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MORPHOLOGICAL CHARACTERIZATION OF SOIL BACTERIA IN NGERE TEA CATCHMENT AREA OF MURANG'A COUNTY, KENYA

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Abstract: **Bacteria are a very diverse group of organisms in soil, and major taxonomic groups are represented in most soils. The extent of the diversity of microorganisms in soil is seen to be critical to the maintenance of soil health and quality, since a wide range of bacteria are involved in the important soil functions. The objectives of this study were to isolate, characterise morphologically bacteria that are associated with soil quality in tea growing areas of Ngere. Thirty eight isolates were obtained using two categories of media, namely dilute nutrient broth agar and Tryptone soy agar. Ngere tea soils had a pH range of 3.9 to 5.0 and organic carbon content that ranged from 3% to 19%. The isolates were characterized using cultural and biochemical techniques. The Gram stain reaction showed that 53% of the isolates were Gram positive while 47% were Gram negative, and they grew well at pH ranging from 5 - 6.5 and temperature range of 25^{o}C to 35^{o}C**

This study demonstrated that Ngere tea soils harbours diverse bacteria with specific biochemical properties like the ability to reduce nitrate to nitrite, nitrogen fixation, ability to produce urease enzyme that splits urea into carbon dioxide and ammonia, ability to hydrolyse starch and to solubilisation phosphate suggests their involvement in the nutrient recycling within the tea soils hence improving soil fertility. The presence of isolates such as D5, D16, S31, D2, and S23 could indicate that they can be used both as bio control agents for weeds. Furthermore, presence of isolates: D61, D19, S30, D1, D78, D13, S55, D60, D79, S48 among others and high percentage of organic carbon content, strongly suggested extend of fertility of this soils because this isolates are important element in mineral recycling hence can be used as indicators of soil health\ biosensors or biofertilizers.

Keywords: **Soil quality, Ngere tea and Bacteria**

I. INTRODUCTION

Tea (*Camellia sinensis*) is a major cash crop in many developing countries, including China, India, Sri Lanka and Kenya (International Tea Committee, 2004). There are about 2.72 million hectares of land under tea cultivation globally (International Tea Committee, 2004) Tea grows in various latitudes from the sea level in Japan to 2700 m above mean sea level (amsl) in Olenguruone, Kenya and Gisovu, Rwanda (Owuor *et al*, 2008). The plant is widely adaptable to geographical areas with large variations in climate and physical features which affect rates of growth, yields and quality (Ng'etich *et al*., 2001). Tea grows well on highland well drained soils having a good depth, acidic pH in the range 4.5 to 5.5 and more than 2% organic matter (KTDA, 2011).

Soil microorganisms are important components of ecosystem functioning as they determine the mineralization of soil organic matter and energy flow (Robertson and Groffman, 2007). Soil microorganisms are important components of ecosystem functioning as they determine the mineralization of soil organic matter and energy flow (Robertson and Groffman, 2007). Microbial recycling of crop residues provides an important component to improve the soil organic matter pool and soil productivity in agricultural management systems, particularly in the tropics, where microbial soil organic matter turnover time is usually shorter as opposed to temperate agro-ecosystems (Oelbermann *et al*., 2004). Soil bacteria are classified according to the morphological appearance into bacillus (rod), coccus (round), Spirilla (spiral) or filamentous. In terms of numbers, *Bacilli* are most numerous followed by Cocci and Spirilla in soil (Tate, 2000). Majority of soil bacteria are heterotrophic in nature and derive their carbon and energy from complex organic substances/organic matter, decaying roots and plant residues (Glick, 1995). Soil microorganisms also influence above-ground ecosystems by contributing to plant nutrition (Timonen *et al*., 1996), plant health (Dodd *et al*., 2000) and soil fertility (O'Donnell *et al*., 2001). Much of the recent studies have focused on tea production in relation to different ecological environment (Carr and Stephens, 1992) with little knowledge on soil microbial composition and their diversity. This research focused on the presence of soil bacteria, their importance as well as their diversity in small scale tea growing soils in Ngere tea catchment area of Murang'a County, Kenya.

II. MATERIALS AND METHODS

2.1 Collection of soil samples

Soil samples were obtained from Ngere tea catchment area. Cross-sectional study involving stratified random sampling was used. The study population was divided into strata based on the tea buying centres. From each stratum a random sample was collected. The soil samples were taken from a depth of 0-20cm and 20-40cm. A zigzag format of sampling was used; soil samples were mixed thoroughly to constitute a composite sample it was then transported intact at ambient temperature of 4^{o} C in sealed polyethylene bags to the laboratory for processing. One gram of soil was serial diluted in normal saline.

2.2 Enrichment of soil bacteria

Dilute Nutrient Broth Agar (DNBA) was used for the cultivation of microorganisms. Difco nutrient broth (DNB) consisted of Difco nutrient broth (BD Diagnostic Systems, Sparks, MD), at a concentration of 8 g per litre of distilled water. For solid media, 15 g of washed Difco technical agar (BD Diagnostic's systems) was added and 10-fold-diluted Tryptone soy agar 0.1 X TSA was prepared as described by (Joseph *et al.*, 2003). In order To adjust the medium pH to 6.0, 0.1M hydrochloric acid solution was added. The media were then autoclaved at 121^{o} C for 15 minutes after which it was then dispensed into 90-mm-diameter polystyrene sterile plastic petri dishes. The freshly sieved soil was carefully mixed and pulverized with spatula on the larger piece of paper. 1 gram of soil was weighed on a sterile aluminium foil and transferred immediately to the conical flask containing 150 ml of normal saline (Janssen *et. al*, 2002). The flask was dispersed by stirring with Teflon-coated magnetic bars for 15 minutes at approximately 200 g. The soil suspension was then serial diluted where 1ml of the soil suspension was added to 9 ml test tube of normal saline. Dilution ratios included: 10^{0}, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}. These preparations were mixed with a vortex mixer at approximately 150 rpm for 1 minute and 1ml of aliquots was rapidly transferred to other 9 ml tubes. For plate count experiments, 200 µl aliquots from different dilutions were transferred to petri dishes containing dilute nutrient broth agar (DNBA) and spread over the surface with a sterile glass spreading rod. This was followed by incubation at 25 ^{o}C for 24 to 72 hours in the dark. Sub culturing was done on dilute nutrient broth agar to isolate pure cultures.

