

## Original Research Article

# Physicochemical Properties of Soils from Different Land Use Systems Influence on the Abundance of *Actinomyces* Populations

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### ABSTRACT

**Aims:** *Actinomyces* are filamentous bacteria which produce bioactive compounds with biomedical potential, making them a potential source of alternative antimicrobial agents. Soil physicochemical properties influences population *Actinomyces* in soil community. Understanding factors of the soil that influence *Actinomyces* population is crucial for harnessing their potential for biotechnological applications. In this study, we assessed the effect of physicochemical properties of soils from different land use systems on the population of *Actinomyces*.

**Study design:** A cross sectional survey design in line transect sampling was used in collection of samples from land use systems. A 4x6x3 factorial experiment laid in completely randomized design was used in determining *Actinomyces* population in land use system and media type culture.

**Place and Duration of Study:** The study was conducted in Meru south sub-county, Kenya, between January 2019 and July 2019.

**Methodology:** Cultural growth of *Actinomyces* was determined using four different media (Luria Bertani Agar, Starch Casein Agar, and International Streptomyces Project). The physicochemical properties of soil (soil temperature, soil pH, soil moisture content, electrical conductivity, organic content, available magnesium, sodium, potassium, and phosphorous) were evaluated using standard laboratory methods. The data obtained were analyzed using analysis of variance and significantly means were separated using Least Significance Difference at  $\alpha = 0.05$  in SAS version 9.4. Correlation analysis was used to determine the relationship between soil physicochemical properties and *Actinomyces* population. *Actinomyces* population varied significantly ( $p < 0.05$ ) among the different land use systems.

**Results:** The type of media used in isolation of *Actinomyces* colonies found to significantly influence the growth and proliferation of *Actinomyces* colonies. The mean number of *Actinomyces* colonies varied significantly ( $p < 0.05$ ) among the different growth media. The highest number of colonies was observed in the Starch casein medium ( $3.4 \times 10^5$  cfu/ml of soil sample) while the lowest was observed in the modified Luria Bertani (M1) ( $1.7 \times 10^5$  cfu/ml). *Actinomyces* populations varied significantly ( $p < 0.05$ ) among the different land use systems. Soils from the dumpsite exhibited the highest *Actinomyces* population ( $3.21 \times 10^5$  cfu/ml of soil sample), while samples from chicken manure had the lowest population ( $2.02 \times 10^5$  cfu/ml of soil sample). The results of this study revealed distinct variations in soil temperature among the different land use systems. The soil samples collected from the dumpsite exhibited the highest temperature ( $30.5^\circ\text{C}$ ) while the soil samples obtained from intact forest soil, registered the lowest temperature of  $19.0^\circ\text{C}$ . There was a moderate positive correlation ( $r = 0.63407$ ;  $p < 0.0001$ ) between *Actinomyces* population and soil pH, a weaker positive correlation ( $r =$

0.3375;  $p = 0.012$ ) between soil moisture content and *Actinomyces* population, as well as between available potassium and actinomyces load ( $r = 0.31483$ ;  $p < 0.0001$ ). On the other hand, no significant correlations were found between Actinomyces and soil available sodium ( $r = 0.2524$ ;  $p = 0.06$ ), available magnesium ( $r = 0.2455$ ;  $p = 0.07$ ), available soil phosphorus ( $r = 0.19217$ ;  $p = 0.16$ ), electrical conductivity ( $r = 0.1296$ ;  $p = 0.35$ ) and between Actinomyces load and organic carbon content ( $r = -0.030$ ;  $p = 0.828$ ).

**Conclusion:** The study concludes soils derived from different land use have potential to be a source of novel Actinomyces and the type of media used in isolation of *Actinomyces* colonies found to significantly influence the growth and proliferation of *Actinomyces* colonies. The abundance and distribution of Actinomyces was affected by soil physicochemical properties and consequently land uses.

*Keywords: Land Use Systems, Actinomyces, Soil Physicochemical Properties, Actinomyces Population*

## 1. INTRODUCTION

Soil microbial communities play a critical role in maintaining ecosystem functions and services. Among the diverse microbial groups *Actinomyces* are known to respond to changes in soil physicochemical characteristics, such as pH, organic matter content, and nutrient availability [1]. The population of *Actinomyces* in soil are influenced by various factors, including land use practices and soil physicochemical characteristics [2] [3]. However, the specific effects of soil physicochemical properties on *Actinomyces* communities remain poorly understood, warranting further investigation.

The population of *Actinomyces* differs in various geographical locations depending on the environmental conditions [4]. The soil pH, soil organic matter, and soil moisture significantly influence the population of *Actinomyces* in different ecological conditions and land uses [5] [6]. Many *Actinomyces* species prefer slightly acidic to neutral pH soil conditions, ranging from pH of 6 to 9 [7] [8]. The population of *Actinomyces* is positively correlated with soil organic matter content and negatively correlated with soil pH [9]. Organic matter content of soil has been identified as critical factor influencing *Actinomyces* populations [10]. Indeed, high organic matter content favors the abundance of *Actinomyces* [6] because *Actinomyces* utilizes organic matter as a carbon source [11]. Soil moisture content also plays a crucial role, as *Actinomyces* generally thrive in soil with moderate moisture levels, while excessive moisture can lead to a decrease in their abundance [12]. Soil electrical conductivity reflects the salinity levels in soil and can directly impact microbial communities. High electrical conductivity can adversely affect *Actinomyces* populations, as they are sensitive to salinity stress [13]. Soil temperature plays a crucial role in determining microbial community composition and activity. Some *Actinomyces* are mesophilic microorganisms, and variations in temperature can either stimulate or inhibit their growth and enzyme production [14].

Several factors influence the population dynamics of *Actinomyces* in soil ecosystems, with nutrient availability being one of the key determinants. Nutrients, such as phosphorus (P), potassium (K), sodium (Na), magnesium (Mg), and organic carbon, are essential for the growth and metabolic activities of *Actinomyces* [15]. These elements influence enzymatic processes, substrate utilization, and cell growth, which collectively affect the abundance and diversity of *Actinomyces* populations in the soil [16]. Magnesium (Mg) is essential for ribosome stability, enzyme catalysis, and phospholipid structure [17]. Furthermore, *Actinomyces* heavily depend on organic carbon sources for energy generation and metabolic processes [18]. Given the ecological importance of *Actinomyces* and the significance of nutrient availability in

their growth and survival, the study investigated the relationship between P, K, Na, Mg, and organic carbon levels in the soil and their impact on the abundance and diversity of *Actinomyces* populations.

