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Impact of Abattoir Effluents on the pH, Organic Matter, Heavy Metal Levels and Microbial Composition of Surrounding Soils in Calabar Municipality

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Authors' contributions

This work was carried out in collaboration between both authors. Author VFE designed the study and managed the analyses of the study and did the statistical analysis Author OBI wrote the first draft of the manuscript and managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

This study examined the impact of abattoir activities on the heavy metal levels and microbial composition in surrounding soils receiving abattoir effluents within Calabar Metropolis. A total of nine composite soil samples were gotten from three locations: Atimbo, Ikot Eneobong and Nasarawa abattoir and environs. The results obtained revealed that the uncontaminated sites (control) had a strongly acidic pH while the abattoir contaminated soils were slightly acidic to slightly alkaline in reaction. Soil organic carbon content was low (< 1.5%) at the control site while the abattoir contaminated sites were high (> 2.0%). The heavy metal levels were highest at the point of direct discharge of abattoir effluents (Cu: 7.27 mg/Kg; Cd: 0.49 mg/Kg; Zn: 47.23 mg/Kg; Pb: 2.62 mg/Kg and Fe: 1071.69 mg/Kg) followed by the surrounding soils (Cu: 6.60 mg/Kg; Cd: 0.40 mg/Kg; Zn: 30.86 mg/Kg; Pb: 1.81 mg/Kg and Fe:871.76 mg/Kg) and the least values were obtained from the

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control (Cu: 5.45 mg/Kg; Cd: 0.30 mg/Kg; Zn: 16.30 mg/Kg; Pb: 1.60 mg/Kg and Fe 586.25 mg/Kg). Bacterial communities such as: *Escherichia coli, Bacillus* spp, *Staphylococcus* spp, *Klebsiella pneumoniae* and *Staphycoccus epidermis* were isolated from the abattoir effluents contaminated sites. Fungal isolates from the abattoir effluents contaminated soils include: *Aspergillus* spp, *Fusarium sporotrichiodes*, *Penicillium erichimultum*, *Absidia* spp. and *Mucor pusillus*. Generally, bacteria and fungi population and diversity were higher in abattoir effluents contaminated soils than the control. The increase in heavy metals levels and the presence of numerous and diverse communities of organisms indicated possible pollution. This calls for concern, as increase levels of heavy metals and invading pathogenic organisms can pollute water bodies as well as affect plant, human and animal health. Therefore, abattoir effluents should be channeled into septic tanks where it can be properly treated before being discharged into the soil environment.

Keywords: Abattoir effluents; heavy metals; microbial composition; soil properties.

1. INTRODUCTION

An abattoir is an approved place where animals are killed and processed for their meat and skin. Abattoir generates large effluents and waste water. The effluents is complex in composition and can alter the chemical composition of the receiving soils [1] which may be harmful to the environment at large.

Various products of livestock have been found to contain heavy metals. Feed materials grown on polluted soils, or treated with mineral and organic fertilizers, direct urination and defecation by animals, pesticides, soil amendment (liming and gypsum application), drinking water, and pharmaceutical etc. account for the major route of heavy metal uptake by livestock. Other sources are accidental access to limed field, mineral supplements with high content of trace metal and licking of soil and painted surfaced containing metallic pigments [2].

Indiscriminate discharges of abattoir effluents into soils have been reported to accumulate metals in receiving soils [3]. Contamination of agricultural land by heavy metals is of critical environmental concern due to their potential adverse ecological effects and undesirable changes in the environment with hazardous consequences. Soil organisms, on the other hand, do not readily adapt to or degrade heavy metals because metals slow down the speed of growth, activities and reproduction of functional microbial population in the soil thus prevailing slower growing organisms with lower diversity and higher resistance to heavy metals, but decreased biological activity [4,5]. Concerns about heavy metals in soils are not just limited to their toxicity to living organisms inhabiting the soil but also because failure to alleviate heavy metal build-up and persistence in soil may result in immobilization within different organic and inorganic colloids and mobilization into the flora and fauna and subsequently become available in food chain with deleterious health effects [6,7]. When these metals accumulate in plant tissues, animals grazing on such contaminated plants and drinking from polluted water also accumulate such metals in their tissues and milk if lactating [8-11]. These metals are ultimately released back to the through the blood, milk, urine faeces, paunch material and other animal parts when slaughtered culminating in a vicious cycle.