To measure the survival efficiency, colonies from 10^{-1} dilutions were counted following the formula adapted by James (1978):

$$\text{Viable cell count(CFU/g soil)} = \frac{N\text{umber of colonies}}{\text{Volume of inoculum}} \text{ X Dilution factor}$$

2.3 Characterization of the isolates

Preliminary characterization was performed using morphological and cultural characteristics as described by (Holt *et al.*, 1994). Morphological identification of the isolate was done under the dissecting and compound microscope to observe cell size, shape and arrangement characteristics after classical staining of bacteria (Bartholomew, 1962). 3% (w/v) KOH test (Gregersen, 1978) was used to determine gram characteristics of isolates. Biochemical tests that were also conducted included; citrate utilization, gelatine liquefaction, methyl red-Voges Proskauer, urease test, nitrate reduction test, motility

at 25^{o} C, starch hydrolysis, H_2S production, catalase test, oxidase test, phosphate solubilization test, nitrogen fixation test and indole production test.

2.4 Soil pH determination

Soil pH was determined by drying the soil then sieving to remove stones, sticks and leaves. Twenty five grams of the soil was measured and put in a conical flask then added 50 ml of distilled water then followed by shaking with a reciprocator machine for 20 minutes. The pH meter was calibrated according to manufacturer's instructions over the appropriate range (Missouri Agricultural Station, 1998).

2.5 Soil organic carbon content determination

The total organic carbon content of the tea soil was determined by Walkley and Black method (Missouri Agricultural Station, 1998) whereby 0.1 g of soil dried and sieve was measured and transferred to the conical flask then 10 ml of potassium dichromate solution was added and swirled gently. In the fume hood 15 ml of concentrated sulphuric acid was added and gently swirled for 1 minute then it was allowed to stand for 30 minutes, followed by addition of 150 ml of distilled water using the measuring cylinder and allowed to cool. Five millimetre of orthophosphoric acid and 10 drops of diphenylamine indicator were added and stirred using a magnetic stirrer. Finally it was titrated with ferrous ammonium solution, the colour changes a bit, then to a dirty green (due to the masking effects of excess dichromate) but shortly before the end point, which is extremely sharp to a ferrous sulphate solution clear green (Missouri Agricultural Station, 1998). The blank titre included all ingredients minus the sample. The calculation was done according to the formula:

%C =B-TX0.3XVX100 / WXBX75

Where B=Blank titre, T=Sample titre, W=Weight of soil and V=Volume of potassium dichromate.

III. RESULTS

3.1 Identification of the isolates

Morphological characterization was based on classical macroscopic techniques of colour, form, shape, and elevation of pure colonies. Most colonies were able to grow within 2-3 days of incubation at 25 $^{\circ}$C. The colony morphology of the isolates ranged from, flat and filamentous or branching (Table 1). They were smooth or rough and the colour ranged from white to cream and brown (Table 1).The ability of the isolates to excrete extracellular enzymes was tested through hydrolysis of starch, and gelatine. The ability of the isolates to excrete intracellular enzymes was determined through tests on catalase reaction; urease, Voges-Proskauer, hydrogen sulphide production, nitrate reduction, methyl red, phosphate solubilization, nitrogen fixation, citrate utilization, oxidase, motility and triple sugar- iron test. The isolates deferred greatly on their ability to excrete various enzymes (Table 2).

3.2 Bacterial viable cell count

The viable cell counts were performed on the bacteria that were able to form visible colonies within 2- 3 days of inoculation. Soil samples were diluted in normal saline, and aliquots from dilutions were plated onto the media in replicates. The amount of variation in colony numbers between replicate plates in counting set decreased with increased dilution factor. The colony forming units were obtained by computing the average among the set of dilution factor (10^{0}, 10^{-1} and 10^{-2}) from different layers of soil. Top soil showed highest count (1.6 x 10^{5}) as compared to the sub-soil (1.42 x 10^{5}) (Figure 1). Each solid bar represents the mean of the three counting sets from the soil sample prepared from top layer and three counting sets prepared from sub- soil (Figure 1). The results also showed that there was statistical support for the differences between the mean counts obtained from top soil and sub-soil by one sample t test (Table 3).

3.3 Relationship between organic carbon, pH and colony counts from top soil

The soil pH ranged from 3.9 to 5.0 (Figure 2) with both tea soils and their adjacent Napier grass having the same pH values. The soil organic carbon concentration was higher in tea soils from top soils ranging from 4.7 to 19 than in the sub soils which ranged from 3.0 to 13.2 (Figure 3). In the tea soils studied, there was no correlation between soil pH and microbial biomass; however there was a linear correlation between organic carbon concentrations and soil microbial colony counts. Soil microbial biomass increased significantly between pH 4 and 4.9 in top soils and between pH 3.9 and 5.0 in sub soils.

IV. DISCUSSION

A wide range of different media have been used to estimate the size of the bacterial community of soil and to isolate representatives of this community (Balestra and Misaghi**,** 1997; Olsen, and, Bakken, 1987). However, it has been known for a long time that the number of bacteria that are able to form colonies on microbiological media is generally only a small part of the total number of bacteria in the soil (Jensen, 1968). Recently, the use of non-traditional media has allowed the isolation of members of some of these previously uncultured groups (Sait, *et al*., 2002). One of these media was dilute nutrient broth agar (DNBA) was formulated to mimic the low concentrations of nutrients and inorganic ions in soils, to allow sufficient biomass formation to produce visible colonies. The pH was adjusted to the pH of the soil at the site being studied (*Singh et al.,* 1999). Based on the results, DNBA was chosen as the growth substrate as it yielded the highest mean viable counts in this study and it has been successfully used in earlier studies (Janssen *et al*., 2002), this medium was compared with 0.1 X TSA which is commonly used to grow soil bacteria.

