Assessment of Physicochemical Parameters and Microbial Quality of Abattoir Effluent-Contaminated Soils in Dei-Dei Abattoir, Federal Capital Territory, Nigeria.

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Abstract: Abattoir effluents continue to contaminate the surrounding environment leading to lots of public health issues. This calls for urgent concern to safeguard environmental health. The assessment of the physicochemical parameters and microbial quality of abattoir effluent-contaminated soil in Dei-Dei Abattoir, Abuja, was investigated using standard laboratory techniques. The range of the mean values of all the parameters measured were: Sand (70.47-87.8%), silt (1.05-23.17%), clay (3.59-4.37%), pH (5.54-5.90mg/L), organic carbon (0.76-1.63mg/L), organic matter (1.24-2.75 mg/L), nitrogen (0.74 -2.09 mg/L), phosphate (2.81-3.38 mg/L), potassium (0.38-0.46 mg/L), sodium (2.09-2.96 mg/L), calcium (0.38-0.54 mg/L), magnesium (0.29-0.55 mg/L), cation exchange capacity (6.68-8.58 mg/L), base saturation (42.27-65.13%), and electrical conductivity (2.06-4.68 µS/cm). The results revealed a high count of 6.75×10^4 cfu/g, 7.43×10^4 cfu/g, and 4.97×10^4cfu/g for bacterial, coliform and fungal counts, respectively. Also, the contaminated soils had higher counts than the control soil. The bacterial and fungi isolated from the contaminated soil in order of frequency of occurrence were *Escherichia coli* (22.86%), *Bacillus* sp. (14.29%), *Klebsiella* sp. and *Aspergillus niger* (11.43%) each, *Aspergillus flavus*, *Penicillin* sp., and *Mucor* sp. had 8.57% each *Staphylococcus aureus* (5.71%). The results of the study revealed that all of the measured parameters showed a significant difference in their mean. The observed high level of microbial contamination of the soils suggests that they could serve as a potential source of pathogenic organisms which could lead to disease outbreak if the abattoir is not well cleaned up.

Keywords: Abattoir Effluent, *Aspergillus niger*, Environmental quality, *Escherichia coli*, Public Health, Soil.

Introduction

Slaughterhouses or abattoirs very important facilities associated with the meat industry in Nigeria. It also creates employment for the people in its immediate vicinity. Nevertheless, such structures can lead to pollution of soils and water bodies if the effluents are not adequately treated before being discharged into the environment. Chukwu *et al.* (2008) explained that waste from abattoirs is dangerous and may cause harm to the environment and human health if not handled properly. The waste generated in the abattoir contains a high organic load with suspended solids, liquids, and fats. The solid wastes include rejected meat, undigested food, bones, horns, hair and aborted calves, while liquid waste may consist of dissolved solids, blood, intestinal content, urine and water (Adeyemo *et al.,* 2002). Environmental pollution occurs when these wastes are dumped on land or water bodies (Ifi *et al*., 2019). This is primarily because of the lack of waste treatment facilities. In many countries, pollution in the meat industry is due to non-adherence to Good Manufacturing Practices (GMP) and Good Hygiene Practices (GHP). Therefore, if the slaughter process is not well controlled, it may be dangerous to the farmers, butchers, the environment and the consumer.

In many Nigerian slaughterhouses, wastewater from animal processing and pen cleaning is often poorly managed, resulting in unhygienic and swampy conditions around these facilities (Asibor 2019). The majority of abattoirs are located near rivers, streams, or other surface water sources to meet their water needs. This guarantees a steady supply of water for operations, but it also gives untreated waste runoff from the production of meat a convenient but hazardous outlet (Omole and Ogbiye, 2013; Enerijiofi *et al*., 2018b). Nigeria, like many developing nations, faces lots of environmental challenges brought on by the uncontrolled discharge of untreated slaughterhouse waste. The wastewater generated by the highly water-intensive meat processing industry often seeps into the nearby soil. This can upset the fragile microbial ecosystems in the soil and drastically change its physical and chemical characteristics over time, including its pH balance (Amisu *et al*., 2003). When construction or flooding disturbs the soil and sediments, harmful bacteria from abattoir waste may lie dormant until they are released back into adjacent water sources. Communities are exposed to long-term health risks as a result, particularly in children, the elderly, and people with compromised immune systems. Pathogens from animal waste can cause a broad spectrum of illnesses, from minor infections to fatal conditions. This contamination will cause irreversible ecological harm and loss if immediate and efficient action is not taken (Osinbanjo and Adie, 2007, Enerijiofi *et al*., 2022)

