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Impact of Different Concentrations of Swine Wastewater on Bacterial Population and Physicochemical Properties of Soil

Aleruchi, Owhonka[1*]., Nengi-Benwari, A. O[2]. and Okparaeke, Chiamaka Cynthia[1]
[1]Department of Microbiology, Rivers State University, Port Harcourt, Nigeria.
[2]Department of Crop and Soil Science, Faculty of Agriculture, University of Port Harcourt, Nigeria.
***Corresponding Author:** *owhonka.aleruchi@ust.edu.ng*

ABSTRACT

This study evaluated the effects of swine wastewater (SWW) on bacteria communities and physicochemical properties of soil over 21 days, with assessments conducted at 7-day intervals. Soil samples were collected from a 0–15 cm depth at the Rivers State University School Farm, Port Harcourt, Nigeria using a sterile hand auger while SWW was collected from the university piggery farm. Collected samples were aseptically transported to the laboratory in an ice-packed cooler. Microbiological and physicochemical analyses of the samples were conducted using standard procedures to ensure accuracy and reliability. The study revealed significant effects of SWW on both microbial and physicochemical properties of the soil. Bacterial counts increased notably with SWW application, peaking at 4.5 × 10^5CFU/g in the Soil + 20 ml SWW treatment on Day 14, before declining to 4.0 × 10^5 CFU/g by Day 21. Eleven bacterial genera were identified, including *Lactobacillus, Streptococcus, Escherichia, Klebsiella, Staphylococcus, Bacillus, Pseudomonas, Enterobacter, Proteus, Salmonella,* and *Shigella.* Physicochemical parameters showed marked changes in response to SWW application. Soil pH fluctuated, with the lowest value (6.09) recorded in the 100 ml SWW treatment on Day 21 and the highest (7.35) in the 40 ml SWW treatment on Day 7. Soil temperature increased with SWW application, peaking at 28.7°C in the 20 ml SWW treatment, compared to the control's 25.4°C. Moisture content ranged from 11.39% in the control on Day 7 to 17.15% in the 40 ml SWW treatment. Organic matter content was lowest in the control with a value of 3.36% on Day 7 and highest (3.83%) in the 100 ml SWW treatment. Nitrogen levels fluctuated, with the control having the highest content (1.7%) on Day 7 and the 40 ml SWW treatment the lowest (0.6%). The carbon-to-nitrogen ratio ranged from 1.15 in the control to 3.38 in the 40 ml SWW treatment. In conclusion, SWW significantly influenced soil microbial activity and physicochemical characteristics, demonstrating its potential as a soil amendment. However, careful management of SWW applications is critical to avoid adverse effects on soil health, and disruption in balance of microbial populations, and to ensure sustainable soil fertility.

Keywords: Swine wastewater, Bacteria, Soil, Moisture content, Organic Matter, Carbon-Nitrogen Ratio.

Introduction

Swine wastewater (SWW) contains high concentrations of pollutants, including suspended solids, organic matter, and nutrients, which can significantly deteriorate the quality of environments where they are discharged (Fischer *et al*., 2018). Traditionally, swine production facilities manage wastewater by flushing it into anaerobic lagoons, with the effluent later sprayed onto agricultural fields. However, continuous effluent application can result in excessive nitrogen and phosphorus accumulation in soils, leading to nutrient imbalances or contamination of surface and groundwater (Lei *et al*., 2013).

While pig slurry is recognized as a valuable fertilizer that enhances crop productivity and reduces the reliance on mineral fertilizers (Khaleel *et al*., 2018), its improper application can adversely affect the soil's physical, chemical, and biological properties. Of particular concern are the biological impacts, as swine wastewater contains a diverse microbiota originating from the gastrointestinal tract of pigs. These microbes, including potential pathogens, are released into the environment via feces (McConnell *et al*., 2012). The introduction of such microbiota into soil systems is a complex, multifactorial process influenced by biotic and abiotic factors, including soil texture, temperature, and moisture.

