Microbial Burden of Air and Soil in the Vicinity of Some Waste Receptacles in Uyo, Nigeria

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Abstract
An investigation of the microbiological quality of air and soil around waste receptacle bins along major streets in Uyo, Nigeria was conducted to assess the presence and pathogenicity of organisms using standard microbiological and culture dependent techniques. A total of 13 microbial isolates identified in this study included bacterial species Bacillus sp, Staphylococcus aureus, Pseudomonas sp, Streptococcus pyogenes, Klebsiella sp, Escherichia coli and Enterobacter sp. and the fungal species Aspergillus niger, Alternaria sp, Fusarium sp, Penicillium sp, Candida albicans, and Rhizopus sp. The isolates generally exhibited γ-haemolysis, with the exception of Streptococcus pyogenes which exhibited β-haemolysis. The Gram positive organisms were susceptible to the antibiotics tested particularly Chloramphenicol whereas the Gram negative bacterial exhibited some resistance to Streptomycin and Gentamicin. Mean microbial counts of THBC (6.48-6.60 log_{10}CFU/g), TCC (5.38-8.84 log_{10}CFU/g), TFC (4.46-5.38 log_{10}CFU/g) were recorded for soil samples obtained from Nwaniba Road (location 1), while the mean microbial counts of THBC (5.47-6.48 log_{10}CFU/g), TCC (6.20-7.65 log_{10}CFU/g), TFC (4.46-2.56 log_{10}CFU/g) were recorded for soil samples collected from waste receptacle sites along Aka Road (location 2). The microbial concentrations of air around location 2 were higher than those of location 1. Coliforms were also present on the settled plate. Generally, the microbial burden at location 1 was greater than that of location 2, thus suggesting the need for frequent evacuation, in order to reduce the pollution menace and public health concerns around the waste receptacle sites.

Keywords: *microbial burden, pathogens, antibiotics, waste receptacle sites*

Introduction

Refuse disposal is one of the major environmental problems that developing countries are faced with. Nigerian cities like most developing countries are inundated with the challenge of improper waste management practices. Garbage and different types of waste litter the urban landscape even where receptacle bins are provided by government as palliatives to assist in the collection and/or disposal of municipal solid wastes. Refuse dumps or receptacle bins receive wastes from several sources with different constituents providing a rich source of microorganisms most of which are pathogenic (Odeyemi, 2012). These wastes which are usually aesthetically unpleasant constitute eyesores and are colonized by microorganisms which transform the degradable (organic fraction) materials emitting ordour and generating complex mixtures of dusts and biological agents with associated health risks (Liao *et al*., 2005). Bioaerosols are airborne biological particles, microbial fragments and constituents of cells, which may be viable or nonviable, and consist of fungi, bacteria, pollen, fragments, particulate matter and/or by-products of cells (Douwes *et al.,* 2003). They are ubiquitous in ambient air, but may be released in elevated concentrations from certain activities and processes. Exposure to these biological agents and dusts occurs in homes, streets and occupational environments (Huang *et al*., 2002) causing adverse health effects, particularly to immuno-compromised persons (Swan *et al*., 2003). There is qualitative evidence which suggests that populations who live or work close to source facilities are at risk to adverse health outcomes including infectious diseases and acute toxic effects (Wang *et al.*, 2010) respiratory symptoms and lung function impairment (Vilavert *et al.*, 2009) allergies and cancer (Ray *et al.*,2005). Besides waste management activities like waste recycling and composting (Jo and Kang,2006), new industrial activities have emerged in recent years with increased exposures to bioaerosols, such as enzymes technology producing highly purified enzymes used in the detergent and food industries (Moletta *et al*.,2007).

Consequently, the present study evaluated the microbial burden of air and soil at different wastes receptacle sites (wastes container bins) by measuring the bacterial and fungal concentrations in air and soil within the immediate environment. This study focuses on viable microorganisms which exist in an airborne state as single cells or clumps and those present in the soil as well as the haemolytic activity and antibiotic susceptibility profile of the pathogenic isolates.