Soil pollution as a result of improper disposal of abattoir effluents and waste in Calabar Metropolis is on the increase, with the last decades witnessing greater quantities of these effluents in soil. This research therefore aims at assessing the impact of abattoir effluents on soil chemical properties, levels of heavy metals and microbial composition in surrounding soils receiving abattoir effluents in Calabar.

2. MATERIALS AND METHODS

2.1 Description of the Study Area

The study area is located in Calabar Municipality. Calabar Municipality lies between latitude 04° 15” and 05° 05” N and longitude 08° 15” and 08° 25” E in southern Cross River State, South-South Nigeria. Calabar municipality is bordered by Odukpani LGA in the North, the great Kwa River in the North-East and Akpabouyo LGA in the West. Its Southern shores are bounded by the Calabar River and Calabar South Local Government Area respectively. The area is characterized by tropical climate, distinct rainy and dry seasons, with an annual rainfall of 2360 mm (ranging from 2290 to 2680 mm), annual temperature range of 21 – 31°C and relative humidity of 82 to 89% [12]. The vegetation is

mainly humid tropical vegetation with vegetables such as *Telferia occidentalis*, *Talinum triangulare*, *Abelmoschus esculentus* and crops such as *Zea maize,* and *Manihot esculenta* grown by farmers. Soils in Calabar are mostly sandy and low in nutrients [13]. The area is characterized by many rivers and streams.

2.2 Field Studies

Representative composite soil samples were collected at depth of 0-30 cm using soil auger from Atimbo, Ikot Eneobong and Nasarawa abattoirs and its environs in Calabar municipality. Three sampling points were mapped out around each abattoir location [14]. Point (1) was the point of direct discharge of the effluents from the abattoir into the soil and served as the reference point. Point (2) was 20 m from point (1) but still within the effluents site. Point 3 was 100 m from point (1) and served as the control. Three composite samples were collected from each abattoir location bringing the total number of samples to nine (9). Soil samples for microbial analyses were collected aseptically in sterile polythene bags, labelled and transported in ice parked coolers to the laboratory for analysis [15].

2.3 Heavy Metal Analyses

Soil samples from the study sites were collected and air dried. Two replicates of 2.5 g sediments were acid-digested in microwave assisted Kjeldahl digester. To each microwave extraction vessel was added with 5 ml of concentrated nitric acid, 2 ml hydrochloride acid and 1 ml of hydrofluoric acid. The vessels were capped and heated in a microwave unit at 800 W to a temperature of 210°C for 20 min with pressure of 40 bar. The digested samples were analyzed for heavy metals by atomic absorption spectrophotometer using flame atomization [16].

2.4 Microbial Analyses

Reagents used in the study: Soil Extract was prepared by suspending 1000 g of soil in 1 litre (1000 ml) of distilled water and stirred vigorously using stirring rod. The mixture was filtered with a Whatman No. 4 filter paper. The filtrate was sterilized by autoclaving at a temperature of 121°C and pressure of 1 b/sq. inch for 15 minutes. The extract was used in the preparation of agar for the estimation of total heterotrophic aerobic bacteria, purification and for stock culture. Malt extract agar was used for the isolation of fungi [17]

2.5 Enumeration of Total Heterotrophic Bacteria and Fungi

Soil samples were serially diluted in ten folds [17]; Dilution factors of 10^{-6} and 10^{-3} were used for bacteria and fungi cultures respectively. Total viable heterotrophic aerobic bacterial and fungal counts were determined using the pour plate technique. Molten soil extract agar and malt extract agar were poured into sterile Petri-dishes containing 1 ml of the appropriate aliquot diluents for the isolation of total heterotrophic bacteria and fungi, however, plating was done in triplicates while observing all precautions. Colony count was taken after incubating the plates at 30°C for 24 and 48 hours for bacteria and fungi respectively. The bacterial and fungal isolates were sub cultured into nutrient agar slants which were then used for biochemical tests [17,18].

