Research Article

Effects of Wastewater Irrigation from Kalshingi Fish Farm on the Microbial and Physicochemical Characteristics of Agricultural Soils

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ABSTRACT
Agriculture and aquaculture are critical for global food security. The use of fish farm wastewater for irrigation raises concerns about soil health and microbial communities; therefore, this study assessed the effects of fish farm wastewater from the Kalshingi Fish Farm on soil bacterial diversity, physicochemical properties, and implications for agricultural productivity. Soil samples were collected from the impacted and control plots, and key soil parameters, including pH, electrical conductivity, salinity, and nutrient concentration, were measured. Bacterial isolates were identified, and their antibiotic sensitivities were evaluated. The results showed that the soils irrigated with wastewater from the Kalshingi fish farm exhibited significant physicochemical alterations relative to the control. Soil pH ranged from 4.72 to 7.00 but was significantly lower on average (5.75 ± 0.68; $p < 0.05$), whereas electrical conductivity (2470–2930 $\mu S\ cm^{-1}$) and salinity (5.82–7.65 mg 100 g^{-1}) were significantly higher ($p < 0.001$). Total heterotrophic bacterial count in impacted samples ranged from 0.007 to 0.075 CFU/mL, with sample E showing the highest count (0.075 CFU/mL). Bacterial species, including *Escherichia coli* (80% occurrence), *Pseudomonas aeruginosa* (80%), and *Klebsiella pneumoniae* (60%), were prevalent in the affected soils. Antibiotic resistance was observed in isolates, particularly *Escherichia coli* and *Klebsiella pneumoniae*, which were resistant to Amoxicillin and Azithromycin. These findings suggest that wastewater can enhance soil fertility but also risks soil degradation and the spread of antibiotic-resistant bacteria. This study highlights the need for sustainable management. Future research should examine the effects of aquaculture wastewater on microbial diversity and antibiotic resistance.

Keywords: Antibiotic resistance*;* Agricultural productivity; Fish farm wastewater; Physicochemical properties; Soil bacterial communities

INTRODUCTION
The integration of aquaculture with crop production is increasingly promoted to enhance food security and nutrient recycling; however, the reuse of fish farm effluents on agricultural land raises unresolved questions about long-term soil health (Chen *et al.,* 2017). Aquaculture wastewater contains nutrients, suspended solids, and diverse microorganisms derived from uneaten feed and fish excreta. When released without adequate treatment, it can drive eutrophication and contamination, with implications for human and environmental health (Ojewole *et al.,*

2024). In many production systems, this wastewater is diverted to nearby fields as a low-cost irrigation source, creating a pathway of influence on soil biota. Previous studies on wastewater irrigation have mainly emphasised changes in soil physicochemical properties, reporting increases in nitrogen, phosphorus, and organic carbon that can improve short-term fertility and crop performance (Drewry *et al.,* 2020; Choudhury *et al.,* 2022). Other studies have documented shifts in microbial abundance or broad community structures after the land application of municipal or industrial effluents (Chen *et al.,* 2017; Obayomi *et al.,* 2020). However, most of these studies treat wastewater as a single category, overlooking the distinctive composition and temporal variability of aquaculture discharges. They rarely examine the specific bacterial taxa that respond to fish farm inputs or the functional traits that they contribute to soil processes (Li *et al.,* 2020). Information is particularly scarce for small-scale fish farms in tropical regions, where regulatory oversight is limited and effluents are commonly channelled to food-producing soils.

Consequently, there is a critical knowledge gap regarding how irrigation with fish farm wastewater reshapes soil bacterial communities at the taxonomic and functional levels and how these changes relate to the measured soil physicochemical conditions. Existing studies rarely compare impacted and non-impacted fields within the same agroecosystem, nor do they isolate dominant bacterial strains to test their responses to wastewater exposure, which constrains the interpretation of potential risks and benefits for soil fertility and crop health (Chen *et al.,* 2017; Li *et al.,* 2020).

This study aimed to address these gaps by utilising the Kalshingi fish farm as a case study. This study quantified the physicochemical properties of soils receiving fish farm effluents in comparison to control soils, isolated and identified representative bacterial taxa from both environments, and evaluated their sensitivity to wastewater treatment. By linking bacterial community composition and isolate-level responses with environmental measurements, this study provides insights into how fish farm wastewater irrigation may restructure soil microbiota and influence the sustainability of integrated aquaculture-agriculture systems.

MATERIALS AND METHODS
Research Design
This study employed an experimental design to assess the impact of fish farm wastewater on soil bacterial communities. Agricultural plots were divided into two groups: control plots, which received no wastewater application, and impacted plots, where wastewater from the fish farm was applied. The design included multiple replicates of both the control and impacted plots to ensure statistical robustness and reliable results.

Study Area
This study was conducted at the Kalshingi Fish Farm in Gombe State, Nigeria, where fish farming and crop cultivation are practiced in close proximity. This setting provides a practical opportunity for wastewater application and subsequent analysis of its effects on agricultural soils.