An abattoir with inadequate waste management facilities is available in almost every town and community in Nigeria (Oboh *et al.,* 2018). Abattoir operations have a detrimental effect on soil, natural water supplies, and the environment as a whole. Although slaughterhouses produce valuable byproducts like meat, leather, and skin, spills of animal waste can contaminate surface waters with excess nutrients and enteric pathogens. There are many microorganisms in abattoir effluents, and the various procedures used to clean the animals generate a lot of waste that is released into the environment untreated, posing a serious risk to public health (Joseph *et al.,* 2021). There are inadequate data on the concentrations of physicochemical parameters and microbial quality of Dei-Dei abattoir effluents in Abuja. Considering its location in Nigeria' capital which is receiving guest worldwide, there is need for adequate data to plan for waste disposal to safeguard public health. This study was set out to evaluate the physicochemical parameters and microbial quality of soil contaminated by Dei-Dei abattoir effluents in the Federal Capital Territory, Abuja, Nigeria.

Materials and Method

Study Area: Data and samples were gathered from Dei-Dei Abattoir in Abuja, Nigeria, a city with approximately 1.857 million residents. Abuja is the Federal Capital of Nigeria and one of the world's fastest-growing cities. It is situated in the North-Central region of Nigeria, with a total land area of 713 km2 (275.3 sqm) and coordinates of $9^0$4'0" N $7^0$29'0" E (Nafarnda *et al.*, 2012).

Soil Sampling and Collection: Abattoir effluent-contaminated soil samples were taken aseptically using a sterilized auger. With pressure applied and using the handle, the auger was rotated after it had been driven into the ground while held vertically. At each 10-cm depth penetration, the auger was removed, and soil samples were taken for analysis separately. At 10, 20, and 30-metreintervals, three replicate samples were taken from the sampling site, with a control sample taken 100 metres away. These samples were taken at the height of activities between 8:00 and 9:00am in sterile polythene bags. They were subsequently transported to the laboratory for processing in an ice box.

Soil Washing: The soil samples were prepared for microbiological analysis by using an ex-situ soil washing technique described by Mohammed *et al.* (2020). Fifty millilitres (50 ml) of distilled water were emptied into 20 g of effluent-contaminated soil sample and vigorously stirred to remove contaminants attached to clay, silt and organic soil particles. The wash water was then poured into a clean tube ready for further analysis.

Physico-chemical Analysis of Soil Samples: Standard techniques for soil analysis were used to ascertain the physicochemical characteristics of the soil samples, using the methods of Enerijiofi and Ekhaise, (2019) and the Association of Official Analytical Chemists (AOAC, 2003). The experimental and control soil samples were placed on sizable wooden trays and the lumps of wet soil were manually broken and thereafter allowed to air dry for 72 hours. Additionally, a 2mm mesh was used to sieve the air-dried samples. Sand, silt, clay, pH, temperature, organic carbon (OC), organic matter (OM), nitrogen, phosphate, potassium, sodium, calcium, magnesium, cation exchange capacity (CEC), percentage base saturation (PBS), and electrical conductivity (EC) were measured from the soil samples. Every chemical and reagent that was used was of analytical quality. The Bouyoucos Hydrometer method was used to determine the soil samples' particle sizes. A conductivity metre was used to measure the electrical conductivity in the filtrate of the water extract, and a pH metre was used to measure the pH of the soil samples in a 1:1 soil-to-water suspension. The chromic acid wet oxidation method was used to calculate the percentage of organic carbon/matter. The complexometric titration of Ca and Mg with EDTA. Flame photometer was used to determine Na and K, while the acid-base titration of exchangeable acidity, and the summation of the exchangeable bases were used to determine the cation exchange capacity.

