals. *Aspergillus* species and *Penicillium* species are well known biodegraders and can degrade the most recalcitrant condensed tannin and lignin (Gadd, 1986; Bennet *et al.,* 2002). Fungi have been successfully used in bioremediation exercises to clean up the environment. Even though fungi are well known to interact and degrade every natural product including plants, animal remains and wood products, the application of fungi in bioremediation is still very limited (Bennet *et al.,* 2002). Fungi have a wider temperature range for growth, being the only eucaryotes that have members with thermophilic (60-62^{o} C) optimum growth temperatures (Thurston, 1994).

2.6.1.2 Bacteria and biodegradation

Bacteria and fungi inhabit the soil but fungi are generally slower growers and in environments where bacteria thrive are easily outgrown by bacteria (Bennet *et al.,* 2002). Bacteria use nutrients to synthesize enzymes which they use to attack and degrade natural products (carbohydrates, proteins, oils and pollutants) through metabolic pathways (Ray, 1994). These bacteria are naturally present in environments, such as soil and water, with specific product degrading bacteria constituting about 1% of natural populations and up to 10% or more in polluted ecosystems (Ray, 1994). Microbial biodegradation is a process by which complex natural products are altered or broken down into other substances such as carbon dioxide, water and partially oxidized inert compounds by microorganisms (Bragg *et al.,* 1992). The process is natural and is an important process responsible for cleaning up the environment. Researchers are exploring the possibility of using biodegradation to convert volumes of agricultural waste generated daily across the world into animal feed. Most of the

organic waste contains anti-nutritional substances that make them unacceptable in animals feed formulations (Mazzafera, 2002; Dei *et al.*, 2008).

Biodegradation can proceed either aerobically, with oxygen being the electron acceptor or anaerobically, with hydrogen being in the centre of the process (Schlegel, 1995). Aerobic biodegradation is carried out by most organisms (fungi, bacteria and protozoa) in well aerated soils with fungi being more successful than bacteria in acid soils (Schlegel, 1995). Anaerobic biodegradation though exists in nature, is insignificant (Schlegel, 1995). When biodegradation is complete it is termed mineralization, that is, total breakdown of organic molecules into water, carbon dioxide and/ or other inorganic substances (Bennet *et al.*, 2002). Bioremediation, the use of microbes to decompose toxic pollutants, including marine oil spills into harmless substances, is actually optimization of biodegradation (Ray, 1994).

2.6.1.3 Substrate concentration and bacteria biodegradation

Naturally high concentration of substrate, in the presence of adequate supply of other factors should enhance microbial multiplication and hasten biodegradation and selecting for microbes capable of utilizing that product as sources of energy and carbon (Ray, 1994). However, high concentration of shea nut cake in the soil may result in paste or cake formation which reduces percolation of water, oxygen and nutrient availability to the microbes, resulting in stress and reduction in biodegradation.

Biodegradation can proceed to the end by one organism or may require other organisms in co-metabolism or synergism (Schlegel, 1995). This happens when an intermediary substance is produced in the degradation process which is not utilized by the degrading organism requiring another organism to complete the degradation. One other limitation to biodegradation relating to substrate is the production of substances which are toxic to the

degrading microbe, which if not removed by another organism or physically may kill the degrading organism and halt biodegradation (Schlegel, 1995).

2.6.1.4 Enumeration of soil microorganisms

The general methods of enumeration of microbes especially bacteria in soil, outlined by Alexander (1982), involve suspending the soil sample in a liquid medium prior to enumeration. Popularly used methods for soil bacteriology are the pour plate and spread plate (flooding) technique. In these methods, a known volume or weight (1 g) of soil containing the bacteria or fungi is suspended in 99 ml of sterile normal saline or water, usually 1 g, well mixed and a serial dilution is made with 1.0 ml of the supernatant to obtain dilutions of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7}.

The microbes are counted either by pour plate or spread plate technique. In the pour plate technique 1.0 ml of the serial dilutions is aseptically pipette into sterile petri dishes and molten agar-based medium poured into the dishes and allowed to solidify. Plate count agar or nutrient agar may be used in the case of bacteria. For fungal counts Potato Dextrose agar with an antibiotic incorporated to prevent bacterial growth is used. Soil has a mixture of fungi and bacteria, so it is imperative to suppress the growth of the unwanted. This is accomplished by incorporating an anti-fungal agent such as Nystatin in the bacteria medium prior to pouring into petri dishes. An anti-bacterial agent (antibiotic) is normally used with fungal cultures. Inoculated media are incubated for 24-48 hours for bacteria and up to 72 hours or more for fungi. Bacterial and fungal growth in the form of colonies are then counted and expressed as colony forming units (cfu) per gram of soil (expressed in dry weight).

Microbial population can also be determined by measuring the optical density in liquid cultures, where increase in optical density represents increase in microbial numbers, growth and activity. Sensitive electronic counters can also be used to count bacterial and fungal cells

as particles as they pass through the sensitive device in a particle count technique. A known volume of broth culture can be stained on a slide and the number of bacterial or fungal cells counted and used to calculate number of cells over time. Fluorescence technique using fluorescent dyes (fluorochromes) is another way of assessing microbial numbers. Measurement of metabolic activity by way of accumulation of metabolic products can also predict bacterial or fungal population as increase in metabolic product is an indication of increase in bacterial or fungal population.

2.7 Selective Isolation of Specific Substrate Degrading Bacteria or fungi

While many bacteria can be seen clearly from colonial characteristics, changes in their environments as a result of their presence or accumulation of substances and be isolated directly, others will need special media or conditions that enable them to compete successfully and outgrow other organisms. Selective isolation is based on the principle that certain bacteria or fungi are capable of degrading and utilizing certain organic compounds as sole source of carbon. In the selective isolation of a substrate degrading microorganism, a special mineral salt medium is prepared with that substrate as the sole source of carbon (Truong, 2005). When a mixed culture is suspected such as in soil and water and where more than one type of organism is involved in the degradation of that substrate, such as bacteria and fungi additional requirements are provided in the medium to eliminate or suppress the growth of the unwanted, while enhancing the growth of the target organism (Schlegel, 1995).

In enrichment cultures, the conditions of cultivation must be suitable for the target organism and as unsuitable as possible for the growth of other organisms (Schlegel, 1995). After incubation for a period of time which may be 24-72 hours or more, the inoculated medium is sub-cultured at time intervals in the same enrichment medium for a number of times and finally onto a solid medium of the same mineral salt medium to which agar has been added as the solidifying agent to obtain colonial growth from which pure colonies can be obtained for

further studies. Repeated sub-culture at short intervals in similar liquid selective medium prevents the growth of accompanying organisms that might utilize excretion products or autolysates of the favoured organism (Schlegel, 1995). The colonies may be purified on nutrient agar or other medium. Fungal growth can be suppressed using anti-fungal agents while bacteria can be suppressed using appropriate antibiotics.

2.8 Nutritional Requirement for Soil Microbial Growth

Bacteria require nutrients to provide energy for cellular activities, cellular material and enzymes for growth. Carbon, Nitrogen, Phosphorus, Hydrogen, Sulphur, Sodium, Calcium, Magnesium and Iron which are components of all cells are referred to as Macro-nutrients. Others present or required in trace quantities are referred to as trace elements or micro-nutrients and include Manganese, Molybdenum, Zinc, Copper, Cobalt and Nickel. Accessory nutrients are part of the cell but cannot be synthesized by the cell and have to be supplied from external source. These are in three categories Amino acids, Nucleotide bases and Vitamins. Amino acids are components of proteins. Nucleotide bases (purines and pyrimidines) are constituents of nucleic acids (DNA and RNA) and Vitamins are components of co-enzymes and prosthetic groups of catalytic functions. Though nutrient requirements of bacteria differ from one bacterial species to the other, all bacteria require Carbon, Nitrogen and Phosphorus.

Carbon, the basic structural element of bacterial cell is required in greatest quantity while the high energy–yielding bonds in organic carbon serve as excellent energy source for bacteria (Ray, 1994). Carbon dioxide is the main source of carbon for photoautotrophic bacteria and organic carbon is the main source of carbon for heterotrophs with soil bacteria depending on soil organic carbon contained in soil organic matter as their main source of carbon. The organic fraction of soil without the non-decomposed plant and animal residues constitute the soil organic matter (Whiting *et al.*, 2010). Naturally occurring soil organic carbon is derived

from the decomposition of plant and animal residues, roots exudates, microorganisms and soil biota (Brian, 2002).. Organic matter may also be added anthropogenically to soil through human activities such as dumping of industrial wastes (Brian, 2002). Nitrogen is a component of bacterial proteins, enzymes, cell wall and nucleic acids. In bacterial nutrition nitrogen is utilized in fixed forms such as organic amino nitrogen, ammonium salts and nitrates which can be scarce in certain environments and become a limiting factor (Ray, 1994).Phosphorous is a major component of the phospholipids bi-layer of the microbial cell membrane, Adenosine Triphosphate (ATP) and nucleic acids.

2.8.1 Carbon

Carbon, required in greatest quantity, is the basic structural element of a microbial cell and an important source of energy with carbon to nitrogen requirement ratio being 10:1 and carbon to phosphorus ratio being 30:1 (Ray, 1994). Additionally, high energy yielding bonds in organic carbon serves as excellent energy source for microorganisms (Ray, 1994). In soil, three forms of carbon exist: elemental carbon, inorganic carbon and organic carbon. Major sources of carbon to microorganisms include carbon dioxide (CO_2) for autotrophs and organic carbon for heterotrophs. Microorganisms may be classified into four main groups based on their carbon and energy sources. These are photoautotrophs, photoheterotrophs, chemoautotrophs and chemoheterotrophs (Table 4). The main sources of carbon for soil microorganisms are the soil organic carbon contained in the soil organic matter (SOM) for heterotrophs and carbon dioxide for autotrophs. Organic carbon enters the soil through the decomposition of plant and animal residues, root exudates, living and dead microorganisms and soil biota.

Soil organic matter refers to the organic fraction of soil without the non-decomposed plant and animal residues; it is heterogeneous, dynamic and varies with respect to particle size, carbon content; decomposition rate and turnover time (Whiting *et al.*, 2010). In addition to

the naturally occurring organic carbon in soil, organic matter may be added through anthropogenic activities, such as dumping of industrial waste which according to Brian (2002), tends to make a significantly dominant fraction of total organic carbon in such sites. The organic carbon of soil resides in the soil organic matter fraction and the ease and speed with which the soil organic carbon becomes available is related to the soil organic matter (Whiting *et al.*, 2010). The accumulation of soil organic carbon is affected by soil texture, climate and time.

Table 4 Major nutritional types of procaryotes.

Nutritional Type	Energy Source	Carbon Source	Examples
1. Photoautotrophs	Light	CO_2	Cyanobacteria, some Purple and Green Bacteria
2. Photoheterotrophs	Light	Organic compounds	Some Purple and Green Bacteria
3. Chemoautotrophs or Lithotrophs (Lithoautotrophs)	Inorganic compounds, e.g. H_2, NH_3, NO_2, H_2S	CO_2	A few Bacteria and many Archaea
4. Chemoheterotrophs or Heterotrophs	Organic compounds	Organic compounds	Most Bacteria, some Archaea

Source: Todar, (2000)

Soil rich in clay protects soil organic matter from decomposition by stabilizing substances that bind to clay surfaces. Enabled aggregation property of clay also protects soil organic matter from mineralization by microorganisms (Whiting *et al.*, 2010). Soil organic matter content is decreased through increased decomposition rates by warm temperatures (Whiting

et al., 2010). According to Whiting *et al.* (2010) level topography tends to have much more soil organic matter content than slope topography, due to erosion losses in the sloppy areas. Soil disturbance, such as ploughing and destruction of aggregation, increases exposure of soil organic matter physically protected in aggregates to biodegradation and affects the distribution of organic matter in the soil depths (USDA, 2007). Dumping or incorporation of organic waste or plant residue in or on soil surface reduces erosion and loss of soil organic carbon (USDA, 2007).Increased soil pH increases microbial activity, organic matter decomposition and carbon dioxide release (USDA, 2007).

Diversity of soil microbial population affects soil organic matter. While bacteria aggressively attack soil organic matter, contributing to carbon loss through mineralization, some fungi (mycorrhizae) slow the decay of soil organic matter by aggregating it with clay and minerals, making soil organic matter and soil organic carbon more resistant inside aggregates than in the free form (USDA, 2007). Soil organic carbon distribution is also affected by soil depth with ploughed deep soils accumulating soil organic carbon in layers beneath the disturbed top soils due to restricted mineralization rates. Humus in soil organic matter contributes to aggregate stability and nutrient and water holding capacity of the soil (USDA, 2007).

Components of soil organic carbon such as polysaccharides bind with mineral particles to form micro-aggregates while glomalin, which accounts for 20% of soil carbon glues aggregates together and stabilizes soil structure making soil resistant to erosion while remaining porous enough to permit air and water to move through the soil (USDA, 2007). Organic acids such as oxalic acid released from decomposing organic matter prevents phosphorus fixation by clay and improves its availability. High soil organic matter increases soil biological diversity. However, poor soil organic carbon content, being the main energy source, reduces microbial biomass activity and nutrients mineralization (Whiting *et al.*, 2010).

The high organic matter content of shea nut cake has been reported by earlier researchers (Hall *et al.*, 1996, Nitiema *et al*., 2010; Oddoye *et al*., 2012; Iddrisu, 2013). Pousiga *et al*. (2007) in their analysis of 36 shea nut cake samples from Bobo-Dioulasso and Sapore in Burkina Faso (Table 5) also noted the high organic matter content of shea nut cake, suggesting a high potential of shea nut cake to increase soil organic matter content.

Table 5 Chemical composition of traditional shea nut cake from two climatic zones of Burkina Faso (% of DM) n= 36

Item.	Bobo	Sapore	Mean	Max.	Min.	SEM	P-Value
Dry matter	95.1	96.1	95.6	98.0	87.3	1.03	0.47
Organic matter	94.5	93.3	93.9	97.3	89.1	0.76	0.30
Ash	5.4	6.7	6.1	10.3	2.6	0.76	0.30
Crude protein	7.4	8.9	6.7	10.3	2.5	0.89	0.29
Ether extraction	6.6	8.0	7.3	9.6	5.3	0.37	0.29
Crude fibre	10.3	11.5	10.9	11.0	7.8	0.33	0.98
ME(MJ/Kg)	13.3	12.9	13.1	14.4	12.4	0.15	0.65

Source: Pousiga *et al*. (2007)

2.8.2 Nitrogen

Nitrogen is an essential component of proteins, enzymes, cell wall and nucleic acids of microbes and is utilized in fixed forms such as organic amino nitrogen, ammonium salts and nitrates which can be scarce in certain environments and thus becomes a limiting factor (Atlas, 1984; Ray, 1994). Numerous amino acids in the protein fraction of shea nut cake should make nitrogen not a limiting factor in soil contaminated with the cake however;

formation of protein-tannin complexes by tannins which are recalcitrant to biodegradation can make nitrogen unavailable and thus a limiting factor to microbial growth.

2.8.3 Carbon/ Nitrogen ratio

The Carbon to nitrogen ratio in crude plant material is said to be as high as 40:1 but conversion of plant material to humus is accompanied by accumulation of nitrogen bringing the carbon/ nitrogen ration to about 10:1, the ratio required for microbial growth (Ray, 1994; Schlegel, 1995).

2.8.4 Phosphorous

Phosphorus is the major component of the phospholipid bi-layer of microbial cell membrane, ATP (universal currency of energy for the cell) and nucleic acids.

2.8.5 Physiological functions of the principal elements in microbial nutrition

Different nutritional elements perform different important physiological roles in the nutrition of organisms. Each nutrient serves either in energy metabolism or as building block or both. Hydrogen plays an important role as a constituent of cellular water, organic cell materials and H^+ as an electron donor in metabolism of certain bacteria. Oxygen is a constituent of cellular water, organic cell materials and an electron acceptor in respiration of aerobic bacteria. Carbon is a constituent of cell materials and an electron donor in energy metabolism. It is an electron acceptor in energy metabolism in fermentation and carbon dioxide reduction in methane fermentation **(**Stanier *et al*., 1986).

Nitrogen is an important **c**onstituent of proteins, nucleic acids and coenzymes. It is an electron donor in energy metabolism of nitrifying bacteria, such as in NH_3 for *Nitrosomonas*; and NO_2^- for *Nitrobacter*. It also acts in NO_3^- or NO_2^- utilization as an electron acceptor in energy metabolism in denitrifying bacteria. Sulphur is a constituent of proteins (as amino acids cystine and methionine) and some Coenzymes (for example, CoA).It is an electron

donor in metabolism of colourless and photosynthetic sulphur bacteria (as in H_2S, S, $S_2O_3^-$)**.** It is a component of SO_4^{2-}acting as an electron acceptor in energy metabolism of sulphate – reducing bacteria.Phosphorusis a **c**onstituent of nucleic acids, phospholipids, coenzymes and a principal agent in energetic coupling of reactions (as in Adenosine Triphosphate). Potassium is one of the principal inorganic cations in cells and is a cofactor for some enzymes. Magnesium is an important cellular cation and an inorganic cofactor for very many enzymatic reactions, including those involving Adenosine Triphosphate and binds enzymes to substrates. It is an important constituent of chlorophylls **(**Stanier *et al*., 1986).

Manganese is an inorganic cofactor for some enzymes, sometimes replacing Magnesium. It is involved in oxidative metabolism. Calcium is a **c**ofactor for some enzymes such as proteinases. It is a major constituent of bacterial endospores. Iron is a **c**onstituent of cytochromes**, c**ofactor for a number of enzymes. Iron ion (Fe^{++}) is an electron donor in the energy metabolism in iron bacteria. Some other minerals such as cobalt, copper, zinc and molybdenum are inorganic constituents of special enzymes. Zinc for instance is a component of alcohol dehydrogenase and molybdenum is a component of the molybdoenzymes necessary for the utilization of nitrate and of molecular nitrogen for biosynthesis **(**Stanier *et al*., 1986)**.**

2.8.6 Growth factors

A growth factor is any essential organic component of cell material that cannot be synthesized by an organism from the principal nutrients and must be provided as a nutrient for the growth of the organism. Growth factors refer to the organic substances required by a given organism in addition to the principal sources of carbon and energy (Stanier *et al*., 1986). Important in this class are the vitamins which are part of coenzymes that perform very important functions. Nicotinic acid (Niacin) is part of the nucleotide coenzymes (DPN and

TPN) involved in dehydrogenations (Stanier *et al*., 1986).Riboflavin (vitamin B_2) is part of flavin nucleotides(FAD and FMN) involved in some dehydrations and electron transport.

Thiamine (vitamin B_1) is part of thiamine pyrophosphate (Co-carboxylase) involved in decarboxylations and some group transfer reactions. Pyridoxine (vitamin B_6) is a component of pyridoxal phosphate which is involved in amino acid metabolism, transamination, deamination and decarboxylation. Pantothenic acid is a component of coenzyme A which functions in keto–acid oxidation and fatty acid metabolism. Folic acid (vitamin B_c) is a component of tetrahydrofolic acid which functions in the transfer of carbon units. Biotin (vitamin H) is part of prosthetic group of biotin enzymes which takes part in carbon dioxide fixation and carboxyl transfer. Cobamide (vitamin B_{12}) is a component of cobamide coenzyme which functions in molecular rearrangement reactions (Stanier *et al*., 1986).Organisms that have requirements for accessory factors are known as autotrophs to differentiate them from prototrophs which do not depend on accessory food factors (Schlegel, 1995).

2.8.7 Nutritional interaction (Synthrophy)

Microorganisms notably some bacteria are more efficient when grown together in a mixed culture and especially when the end product of metabolism of a substrate by one organism serves as substrate for another organism. Such a phenomenon is known as synergism from the Greek word syntrophy (together and nourish). In this situation one organism digests the original substrate, growing slowly at the expense of the products of digestion which it accumulates in the medium. A metabolic waste product of this first organism which cannot be utilized by the producing organism accumulates. The presence of a second organism which cannot utilize and develop alone in the original substrate but can grow rapidly on the metabolic waste products of the first organism will result in a mixed culture in which the second organism and its end metabolic products predominate (Stanier *et al*., 1986). In the

same way the end products of metabolism of one organism may serve as nutrients for a second organism. This second organism may produce a metabolic end product from such product from the first organism which both organisms cannot utilize, requiring a third organism. This type of collaborative decomposition of organic matter is encountered in the natural environment which contributes to the recycling of matter (Stanier *et al*., 1986).

Very often in mixed cultures some bacteria may require growth factors for development. Microorganisms that can synthesize their own cell materials from simple organic constituents of the medium may excrete some amounts of vitamins and amino acids essential for the growth of others, resulting in satellite colony formation in the vicinity of another which provides them with essential growth factors (Satellitism), for example *Haemophilus* in the presence of *Staphylococcus aureus* on blood agar. The *Staphylococcus* haemolyses the blood releasing the X and V factors in the blood for the growth of the *Haemophilus*. Cross–feeding or mutualism is a complex type of nutritional relationship between organisms in which each type depends on the other for some essential nutrients nutritionally deficient for either one alone (Stanier *et al*., 1986).

2.9 Environmental requirement for microbial growth

Microbes will require favourable environmental conditions for growth in the absence of which stress will set in resulting in death and biodegradation will eventually stop. Microbial environment for growth is influenced by many factors inherent in soil thus, aeration, moisture, temperature, pH, soil texture and substrate concentration.

2.9.1 Oxygen

Oxygen is very essential for microbial metabolism and biodegradation which is essentially aerobic. Shea nut cake contains both aliphatic compounds in fatty acids and aromatic compounds in polyphenols, including tannins. Degradation of shea nut cake will require

oxygen since the metabolic pathways for the degradation of fatty acids and aromatic compounds of polyphenols involve molecular oxygen and oxygenases. Based on oxygen requirement microorganisms may be classified as obligate (strict) aerobes, obtaining energy only via aerobic respiration in the presence of oxygen; obligate (strict) anaerobes which do not require oxygen and molecular oxygen is toxic to them; facultative anaerobes, which can use oxidative or fermentative metabolic pathway depending on prevailing conditions (availability or absence of oxygen respectively). Microaerophiles require oxygen but cannot tolerate the partial pressure in air (0.2 bar). Aerobic biodegradation requires oxygen and is essentially an oxidation process (Ray, 1994).

Bacteria enzymes will catalyse the insertion of oxygen into the hydrocarbon so that the molecule can subsequently be consumed by cellular metabolism, via the oxygenases (Ray, 1994). Anaerobic degradation in natural ecosystem is negligible (Atlas, 1981; Schlegel, 1995). Major source of oxygen to microbes is atmospheric oxygen which can be a limiting factor in soils especially deeper depths of compact soils (Ray, 1994) and if soil is heavily contaminated with shea nut cake at the top soil level it can reduce percolation and the amount of oxygen, water and nutrients supply to microbes at deeper levels.

2.9.2 Water

Water which constitutes a large proportion of microbial cell cytosol, is a medium for enzymatic reactions, an important vehicle for transport of substances across the cell membrane and a source of oxygen for some microorganisms. Water in soil ecosystem is measured in terms of soil moisture content. The measurement of water content is one of the most common soil analysis performed. The potential effects and influence of soil water content on the behaviour and application of soil make it an important measurement in every type of soil study (Carter, 1993).

The amount of water found in soil varies with soil type, climate and organic matter content of the soil, and that quantity available to microbes in soil determines the type of microorganisms that can survive in it. Since water acts as a medium of nutrient transport it is an important cellular component necessary for cell survival (Carter, 1993). The term soil water content is defined as the amount of water incorporated within soil pores. Soil water content can be defined as either gravimetric water content, or volumetric water content. Gravimetric water content is defined on a mass of soil basis and volumetric water content is defined as a percentage by volume of soil (Carter, 1993).

Soil water can be a serious limiting factor for microbial growth and biodegradation because its availability depends on so many factors that are not easily, such as rainfall pattern, soil characteristics and soil depth (Carter, 1993). During biodegradation in the natural environment, water is an important limiting factor in soil especially during the long periods of draught, in deeper depths of soil especially compact soils (Carter, 1993). Soils low in organic matter and areas with low rainfall, drought and periods of dry season record low moisture content (Carter, 1993).These are often associated with reduced nutrient availability, microbial numbers and metabolism, slowing down biodegradation. Moisture content and water holding capacity are some of the parameters that measure the availability of water in soil for microbial activities (Carter, 1993).

2.9.3 Temperature

Temperature has some influence on enzymatic reaction and biodegradation. Low temperature slows down enzymatic reactions while high temperature accelerates enzymatic reactions and rate of biodegradation provided the microbial enzymes are not denatured. In Northern Ghana, soil temperature is not a limiting factor for microbial life and biodegradation since atmospheric temperatures in the area are generally high throughout the year, 25^{o} C to 39^{o} C.

2.9.4 Hydrogen ion concentration and Soil pH

High mobility of hydroxyl and hydrogen ions give significant effect even with small change in their concentrations. Most organisms grow best at neutral pH (pH 7) where both H^+ and OH^-concentrations are equal (Schlegel, 1995). Some bacteria prefer slightly higher (alkaline pH) while only a few are acid tolerant such as lactobacilli. Fungi on the other hand prefer lower pH ranges. Culturing soil samples at different pH values will give predominantly fungi at pH 5 and bacteria at pH 8 (Schlegel, 1995). To avoid self-poisoning from acid accumulation by those bacteria that produce acids, but are not acid tolerant, the media must be buffered using inorganic phosphates which provide a narrow range around pH 7.2 (Schlegel, 1995). Organisms that produce large volumes of acid such as lactobacilli require the addition of calcium carbonate to the medium. The damage to bacteria cell by unsuitable pH is not as a result of H^+ and/or OH^-. These ions only increase the undissociated proportions of weak acids or bases which are physiologically more active and therefore penetrate more readily into the cell than their dissociated products (Schlegel, 1995).

Soil pH gives the acidity or alkalinity of the soil and is related to the hydrogen ion concentration of the soil. Values below 7 are described as acidic, pH value of 7 is neutral and values above 7 are classified as alkaline. Initially, each type of soil has a certain level of acidity depending on its composition, native vegetation, and rainfall amounts. However, various factors over time cause changes in soil pH. Leaching, erosion, and crop uptake of basic cations (calcium, Ca^{2+}; magnesium, Mg^{2+}; potassium, K^+), decay of plant residues, and plant root exudates are all means by which the soil acidity is increased. Soil pH can have some influence on rates of biodegradation. This has been reported not to pose a major problem in natural environments where microorganisms naturally exist (Ray, 1994). The pH of polluted soil is influenced by the nature and degree of pollution (Berry *et al.*, 2007). Cement contamination of soil and soils high in organic carbon have higher pH which

increases towards alkaline with increase in pollution (Hamida, 2005; Berry *et al.*, 2007), while hydrocarbon-polluted soils have acidic pH (Hamzah *et al.*, 2010).

In addition to the naturally occurring organic carbon in soil, the major source being plants, organic matter may be added through human activities, such as dumping of industrial waste. This has been reported to form a significantly dominant fraction of total organic carbon in such sites (Brian, 2002). This study was therefore carried out to investigate the physico-chemical characteristics of shea nut cake polluted soils and how shea nut cake pollution influences the physico-chemical characteristics of soil.

2.10 Application of biodegradation and Bioremediation

The disposal of toxic industrial, agricultural and agro-based industrial wastes high in polyphenolic compounds has become a global concern. Around the world microbes are being investigated as agents that can turn vast agricultural and agro-based industrial wastes into animal feed and toxic pollutants into harmless substances without producing new toxic substances (Ray, 1994; Mazzafera, 2002). This innovation has worked for some, such as converting tannin-rich coffee husks and pulp to animal feed (Mazzafera, 2002) and clearing oil spills from hydrocarbon polluted soils and water (Ray, 1994). Very often, a polluted environment had been the source of the microorganism to degrade the pollutants (Ray, 1994; Mazzafera, 2002), hence the need to test the technology on the management of shea nut cake. Microbial biodegradation is cost-effective and more environmentally friendly than physical and chemical methods of organic waste disposal, especially if the waste is biodegradable. Biodegradation, which is gradually gaining ground in the scientific world is the use of microorganisms to break down complex substances into simple ones. This is a common process by which microorganisms obtain nutrients and energy from natural products. In nature biodegradation can occur aerobically or anaerobically (Schlegel, 1995).

Governments, industries and environmentalists are worried about industrial chemicals and wastes discharged into the environment which eventually affect the ecosystem and humans adversely (Wackett and Ellis, 1999; Nitiema *et al*., 2010). Bioremediation, the use of microbes or microbial enzymes to decompose toxic pollutants, into harmless substances, is optimization of biodegradation (Ray, 1994). Bioremediation can involve indigenous microbial population with or without the addition of nutrients in which case modified strains of bacteria emerge, characterized by the ability to degrade that pollutant which induced the modification (Ray, 1994; Aluyor *et al*., 2009). Biodegradation can also be carried out by the inoculation of exogenous microbes from outside (bioaugmentation) (Ray, 1994; Aluyor *et al*., 2009). In bioremediation the target compound may be used as a carbon source, enzymatically attacked but not used as carbon source (co-metabolism) or absorbed and concentrated within the organism but not metabolized (bioaccumulation). In all forms of application of microbial biodegradation including bioremediation microbes may be added to substrate (Seeding) to promote degradation if such microbes are found to be absent. Such microbes may be grown from already contaminated systems or genetically engineered. If however, microbes are found to exist in the system, sufficient nutrients and other growth conditions may be added (Fertilization) to increase microbial numbers and hasten biodegradation (Ray, 1994).

Biodegradability of a product however is a function of the chemical composition of the product. Since the discovery of the use of microorganisms to treat waste water by the Romans, as far back as 600BC, efforts have been made with tremendous success to use microbes to clear oil spills, industrial discharges and pesticides and have been found to be a more environmentally friendly and cost effective measure of dealing with wastes than chemical and physical methods (Ray, 1994). The idea of converting agricultural and agro–based industrial wastes to other usable forms using microbial biotechnology instead of allowing it to go waste, has become a major subject of concern worldwide (Mazzafera, 2002).

Biodegradability may be assessed by monitoring certain parameters considered indicative of consumption of the test substance by microorganisms, or production of simple compounds indicative of mineralization (Aluyor *et al*., 2009). Several methods have been developed to measure the biodegradability and biodegradation of substances, most of which are laboratory based (Aluyor *et al*., 2009). One of such methods measures the degree of degradability by calculating the rate of conversion of the organic substance to CO_2 by a microbial inoculum. One other method measures the uptake of oxygen by the activities of microorganisms (Biochemical Oxygen Demand–BOD), while another, the CEC-L-33-A-94 method developed by the Coordinating European Council (CEC), measures the disappearance of the substance or a component of the substance by analysing the test material at various incubation times through infrared spectroscopy (Aluyor *et al*., 2009).

2.11 Metabolic pathways for biodegradation of major organic components of shea nut cake

2.11.1 Carbohydrate metabolism

The biodegradation of carbohydrate is mainly through the Embden Meyerhof glycolytic pathway. Plant polysaccharides of various forms (principally lignocellulose and cellulose) are first hydrolysed by respective hydrolytic enzymes of degrading soil microbes into glucose, maltose and oligomers which in the presence of permeases are transported into the cell and converted into glucose units (Schlegel, 1995). The glucose through glycolytic pathway is converted to pyruvic acid anaerobically, generating ATP and providing building blocks for biosynthesis (Fig. 10).

An initial investment of two molecules of ATP per molecule of glucose is used and pyruvate and four (4) molecules of ATP are produced, a net gain of two (2) molecules of ATP. The process does not require the use of oxygen (Schlegel, 1995). In the absence of molecular

oxygen pyruvate is converted into lactic acid with the production of a few more ATP (Schlegel, 1995).

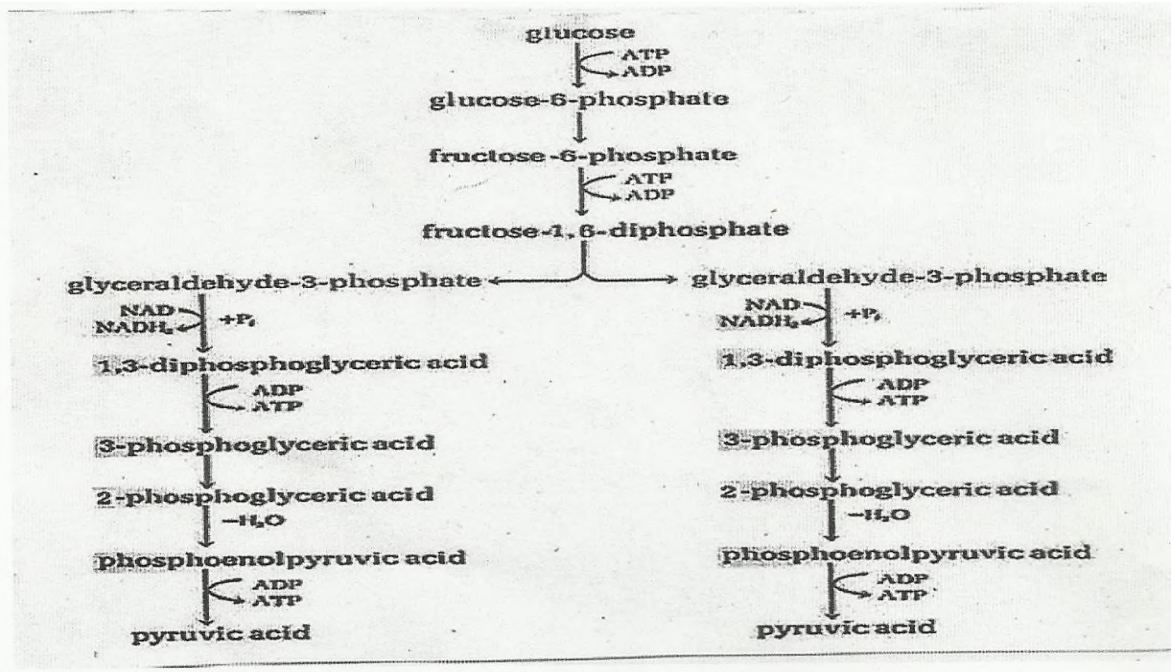
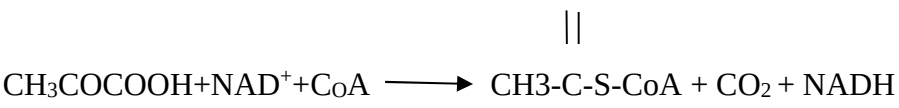


Figure 10 The Embden Meyerhof pathway for glucose dissimilation (Source: Todar, 2012)

In the presence of molecular oxygen pyruvate enters the tri-carboxylic acid (Krebs) cycle as Acetyl coenzyme A (aerobic oxidative process of pyruvate) indicated below.



Pyruvic acid (oxidized) Acetyl-CoA

In the series of reaction more ATP molecules are given off for biosynthesis, and electrons (H^+) generated in the process are carried through electron transport chain for the production of more ATP as it is passed down descending energy levels and finally the hydrogen electron

is accepted by oxygen to form water. Acetyl Co-A, one of the products of the Krebs cycle, initiates another cycle.

2.11.2 Protein degradation

Microbial biodegradation of proteins is via their numerous proteases and hydrolytic enzymes that cleave respective amino acid units from the protein molecules under favourable conditions. These enzymes abound in microorganisms (Schlegel, 1995). However in tannin-protein complexes these proteins in the complexes may not be digestible unless the tannin is first degraded to free the protein for degradation (Hagerman, 2002). Any microorganism capable of degrading the tannin in the tannin-protein complex and tannin polysaccharide complex in shea nut cake can degrade shea nut cake.

2.11.3 Fatty acids degradation

Shea butter contains up to 16 saturated and unsaturated fatty acids with the most predominant fatty acids being palmitic, strearic, oleic, linoleic and arachidic acids with values greater than 0.1% (OKullo *et al.*, 2010). The extraction of butter oil from shea nut is not a perfect process and quite a substantial amount of these fatty acids (Table 6) remain in the wastes. These fatty acids are relatively simple aliphatic compounds in structure, of a general formula $C_nH_{2n+1}COOH$. The simplest fatty acid components of shea butter are the saturated straight chain fatty acids without double bonds (1) or unsaturated linkages that cannot be altered by hydrogenation or halogenations (2). A fatty acid is unsaturated when double bonds are present: monounsaturated if only one double bond is present (3) and polyunsaturated (polyenoic) if more than one double bond is present (4).

The metabolic pathway for the degradation of straight chain fatty acids of the type found in shea butter and shea nut cake is beta-oxidation pathway.

Table 6 Types of fatty acid in Shea nut cake

1.	Palmitic acid: (16 C chain) Hexadecanoic acid; saturated fatty acid; $CH_3(CH_2)_{14}COOH$
2.	Stearic acid: (18 C chain) Octadecanoic acid; saturated; $C_{18}H_{36}O_2$;$CH_3(CH_2)_{16}COOH$:
3.	Oleic acid: (18.1) Monounsaturated omega-9-fatty acid; $CH_3(CH_2)_7CH=CH(CH_2)_7COOH$
4.	Linoleic acid: (18.2 18c- chain with 2 cis double bonds at C-9 and C-12) Unsaturated n-6 fatty acid; a carboxylic acid
5.	Arachidic acid: (20 C- chain); eicosanoic acid; saturated fatty acid

Source: OKullo *et al.*, 2010

In beta-oxidation acetate is formed along with a new fatty acid of two carbons less than the original (previous) and the process will repeat until the compound is completely broken down to water and CO_2, a process known as mineralization. Fatty acid biodegradation can proceed under anaerobic conditions.

