

Original Research Article

IMPACT OF ABATTOIR EFFLUENT ON SOIL PROPERTIES, HEAVY METAL LEVELS AND MICROBIAL COMPOSITION OF SURROUNDING SOILS IN CALABAR MUNICIPALITY

ABSTRACT

This study examined the impact of abattoir activities on the heavy metal levels and microbial composition in surrounding soils receiving abattoir effluent 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 acid pH while the abattoir contaminated soils were slightly acid 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 point of direct discharge of abattoir effluent (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 soil (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 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 *Staphylococcus epidermis* were isolated from the abattoir effluent contaminated sites. Fungal isolates from the abattoir effluent contaminated soil include: *Aspergillus spp*, *Fusarium sporotrichiodes*, *Penicillium erichimultum*, *Absidia spp*. and *Mucor pusillus*. Generally, bacteria and fungi population and diversity were higher in abattoir effluent contaminated soils than the control. The increase in heavy metals level and the presence of numerous and diverse communities of organisms indicates possible pollutant. 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 effluent should be channeled into septic tanks where it can be properly treated before being discharged into the soil environment.

Keywords: Abattoir effluent, 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 effluent and waste water. The effluent is complex in composition and can alter the chemical composition of the receiving soil [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 effluent into soil 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

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to heavy metals, but decreased biological activity [4, 5]. Concerns about heavy metal in soils are not just limited to their toxicity to living organisms inhabiting the soil but also because failure to alleviate heavy metal buildup and persistence in soils 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 this metal 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, 9, 10, 11]. These metals are ultimately released back to the soil 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 effluent 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 effluent on soil chemical properties, levels of heavy metals and microbial composition in surrounding soil receiving abattoir effluent in Calabar.

2. MATERIAL 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, Southeast Nigeria. Calabar municipality is bordered by Odukpani LGA in the North, the great Kwa River in the North-East and Akpabuyo 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 (range 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. 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. Point (1) was the point of direct discharge of the effluent from the abattoir into the soil and served as the reference point. Point (2) was 20 m from point (1) but still within the effluent 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 poly bags, labelled and transported in ice parked coolers to the laboratory for analysis.

2.3 Heavy metal Analyses

Soil samples from the study sites were collected and air dried. Two replicates of 0.25 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.

2.4 Microbial Analyses

Reagents used in the study: Soil Extract was prepared by suspending 1000 g of soil in 1 liter (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.

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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.

2.5 Enumeration of total heterotrophic Bacteria and Fungi

Soil samples were serially diluted in ten folds [13]; 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 counts were taken after incubating the plates at 30 °C for 24 and 48 hours for bacteria and fungi respectively. The bacterial isolates were sub cultured into nutrient agar slants which were then used for biochemical tests.

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 [13, 14, 15, 16, 17, 18, 19]. Microbial identification was performed as outlined in [20]. Fungal isolates were examined macroscopically and microscopically using the needle mounts technique [21].

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 soil contaminated with abattoir effluent in Atimbo, Ikot Eneobong and Nasarawa are presented in Table 1. The result indicates that particle size distribution was dominated by sand (Table 1), with values ranging from 87.38 – 87.81 %, 86.85 – 87.75 % and 81.70 – 86.75 % for Points 3, 1 and 2 respectively. Silt fraction ranged between 10.91 and 11.09 % at the control site, 11.00 – 11.75 % at Point 1 and 12.0 -16.75 % 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 effluent had no effect on the textural class. Such sand dominated soils will allow for high rate of leaching and infiltration of the effluent through the soil fractions probably down to the water aquifer.

The soil 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.

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Table 1: Mean physicochemical properties and heavy metals (mg/kg) in soils contaminated with abattoir effluent in Calabar Metropolis

Location	Sand %	Clay %	Silt %	Text.	pH	Org. C %	Cu	Cd	Zn (mg/kg)	Pb	Fe
Point 1 (Point of direct discharge)											
Atimbo	87.75	1.25	11.00	LS	6.40	7.89	7.25	0.57	52.25	2.55	1078.18
Ikot Eneobong	87.70	1.17	11.13	LS	6.85	7.99	6.95	0.45	45.15	2.95	991.64
Nasarawa	86.85	1.40	11.75	LS	7.35	7.30	7.60	0.45	44.30	2.35	1145.27
Mean	87.43	1.27	11.29		6.87	7.73	7.27	0.49	47.23	2.62	1071.69
Point 2 (20 m from point 1)											
Atimbo	86.75	1.25	12.00	LS	6.30	6.85	6.95	0.55	43.15	2.15	878.38
Ikot Eneobong	86.70	1.17	12.13	LS	6.75	7.20	6.65	0.40	25.15	1.95	791.44
Nasarawa	81.70	1.55	16.75	LS	7.30	6.30	6.20	0.25	24.30	1.35	945.47
Mean	85.05	1.32	13.63	LS	6.78	6.78	6.60	0.40	30.86	1.81	871.76
Point 3 (Control)											
Atimbo	87.38	1.71	10.91	LS	5.00	1.10	5.54	0.24	15.80	1.54	479.90
Ikot Eneobong	87.81	1.19	11.00	LS	4.8	0.90	5.38	0.30	17.00	1.61	579.99
Nasarawa	87.51	1.40	11.09	LS	5.2	1.00	5.43	0.36	16.10	1.65	698.86
(Mean)	87.57	1.43	11.00	LS	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							12.90	30.76	46.34	26.36	26.68

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The soils of the control soil were strongly acid while the abattoir contaminated soils were slightly acid 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.

