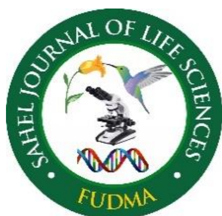

Sahel Journal of Life Sciences FUDMA (SAJOLS)
June 2026 Vol. 4(2): 76-83
ISSN: 3027-0456 (Print)
ISSN: 1595-5915 (Online)
DOI: https://doi.org/10.33003/sajols-2026-0402-10

Research Article

Microbiological Evaluation of Soil and Wastewater from Major Abattoir Sites in Umuahia, Abia State

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ABSTRACT
Abattoirs with their associated activities produces enormous amount of wastewater that can alter the microbiota of the immediate environment. This study evaluated the microbial profile of wastewater and soil within two major Abattoirs, in Umuahia, Abia state, Nigeria. Wastewater and soil samples within the immediate environment (at different depths) were collected aseptically from different location (Ubakala and Ndioru). The samples were inoculated into culture media for isolation of microorganisms and identified accordingly using standard techniques. The total heterotrophic bacterial count ranged from $8.7 \times 10^6 – 1.58 \times 10^7$ cfu/g and $8.1 \times 10^6 – 1.33 \times 10^7$ cfu/g for the on-site and offsite soil samples with the Ubakala samples significantly recording higher counts than Ndioru. The wastewater samples from both locations had counts in the range of $9.6 \times 10^6 – 1.36 \times 10^7$ cfu/mL while coliform counts ranged between $3.7 \times 10^4 – 5.3 \times 10^4$ cfu/mL. Diverse microorganisms were isolated as *Bacillus subtilis Lysinibacillus* species, *Pseudomonas aeruginosa,* CONs, *Micrococcus* species, *Escherichia. coli, Acetobacter* species and *Enterobacter* species while the fungal isolates included *Mucor* sp., *Rhizopus* sp. and *Penicillium* sp., *Curvularia* species, *Cladosporium* species, *Aspergillus fumigatus*. The results as obtained in this study revealed that bacterial and fungal diversity was largely higher in onsite samples compared to offsite samples. This is a clear indication of the direct influence of abattoir effluent on soil microbial communities. The contamination level especially with pathogenic species as seen from this study shows that the study sites could be breeding grounds conducive for silent transmission of pathogen.

Keywords: Abattoir; *Escherichia coli;* Pathogen; Wastewater

Citation: Ekundayo, C.I., Okafor, A.T., & Enya, E. (2026). Microbiological Evaluation of Soil and Wastewater from Major Abattoir Sites in Umuahia, Abia State. *Sahel Journal of Life Sciences FUDMA*, 4(2): 76-83. DOI: https://doi.org/10.33003/sajols-2026-0402-10

INTRODUCTION

Abattoir wastewater usually has a complex composition that could be potentially detrimental to the environment and endanger public health (Emeh *et al.,* 2020). In many developing nations including Nigeria, abattoir business is an important component of the livestock industry, as it provides constant meat supply to people and employment opportunities for a good number of the population (Nafaranda *et al.,* 2012). However, this industry is ravaged with poor facilities for the treatment of abattoir effluents (Ogbonnaya, 2011). Thus, large amounts of solid waste and effluent such as rumen contents, blood and wastewater are generated from abattoirs and they pollute surface and ground water with pathogens and undesirable chemical compounds (Olaiya *et al.,* 2016).

Different types of animals are usually slaughtered in the abattoir, with their blood, part of the dung and abdominal content washed on cemented pavements. The animal blood and most other wastes are most often released untreated into the flowing stream while the consumable parts of the slaughtered animal are washed directly into the flowing water (Omole and Longe, 2008). The release of this wastewater into water bodies is of public health concern because the water bodies into which these effluents are discharged is used by the locals for innumerable purposes including being a source of potable water, domestic water and for irrigation of farm lands (Emeh *et al.,* 2020).