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These factors affect microbial community structure, diversity, and activity (Mabuduike *et al.*, 2010). Disposal of SWW in agricultural soils is known to alter microbial community dynamics, as intestinal-origin microorganisms initially thrive but later decline due to environmental pressures. However, some subpopulations may adapt to soil conditions and establish stable communities through interactions with other soil organisms (Mielke and Mazurak, 2016). Effective utilization of SWW in agriculture requires balancing its nutrient benefits with minimizing environmental risks. Maximizing the recycling of manure-derived nitrogen and phosphorus while reducing adverse environmental impacts is critical for sustainable agricultural practices (Mielke and Mazurak, 2016). Characterization of soil microbial communities under SWW treatment is essential for understanding its impact on nutrient cycling, decomposition, and mineralization processes. Microbial indicators are particularly valuable as they respond more rapidly to environmental changes than chemical or physical soil properties, making them early markers of soil health (Overcash and Humenik, 2016). While organic amendments like SWW are known to increase microbial biomass and activity (Hurt *et al.*, 2012), there remains limited understanding of how these amendments specifically influence microbial diversity, abundance, and community structure, especially under the influence of varying environmental conditions (Overcash and Humenik, 2016). Population growth and climate change have increased the demand for sustainable waste management practices, particularly in agriculture where maintaining a balanced microbial community is essential (Jongbloed and Lenis, 2012).

The expansion of pig farming to meet the rising demand for pork products has resulted in significant SWW production. This wastewater contains not only nutrients and organic matter but also potential contaminants, such as heavy metals and pathogens, which can profoundly impact soil ecosystems if not properly managed (Nkoa, 2014). Introducing SWW into agricultural soils can alter critical soil properties, including pH, electrical conductivity, organic matter content, and nutrient availability, while simultaneously reshaping the microbial community. These changes may influence nutrient cycling, soil fertility, and overall soil health, with broader implications for plant growth and environmental sustainability (Wang *et al.*, 2014). Despite its agricultural benefits, there is limited understanding of how different concentrations of SWW influence the diversity, activity, and functionality of soil bacteria.

This present study addresses this gap by evaluating the effects of varying SWW concentrations on soil bacterial communities and key physicochemical properties. The findings are critical for developing guidelines to optimize SWW use in agricultural systems, ensuring sustainable soil management while mitigating environmental risks. By examining the microbial and physicochemical responses to SWW applications, this study aims to inform practices that balance agricultural productivity with environmental protection, contributing to more sustainable livestock farming systems.

Materials and Methods

Study Area and Sampling Locations

The samples were collected at the Rivers State University School Farm in Port Harcourt, Nigeria. Soil samples were collected from LAT N4°48'3.84640", LONG E6°58'36.13640", while swine wastewater (SWW) samples were collected from the university piggery farm at LAT N4°48'12.99620", LONG E6°58'34.99730".

Sample Collection

Soil samples were collected from a depth of 0–15 cm using a sterile hand auger and placed into sterile containers. Samples from various areas across the farm were combined to form a composite sample and transported to the laboratory in an ice-packed cooler.

Swine wastewater (SWW) was aseptically collected using a sterile ladle from the drainage channels within the piggery. The samples were transferred into a sterile 1500 mL screw-capped container and transported in an ice-packed cooler to the microbiology laboratory within 2 hours. Samples not immediately analyzed were stored at 4°C.

Experimental Design

The experiment was designed to assess the effects of different concentrations of SWW on soil properties over 21 days. Soil samples were divided into four treatment groups:

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Control - No SWW application; 10 ml SWW per 100 g soil; 20 ml SWW per 100 g soil; and 40 ml SWW per 100 g soil (Maciel *et al*., 2024). Each treatment was replicated three times and incubated at room temperature in sterile containers**.**

Microbiological Analysis

Standard microbiological procedures were followed to determine bacterial counts and identify bacterial genera in the soil samples:

Bacterial Enumeration: The total heterotrophic bacterial count (THBC) was determined using the pour plate method on nutrient agar. Plates were incubated at 37°C for 24–48 hours, and colonies were counted and expressed as colony-forming units per gram (CFU/g) of soil.

Bacterial isolates were identified using colonial, morphological, biochemical and physiological tests, such as Gram staining, catalase test, oxidase test, and sugar fermentation profiles. Identification was carried out using Bergey's Manual of Systematic Bacteriology (Krieg & Holt, 1984).

Physicochemical Analysis

The physicochemical properties of the soil were analyzed using standard procedures (Sparks, 1996). The procedures are summarized as follows:

pH Measurement: A 1:1 soil-to-water suspension was prepared by mixing equal parts of soil and distilled water. The mixture was stirred thoroughly and allowed to equilibrate before measuring the pH using a Hanna HI 2211 pH meter (Hanna Instruments, Woonsocket, Rhode Island, USA). The pH meter was calibrated using standard buffer solutions (pH 4.0, 7.0, and 10.0) before measurement (Sparks, 1996).