Soil sample collection
Soil samples were collected from both the control and impacted plots of the fish farm, and the Global Positioning System (GPS) coordinates of each sampling location were recorded for accuracy. Data collection followed a standardized protocol to ensure consistency and reliability. Soil cores were collected from the top 15 cm of the soil profile, as this depth typically contains the highest level of microbial activity. To obtain a representative sample, two random locations within each plot were sampled, and the samples were combined to form a composite sample for each plot. Sterile tools and containers were used throughout the process to prevent soil sample contamination. Additionally, soil samples from the aquaculture system of the fish farm were collected two days after wastewater was discharged into the agricultural plots to analyze nutrient content and microbial composition.

Physicochemical Analysis
The physicochemical properties of the soil were determined using standard analytical procedures. Soil pH and electrical conductivity were measured in a soil water suspension prepared by mixing 1 g of soil with distilled water using calibrated pH and conductivity meters after equilibration (Merl *et al.,* 2022; Pipa and Brandenburg, 2019). Electrical conductivity was used as an index of salinity (Corwin and Yemoto, 2020; Wilson, 2025). Nitrate was quantified after drying the soil to inhibit microbial transformation, and the results were expressed as nitrate nitrogen (Garmay *et al.,* 2024). Phosphate was determined colorimetrically following Bray 1 extraction (Anjum, 2024). Sulfate and chloride were analysed using turbidimetric and colorimetric methods, respectively (Mukhopadhyay 2020; Muir and Innes 2024). Sodium, potassium, magnesium, and calcium were quantified using atomic absorption spectroscopy or element-

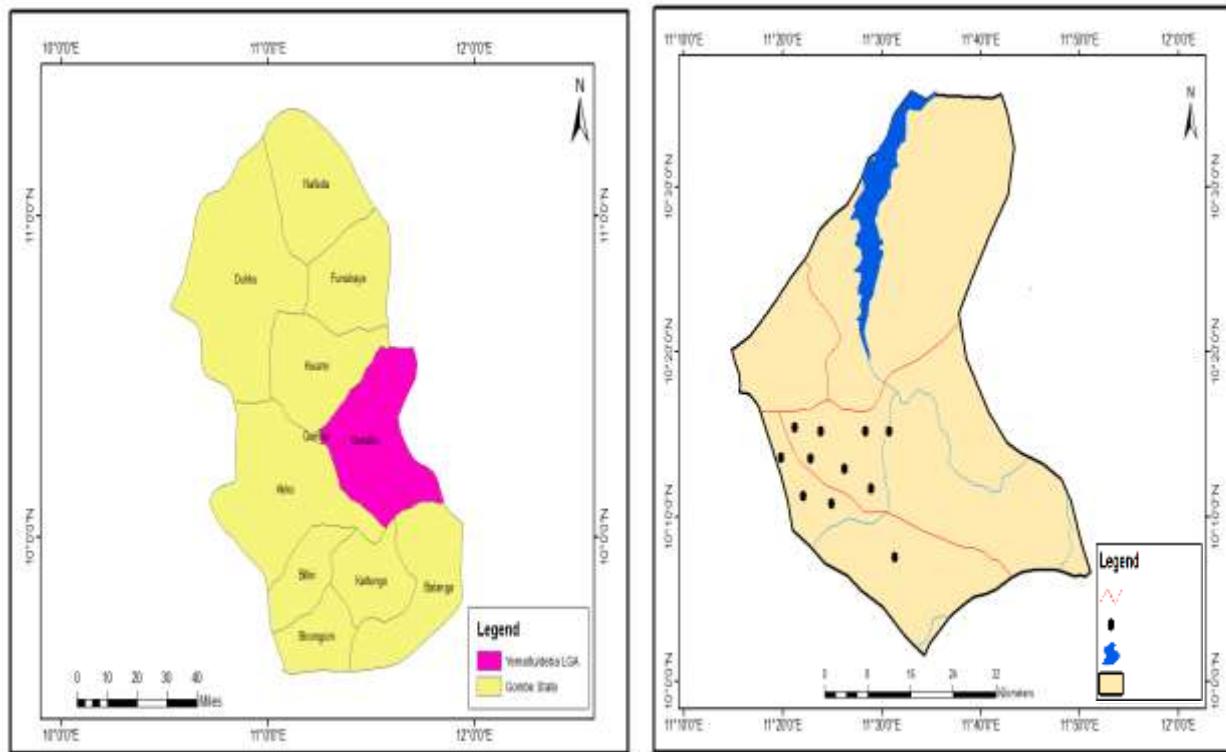specific analysers (Mukhopadhyay, 2020; Kianira, 2025; Pierzynski *et al.,* 2025; Singhal and Singh, 2024).

Fig. 1. Map showing Gombe State, Yemaltu Deba LGA, and the sampling site.

