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Bacteriological and Physicochemical Properties of Abattoir Wastewater and Receiving Soils in Agbor, Nigeria

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ABSTRACT

This study was carried out to determine the degree of contamination, identify microbial and physicochemical composition and attendant impacts on soil quality brought on by abattoir wastewater exposure. Polluted and unpolluted soil samples were examined, and wastewater samples were collected from three diifferent abattoir locations. Physicochemical parameters, including pH, chemical oxygen demand (COD), biochemical oxygen demand (BOD), total hydrocarbons, nitrate, phosphate, ammonium, heavy metals (iron, cadmium, and lead), and electrical conductivity, were measured using standard laboratory procedures. Total viable counts, coliforms, and antibiotic susceptibility testing of bacterial isolates were all part of the bacteriological evaluation. The findings showed that the levels of heavy metals, nutrients, and organic pollutants in wastewater samples were beyond permissible limits, compared to control soil samples. Polluted soils showed elevated pH, nutrient enrichment, and microbial composition. Numerous pathogenic bacteria that were isolated showed multidrug resistance to widely used antibiotics, including *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella spp.*, and *Pseudomonas spp.* This study revealed that use and discharge of untreated abattoir wastewater poses health risks. To minimize pollution and guarantee sustainable agriculture and environment, proper wastewater treatment, regulation, and public awareness are advised.

Keywords: Abattoir wastewater, Soil contamination, Polluted soil and unpolluted soil, Physicochemical parameters and Microbial quality

INTRODUCTION

In Agbor, the disposal of untreated abattoir wastewater directly into the environment has become a major concern. This wastewater, rich in organic matter, blood, fats, and faeces, seeps into surrounding soils, leading to significant soil pollution and degradation. Polluted soils exhibit altered pH, distorted microbial composition, and increased heavy metal concentrations, which affect soil fertility and reduce agricultural productivity over time [1]. Moreover, the contamination of soil and nearby water sources has dire environmental consequences, including surface and groundwater pollution, offensive odour, and disruption of aquatic life. Crops cultivated on such contaminated soils may bioaccumulate harmful substances, posing risks to consumers and reducing the quality of farm produce [2]. From a public health standpoint, abattoir wastewater contains various pathogenic microorganisms, including *Escherichia coli*, *Salmonella spp.*, *Shigella spp.*, *Clostridium perfringens*, and *Staphylococcus aureus*. These pathogens can lead to severe diseases including diarrhoea, typhoid, dysentery, and food poisoning, particularly when they infiltrate the food chain [3, 4]. There is insufficient comprehensive data on the bacteriological and physicochemical characteristics of abattoir wastewater and its effects on adjacent soils in Agbor, despite the associated risks [5, 6]. This study aims to fill

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this gap by evaluating the level of pollution and pinpointing the microbial and physicochemical risks linked to abattoir operations in the area.

MATERIALS AND METHODS

Materials

For the physicochemical investigations, the materials comprised both standard laboratory apparatus and analytical instruments and procedures. Beakers, conical flasks, volumetric glassware, test tubes, and sterile distilled water were employed for sample preparation and handling. A digital electrical conductivity (EC) meter, standardized with 0.01 M potassium chloride solution to 1413 µS/cm, was used to determine electrical conductivity, while salinity estimations were extrapolated from EC–salinity calibration standards. A biological oxygen demand (BOD) meter with calibrated probes was utilized for BOD measurements, and other supporting accessories included cotton wool, aluminum foil, and sterile containers for sample storage. All reagents and chemicals were of analytical grade and prepared in accordance with standard environmental analytical procedures.

For the bacteriological assessments, the materials consisted of sterile Petri dishes, test tubes, inoculating loops, needles, sterile forceps, glass slides, cover slips, and meter rules for measurement of inhibition zones. An autoclave was used to sterilize media and reagents at 121 °C for 15 minutes, while an incubator maintained at 37 °C facilitated bacterial growth. Colony counts were obtained using an electric colony counter, and microscopic examinations were performed with a compound light microscope fitted with an oil immersion objective. The culture media employed included Nutrient Agar, MacConkey Agar, Eosin Methylene Blue Agar, Mannitol Salt Agar, and Mueller–Hinton Agar, all prepared according to manufacturer's specifications. Biochemical characterization of isolates utilized Gram staining reagents (crystal violet, Gram's iodine, 95% ethanol, and safranin), 3% hydrogen peroxide for catalase testing, urea medium, Simon's citrate medium, Kovac's reagent, peptone-water sugar broths with bromocresol purple indicator, and EDTA-treated plasma for coagulase testing. A wide range of commercial antibiotic discs, including pefloxacin, gentamicin, ampiclox, cefotaxime, ciprofloxacin, amoxicillin, augmentin, azithromycin, levofloxacin, streptomycin, septrin, tarivid, erythromycin, zinnacef, and rocephin, were used for susceptibility profiling. All microbiological reagents and antibiotic discs were sourced from certified suppliers to ensure reliability and reproducibility.

Study Area

This research was conducted in Agbor, Delta State of Nigeria. The residents of Agbor are agrarians, civil servants, and commence-oriented. According to Nigeria Population Commission Survey in 2021, Agbor is projected to be estimated at four hundred thousand people. The residents get water from rivers, lakes, and boreholes. Agbor has social amenities and educational institutions including University of Delta, Agbor, College of Nursing Sciences, Agbor, Nigeria Police Area Command, Central Hospital, Nigeria Correctional Service, and 181 Amphibious Battalion Nigeria Army. There are also sport facilities such as waterpark, Agbor township stadium. One of the oldest landmarks in Agbor is the Dein Royal Palace Agbor. The Dein of Agbor is the paramount ruler of Agbor. A drive around abattoir locations was undertaken to enable proper capture of the accurate abattoir geographical point using the handheld Global Positioning System [7]. The coordinates of the actual positions were acquired and imputed on the Google map.