Temperature Monitoring: Soil temperature was measured using a mercury thermometer. The thermometer was inserted into the soil at the desired depth, and the temperature reading was recorded once stabilized.

Moisture Content Determination: Fresh soil samples were weighed to obtain their wet weight. The samples were then oven-dried at 105°C for 24 hours to remove all moisture. After drying, the samples were reweighed to obtain their dry weight. The moisture content was calculated as the percentage loss in weight relative to the original wet weight (Sparks, 1996).

Organic Matter Content Assessment (Walkley-Black Method): A known weight of soil was treated with a potassium dichromate and sulfuric acid solution, which oxidizes the organic carbon present. The amount of dichromate reduced during the reaction, corresponding to the organic carbon content, was determined by titration. The organic matter content was then calculated by multiplying the organic carbon content by a factor (commonly 1.724, based on the assumption that organic matter contains approximately 58% carbon) (Sparks, 1996).

Nitrogen Content Determination (Kjeldahl Method): Soil samples were digested with concentrated sulfuric acid, converting organic nitrogen to ammonium sulfate. After digestion, the solution was made alkaline, and the released ammonia was distilled into a boric acid solution. The amount of ammonia was then determined by titration with a standard acid solution, allowing for the calculation of total nitrogen content in the soil (Sparks, 1996).

Carbon-to-Nitrogen Ratio (C:N) Calculation: The C:N ratio was determined by dividing the organic carbon content (obtained from the Walkley-Black method) by the total nitrogen content (obtained from the Kjeldahl method). This ratio provides insight into the balance between carbon and nitrogen in the soil, which is important for understanding nutrient availability and microbial activity (Sparks, 1996).

Data Collection and Analysis

Measurements were taken at 7-day intervals (Days 7, 14, and 21). Data were analyzed using descriptive and inferential statistics. Significant differences between treatments were determined using one-way analysis of variance (ANOVA), with a significance level set at $p<0.05$ (Maciel *et al*., 2024).

Impact Assessment Parameters:

The impact of SWW on soil was assessed based on the following parameters: Microbial Response: Changes in total bacterial counts and identification of bacterial genera. Soil Physicochemical Properties: Variations in pH, temperature, moisture content, organic matter content, nitrogen levels, and C:N ratio.

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Results

The results of the Bacterial population and Physicochemical characteristics of the soil and swine wastewater samples before treatment are presented in Table 1.

The bacterial count of the soil and swine wastewater samples before treatment was 3.6 x 10^5 CFU/g and 4.2 x 10^5 CFU/ml, respectively as shown in Table 1. The physicochemical properties of the soil sample before treatment indicate a slightly acidic pH of 6.19, a typical temperature for tropical environments at 26.7°C, and a relatively low moisture content of 7.14%. The organic matter present in the soil was 3.11%, with nitrogen content at 1.5%, and a carbon to nitrogen (C) ratio of 1.01. These baseline characteristics provide a reference point for assessing the impact of swine wastewater (SWW) on the soil in subsequent treatment phases (Table 1).

The swine wastewater used in the treatment had a neutral pH of 7.35 and an electrical conductivity of 760.31 µS/cm, which suggests a moderate concentration of ions. It contained 2.29 mg/l of total suspended solids, indicating low levels of particulate matter, and 0.084 mg/l of ammonia, reflecting minimal nitrogen pollution. The chemical oxygen demand (COD) was 101.36 mg/l, highlighting the organic matter content, while the biological oxygen demand (BOD) was 18.45 mg/l, pointing to the presence of biodegradable material (Table 1).

Table 1: Bacterial count and physicochemical characteristics of soil and swine wastewater before treatment

Parameter	Farm Soil	Parameter	Swine Wastewater
Bacterial Count	3.6×10^5 CFU/g	Bacterial Count	4.2×10^5 CFU/ml
pH	6.19	pH	7.35
Temperature (°C)	26.7	Electrical Conductivity (µS/cm)	760.31
Moisture content (%)	7.14	Total Suspended Solid (mg/l)	2.29
Organic matter (%)	3.11	Ammonia (mg/l)	0.084
Nitrogen (%)	1.5	Chemical Oxygen Demand (mg/l)	101.36
Carbon-Nitrogen ratio	1.01	Biological Oxygen Demand (mg/l)	18.45