2.6 Characterization and Identification of Isolates

Characterization and identification was carried out at a magnification of X40 using an objective lens. Gram positive (+ve) organisms were seen as blue or violet colourations while red colours indicate gram negative (-ve) bacteria, however, spore formation, motility, oxidase and catalase production; Citrate utilization, oxidative /fermentation (O/F) utilization of glucose; indole and coagulase production, starch hydrolysis, sugar fermentation, methyl red, Voges Proskaur reaction and urease production tests were also performed following standard procedures [15-21]. Microbial identification was performed as outlined in [22]. Fungal isolates were examined macroscopically and microscopically using the needle mounts technique [18,23].

3. RESULTS AND DISCUSSION

3.1 Physicochemical Properties and Heavy Metal Levels

The results of the means of the physicochemical properties and heavy metals levels of soils contaminated with abattoir effluents in Atimbo, Ikot Eneobong and Nasarawa are presented in Table 1. The result indicated that particle size distribution was dominated by sand (Table 1), with values ranging from 87 – 88%, 87 – 88% and 82 – 87% for Points 3, 1 and 2 respectively.

Silt fraction was 11.00 % at the control site, 11–12% at Point 1 and 12 -17% at Point 2. Clay was less than 2% across the entire study sites (Table 1). The soils were mainly loamy sand, indicating that the abattoir effluents had no effect on the textural class. Such sand dominated soils will allow for high rate of leaching and infiltration of the effluents through the soil fractions probably down to the water aquifer.

The soils pH values ranged between 4.8 - 5.2, 6.3 – 7.3 and 6.4 – 7.35 with means of 5.00, 6.78 and 6.87 for the Control, Points 1 and 2 respectively.

The soils of the control site were strongly acidic while the abattoir contaminated soils were slightly acidic to slightly alkaline (Table 1). Such pH conditions as observed for the abattoir sites will favour the proliferation of soil microbes as well as the growth of various crops.

Soil organic carbon content was low (< 1.5%) at the control site (Point 3) with mean value of 1.00% while values recorded for the abattoir contaminated sites were high (> 2.0%) with values ranging between 7.00 – 8.00% and 6.00 – 7.00% for Points 1 and 2 respectively. The high organic carbon content in the abattoir effluents contaminated sites suggests high organic matter content.

Various products of livestock have been reported to contain heavy metals [2]. Leaving us to hypothesize that the effluents will invariably contain heavy metals. Assessing the heavy metals status of the study area is of great concern as a lot of vegetables are cultivated within these sites. Although some metals are considered as micro nutrients and required in minute quantities for plant uptake, nevertheless, excess of these metals can lead to plant and eco-toxicity [24]. Copper (Cu) for instance is considered micronutrient in plant nutrition. Despite the essential nature of Cu, excess of it in soil plays a cytotoxic role, induces stress, retards growth and causes leaf chlorosis and injury to plants [25]. Results obtained from the study site for Cu ranged between 5.38 – 5.54 mg/Kg, 6.95 – 7.60 mg/Kg and 6.20 – 6.95 mg/Kg with means of 5.45 mg/Kg, 7.27 mg/Kg and 6.60 mg/Kg for Points 3, 1 and 2 respectively (Table 1). Nasarawa recorded the highest value (7.60 mg/Kg) (Fig. 3) for Cu while the point of direct discharge of the abattoir effluents (Point 1) recorded the highest mean (7.27 mg/Kg). The data obtained for Cu from the sampling points differed from each other with a standard deviation (SD) of $\pm$ 0.83 with a coefficient variability of 13.00% suggesting the closeness of the results of the contaminated sites to the control.