Abattoirs by their nature serve as breeding grounds for pathogens and contaminants, polluting the environment and threatening public health (Anele *et al.,* 2023). This is so because these wastes are generated from unscreened animals that are slaughtered in the abattoirs which are hosts to microorganisms including bacteria (Constance *et al*., 2019). Microorganisms present in abattoir effluents may include *Cryptosporidium parvum*, *Campylobacter* spp., *Yersinia enterocolitica*, hepatitis E virus, *Salmonella* spp., rotaviruses, *Escherichia coli* O157: H7, *Listeria monocytogenes* and *Giardia lamblia* which are known pathogens (Ubwa *et al*., 2013). The discharge of effluents from abattoirs into a water body introduces some potentially pathogenic microorganisms into the water body which is used either for domestic, recreation or irrigation purposes thus constituting environmental and public health challenge. Several studies including those of Ovuru *et al*. (2024) as well as Fasanmi *et al*. (2017) have revealed that abattoirs in developing countries have an unhygienic environment and detected the presence of pathogens that are known causes of diarrheal diseases (Adeyemo, 2002; Nwanta *et al*., 2010).

There are increased chances of portions of these wastewaters been washed directly to the soil within the vicinity which may affect microbial species diversity and buildup of pathogens in the food chain. This study hence, evaluated the microbiological profile of wastewater effluents generated from the abattoir as well as the surrounding soil.

MATERIALS AND METHODS

Sample collection

Wastewater and soil samples within the immediate environment (at different depths) were collected from abattoirs in two different locations (Ubakala and Ndioru) of Abia State into sterile bottles. The samples were immediately transported in an ice box to the laboratory for analyses within 6 hours of collection.

Media Preparation

The culture media (Nutrient agar, MacConkey agar and Sabouraud dextrose agar) used for this study were prepared by weighing out specific grams of the agar powder and dissolving in equivalent volume of distilled water following the directives stipulated by the manufacturers of the medium. They were sterilized by autoclaving at 121ºC for 15minutes, allowed to cool before pouring into plates.

Isolation of Microorganisms from the samples

The serial dilution technique was employed in the inoculation of the soil/water samples. Each of the samples was diluted in the 10-fold serial dilution technique described by Gurung *et al.* (2009). Six test tubes were set up in a rack and filled with 9ml of sterile distilled water labelled stock, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} respectively. One (1) g and 1ml each of the soil/water sample was added to the tube labelled stock and properly mixed together. An Aliquot (1ml) was transferred from this tube into the one labelled 10^{-1} and carefully mixed. Similarly, 1ml was drawn up from the 10^{-1} tube and transferred to the 10^{-2} tube. This was continued up to the last tube labelled 10^{-5} from where 1ml was discarded. Dilutions from 10^{-3} and 10^{-4} were inoculated onto freshly nutrient agar, MacConkey agar and Sabouraud Dextrose agar plates. The Spread plate method of inoculation as described by Prescott *et al. (*2007) was used where 0.1ml of the respective dilutions (10^{-3} and 10^{-4}) were plated on various agar plates and evenly spread over the entire plate using a flame sterilized glass rod. The inoculated plates were incubated at 35^0C for 24hrs for bacteria and at room temperature ($25+2^0$C) for fungi.

Characterization and Identification of the Isolates

Gram staining

The method of Green-Berge *et al.* (1992) was adopted in identification of bacteria Gram reaction (Gram positive and Gram negative). Bacteria smear was made on a clean grease-free slide, air dried and heat fixed. The slide was flooded with crystal violet for 1 minute, decanted and rinsed with water. Lugol's

iodine (mordant) was applied for 60 seconds and rinsed. Acetone was used in decolorizing and washed immediately then counter stained with neutral red for 1 minute. It was then rinsed with water, blotted carefully and air dried. Finally, the slides were observed under the microscope using oil immersion objectives (x100).

Biochemical Tests

Isolated organisms were identified by standard microbiology identification techniques including catalase test, citrate, hydrogen sulphide test, starch hydrolysis test, methyl-red test, voges-proskauer test, oxidase test as well as the urease and indole tests.

RESULTS

The results presented in Table 1 shows the microbial counts across different sample types (soil at two depths, wastewater, and clean water controls) for two abattoir locations (Ndoru and Ubakala). The total heterotrophic bacterial count (THBC) ranged from 8.7 x 10^6 cfu/g – 1.58 × 10^7 cfu/g and 8.1 x 10^6 cfu/g – 1.33 × 10^7 cfu/g for the on-site and offsite soil samples with the Ubakala samples significantly recording higher counts than Ndioru. Clean water controls had lower counts between 3.8 x 10^6 cfu/mL – 4.3 × 10^6 cfu/mL. Coliforms were **d**etected in most onsite soils and wastewater samples, but absent in several offsite soils while the fungal load **r**anged from 3.4 x 10^4 cfu/g – 7.6 × 10^4 cfu/g at the Ndioru site and within 2.8 x 10^4 cfu/g – 6.8 × 10^4 cfu/g at Ubakala. The bacterial isolates recovered from the samples in this study were identified as *B. subtilis, Lysinibacillus* species, *Pseudomonas aeruginosa,* CONs, *Micrococcus* species, *E. coli, Acetobacter* species and *Enterobacter* species while the fungal isolates included *Mucor* sp., *Rhizopus* sp. and *Penicillium* sp., *Curvularia* species, *Cladosporium* species, *Aspergillus fumigatus*. The results as obtained in this study revealed that bacterial and fungal diversity was largely higher in onsite samples compared to offsite samples.