Microbiological Analysis

Microbiological Analysis was conducted using standard serial dilution and culture-based techniques. Briefly, 1 g of soil was aseptically transferred into a sterilised test tube containing 9 mL of distilled water (autoclaved at 121°C for 15 min and cooled to 35–37°C) to obtain the stock suspension, followed by tenfold serial dilutions to 10^{-3}. From the 10^{-3} dilution of each sample and control, 1 mL was plated into sterile petri dishes. Plate Count Agar (PCA) was prepared by dissolving 4.6 g in 200 mL distilled water, sterilising at 121°C for 15 min, cooling to 35–37°C, and pouring over the inoculated plates, which were gently swirled, allowed to solidify, inverted, and incubated at 37°C for 24 h prior to colony enumeration. For selective isolation, Eosin Methylene Blue (EMB) agar (36 g/L) and MacConkey (36 g/L) agars were prepared in distilled water, autoclaved at 121°C for 15 min, cooled to ~37°C, dispensed into sterile Petri dishes, and solidified. Diluted soil suspensions were then inoculated and incubated at 37°C for 24 h. Colonies on EMB and MacConkey plates were preliminarily differentiated based on morphology (size, shape, and colour), after which representative colonies were subcultured onto

nutrient agar (28 g/L in distilled water, autoclaved at 121°C for 15 min, cooled, and poured into Petri dishes) using a sterile loop and incubated at 37°C for 24 h to obtain pure cultures for further characterisation (Ataikiru & Ajuzieogu,2023).

Biochemical characterisation and gram staining

Biochemical characterisation and gram staining were performed using standard procedures. For catalase testing, a colony portion was placed on a glass slide with hydrogen peroxide, and immediate bubbling indicated a positive result. Indole production was tested by inoculating isolates into Motility Indole Ornithine medium at 37°C for 24 h and adding Kovacs reagent; a red or pink ring at the surface indicated a positive reaction. Citrate utilisation was tested using Simmons citrate agar slants at 37°C for 24 h, with a colour change from green to blue indicating citrate-positive isolates. Oxidase activity was tested by smearing colonies onto oxidase reagent-impregnated filter paper and observing for blue or purple colour within 10–30 s. Coagulase production was tested by mixing the bacterial suspension with citrated plasma, with clot formation indicating a positive result. Gram staining involved heat-fixing bacterial smears and applying crystal violet, iodine, ethanol decolorizer,

and safranin, followed by microscopic examination to differentiate Gram-positive (purple) from Gram-negative (pink to red) cells. For Methyl Red testing, isolates were grown in glucose broth for 18–24 h, with Methyl Red indicator added; a stable red colour indicated a positive result. Urease activity was tested by inoculating the isolates into a urea-containing medium for 18–24 h, with pink or red indicating urease production. Lipase activity was tested by inoculating cultures into lipase reagent medium and monitoring for colour change after 18–24 h. Triple Sugar Iron reactions were assessed by streaking TSI agar slants, incubating at 37°C, and examining for butt and slant colour changes, gas bubbles, and carbohydrate fermentation indicators (Türkay *et al.*, 2024; Mohite, 2013; Mahe *et al.*, 2021; Hafezi and Khamar, 2024; Tegegn *et al.*, 2025).

Sensitivity Test

Mueller-Hinton agar (MHA) was used to culture the bacterial isolates. The inoculated plates were exposed to antibiotic discs, and after incubation, the zones of inhibition were measured to assess antibiotic effectiveness. The results were classified as resistant, intermediate, or susceptible based on the size of the inhibition zone (Wayne, 2017).

Data Analysis

Soil physicochemical parameters from wastewater-impacted locations (A–J) were pooled (n = 10) and expressed as mean ± standard deviation. Differences between the impacted soils and the control soil (CS) were evaluated using a one-sample t-test, in which the mean of the impacted soils was compared with the control value. All statistical analyses were performed using IBM SPSS Statistics, and statistical significance was set at p < 0.05.

RESULTS

Physicochemical Analysis of Soil

The physicochemical characteristics of the soil samples are presented in Tables 1 and 1a, respectively. The soil pH ranged from 4.72 to 7.00 and was generally suitable for soil health, whereas the electrical conductivity (2470–2930 μS/cm) and salinity (5.82–7.65 mg/100 g) indicated elevated salinity. Nutrient concentrations were high, with nitrate (348.62–479.65 mg/100 g), phosphate (62.25–

85.51 mg/100 g), and sulfate (121.35–267.25 mg/100 g) concentrations exceeding the recommended limits. Compared to the control, wastewater-irrigated soils exhibited significantly lower pH and higher electrical conductivity, salinity, macronutrients, and major ions (p < 0.05–0.001), indicating increased ionic loading from fish-farm wastewater.