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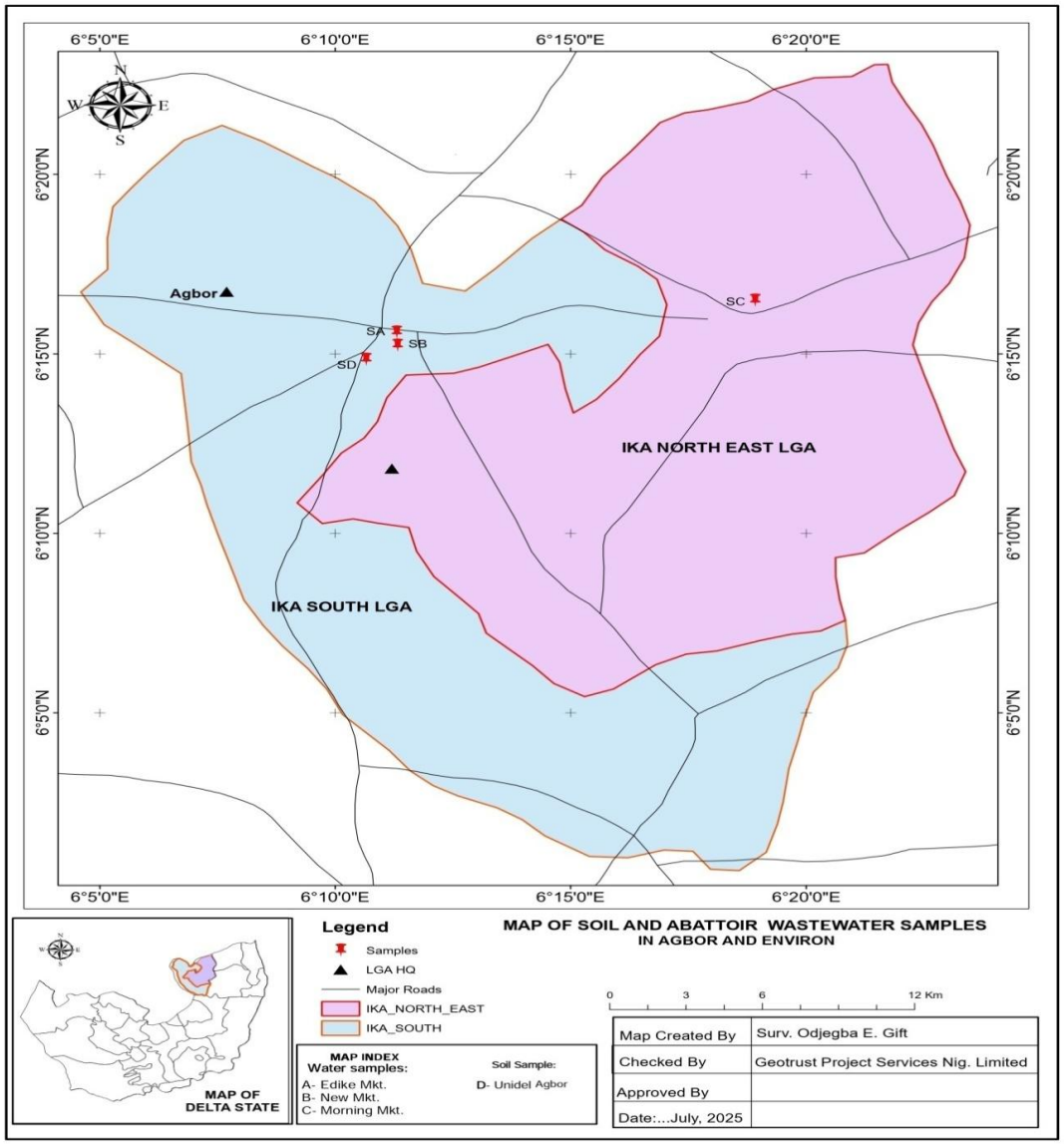

Figure 1 **Map of soil and abattoir water waste samples in Agbor.**

Research centre

The research was conducted in Biological Sciences Laboratory, University of Delta, Agbor.

Physicochemical analysis of soil sample

Using standard methods, analyses was done for the following physicochemical parameters, **pH, particle size**, **organic carbon, nitrogen, phosphorus, exchange acidity, exchangeable Na and K.**
Similarly, using standard methods, physicochemical parameters was determined for abattoir wastewater samples.

The parameters include; **total hydrocarbons, moisture content, ammonium (nitrogen), Nitrate (NO_3), sulphate (SO_4), chloride, chemical oxygen demand (COD), turbidity, colour, suspended solids, carbonate, bicarbonate and hydroxyl ions (Alkalinity), salinity, biological oxygen demand (BOD).**

Bacteriological analysis
Preparation of Culture Media

Preparation of Nutrient Agar

28 grams of nutrient agar (NA) powder was dissolved in 1 litre of distilled water in a conical flask covered with cotton wool and aluminium foil paper. It was mixed thoroughly and sterilized by autoclaving at 121°C for

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15 minutes. The medium was cooled to 45-50°C and then dispensed aseptically into sterile petri dishes in the laminar flow.

Preparation of MacConkey Agar

55 grams of MacConkey agar (MCA) powder was dissolved in 1litre of distilled water in a conical flask covered with cotton wool and aluminium foil paper. It was mixed thoroughly and sterilized by autoclaving at 121°C for 15 minutes. The medium was cooled to 45-50°C and then dispensed aseptically into sterile petri dishes in the laminar flow.

Preparation of Eosin Methylene Blue Agar

36 grams of eosin methylene blue (EMB) Agar powder was dissolved in 1litre of distilled water in a conical flask covered with cotton wool and aluminium foil paper. It was mixed thoroughly and sterilized by autoclaving at 121°C for 15 minutes. The medium was cooled to 45-50°C and then dispensed aseptically into sterile petri dishes in the laminar flow.

Preparation of Mannitol Salt Agar

111 grams of MSA agar was dissolved in 1000ml distilled water in a conical flask covered with cotton wool and aluminum foil paper. It was mixed thoroughly sterilized by autoclaving at 121°C for 15 minutes. The medium was cooled to 45-50°C and dispensed aseptically into sterile petri dishes in the laminar flow.

