Original Research Article

Incidence of alarming microbial pathogens over Refuse Dump Sites and its 'Harmful Distance' In Port Harcourt, Southern Nigeria

ABSTRACT

Aim: Microbial air quality over illegal refuse dump sites in Port Harcourt, Nigeria, was conducted to assess the aero-microbial contaminant of dumpsite to the closest neighbourhood and the harmful distance.

Place and Duration of Study: The dump sites were located at oil mill market (Latitude 4.8578 N4°51'28.06344" Longitude 7.06653 E7°3'59.50152") and Iloabuchi Timber market (longitude N4.790191, latitude E6.988416) all in Port Harcourt, South South Nigeria. The samplings were carried out between June (dry season) and July (wet season) 2018.

Methodology: The microbial concentration of air around the dump sites were measured using the "sedimentation method" that involved exposing different sterile Petri dishes containing nutrient agar, Mac Conkey agar, and sabauroud dextrose agar to the air for ten minutes. The exposures were carried out at different locations within and around the dump site viz; Top of the dumpsite at different altitude (3ft, 6ft and 9ft above dump surface), 0m, 10m away from the dumpsite, and at the nearest neighbourhood which is about 100m away from the dumpsite. These samplings were carried out to the left and right sides of the dump sites. The samplings were carried out between June and July 2018, so as to compare the microbial load between the dry and wet seasons.

Result: The microbes at the dump sites were in most cases higher than the microbes at the neighbourhood (100m away to the left and right). Seasonal occurrence revealed that microbial load in air during the dry season (6.037±0.92 cfu/min-m^2) is higher than during the wet season (1.814±0.19 CFU/min-m^2). Percentage variation amongst heterotrophic bacterial isolates revealed, *Staphylococcus massiliensis* (47.90%) > *Erwinia psidii (18.24%)* > *Shigella dysenteriae (18.17%)* > *Bacillus simplex (6.08%)* > *Saminicoccus kunminingensis (3.23%)* > *Corynebacteriun afermentans (3.00%)* > *Paenibacillus celluositrophycus (2.25%)* > *Streptococcus parasuis (5.26%)*; percentage variation amongst enteric bacterial isolates revealed, *Staphylococcus aureus (28.57%)* > *Geobacillus stearothermophilus (20.82%)* > *Escherichia coli (8.16%)* and *Bacillus carboniphilus (8.16)* > *Salmonella enterica (6.94%)* > *Bacillus smithii (6.12%)* > *Macrococcus brunensis (4.49%)* > *Lactobacillus kitasatonis (3.67%)* > *Klebsiella pneumonia (2.86%)* > *Staphylococcus saccharolyticus (2.45%)* > *Bacillus badius (2.04%)* = *Paenibacillus lautus (2.04%)* > *Brevibacillus laterosporus (1.63%).* The fungal distribution revealed, *Aspergillus fumigatus (16.62%)* > *Microsporium canis (15.40%)* > *Aspergillus flavus (14.75%)* > *Aspergillus niger (10.99%)* > *Conidiobolus coronatus (10.19%)* > *Pheaocremonium parasiticum (6.97%)* > *Fusarium chlamydosporium (6.70%)* > *Trychophyton etriotrephon (5.63%)* > *Trychophyton quinckeanum (4.02%)* > *Lichtheeimia corymbifera (3.57%)* > *Cladosporium cladosporioides (2.95%)* > *Saccharomyces spp (2.68]%).*

Conclusion: The presence of microbial pathogens such as *Escherichia coli, Staphylococcus aureus, Bacillus* spp*, Klebsiella pneumonia, Salmonella enterica* and *Aspergillus species,* is alarming and of great health concern. The harmful distance exceeds 100m away from the dump site which encroached 30 meters into residential areas. This research work revealed the relevance of Environmental air monitoring in any Governmental Waste Management System and the potential hazard of open dump system of waste disposal around residential area.

Keywords: Air Quality**,** Aero**-**microbial contaminant, Sedimentation method, Harmful distance, *Staphylococcus aureus, Klebsiella pneumonia, Salmonella enterica* and *Aspergillus sp.*

1. INTRODUCTION

In Nigeria as well as in most developing countries, the urban landscapes are littered with garbage, plastics, bottles, disposable cups, discarded tires and even human and livestock faeces. These wastes are aesthetically unpleasant, constitute eyesores, produce unpleasant odour especially when their organic compositions are acted upon by putrefying bacteria. These refuse dumps thus constitute a habitat for vector and other nuisance organisms capable of transmitting or causing diseases such as typhoid, infantile diarrhea and cholera in humans and animals [1][2].

Refuse dumps refer to areas or land sites where material wastes from several sources and processes are deposited. Refuse dumps include both municipal solid wastes and in dustrial wastes including liquid effluents containing heavy metals [3]. Refuse dumps provide a rich source of microorganisms most of which are pathogenic [4]. This is usually as a result of the attraction of rodents and vector insects for which the dump serves as shelter and food source [5]. Although it is known that vector insects and rodents can transmit various pathogenic agents of diseases such as amoebic and bacillary dysentery, typhoid fever, salmonellosis, cholera, plague and so on. A good percentage of these infections are caused by bacteria which are suspended in air around these refuse dumps which may later settle and cause contamination. Activities involving the disposal of solid wastes even if properly controlled with proper precautionary measures adopted may have adverse impact on the environment especially air since most of the dumps are open. Microorganisms present in the refuse use the refuse as a food source [17]. Under the anaerobic conditions typical in most dumps, these microorganisms convert the organic material in the refuse to methane and carbon dioxide. As the gas rises through the dump and escapes into the atmosphere, it sometimes picks up other compounds[18]. The presence of large amounts of methane in this uncontrolled environment may result in explosions and fires. Additionally, this untreated gas may contain other compounds that pose a substantial health risk to nearby communities [6][7][8]. Many microbes can remain viable even after extended periods of time aloft despite the challenges associated with surviving in the atmosphere, including extended UV exposure, low moisture levels and extremely oligotrophic conditions [9]. Atmosheric transport is a key mode of microbial dispersal [10] and the transmission of airborne plant and animal pathogens can have significant impacts on ecosystems, human health and agricultural productivity.