Table 1: Total Heterotrophic bacterial and fungal counts (in cfu/g & cfu/ml) of the samples

Samples	THBC	Coliform count	Fungal load
Ndoru@15cm offsite (Soil)	8.1 x 10^6	NG	3.4 x 10^4
Ndoru@10cm offsite (Soil)	1.08 x 10^7	1.8 x 10^4	6.1 x 10^4
Ndoru@15cm onsite (Soil)	8.7 x 10^6	3.3 x 10^4	3.8 x 10^4
Ndoru@10cm onsite (Soil)	1.27 x 10^7	4.1 x 10^4	7.6 x 10^4
Wastewater Ndoru1	1.08 x 10^7	3.7 x 10^4	3.8 x 10^4
Wastewater Ndoru2	1.36 x 10^7	5.3 x 10^4	4.6 x 10^4
Clean water (Control)	4.3 x 10^6	1.8 x 10^4	2.1 x 10^4
Ubakala@15cm offsite (Soil)	1.19 x 10^7	NG	2.8 x 10^4
Ubakala@10cm offsite (Soil)	1.33 x 10^7	NG	4.2 x 10^4
Ubakala@15cm onsite (Soil)	1.03 x 10^7	NG	3.8 x 10^4
Ubakala@10cm onsite (Soil)	1.58 x 10^7	4.6 x 10^4	6.8 x 10^4
Wastewater Ubakala1	1.12 x 10^7	3.8 x 10^4	3.8 x 10^4
Wastewater Ubakala2	9.6 x 10^6	4.6 x 10^4	4.6 x 10^4
Clean water (Control)	3.8 x 10^6	2.6 x 10^4	NG

THBC: Total heterotrophic bacterial count; NG: No Growth

Table 2: Macroscopic and Microscopic Description of Isolated Fungi

Macroscopic Appearance	Microscopic features	Probable fungi
White fluffy colonies that turned grey with age	Long branching sporangiophores with non-septate hyphae bearing spherical spores at the tip	*Mucor* species
Bright-green colonies with white edges	Long ellipsoidal conidia with smooth walled conidiophores borne on septate hyphae	*Penicillium* species
Rapidly growing white cottony colonies on PDA plate	Upright sporangiophores borne on aseptate hyphae with numerous spiral spores.	*Rhizopus* species
Brown to blackish brown fluffy colonies with a black reverse pigmentation	Erect conidiophores borne on septate hyphae	*Curvularia* species
Colonies were dark olive and flat. The reverse side was black	Hyphae were septate, with erect Conidiophores bearing Conidia	*Cladosporium* species
Yellow or yellowish green colonies with distinct margin	Hyphae were septate, simple and thick-walled. Conidiophores bearing conidial heads containing conidia were seen	*Aspergillus fumigatus*

Table 3: Frequency of Isolation of each isolate

Bacterial Isolates	No. (%) Isolated	Fungal Isolates	No. (%) Isolated
Micrococcus species	6 (42.8)	*Cladosporium* species	4 (28.6)
Bacillus subtilis	4 (28.6)	*Aspergillus fumigatus*	7 (50.0)
Coagulase Negative Staphylococci (CONs)	9(64.2)	*Curvularia* species	6 (42.8)
Pseudomonas aeruginosa	4 (28.6)	*Penicillium* species	6 (42.8)
Enterobacter species	5 (35.7)	*Mucor* species	8 (57.1)
Escherichia coli	6 (42.8)	*Rhizopus* species	8 (35.7)
Salmonella species	6 (42.8)		
Bacillus megaterium	6 (42.8)		
Lysinibacillus species	5 (35.7)		
Acetobacter species	4 (28.6)		

