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Influence of Pesticides Contamination on Microbial Population of Selected Farmlands

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ABSTRACT: *Pesticides play a pivotal role in agriculture by combating various pests and increasing crop yields. However, extensive use of pesticides can result in unintended consequences, including potential impact on soil microbes. This study was aimed at investigating the influence of pesticides contamination on microbial population of farmlands in Otuoke, Nigeria. Soil samples were collected from four pesticide treated farmlands designated Bakery 1, Bakery 2, Dorcas, and PGS. A farmland without pesticide treatment served as Control. Microbial population, physiochemical parameters and pesticide residue of samples were investigated using standard techniques. Results revealed significant differences in microbial populations between pesticide-contaminated soils and control. The highest bacterium isolated and occurrence (%) in each location was; Streptomyces coelicolor 42(45.7%), Proteus vulgaris 179(59.9%), Streptomyces scabies 41(38.7%), Streptococcus pyrogenes 101(44.7%), and Pseudomonas aeruginosa 69(40.6%) for Bakery 1, Bakery 2, Dorcas, PGS, and control respectively. Highest fungus isolated and occurrence (%) was; Rhodotorula glutini 51(82.3%), 31(77.5%), and 43(81.1%) for Bakery 1, Bakery 2, and Dorcas respectively; and Candida tropicalis 25(80.6%) and Lichtheimia hyalospora 4(28.6%) for PGS and Control respectively. Pesticide analysis showed that Paraquat dichloride, Endosulfan, Diazinon, and N-(phosphonomethyl) glycine were present in the soil samples with about 75% residue. Site-specific pesticide concentrations varied in soil samples, with Bakery 1 having the highest concentrations Endosulfan and Diazinon, bakery 2 had highest concentration of N-(phosphonomethyl) glycine, and PGS had the highest concentration of Paraquat. Physiochemical characteristics showed that temperature ranged from 28.70 – 26.70°C, electrical conductivity 508 – 365µS/cm, moisture content 7.50 - 3.10%, pH 6.90 -3.90, and organic matter 4.70 – 3.00%. Decreasing order of cation exchange capacity (CEC) in farmlands was PGS > Bakery 2 > Bakery 1 > Dorcas > Control. There was no significant difference (p > 0.05) in each parameter between locations.*

KEYWORDS: Pesticides, soil, cation exchange capacity, bacteria, fungi, population.

INTRODUCTION

Pesticides are chemical substances used for the prevention of plant diseases, weeds and for increasing the yield as well as productivity of food products. More so, these pesticides interact with environment by altering the properties of microbial population by producing adverse impacts because most farmers apply them excessively on their farmlands. Pesticides are absorbed by soil particles which are transported to plants as well as animals through food chain and severely affect the ecosystem by causing acute or chronic disorders in people of all ages. Similarly, these

pesticides can likely cause serious threats to the aquatic ecosystem after their release by comprising different toxic substances, heavy metals and contaminants. However, pesticides are a diverse group of chemical compounds designed to manage and control pests that can threaten agriculture, public health, and ecosystems (Khalid *et al*., 2020). They play a crucial role in modern agriculture, safeguarding crop yields and minimizing the impact of diseases and pests on food production. Pesticides come in various forms, each with distinct properties and uses, and they are employed across different sectors, including agriculture, public health, and forestry (Tripathi *et al*., 2020). The use of pesticides in agriculture has become ubiquitous as a means to combat pests that threaten crop productivity. Pesticides are classified into several categories based on their intended target organisms and modes of action (Kaur *et al*., 2019). Classes of pesticides used in agriculture, horticulture, public health, and pest management include insecticides, herbicides, fungicides, rodenticides, nematicides, bactericides and virucides (Tadeo *et al*., 2019; Abubakar *et al*., 2020) each designed to target specific groups of pests (Khalid *et al*., 2020; Raffa and Chiampo, 2021). Their application methods encompass a range of techniques, such as aerial spraying, soil treatments, and seed coatings. This extensive use of pesticides reflects their role in modern agriculture to ensure food security and high crop yields. Numerous studies have explored the impact of pesticides on soil microorganisms. These investigations have revealed that pesticides can have both short-term and long-term effects on soil microbial communities. Short-term impacts include shifts in microbial composition, alterations in diversity and richness, and disruptions in microbial activities, such as enzyme inhibition. The mechanisms underlying these effects are multifaceted, with some pesticides directly toxic to microorganisms and others indirectly affecting them through modifications of the soil environment (Tripathi *et al*., 2020).

Despite the benefits of pesticide use in pest management and increased agricultural production, concerns have emerged regarding their potential impact on the environment (Dugan *et al*., 2023). Of particular concern is the contamination of soils by pesticide residues. Pesticides can persist in the environment for varying durations, leading to soil contamination. This contamination is the focal point of apprehension due to its potential consequences for human health, soil health, microbial communities, agricultural sustainability, and environmental integrity (Ikpesu and Ariyo, 2013). A growing body of research indicates that pesticides contamination can have long-term consequences for soil health. Pesticide residues can accumulate in soil over time, potentially leading to chronic effects on microbial populations (Kaur *et al*., 2019). These effects may include changes in community stability or resilience, selection for pesticide-resistant microorganisms, impacts on long-term soil fertility, and even soil degradation and erosion.

The soil is a fundamental component of ecosystems, serving as a habitat for countless microorganisms and playing a central role in maintaining biodiversity and ecological balance (Lehmann *et al*., 2020). Soil is inhabited by a diverse community of microorganisms, including bacteria, fungi, archaea, protozoa, and nematodes, each contributing to the soil's health and functioning. Microorganisms in soil are pivotal in nutrient cycling, organic matter decomposition, and the suppression of plant pathogens, and symbiotic interactions with plants; making them essential to the soil's vitality (Hayatsu *et al*., 2021). Pesticides are chemicals designed to control or eliminate pests that can harm crops, but their use has raised significant concerns about their impact on the environment. The widespread use of pesticides has several unintended consequences, including their significant impact on soil microorganisms. Some of these consequences include alterations in microbial community composition, reduction in microbial biomass, impact on beneficial microbes, and disruption of nutrient cycling, reduced enzyme activity, and soil microbial resilience (Tripathi *et al*., 2020). Some microorganisms are more sensitive to pesticides than others, leading to shifts in community composition. These shifts can result in a decrease in the diversity of soil microorganisms, which may have long-term consequences for soil health and ecosystem stability (Cycoń *et al*., 2019).

In addition, pesticides can persist in the environment for extended periods. The persistence of pesticides in soil and their potential to accumulate pose significant ecological challenges (Dugan *et al*., 2023). This contamination can impact the composition, diversity, and function of soil microbial populations. Microorganisms play a fundamental role in soil health, nutrient cycling, and overall ecosystem sustainability. Pesticides have the potential to disrupt the equilibrium of microbial communities in soil, which can have far-reaching consequences for agricultural productivity and environmental sustainability. The persistence and breakdown of pesticides in soil are critical factors that influence the environmental impact and effectiveness of pesticide applications (Khalid *et al*., 2020). It involves a complex and multifaceted process that is influenced by various factors, including the chemical properties of the

pesticide, soil composition, environmental conditions, and microbial activity. Pesticides can breakdown in soil through various processes such as microbial degradation, chemical hydrolysis, and so on (Sharma *et al*., 2020). Microbial degradation is a primary mechanism for breaking down organic pesticides using microorganisms. These microorganisms utilize pesticides as a source of carbon and energy, converting them into non-toxic metabolites. The rate of degradation depends on the pesticide's chemical structure and the microbial population in the soil (Khalid *et al*., 2020). Some pesticides can undergo hydrolysis, a chemical reaction in which water molecules break down the pesticide into simpler, less toxic compounds (Sharma *et al*., 2020). The pH of the soil can significantly affect the rate of hydrolysis. In most scenarios, pesticides exposed to sunlight can undergo photo-degradation (Khalid *et al*., 2020). Certain plants can uptake pesticides from the soil, and metabolize the pesticide within itself, helping to reduce their concentration in the soil (Sharma *et al*., 2020).