This study aimed at isolating bacteria and fungi present in air around specific dump sites in Port Harcourt metropolis, identifying the isolated organisms, determining their concentration in the air and assessing the harmful distance.

2. MATERIAL AND METHODOLOGY

2.1 Description of the Study Area and Sampling Location

The study area are oil mill, a Port Harcourt mid-week market located at Rumuchorlu Community in Obio Akpor Local Government Area of Rivers State and Iloabuchi (Timber market) behind Rivers State University all in Port Harcourt. The dump sites were located at oil mill market (Latitude 4.8578 N4°51'28.06344" Longitude 7.06653 E7°3'59.50152") and Iloabuchi Timber market (longitude N4.790191, latitude E6.988416) all in Port Harcourt, Southern Nigeria.

2.2 Collection of Samples.

The “sedimentation method” that involved exposing different sterile Petri dishes containing nutrient agar, Mac Conkey agar, and sabauroud dextrose agar to the air for ten minutes was used for sample collection. The different sampling points (SP) on and around the dump sites include SP1 (100m to the dump from left), SP2 (10m to the dump from left), SP3 (edge of the dump from left), SP4 at the center of the dump (3ft, 6ft, and 9ft high), SP5 (edge of the dump to the right), SP6 (10m to the right), and SP7 (100m to the right).

2.3 Isolation and Identification of Bacteria and fungi in the Air Samples

Bacteria: Representative colonies were picked and inoculated onto nutrient agar to obtain pure cultures. The pure cultures were stored as frozen 10% (v/v) glycerol suspensions at -35^{0}C in a refrigerator [9]. This glycerol serves as a means for fresh working cultures. Further inoculations of pure cultures onto appropriate media to check for consistency were done. Identification of the isolates were carried out according to the schemes of Buchanam and Gibbon [11]; for bacterial isolates.

Fungi: Isolation and identification of fungi was based on their macroscopic morphology - best growth temperature, growth rate, colour on SDA, colour on reverse side, texture and special feature while the microscopic morphologies and identities of the different species of the fungal isolates based on characteristic features of conidiopore, phialides, vesicle, sclerotia, hulle cells, sporangiophore, apophysis, columella, sporangium and rhizoids according to schemes of Cheesbrough [12].

2.4 Analytical formula for *Direct Sedimentation method*:

The standard time used for bioaerosol sampling is ten minutes (10 mins)

$$\text{Cfu/min-m}^2 = \frac{\text{No. of colonies x } 3.142r^2}{\text{Time of exposure}}$$

Where: r = radius of media plate used (in meters).

3. RESULTS

The microbial load from station 1 (Oil Mill), varies in concentrations at different sampling points (Fig. 1); At the top of the dump site, Total Heterotrophic Bacteria (THB) 7.26 > Total Aerobic Fungi (TAF) 2.196 > Enteric Bacteria (EB) 1.438 during dry season, and TAF (0.720) > EB (0.664) > THB (0.242) during wet season. At 0(zero) meter away from the dump, THB (4.290) > TAF (2.058) > EB (0.884) during dry season, and TAF (0.646) > EB (0.624) > THB (0.216) during wet season. At 10 meters away from dump site, THB (6.260) > TAF (1.752) > EB (0.370) during dry season and TAF (0.414) > EB (0.370) > THB (0.142) during wet season. At 100 meters away from the dump site, during dry season, THB (6.16) > EB (0.964) > TAF (0.685), and TAF (0.336) > EB (0.296) > THB (0.078) during wet season. This results revealed that Heterotrophic Bacteria were more dominant during the dry season than wet season, and Aerobic Fungi more dominant during wet season. It also reveals that microbial concentration decreases in relation to distance from the refuse dump site.