Values recorded for Cu were within the range of 2 - 100 mg/Kg recommended by [26] and 2- 250 mg/Kg given by [27] as normal range in soil.

Cadmium (Cd), had values ranging between 0.24 – 0.36 mg/Kg, 0.45-0.57 mg/Kg and 0.25 – 0.55 mg/Kg with means of 0.30 mg/Kg, 0.49 mg/Kg and 0.40 mg/Kg for Points 3, 1 and 2 respectively with a percentage deviation of 31.00%. The values recorded for Cd at the abattoir sites in Calabar were however, lower than the 13.21 - 30.02 mg/Kg reported by [3] for abattoir environs in Sokoto and below the 1.1 mg/Kg (maximum) standard for the regulatory limit of Cd in agricultural soils [28,29].

Zinc (Zn) is another metal of concern because high levels of Zn in soil inhibit many plant metabolic functions and limits the growth of both root and shoot [28-31]. The mean analytical determination for Zn from the study sites ranged from 15.80 – 17.0 mg/Kg, 44.30 – 52.25 mg/Kg and 24.30 – 43.15 mg/Kg with means of 16.3 mg/Kg, 47.23 mg/Kg and 30.86 mg/Kg ($\pm$30.86) for locations 3, 1 and 2 concurrently. The least content of Zn was recorded for the control site while the highest value was recorded at the point of direct discharge of the abattoir effluents into the soil (Table 1). The mean values for Zn from the study site varied with a CV of 46.00%, indicating a wide spread of the data with each other. The values obtained for Zn in this research were lower than 56.31-92.50 mg/Kg recorded by [3] for abattoir effluents contaminated soils in Sokoto and were within permissible limits of 10-300 mg/Kg recommended by [26] but however, higher than the 5 mg/Kg quantity required as micronutrient level.

Lead (Pb) from the study sites had values ranging from 1.53 -1.64 mg/Kg, 2.35 -2.95 mg/Kg and 1.35- 2.15 mg/Kg with means of 1.60 mg/Kg, 2.62 mg/Kg and 1.81$\pm$0.36 mg/Kg for the control, Points 1 and 2 correspondingly. The values recorded for Pb from the study sites were within the recommended soil range (2-200 mg/Kg) for uncontaminated soils as recorded by [26]. High level of Pb could inhibit enzymatic activities, alter water imbalance, membrane permeability and disturbs mineral nutrition [32].

Table 1. Mean physicochemical properties and heavy metals (mg/Kg) in soils contaminated with abattoir effluents in Calabar Metropolis

Location	Sand %	Clay %	Silt %	Text.	pH	Organic C %	Cu	Cd	Zn (mg/kg)	Pb	Fe
Point 1 (Point of direct discharge)											
Atimbo	88.00	1.00	11.00	LS	6.40	7.89	7.25	0.57	52.25	2.55	1078.18
Ikot Eneobong	88.00	1.00	11.00	LS	6,85	7.99	6.95	0.45	45.15	2.95	991.64
Nasarawa	87.00	1.00	12.00	LS	7.35	7.30	7.60	0.45	44.30	2.35	1145.27
Mean	**87.60**	**1.00**	**11.30**		**6.87**	**7.73**	**7.27**	**0.49**	**47.23**	**2.65**	**1071.69**
Point 2 (20 m from Point 1)											
Atimbo	87.00	1.00	12.00	LS	6.30	6.85	6.95	0.55	43.15	2.15	878.38
Ikot Eneobong	88.00	1.00	12.00	LS	6.75	7.20	6.65	0.40	25.15	1.95	791.44
Nasarawa	82.00	2.00	17.00	LS	7.30	6.30	6.20	0.25	24.30	1.35	945.47
Mean	85.30	1.30	13.66		6.78	6.78	6.60	0.40	30.86	1.81	871.76
Point 3 (Control)											
Atimbo	87.00	2.00	11.00	LS	5.00	1.10	5.54	0.24	15.80	1.54	479.90
Ikot Eneobong	88.00	1.00	11.00	LS	4.80	0.90	5.38	0.30	17.00	1.61	579.99
Nasarawa	87.00	1.00	11.00	LS	5.20	1.00	5.43	0.36	16.10	1.65	698.86
Mean	**87.30**	**1.30**	**11.00-**		**5.00**	**1.00**	**5.45**	**0.30**	**16.30**	**1.60**	**586.25**
SD							**0.83**	**0.12**	**14.58**	**0.53**	**224.99**
Variance							**0.69**	**0.01**	**212.81**	**0.28**	**50621.58**
CV							**13.00**	**31.00**	**46.00**	**26.00**	**27.00**