2.11.4 Tannin Degradation

Shea nut cake contains carbohydrates, proteins, fatty acids, tannins and saponins (Dei *et al.*, 2008). Complete degradation of shea nut cake will therefore involve the breakdown of these compounds to yield cell building blocks and energy. The complex formation of proteins and some carbohydrates in shea nut cake with tannins and other polyphenolic compounds makes metabolism of shea nut cake by most microorganism very problematic apart from the negative impact of tannin on metabolic enzymes. Plant tannins (Hydrolysable and Condensed) easily form complexes with proteins and some polysaccharides making them recalcitrant to biodegradation (Nitiema *et al.*, 2010). Hydrolysable tannins are more easily degraded by some bacteria and fungi which are able to synthesize the enzyme tannase. Complex organic substances with five or more aromatic rings are not easily degraded by microorganisms and tend to persist in the environment for long periods of time (Ray, 1994).

Plant tannins have five or more aromatic rings and therefore pose a problem to the environment (Ray, 1994).

Using phenol as an example of the aromatic group, cleavage of carbon is initiatedby opening of the aromatic ring with the process ending in acetyl-CoA or Pyruvic Acid. Cleavage of the ring is by di-oxygenases with the incorporation of molecular oxygen. Mono substituted or 1,2 disubstituted aromatic rings such as phenol and phenylalanine are degraded to catechol. 1,3;1,4. Multi or poly substituted aromatic rings are degraded to protocatechuate (Schlegel, 1995). In aromatic degradation, hydroxyl group is incorporated into the ring with the oxygen coming from molecular oxygen (Schlegel, 1995).

Phenolic aromatics are further hydrogenated by mono-oxygenase with one of the atoms of molecular oxygen incorporated and the other reduced to water by hydrogen from reduced pyridine (Schlegel, 1995). Catechol is cleaved by ortho-pyrocatechase to cis, cis-muconate which is further split into succinyl co-A or Acetyl co-A which then enters the intermediate metabolism (Schlegel, 1995). Cleavage may also occur between hydroxylated carbon and neighbouring non-hydroxylated carbon atoms. The process is meta or extradiol cleavage catalysed by dioxygenases into 2-hydroxy-muconate-semi aldehydes which then enter the intermediary metabolism via pyruvate or similar products (Fig. 11).

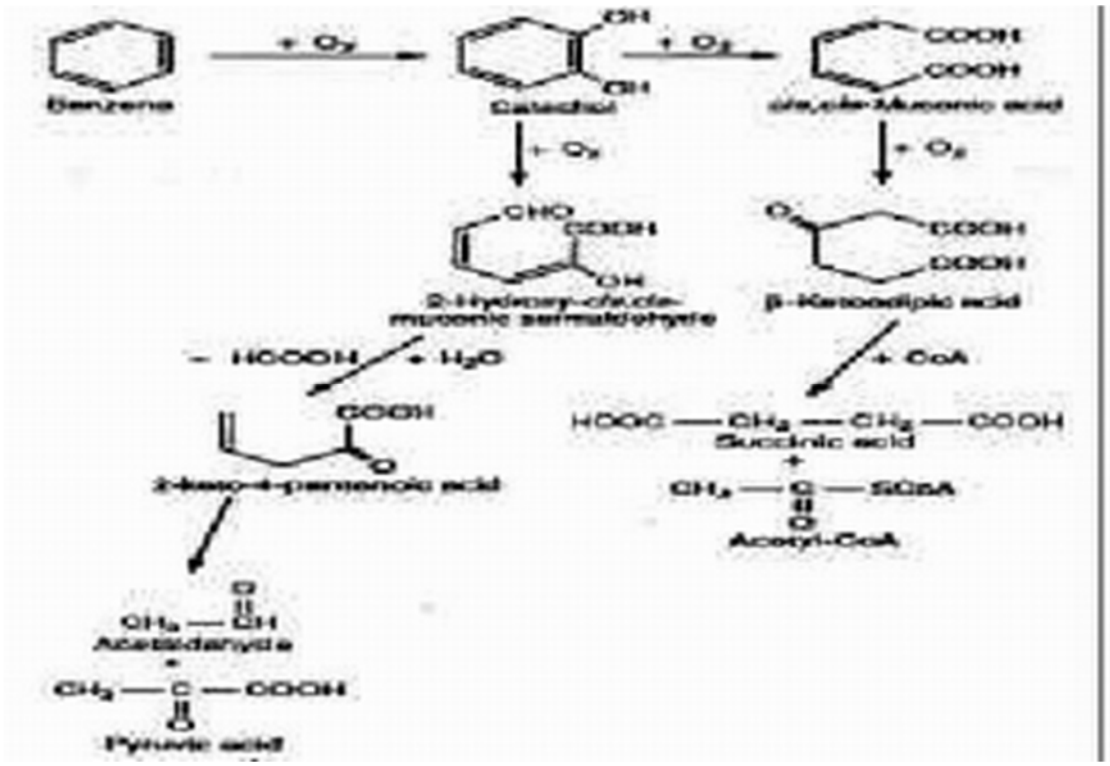


Figure 11Pathway for Benzene biodegradation Source: Truong (2005)

Microorganisms (fungi and bacteria) capable of utilizing toxic agricultural wastes of high tannin content similar to shea nut cake, such as coffee husks, as carbon and energy sources have been reported to abound in soils contaminated with such wastes (Mazzafera, 2002). Many hydrocarbon degrading bacteria have also been characterized from various hydrocarbon polluted environments including soil and water and applied in bioremediation to clear oil spills (Ray, 1994). Where hydrocarbon degrading microbes were found to abound, nutrients and growth factors were added (fertilization) to increase bacteria population and hasten biodegradation (Bragg *et al.*, 1992; Ray, 1994; Adebusoye *et al.*, 2006; Hawle-Ambrosch *et al.*, 2007; Hamzah *et al.*, 2010). Hydrocarbon degrading microbes are reported to be naturally present in soil and rapidly increase in number in contaminated soils (Ray, 1994; Truong, 2005). Hydrocarbon degrading bacteria identified in hydrocarbon contaminated beaches include *Pseudomonas* spp, *Pseudomonas fluorescens, Pseudomonas petida*, *Trichosporon* spp., and *Aeromonas* spp. (Ray, 1994; Hamzah *et al.*, 2010).

Pseudomonas spp. has been reported as the predominant bacteria isolated from soil from coffee farms (Mazzafera, 2002). Mazzafera, (2002) reported that a strain of *Lactobacillus plantarum* was able to degrade up to 90% of tannin present in coffee pulp.

The most remarkable and notable physiological property of *Pseudomonas*, especially fluorescent *Pseudomonas*, is the wide range of different organic compounds including heterocyclic and aromatic compounds not attacked by other bacteria, that they can metabolise as sources of carbon and energy. This versatility of *Pseudomonas* over all biological groups is because most of these special enzymes used by the organism are inducible and are only formed in response to specific induction by the substrate via plasmids (Bhatia and Ichhpujani, 2005). The isolation of tannin-protein complex degrading bacteria from gut contents of animals and humans feeding on tannin-rich plants has also been reported (Osawa *et al.*, 2000; Sasaki *et al.*, 2005; Nitiema *et al.*, 2010). Even though the potential of shea nut cake as animal and poultry feed, has been recognized and the potential in bacteria to remove tannin from the waste is beginning to attract public attention (Mazzafera, 1992; Nitiema *et al.*, 2010), information on suitable aerobic microorganisms capable of degrading tannin complexes in shea nut cake is unavailable. Some earlier studies cited above centred on gut contents of animals feeding on tannin-rich plant products as possible microbial sources (Sasaki *et al.*, 2005; Nitiema *et al.*, 2010), while available literature has not reported any information on indigenous soil, with its myriad of microorganisms and constantly receiving shea nut cake as a source of shea nut cake degrading bacteria. This study was therefore aimed atisolating and using indigenous aerobic soil bacteria to degrade or manage shea nut cake under the organism's optimum conditions of growth.

2.12: Aerobiosis and anaerobiosis in microorganisms

Microorganisms can be classified based on their oxygen requirement for survival. Some require strictly the presence of molecular oxygen with aerobic respiration as mechanism of obtaining energy for survival, the absence of which results in death; these are obligate aerobes. Some microorganisms grow best at partial pressures of oxygen lower than that in air and are referred to as microaerophiles. The phenomenon is the result of the sensitivity of some of their enzymes to strongly oxidizing conditions despite the requirement for molecular oxygen in energy generation (Stanier *et al*., 1986). Strict or obligate anaerobes are so sensitive to the presence of minute amounts of molecular oxygen that the presence of oxygen inactivates the vital enzymes which must remain in a reduced state to function. Such enzymes become oxidized in the presence of oxygen and some metabolic processes are prevented resulting in death (Stanier *et al*., 1986).

Anaerobes lack the enzymes that can react with oxygen to produce hydrogen peroxide which acts as the oxidising agent and inhibits growth (Stanier *et al.*, 1986).Facultative anaerobes can grow either in the presence or absence of air. They have anaerobic type of metabolism but are not sensitive to the presence of oxygen. These organisms can shift from aerobic to anaerobic metabolism depending on the presence or absence of oxygen. Many bacteria are in this category and can carry out respiration or fermentation, switching from one to the other depending on the availability of oxygen (Stanier *et al*., 1986).

2.13 Removal of Tannins in shea nut cake

Anti–nutritional factors reported in shea nut cake are saponins (Atuahene, 1998), tannins (Okai, 1990; Oddoye *et al*., 2012) and theobromine (Atuahene, 1998; Oddoye *et al*., 2012). Though the potential of shea nut cake in animal nutrition has been recognised due to its high content of protein, carbohydrates, fatty acids and minerals (Oddoye *et al.*, 2012, Iddrisu, 2013, Dei *et al*., 2008), published works suggested the inclusion of limited amounts in feed of

various animals and poultry (Atuahene *et al*., 1998; Adeogun, 1989; Osei–Amaning, 1993; Olorede and Longe, 1999; Okai, 1990; Konlan, 2010). Some forms of intervention to minimize the effects of these anti–nutritional factors in shea nut cake have been attempted. These include treatment of shea nut cake with water, chemicals such as sodium hydroxide and sodium chloride (Okai, 1989, Oddoye *et al*., 2012).

These treatments reported that soaking of shea nut cake in the ratio of 1 g of shea nut cake to 20 ml of water and boiling for one hour reduced the tannin content of shea nut cake from almost 10% to 3%. When rats were fed on the products, they gained weight (Okai, 1990). Oddoye *et al*. (2012) investigated various methods of tannin removal. They reported that tannin in shea nut cake are highly soluble in water due to their free hydrogen bond interaction with water molecules. From the various methods of tannin removal from shea nut cake investigated, boiling shea nut cake in water (1 g: 20 ml of water) at 75^{o} C for 1hour,wetting of shea nut cake with 0.01M aqueous solution of sodium hydroxide and with 3% sodium chloride,

Oddoye *et al*. (2012) reported that boiling reduced tannin concentration by 70% and sodium hydroxide reduced both tannin and theobromine by 69.9% and 25% respectively, making sodium hydroxide treatment a more efficient way of reducing both anti–nutritional agents as compared to boiling. Oddoye *et al*. (2012) noted that increase in treatment time and temperature resulted in significant reduction in total tannins. Similar studies with other vegetable tannins gave enhanced tannin reduction with increase in length of time and temperature (Onwuka, 2006; Oladele *et al*., 2009).

Oddoye *et al*. (2012) attributed this high removal rate of tannin by sodium hydroxide to alkaline hydrolysis and solubilisation of tannins leading to the breaking of the C-C bonds linking their monomers. Importantly Oddoye *et al*. (2012) noted that elimination of anti-

nutritional agents including theobromine by chemical means is expensive in terms of high cost of chemicals, and sophisticated equipment required. Side reactions in chemical treatment pose another problem as to how to deal with such end products; the removal of which comes with additional cost especially in large scale production. This makes chemical treatment not practicable or sustainable in low income groups in developing countries (Oddoye *et al.*, 2012). The researchers suggested the use of simple, effective, affordable and environmentally friendly procedures such as biological treatment. They suggested that the use of microorganisms to remove anti-nutritional agents would be more environmentally safe and cost effective compared to chemical methods. This experiment was conducted to investigate the use of soil microorganisms to reduce concentration of tannins in shea nut cake.

3.0 CHAPTER THREE

MATERIALS AND METHODS

3.1 Soil sampling and Experimental design

3.1.1 Selection of sites

Selection of study sites was purposive, and location of Sagnarigu was of interest. In Sagnarigu there are certain locations in which activities of shea butter extraction are carried out in an organized way. The selection of locations was based on practice. The following three centres were selected for study: Tungteiya Women Association Shea Butter Extraction Centre (Christian Mothers Association project) in Jisonayilli; Tiehisuma Shea Butter Processing Centre in Gurugu and Sekaf Shea Butter Village in Kasalgu. The selected extraction centres are well established shea butter extraction and exporting centres that operate all year round, with high mass production levels.

All the selected areas have a period of not less than five years of continuous shea butter extraction and shea nut cake dumping sites have received shea nut cake pollution constantly and heavily over that period with an annual average shea kernel consumption of 250 to 300 metric tonnes per location. Soils of shea nut cake dumping sites receiving shea nut cake consistently for this period are more likely to be good sources of stable shea nut cake degrading bacterial population. Jisonayilli is a human settlement located between Kanvilli and Gurugu. Gurugu is located along Tamale-Kumbugu road and Kasalgu is located along the Tamale- Nyankpala road, all in the Sagnarigu District. The lands in Jisonayilli and Gurugu are flat while that of Kasalgu slopes from north to south.

3.1.2 Soil sampling

A land size of $24\,m \times 6\,m$ was mapped out on lands in front and at the back of each of the three selected shea butter processing centres which served as shea nut cake dumping sites. A similar land size was measured from unpolluted land 100 metres away from the shea nut cake polluted area for each location. Each parcel of measured land was divided into three (3) plots of $8\,m \times 6\,m$ each. Three spots were selected randomly from each plot from which samples were taken at three soil depths (0-20 cm, 21-40 cm, 41-60 cm). Lands were considered as unpolluted areas based on discussions with key-informants such as traditional rulers, Assemblymen, opinion leaders and women in shea butter extraction business in the areas concerned, which revealed that those areas have never received shea nut cake. A total of fifty four (54) samples from each of the three selected polluted locations making up a total of one hundred and sixty two (162) samples from the three polluted locations and one hundred and sixty two (162) from sites with no history of shea nut cake pollution (unpolluted) in Gurugu, Jisonayilli and Kasalgu were collected. The samples were collected in alternate months from September, 2010 to July, 2011 into sterile polythene bags, using a graduated steel soil auger, stored in a fridge at 4^{o}C in the Laboratory of the School of Medicine and Health Sciences of the University for Development Studies, and analysed within 24 hours.

3.1.3 Experimental design

Both factorial design and single factor experiments were used for the investigations. The experimental design to study the physico-chemical characteristics and microbial populations of shea nut cake polluted soils was a completely randomized 4-way$3 \times 3 \times 2 \times 3 \times 6$ multi-factorial structure representing the following independent variables respectively 3 replicates, 3 locations, 2 soil types, 3 soil depths and 6 sampling times and 324 samples and data points followed by multiple comparison (multifactor Analysis of Varience-ANOVA) using least significant difference at 5% level of probability for analysis of the data. The dependent

variables measured were pH, moisture content, nitrogen content, carbon content, fungi population and bacteria population. Microbial populations were transformed using $\log_{10}$.

Single factor experiments were used to determine the optimum growth conditions of the isolated strain of *Pseudomonas* species and degradation of tannins in shea nut cake. T-test was used to compare the means for Gram reaction of bacteria isolated from shea nut cake polluted soil.

3.2 General Methods

3.2.1 Soil colour Determination

Soil colour was determined using the Munsell chart below based on a method described by Lynn and Pearson (2000). To 10 g of soil was added 1.0 ml of distilled water and well mixed against a white tile background. The colour of the moistened soil was then determined by matching with standard colours on Munsell soil colour chart (Fig. 12) to select the colour that most matched the colour of the moistened soil test sample (Lynn and Pearson, 2000). From the managements of the shea butter extraction centres, production and pollution of the soil of sampling sites with shea nut cake started five years ago hence the 5 years Munsell colour chart was used for shea nut cake polluted soil, while the 10 years chart was used for the unpolluted soil.

10 years

5 years

Figure 12 Munsell Soil Colour Chart

3.2.2 Soil texture reading

The soil texture triangle (Fig. 13) is divided into regions. Each region represents a soil texture class and is designated as such. Each of the three sides of the triangle represents one of the three soil particles (sand, silt and clay). Soil texture reading was carried out in accordance with the method of the American Soil Science Society described by Berry *et al.,* (2007). Below each of the three sides of the soil texture triangle labelled 1-100 is a standard arrow showing the direction the line from the point representing the percentage of the respective soil particle and parallel to the arrow should be drawn across the triangle. The labelled region of the triangle in which the lines from the three sides of the triangle, representing the percentages of the three soil particles intersected gave the texture class of the soil.

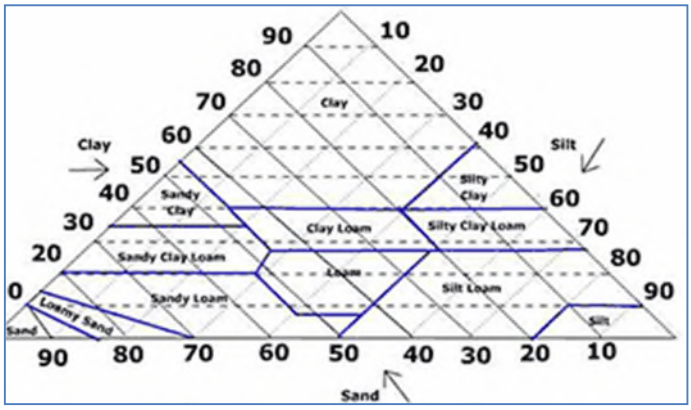


Figure 13 Soil Texture Triangle

3.3 Preparation of laboratory materials

3.3.1 Cleaning and Sterilization of laboratory materials

3.3.1.1 Glass ware

Petri dishes, test tubes and slides were sterilized by hot air oven. The materials were washed clean with detergent, dried, wrapped individually with aluminium foil and placed in a 60-230^{o}C Hot Air oven, model WTB Binder E 28 of Binder Co. U.K., and subjected to a temperature of 160^{o} C for two hours.

3.3.1.2 Bacteriological Inoculation wires

These were sterilized by dipping in 70% ethanol before flaming to red hot in Bunsen burner flame prior to use.

3.3.1.3 Sterilization of Culture Media

Media were prepared as described below and placed in a Gallenkamp Express Bucket water jacketed autoclave from Gallenkamp Co. U.K. with caps loosened. The materials were tagged with a strip of Bowie-Dick autoclave tape to check the efficiency of sterilization (Ochei and Kolhatkar, 2000). The materials were then subjected to steam at 15 pounds pressure which gave a temperature of 121^{o} C for 15 minutes. After the autoclaving cycle the autoclave was

switch off, steam gradually released until pressure gauge reading was zero (0) before materials were removed from the autoclave. The autoclave tape was inspected for uniform colour change as indication that sterilization was complete.

3.3.2 Preparation of Media

3.3.2.1 Nutrient Agar

Nutrient agar is a basic or general purpose medium. The medium was prepared by mixing the ingredients listed below (Table 7) in one litre (1.0 L) of distilled water and sterilizing at 121^{o} C for 15 minutes. The sterilized medium was cooled to 50^{o} C and aseptically poured 10-12 ml volumes into sterile petri dishes, placed on flat undisturbed bench to solidify. Sterility was tested by incubating at 37^{o} C for 24 h for bacterial growth. Any plate showing growth was contaminated and was to be discarded.

Table 7 Recipe for Nutrient Agar

Ingredients	**Amount (g L^{-1} Distilled water)**
Lab-Lemco powder	1
Yeast extract	2
Peptone	5
Sodium chloride	5
Agar No. 3	15

Source: Oxoid, (2000)

3.3.2.2 Nutrient Agar for counting bacteria

The above sterilized Nutrient agar medium (2.5.2) was cooled to 50^{o}C and Nystatin U.S.P. 100,000 I.U.L^{-1} from Biomedicine S.P.R.L, Brussels/Belgium aseptically added to prevent

fungal growth.The mixture was thoroughly mixed, poured in 10-12 ml volumes into sterile petri dishes and placed on flat undisturbed bench to solidify. Test of sterility was performed by incubating at 37^{o} C for 24 h for bacterial growth. Any plate showing growth was contaminated and therefore discarded.

3.3.2.3 Nutrient broth preparation

The ingredients below (Table 8) were dissolved in distilled water and the pHwas adjusted to 7.4. The medium was dispensed into clean screw-cap test tubes. The caps of the tubes were loosened and sterilized at 121^{o} C for 15 minutes. The media were then cooled to 50^{o} C and caps tightened.

Table 8 Recipe for Nutrient broth

Ingredients	**Amount (gL^{-1} Dist water)**
Lab-Lemco powder	1
Yeast extract	2
Peptone	5
Sodium chloride	5

Source: Oxoid Limited, (2000)

3.3.2.4 MacConkey agar

The ingredients indicated below (Table 9) were added to the distilled water, boiled to dissolve and pH adjusted to 7.4. The medium was sterilized at 121^{o} C for 15 minutes, cooled to 50^{o} C, dispense in 10-12 ml volumes into sterile petri dishes and allowed to solidify.

Table 9 Recipe for MacConkey agar

Ingredients	Amount (g L^{-1} Distilled water)
Peptone	20.0
Lactose	10.0
Bile salts	5.0
Sodium chloride	5.0
Neutral red	0.07
Agar	12.0

Source: Oxoid Limited (2000)

3.3.2.5 Blood Agar

Blood Agar is an enriched medium for the cultivation of all bacteria including fastidious ones. It is also used to demonstrate haemolysis. The blood agar base ingredients below (Table 10) were boiled in the distilled water to dissolve. The pH of the medium was adjusted to 7.4. The medium was sterilized at 121^{o} C for 15 minutes and cooled to 48^{o} C. Five per cent (5%) of defibrinated blood was added, rolled to mix thoroughly, poured aseptically in 10 ml amounts into sterile petri dishes and left under sterile conditions to solidify.

Table 10 Recipe for Blood Agar base

Ingredients	Amount (g L^{-1} Distilled water)
Lab-Lemco powder	1
Yeast extract	2
Peptone	5
Sodium chloride	5
Agar	15

Source: Oxoid Limited, (2000)

3.3.2.6 Motility Agar

Motility Agar is soft medium that permits motile organisms growing in it to migrate away from point of inoculation. The ingredients for the medium (Table 11) were dissolved in the distilled water, pH adjusted to 7.4, dispensed in 8 ml volumes in screw-cap test tubes and sterilized at 121^{o} C for 15 minutes. Inoculation was by deep stabbing with colony or broth culture with a sterilized inoculation straight wire. Motile organisms were expected to show diffused growth away from line of inoculation while non-motile organisms showed growth only along the line of inoculation.

Table 11 Recipe for Motility Agar

Ingredients	Amount (g L^{-1} Distilled water)
Beef extract	3.0
Peptone	10.0
Sodium chloride	5.0
Agar	4.0

Source: Ovoid Limited (2000)

3.3.2.7 Kligler Iron Agar (KIA)

Thisis a differential medium for the identification of *Enterobacteriaceae* on the basis of double sugar fermentation, gas and H_2S production. The ingredients (Table 12) were mixed, boiled to dissolve, pH adjusted to 7.4 and distributed into screw cap tubes. The media in the tubes were sterilized at 121^o C for 15 minutes and slanted to set as slopes with 1 inch butt. Inoculation was by stabbing of butt deep and smearing slant with pure culture or colony. A yellow butt (acid production) and pink slope indicated fermentation of glucose only. Cracks and bubbles in the medium indicated gas production. Blackening of medium indicated H_2S production.

Table 12 Recipe for Kligler Iron Agar (KIA)

Ingredients	Amount (gL^{-1} distilled water)
Lab-Lemco powder	3.0
Yeast extract	3.0
Peptone	20.0
Sodium chloride	5.0
Lactose	10.0
Dextrose	1.0
Ferric citrate	0.3
Sodium thiosulphate	0.3
Phenol red	0.05
Agar	12.0

Source: Oxoid Limited (2000)

3.3.2.8 Simmons Citrate Agar

Simmons Citrate Agar is a biochemical medium containing citrate as sole source of carbon. It is used to demonstrate the ability of an organism to utilize citrate as sole source of carbon. The medium is green in colour but when positive turns blue. The medium was prepared by mixing the ingredients (Table 13) in the one litre of distilled water, boiledto dissolve and pH adjusted to 7.0. The medium was dispensed in 3-5 millilitre volumes into screw-cap tubes, sterilized at 121^{o} C for 15 minutes, cooled to room temperature and allowed to solidify.

Table 13 Recipe for Simmons Citrate Agar

Ingredients	Amount (g L^{-1} Distilled water)
Sodium chloride	5.0
Sodium ammonium phosphate	0.8
Ammonium dihydrogen phosphate	0.2
Magnesium sulphate	0.2
Sodium citrate, tribasic	2.0
Bromo-thymol blue	0.08
Agar	15.0

Source: Oxoid Limited (2000)

3.3.2.9 Peptone Water

This is a basic or general purpose medium popularly used for investigating indole production by bacteria. The under stated reagents of the medium (Table 14) were added to the distilled water and boiled to dissolve. The pH was adjusted to 8.0. The medium was dispensed in 5.0 ml volumes into screw-cap tubes and sterilized at 121^{o} C for 15 minutes.

Table 14 Recipe for Peptone Water

Ingredients	Amount (g L^{-1} Distilled water)
Peptone	10.0
Sodium chloride	5.0

Source: Oxoid Limited (2000)

3.3.2.10 Urea broth

The ingredients (Table 15) were added to 950 ml distilled water, sterilized by autoclave at 121º C for 15 minutes. It was cooled to 55º C and 50 ml of sterile 40% urea solution was added aseptically. The medium was then mixed and distributed in 10 ml amounts into sterile tubes or bottles. Inoculation was by washing inoculum culture in broth and incubating at 35º C for 24 hours. Urease production was indicated by pink colour of medium with bacterial growth.

Table 15 Recipe for Urea broth base

Ingredients	Amount (g L^{-1} Distilled water)
Peptone	1.0
Dextrose	1.0
Disodium phosphate	1.2
Potassium dihydrogen phosphate	0.8
Sodium chloride	5.0
Phenol red	0.004

Source: Oxoid Limited (2000)

3.3.2.11 Potato Dextrose Agar (PDA)

The ingredients for the medium (Table 16) were mixed in one litre (1.0L) of distilled water and boiled to dissolve, sterilized at 121º C for 15 minutes and pH adjusted to 3.5 with 10 ml of 10% sterile Tartaric acid commercially available, without reheating. The medium was then cooled to 50º C and Benzylpenicillin 1MIU from Letap Pharmaceuticals Ltd, Accra, Ghana per litre of sterile medium added to inhibit the growth of bacteria. The medium was

poured aseptically in 15ml volumes into sterilized petri dishes and allowed to solidify. Plates were inoculated by spread plate technique.

Table 16 Recipe for Potato Dextrose Agar

Ingredients	**Amount (g L^{-1} Dist. water)**
Potato extract	4
Dextrose	20
Agar	15

Source: Oxoid Limited (2000)

3.3.2.12: Mineral salt medium (MSM) for isolation of bacteria associated with shea nut cake.

The ingredients for the medium (Table 17) were dissolved in a litre of distilled water. The pH was adjusted to 7.0 with 1M Sodium Hydroxide and sterilized at 121^{o} C, 15 pounds pressure, for 15 minutes. The medium was cooled to room temperature and 20.0 g/L (2%) shea nut cake was added as sole source of carbon. Prior to inoculation Nystatin, U.S.P. 100,000 I.U. from Biomedicine S.P.R.L, Brussels, Belgium was added per litre of sterile medium to prevent fungal growth.

Table 17 Recipe for Mineral salt medium

Ingredient	Amount (g) in 1L of distilled water
Dipotassium hydrogen phosphate	1.0
Potassium dihydrogen phosphate	0.2
Sodium chloride	1.0
Calcium chloride	0.001
Magnesium sulphate ($MgSO_4.7H_2O$)	0.5
Ferrous sulphate ($FeSO_4.6H_2O$)	0.01
Cupper sulphate ($CuSO_4.5H_2O$)	0.001
Zinc sulphate ($ZnSO_4.7H_2O$)	0.001
Manganese sulphate ($MnSO_4.H_2O$)	0.001

Source: Murad *et al*. (2007)

3.3.2.13 Shea nut cake agar medium

To one (1.0) litre of the above mineral salt medium (2.5.2.12) was added 15.0 grams of agar as a solidifying agent, sterilized at 121^o C for 15 minutes, cooled to 50^o C before adding the 2% shea nut cake and the antibiotic as in 2.5.2.12. The medium was poured into sterile petri dishes (20 ml each) and allowed to solidify.

3.4 Reagents preparation and use

3.4.1 Gram's Staining Reagents (Source: Cheesbrough, 2002).

(a) Crystal violet stain: An amount of 20 g crystal violet and 95 ml absolute ethanol, 9 g ammonium oxalate were dissolved in 1.0 litre of distilled water.

(b) Gram's Iodine

One gram (1.0 g) of Iodine crystals and 2.0 grams of Potassium iodide were ground together, dissolved in 5.0 ml of distilled water, followed by 240 ml of distilled water and then 60.0 ml of 5% sodium bicarbonate aqueous solution.

(c) Acetone-Alcohol (Decolorizer for Gram's staining)

Acetone and ethanol (95%) were mixed in equal volumes and stored for use.

(d) Neutral Red (1g/L = 0.1%)

One gram (1g) neutral red powder was dissolved in 1L of distilled water.

3.4.2 Gram Staining Technique (Source: Cheesbrough, 2002)

1. Dried smears on the slides were fixed by passing the smear through gentle flame 3 to 4 times and allowed to cool.
2. Fixed smear was flooded with crystal violet stain for 30 seconds, rapidly washed off with clean running water.
3. The smear was then flooded with Gram's iodine for 60 seconds.
4. Iodine was washed off with clean running water.
5. The smear was decolorize rapidly (few seconds) with acetone-alcohol, washed immediately with clean running water and counter stained with neutral red stain for 2 minutes. The counter stain was washed off, smear drain dried and examined under oil immersion (X 100) objective.

Gram positive bacteria appeared dark purple and Gram negative red.

3.4.3 Oxidase test

Oxidase reagent: Tetramethyl–p–phenylenedianine dihydrochloride (commercial preparation available)

One gram (1 g) was dissolved into 10 ml of distilled water. Prepared fresh for the day's use as prepared reagent is not stable. A piece of filter paper was soaked with a few drops of oxidase reagent. A colony of the test organism was then smeared on the filter paper. If the organism was oxidase-producing, the phenylenediamine in the reagent would be oxidized to a deep purple colour.

3.4.4 Catalase test

The reagent for the test is Hydrogen Peroxide (commercially available). Catalase acts as a catalyst in the breakdown of hydrogen peroxide to oxygen and water. Into a sterile test tube were added 2.0 ml of hydrogen peroxide reagent. Using a sterile wooden stick or a sterile glass rod, a good growth of a 24 hours Nutrient agar culture of the test organism was removed and added to the hydrogen peroxide solution, and observed for immediate bubbling of gas. Active gas bubbling was an indication of catalase positive and no release of gas bubbles indicated catalase negative.

3.4.5 Indole Test

One millilitre (1 ml) of Kovac's reagent was added to 5.0 ml of 24 hours peptone water culture of test organism and observed for red colour between reagent and culture broth for indole producing organisms. Negative test does not show any red colour.

3.4.6: Methyl-Red (MR) and Vokes-Proskauer (VP) test (Source:Baron *et al.*, 1994)

Principle: The test differentiates mixed fermentation in glucose metabolism by bacteria. Acid production from glucose metabolism by mixed fermentation pathways (Positive test)

is detected by development of red colour with methyl red indicator: production of acetyl methyl carbinol –butanediol fermentation) (indicating pH < 4.5) (MR).

Acetoin and butanediol products of butanediol fermentation yield a pink or red colour with α-naphthol in the alkaline environment (VP).

Reagents:

Methyl-Red reagent

Methyl Red powder - 0.1 g

Absolute ethanol – 300 ml

Distilled water – 200 ml

The methyl red powder was dissolved in the alcohol before adding the distilled water.

Voges-Proskauer reagents

Reagent A

α-Naphthol – 5.0 g

Ethanol (absolute) – 100 ml

The Naphthol was dissolved in a small amount of alcohol and made up to 100 ml with the alcohol.

Reagent B

Potassium Hydroxide – 40 g

Distilled water – 100 ml

The Potassium hydroxide was dissolved in a small amount of alcohol in a cool water bath to avoid overheating and made up to 100 ml with distilled water.

Test procedure

Test organisms were inoculated in Voges-Proskauer broth base (Commercially available) and incubated at 35 ^{o}C for 48 hours.

Voges-Proskauer (VP) test

To a 2.5 ml 48 hours culture above were added 0.6 ml (6 drops) of VP reagent A and 0.2 ml (2 drops) of VP reagent B. The above were mixed and allowed to stand on the bench for 15 minutes, observing for colour development. Positive test is indicated by the development of pink to red colour. Colourless or yellow colour indicates negative test for VP test.

Methyl red (Acidity) test

To 2.5 ml of fresh 48 hours VP culture of test organism was added 0.5 ml (5 drops) of MR reagent and observed for colour change. If indicator (Medium) remained red it was an indication of positive test (indicating pH < 4.5). A yellow colour indicated a negative test (pH > 6). Control organisms used were: *Escherichia coli* which gave MR = +ve and VP = -ve;

And *Klebsiella pneumoniae*: MR= -ve and VP = +ve

3.5 Soil microbial count

One gram (1 g) of soil was suspended in sterile normal saline and votexed for 2 hrs.Seven sterile test tubes were added 9.0 ml of sterile normal saline. One millilitre (1ml) of soil suspension was added to the first tubes and mixed. One millilitre (1ml) was transferred from

the first tube to the second tube. The process was continued until the last tube was served. This gives serial dilutions of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7}.

For each transfer a separate sterile pipette was used. One millilitre (1ml) of each of the above tubes was inoculated onto nutrient agar for bacteria and potato dextrose agar for fungi by spread plate method. The suspension was placed on the sterile medium and allowed to flow over the entire surface of the medium. Excess suspension was decanted by turning plate over a receptacle. The plates were then incubated at 25^{o} C for 24 hours for bacteria and up to 48 hours for fungi.

Each plate was divided into quadrants and colonies in each quadrant counted in a manual colony counter using tally counters. Inoculation and counting were done in triplicates and a mean calculated. The colony forming units (cfu) per gram dry weight of soil was calculated as indicated in Table 3.5 below.

Table 18Form for reading and calculating microbial numbers (cfu)

Soil depth:

sampling	JISONAYILLI			GURUGU			KASALGU		
Month	Wet wght of soil gm^{-1} dry weight	Cfu gm^{-1} wet wght of soil (100ml=cfu/ml x 100)	Cfu gm^{-1} dry wght soil	Wet wght of soil gm^{-1} dry weight	Cfu gm^{-1} wet wght of soil (100ml)	Cfu gm^{-1} dry wght soil	Wet wght of soil gm^{-1} dry weight	Cfu gm^{-1} wet wght of soil (100ml)	Cfu gm^{-1} dry wght soil
		$X\ 10^x$	$X\ 10^x$		$X\ 10^x$	$X\ 10^x$		$X\ 10^x$	$X\ 10^x$
	a	b	a x b	a	b	a x b	a	b	a x b
1. Sept 2010									
2. Nov 2010									
3. Jan. 2011									
4. Mar 2011									
5. May 2011									
6. July 2011									

3.6 Determination of physico-chemical characteristics

3.6.1 Soil preparation

Soil sampling was as described in page 63. Therefore a total of 324 samples were used for the determination of physico-chemical characteristics. The samples were sieved through a sterile sieve of 1.7 mm pore diameter to remove large objects and used for physico-chemical analysis. Depth-wise distributions of physico-chemical characteristics of shea nut cake polluted soils were compared with unpolluted soils.