The bacterial counts for different soil treatments with swine wastewater (SWW) over 21 days are presented in Figure 1. On Day 7, the control soil has the lowest bacterial count of 1.5 × 10^5 CFU/g. In contrast, Soil + 20ml SWW had a higher count of 3.0 × 10^5 CFU/g, while Soil + 40ml SWW records a slightly higher count, of 3.5 × 10^5 CFU/g. The highest bacterial count on Day 7 is seen in the Soil + 100ml SWW treatment, reaching 4.0 × 10^5 CFU/g. On Day 14, the bacterial counts rise across all treatments. Soil + 20ml SWW records the highest count of 4.5 × 10^5 CFU/g, followed closely by Soil + 40ml SWW with 4.0 × 10^5 CFU/g. Soil + 100ml SWW shows a bacterial count of about 3.8 × 10^5 CFU/g, while the control (Soil only) reaches 2.0 × 10^5 CFU/g. By Day 21, bacterial counts decrease in all treatments. Soil + 20ml SWW still maintains the highest count at 4.0 × 10^5 CFU/g, with Soil + 40ml SWW slightly lower at 3.5 × 10^5 CFU/g. The Soil + 100ml SWW treatment has a bacterial count of approximately 3.0 × 10^5 CFU/g, while the control had the lowest count at 1.5 × 10^5 CFU/g.

Results of the colonial, morphological, biochemical and physiological tests showed that the bacteria isolated belong to the following genera: *Lactobacillus*, *Streptococcus*, *Enterobacter*, *Escherichia*, *Klebsiella*, *Staphylococcus*, *Bacillus*, *Pseudomonas*, *Proteus*, *Salmonella*, and *Shigella*.

The results of the physicochemical parameters of soil treated with various concentrations of swine wastewater (SWW) are presented in Fig. 2 to Fig. 7. In Figure 2, the lowest value of pH (6.14) was recorded in the Soil + 20ml SWW treatment on Day 7, while the highest value of pH (7.35) was recorded in the Soil + 40ml SWW treatment. On Day 14, the lowest value of pH (6.84) was recorded in the Soil + 20ml SWW treatment, while the highest value of pH (7.08) was recorded in the Soil + 100ml SWW treatment. On Day 21, the lowest value of pH (6.09) was recorded in the Soil + 100ml SWW treatment, while the highest value of pH (6.84) was recorded in the Soil + 40ml SWW treatment.

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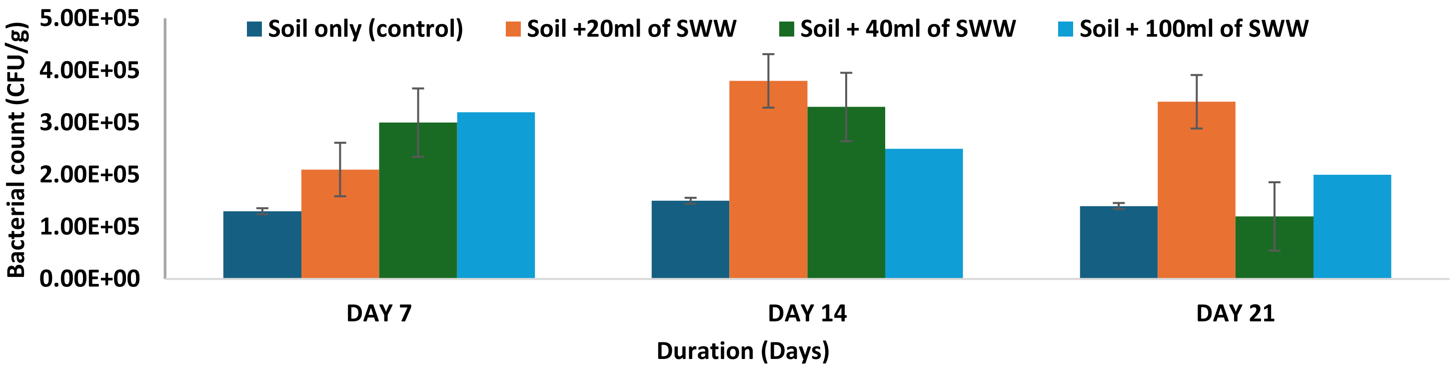