Total Heterotrophic Bacterial Count

The total heterotrophic bacterial counts of soil samples from the agricultural farmland at the Kalshigi Fish Farm are summarised in Table 2. Bacterial counts varied across the sampling locations, with the highest value recorded in sample E (0.075 CFU/mL), followed by samples H (0.068 CFU/mL), I (0.047 CFU/mL), and G (0.043 CFU/mL). Moderate counts were observed in samples D (0.038 CFU/mL), F (0.037 CFU/mL), J (0.033 CFU/mL), and B (0.014 CFU/mL). The control soil exhibited a bacterial count of 0.040 CFU/mL, whereas sample C recorded 0.020 CFU/mL. The lowest bacterial count was observed in sample A (0.007 CFU/mL).

Identification and Biochemical Characteristics of the Isolates

The biochemical characteristics of bacterial isolates are summarized in Table 3. Isolate A tested positive for catalase, indole, methyl red, and triple sugar iron and was identified as *Escherichia coli*. Isolate B showed positive reactions for catalase, indole, citrate, motility, methyl red, lipase, urease, and triple sugar iron and was identified as *Proteus mirabilis*. Isolate C was identified as *Pseudomonas aeruginosa*, while isolates D, E, and F were identified as *Klebsiella pneumoniae*, *Bacillus subtilis*, and *Enterobacter cloacae*, respectively. All isolates were catalase positive and coagulase negative, indicating the absence of plasma-clotting activity.

Frequency of occurrence

The frequency of occurrence of bacterial isolates across the soil samples is presented in Table 4. *Escherichia coli* and *Pseudomonas aeruginosa* showed the highest occurrence, with each detected in 80% of the samples. *Proteus mirabilis* was observed in 70% of samples, followed by *Klebsiella pneumoniae* in 60% of samples. *Bacillus subtilis* was detected in 50% of the samples, whereas *Enterobacter cloacae* exhibited the lowest occurrence, being present in 40% of the samples.

Table 1: Physicochemical Properties of Agricultural Soils Irrigated with Wastewater from Kalshingi Fish Farm

Sample/Parameter	A	B	C	D	E	F	G	H	I	J	Control (CS)	Standard (STD)
pH	5.28±0.98	4.72±0.11	5.92±0.78	6.35±0.12	7.00±0.74	5.90±0.66	5.79±0.51	4.82±0.92	5.72±2.76	5.95±1.41	6.35	6.0-7.5
E. conductivity (µS/cm)	2925±1.09	2892±0.29	2930±0.54	2890±2.32	2535±0.06	2470±2.38	2463±1.19	2592±2.73	2497±0.72	2680±1.25	846	0-4
Salinity (mg/100g)	7.65±0.57	7.55±1.87	7.40±2.45	6.90±1.44	7.25±0.23	6.70±1.67	6.81±1.91	7.35±0.53	5.90±0.56	5.82±2.34	3.79	0-4.0
NO_3^- (mg/100g)	438.82±0.23	434.81±0.76	452.72±1.44	429.90±1.32	387.52±2.32	348.62±1.10	379.56±0.06	479.65±2.34	384.45±1.90	428.55±1.71	154.25	20-100
PO_4^{3-} (mg/100g)	85.51±0.45	85.25±1.75	84.92±3.22	84.45±0.77	75.75±1.67	65.73±0.74	62.25±1.82	63.55±2.57	72.75±2.98	64.80±2.76	34.37	10-60
SO_4^{2-} (mg/100g)	261.75±1.22	256.65±2.10	267.25±0.94	349.35±1.54	224.95±2.23	232.25±2.96	121.35±0.56	224.75±2.69	189.35±1.72	261.75±1.43	82.45	50-200
Cl^- (mg/100g)	765.35±2.12	755.70±1.45	769.95±1.56	748.45±0.81	682.65±1.93	647.55±1.65	628.75±1.69	729.65±1.53	619.45±0.68	674.95±0.97	253.65	10-50
Na (mg/100g)	1825.45±0.42	1847.35±2.23	1652.25±1.86	1725.50±2.54	1457.35±1.43	1463.65±1.95	1362.90±0.53	1495.45±2.21	1374.35±2.63	1320.45±2.56	368.40	20-100
K (mg/100g)	2751.35±1.32	2751.30±1.67	2653.45±1.53	2574.65±0.86	2491.55±3.21	2345.85±0.95	2248.55±1.49	2391.65±0.63	2145.75±1.42	2265.87±1.45	729.45	50-200
Mg (mg/100g)	8683.95±0.89	8645.65±0.06	8548.75±0.42	8475.74±2.65	7684.65±0.34	6954.65±0.45	6454.85±3.32	6453.86±0.83	6345.45±1.81	6324.75±0.73	2349.65	50-200
Ca (mg/100g)	5847.35±1.32	5834.54±0.07	5765.65±0.31	5204.76±1.43	4845.35±1.87	4851.75±2.67	4732.79±1.42	4753.54±0.56	4675.65±2.21	4564.76±2.86	1235.35	500-2000

Table 1a. Physicochemical characteristics of wastewater-impacted soils compared with control soil