Materials and Methods

Study site

This research work was carried out at three different wastes receptacle sites (A, B, C) along two major streets: Nwaniba Road (location 1) leading to the University of Uyo main campus with large human population, hospitals, markets and hotels/fast food centers and Aka Road (location 2) a less populated area with few residential buildings, companies, office complexes and business centers within Uyo metropolis. These bins receive wastes from kitchen/household, agricultural, industrial, hospital and market.

Sample collection and Microbiological Analysis

The microbiological analysis was carried out to determine the microbial load of air and soil samples around the various waste receptacle sites. The microbial concentration in air was measured using the ‘passive method’ settled plate technique (sedimentation technique) which involved the exposure of sterilized media plates for a specific time at designated locations whereas the microbial burden of soil samples were determined using pour plate technique. Soil samples were collected using sterile auger, transported to the laboratory and a Ten-fold

serial dilution was done. From the dilutions, pour plate procedure was carried out using Nutrient agar, MacConkey agar, Sabouraud dextrose agar and Brilliance agar as analytical media respectively. The plates were incubated at 28±2^{o}C and 37^{o}C for between 24 - 120hours. After incubation the organisms were counted with the aid of a Quebec colony counter and the results for bioaerosols were recorded as CFU/20mins and CFU/g for soil samples.

Representative colonies on the culture plates were picked and repeatedly sub-cultured on freshly prepared Nutrient agar and Sabouraud dextrose agar plates. Pure isolates were characterized based on their cultural, morphological, biochemical, macroscopic and microscopic characteristics (Etok *et al*., 2004). Identification was done by comparing with known taxa using the schemes of Cowan (1988) and Holt *et al*., (1994).

Antibiotic Susceptibility Test

Susceptibility of the various bacterial isolates from the air and soil samples to different antibiotics were determined using Bauer disc-diffusion technique (Bauer *et al*., 1996) The zones of inhibition were measured in millimeter at the end of the incubation. Interpretation of the measurement as resistant, intermediate and susceptible (sensitive) was done based on the manufacturer's standard zone size interpretative manual.

Determination of Haemolytic Activity

The haemolytic activity tests were performed on blood agar containing 5% sheep red blood cells. Haemolysis test is the breakdown of red blood cell walls. The presence of haemolysis in agar plates was detected after 24 hour incubation at 37 ^{o}C according to Singh and Sanyal (1992).

Results

Figures 1 and 2 presents the results of the microbial concentration of air around the three waste receptacle sites (A, B, C) at locations 1 and 2 respectively. The mean total heterotrophic bacterial counts (THBC) ranging from 1.8 – 2.3 $\log_{10}$CFU/20mins were generally higher than the total coliform counts (TCC) 0.7 – 1.5$\log_{10}$CFU/20mins and total fungal counts (TFC) 0.3 – 1.0 $\log_{10}$CFU/20mins. The bioburden from soil samples THBC (6.48-6.60 $\log_{10}$CFU/g), TCC (5.38-8.84 $\log_{10}$CFU/g), TFC (4.46-5.38 $\log_{10}$CFU/g) around waste container bins along Nwaniba road (location 1) and THBC (5.47-6.48 $\log_{10}$CFU/g), TCC (6.20-7.65 $\log_{10}$CFU/g), TFC (4.46-5.56 $\log_{10}$CFU/g) at Aka road (location 2) are presented in figures 3 and 4 respectively. The results indicated the presence of *E. coli* suggesting faecal contamination of the soil. The mean microbial counts for THBC, TCC and TFC in the soil samples were higher than those obtained in the air samples. The haemolytic activity of the bacterial isolates presented in table 1 indicated that the isolates generally exhibited γ-haemolysis, with the exception of *Streptococcus pyogenes* which exhibited β-haemolysis. Tables 2 and 3 shows the results of the antimicrobial susceptibility of Gram negative and Gram positive organisms respectively.