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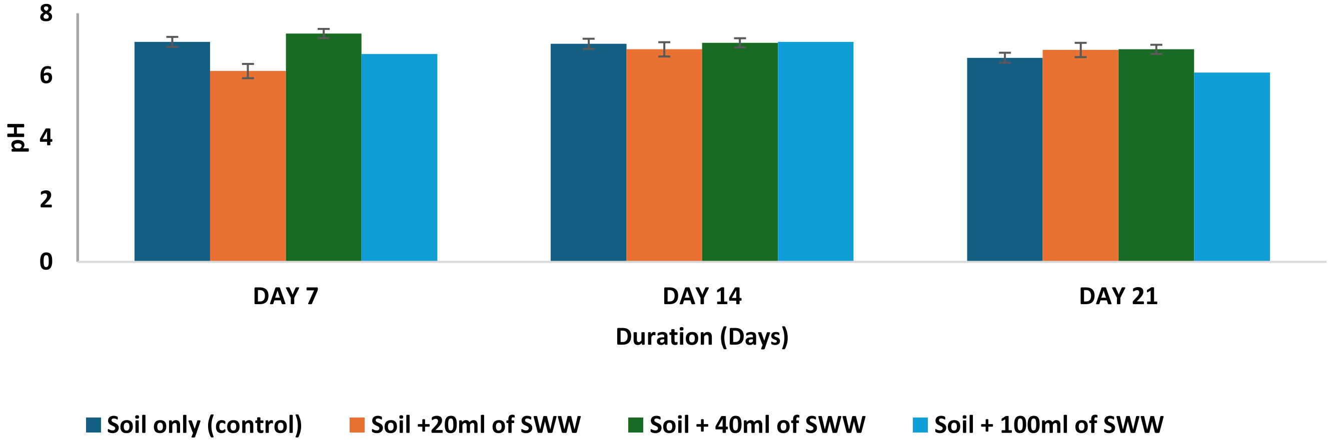

Fig. 2: Trends in pH of soil treated with various concentrations of Swine wastewater (SWW)

Figure 3 shows that the lowest temperature (25.4°C) was recorded in the control soil on Day 7, while the highest temperature (28.7°C) was recorded in the Soil + 20ml SWW treatment. On Day 14, the lowest temperature (25.2°C) was recorded in the Soil only (control) treatment, while the highest temperature (26.8°C) was recorded in the Soil + 100ml SWW treatment. On Day 21, the lowest temperature (26.2°C) was recorded in the Soil + 20ml SWW treatment, while the highest temperature (26.8°C) was recorded in the control soil.

Figure 4 show that the control soil had the lowest moisture content at 11.39% on Day 7 while the Soil + 40ml SWW treatment had the highest moisture content at 17.15%. On Day 14, the Soil only (control) treatment again had the lowest moisture content at 12.51%, while the Soil + 40ml SWW treatment maintained the highest moisture content at 14.76%. On Day 21, the Soil only (control) treatment had the lowest moisture content at 11.23%, while the Soil + 100ml SWW treatment had the highest moisture content at 12.39%.

Result of organic matter presented in Fig. 5 show that, the lowest value (3.36 %) was recorded in the control soil on Day 7, while the highest value (3.83 %) was recorded in the Soil + 100ml SWW treatment. On Day 14, the lowest value (0.78 %) was recorded in the Soil + 20ml SWW treatment, while the highest value (1.58 %) was recorded in the Soil + 40ml SWW. On Day 21, the lowest value (0.35 %) was recorded in the Soil + 40ml SWW, while the highest value (0.66 %) was recorded in the Soil + 100ml SWW treatment.

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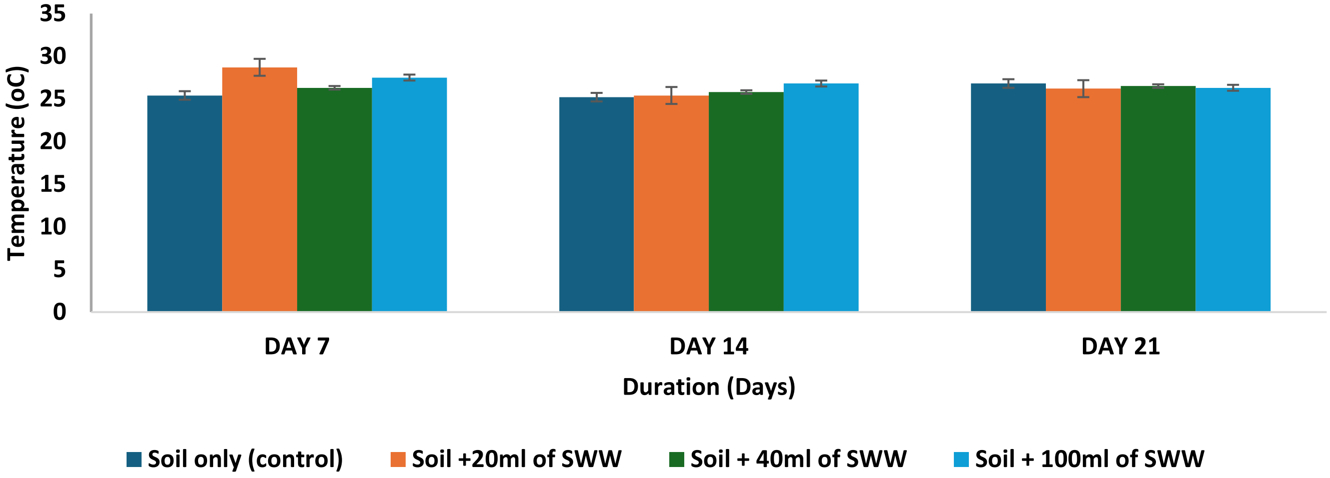