Different land use systems, such as agricultural fields, forests, cattle manure, chicken manure and urban areas, exhibit distinct physicochemical properties, nutrient availability, and microbial interactions. Forest soils are unique ecosystems with distinct physicochemical characteristics. *Actinomyces* are vital components of forest soil microbial communities, contributing to organic matter decomposition and nutrient cycling. Understanding the relationship between soil properties and *Actinomyces* populations in forest soils can provide insights into the functioning and resilience of these ecosystems [19]. Cattle and chicken manure are commonly used as organic fertilizers in agriculture. *Actinomyces* are known to be involved in the decomposition of organic matter in manure and the transformation of nutrients. Examining the influence of physicochemical properties on *Actinomyces* populations in manure can help optimize the use of these organic inputs in sustainable agriculture practices [20]. Dumpsites are known to contain a wide range of contaminants that can alter the physicochemical properties of soils. Understanding how these properties influence *Actinomyces* populations can provide valuable insights into the impact of waste disposal on soil microbial communities and overall soil health [21]. While there have been some studies investigating the relationship between soil physicochemical properties and *Actinomyces* populations, this study assessed the effect of physicochemical properties of soils from different land use systems on the population of *Actinomyces*.

## 2. MATERIAL AND METHODS

### 2.1 STUDY SITE

The study was conducted in Meru South Sub-County, which is located within Tharaka Nithi County in the Eastern Region of Kenya. The soil samples for the study were collected from Mt. Kenya forest (S0° 18.247' E37° 34.111'), farm lands (S0° 19.347' E37° 39.483') and solid waste dump site in Meru Sub-county (S0° 19.699' E37° 39.176') in Tharaka-Nithi County in Kenya. The sampling locations were marked using the Etrex 30x Garmin Global Positioning System to ensure accurate spatial referencing (Table 1). The temperature within Meru south sub-county ranges from 14 °C to 30 °C [22]. The soils in Meru South Sub-County are generally acidic, with pH values ranging from 4.2 to 6.2. Additionally, the study found that the soils had low organic matter content, ranging from 0.6% to 1.7% [22]. These soil characteristics provide insights into the soil fertility status and potential nutrient deficiencies that may influence the development of soil microorganisms in the study area. The specific sampling locations are shown (Figure 1).

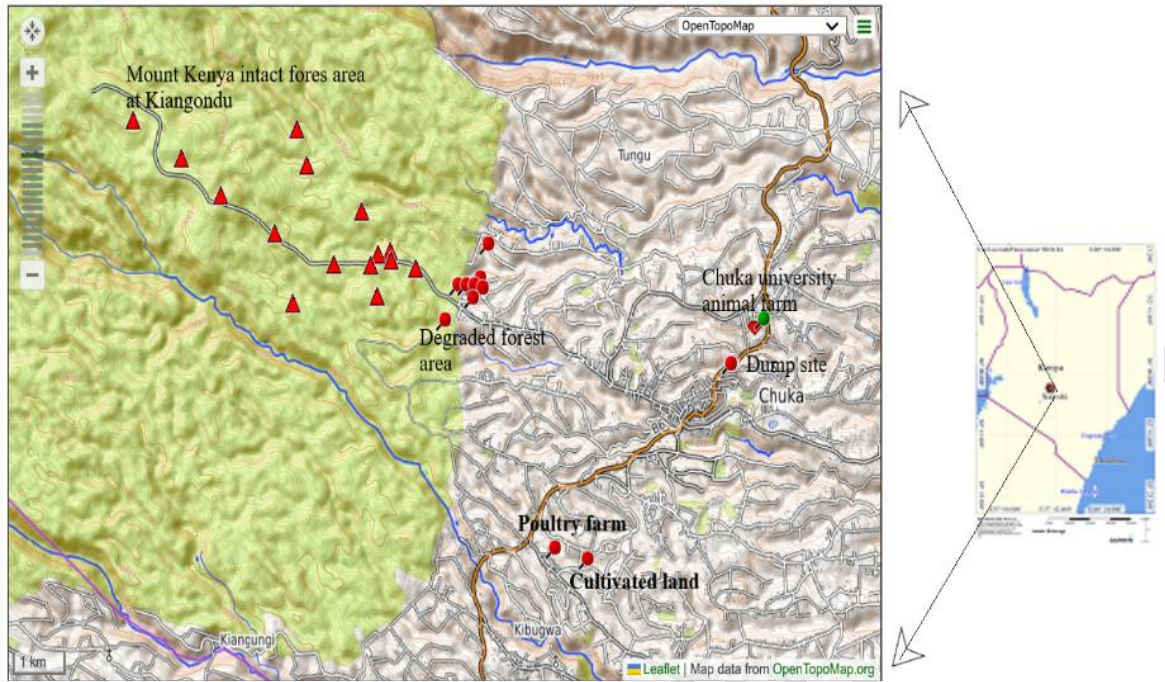


Figure 1: Soil of sample sampling sites in Meru-South Sub-County, Tharaka-Nithi County.

Table 1: Soil sampling from different land use systems of Meru South Sub-county

Site(s) <sup>1</sup>	Soil sampling area	Ecological importance	Sd <sup>2</sup> (cm)	Elevation	Latitude	Longitude
A	Chuka forest zone	Intact soils	5	1593m	0°19.30'S	37°36'0''E
B	Ikuu farm	Cultivated soils	5	1321m	0°11.0'S	37°19'00'E
C	Chuka forest zone	Destructed area	5	1558m	0°19.30'S	37°36'00''E
D	Chuka town	Dump site soils	5	1356m	0°19.699'S	37°39.17'E
E	Chuka University	cattle manure	5	1406m	0°19.347'S	37°39.483'E
F	Ikuu farm	Chicken manure	5	1321m	0°11.0'S	37°19.00'E

<sup>1</sup>Sampling sites, <sup>2</sup> Soil depth

## 2.2 Study Design

The study was conducted using survey design to collect soil samples for isolation of *Actinomycetes* and experimental design for laboratory work.

### 2.2.1. Field Survey

A cross sectional survey design in line transect was used to collect soil samples for investigating soil physicochemical characteristics from diverse land use systems. The cross-sectional survey method was suitable for this study because it allows for the collection of data at a single point in time from a representative sample of the population being studied (Cherry, 2022). This method is useful for evaluating the prevalence of a particular characteristic or condition within a population under its existing conditions and can be used to study the relationships between different variables [23].

## 2.2.2 Experimental design

A 4x6x3 factorial experiment laid in completely randomized design was used in determining *Actinomycetes* population in land use system and media type culture.