**LS: Loamy sand*

Amongst the various metals studied, Iron (Fe) yielded the highest values across locations (Figs. 1,2 and 3). Values recorded for Iron (Fe) from the study sites ranged between 479.90 – 698.86 mg/Kg, 991.64 – 1145.27 mg/Kg, 791.44 -945.47 mg/Kg with means of 586.25 mg/Kg, 1071.69 mg/Kg, and 871.76 mg/Kg for the control, Points 1 and 2 (Table 1) respectively. The least mean value (586.25 mg/Kg) was recorded in the control while the highest mean (1071.69 mg/Kg) was recorded at the point of direct discharge (Table 1). This research observed a wide spread between data point for Fe with a coefficient variability of 225.00%. The levels of Fe recorded for the abattoir contaminated soils were slightly higher in values than the control soils but lower than values (2569.1 - 4130.0 mg/Kg) recorded by [3] and far below the typical background concentrations of 7000 – 55.000 mg/Kg levels outlined by [26].

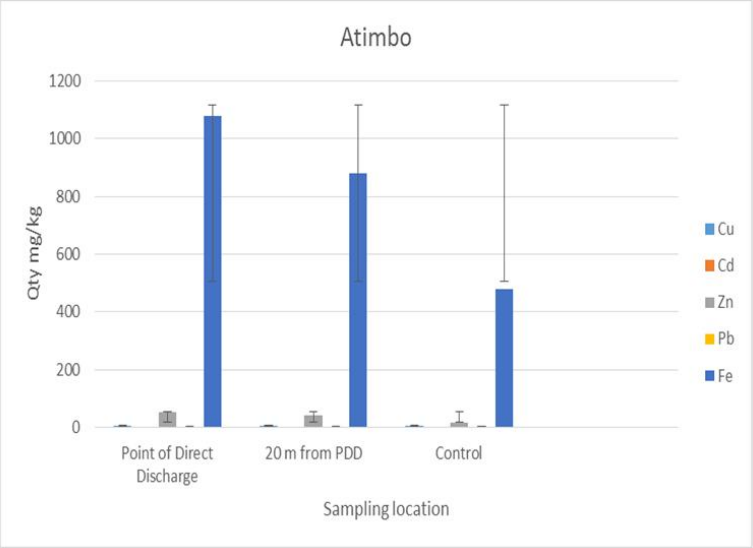

Fig. 1. Mean heavy metals (mg/kg) in soils contaminated with abattoir effluent in Atnimbo

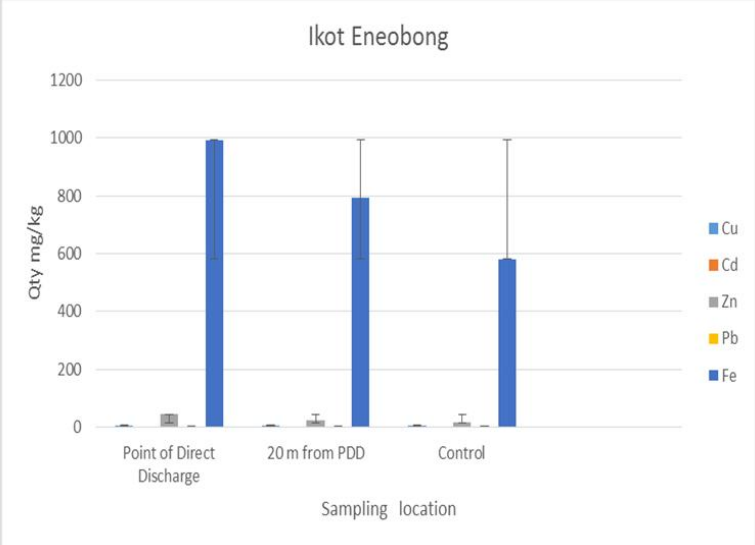

Fig. 2. Mean heavy metals (mg/kg) in soils contaminated with abattoir effluent in Ikot Eneobong