Parameter group	Parameter	Impacted soils (A–J) Mean ± SD	Control (CS)	p-value
General properties	pH	5.75 ± 0.68	6.35	0.020
	Electrical conductivity (µS/cm)	2687.40 ± 201.24	846.00	<0.001
	Salinity (mg/100 g)	6.93 ± 0.65	3.79	<0.001
Macronutrients	NO_3^- (mg/100 g)	416.46 ± 39.86	154.25	<0.001
	PO_4^{3-} (mg/100 g)	74.50 ± 9.94	34.37	<0.001
	SO_4^{2-} (mg/100 g)	238.94 ± 58.76	82.45	<0.001
Major ions / base cations	Cl^- (mg/100 g)	702.25 ± 58.39	253.65	<0.001
	Na (mg/100 g)	1552.47 ± 195.24	368.40	<0.001
	K (mg/100 g)	2462.00 ± 216.05	729.45	<0.001
	Mg (mg/100 g)	7457.23 ± 1053.06	2349.65	<0.001
	Ca (mg/100 g)	5107.61 ± 516.34	1235.35	<0.001

Values for wastewater-impacted soils represent mean ± standard deviation of samples A–J (n = 10). Differences between impacted soils and the control soil (CS) were assessed using a one-sample t-test. Statistical significance was set at p < 0.05.

Table 2: Total Heterotrophic Bacterial Counts in Soil Samples from Kalshingi Fish Farm

S/N	Sample Code	Number of Colonies	Dilution Factor	CFU/mL	Log_{10}
1	CS	40	10^{-3}	0.4	-0.39 ± 0.45
2	A	7	10^{-3}	0.07	-1.15 ± 0.09
3	B	14	10^{-3}	0.14	-0.85 ± 0.01
4	C	20	10^{-3}	0.2	-0.69 ± 0.07
5	D	38	10^{-3}	0.38	-0.42 ± 0.23
6	E	75	10^{-3}	0.75	-0.12 ± 0.04
7	F	37	10^{-3}	0.37	-0.43 ± 0.89
8	G	43	10^{-3}	0.43	-0.37 ± 0.07
9	H	68	10^{-3}	0.68	-0.17 ± 0.56
10	I	74	10^{-3}	0.47	-0.33 ± 0.14
11	J	33	10^{-3}	0.33	-0.48 ± 0.02

Table 3: Biochemical and Morphological Characteristics of Bacterial Isolates Recovered from Wastewater-Irrigated Soils

Characteristics	*Escherichia coli*	*Proteus mirabilis*	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*	*Bacillus subtilis*	*Enterobacter cloacae*
Colony Morphology						
Shape	Rod	Rod	Rod	Rod	Rod	Rod
Elevation	Flat	Convex	Flat	Convex	Flat	Flat
Texture	Smooth	Smooth	Smooth	Mucoid	Rough	Smooth
Color	Pale pink	White opaque	Blue-green	White	White	Pale yellow
Gram Staining						
Gram Reaction	-	-	-	-	+	-
Arrangement	Single	Single	Single	Single	Chain	Single
Shape	Rod	Rod	Rod	Rod	Rod	Rod
Flagella	Peritrichous	Peritrichous	Peritrichous	Peritrichous	Peritrichous	Peritrichous
Biochemical Tests						
Catalase	+	+	+	+	+	+
Indole	+	+	-	+	-	+
Citrate	-	+	+	+	-	+
Oxidase	-	-	+	-	+	-
Coagulase	-	-	-	-	-	-
Methyl Red	+	-	-	+	-	+
Lipase	-	+	-	-	+	-
Urease	-	+	+	-	-	-
Motility	-	+	+	-	-	-
Triple Sugar Iron	++	-+	--	++	--	++

Key: "+" = Positive (Presence) "-" = Negative (Absence); "++" = Acidic/Acidic; "+-" = Alkaline/Alkaline; "--" = No change

Table 4: Frequency of Occurrence of Bacterial Isolates Identified in Soil Samples

S/N	Organism	Number of Samples	Frequency	Percentage (%)
1	*Escherichia coli*	10	8	80%
2	*Proteus mirabilis*	10	7	70%
3	*Pseudomonas aeruginosa*	10	8	80%
4	*Klebsiella pneumonia*	10	6	60%
5	*Bacillus subtilis*	10	5	50%
6	*Enterobacter cloacae*	10	4	40%

Table 5: Comparison of Bacterial Isolates Between Wastewater-Impacted and Control Soil Samples

S/N	Control Sample Isolates	Impacted Sample Isolates
1	*Escherichia coli*	*Escherichia coli*
2	*Bacillus subtilis*	*Proteus mirabilis*
3	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*
4	*Klebsiella pneumonia*	*Klebsiella pneumonia*
		Bacillus subtilis
		Enterobacter cloacae

Sensitivity Test

The antibiotic resistance profiles of bacterial isolates from wastewater-irrigated soils are presented in Table 6, while those of the control soils are shown in Table 8. In wastewater-irrigated soils, *Escherichia coli* and *Enterobacter cloacae* were not susceptible to pefloxacin or amphiclox/pefloxacin. *E. coli* showed resistance to ampiclox (0 mm) and amoxicillin (12.45 mm), whereas *Klebsiella pneumoniae* was resistant to pefloxacin (10.70 mm) and tetracycline (10.50 mm). High susceptibility was observed for *E. coli* to levofloxacin (27.34 mm) and ciprofloxacin (25.75 mm) and for *Bacillus subtilis* to chloramphenicol (25.67 mm). The control isolates exhibited varied resistance patterns, with *K. pneumoniae* showing resistance to multiple antibiotics and *Pseudomonas aeruginosa* displaying intermediate susceptibility to ciprofloxacin (20.70 mm), azithromycin (14.47 mm), chloramphenicol, and cefotaxin.

