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Microbial Population and Hydrocarbon Utilizing Micro-organisms from Abattoir Soils in the Niger Delta

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Abstract

In Nigeria, adequate abattoir waste management is lacking in all public abattoirs such that large solid wastes and untreated effluents are common sites which can serve as a source of disease transmission. The microbial population and hydrocarbon utilizing microorganisms from seven abattoir soils within Yenagoa and Port Harcourt metropolis was investigated. This was carried out by the determination of the total count and characterization of the heterotrophic bacteria and fungi in the abattoir soil samples. Characterization of hydrocarbon-utilizing bacteria and fungi with bioremediation potentials was also carried out in accordance with standard procedures. The result of the mean density (population) of total culturable heterotrophic bacteria (THB), hydrocarbon utilizing (HUB) bacteria and HUB/THB percent of the abattoir soil ranged from 1.2×10^7 to 8.0×10^7cfu/g soil, 1.6×10^5 to 3.8×10^5 cfu/g soil, and 0.3% to 1.76% respectively. While the mean density of total fungi (TF), hydrocarbon utilizing (HUF) fungi and HUF/TF percent of the abattoir soils ranged from 1.5×10^5 to 2.4×10^5 sfu/g soil, 1.3×10^3 to 1.6×10^3 sfu/g soil and 0.62% to 0.89% respectively. Generally, the abattoir soils had a higher microbial density than the control soil. The heterotrophic bacteria isolated include *Bacillus* sp., *Pseudomonas* sp., *Micrococcus sp*., *Escherichia coli sp*. and *Staphylococcus sp*., *Proteus sp, Alcaligenes sp. Salmonella sp. and Enterobacter sp*. Total hydrocarbon utilizing bacteria characterized belong to the genera *Alcaligenes*, *Bacillus*, *Escherichia*, *Enterobacter*, *Proteus*, *Pseudomonas*, *Staphylococcus and Micrococcus*. Both *Bacillus* and *Pseudomonas* species were the most occurring bacteria while *Micrococcus* sp. *and Alcaligenes* were the least occurring bacteria. The five genera of fungi isolated were *Aspergillus*, *Fusarium*, *Geotrichum*, *Mucor* and *Penicillium*. All the fungi isolated with the exception of *Mucor* demonstrated hydrocarbon utilizing potentials. The presence of bacteria which are indicators of recent faecal contamination as observed in this study are pointers to the dangers associated with the discharge of untreated abattoir wastes and effluent into the soil. On the other hand, the abattoir soils are rich in hydrocarbon utilizing microbes that can be harnessed for the clean-up of hydrocarbon contaminated soils.

Keywords: Niger-Delta, Abattoir, soil, hydrocarbon utilizing bacteria, fungi

Introduction

The continuous drive to increase meat production for the protein needs of the ever increasing world population comes with some attending pollution problems.

Abattoir operation produces a characteristic highly organic waste with relatively high levels of suspended solids, liquid and fats. Such waste generated is usually composed of dissolved solids, blood, gut contents, bones, urine and process water which is typically characterized with high organic matter level (Coker *et al*., 2005, Osibanjo and Adie, 2007). In many countries, pollution arising from activities in meat production is as result of failure to adhere to Good Manufacturing Practices (GMP) and Good Hygiene Practices (GHP) (Adesemoye *et al.,* 2006). Consideration is hardly given to safety practices during animal transport to the abattoir, during slaughter and during dressing. Yet, contamination from slaughter slabs and within the abattoir is of a high significance as it can occur at each step employed during the carcass dressing.

In Nigeria, adequate abattoir waste management is lacking in all public abattoirs such that large solid wastes and untreated effluents are common sites unlike in developed countries where these facilities are adequately provided (Odeyemi, 1991; Adeyemo, 2002; Ogbonnaya, 2008; Adebowale *et al*., 2010). Human micro biota could also contaminate the carcasses during slaughtering and distribution. Also the animal involved, such as cow, sheep, goats and birds; can also serve as sources of contaminants. Contamination of river body and land with abattoir wastes could constitute a significant environmental and health hazard (Coker *et al*., 2005; Osibanjo and Adie, 2007).

Similarly, the physicochemical properties of the soil such as the pH become altered, due to the uncontrolled discharge of untreated abattoir waste water resulting in the loss of certain soil microbes (Edward, 1990). Tortora *et al.*, (2007) reported that following the discharge of untreated wastewater into the soil, certain elements (for example, iron, lead, phosphorus, calcium, and zinc) previously absent or present in minute quantities will be introduce into the 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 (flora and fauna) communities of the soil. Some of the wastewater from abattoir drains into the surrounding soil environment while the remaining is channeled through the abattoir drainages into rivers. The resultant 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 farmlands, in addition to damages and destructions of aquatic lives.