## 2.3 Soil Samples Collection

Soil samples were collected from six different land use systems within the study area. These land use systems included uncultivated and degraded soils in the Mt. Kenya forest, cultivated soils from agricultural land, cattle manure from Chuka University, chicken manure from Ikuu farm, and soils from Chuka municipal solid waste dumpsite. A spiral soil auger was employed to collect soil samples, with the auger cleaned using 10% formalin after each sampling to prevent cross-contamination. Sampling was conducted at soil depth of 7.5, 5 and 2.5 cm, taking into account factors such as organic matter content, microbial abundance, and soil property variations [24]. For each designated depth and land use system, approximately 100 g of soil was collected using sterile brown paper bags to avoid contamination. After collection, each soil sample was labelled with appropriately indicating the sampling depth, land use system, and sampling location to ensure proper identification and traceability of the samples throughout the study. Subsequently, the labelled soil samples were packed in ice box and were transported to the microbiology laboratory at Chuka University. In the laboratory, the samples were maintained at a 4° C until they were ready for further analysis. This storage temperature was chosen to minimize microbial activity and maintain the physicochemical characteristics of the soil samples.

## 2.4 DETERMINATION OF SOIL PHYSICOCHEMICAL PROPERTIES

The soil samples collected were analyzed for various parameters, including soil pH, electrical conductivity (EC), soil moisture content (SMC), organic carbon (OC), soil available sodium (Na), phosphorus (P), potassium (K), and magnesium (Mg). The electrical conductivity of the soil sample was determined following the method described by Oyetola et al. [25]. Ten grams of air-dried soil sample were placed in nine beakers, and then 50 mL of distilled water was added to achieve a ratio of 1:5 (soil to water). The mixtures were stirred with a glass rod for 30 minutes and left undisturbed for 1 hour. The soil particles settled down, and the EC value was measured using an electrical conductivity meter (ELMEIRON, EC-60, Germany) in  $EC(dSm^{-1})$ . Conductivity meter was calibrated using KCl solution before conducting the actual measurements to ensure the accuracy and reliability of the readings obtained from the meter.

To determine the soil temperature across different land use systems, representative sampling points were randomly selected. At each sampling point, soil temperature was measured at a depth of 5cm using a measuring tape. The consistent 5cm depth was applied to all land use systems for standardized comparisons. A mercury bulb thermometer was inserted into the soil at the predetermined depth, allowing it to adjust and stabilize to the surrounding soil temperature for 3 minutes before recording the temperature.

To determine soil organic carbon,  $K_2Cr_2O_7-H_2SO_4$  oxidation method was adopted as described by Aregahegn [26]. One gram of soil sample was treated with 5 ml of concentrated  $H_2SO_4$  for 4 hours, then with 5 ml of 0.5 M  $K_2Cr_2O_7$ . The mixture was then heated at 150 - 160°C for 5 minutes and then cooled at room temperature. The solution was transferred into a conical flask with 100 mL deionized water. The unreacted  $K_2Cr_2O_7$  was determined by titrating with 0.25 M  $FeSO_4$ .

The endpoint approached when the solution changed from dark green colour to blue to reddish-brown colour [27]. Percentage organic carbon was calculated using the formula,

$$\text{Organic carbon (\%)} = M \times \left( \frac{V1 - V2}{\text{mass of soil}} \right) \times 0.39$$

where, M= concentration of FeSO<sub>4</sub>, V1 =Volume of blank, V2 =Volume of FeSO<sub>4</sub>, 0.39 = constant.

To determine the pH of the soil sample, 10 grams of air-dried soil was added to nine 100 mL beakers. Then, 50 mL of distilled water was added to each beaker to create a soil-water slurry in a ratio of 1:5. After allowing the soil to settle, the pH of the water suspension was measured using a potable pH meter (TS-PC200) calibrated with buffer solutions of pH 4.0, 7.0, and 9.0, following the method described by Hegazy [28]. The experiment was repeated three times using soil samples collected from different sites. To determine the soil moisture content, the air-dried soil samples collected from different regions were placed in a hot air oven at 110°C. The soil samples were wrapped in pre-weighed aluminum foil (recorded as W1), and the final weight was recorded (W2). The samples were then dried in the oven until the soil was completely dry. The aluminum foil containing each dried soil sample was re-weighed, and the weight recorded (W3). The percentage of soil moisture content (% MC) was calculated according to Oyeyiola and Agbaje [29].

$$\text{MC (\%)} = \frac{\text{Weight of water}}{\text{weight of moist soil}} \times 100 = \frac{W2 - W3}{W2 - W1} \times 100$$

where; MC =moisture content , W1 = pre-weighed aluminum foil weight ; W2 = final weight, and W3 = reweighed soil weight

To determine the available soil potassium and sodium from soil samples, the shaking and centrifugation method was used after extraction with 0.5 M ammonium acetate, following the procedure described by [30]. Firstly, 5 g of air-dried soil was mixed with 20 ml of 1.0 M NH<sub>4</sub>OAc at pH 7.0 in a 50 ml falcon tube and shaken for 10 minutes. The supernatant was then decanted into a clean 100 ml flask to extract the potassium, and the extract was diluted to 100 ml with ammonium ethyl ethanoate and mixed. The available soil potassium was then measured using a flame photometer at 5 lbs. air pressure with the scale adjusted to zero reading, following the procedure of Mostar and Roy [31]. A standard solution of potassium chloride (20 ppm) was fed to the instrument, and the flame was adjusted to 100 reading. The readings of the standard solution were compared with the reading for potassium in the sample using a standard curve [30]. Finally, the concentrations of sodium and potassium were calculated using the formulae,

$$\text{K and Na cmolKg/l} = \frac{(Vs - Vb) \times V \times mcf}{10 \times M \times W}$$

where, Vs is concentration of Na or K in sample (mgL<sup>-1</sup>), Vb is concentration of K or Na in blank (ammonium acetate) (mg<sup>-1</sup>), V is total volume of ammonium leached (250mL), M is mass of airy dried soil (5 g) and W is atomic weights of Na or K.

To determine the available soil phosphorus and sodium, ICP-AES was used to measure the concentrations in parts per million (ppm). The test solution was prepared by diluting 1.5 L of lactic acid (90%) with 4.5 L of ultra-pure water and heating it at 96 - 98°C for 48 hours before cooling. Next, a 1.000 M NaOH solution was prepared by dissolving 20.27 g of 98.6% NaOH in 519.81 g of water. A third solution was prepared by dissolving 0.10 g of phenolphthalein in 100 mL of 95% ethanol. The molality of the lactic acid was calculated by titrating 1 mL of it with the NaOH solution. Titration of 4.99 g of lactic acid with 21.93 g of 1.000 M NaOH resulted in a lactic acid solution with a concentration of 4.22 moles/Kg. Acetic acid was also titrated with the NaOH solution. Finally, a stock solution (Al-solution) was prepared by mixing 392.07 g of

98.3% NH<sub>4</sub>OAc, 897.81 g of acetic acid, and 1183.60 g of lactic acid, which was then diluted to 5 litres using ultra-pure Millipore water. The final solution of 1 M ammonium lactate and 4 M acetic acid was further diluted 10 times and its pH adjusted to 3.75 ±0.05 before use. For the test, 5 g of dried soil sample was weighed and placed into a container to which 100.0 ml of the AL-solution was added. The samples were shaken for 1 hour and 30 minutes, and then filtered using a 150 mm filter paper. The soil's available phosphorus was extracted using 0.025 M HCl–0.03 M NH<sub>4</sub>F and measured by ammonium molybdate colorimetry.