Soil is a vital component of ecosystems, playing a key role in maintaining biodiversity and ecological balance. Pesticide contamination has the potential to disrupt this ecological balance by affecting soil microbial communities, which, in turn, can have cascading effects on the entire ecosystem. The contamination of soil with pesticides is a significant form of environmental pollution. Pesticides, while designed to target specific pests, often have unintended consequences for non-target organisms, including soil microorganisms. Microorganisms in soil are responsible for nutrient cycling, making essential elements like nitrogen and phosphorus available to plants. Investigating the effects of pesticides on soil microorganisms is crucial to understanding the environmental impact of pesticide use. Hence, this study is important in the determination of the influence of pesticide contamination on the microbial population of selected farmlands in Otuoke.

The aim of this study is to determine the influence of pesticide contamination on the microbial population, physiochemical parameters and pesticide residue of soil of selected farmlands in Otuoke, Bayelsa State, Nigeria.

MATERIALS AND METHODS

Description of Study Area

The study area is Otuoke Community in Ogbia Local Government Area (LGA) of Bayelsa State, Nigeria, approximately at longitude 3^0 23’ 32” East and latitude 8^0 8’ 36” North. The community is one of the semi urban communities situated in Ogbia LGA of Bayelsa State in the Niger Delta region.

Materials and Reagents

Soil samples, cotton wool, incubator, autoclave, hot air oven, bunsen burner, weighing balance, wire loop, measuring cylinder, distilled water, test tubes, test tube rack, Pseudomonas Cetrimide agar (PCA), Starch casein agar (SCA), Potato dextrose agar (PDA), aluminium foil or foil paper, methylated spirit, beaker, conical flask, Petri Dish, syringe and needle, Nutrient agar (NA), Bijou bottles, Hydrogen peroxide, Iodine, Alcohol, Crystal violet, Gloves, Microscope, Safranine, Oil immersion, Triple sugar iron (TSI), Kovac’s reagent, spatula, Slides, Sterile container. Pesticides used were Diazinon ($C_{12}H_{21}N_{203}PS$), Paraquat ($C_{12}H_{14}N_2C_{l2}$), Endosulfan ($C_9H_6Cl_6O_3$) and N-(phosphonomethyl) glycine ($C_3H_8NO_5P$).

Sample collection

Loamy Soil samples were collected from four pesticide treated farmlands designated Bakery 1, Bakery 2, Dorcas, and PGS. A farmland without pesticide treatment in Esosie served as Control. All the 5 different farmlands were located in Otuoke. The first three farmlands were cultivated with cassava, pepper, maize, and vegetables, the fourth farmland was a flower bed, while the last farmland being the control, was a small vegetable garden. Soil samples were aseptically collected from a depth at 10 - 15cm of the soil with the aid of a sterile hand trowel and placed into a sterile container. Collected samples were appropriately labeled and put into an ice-packed cool box and immediately transported to the laboratory for microbiological and physicochemical analysis.

Media preparation and sample processing

Different media were prepared and used to culture and obtain microbial growth from the farmland soils and control samples. Nutrient agar is a general purpose, nutrient medium used for the cultivation of microbes supporting growth of a wide range of non-fastidious organisms. and contains many nutrients needed for the bacterial growth Cetrimide agar is a selective differential medium used for the isolation and identification of *Pseudomonas aeruginosa*. Starch casein agar (SCA) was used for cultivation and detection of saccharolytic marine bacteria and mostly Actinomycetes. Potato dextrose agar (PDA) is used for the cultivation of fungi, which is mold and yeast.

Serial dilution

In serial dilution, the density of cells is reduced in each step so that it is easier to calculate the concentration of the cells in the original solution by calculating the total dilution over the entire series. Eight sterile test tubes with 9ml of sterile distilled water was prepared and labelled 10^1 to 10^8. A quantity of 1gm of the soil sample was aseptically weighed and transferred into the test tube labelled as 10^1 and thoroughly mixed to uniformly disperse the soil microbes in the sterile distilled water. With the aid of a sterile glass pipette 1ml of the stock was aseptically transferred from the test tube labelled 10^1 into the test tube labelled as 10^2. Another 1ml from the test tube labelled as 10^2 was transferred into the test tube labelled as 10^3. This dilution was serially performed to 10^8. An aliquot of 1.0ml of the dilution folds 5 and 8 (10^5 and 10^8) were separately cultured using pour plate method.

Pour plate

This is one of the isolation methods used for counting the number of colony forming unit (CFU) present in a sample. CFU is a unit used to count the number of viable microorganisms. The sample prepared by serial dilution was set for use. The Petri dish was labelled with the name of culture, dilution fold/factor and date of inoculation. An aliquot of 1.0ml from the 10^5 dilution of the sample was aseptically inoculated into sterile Petri plates using a sterile pipette. The molten agar (40-45°C) was slowly poured into the inoculated plates and gently rotated in all directions. Cultured plates from all the soil samples were incubated at 37°C for 24-96 hours.

Isolation, Purification, Biochemical Characterization and Identification of Isolated Bacteria

After an incubation period of 24-48 hours, observed distinct colonies from the different media were sub-cultured on freshly prepared Nutrient agar in Petri dishes and incubated for 24hrs to obtain pure cultures. Many bacteria have inherent abilities to produce different types of enzymes and substances, or to utilize or ferment different types of sugars. Based on these abilities that distinguish them, Gram staining and different biochemical tests were performed on the isolated bacteria to properly identify the isolates.

Gram staining is used to differentiate bacteria based on the structure and composition of the cell wall (Ogodo *et al*., 2021). Gram positive bacteria appeared dark purple while Gram negative bacteria appeared pink. Other biochemical tests performed using the pure isolates were; catalase test (reiner, 2010), oxidase test, indole production test**,** methyl red test, citrate utilization test, Voges-Proskauer test, urease test, starch hydrolysis test, and triple sugar (TSI) test

Determination of soil Physicochemical Parameters

Determination of soil temperature

The gardener's thermometer is used to determine the temperature. The gardener's thermometer was placed into the soil sample and left to stay for around 5 minutes before reading the temperature of the soil sample.

Determination of the soil pH

This is accomplished by using a JENWAY 3510 pH meter that has been calibrated with buffers 4 and 7. 10 g of the sample was weighed into a 250 ml beaker, and 100 ml of distilled water was added and swirled for around 30 minutes

before being left for 2 hours. The pH electrode was immersed in the beaker for around 5 minutes before getting the reading.

Determination of electrical conductivity

The HANNA HI8733 electrical conductivity was used to determine the electrical conductivity. The electrical conductivity meter was calibrated first with a potassium chloride solution. 20 g of the sample was weighed into a 500 ml beaker, 200 ml of water was added, and the mixture was allowed to sit for 30 minutes. The electrode was dipped inside the beaker, and the electrical conductivity screen was used to obtain the reading.