The enumeration of bacteria viable cell count against two layers of soil i.e. top-soil (0-20 cm) and sub-soil (20-40c m) (Figure 1.) showed that the viable cell counts were significantly different between the two soil layers. The effect of two different soil layers was investigated by determining the number of colonies visible on plates after 24 to 72 hours. The viable counts after 72 hours of incubation showed that the counts with top layer reached their maximum of 1.6 X 10^5 while the counts reached 1.42 X 10^5 for sub-soil (Figure 1). Using student's t- test, the results showed significant difference between the mean of the soil layers in relation to viable counts. The counts were higher for the top layer as compared to those obtained with sub-soil (Table 3). The top soil consists of high amount of organic matter which is produced originally by living organisms such as plant or animals (Torsvik *et al*., 2002). This organic matter is returned to the soil and goes through decomposition process by soil microorganisms. The composition of soil microorganisms depends on the food source. Each species and groups exists where it can find appropriate nutrient supply, space, nutrients and moisture. Soil microorganisms occur where organic matter occurs (Ingham, 2000). Therefore, soil microorganisms are concentrated around roots, in litter, on humus, on the surface of soil aggregates and in spaces between aggregates. For this reason, they are most prevalent in forested areas and cropping systems that leave a lot of biomass on the surface. Furthermore, top soils are well aerated which is ideal for aerobic and facultative anaerobic bacteria. The soil microbial biomass is a significant component of terrestrial ecosystems. Its activity contributes to the regulation of soil carbon sequestration, carbon mineralization, nutrient recycling and ecosystem productivity. Soil pH is a significant controlling parameter for the microbial biomass in both laboratory and field studies (Kemmitt *et al*., 2006). The biomass, microbial activity and microbial community structure have been shown to change significantly in soils under tea plants (Yao *et al*., 2000; Yu *et al*., 2003). Nioh *et al*., (1993) found that, despite low pH, tea soils contained more microbial biomass concentrations than in cultivated soil of pH 7.

This study agrees with Yan *et al*. (2003) and Tokuda and Hayastu (2002) found no significant differences in microbial biomass concentrations between tea soils and forest or arable soils as controls.

Total carbon was significantly high in this soil probably because the carbon and nitrogen inputs might be large (figure 3). Also, it is possible that the pH had declined to threshold where mineralization were seriously impeded, allowing accumulation of organic matter Pansombat *et al*. (1997a) demonstrated that long term tea cultivation resulted in accumulation of organic carbon and total nitrogen. This relationship may have been caused by the different rates of fertilizer application which affected both nutrient availability to plants thus influencing the amount and quality of litter inputs to the soil and the soil pH. According to Martin and Nolin (1991), soils are distributed among eight organic matter content classes as follows; less than 3% are classified as very low, 3-4% low, 4-5% moderately low, 5-7.5% moderate, 7.5-9% high, 15-30% very high and those equal or above 30% are extremely high. However, according to this study Ngere tea soils had moderately high carbon content; this could be due to the fact that most farmers use both plant and animal materials as manure this in turn promotes biological and microbial activities that facilitates the formation of soil humus which is suitable for uptake by plants (Wang *et al.*, 2007b). The taxonomic classification of the isolates performed using morphological characteristics; biochemical tests placed the isolates to the genera *Bacillus, Pseudomonas, Burkholderia, Chryseobacterium, Acinetobacter, Enterobacter, Serratia and Micrococcus.* The nitrogen fixation test was performed to establish the ability of the isolates to fix nitrogen. Organisms that are able to fix atmospheric nitrogen possess the enzyme nitrogenase, which reduces nitrogen to ammonia (Cappuccino and Sherman, 2002). Nitrogenase enzyme catalyzes the reduction of not only nitrogen but also a variety of other substrates (Cappuccino and Sherman, 2002). This test showed that most isolates were positive thus their potential to fix nitrogen into the soil. This is a crucial aspect for tea soils as nitrogen is one of key component in soil fertility. The ability of the isolates to reduce nitrate

indicates their ability to produce enzyme nitrate reductase which reduces nitrates that the cell uses as a final hydrogen acceptor during anaerobic respiration to nitrites or free nitrogen gas and water (Harold, 2002). Most isolates were positive for this test. This is an important factor to help maintain the nitrogen cycle in the three phases namely the atmosphere, water, and soil. The release of inorganic phosphate from organic phosphates is called mineralization and is caused by microorganisms breaking down organic compounds. The ability of bacteria to solubilize insoluble Phosphorous minerals has been attributed to their capacity to reduce pH by the excretion of organic acids and protons during the assimilation of ammonia (Mullen, 2005). The *Bacillus, Enterobacter, Serratia, Micrococcus, Burkholderia, Chryseobacterium, Acinetobacter and Pseudomonas* genera identified in this study were also shown to have potential to solubilize phosphorous. Phosphorus is an essential plant nutrient with low availability in many agricultural soils (Wakelin *et al.*, 2004). The urease test was done to determine the ability of the isolates to break down urea, to simple forms of nitrogen which can be readily absorbed by the plants to promote growth. The positive implication is an important aspect in growth and development of tea in the case where fertilizers are applied, as the bacteria have shown potential to convert urea to simpler forms of nitrogen which are readily absorbed by plants. For plants to absorb nitrogen from urea it must first be broken down. Urease catalyzes the hydrolysis of urea to unstable carbamic acid.

Bacilli are described as aerobic or facultative anaerobic, Gram positive, rod shaped, flagellated motile bacteria, catalase positive belonging to the division Firmicutes with a wide ecological diversity mostly saprophytic they are commonly found in soil, dust, milk, plant surfaces, a few are animal or insect parasites or pathogens. Morphological and biochemical assignments of the isolates D16, D61 and D51 also suggested their close relatedness with *Bacillus cereus*; they are catalase positive, liquefied gelatine, citrate positive, Voges Proskauer positive and motile and had the ability to reduce nitrate to nitrite, and oxidase positive. Most isolates were negative with indole, methyl red test and hydrogen sulphide gas production. Isolates D5, D2, S30 and D19 were biochemically and morphologically closely related to *Bacillus thuringiensis* with the same characteristics as *Bacillus cereus* only that they had large rods upon gram staining. Isolate S31 was closely related to *Bacillus subtilis* it was catalase positive, motile and did not produce hydrogen sulphide gas. Isolate D29 closely related to *Bacillus mycoides* and it was a non- motile rod all isolates were phosphate solubilization and nitrogen fixation tests positive (Table 2). Some of the *Bacillu*s species have been classified as plant growth promoting Rhizobacteria (Probanza *et al*., 2002). There are a number of metabolites that are released by these strains (Charest *et al*., 2005) which strongly affect the environment by increasing availability of the plants nutrients (Barriusso *et al*., 2008). Naturally present in the immediate vicinity of plant roots, *Bacillus subtilis* is able to maintain stable contact with higher plants and promote their growth. *Pseudomonas* are described as aerobic, rod shaped, Gram negative bacteria with one or more polar flagella providing motility. Morphological and biochemical signatures of isolate D28 and D63 indicated that they were highly closely related to *Pseudomonas tolaasii,* it was slightly indole positive, did not reduced nitrate to nitrite, liquefied gelatine, hydrolysed starch and showed positive results with Voges- Proskauer while isolate S20 and D69 were closely related to *Pseudomonas putida*, it was negative with indole, starch gelatine, and urea. It was positive with nitrate all isolates were phosphate solubilization and nitrogen fixation tests positive (Table 2). The isolates had multiple polar flagella for motility and grew optimally at a temperature of 25^{o} C to 35^{o} C. The presence of most *Pseudomonas sp* helps in the maintenance of soil health (Lata *et al*., 2002). The presence of *Pseudomonas fluorescence* plays an effective role in stimulating yields and growth of various plants (Rokhzadi *et al.,* 2008). Specific strains of *Pseudomonas putida* have recently been used as seed inoculants on crop plants to promote growth and increase yields (Johri, 2001)

