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ASSESSMENT OF MICROBIAL POPULATION AND PHYSICO-CHEMICAL PROPERTIES OF ABATTOIR EFFLUENT-CONTAMINATED SOILS IN BENIN CITY, NIGERIA

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ABSTRACT

The research was conducted in order to determine the effect of abattoir effluent on the microbiological and physico-chemical characteristics of the receiving soils in Ikpoba Hill and Oluku located in Benin City, Nigeria. Microbial analysis was carried out using standard microbiological techniques. The microorganisms isolated from the contaminated soil in this study were: Escherichia coli, Pseudomonas aeruginosa, Bacillus sp, Staphylococcus. epidermidis, Staphylococcus aureus, Alcaligenes sp, Klebsiella sp, Aspergillus flavus, Aspergillus niger, Penicillium sp, Geotrichum sp and Mucor sp. The results revealed high count of 4.77 x 10^6 ± 0.001 cfu/g, 7.31 x 10^4 ± 0.01 cfu/g and 2.53 x 10^3 ± 0.001 cfu/g for bacteria, coliform and fungal count, respectively in Oluku abattoir effluent-contaminated soil, while Ikpoba Hill effluent-contaminated soil had 6.64 x 10^6 ± 0.001 cfu/g, 6.68 x 10^4 ± 0.001 cfu/g and 3.47 x 10^3 ± 0.001 cfu/g for bacteria, coliform and fungal count, respectively. The microbial counts were higher in the contaminated samples than in the control, having 2.35 x 10^4 ± 0.1 cfu/g, 1.15 x 10^2 ± 0.1 cfu/g and 1.25 x 10^3 ± 0.1 cfu/g for bacteria, coliform and fungal counts, respectively. The physico-chemical parameters showed that the contaminated soil had pH of 7.70 and 7.80, total organic Carbon of 30.66 ppm and 33.33 ppm, Potassium of 8.84 ppm and 8.90 ppm, Phosphorus of 7.10 ppm and 7.45 ppm, Nitrogen of 0.39 ppm and 0.40 ppm for Oluku and Ikpoba hill abattoirs, respectively. The presence of the coliform organisms is an indication of recent faecal contamination of the abattoir effluent, which can cause significant environmental and health hazard. Overall, the observed high level of microbial contamination of the soils suggests that they are not just contaminated but possibly polluted, and this situation could lead to transmission of pathogens to humans.

Key words: Effluent, abattoir, bacteria, fungi, health risk

INTRODUCTION

An abattoir or slaughterhouse is a facility where animals are killed and prepared fresh for traders and consumers to buy for various types of food products (Eze *et al.,* 2013). They act as the starting point of the meat processing industry where stock comes from market or farms to enter the food chain. The abattoir industry is an important component of livestock industry in Nigeria, providing domestic meat supplies to over 150 million people and employment opportunities for the teeming population (Neboh *et al.,* 2013). They pollute the environment either directly or indirectly from their various processes, if not handled properly (Kosamu *et al.,* 2011). Wastewater from an abattoir is a concentrated source of oxygen-consuming waste (Girards, 2005). It contains high levels of organic matter due to presence of faeces, manure, blood, fats, grease, hair, grit and undigested feeds. It can also contain high level of salts, phosphate and nitrates. Blood and fats contribute mostly to organic load (Food Standard Agency, 2013).

Abattoirs generally use large quantities of water for washing meat and cleaning cutlasses used for cutting meat and they are usually located near water bodies in order to gain access to water for processing (Aimisu *et al.,* 2003; Rabah *et al.,* 2010). Contamination of river body and land from abattoir wastes could constitute a significant environmental and health hazard (Nafarnda *et al.,* 2006; (Osibanjo and Adie, 2007). One of the effects of wastewater source draining into soil is making the soil oxygen to become less available as an electron acceptor, prompting denitrifying bacteria to reduce available nitrate to gaseous