Determination of organic matter

The muffle furnace is used to ascertain this 10 g of the sample was weighed into a porcelain dish and placed inside a muffle furnace set to 420 °C for 2 hours. The organic matter content was calculated using the formula below.

$$SOM = Mo\ /MD \times 100$$

Where: SOM = Soil organic matter, Mo is organic matter, MD is mass of dry soil, MA is mass of burned soil

$$Mo = MD - MA$$

Determination of Moisture content

A 10 g sample was weighed onto a porcelain dish and heated in a 105 °C hot air oven. The weight of the sample was examined every 30 minutes until it reached a steady weight. The soil moisture is computed using the formula:

$$\text{Moisture content} = \frac{\text{wet weight} - \text{dry weight}}{\text{Dry weight}}$$

Pesticide Extraction and Analysis

The procedure applied for the extraction of pesticides was similar to those reported by; Laab *et al* (2000), Steinwandter (1990s), and Von Duszeinj (1989). The samples were homogenized and dried with anhydrous sodium sulphate. A sub – samples of the sediment (10 – 20g) was weighed into a clean extraction bottle. Pesticides were extracted by ultrasonic extraction using a mixture of dichloromethane and n – hexane in a ratio 2:3, having been subjected to a vigorous shaking in a sonication bath for 5hrs. The solvent was separated and concentrated with a rotatory evaporator. Pre – elution was carried out with HPLC methanol. The concentrated extract was then analyzed for pesticides.

Sample Preparation for Analysis

Chemicals/Reagents

Methanol (HPLC analytical grade), Lindane (95.5% purity), Endosulfan (99% purity), Diazinon (98.5% purity) and N-(phosphonomethyl) glycine (99% purity) which were used as internal standard in HPLC analysis were obtained from chemical Service, West Chester, U.K.

Equipment/ Glass Wares

Cecil High performance Liquid Chromatography (HPLC) system comprised of CE 1200 High performance variable wavelength monitor and CEII00 liquid chromatography pump, UV detector with variable wavelength and stainless-steel column (C_{18} Reverse phase) packed with Octasilica; vacuum pump, ultrasonic check, weighing balance, 250ml Buckner flask, conical flask, separating funnels, funnels, volumetric flask (10, 100, 250 and 250ml), measuring cylinders, test tubes, pipettes, and filter paper.

Preparation of Internal Standards Stock Solution

Two 10mg/g stock solutions of each of the two internal standards were prepared. This was prepared by measuring 0.1g of each of the Lindane, Endosulfan, Diazinon and N-(phosphonomethyl) glycine standards into a 100ml volumetric flask. A small quantity of methanol was then added to each of the volumetric flasks to dissolve the standards and then shaken to mix properly. The solution was made up of the 100ml mark on the flask. The following concentrations (80, 60, 40, 20, and 10 mg/g) were prepared subsequently from the 100 mg/g stock solution. Then, from the 10 gm/g concentrations, lower concentrations up to 0.01 were prepared.

HPLC Mobile Phase Preparation

The solvent of the mobile phase of the HPLC is methanol and water (1:1). This was prepared by measuring 250ml of HPLC grade methanol into a 500ml flask and made up with 250ml of distilled water.

Activation of the HPLC System

The HPLC model CECIL 1010 was switched on. The wavelength of the system was determined by using UV visible equipment. Little quantity of the stock solution was diluted with methanol and its wavelength determined by scanning. A pack of 202nm was reached. The system wavelength was then set at 202nm and the sensitivity of the 0.05nm if the uv detector component set. The flow rate was set at 1ml/min. afterwards, the purging of the system commenced by allowing the system to run for some time. This was done to remove air from the system and also to make the colon charged. The purging was carried out through a washing solution of 30% methanol, 70% water.

Degassing the Mobile Phase Solution

Bubbling helium gas into the solution carried out degassing of the mobile phase. This was done to remove the air from the solution. The mobile phase was then set up and connected with HPLC system and allowed to run through the system of 20 minutes. The system was then separated following the procedure as contained in the operating manual.

Determination of Retention Time for Internal Standard

The internal standards for each of the pesticide were loaded and injected into system to determine their various retention time. The series of concentrations starting from 100ppm to 0.025ppm were loaded and injected and their chromatograph printed out. The resulting peak areas were then used to plot a graph against concentration to determine the linearity of each of the standard chromatographs. The retentions time for each of the standard are as follows: Lindane (4.90) Endosulfan (5.10), Diazinon (5.30) and N-(phosphonomethyl) glycine (5.88).

Statistical analysis

The data recorded during the investigation were subjected to statistical analysis. The analysis of variance (ANOVA) method was used to conduct a statistical analysis of the data using a two-factor analysis with replications.

RESULTS AND DISCUSSION

The result of the microbial counts obtained from the different farmlands is shown in Table 1. The total heterotrophic bacteria count shows considerable variation ranging from 3.0×10^9 CFU/g to 23.1×10^9 CFU/g. This variability may be attributed to the influence of pesticide contamination and the ability of the microorganisms to resist or tolerate the presence of pesticides. Studies by Aleruchi *et al*. (2020) demonstrated that pesticide contamination resulted in a reduction in the number of soil microorganisms present in the contaminated soil, thereby leading to a variation in the total heterotrophic count.

The Pseudomonal counts varied significantly across the farmlands, with the highest count observed in Control (6.9×10^6 CFU/g). The high prevalence of *Pseudomonas* species in the control could be attributed to their resilience to

environmental changes or their adaptability to the natural conditions in this farmland leading to a higher count (Laborda *et al*., 2021). This is also a proof that the pesticides have an influence on the microbial diversity. The highest Actinomycetes count was seen in PGS (11.3 x 10^6 CFU/g) which is likely due to its ability to thrive in this farmland despite the pesticide contamination.

Table 1: Microbial counts of the farmlands in the different locations

Farmland/ Location	**Microbial counts (CFU/g) of the farmlands**			
	Total heterotrophic Bacteria	**Pseudomonal**	**Actinomycetes**	**Fungi**
Bakery 1	3.0 x 10^9	-	6.2 x 10^6	7.6 x 10^6
Bakery 2	23.1 x 10^9	3.1 x 10^6	3.7 x 10^6	4.0 x 10^6
Dorcas	5.0 x 10^9	-	5.6 x 10^6	5.3 x 10^6
PGS	11.3 x 10^9	-	11.3 x 10^6	3.1 x 10^6
Control	3.7 x 10^9	6.9 x 10^6	6.4 x 10^6	1.4 x 10^6

On the other hand, the total fungal count shows considerable variation ranging from 1.4 x 10^6 CFU/g to 7.6 x 10^6 CFU/g. This variability may be attributed to the influence of pesticide contamination and the ability of the microorganisms to resist or tolerate it. Aleruchi *et al*. (2020) demonstrated that pesticide contamination resulted in a reduction in the number of soil microorganisms present in the contaminated soil, thereby leading to a variation in the total fungal count.

The presence of organophosphate and other pesticides which are designed to control pests and weeds, can have unintended consequences on non-target organisms, including fungi. A study by Srinivasulu and Ortiz (2017) demonstrated the effect of induced oxidative stress caused by pesticide contamination on fungal population. Sidhu *et al*. (2019) investigated the toxic effects of organophosphates on microbial population and demonstrated that some organophosphates interfere with the activities of enzymes involved in fungal cell metabolism, leading to impaired growth and reproduction.

The results of the cultural, morphological, biochemical characteristics and probable identity of bacteria isolated from the farmland soils are presented in Table 2.