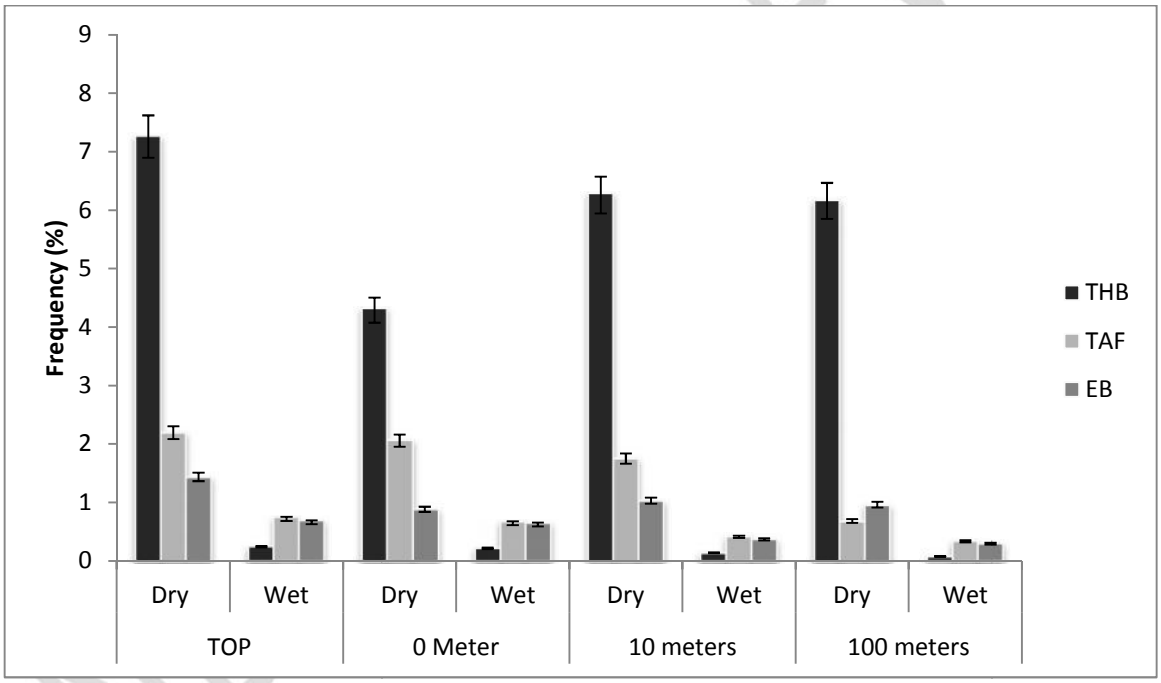

Fig.1: Total Microbial Load from Station 1 (Oil Mill) at Different Sampling Points. (Key: THB= Total Heterotrophic bacteria, TAF= Total Aerobic Fungi, EB= Enteric Bacteria)

From station 2 (Iloabuchi), the variation is shown in Fig. 2. This also shows that the microorganisms isolated from the dumpsites and distances (10 meters, and 100 meters away) were different in composition and distribution. Generally the microbial load decreased with distances away from the dumpsite. The bacterial load for the air samples showed that the bacteria counts decreased with distance from the dumpsite. It was equally observed that the bacterial counts were higher in the dry season (1.95±0.919 and 0.466±0.464 from stations 1 and 2 respectively) and decreased slightly in the wet season (0.255±0.197 and 0.326±0.179).

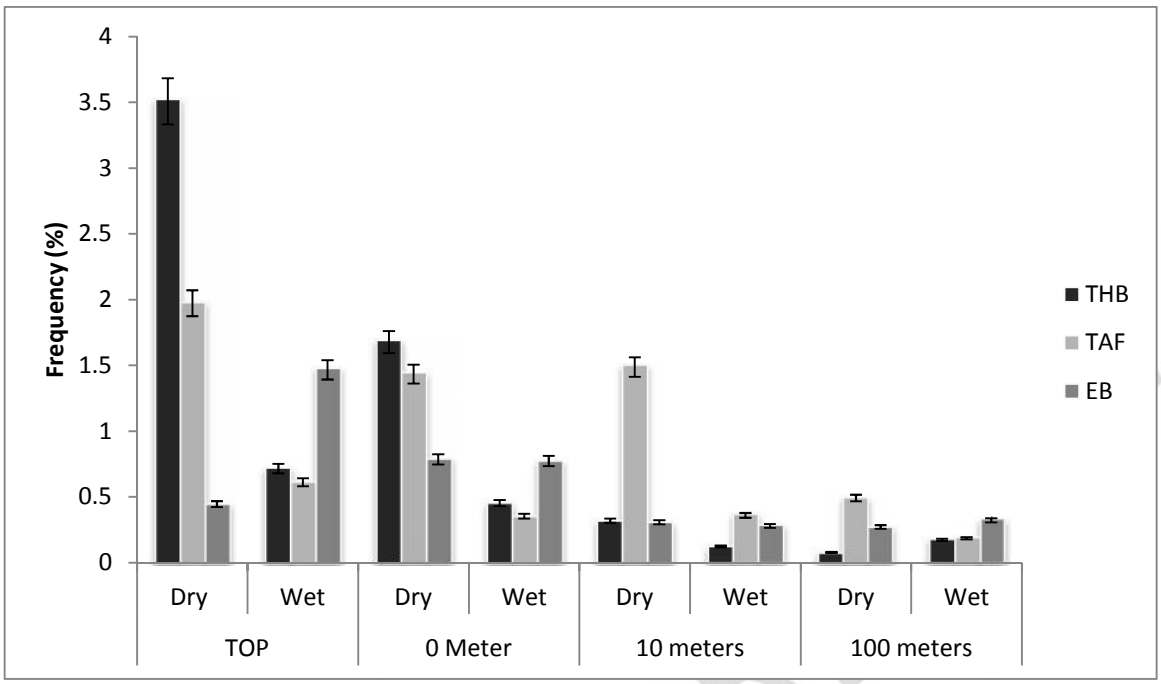

Fig. 2: Total Microbial Load from Station 2 (Iloabuchi Timber) at Different Sampling Points. (Key: THB= Total Heterotrophic bacteria, TAF= Total Aerobic Fungi, EB= Enteric Bacteria)

The bacterial load of the air samples from the dumpsites at oil mill and Timber markets are presented in table 1-3. It was observed that the bacterial counts decreased with distance from the dumpsite and were higher in the months of June (dry season), but decreased slightly in the months of July (wet season). The Heterotrophic bacterial counts in the air samples from station 1 (Oil mill market) in June and July were 1.332±0.418 cfu/min-m^2 and 0.038±0.025 cfu/min-m^2 respectively for dry and wet season, 0.245±0.332 cfu/min-m^2 and 0.099±0.128 cfu/min-m^2 respectively, for enteric bacteria.

Table 1: Average values of Heterotrophic Bacteria from Station 1 (Oil Mill market) and Station 2 (Iloabuchi Timber market) during Dry and wet Season.