Abattoir activities in Nigeria is not excluding the use of intense fire generated from combusting fuel sources to roast cowhide in the course of processing it for human consumption thus generating Polycyclic Aromatic Hydrocarbons. Pyrogenic/ anthropogenic sources of polycyclic aromatic hydrocarbons (PAHs) include processes of incomplete combustion. Different types of PAHs are formed based on combustion temperature; where high temperatures (i.e. cooking process) create simple PAHs, and low temperatures (i.e. smoldering) result in more complex PAHs. Existing literatures do support the fact that Polycyclic Aromatic Hydrocarbons are one of the key components of woodsmoke. PAHs are a member of the Persistent Organic Pollutants (POPs) inclusive of PCBs, DDTs and others.

The continuous discharge of pollutants such as PAHs into the abattoir environment has resulted in a proliferation of a microbial community capable of utilizing such toxicant. Because microbes are sensitive to changes in the environment there is a continuous experience of microbial succession to give room to organisms that can

survive the changes. Consequently, information on the composition of microorganisms in a polluted site is of valuable importance in order to estimate the self-purification capability of the ecosystem and the feasibility of biological decontamination if engineered bioremediation should be considered (Allen *et al*., 2007; Said *et al*., 2008).

The aim of this study therefore is to assess the distribution of microbial contamination of soils in some abattoirs in Yenagoa and Port Harcourt cities in the Niger-Delta region, considering the environmental and public health implications of such indiscriminate discharge of abattoir wastes onto surrounding soil and also to assay for microbes with bioremediation potentials since the search for such will be beneficial to mankind and the Niger Delta region where crude oil exploration and production activities are carried out.

Materials and Methods

Study areas and Sample collection

The abattoirs from which soil samples were collected for the study are Igbogene, Tombia, Swale, and Opolo in Yenagoa metropolis and from Rumuokoro and Rupokwu in Port Harcourt metropolis. While the abattoirs are still in active use, the Rukpokwu site is an abandoned tire flaring site for roasting cowhide.

A total of 21 (twenty one) soil samples were collected from the designated abattoirs in Port Harcourt and Yenagoa cities with the aid of soil auger. One kilogram of soil sample was collected at 10 - 20cm depth from each abattoir into appropriately labeled sterile polythene bags. The sample bags were placed in ice – packed coolers and immediately transported to the laboratory for analyses. Soil samples collected from a distance of 400m away from the abattoir served as control.

Cultivation, enumeration and isolation of culturable bacteria and fungi from abattoir soils

The microbiological analysis of the soil samples were carried out according to the method described by Prescott *et al*., (2002) since microbial activities are limited to and is in the range of 0-15cm depth. One (1) gram of soil sample was weighed aseptically and poured into 9ml of sterile distilled water. Six fold serial dilutions were carried out. An aliquot (0.1ml) of the 10^{-4} and 10^{-5} dilutions were plated separately by spread plate method on freshly prepared sterile Nutrient agar. Plating was done in duplicates. Cultured plates were incubated in inverted positions at 37^0C for 24hours. On the other hand, an aliquot (0.1ml) of the 10^{-3} dilution was transferred with sterile pipette onto Petri dishes containing freshly prepared Potato Dextrose agar acidified with 0.1% lactic acid (to inhibit bacterial growth) for the cultivation of fungi and the plates were incubated in inverted position at room temperature ($\pm 26^0$C) for 5-7 days.

Discrete colonies of between 30 and 300 colonies which developed in cultured plates of bacteria were counted, calculated and recorded as colony forming unit (cfu) per gram soil. While discrete colonies which developed in cultured plates of fungi were counted, calculated and recorded as spore forming unit (sfu) per gram

soil. Pure bacteria isolates were obtained from these plates by streaking discrete colonies onto freshly prepared sterile media in Petri dishes. Pure cultures were stored on respective agar slants and refrigerated at 4^0C for further studies.

The pure bacterial isolates were identified and characterized using standard biochemical tests described by Cheesebrough (2006). The pure bacterial isolates were characterized using standard biochemical tests described by Cheesebrough (2006). Bacterial isolates were identified with the reference to the Bergey's Manual of Determinative Bacteriology (Holt *et al*, 1994). Pure fungal isolates were identified based on their morphological features followed by microscopic examination of their wet mounts prepared with lactophenol-cotton blue and with reference made to a fungal identification atlas by Barnett and Hunter (1998).