Isolation of Bacteria

Results per dilution count were recorded. The number of colony forming unit per millilitre Weighed into 9ml of sterile distilled water and allowed to stand for 30 minutes. The aliquot growth of microorganisms, the colonies were counted with a colony counter and the Growth) was poured in aseptically and incubated at 37°C for 24 hours. After successful 1ml of abattoir waste water sample were measured and placed in 9ml sterile distilled water and allowed to stand for 30 minutes and also one gram(1g) of soil samples were Was then transferred aseptically to sterile petri plates. The prepared agar (for bacteria

Was calculated with the formula:

$$\mathbf{CFU/g} = \frac{\boldsymbol{Numbers\ of\ Colonies}}{\boldsymbol{Volume\ plate\ x\ dillution\ factor}}$$

Pure culture

One single colony was identified and re-streaked as a primary inoculant on the surface of a nutrient agar plate medium. Pure cultures were checked from nutrient agar plates. After achieving a pure culture, the same colony was streaked onto a nutrient agar slant. These cultures were incubated at 37°C for 24 hours.

Biochemical and Morphological Identification of Bacterial Isolates

Using standard methods biochemical and morphological characterisation was done to identify the bacterial isolates. The identification followed these observations;

Each colony morphology e.g., size, shape, margin, elevation, consistency, colour, transparency and Gram stain was determined.

The biochemical tests include; catalase, urease, citrate utilization, hydrogen sulphide (H_2S), indole, sugar fermentation, coagulase.

Antibiotics susceptibility test

Test organisms will be subjected to antibiotics sensitivity test using the Kirby Bauer disc diffusion on prepared media. Ten (10) different commercial antibiotic discs will be used. The antibiotic discs will be carefully and firmly placed on the inoculated plates using a sterile pair of forceps. The plates will be inverted and incubated for 37°C for 24 hours. The diameter of the zone of inhibition will be measured in millimeters (mm) using a meter rule. The experiments will be carried out in triplicates to minimize probability of error.

Preparation of Mueller Hinton Agar

Suspend 38 grams of Mueller Hinton agar powder in 1L of distilled water. Mix and dissolve them completely. Sterilize by autoclaving at 121°C for 15 minutes. Pour the liquid into the petri dish and wait for the medium to solidify. Be sure to prepare the agar in the clean environment to prevent any contamination.

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Determination of bacteria total count

1ml of abattior waste water sample were measured and placed in 9ml sterile distilled water and allowed to stand for 30 minutes and also one gram(1g) of soil samples were weighed into 10ml of distilled deionized water as stick solution. Six flask containing 9ml each of sterile distilled water was used for the dilution. One millilitre of the initial dilution was introduced into the first 9ml test tube to give 0.1^1 suspension up to 10^3 (for wastewater) and 10^5(soil samples) suspension. suspension. Aliquot of 0.1ml of the appropriate dilution from each wastewater samples and soil samples (polluted and unpolluted) was plated in nutrient Agar. Aerobically, the number of discrete colonies were counted in colony forming units per gram (cfu/g),(cfu /mL) using an electric colony counter machine. The viable count was calculated from the values as follows:

For waste water:

CFU/mL =No of colonies x dilution factor

For soil samples

CFU/g =No of colonies x dilution factor

Examination of total and fecal coliform

1ml of abattior waste water sample were measured and placed in 9ml sterile distilled water and allowed to stand for 30 minutes and also one gram(1g) of soil samples were weighed into 10ml of distilled deionized water as stick solution. Six flask containing 9ml each of sterile distilled water was used for the dilution. One millilitre of the initial dilution was introduced into the first 9ml test tube to give 0.1^1 suspension up to 10^3(for wastewater) and 10^4 (soil samples) suspension. Aliquot of 0.1ml of the appropriate dilution from each wastewater samples and aliquot of 0.5ml of the appropriate dilution from each soil samples (polluted and unpolluted) was plated in Mac Conkey Agar . Aerobically, the number of discrete colonies were counted in colony forming units per gram (cfu/g),(cfu /mL) using an electric colony counter machine . The viable count was calculated from the values as follows:

For waste water

CFU/mL =No of colonies x dilution factor

For soil samples

CFU/g =No of colonies x dilution factor

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RESULTS

Table 1: Calculation of Hydroxide, Carbonate, and Bicarbonate Alkalinity from Titration Data

Result of Titration	Titration Value Related to each ION		
	Hydroxide	Carbonate	Bicarbonate
P = 0	0	0	T
P<1/2T	0	2P	T-2P
P=1/2T	0	2P	0
P>1/2T	2P-T	2(T-P)	0
P=T	T	0	0

P= Titration to the phenolphthalein end point
T= Total Titration to the methyl orange end point
Molarity x titre x mol. Wt x 1000) mg per litre
Aliquot
T x 61 = ppm $HCO3^{2-}$

Table 2: Correlation between Molarity, Salinity, and Electrical Conductivity in Aqueous Solutions

Morality (M)	Salinity (g/l)	Electrical Conductivity (mS/cm)
0.001	0.055	0.156
0.002	0.117	0.341
0.003	0.175	0.485
0.004	0.234	0.638
0.005	0.292	0.774
0.006	0.351	0.915
0.007	0.409	1.141
0.008	0.468	1.288
0.009	0.526	1.398
0.010	0.585	1.416
0.020	1.169	2.876
0.030	1.754	4.216
0.040	2.338	5.616
0.050	2.923	7.011
0.060	3.507	7.621
0.070	4.092	9.103
0.080	4.676	10.351
0.090	5.261	11.600
0.100	5.845	12.859
0.200	11.691	22.447

Table 3: Distribution patterns of bacterial isolates in wastewater

Organism	A	B	C
***Enterobacter*sp.**			+
***Klebsiella*sp.**	+		
***Salmonella* sp.**		+	
***Enterobacter*sp.**		+	
***Streptococcus* sp.**			+
***Yersinia* sp.**		+	
***Enterococcus* sp.**	+		
***Bacillus* sp.**	+		
Escherichia coli	+		
Escherichia coli	+		
Escherichia coli		+	
Escherichia coli			+
Escherichia coli			+
Escherichia coli			+
Staphylococcus epidermidis	+		
Staphylococcus epidermidis	+		
Staphylococcus aureus		+	
Staphylococcus aureus		+	
Staphylococcus epidermidis			+
Staphylococcus aureus			+

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Table 4: Distribution pattern of bacterial isolates in soil samples