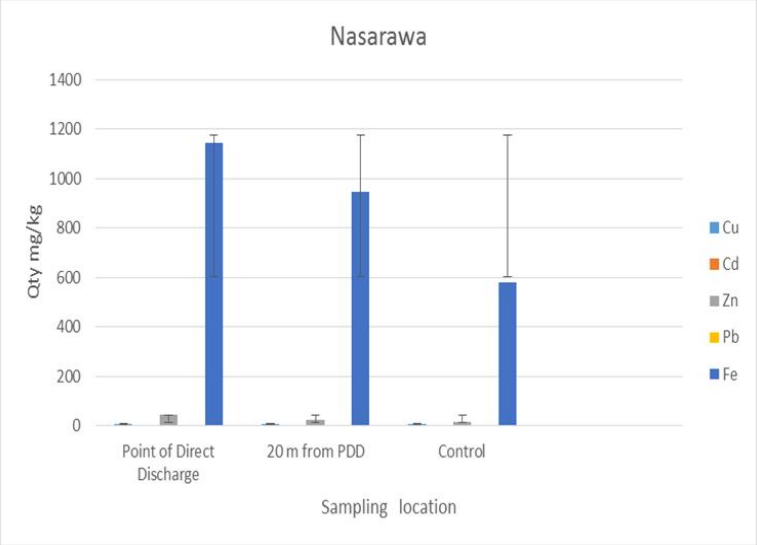

Fig. 3. Mean heavy metals (mg/kg) in soils contaminated with abattoir effluent in Nasarawa

Generally, the control site recorded the least values for heavy metals when compared with values from the abattoir effluents contaminated soils across the sampling locations (Figs. 1, 2 and 3). The analytical determinations portrayed the sequence of heavy metal in the study site as: Fe > Zn > Cu > Pb > Cd. Heavy metal values recorded in this study for the abattoir contaminated soils in Calabar metropolis are still within the permissible limits probably because the abattoirs are relatively new or as a result of high rate of leaching owing to the sandy nature of the soils and leaching due to high amount of rainfall. The high organic C (< 6.0%) observed for the effluents contaminated site (Points 1 and 2) suggests high organic matter content in the soils. This could reduce metal availability due to metal – organic complexation [33].

3.2 Microbial Analysis

The results of the percentage occurrence of the microbial isolates are presented in Table 2. The result revealed enrichment in the diversity and population of microorganism in the soils contaminated with abattoir effluents. Although most of the microbes isolated were native to the soils, others were invading organisms attracted by the contaminant or associated with food and beef decay. Amongst the bacterial isolates, *Escherichia coli* and *Bacillus subtilis* showed the highest frequency (15%) of occurrence and closely followed by *Staphylococcus* (14%), *Bacillus anthracis* (13%), *Bacillus polymyxa* and *Klebsiella pneumoniae* (8%). The least frequency of occurrence (4%) was observed for *Staphylococcus epidermides*. The prevalence of various species and communities of organisms in the contaminated soils could be attributed to the continuous discharge of the effluents, various stages of decomposition of the effluents/ inclusions (blood, paunch manure, urine, body parts, dung and undigested grains and grasses). The decomposition of the dung explains the high prevalence of *Escherichia coli* and *Streptococcus faecalis* in the effluents contaminated soils.

Escherichia coli and *Streptococcus faecalis* are known to be associated with faecal contaminants while the main microorganisms responsible for fermentation of effluents are *Bacillus spp*, *Staphylococcus spp* and *Micrococcus spp.* Similar, findings of high microbial prevalence have been reported from soil samples contaminated with waste-water from various locations in Nigeria [34,35,36].