To determine the available magnesium in soil, the method used was based on Sanledhin and Taye [32]. Firstly, a 10 mL portion of the ammonium acetate soil extract, obtained from cation exchange capacity and exchangeable bases extraction, was taken and transferred into a 250 mL Erlenmeyer flask. Then, 40 mL of distilled water was added to make the total volume 50 mL. Methyl orange (3 drops) and 1 M HCl were added until the color of the solution turned orange. The mixture was boiled for 3 minutes and allowed to cool to 60°C. Next, 2 mL of KCN solution and 7 mL of buffer solution were added to adjust the pH to approximately 10. Finally, the mixture was titrated with 0.1 M EDTA disodium salt until a pure blue color was obtained without any trace of red. The magnesium value was then calculated using the formula described by Sanledhin and Taye [32],

$$\text{Mg}^{2+} = \left( \frac{\text{meq}}{100\text{g}} \right) = \frac{N \times V \times T \times 100 \times \text{mcf}}{A \times S}$$

where, N is normality of EDTA, V is volume of EDTA required for sample, T is total volume of extract (250mL), A is sample taken for titration (10mL), and S is air dry weight of sample, Mcf is moisture correction factor.

## 2.5 Isolation of *Actinomycetes*

*Actinomycetes* were isolated by serial dilution method from the composite soil samples according to Gebreyohannes *et al.* [33]. After serial dilution, 0.1 ml of each sample was separately plated using spread plate technique in Luria Bertani Agar (M1), Starch Casein Agar and International Streptomyces Project (ISP-1) and (ISP-4) media. The plates were incubated at 28°C and colony growth observed on the 7<sup>th</sup> day after inoculation. After incubation, *Actinomycetes* isolates were distinguished from other microbial colonies using morphological characteristics [34]. Sub-culturing of isolates was done using nutrient agar. For the purpose of conducting further analysis, the pure cultures were stored at a temperature of 4°C in slant culture on Starch Casein Agar and in glycerol broth [35].

## 2.6 Enumeration of *Actinomycetes* population from Diverse Land Use System

Soil sample preparation from six land use systems was done by weighing 10 g of each soil sample and mixing it with 90 ml of sterile distilled water in a flask. The mixture was vortexed to make a suspension. The culture media used for enumeration included starch casein agar (SCA), Luria Bertani (M1) medium, ISP (Medium 1) and ISP (Medium 4). The media were supplemented with fluconazole (25 µg ml<sup>-1</sup>) and streptomycin (25 µg ml<sup>-1</sup>) to inhibit the growth of fungi and gram negative bacteria, respectively. All the plates were incubated at 30 °C for 14 days. After incubation, *Actinomycetes* colonies were counted using colony counter and the population of *Actinomycetes* was expressed as number of colony forming units (cfu/ml). The number of colonies to determine *Actinomycetes* population was obtained from six different land use systems and on four selective media type culture. Sub-culturing of isolates was done using nutrient agar. For the purpose of conducting further analysis, the pure cultures were stored at a temperature of 4°C in slant culture on Starch Casein Agar and in glycerol broth [35].

## 2.7 STATISTICAL ANALYSIS

The data obtained on *Actinomyces* population from soil samples collected in various land use systems, along with the *Actinomyces* count in different culture media, were subjected to analysis of variance (ANOVA) at significance value ( $P < 0.05$ ) using SAS version 9.4 [36]. Significant means were separated using the Least Significant Difference (LSD) at  $\alpha = 0.05$ . Correlation analysis was used to evaluate the relationship between *Actinomyces* population and soil physicochemical properties of the soil. Data obtained on the soil physicochemical parameters was also subjected to ANOVA and significant means separated using LSD at  $\alpha = 0.05$ .

## 3. RESULTS

### 3.1 Influence of Soil Physicochemical Characteristics across Diverse Land Use Systems

The results of this study revealed distinct variations in soil temperature among the different land use systems (Table 2). The soil samples collected from the dumpsite exhibited the highest temperature (30.5°C) while the soil samples obtained from intact forest soil, representing the forest zone, registered the lowest temperature of 19.0°C. The study findings presented a significant difference ( $P < 0.05$ ) in soil parameters across different land use systems. Specifically, the mean soil pH ranged from 4.37 in the intact soil to 7.42 in the dumpsite. Moreover, the dumpsite exhibited the highest available soil potassium concentration (76.89 ppm), while the intact soil had the lowest (20.67 ppm) (Table 2). In terms of available soil sodium, the mean ranged from 12.22 ppm in the intact soil to 32.17 ppm in the chicken manure. Additionally, the mean available soil magnesium ranged from 0.17 ppm in the intact soil to 0.54 ppm in the dumpsite soil. As for available phosphorus, the mean ranged from 0.105 ppm in the chicken manure to 0.307 ppm in the dumpsite soil. The study also assessed the mean organic carbon content, which varied from 0.034% in the cultivated soil to 0.128% in the cattle manure. Furthermore, the electrical conductivity values ranged from 14.0 dSm<sup>-1</sup> in soils from deforested forest areas to 1548 dSm<sup>-1</sup> in cattle manure. Lastly, the mean soil moisture content ranged from 16.18% in uncultivated soils to 53.46% in chicken manure (Table 2)

Table 2: Influence of Soil Physicochemical Characteristics on different Land Uses in Meru South Sub-County in Kenya

Land uses	pH	Mg (ppm)	K (ppm)	P (ppm)	Na (ppm)	OC (%)	MC (%)	EC(dSm <sup>-1</sup> )	Temp(° C)
DS	7.42 a	0.54a	76.89a	0.307b	30.01a	0.076b	31.85b	613.00b	30.5
CM1	6.33ab	0.25bc	51.44b	0.105d	32.17a	0.079b	53.46a	14.00c	26.0
CS	5.95b	0.21cd	42.89c	0.106cd	23.67b	0.034d	16.18d	17.93c	25.0
DFZ	5.46abc	0.22cd	39.67d	0.107c	16.50c	0.082b	20.31c	513.67b	24.5
CM2	5.29bc	0.30b	31.33e	0.333a	14.18cd	0.128a	22.37c	1548.00a	27.5
IS	4.38c	0.17d	20.67f	0.106c	12.22d	0.071c	20.02c	31.44c	19.0
Mean	5.81	0.279	43.81	0.178	0.078	0.078	27.37	456.34	
LSD	1.447	0.064	3.054	0.001	0.030	0.0204	2.991	223.37	

CV(%) 13.69 9.21 3.831 0.43 21.46 27.39 6.007 20.871

<sup>a</sup>Means followed by the same letters are not significantly different at 5% probability level. DS = Dumpsite, CM1 = Chicken manure, CS = Cultivated soil, DFZ = Deforested zone, CM2 = Cattle manure, IS = Intact soil.