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Table 2: Cultural, morphological, biochemical characteristics and probable identity of bacteria isolated from the farmland soils

Isolate code	Macroscopy (Cultural morphology)	Microscopy	Gram Reaction	Motility	Urease	Citrate	Voges-Proskauer	Methyl Red	Catalase	Oxidase	Indole	Starch Hydrolysis	TSI	Probable organism
PS1	Medium-sized, irregular margins with yellow-green appearance on PCA	Rod-shaped	-	+	-	+	-	-	+	+	-	-	-	*Pseudomonas aeruginosa*
SC1	Powdery, dry, filamentous, branched growth with blue pigment and fuzzy appearance on SCA	Elongated thread-like filamentous cells with aerial hyphae (chains of spores)	+	-	-	-	-	+	+	-	-	+	Nil	*Streptomyces coelicolor*
SC2	Irregular cream rough with raised elevation on SCA	Elongated filamentous cells with irregular filaments	+	-	-	-	-	+	+	-	-	+	Nil	*Streptomyces scabies*
SC3	Cream-colored with raised elevation, small in size, circular with irregular edge and a fine texture on SCA.	Irregular, branching filamentous cells that form clusters of threadlike structures that resemble fungal hyphae	+	-	-	-	-	+	-	-	-	+	+	*Actinomyces isrealii*
SC4	Golden brown with powdery texture and exhibits filamentous and branching growth pattern on SCA	Elongated, filamentous cells with a branching growth pattern	+	-	-	-	-	+	+	-	-	+	Nil	*Streptomyces aureofaciens*
SC5	Grey colored with powdery texture, irregular shaped, and exhibits filamentous and branching growth pattern on SCA	Filamentous, elongated threadlike cells that form complex branches of hyphae	+	-	-	-	-	+	+	-	-	+	Nil	*Streptomyces griseus*
SC6	White, dry, and powdery texture with raised irregular margin on SCA	Filamentous, branching cells like fungal hyphae	+	-	-	-	-	+	+	-	-	+	Nil	*Nocardia asteroides*
N1	Yellowish brown, undulate margin, transparent spread across the surface NA exhibiting swarming growth	Short rods with pink color	-	+	+	+	-	+	+	-	+	+	+	*Proteus vulgaris*
N2	Small, round, smooth and translucent with a raised elevation on NA	Cocci arranged in chains	+	-	-	-	-	+	-	-	-	-	+	*Streptococcus pyogenes*

N3	Cream colored, large, smooth, mucoid, and slightly raised glistening appearance on NA	Non-motile and non-spore forming rod shaped bacterium	-	-	+	+	+	-	+	-	-	+	+	*Klebsiella pneumoniae*
N4	Cream, large, round with raised elevation and having a convex appearance on NA	Rod-shaped organism. The vegetative cells appeared white, and the endospores appeared green in color	+	+	-	+	+	+	+	-	-	+	-	*Bacillus subtilis*

A total of 11 bacteria were isolated and identified with reference to the Bergey's manual of Determinative Bacteriology (Bergey, 1994). These organisms were identified as *Pseudomonas aeruginosa*, *Streptomyces coelicolor*, *Streptomyces scabies*, *Actinomyces isrealii*, *Streptomyces aureofaciens*, *Streptomyces griseus*, *Nocardia asteroids*, *Proteus vulgaris*, *Streptococcus pyogenes*, *Klebsiella pneumoniae*, and *Bacillus subtilis*. Some of these bacteria have been studied and realized to thrive as a response to environmental stress, possibly induced by pesticide contamination (Harir *et al*., 2018).

The distribution and occurrence (%) of bacteria isolated from the different farmlands is presented in Figure 1.

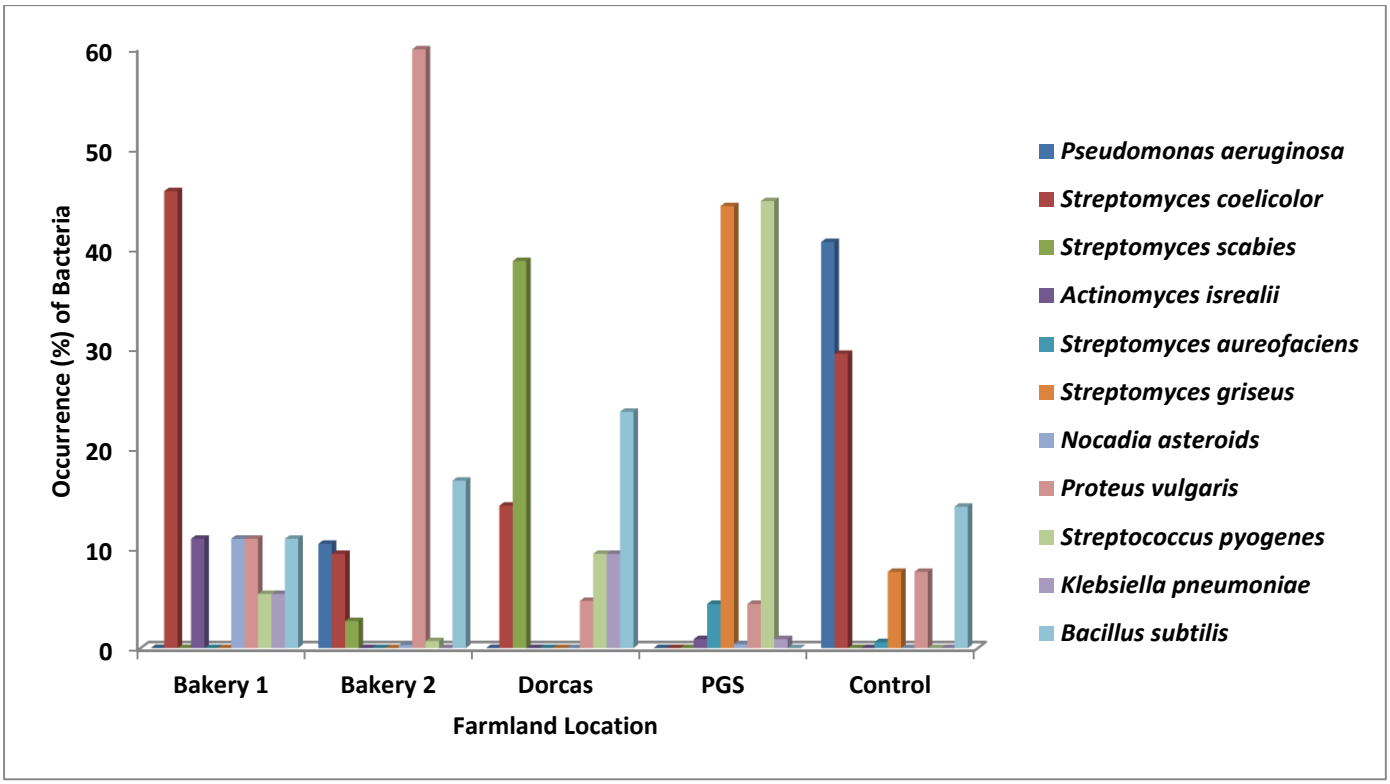

Fig. 1: Distribution and occurrence (%) of bacteria isolated from the different farmlands

Pseudomonas aeruginosa shows a significant presence at Bakery 2 (10.4%) and Control (40.6%), but it is absent at the other farmlands. Since *Pseudomonas aeruginosa* is known for its adaptability and can thrive in various conditions, its prevalence at Bakery 2 might indicate its ability to thrive despite the contamination of the pesticides (Diazinon) and environmental conditions in this farmland (Cabot *et al*., 2016). Research by Borad *et al*. (2022) has shown that *Pseudomonas* species can degrade Organophosphates (Diazinon) and thrive despite its presence in a farmland. The presence of *Pseudomonas aeruginosa* in the Control could be because of its ability to thrive in diverse environments and as part of the normal soil microbiota. This is in agreement with a study by Crone *et al*. (2020) where the ubiquitous nature of *Pseudomonas aeruginosa* was investigated, with results showing that it was present in all habitats. There could also be a possibility of runoffs into the control farmland from pesticide contaminated farmlands, introducing Organophosphates into the control farmland.