Fig. 3: Trends in Temperature of soil treated with various concentrations of Swine wastewater (SWW)

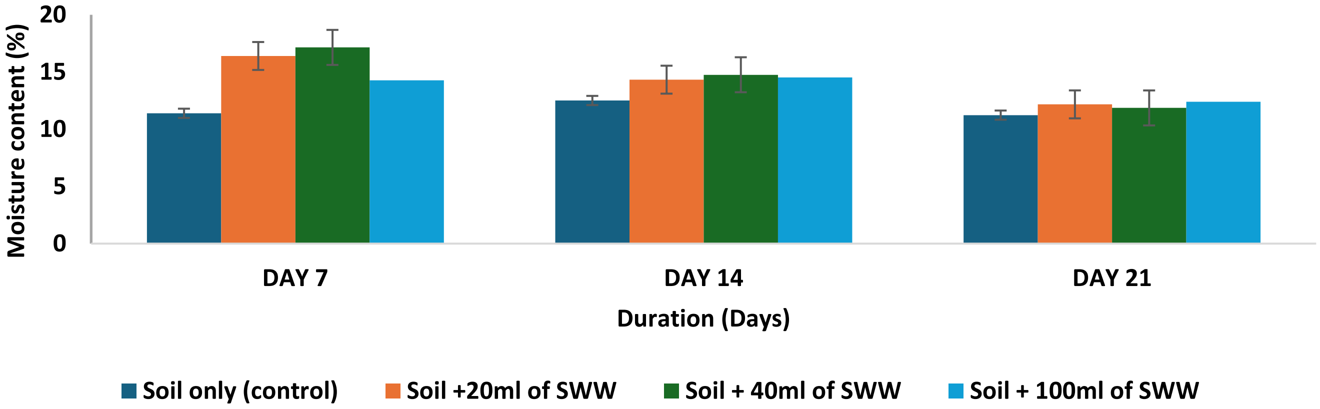

Fig. 4: Trends in moisture content of soil treated with various concentrations of Swine wastewater (SWW)

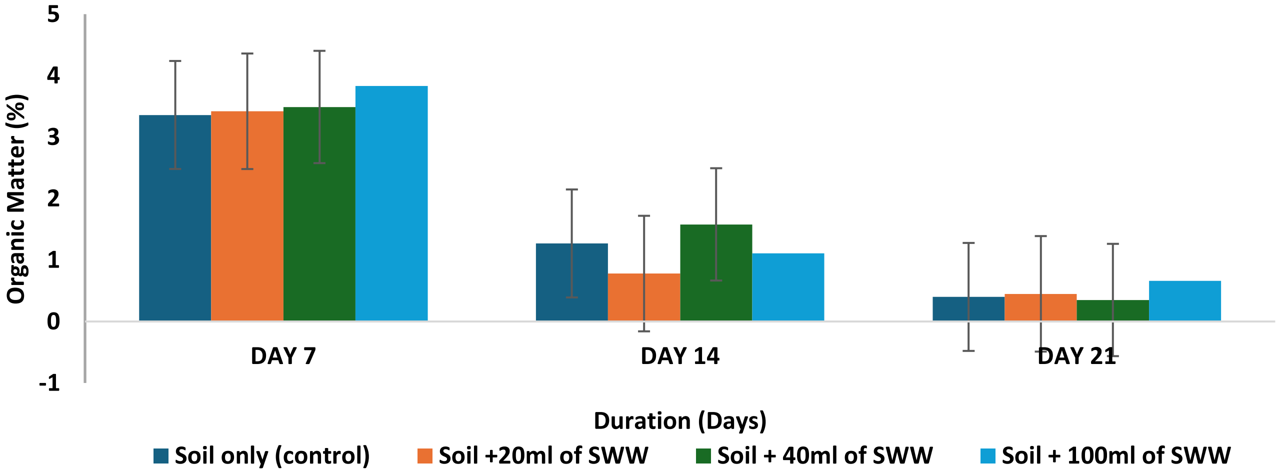

Fig. 5 Trends in organic matter of soil treated with various concentrations of Swine wastewater (SWW)