Polluted soil			
Organism	**A**	**B**	**C**
Bacillus cereus	+		
Staphylococcus aureus	+		
***Bacillus* sp.**		+	
***Enterobacter* sp.**			+
Bacillus subtilis			+
***Klesbsiella*sp.**	+		
***Citrobacter*sp.**			+
***Klebsiella* sp.**	+		
***Enterobacter* sp.**		+	
Escherichia coli	+		
Escherichia coli		+	
Escherichia coli			+
Staphylococcus aureus	+		
Staphylococcus aureus		+	
Staphylococcus aureus			+

Pristine (unpolluted)soil
***Yersinia* sp.**
***Salmonella* sp.**
***Klebsiella* sp.**
***Citrobacter* sp.**
***Shigella* sp.**
***Enterobacter* sp.**
Staphylococcus aureus
Staphylococcus aureus
Staphylococcus epidermidis

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Table 5: Colony count on bacterial isolates from wastewater

	Heterotrophic Bacteria (NA) count	Mannitol salt (MSA) Agar	Total coliform (MCA) count	Eosin methylene blue (EMB) agar	Faecal coliform count
SAMPLE NAME	R	R	R	R	R
Wastewater A	16.67	19.33	5.00	27.67	1.67
Wastewater B	75.33	42.33	28.33	27.34	4.67
Wastewater C	23.00	11.67	19.67	14.67	7.33

Table 6: Colony count on bacterial isolates from soil sample

	Heterotrophic Bacteria (NA) count	Mannitol salt (MSA) Agar	Total coliform (MCA) count	Eosin methylene blue (EMB) agar	Faecal coliform count
SAMPLE NAME	R	R	R	R	R
Pristine (unpolluted)	22.33	5.67	2.33	0.00	1.67
Polluted A	24.67	0.00	10.33	9.67	7.00
Polluted B	56.00	4.67	97.33	14.67	12.00
Polluted C	101.33	12.67	59.33	12.33	10.00

Table 7: Characteristics of abattoir wastewater isolates

Organism	Shape	Size	Elevation	Transparency	Colour Nutrient Agar	Mannitol Salt Agar	MacConkey Agar	Eosin Methylene Blue Agar
***Enterobacter*sp.**	Round	Medium	Raised	Opaque			Cream	
***Klebsiella*sp.**	Round	Small	Raised	Opaque			Pink	
***Salmonella* sp.**	Round	Small	Raised	Opaque			Pink	
***Enterobacter*sp.**	Round	Small	Raised	Translucent			Pink	
***Streptococcus* sp.**	Round	Small	Flat	Opaque	Cream			
***Yersinia* sp.**	Round	Small	Flat	Opaque	Cream			
***Enterococcus* sp.**	Round	Small	Flat	Opaque	Cream			
***Bacillus* sp.**	Irregular	Large	Raised	Opaque	Cream			
Escherichia coli	Round	Small	Raised	Opaque				Pink
Escherichia coli	Round	Small	Flat	Translucent				Pink
Escherichia coli	Irregular	Small	Flat	Opaque				Pink
Escherichia coli	Round	Small	Raised	Opaque				Pink
Escherichia coli	Irregular	Medium	Flat	Translucent				Cream
Escherichia coli	Round	Small	Flat	Translucent				Cream
Staphylococcus epidermidis	Irregular	Large	Flat	Translucent		Green		
Staphylococcus epidermidis	Round	Medium	Raised	Opaque		Green		
Staphylococcus aureus	Round	Small	Flat	Opaque		Cream		
Staphylococcus aureus	Irregular	Large	Flat	Translucent		Green		
Staphylococcus epidermidis	Round	Small	Flat	Opaque		Cream		
Staphylococcus aureus	Irregular	Large	Flat	Translucent		Green		

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Table 8: Characteristics of bacterial isolates from soil samples

Polluted Soil								
Organism	**Shape**	**Size**	**Elevation**	**Transparency**	**Colour Nutrient Agar**	**Mannitol Salt Agar**	**MacConkey Agar**	**Eosin Methylene Blue Agar**
Bacillus cereus	Round	Medium	Flat	Opaque	Cream			
Staphylococcus aureus	Irregular	Large	Flat	Opaque	Cream			
***Bacillus* sp.**	Irregular	Small	Flat	Opaque	Cream			
***Enterobacter* sp.**	Round	Small	Flat	Opaque	Cream			
Bacillus subtilis	Irregular	Large	Flat	Opaque	Cream			
***Klesbsiella*sp.**	Irregular	Medium	Flat	Opaque			Pink	
***Citrobacter*sp.**	Small	Round	Flat	Opaque			Pink	
***Klebsiella* sp.**	Irregular	Medium	Raised	Opaque			Cream	
***Enterobacter* sp.**	Round	Small	Flat	Opaque			Pink	
Escherichia coli	Round	Small	Raised	Opaque				Pink
Escherichia coli	Round	Medium	Raised	Opaque				Pink
Escherichia coli	Round	Medium	Raised	Opaque				Pink
Staphylococcus aureus	Irregular	Large	Flat	Translucent			Cream	
Staphylococcus aureus	Irregular	Medium	Flat	Opaque			Cream	
Staphylococcus aureus	Irregular	Medium	Raised	Opaque			Cream	
Pristine (Unpolluted)**Soil**								
***Yersinia* sp.**	Round	Punctiform	Flat	Opaque	Cream			

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***Salmonella* sp.**	Round	Punctiform	Flat	Translucent	Cream		
***Klebsiella* sp.**	Round	Punctiform	Flat	Opaque	Cream		
***Citrobacter* sp.**	Irregular	Large	Flat	Opaque		Cream	
***Shigella* sp.**	Irregular	Medium	Flat	Opaque		Cream	
***Enterobacter* sp.**	Irregular	Medium	Flat	Opaque		Cream	
Staphylococcus aureus	Irregular	Medium	Raised	Opaque			Cream
Staphylococcus aureus	Round	Small	Raised	Translucent			Cream
Staphylococcus epidermidis	Round	Small	Raised	Opaque			Cream

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Table 9: Morphology and biochemical tests for bacterial isolates from abattoir wastewater