The genus *Burkholderia* is described as aerobic, Gram negative, rod–shaped, motile with multitrichous polar flagellated bacteria (Gilligan and Whittier, 1999). It contains organisms that are important causes of human, animal and plant disease, as well as organisms useful in promoting plant growth and bioremediation. Isolate S23 adheres to the entire signature phenotypic and biochemical characteristics of *Burkholderia sp* which is a rod-shaped, gram negative, oxidase positive, motile, positive with Phosphate solubilization, nitrogen fixation, and citrate, nitrate, and starch tests. It is indole, urease and methyl-red negative and unable to reduce gelatine (Table 2). A variety of *Burkholderia* spp. have been characterized as important environmental strains, with phenotypes that include biological control of plant root-infecting fungi, plant growth promotion, nitrogen fixation, and biodegradation of recalcitrant compounds in soil (Parke and Gurian Sherman, 2001). This genus *Enterobacter* is described as facultative anaerobe, rod-shaped, Gram negative bacteria with peritrichous flagella providing motility. *Enterobacter* species are found in the natural environment in habitats such as water, sewage, vegetables, and soil. The morphological, and biochemical signatures for isolates D79, S48, and S49 indicated that they are closely related to genus *Enterobacter cloacae* they were citrate positive, nitrate positive were positive with Voges-Proskauer, phosphate solubilization, nitrogen fixation and showed negative results with indole, urea and hydrogen

sulphide gas production. The isolates were motile (Table 2) and grew optimally at a temperature range of 25°C to 35°C. Recent studies have indicated that the genus *Enterobacte*r is associated with the plant rhizosphere and are able to exert a beneficial effect on plant growth (Tilak *et al*., 2005 and Egamberdiyeva, 2005). The important role is played by plants in selecting and enriching the types of bacteria by the constituents of their root exudates. *Chryseobacterium* are described as Gram- negative, non-motile rods, catalase, gelatine hydrolysis and oxidase positive (Bernardet *et al*., 2002) Many *Chryseobacterium* strains occur in soil, freshwater, and marine environments, while others are found in dairy products; others are opportunistic pathogens of humans and animals. Isolate D60 displayed the typical characteristics and biochemical properties of members of the genus *Chryseobacterium*. They were Gram- negative, non-motile rods, oxidase, catalase, indole and gelatine positive and were negative for urease, nitrate reduction and hydrogen sulphide gas production (Table 2) with an optimal growth at temperatures 25° C to 35°C. Recent works suggest that *Chryseobacterium sp* would be used as Plant growth promotion Rhizobacteria are universal symbionts of higher plants, which enhance the adaptative potential of their hosts through a number of mechanisms, such as the fixation of molecular nitrogen, the mobilization of recalcitrant soil nutrients and the synthesis of phytohormones and the control of phytopathogens (Weller and Thomashow, 1994). *Acinetobacter* can be described as strictly aerobic, Gram negative, rod-shaped, oxidase negative and non-motile bacteria. The species of *Acinetobacter* are common, free- living saprophytes found in soil, water, sewage and foods. The morphological and biochemical signatures for isolates D1 and S55 indicated that they are closely related to *Acinetobacter sp*. Members of the genus *Serratia* are Gram-negative, non-spore forming rods belonging to the family Enterobacteriaceae. These facultative anaerobes typically are motile by means of peritrichous flagella. Biological phosphate removal from wastewater is an efficient cost-effective alternative to chemical phosphorus precipitation. This biological process is obtained by recycling the sludge through anaerobic and aerobic zones. It is dependent on the enrichment of activated sludge with polyphosphate accumulating strictly aerobic *Acinetobacter sp*. which could absorb phosphate up to 100 mg phosphorus per gram of dry biomass during aerobic conditions and release it anaerobically (van Groenestijn *et al*., 1989;). It was confirmed that *Acinetobacte*r are primarily responsible for biological phosphate removal (Wagner *et al*., 1994). *Micrococcus* can be described as Gram positive, non-motive, coccus-shaped, with colonies circular, Smooth, convex, entire and pale yellow in colour bacteria. These organisms occur in a wide range of environments including dusts, water, milk, dairy products and soil. Many Micrococcus species have been described including. *M ureae*, *M. flavus*, *M. luteus* and other related modern members of the genus have numerous genetic adaptations for survival. This includes extreme, nutrient-poor conditions. These phenotypes have assisted the microbe in persistent and prevalent dispersal within the environment. This species has an ability to utilize succinate and terpine related compounds to enhance and ensure its survival in oligotrophic environments (Greenblatt, *et al.*, 2004). It is also mobile in soil and is considered an environmental teratogen (Sims *et al*., 1986). Microorganisms play an important role in effecting the availability of soil phosphorous to plant roots, and increasing P-mobilization in soil. The ability of soil microorganisms to convert insoluble forms of phosphorus to a soluble form is an important trait in plant growth-promoting bacteria for increasing plant yields a potential *Micrococcus* strain NII-0909 isolated from Western *ghat* forest possessed multiple plant growth traits, like P-solubilisation, and Siderophore production (Berleth and Sachs, 2001). Isolates D13 and D78 adheres to all the signature phenotypic and biochemical characteristics of genus *Micrococcus* both isolates formed circular, Smooth, convex, entire cream colonies and were gram positive non-motile Coccus, catalase and citrate positive, bacteria negative with indole production, starch hydrolysis, hydrogen sulphide gas production, nitrate reduction, methyl red and Voges Proskauer negative. However isolate D78 was urease positive which suggested it was *Micrococcus ureae* while isolate D13 did not hydrolyse urea hence *Micrococcus luteus* (Robert *et al*., 1957) (Table 2).