Multiple Drug Resistance

Table 7 summarises the antibiotic resistance profiles of bacterial isolates from Kalshingi Fish Farm soil, showing the number of antibiotics ssto which, each isolate was resistant. *Klebsiella pneumoniae* exhibited the highest level of resistance, being resistant to six antibiotics, followed by *Proteus mirabilis*, which showed resistance to four antibiotics. *Escherichia coli* and *Bacillus subtilis* were resistant to three antibiotics, whereas *Pseudomonas aeruginosa* was resistant to two antibiotics. *Enterobacter cloacae* was resistant to one antibiotic.

Table 6: Antibiotic Sensitivity Profiles of Bacterial Isolates from Wastewater-Irrigated Soils

S/N	Organism	Antibiotics	Code	Zone of Inhibition (mm)	Remarks
1	*Escherichia coli*	Pefloxacin,	PEF, GN, APX,	24.02 ± 0.24,	S,
		Gentamicin,	T,	12.50 ± 1.08,	R,
		Ampiclox,	AM, CHL, CPX,	NIL,	NA,
		Tetracycline,	AZ, LEV,	20.85 ± 0.07,	S,
		Amoxicillin, Chloramphenicol,	E	12.45 ± 1.04,	R,
		Ciprofloxacin, Azithromycin,		15.43 ± 1.54,	I,
		Levofloxacin,		25.75 ± 0.78,	S,
		Erythromycin		12.76 ± 1.32,	R,
				27.34 ± 0.06,	S,
				NIL	NA
2	*Proteus mirabilis*	Pefloxacin,	PEF, GN, APX,	18.54 ± 2.09,	S,
		Gentamicin,	T,	10.56 ± 0.85,	R,
		Ampiclox,	AM, CHL, CPX,	12.88 ± 0.28,	R,
		Tetracycline,	AZ, LEV,	19.08 ± 1.17,	S,
			E	12.65 ± 1.12,	R,

				17.56 ± 0.51,	I,
		Amoxicillin, Chloramphenicol, Ciprofloxacin, Azithromycin, Levofloxacin, Erythromycin		19.50 ± 0.70,	S,
				12.20 ± 0.83,	R,
				18.42 ± 0.67,	S,
				19.87 ± 0.86	I
3	*Pseudomonas aeruginosa*	Pefloxacin,	PEF,	22.54 ± 0.97,	S,
		Gentamicin,	GN,	16.50 ± 0.55,	S,
		Augmentin,	AU,	12.56 ± 0.50,	R,
		Tetracycline,	T,	20.70 ± 1.30,	S,
		Amoxicillin, Chloramphenicol,	AM, CHL, CPX,	12.50 ± 0.60,	R,
		Ciprofloxacin, Azithromycin,	AZ, LEV,	16.45 ± 0.45,	I,
		Levofloxacin,	CF	20.70 ± 0.98,	I,
		Cefotaxin		14.47 ± 0.45,	I,
				25.50 ± 1.25,	S,
				16.60 ± 0.65	I
4	*Klebsiella pneumoniae*	Pefloxacin,	PEF, CN, APX,	10.70 ± 0.55,	R,
		Gentamicin,	T,	12.40 ± 1.56,	R,
		Amphiclox,	AM, CHL, CPX,	17.30 ± 0.67,	I,
		Tetracycline,	AZ,	10.50 ± 0.08,	R,
		Amoxicillin, Chloramphenicol,		12.65 ± 1.45,	R,
		Ciprofloxacin, Azithromycin,	LEV,	16.50 ± 0.70,	I,
		Levofloxacin, Erythromycin	E	14.70 ± 0.44,	R,
				10.50 ± 1.40,	R,
				.	
				15.50 ± 0.70,	I,
				12.30 ± 0.80	I
5	*Bacillus subtilis*	Pefloxacin,	PEF, GN, APX,	NIL,	NA,
		Gentamicin,	T,	14.67 ± 0.52,	I,
		Ampiclox,	AM, CHL, CPX,	10.67 ± 0.63,	R,
		Tetracycline,	AZ, LEV,	23.79 ± 0.75,	S,
		Amoxicillin, Chloramphenicol,	E	18.67 ± 0.55,	I,
		Ciprofloxacin, Azithromycin,		25.67 ± 0.54,	S,
		Levofloxacin, Erythromycin		24.54 ± 0.67,	S,
				10.45 ± 0.65,	R,
				15.55 ± 0.56,	I,
				12.45 ± 0.54	R
6	*Enterobacter cloacae*	Pefloxacin,	PEF,	NIL,	NA,
		Gentamicin,	GN, APX,	18.75 ± 0.45,	S,
		Ampiclox,	T,	18.45 ± 0.86,	I,
		Tetracycline,	AM, CHL, CPX,	24.45 ± 0.67,	S,
		Amoxicillin, Chloramphenicol,	AZ, LEV,	13.79± 0.05,	R,
		Ciprofloxacin, Azithromycin,	E	20.65±0.75,	S,
		Levofloxacin,		22.54±0.78, 14.45±0.76,	S,
		Erythromycin		25.45±0.75, 14.50 ± 0.70	I,
					S,
					I