Gram stain	-	-	-	-	+	-	+	+	-	-	-	-	-	-	+	+	+	+	+	+
Cell type	R	R	R	R	C	R	C	R	R	R	R	R	R	R	C	C	C	C	C	C
Cell arrangement	Ch	S	S	Cl	Cl	S	Cl	Cl	Cl	Cl	S	S	S	S	Cl	S	Cl	Ch	Cl	Cl
Urease	-	+	-	-	-	+	-	-	-	-	-	-	-	-	+	+	+	+	+	+
Indole	-	-	-	-	-	-	+	-	+	+	+	+	+	+	-	-	-	-	-	-
Citrate	+	+	-	+	-	-	-	+	-	-	-	-	-	-	-	-	+	+	-	+
Catalase	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
H_2S	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-
Coagulase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+
Lactose	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
Sucrose	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fructose	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Starch	-	+	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
Sorbitol	+	+	+	+	-	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-

	***Enterobacter* sp.**	***Klebsiella* sp.**	***Salmonella* sp.**	***Enterobacter* sp.**	***Streptococcus* sp.**	***Yersinia* sp.**	***Enterococcus* sp.**	***Bacillus* sp.**	***Escherichia coli***	***Escherichia coli***	***Escherichia coli***	***Escherichia coli***	***Escherichia coli***	***Escherichia coli***	***Staphylococcus epidermidis***	***Staphylococcus epidermidis***	***Staphylococcus aureus***	***Staphylococcus aureus***	***Staphylococcus aureus***	***Staphylococcus aureus***

Keys : R = Rod, C = Cluster, Ch = Chain, Cl = Cluster, S = Single

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Table 10: Morphology and biochemical tests for bacterial isolates from soil samples

Polluted soil															
Gram stain	+	+	+	-	+	-	-	-	-	-	-	-	+	+	+
Cell type	R	C	R	R	R	R	R	R	R	R	R	R	C	C	C
Cell arrangement	Ch	Cl	Cl	Ch	Cl	S	Ch	Cl	Cl	Ch	S	Ch	Ch	Ch	Cl
Urease	+	+	+	-	-	+	-	+	-	-	-	-	+	+	+
Indole	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-
Citrate	+	+	+	+	+	+	+	+	+	-	-	-	+	+	+
Catalase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
H_2S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Coagulase	-	+	-	-	-	-	-	-	-	-	-	-	+	+	+
Lactose	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fructose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Starch	+	-	+	-	+	-	-	-	-	-	-	-	-	-	-
Sorbitol	-	-	-	+	+	+	+	+	+	+	+	-	-	-	-
	Bacillus cereus	***Staphylococcus aureus***	***Bacillus* sp.**	***Enterobacter* sp.**	**Bacillus subtilis**	***Klesbsiella*sp.**	***Citrobacter*sp.**	***Klebsiella* sp.**	***Enterobacter* sp.**	***Escherichia coli***	***Escherichia coli***	***Escherichia coli***	***Staphylococcus aureus***	***Staphylococcus aureus***	***Staphylococcus aureus***

Keys : R = Rod, C = Cluster, Ch = Chain, Cl = Cluster, S = Single

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Table 11: Morphological, biochemical and sugar tests of bacterial isolates

	Control soil								
Gram stain	-	-	-	-	-	-	+	+	-
Cell type	Rod	Rod	Rod	Rod	Rod	Rod	Cocci	Cocci	Cocci
Cell arrangement	Single	Cluster	Pairs	Cluster	Single	Chains	Chains	Cluster	Chains
Urease	+	-	+	-	-	-	+	+	+
Indole	-	-	-	-	+	-	-	-	-
Citrate	-	-	+	+	-	+	+	+	-
Catalase	+	+	+	+	+	+	+	+	+
H_2S	-	-	-	-	-	-	-	-	+
Coagulase	-	-	-	-	-	-	+	+	-
Lactose	-	-	+	+	-	+	+	+	+
Sucrose	+	-	+	+	-	+	+	+	+
Glucose	+	+	+	+	+	+	+	+	+
Fructose	+	-	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+	+
Starch	-	-	-	-	-	-	-	-	-
Sorbitol	+	+	+	+	-	+	-	-	-

	***Yersinia* sp.**	***Salmonella* sp.**	***Klebsiella* sp.**	***Citrobacter* sp.**	***Shigella* sp.**	***Enterobacter* sp.**	***Staphylococcus aureus***	***Staphylococcus aureus***	***Staphylococcus epidermidis***

Table 12: Antibiotic susceptibility for abattoir wastewater isolates

Positive disc

Isolates	**R**	**CPX**	**AZ**	**LEV**	**E**	**PEF**	**CN**	**APX**	**Z**	**AM**	**R.I**
***Streptococcus* sp.**	16(I)	14(I)	16(I)	16(I)	16(I)	2(R)	10(R)	0(R)	0(R)	18	0.4
***Enterococcus* sp.**	18(S)	18(S)	10(R)	20(S)	18(S)	18(S)	18(S)	8(R)	6(R)	16(I)	0.3
***Bacillus* sp.**	20(S)	18(S)	12(I)	20(S)	18(S)	18(S)	18(S)	16(I)	14(I)	16(I)	0
Staphylococcus epidermidis	10(R)	8(R)	8(R)	10(R)	16(I)	20(S)	12(I)	4(R)	0(R)	0(R)	0.7

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Staphylococcus epidermidis	10(R)	0(R)	16(I)	16(I)	12(I)	10(R)	12(I)	14(I)	18(S)	20(S)	0.3
Staphylococcus aureus	6(R)	14(I)	12(I)	10(R)	10(R)	14(I)	10(R)	0(R)	0(R)	6(R)	0.7
Staphylococcus aureus	20(S)	18(S)	12(I)	22(S)	6(R)	8(R)	12(I)	6(R)	0(R)	16(I)	0.4
Staphylococcus epidermidis	18(S)	16(I)	14(I)	14(I)	12(I)	12(I)	8(R)	0(R)	0(R)	0(R)	0.4
Staphylococcus aureus	0(R)	12(I)	0(R)	12(I)	12(I)	14(I)	4(R)	0(R)	0(R)	0(R)	0.6