V. CONCLUSION AND RECOMMENDATIONS

There is a diverse and complex prokaryotic community that is resident in Ngere tea catchment area. The biochemical properties of some isolates, like ability to reduce nitrate to nitrite nitrogen fixation and phosphate solubilization, suggests their involvement in the nitrogen cycle within the tea soils.

The following are recommendations drawn from this study to aid further research in this area**:**

- More studies should be carried out to target Specific groups of microbes that are important in tea soils e.g. Phosphate solubilisers, Nitrogen fixers and disease suppressors
- Further studies should be carried out to determine possible application of the isolates as Biofertilizers, biopesticides or biocontrol agents against the weeds

- Further analysis of bacteria is necessary for total characterization and identification of more strains from other tea regions
- More research is required to design studies that would compare the diversity of Bacteria in different seasons of the year such as the rainy and dry seasons

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APPENDIX - A

TABLES:

Table 1: Morphological characteristics of isolates obtained from Ngere tea catchment area

Isolate	Colony Characterization				Cell characterization	
	Colour	form	elevation	margin	Cell arrangement	Gram reaction
D16an	Cream	Irregular	Flat	Ciliate	Large rods	Positive
D19	White	Irregular	Flat	Undulate	Small rods	Positive
D3	Cream	Irregular	Flat	Undulate	Short rods	Positive
D5	White	Irregular	Flat	Whip- like	Large rods	Positive
D72	Cream	Irregular	Flat	Undulate	Large rods	Positive
D62	Cream	Irregular	Flat	Branching	Large rods	Positive
S6	Cream	Irregular	Flat	Undulate	Large rods	Positive
D61	Cream	Irregular	Flat	Branching	Small rods	Positive
D70	Brown	Irregular	Flat	Undulate	Large rods	Positive
D2	Brown	Irregular	Flat	Ciliate	Large rods	Positive
D29	Cream	Irregular	Flat	Undulate	Large rods	Positive
D51	Cream	Irregular	Flat	Ciliate	Short rods	Positive
C5	Cream	Irregular	Flat	Undulate	Short rods	Positive
S42	Brown	Irregular	Flat	Ciliate	Large rods	Positive
S31	Cream	Irregular	Flat	Ciliate	Large rods	Positive
S12	White	Irregular	Flat	Ciliate	Short rods	Positive
D68an	Brown	Irregular	Flat	Undulate	Large Rods	Positive
S30	White	Irregular	Flat	Entire	Large rods	Positive
D49an	Brown	Circular	Raised	Entire	Short rods	Negative
S48	Cream	Circular	Flat	Smooth	Large rods	Negative
S49	Cream	Irregular	Raised	Smooth	Large rods	Negative
D79	Brown	Circular	Flat	Entire	Short rods	Negative
D60	Yellow	Circular	Flat	Entire	Large rods	Negative
D28	Brown	Circular	Raised	Smooth	Short rods	Negative
D63an	Cream	Circular	Flat	Smooth	Short rods	Negative
S20	Brown	Circular	Raised	Smooth	Short rods	Negative
D66an	Brown	Irregular	Raised	Entire	Short rods	Negative
C53	Cream	Circular	Flat	Entire	Short rods	Negative
D69	Cream	Irregular	Raised	Smooth	Short rods	Negative
D13an	Cream	Irregular	Flat	Entire	Coccus	Positive
D78	Cream	Circular	Raised	Smooth	Coccus	Positive
D64an	Cream	Circular	Flat	Entire	Large rods	Negative
D80	Cream	Irregular	Flat	Raised	Short rods	Negative
S55	Cream	Circular	Flat	Smooth	Large rods	Negative
D1	Brown	Irregular	Flat	Undulate	Short Rods	Negative
S50	Cream	Round	Flat	Smooth	Short rods	Negative
S23	White	Round	Raised	Smooth	Short rods	Negative
D77	Cream	Round	Raised	Smooth	Large rods	Negative

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Table 2: Biochemical characteristics of the isolates obtained from Ngere tea catchment