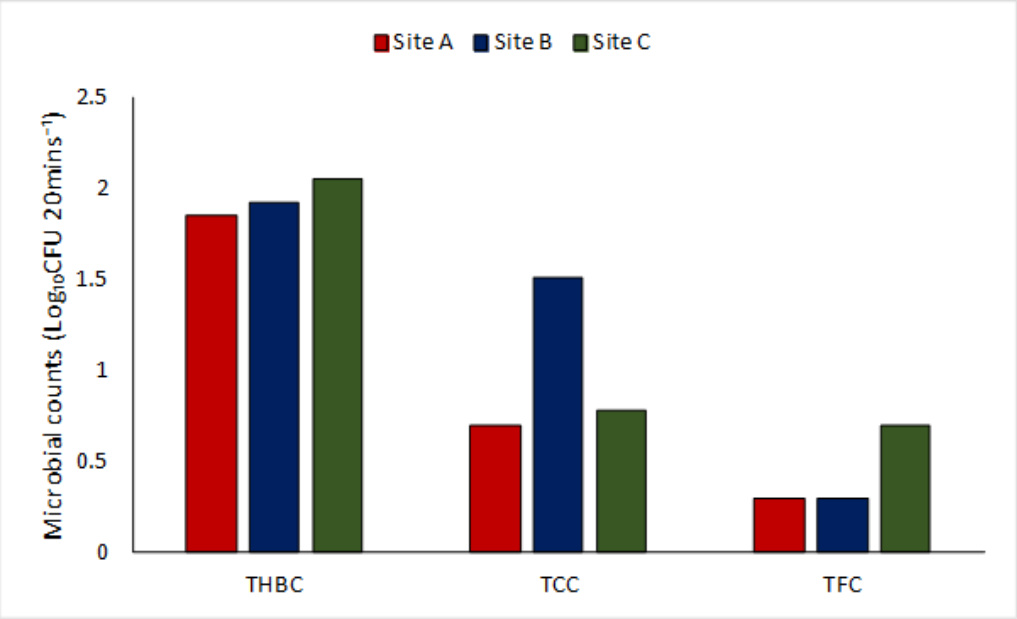

Figure 1: Microbial concentration of air at Nwaniba road (location 1) waste receptacle Sites.
Keys: THBC-Total heterotrophic bacteria counts, TCC-Total coliform counts and TFC-Total fungal counts.

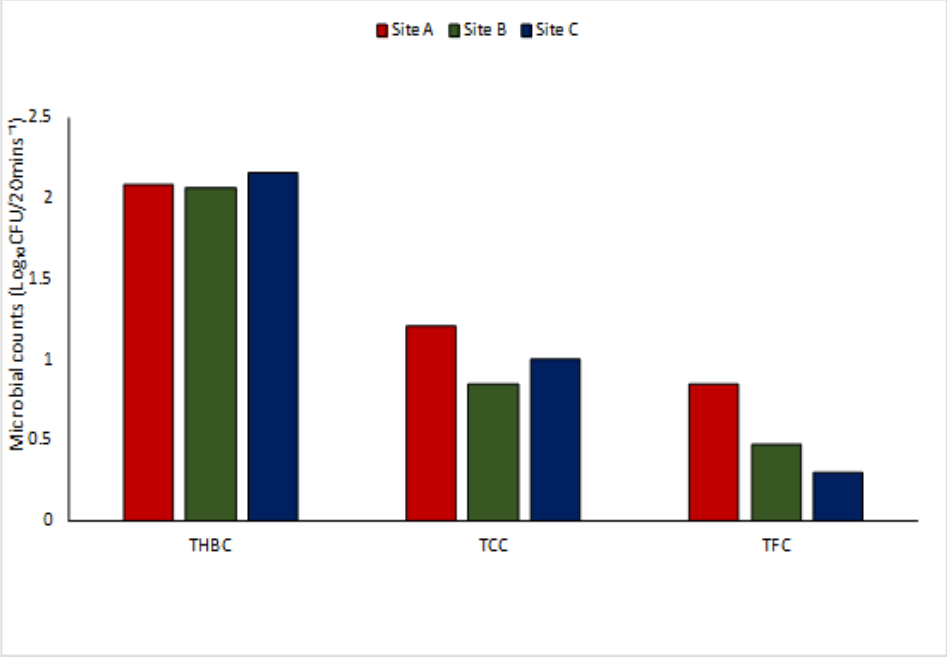

Figure 2: Microbial concentration of air at Aka road (location 2) waste receptacle sites.
Keys: THBC-Total heterotrophic bacteria counts, TCC-Total coliform counts and TFC-Total fungal counts.