Table 4: Distribution of the Fungal isolates across the sample types

Samples	*Rhizopus* species	*Penicillium* species	*Aspergillus fumigatus*	*Curvularia* species	*Mucor* species	*Cladosporium* species
A	-	+	+	-	+	-
B	+	+	+	-	+	+
C	+	-	+	-	-	-
D	+	+	-	+	+	-
E	-	-	+	+	+	-
F	-	-	-	-	+	-
G	-	-	-	+	-	-
H	-	+	+	-	-	-
I	+	-	+	-	-	+
J	-	+	-	-	+	-
K	+	+	-	+	-	+
L	-	-	-	+	+	+
M	-	-	+	+	+	-
N	-	-	-	-	-	-

A: Ndoru@15cm offsite; B: Ndoru@10cm offsite; C: Ndoru@15cm onsite; D: Ndoru@10cm onsite; E: Wastewater Ndoru1; F: Wastewater Ndoru2; G: Clean water Control Ndoru; H: Ubakala@15cm offsite; I: Ubakala@10cm offsite; J: Ubakala@15cm onsite; K: Ubakala@10cm onsite; L: Wastewater Ubakala1; M: Wastewater Ubakala2; N: Clean water control Ubakala.

Table 5: Distribution of the Bacterial isolates across the sample types

Samples	*B. subtilis*	*Lysinibacillus* species	*Pseudomonas aeruginosa*	CONs	*Micrococcus* species	*E. coli*	*Acetobacter* species	*Enterobacter* species	*Salmonella* species
A	+	+	+	-	+	-	-	-	-
B	+	-	-	+	+	-	-	-	+
C	-	-	+	-	-	+	-	+	-
D	-	+	-	+	+	+	-	-	+
E	-	-	-	+	-	-	+	+	+
F	-	-	-	-	-	+	+	-	+
G	-	-	-	+	+	-	-	+	-
H	+	+	-	-	-	-	-	-	-
I	-	+	+	+	-	-	+	-	-
J	+	-	+	+	-	-	-	-	-
K	-	+	-	+	+	+	-	-	+
L	-	-	-	+	-	+	+	-	-
M	-	-	-	-	+	+	-	+	+
N	-	-	-	+	-	-	-	+	-

A: Ndoru@15cm offsite; B: Ndoru@10cm offsite; C: Ndoru@15cm onsite; D: Ndoru@10cm onsite; E: Wastewater Ndoru1; F: Wastewater Ndoru2; G: Clean water Control Ndoru; H: Ubakala@15cm offsite; I: Ubakala@10cm offsite; J: Ubakala@15cm onsite; K: Ubakala@10cm onsite; L: Wastewater Ubakala1; M: Wastewater Ubakala2; N: Clean water control Ubakala.

DISCUSSION

The total heterotrophic bacterial count (THBC) ranged from 8.7 x 10^6 cfu/g – 1.58 × 10^7 cfu/g and 8.1 x 10^6 cfu/g – 1.33 × 10^7 cfu/g for the on-site and offsite soil samples with the Ubakala samples significantly recording higher counts than Ndioru. The wastewater samples from both locations had counts in the range of 9.6 x 10^6 cfu/mL – 1.36 × 10^7 cfu/mL while coliform counts ranged between 3.7 × 10^4 cfu/mL – 5.3 × 10^4 cfu/mL. Anele *et al.* (2023) reported a THBC of 3.3 x 10^5 – 5.0 x 106 cfu/g in a related study on environmental impact assessment of abattoirs in Rivers State, Nigeria. Equally, similar counts have been reported by (Nafarnda *et al.,* 2012; Ogbonnaya, 2011).

The total heterotrophic bacterial counts (THBC) in the soil samples at Ubakala recorded higher bacterial counts more than those of Ndioru probably due to the degree of movement and activity at the Ubakala site. Also, the abundance of microorganisms can be attributed to the abundance of nutrients in the wastewater, which are suitable for optimal microbial metabolism (Neboh *et al*., 2013; Darbar and Saha, 2023). It can be seen that the value 9.6 x 10^6 cfu/mL – 1.36 × 10^7 cfu/mL was high, indicating that the wastewater ought to be treated before onward discharge into the environment. The counts as obtained in this study is in contrast to the WHO allowable limit (1×10^2 cfu/mL) reported by Dankaka *et al.* (2018). Rabah *et al.* (2008) reported a mean (6.4×10^7 cfu/mL) for the total viable count of bacteria isolated in Sokoto abattoir effluent, which is consistent with the result of our study.