3.6.2 Soil colour determination

Soil colour was determined by the Munsell System of colour notation described in page 64

3.6.3 Soil Texture

Soil particle proportions expressed in percentages, were determined usinga method described by Berry *et al*. (2007). Five hundred grams (500 g) of well mixed, air dried soil samples described in immediate paragraph page 90 were transferred into a wide mouth, screw capped graduated jar. The jar was filled with water to the ¾ mark to allow some space for air. The cap of the jar was tightly screwed on and the jar shaken vigorously for two (2) minutes for all soil particles to form a homogenous suspension. The jar containing the suspended soil in water was placed on a bench to settle by gravity. The soil particles settled in the order sand, silt and clay from bottom to top of the jar. The volume occupied by each was recorded at the following time intervals (1 minute for sand, 60 minutes for silt and 48 hours for clay) and converted to percentages and the texture class determined from a soil texture triangle described in page 65 and stated in Appendix I.

3.6.4 Soil pH measurement

The pH values of the soil samples were measured with a pH meter, (Gallenkamp Model 640 U.K.) and combination electrode (a set of glass electrode and reference electrode), (Type No. PHM-110-010Y), after the electrode was calibrated against pH buffer 4, 7 and 9 to adjust the response of the glass electrode. To one gram of sieved air-dried soil in a beaker was added 10 ml of distilled water, mixed and allowed to stand foe 1 hr before calibrated electrodes were inserted for pH measurement. All chemicals, solutions and calibration standards of ANALAR grade were from BDH Chemical Supply Ltd., UK.

3.6.5 Soil Moisture Content determination

Soil moisture content was determined by the Gravimetric method described by Hausenbuiller (1975). Water content measurement by gravimetric method involves weighing a wet sample, removing the water via drying in an oven, and reweighing the sample at intervals to obtain a constant dry weight which is then used to determine the amount of water removed, expressed as a percentage. Fifty grams (50 g) of sieved (pore diameter less than 1.7 mm) fresh soil was weighed in a clean dry beaker. The soil was dried in a hot air oven (Model WTB Binder E 28 of Binder Co. U.K.) at 105^{o} C and weighed at 24 hours intervals until a constant weight was obtained for three consecutive times, representing the dry weight of soil. The soil dry weight was used to calculate the moisture content as indicated in the equation below.

$$\%M = (W_s|W_d) \times 100$$

M = represents soil moisture content;

W_S = represents difference between wet soil and dry soil;

W_d= weight of oven dry soil.

Triplicate results converted to $\log_{10}$ were expressed as mean.

3.6.6 Soil Organic Carbon Content measurement

The Potassium dichromate volumetric method described by Walkley and Black (1934) was used in this study to determine the organic carbon content of the soil samples. One gram (1 g) of soil was weighed into a 500 ml conical flask to which 10ml of 0.1667 M Potassium dichromate solution and 20 ml concentrated Sulphuric acid containing Silver Sulphate (Ag_2SO_4) were added. The suspension was thoroughly mixed and reaction allowed to continue for 30 minutes, diluted with 200 ml of water and 10 ml Phosphoric acid (H_3PO_4), followed by 10 ml of Sodium Fluoride (NaF) solution and 2ml of diphenylamine indicator.

This final solution was then titrated with 0.5 M Ferrous Sulphate ($FeSO_4$) solution to a brilliant green colour. A blank without sample was run simultaneously. The percentage of carbon was then calculated from the equation below:

$$\%\ carbon = \frac{(S-T)\times N\times 0.003\times 100}{W}$$

S = Millilitres of $FeSO_4$required for blank;

T = Millilitres of $FeSO_4$ required for soil sample;

W = Weight of soil sample (g)

N = Normality of $FeSO_4$

0.003 = constant

All reagents were ANALAR grade from Merck/BDH Chemical Merck House, Poole, BH15 1TD, UK.

3.6.7 Total Nitrogen determinaion

Total Nitrogen was determined by the Kjeldahl Nitrogen method described by Janssen and Koopmann (2005). The sieved soil sample was digested in a suitable Kjeldahl tube with concentrated H_2SO_4. Copper sulphate ($CuSO_4$) served as catalyst and potassium sulphate (K_2SO_4) used to maintain the temperature at 400^{o}C. Sodium hydroxide was then added to the digested solution to produce ammonium from all nitrogen species which was evaporated and distilled as Ammonia, condensed with boric acid into a flask and titrated against H_2SO_4 with indicator (Bromocresol Green and Methyl Red mixture) to a violet end point.

As done for the test, two blanks were run and average taken for calculation of the Total Nitrogen thus:

$$W_N = (V_1 - V_0) \times c(H^+) \times M_N \times \frac{100}{m} \times m_t$$

Where:

V_1 = volume in ml of H_2SO4 used in titration of the sample;

V_0 = volume in ml of H_2SO_4 used in titration of blank test;

c (H^+) = Concentration of H^+ in H_2SO_4 in moles/L (e.g. if 0.01 mol /L H_2SO_4 c(H^+) = 0.02mol/L; M_N = molar mass of Nitrogen in g/mole (= 14);

m = Mass of test sample;

m_t = dry residue expressed as g/100g on the basis of oven dried material.

All reagents were of ANALAR grade from Merck/ BDH Chemical Merck House, Poole, BH15 1TD, UK.

3.7 Determination of Microbial population

3.7.1 Soil sampling and experimental design

Soil sampling was as described in page 63, sieved with mesh of 1.7 mm diameter to remove large pieces of debris and plant materials and cultured for bacteria and fungi colony forming units within 24 hours. The experimental designused for the study was as described in page 63. The effects of the different locations, soil types, soil depths and sampling time on bacterial and fungal populations were investigated

3.7.2 Microbial count

Standard procedures for determining total soil microbial numbers described by Alexander (1982) and Truong (2005) and described below were adapted for bacteria and fungi counts. Bacterial and fungal populations were expressed as mean of log of colony forming units (cfu).

3.7.2.1 Total Bacterial count

One gram (1g) of sieved soil was suspended in 100 ml of sterile distilled water in a sterile 500 ml Erlenmeyer flask, vortexed for 60 minutes and allowed to settle for one hour. A serial dilution of the following strengths was made with 1.0 ml of the supernatant: 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7}. One millilitre (1 ml) of each dilution was inoculated by spread plate technique in triplicates onto sterile Nutrient agar plates, supplemented with Nystatin U.S.P. 100,000 I.U.L^{-1} from Biomedicine, S.P.R.L, Brussels/Belgium to prevent fungal growth, (page 67), incubated aerobically at 25^{o} C for 24 hours. Plates that recorded 30-300 colonies were selected, counted and the average of total bacteria count for the three replicated plates expressed as mean of colony forming units per dry weight of soil as explained in page 80.

3.7.2.2 Total Fungal population

One gram (1 g) of soil was suspended in 100 ml of sterile distilled water in a sterile 500 ml Erlenmeyer flask, vortexed for 60 minutes and allowed to settle for one hour. Serial dilutions of the following strengths were made with 1.0 ml of the supernatant: 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7}.

One millilitre (1.0 ml) each of the dilutions of soil supernatant was inoculated onto Potato Dextrose Agar (PDA) plates by spread plate method, incubated at 25^{o} C aerobically for 48 hours and observed daily for growth. Inoculation was done in triplicates. Prior to pouring the sterilized PDA medium it was cooled and supplemented with Benzylpenicillin 1MIU per litre of sterile medium to inhibit the growth of bacteria. The antibiotic was from Letap Pharmaceuticals Ltd, Accra, Ghana. Plates that recorded 30-300 colonies were counted and average of total fungi population for the three replicated plates expressed as colony forming units per gram dry weight of soil. Results were expressed in $\log_{10}$ of colony forming units as in page 80.

3.8 Isolation, identification and microbial degradation of tannins in shea nut cake

3.8.1 Sample collection

One hundred and sixty two shea nut cake polluted soil samples taken from shea nut cake polluted soils in the shea nut cake dumping sites of the three shea butter extraction centres in Gurugu, Jisonayilli and Kasalgu in the Sagnarigu District described in page 63 were used for the selective isolation of shea nut cake degrading bacteria.

3.8.2 Isolation and identification of tannin degrading bacteria from shea nut cake polluted soil.

Shea nut cake degrading bacteria were isolated by the enrichment method. A mineral salt medium modified from that described by Murad *et al. (*2007) for the isolation of soil bacteria was prepared as described in page 76. One gram (1 g) each of the one hundred and sixty two shea nut cake polluted soil samples was weighed into 100 ml of sterile mineral salt shea nut cake medium in a 250 ml Erlenmeyer flask, mixed and incubated at 25^{o} C for 72 hours. The inoculated medium was subcultured into similar mineral salt shea nut cake medium after every 72 hours. After a third successive subculture, a final subculture was made onto mineral salt medium supplemented with 2% shea nut cake to which 15 grams of agar was added as a solidifying agent described on page 76. The bacteria isolates were subcultured on 5% and 10% shea nut cake agar to isolate bacteria that can tolerate high concentration of shea nut cake.

Colonies were isolated, purified on nutrient agar prepared as described on page 71 and stored in glycerol broth at minus seventy degrees (-70^{o} C) in the Public Health Laboratory atTamale TeachingHospital (TTH) for further studies. The isolated bacteria were subjected to Gram staining as described on page 77. Suspected colonies were studied morphologically and

biochemically as described by Cheesbrough (2002) to select one for tannins degradation in shea nut cake.

Two colonies of GUR/09 and GUR/38 on Nutrient agar plates were inspected for pigmentation. *Pseudomonas* produces two types of pigments, fluorescent pigment pyoverdin and the blue pigment pyocyanin. Pyocyanin functions in iron metabolism and its production is associated with media of low iron content. Blood agar plates were inspected for haemolysis. MacConkey agar plates were inspected for lactose fermentation. Motility was tested by inoculating motility agar tubes prepared as described on page 70 with 24 hours colonies from nutrient agar. Gas, hydrogen sulphide and acid production with carbohydrate metabolism was tested with Kligler Iron Agar (KIA) which contains two sugars, lactose and glucose, and was prepared as described in page 71**.** Citrate utilization test was performed as described in page 76.

Urease production was tested by inoculating urea broth as described in page 74. Oxidase test was performed as on page 78. Catalase production was tested for as described in page 78. Indole production was investigated as described on page 78. Methyl-red and Voges-Proskauer tests were performed to established whether isolates produce acid from glucose metabolism through the mixed fermentation pathways as outlined in page 78.

Ability to form spores, heat resistance, ability to grow under anaerobic conditions, effect of pH, temperature and yeast extracts on the growth of GUR/09 and GUR/38 were conducted as described below**.**

3.8.3 Determination of Growth characteristics of GUR/09 and GUR/38

3.8.3.1 Ability to form Spores

Microscopic examinations of cultures of GUR/09 and GUR/38 were carried out at various stages of growth for spores.

3.8.3.2 Resistance to Heat

Test of resistance to heat was conducted on GUR/09 and GUR/38 in a method described by Nitiema *et al.* (2010). Twenty four (24) hour broth cultures of GUR/09 and GUR/38 were heated in mineral salt medium supplemented with 2% glucose at 80^{o} C, 90^{o} C and 100^{o} C for 10 minutes, cooled to room temperature and sub-cultured in fresh mineral salt medium, and on nutrient agar. The inoculated media were incubated at 25^{o} C and observed daily for up to 5 days for bacteria growth.

3.8.3.3 Anaerobiosis

This phenomenon was investigated using a fresh 24 hour nutrient broth culture of GUR/09 and GUR/38, inoculated onto sterile nutrient agar plates, of pH 7.0 by the streak method and incubated anaerobically at 25^{o} C using Anaerocult P gas generation kit and Anaerobiosis indicator, Anaerotest (Cat. No. 1.15112) from Merck KGaA Darmstadt, Germany. The plates were observed after 48 hours for bacterial growth. The blue indicator portion of the Anaerobiosis indicator strip, after 48 hours incubation turned colourless, indicating anaerobic conditions were met as stated by the manufacturer. The plates were inspected for colonial growth, after the indicator strip showed that anaerobic conditions were met.

3.8.3.4 Effect of pH on the growth of GUR/09 and GUR/38

The colorimetric method described by Nitiema *et al.* (2010) on shea nut cake degrading bacteria was used. For the determination of the effect of pH on growth, basal medium containing 2% glucose was used and pH adjusted to different pH values between 5 and 10 (5.0, 6.0, 7.0, 8.0, 9.0 and 10). One hundred millilitres (100 ml) per flask of Mineral salt medium were prepared as described above in triplicates in 250 ml Erlenmeyer flasks and pH adjusted to the above values. One millilitre (1.0 ml) of 24 hour broth culture of each of the selected bacteria was inoculated into each of the prepared mineral salt medium after adjusting the pH to the respective values. The inoculated media were incubated at 25^{o} C for 24 hours.

Microbial growth was determined using a spectrophotometer, Model: Therm Spectronic, Model:Helios Epsilo, CAT: 8523UVE1200E; SN: 3SGG162001 from USA, at 580 nm (Nitiema *et al.*, 2010). Optical Density (O.D.) reading was taken immediately after inoculation and after 24 hours against uninoculated Mineral Salt Medium amended with 2% glucose as reagent blank. Microbial growth was indicated by increase in optical density. Uninoculated media of the 6 pH values incubated along with media inoculated with bacteria served as controls.

3.8.3.5 Effect of Temperature on the Growth of GUR/09 and GUR/38

Like pH, temperature is very critical for bacteria biodegradation of natural products. The effect of temperature on growth of GUR/09 and GUR/38 was investigated. Basal medium containing 2% glucose and pH adjusted to 7.0 with N/10 sodium hydroxide was inoculated with isolates and incubated at the following temperatures: 25^{o} C, 35^{o} C, 45^{o} C, and 55^{o} C for 24 hours and growth measured in terms of increasing optical density at 580 nm against medium blank, using uninoculated medium incubated at respective temperatures as Unpolluted.

3.8.3.6 Effect of Yeast extracts on the Growth of GUR/09 and GUR/38

The growth of GUR/09 and GUR/38 in the presence of yeast extracts was investigated to find out if yeast extracts, which have been reported to contain sufficient vitamins and growth factors needed for bacteria growth (Stanier *et al.*, 1986), will have any effect on the growth of the bacteria. Stanier *et al.* (1986) stated that it is superfluous to add vitamins and growth factors to medium containing yeast extracts. To sterile tubes of mineral salt medium containing 2% glucose were added 0.5% yeast extracts. The pH was adjusted to 7.0 with N/10 sodium hydroxide and the media in triplicates were inoculated with GUR/09 and GUR/38, incubated at 25^{o} C and growth measured after 24 hours (Nitiema *et al.*, 2010). Bacterial growth was indicated by increase in optical density at 580 nm. for GUR/09 was

selected for biodegradation of tannins in shea nut cake based on better growth performance than GUR/38 outlined above.

3.8.4 Degradation of tannin in shea nut cak by Pseudomonas aeroginosa strain GUR/09

The method used in this study measured the disappearance of polyphenolic compounds (Tannins) from fresh and boiled shea nut cake as an indication of biodegradation of tannins in shea nut cake.

3.8.4.1 Preparation of shea nut cake for biodegradation of tannin in shea nut cake

A forty (40) kilogram shea nut cake sample was obtained from Tungteiya Women Association Shea Butter Extraction Centre in Jisonayilli. The sample was divided into two portions of 20 kg each. One part consisting of 20 kg of shea nut cake was divided into 1 kg each and boiled at 75^{o}C for 1hr, cooled overnight, strained, dried and used for tannin degradation (Oddoye *et al*., 2012). The ratio of shea nut cake to water was 1.0 Kg in 20 L water (representing 1.0 g: 20 mg) (Okai, 1990). The other 20 kg shea nut cake was divided into 1 kg each and used fresh (without boiling). To each kilogram treatment was added one litre of sterile distilled water. The ten treatment lots were labelled as indicated in Table 19.

Substrate preparation for each treatment was a modification of that described by Mazzafera (2002) for the removal of tannin from coffee pulp. The pH of each treatment was checked. To each kilogram treatment 5% yeast extracts and 1% glucose were added. The preparation was homogenized with one hundred millilitres (100 ml) of fresh 24 hour nutrient broth culture of *Pseudomonas aeroginosa* strain GUR/09, incubated at room temperature. The preparation was agitated twice daily to improve aeration (air supply to the organism). At the end of the incubation period of each preparation the treatment was strained, dried, ground to fine powder and sampled, 250 mg each for polyphenol assay.

Table 19 Preparation of samples for degradation of tannin in shea nut cake

Treatment	Treatment description	Incubation period (Days)
T_O	Fresh, unboiled, without bacteria serving as Unpolluted	
T_1	Boiled at 75° C without bacteria	
T_2	Fresh, unboiled inoculated with Pseudomonas *aeroginosa* GUR/09	10
T_3	Fresh, unboiled inoculated with Pseudomonas *aeroginosa* GUR/09	20
T_4	Fresh, unboiled inoculated with Pseudomonas *aeroginosa* GUR/09	30
T_5	Fresh, unboiled inoculated with Pseudomonas *aeroginosa* GUR/09	40
T_6	Fresh, boiled inoculated with Pseudomonas *aeroginosa* GUR/09	10
T_7	Fresh, boiled inoculated with Pseudomonas *aeroginosa* GUR/09	20
T_8	Fresh, boiled inoculated with Pseudomonas *aeroginosa* GUR/09	30
T_9	Fresh, boiled inoculated with Pseudomonas *aeroginosa* GUR/09	40

Each treatment was duplicated and mean of results recorded. Reduction in total phenolic contents expressed as tannic acid equivalent (TA) in % Dry Matter (DM) was a measure of biodegradation of tannins in shea nut cake. Percentage Dry Matter (% DM) was determined by subjecting 100 g air-dried ground shea nut cake to 100° C and weighing daily to obtain a constant weight for three consecutive times, and calculated using the formula:

$$\% DM = \frac{Constant\ dry\ weight}{Total\ (initial)\ weight} \times 100$$

3.8.4.2 Extraction of polyphenolic compounds

Phenolic compounds were extracted by a method described by FAO/IAEA (2000). Air-dried shea nut cake was ground to fine powder to pass through 0.5 mm sieve. To 250 mg of the powder from each of the 40 treatments was added 10 ml of 70% aqueous acetone (70:30) in a 50ml flask with lid, vigorously shaken and subjected to ultrasonic treatment for 20 minutes at 25° C. The contents of the flasks were then transferred to centrifuge tubes, cooled on ice and

then centrifuged at 3,000 g for 10 minutes. The supernatant was collected. The brown colour of the supernatant was removed by evaporating the acetone at 50° C and washing residue with diethyl ether containing 1% acetic acid until no brown colour appeared. The diethyl ether was evaporated at 50° C and the deposit dissolved in 10 ml of 70% aqueous acetone and used for polyphenol estimation. Folin-Ciocalteu method described by Makkar *et al*. (1993)was used for the determination of total phenols.

3.8.4.3 Preparation of Tannin Reagents

(1) **Folin Ciocalteu reagent (1 N):** Five millilitres (5 ml) of commercially available Folin-Ciocalteu reagent (2N) was diluted with an equal volume of distilled water.

(2) **Sodium carbonate (20%)** was prepared by dissolving forty grams (40 g) sodium carbonate in 150 ml of distilled water and made up to 200 ml with distilled water.

(3) **Standard tannic acid solution** (0.1 mg/ml) was prepared by dissolving 25 mg tannic acid (TA) in 25 ml distilled water. The preparation was diluted 1:10 in distilled water and used fresh.

All reagents were obtained from Hatch Co. USA.

3.8.4.4 Estimation of Tannin concentration

Determination of total phenolics is based on the fact that phenols are reducing agents (FAO/IAEA, 2000). Tannin extract (0.05 ml) was added to 0.45 ml of distilled water followed by addition of 0.25 ml of 1N Folin-Ciocalteu reagent. After three minutes, 1.25 ml of 20% aqueous solution of Sodium carbonate ($Na_2CO_3.10H_2O$) was added, vortexed and allowed to stand at room temperature for 40 minutes. The absorbance representing the intensity of the blue colour was measured at 725 nm after 40 minutes against a reagent blank. Total phenol concentration was estimated against the standard 0.1mg/ml tannic acid solution as directed by Hatch, Loveland CO., USA. Test and standard samples were treated the same way and calculations were done using the formula below (US AIRFORCE, 1997):

$$Concentration\ of\ Test\ (\mu g) = \frac{Optical\ Density\ of\ Test\ (nm)}{Optical\ Density\ of\ Standard\ (nm)} \times Concentration\ of\ Standard\ (\mu g)$$

All tannin and lignin test reagents were obtained from Hatch, Loveland Co., USA.

3.8.4.5 Interpretation of Table 5.5

STD represents the standard tannic acid used.

T0 –T9 Represents Treatments 0 to 9

Column (a) = Optical Densities

Column (b) = Concentration of Tannic acid in 0.05 ml of sample used for the test.

Since shea nut cake tannin extract and Tannin standard were treated the same way in the test procedure, column (b) was obtained using the formula (US AIRFORCE, 1997):

$$Concentration\ of\ Test\ (\mu g) = \frac{Optical\ Density\ of\ Test}{Optical\ Density\ of\ Standard} \times Concentration\ of\ Standard$$

From the preparation of the Tannic acid standard above 1.0 ml of standard tannic acid contained 0.1 mg Tannic acid (0.1mg per ml).

Therefore 0.05 ml of standard tannic acid used in the test will contain

$0.05\ ml = \frac{0.05}{1} \times 0.1 = 0.005\ mg\ TA$ (Tannic Acid)

Since $1.0\ mg = 1000\ \mu g$

$$0.005\ mg = 0.005 \times 1000 = 5.0\ \mu g$$

Applying the formula above

$$Concentration\ of\ Test\ (\mu g) = \frac{Optical\ Density\ of\ Test}{Optical\ Density\ of\ Standard} \times Concentration\ of\ Standard$$

$$Let\ Optical\ Density\ of\ test\ = y$$

And $Optical\ Density\ reading\ of\ Standard\ in\ Table\ above\ = 0.540$

$$Concentration\ of\ standard\ = 5.0 \mu g$$

Therefore

$$Concentration\ of\ test\ treatment\ (\mu g)\ (Column\ (b)) = \frac{y}{0.540} \times 5.0\ \mu g = 9.259y$$

If 0.05 ml of shea nut cake extract contains yµg of Tannin then

$$1.0\ ml\ shea\ nut\ cake\ extract\ (Column\ (c))\ = \frac{1.0}{0.05} y = 20y$$

$$Concentration\ of\ Tannic\ Acid\ per\ ml\ of\ shea\ nut\ cake\ extract\ \left({}^{mg}/_{ml}\right) column\ (d) = \frac{Column\ (c)}{1000}$$

$$Concentration\ of\ Tannic\ Acid\ per\ 100\ ml\ of\ shea\ nut\ cake\ extract\ (Column\ e)$$

$$= column\ d \times \frac{100}{250} \times 10\ = 4 \times column\ d$$

Shea nut cake contained 45% dry matter, therefore

$$Tannic\ acid\ Equivalent\ in\ \%\ Dry\ Matter\ = \frac{Column\ e}{0.45}$$

$$Tannic\ Acid\ equivalent\ in \frac{g}{Kg}; Column\ g = Column\ f\ \times 10$$

$$\%\ Reduction\ in\ Tannic\ Acid\ equivalent = \frac{(e) of\ fresh\ Standard - (e)\ of\ treatment}{(e)\ of\ fresh\ standard} \times 100$$

Where (**e)** is Concentration (g/kg)

3.9 Data Analysis

The values from the physico-chemical properties, bacterial and fungal populations for each sample were subjected to analysis of variance (ANOVA) using GENSTAT Discovery Version 4 (2013) software, followed by multiple comparisons using least significant difference at 5% level of probability. The data were analysed on the basis of soil type, location, soil depth and month sampled. . Significantly different means ($p < 0.05$) were separated using the least significant difference (LS.D.) at 5% level.

T-test (Genstat Release 10.3DE PC/Windows 7, 2016 soft ware) was used to compare the means for Gram reaction of bacteria isolated from shea nut cake polluted soil, growth conditions of the isolated *Pseudomonas* species (GUR/09 and GUR/38) and the concentration of tannin in treatments of shea nut cake. For Gram reaction Gram positive and Gram negative bacteria were compared. For the effect of heat, pH, temperature, yeast extracts, the growth of *Pseudomonas* GUR/09 and GUR/38 were compared. For tannin degradation the concentrations of tannin in the various treatments were compared

4.0 CHAPTER FOUR

RESULTS

4.1 Soil colour

The colour of the soils were reddish for both polluted (5YR 5/3) and unpolluted (10YR 6/4) soils in Gurugu, Black(5 YR 3/2) for polluted and reddish (10YR 6/4) for unpolluted in Jisonayilli and white for both unpolluted (10YR 8/2) and polluted soils (5YR 6/2) in Kasalgu.

4.2 Soil texture

Proportions of sand ranged from 45% to 80%, silt from 10% to 45% and clay from 10% to 55% in shea nut cake polluted soils and 30% to 80%, 15% to 30% and 5% to 40% for sand, silt and clay respectively for unpolluted soils (Table 20). Gurugu had the highest sand content (80%) in both polluted and unpolluted soils followed by Jisonayilli, 45% and 75% for polluted and unpolluted respectively. Kasalgu recorded the least percentage of sand, 25% and 30% for polluted and unpolluted soils respectively. Silt proportion was higher in unpolluted soil (15%) than polluted soil (10%) in Gurugu and higher in polluted soil than unpolluted soil in Jisonayilli and Kasalgu. Clay was highest in Kasalgu among the three sites studied, 55% in polluted soil and 45% in unpolluted soil. Gurugu and Jisonayilli recorded the same clay percentage in polluted soil (10%) and unpolluted soils (5%).

There were significant differences ($P < 0.05$) in the proportions (%) of the three soil particles (sand, silt and clay) of the soil studied (Fig. 14).

Table 20Proportions of soil particles in the study

Soil	Gurugu		Jisonayilli		Kasalgu	
Particle	**Unpolluted**	**Polluted**	**Unpolluted**	**Polluted**	**Unpolluted**	**Polluted**
Sand	80%	80%	75%	45%	30%	25%
Silt	15%	10%	20%	45%	30%	20%
Clay	5%	10%	5%	10%	40%	55%

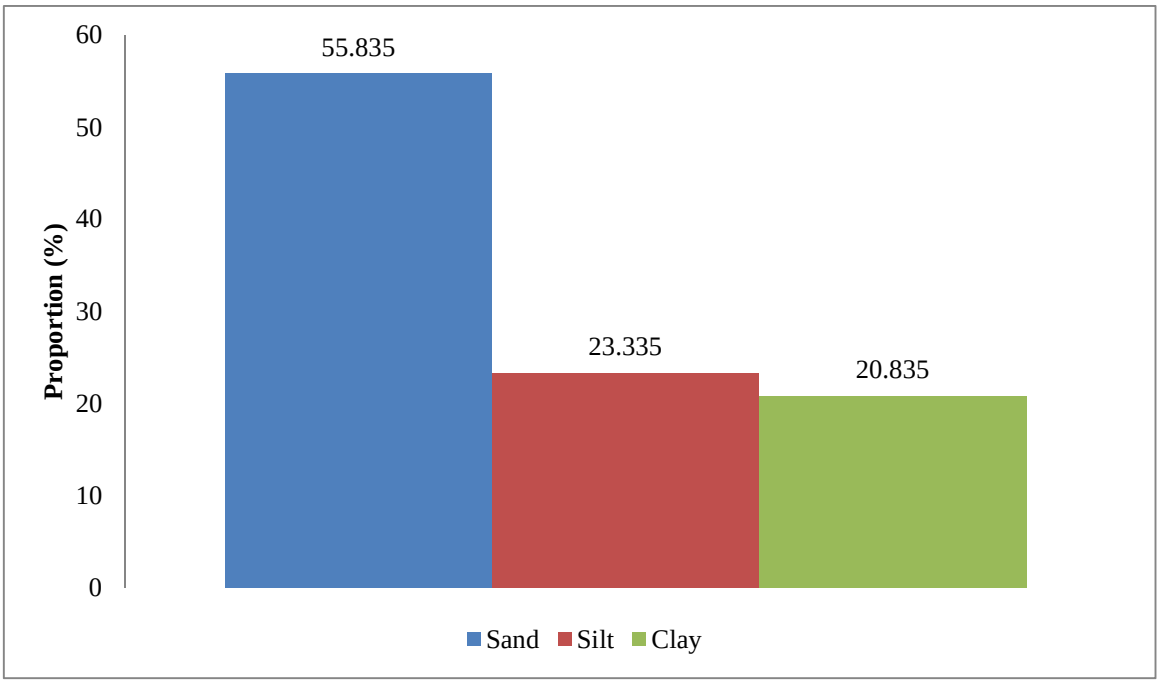


Figure 14 Interaction between location and soil type on soil particle size.

Soil texture classes were determined from the soil texture triangle as described in page 68 and results indicated below (Table 21).

Table 21 Texture class by soil type and location

	Gurugu		Jisonayilli		Kasalgu	
	Polluted	Unpolluted	Polluted	Unpolluted	Polluted	Unpolluted
Texture	Loamy sand	Sandy loam	Sandy loam	Loam	Clay	Clay

4.3 Main effect on dependent variable: Soil property by soil type

Values of parameters quoted below are means.

All the soil properties (dependent variables) studied (soil pH, moisture, organic carbon, total nitrogen, bacteria and fungi counts) were significantly higher ($P < 0.05$) in shea nut cake polluted soil than unpolluted soil (Table 22). The results of the above parameters for both shea nut cake polluted and unpolluted soils were also significantly different ($P < 0.05$) for the three locations (Gurugu, Jisonayilli and Kasalgu) (Table 23) and for the three soil depths studied (Table 24)

Table 22 Soil property by soil type (P < 0.05)

Soil property	Unpolluted	Polluted	Sig. effect
Moisture (mean %)	2.035	3.183	< 0.001
pH	4.93	6.83	< 0.001
Org Carbon (%)	0.954	1.692	< 0.001
Nitrogen (%)	0.08	0.137	< 0.001
C/N Ratio	12	11	<0.001
Bacteria (Log of cfu)	6.341	6.690	< 0.001
Fungi (Log of cfu)	3.415	3.618	<0.001

Table 23 Soil property by location

Soil property	Gurugu	Jisonayilli	Kasalgu	Sign.
Moisture (log of %)	2.474	2.590	2.762	<0.001
Mean pH	5.13	6.75	5.77	< 0.001
Carbon (%)	1.79	1.254	0.926	<0.001
Nitrogen (%)	0.162	0.093	0.072	<0.001
C/N Ratio	11.361	11.917	11.556	0.001
Bacteria (log of cfu)	6.702	6.703	6.142	<0.001
Fungi (log of cfu)	3.539	3.489	3.522	<0.001

Table 24 Soil property by soil depth

Soil property	0-20 cm	21-40 cm	41-60 cm	Significant
Moisture (log of %)	2.632	2.629	2.566	<0.001
Mean pH	6.01	5.92	5.72	<0.001
Carbon (%)	1.860	1.489	0.631	< 0.001
Nitrogen (%)	0.163	0.108	0.056	<0.001
C/N Ratio	11	12	11	<0.001
Bacteria (log of cfu)	7.415	6.502	5.630	<0.001
Fungi (log of cfu)	3.540	3.537	3.472	<0.001

The analysis of varience (ANOVA) table of "Tests of Between Subject Effects" for dependent variables showed that sampling time had no significant effect on carbon/nitrogen ratio (Sign. = 0.708), However for the other dependent variables investigated (Soil pH, moisture organic carbon, nitrogen contents, fungi and bacteria populations), sampling time had significant effect ($P < 0.05$) on their levels in the soil studied (Table 25).

Table 25 Effect of sampling time on soil property

Soil property	Sept. 2010	Nov. 2010	Jan. 2011	March. 2011	May. 2011	Jul. 2011	Sign. level	
Moisture (log_{10} of %)	2.745	2.693	2.533	2.439	2.591	2.652	< 0.001	Significant
Mean pH	5.89	5.9	5.89	5.87	5.86	5.89	< 0.001	Significant
Carbon (%)	1.33	1.335	1.328	1.32	1.322	1.304	0.026	Significant
Nitrogen (%)	0.11	0.109	0.11	0.109	0.108	0.108	0.026	Significant
Carbon/Nitrogen Ratio	12	12	12	12	12	12	0.708	Not Significant
Bacteria (log of cfu)	6.604	6.581	6.466	6.425	6.489	6.531	<0.001	Significant
Fungi (log of cfu)	3.534	3.554	3.5	3.48	3.509	3.523	<0.001	Significant

4.4 Interaction Effect on Dependent variables: Soil Moisture content

Soil moisture content values were statistically analysed and results summarised below (Table 26). Detailed interactive results are given in Appendix A.

Table 26 Summary of Analysis of variance (Moisture)

Variate: log_moisture

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
month	5	3.372E+00	6.743E-01	7392.34	<.001
location	2	4.536E+00	2.268E+00	24862.57	<.001
month.location	10	1.008E+00	1.008E-01	1104.56	<.001
location.soiltype	3	1.160E+02	3.866E+01	4.238E+05	<.001
month.location.soiltype	15	1.920E+00	1.280E-01	1403.00	<.001
location.soiltype.depth	12	1.842E+00	1.535E-01	1683.14	<.001
month.location.soiltype.depth	60	1.257E+00	2.095E-02	229.63	<.001
Residual	216	1.970E-02	9.122E-05		
Total	323	1.299E+02			

4.4.1 Effect of soil type on moisture content of soil locations

Moisture contents were significantly higher ($P < 0.05$) in shea nut cake polluted soils than unpolluted (control) soils in all the three locations (Fig. 15) Jisonayilli recorded the highest moisture in shea nut cake polluted soil, while Kasalgu recorded the highest. in unpolluted soil. The lowest moisture was observed in unpolluted soil in Jisonayilli.

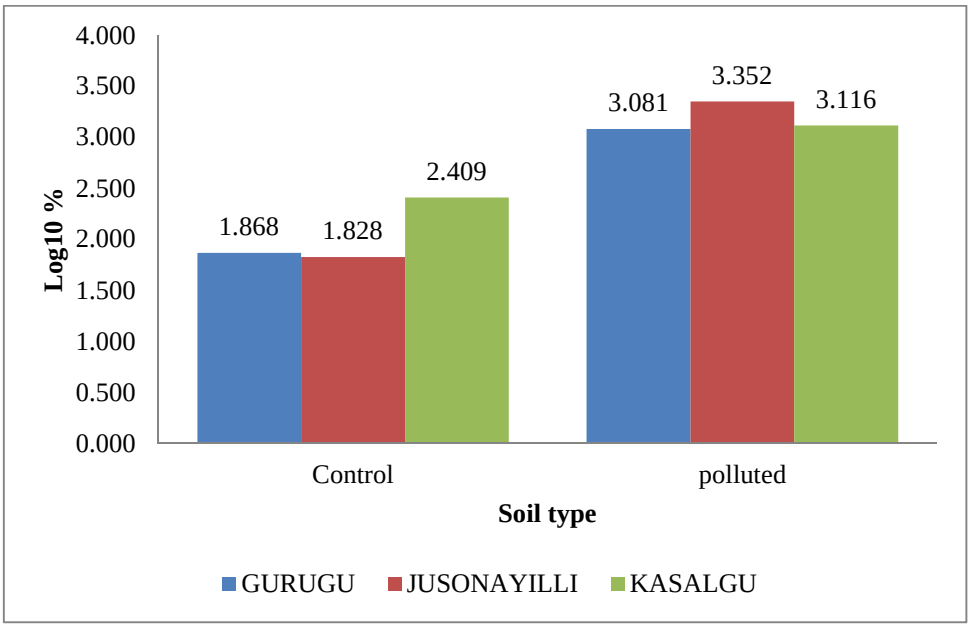


Figure 15Effect of soil type on moisture content of soil locations

4.4.2 Effect of soil depth on moisture content of soil locations

For each location soil depth had significant effect on moisture content with mean moisture contents being significantly different (Fig. 16). Highest mean of moisture content by soil depth and location was recorded in the 21-40 cm soil depth in Kasalgu and the least was recorded at the 41-60 cm soil depth in Gurugu. Gurugu recorded the least soil moisture contents in all the soil depths. Moisture content decreased with increasing soil depth in Gurugu and Jisonayilli

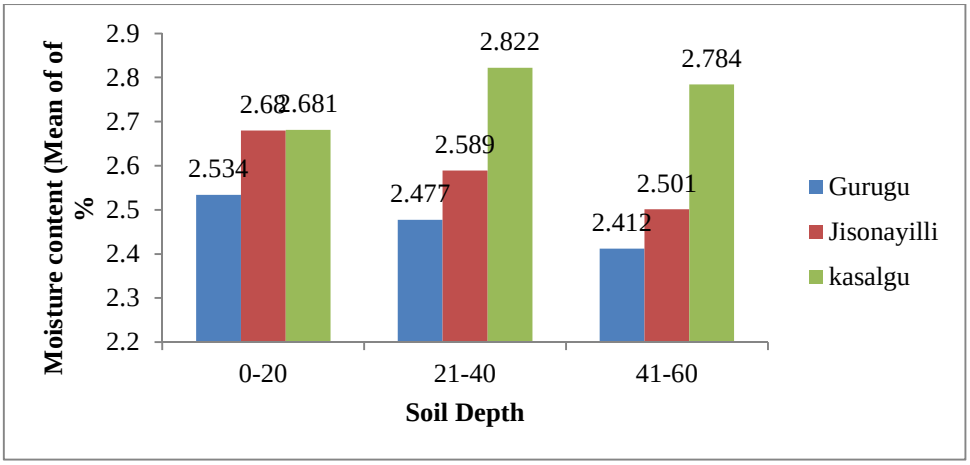


Figure 16 Effect of soil depth on moisture content of soil location

4.4.3 Effect of time on moisture content of soil locations

Sampling time had significant effect on soil moisture content of the studied locations. With the exception of September and November in Kasalgu where no significant difference was observed in moisture contents, mean moisture contents were significantly different ($P < 0.05$) for all the months sampled in the three locations with the highest recorded in September for all the three locations. Kasalgu recorded the highest mean of moisture content in September with no significant difference between September and November and Gurugu the least in March (Table. 27).