Cultivation, enumeration and identification of total hydrocarbon utilizing bacteria and fungi

The Mineral Salt Agar (MSA) was used to isolate hydrocarbon utilizing bacteria and fungi but 0.1% lactic acid was added to the medium for fungal isolation. The hydrocarbon utilizing bacteria and fungi was cultivated by inoculating 0.1ml aliquot of 10^{-3} serially diluted samples onto mineral salt agar media prepared as described by Joythi *et al*., 2012. The vapor phase transfer method of Mills and Colwell (1978) was adopted. Whatman filter of 99mm diameter was sterilized in the oven, cooled and impregnated with Bonny light crude oil. This was placed in the lid of the plate aseptically. Media for fungal isolation was acidified with 0.1% lactic acid before inoculation and incubation. Cultured plates were incubated in inverted position at room temperature ($\pm 26^0$C) for 5 - 7 days.

After the incubation period, discrete colonies which developed were counted from which pure cultures were obtained by streak method. Pure isolates were stored on agar slants and refrigerated for further study. The pure bacterial isolates were characterized using standard biochemical tests described by Cheesebrough (2006). Bacterial isolates were identified with the reference to the Bergey's Manual of Determinative Bacteriology (Holt *et al*., 1994).

Pure fungi isolates were identified based on their morphological features followed by microscopic examination of their wet mounts prepared with lactophenol -cotton blue and reference made to a fungal identification atlas by Barnett and Hunter (1972).

Results

The result of the mean density (population) of total culturable heterotrophic bacteria (THB), hydrocarbon utilizing (HUB) bacteria and HUB/THB percent; and of total fungi (TF), hydrocarbon utilizing (HUF) fungi and HUF/TF percent of the abattoir soil samples is shown in Table 1. The mean density of THB, HUB and HUB/THB percent of the abattoir soil samples ranged from 1.2×10^7 to 8.0×10^7cfu/g soil, 1.6×10^5 to 3.8×10^5 cfu/g soil, and 0.3% to 1.76% respectively. While the mean density of THB, HUB and HUB/THB percent of the control soil was 6.0×10^5 cfu/g soil, 4.8×10^4 cfu/g soil, and 8.0% respectively. On the other hand, the mean density of TF, HUF and HUF/TF percent of the abattoir soil samples ranged

from 1.5×10^5 to 2.4×10^5 sfu/g soil, 1.3×10^3 to 1.6×10^3 sfu/g soil and 0.62% to 0.89%. While the mean density of TF, HUF and HUF/TF percent of the control soil was 1.3×10^3 sfu/g soil, 1.0×10^2 sfu/g soil, and 7.69% respectively. The highest populations of all the microbial groups were recorded from abattoir soil from Swale.

Table 1: Mean density of total culturable heterotrophic (THB), hydrocarbon utilizing (HUB) bacteria and HUB/THB percent; and total fungi (TF), hydrocarbon utilizing (HUF) fungi and HUF/TF percent for abattoir soil samples in the Niger Delta

Abattoir location	THB (cells/g)	HUB (cells/g)	HUB/ THB (%)	TF (spores/g)	HUF (spores/g)	HUF/ TF (%)
Opolo	1.2×10^7	1.8×10^5	1.5	2.1×10^5	1.3×10^3	0.62
Swale	8.0×10^7	3.7×10^5	0.46	2.4×10^5	1.6×10^3	0.67
Rupokwu	4.2×10^7	1.6×10^5	0.38	1.5×10^5	1.3×10^3	0.87
Tombia	4.0×10^7	3.8×10^5	0.95	2.4×10^5	1.5×10^3	0.63
Igbogene	2.1×10^7	3.7×10^5	1.76	2.14×10^5	1.6×10^3	0.75
Rumuoko-ro	2.2×10^7	3.6×10^5	1.64	1.8×10^5	1.6×10^3	0.89
Control	6.0×10^5	4.8×10^4	8.00	1.3×10^3	1.0×10^2	7.69

One way ANOVA test showed there was no significant difference ($p\leq 0.05$) between bacterial populations and isolates at the various locations. However, there was significant difference between the abattoir soils and the control for both the bacterial and fungal populations.

The frequency of occurrence (%) of total heterotrophic bacteria isolates in the various abattoir soil samples is shown in Figure 1. The percentage of occurrence of hydrocarbon utilizing bacteria isolates throughout the investigation was *Alcaligenes* (3%), *Bacillus* (27%), *E. coli* (17%), *Proteus* (6%), *Pseudomonas* (22%) *and Staphylococcus* (22%) *and Micrococcus* (3%).