Streptomyces species are important for soil health, nutrient cycling, and usually associated with antibiotic production (Javed *et al*., 2021). *Streptomyces coelicolor* is found at varying percentages across all farmlands, but it's most dominant at Bakery 1 (45.7%) and Control (29.4%). Their presence suggests that these farmlands may have relatively healthier soil compared to others, possibly due to reduced pesticide contamination.

The dominance of *Streptomyces scabies* at Dorcas (38.7%) might indicate its adaptation to counteract the stress from pesticides. *Streptomyces aureofaciens* is more prevalent at PGS (4.4%) suggesting that this farmland might have a relatively healthier environment for these organisms. The prevalence of *Streptomyces griseus* at PGS (44.2%) could indicate a response to environmental stress, possibly induced by pesticide contamination (Harir *et al*., 2018).

The presence of *Actinomyces israelii* at Bakery 1 (10.9%) and PGS (0.9%) might be an indication of their resistance to the pesticides at these farmlands. *Nocardia asteroides* was found at varying low percentages at multiple farmlands. *Nocardia* species are involved in decomposing complex organic compounds, and their presence might indicate some adaptation to the soil conditions, including pesticide contamination (Traxler *et al*., 2022). *Proteus vulgaris* is dominant at Bakery 2 (59.9%) and present at other farmlands. The high presence of *Proteus vulgaris* at Bakery 2 might suggest that this species is more resistant to the pesticides used in that area. *Streptococcus pyogenes* is dominant at PGS (44.7%) and present at Bakery 1 (5.4%). Since *Streptococcus pyogenes* is not typically associated with soil health, its dominance at PGS might indicate some adaptation to the local conditions (Harir *et al*., 2018).

The variations in bacterial populations across different sampling sites reflect the influence of pesticide contamination, as well as other local environmental factors. Research studies on the impact of pesticides on soil microbiota have demonstrated changes in bacterial diversity and community composition in response to pesticide exposure (Shahid and Khan, 2022). This could also be because different microorganisms have varying environmental requirements and tolerances. Some may be more adapted to specific soil conditions, such as pH, moisture, organic matter content, and nutrient availability (Puissant *et al*., 2019). Also, as suggested in a study by Shahid and Khan (2022), some of the microorganisms may have developed resistance or tolerance to the pesticides used in these sampling sites, or due to reduced pesticide contamination. This resistance could be due to genetic adaptations that allow them to survive and proliferate in the presence of these chemical stressors.

A review by Imfeld and Vuilleumier (2012), discussed the effects of pesticides on the bacterial communities in soil, highlighting that pesticides can have both direct toxic effects on certain bacteria and indirect effects by altering the availability of nutrients in the soil, leading to shifts in bacterial populations. The dominance of specific bacterial species at certain farmlands could indicate their adaptability or resilience to the pesticides used in those farmlands, or their ability to thrive in diverse environments (Crone *et al*., 2020; Shahid and Khan, 2022).

Table 5: Morphological characteristics of fungal isolates

Isolate Code	Morphological characteristics	Probable organism
ISO1	Fast growing, grey and white colored and cottony colonies	*Alternaria alternata*
ISO2	White cottony appearance with a round (large) shape	*Fusarium oxysporum*
ISO3	Cream colored, smooth, moist, and glistening and round	*Candida tropicalis*
ISO4	Pink, smooth, moist, yeast like colonies and round	*Rhodotorula glutinis*
ISO5	Fast growing white long cottony appearance	*Lichtheimia hyalospora*
ISO6	White, dense, fluffy cottony appearance with brown reverse	*Rhizopus arrhizus*
ISO7	Irregular white cottony appearance with radial growth	*Fusarium chlamydosporium*
ISO8	White and black fluffy cottony appearance with dense growth	*Rhizopus stolonifera*

ISO9	White wrinkled velvety, raised elevation with light green center	*Penicillium camemberti*
ISO10	White large cottony appearance with smooth reverse	*Fusarium fumonisin*
ISO11	Filamentous white cottony appearance with spores	*Trichoderma* spp
ISO12	White with black spores with flat margin, raised center & yellow reverse	*Aspergillus flavus*
ISO13	Filamentous white opaque raised elevation and rough surface	*Phytophthora occultans*
ISO14	Fast growing, white with green center, dense spores with radial growth	*Penicillium italicum*
ISO15	White with black spores with pale yellow reverse	*Aspergillus niger*
ISO16	Irregular black cottony appearance with opaque edge and smooth reverse	*Chladosporium hyalospora*

The result of fungal population across the farmlands is shown on Figure 2. The fungi isolated were *Alternaria alternata*, *Fusarium oxysporum*, *Candida tropicalis*, *Rhodotorula glutinis*, *Lichtheimia hyalospora*, *Rhizopus arrhizus*, *Fusarium chlamydosporium*, *Rhizopus stolonifera*, *Penicillium camemberti*, *Fusarium fumonisin*, *Trichoderma* spp, *Aspergillus flavus*, *Phytophthora occultans*, *Penicillium italicum*, *Aspergillus niger*, and *Cladosporium hyalospora*.