### 3.2 Association between Soil Physicochemical Characteristics and *Actinomyces* Population

The correlation analysis between soil physico-chemical characteristics and *Actinomyces* population revealed that there was a moderate positive and significant correlation between *Actinomyces* population and soil pH ( $r = 0.63407$ ;  $p < 0.0001$ ; Table 3). Additionally, weaker positive and significant correlations were observed between *Actinomyces* load and soil available potassium ( $r = 0.31483$ ;  $p < 0.0001$ ), as well as between soil moisture content and *Actinomyces* load ( $r = 0.3375$ ;  $p = 0.012$ ). On the other hand, no significant correlations were found between *Actinomyces* and soil available sodium ( $r = 0.2524$ ;  $p = 0.06$ ), available magnesium ( $r = 0.2455$ ;  $p = 0.07$ ), available soil phosphorus ( $r = 0.19217$ ;  $p = 0.16$ ), electrical conductivity ( $r = 0.1296$ ;  $p = 0.35$ ) and between *Actinomyces* load and organic carbon content ( $r = -0.030$ ;  $p = 0.828$ ).

	pH	K (Kg/ha)	Na (kg/ha)	Mg (ppm)	P(ppm)	SOC (%)	EC(ds/m)	SMC	TAP (cfu/ml) $\times 10^5$
pH	1								
K (Kg/ha)	0.52153 ( $<.0001$ )	1							
Na (kg/ha)	0.54201 ( $<.0001$ )	0.80551 ( $<.0001$ )	1						
Mg (ppm)	0.54866 ( $<.0001$ )	0.70977 ( $<.0001$ )	0.52028 ( $<.0001$ )	1					
P(ppm)	0.19217 (0.1639)	-0.14007 (0.3124)	-0.23573 (0.0862)	-0.00624 (0.9643)	1				
SOC (%)	-0.05775 (0.6783)	0.03817 (0.7841)	-0.07893 (0.5705)	0.23192 (0.0915)	0.33047 (0.0147)	1			
EC (ds/m)	0.24257 (0.0772)	0.00012 (0.9993)	-0.03564 (0.7981)	0.19564 (0.1563)	0.29003 (0.0334)	0.55211 ( $<.0001$ )	1		
SMC	0.30330 (0.0258)	0.45650 (0.0005)	0.60473 ( $<.0001$ )	0.35023 (0.0094)	-0.04108 (0.7681)	0.07493 (0.5902)	0.1960 (0.1553)	1	
TAP(cfu/ml* $10^5$ )	0.63407 ( $<.0001$ )	0.31483 (0.0204)	0.25247 (0.0655)	0.50698 ( $<.0001$ )	0.24559 (0.0735)	-0.03015 (0.8287)	0.1296 (0.3502)	0.3375 (0.0126)	1
Minimum	4.32	13.00	7.400	0.138	0.097	0.0297	11.6	14.199	100000
Maximum	7.85	123.0	72.80	0.714	0.804	0.158	2440	81.96	610000
Mean $\pm$ SD	5.81 1.09	43.81 30.67	21.48 16.68	0.278 0.160	0.178 0.209	0.078 0.034	456.34 681.39	27.37 17.20	309815 10881

Table 3: Correlation coefficient between soil physicochemical characteristics and *Actinomyces* Population in Meru South Subcounty

NOTE: TAP=Total *Actinomyces* population; EC=Electrical conductivity; SMC=soil moisture content; SOC=Soil organic content; P=phosphorous; Mg=Magnesium; Na=Sodium; K=Potassium

### 3.3 Morphological Characterization of *Actinomyces* Isolates using Selective Culture Media

There was variability in the morphology and colour of the isolates, which ranged from grey, pink, brown, cream, yellow, red, green-yellow, and cream-white (Plate 1). Although some cultures appeared similar on the surface, they were different when viewed from the reverse side of the plate. For instance, some had grey, white, or cream on the front side but had

brown, yellow, pink, or grey on the reverse side. The mycelium of *Actinomycetes* exhibited diverse colours in colonies, and both substrate and aerial mycelium were capable of producing pigments that caused the colour variations in some colonies.