Negative disc

Isolates	**CPX**	**AM**	**AU**	**CN**	**PEF**	**OFX**	**AZ**	**LEV**	**CF**	**SP**	**R.I**
***Enterobacter*sp.**	12(I)	16(I)	16(I)	8(R)	16(I)	0(R)	14(I)	12(I)	0(R)	0(R)	0.4
***Klebsiella*sp.**	12(I)	10(R)	16(I)	16(I)	20(S)	21(S)	12(I)	12(I)	10(R)	12(I)	0.2
***Salmonella* sp.**	16(I)	18(S)	18(S)	18(S)	18(S)	18(S)	12(I)	0(R)	0(R)	18(S)	0.2
***Enterobacter*sp.**	6(R)	0(R)	0(R)	12(I)	0(R)	8(R)	18(S)	18(S)	0(R)	0(R)	0.7
***Yersinia* sp.**	2(R)	18(S)	6(R)	14(I)	2(R)	2(R)	8(R)	18(S)	16(I)	16(I)	0.5
Escherichia coli	10(R)	16(I)	14(I)	10(R)	10(R)	12(I)	12(I)	6(R)	0(R)	4(R)	0.6
Escherichia coli	0(R)	10(R)	8(R)	24(S)	10(R)	12(I)	6(R)	0(R)	0(R)	0(R)	0.8
Escherichia coli	6(R)	20(S)	12(I)	16(I)	12(I)	12(I)	10(R)	6(R)	0(R)	0(R)	0.5
Escherichia coli	4(R)	24(S)	8(R)	16(I)	10(R)	10(R)	10(R)	9(R)	0(R)	0(R)	0.8
	S	**SXT**	**CH**	**LP**	**CPX**	**AM**	**AU**	**CN**	**PEF**	**OFX**	**R.I**
Escherichia coli	0(R)	0(R)	10(R)	0(R)	0(R)	6(R)	0(R)	0(R)	6(R)	0(R)	1.0
Escherichia coli	0(R)	2(R)	8(R)	0(R)	0(R)	10(R)	6(R)	6(R)	6(R)	2(R)	1.0

KEYS: R.I = Resistance Index, Resistant (R) = 0-10mm, Intermediate (I) = 11-16mm, Sensitive (S) =17mm and above

Key: Positive Disc	**Key: Negative Disc**

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Abbreviation	Antibiotics	Concentration	Abbreviation	Antibiotics	Concentration
PEF	Pefloxacin	10µg	LEV	Levofloxacin	20µg
CN	Gentamycin	10µg	CF	Cefotaxim	10µg
APX	Ampiclox	30µg	SP	Sparifloxacin	10µg
Z	Zinnacef	20µg	CPX	Ciprofloxacin	30µg
AM	Amoxacillin	30µg	AM	Amoxacillin	30µg
R	Rocephin	25µg	AU	Augmentin	10µg
CPX	Ciprofloxacin	10 µg	CN	Gentamycin	30µg
AZ	Azithromycin	12 µg	PEF	Pefloxacin	30µg
LEV	Levofloxacin	20µg	OFX	Tarivid	10µg
E	Erythromycin	10µg	AZ	Azithromycin	12µg

Key: Positive disc			**Key: Negative Disc**		
Abbreviation	**Antibiotics**	**Concentration**	**Abbreviation**	**Antibiotics**	**Concentration**
PEF	Pefloxacin	10µg	SXT	Septrin	30µg
CN	Gentamycin	10µg	CH	Chloranphenicol	30µg
APX	Ampliclox	30µg	SP	Sparifloxacin	10µg
Z	Zinnacef	20µg	CPX	Ciprofloxacin	30µg
AM	Amoxacillin	30µg	AM	Amoxacillin	30µg
R	Rocephin	25µg	AU	Augmentin	10µg
CPX	Ciprofloxacin	10µg	CN	Gentamycin	30µg
S	Streptomycin	30µg	PEF	Pefloxacin	30µg
SXT	Septrin	30µg	OFX	Tarivid	10µg
E	Erythromycin	10µg	S	Streptomycin	30µg

Table 13: Antibiotic susceptibility test for soil samples isolates

Positive disc

	Polluted Soil										
ISOLATES	**CN**	**PEF**	**E**	**LEV**	**AZ**	**CPX**	**R**	**AM**	**Z**	**APX**	**R.I**
Bacillus cereus	8(R)	14(I)	10(R)	14(I)	8(R)	20(S)	12(I)	0(R)	0(R)	0(R)	0.6
Staphylococcus aureus	16(I)	18(S)	14(I)	18(S)	14(I)	14(I)	4(R)	8(R)	0(R)	8(R)	0.4
***Bacillus* sp.**	16(I)	14(I)	14(I)	18(S)	18(S)	20(S)	16(I)	16(I)	16(I)	6(R)	0.1
Bacillus subtilis	8(R)	16(I)	12(I)	8(R)	0(R)	12(I)	0(R)	8(R)	0(R)	0(R)	0.7
Staphylococcus aureus	14(I)	18(S)	20(S)	20(S)	18(S)	16(I)	16(I)	18(S)	10(R)	12(I)	0.1
Staphylococcus aureus	16(I)	18(S)	16(I)	16(I)	12(I)	10(R)	12(I)	16(I)	14(I)	14(I)	0.1
Staphylococcus aureus	18(S)	20(S)	22(S)	22(S)	14(I)	18(S)	12(I)	10(R)	0(R)	8(R)	0.3
	Pristine (Unpolluted)Soil										
	CN	**APX**	**Z**	**AM**	**R**	**CPX**	**AZ**	**LEV**	**E**	**PEF**	**R.I**
Staphylococcus aureus	16(I)	16(I)	16(I)	18(S)	12(I)	10(R)	20(S)	16(I)	22(S)	18(S)	0.1
Staphylococcus aureus	12(I)	2(R)	2(R)	18(S)	16(I)	18(S)	12(I)	14(I)	12(I)	12(I)	0.2
Staphylococcus epidermidis	8(R)	22(S)	20(S)	18(S)	12(I)	10(R)	10(R)	12(I)	20(S)	18(S)	0.3