The fungal species that were isolated during this investigation from Swale and Tombia abattoirs were *Aspergillus*, *Geotrichum* and *Penicillium* species; from Igbogene and Rukpokwu were *Aspergillus*, *Mucor* and *Penicillium* species; from Opolo were *Fusarium* and *Penicillium* species; from Rumuokoro were *Aspergillus*, *Fusarium* and *Penicillium* species and from the control was only *Penicillium* species. All the fungi isolated with the exception of *Mucor* demonstrated hydrocarbon utilizing potentials. The frequency of occurrence (%) of hydrocarbon utilizing fungi is *Aspergillus* (35%), *Fusarium* (10%), *Geotrichum* (10%) and *Penicillium* (45%).

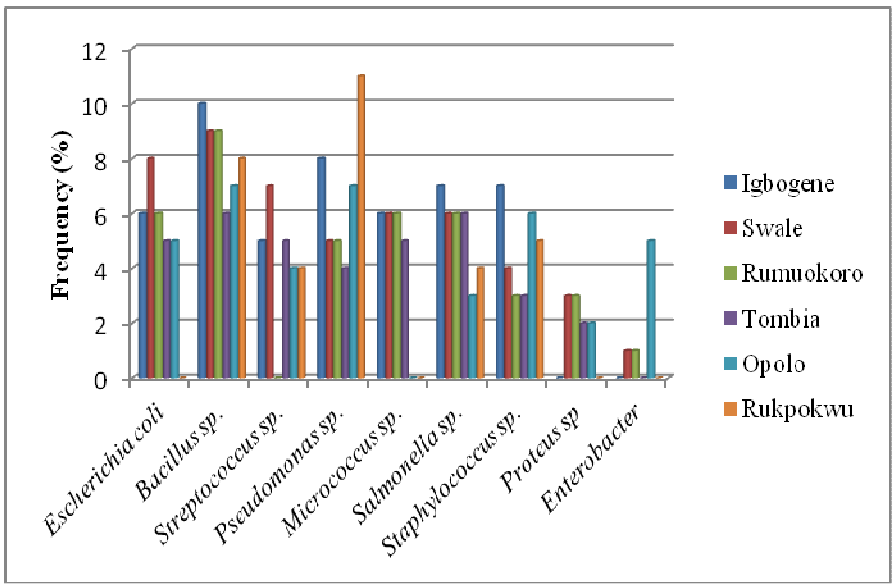

Fig 1: Frequency of occurrence (%) of total heterotrophic bacteria isolates

Discussion

This present study has revealed the microbial population and hydrocarbon utilizing microorganisms from abattoir soils in the Niger Delta. The high counts of both bacteria and fungi obtained from all the abattoir soil samples indicated that the abattoir soils had a higher microbial density than the control soil. Also, there was significant difference between the counts in the contaminated soil and the control soil. Bacterial load was highest in the Swale soil samples and lowest in Opolo soil samples. This fact may be due to the greater slaughtering activities of the Swale abattoir as compared to the slaughtering activities in the other abattoirs. Consequently, more abattoir wastes are generated and discharged directly into the soil in Swale abattoir. These abattoir wastes provided the required utilizable microbial nutrients and growth factors which resulted in higher proliferation of microorganisms in the abattoir soils as compared to the uncontaminated control soil. The destabilization of the soil ecological balance as a result of the contamination due to the discharged of the abattoir waste water into the soil ecosystem was also reported by Atlas and Bartha (2007).

The presence and abundance of *Bacillus* observed in the contaminated soils may not be surprising as these organisms are indigenous to soil environment and are known to persist in such environment (Atlas and Bartha, 2007). However, the presence of *E. coli*, *Salmonella* and *Micrococcus* in the abattoir soils may be attributable to the discharge of the content of animal bowels onto the soil. The presence of these bacteria is also an indication of recent faecal pollution of the soils. The presence of these organisms is a pointer to possible human pollution and may have an effect on the soil ecological balance and preservation. These organisms are also of public health concerns and pathogenic in nature too. These findings are in conformity with

that of Adesemoye *et al.,* (2006) as well as Ogbonna and Igbenijie (2006) who also recorded the above mentioned organisms in their study. The presence of *Pseudomonas* in the abattoir soils is probably due to the availability of PAHs in these soils. *Staphylococcus* and *Streptococcus* isolated in this present study is in agreement with the report of Adeyemo *et al.,* (2002) who also isolated these organisms from the abattoir environment in their study. This study also confirmed the presence of hydrocarbon utilizing bacteria belonging to the genera *Alcaligenes*, *Bacillus*, *Escherichia*, *Micrococcus*, *Proteus*, *Pseudomonas*, and *Staphylococcus* in the abattoir soil samples.