Table 27 Effect of time on moisture content of soil locations

Location	Sept. 2010	Nov. 2010	Jan. 2011	March. 2011	May. 2011	Jul. 2011
Gurugu	2.645[a]	2.631[b]	2.347[c]	2.296[d]	2.376[e]	2.551[f]
Jisonayilli	2.785[a]	2.640[b]	2.487[c]	2.480[d]	2.586[e]	2.562[f]
Kasalgu	2.808[a]	2.805[a]	2.766[b]	2.541[c]	2.812[d]	2.843[e]

lsd0·006

[a-f]Mean values with the same superscript letter across the same row are not significantly different.

Lower moisture contents were observed in the dry season months (November, January and March) than the raining season months (May, July and Sept) for all the locations.

4.4.4 Effect of soil depth on moisture content of soil types.

Generally soil depth had significant effect on soil moisture content of the soil types studied. With the exception of 21-40 and 41-60 cm soil depths in shea nut cake polluted soil which were not significantly different in moisture contents, the moisture contents of the shea nut cake polluted and unpolluted soils investigated were significantly different across soil depths (Table 28). Highest moisture content was recorded in 0-20 cm depth in shea nut cake polluted soil, decreasing with increasing soil depth, while moisture content in unpolluted soils was lowest in 0-20 cm depth, increasing with increasing soil depth.

Table 28 Effect of soil depth on moisture content of soil types

Soil type	0-20	21-40	41-60
Unpolluted	2.017[a]	2.041[b]	2.046[b]
Polluted	3.246[a]	3.218[b]	3.086[c]

Lsd 0.006

[a-c]Mean values with the same superscript letters across the same row are not significantly different

4.4.5 Effect of sampling time on moisture content of soil type

Sampling month had significant effect on moisture content in the two soil types, with highest recorded in September for both soil types (Table 29). Apart from July and November which showed no significant difference in moisture content in unpolluted soil, all other months sampled showed significant differences in moisture content, with raining season months (May, July and September) recording significantly higher moisture contents than the dry

season months (November, January and March) with peak in September in both shea nut cake polluted and unpolluted soils.

Table 29Effect of time on moisture content of soil type

Soil type	Sept. 2010	Nov. 2010	Jan. 2011	Mar.2011	May. 2011	Jul.2011
Unpolluted	2.132[a]	2.041[b]	1.975[c]	2.000[d]	2.027[e]	2.034[b]
polluted	3.358[a]	3.346[b]	3.091[c]	2.878[d]	3.156[e]	3.269[f]

L.s.d: 0.0089

[a-f]Mean values with the same superscript letters across the same row are not significantly different

4.4.6 Effect of sampling time on moisture content of soil depths

Moisture contents of the various soil depths sampled were significantly affected by sampling month and were significantly different ($P < 0.05$) with each soil depth for all the months sampled. The highest moisture content was recorded in the 0-20 cm and 21-40 cm depths in September, which showed no significant difference (Fig. 17). Least moisture contents were recorded for all soil depths in March. Highest moisture contents for the dry season months (November, January and March) were recorded at the 21-40 cm depth.

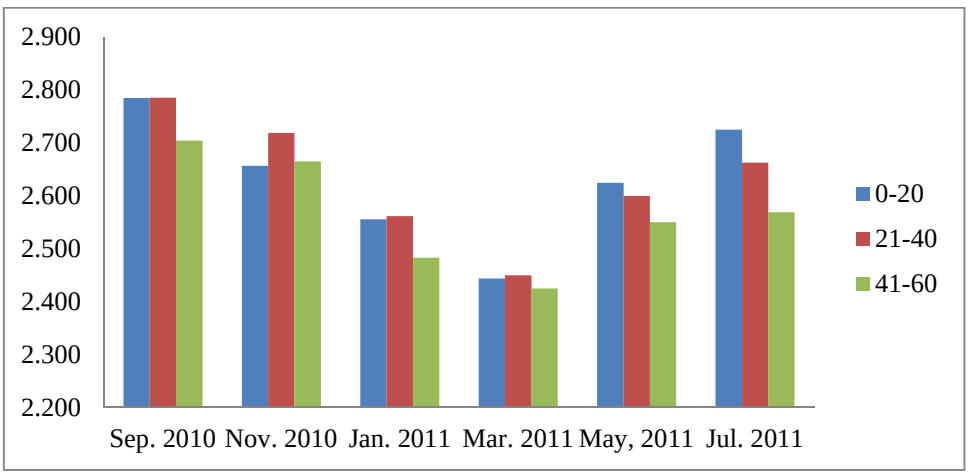


Figure 17Effect of time on moisture content of soil depths

4.5 Interaction Effect on Dependent variable: Soil pH

Soil pH values were statistically analysed and results summarised below (Table 30). Detailed interactive results are given in Appendix D.

Table 30 Summary of ANOVA: Tests of Between-Subjects Effects (Soil pH).

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	207.846[a]	87	2.389	8714.462	.000
Intercept	3738.113	1	3738.113	1.364E7	.000
Sample	97.533	1	97.533	355771.419	.000
location	48.221	2	24.111	87948.280	.000
Date	.016	5	.003	11.922	.000
Depth	1.586	2	.793	2893.232	.000
Sample * location	56.076	2	28.038	102274.413	.000
Sample * Date	.004	5	.001	2.675	.052
Sample * Depth	1.236	2	.618	2253.418	.000
location * Date	.008	10	.001	3.085	.015
location * Depth	1.894	4	.474	1727.461	.000
Sample * Date * Depth	.003	10	.000	1.114	.399
location * Date * Depth	.009	20	.000	1.653	.135
Sample * location * Date	.020	10	.002	7.326	.000
Sample * location * Depth	1.233	4	.308	1124.540	.000
Error	.005	20	.000		
Total	3945.965	108			
Corrected Total	207.852	107			

R Squared = 1.000 (Adjusted R Squared = 1.000)

4.5.1 Effect of soil type on the pH of soil location (Extracted from Appendix D 5)

Soil type had significant effect on pH of the locations sampled. Soil pH values were significantly higher ($P < 0.05$) in shea nut cake polluted soils than unpolluted soils in all the three locations with Jisonayilli recording the highest and Kasalgu the least for shea nut cake polluted soils (Fig. 18). Kasalgu recorded the highest and Gurugu the least pH values for unpolluted soils.

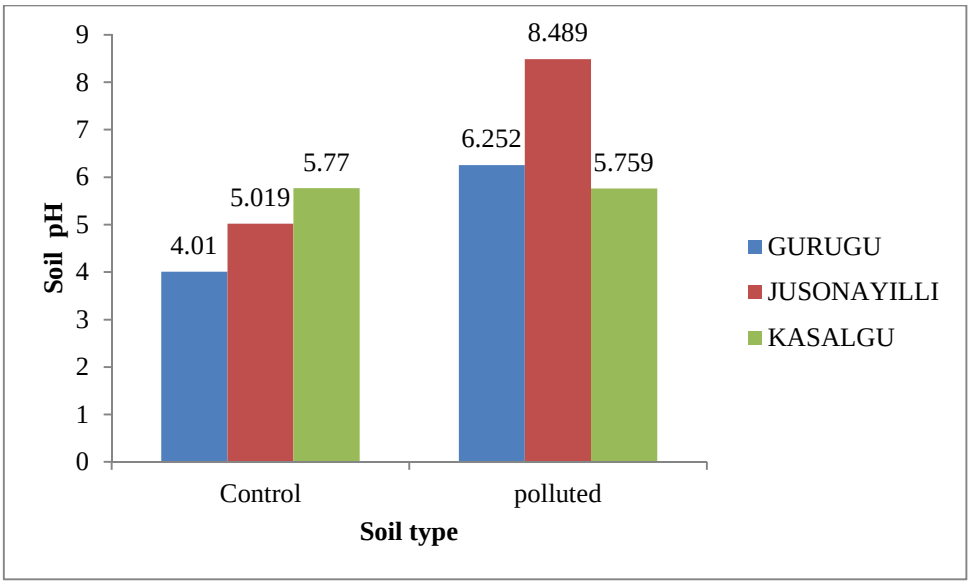


Figure 18Effect of soil type on the pH of soil location

4.5.2 Effect of soil depth on pH of soil location (Extractedfrom Appendix D 9)

Soil depth had significant effect on soil pH in the three locations studied. Soil mean pH values were significantly different ($P < 0.05$) for all soil depths in all locations, with Jisonayilli recording the highest mean pH in all the soil depths (Fig. 19). Soil pH values were highest in the 0-20 cm depth in Jisonayilli and Gurugu, decreasing with increasing soil depth. The highest mean pH value in Kasalgu was recorded in the 21-40 cm soil depth. The least pH values were recorded in the 41-60 cm soil depth in all the locations.

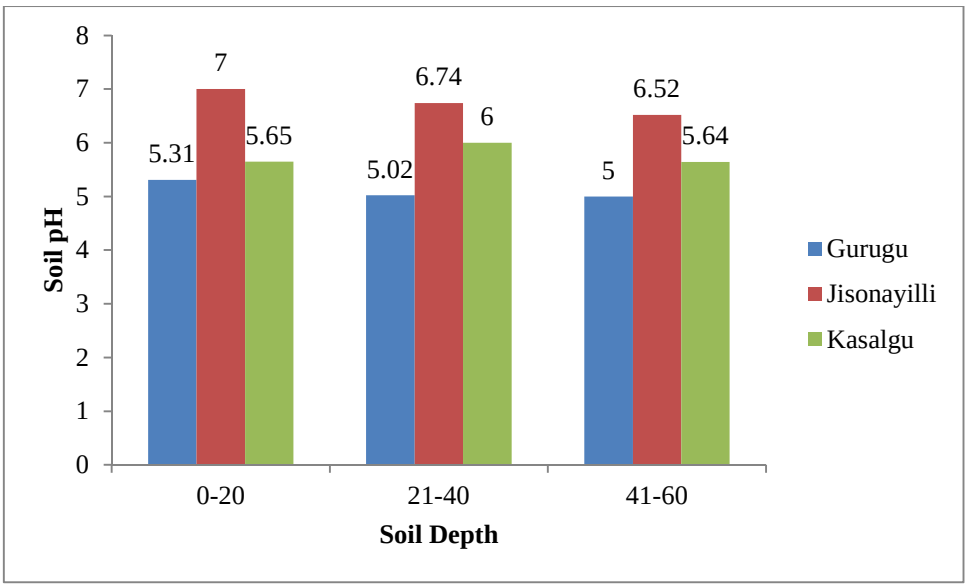


Figure 19Effect of soil depth on pH of sample location

4.5.3 Effect of depth on pH of soil type (Extracted from Appendix D 7)

Soil depth had significant effect on pH of the two soil types sampled. Mean soil pH of the two soil types studied were significantly different ($P < 0.05$) for all soil depths and highest in the 0-20 cm depth in shea nut cake polluted soil, decreasing with increasing soil depth and in the 21-40 cm depth in unpolluted soil (Fig. 20). The least pH values for both soil types were recorded in the 41-60 cm depth.

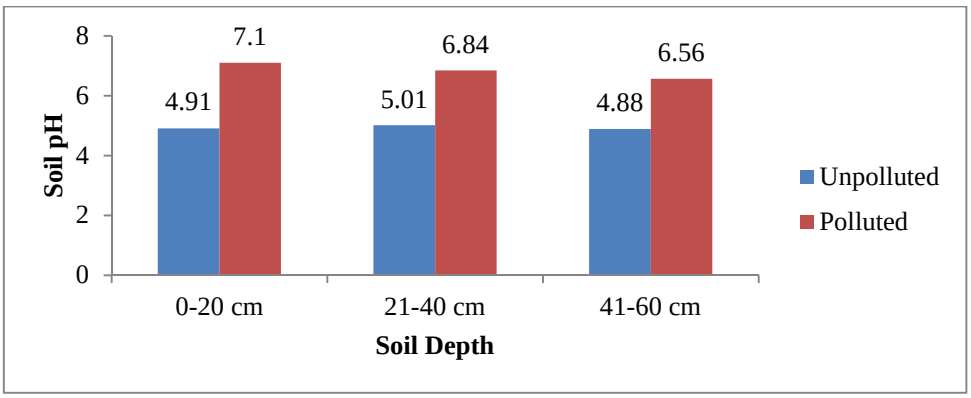


Figure 20Effect of depth on pH of soil type

4.5.4 Effect of sampling time on pH of soil location (Sig. 0.015)

With significant level of 0.015, time of sampling had significant effect ($P < 0.05$) on the soil pH for the three locations (Table 30).

4.5.5 Effect of sampling time on pH of soil type (Sig. 0.052)

Time of sampling had no significant effect ($P > 0.05$) on the pH of the two soil types (Sig. 0.052) from Table 30

4.6 Interaction Effect on Dependent variables: Soil organic carbon:

Organic carbon values of soil samples were statistically analysed and results summarised below (Table 31). Interactive results are given in Appendix E

Table 31 Summary of ANOVA of soil Organic carbon

Tests of Between-Subjects Effects

Dependent Variable: Org. Carbon %

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	87.691[a]	87	1.008	1546.383	.000
Intercept	189.042	1	189.042	290026.205	.000
Sample	14.741	1	14.741	22614.885	.000
location	13.685	2	6.842	10497.635	.000
Date	.011	5	.002	3.242	.026
Depth	28.225	2	14.113	21651.493	.000
Sample * location	5.035	2	2.517	3862.247	.000
Sample * Date	.011	5	.002	3.254	.026
Sample * Depth	6.573	2	3.286	5041.920	.000
location * Date	.014	10	.001	2.164	.068
location * Depth	13.010	4	3.253	4989.984	.000
Date * Depth	.006	10	.001	.860	.582
Sample * Date * Depth	.006	10	.001	.960	.505
location * Date * Depth	.011	20	.001	.827	.663
Sample * location * Date	.012	10	.001	1.898	.107
Sample * location * Depth	6.353	4	1.588	2436.522	.000
Error	.013	20	.001		
Total	276.746	108			
Corrected Total	87.704	107			

a. R Squared = 1.000 (Adjusted R Squared = .999)

b. N = 324 Samples

4.6.1 Effect of soil type on organic carbon content of sampled locations

Soil type has significant effect on organic carbon content of soil location. Soil organic carbon contents were significantly higher ($P < 0.05$) in shea nut cake polluted soils than unpolluted

soils in all the three locations with Gurugu recording the highest for both shea nut cake polluted and unpolluted soils (Fig. 21). Kasalgu recorded the least organic carbon content for shea nut cake polluted soils while Jisonayilli recorded the least for the unpolluted soil.

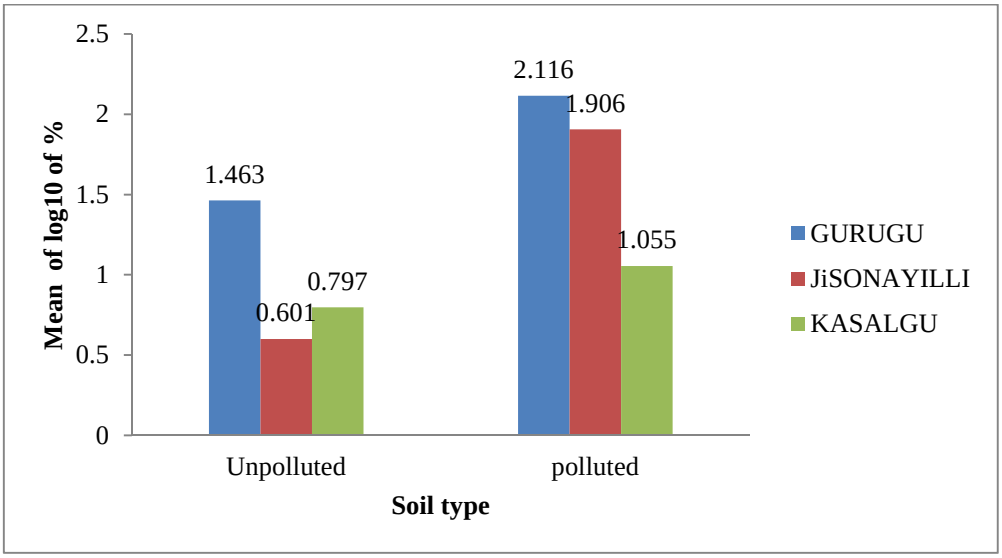


Figure 21Effect of soil type on organic carbon content of sampled location

4.6.2 Effect of soil depth on soil organic carbon content of soil location

Soil organic carbon contents of sample locations were significantly affected by soil depth. The mean carbon contents of the three soil depths were significantly different ($P < 0.05$) in all locations investigated, with Gurugu recording the highest mean carbon in all the soil depths (Fig. 22). The highest mean carbon content in soils in Gurugu and Jisonayilli were recorded in the 0-20 cm depth, decreasing with increasing soil depth. The highest mean organic carbon content in Kasalgu was recorded in the 21-40 cm soil depth. The least organic carbon contents were recorded in the 41-60 cm soil depth in all the locations.

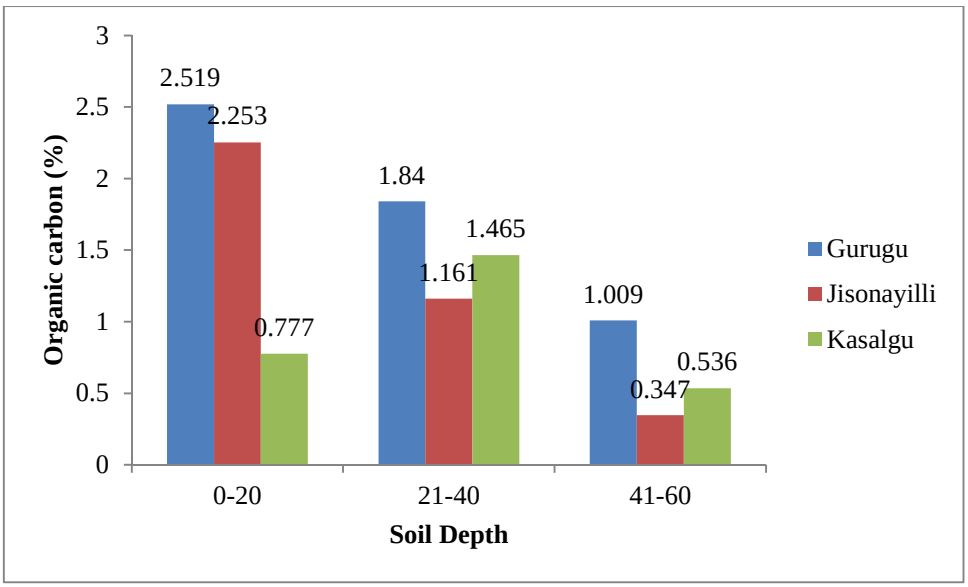


Figure 22Effect of soil depth on soil organic carbon content of soil location

4.6.3 Effect of soil depth on soil organic carbon content of soil type (Sing. 0.000)

Soil depth had significant effect on soil organic matter of soil type. Mean organic carbon contents of the two soil types studied were significantly different ($P < 0.05$) for all soil depths and highest in the 0-20 cm depth in shea nut cake polluted soil, decreasing with increasing soil depth and in the 41-60 cm depth in unpolluted soil (Fig. 23). Lowest carbon contents were recorded in the 41-60 cm soil depth for polluted soil and the 21-40 cm depth in unpolluted soil.

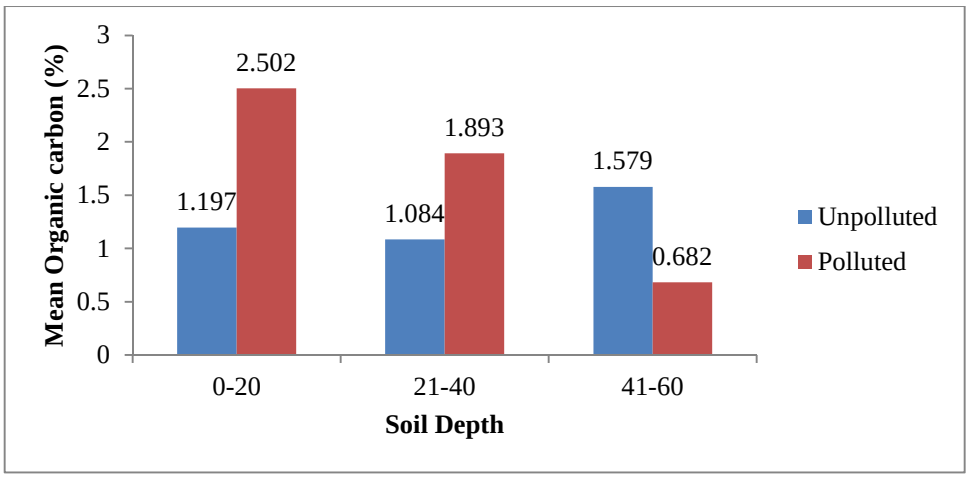


Figure 23Effect of soil depth on soil organic carbon content of soil type

4.6.4 Effect of sampling time on organic carbon content of soil type (sig. 0.026).

From Table 31, time of sampling had significant effect ($P < 0.05$) on the organic carbon contents of the two soil types.

4.6.5 Effect of sampling time on organic carbon content of soil location (sign. 0.068)

From Table 31, sampling time has no significant effect ($P > 0.05$) on mean organic carbon contents for each location (Sign. 0.068) and soil depths (Sig. 0.0582).

4.7 Interaction Effect on Dependent variables: Soil nitrogen

Nitrogen results of soil samples were statistically analysed and results summarised below (Table 32). Interactive results are given in Appendix F.

Table 32 Summary of ANOVA (Soil nitrogen):Tests of Between-Subjects Factors

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.696[a]	87	.008	2088.967	.000
Intercept	1.280	1	1.280	334129.878	.000
Sample	.088	1	.088	22938.126	.000
Location	.159	2	.080	20784.320	.000
Date	6.238E-5	5	1.248E-5	3.256	.026
Depth	.203	2	.102	26542.528	.000
Sample * location	.024	2	.012	3101.356	.000
Sample * Date	9.053E-5	5	1.811E-5	4.725	.005
Sample * Depth	.064	2	.032	8356.222	.000
location * Date	9.540E-5	10	9.540E-6	2.490	.040
location * Depth	.095	4	.024	6225.240	.000
Date * Depth	4.320E-5	10	4.320E-6	1.128	.390
Sample * Date * Depth	2.654E-5	10	2.654E-6	.693	.720
location * Date * Depth	5.960E-5	20	2.980E-6	.778	.710
Sample * location * Date	7.466E-5	10	7.466E-6	1.948	.098
Sample * location * Depth	.062	4	.016	4053.545	.000
Error	7.664E-5	20	3.832E-6		
Total	1.977	108			
Corrected Total	.696	107			

a. R Squared = 1.000 (Adjusted R Squared = .999)

4.7.1 Effect of soil type on nitrogen content of soil location

Soil type was observed to have significant effect on nitrogen content of the different soil locations sampled. Soil total nitrogen contents were significantly higher ($P < 0.05$) in shea nut cake polluted soils than unpolluted soils in all the three locations with Gurugu recording the highest for means of the three soil depths for both shea nut cake polluted and unpolluted soils (Fig. 24). Kasalgu recorded the least nitrogen content for shea nut cake polluted soils while Jisonayilli recorded the least for the unpolluted soil.

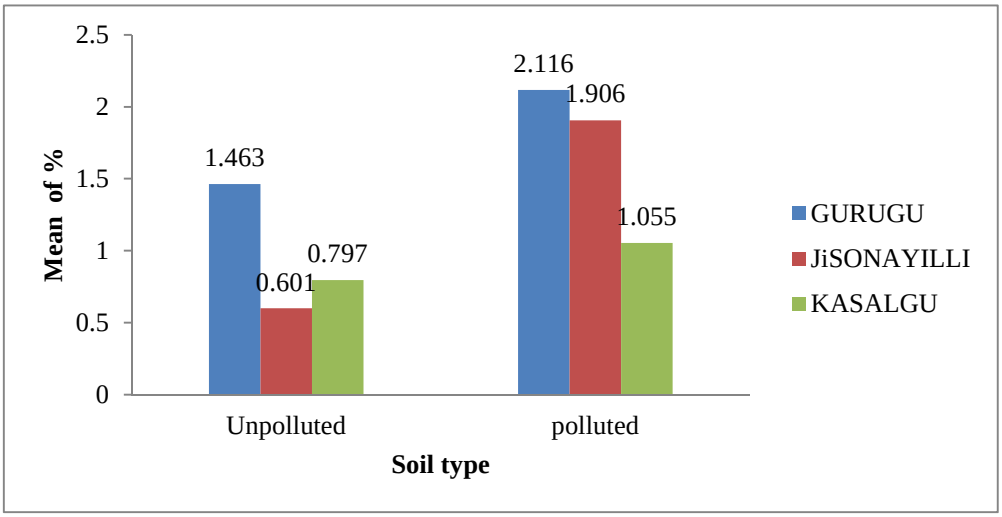


Figure 24Effect of soil type on nitrogen content of soil location

4.7.2 Effect of soil depth on nitrogen content of soil location

Soil depth was observed to have significant effect on total soil nitrogen of different soil locations. Soil mean total nitrogen contents by location and soil depth were significantly different ($P < 0.05$) in all locations and soil depths investigated, with Gurugu recording the highest mean nitrogen in all the soil depths (Fig. 25). The highest mean nitrogen content in polluted soils in Gurugu and Jisonayilli were recorded in the 0-20 cm depth. The highest mean nitrogen content in Kasalgu was recorded in the 21-40 cm soil depth. The least nitrogen contents were recorded in the 41-60 cm soil depth in all the locations. Kasalgu recorded the

least in the 0-20 cm depth for shea nut cake polluted soil, while Jisonayilli recorded the least in the 41-60 cm depth.

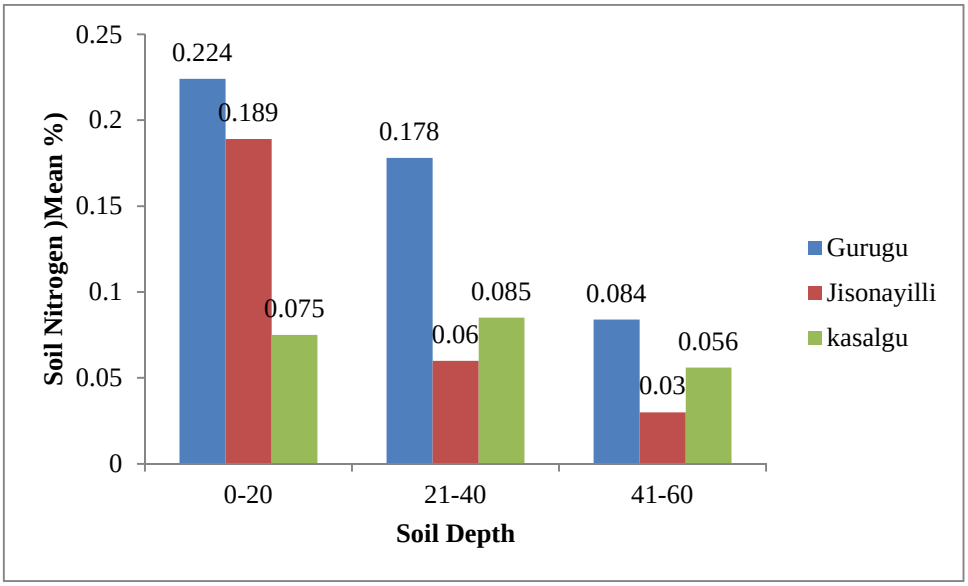


Figure 25Effect of soil depth on nitrogen content of soil location

4.7.3 Effect of soil depth on nitrogen content of soil type

Total nitrogen contents of the two soil types studied were directly affected by soil depth with nitrogen content being significantly higher in shea nut cake polluted soil than unpolluted at all soil depths sampled. Mean total nitrogen contents of the two soil types studied were significantly different ($P < 0.05$) for all soil depths and highest in the 0-20 cm depth in both shea nut cake polluted and unpolluted soils (Fig. 26). The least nitrogen contents were found in the 41-60 cm soil depth for shea nut cake polluted soil and in the 21-40 cm depth for unpolluted soil.

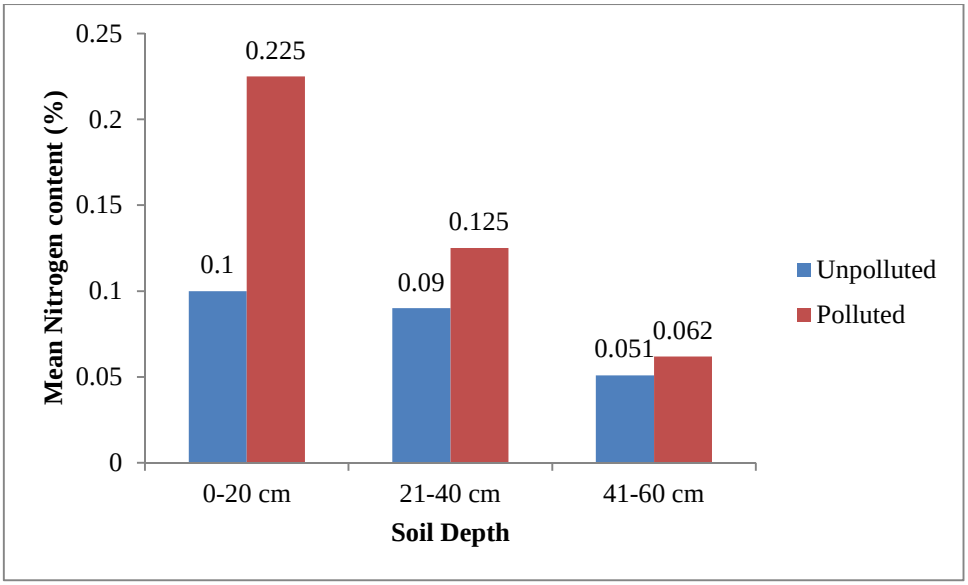


Figure 26Effect of soil depth on nitrogen content of soil type

4.7.4 Effect of sampling time on nitrogen content of soil type (Sign. 0.005)

Time of sampling had significant effect ($P < 0.05$) on the total nitrogen contents for the two soil types (Table 32)

4.7.5 Effect of sampling time on nitrogen content of soil location (Sign. 0.04)

Analysis of interactive effect showed that sampling time had significant effect ($P < 0.05$) on mean total nitrogen contents for the three locations (Table 32).

4.7.6 Effect of sampling time on nitrogen content of soil depth (Sign. 0.392).

Table of interaction between subject effect (Table 32) showed that sampling time had no significant effect ($P > 0.05$) on mean total nitrogen contents for each soil depth (Sig. 0.392).

4.8 Interaction Effect on Dependent variables: Carbon-Nitrogen Ratio

Carbon/nitrogen ratios of test soil samples were statistically analysed and results summarised below (Table 33). Interactive results are given in Appendix G.

Table 33 Summary of ANOVA: Tests of Between-Subjects Effects:

Dependent Variable: C/N Ratio

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	218.389a	87	2.510	9.512	.000
Intercept	14560.333	1	14560.333	55176.000	.000
Sample	21.333	1	21.333	80.842	.000
location	5.722	2	2.861	10.842	.001
Date	.778	5	.156	.589	.708
Depth	19.500	2	9.750	36.947	.000
Sample * location	17.167	2	8.583	32.526	.000
Sample * Date	1.556	5	.311	1.179	.354
Sample * Depth	.056	2	.028	.105	.901
location * Date	3.167	10	.317	1.200	.348
location * Depth	116.611	4	29.153	110.474	.000
Date * Depth	2.389	10	.239	.905	.546
Sample * Date * Depth	2.722	10	.272	1.032	.453
location * Date * Depth	4.500	20	.225	.853	.638
Sample * location * Date	2.611	10	.261	.989	.483
Sample * location * Depth	20.278	4	5.069	19.211	.000
Error	5.278	20	.264		
Total	14784.000	108			
Corrected Total	223.667	107			

a. R Squared = .976 (Adjusted R Squared = .874)

4.8.1 Effect of location on carbon/nitrogen ratio of soil type (Sign. 0.000)

Soil location had significant effect ($P < 0.05$) on soil carbon/nitrogen ratio of the two soil types being highest in Kasalgu in unpolluted soil and in Jisonayilliin shea nut cake polluted soil (Fig. 27).

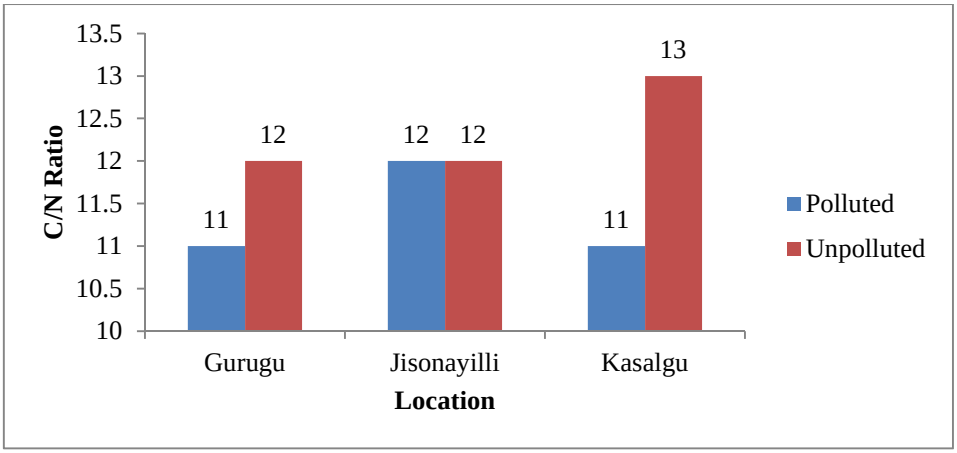


Figure 27Effect of location on carbon/nitrogen ratio of soil type

4.8.2 Effect of sampling depth on carbon/nitrogen ratio of soil location (Sign. 0.000)

Soil sampling depth had significant effect ($P < 0.05$) on carbon/nitrogen ratio of location. The highest carbon-nitrogen ration was recorded at 21-40 cm soil depth in Kasalgu. The highest carbon/nitrogen ratio at the 0-20 cm depth was recorded in Jisonayilli, which decreased with increasing soil depth (Fig. 28). The highest carbon/nitrogen ratio in Kasalgu was recorded in the 21-40 cm depth, which was the highest at that soil depth. There was no significant difference in carbon-nitrogen ratio in 0-20 cm and 41-60 cm depths in Kasalgu. The highest in the 41-60 cm depth was recorded in Gurugu.

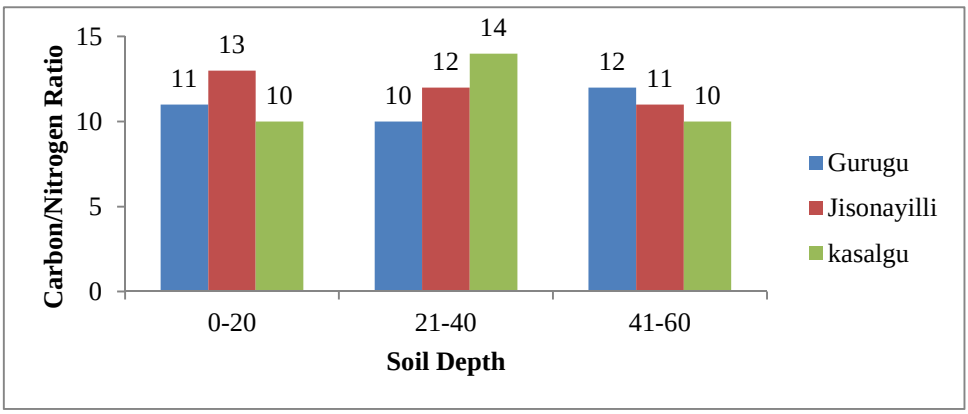


Figure 28Effect of soil depth on soil carbon/nitrogen ratio in soil location

4.8.3 Effect of sample depth on carbon/nitrogen ratio of the sample types (Sig. 0.901)

ANOVA analysis (Table 33) showed that soil sampling depth had no significant effect (P > 0.05) on carbon/nitrogen ration of the sample types studied.

4.8.4 Effect of sampling time on carbon/nitrogen ratio of the sample location (Sign. 0.348)

Time of soil sampling had no significant effect (P > 0.05) on soil carbon-nitrogen ratios of the studied locations (Table 33).