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nitrogen which enters the atmosphere with resultant negative effects. Also, the anaerobic methanogens may produce excessive methane at a higher rate than aerobic methane-oxidizing bacteria (methanotrophs) could cope with, thus contributing to greenhouse effect and global warming. Similarly, the physicochemical properties of the soil may become altered, such as the pH, due to the uncontrolled discharge of untreated abattoir wastewater resulting in the loss of certain microbes (Edward, 1990). Disposal of untreated effluent into the surrounding soil and water could lead to the transmission of pathogens to humans, and this often times causes zoonotic diseases (Cadmus *et al.*, 1999). Following the discharge of untreated wastewater into soil, certain elements such as iron, lead, phosphorus, calcium and zinc, previously absent or present in minute quantities will be introduced, leading to the magnification of these chemicals and thus altering the physicochemical nature of the soil. Some of these chemicals may be toxic to the microbial, floral, and faunal communities of the soil.

Since abattoirs produce large quantities of solid wastes, washwater and process wastewater containing organic matter, suspended solids and a wide variety of contaminants are generated during different processing stages (Eze *et al.*, 2013). Pathogens present in animal carcass or shed in animal wastes may include rotaviruses, *Salmonella* spp., *Escherichia coli* 0157:H7, *Yersinia enterocolitica*, *Campylobacter* spp., *Cryptosporidium parvum* and *Giardia lamblia* (Jackson *et al.*, 1998). These zoonotic pathogens can exceed millions to billions per gram of faeces, and may infect humans through various routes such as contaminated air, contact with livestock animals or waste products, swimming in water impacted by animal faeces, exposure to potential vectors such as flies and rodents or consumption of food or water contaminated by animal wastes (Armand-lefevre *et al.*, 2005; Schlech *et al.*, 2005).

In Nigeria, facilities for the treatment of abattoir effluents are lacking, unlike in developed countries where these facilities are adequately provided (Ogbonnaya, 2008), and as such immediate remedy is required as the abattoir wastes are sources of embarrassment. Animals slaughtered in Ikpoba hill and Oluku abattoirs accounts for a large percentage of the total animals slaughtered in Benin metropolis of Nigeria. The waste from the slaughtering and dressing ground in the abattoirs drains into the surrounding soil environment untreated, while the remaining is chanelled through the abattoir drainages into Ikpoba River and rivers in Oluku and environs. Leachate from the series of decomposition processes of these wastes can introduce pathogens and excess nutrients into the surrounding soils, surface water and percolate into the underlying aquifers to contaminate the hand-dug wells which serve the dual purpose of drinking water for butchers and the entire neighbourhood. The consequences could be the degradation of soil fertility due to the accumulation of certain nutrients and heavy metals that may lead to low productivity in the surrounding farmland, in addition to damage and destruction of aquatic lives. Since the water from the river is used for irrigation and the soil for farming, the possibility of zoonotic disease amongst consumers of produce from such irrigated fields cannot be ruled out.

The aim of this study therefore was to investigate the diversity of microorganisms and the physico-chemical properties of the soil surrounding the Ikpoba Hill and Oluku abattoirs contaminated with the discharged abattoir effluents.

MATERIALS AND METHODS

Description of Study Area

The study was conducted at the Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria. Benin City is located at 6.34^{o}N and 5.63^{o}E and is on an elevation of 80 m above the sea level. The city has a population of about 1,125,058 people, has lowland rainforest towards the south and the guinea savanna in the north, as well a mixture of soil types.

Sample Collection

This was carried out according to the methods of described by Adesemoye *et al.* (2006). Twenty grammes of soil contaminated with abattoir wastewater were collected from Ikpoba Hill and Oluku abattoirs. The samples were placed in sterile polythene bags and transported to the laboratory for processing. Sampling was done in replicates. Samples used as control were collected from the Botanic garden of the University of Benin, Benin City (06^{0} 23$^{'}$ 85.0$^{''}$; N 005^{0} 36$^{'}$ 96.8$^{''}$ East), located 6 km away from the abattoirs.