Negative Disc

	Polluted soil										
ISOLATES	**SXT**	**CH**	**SP**	**CPX**	**AM**	**AU**	**CN**	**PEF**	**OFX**	**S**	**R.I**
***Enterobacter* sp.**	6(R)	12(I)	14(I)	18(S)	8(R)	2(R)	0(R)	10(R)	18(S)	4(R)	0.6

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***Klesbsiella*sp.**	6(R)	12(I)	14(I)	16(I)	0(R)	0(R)	2(R)	16(I)	18(S)	0(R)	0.5
***Enterobacter* sp.**	14(I)	10(R)	10(R)	14(I)	0(R)	0(R)	2(R)	18(S)	20(S)	6(R)	0.6
Escherichia coli	8(R)	12(I)	10(R)	14(I)	6(R)	0(R)	6(R)	8(R)	16(I)	0(R)	0.7
Escherichia coli	4(R)	18(S)	18(S)	18(S)	0(R)	2(R)	12(I)	16(I)	18(S)	0(R)	0.4
Escherichia coli	12(I)	12(I)	14(I)	16(I)	18(S)	20(S)	18(S)	16(I)	12(I)	14(I)	0
***Citrobacter*sp.**	8(R)	12(I)	12(I)	14(I)	2(R)	12(I)	0(R)	4(R)	0(R)	0(R)	0.6
***Klebsiella* sp.**	20(S)	16(I)	12(I)	12(I)	14(I)	14(I)	8(R)	10(R)	6(R)	16(I)	0.3

Pristine (unpolluted)soil

	AU	**CN**	**PEF**	**OFX**	**AZ**	**LEV**	**E**	**SP**	**CPX**	**AM**	**R.I**
***Yersinia* sp.**	12(I)	22(S)	20(S)	18(S)	14(I)	14(I)	14(I)	12(I)	18(S)	22(S)	0
***Salmonella* sp.**	10(R)	22(S)	18(S)	18(S)	14(I)	14(I)	8(R)	14(I)	16(I)	12(I)	0.2
Klebsiella sp.	10(R)	16(I)	18(S)	22(S)	20(S)	20(S)	8(R)	14(I)	10(R)	12(I)	0.3

Pristine (unpolluted) soil

	AU	**CN**	**PEF**	**OFX**	**S**	**SXT**	**CH**	**LP**	**CPX**	**AM**	**R.I**
***Citrobacter* sp.**	0(R)	20(S)	22(S)	22(S)	0(R)	0(R)	6(R)	8(R)	14(I)	0(R)	0.6
***Shigella* sp.**	10(R)	24(S)	24(S)	22(S)	0(R)	8(R)	12(I)	12(I)	14(I)	0(R)	0.4
***Enterobacter* sp.**	10(R)	20(S)	20(S)	20(S)	0(R)	0(R)	4(R)	14(I)	18(S)	18(S)	0.4

KEYS: R.I = Resistance Index, Resistant (R) = 0-10mm, **Intermediate (I)** = 11-16mm, **Sensitive (S)** =17mm and above

Key: Positive Disc			**Key: Negative Disc**		
Abbreviation	**Antibiotics**	**Concentration**	**Abbreviation**	**Antibiotics**	**Concentration**
PEF	Pefloxacin	10µg	**SXT**	Septrin	30µg
CN	Gentamycin	10µg	**CH**	Chloranphenicol	30µg
APX	Ampliclox	30µg	**SP**	Sparifloxacin	10µg
Z	Zinnacef	20µg	**CPX**	Ciprofloxacin	30µg
AM	Amoxacillin	30µg	**AM**	Amoxacillin	30µg
R	Rocephin	25µg	**AU**	Augmentin	10µg
CPX	Ciprofloxacin	10µg	**CN**	Gentamycin	30µg
S	Streptomycin	30µg	**PEF**	Pefloxacin	30µg
SXT	Septrin	30µg	**OFX**	Tarivid	10µg
E	Erythromycin	10µg	**S**	Streptomycin	30µg

Key: Positive Disc			**Key: Negative Disc**		
Abbreviation	**Antibiotics**	**Concentration**	**Abbreviation**	**Antibiotics**	**Concentration**
PEF	Pefloxacin	10µg	**LEV**	Levofloxacin	20µg
CN	Gentamycin	10µg	**CF**	Cefotaxim	10µg

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APX	Ampiclox	30µg	**SP**	Sparifloxacin	10µg
Z	Zinnacef	20µg	**CPX**	Ciprofloxacin	30µg
AM	Amoxacillin	30µg	**AM**	Amoxacillin	30µg
R	Rocephin	25µg	**AU**	Augmentin	10µg
CPX	Ciprofloxacin	10 µg	**CN**	Gentamycin	30µg
AZ	Azithromycin	12 µg	**PEF**	Pefloxacin	30µg
LEV	Levofloxacin	20µg	**OFX**	Tarivid	10µg
E	Erythromycin	10µg	**AZ**	Azithromycin	12µg

Table 14: Wastewater physicochemical analysis

Parameter	A	B	C	WHO Limit
pH	7.1	7.0	6.9	6.5–8.5
EC (µS/cm)	341	1145	337	<750–1000
Sal. (g/l)	0.154	0.518	0.152	<0.5
Col. (Pt.Co)	0.03	0.04	0.03	<15
Turb. (NTU)	0.02	0.03	0.01	<5.0
TSS (mg/l)	0.06	0.06	0.05	<30
TDS (mg/l)	170	570	169	<1000
DO (mg/l)	3.8	3.7	4.3	>4.0
BOD (mg/l)	3.3	4.0	3.1	<30
COD (mg/l)	83.2	50.4	80.8	<250
HCO_3 (mg/l)	457.5	176.9	512.4	500
Na (mg/l)	7.7	10.4	6.4	200
K (mg/l)	2.8	5.0	1.7	12
Ca (mg/l)	18.2	33.4	15.3	75
Mg (mg/l)	11.3	17.7	10.7	50
Cl (mg/l)	53.2	118.6	53.2	250
P (mg/l)	3.48	5.30	2.35	5
NH_4-N (mg/l)	1.21	4.45	1.28	0.5–1.0
NO_2 (mg/l)	0.04	0.08	0.03	0.1
NO_3 (mg/l)	1.01	1.81	1.15	50
SO_4 (mg/l)	1.29	3.07	1.89	250