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Soil organic carbon content was low (< 1.5 %) at the control site (Point 3) with mean value of 1.00 % while value recorded for the abattoir contaminated site were high (> 2.0 %) with values ranging between 7.30 – 7.73 % and 6.30 – 7.20 % for Points 1 and 2 respectively. The high organic carbon content in the abattoir effluent contaminated sites suggests high organic matter content.

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Various products of livestock have been reported to contain heavy metals [2]. Leaving us to hypothesize that the effluent 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 [22]. Copper (Cu) for instance is considered micronutrient in plants 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 [23]. 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 effluent (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 ± 0.83 with a coefficient variability of 12.90 % 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 [24] and 2- 250 mg/kg as given by [25] as normal range in soils.

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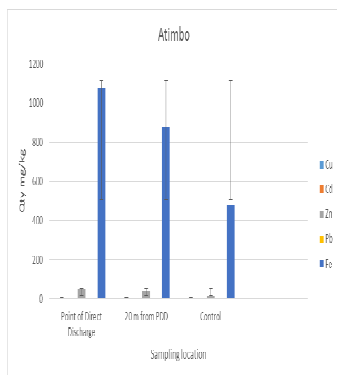


FIG 1: Mean heavy metals (mg/kg) in soils contaminated with abattoir effluent in Atimbo

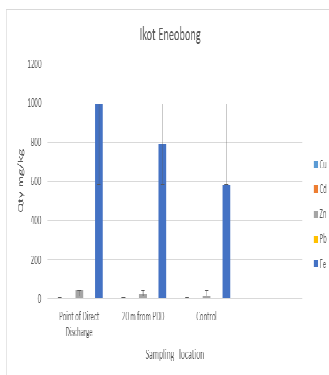


FIG 2: Mean heavy metals (mg/kg) in soils contaminated with abattoir effluent in Ikot Eneobong

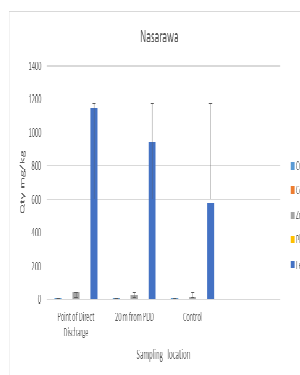


FIG 3: Mean heavy metals (mg/kg) in soils contaminated with abattoir effluent in Nasarawa

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 30.76 %. The values recorded for Cd at the abattoir sites in Calabar were however, lower than the 13.21 mg/kg - 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 soil [26, 27].

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, 29,30]. 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 (± 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 effluent into the soil (Table 1). The mean values for Zn from the study site varied with a CV of 46.34 %, 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 effluent contaminated soils in Sokoto and were within permissible limits of 10-300 mg/kg recommended by [24] but however, higher than the 5 mg/kg quantity required as micronutrient level.

Lead (Pb) from the study site 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 (± 0.36) for the control, Points 1 and 2 correspondingly. The values recorded for Pb from the study sites were within the soil range (2-200 mg/kg) for uncontaminated soils as recorded by [24]. High level of Pb could inhibit enzymatic activities, alter water imbalance, membrane permeability and disturbs mineral nutrition [31].

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 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 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 224.99 %. 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 – 5500.00 mg/kg levels outlined by [24].

Generally, the control site recorded the least values for heavy metals when compared with values from the abattoir effluent contaminated soils across the sampling locations (Figs

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1,2&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 effluent contaminated site (Points 1 & 2) suggests high organic matter content in the soils. This could reduce metal availability due to metal – organic complexation [32].

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 effluent. Although most of the microbes isolated were native to the soil, 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 soil could be attributed to the continuous discharge of the effluent, various stages of decomposition of the effluent/ 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 effluent contaminated soil. *Escherichia coli* and *Streptococcus faecalis* are known to be associated with faecal contaminants while the main microorganisms responsible for fermentation of effluent 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 [33, 34].

Aspergillus niger (10) was the most prevalent fungi followed by *Fusarium sporotrichoides* (20%) while *Mucor pusillus* showed the lowest occurrence of 6% for the abattoir contaminated soil (Table 2). The diverse microbial communities observed in the abattoir effluent contaminated soil 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: Percentage occurrence of the microbial isolates in abattoir effluent contaminated soils in Calabar Metropolis.

Isolates	Frequency of occurrence	
	(%)	
	Abattoir effluent soil	Control
1) Bacteria		
<i>Escherichia coli</i>	15	-
<i>Clostridium spp.</i>	5	-
<i>Bacillus anthracis</i>	13	5
<i>Bacillus polymyxa</i>	8	-
<i>Bacillus subtilis</i>	15	13
<i>Streptomyces spp</i>	-	12
<i>Streptococcus faecalis</i>	6	-
<i>Salmonella spp</i>	5	-
<i>Staphylococcus aureus</i>	14	-
<i>Staphylococcus epidermides</i>	4	-
<i>Klebsiella pneumoniae</i>	8	10
<i>Pseudomonas aeruginosa</i>	7	10
<i>Micrococcus spp.</i>	-	15
<i>Arthrobacta spp.</i>	-	20
<i>Nocardia spp.</i>	-	15

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Total	100	100
2) Fungi		
<i>Aspergillus fumigates</i>	10	-
<i>Aspergillus niger</i>	30	20
<i>Aspergillus flavus</i>	12	18
<i>Absidia spp.</i>	8	-
<i>Mucor pusillus</i>	7	7
<i>Penicillium endrinulatum</i>	13	15
<i>Fusarium sporotrichoides</i>	20	20
<i>Microsporium spp</i>	-	20
Total	100	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 level can considerably be increased by accumulation from the continuous discharge of the effluent. 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 effluent contaminated sites. It is recommended that abattoir effluent be properly channeled into septic tanks to avoid accumulation of heavy metals in plants and surrounding soil 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.

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