Preparation of Culture Media

Nutrient agar and potato dextrose agar were used for the isolation of bacteria and fungi, respectively. All media were prepared according to manufacturer's instruction.

Isolation and Enumeration of Bacteria

The bacteriological analyses of the soil samples were carried out according to the methods of Oyeleke and Manga (2008) and Rabah *et al.* (2008). The bacterial isolates were identified and characterized using standard biochemical tests (Jolt *et al*., 1994; Cheesebrough, 2006). The tests carried out included colonial, morphological characteristics, Gram stain, motility, catalase, methyl red, Voges-Proskauer, indole production, urease activity, citrate utilization, glucose, sucrose and lactose utilization tests. Coliform count was carried out according to the methods in APHA (1998).

Identification/Characterization of Fungal Isolates
The fungal species were identified and characterized based on their morphological characteristics, including the colour of aerial hyphae, substrate mycelium, arrangement of hyphae and conidial arrangement. Microscopic analysis using taxonomic guides and standard procedures was also carried out (Gilman, 1944; Barnett and Hunter, 1972; Ellis, 1976; Domsch *et al*., 1980).

Physico-chemical Analyses
The physico-chemical analyses of the soil samples were determined using the methods of Udo and Ogunwale (1986) and the Association of Official Analytical Chemists (AOAC, 1990).

Statistical Analysis
Statistical analysis of variance (ANOVA) using SPSS statistical program (Ogbeibu, 2005), was carried out with the replicate values to determine whether there were significant differences in the counts obtained.

RESULTS AND DISCUSSION

The results of this study are presented in Tables 1-5. Table 1 shows the microbial counts. Table 2 shows the bacteria isolated from the abattoir effluent-contaminated soil. Table 3 shows the fungi isolated from the Abattoir effluent-contaminated soil. Table 4 shows the microbial isolates from the uncontaminated soil (control). Table 5 shows the physico-chemical analysis of the samples.

The results revealed high count of $4.77 \times 10^6 \pm 0.001$ cfu/g, $7.31 \times 10^4 \pm 0.01$ cfu/g and $2.53 \times 10^3 \pm 0.001$ cfu/g for bacteria, coliform and fungi respectively, in Oluku abattoir effluent-contaminated soil, while Ikpoba Hill effluent-contaminated soil had $6.64 \times 10^6 \pm 0.001$ cfu/g, $6.68 \times 10^4 \pm 0.001$ cfu/g and $3.47 \times 10^3 \pm 0.001$ cfu/g cfu/g for bacteria, coliform and fungi respectively (Table 1). The microbial counts were higher in the samples than in the control, having $2.35 \times 10^4 \pm 0.1$ cfu/g, $1.15 \times 10^2 \pm 0.1$ cfu/g and $1.25 \times 10^3 \pm 0.1$ cfu/g for bacteria, coliform and fungi respectively. This could be because the effluents may contain many growth factors that could be easily utilized by the organisms which are not available in the uncontaminated soil. It could also be due to the destabilization of the soil's ecological balance as a result of the contamination from the discharge of abattoir wastewater into the soil environment. These results agree with the reports of Rabah *et al.* (2010) and Eze *et al.* (2013).