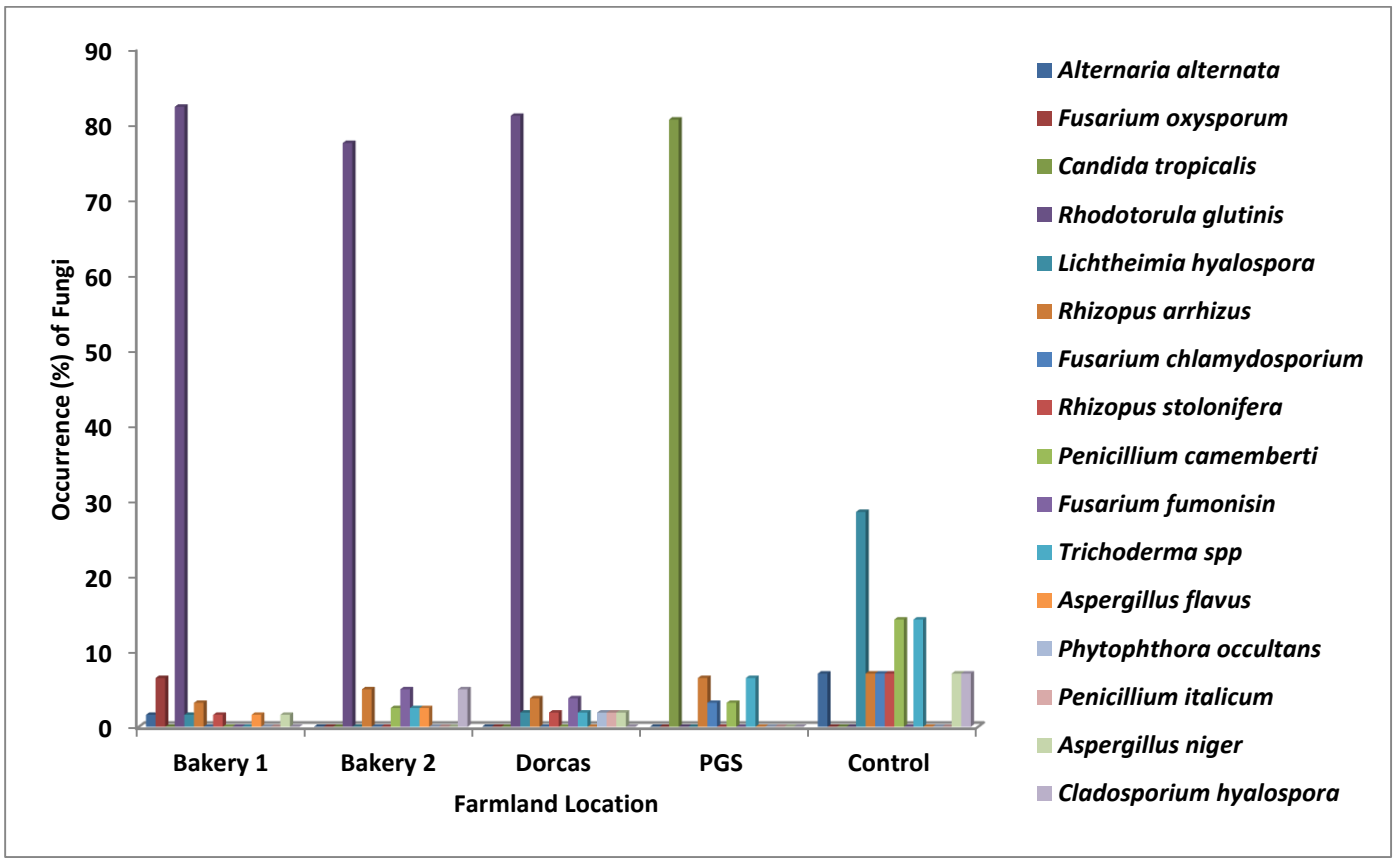

Fig. 2: Distribution and occurrence of fungi isolated from the farmland soils

The percentage of occurrence of various fungi population across the farmlands is displayed on Figure 2. *Alternaria alternata* is present at low percentages in multiple farmlands, with the highest occurrence at Control (7.1%). Its prevalence at this farmland may be because of its ability to exist as an endophytic fungus (Audenaert *et al*., 2014). It

may have established endophytic associations with the vegetable plants cultivated in this farmland, thereby residing within the plant tissues without causing apparent disease symptoms. *Fusarium oxysporum* is mostly absent in all farmlands, suggesting it may be less responsive to the pesticides used in these areas. *Rhodotorula glutinis* is prevalent at Bakery 1 (82.3%), Bakery 2 (77.5%), and Dorcas (81.1%). *Rhodotorula* species are known to be resilient to various stress factors, including pesticides (Suleiman *et al*., 2020). Their dominance in these farmlands might suggest their ability to thrive despite pesticide contamination. *Candida tropicalis* is dominant at PGS (80.6%). This might be due to its resilience to the environmental conditions and pesticides in use at these farmlands. On the other hand, *Lichtheimia hyalospora* (28.6%) had the highest population in the Control farmland. *Rhizopus arrhizus* is distributed across multiple farmlands but does not dominate in any, probably because it can adapt to varying soil conditions and pesticide levels (Kaerger *et al*., 2015). *Fusarium* species can be plant pathogens and may thrive where the environmental conditions are conducive to their growth. *Penicillium* species are diverse and may exhibit different responses to pesticides. The dominance of *Penicillium camemberti* at Control (14.3%) and its presence in other farmlands suggests adaptation to the environmental conditions at these farmlands. *Fusarium* species can produce mycotoxins and may be more resilient to certain pesticides.

The presence of *Fusarium fumonisin* at Bakery 2 (5%) and Dorcas (3.8%) suggests that they may be resilient to the pesticides used in these farmlands. *Trichoderma* species are found in multiple farmlands, with the highest occurrence at Control (14.3%). They are known for their biocontrol and plant growth-promoting properties and may be thriving in this farmland as part of the native soil microbial community (Manzar *et al*., 2022).

The soil was checked for pesticides (Paraquat dichloride, Endosulfan, Diazinon, and N-(phosphonomethyl) glycine). Seventy-five percent (75%) of pesticide residue was detected in the soil samples and presented on Table 8.

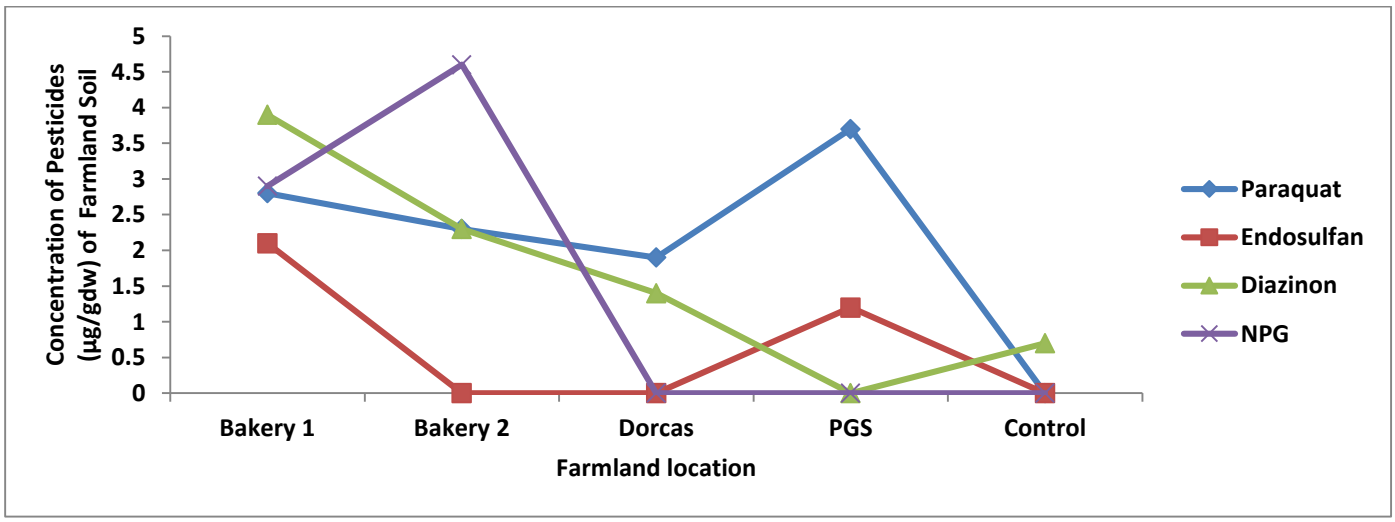

Fig. 3: Occurrence and distribution of pesticide residues in the various farmlands

The pesticide residues in migro gramme per gramme dry weight (µg/gdw) of soil across the farmlands are presented in Figure 5. Site-specific pesticide concentrations (NPF, diazinon, endosulfan, and paraquat dichloride) vary in sediment samples, with Bakery 1 station having the highest pesticide concentrations in increasing order; diazinon > paraquat > endosulfan > N-(phosphonomethyl) glycine.

