International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2016): 79.57 | Impact Factor (2017): 7.296

Black Soot: Percentage Source and Aeromicrobiology

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Abstract: ***Black soot in aerosols of Niger Delta, Nigeria especially Port Harcourt is attracting global concern. Six major sources of black soot have been identified; Burning of local "kpo-fire" crude oil for production of diesel and Kerosene 67.5%, Smoke from refinery and petro-chemical industries 20%, Gas flaring from flow station of oil companies 5%, Smoke from generators both industrial and domestic 3%, Smoke from exhaust of vehicles of all types 1.5%, Burning of vehicle tyres either at animal slaughter abbatoir or for other purposes 3%. This present research aimed at analyzing and comparing the variations of aero-microbiological status of Black soot in campus lecture halls located in different altitudes (Ground, first and second floor) of 36feet height; 10feet each floor. The study area was lecture halls in Rivers State University in three departments namely: Microbiology (MCR), Lecture halls I - IV, ground floor; Animal and Environmental Biology (AEB), Lecture halls I – IV, first floor and Plant Science and Biotechnology (PSB), Lecture halls I – IV, second floor. It was conducted in the month of June, 2017; Monitored in all the lecture halls morning 7:00am and afternoon 12:00pm on 7th, 10th and 13th June, 2017 . Standard microbiological techniques were used; Bacteria and fungi load of air of the selected departments were determined using Settle Plate Technique (Sedimentation method). In this technique, air microbes settled directly on the prepared agar plates (Nutrient Agar NA, MacConkey Agar MCA, Sabouraud Dextrose Agar SDA) exposed for a period of 10 minutes. Samples were collected twice daily at 7:00 am, and 12:00 pm in each class room in the various departments. After exposure, the samples were taken to the laboratory and incubated at 37^{0}C for 24 hrs for NA, 42^{0}C for 24hrs for MCA and SDA plates were incubated at 25^{0}C (room temperature) for 4 days. Once colony forming units (CFU) were enumerated, CFU/10mins were determined. Identification of isolates was done using standard methods. Microbial Percentage (%) frequency associated with black soot at different altitude Ground floor (GF), First floor (FF) and Second floor (SF) were; Total Heterotrophic Bacteria: FF (38.7) > GF (34.1) > SF (27.1); Enteric Bacteria: GF (44.9) > FF (27.9) > SF (27.2); Total Fungi: GF (38.8) > FF (36.6) > SF (27.5) (Fig. 1). The overall percentage (%) microbial load revealed that Ground floor sampled at a height of 2m form the ground has the highest load 39% (117.8), followed by First floor 34% (110.3) while Second floor had the least 27% (81.8) The colonies formed during the afternoon sampling were higher the ones for morning sampling. This was due to the atmospheric condition in relation to increased wind/air flow; increased activities from sources of black soot in afternoon more than morning hours. The percentage (%) frequency of bacteria genera isolated were Bacillus sp (39.0%) > Staphylococcus sp (21.2%) > Pseudomonas sp (14.9%) > Streptococcus sp (14.4%) > Klebsiella sp (6.0%) > Micrococcus sp (4.5%) while fungal isolates were Candida sp (34.9%) > Penicillium sp (21.4%) > Mucor sp (21.4%) > Aspergillus niger (11.3%) Aspergillus flavus (10.4%). The enteric organisms were Klebsiella sp (34.3%) > Escherichia coli (32.2%) > Enterobacter sp (25.5%) > Shigella sp (8%). Conclusively, the occurrence of pathogenic microorganisms (Escherichia coli, Staphylococcus and Bacillus) mixed with black soot is of great health importance. Also, long exposure to black soot could cause cancer.***

Keywords: Aeromicrobiology, Black soot, *Percentage* (%) source, Sedimentation method, enteric bacteria, Pathogenic microorganisms, *Staphylococcus* sp., *Aspergillus* sp.

1. Introduction

Microorganisms are ubiquitous in nature. One of the places they are found is air. The study of living microbes which are suspended in the air is referred to as Aeromicrobiology. They are grouped as Bioaerosols.

Soot is a cancer-causing particle in the atmosphere linked to oil exploration and gas flaring in the Niger Delta region of the country. It is also a general term that covers pollutants derived from the incomplete or inefficient burning of fossil fuels or biomass – plants or plant-based materials used as source of energy (Meadow *et al*., 2014). The air inhaled by people is numerously populated with microorganisms which form bio aerosol (Nrior and Adiele, 2015). Bioaerosol is colloidal suspension, formed by liquid droplets and particles of solid matter in the air, whose components contains or have attached to the viruses, fungal spores conidia bacteria endospores, plant pollen and fragment of plant tissues (Karwowska, *et al*., 2005).
Numerous studies have been carried out worldwide assessing indoor microbiological air quality, pointing at the important role air quality plays in impacting the general health and well being of humans as a whole. A World Health document outlining the WHO guidelines for indoor air quality, describes healthy indoor air as a fundamental human right. This is particularly important considering that a significant number of people have been reported to spend the majority of their time (between 80% and 90%) indoors (Hospodsky *et al*., 2012; Hayleeyesus and Manaye, 2014).