S/N	**Heterotrophic Bacterial (HB) Isolates**	STATION 1 (Oil Mill Market)		STATION 2 Iloabuchi Timber market	
		Dry season (cfu/min-m^2)	**Wet season** (cfu/min-m^2)	**Dry season** (cfu/min-m^2)	**Wet season** (cfu/min-m^2)
1	*Staphylococcus massiliensis*	0.638±0.290	0.003±0.007	0.102±0.101	0.020±0.030
2	*Erwinia psidii*	0.243±0.179	0.015±0.017	0.072±0.113	0.016±0.020
3	*Bacillus simplex*	0.081±0.061	0.007±0.008	0.039±0.053	0.011±0.012
4	*Shigella dysenteriae*	0.242±0.108	0.003±0.005	0.041±0.053	0.006±0.008
5	*Saminicoccus kunmingensis*	0.043±0.057	0.001±0.003	0.036±0.037	0.009±0.014
6	*Paenibacillus cellulositrophycus*	0.030±0.036	0.003±0.007	0.013±0.022	0.004±0.006
7	*Streptococcus parasuis*	0.015±0.035	0.002±0.005	0.01±0.013	0.006±0.007
8	*Corynebacterium afermentans*	0.040±0.083	0.004±0.006	0.003±0.004	0.010±0.010

The bacterial counts in the air samples from station 2 (Timber) in June and July were 0.316±0.354 cfu/min-m^2 and 0.082±0.052 cfu/min-m^2 respectively, for heterotrophic bacteria, and 0.101±0.067 cfu/min-m^2 and 0.158±0.095 cfu/min-m^2 respectively, for enteric bacteria.

The observed decreased trend in the bacterial counts in wet season could be as a result of increased rainfall in the months of July and decreased rainfall in June leading to reduced water activity. These results agree with the reports of Obire and Aguda [13] who stated that seasonal variations favour physiological types.

Table 2: Average values of Enteric Bacteria from Station 1 (Oil Mill market) and Station 2 (Iloabuchi Timber market) during Dry and wet Season.

S/N	**Enteric Bacterial (EB) Isolates**	STATION 1 (Oil Mill Market)		STATION 2 Iloabuchi Timber market	
		Dry season (cfu/min-m^2)	**Wet season** (cfu/min-m^2)	**Dry season** (cfu/min-m^2)	**Wet season** (cfu/min-m^2)
1	*Geobacillus stearothermophilus*	0.051±0.070	0.002±0.005	0.024±0.033	0.017±0.028
2	*Paenibacillus lautus*	0.005±0.007	0.003±0.006	0.001±0.003	0.010±0.016
3	*Bacillus badius*	0.005±0.011	0.005±0.007	0.003±0.006	0.015±0.018
4	*Bacillus carboniphilus*	0.020±0.028	0.011±0.015	0.004±0.004	0.016±0.020
5	*Salmonella enterica*	0.017±0.024	0.010±0.011	0.006±0.008	0.007±0.010
6	*Staphylococcus saccharolyticus*	0.006±0.007	0.004±0.010	0.0003±0.001	0.009±0.013
7	*Brevibacillus laterosporus*	0.004±0.006	0.013±0.015	0.013±0.012	0.008±0.011
8	*Staphylococcus aureus*	0.070±0.080	0.007±0.007	0.003±0.006	0.025±0.033
9	*Lactobacillus kitasatonis*	0.009±0.016	0.004±0.014	0	0.011±0.012
10	*Macrococcus brunensis*	0.011±0.020	0.007±0.007	0.009±0.008	0.012±0.022
11	*Bacillus smithii*	0.015±0.032	0.012±0.011	0.009±0.012	0.003±0.005
12	*Escherichia coli*	0.020±0.021	0.012±0.010	0.014±0.020	0.018±0.027
13	*Klebsiella pneumonia*	0.007±0.010	0.009±0.010	0.015±0.013	0.007±0.010

The average fungal counts of the air samples from the dumpsites in Oil mill and Iloabuchi timber are presented in table 3. It was observed that the fungal counts decreased with distance from the dumpsites and increased in the months of June. The fugal counts in the air of the dumpsites in June ranged from 0.373±0.169 cfu/10mins/m^2 to 0.049±0.043 cfu/10mins/m^2 in June and 0.0118±0.044 cfu/10mins/m^2 to 0.086±0.032 cfu/10mins/m^2 in July.

Table 3: Average values of Fungal isolates from Station 1 (Oil Mill market) and Station 2 (Iloabuchi Timber market) during Dry and wet Season.

S/N	**Fungal (EB) Isolates**	STATION 1 (Oil Mill Market)		STATION 2 Iloabuchi Timber market	
		Dry season (cfu/min-m^2)	**Wet season** (cfu/min-m^2)	**Dry season** (cfu/min-m^2)	**Wet season** (cfu/min-m^2)
1	*Microsporium canis*	0.056±0.049	0.031±0.034	0.049±0.043	0.012±0.011
2	*Conidiobolus coronatus*	0.038±0.040	0.009±0.013	0.032±0.035	0.008±0.008
3	*Aspergillus niger*	0.041±0.029	0.004±0.005	0.036±0.021	0.008±0.009
4	*Phaeocremonium parasiticum*	0.026±0.024	0.006±0.006	0.021±0.015	0.007±0.008
5	*Trychophyton etriotrephon*	0.020±0.030	0.008±0.009	0.021±0.025	0.011±0.014
6	*Saccharomyces spp*	0.010±0.010	0.008±0.010	0.009±0.007	0.006±0.010
7	*Aspergillus fumigatus*	0.062±0.035	0.007±0.006	0.039±0.027	0.002±0.004
8	*Aspergillus flavus*	0.055±0.040	0.009±0.008	0.048±0.034	0.011±0.013
9	*Trychophyton quinckeanum*	0.015±0.016	0.013±0.015	0.014±0.010	0.004±0.004
10	*Lichtheimia corymbifera*	0.014±0.017	0.005±0.005	0.010±0.014	0.005±0.007
11	*Cladosporium cladosporioides*	0.011±0.012	0.013±0.015	0.015±0.018	0.008±0.013
12	*Fusarium chlamydosporum*	0.025±0.029	0.005±0.008	0.024±0.028	0.004±0.005