4.8.5 Effect of sampling time on carbon/nitrogen ratio of the sample type (Sign. 0.354) and sample depth (0.546).

Sampling time had no significant effect (Table 33) on carbon-nitrogen ratio of soil type (Sign. 0.354) and soil depth (0.546).

4.9 Interaction Effect on Dependent variables: Soil microbial populations

4.9.1 Summary of ANOVA: Bacteria population

Bacteria population values of soil samples were statistically analysed and results summarised below (Table 34). Detailed interactive results are given in Appendix B

Table 34 Summary of ANOVA on bacteria population

GenStat Release 10.3DE (PC/Windows Vista) 04 December 2013 13:47:40

Analysis of variance

Variate: bacterial_log

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
month	5	1.287E+00	2.574E-01	5825.90	<.001
location	2	2.268E+01	1.134E+01	2.567E+05	<.001
month.location	10	2.642E-02	2.642E-03	59.80	<.001
location.soiltype	3	1.148E+01	3.826E+00	86588.70	<.001
month.location.soiltype	15	1.183E-01	7.886E-03	178.50	<.001
location.soiltype.depth	12	1.895E+02	1.579E+01	3.574E+05	<.001
month.location.soiltype.depth	60	3.917E-01	6.529E-03	147.77	<.001
Residual	216	9.543E-03	4.418E-05		
Total	323	2.255E+02			

4.9.2 Effect of soil type on bacteria population in soil location

Soil type was observed to have significant effect on bacteria population of the different soil locations sampled. Soil bacteria counts were significantly higher ($P < 0.05$) in shea nut cake polluted soils than unpolluted soils in all the three locations with Gurugu recording the highest in shea nut cake polluted soil and Jisonayilli in unpolluted soils (Fig. 29). Kasalgu recorded the least bacteria population in both shea nut cake polluted and unpolluted soils.

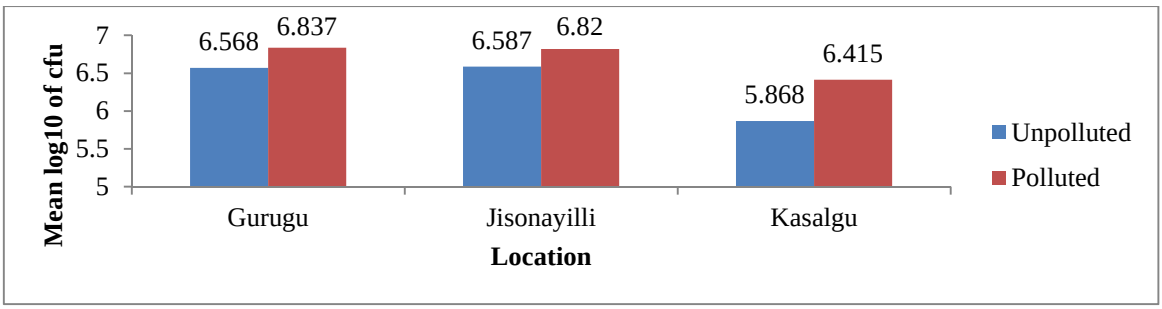


Figure 29Effect of soil type on bacteria population in soil location

4.9.3 Effect of soil depth on bacteria population in soil locations

Soil depth was found to have significant effect on bacteria count in different soil locations with the highest count occurring in the 0-20 cm depth, decreasing with increasing soil depth in all the three locations sampled while the least counts were recorded in the 41-60 cm depth in all the locations (Fig. 30)

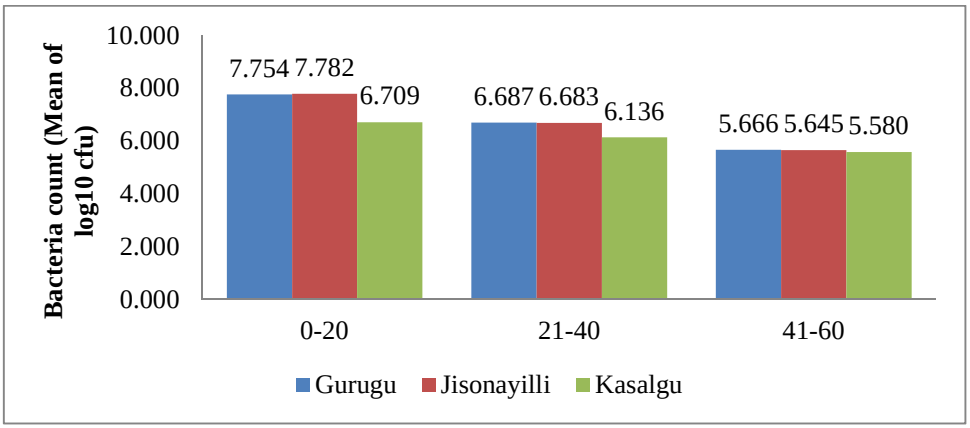


Figure 30Effect of soil depth on bacteria population in soil location

4.9.4 Effect of time on bacteria population in location

Sampling time had significant effect on soil bacteria population of the studied locations, bacteria counts being significantly different ($P < 0.05$) for all the months sampled in the three locations with the highest recorded in September and the least in March for all the three locations (Table. 35).

Table 35 Effect of time on bacteria population in soil location

	Sept. 2010	Nov. 2010	Jan. 2011	Mar. 2011	May. 2011	Jul. 2011
Gurugu	6.796[a]	6.777[b]	6.649[c]	6.588[d]	6.686[e]	6.723[f]
Jisonayilli	6.796[a]	6.773[b]	6.655[c]	6.615[d]	6.669[e]	6.713[f]
Kasalgu	6.225[a]	6.195[b]	6.093[c]	6.070[d]	6.111[e]	6.156[f]

Lsd: 0.002

[a-f]Mean values with different superscript letters across the same row are significantly different.

4.9.5 Effect of soil depth on bacteria population in soil types

Soil depth had significant effect on bacteria population in the different soil types, being highest in 0-20 cm soil depth in both shea nut cake polluted and unpolluted soils, decreasing with increasing soil depth (Fig. 31).

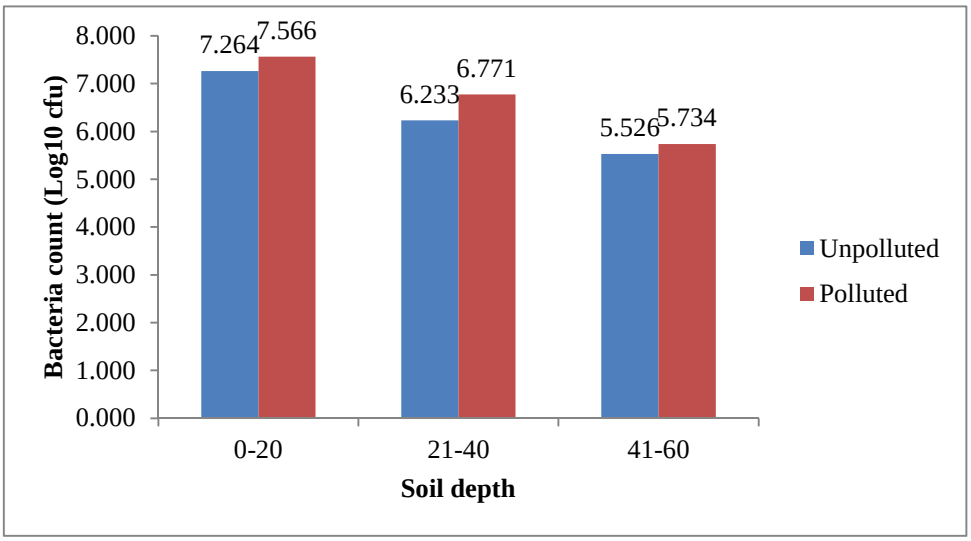


Figure 31Effect of soil depth on bacteria population in soil types

4.9.6 Effect of sampling time on bacteria population in soil types

Sampling time had significant effect on bacteria population in the two soil types. The highest counts were obtained in September and the least in March for both shea nut cake polluted and unpolluted soils (Fig. 32). Counts were generally higher in the raining season months (May, July, Sept.) than the dry season months (Nov., Jan., March).

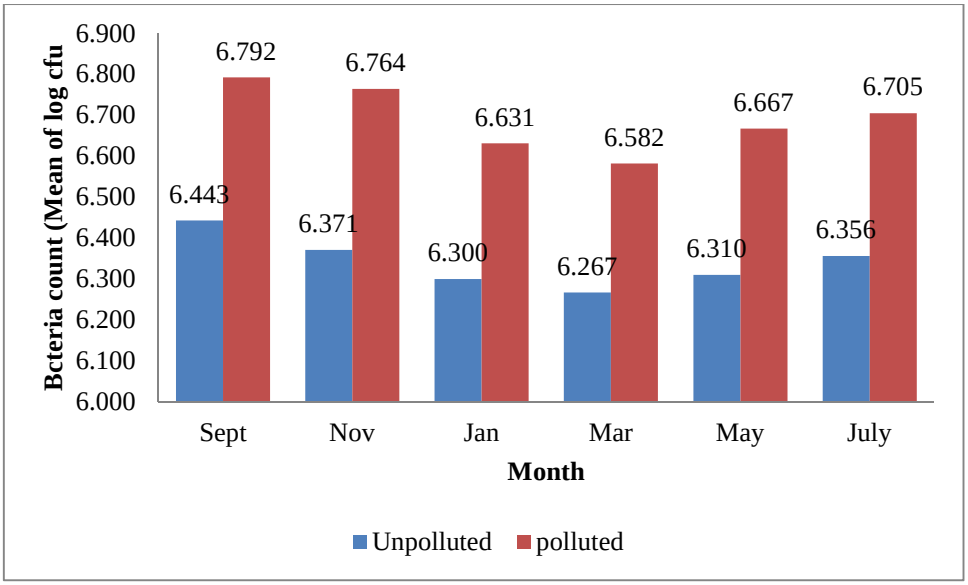


Figure 32Effect of time on bacteria population in soil type

4.9.7 Effect of time on bacteria population in soil depths

Sampling time had significant effect on bacteria population in the three soil depths sampled. The highest counts were obtained in September and the least in March in all the three soil depths (Table.36). With the exception of July and September in the 41-60 cm soil depth where bacteria counts were not significantly different, bacteria populations in all other months sampled from all soil depths were significantly different.

Table 36Effect of time on bacteria population in soil depth

Soil depth	Sept. 2010	Nov. 2010	Jan. 2011	Mar. 2011	May. 2011	Jul. 2011
0-20	7.589^{e}	7.506^{f}	7.330^{a}	7.282^{b}	7.365^{c}	7.418^{d}
21-40	6.582^{e}	6.543^{f}	6.469^{a}	6.419^{b}	6.473^{c}	6.527^{d}
41-60	5.682^{e}	5.654^{d}	5.598^{a}	5.573^{b}	5.628^{c}	5.647^{d}

l.s.d.: 0.010697

$^{a-f}$Mean values with the same superscript across the same row are not significantly different.

4.10 Soil fungi population

4.10.1 Summary of ANOA

Fungi population values of soil samples were statistically analysed and results summarised below (Table 37). Detailed interactive results are given in Appendix C.

Table 37 Summary of ANOVA on fungi population

GenStat Release 10.3DE (PC/Windows Vista) 04 December 2013 13:53:30

Analysis of variance

Variable: fungi_log

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
month	5	0.18588263	0.03717653	889.26	<.001
location	2	0.14056310	0.07028155	1681.14	<.001
month.location	10	0.01723321	0.00172332	41.22	<.001
location.soiltype	3	3.52051919	1.17350640	28070.35	<.001
month.location.soiltype	15	0.03830083	0.00255339	61.08	<.001
location.soiltype.depth	12	0.84813138	0.07067761	1690.61	<.001
month.location.soiltype.depth	60	0.09208039	0.00153467	36.71	<.001
Residual	216	0.00903008	0.00004181		
Total	323	4.85174080			

4.10.2 Effect of soil type on fungi population in soil location

The study revealed that soil type had significant effect on fungal population in the soil locations studied, with shea nut cake polluted soils having significantly higher fungal counts than unpolluted soils in all the locations (Fig. 33). The highest counts in both shea nut cake polluted and unpolluted soils were obtained in Gurugu, while the least counts for both soil types were obtained in Jisonayilli.

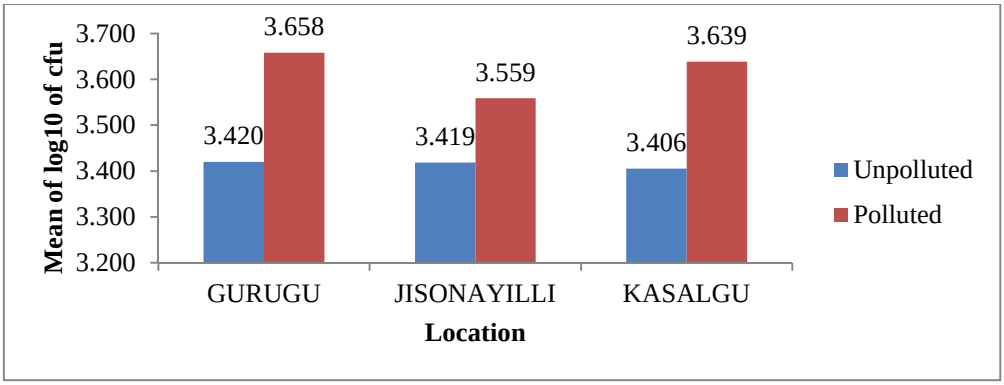


Figure 33Effect of soil type on fungi population in soil location

4.10.3 Effect of soil depth on fungi population in soil locations

Soil depth was found to have significant effect on fungi count in different soil locations with the highest counts occurring in the 0-20 cm depth in Gurugu and Jisonayilli, decreasing with increasing soil depth and in the 21-40 cm soil depth in Kasalgu (Fig. 34). The least fungi counts were recorded in the 41-60 cm depth in all the locations. Gurugu recorded the highest in the 0-20 cm and 41-60 cm soil depths, while the least fungi counts in the 21-40 cm and 41-60 cm soil depths were recorded in Jisonayilli.

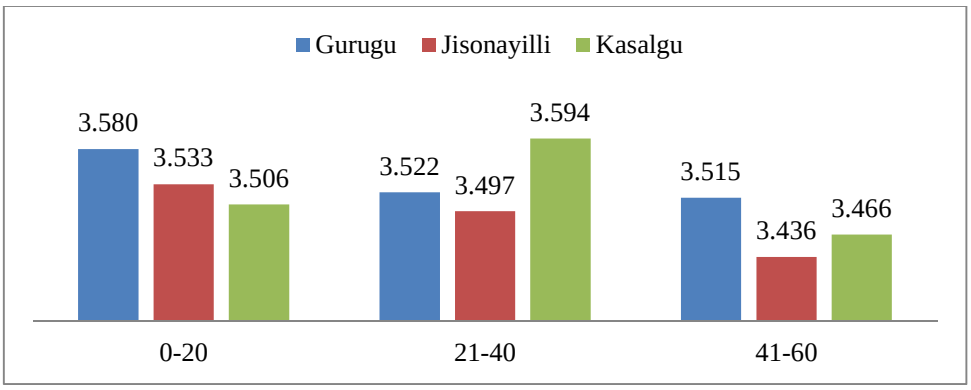


Figure 34Effect of soil depth on fungi population in soil locations

4.10.4 Effect of time on fungi population in soil locations

Sampling time had significant effect on fungi population in the sampled soil locations, with counts being significantly different for all months (Fig. 35). The highest fungi population was

recorded in November and the least in March. The overall highest fungi count which occurred in November was in Gurugu while the least which occurred in March was recorded in Jisonayilli.

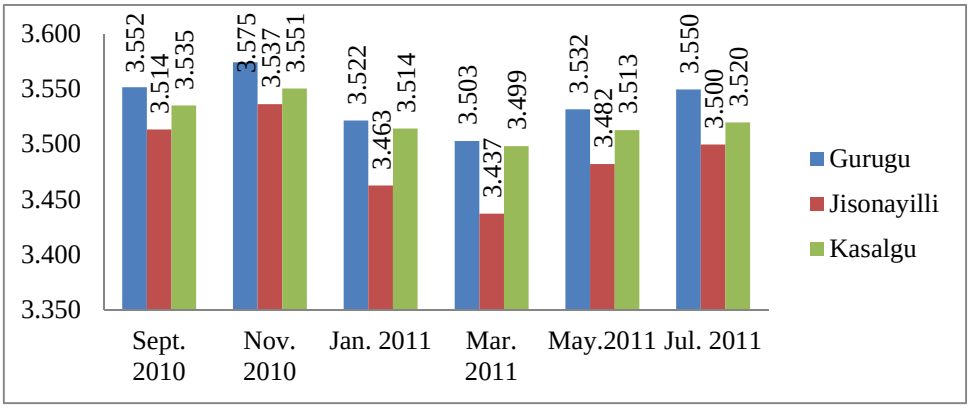


Figure 35Effect of time on fungi population in soil location

4.10.5 Effect of soil depth on fungi population in soil types

Soil depth had significant effects on fungi population in the two sample types studied. There were significant differences in fungi populations in all the sampled soil depths in shea nut cake polluted soil and significantly lower population in 41-60 cm depth than 21-40 cm and 0-20 cm depths for unpolluted soil (Table 38).

Table 38 Effect of soil depth on fungi population in soil types

Soil type	0-20 cm	21-40 cm	41-60 cm
Unpolluted	3.423[a]	3.423[a]	3.398[b]
Polluted soil	3.6565[a]	3.6523[b]	3.546[c]
lsd 0.004			

[a-c]Mean values with the same superscript letters across the same row are not significantly different.

4.10.6 Effect of time on fungi population in soil type

Sampling time had significant effect on fungal population in soil types wit h counts for soil types being significantly different in all the months (Fig. 36). November recorded the highest population while March recorded the least in both shea nut cake polluted and unpolluted soils.

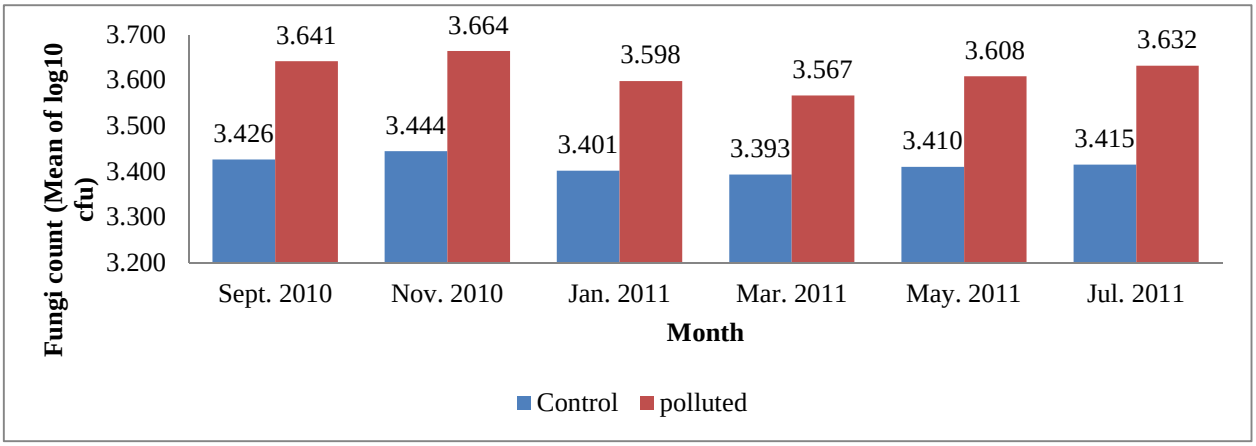


Figure 36Effect of time on fungi population in soil type

4.10.7 Effect of time on fungi population in soil depths

Fungi populations in the three soil depths were significantly affected by time, being significantly different for all the months in the various soil depths with the highest population occurring in November in the 0-20 cm depth and the least in the 41-60 cm depth in March. Fungi counts were highest in the 0-20 cm depth in the dry season months (November, January and March and highest in the 21-40 cm depth in the rainy season months of May, July and September (Fig. 37).

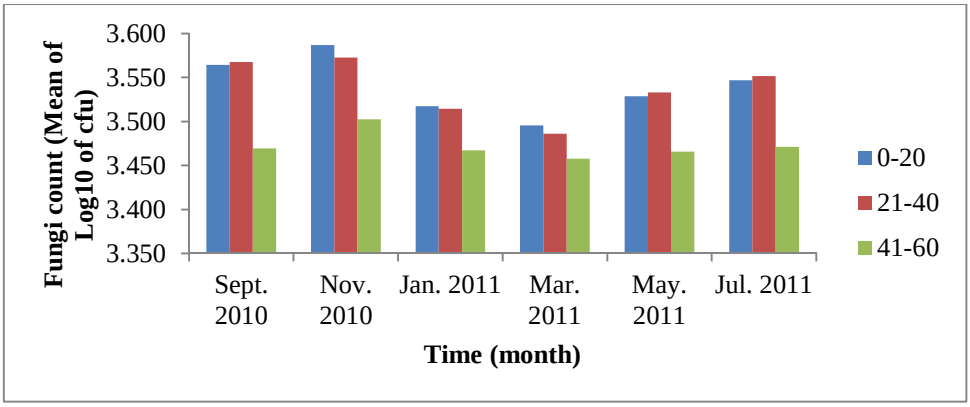


Figure 37Effect of time on fungi population in soil depth.

4.11 Isolation, identification and microbial biodegradation of tannins in shea nut cake

4.11.1 Gram reaction of bacteria isolated from shea nut cake polluted soil.

Using one-sample t-test (Paired sample) with the null hypothesis that the mean of GNR - GPR is equal to 0 gave $P < 0.05$ above, indicated that Gram Negative bacteria were significantly higher ($P < 0.05$) than Gram Positive bacteria (Table 39).

Table 39 Summary of T-test results of Gram results of bagteria isolated from shea nut cake polluted soil.

Summary : Variable: Y[1].

Sample	Size	Mean	Variance	deviation	of mean
GNR-GPR	9	16.67	20.75	4.555	1.518

Test of null hypothesis that mean of GNR-GPR is equal to 0

95% confidence interval for mean: (13.17, 20.17). Test statistic t = 10.98 on 8 d.f.

Probability < 0.001

Out of one hundred and thirty (130) bacteria isolated from shea nut cake contaminated soil examined by Gram staining, ninety (90) representing 69.23% were Gram Negative Bacilli and forty (40) representing 30.77% were Gram positive bacilli (Fig. 38). Gurugu recorded the highest in both Gram negative bacteria and Gram positive bacteria (49 representing 37.69%), while those from Kasalgu recorded the least, 38 (29.23%).

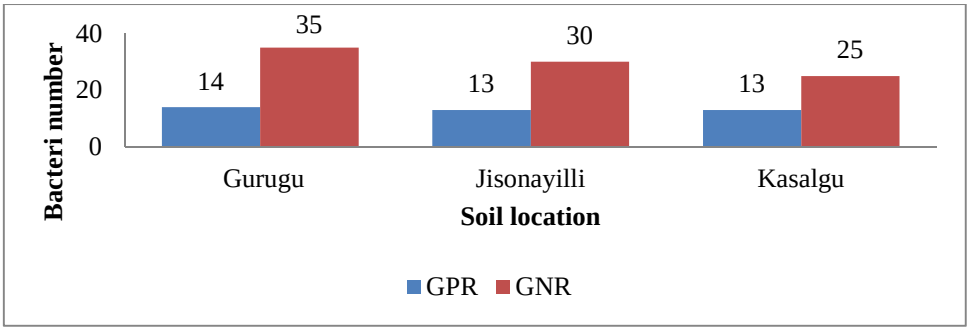


Figure 38 Gram results of bacteria by location

KEY: GPR = GRAM POSITIVE RODS; GNR = GRAM NEGATIVE RODS

Out of one hundred and thirty bacteria studied 88 (67.69%) were from the top (0-20 cm) soil depth, 32 (24.62%) from the 21-40 cm depth and 10 (7.69%) from 41-60 cm depth (Fig. 39).

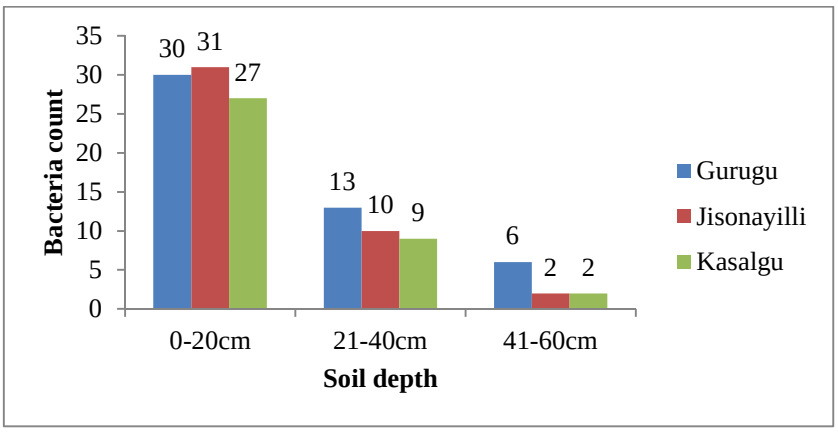


Figure 39 Distribution of Gram results of isolates by location and soil depth

Statistical analysis (Appendix B) showed that the interaction between site and depth was significant at $P < 0.05$ with Jisonayilli having the highest number of bacteria at 0-20 cm soil depth which was significantly higher ($P < 0.05$) than Gurugu and Kasalgu. However at the 21-40 cm depth Gurugu had the highest bacteria number which was significantly higher than ($P < 0.05$) Jisonayilli and Kasalgu. The least counts for all the locations were observed in the 41-60 cm depth.

4.11.2 Identification of GUR/O9 and GUR/38

Morphological and biochemical characteristics of Pseudomonas species GUR/09 and GUR/38 are shown in Table 4.22. GUR/O9 and GUR/38 were Gram negative rods.GUR/09 and GUR/38 were motile giving diffuse growth away from point of inoculation in motility agar. They were oxidase positive within 30 seconds, catalase positive and non-spore forming. On nutrient Agar GUR/09 produced large (4 mm in diameter), flat mucoid colonies which exhibited blue pigmentation with fruity smell, while GUR/38 produced large flat mucoid colonies that showed yellow pigmentation.

GUR/09 and GUR/38 were β-Haemolytic on blood agar and non-lactose fermenters, producing colourless colonies on MacConkey agar. GUR/09 and GUR/38 tested positive for citrate utilization as sole source of carbon, changing the medium from green to blue, which was more rapid in GUR/09 than GUR/38 (Fig. 40).

a: After 24 hours **b: After 48 hours**

Figure 40 Citrate utilization test.

The two organisms produced the enzyme urease by showing positive (pink colour) for the test which was more intense in GUR/09 than GUR/38 (Fig. 41).

Figure 41 Urease production test; JU 01 here is a negative test.

GUR/09 and GUR/38 tested negative for both Methyl Red and Voges Proskauer tests

On Kligler Iron Agar slants, both grew producing red slants and red butts with no gas and no H_2S production. The two isolates tested negative for indole.

The morphological and biochemical characteristics tested for GUR/09 and GUR/38 are summarised in Table 40 below.

Table 40 Summary of Characteristics of shea nut cake degrading bacteria Isolates (GUR/09 and GUR/38)

	Biochemical tests														
												TSI			
Isolate	N.A	B.A.	M. A	Gram	Mot	Oxid	Cat	Cit	Ind	MR	VP	S	B	Gas	H_2S
GUR/09	Mu BP	β- H	NLF	GNR	+ve	+ve <30 sec	+ve	+ve	-ve	- ve	- ve	R	R	- ve	- ve
GUR/38	Mu YP	β- H	NLF	GNR	+ve	+ve <30 sec	+ve	+ve	-ve	-ve	- ve	R	R	- ve	- ve

Key: N.A = Nutrient Agar; MuBP = Mucoid colonies Blue Pigmentation; MuYP= Mucoid colonies Yellow Pigmentation; B.A.= Haemolysis on Blood Agar; β- H= Beta Haemolytic; M. A = MacConkey Agar; NLF = Non-Lactose Fermenter; Gram = Gram Reaction; GNR = Gram Negative Rods; Mot = Motility; Oxidase production test = Oxidase; Cat= Catalase; Cit = Citrate; Ure = Urease test; In d= Indole; MR = Methyl Red; VP = VogesProskaeur; TSI = TripleSugar Iron Agar; Gas = CO_2; H_2S = HydrogenSulphide; R = Red (alkaline);

+ve = Positve; -ve = Negative.

GUR/09 and GUR/38 were identified morphologically and biochemically as *Pseudomonas aeruginosa* and *Pseudomonasputida* respectively with the help of Bergy's Manual of Determinative Bacteriology (Holt *et al*., 1994).

4.11.3 Growth characteristics of GUR/09 and GUR/38

4.11.3.1 Spore formation

No spores were found with the isolates. GUR/09 and GUR/38 were therefore non-spore formers.

4.11.3.2 Anaerobiosis

GUR/09, GUR/38 failed to grow on nutrient agar under anaerobic conditions.

4.11.3.3 Resistance to heat

GUR/09 and GUR/38 failed to grow after heat treatment at 80^{o}C and above.

4.11.3.4 Effect of pH on growth of GUR/09 and GUR/38

GUR/09 and GUR/38 grew well between pH 5 and pH 10 with optimum growth at pH 7 (Fig. 42). The pH of choice for high rapid growth and high bacterial activity will therefore be pH 7.0. GUR/09 recorded higher bacterial growth at pH 7 than GUR/38.

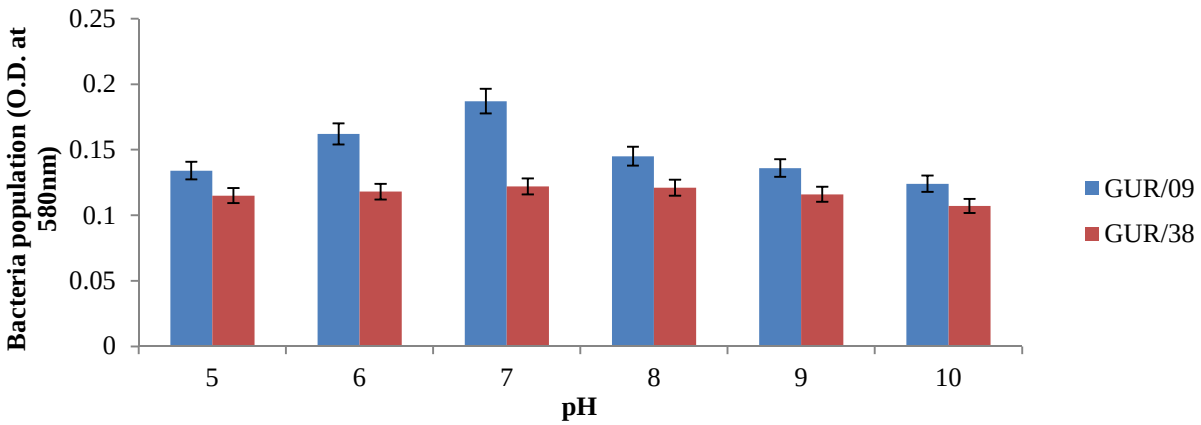


Figure 42 Effect of pH on growth of GUR/09 and GUR/38.

4.11.3.5 Effect of temperature on growth of GUR/09 and GUR/38

GUR/09 and GUR/38 produced good growth between 25° C and 40° C (Fig. 43) with optimum at 35° C. GUR/09 grew better than GUR/38 at all the temperatures tested especially at 35° C their optimum temperature for growth. Even though their optimum temperature for growth was 35° C, they also grew well at 25° C and biodegradation can easily be carried out at room temperature without the need for an incubator or special temperature arrangement.

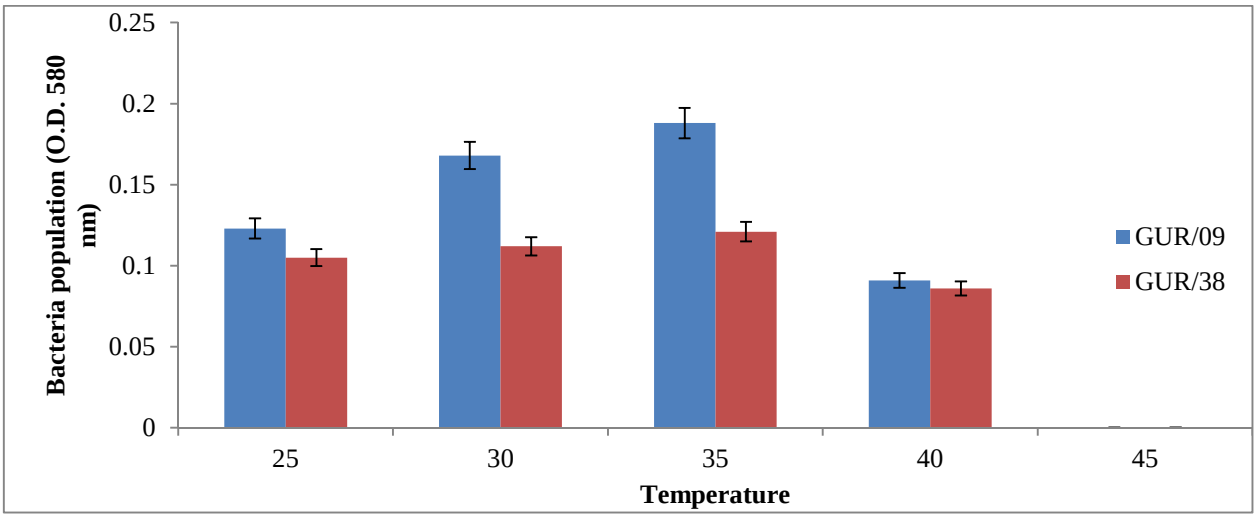


Figure 43 Effect of temperature on growth of GUR/09 and GUR/38.

4.11.3.6 Effect of yeast extracts on growth of GUR/09 and GUR/38

GUR/09 and GUR/38 grew well without yeast extract but manifested a faster growth in the presence of 0.5% yeast extracts (Fig. 44). GUR/09 grew better than GUR/38.

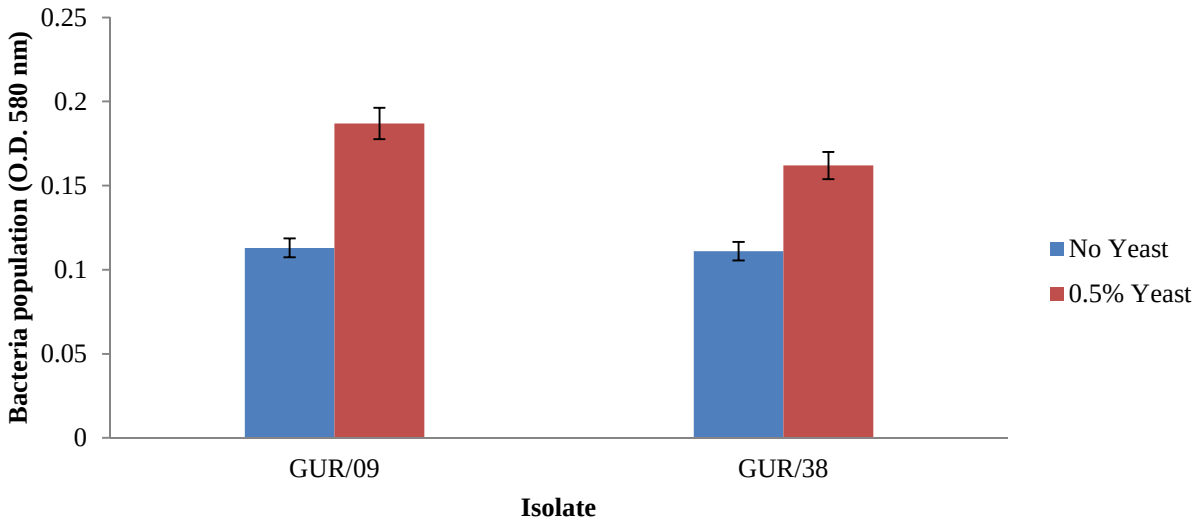


Figure 44 Effect of yeast extracts on growth of GUR/09 and GUR/38

4.11.4 Biodegradation of tannins in shea nut cake by Pseudomonas aeroginosa strain GUR/09

The pH of shea nut cake prio to inoculation with the test organism was 5.0.

Optical Density reading and calculation of Concentration of Polyphenols in Tannic acid equivalent are indicated in Table 41 and final results of microbial biodegradation of tannin in shea nut cake in Table 42.