Table 15: Soil physicochemical analysis with WHO limits

Parameter	PA	PB	PC	UP (Ctrl)	WHO Limit
pH	6.0	5.7	5.6	6.1	6.0–8.5[1]
EC (µS/cm)	747	566	577	560	≤1000[2]
Org. C (%)	2.14	1.38	1.91	1.21	1–3[3]
Org. M (%)	3.68	2.37	3.29	2.08	—
T. N	0.19	0.13	0.17	0.11	0.2–2.0[4]
EA (meq/100g)	0.5	0.9	0.7	1.1	≤2.0[5]
Na (meq/100g)	0.48	0.27	0.33	0.19	≤2.0[6]
K (meq/100g)	0.21	0.11	0.17	0.10	≤0.8[7]
Ca (meq/100g)	1.01	0.71	0.88	0.70	≤2.0[6]
Mg (meq/100g)	0.74	0.55	0.60	0.51	≤1.5[6]
Av. P (mg/kg)	12.5	7.62	8.68	5.80	≤15.0[8]
Cl^- (mg/kg)	354	177	212	177	≤250[9]

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NH_4-N (mg/kg)	4.90	3.63	3.98	2.85	≤10.0[10]
NO_3 (mg/kg)	10.2	6.23	8.10	4.98	≤50.0[11]
NO_2 (mg/kg)	0.104	0.071	0.087	0.063	≤0.1[12]
SO_4 (mg/kg)	0.66	0.55	0.56	0.54	≤1.0[13]
Clay (%)	14.1	13.3	13.8	12.4	0
Silt (%)	1.0	0.6	0.7	0.6	0
Sand (%)	85.9	86.7	85.5	87.0	0

DICUSSION

Microbial findings

The microbial analysis of abattoir wastewater and contaminated soils in Agbor revealed high total viable counts (TVCs), significantly exceeding WHO permissible limits for environmental safety, this agrees with Oghonim [21] comparative findings on Total Viable Counts (TVCs) in borehole and other water samples in Agbor. Pathogenic bacteria such as *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella spp*., and *Pseudomonas spp*. were isolated, many of which displayed resistance to commonly used antibiotics. These results align with the findings of [11], who reported that untreated abattoir effluents substantially increase the microbial burden of soils, raising the risk of zoonotic disease transmission. Previously Oghonim P.AN [22] has reported antimicrobial resistance in water samples in Agbor. Similarly, [12] documented the presence of protozoa and helminths in abattoir-polluted soils, reinforcing concerns about the sanitary risks posed to surrounding communities.

The high microbial load observed in this study can be attributed to the discharge of blood, intestinal contents, and organic residues into the environment, which provide favorable substrates for bacterial growth. This agrees with [13, 14], who identified slaughterhouse wastes as rich sources of organic nutrients that facilitate microbial proliferation. The detection of multidrug-resistant isolates further highlights the public health implications of indiscriminate antibiotic use in livestock, echoing the observations of [15] that abattoir environments act as reservoirs for antimicrobial resistance genes.

Oghonim et.al. [7] emphasized similar concerns in his study of abattoir wastewater in Delta State, noting that microbial contamination extends beyond immediate soil sites to groundwater sources through leaching and runoff. He further observed that resistant strains of *E. coli* and *Klebsiellapneumoniae* in such environments represent a major health hazard, given their ability to enter the human food chain. This corroborates the present findings, underscoring the need for strict regulation and monitoring.

Physicochemical findings

The physicochemical analysis showed significant alterations in soil quality in areas exposed to abattoir wastewater. Polluted soils recorded elevated pH, total organic carbon, nitrogen, and phosphorus levels compared to unpolluted controls. These findings are consistent with [16], who reported that soils impacted by abattoir discharges exhibit nutrient enrichment and higher organic matter content. While such changes may temporarily improve fertility, long-term impacts include soil salinization, reduced microbial balance, and impaired crop productivity. Oghonim et.al., [7] found that total chloride, phosphate, alkalinity, calcium hardness and other chemical parameters of water samples in Agbor are within recommended limits unless impacted on by additional pollutants like abattoir wastewater.

Heavy metal analysis revealed the presence of lead (Pb), cadmium (Cd), and iron (Fe) at concentrations above WHO permissible limits. These results align with [17, 18], who confirmed that abattoir effluents are key contributors to heavy metal accumulation in Nigerian soils. Continuous exposure to these metals can lead to bioaccumulation in crops, posing chronic toxicity risks to both livestock and humans.

The increase in electrical conductivity (EC) observed in polluted soils indicates higher salinity levels, a condition known to impair plant water uptake and reduce agricultural yield [19]. Although organic matter enrichment may initially promote soil structure and water retention, the imbalance in nutrient dynamics undermines soil sustainability, as also highlighted by [20].

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Ogunlade *et al.* [14] similarly reported elevated heavy metal concentrations and salinity in abattoir-contaminated soils within Delta State. His work emphasizes that without effective wastewater treatment, pollutants accumulate progressively, causing irreversible soil degradation and raising public health risks through the food chain. He further advocates for low-cost remediation strategies, including the use of constructed wetlands and biofiltration systems, to reduce both organic and inorganic contaminants. Orji and Oghonim [23] affirmed that physical characteristics like electrical conductivity and temperature of water samples in Agbor are within recommended limits unless impacted on by additional pollutants like abattoir wastewater.

CONCLUSION

The results of this research confirm that the discharge of untreated abattoir wastewater into soil environments in Agbor has detrimental effects on both soil quality. The effluents are rich in organic pollutants, heavy metals, and pathogenic bacteria, many of which exhibit resistance to antibiotics, thus posing significant threats to food safety, groundwater quality, and community health. Polluted soils exhibited higher levels of nutrients and organic matter, which might suggest short-term improvement in fertility. However, this is outweighed by long-term degradation risks such as heavy metal accumulation, salinity increase, microbial imbalance, and reduced soil productivity. The presence of resistant and pathogenic bacteria in the soil also underscores the danger of zoonotic disease transmission and environmental antimicrobial resistance. Without immediate intervention through proper wastewater treatment, environmental monitoring, and regulatory enforcement, the situation is likely to worsen, leading to ecological alterations and increased disease burdens in affected communities.

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