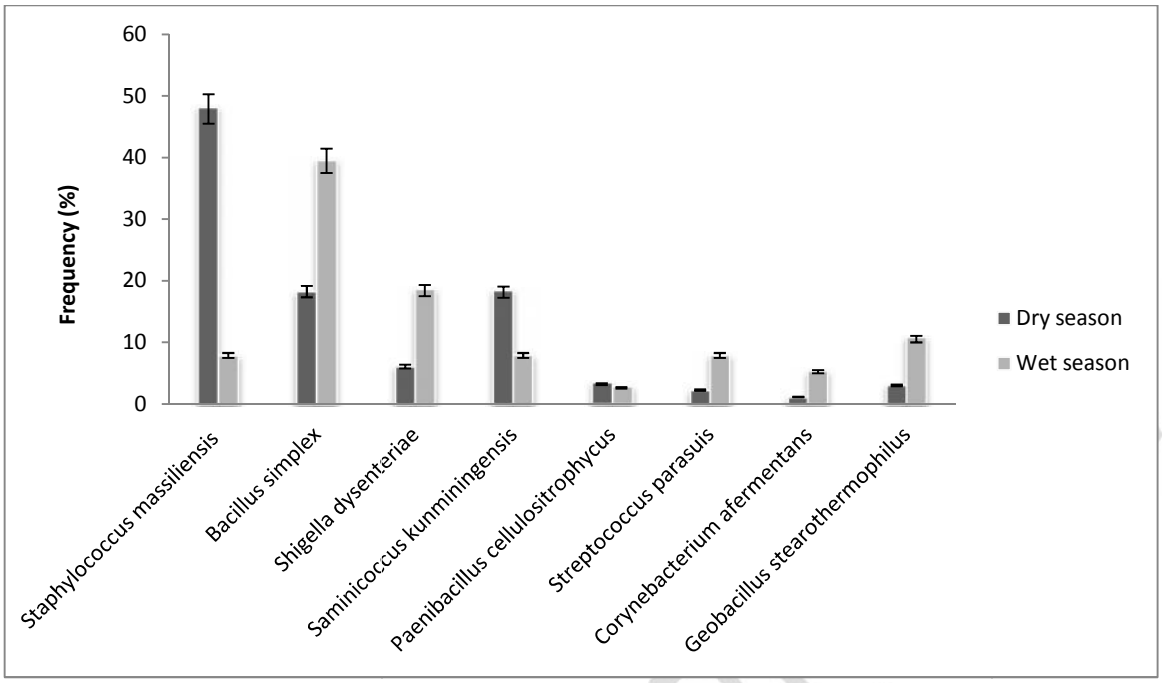

Fig 1: Percentage (%) frequency of different isolates of Heterotrophic Bacteria from Station 1 (Oil Mill narket)

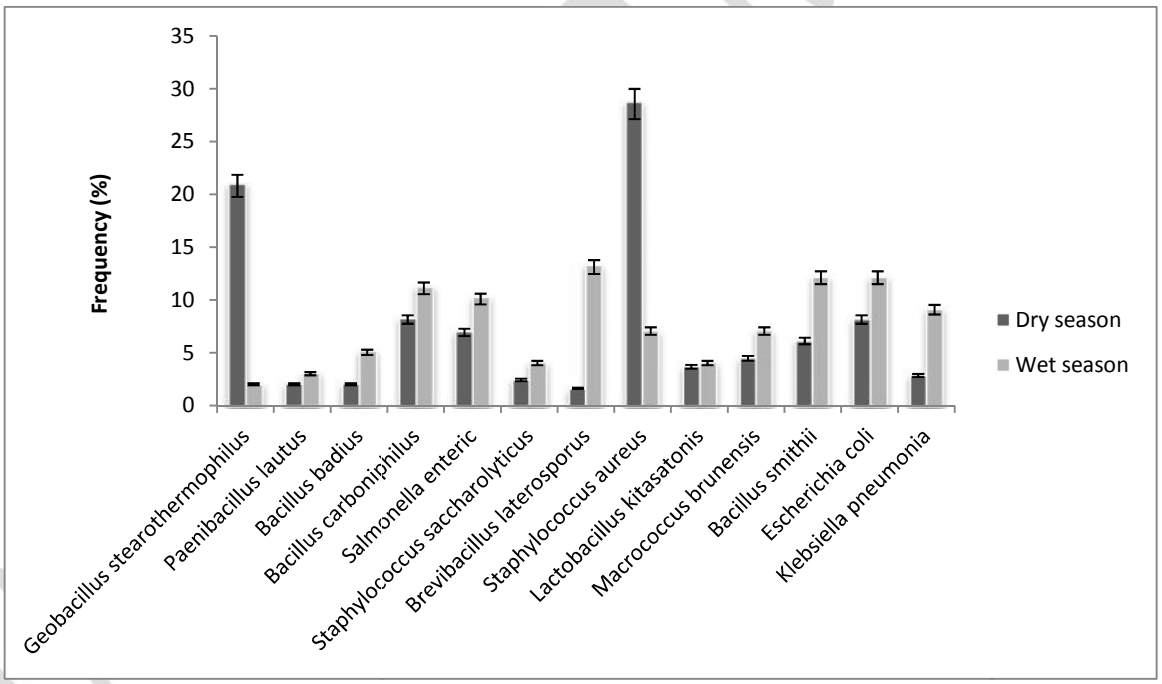

Fig. 2: Percentage (%) frequency of different isolates of Enteric Bacteria from Station 1 (Oil Mill) during Dry and Wet Season.