The bacterial isolates recovered from the samples in this study were identified as *B. subtilis, Lysinibacillus* species, *Pseudomonas aeruginosa,* CONs, *Micrococcus* species, *E. coli, Acetobacter* species and *Enterobacter* species while the fungal isolates included *Mucor* sp., *Rhizopus* sp. and *Penicillium* sp., *Curvularia* species, *Cladosporium* species, *Aspergillus fumigatus*. Anele *et al.* (2023) also identified similar bacterial isolates in wastewater samples in abattoirs within Port Harcourt. The diversity of microorganisms as obtained in this study is in line with the results of Obire and Ariyo (2021), who identified *Salmonella* sp. (13.16%), *Bacillus* sp. (20.16%), *Pseudomonas* sp. (16.04%), *Escherichia coli* (12.34%), *Micrococcus* sp. (9.46%) and *Staphylococcus* sp. (22%), from Niger Delta abattoir soils.

The high frequency of CONs (64.2%) is particularly noteworthy. *Staphylococcus aureus* was also the most common isolate (28.5%) from Akure abattoirs, according to Adegunloye (2013), demonstrating the prevalence of staphylococci in these kinds of environment. The presence of *Enterobacter* species (35.7%) and *Pseudomonas aeruginosa* (28.6%) is of significance, as both are capable of causing a range of infections. *Salmonella* species were found in 42.8% of samples from several sites in this study. This is a crucial finding with major consequences for environmental and public health. Ijah *et al.* (2022) also reported *Salmonella* as prevalent pathogens in Nigerian abattoir environments, with counts ranging from 1.8 × 10^3 to 2.1 × 10^4 cfu/mL in Minna abattoir wastewater. It was also found that at both onsite soils and wastewater, 42.8% of samples had *Escherichia coli*. This aligns with findings from Oladipo *et al.* (2022), who identified *E. coli* as a predominant organism in abattoir wastewater and contaminated soils. Equally, Akindele *et al.* (2015) reported *E. coli* as one of the key bacterial isolates from Ijebu-Igbo abattoir. The presence of these bacteria especially *Salmonella* species and *Escherichia coli* portends a grave public health concern as these microorganisms are linked with water borne diseases as they can persist in the environment and could contaminate nearby water sources through run-offs (Olaiya *et al*. 2016).

There were six different fungus genera identified in this study with *Aspergillus fumigatus* having the highest dominance (50%). The presence of this organism is significant as it is a known opportunistic pathogen that can cause aspergillosis in immunocompromised persons. Similar fungal profiles were found in Niger Delta abattoir soils, according to the findings of Obire and Ariyo (2021), with *Penicillium* (43.3%) being the most common isolate, followed by *Aspergillus* sp. (26.8%), *Fusarium* sp. (11.6%), and Mucor (9.2%). Related studies in different regions of Nigeria have recorded similar microbial profile especially those of Ogbomida *et al*., (2016), Ogunnusi and Dahunsi (2014), Shukri *et al*. (2017) Njoku *et al*. (2018) and Awari *et al.* (2020) who also identified bacterial pathogens in effluent samples from abattoirs.

The results as obtained in this study revealed that bacterial and fungal diversity was largely higher in onsite samples compared to offsite samples. This is a clear indication of the direct influence of abattoir effluent on soil microbial communities and is consistent with findings from Ekpenkhio *et al.* (2025), who reported increased bacterial and fungal counts in abattoir-impacted soils compared to control sites. Meanwhile, clean water controls showed limited microbial diversity, with only Coagulase negative

Staphylococcus (CONs) and *Micrococcus* detected at Ndoru, and CONs alone at Ubakala affirming that the high microbial loads observed at abattoir sites are directly attributable to contamination from slaughter activities.

CONCLUSION

The microbiological evaluation of soil and wastewater from Ndoru and Ubakala abattoir sites reveals heavy contamination with diverse bacterial and fungal populations, including multiple pathogenic species. The contamination level especially with pathogenic species as seen from this study shows that the study sites could be breeding grounds conducive for silent pathogen transmission. It is thus suggested improved sanitation infrastructures, and enhanced surveillance around abattoir sites be enforced to preserve the environment and protect the public.

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