Determination of Total Microbial Load: The wash-off water was serially diluted using the procedures outlined by Oyeleke and Manga (2008) and Rabah *et al.* (2008). The soil samples were serially diluted in tenfold increments up to 10^{-6} tubes using a measure of 1 ml. Then 0.1ml aliquots from the 10^{-4} tubes were aseptically inoculated onto freshly prepared plates of Nutrient agar (NA), Eosin Methylene Blue Agar (EMBA) and Potato Dextrose Agar (PDA) using the pour plate method. The Nutrient and Eosin Methylene Blue Agars were used to grow and enumerate the mean heterotrophic and enteric coliform bacterial counts However, potato dextrose agar was used to grow and enumerate the mean heterotrophic fungal count (Aneja, 2003). Every culture medium was prepared following the manufacturer's instructions before being poured. After plating in triplicate, swirling, and allowing to solidify, the plates were incubated for 48 hours at 37^0C for NA and EMBA, and for 72 hours at 28 ± 2^0C for PDA and thereafter counted and recorded in CFU/g.

Identification and Characterization of Bacterial Isolates: Freshly made Nutrient Agar, EMBA plates were used to subculture distinct representative bacterial colonies. The plates were incubated at 37°C for 24 to 48 hours. The sub-cultured bacterial colonies' colonial features were noted. The bacterial isolates were further characterized and identified using phenotypic features (Cheesbrough, 2005).

Identification and Characterization of Fungal Isolates: The fungal isolates were identified and characterized by sub-culturing them on Potato Dextrose Agar plates. These were incubated at room temperature of 25^0C for 3–5 days. The fungal isolates were identify by their morphological and microscopic characteristics. The wet mount technique was used to analyze their microscopic characteristics. Distilled water and lactophenol cotton blue were employed as mountants, respectively. The fungal isolates were also identified using the techniques (Aneja, 2003)

Determination of Percentage Occurrence of Bacterial and Fungal Isolates: The percentage frequency of occurrence for each isolated species of bacteria and fungus was calculated using the formula = A/B x 13100

Where A is the number of plates on which the species was found and,

B is the total number of plates incubated for each site.

Statistical Analysis: The replicate values were subjected to statistical analysis of variance (ANOVA) using the SPSS (Version 29) statistical program to determine if there is a statistically significant difference (Ogbeibu, 2005).

Results

The results obtained for the physicochemical properties of the abattoir effluent-contaminated soil revealed the mean pH value, organic carbon and organic matter to be 5.73±0.03, 1.96±0.02 %, %, and 2.24±0.09 %, respectively. The mean value of 1.98±0.04 mg/l and 3.31±0.01 mg/l was observed for total nitrogen and phosphate respectively. The mean values for potassium, sodium, calcium and magnesium were 0.41±0.01mg/l, 2.20±0.02mg/l, 0.43±0.01mg/l and 0.47±0.01 mg/l, respectively. The electrical conductivity, mean cation exchange capacity, and percentage base saturation were found to be 4.25±0.074 µS/cm, 8.39±0.01 mg/l and 42.78±0.33 % respectively (Table 1). The highest bacterial counts were observed in soil samples from the 10-metre area ($6.75x10^4$ cfu/g), followed by soil samples from the 20-metre area ($5.03x10^4$ cfu/g), while the least count was observed in soils from the 30-metre area ($2.84x10^4$ cfu/g). The highest coliform counts were recorded in soil samples from 10-metre area ($7.43x10^4$ cfu/g), followed by soil samples from