Currently, though no single international standard exists as a guide for bacterial contamination of indoor air, several recommendations have been made. A WHO publication asserts that environments with a greater than 1000CFU/m3 microbial load should be considered contaminated (WHO, 2009). Most other reports have set thresholds ranging from 500 to 1000 CFU/m3 as acceptable (Borrego et al., 2012; Naruka and Gaur, 2013; Kabir *et al*., 2016). The quality of indoor air may be affected by several factors. One of such factors is the influence of the external environment, with reports that in a well-ventilated room, the indoor air quality is similar to that of the outdoor air quality (Meadow et al., 2014). The air inhaled by people is numerously poluted with microorganisms form so-called bio aerosol (Nrior and Chioma, 2017). Bio aerosol is collodial suspension, formed by liquid droplets and particles of solid matter in the air, whose components contains or have attached to them

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viruses, fungal spores and conidia bacteria endospores plant pollen and fragment of plant tissues (Karwowska, et al 2005).

Since November 2016, Port Harcourt has been affected by a "strange black soot" of relatively unknown source (The Guardian, 2017). Port Harcourt, the capital city of Rivers State, Nigeria, is part of the oil producing Niger Delta region of the country. Several speculations on the source of the soot have pointed at illegal refineries and asphalt burning by asphalt firms. The presence of the black soot has been suspected of being linked with respiratory tract infections. There have however been no documented confirmatory reports so far. One important facet of air quality is the level and composition of airborne bacterial load.

Radio report of black soot in port harcourt

A report of the analysis on soot as monitored by an interview granted a radio station by the Commissioner of Environment Rivers state:

- **Sampling:** Sample collected from Abuloma area and Peter Odili area of PH. Sample collected in two-time frames; 12midnight – 6am and 6am – 8am – Date of collection of sample 23rd December 2016
- **Result:** Particulate Matter Size; – Average of 270 micron/m3 for Abuloma& Peter Odili area between 12midnight – 6am –Abuloma area 6am -8am; 125 micron/m3 – Peter Odili area 6am -8am; 62 micron/m3 Acceptable size is 25 micron/m3
- **Chemical substance:** – Additive for making tyres – Nickel – high, Lead – Low. Test shows that the soot is petroleum based as a result of incomplete combustion of petroleum products
- **Suspected source of soot:**– Refineries – Petro-Chemical companies– LNG– Activities of Illegal refineries– Gas flaring– Burning of tyre (to access copper embedded in the tyres). The Rivers State government cannot, for now, ascertain which of the above activities or any company or group of persons responsible for the soot.

The report as monitored indicates that the activity mainly takes place at night which may indicate that the source of the soot will likely be from an illegal activity (The Guardian, 2017).

A PUBMED and Google Scholar search for articles on microbial air quality in Port Harcourt found five articles published between 2012 and 2016. Majority (4/5) of these studies assayed indoor air quality. These studies all used a similar sampling method, the settle plate or passive natural sedimentation method. They however differed in their mode of result presentation. Of the 5 studies, only 2 represented the results of their study as CFU/m3 (Udochukwu *et al.*, 2015; Emuren and Ordinioha, 2016).

These ranges were similar to those of several studies carried out in Ethiopia, which noted microbial loads with ranges of 117 – 7284, 397 – 2595, 511 – 9960 and 3106 – 9733 (Kabir *et al.*, 2016; Hayleeyesus and Manaye, 2014; Hayleeyesus *et al.*, 2015). These studies noted a variation in microbial load based on sampling location (i.e. indoor or outdoor), function of the indoor environments and month of the year. All these reports however differed from some reports from the rest of Nigeria, which noted low microbial loads with ranges such as 42 – 100, 422 – 1386 and 45 – 1125 CFU/m3 (Makut *et al.*, 2014; Ambrose *et al.,* 2015; Awosika *et al.*, 2012). One common observation of the various studies was the effect of human activity on microbial load. The analysis of current literature on microbial air quality in Port Harcourt yielded data that could serve as a baseline for further comparison. Additionally though, this assessment of the literature revealed an immediate need for standardization and clear representation of methodology, despite the fact that 5 different articles reported on microbial air quality in Port Harcourt, Rivers State, Nigeria, data comparison could only be clearly made from two papers due to a lack of standardized methodology. Thus, this research aims at assessing the Aeromicrobiology of Black soot in Campus Lecture Halls as indices for further microbiological work on indoor and outdoor bioaerosol associated with black soot.