		Biochemical Tests													
Isolates	Starch	Indole	Catalase	Nitrate	Motility	Urea	Citrate	MR	VP	Gelatine	Oxidase	NFT	H_2S	Phosp	**Bacteria Spp**
D1	+	-	+	-	-	+	+	+	-	-	-	+	-	+	*Acinetobacter*
S55	+	-	+	-	-	-	+	-	-	-	-	-	-	+	*Acinetobacter*
D13an	-	-	+	-	-	-	+	-	-	-	+	+	-	+	*Micrococcus lateus*
D78	-	-	+	-	-	+	+	-	-	-	+	+	-	+	*Micrococcus urease*
S48	+	-	+	-	+	-	+	-	+	-	+	+	-	+	*Enterobacter cloacae*
D49an	+	-	+	+	+	-	+	+	-	-	+	+	-	+	*Enterobacter cloacae*
S49	+	-	+	+	+	-	+	-	-	-	+	+	-	+	*Enterobacter cloacae*
D79	+	-	+	+	+	-	+	+	-	-	-	+	-	+	*Enterobacter cloacae*
D60	+	+	+	-	-	-	+	-	-	+	+	+	-	+	*Chryseobacterium sp*
D80	-	-	+	+	+	-	+	-	+	+	-	+	-	+	*Serratia sp*
D64an	-	+	+	-	+	-	+	-	+	+	+	+	-	+	*Pseudomonas sp*
D63an	+	+	+	-	+	-	+	+	-	+	+	+	-	+	*Pseudomonas tolaasii*
D28	+	+	+	-	+	-	+	-	-	+	+	+	-	+	*Pseudomonas. tolaasii*
D66an	+	-	+	+	+	-	+	+	-	+	+	+	-	+	*Pseudomonas fluorescence*
C53	+	-	+	+	+	-	+	+	-	+	+	+	-	+	*Pseudomonas fluorescence*
D69	-	-	+	+	+	+	+	-	-	-	+	+	-	+	*Pseudomonas putida*
S20	-	-	+	+	+	-	+	-	-	-	+	+	-	+	*Pseudomonas putida*
D77	+	-	+	+	+	+	+	+	-	-	+	+	-	+	*Burkholderia sp*
S23	+	-	+	+	+	-	+	-	-	-	+	+	-	+	*Burkholderia sp*
S50	+	-	+	+	+	-	+	-	-	-	+	+	-	+	*Bacillus cereus*
D70	+	-	+	+	+	+	+	-	+	+	+	+	+	+	*Bacillus thuringiensis*
D2	+	-	+	-	+	-	+	-	+	-	+	+	+	+	*Bacillus thuringiensis*
D68an	+	-	+	-	+	-	+	-	+	+	+	+	+	+	*Bacillus cereus*
D62an	+	-	+	-	+	-	+	-	-	+	+	+	-	+	*Bacillus cereus*
D29	+	+	+	-	-	-	+	-	+	-	+	+	-	+	*Bacillus mycoides*
D51	+	-	+	+	+	+	+	-	+	+	+	-	+	+	*Bacillus cereus*
S30	+	-	+	+	+	+	+	-	+	-	+	+	+	+	*Bacillus thuringiensis*
S31	+	-	+	+	+	+	+	-	+	+	+	+	+	+	*Bacillus subtilis*
S12	+	-	+	+	+	+	+	-	+	+	+	+	+	+	*Bacillus subtilis*
C5	+	-	+	-	+	+	+	+	+	+	+	+	+	+	*Bacillus sp*
S6	+	-	+	-	-	-	+	-	+	+	+	-	-	+	*Bacillus sp*
S42	+	-	+	+	+	+	+	-	+	-	+	+	+	+	*Bacillus thuringiensis*
D16an	+	-	+	+	+	+	+	-	+	+	+	+	+	+	*Bacillus cereus*
D3	+	-	+	+	+	+	+	-	+	+	+	+	+	+	*Bacillus thuringiensis*
D5	+	-	+	+	+	+	+	-	+	+	+	+	+	+	*Bacillus thuringiensis*
D61an	+	-	+	+	+	+	+	-	+	+	+	+	+	+	*Bacillus cereus*
D19	+	-	+	+	+	+	+	-	+	+	+	+	+	+	*Bacillus subtilis*
D72	+	-	+	-	+	+	+	+	+	+	+	+	-	+	*Bacillus sp*

Key: (+) Positive, (-) Negative, MR: Methyl Red, VP: Voges-Proskauer, NFT: Nitrogen fixation test and H_2S: Hydrogen Sulphide gas, Phosp: Phosphate

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Table 3: The mean difference of Top soil and sub soil determined by one- sample T test using Portable_PASW_Statistics_18 (SPSS) at 95% confidence interval

	Test Value = 0					
					95% Confidence Interval of the Difference	
	T	Df	Sig. (2-tailed)	Mean Difference	Lower	Upper
Top-Soil(0-20 cm)	36.047	30	.000	1.3605	1.283	1.438
Sub-Soil (20-40 cm)	23.286	30	.000	1.15645	1.0550	1.2579

Key: T: test, DF**:** Degrees of Freedom

PLATES

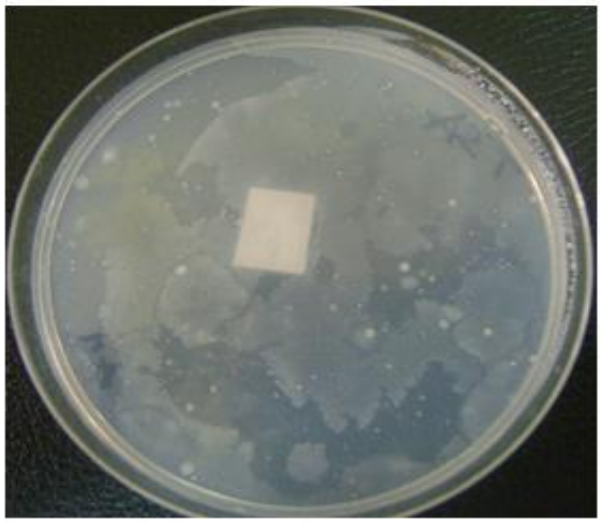

Plate 1 a

Plate 1 b

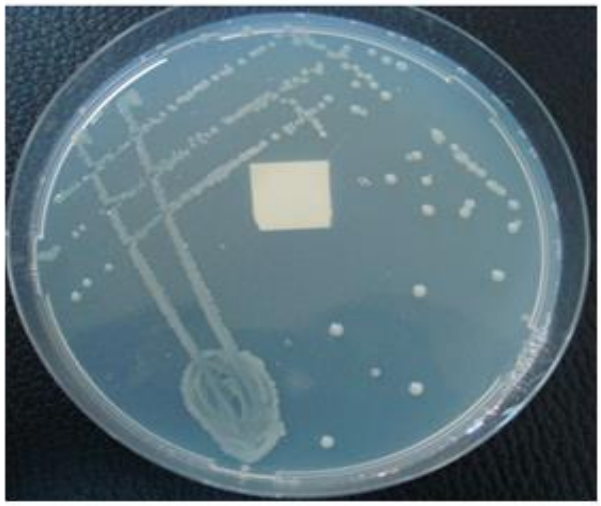

Plate 1 c

Plate 1: (1a) Tryptone soy agar medium plate with different colonies before isolation of individual colonies (low diversity with a few colonies). (1 b) Dilute nutrient broth agar medium culture plate with different colonies before isolation of individual colonies (high diversity). (1 c) Culture plate with individual colonies.