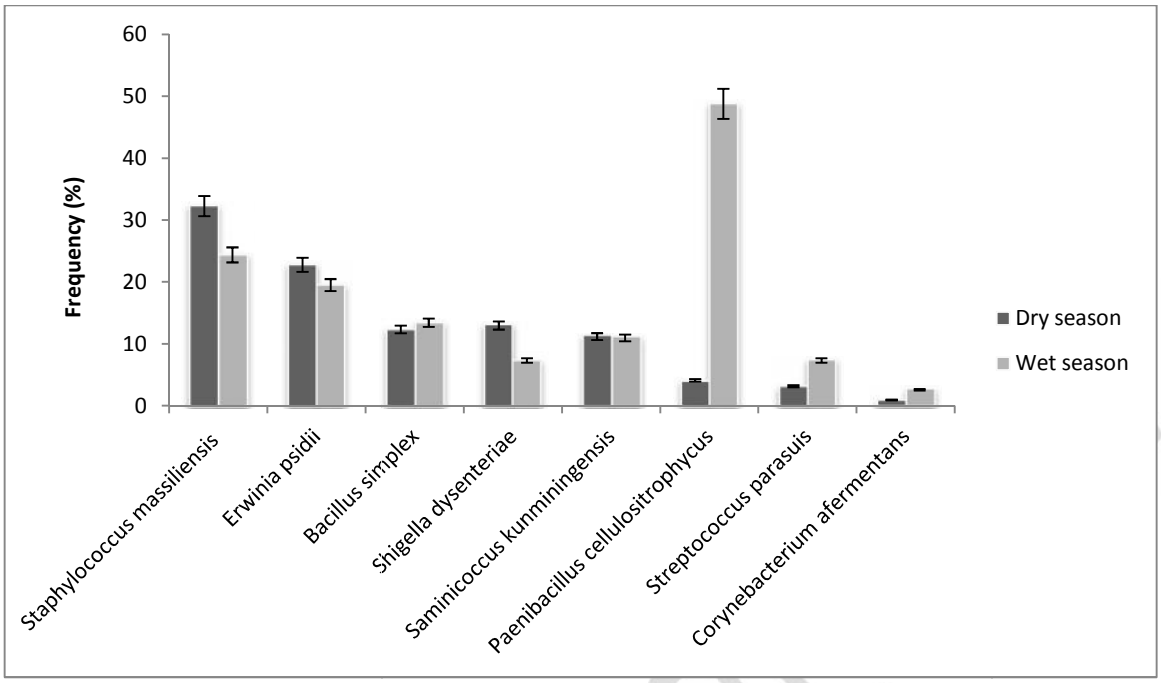

Fig. 3: Percentage (%) frequency of different isolates of Heterotrophic Bacterial isolates from Station2 (Timber market Iloabuchi)

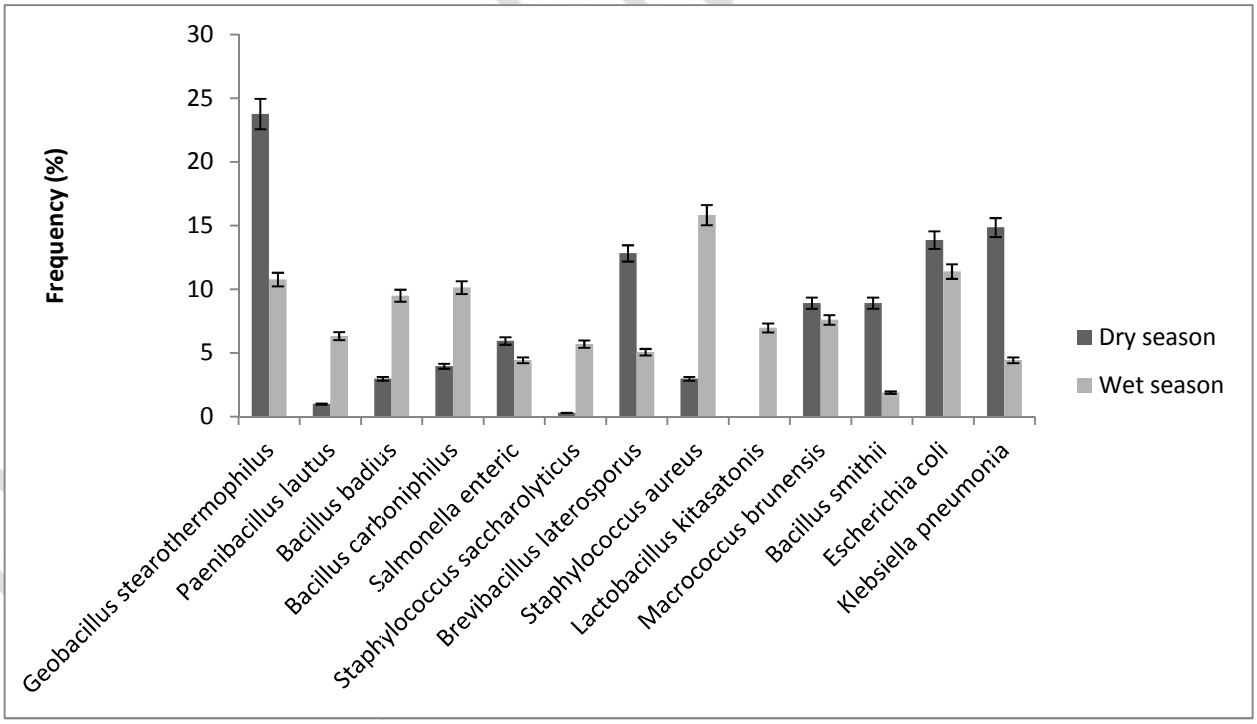

Fig 4: Percentage (%) frequency of different isolates of Enteric Bacterial isolates from Station 2 (Timber Iloabuchi)