The microorganisms isolated from the contaminated soil in this study were: *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus* sp, *Staphylococcus. epidermidis*, *Staphylococcus aureus*, *Alcaligenes* sp, *Klebsiella* sp, *Aspergillus flavus*, *Aspergillus niger*, *Penicillium* sp, *Geotrichum* sp and *Mucor* sp (Tables 2 and 3). This agrees with the results of Adesemonye *et al.* (2006) and Ezeronye and Ubalua (2005), who also isolated similar organisms. The uncontaminated soil (control) had fewer microorganisms (Table 4), compared to the contaminated samples. This could be as a result of more organic matter in the contaminated samples. The presence of the coliform organisms is an indication of recent faecal contamination of the abattoir effluent from the high load of animal excreta in the wastewater. Faecal coliforms live in the digestive tract of warm-blooded animals; their counts are often used as a surrogate measurement for gastro-enteric pathogens, since the presence of faecal coliform bacteria is an indication of contamination by human and/or animal wastes. *E. coli* is the most prevalent member of the faecal coliform group; livestock harbour the bacteria and release it in their faeces. The presence of *E. coli* in water is considered a specific indicator of faecal contamination and the presence of enteric pathogens; it is used as the general indicator organism that signals whether there has been faecal contamination or not. The high levels of total coliforms and *E. coli* in the abattoir effluent-contaminated soil therefore, is an indication of the contamination of water sources with feacal material and possibly pathogenic organisms from abattoir wastewater discharged untreated (Cadmus *et al.*, 1999; 1966; Jansen *et al.*, 1974; Doran *et al.*, 1979; Giddens and Barnett, 1980). The discharge of untreated abattoir wastewater could result in outbreaks of *E. coli* infection as observed by (Millard *et al.*, 1994). Bacterial pathogens such as Salmonella (Barros *et al.*, 2007), *Campylobacter* and *Listeria monocytogenes* (Pepperell *et al.*, 2003) have been isolated from abattoir wastewater. The presence of *Bacillus* sp in the contaminated soil is expected as this organism is indigenous to the soil environment, and are known to persist in such environment (Atlas and Bartha, 2007). Similar observations were made by Ezeronye and Ubalua (2005), Bala (2006) and Rabah *et al.* (2010).

Table 1: *Microbial counts of effluent-contaminated and non-contaminated soils

Paramaters	Oluku contaminated soil (cfu/g)	Ikpoba Hill contaminated soil (cfu/g)	Control (cfu/g)	*p-value*
Bacterial count	$4.77 \times 10^6 \pm 0.001^b$	$6.64 \times 10^6 \pm 0.001^c$	$2.35 \times 10^4 \pm 0.1^a$	0.00
Coliform count	$7.31 \times 10^4 \pm 0.01^b$	$6.68 \times 10^4 \pm 0.001^{ab}$	$1.15 \times 10^2 \pm 0.1^a$	0.07
Fungal count	$2.53 \times 10^3 \pm 0.001^{ab}$	$3.47 \times 10^3 \pm 0.001^b$	$1.25 \times 10^3 \pm 0.1^a$	0.05

*Values are means ± standard error. Values followed by the same letter in the same row are statistically similar at $p < 0.05$.

Pseudomonas spp. is liable to survive and multiply in almost any moist situation and cause extensive rashes. Food-borne diseases caused by *E. coli* occurs in most slaughter houses due to their unhygienic practices especially when the cow meat comes in contact with faecal material. The fungi isolated have been known as common spoilage organisms associated with the beef industry (Alonge, 1991), and they are also soil-inhabiting microorganisms (Atlas and Bartha, 2007). They are opportunistic fungi. The *Aspergillus* spp. are usually found when organic debris abounds and can cause Aspergillosis in human, cattle, and poultry.

Table 2: Bacterial isolates from the abattoir effluent-contaminated soil

Oluku Abattoir	**Ikpoba Hill Abattoir**
Pseudomonas aeruginosa	*Staphylococcus Aureus*
Bacillus spp.	*Staphylococcus epidermidis*
Alcaligenes spp.	*Pseudomonas aeruginosa*
Escherichia coli	*Escherichia coli*
Staphylococcus epidermidis	*Bacillus* spp.
Staphylococcus Aureus	*Serratia* spp.
Klebsiella spp.	*Micrococcus* spp.

Table 3: Fungal isolates from abattoir effluent-contaminated soil

Oluku Abattoir	Ikpoba-Hill Abattoir
Aspergillus flavus	*Aspergillus niger*
Mucor spp.	*Aspergillus flavus*
Aspergillus niger	*Penicillium* spp.
Penicillium spp.	*Geotrichum* spp.