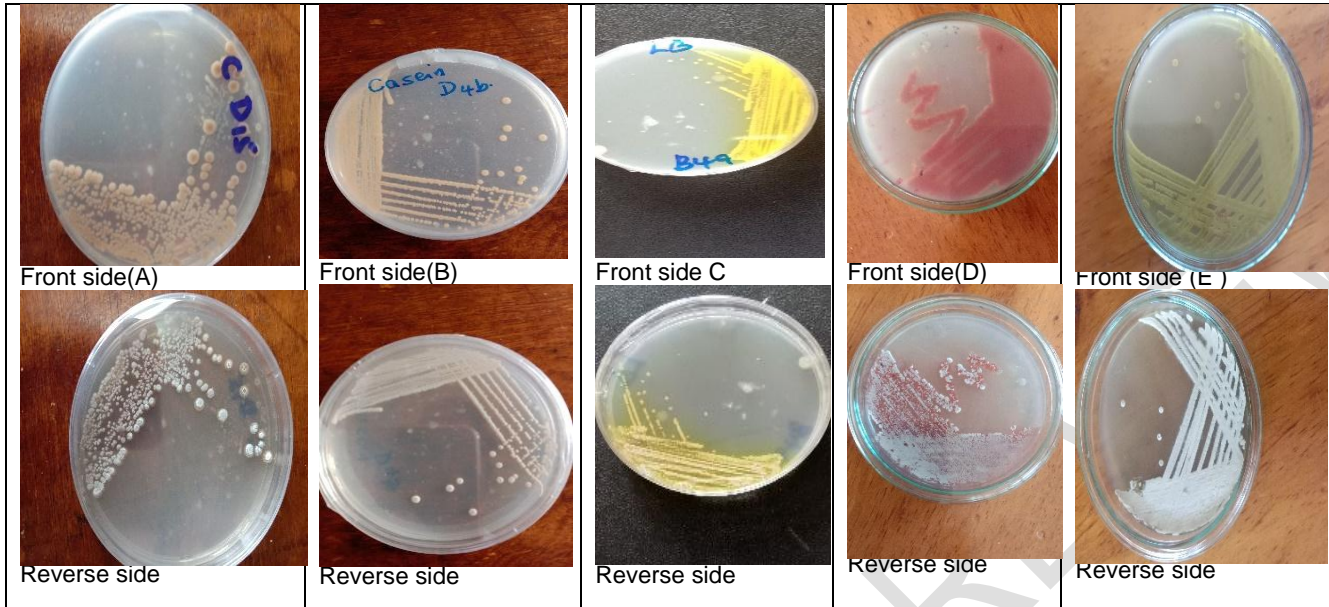


Plate 1: Colony morphology of some isolate showing sporulation on front and reverse side; Medium used: A and B=(Starch casein Agar); C=(Modified Luria Bertani); D=(ISP-1); E=(ISP-4)

### 3.4 Effect of Culture Media on Number of *Actinomycetes* Colonies

The mean number of *Actinomycetes* colonies varied significantly ( $P < .05$ ) among the different growth media. The highest number of colonies was observed in the Starch casein medium ( $3.4 \times 10^5$  cfu/ml of soil) while the lowest was observed in the modified Luria Bertani ( $1.7 \times 10^5$  cfu/ml; Table 4).

Table 4: Effect of culture media on number of *Actinomycetes* isolates

Media type cultures	Population (cfu/ml; $1 \times 10^5$ )
Starch casein agar (SCA)	3.4037a
International Streptomyces Project (ISP-4)	2.7500b
International Streptomyces Project (ISP-1)	2.3240c
Modified Luria Bertani (M1)	1.7019d
Mean	2.5439
LSD	0.1015
CV (%)	5.9456

<sup>a</sup>Means followed by the same letters are not significantly different at 5% probability level.

### 3.5 Population of *Actinomycetes* in Soils from different Land Use System

The results revealed a significant difference ( $P < .05$ ) in *Actinomycetes* population among the land use systems. The dumpsite exhibited the highest *Actinomycetes* population, with an average count of  $3.21 \times 10^5$  cfu/ml of soil sample (Table 5). In contrast, chicken manure had the lowest *Actinomycetes* population, with an average count of  $2.02 \times 10^5$  cfu/ml of soil sample, but not significantly different from deforested zone and cattle manure.

Table 5: Population of *Actinomycetes* in different land use systems

Land use systems	Population (cfu/ml; $1 \times 10^5$ )
Dumpsite	3.2194a
Cultivated soil	3.0583b
Intact soil(forest zone)	2.4722c
Deforested zone	2.3028cd
Cattle manure	2.1889d
Chicken manure	2.0222d
Mean	2.5439
LSD	0.1243
CV(%)	5.9456

<sup>a</sup>Means followed by the same letters are not significantly different at 5% probability level. Where cfu = colony forming units.

### 3.6 Combined effect of land use system and culture media on *Actinomycetes* Population

The results of this study revealed that there was significant combined effect ( $P < .05$ ) of culture media and land use system on the *Actinomycetes* population (Table 6). A combination of starch casein agar (culture media) and dumpsite (land use system) gave the highest number of colonies ( $5.54 \times 10^5$  cfu/ml of soil) while a combination of MI (culture media) and cultivated soils (land use system) gave the lowest number of colonies ( $1.33 \times 10^5$  cfu/ml of soil) of the *Actinomycetes*.

Table 6: Combine effect of land use system and culture media on *Actinomycetes* population

Land use systems	Media type culture	<i>Actinomycetes</i> population (cfu/ml; $1 \times 10^5$ )
Dumpsite	SCA	5.54444a
Cultivated soil	SCA	4.2222b

Cultivated soil	ISP-4	3.9000c
Intact soil	SCA	2.9444d
Intact soil	ISP-4	2.8899de
Deforested zone	SCA	2.7778de
Cultivated soil	ISP-1	2.7778de
Dumpsite	ISP-4	2.7222de
Cattle manure	SCA	2.6778e
Dumpsite	ISP-1	2.6667e
Chicken manure	ISP-4	2.3889f
Deforested zone	ISP-4	2.3111f
Cattle manure	ISP-4	2.2889fg
Chicken manure	SCA	2.2556fg
Cattle manure	ISP-1	2.2444fgh
Deforested zone	ISP-1	2.2335fgh
Intact soil	ISP-1	2.0566fghi
Intact soil	M1	2.0000hi
Chicken manure	ISP-1	1.9445i
Dumpsite	M1	1.9444i
Deforested zone	M1	1.8889i
Cattle manure	M1	1.5444j
Cattle manure	M1	1.5000j
Cultivated soil	M1	1.3333j
Mean		2.5439
LSD		0.2486
CV(%)		5.9456

<sup>a</sup>Means followed by the same letters are not significantly different at 5% probability level. NOTE: cfu = colony forming units. Note: SCA= Starch casein agar; ISP-4= International Streptomyces Project-4; ISP-1= International Streptomyces Project-1 and M1= Modified Luria Bertani.

#### 4. DISCUSSION

This study showed that the population of *Actinomycetes* is significantly influenced by physiochemical properties of the soil. Soil temperature is important environmental factor that affects *Actinomycetes* populations. The findings of this study indicated that the largest population of *Actinomycetes* was observed in the soil obtained from a dumpsite, where the temperature recorded was 30.5°C. Additionally, soil samples from chicken manure, which had a slightly lower temperature of 27.5°C, also exhibited a considerable presence of *Actinomycetes*. The dumpsite environment might have provided favorable conditions for *Actinomycetes* growth, such as organic matter and nutrient availability. The decomposition of waste materials in the dumpsite could have supplied a rich source of organic compounds that *Actinomycetes* thrive on. Moreover, the relatively higher temperature at the dumpsite compared to the chicken manure samples might have offered an optimal range for the growth and metabolic activities of *Actinomycetes*. Overall, the combination of nutrient availability and suitable temperature conditions likely played a significant role in the high population of *Actinomycetes* observed in the soil from the dumpsite.