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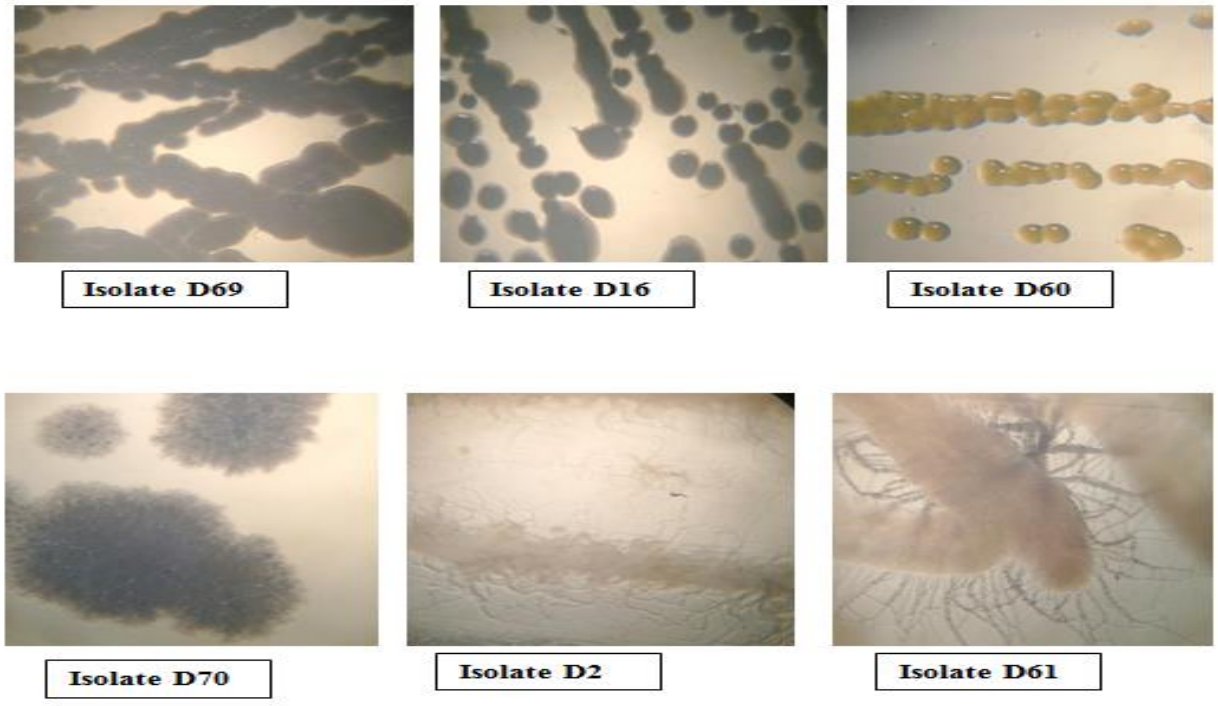

Plate 2: Some of the bacterial colonies, as seen under dissecting microscope
Legend: Irregular, raised and smooth (**D69**), irregular, flat and ciliate (**D16**), circular, flat and entire (**D60**), Irregular, flat and undulate (**D70**), irregular, flat and ciliate (**D2**), irregular, flat and branching (**D61**)

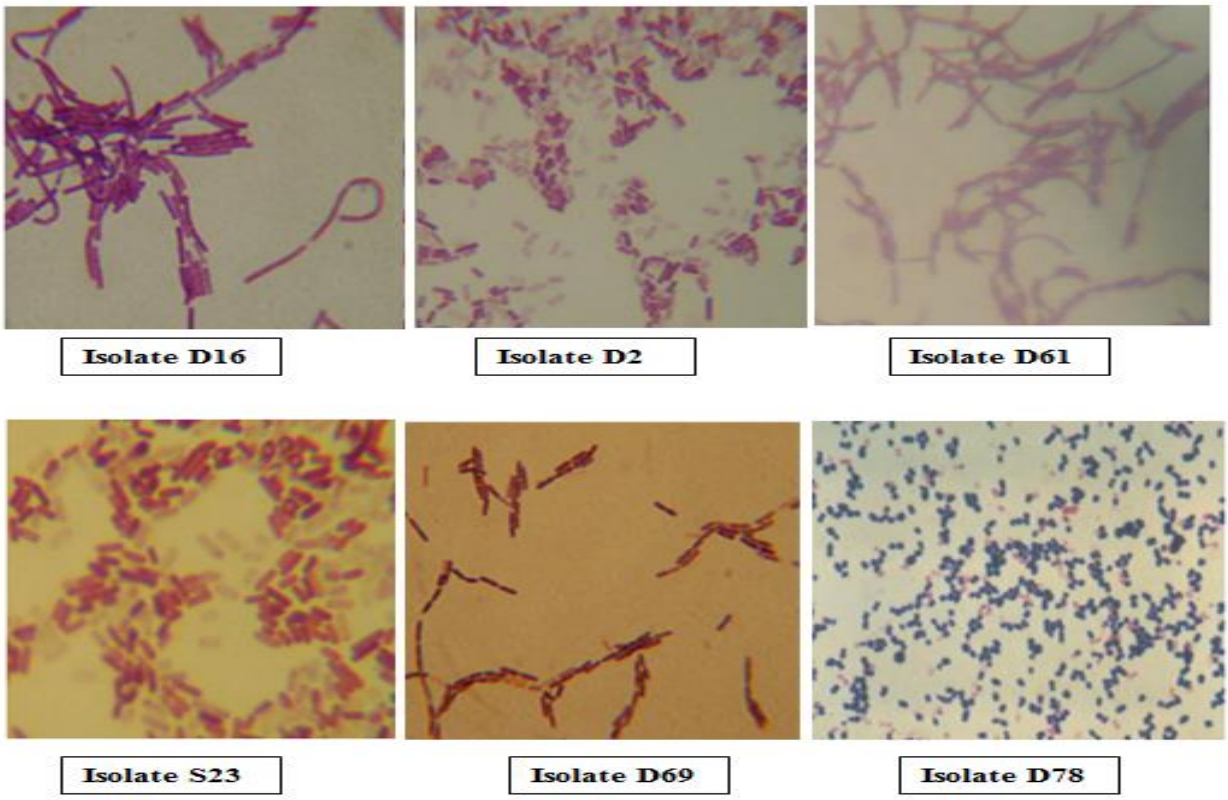

Plate 3: Gram reaction of selected bacterial isolates
Legend: Gram positive rods [(**D16**), (**D2**), (**D61**)],gram negative rods [(**S23**), (**D69**)], gram positive coccus [(**D78**)]