Table 4: Microbial isolates from uncontaminated soil (control)

Bacteria	Fungi
Enterobacter aerogenes	*Aspergillus niger*
Micrococcus spp.	*Penicillium* spp.
Bacillus spp.	
Staphylococcus epidermidis	

Inhalation of *Aspergillus* sp. can result in Asthma with difficulty in breathing. A large Aspergilloma in the lungs can block respiratory gas exchange and cause death due to asphyxiation (Ronald, 2003). The microbial contamination observed in this study is an indication of possible pollution and this may have effect on the ecological balance of the soil (Ogbonna and Igbenijie, 2006). Contaminated abattoir effluent is neither good for domestic use nor is supposed to be discharged directly into the environment without treatment (APHA, 1998).

The mean values of soil pH obtained (Table 5) ranged from 7.70 to 7.80. They were also within the recommended range (6.5-9.5) (Sawyer, 2003). The pH of the contaminated soil samples plays a part in determining both the qualitative and quantitative abundance of micro-flora. It could be inferred then that the pH (Table 5) of the contaminated soils affected their pattern of microbial population. This is supported by the report of (Nazina *et al.*, 2002), that abundance and activity of micro-flora in soil strata are controlled by the availability of water, nutrients, pH, concentration of metal ions and hydrogen dynamic communication with the ground surface. The presence of nitrogen and phosphorus can be attributed to the feed given to the cow. The high concentration of nitrogen can be attributed partly to the high concentration of organic matter in the samples and partly to decomposition of proteinous and nitrogenous compounds to simpler substances like ammonia. When the nitrogenous content of the soil is high, microbial presence is greatly enhanced and this will promote plant's growth, a shortage in nitrogen content or poor organic matter content causes otherwise (Norton *et al.*, 2002). Phosphorus is not an abundant component of the ecosphere (Atlas and Bartha, 2007) and its availability can further be restricted by its tendency to precipitate in the presence of bivalent metals (Ca^{2+}, Mg^{2+}) and ferric (Fe^{3+}) ion at neutral to alkaline pH. Phosphates are combined with calcium within many habitats rendering them insoluble and unavailable to plants. Many microorganisms can also solubilize phosphates from such sources and assimilate and release them for use by other organisms (Eze *et al.*, 2013).

Table 5: Physico-chemical analysis of samples

Parameters	Oluku Abattoir	Ikpoba Hill Abattoir	*FMEnv limit
pH	7.70	7.80	6.00 – 9.00
Total organic Carbon (ppm)	30.66	33.33	NA
K (ppm)	8.84	8.90	NA
P (ppm)	7.10	7.45	5.00
N (ppm)	0.39	0.40	NA
Sand (%)	68.00	56.33	NA
Silt (%)	22.33	23.33	NA
Clay (%)	9.66	20.33	NA

Key: NA = Not available, ppm = parts per million, FMEnv = Federal Ministry of Environment, * = FEPA(1991)

The high level of contamination of the abattoir effluent-contaminated soil as obtained in this study support existing literature pointing to the dangers associated with discharging untreated waste-water to surrounding soils and rivers. There is thus the need for adequate treatment of abattoir effluent to ensure decontamination. Considering the present demand for livestock due to growth in population and requirement on health grounds for meeting up with the calcium and protein requirements of the population, sustainability in meat production should be given priority of place since it intertwines with public health and economic development.

CONCLUSION/RECOMMENDATIONS

The physico-chemical parameters and microbial population analysed in this study showed, to a large extent, the quality and types of pollutants and micro-organisms present as a result of the abattoir effluent discharged into the soil from the slaughter house. From the findings of this work, it has been shown that the abattoir effluent is responsible for the contamination of soil within its vicinity. Better inspection of abattoirs and strict enforcement of law should be made to reduce environmental contamination and incidences of related diseases especially zoonotic diseases. Attempts should also be made to control the hygiene of abattoirs using visual assessment of premises and animals themselves, and those that are visibly unacceptably dirty or affected by diseases should not be allowed for slaughter. Government agencies and other stake holders should develop methods of abattoir waste treatments for reasons of environmental conservation and public health.

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