Percentage variation amongst individual bacterial isolates shows that *Staphylococcus massiliensis* (47.90%) > *Erwinia psidii* (39.47%) > *Staphilococcus aureus* (28.57%) > *Geobacillus stearothermophilus* (20.816%) >*Bacillus simplex*

(18.42%) > *Shigella dysenteriae* (18.17%) > *Escherichia coli* (12.12%) > *Corynebacteriun afermentans* (10.82%) > *Salmonella enterica* (10.101%) > *Klebsiella pneumonia* (9.09%) > *Paenibacillus celluositrophycus* (7.89%) > *Streptococcus parasuis* (5.26%) > *Saminicoccus kunminingensis* (3.23%) (Fig. 1-4)

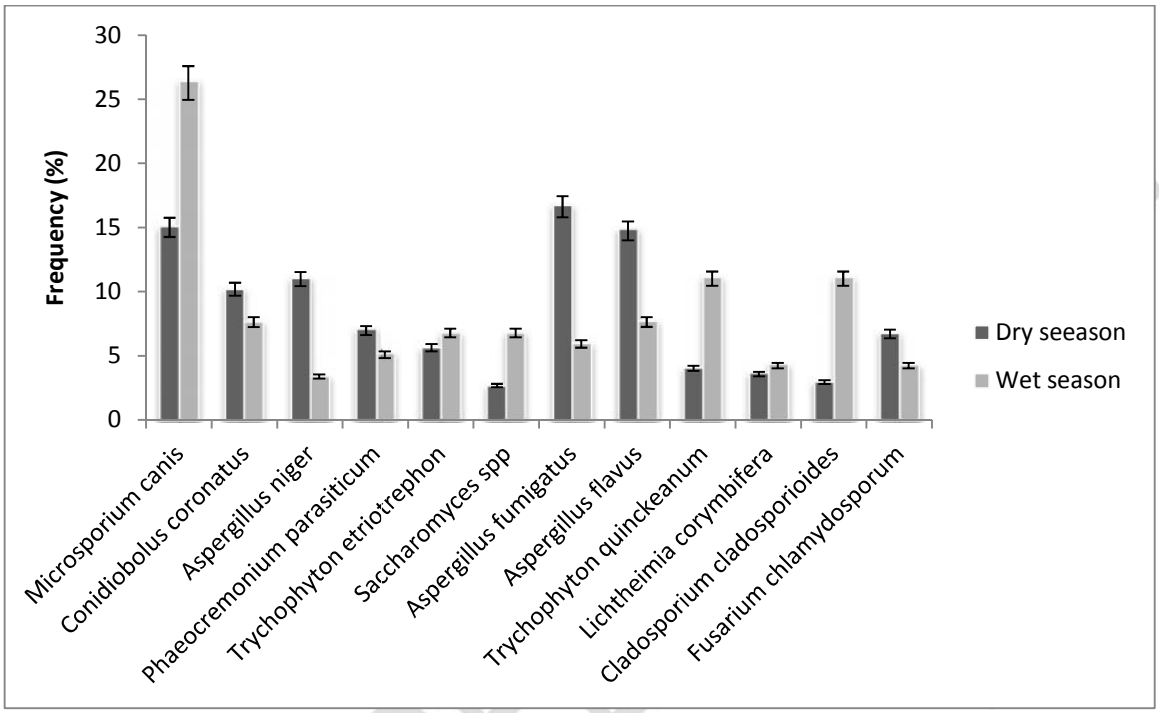

Fig. 5: Percentage (%) frequency of different isolates of aerobic fungi isolated from station 1 (oil mill)