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Results of the nitrogen are shown in Fig. 6. On Day 7, the lowest value (0.6%) was recorded, in the Soil + 40ml SWW treatment and the Soil only (control) had the highest value (1.7 %). On Day 14, the lowest value (1.58 %) was recorded in the Soil + 40ml SWW treatment, while the highest value (2.24 %) was recorded in the Soil only (control) treatment. On Day 21, the lowest value (1.51 %) was recorded in the Soil + 40ml SWW treatment, while the highest value (2.24 %) was recorded in the control soil.

Carbon nitrogen ratio lowest value (1.15) was recorded in the control soil on day 7, while the highest value (3.38) was recorded in the Soil + 40ml SWW treatment. On Day 14, the lowest value (0.27) was recorded in the Soil + 20ml SWW treatment, while the highest value (0.57) was recorded in Soil + 40ml SWW treatment. On Day 21, the lowest value (0.18) was recorded in the Soil only (control) treatment, while the highest value (0.36) was recorded in the Soil + 100ml SWW treatment (Fig 7).

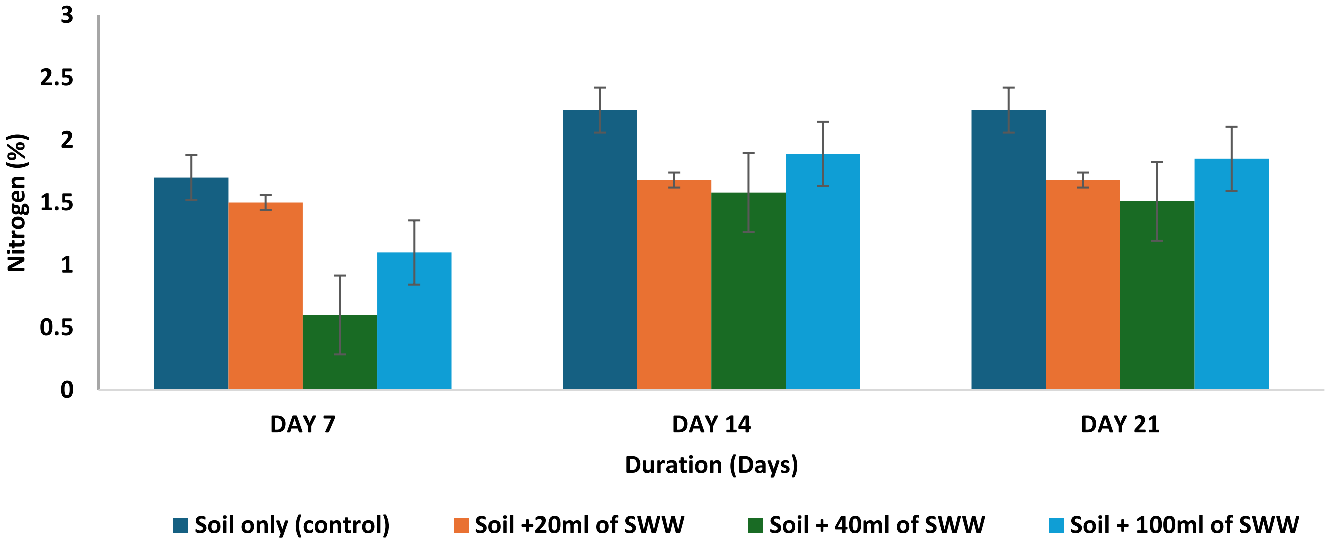

Fig. 6: Trends in nitrogen of soil treated with various concentrations of Swine wastewater (SWW)