Table 41 Estimation of Tannin concentration in Shea Nut Cake (gkg^{-1}) on dry matter basis (Mean of triplicate tests)

	Optical Density (a)	µg/0.05ml (b)	1.0ml extract (c)	mg/ml (d)	mgTA/ 100mgSNC (e)	%DM (f)	g/Kg (g)
STD. Standard tannic acid	**0.540**	**5.00000**					
T0 Unpolluted Fresh	1.657	15.34216	306.8433	0.307	1.228	2.729	27.29
T1 Unpolluted boiled	0.497	4.601723	92.03446	0.092	0.368	0.818	8.18
T2 Fresh 10 days	0.265	2.453635	49.0727	0.049	0.196	0.436	4.36
T3 Fresh 20 days	0.133	1.231447	24.62894	0.025	0.1	0.222	2.22
T4 Fresh 30 days	0.081	0.749979	14.99958	0.015	0.06	0.133	1.33
T5 Fresh 40 days	0.054	0.499986	9.99972	0.01	0.04	0.089	0.89
T6 Boiled 10 days	0.135	1.249965	24.9993	0.025	0.1	0.222	2.22
T7 Boiled 20 days	0.081	0.749979	14.99958	0.015	0.06	0.133	1.33
T8 Boiled 30 days	0.05	0.46295	9.259	0.009	0.036	0.08	0.80
T9 Boiled 40 days	0.05	0.46295	9.259	0.009	0.036	0.08	0.80

Table 42 Tannin Concentration in shea nut cake

Treatments	T_O	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	l.s.d
Tannins (g/Kg)	27.29^{a}	8.18^{b}	4.36^{c}	2.22^{d}	1.33^{e}	0.89^{f}	2.22^{d}	1.33^{e}	0.80^{g}	0.80^{g}	0.006

$^{a-g}$Means with the same superscript letter across the same row are not significantly different from each other.

Pseudomonasaeroginosa strain GUR/09 degraded 92 % of tannin in fresh shea nut cake in 20 days and 95% in 20 days when the shea nut cake was boiled in water for one hour. There was no significant difference in shea nut cake tannin reduction by the *Pseudomonasaeroginosa* strain GUR/09 between T3 and T6 and between T4 and T7. This suggests that boiling shortened the time taken to reduce the tannin concentration in 27.29 g/Kg shea nut cake to 2.22 g/Kg by the *Pseudomonas aeroginosa* strain GUR/09 from 20 days (T3) to 10 days (T6) and from 27.29 g/Kg to 1.33 g/Kg in 30 days (T4) to 20 days (T7). Boiling of shea nut cake

reduced tannin content in shea nut cake, from 27.29 $gKg^{-1}$to 8.18 gKg^{-1} representing 70.03% (Table 42). Tannin concentration was the same for T8 and T9 indicating no significant degradation of boiled shea nut cake was taking place between day 30 and day 40.

4.12 Discussion

Proportions of the three soil particles, sand, silt and clay which determine the texture of soil were significantly different Gurugu, Jisonayilli and Kasalgu sampled. The darker colours of shea nut cake polluted soils compared to their respective unpolluted samples agreed with the report of Cathcart, (2010) report that dark colours of soil are associated with soil high in organic matter. The environmental and nutritional requirements of soil bacteria are inherent in the physical and chemical properties of the soil (Berry *et al*., 2007). Many soil properties are influenced by the texture and other physical conditions of the soil, which also influence nutrient availability and bacterial population and diversity.

Soil organic carbon, nitrogen, moisture content, pH, fungi and bacteria counts in this study were significantly higher in shea nut cake polluted soils than unpolluted soils. They were highest in the 0-20 cm depth decreasing with increasing soil depth in Gurugu and Jisonayilli and with the exception of bacteria, highest in the 21-40 cm soil depth in Kasalgu. This agreed with Cathcart (2010) who reported high organic carbon, moisture, pH, and nutrients in soil high in organic matter. Tangjang*et al*. (2009) reported high organic carbon, moisture, pH, bacteria and fungi counts in soil high in organic matter. Significantly higher organic matter in shea nut cake polluted soil than unpolluted soil was consistent with the report of Pausing*et al*. (2007) that shea nut cake has high organic matter content up to about 93.7% which in this study could account for the additional organic matter in shea nut cake polluted soils. Brian et al. (2002) also reported that organic matter added through anthropogenic activities, such as dumping of industrial waste tends to make a significantly dominant fraction of total organic carbon in such sites.

Organic carbon, pH, moisture, nitrogen and fungi were found to be concentrated in the 0-20 cm soil depth in Gurugu and Jisonayilli, decreasing with increasing soil depth and highest in the 21-40 cm depth in Kasalgu.This was consistent with the report of Cathcart (2010) that on level grounds organic matter is more concentrated in the top soil than deeper depths, and that top soil of sloppy lands tends to record lower organic matter content due to erosion losses from the hill slope during heavy rains. Tangjang *et al*. (2009) also reported a similar trend. High organic carbon in the deeper soils in Gurugu in this study agreed with Tangjang *et al.* (2009) report which found high concentration of organic matter in deeper depths of loamy sand soil, which they attributed to higher rate of infiltration.

Significantly higher moisture content observed in shea nut cake polluted soil as compared to unpolluted soil was in agreement with Cathcart (2010), who reported that soil high in organic matter has increased water holding capacity and moisture content. Highest moisture content in Kasalgu in this study was observed in all soil depths. This is consistent with Cathcart (2010) report, that soil high in clay content has high moisture holding capacity and high moisture content than sandy loam and loamy soils as that found in Gurugu and Jisonayilli respectively. Lower moisture content of soil from Gurugu in this project agreed with Tangjang*et al*. (2009) and Cathcart (2010), who reported low moisture content for sandy loam soil which they attributed to high rate of percolation and low water holding capacity for sandy soil.

Soil sampling time was observed in this study to have significant effect on moisture content as seasonal variations in moisture content was observed with significantly higher moisture values recorded in the rainy season (May to October) with peak in September and least in March for all soil types, locations and soil depths. The results were consistent with Tangjang*et al*. (2009) who reported seasonal variations in soil moisture content with highest figures in summer.

Significantly higher pH values in shea nut cake polluted soil with higher organic carbon than unpolluted soils, agreed with Berry *et al.* (2007) report, which associated higher pH values with soil rich in organic matter. Tangjang *et al.* (2009) also reported higher pH in soil high in organic matter. Hamida, 2005; Berry *et al.* (2007) and Hamzah *et al.* (2010) observed that soil pH is influenced by the nature and degree of pollution. Hydrocarbon-polluted soils have been reported to have acidic pH (Hamzah *et al.,* 2010), while cement polluted soil and soil high in organic matter increase soil pH towards alkalinity (Hamida, 2005; Berry *et al.*, 2007).Tangjang *et al.* (2009) also reported higher pH in soil high in organic matter. Lowest soil pH in Gurugu despite high organic carbon content agreed with Tangjang *et al.* (2009) who reported low pH in sandy loam soil which they attributed to loss of buffering properties through leaching.

The study revealed a direct positive influence of soil organic matter from shea nut cake on soil nutrients (e.g. nitrogen) availability in the three locations, soil types and soil depths, which was found to increase with increase in pollution. Higher nitrogen contents in shea nut cake polluted soil agreed with Iddrisu (2013) report that shea nut cake has high nitrogen content and other minerals such as phosphorous and magnesium which could favour the use of shea nut cake as fertilizer and has the potential to increase the availability of nutrients to soil microorganisms. Soil nitrogen contents were observed to follow directly the trend of organic carbon contents in the soil types, location and soil depths studied, increasing with high carbon content. This agreed with Cathcart (2010) report that the concentration of nitrogen in soil is associated with that of soil organic carbon, increasing as the organic carbon concentration increases.

The Carbon/Nitrogen ratio in shea nut cake is lower than that for crude plant material which is reported to be 40:1 (Schlegel, 1995). This might be as a result of the shea butter extraction

during which a lot of carbon is extracted as fatty acids and other organic components. Lower Carbon/Nitrogen ratios in polluted soils is indication of a high rate of microbial conversion of organic matter to humus in which carbon is bound in organic complexes thus releasing more nitrogen into the soil (Schlegel, 1995). The generally higher Carbon/Nitrogen ratios in unpolluted soils could also be due to lower microbial populations and activities. High rate of pollution or addition of shea nut cake in Jisonayilli could account for the generally high carbon/nitrogen ratios in Jisonayilli. The observed highest Carbon/Nitrogen ratio in unpolluted soil in Kasalgu could be due the clay factor which forms stable complexes with organic matter preventing it from mineralizatiom by microorganisms. Generally high carbon/nitrogen ratio in the 21-40 cm depth of Kasalgu could be due to high organic matter with low microbial activity. This agrees with the findings of Berry *et al*. (2007), who attributed the phenomenon to low microbial population/activity due to unfavourable growth conditions and formation of stable aggregates with organic matter by clay thus protecting the organic matter from mineralization. The result also agrees with Whiting *et al*. (2010) report that soil rich in clay protects soil organic matter from decomposition by stabilizing substances that bind to clay surfaces. According to them enabled aggregation property of clay protects soil organic matter from mineralization by microorganisms

A Carbon/Nitrogen ratio of 11:1 to 12:1 in shea nut cake polluted soil in the locations studied met the Carbon/Nitrogen ratio of 10:1 required for microbial growth stated by Ray, (1994) and Schlegel, (1995). The observed significantly lower Carbon/Nitrogen ratio at 21-40 cm soil depth in Gurugu than Jisonayilli could be due to increased microbial population and activities as a result of high nutrient content resulting from high rate of percolation in the presence of favourable conditions of temperature and aeration in deeper soil depth of loamy sand soils. Tangjang *et al*. (2009) attributed high microbial growth across deeper depths of loamy sand soils to high rate of infiltration; surface temperatures are generally higher in

sandy soils in the rainy season. Significantly lower Carbon/Nitrogen ratios in 0-20 cm depth in shea nut cake polluted soil in Kasalgu could be due to lower concentration of organic carbon attributable to run-off losses during erosion.

Bacteria populations were significantly affected by soil type and depth, increasing significantly with shea nut cake pollution than unpolluted soils and highest at the 0-20 cm depth decreasing with increasing soil depth. This agreed with Ebuehi *et al.* (2005) who reported high bacteria population in hydrocarbon polluted than unpolluted soil. Truong, (2005) reported higher bacteria populations in hydrocarbon-polluted soil than unpolluted soil. Bahig *et al.* (2008) noted higher bacteria counts in soil irrigated with waste water than soil irrigated with canal water. Higher bacteria populations in the top 0-20 cm soil which decreased with increasing soil depth in the present study agreed with Bahig *et al*. (2008) who reported that top soil harbours higher bacterial populations than soil of deeper depth. In their study, bacteria counts ranged from 52.3×10^3cfu and 10.97×10^3cfu g^{-1} to 71.1×10^2cfu g^{-1} and 15.4×10^2cfu g^{-1} in surface soil and deeper soil depths respectively for agricultural soil irrigated with canal water, while bacterial counts for agricultural soil irrigated with waste water ranged from12.1×10^3 cfug^{-1} to 19.9×10^4 cfu g^{-1} for surface and from $10.65 \times 10^3$to 81×10^3cfug^{-1} for deeper soil depth. Maler *et al.* (2004) concluded from their data that total cultivable bacterial count from surface soils were more than in deeper soil depth. Shamir and Steinberger (2007) and Tangjang *et al.* (2009) observed a similar trend in the distribution of bacteria across soil depth, which they attributed to the compact nature of soil at deeper depth, limiting air, water and nutrients supply and increased carbon dioxide in deeper soil depth.

Increase in bacterial populations in shea nut cake polluted soil above that of unpolluted soil observed in the study was an indication that bacteria capable of utilizing shea nut cake as

source of carbon and energy were naturally present in the soil studied, increasing in population in shea nut cake polluted soil. This agreed with the reported of Truong (2005) that hydrocarbon polluted soil recorded higher bacteria population than unpolluted soil. Ray (1994) reported that natural product degrading bacteria are present in soil, with specific product degrading bacteria rapidly increasing in population with pollution with that product. Significantly higher bacteria count recorded in Gurugu and Jisonayilli than Kasalgu could be due to high organic matter, nutrients, pH and other favourable growth conditions in Gurugu and Jisonayilli. This agreed with Cathcart (2010) report that the main source of carbon and other nutrients for soil microorganisms is soil organic matter and soil rich in organic matter increases soil microbial populations by providing more nutrients. Low bacterial count in Kasalgu could be due to low organic matter and associated nutrients, which was observed in the study, probably due to run-off losses along the hill slop during heavy rains as reported by Tangjang *et al*. (2009). The high clay content could also be preventing mineralization of organic matter in the soil,supported by high carbon/nitrogen ratio observed in the study as reported by Cathcart (2010)

Bacteria populations in soil type, location and depth were significantly affected by sampling time. Seasonal variation was observed with counts being higher in the rainy season than the dry season with peak in September and least in March for all soil types, depths and locations. These agreed with Jha *et al.* (1992) who reported higher bacteria population in summer than winter, which they attributed to favourable growth conditions in the rainy season. Lower counts in dry season, especially March, could be due to low moisture stress. This is supported by Tangjang *et al*. (2009) who reported low bacteria count in winter which they attributed to moisture stress.

Fungi populations in locations were significantly affected by soil type and soil depth, being higher in polluted soil than unpolluted in all locations. This is in agreement with Bennet *et al*. (2002) who reported higher fungi population in polluted soil than unpolluted soil. Fungi counts were significantly different in all the soil depths in the locations studied, with highest count in the 0-20 cm depth in Gurugu and Jisonayilli decreasing with increasing soil depth and in the 21-40 cm depth in Kasalgu. For the two soil types studied fungi counts were significantly different in the different soil depths in shea nut cake polluted soil, decreasing with increasing soil depth, though no significant difference was observed between 0-20 cm and 21-40 cm depth in unpolluted soil.This agreed with Bahig (2008) who reported lower counts at deeper depths Shukla *et al.* (1989). Arunachalam *et al*. (1997) however reported that negligible differences were observed in fungal counts across soil depths.

Higher fungi population in the 21–40 cm depth soil in Kasalgu than top 0-20 cm depth observed in this study agreed with Tangjang *et al.* (2009), report which recorded higher fungal counts in the subsoil of slope land than the top soil, which they attributed to run–off losses of fungal propagules with plant residue from the hill slope. This was further confirmed by the lower organic matter content observed in the top 0–20 cm soil than the 21–40 cm soil at Kasalgu, a phenomenon attributed to erosion losses that often result in higher organic matter in deeper soil than surface (Tangjang *et al*., 2009). Significantly higher fungi counts in shea nut cake polluted soil in Gurugu, sandy loam soil, compared to Jisonayilli and Kasalgu agreed with Tangjang *et al.* (2009) report which observed a similar trend which they attributed to adequate nutrients supply in the presence of favourable conditions of aeration and moisture across soil depths associated with sandy loam soils.

The study revealed lower fungal counts in the 0-20 cm soil depth during the raining season months (May, July, September) than the dry season months (November, January and March).

This agrees with Tangjang *et al*. (2009) report which attributed the phenomenon to unfavourably high soil surface temperatures during summer. The higher fungi colony forming units in shea nut cake polluted soil observed in the study could be attributed to high organic matter which supplied more nutrients for fungal growth. The result agreed with Bennet *et al.* (2002) who reported higher fungi counts in polluted soils than unpolluted soils. The significantly higher fungal counts in Gurugu and Kasalgu which have acid soils than the alkaline soils of Jisonayilli agreed with Schlegel (1995) that fungi grow better in acid soils than alkaline soils. Generally higher bacteria counts than fungal counts observed in the study agreed with Bennet *et al*. (2002) report that bacteria are faster growers than fungi.

The isolation of bacteria from shea nut cake polluted soils agreed with earlier report that organic pollutant-degrading bacteria are naturally present in soil (Ray, 1994; Troung, 2005). Gram reaction results of bacteria isolated from shea nut cake polluted soil showed that different types of bacteria were involved in shea nut cake degradation in soil and that more Gram negative bacilli were involved in shea nut cake degradation in soil than Gram positive bacilli. This agreed with Troung (2005) reported that more Gram negative bacilli are involved in hydrocarbon degradation in soil than Gram positive bacilli. In this study, top soil (0-20 cm) harboured the highest number of shea nut cake degrading bacteria. This agreed with Hamzah *et al.* (2010) report that higher crude oil-degrading bacteria were found in the upper layers of crude oil-polluted soil than deeper depths.

Traditional shea butter extraction with 25% efficiency (Iddrisu, 2013), results in large amount of unextracted shea butter in shea nut cake. The isolation of *Pseudomonas* in shea nut cake polluted soil therefore agreed with Hamzah *et al*. (2010) report which associated *Pseudomonas* species with oil polluted environment, including soil. Ray, (1994) reported *Pseudomonas* as the most predominant isolate in crude oil polluted soils and water.

Pseudomonas has been reported to have a high capability of degrading many natural products, including cyclic compounds, a versatility attributed to the presence of plasmids coding for the production of inducible enzymes (Bhatia and Ichhpujani 2008). This versatility characteristic of *Pseudomonas* reported by Bhatia and Ichhpujani (2008) confirms the urease positive results obtained for GUR/09 and GUR/38 in this study, since *Pseudomonas* isolated from humans are often reported negative for urease test (Departments of The Air force and The Army, USA, 1977)

Bioremediation utilizes the ability of microbes to degrade or detoxify organic pollutants with the new concept being an efficient, cost effective and environmentally friendly, biological treatment method (Ray, 1994). It depends on the manipulation of nutritional and environmental factors to increase bacterial numbers to hasten biodegradation (Ray, 1994). The first report on soil bacteria degrading shea nut cake was by Quattara *et al.* 1992, who isolated anaerobic *Streptococcus* from rice field capable of degrading hydrolysable tannin. Growth of the strain of *Pseudomonas aeroginosa* GUR/09 in the pH range of 5 to 10 agreed with Bhatia and Ichhpugan (2008) report that *Pseudomonas* has a wide pH growth range above 4.5. Optimum pH 7 for growth of *Pseudomonas aeroginosa* strain GUR/09 isolated in the present study agreed with Hamzah*et al.* (2010) report which gave the optimum pH for growth of a species of *Pseudomonas* used for the degradation of petroleum hydrocarbonas 7.0. However, the above report of Bhatia and Ichhpugan (2008) on the wide pH of growth of *Pseudomonas* above pH 4.5 supported the use of shea nut cake with an observed pH 5.0 in the study without adjustment. Good growth of GUR/09 at 25^{o} C with optimum temperature of 35^{o} C also agreed with Hamzah *et al.* (2010) report that *Pseudomonas* grows well at 35^{o} C and at room temperature. Susceptibility of GUR/09 to high temperatures (≥80^{o} C) is an advantage in bioremediation as the organism can easily be destroyed after successful use,

supporting the fact that the use of the organism for managing shea nut waste is environmentally friendly.

Yeast extract has been reported to contain nicotinic acid and sufficient quantities of most essential elements in utilizable form, which enhance the growth of most soil bacteria (Stanier *et al.*, 1963; Hamzah *et al.*, 2010). Increase in populations of *Pseudomonas aeroginosa* strain GUR/09 and *Pseudomonas putida* strain GUR/38 in the presence of yeast extracts in the present study confirmed that yeast extract enhanced growth of the isolates. These results agreed with Hamzah *et al.* (2010), report that *Pseudomonas aeruginosa* and *Pseudomonas putida* isolated from soil showed better growth with addition of yeast extracts. However, significant growth of GUR/09 and GUR/38 in the absence of yeast extracts suggested that yeast extract was not an absolute requirement for growth and that the isolates can grow without yeast extract, but will require it for accelerated growth and rapid degradation of shea nut cake.

Pseudomonas aeroginosa strain GUR/09 and *Pseudomonas putida* strain GUR/38 exhibited different pigmentations on nutrient agar. The exact identity of the strains can be confirmed with molecular biology techniques such as Polymerase Chain Reaction (PCR), G-C ratio and DNA hybridization. Higher growth of *Pseudomonas aeroginosa* strain GUR/09 than *Pseudomonas putida* strain GUR/38 under the conditions investigated led to the selection of *Pseudomonas aeroginosa* strain GUR/09 for the management of shea nut waste. There was no significant difference between T3 andT6. This could be due to the boiling inT6 which reduced the tannin concentration and enhanced biodegradation, achieving the same result as T3 within a shorter time, 10 days instead of 20 days in T3. Boiling shea nut cake in this study reduced the tannin content from 27.29 gKg^{-1} to 8.18 gKg^{-1} (70.03%). This agreed with Okai (1989) and Oddoye *et al.* (2012) reports that boiling of shea nut cake in water for one hour reduces tannin content by about 70%.

A strain of *Pseudomonas aeroginosa* strain GUR/09 degraded 91.87% tannin in fresh shea nut cake in 20 days and 95.13% in 30 days. This agrees with Oddoye *et al*. (2012) who reported that increase in treatment time resulted in significant reduction in tannins concentration. Similar studies with other vegetable tannins gave enhanced tannin reduction with increase in length of time (Rehman *et al*., 2002; Oladele *et al*., 2009). High degradation of about 92% tannin in shea nut cake in less than 28 days suggested that shea nut cake is biodegradable. This agreed with Aluyor *et al*. (2009) report that plant products are biodegradable when degradation by microorganisms is within 70-100% within a period of 28 days. The high biodegradability of shea nut cake, above 90% in 20 days, observed in this study also agreed with Bhatta *et al*. (2012) report that shea nut cake has a higher amount of hydrolysable tannin phenol which is more easily degraded by bacteria than condensed tannin which, according to them is less than 0.1%. It was observed in this study that boiling of shea nut cake enhanced bacterial degradation of shea nut cake probably by reducing significantly.

4.13 Conclusion

1. Shea nut cake polluted soils had significantly higher pH, moisture, organic carbon and nitrogen contents than unpolluted soils. Soil pH, moisture, organic carbon and nitrogen contents were generally more concentrated at the top (0-20 cm) soil depth and decreased as the soil depth increased. In terms of location (Gurugu, Jisonayilli, Kasalgu) these parameters were significantly different.
2. Bacteria count was higher than fungi and higher in shea nut cake polluted soil than unpolluted soil. Both bacteria and fungi counts were generally highest in the top soils.
3. Seasonal variations were observed in moisture content and bacteria count with soil moisture contents and bacteria populations being generally higher in the rainy season than dry season with the peak in September.

4. Shea nut cake degrading bacteria were naturally present in the studied soil, increasing in population with pollution and more concentrated in the top 0-20 cm soil depth.
5. Different types of bacteria were involved in shea nut cake degradation in soil with Gram negative bacilli dominating in shea nut cake polluted soil studied.
6. A strain of *Pseudomonas aeroginosa* GUR/09 capable of degrading tannins in shea nut cake was identified. Shea nut cake was biodegradable, with the strain of *Pseudomonas aeroginosa* GUR/09 degrading 92% of tannins in fresh shea nut cake in 20 days and 95% in 20 days when the shea nut cake was boiled in water for one hour Boiling shea nut cake for one hour reduced tannins by 70%.
7. *Pseudomonas aeroginosa* strain GUR/09 with a high ability to remove polyphenols from shea nut cake can be used to remove tannin, an anti-nutritional factor from shea nut cake, a waste product of shea butter extraction and permit its inclusion in animal feed and conversion to other economically useful .products.

5.0 CHAPTER FIVE

SUMMARY AND RECOMMENDATION

5.1 Summary

Shea butter extraction is on the increase as a result of high demand locally and internationally and support from Governments of countries in the shea belt to develop the industry as an important source of foreign exchange and employment for the rural communities especially women, thus increasing shea nut cake production. Shea nut cake is a waste product generated from the extraction of shea butter from the nuts of the shea tree (*Vitellaria paradoxa)*. It is a brown amorphous suspension containing nut deposits. It is obtained when the crude shea butter which has separated from the brown paste as a result of vigorous kneading is whisked out for clarification. Other waste products of the industry, Shea seed husks and black cake (black residue being charred deposit after clarification) have found uses such as domestic lighting. Shea nut cake is of no use to the shea butter industry. The product is too bulky, toxic and recalcitrant to biodegradation by most soil bacteria to be used for soil fertility. However it has a potential in animal industry as feed because of its high content of protein, carbohydrates, fatty acids and mineral salts. An important draw back in this direction has been the presence of anti-nutritional factors such as tannins. Numerous attempts to use the product as feed for animals and poultry have recommended the addition of only small amounts above which growth response and acceptability of animals are very poor. Hence the soil is the ultimate recipient of the waste in an environmentally unacceptable way. Attempts to remove or reduce anti-nutritional factors have employed physical and chemical means which have been considered expensive and have recommended the use of micro-organisms as the most environmentally friendly and cost effective way, hence this study.

This study involved six main chapters; Introduction, literature review, Materials and Methods, Results.

Chapter one (1) Introduction dealt with background information on the shea butter industry, shea waste, Challenges of the shea industry, the study area and people, problem statement, study objectives and significance of study.

Chapter two (2) Literature review dealt with literature on the industry and microbial biodegradation to bring experience to bear on the study. The chapter dealt with the shea butter industry, benefits and potential; the shea tree and uses of its parts; shea butter extraction processes; wastes generated from shea butter extraction and their uses; composition of shea nut cake, uses and efforts to solve the anti-nutritional problems of shea nut cake. The chapter also dealt with tannin as an important anti-nutritional component of plant products in general and shea nut cake in particular which make them recalcitrant to biodegradation. The literature also reviewed some physicochemical properties of soil, soil microorganisms and biodegradation, nutritional and environmental requirements for microbial growth, biodegradation of major components of shea nut cake, removal of anti-nutritional factors from shea nut cake.

Chapter three (3) Materials and methods discussed an over view of the general methods used in the study. These included the experimental design employed in the study, the selection of locations, sample collection and processing. The emphasis was on the general microbiological techniques while reserving very technical and specific techniques to the respective topics.

The chapter dealt with analysis of physico-chemical characteristics of shea nut cake polluted and unpolluted soils. The study was conducted in shea nut cake dumping sites of three shea nut cake processing centres in Sagnarigu using standard procedures and results were

compared with those of soil from lands without any history of shea nut cake pollution. These lands were located 100 metres away from the shea nut cake polluted soils and served as controls. The shea butter extraction centres were Tiehisuma Shea Butter Processing Centre in Gurugu, Tungteiya Women Association Shea butter Extraction Centre in Jisonayilli and Sekaf Shea Butter Village in Kasalgu. Soil depths studied were 0-20 cm, 21-40 cm and 41-60 cm. Effect of the following dependent variables location, soil type, depth and sampling time on the following dependent variables, soil moisture content, pH, soil organic carbon, total nitrogen content, carbon/nitrogen ratio and microbial (fungi and bacteria) populations were investigated.

Chapter four dealt with the results of the study. The chapter noted that shea nut cake added organic matter to the soil which changed the soil parameters as compared to unpolluted soils. Soil colours were darker in shea nut cake polluted soils. Soil pH, moisture and nutrient contents were significantly higher in shea nut cake polluted soils than unpolluted soils. The highest concentrations were in the top soil decreasing with increasing soil depth and in sloppy grounds the highest concentration was in the sub-soils (21–40 cm) in sloppy grounds. Loamy sand soils were found to have significantly higher concentrations of organic matter and nutrients across soil depth than the clay and loamy soils, due to high percolation associated with sandy soils. The highest moisture content and pH values were recorded in the top 0 – 20 cm soil depth in Jisonayilli shea nut cake polluted soils where organic matter content was highest. In unpolluted soils however highest moisture content was recorded in Kasalgu which has high clay content, with highest moisture content in the 21-40 cm depth. For total organic carbon for all the three soil depths studied, Gurugu recorded the highest in both soil types, with Jisonayilli recording the least in unpolluted soil while Kasalgu recorded the least in shea nut cake polluted soil. It was observed that shea nut cake increased organic matter content of the polluted soil which improved the physical and chemical properties including nutrients

availability in the soil. The bacterial and fungal populations across soil depths and locations of both shea nut cake polluted and unpolluted soils were compared. Bacterial and fungal counts were significantly higher in shea nut cake populated soils. Bacterial populations were significantly higher in the top soil in all locations sampled and decreased with increasing soil depths. Fungal counts were highest in the top soil on level grounds and highest in the sub-soil (21 – 40 cm) in sloppy grounds of Kasalgu where organic carbon, pH, and nutrient contents were highest. Loamy sand of Gurugu recorded the highest bacterial counts due to high concentration of organic matter, nutrients and good aeration across soil depths. Bacteria being faster growers than fungi recorded higher populations than fungi in all the samples studied. Shea nut cake added organic matter which increased moisture content, pH, nutrient availability and microbial populations in the soil. Seasonal variation in bacteria population was observed to be related to that of soil moisture content, suggesting that bacteria growth in the presence of adequate supply of nutrients was influenced by moisture content. Soil moisture content, bacteria and fungi counts were significantly affected by soil sampling time which experienced seasonal variations with significantly higher concentrations in the raining season and peak in September for moisture and bacteria and November for fungi.

Shea nut cake degrading bacteria were isolated from shea nut cake polluted soils using mineral salt medium supplemented with 2% shea nut cake as sole source of carbon. This suggested that shea nut cake degrading bacteria were naturally present in the soil, which increased in population with shea nut cake pollution and probably accounted for the significantly higher bacteria counts in shea nut cake polluted soils than unpolluted soils. Different types of bacteria were involved in shea nut cake degradation in soil with Gram negative bacilli being significantly higher than Gram positive bacteria. Bacterial isolate GUR/09 which gave good growth in 5% shea nut cake medium was identified morphologically and biochemically as *Pseudomonas aeroginosa*. Optimum growth

conditions to increase population were investigated. Increase in Optical Density at 580 nm indicated increase in bacteria numbers.

Optimum temperature for growth was 35^0 C and pH 7.0. The organism however, grew equally well at 25^0C (Room Temperature). Yeast extracts enhanced growth of GUR/09. For field practical purposes aerobic shea nut cake degrading bacteria that grow well at room temperature will permit large scale practical application without additional cost to maintain a different temperature for accelerated growth and degradation. Tannin, an anti-nutritional polyphenolic agent which forms indigestible complexes with proteins and some polysaccharides in shea nut cake and removal by degradation of the tannins will permit easy break down of the shea nut cake by the microorganism for energy and cell building materials was the target as indicator of biodegradation of shea nut cake in this study. Tannin removal from shea nut cake can permit rapid degradation of shea nut cake by soil bacteria and favour its use as organic fertilizer as well as animal feed. The disappearance or reduction of Tannin concentration in the substrate incubated with the shea nut cake degrading bacteria over time was therefore an indication of biodegradation of shea nut cake as indicated by Coordinating European Council (Aluyor *et al*., 2009). GUR/09 degraded 92% of Tannin in shea nut fresh (unboiled) cake in 20 days and 95% in 20 days when shea nut cake (1 g shea nut cake : 20 ml water) was boiled for one hour. Boiling of shea nut cake reduced tannin concentration in shea nut cake by 70% and enhanced biodegradation of shea nut cake by the microorganism.

Shea nut cake is biodegradable and *Pseudomonas aeroginosa* strain (GUR/09) can be used for the management of shea nut waste under the optimum growth conditions of the organism. The biodegradation of shea nut cake by GUR/09 was observed to increase with longer incubation period, degrading 95% of the fresh waste in 30 days. The aerobic organism that is a non-spore former and is destroyed at 80^0 C and above can easily be destroyed after use making the application environmentally safe.

Microbial biodegradation is an environmentally friendly way of disposing of the waste and soil polluted with such waste is a good and reliable source of waste degrading microorganisms. The choice of a suitable organism should in addition to having a high degrading ability, take into consideration how to dispose of the organism in an environmentally safe way after use.

5.2 Recommendations

1. District Assemblies should Unpolluted the disposal of shea nut cake into the soil.
2. Assemblies should provide funds for the development and testing of management strategies for plants growing in the area, especially around the polluted fields for bench type experiments.
3. In the absence of facilities to carry out detailed study of the tannin degrading organism in this study, it is recommended that detailed molecular typing of Pseudomonas GUR/09 and tannin degrading enzyme will permit detailed characterization and large scale removal of anti-nutritional factors from shea nut cake for commercial use.
4. Government should encourage the development and use of techniques of seeding shea nut cake polluted soil to make the soil environmentally safe.
5. Shea nut cake should be boiled before disposal since boiling reduces tannin concentration in shea nut cake.
6. Boiling of shea nut cake and seeding with appropriate microorganisms should eliminate anti-nutritional factors from shea nut cake and permit its addition in the formulation of feed for the animal husbandry and poultry industry.
7. Sustainable education of processors in converting shea nut cake into useful products or establishing enterprises or industries to use safe shea nut cake for the production

feed for the benefit of animal and poultry farmers and fertilizer for soil fertility to enhance crop production.

Chapter five gave an overview of the entire study and recommendations

References

Abbiw D. (1990)*The useful plants of Ghana*. Intermediate Technology Publications/Royal Botanic Gardens, London/Kew. Pp337

Adebusoye S.A., Ilori M.O., Amund O.O., Teniola O.D. and Olatope S.O. (2006) Microbial degradation of petroleum hydrocarbons in a polluted tropical stream. *Jr. of American Sci*. 2(3): 48-56

Adeduntan S.A..(2009) Diversity and abundance of soil mesofauna and microbial population in South-Western Nigeria.*African Journal of Plant Sci* .Vol. **3** (9): 210-216

Adesemoye A.O., Opere B.O. and Makinde S.C.O. (2006) Microbial content of abattoir waste water and its contaminated soilin Lagos, Nigeria. African Journal of Biotechnology **5**:1963-1968

Adeogun W.O. (1989)Sheabutter cake as an ingredient in broiler chick rations. M.Sc. Thesis, Department of Animal Science, University of Ibadan, Nigeria

Adu-Ampomah Y, Amponsah J.D. and Yidana J. A.(1995) Collecting germplasm of shea nut (*Vitellariaparadoxa*) in Ghana. *Plant Genetic Resources Newsletter*, **102**:37-38

Alexander M. (1982)Most probable method for microbial populations. In: Page A. C. R. H. Miller and D. R. Keeney (ed). Methods of soil analysis: Part 2. Chemical and microbiological properties 2nd Edn. Madson, ASA PP. 815-820.

Aluyor E.D.,Obuhiagbon K.O. and Ori-jesu M (2009) Biodegradation of Vegetable oils: Areview.Sci. Res. Essays Vol. 4(6): 543-548

Arunachalam K., Arunachalam A., Tripathi R.S., and Pandey H.N (1997) Dynamics of microbial population during the aggradations phase of a selectively logged subtropical humid forest in Northeast India. *Trop. Ecol*. **38**:333-41

Atlas, R. M. (ed.) (I984) Petroleum Microbiology. Macmillan, New York, N.Y.

Atlas, R.M. and Bartha R.(1981) Microbial Ecology: Fundamentals and Applications. Reading, MA: Addison-Wesley publishing Company.

Atuahene C.C., Asante, F. and Opoku R.S. (1998)The value of shea nut cake (SNC) as a feed ingredient in broiler finisher diets. *Proceedings of the 19th Animal Science Symposium*, Kumasi, Ghana, 22-26th. October 1989 pp 111-114.

Bahig A. E., Aly E. A., Khaled A. A. and Amel K. A. (2008) Isolation, characterization and EcoI application of bacterial population from agricultural soil at Sohag Province, Egypt. *Malaysian j. Microb.***4**(2): 42-50.

Baron E. J. O., Peterson L. R., Finegold S. M. (1994) Bailey & Scott's Diagnostic Microbiology. 9th Ed. Mosby-year book Inc. 11830 Westline Industrial Drive, St.

Louis, Missouri 63146, U.S.A pp 376-377

Bennet J.W, Wunch K.A. and Fauson B.O. (2002) Use of fungi in Biodegradation.*Manual of environmental microbiology*. ASM press Washington DC. P8 960-970

Berry W., Keterings E., Antes S., Page S., Russell-Anelli J., Rao R. and De Gloria S. (2007) soil texture; *Agronomy fact sheet series;* Cornell University Co-operative Extension; Fact sheets3, 6, 22, 29. http://nmsp.css.cornell.edu/publications/factsheets.asp

Bhatia R. and Ichhpujani R. (2008) Pseudomonas In: Essentials of Medical Microbiology. 4th Ed. Jaypee Brothers Medical Publisher (P) Ltd. pp 258-260.

Bhatta R., Mani S., Baruah L. and Sampath K. T. (2012) Phenolic composition, Fermentation profile, Protozoa population and methane production from sheanut (Butryospermumparki) By-products invitro. *Asian-Austral E.S. J. Anim. sci*Vol..25(10) 1389-1394.

Bragg J.R., Prince R. C., Wilkinson J.B., Atlas R.M. (1992) Bioremediation for Shoreline Cleanup Following the 1989 Alaskan Oil Spill. *Houston, TX*: Exxon Co.; 1992. p6

Brian A.S. (2002) Total Organic Carbon (TOC) in soils and sediments. *Ecological Risk Assessment Centre, USA*

Busson I. (1965) Plantes alimentaires de l`OuestAfricain.'etudebotanique, biologique et chimique.*Leconte,* Marseilles. 568pp

Carter, M. R. (1993) Soil Sampling and Methods of Analysis, CSSS, Canadian Society of Soil Science, Lewis Publishers,Boca Raton, FA.

Cathcart J. (2010) Soil organic matter. AESA Soil quality Programme Resource Science and Branch Alberta and Agriculture and food.*Deb Sutton.*

Cheesbrough M. (2002) Microbiology Tests.In: District Laboratory Practice in Tropical Countries Part 2. Cambridge University Press.