20-metre area ($4.58x10^4$ cfu/g) and the least in soils from 30-metre area ($3.84x10^4$ cfu/g). The total coliform count ranged from $3.84x10^4$ cfu/g in the 30-metre area to $7.43x10^4$ cfu/g in the 10-metre area respectively. The highest total fungal counts were observed in soils from the 10-metre area ($4.97x10^4$ cfu/g) and the lowest from the 30-metre area ($2.24x10^4$ cfu/g). For the control sites, the highest counts were observed for the coliform organisms ($1.82x10^4$ cfu/g) while the lowest was for the fungal count ($1.25x10^4$ cfu/g). (Table 2). Among the bacteria isolates, *Escherichia coli* recorded the highest frequency of occurrence (22.86%), followed by *Bacillus* sp. (14.29 %), and *Klebsiella* sp. (11.43 %) while the least was *Staphylococcus aureus* (5.71 %). However, among the fungal isolates, *Aspergillus niger* had the highest frequency of occurrence of 11.43 % while *Aspergillus flavus*, *Penicillin* sp., and *Mucor* sp. had the least of 8.57 % each (Table 3).

Table 1: Physicochemical Properties of the Dei-Dei Abattoir Effluent-Contaminated Soil

Parameters	10 metres	20 metres	30 metres	Control	Mean/S.D	FEPA Limit
Sand (%)	70.84	72.47	74.57	87.85	72.63±0.54	NA
Silt (%)	15.4	18.25	23.19	1.03	18.95±0.08	NA
Clay (%)	4.27	4.39	3.58	3.84	4.08±0.02	NA
pH	5.73	5.88	5.57	5.76	5.73±0.03	6.0-9.0
OC (%)	1.64	1.21	1.06	0.77	1.96±0.02	NA
OM (%)	2.05	1.80	2.88	1.22	2.24±0.09	NA
N (%)	1.96	2.11	1.88	0.76	1.98±0.04	NA
PO_4^{3-} (mg/L)	3.32	3.21	3.39	2.81	3.31±0.01	NA
K^+(mg/L)	0.41	0.44	0.39	0.47	0.41±0.01	5.0
Na^+ (mg/L)	2.11	2.35	2.13	2.97	2.20±0.02	NA
Ca^{2+}(mg/L)	0.50	0.39	0.41	0.52	0.43±0.01	200
Mg^{2+}(mg/L)	0.53	0.31	0.57	0.29	0.47±0.02	200
CEC (mg/L)	8.08	8.60	8.48	6.69	8.39±0.01	200
PBS (%)	43.25	42.29	42.80	65.14	42.78±0.33	NA
EC (µS/cm)	3.65	4.42	4.68	2.06	4.25±0.07	NA 1000

Key: CEC - Cation Exchange Capacity, PBS – Percentage Base Saturation, EC – Exchange Capacity, OM – Organic Matter, OC - Organic Carbon, N- Nitrogen, PO_4^{3-} - Phosphate, K^+ - Potassium, Na^+ - Sodium, Ca^{2+} - Calcium, Mg^{2+} - Magnesium, NA: not available;
FEPA (1991)

Table 2: Determination of Total Bacterial and Fungal Load of Dei-Dei Abattoir Effluent-Contaminated Soil

Parameters	Bacterial count ($x10^4$ cfu/g)	Coliform count ($x10^4$ cfu/g)	Fungal count ($x10^4$ cfu/g)	P-Value
10-metre	6.75^b	7.43^c	4.97^a	0.03
20-metre	5.03^{ab}	4.58^b	3.67^a	0.01
30-metre	2.84^{ab}	3.84^c	2.24^a	0.00
Control	1.68^b	1.82^b	1.25^a	0.00

*Counts represent means of triplicate samples
Values are average mean. Values on the same row that have the same letter subscript are statistically similar at $p<0.05$.