Paraquat dichloride was detected in all the farmlands except the farmland that served as Control (Esosie), and it varies significantly ($p < 0.05$) between the sample farmlands and the control, between the PGS and the Bakery 1 and Bakery 2. No significant difference ($p > 0.05$) between the two farmlands (Table 7). Endosulfan was detected only in PGS and Bakery 1. Not detected in Bakery 2, Dorcas, and Control. There was no significant ($p > 0.05$) difference in the pesticide concentrations between PGS and Bakery 1.

Diazinon was not detected in PGS but in all the farmlands, including the control. The order of the occurrences of the diazinon is Bakery 1 > Bakery 2 > Dorcas > Control (Figure 5). The pesticide varies significantly ($p < 0.05$) between the sampling sites and the control. N-(phosphonomethyl) glycine was only detected in Bakery 1 and Bakery 2 and was not significant ($p > 0.05$).

Table 9: Physiochemical characteristics of the farmland soil samples (Given as mean ± standard error)

Farmland/Location	Temperature (°C)	Electrical conductivity (µS/cm)	Moisture content (%)	pH	Organic matter (%)
PGS	28.02± 0.10	478± 0.78	6.50 ± 0.90	5.80 ± 0.18	4.10 ± 0.20
Bakery 1	27.60 ± 0.30	506± 2.17	7.10 ± 1.06	4.58 ± 0.30	3.80 ± 0.17
Bakery 2	28.27 ± 0.19	389± 5.02	3.50 ± 0.20	5.70± 0.28	3.20 ± 0.10
Dorcas	28.16 ± 0.25	467± 1.80	4.37 ± 0.46	6.54 ± 0.20	4.06 ± 0.40
Control	28.80 ± 0.13	598± 2.54	5.90 ± 0.33	6.21 ± 0.12	3.10 ± 0.10

The physiochemical characteristics of the soil samples from the different sampling sites are shown in Table 9. The temperature ranged from 28.70 – 26.70°C. The ranges of electrical conductivity (µS/cm), moisture content (%), pH, and organic matter of the soil samples from the borough were 508 – 365, 7.50 -3.10, 6.90 -3.90, and 4.70 – 3.00, respectively. There was no significant difference ($p > 0.05$) in each parameter between the different selected farmlands in Otuoke.

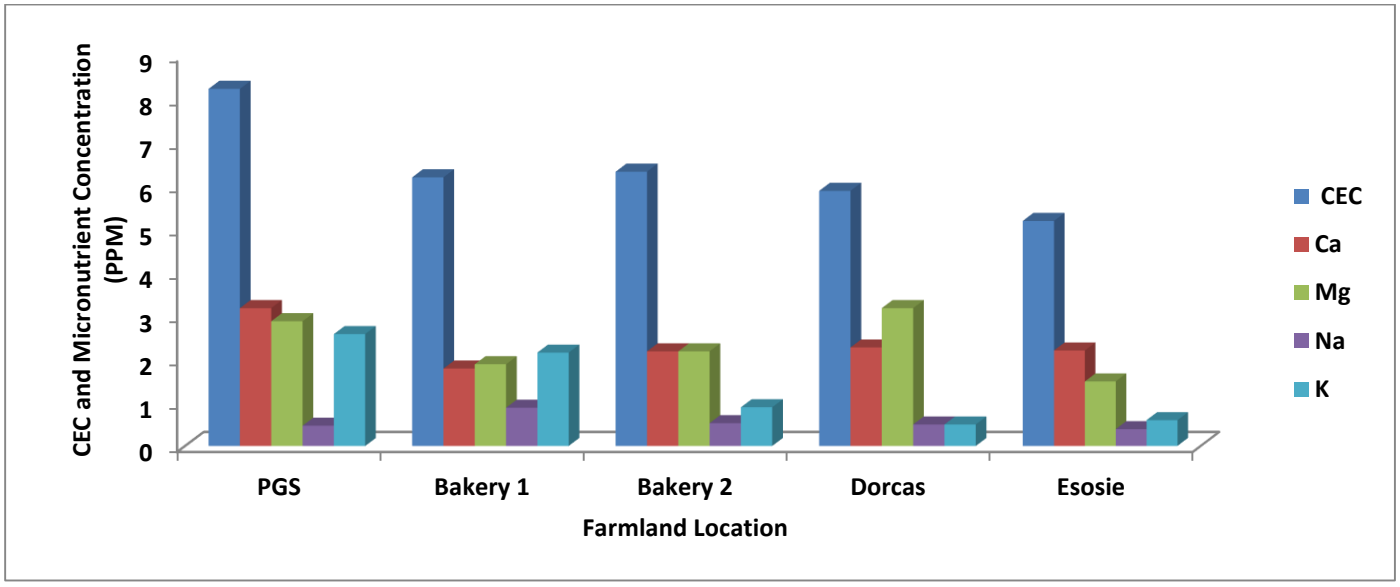

Figure 4: Cation exchange capacity and micronutrient concentration of the soil samples

The cation exchange capacity and micronutrients of the soil samples of the selected farmlands in the Otuoke are shown in Table 10, with further illustration in Figure 6.

The cation exchange capacity order of increase is PGS > Bakery 2 > Bakery 1 > Dorcas > Control. There was no significant difference ($p > 0.05$) in the CEC of soil samples in these farmlands. Calcium content was highest in PGS and least in bakery 2. Ca content varies significantly ($P < 0.05$) between PGS and bakery 1. Mg content was highest in PGS (2.90± 0.08) ppm and least in Control (1.50 ± 0.17) ppm. It also varies significantly between the two sites. Sodium was detected in all the investigated sites in the order Bakery 1 (0.89 ± 0.04) > Bakery 2 (0.53 ± 0.16) > Dorcas (0.50 ± 0.10) > Control (0.39 ± 0.22). No significant difference ($p > 0.05$) in the sodium contents between the farmlands.

Potassium contents in the soil samples from investigated sites are PGS (2.60 ± 0.08), Bakery 1 (2.17 ± 0.15), Bakery 2 (0.90 ± 0.03), Dorcas (0.50 ± 0.01), and Control (0.60 ± 0.24). Statistical analysis revealed significant differences ($p < 0.05$) between PGS and Dorcas, PGS and Control, Bakery 1 and Bakery 2, PGS and Bakery 2, Bakery 2 and Dorcas, Bakery 2 and Control (Table 10).

CONCLUSION AND RECOMMENDATIONS

In conclusion, the findings of this study revealed that the various bacterial isolates showed variations in their populations across the different farmlands, reflecting the influence of pesticide contamination. Only few bacterial and fungal species showed dominance at certain farmlands due to their ability to adapt or show resistance to the pesticides used in those farmlands, further establishing the fact that pesticide contamination exerts a profound influence on microbial population of the Otuoke farmlands. From the findings of this study, it is recommended that integrated pest management strategies that focus on reducing pesticides usage through the implementation of alternative pest control methods, such as biological control, crop rotation, and pest-resistant crop varieties should be adopted. will help minimize the negative impact of pesticides on soil microbial communities. This will help minimize the negative impact of pesticides on soil microbial communities.

This study can be undertaken further in other parts of Nigeria as well as in other regions of the Global South to ascertain the influence of pesticide contamination on the soil.

REFERENCES

Abubakar, Y., Tijjani, H., Egbuna, C., Adetunji, C. O., Kala, S., Kryeziu, T. L. and Patrick-Iwuanyanwu, K. C. (2020). Pesticides, history, and classification. In *Natural remedies for pest, disease and weed control* (*pp*. 29-42). Academic Press.