The present study revealed significant differences ( $P < 0.05$ ) in soil pH studied among the various land use systems. The mean soil pH ranged from 4.37 in the intact soil to 7.42 in the dumpsite, suggesting that the dumpsite soil exhibited higher alkalinity than the intact soil. This alkalinity can be attributed to the deposition of alkaline materials in the dumpsite, resulting in higher pH values. Similar findings have been reported by Karanja *et al.* [37] the *Actinomyces* population was negatively correlated with soil pH, with higher populations observed in soils with pH values between 6.5 and 7.5 on the study on microbial diversity and abundance in the soils of the Nairobi city dumpsite, Kenya. The findings of the present study indicate that *Actinomyces* populations were at their lowest within forested areas, which are characterized by slightly acidic soil conditions with a pH of 4.4. Forested areas typically exhibit acidic soil due to the accumulation of organic matter and litter on the forest floor.

The concentrations of available sodium, magnesium, and phosphorus also varied among the different land uses. The higher concentration of available sodium in chicken manure suggests that the input of sodium-rich organic matter from manure can influence the soil's sodium content. Similarly, the dumpsite soil exhibited higher concentrations of available magnesium and phosphorus compared to the intact soil (forest zone), indicating the influence of waste deposition on these nutrient levels. *Actinomyces* are known to play a crucial role in nutrient cycling, including the decomposition of organic matter and mineralization of nutrients [38]. Therefore, variations in nutrient availability among land uses may affect the abundance and functional potential of *Actinomyces*. The organic carbon content in soils varied among land uses, with higher values observed in cattle manure compared to cultivated soil. This highlights the contribution of organic matter from animal waste, such as cattle manure, to soil fertility. Therefore, the higher organic carbon content in cattle manure may support the growth and activity of *Actinomyces* in these soils.

Electrical conductivity, a measure of dissolved salts, varied greatly among the land use systems. The values ranged from  $14.0 \text{ dSm}^{-1}$  in the soils from deforested forest areas to  $1548 \text{ dSm}^{-1}$  in cattle manure. The high electrical conductivity in cattle manure indicates a higher concentration of dissolved salts compared to the deforested forest soils. Soil electrical conductivity (EC) can affect the growth and survival of microorganisms, including *Actinomyces*, which are important decomposers of organic matter in soil. A study by Devi *et al.* [39] in India found that *Actinomyces* population and load in cattle and chicken manure were positively correlated with soil electrical conductivity, possibly due to the increased nutrient availability. In a dumpsite, high soil electrical conductivity can result from the accumulation of salts and other pollutants, which can negatively impact the growth and activity of *Actinomyces*. This is contrast to the present study findings where population in the dumpsite was higher regardless of high electrical conductivity. A study report by Kioko *et al.* [40] in Kenya found that *Actinomyces* population in a dumpsite was significantly reduced compared to a control site with lower soil electrical conductivity.

The mean soil moisture content ranged from 16.18% in cultivated soils to 53.46% in chicken manure. The higher moisture content in chicken manure can be attributed to its organic nature and water-holding capacity. *Actinomyces* are aerobic bacteria and require sufficient oxygen for their growth and activity. Soil moisture content affects the availability of oxygen in soil, hence, it influences the growth and activity of *Actinomyces*. Similar findings were reported study by Jaiswal *et al.* [41], on the study on influence of farmyard manure moisture content on *Actinomyces* population and diversity.

The study also investigated the association of soil physico-chemical characteristics on the population of *Actinomycetes*. The findings of the study revealed several significant correlations between *Actinomycetes* population and different soil properties, shedding light on the factors that may affect the abundance of *Actinomycetes* in soil. There was a moderate positive correlation was observed between *Actinomycetes* population and soil pH ( $r = 0.63407$ ;  $p < 0.0001$ ). This indicates that as soil pH increases, the abundance of *Actinomycetes* tends to increase as well. This finding aligns with previous research on soil pH and plant diversity shape soil bacterial community structure in Northeastern China that has shown *Actinomycetes* to be more prevalent in alkaline soils [42]. The pH of soil plays a vital role in influencing microbial communities and their metabolic activities, and *Actinomycetes* seem to favor slightly alkaline environments. Another study demonstrated that, *Actinomycetes* populations tend to increase in acidic soils due to the favorable conditions for their growth [9].

There was a weaker positive correlation was found between *Actinomycetes* population and soil available potassium ( $r = 0.31483$ ;  $p < 0.0001$ ). This suggests that higher levels of available potassium in the soil are associated with increased *Actinomycetes* abundance. This finding aligns with the work of Johnson *et al.* [43], who reported a positive relationship between *Actinomycetes* populations and potassium levels in agricultural soils. Potassium is an essential macronutrient for microbial growth and metabolism, and its availability in the soil can affect microbial community composition [44]. *Actinomycetes* may benefit from potassium availability, leading to their higher abundance in potassium-rich soils.

On the other hand, no significant correlations were observed between *Actinomycetes* population and soil available sodium ( $r = 0.2524$ ;  $p = 0.06$ ). These results suggest that changes in available sodium levels do not have a substantial influence on *Actinomycetes* abundance. Previous studies have reported mixed results regarding the effects of sodium on microbial communities, indicating that *Actinomycetes* may be less sensitive to variations in this specific soil properties [45]. Similarly, no correlation was observed between *Actinomycetes* population and soil available magnesium ( $r = 0.2455$ ;  $p = 0.07$ ). This indicates that magnesium levels in the soil may not be a critical factor influencing *Actinomycetes* populations. The lack of correlation between *Actinomycetes* and magnesium is supported by the study of Li *et al.* [46], which found no significant relationship between *Actinomycetes* abundance and magnesium content in forest soils. There was no correlation was found between *Actinomycetes* population and organic carbon content ( $r = -0.030$ ;  $p = 0.828$ ). This suggests that the organic carbon levels in the soil may not be a significant effect on *Actinomycetes* abundance. The lack of correlation between *Actinomycetes* and organic carbon is consistent with the study conducted by Wu *et al.* [47], which found no significant relationship between *Actinomycetes* populations and organic carbon content in arable soils.

A weaker positive and significant correlation was observed between *Actinomycetes* population and soil moisture content ( $r = 0.3375$ ;  $p = 0.012$ ). This implies that *Actinomycetes* populations may be influenced by the moisture levels in the soil. This finding is supported by the work of Zhang *et al.* [48] which reported a positive relationship between *Actinomycetes* abundance and soil moisture content in grassland soils. There was no correlation found between *Actinomycetes* population and electrical conductivity ( $r = 0.1296$ ;  $p = 0.35$ ). This suggests that the electrical conductivity of the soil may not be a determining factor for *Actinomycetes* abundance. The lack of correlation between *Actinomycetes* and electrical conductivity is consistent with the findings of Liu *et al.* [48], who reported no significant relationship between *Actinomycetes* populations and electrical conductivity in paddy soils. However, the study findings was in contrast with a

study by Otieno *et al.* [49] in Kenya found that *Actinomycetes* population in agricultural soil were positively correlated with soil electrical conductivity, likely due to the increased nutrient availability from fertilizer application

The study revealed no correlation between *Actinomycetes* population and available soil phosphorus ( $r = 0.19217$ ;  $p = 0.16$ ). This indicates that the presence or abundance of *Actinomycetes* may not be strongly influenced by phosphorus availability in the soil. This finding aligns with the research conducted by Wang *et al.* [50] which found no significant correlation between *Actinomycetes* populations and phosphorus levels in agricultural soils. Changes in available phosphorus levels did not appear to be associated with *Actinomycetes* abundance. Phosphorus is an essential nutrient for microbial growth, and its availability often limits microbial activities in soil [51]. However, *Actinomycetes* may have different strategies for phosphorus acquisition or may not be as influenced by the available phosphorus levels as other microorganisms.