Aspergillus niger (30%) was the most prevalent fungi followed by *Fusarium sporotrichoides* (20%) while *Mucor pusillus* showed the lowest occurrence of 6% for the abattoir contaminated soils (Table 2). The diverse microbial communities observed in the abattoir effluents contaminated soils could be attributed to the availability of substrates that are easily utilized by the organisms for food and energy source as well as the favourable pH.

Table 2. Frequency of occurrence of the microbial isolates in abattoir effluents contaminated soils in Calabar Metropolis

Isolates	Abattoir effluent soils Mean count (cfu/g)	Frequency of occurrence (%)	Control soils Mean count (cfu/g)	Frequency of occurrence (%)
1) Bacteria				
Escherichia coli	16.2 $X10^6$	15	--	--
Clostridium spp.	5.4 $X10^6$	5	--	--
Bacillus anthracis	14.1 $X10^6$	13	4.0 $X10^6$	5
Bacillus polymyxa	8.7 $X10^6$	8	--	--
Bacillus subtilis	16.2 $X10^6$	15	10.4 $X10^6$	13
Streptomyces spp	--	--	9.6 $X10^6$	12
Streptococcus faecalis	6.5 $X10^6$	6	--	--
Salmonella spp	5.4 $X10^6$	5	--	--
Staphylococcus aureus	15.2 $X10^6$	14	--	--
Staphylococcus epidermides	4.0 $X10^6$	4	--	--
Klebsiella pneumoniae	8.7 $X10^6$	8	8.0 $X10^6$	10
Pseudomonas aeruginosa	7.6 $X10^6$	7	8.0 $X10^6$	10
Micrococcus spp.	--	--	12.0 $X10^6$	15
Arthrobacta spp.	--	--	16.0 $X10^6$	20
Nocardia spp.	--	--	12.0 $X10^6$	15
Total (CFU)	**108**	**100**	**80**	**100**
2) Fungi				
Aspergillus fumigates	9.0 $X10^4$	10	--	--
Aspergillus niger	27.0 $X10^4$	30	12.0 $X10^4$	20
Aspergillus flavus	11.0 $X10^4$	12	11.0 $X10^4$	18
Absidia spp.	7.2 $X10^4$	8	--	--
Mucor pusillus	6.3 $X10^4$	7	4.0 $X10^4$	7
Penicillum endrinulatum	11.5 $X10^4$	13	9.0 $X10^4$	15
Fusarium sporotrichoides	18.0 $X10^4$	20	12.0 $X10^4$	20
Microsporium spp	--	--	12.0 $X10^4$	20
Total (CFU)	**90**	**100**	**60**	**100**

4. CONCLUSION

This research observed increased heavy metal values at the abattoir environs when compared with the control sites giving a trend of: Fe > Zn > Cu > Pb > Cd. However, heavy metal values recorded in this study for the abattoir contaminated soils in Calabar metropolis are still within the permissible limits however, precaution needs to be applied as these levels can considerably be increased by accumulation from the continuous discharge of the effluents. The microbial analysis revealed an enrichment of bacterial isolates: *Escherichia coli, Clostridium spp*., *Bacillus anthracis, Bacillus polymyxa*, *Bacillus subtilis*, *Streptococcus faecalis*, *Salmonella spp, Staphylococcus aureus, Staphylococcus epidermides and Klebsiella pneumoniae, Pseudomonas aeruginosa and* fungal isolates: *Aspergillus fumigates, Aspergillus niger, Aspergillus flavus, Absidia spp., Mucor pusillus, Penicillium spp and Fusarium spp* at the effluents contaminated sites. It is recommended that abattoir effluents be properly channeled into septic tanks to avoid accumulation of heavy metals in plants and surrounding soils as well as prevent the contamination of water bodies and possible disease outbreak, since most of the organisms found there are very harmful to man.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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