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FIGURES

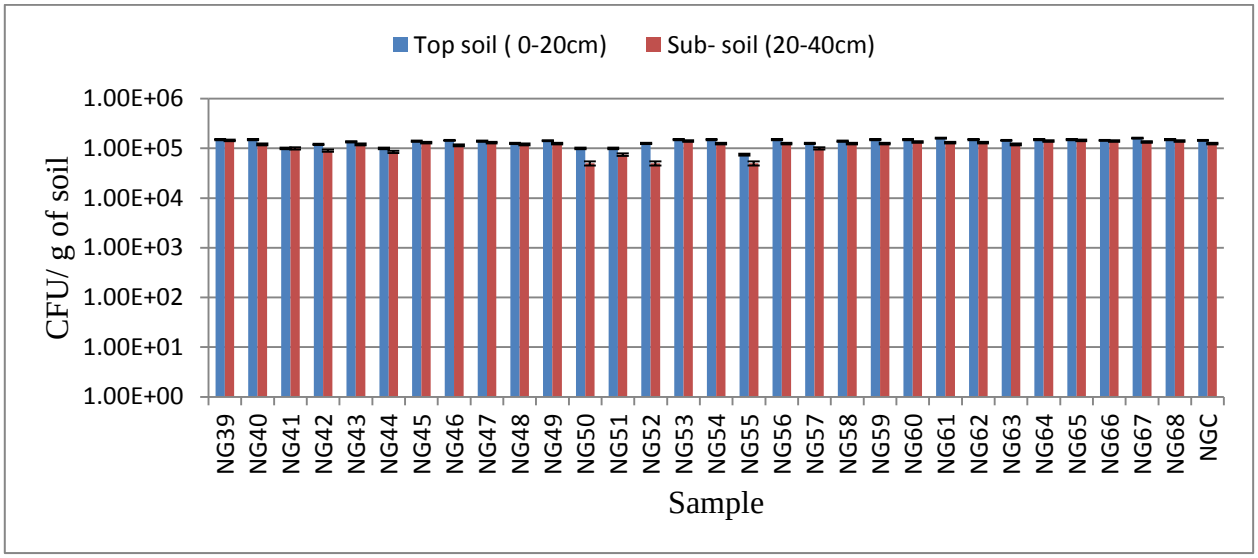

Figure 1: Showing bacteria viable cell counts from top-soil (0-20 cm) and sub-soil (20-40 cm

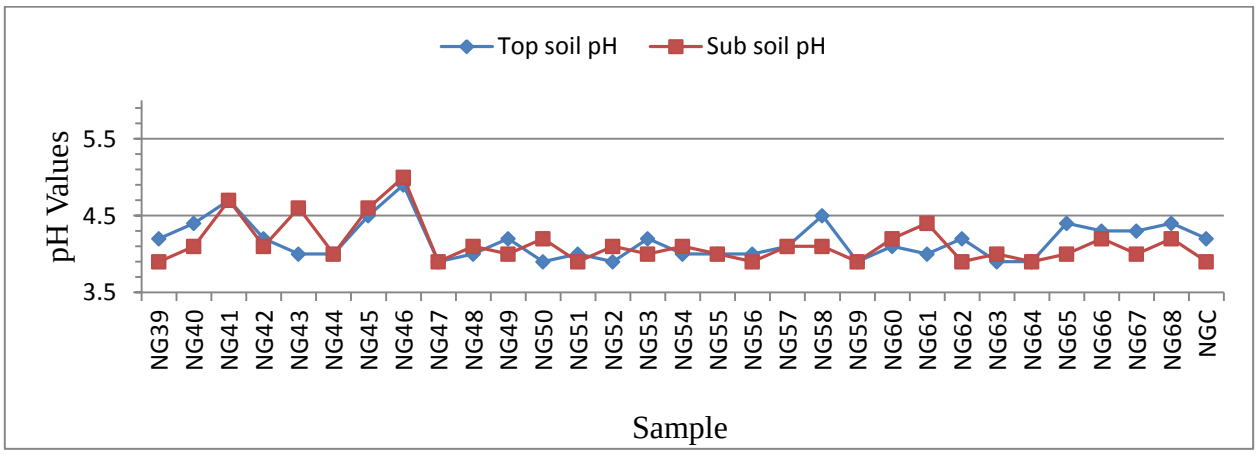

Figure 2: Relationship between the pH of the top (0-20cm) and sub-soil (20-40cm)

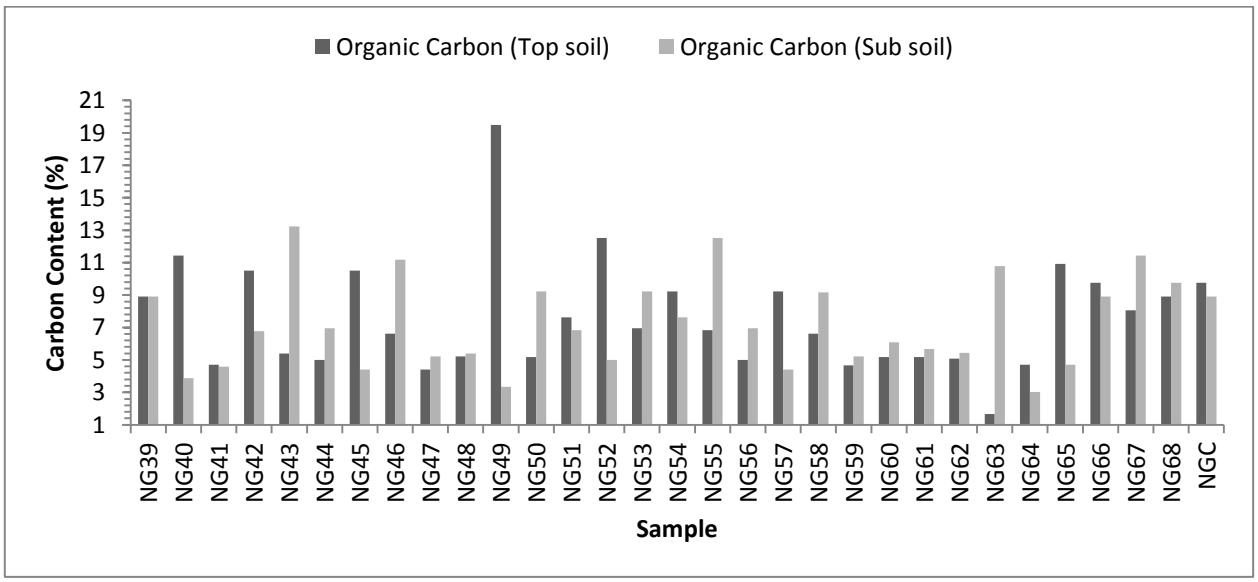

Figure 3: Relationship between the organic carbon content of the top (0-20cm) and sub-soil (20-40cm)