This study revealed that the populations of heterotrophic bacteria and fungi were higher in abattoir soils than the control. Also the population of culturable hydrocarbon utilizing bacteria and fungi were higher in abattoir soils than the control. Walker and Colwell (1976) suggested that counts of petroleum degraders be expressed as a percentage of the total population rather than as total number of petroleum degraders per se. The percentage of culturable hydrocarbon utilizing fungi obtained ranged from 0.62% - 0.89% in the abattoir soil samples while the percentage of culturable hydrocarbonoclastic bacteria ranged from 0.38% to 1.76%. This observation is similar to the result obtained by Udotong and Udotong (2015). The percentages of hydrocarbon utilizing bacteria obtained from oil polluted sites in Ibeno LGA, Nigeria were above 1% (1.4% - 1.7%). The higher proliferation of bacteria in such sites may be due to the fact that pollution was by crude oil (which contains aliphatic, aromatic and asphaltic hydrocarbons) unlike the abattoir environment covered by this study. The wide ranges of population of hydrocarbon degraders thus indicate proliferation due to pollution by Polycyclic Aromatic Hydrocarbons being emitted through the burning of tyres used for the smoking and roasting processes in the abattoirs (Nwachukwu *et al*., 2015). Numerous laboratory studies have indicated sizeable increases in the population of hydrocarbon utilizing microorganisms when environmental samples are exposed to petroleum hydrocarbons (Atlas and Bartha, 1972; Eze *et al*., 2014).

The abundance of these degraders is encouraged by the huge deposit of Polycyclic Aromatic Hydrocarbons into the soil during the process of roasting cowhide for human consumption. This study corroborates that of Said *et al*., 2008 who isolated *Bacillus*, *Staphylococcus*, *Pseudomonas* and *Acinetobacter* spp. capable of degrading PAHs from polluted sediment. Chikere *et al*., (2009) also characterized hydrocarbon utilizing bacteria belonging to the following genera; *Bacillus*, *Nocardia*, *Staphylococcus*, *Pseudomonas*, *Flavobacterium*, *Escherichia*, *Acinetobacter* and *Enterobacter* from Nembe water sediments. Akinde and Obire (2008) also reported the ability of *Pseudomonas* and *Bacillus* from cow dung to utilize hydrocarbons in the environment.

This present study has also reported that, out of the five genera of fungi isolated, four demonstrated hydrocarbon utilizing potentials. These are members of the genera *Aspergillus, Fusarium, Geotrichum* and *Penicillium*. This is similar to the observation of George-Okafor *et al.,* 2009. The ability of fungi to also degrade hydrocarbons is of immense importance in bioremediation procedures. Uzoamaka *et al*., 2009, isolated twelve fungal isolates from petroleum-contaminated soils, out if which eight showed potentials for biodegradation. The isolates that showed potentials for hydrocarbon biodegradation were identified as *Aspergillus versicolor, As-*

pergillus niger, Aspergillus flavus, Syncephalastrum spp., Trichoderma spp., Neurospora sitophila, Rhizopus arrhizus and Mucor spp. Most of the fungal isolates were also soil-inhabiting microorganisms (Atlas and Bartha, 2007). Some of these organisms have earlier been reported as hydrocarbon bio-degraders by April *et al*., (2000) and Oudot *et al*., (1993). Hydrocarbon-utilizers isolated from the abattoir effluent by Goddey and Umaru (2014) included *Candida sp., Rhodotorula sp., Fusarium sp., Penicillium chrysogenus and Aspergillus niger.*

Conclusion

The high level of microbial contamination of the abattoir soil samples and especially the presence of bacteria which are indicators of recent faecal contamination as observed in this study are pointers to the dangers associated with the discharge of untreated abattoir wastes and effluent into the soil. The discharge of untreated abattoir wastes and effluent may lead to health hazards because these organisms are of public health concerns.

It is therefore recommended that there should be swift inspection of abattoir facilities and the stakeholders should be compelled to adhere strictly to Environmental and safety regulations. Also the exposure of hydrocarbon contaminants to the soil in the abattoir environment has led to the proliferation of microbes that are adapted to utilizing these contaminants thus cleaning up the environment. The abattoir soils are rich in hydrocarbon utilizing microbes that can be harnessed for the clean up (bioremediation) of hydrocarbon contaminated environments.

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