Table 3: **Percentage of Occurrence of Bacterial and Fungal Isolates in Dei-Dei Abattoir Effluent-Contaminated Soil**

Bacterial Species	10m	20m	30m	F – Total	% F
Escherichia coli	3	4	-	8	22.86
Bacillus sp.	4	-	1	5	14.29
Klebsiella sp.	2	-	2	4	11.43
Salmonella sp.	2	1	-	3	8.57
Staphylococcus aureus	2	-	-	2	5.71
Fungal Species					
Aspergillus niger	3	1	1	4	11.43
Aspergillus flavus	3	-	-	3	8.57
Penicillin sp.	2	2	-	3	8.57
Mucor sp.	3	1	-	3	8.57
Total	24	9	4	35	100

Key: F-Total = Total Frequency of Occurrence, F% = Percentage Occurrence, m = metres

Discussion

The pH ranged from 5.57 to 5.88 mg/L, which was close to neutral and below the recommended range of 6.0 to 9.5. The pH values found by Chukwu (2008) are quite similar to the range reported in this study. The decomposition of abattoir waste products, including blood, meat trimmings, and cattle excreta, may contribute to the acidic pH. The decomposition is linked to have an influence on the soil pH which is observed to be low. This aligns with the findings of Omole and Ogbiye (2013), who reported that soil acidity significantly affects nutrient availability, thereby influencing crop yield and seed germination. All the soil samples had low pH values, which suggested that abattoir effluent could reduce soil pH. Furthermore, the pH level might influence the quantity and quality of microorganisms present in the contaminated soil. It can be deduced from Akinnibosun and Ayejuyoni (2015) that the microbial population's pattern was influenced by the contaminated soil pH. Since microorganisms regularly alter the pH of their habitat by generating acidic or basic metabolic waste products, the pH serves as an indicator of their activity.

The higher total organic carbon value in the contaminated soil could be suggestive of the influence of organic components of abattoir effluent. The waste from cattle in the abattoir was mostly cellulolytic components of foraged feeds (grasses and other weeds, digested and undigested). This helped to add extra carbon to the soil. This result aligns with that of Chikwendu *et al*. (2019). The Organic matter (OM) ranged between 1.22 - 2.88.00% with the highest value recorded at 30 metres while the control sample had the lowest value. Organic matter is an important soil property that may influence metal availability, cation exchange, and complex formation. The OM range obtained is higher than the 0.7 - 7.4% reported by Yahaya *et al.* (2009) and lower than the 5.6 - 24.1% reported by Ubwa *et al.* (2013) and Ojo *et al*. (2016). This could be a result of a low volume of biodegradable wastes at the studied sites. Hence, the high amounts of biodegradable wastes in the abattoir soils may have affected the organic content of the contaminated soils considerably.

The Nitrogen values ranged between 0.76 - 2.11mg/L with the highest value recorded at 20 metres while the control sample had the lowest value. This may be attributed to the washing away of faeces that are known to contain undigested protein and excess nitrogen. The high nitrogen content in the effluent-contaminated soil enhances microbial proliferation and promotes plant growth (Norton *et al.,* 2002). The value of CEC obtained in the studied abattoir soils is significantly higher than 6.69 (mg/L) obtained from the control site. This is similar to the findings by Iwegbue *et al.* (2006). This may be attributed to the high organic content of the contaminated soils. The EC values obtained in the studied abattoir soils were higher than values in the control site. This is consistent with the results reported for EC in abattoir waste-impacted soils as reported by Chikwendu *et al.* (2019). However, the values recorded could be attributed to the variations in the rate at which organic matter complexes were formed.

The effluent-contaminated soil samples had significantly higher levels of bacteria and fungi compared to the control. According to Rabah *et al* (2010), this might be because the effluent includes a large number of growth-promoting factors that microorganisms could readily use to enhance their growth and proliferation but are absent in the control soil samples. Another possibility is that the contamination from the release of abattoir wastewater into the soil environment has caused the ecological balance of the soil to become unstable. These findings concur with those of Akinnibosun and Ayejuyoni (2015), who evaluated the microbial population and physicochemical characteristics of soils contaminated by abattoir effluent in Benin City, Nigeria. This demonstrates that any water contaminated by the abattoir effluent is not suitable for household use and should not be released untreated into the environment as a failure to follow Good Hygiene Practices (GHP) and Good Manufacturing Practices (GMP).