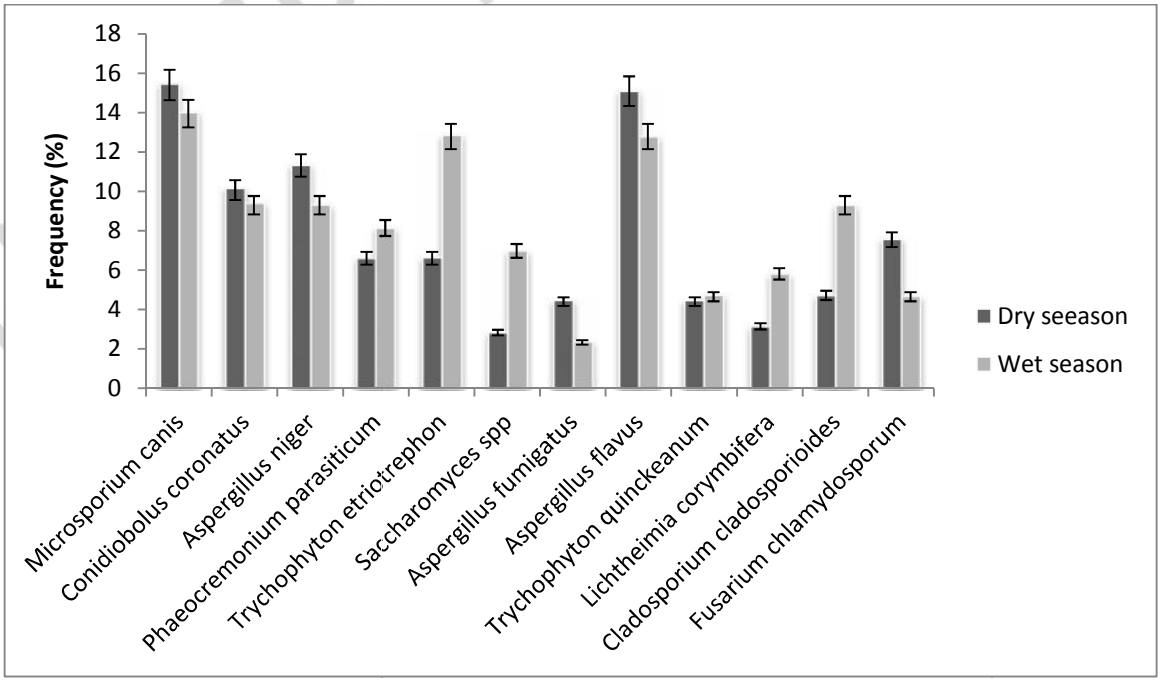

Fig. 6: Percentage (%) frequency of different isolates of aerobic fungi isolated from station2 (Timber Iloabuchi)

Percentage variation amongst individual fungal isolates *Microsporium canis, Conidiobolus coronatus, Aspergillus niger, Phaeocremonium parasiticum, Trychophyton etriotrephon, Saccharomyces spp, Aspergillus fumigates, Aspergillus flavus, Trychophyton quinckeanum, Lichtheimia corymbifera, Cladosporium cladosporioides,* and *Fusarium chlamydosporum* were shown in Fig. 5-6. Overall assessment revealed higher percentage during wet season than dry season, though some isolates especially species of *Aspergillus* showed alternate variations.

4. DISCUSSION

This result from station 1 reveals that Heterotrophic Bacteria were more dominant during the dry season, and Aerobic Fungi more dominant during wet season. It also reveals that microbial concentration decreases in relation to distance from the refuse dump site. The microbial loads of the air samples taken from the dumpsites were higher than the normal atmospheric concentration of the microorganisms as the reported average level of the microbes in the ambient air [14][15]. This is an indication of the extent of microbial pollution of waste dump sites in the study sites.

The decreasing bacterial counts with distance away from the dumpsite could be due to increased microbial activity in the dumpsite as a result of putrefaction and increased decomposition of organic matter in the vicinity of the dumpsite. It could also be as a result of household and industrial wastes deposited in the waste dumpsites as well as contaminants generated naturally that were propelled through the air, such as particles of dust and soil microbial spores in the air within the dumpsites. These results agree with the report of McCarthy [16]; Nrior and Adiele [2] who listed these amongst others as possible sources of air contaminants.

From station 2 (Iloabuchi), the variation is shown in Fig. 2. This also shows that the microorganisms isolated from the dumpsites and distances (10 meters, and 100 meters away) were different in composition and distribution. Generally the microbial load decreased with distances away from the dumpsite. The bacterial load for the air samples showed that the bacteria counts decreased with distance from the dumpsite. It was equally observed that the bacterial counts were higher in the dry season (1.95±0.919 and 0.466±0.464 from stations 1 and 2 respectively) and decreased slightly in the wet season (0.255±0.197 and 0.326±0.179). The decreasing bacterial counts with distance away from the dumpsite could be due to increased microbial activity in the dumpsite as a result of putrefaction and increased decomposition of organic matter in the vicinity of the dumpsite. It could also be as a result of household and industrial wastes deposited in the waste dumpsites as well as contaminants generated naturally that were propelled through the air, such as particles of dust and soil microbial spores in the air within the dumpsites. These results agree with the report of McCarthy [16]; Nrior and Adiele [2] who listed these amongst others as possible sources of air contaminants.

The observed decreased trend in the bacterial counts in wet season could be as a result of increased rainfall in the months of July and decreased rainfall in June leading to reduced water activity. These results agree with the reports of Obire and Aguda [13] who stated that seasonal variations favour physiological types.

The fungal load for the air samples from the dumpsites showed that the fungal counts decreased with distance away from the dumpsites. It was equally observed that the fungal counts were lower in the wet season and increased slightly in the dry season as represented in table 2 and its graph above. The decreasing fungal count with distance away from the dumpsite could be due to same reasons propounded for bacterial counts above. These results agreed with the report of McCarthy [16] who reported similar suggestions. The observed trend in the fungal counts could be due to spore formation due to increased rainfall in the months of July and decreased rainfall in the months of June. These findings also agree with the reports of earlier researchers Obire and Aguda [13] who opined that seasonal variations favor physiological types.

5. CONCLUSIONS

From this study, it can be concluded that the open dump system of waste disposal is indeed a potential environmental quality problem which takes the form of unsightliness, land and water pollution, it reduces the quality of air by the

emission of foul odours and different gases derived from the anaerobic decomposition as well as occasional burning. It also serves as a potential source of air pollution and contamination as it promotes the dispersion of bacterial pathogens either as free entities or attached to particles into the air. These pathogens when suspended in air are of less importance but become a source of immediate concern when they settle on surfaces as they cause varying kinds of infectious diseases, respiratory symptoms and lung function impairment which can range from acute mild conditions that hardly affect daily life to severe chronic respiratory diseases, cancer, and so on, that require specialist's care.

In order to checkmate these high air pollution profile and protect the lives of people, it is recommended that land fill waste disposal system should replace the open system of waste disposal. In case of limited land availability, the wastes can be incinerated under high heat in a controlled environment. More so waste management practices of waste reduction, waste re-use and recycling should be encouraged; and public awareness.

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