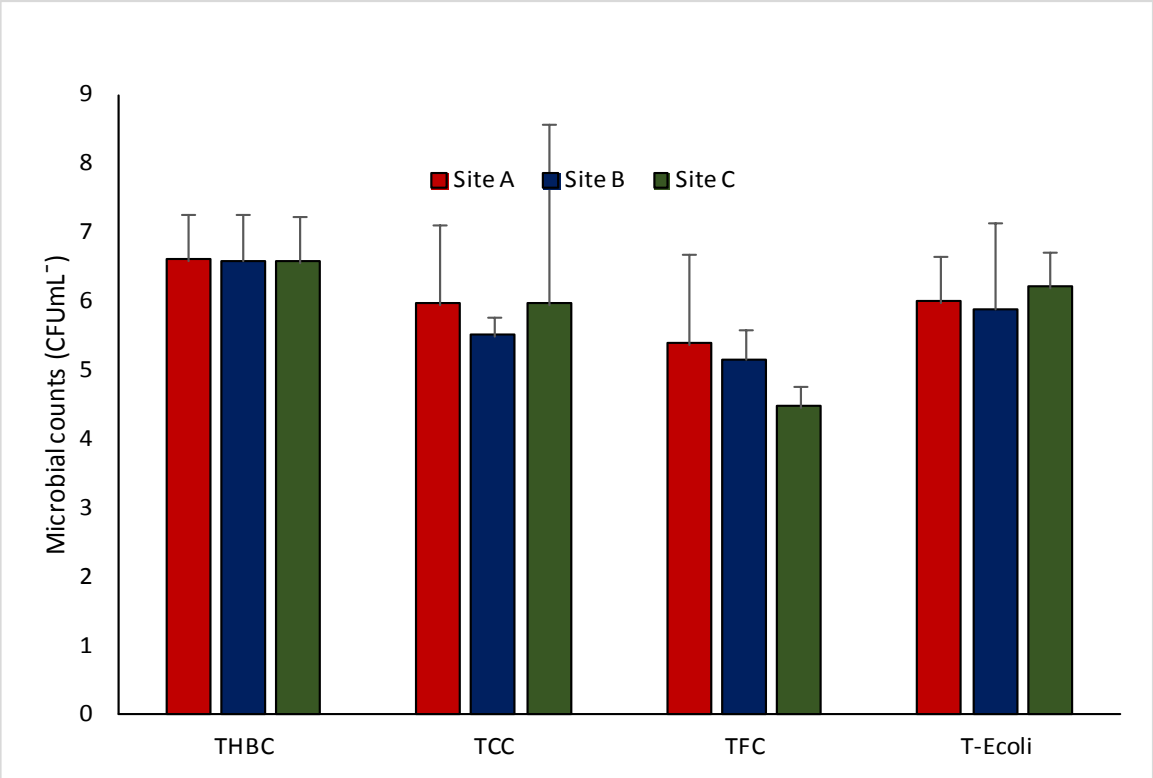

Figure 3: Microbial counts from Soil samples around waste receptacle sites along Nwaniba Road (location 1).

Keys: THBC-Total heterotrophic bacteria counts, TCC-Total coliform counts, TFC-Total fungal counts and T.*E.coli*-Total *E.coli* count