Key: **S** = Sensitive; **R** = Resistance; **I** = Intermediate; **NA** = Nonapplicable

Table 7: Multiple Antibiotic Resistance Patterns of Bacterial Isolates from Kalshingi Fish Farm Soils

Isolate	Drugs Resistance
Escherichia coli	+ + +
Proteus mirabilis	+ + + +
Pseudomonas aeruginosa	+ +
Klebsiella pneumoniae	+ + + + + +
Bacillus subtilis	+ + +
Enterobacter cloacae	+

Key: **+ + + + + +** = Resistance to six antibiotics; **+ + + +** = Resistance to four antibiotics; **+ + +** = Resistance to three antibiotics; **+ +** = Resistance to two antibiotics

Table 8: Antibiotic Susceptibility of Control Soil Isolates from Kalshingi Fish Farm

S/N	Organism	Antibiotics	Code	Zone of Inhibition (mm)	Remarks
1	*Escherichia coli*	Pefloxacin,	PEF,	-	S,
		Gentamicin,	CN,		R,
		Ampiclox,	APX,		NA,
		Tetracycline,	T,		S,
		Amoxicillin,	AM, CHL, CPX,		R,
		Chloramphenicol,	AZ,		I,
		Ciprofloxacin,	LEV,		S,
		Azithromycin,	E		R,
		Levofloxacin,			S,
		Erythromycin			NA
2	*Pseudomonas aeruginosa*	Pefloxacin,	PEF,	-	S,
		Gentamicin,	CN,		S,
		Augmentin,	AU,		R,
		Tetracycline,	T,		S,
		Amoxicillin,	AM, CHL, CPX,		R,
		Chloramphenicol,	AZ,		I,
		Ciprofloxacin,	LEV,		I,
		Azithromycin,	CF		I,
		Levofloxacin,			S,
		Cefotaxin			I
3	*Klebsiella pneumoniae*	Pefloxacin,	PEF,	-	R,
		Gentamicin,	CN,		R,
		Amphiclox,	APX,		I,
		Tetracycline,	T,		R,
		Amoxicillin,	AM, CHL, CPX,		R,
		Chloramphenicol,	AZ,		I,
		Ciprofloxacin,	LEV,		R,
		Azithromycin,	E		R,
		Levofloxacin,			I,
		Erythromycin			I
4	*Bacillus subtilis*	Pefloxacin,	PEF,	-	NA,
		Gentamicin,	CN,		I,
		Ampiclox,	APX,		R,
		Tetracycline,	T,		S,
		Amoxicillin,	AM, CHL, CPX,		I,
		Chloramphenicol,	AZ,		S,
		Ciprofloxacin,	LEV,		S,
		Azithromycin, Levofloxacin,	E		R,
		Erythromycin			I,
					R

Key: **S** = Sensitive **R** = Resistant **I** = Intermediate **NA** = non-applicable

DISCUSSION

Soils irrigated with wastewater from the Kalshingi fish farm exhibited pronounced alterations in physicochemical properties relative to the control group. Although the soil pH ranged from 4.72 to 7.00 and remained within a tolerable acidic to neutral range for most crops, the mean pH of wastewater-impacted soils was significantly lower (5.75 ± 0.68; p < 0.05). In contrast, salinity indicators exceeded the recommended agronomic limits, with electrical conductivity values of 2470–2930 µS cm^{-1} (2.47–2.93 dS m^{-1}) and total soluble salts of 5.82–7.65 mg 100 g^{-1} (58–76 g kg^{-1}), both significantly higher than those in the control (p < 0.001). Nutrient enrichment was substantial, as the nitrate, phosphate, and sulfate concentrations were significantly elevated relative to the control (p < 0.001). Comparable increases in electrical conductivity and nitrate levels have been reported in wastewater-irrigated soils by Saleh *et al.* (2025) and Ofori *et al.* (2024). These changes are attributable to high inputs of sodium, chloride, nitrogen, and phosphorus combined with limited leaching, as reflected by the elevated major ion and base cation concentrations (p < 0.001). Prolonged exposure may impair soil structure, water infiltration, microbial function, and groundwater quality.