Dalziel, J.M. (1937)*Useful plants of West Africa*.Crown Agents, London. Pp612

Danikuu F.M. and Sowley E.N.K (2014) Biodegradation of Shea nut cake by indigenous soil bacteria. *Journal of Medical and Biomedical Sciences* (2014)3 (3), 9-15

De Muelenaere G. (1997) Proposal for a Directive Relating to Cocoa and Chocolate Products.Consequences for Exporting and Importing Countries.*European Union.* pp34

Department of the Air force- Medical Service (1977)*Clinical Laboratory Procedures-Bacteriology*

Dei H.K., Rose S. P., Mackenzie A.M., Pirgozhev V. (2008) Metabolizable energy in different shea nut (*Vitellaria paradoxia*) Meal samples for Broiler chicken.*Poultry Science 2008*. Poultry Science Association, **87**: 694-699

Diaz E. (2004) Bacterial degradation of aromatic pollutants: a paradigm of metabolic versatility. *Int. Microbiol***7**: 173-180

Dkhar M.S. (1983) Studies on population of edaphic microbial populations and their activities in maize fields. *PhD Thesis*, Northeastern Hill University, Shilong, India

Ebuehi O. A. T., Abibo I. B., Shekwolo P.D., Sigismund K. I., Adoki A. and Okoro I. C. (2005) Remediation of crude oil contaminated soil by enhanced natural attenuation technique. *J. Appl. Sci. Environ.* Mgt. **9**:103-106

FAO (2007) Minor oil crops.Corporate Document. http:www.fao.org/docrep/X5043/x5043Eob.htm.

FAO/IAEA (2000)Quantification of Tannins in Tree Foliage.FAO/IAEA Working Document IAEA, VIENNA

Fobil J.N. (2002) Research by the Cocoa Research Institute of Ghana (CRIG) into the cultivation and processing of nuts as an alternative to cocoa products.*Solution Site Case Study*.http://www.solutions-site.org/cat//-sol/19.htm

Gadd G. M. (1986) Fungal response towards heavy metals,p. B3-110. In R. A. Herbert and G. A. Gadd (ed.),Microbes in Extreme Environments. Academic Press,L td.,London, United Kingdom.

GenStat (2013) Release 10.3DE (PC/Windows Vista) 04 VSN International ltd (Rotamsted Experimental Station) Genstat Co. U.K.

GenStat (2016) Release 10.3DE (PC/Windows Vista) 04 VSN International ltd (Rotamsted Experimental Station) Genstat Co. U.K.

Ghana Statistical Service (2010) Ghana export promotion council report in 2007 report in 2010 Population and Housing Census.

Ghana Statistical Service (2010) Bank of Ghana report in 2007 report in 2010 Population and Housing Census.

Hagerman AE (2002) Tannin Chemistry, *MUOHIO.edu*

Hall J.B., Aebischer D.P., Tomlison H.F.,Osei-Amaning E. and Hindle J.R. (1996)*Vitellariaparadoxa*. *A Monograph*.School of Agricultural and Forest Sciences. University of Wales, Bangor, **8:**105pp

Hamida S.K. (2005) Fungal and bacterial population in cement incorporated soil. International *Jn. Of Agric and Biology*.**1**:561-853

Hamzah A., Rabu A., Farzarul R., Azmy H.R.J., Yussor N. A. (2010) Isolation and characterization of Bacteria degrading Sumandak and south Angsi oils. *Sains Malaysiana* 39(2) 161-168

Harsch E. E. (2001) Making Trade Work For Poor Women. *Africa Recovery* Vol. 15 (4) p6

Hausenbuiller R. L. (1975) Soil Science Principles and Practice.4th.Ed Wm. Brown Co., Dubuque, Iowa. Page 40

Hawle-Ambrosch E., Riepe W., Dornmayr-Pfaffenhuemer M., Radax C., Holzinger A. and Stan-Lotter H. (2007) Biodegradation of fuel oil hydrocarbon by a mixed bacterial consortium in sandy and loamy soils. *Biotechnology Journal* **2**:1564-1568

Holt, J.G., Krieg N.R., Sneath P.H.A., Stanley J.T. and William S.T. (1994)*Bergey`s Manual of Determinative Bacteriology.* Baltimore*:* William and Wilkins.

Iddrisu A. (2013) Biochemical and Microbiological Analysis of Shea nut cake: A waste product from Shea butter processing. MPhil Thesis, KNUST, Kumasi. *African Journal of Biotechnology*

Janssen and Koopman R. (2005) Determination of Kjeldahl Nitrogen in soil, biowaste and sewage sludge. *STD6161_Kj-N.pdf*

Jha D.K., Sharma G. D. and Mishra R. R. (1992) Soil microbial population numbers and enzyme activities in relation to altitudes and forest degradation. *Soil Biol. Biochem*. **24**: 761-767

Kennedy N., Gleeson D. E., Connolly J. and Clipson N. J. W. (2005) Seasonal and management influences on bacterial community structure in an upland grassland soil. *FEMS Microb. Ecol*., **53**: 329-337.

Kunlun S.P. (2010) Shea nut cake in supplemental concentration for growing Gjallonke Rams fed a base diet of rice straw and groundnut haulms in the dry season. PhD Thesis, KNUST, Kumasi. *GEZ* Publication magazine.

Kummerer K. (2004) Resistance in the environment. *J. Antimicrob. Chemoth***45**: 311-320.

Laukova A., Marekova M., Vasilkova Z., Papajova I. and Juris P. (2002) Selected microbial consortium of raw and digested slurry and its susceptibility to enterocins. *World Journal of Microbiology and Biotechnology***18**: 11-15

Lynn W.C.and Pearson M.J. (2000)The color of soil. The science teacher.www.munsell.com.

Makkar H.P.S., Bluemmel M., Borowy N.K., Becker K., (1993) Gravimetricdetermination of tannins and their correlations with chemical and protein precipitation methods, *J. Sci. Food Agric.* **61** (1)

Maler R. M., Drees K. P., Neilson J. L., Navarro-Gonalez R., Rainey F. A. and McKay C. P. (2004)Microbial life in the Atacama Desert. *Science***306**:1289-90.

Marchant D. (1988) Extracting profits with a shea butter press. International Development Research Centre Reports, 17: 14-15.

Mazzafera P. (2002) Degradation of caffeine by Microorganisms and potential use of decaffeinated coffee husks and pulp in animal feeding.*Sci. agric. (Piracicaba, Braz.) vol. 59(4)* ISSN 0103-9016

Mellifera (2009) Raw Science Tannins-Give it to me raw *mht*.

Millee J.K. (1984) Secondary products of species native to the Dinderesso Forest Reserve, Forestry Education and Development Project USAID, Ouagadougou.

Murad S., Hasan F., Shah A.A., Hameed A. and Ahmed S. (2007) Isolation of phthalic acid degrading *pseudomonas* sp. p1 from soil.*Pak. J. Bot*., 39(5): 1833-1841

Nitiema, L.W., Dianou, D., Simpore, J., Karou, S.D., Savadogo, P.S. and Traore A.S. (2010) Isolation of Tannic Acid-Degrading *Streptococcu*s sp. From an Anaerobic Shea cake Digester. *Pak. Biol. Sciences*, **12**: 46-50.

Ochei J., Kolhatkar A. (2000) Medical Laboratory Science Theory and Practice. Tata MacGraw-Hill Pub. Co. Ltd. New Delhi. 1242pp

Oddoye E.O.K., Alemawor F., Agyente-Badu K. and Dzogbefia V.P. (2012) Proximate analysis of shea nut kernel cake cake/meal samples from industry and cottage industry and some methods of removal of anti-nutritional factors. International J. of Biochemistry and Biotechnology.vol. 1(9): 239-242.

Ofosu M. A. (2009) Anaerobic Digestion ofShea Waste for Energy Generation.PhD Thesis submitted to the *University of Cape Coast*. Cape Coast.

Okai D. B. (1990) Seed cake tried in Ghana. Pig Int., 20: 28.

Okullo J.B., Omujal F., Agea G.I., Vuzi P.C., Namutebi A, Okello J.B.A., N.yanzi SA. (2010) Physico-chemical characteristics of sheabutter(V.paradoxa CF Gearth) oil from the shea Districts of Uganda. *Afrid.Food, Agric, nutria.and development at The Free Library by Farlex*

Olorede B.R., Longe O.G. (1999)Growth, nutrient retention, haematology and serum chemistry of pullet chicks fed shea butter cake in the humid tropics. *Arch. Zootechnol.*, 49: 441-444.

Onwuka G. I. (2006) Soaking, boiling and anti-nutritional factors in Pigeon pea (*Cajanuscajan*) and Cowpea (*Vignaunguiculata*). *J. Food Process. Preserv.*, 30: 616-630.

Orkorley E.L., Fofoe F.K., Nashiru S. (2005) Technology changes in shea butter production in Ghana- A case study of shea butter production in the Yendi District of Northern Ghana. *Journal of Agricultural Science* ISSN0855-0042.

Osawa, Keikakuroiso, Goto S, Shimizu A. (2000) Isolation of tannin-degrading lactobacilli from humans and fermented foods. *J.American Society for Microbiology. Applied and env. Microbio.* pp 3093-3097, vol.7.http:acm.org/cgi/content/full/66/7/3093/T12

Osei-Amaning E. (1993) Shea nut expeller cake utilization; integrated livestock shea nut farming experiment. Cocoa Research Institute, Ghana, Annual Report 1989/90:148.

Pausing S., Boly H., Lindberg J. E. and Ogle B. (2007)Evaluation of Traditional Sorghum (Sorghum bicolor) Beer Residue, Shea-Nut (*Vitellaria paradoxa*) Cake and

Cottonseed (Gossypium spp) Cake for Poultry in Burkina Faso: Availability and Amino Acid Digestibility. *International Journal of Poultry Science* 6 (9): 666-672.

Quattara, A.S., Traore S.A. and Garcia L.J. (1992) Characterization of Anaerovibrio burk-inabensis sp. Nov., a lactate fermenting bacterium isolated from rice field soils. *Int. J. Syst. Bacteriol*., 42:390-397

Ray G. (1994) Bioremediation and its application to Exxon Valdez oil spill in Alaska. *Ray's Environmental sci. Web Site*

Salunkhe D. K., Shavan J. K., Adsule R. N., Kadam S. S. (1992) World oil seeds. Chemistry, technology and utilization. Van Nostrand Reinhold, NY. pp 554

Sasaki E, Shimada T, Osawa R, Nishitani Y, Spring S, lang E. (2005) Isolation of Tannin degrading bacteria from feces of the Japanese large wood mouse, Apodemus species, feeding on tannin-rich acons. *Systemic and applied biology* 28(4) 358-365

Schlegel H.G. (1995) Degradation of Natural substances. In: General Microbiology, 7th.edition, Cambridge University Press. pp447-483

Schreckenberg, K. (1996) Forests, fields and markets: a study of indigenous tree products in the woody savannahs of the Bassila region, Benin. *PhD thesis*, University of London. pp 326

Shamir I. and Steinberger Y.(2007) Vertical distribution and activity of soil microbial population in a sandy desert ecosystem.*Microb. Ecol*., **53**:340-47

SDAPU (2013)The Profile of Sagnarigu District. Sagnarigu District Assembly Planning Unit.

Shukla A.K., Tiwan B.K., Mishra R. R. (1989) Temporal and depth-wise distribution of microbes, enzyme activities and soil respiration in potato field soil under different

agricultural systems in Northeastern Hill region of India. *Revue Ecologie et de Biologie du sol***26**:249-65.

Stanier R. Y., Doudoroff M., Adelberg E. (1963) Microbial Nutrition and Physiology in: The Microbial World. 2nded.

Stanier R. Y; Doudoroff M.; Adelberg E. (1986) Microbial Nutrition and Physiology in: The Microbial World. 5thed.

Tangjang S., Arunachalam K., Arunachalam A. and Shukla A.K. (2009) Microbial population dynamics of soil under traditional agroforestry systems in Northeast India. *Res. J. Soil Biol*. **1**:1

Thurston C.E. (1994)The structure and function of fungal Laccases. Microbiology 160:19-26

Todar K. (2012) Microbial Metabolism InTodas online Textbook of Bacteriology.Dept.Of bacteriology, University of Wisconsin, Madison.

Todar K., (2000) Nutrition and Growth of Bacteria, *Frontier on a Macintosh*, University of Wisconins-Madison.

Truong S. P. (2005) Hydrocarbon degrading bacteria- in the search for potential species.TIN TÚ`C Tin Tú`c/Tin nôibô/ Chi tiêt.

USDA (2007) Soil quality indicators.USDA Natural Resources Conservation Services Report.

US AIRFORCE (1977) Introduction to bacteriology.Headquarters US Air Force, Washington DC 20330

US AIRFORCE (1997):Clinical Laboratory Procedures-Chemistry and Urinalysis. Headquarters US Air Force, Washington DC 20330 PP 7-12

Vivien J. (1990) Fruitierssuavages du Cameroun.*Fruits***. 45**: 291-30

Wackett L. P., Ellis L. B. M. (1999) Predicting Biodegradation. *Environmental Microbiology* 1(2): 119-124

Walkley A. and Black I.A.(1934) An Examination of the Deggareff method for the determination of organic carbon in soils.Effect of variations in digestion conditions and of inorganic soil constituents.*Soil Sci*. **63**: 251-263.

Wallace-Bruce Y. (1995) Shea butter extraction in Ghana. In Do it Herself. Women and Technical innovation.H. Appleton(ed.) pp157-161 *Intermediate Technical Publication,* London 310pp

Whiting D., Card A., Wilson C. and Jean (2010) CMG Garden notes: http:Estimating Soil Texture Sand, Silt, Clay?*,//cmg.colostate.edu/gardennotes/214.pdf*

Whittaker R. H, 1969 New concepts of Kingdom of organisms.*Science.* 163:150-169.

Yidana J. A. (2004) Progress in Developing Technologies to Domesticate the Cultivation of Shea Tree (*Vitellariaparadoxa*) in Ghana.*Agricultural and Food Science Journal of Ghana* **3**:249.

Zwolinski J., Olszowski J., Olszowska G.and Zwolinska B. (1988) The effect of industrial dust from different emission sourceson the biological activity of soils. *ZeszytyNaukoweAkademilRolniczejim H. Kallataja W. Krakowie, Lesnictwo,* 18:105-25

APPENDIX

APPENDIX A

Detailed computer Analysis of Variance (ANOVA) output of soil moisture content.

GenStat Release 10.3DE (PC/Windows Vista) 04 December 2013 14:01:15

GenStat Discovery Edition 4

GenStat Procedure Library Release PL18.2

```
 1                          %CD                    'C:/Users/hb/Documents'
 2              "Data       taken       from       File:          \
-3   C:/Users/hb/Desktop/lecture  notes/Research/Francis_Danikuu_data/moisture.gsh\
-4                                                                          "
 5        DELETE    [REDEFINE=yes]    _stitle_:    TEXT    _stitle_
 6          READ    [PRINT=*;    SETNVALUES=yes]    _stitle_
 9           PRINT    [IPRINT=*]    _stitle_;    JUST=left
```

Data imported from Clipboard

on: 4-Dec-2013 14:01:49

```
10     DELETE  [REDEFINE=yes]  soiltype,location,month,depth,moisture,log_moisture
11                                    UNITS                    [NVALUES=*]
12              FACTOR    [MODIFY=yes;    NVALUES=324;    LEVELS=2;
LABELS=!t('Unpolluted','polluted')\
13                        ;              REFERENCE=1]              soiltype
14                READ      soiltype;      FREPRESENTATION=ordinal
```

Identifier	Values	Missing	Levels
soiltype	324	0	2

```
24              FACTOR    [MODIFY=yes;    NVALUES=324;    LEVELS=3;
LABELS=!t('GURUGU','JISONAYILLI',\
25                        'KASALGU');    REFERENCE=1]              location
26                READ      location;      FREPRESENTATION=ordinal
```

Identifier	Values	Missing	Levels
location	324	0	3

```
36  FACTOR [MODIFY=yes; NVALUES=324; LEVELS=6; LABELS=!t('Jan','Mar','May',\
37                        'Jul','Sep','Nov');    REFERENCE=1]              month
38                READ      month;      FREPRESENTATION=ordinal
```

Identifier	Values	Missing	Levels
month	324	0	6

```
48  FACTOR [MODIFY=yes; NVALUES=324; LEVELS=3; LABELS=!t('0-20','21-40','41-
60')\
 49                              ;              REFERENCE=1]          depth
 50                    READ          depth;          FREPRESENTATION=ordinal

      Identifier      Values     Missing      Levels
           depth         324           0           3

60                  VARIATE                [NVALUES=324]                 moisture
61                                         READ                          moisture

      Identifier     Minimum        Mean     Maximum      Values     Missing
        moisture      0.4700       9.485       46.09         324           0  Skew

86                        VARIATE              [NVALUES=324]          log_moisture
 87                                          READ                    log_moisture

      Identifier     Minimum        Mean     Maximum      Values     Missing
   log_moisture       1.672       2.609       3.664         324           0
```

```
169
170 "General Analysis of Variance."
171 BLOCK "No Blocking"
172 TREATMENTS month*(location/soiltype/depth)
173 COVARIATE "No Covariate"
174 ANOVA [PRINT=aovtable,information,means; FACT=32; CONTRASTS=7;
PCONTRASTS=7; FPROB=yes;\
175 PSE=diff,lsd; LSDLEVEL=5] log_moisture
```

Analysis of variance

Variate: log_moisture

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
month	5	3.372E+00	6.743E-01	7392.34	<.001
location	2	4.536E+00	2.268E+00	24862.57	<.001
month.location	10	1.008E+00	1.008E-01	1104.56	<.001
location.soiltype	3	1.160E+02	3.866E+01	4.238E+05	<.001
month.location.soiltype	15	1.920E+00	1.280E-01	1403.00	<.001
location.soiltype.depth	12	1.842E+00	1.535E-01	1683.14	<.001
month.location.soiltype.depth	60	1.257E+00	2.095E-02	229.63	<.001
Residual	216	1.970E-02	9.122E-05		
Total	323	1.299E+02			

Tables of means

Variable: log_moisture

Grand mean 2.60895

month	Jan	Mar	May	Jul	Sep	Nov
	2.53317	2.43892	2.59140	2.65189	2.69320	2.74510

location	GURUGU	JISONAYILLI	KASALGU
	2.47447	2.58994	2.76243

month	location	GURUGU	JISONAYILLI	KASALGU
Jan		2.34701	2.48683	2.76568
Mar		2.29591	2.47951	2.54134
May		2.37628	2.58623	2.81168
Jul		2.55118	2.56191	2.84259
Sep		2.63131	2.63999	2.80831
Nov		2.64516	2.78517	2.80496

location	soiltype	Unpolluted	polluted
GURUGU		1.86785	3.08110
JISONAYILLI		1.82779	3.35209
KASALGU		2.40868	3.11617

month	location	soiltype	Unpolluted	polluted
Jan	GURUGU		1.80135	2.89267
	JISONAYILLI		1.70862	3.26504
	KASALGU		2.41570	3.11567
Mar	GURUGU		1.83548	2.75634
	JISONAYILLI		1.81432	3.14470
	KASALGU		2.34977	2.73290
May	GURUGU		1.83408	2.91847
	JISONAYILLI		1.80949	3.36298
	KASALGU		2.43641	3.18695
Jul	GURUGU		1.86595	3.23641
	JISONAYILLI		1.75003	3.37378
	KASALGU		2.48716	3.19803
Sep	GURUGU		1.91167	3.35096
	JISONAYILLI		1.83405	3.44592
	KASALGU		2.37631	3.24031
Nov	GURUGU		1.95856	3.33176
	JISONAYILLI		2.05021	3.52013
	KASALGU		2.38673	3.22318

	Soiltype	Unpolluted			polluted		
location	depth	0-20	21-40	41-60	0-20	21-40	41-60
GURUGU		1.91667	1.85253	1.83435	3.15142	3.10157	2.99031
JISONAYILLI		1.86037	1.80473	1.81826	3.49886	3.37391	3.18351
KASALGU		2.27425	2.46654	2.48525	3.08762	3.17727	3.08362

month	location	soiltype	depth	0-20	21-40	41-60
Jan	GURUGU	Unpolluted		1.83462	1.80604	1.76338
		polluted		2.92737	2.91558	2.83505
	JISONAYILLI	Unpolluted		1.76313	1.68154	1.68118
		polluted		3.39789	3.27948	3.11776
	KASALGU	Unpolluted		2.36361	2.40488	2.47861
		polluted		3.04584	3.27997	3.02119
Mar	GURUGU	Unpolluted		1.79216	1.84507	1.86921
		polluted		2.82715	2.80072	2.64114
	JISONAYILLI	Unpolluted		1.77839	1.77811	1.88647
		polluted		3.23971	3.18893	3.00547
	KASALGU	Unpolluted		2.30970	2.35217	2.38745
		polluted		2.71184	2.73024	2.75663
May	GURUGU	Unpolluted		1.86312	1.83272	1.80640
		polluted		2.90633	2.93935	2.90973
	JISONAYILLI	Unpolluted		1.83229	1.80438	1.79179
		polluted		3.55469	3.38709	3.14716
	KASALGU	Unpolluted		2.41670	2.43616	2.45637
		polluted		3.17436	3.19700	3.18949

Jul	GURUGU	Unpolluted	1.95899	1.83272	1.80614
		polluted	3.35031	3.29884	3.06007
	JISONAYILLI	Unpolluted	1.80347	1.76539	1.68124
		polluted	3.56804	3.39777	3.15553
	KASALGU	Unpolluted	2.48435	2.48996	2.48718
		polluted	3.18213	3.18949	3.22246
Sep	GURUGU	Unpolluted	1.96836	1.86921	1.89744
		polluted	3.39800	3.35602	3.29885
	JISONAYILLI	Unpolluted	1.77811	1.79463	1.92940
		polluted	3.57019	3.46284	3.30474
	KASALGU	Unpolluted	1.99549	2.55141	2.58203
		polluted	3.22943	3.27990	3.21161
Nov	GURUGU	Unpolluted	2.08278	1.92940	1.86352
		polluted	3.49937	3.29892	3.19700
	JISONAYILLI	Unpolluted	2.20682	2.00431	1.93950
		polluted	3.66263	3.52737	3.37039
	KASALGU	Unpolluted	2.07567	2.56466	2.51987
		polluted	3.18214	3.38703	3.10037

Standard errors of differences of means

Table	month	location	month location	location soiltype
rep.	54	108	18	54
d.f.	216	216	216	216
s.e.d.	0.001838	0.001300	0.003184	0.001838

Table	month location soiltype	location soiltype depth	month location soiltype depth
rep.	9	18	3
d.f.	216	216	216
s.e.d.	0.004502	0.003184	0.007798

Least significant differences of means (5% level)

Table	month	location	month location	location soiltype
rep.	54	108	18	54
d.f.	216	216	216	216
l.s.d.	0.003623	0.002562	0.006275	0.003623

Table	month location soiltype	location soiltype depth	month location soiltype depth
rep.	9	18	3
d.f.	216	216	216
l.s.d.	0.008874	0.006275	0.015371

APPENDIX B: Analysis Of Variance (ANOVA) Output Of Soil Bacteria Population

GenStat Release 10.3DE (PC/Windows Vista) 04 December 2013 13:47:40

GenStat Discovery Edition 4

GenStat Procedure Library Release PL18.2

```
1                    %CD                    'C:/Users/hb/Documents'
 2        "Data    taken    from    unsaved    spreadsheet:    New    Data;1"
 3          DELETE    [REDEFINE=yes]    _stitle_:    TEXT    _stitle_
 4            READ    [PRINT=*;    SETNVALUES=yes]    _stitle_
 7             PRINT    [IPRINT=*]    _stitle_;    JUST=left
```

Data imported from Clipboard

on: 4-Dec-2013 13:48:18

```
 8      DELETE  [REDEFINE=yes]  soiltype,location,depth,month,bacteria,bacteria_,\
 9                                                                  bacterial_log
10                                          UNITS                  [NVALUES=*]
11          FACTOR  [MODIFY=yes;  NVALUES=324;  LEVELS=2;
LABELS=!t('Control','polluted')\
12                          ;           REFERENCE=1]               soiltype
13              READ        soiltype;       FREPRESENTATION=ordinal


      Identifier    Values    Missing    Levels
       soiltype     324       0          2
23          FACTOR  [MODIFY=yes;  NVALUES=324;  LEVELS=3;
LABELS=!t('GURUGU','JISONAYILLI',\
24                      'KASALGU');         REFERENCE=1]           location
25              READ        location;       FREPRESENTATION=ordinal


      Identifier    Values    Missing    Levels
       location     324       0          3
35  FACTOR [MODIFY=yes; NVALUES=324; LEVELS=3; LABELS=!t('0-20','21-40','41-
60')\
36                          ;           REFERENCE=1]               depth
37              READ        depth;          FREPRESENTATION=ordinal


      Identifier    Values    Missing    Levels
        depth       324       0          3
```

```
47  FACTOR [MODIFY=yes; NVALUES=324; LEVELS=6; LABELS=!t('Jan','Mar','May',\
48                  'Jul','Sep','Nov');          REFERENCE=1]          month
49                  READ          month;          FREPRESENTATION=ordinal

          Identifier      Values     Missing      Levels
               month         324           0           6

59                  VARIATE              [NVALUES=324]              bacteria
60                                       READ                       bacteria

          Identifier     Minimum        Mean     Maximum      Values     Missing
            bacteria     0.02900       1.661       13.04         324           0  Skew

88                  VARIATE              [NVALUES=324]              bacteria_
89                                       READ                       bacteria_

          Identifier     Minimum        Mean     Maximum      Values     Missing
            bacteria_      290000    16607429   1.304E+08         324           0  Skew

125                 VARIATE              [NVALUES=324]              bacterial_log
126                                      READ                       bacterial_log

          Identifier     Minimum        Mean     Maximum      Values     Missing
       bacterial_log       5.462       6.516       8.115         324           0
```

```
208
209 "General Analysis of Variance."
210 BLOCK "No Blocking"
211 TREATMENTS month*(location/soiltype/depth)
212 COVARIATE "No Covariate"
213 ANOVA [PRINT=aovtable,information,means; FACT=32; CONTRASTS=7;
PCONTRASTS=7; FPROB=yes;\
214 PSE=diff,lsd; LSDLEVEL=5] bacterial_log
```

Analysis of variance

Variable: bacterial_log

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
month	5	1.287E+00	2.574E-01	5825.90	<.001
location	2	2.268E+01	1.134E+01	2.567E+05	<.001
month.location	10	2.642E-02	2.642E-03	59.80	<.001
location.soiltype	3	1.148E+01	3.826E+00	86588.70	<.001
month.location.soiltype	15	1.183E-01	7.886E-03	178.50	<.001
location.soiltype.depth	12	1.895E+02	1.579E+01	3.574E+05	<.001
month.location.soiltype.depth	60	3.917E-01	6.529E-03	147.77	<.001
Residual	216	9.543E-03	4.418E-05		
Total	323	2.255E+02			

Message: the following units have large residuals.

units 90	0.01768	s.e.	0.00543
units 91	0.02952	s.e.	0.00543
units 92	-0.02251	s.e.	0.00543

units 275	0.02482	s.e.	0.00543
units 276	-0.02078	s.e.	0.00543
units 316	-0.04096	s.e.	0.00543
units 318	0.05295	s.e.	0.00543

Tables of means

Variable: bacterial_log

Grand mean 6.51575

month	Jan	Mar	May	Jul	Sep	Nov
	6.46554	6.42462	6.48865	6.53055	6.58140	6.60375

location	GURUGU	JISONAYILLI	KASALGU
	6.70230	6.70337	6.14158

month	location	GURUGU	JISONAYILLI	KASALGU
Jan		6.64905	6.65456	6.09300
Mar		6.58848	6.61494	6.07043
May		6.68618	6.66906	6.11072
Jul		6.72296	6.71295	6.15574
Sep		6.77659	6.77256	6.19505
Nov		6.79056	6.79617	6.22452

location	**soil type**	**Control**	**polluted**
GURUGU		6.56792	6.83669
JISONAYILLI		6.58698	6.81977
KASALGU		5.86834	6.41482

month	**location**	**soiltype**	**Control**	**polluted**
Jan	GURUGU		6.52378	6.77433
	JISONAYILLI		6.54520	6.76393

	KASALGU	5.83078	6.35523	
Mar	GURUGU	6.48839	6.68857	
	JISONAYILLI	6.50716	6.72272	
	KASALGU	5.80540	6.33545	
May	GURUGU	6.53693	6.83543	
	JISONAYILLI	6.55316	6.78496	
	KASALGU	5.84027	6.38117	
Jul	GURUGU	6.58290	6.86302	
	JISONAYILLI	6.60545	6.82044	
	KASALGU	5.87913	6.43235	
Sep	GURUGU	6.59994	6.90557	
	JISONAYILLI	6.61960	6.90106	
	KASALGU	5.89196	6.48657	
Nov	GURUGU	6.67556	6.95325	
	JISONAYILLI	6.69129	6.92552	
	KASALGU	5.96247	6.49814	

	soiltype	Control			polluted		
location	depth	0-20	21-40	41-60	0-20	21-40	41-60
GURUGU		7.60198	6.57494	5.52683	7.90607	6.79878	5.80523
JISONAYILLI		7.62879	6.58233	5.54981	7.93555	6.78397	5.73979
KASALGU		6.56140	5.54105	5.50256	6.85582	6.73157	5.65708

month	location	soiltype	depth	0-20	21-40	41-60
Jan	GURUGU	Control		7.52891	6.55388	5.48854
		polluted		7.79518	6.76492	5.76288
	JISONAYILLI	Control		7.55388	6.56584	5.51587

		polluted	7.86707	6.73718	5.68752
	KASALGU	Control	6.46684	5.53275	5.49276
		polluted	6.76715	6.65706	5.64147
Mar	GURUGU	Control	7.50242	6.48855	5.47420
		polluted	7.70415	6.68124	5.68033
	JISONAYILLI	Control	7.54158	6.48995	5.48996
		polluted	7.75638	6.73024	5.68154
	KASALGU	Control	6.46794	5.46686	5.48141
		polluted	6.71767	6.65928	5.62941
May	GURUGU	Control	7.54157	6.54033	5.52891
		polluted	7.85722	6.81976	5.82930
	JISONAYILLI	Control	7.57633	6.54158	5.54158
		polluted	7.87699	6.75663	5.72126
	KASALGU	Control	6.50785	5.50650	5.50647
		polluted	6.82866	6.67240	5.64246
Jul	GURUGU	Control	7.60959	6.59878	5.54032
		polluted	7.90327	6.83884	5.84694
	JISONAYILLI	Control	7.63043	6.60959	5.57634
		polluted	7.93146	6.77476	5.75510
	KASALGU	Control	6.56702	5.56702	5.50335
		polluted	6.86915	6.77085	5.65705
Sep	GURUGU	Control	7.64836	6.59879	5.55266
		polluted	8.07041	6.82478	5.82151
	JISONAYILLI	Control	7.67394	6.60852	5.57633
		polluted	8.06670	6.84385	5.79262

	KASALGU	Control	6.60745	5.56466	5.50376
		polluted	6.96864	6.81622	5.67486
Nov	GURUGU	Control	7.78104	6.66931	5.57632
		polluted	8.10619	6.86313	5.89042
	JISONAYILLI	Control	7.79657	6.67851	5.59878
		polluted	8.11472	6.86113	5.80072
	KASALGU	Control	6.75128	5.60851	5.52762
		polluted	6.98363	6.81358	5.69723

Standard errors of differences of means

Table	month	location	month location	location soiltype
rep.	54	108	18	54
d.f.	216	216	216	216
s.e.d.	0.001279	0.000905	0.002216	0.001279

Table	month location soiltype	location soiltype depth	month location soiltype depth
rep.	9	18	3
d.f.	216	216	216
s.e.d.	0.003133	0.002216	0.005427

Least significant differences of means (5% level)

Table	month	location	month location	location soiltype
rep.	54	108	18	54

d.f.	216	216	216	216
l.s.d.	0.002521	0.001783	0.004367	0.002521
Table	month	location	month	
	location	soiltype	location	
	soiltype	depth	soiltype	
			depth	
rep.	9	18	3	
d.f.	216	216	216	
l.s.d.	0.006176	0.004367	0.010697	

APPENDIX C: Analysis Of Variance (ANOVA) Output Of Soil Fungi Population

GenStat Release 10.3DE (PC/Windows Vista) 04 December 2013 13:53:30

GenStat Discovery Edition 4

GenStat Procedure Library Release PL18.2

```
1                    %CD                    'C:/Users/hb/Documents'
 2    "Data taken from unsaved spreadsheet: New Data;1"
 3    DELETE [REDEFINE=yes] _stitle_: TEXT _stitle_
 4    READ [PRINT=*; SETNVALUES=yes] _stitle_
 7    PRINT [IPRINT=*] _stitle_; JUST=left
```

Data imported from Clipboard

on: 4-Dec-2013 13:53:59

```
 8      DELETE  [REDEFINE=yes]  soiltype,location,depth,month,fungi,fungi_,fungi_log
 9                                        UNITS                    [NVALUES=*]
 10              FACTOR      [MODIFY=yes;      NVALUES=324;      LEVELS=2;
LABELS=!t('Unpolluted','polluted')\
 11                          ;                 REFERENCE=1]              soiltype
 12                  READ          soiltype;          FREPRESENTATION=ordinal

        Identifier      Values      Missing      Levels
         soiltype       324           0           2

22              FACTOR      [MODIFY=yes;      NVALUES=324;      LEVELS=3;
LABELS=!t('GURUGU','JISONAYILLI',\
 23                       'KASALGU');          REFERENCE=1]              location
 24                  READ          location;          FREPRESENTATION=ordinal

        Identifier      Values      Missing      Levels
         location       324           0           3
34  FACTOR [MODIFY=yes; NVALUES=324; LEVELS=3; LABELS=!t('0-20','21-40','41-
60')\
 35                          ;                 REFERENCE=1]              depth
 36                  READ          depth;          FREPRESENTATION=ordinal

        Identifier      Values      Missing      Levels
          depth         324           0           3
```


```
46  FACTOR [MODIFY=yes; NVALUES=324; LEVELS=6; LABELS=!t('Jan','Mar','May',\
47                  'Jul','Sep','Nov');          REFERENCE=1]          month
48                  READ          month;          FREPRESENTATION=ordinal
```

Identifier	Values	Missing	Levels
month	324	0	6

```
58                  VARIATE              [NVALUES=324]              fungi
59                                       READ                       fungi
```

Identifier	Minimum	Mean	Maximum	Values	Missing
fungi	0.2370	0.3425	0.5980	324	0

```
86                  VARIATE              [NVALUES=324]              fungi_
87                                       READ                       fungi_
```

Identifier	Minimum	Mean	Maximum	Values	Missing
fungi_	2370	3425	5980	324	0

```
110                 VARIATE              [NVALUES=324]              fungi_log
111                                      READ                       fungi_log
```

Identifier	Minimum	Mean	Maximum	Values	Missing
fungi_log	3.375	3.517	3.777	324	0

193

194 "General Analysis of Variance."