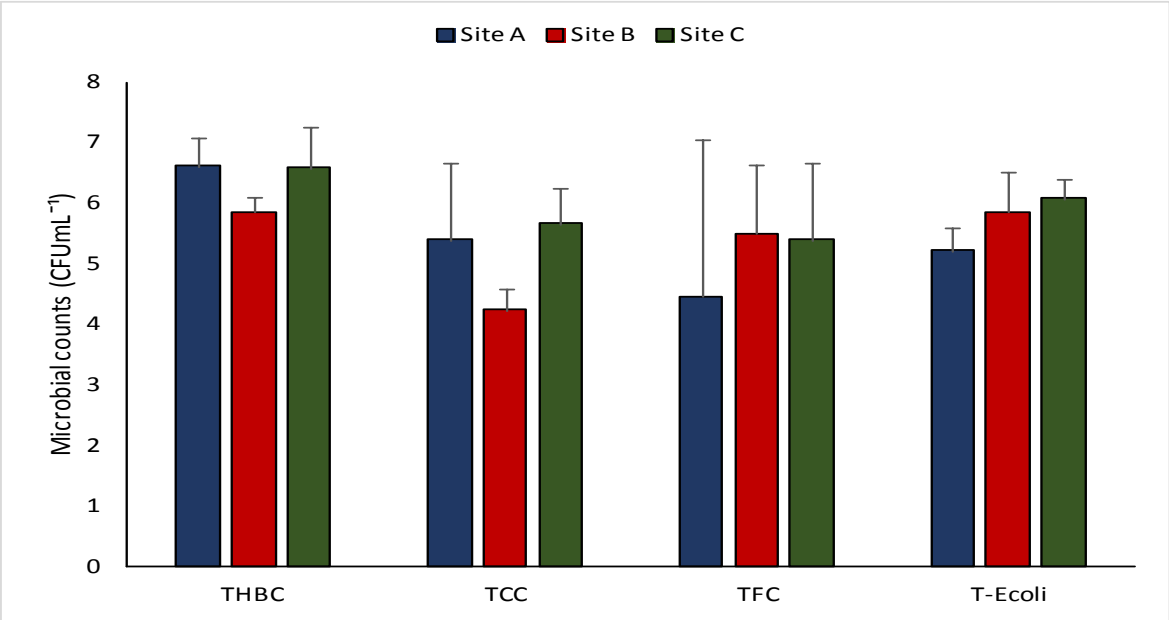

Figure 4: Microbial counts from soil samples around waste receptacle sites on Aka road (location 2).

Keys: THBC-Total heterotrophic bacteria counts, TCC-Total coliform counts, TFC-Total fungal counts and T *E.coli*-Total *E.coli* counts

Table 1: Haemolytic activity of Bacteria isolated from waste receptacle sites

Isolate code	Probable organism	Haemolysis
1	*Bacillus* sp	γ
2	*Staphylococcus aureus*	γ
3	*Pseudomonas* sp	γ
4	*Streptococcus pyogenes*	β
5	*Escherichia coli*	γ
6	*Enterobacter* sp	γ
7	*Klebsiella* sp	γ

Table 2: Antimicrobial Susceptibility of Gram Negative Organism

	Diameter zones of inhibition								
Organism	**CEP (mm)**	**OFX (mm)**	**NA (mm)**	**PEF (mm)**	**CN (mm)**	**CPX (mm)**	**SXT (mm)**	**S (mm)**	**PN (mm)**
Escherichia coli	21	20	21	29	22	16	30	13	17
Enterobacter sp	24	16	18	25	20	30	27	14	18
Klebsiella sp	22	18	16	17	11	18	17	22	21
Pseudomonas sp	15	28	19	14	12	26	22	25	12

CEP=Cephalosporin, CPX=Ciprofloxacin, CN= Gentamicin, OFX=Ofloxacin, NA=Nalidixic Acid, PEF=Pefloxacin, SXT= Sulphamethoxazole/ Trimethoprim, S=Streptomycin, PN= Penicillin.