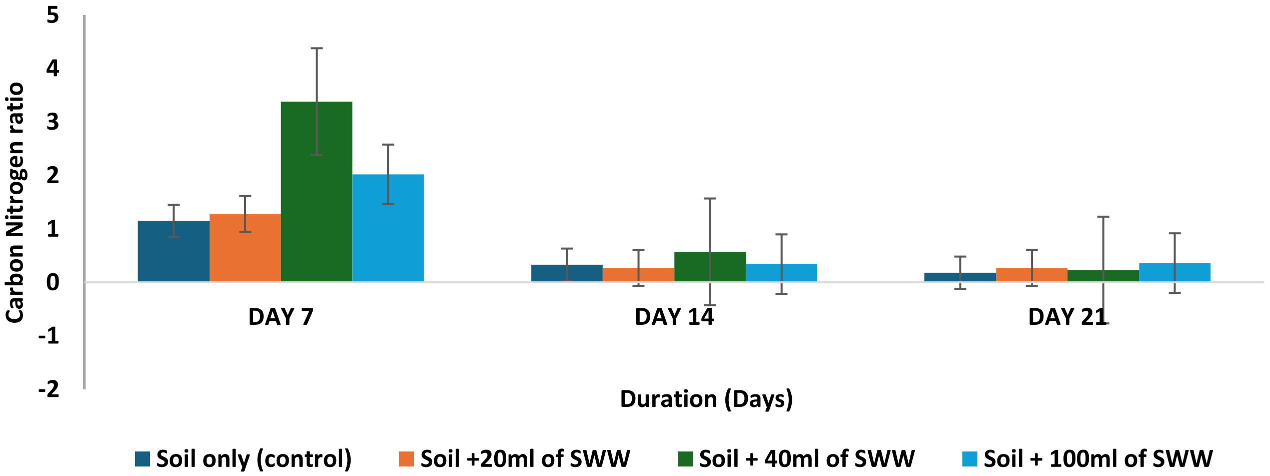

Fig. 7: Trends in carbon nitrogen ratio of soil treated with various concentrations of Swine wastewater (SWW)

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Discussion

The results of this study highlight the significant impact of swine wastewater (SWW) on soil bacterial populations and physicochemical properties. The initial bacterial counts in the untreated soil and SWW suggest that the nutrient-rich composition of SWW supports microbial proliferation. This aligns with previous studies showing that wastewater application introduces diverse microbial communities into the soil, often altering its microbial structure and activity (Liu et *al.*, 2019; Smith *et al.*, 2019). The physicochemical characteristics of the soil before treatment reflect typical tropical soil properties, including slight acidity and moderate fertility. However, the application of SWW introduced organic matter and nutrients, resulting in observable changes. Notably, the pH of the soil shifted closer to neutral, which is known to create a more favorable environment for many soil microbes. This supports findings from earlier research, which documented increases in soil pH following organic waste applications (Zhang et al., 2020). Similarly, changes in temperature and moisture content were observed, likely due to the thermal and hydrological effects of wastewater. These changes can influence microbial activity, as microorganisms are highly sensitive to environmental conditions. Comparable trends have been reported in other studies investigating the impact of organic amendments on soil properties (Liu *et al*., 2019; Smith *et al.,* 2019). The organic matter and nitrogen content of the soil increased with SWW application, reflecting enhanced fertility. These changes are consistent with research demonstrating that organic waste improves nutrient cycling and boosts microbial activity (Huang *et al*., 2019; Chen *et al*., 2018).

However, the observed changes in this study were more pronounced, potentially due to differences in the composition or concentration of the SWW used. Microbial diversity analyses revealed the presence of several bacterial genera, including those associated with organic matter decomposition and nutrient cycling. The introduction of SWW likely stimulated the growth of specific bacteria adapted to the nutrient influx, creating dynamic shifts in the microbial community structure. However, over time, the bacterial populations stabilized or declined, suggesting nutrient depletion or competition among microorganisms. This phenomenon has been previously described in studies examining microbial dynamics in soils treated with organic amendments (Torsvik *et al*., 2002). The overall findings indicate that SWW can significantly enhance soil fertility by increasing key parameters such as organic matter, nitrogen, and microbial activity. However, these benefits must be balanced against the potential risks of overloading the soil with nutrients, which could lead to microbial imbalances or environmental degradation.

In conclusion, this study demonstrates that SWW has a profound influence on soil bacterial communities and physicochemical properties. The initial nutrient and microbial influx promote increased microbial activity and improved soil fertility, particularly in the early stages of application. However, the changes observed suggest that prolonged or excessive SWW use could alter microbial dynamics and soil health over time. It is recommended that, Careful regulation of the volume and frequency of Swine Wastewater (SWW) application is essential to maximize benefits while preventing nutrient imbalances or microbial competition; Post-treatment monitoring of key soil parameters, including pH, organic matter, and microbial diversity, is also recommended to ensure sustainable soil management practices and that further research should focus on the cumulative effects of repeated SWW application on soil health and microbial communities, particularly under varying environmental conditions.

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