Aleruchi, O., Awari, V. G. and Emmanuel, E. M. (2020). Effect of organochlorine pesticide consortium on the indigenous farm soil microorganisms. *Journal of Advances in Microbiology Research*, 1(2), 29-36.

Audenaert, K., De Meyer, G. B. and Höfte, M. M. (2014). Abscisic acid determines basal susceptibility of tomato to *Botrytis cinerea* and suppresses salicylic acid-dependent signaling mechanisms. *Plant Physiology*, 166(1), 166-176.

Bergey, D. H. (1994). *Bergey's manual of determinative bacteriology*. Lippincott Williams & Wilkins.

Borad, P., Chaudhari, M., Harkhani, K. and Sharma, A. K. Pseudomonas Species Potential for Organophosphorus Degradation. *International Journal of Agriculture, Environment and Biotechnology*, 15, 653-662.

Cabot, G., Zamorano, L., Moyà, B., Juan, C., Navas, A., Blázquez, J. and Oliver, A. (2016). Evolution of *Pseudomonas aeruginosa* antimicrobial resistance and fitness under low and high mutation rates. *Antimicrobial agents and chemotherapy*, 60(3), 1767-1778.

Crone, S., Vives-Flórez, M., Kvich, L., Saunders, A. M., Malone, M., Nicolaisen, M. H. and Bjarnsholt, T. (2020). The environmental occurrence of *Pseudomonas aeruginosa*. *Apmis*, 128(3), 220-231.

Cycoń, M., Żmijowska, A. and Piotrowska-Seget, Z. (2019). Enhancement of biodegradation of pesticides in agricultural soil by introducing a multi-substrate environment. *Environmental Pollution*, 245, 152-160.

Dugan, S. T., Muhammetoglu, A. and Uslu, A. (2023). A combined approach for the estimation of groundwater leaching potential and environmental impacts of pesticides for agricultural lands. *Science of the Total Environment*, 901, 165892.

Harir, M., Bendif, H., Bellahcene, M., Fortas, Z. and Pogni, R. (2018). Streptomyces secondary metabolites. *Basic biology and applications of actinobacteria*, 6, 99-122.

Hayatsu, M., Katsuyama, C. and Tago, K. (2021). Overview of recent researches on nitrifying microorganisms in soil. *Soil Science and Plant Nutrition*, 67(6), 619-632.

Ikpesu, T. O. and Ariyo, A. B. (2013). Health implication of excessive use and abuse of pesticides by the rural dwellers in developing countries: the need for awareness. *Greener journal of environment management and public safety*, 2(5), 180-188.

Imfeld, G. and Vuilleumier, S. (2012). Measuring the effects of pesticides on bacterial communities in soil: a critical review. *European journal of soil biology*, 49, 22-30.

Kaerger, K., Schwartze, V. U., Dolatabadi, S., Nyilasi, I., Kovács, S. A., Binder, U. and Voigt, K. (2015). Adaptation to thermotolerance in Rhizopus coincides with virulence as revealed by avian and invertebrate infection models, phylogeny, physiological and metabolic flexibility. *Virulence*, 6(4), 395-403.

Kaur, R., Mavi, G. K., Raghav, S. and Khan, I. (2019). Pesticides classification and its impact on environment. International *Journal of Current Microbiology and Applied Sciences*, 8(3), 1889-1897.

Khalid, S., Shahid, M., Murtaza, B., Bibi, I., Naeem, M. A. and Niazi, N. K. (2020). A critical review of different factors governing the fate of pesticides in soil under biochar application. *Science of the Total Environment*, 711, 134645.

Laabs VW, Amelung A Pinto AZ and Zech W. 2000. Multi residues analysis of Corn and soyabean pesticides inBrazilian Oxisols using gas chromatography and mass selective detection. J. Environ Qual. 28:1998-1786.

Laborda, P., Sanz-García, F., Hernando-Amado, S. and Martínez, J. L. (2021). *Pseudomonas aeruginosa*: An antibiotic resilient pathogen with environmental origin. *Current Opinion in Microbiology*, 64, 125-132.

Lehmann, J., Bossio, D. A., Kögel-Knabner, I. and Rillig, M. C. (2020). The concept and future prospects of soil health. *Nature Reviews Earth & Environment*, 1(10), 544-553.

Manzar, N., Kashyap, A. S., Goutam, R. S., Rajawat, M. V. S., Sharma, P. K., Sharma, S. K. and Singh, H. V. (2022). Trichoderma: Advent of versatile biocontrol agent, its secrets and insights into mechanism of biocontrol potential. *Sustainability*, 14(19), 12786.

Ogodo, A. C., Narayana, M. S. V., Vardhan, P. S., Gupta, R. K., Gautam, A. K., Egbuna, C. and Kumar, S. (2021). Principles of applied microbiology and biotechnology: Technique for the screening of antimicrobial herbs. In *Preparation of Phytopharmaceuticals for the Management of Disorders* (*pp*. 185-214). Academic Press.

Puissant, J., Jones, B., Goodall, T., Mang, D., Blaud, A., Gweon, H. S. and Griffiths, R. (2019). The pH optimum of soil exoenzymes adapt to long term changes in soil pH. *Soil Biology and Biochemistry*, 138, 107601.

Raffa, C. M. and Chiampo, F. (2021). Bioremediation of agricultural soils polluted with pesticides: A review. *Bioengineering*, 8(7), 92.

Shahid, M. and Khan, M. S. (2022). Tolerance of pesticides and antibiotics among beneficial soil microbes recovered from contaminated rhizosphere of edible crops. *Current research in microbial sciences*, 3, 100091.

Sharma, A., Kumar, V., Kohli, S. K., Kaur, R., Kaur, T., Arora, S. and Bhardwaj, R. (2020). Pesticide metabolism in plants, insects, soil microbes and fishes: an overview. *Pesticides in Crop Production: Physiological and Biochemical Action*, 35-53.

Sidhu, G. K., Singh, S., Kumar, V., Dhanjal, D. S., Datta, S. and Singh, J. (2019). Toxicity, monitoring and biodegradation of organophosphate pesticides: a review. *Critical reviews in environmental science and technology*, 49(13), 1135-1187.

Srinivasulu, M. and Ortiz, D. R. (2017). Effect of pesticides on bacterial and fungal populations in Ecuadorian tomato cultivated soils. *Environmental Processes*, 4, 93-105.

Steinwandter H. 1990. Contributions to the on-line method. For the extraction and isolating of pesticide residues and environmental chemicals II. Miniaturization of the on-linemethod. *Fresenius J. Anal Chem*. 336, 8-11.

Suleiman, A. K., Manafi, M. and Najafi, M. F. (2020). Evaluation of *Rhodotorula glutinis* for its potential as a biocontrol agent against postharvest blue mold decay of citrus fruits. *Biological Control*, 150, 104365.

Tadeo, J. L., Albero, B. and Pérez, R. A. (2019). Pesticides: classification and properties. In *Analysis of Pesticides in Food and Environmental Samples*, Second Edition (pp. 1-40). CRC Press.

Tripathi, S., Srivastava, P., Devi, R. S. and Bhadouria, R. (2020). Influence of synthetic fertilizers and pesticides on soil health and soil microbiology. *In*: *Agrochemicals detection, treatment and remediation* (pp. 25-54). Butterworth-Heinemann.

Von Duszeinj. 1995. Analysis of pesticides in Costa Rica with special emphasis on water and fish samples. *Technical report. Duetsche Gesellschaft Fur Technische Zusammenarbeit*, Bremen, Germany.