195 BLOCK "No Blocking"

196 TREATMENTS month*(location/soiltype/depth)

197 COVARIATE "No Covariate"

198 ANOVA [PRINT=aovtable,information,means; FACT=32; CONTRASTS=7; PCONTRASTS=7; FPROB=yes;\

199 PSE=diff,lsd; LSDLEVEL=5] fungi_log

Analysis of variance

Variable: fungi_log

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
month	5	0.18588263	0.03717653	889.26	<.001
location	2	0.14056310	0.07028155	1681.14	<.001
month.location	10	0.01723321	0.00172332	41.22	<.001
location.soiltype	3	3.52051919	1.17350640	28070.35	<.001
month.location.soiltype	15	0.03830083	0.00255339	61.08	<.001
location.soiltype.depth	12	0.84813138	0.07067761	1690.61	<.001
month.location.soiltype.depth	60	0.09208039	0.00153467	36.71	<.001
Residual	216	0.00903008	0.00004181		
Total	323	4.85174080			

Message: the following units have large residuals.

units 208	0.02330	s.e.	0.00528
units 210	-0.01857	s.e.	0.00528
units 304	-0.03034	s.e.	0.00528
units 306	0.02965	s.e.	0.00528

Tables of means

Variable: fungi_log

Grand mean 3.51653

month	Jan	Mar	May	Jul	Sep	Nov
	3.49960	3.47965	3.50911	3.52319	3.53369	3.55397

location	GURUGU	JISONAYILLI	KASALGU
	3.53885	3.48873	3.52202

month	location	GURUGU	JISONAYILLI	KASALGU
Jan		3.52165	3.46278	3.51435
Mar		3.50315	3.43723	3.49858
May		3.53187	3.48233	3.51312
Jul		3.54976	3.49993	3.51988
Sep		3.55207	3.51351	3.53548
Nov		3.57462	3.53658	3.55071

location	soiltype	Unpolluted	polluted
GURUGU		3.41995	3.65776
JISONAYILLI		3.41879	3.55866
KASALGU		3.40554	3.63850

month	location	soiltype	Unpolluted	polluted
Jan	GURUGU		3.40349	3.63981
	JISONAYILLI		3.40754	3.51802
	KASALGU		3.39259	3.63612
Mar	GURUGU		3.39344	3.61285
	JISONAYILLI		3.39633	3.47813
	KASALGU		3.38854	3.60862

May	GURUGU		3.41425	3.64948
	JISONAYILLI		3.41229	3.55236
	KASALGU		3.40286	3.62338
Jul	GURUGU		3.41974	3.67978
	JISONAYILLI		3.41696	3.58290
	KASALGU		3.40734	3.63242
Sep	GURUGU		3.43706	3.66709
	JISONAYILLI		3.42808	3.59895
	KASALGU		3.41262	3.65835
Nov	GURUGU		3.45171	3.69752
	JISONAYILLI		3.45153	3.62162
	KASALGU		3.42929	3.67213

soiltype	Unpolluted			polluted		
location	depth	0-20	21-40	41-60	0-20	21-40	41-60
GURUGU		3.43939	3.41948	3.40097	3.72104	3.62389	3.62834
JISONAYILLI		3.43007	3.41973	3.40656	3.63692	3.57335	3.46572
KASALGU		3.40040	3.42851	3.38771	3.61140	3.75960	3.54451

month	location	soiltype	depth	0-20	21-40	41-60
Jan	GURUGU	Unpolluted		3.41154	3.41158	3.38734
		polluted		3.72591	3.57864	3.61490
	JISONAYILLI	Unpolluted		3.41323	3.41322	3.39618
		polluted		3.57749	3.52634	3.45024
	KASALGU	Unpolluted		3.38736	3.40482	3.38559
		polluted		3.58771	3.75128	3.56937
Mar	GURUGU	Unpolluted		3.39440	3.39618	3.38974

		polluted	3.70415	3.53020	3.60422
	JISONAYILLI	Unpolluted	3.39615	3.39613	3.39670
		polluted	3.53147	3.46834	3.43456
	KASALGUU	Unpolluted	3.38910	3.38915	3.38736
		polluted	3.55750	3.73560	3.53275
May	GURUGUU	Unpolluted	3.42810	3.41156	3.40310
		polluted	3.68395	3.64542	3.61908
	JISONAYILLI	Unpolluted	3.42864	3.39673	3.41151
		polluted	3.63749	3.55871	3.46089
	KASALGUU	Unpolluted	3.40308	3.41992	3.38559
		polluted	3.58994	3.76567	3.51454
Jul	GURUGUU	Unpolluted	3.44398	3.41214	3.40309
		polluted	3.73878	3.67117	3.62940
	JISONAYILLI	Unpolluted	3.42807	3.41154	3.41127
		polluted	3.66276	3.61172	3.47421
	KASALGUU	Unpolluted	3.40136	3.43456	3.38611
		polluted	3.60637	3.76715	3.52374
Sep	GURUGUU	Unpolluted	3.47273	3.44402	3.39442
		polluted	3.72916	3.65896	3.61314
	JISONAYILLI	Unpolluted	3.44456	3.42809	3.41159
		polluted	3.69548	3.63749	3.46388
	KASALGUU	Unpolluted	3.39501	3.46089	3.38196
		polluted	3.64835	3.77524	3.55145
Nov	GURUGUU	Unpolluted	3.48562	3.44139	3.42812
		polluted	3.74429	3.65896	3.68931

JISONAYILLI	Unpolluted	3.46979	3.47270	3.41211
	polluted	3.71683	3.63749	3.51054
KASALGU	Unpolluted	3.42650	3.46171	3.39966
	polluted	3.67852	3.76268	3.57518

Standard errors of differences of means

Table	month	location	month location	location soiltype
rep.	54	108	18	54
d.f.	216	216	216	216
s.e.d.	0.001244	0.000880	0.002155	0.001244

Table	month location soiltype	location soiltype depth	month location soiltype depth	
rep.	9	18	3	
d.f.	216	216	216	
s.e.d.	0.003048	0.002155	0.005279	

Least significant differences of means (5% level)

Table	month	location	month location	location soiltype
rep.	54	108	18	54
d.f.	216	216	216	216
l.s.d.	0.002453	0.001734	0.004248	0.002453

Table	month location	location soiltype	month location	

	soiltype	depth	soiltype depth
rep.	9	18	3
d.f.	216	216	216
l.s.d.	0.006008	0.004248	0.010405

APPENDIX D

Detailed computer Analysis of Variance (ANOVA) output of soil pH.

GenStat Discovery (PC/Windows Vista) 04 December 2015 VSN International Ltd. (Rothamsted Experimental Station).

Summary of ANOVA: Tests of Between-Subjects Effects (Soil pH).

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	207.846a	87	2.389	8714.462	.000
Intercept	3738.113	1	3738.113	1.364E7	.000
Sample	97.533	1	97.533	355771.419	.000
location	48.221	2	24.111	87948.280	.000
Date	.016	5	.003	11.922	.000
Depth	1.586	2	.793	2893.232	.000
Sample * location	56.076	2	28.038	102274.413	.000
Sample * Date	.004	5	.001	2.675	.052
Sample * Depth	1.236	2	.618	2253.418	.000
location * Date	.008	10	.001	3.085	.015
location * Depth	1.894	4	.474	1727.461	.000
Sample * Date * Depth	.003	10	.000	1.114	.399
location * Date * Depth	.009	20	.000	1.653	.135
Sample * location * Date	.020	10	.002	7.326	.000
Sample * location * Depth	1.233	4	.308	1124.540	.000
Error	.005	20	.000		
Total	3945.965	108			
Corrected Total	207.852	107			

R Squared = 1.000 (Adjusted R Squared = 1.000)

Between-Subjects Factors

		N
Sample	Unpolluted	54
	Polluted	54
location	Guru	36
	Juso	36
	kasal	36
Date	Jan. 2011	18
	Jul. 2011	18
	Mar. 2011	18
	May. 2011	18
	Nov. 2010	18
	Sept. 2010	18
Depth	0-20 cm	36
	21-40 cm	36
	41-60 cm	36

Estimated Marginal Means

1. Sample

Dependent Variable: SOIL pH

Sample	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Unpolluted	4.933	0.002	4.928	4.938
Polluted	6.834	0.002	6.829	6.838

2. location

Dependent Variable :SOIL pH

location	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Gurugu	5.131	.003	5.125	5.136
Jisonayilli	6.754	.003	6.749	6.760
kasalgu	5.765	.003	5.759	5.770

3. Date

Dependent Variable: SOIL pH

Date	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Sept. 2010	5.886	0.004	5.877	5.894
Nov. 2010	5.898	0.004	5.89	5.906
Jan. 2011	5.885	0.004	5.877	5.893
Mar. 2011	5.874	0.004	5.866	5.882
May. 2011	5.862	0.004	5.854	5.87
Jul. 2011	5.894	0.004	5.886	5.903

4. Depth

Dependent Variable:SOIL pH

Depth	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
0-20 cm	6.009	.003	6.003	6.015
21-40 cm	5.921	.003	5.916	5.927
41-60 cm	5.719	.003	5.714	5.725

5. Sample * location

Dependent Variable:SOIL pH

Sample	Location	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Unpolluted	Gurugu	4.01	0.004	4.001	4.018
	Jisonayilli	5.019	0.004	5.011	5.028
	kasagul	5.77	0.004	5.761	5.778
Polluted	Gurugu	6.252	0.004	6.244	6.26
	Jisonayilli	8.489	0.004	8.481	8.498
	kasagul	5.759	0.004	5.751	5.768

6. Date * Sample

Dependent Variable :SOIL pH

Date	Sample	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Sept. 2010	Unpolluted	4.941	0.006	4.93	4.953
	Polluted	6.83	0.006	6.818	6.842
Nov. 2010	Unpolluted	4.939	0.006	4.927	4.95
	Polluted	6.858	0.006	6.846	6.869
Jan. 2011	Unpolluted	4.931	0.006	4.92	4.943
	Polluted	6.839	0.006	6.827	6.85
Mar. 2011	Unpolluted	4.925	0.006	4.914	4.937
	Polluted	6.823	0.006	6.812	6.835
May. 2011	Unpolluted	4.909	0.006	4.897	4.92
	Polluted	6.814	0.006	6.803	6.826
Jul. 2011	Unpolluted	4.952	0.006	4.941	4.964
	Polluted	6.837	0.006	6.825	6.848

7 Sample*Depth

Dependent Variable: SOIL pH

epth	Sample	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
)-20 cm	Unpolluted	4.913	.004	4.905	4.921
	Polluted	7.104	.004	7.096	7.113
21-40 cm	Unpolluted	5.007	.004	4.999	5.015
	Polluted	6.836	.004	6.827	6.844
41-60 cm	Unpolluted	4.878	.004	4.870	4.886
	Polluted	6.561	.004	6.552	6.569

8. Date * location

Dependent Variable:Soil pH

Date	Location	Mean	Std Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Sept. 2010	Gurugu	5.132	0.007	5.118	5.146
	Jisonayilli	6.755	0.007	6.741	6.769
	kasalgu	5.769	0.007	5.755	5.784
Nov. 2010	Gurugu	5.135	0.007	5.121	5.149
	Jisonayilli	6.777	0.007	6.763	6.791
	kasalgu	5.783	0.007	5.769	5.797
Jan. 2011	Gurugu	5.135	0.007	5.121	5.149
	Jisonayilli	6.755	0.007	6.741	6.769
	kasalgu	5.765	0.007	5.751	5.779
Mar. 2011	Gurugu	5.132	0.007	5.118	5.146
	Jisonayilli	6.742	0.007	6.728	6.756
	kasalgu	5.749	0.007	5.735	5.764
May. 2011	Gurugu	5.125	0.007	5.111	5.139
	Jisonayilli	6.717	0.007	6.703	6.731
	kasalgu	5.743	0.007	5.729	5.757
Jul. 2011	Gurugu	5.125	0.007	5.111	5.139
	Jisonayilli	6.782	0.007	6.768	6.796
	kasalgu	5.777	0.007	5.763	5.791

9. Depth * location

Dependent Variable: soil pH

Soil Depth	Location	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
0-20 cm	Gurugu	5.372	0.005	5.362	5.382
	Jisonayilli	7.002	0.005	6.992	7.012
	kasalgu	5.653	0.005	5.643	5.663
21-40 cm	Gurugu	5.017	0.005	5.007	5.027
	Jisonayilli	6.744	0.005	6.734	6.754
	kasalgu	6.003	0.005	5.993	6.013
41-60 cm	Gurugu	5.004	0.005	4.994	5.014
	Jisonayilli	6.518	0.005	6.508	6.527
	0kasalgu	5.637	0.005	5.627	5.647

APPENDIX E

Computer Cmputer Analysis of Varience (ANOVA) output of soil organic carbon.

Summary of ANOVA of soil Organic carbon

Tests of Between-Subjects Effects

Dependent Variable: Org. Carbon %

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	87.691[a]	87	1.008	1546.383	.000
Intercept	189.042	1	189.042	290026.205	.000
Sample	14.741	1	14.741	22614.885	.000
location	13.685	2	6.842	10497.635	.000
Date	.011	5	.002	3.242	.026
Depth	28.225	2	14.113	21651.493	.000
Sample * location	5.035	2	2.517	3862.247	.000
Sample * Date	.011	5	.002	3.254	.026
Sample * Depth	6.573	2	3.286	5041.920	.000
location * Date	.014	10	.001	2.164	.068
location * Depth	13.010	4	3.253	4989.984	.000
Date * Depth	.006	10	.001	.860	.582
Sample * Date * Depth	.006	10	.001	.960	.505
location * Date * Depth	.011	20	.001	.827	.663
Sample * location * Date	.012	10	.001	1.898	.107
Sample * location * Depth	6.353	4	1.588	2436.522	.000
Error	.013	20	.001		
Total	276.746	108			
Corrected Total	87.704	107			

c. R Squared = 1.000 (Adjusted R Squared = .999)
d. N = 324 Samples

Estimated Marginal Means.

1. Sample

Dependent Variable: Organic Carbon (Mean of %)

Sample	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Unpolluted	0.954	0.003	0.946	0.961
Polluted	1.692	0.003	1.685	1.7

2. location

Dependent Variable: Organic Carbon (Mean of %)

location	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Gurugu	1.790	.004	1.781	1.798
Jisonayilli	1.254	.004	1.245	1.262
kasalgu	.926	.004	.917	.935

3. Date

Dependent Variable: Organic Carbon (Mean of %)

Date	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Sept. 2010	1.33	0.006	1.317	1.342
Nov. 2010	1.335	0.006	1.323	1.348
Jan. 2011	1.327	0.006	1.315	1.34
Mar. 2011	1.32	0.006	1.308	1.333
May. 2011	1.322	0.006	1.31	1.335
Jul. 2011	1.304	0.006	1.291	1.316

4. Depth

Dependent Variable: Organic Carbon (Mean of %)

Depth	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
0-20 cm	1.850	.004	1.841	1.859
21-40 cm	1.489	.004	1.480	1.497
41-60 cm	.631	.004	.622	.640

5. Sample * location

Dependent Variable: Organic Carbon (Mean of %)

Sample	Location	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Unpolluted	Gurugu	1.463	0.006	1.45	1.475
	Jisonayilli	0.601	0.006	0.588	0.613
	Kasalgu	0.797	0.006	0.784	0.81
Polluted	Gurugu	2.116	0.006	2.104	2.129
	Jisonayilli	1.906	0.006	1.894	1.919
	Kasalgu	1.055	0.006	1.042	1.067

6. Date * Sample

Dependent Variable: Organic Carbon (Mean of %)

Date	Sample	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Sept. 2010	Unpolluted	0.954	0.009	0.936	0.971
	Polluted	1.706	0.009	1.688	1.723
Nov. 2010	Unpolluted	0.954	0.009	0.936	0.972
	Polluted	1.716	0.009	1.698	1.734
Jan. 2011	Unpolluted	0.954	0.009	0.936	0.972
	Polluted	1.701	0.009	1.683	1.719
Mar. 2011	Unpolluted	0.952	0.009	0.935	0.97
	Polluted	1.688	0.009	1.67	1.706
May. 2011	Unpolluted	0.953	0.009	0.935	0.971
	Polluted	1.691	0.009	1.673	1.709
Jul. 2011	Unpolluted	0.954	0.009	0.936	0.972
	Polluted	1.653	0.009	1.636	1.671

7. Depth * Sample

Dependent Variable: Organic Carbon (Mean of %)

Depth	Sample	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
0-20 cm	Unpolluted	1.197	0.006	1.185	1.21
	Polluted	2.502	0.006	2.49	2.515
21-40 cm	Unpolluted	1.084	0.006	1.071	1.097
	Polluted	1.893	0.006	1.881	1.906
41-60 cm	Unpolluted	0.579	0.006	0.567	0.592
	Polluted	0.682	0.006	0.67	0.695

8. Date * location

Dependent Variable: Organic Carbon (Mean of %)

Date		Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Sept. 2010	Gurugu	1.805	0.01	1.783	1.827
	Jisonayilli	1.256	0.01	1.234	1.277
	kasalgu	0.928	0.01	0.906	0.95
Nov. 2010	Gurugu	1.814	0.01	1.792	1.836
	Jisonayilli	1.259	0.01	1.237	1.28
	kasalgu	0.933	0.01	0.911	0.954
Jan. 2011	Gurugu	1.81	0.01	1.788	1.832
	Jisonayilli	1.25	0.01	1.228	1.271
	kasalgu	0.923	0.01	0.901	0.945
Mar. 2011	Gurugu	1.785	0.01	1.763	1.807
	Jisonayilli	1.249	0.01	1.227	1.271
	kasalgu	0.926	0.01	0.905	0.948
May. 2011	Gurugu	1.783	0.01	1.762	1.805
	Jisonayilli	1.264	0.01	1.242	1.285
	kasalgu	0.919	0.01	0.898	0.941
Jul. 2011	Gurugu	1.74	0.01	1.718	1.762
	Jisonayilli	1.245	0.01	1.223	1.267
	kasalgu	0.926	0.01	0.905	0.948

9. Depth * location

Dependent Variable: Organic Carbon (Mean of %)

Depth	Location	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
0-20 cm	Gurugu	2.519	0.007	2.504	2.535
	Jisonayilli	2.253	0.007	2.237	2.268
	kasalgu	0.777	0.007	0.762	0.793
21-40 cm	Gurugu	1.84	0.007	1.825	1.855
	Jisonayilli	1.161	0.007	1.145	1.176
	kasalgu	1.465	0.007	1.449	1.48
41-60 cm	Gurugu	1.009	0.007	0.994	1.025
	Jisonayilli	0.347	0.007	0.332	0.363
	kasalgu	0.536	0.007	0.52	0.551

APPENDIX F: ANOVA Output Of Soil Nitrogen (%). Tests Of Between-Subjects Factors

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.696[a]	87	.008	2088.967	.000
ntercept	1.280	1	1.280	334129.878	.000
Sample	.088	1	.088	22938.126	.000
ocation	.159	2	.080	20784.320	.000
Date	6.238E-5	5	1.248E-5	3.256	.026
Depth	.203	2	.102	26542.528	.000
Sample * location	.024	2	.012	3101.356	.000
Sample * Date	9.053E-5	5	1.811E-5	4.725	.005
Sample * Depth	.064	2	.032	8356.222	.000
ocation * Date	9.540E-5	10	9.540E-6	2.490	.040
ocation * Depth	.095	4	.024	6225.240	.000
Date * Depth	4.320E-5	10	4.320E-6	1.128	.390
Sample * Date * Depth	2.654E-5	10	2.654E-6	.693	.720
ocation * Date * Depth	5.960E-5	20	2.980E-6	.778	.710
Sample * location * Date	7.466E-5	10	7.466E-6	1.948	.098
Sample * location * Depth	.062	4	.016	4053.545	.000
Error	7.664E-5	20	3.832E-6		
Total	1.977	108			
Corrected Total	.696	107			

a. R Squared = 1.000 (Adjusted R Squared = .999)

Estimated Marginal Means: Dependent Variable: Nitrogen

1. Sample

Dependent Variable: Nitrogen (Mean of %)

Sample	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Unpolluted	0.08	0.000	0.08	0.081
Polluted	0.137	0.000	0.137	0.138

2. location

Dependent Variable: Nitrogen (Mean of %)

Location	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Gurugu	.162	.000	.161	.163
Jisonayilli	.093	.000	.092	.094
kasalgu	.072	.000	.071	.073

3. Date

Dependent Variable: Nitrogen (Mean of %)

Date	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Sept. 2010	0.11	0	0.109	0.111
Nov. 2010	0.109	0	0.108	0.11
Jan. 2011	0.11	0	0.109	0.111
Mar. 2011	0.109	0	0.108	0.11
May. 2011	0.108	0	0.107	0.109
Jul. 2011	0.108	0	0.107	0.109

4. Depth

Dependent Variable: Nitrogen (Mean of %)

Depth	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
0-20 cm	.163	.000	.162	.163
21-40 cm	.108	.000	.107	.108
41-60 cm	.056	.000	.056	.057

5. Sample * location

Dependent Variable: Nitrogen (Mean of %)

Sample	Location	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Unpolluted	Gurugu	0.127	0	0.126	0.128
	Jisonayilli	0.05	0	0.049	0.051
	kasalgu	0.064	0	0.063	0.065
Polluted	Gurugu	0.197	0	0.196	0.198
	Jisonayilli	0.136	0	0.135	0.137
	kasalgu	0.08	0	0.079	0.081

6. Date * Sample

Dependent Variable: Nitrogen (Mean of %)

Date	Sample	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
dimension2	Unpolluted	0.08	0.001	0.079	0.082
	Polluted	0.139	0.001	0.138	0.14
Nov. 2010	Unpolluted	0.08	0.001	0.079	0.081
	Polluted	0.139	0.001	0.138	0.14
Jan. 2011	Unpolluted	0.081	0.001	0.079	0.082
	Polluted	0.139	0.001	0.138	0.14
Mar. 2011	Unpolluted	0.081	0.001	0.079	0.082
	Polluted	0.137	0.001	0.135	0.138
May. 2011	Unpolluted	0.08	0.001	0.079	0.081
	Polluted	0.137	0.001	0.135	0.138
Jul. 2011	Unpolluted	0.081	0.001	0.079	0.082
	Polluted	0.134	0.001	0.133	0.136

7. Depth * Sample

Dependent Variable:Nitrogen (Mean of %)

Depth	Sample	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
0-20 cm	Unpolluted	0.1	0	0.099	0.101
	Polluted	0.225	0	0.224	0.226
21-40 cm	Unpolluted	0.09	0	0.089	0.091
	Polluted	0.125	0	0.124	0.126
41-60 cm	Unpolluted	0.051	0	0.05	0.052
	Polluted	0.062	0	0.061	0.063

8. Date * location

Dependent Variable: Nitrogen (Mean of %)

Date	Location	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Sept. 2010	Gurugu	0.164	0.001	0.162	0.165
	Jisonayilli	0.093	0.001	0.092	0.095
	kasalgu	0.072	0.001	0.07	0.073
Nov. 2010	Gurugu	0.164	0.001	0.162	0.165
	Jisonayilli	0.093	0.001	0.092	0.095
	kasalgu	0.072	0.001	0.07	0.073
Jan. 2011	Gurugu	0.164	0.001	0.162	0.165
	Jisonayilli	0.093	0.001	0.092	0.095
	kasalgu	0.072	0.001	0.071	0.074
Mar. 2011	Gurugu	0.162	0.001	0.16	0.164
	Jisonayilli	0.092	0.001	0.09	0.093
	kasalgu	0.072	0.001	0.071	0.074
May. 2011	Gurugu	0.16	0.001	0.158	0.162
	Jisonayilli	0.093	0.001	0.092	0.095
	kasalgu	0.072	0.001	0.07	0.073
Jul. 2011	Gurugu	0.159	0.001	0.157	0.16
	Jisonayilli	0.092	0.001	0.09	0.094
	kasalgu	0.072	0.001	0.071	0.074

9 Depth*Location

Dependent Variable: Nitrogen (Mean of %)

Depth	Location	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
0-20 cm	Gurugu	.224	.001	.223	.226
	Jisonayilli	.189	.001	.187	.190
	kasalgu	.075	.001	.074	.076
21-40 cm	Gurugu	.178	.001	.176	.179
	Jisonayilli	.060	.001	.059	.061
	kasalgu	.085	.001	.084	.086
41-60 cm	Gurugu	.084	.001	.082	.085
	Jisonayilli	.030	.001	.029	.031
	kasalgu	.056	.001	.054	.057

APPENDIX G: ANOVA Output Of Carbon-Nitrogen Ratio

Tests of Between-Subjects Effects; Dependent Variable: C/N Ratio

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	218.389[a]	87	2.510	9.512	.000
Intercept	14560.333	1	14560.333	55176.000	.000
Sample	21.333	1	21.333	80.842	.000
location	5.722	2	2.861	10.842	.001
Date	.778	5	.156	.589	.708
Depth	19.500	2	9.750	36.947	.000
Sample * location	17.167	2	8.583	32.526	.000
Sample * Date	1.556	5	.311	1.179	.354
Sample * Depth	.056	2	.028	.105	.901
location * Date	3.167	10	.317	1.200	.348
location * Depth	116.611	4	29.153	110.474	.000
Date * Depth	2.389	10	.239	.905	.546
Sample * Date * Depth	2.722	10	.272	1.032	.453
location * Date * Depth	4.500	20	.225	.853	.638
Sample * location * Date	2.611	10	.261	.989	.483
Sample * location * Depth	20.278	4	5.069	19.211	.000
Error	5.278	20	.264		
Total	14784.000	108			
Corrected Total	223.667	107			

[a]. R Squared = .976 (Adjusted R Squared = .874)

Estimated Marginal Means: Dependent Variable: C_N Ratio

1. Sample

Dependent Variable: C_N Ratio

Sample	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Control	12.056	0.07	11.91	12.201
Treated	11.167	0.07	11.021	11.312

2. location

Dependent Variable: C_N Ratio

Location	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Gurugu	11.361	.086	11.183	11.540
Jisonayilli	11.917	.086	11.738	12.095
kusalgu	11.556	.086	11.377	11.734

3. Date

Dependent Variable: C_N Ratio

Date	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Jan. 2011	11.611	.121	11.359	11.864
Jul. 2011	11.722	.121	11.470	11.975
Mar. 2011	11.500	.121	11.247	11.753
May. 2011	11.667	.121	11.414	11.919
Nov. 2010	11.500	.121	11.247	11.753
Sept. 2010	11.667	.121	11.414	11.919

4. Depth

Dependent Variable: C_N Ratio

Depth	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
0-20 cm	11.444	.086	11.266	11.623
21-40 cm	12.194	.086	12.016	12.373
41-60 cm	11.194	.086	11.016	11.373

5 Sample*Location

Dependent Variable: C_N Ratio

Sample	Location	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Control	Gurugu	11.611	.121	11.359	11.864
	Jisonayilli	12.000	.121	11.747	12.253
	kusalgu	12.556	.121	12.303	12.808
Treated	Gurugu	11.111	.121	10.859	11.364
	Jisonayilli	11.833	.121	11.581	12.086
	kusalgu	10.556	.121	10.303	10.808

6. Date * Sample

Dependent Variable: C_N Ratio

Date	Sample	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Sept. 2010	Control	12	0.171	11.643	12.357
	Treated	11.333	0.171	10.976	11.691
Nov. 2010	Control	12.111	0.171	11.754	12.468
	Treated	10.889	0.171	10.532	11.246
Jan. 2011	Control	12	0.171	11.643	12.357
	Treated	11.222	0.171	10.865	11.579
Mar. 2011	Control	12.111	0.171	11.754	12.468
	Treated	10.889	0.171	10.532	11.246
May. 2011	Control	12	0.171	11.643	12.357
	Treated	11.333	0.171	10.976	11.691
Jul. 2011	Control	12.111	0.171	11.754	12.468
	Treated	11.333	0.171	10.976	11.691

7. Depth * Sample

Dependent Variable:C_N Ratio

Depth	Sample	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
0-20 cm	Control	11.889	0.121	11.636	12.141
	Treated	11	0.121	10.747	11.253
21-40 cm	Control	12.611	0.121	12.359	12.864
	Treated	11.778	0.121	11.525	12.03
41-60 cm	Control	11.667	0.121	11.414	11.919
	Treated	10.722	0.121	10.47	10.975

8. Date * location

Dependent Variable: C/N Ratio

Date	Location	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Sept. 2010	Gurugu	11.333	0.21	10.896	11.771
	Jisonayilli	12	0.21	11.563	12.437
	Kusalgu	11.667	0.21	11.229	12.104
Nov. 2010	Gurugu	11.333	0.21	10.896	11.771
	Jisonayilli	11.5	0.21	11.063	11.937
	Kusalgu	11.667	0.21	11.229	12.104
Jan. 2011	Gurugu	11.167	0.21	10.729	11.604
	Jisonayilli	12.167	0.21	11.729	12.604
	Kusalgu	11.5	0.21	11.063	11.937
Mar. 2011	Gurugu	11.167	0.21	10.729	11.604
	Jisonayilli	11.667	0.21	11.229	12.104
	Kusalgu	11.667	0.21	11.229	12.104
May. 2011	Gurugu	11.667	0.21	11.229	12.104
	Jisonayilli	12	0.21	11.563	12.437
	Kusalgu	11.333	0.21	10.896	11.771
Jul. 2011	Gurugu	11.5	0.21	11.063	11.937
	Jisonayilli	12.167	0.21	11.729	12.604
	Kusalgu	11.5	0.21	11.063	11.937

9. Depth * location

Dependent Variable: C_N Ratio

Depth	Location	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
0-20 cm	Gurugu	11.417	0.148	11.107	11.726
	Jisonayilli	12.5	0.148	12.191	12.809
	kusagul	10.417	0.148	10.107	10.726
21-40 cm	Gurugu	10.417	0.148	10.107	10.726
	Jisonayilli	12.25	0.148	11.941	12.559
	kusagul	13.917	0.148	13.607	14.226
41-60 cm	Gurugu	12.25	0.148	11.941	12.559
	Jisonayilli	11	0.148	10.691	11.309
	kusagul	10.333	0.148	10.024	10.643

10. Date * Depth

Dependent Variable: C/N Ratio

Date	Depth	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Sept. 2010	0-20 cm	11.5	0.21	11.063	11.937
	21-40 cm	12.167	0.21	11.729	12.604
	41-60 cm	11.333	0.21	10.896	11.771
Nov. 2010	0-20 cm	11.5	0.21	11.063	11.937
	21-40 cm	12.167	0.21	11.729	12.604
	41-60 cm	10.833	0.21	10.396	11.271
Jan. 2011	0-20 cm	11.5	0.21	11.063	11.937
	21-40 cm	12.167	0.21	11.729	12.604
	41-60 cm	11.167	0.21	10.729	11.604
Mar. 2011	0-20 cm	11.333	0.21	10.896	11.771
	21-40 cm	12.333	0.21	11.896	12.771
	41-60 cm	10.833	0.21	10.396	11.271
May. 2011	0-20 cm	11.333	0.21	10.896	11.771
	21-40 cm	12.167	0.21	11.729	12.604
	41-60 cm	11.5	0.21	11.063	11.937
Jul. 2011	0-20 cm	11.5	0.21	11.063	11.937
	21-40 cm	12.167	0.21	11.729	12.604
	41-60 cm	11.5	0.21	11.063	11.937

APPENDIX H: ANOVA Output of T-Test (Bacteria Distribution0

Variable:Y[1].

Summary

Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
GNR-GPR	9	16.67	20.75	4.555	1.518

95% confidence interval for mean: (13.17, 20.17)

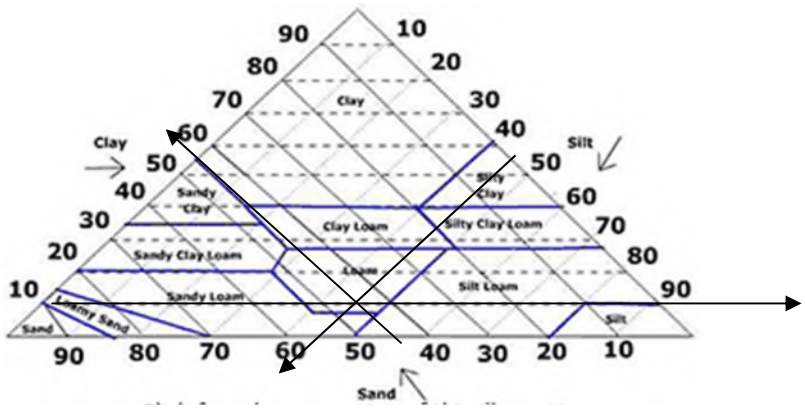
Test of null hypothesis that mean of GNR-GPR is equal to 0

Test statistic t = 10.98 on 8 d.f.

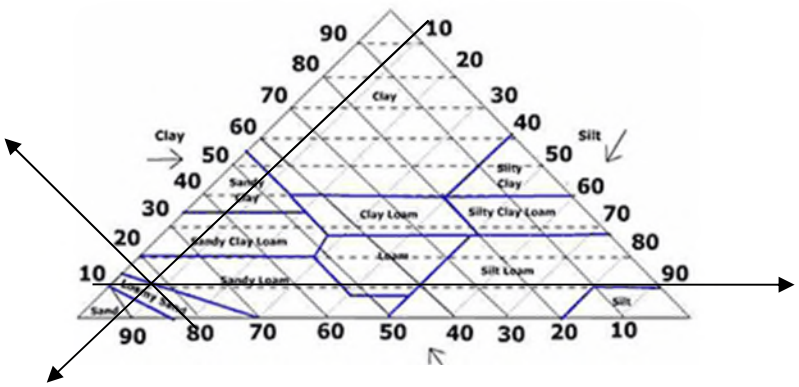
Probability < 0.001

APPENDIX I: Soil Texture Class Reading

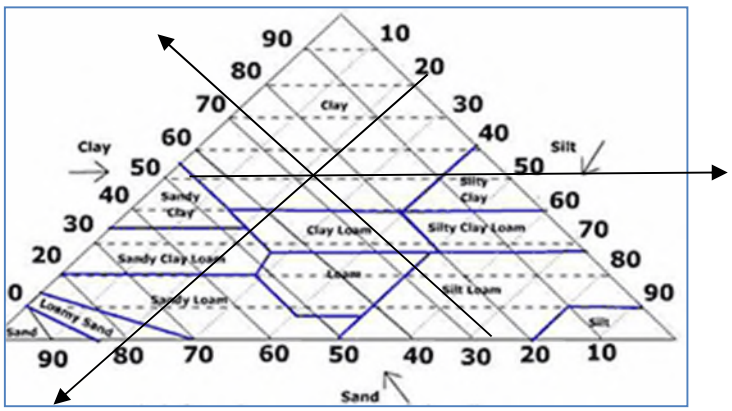
Shea nut cake polluted soils in Jisonayilli was loam (a), Gurugu was loamy sand (b)and in Kasalgu was clay (c). Unpolluted soils were sandy loamfor Jisonayilli (d), loamy sand for Gurugu(e) and clay for Kasalgu (f).



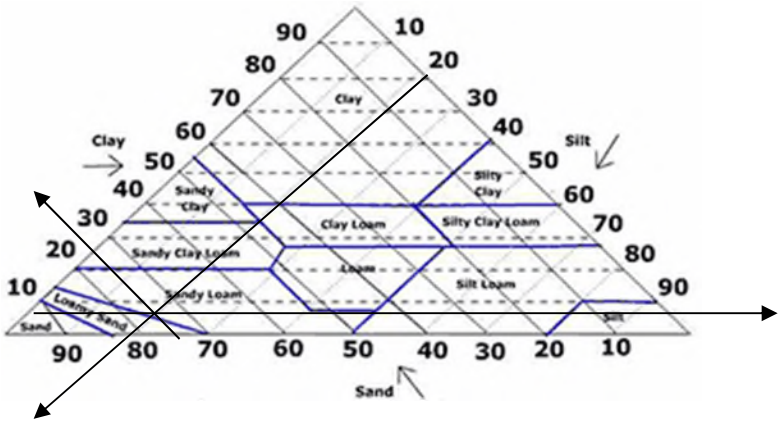
a: Jisonayilli Texture class of shea nut cake polluted soil



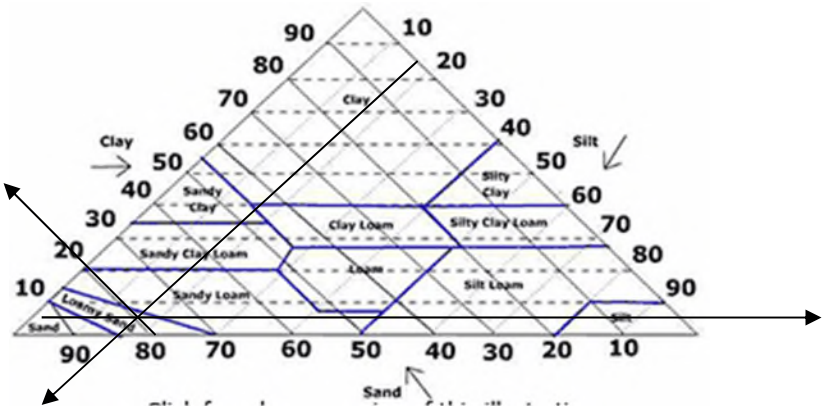
b: Gurugu Texture class of shea nut cake polluted soil



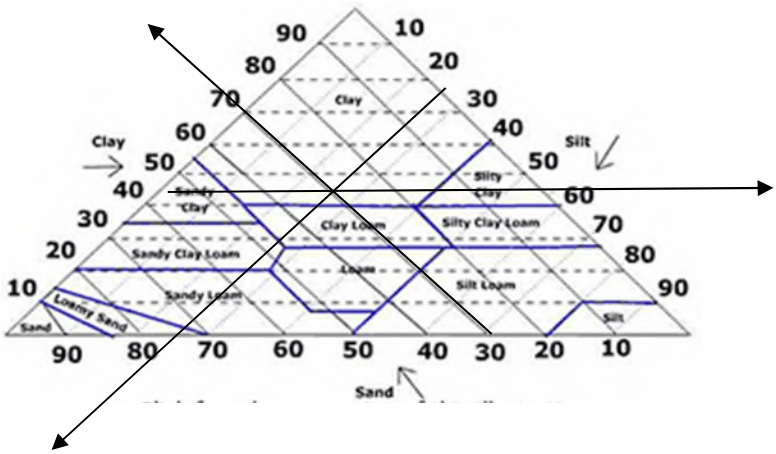
c: Kasalgu Texture class of shea nut cake polluted soil



d: Jisonayilli Texture class of unpolluted soil



e: Gurugu Texture class for unpolluted soil



f: KasalguTexture class of unpolluted soil

APPENDIX J: Definition Of Terms

GURUGU –Tiehisuma Shea butter processing Centre

JISINAYILLI – Tungteiya Women Association Shea butter Extraction Centre

KASALGU – Sekaf Shea butter Village

GNL – Ghana Nut Company Ltd.

SNC – Shea Nut Cake

Clarification – Boiling of crude shea butter in water to release shea oil.