Interpretation: 0-13mm (Resistant), 14-19mm (Intermediate), 20-above (Susceptible)

Table 3: Antimicrobial Susceptibility of Gram Positive Organism
Diameter zones of inhibition

Organism	S(mm)	CH(mm)	CPX(mm)	LEV(mm)	CN(mm)	APX(mm)	AMX(mm)	E(mm)
Bacillus sp	26	29	20	14	22	15	20	25
S.aureus	25	30	27	29	20	18	19	26

S=Streptomycin, CH= Chloramphenicol, CPX=Ciprofloxacin, LEV= Levofloxacin, CN= Gentamicin, APX= Ampiclox, AMX= Amoxicillin, E= Erythromycin,
Interpretation: 0-13mm (Resistant), 14-19mm (Intermediate), 20-above (Susceptible)

Discussion and Conclusion

The results of this work have highlighted the extent of environmental pollution and/or contamination and the potential public health hazard sanitation workers and residents in close proximity to the receptacle sites are exposed to while evacuating the waste. Since these wastes are often dumped unprocessed, they decompose by means of microbiological mineralization producing toxic gases, putrefying ordour and potential pathogens. These results collaborate that of other researchers (Noah *et al*., 2008, Odeyemi 2012, Igborgbor and Ogu, 2015) who observed the proliferation of organisms (particularly pathogens) around dumpsites and in areas close to the dumpsites.

The differences in the bacterial, coliform and fungal counts could be attributed to the fact that fungi are better adapted to survive in the air by forming spores. The spores of common air borne and soil borne fungi have thick melanized walls which contain complex carbohydrates. These carbohydrates are hydrophobic and waxy which enables the control of water loss. The higher microbial counts for both air and soil samples from waste receptacle sites at location 1 could be attributed to the high population, traffic and increased human activities of the inhabitants of that area as well as proximity of the receptacle bins to markets and schools. Bacteria occur in dusty, dirty places inhabited by humans (Yassin and Almouquatea, 2010). On the other hand, the decreased microbial burden around waste receptacle sites at location 2, could be due to the better sanitary condition of the area and the hygienic standards of the residents.

The high number of bacteria from the family *Enterobacteriace* and considerable number of coliform bacteria is indicative of the hazardous nature of the environment and constitute serious health risk and threat to both waste workers and those living in close proximity to the sites as they are bound to suffer personal discomfort due to the unpleasant ordour emanating from the dumpsites (Odeyemi, 2012).

Among the microbial isolates which occurred at varying frequencies at the different locations, *Staphylococcus aureus* a normal human skin flora known to form aggregates in nature, gave higher colony counts because of the possible breaking of the clusters. *Bacillus* sp endospores have a usual resistance to chemical and physical agents. This makes them

predominant in soils and explains their aerial distribution. *Escherichia coli, Klebsiella* sp and *Enterobacter* sp are coliforms and are found in faecal matter and untreated sewage. These organisms can be carried by air movement particularly in dried faecal matter. Among the most common fungal species were those of the genera *Aspergillus* and *Penicillium* which are broadly present in nature, including soil, cereal grains, hay and plant material or food stuff (Burge, 1990). Exposure to these moulds has been associated with a variety of adverse health outcomes including respiratory, haematological, immunological and neurological symptom disorder and/or diseases (Yassin and Almouquatea, 2010). According to Donderski *et al*., (2005) another significant source of microorganisms are land organism themselves, acting as carriers of saprophytic and pathogenic microflora causing illness in individuals of a given species. The Gram positive bacteria *Bacillus* sp. and *Staphylococcus aureus* were generally susceptible to the antibiotics tested with highest diameter zones of inhibition (29 and 30mm) observed for Chloramphenicol. For the Gram negative bacteria, *E. coli* and *Enterobacter* sp were moderately resistant to Streptomycin while *Klebsiella* sp and *Pseudomonas* were resistant to Gentamicin antibiotics.

Conclusion

This study and previous researches have revealed that waste is a concentrated source of infectious agents; it also offers an attractive contact for pest and rodent. Pathogens in waste may enter the human body via wound present on the skin and may be acquired by inhaling if improperly disposed. The presence of some opportunistic pathogens (*Staphylococcus aureus, Pseudomonas* sp, *Escherichia coli, Streptococcus pyrogenes, Enterobacter* sp and *Klebsiella* sp) identified in this study within the vicinity of the waste receptacles poses a significant health hazard. Therefore proper packaging of waste before disposal, minimizing of waste through reduction of material, reuse and recycling as well as reduction of human activities (e.g. markets, residence and schools) around waste receptacle sites is recommended.

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