Wastewater irrigation resulted in elevated heterotrophic bacterial counts, although spatial variability was observed. Sample E recorded the highest bacterial load at 0.075 CFU mL^{-1}, compared with approximately 0.040 CFU mL^{-1} in the control. Similar observations were reported by Perulli *et al.* (2024), who found that wastewater irrigation introduced faecal bacteria without necessarily causing a uniform increase in total bacterial counts. In the present study, the elevated nutrient content likely stimulated microbial proliferation, whereas high salinity and potentially toxic constituents may have suppressed sensitive taxa, resulting in reduced microbial diversity. Chen *et al.* (2025) similarly reported that wastewater induced salinity and heavy metal stress can lower microbial diversity even when overall bacterial abundance remains high. Consistent with Soufi *et al.* (2025), organic matter and ammonia inputs promoted microbial biomass, whereas chemical stressors selected resilient populations. These dynamics suggest a metabolically active but ecologically stressed microbial community, increasing the likelihood of pathogen persistence and resistance development in wastewater-irrigated soil.

Biochemical characterisation revealed the dominance of bacteria that are typically associated with wastewater environments. The identified species included *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Bacillus subtilis*, and *Enterobacter cloacae*. All isolates were catalase-positive and coagulase-negative, confirming non-Staphylococcus identities. *Escherichia coli* and *Pseudomonas aeruginosa* were detected in 80 percent of the samples, *Proteus mirabilis* in 70 percent, *Klebsiella pneumoniae* in 60 percent, *Bacillus subtilis* in 50 percent, and *Enterobacter cloacae* in 40 percent. Similar bacterial assemblages have been reported in wastewater-impacted soils by Barnwal and Saleh (2025) and Hidri *et al.* (2020). The high prevalence of enteric bacteria indicates persistent faecal contamination despite soil filtration. Biochemical profiles, such as indole positivity and acid-producing TSI reactions, supported species identification. The co-occurrence of these taxa reflects the selection of fast-growing copiotrophic organisms favoured by organic-rich wastewater, as observed by Abdelkader *et al.* (2025). This shift toward human-associated bacteria suggests that wastewater irrigation transforms soil into reservoirs of opportunistic pathogens.

Antibiotic susceptibility testing revealed widespread resistance among the soil isolates. *Escherichia coli* showed resistance to beta-lactam antibiotics, with no inhibition observed for ampiclox and a reduced inhibition zone of 12.4 mm for amoxicillin, while remaining sensitive to fluoroquinolones such as levofloxacin (27.3 mm) and ciprofloxacin (25.8 mm). *Enterobacter cloacae* exhibited complete resistance to pefloxacin and ampiclox. *Klebsiella pneumoniae* showed reduced inhibition zones of approximately 10.5 to 10.7 mm for tetracycline and pefloxacin, indicating resistance. *Pseudomonas aeruginosa* displayed intermediate susceptibility to ciprofloxacin (20.7 mm) and azithromycin (14.5 mm), whereas *Bacillus subtilis* was highly susceptible to chloramphenicol and ciprofloxacin, with inhibition zones exceeding 24 mm. These patterns are consistent with those reported by Tang *et al.* (2021) and Barnwal and Saleh (2025). The data indicate enrichment of the soil antibiotic resistome due to wastewater input, creating potential pathways for resistance transfer to crops and humans.

The high prevalence of multidrug-resistant bacteria in wastewater-irrigated soils poses significant public health concerns. All identified genera exhibited

resistance to multiple antibiotics, with *Klebsiella pneumoniae* and *Proteus mirabilis* resistant to six and four antibiotics, respectively. These resistance profiles exceed those commonly reported in non-impacted agricultural soils. Loh *et al.* (2018) identified soils and wastewater as major reservoirs of antibiotic resistance genes, a finding reinforced by Barnwal and Saleh (2025), who documented extreme beta-lactam resistance in sewage-derived bacteria. Soufi *et al.* (2025) further demonstrated that wastewater pollutants promote the persistence and mobility of resistance genes without necessarily altering the overall bacterial composition. The presence of multidrug-resistant bacteria in agricultural soils increases the risk of resistance transmission through the soil-crop-human continuum. Without adequate wastewater treatment and management strategies, such practices may undermine food safety, reduce crop quality and exacerbate the global antimicrobial resistance crisis.

CONCLUSION

This study highlights the dual impact of fish farm wastewater on soil health, showing that while it provides beneficial nutrients that can enhance soil fertility, its long-term application can disrupt soil properties and alter microbial communities. Elevated electrical conductivity, salinity, and nutrient levels were observed, which could impair soil fertility and reduce microbial activity. The identification of pathogenic and antibiotic-resistant bacteria, such as *Klebsiella pneumoniae*, raises concerns about the spread of resistance genes and the potential risks to both plant and human health. Overall, although wastewater irrigation offers nutrient recycling benefits, its unregulated use poses significant environmental and public health risks, necessitating careful management and further research on sustainable practices.

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