The high load of animal excreta in the wastewater is associated with coliform organisms, *Salmonella* species, *Escherichia coli*, and *Klebsiella sp*. isolated in the contaminated soil. These coliform organisms showed recent contamination of the abattoir effluent with the high load of animal excreta released before and during slaughtering. The warm-blooded animals' digestive tract is home to faecal coliforms, which are frequently used as an indicator for gastro-enteric pathogen counts. *Escherichia coli*, which is also the most common member of the faecal coliform group was the most frequent isolate in the abattoir effluent. According to Chukwu *et al.* (2021), the most frequent pathogens linked to the contamination of food crops that are cultivated using organic fertilizers are *Salmonella sp.* and *Escherichia coli*. To cultivate their crops, farmers typically collect animal manure from slaughterhouses. Food crops may become contaminated when plant surfaces come into direct contact with animal manure (Chukwu *et al.,* 2021; Burris *et al.,* 2020). Therefore, the presence of *E. coli* in the soil contaminated by the abattoir effluent is a sign that the immediate and surrounding environment of the Dei-Dei abattoir, as well as water sources, are contaminated with faeces and potentially harmful organisms (Akinnibosun and Adejuyoni, 2015). If precautions are not taken, the release of untreated abattoir wastewater may lead to outbreaks of *Escherichia coli* infections, including pneumonia, bacteremia, meningitis, pelvic and abdominal infections, and urinary tract infections. These infections could be lethal. Additionally, *Bacillus sp*. is expected in the contaminated soil as this organism is native to the soil environment and has been shown by Rabah *et al*. (2010) to persist in such an environment. As opportunistic pathogens, *Staphylococcus aureus* and others can cause wound infections that can result in sepsis and chronic septicemia-related death. A common cause of human illnesses, *Staphylococcus aureus* is a gram-positive opportunistic bacterium that can cause food poisoning when consumed in food or water tainted with abattoir effluent. Their high abundance in the soil samples under study is most likely caused by the high organic matter in abattoir effluent as reported earlier (Enerijiofi *et al*, 2018a). Humans, cattle, and poultry can contract Aspergillosis from *Aspergillus niger*, which is typically found in areas with a lot of organic debris. Breathing problems like asthma can also be caused by inhaling *Aspergillus niger*. Large lung aspergillomas can obstruct respiratory gas exchange and result in asphyxiation-related death (Eze *et al*., 2013). Aflatoxin, a strong carcinogen, is produced by *Aspergillus flavus.* By causing the liver cells to undergo fatty acid metamorphosis, potentially leading to death - aflatoxin damages the liver. More than 4 billion people worldwide are thought to develop liver cancer linked to aflatoxin, and over 5 billion people in developing nations are thought to be at risk of chronic aflatoxin exposure from eating food contaminated with aflatoxin. Although food tainted with *Mucor* sp. constitutes a limited potential health hazard, it continues to be harmful and has been identified as the causative agent of some mycotic infections (Enerijiofi *et al.,* 2018a; Ikhajiagbe *et al*., 2021). These contaminated abattoir wastewater should not be released into the environment untreated or used for household purposes due to the possible public health issues that may arise.

Conclusion and Recommendation

The results of this investigation have demonstrated that abattoir effluent contains coliforms like *E. coli* and *Salmonella* sp. which are indications of recent faecal contamination of soil within its vicinity. Also, reported of note is *Aspergillus flavus* which is the causative agent of Aflatoxin. There is need for continuous education and awareness programs for butchers on Good Hygiene Practices to prevent major public health issues within the Dei-Dei abattoir in particular and Abuja residence in general. Finally, the study recommends proper treatment of abattoir effluents to meet acceptable environmental standards before discharge.

Authors Contribution

This work was carried out in collaboration between all Authors. OSO did the laboratory analyses, SOO designed and approved the study. KEE wrote the first draft of the manuscript, ensured its originality and revised it severally including after editorial review comments and gallery proof correction. OSO and KEE managed the literature searches. All authors read and approved the final manuscript. SSM and JMO managed the literature searches and gave magnanimous contributions in the laboratory analysis

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