The study findings indicate significant variability in the morphology and color of the isolates of *Actinomycetes*. The observed colors of the colonies ranged from grey, pink, brown, cream, yellow, red, green-yellow, to cream-white. This diversity in coloration suggests the presence of different pigments produced by the mycelium of *Actinomycetes*. The study also noted that even though some cultures appeared similar on the surface, they were actually different when viewed from the reverse side of the plate. For example, certain colonies had grey, white, or cream color on the front side, but on the reverse side, they exhibited different colors such as brown, yellow, pink, or grey. This observation suggests that there might be complex interactions between the mycelium and the growth medium or environmental factors that influence the pigmentation on different sides of the colonies. Sowndhararajan and Kang [52] described that the colour of the aerial and substrate mycelia produced by *Actinomycetes* varied with different media. Environmental factors such as nutrient availability, pH, temperature, and light exposure have been reported to influence pigment production in *Actinomycetes* [53]. In addition, the present study revealed growth of colonies characterized by small compact, soft to hard colonies tenaciously adhering to the medium, the surface being either flat or elevated. Those characters were in line with the study conducted by Quinyuan *et al.* [54], that showed that colonies of *Actinomycetes* were powdery, consistency and stick firmly to agar surface; in culture media and produced hyphae and spore-like fungi from isolates obtained from terrestrial ecosystem in China.

The *Actinomycetes* population from different media type cultures showed that there was a significant difference ( $P < .05$ ) among the *Actinomycetes* isolates when exposed to selective growth media. The starch casein medium demonstrated the highest number of colonies, with a mean of  $3.4 \times 10^5$  cfu/ml of soil sample among the tested growth media. The findings of this study demonstrate that the choice of growth medium significantly impacts the growth and colony formation of *Actinomycetes*. These findings are consistent with previous studies that have reported the stimulatory effect of starch and casein on *Actinomycetes* growth [55]. The higher number of colonies observed in ISP-4 and ISP-1 media, though slightly lower than Starch casein medium, further highlights the importance of nutrient composition for *Actinomycetes* growth. On the other hand, the modified Luria Bertani (M1) medium exhibited the lowest number of colonies, with a mean of  $2.02 \times 10^5$  cfu/ml of soil sample. The Modified Luria Bertani (M1) medium, commonly used for the growth of bacteria, exhibited lower colony counts compared to Starch Casein Medium. This could be attributed to differences in nutrient availability and composition between the two media. According to study conducted by Chen *et al.* [56] on isolation *Actinomycetes* from soil samples obtained from Yunnan province, China, the M1 medium contains glucose, tryptone, and

yeast extract, which may not be as conducive to *Actinomyces* growth as the starch and casein present in Starch Casein Medium. The study findings also aligns with previous studies that have reported differential growth responses of *Actinomyces* to various media formulations [57].

The present study revealed that the number of *Actinomyces* isolated differed significantly amongst different land use system. The dumpsite, characterized by anthropogenic waste deposition, exhibited the highest *Actinomyces* population. The lowest *Actinomyces* population observed in chicken manure, a highly managed agricultural land use system, could be attributed to various factors such as frequent disturbances, high ammonia levels chemical inputs, and altered soil properties due to agricultural practices. These disturbances might have adversely affected the *Actinomyces* community, leading to a reduced population compared to other land use systems. The soil samples from intact soil from the forest zone exhibited the less *Actinomyces* population compared to the dumpsite. Forest ecosystems typically have well-established soil microbial communities that are adapted to low-nutrient conditions. The lower *Actinomyces* abundance in the forest soil could be attributed to the availability of limited organic inputs and the dominance of other microbial groups, such as bacteria and fungi, in nutrient cycling processes [58].

The findings of this study provide valuable insights into how culture media and land use systems influence the population of *Actinomyces* in soil. The significant combined effect ( $P < .05$ ) suggests that both factors work together to shape the microbial community. This could be attributed to the varying nutrient compositions and environmental conditions offered by different culture media and land use systems. The combination of starch casein agar as a culture medium and dumpsite as a land use system resulted in the highest number of *Actinomyces* colonies ( $5.54 \times 10^5$  cfu/ml of soil). Dumpsites are known to contain various organic wastes and pollutants, which may have provided a rich source of nutrients for *Actinomyces* growth. Additionally, the specific physicochemical properties of starch casein agar might have been particularly suitable for supporting *Actinomyces* proliferation. Conversely, the combination of MI culture medium and cultivated soils as the land use system yielded the lowest number of *Actinomyces* colonies ( $1.33 \times 10^5$  cfu/ml of soil). Cultivated soils often undergo regular tillage, pesticide applications, and other agricultural practices that could potentially affect the soil microbial community negatively. The MI culture medium might not have provided the necessary nutrients or conditions for the robust growth of *Actinomyces* in this particular environment.

## 5. CONCLUSION

The colony morphology, spore arrangement, and pigmentation of the isolated *Actinomyces* enabled preliminary identification and differentiation of the species. The type of media used in isolation of *Actinomyces* colonies found to significantly influence the growth and proliferation of *Actinomyces* colonies. The study assessed the impact of soil physicochemical characteristics on the load and distribution of *Actinomyces*. The concentrations of soil available potassium, sodium, magnesium, phosphorus, pH, organic carbon content, electrical conductivity, and moisture was content varied across different land use systems. The soil physico-chemical properties influence significantly the diversity and composition of *Actinomyces* communities. The land use systems influenced significantly the diversity and composition of *Actinomyces* communities.

## 6. RECOMMENDATION

The study recommends use of Starch casein medium in isolating *Actinomyces* in order to obtain a higher yield of colonies. Furthermore, the study recommends continued monitoring of soil physicochemical properties and *Actinomyces*

abundance in different land uses to understand the relationship between soil health and microbial diversity, which is vital for biotechnological applications.

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