2. Material and Methods

Place and Duration of Study

The study area was lecture halls in Rivers State University in three departments namely: Microbiology (MCB), Lecture halls I - IV, ground floor; Animal and Environmental Biology (AEB), Lecture halls I – IV, first floor and Plant Science and Biotechnology (PSB) Lecture halls I – IV, second floor. It was conducted in the month of June, 2017; Monitored in all the lecture halls morning 7:00am and afternoon 12:00pm on 7th, 10th and 13th June, 2017 .

Analytical Media

The culture media used for the research work were nutrient agar (NA) for total hererotrophic bacterial count, MacConkey agar (MCA) for total enteric bacterial count and Sabouraud Dextrose Agar (SDA) for total fungal count. They were prepared according to the manufacturer's prescription and sterilized for 15 minutes at 121^{0}C. The media was cooled to 40-45^{0}C, poured into sterile petri dishes and allowed to solidify for about 10-15 minutes before use.

Sampling Procedures

Bacteria and fungi load of air of the selected departments were determined using Settle Plate Technique. In this technique, air microbes settled directly on the prepared agar plates and exposed for a period of 10 minutes. Samples were collected three times daily at 7:00 am, and 12:00 pm in each class room in the various departments. After exposure, the samples were taken to the laboratory and incubated at 37^{0}C for 24 hrs for NA, 42^{0}C for 24hrs for MCA and SDA plates were incubated at 25^{0}C (room temperature) for 4 days. Once colony forming units (CFU) were enumerated, CFU/5mins were determined. Then, identification of isolates was done using standard methods.

Equipments and Reagents

All equipments and reagents used were supplied by the laboratory of the Department of Microbiology. Glass wares such as pipettes, glass rods, etc. were sterilized in hot air oven for 160^{0}C for 1 h.

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Enumeration of Microbial Isolates
Duplicate samplings were carried out and incubated at 37^0C for 24 hrs for NA, 42^0C for 24hrs for MCA and SDA plates were incubated at 25^0C (room temperature) for 4 days, discrete colonies were counted and the average count were recorded.

Isolation and Identification of Bacteria isolates in Air
Pure cultures of bacteria were obtained by aseptically streaking representative colonies of different cultural types which appeared on the plates to a freshly prepared nutrient agar plates which were incubated at 37^0C for 24 hours. Discrete bacteria colonies which developed were sub-cultured on nutrient agar and incubated at 28^0C for 24 hours. These served as pure stock cultures for subsequent characterization tests. Identification of isolates was based on their cultural morphology, microscopic examination, coagulase test, and other biochemical tests in accordance with methods described by Cowan (2004).

Isolation and Identification of Fungi
After sampling and incubation, discrete colonies were formed and counted and the average count for the duplicate cultures was recorded as total heterotrophic fungi. Pure cultures of fungi were obtained by sub-culturing discrete colonies into freshly prepared sterile SDA plates and incubated for 3-5 days.

3. Results and Discussion

Microbial Load in different halls at different altitudes
The principal results of the aeromicrobiological analysis of lecture halls (Ground floor - Department of Microbiology (MCB) halls I-IV, First floor - Department of Animal and Environmental Biology (AEB) halls I-IV and Second floor – Department of Plant Science and Biotechnology (PSB) halls I-IV in Rivers State University (RSU) showed variations in total heterotrophic bacteria (THB), total fungi (TF) and total enteric bacteria (TEB) for morning and afternoon by direct sampling using open plate technique. The highest value of THB (54 cfu/10min) was obtained in the afternoon at 7m height (First floor AEB II) while the least (2 cfu/10min) was obtained in the morning at 10m height (Second floor PSB IV). The THB mean (average)(log 10cfu/10min) reveals the same trend; first floor AEB II having highest value 1.30±1.27 while Second floor PSB II had the least 0.91±0.65 (Table 1). Enteric bacteria (EB) showed significant reduced value compared to total heterotrophic bacteria (THB) (Table 1-2).

Assessing the mean EB (log10 cfu/10min), Ground floor MCB IV had the highest load (1.03±0.92) while MCB III had the least (0.30±0.19) (Table 2). Nrior and Adiele, 2015 had earlier showed that different sampling points/halls in the same station/building or area at different altitude could results in significant variation in microbial load.

Table 1: Total Heterotrophic Bacteria in Lecture Halls of three Departments in RSU

Altitude Per 3.5m Height	Dept	7th June		10th June		13th June		Average (Log10Cfu/10mins)
		Morning (Cfu/10ms)	Afternoon (Cfu/10mins)	Morning (Cfu/10mins)	Afternoon (Cfu/10mins)	Morning (Cfu/10mins)	Afternoon (Cfu/10mins)	
Ground floor	MCB I	7	21	5	13	4	12	1.01±0.80
	MCB II	20	24	7	20	12	15	1.21±0.79
	MCB III	20	27	8	30	10	18	1.27±0.95
	MCB IV	11	20	4	21	15	21	1.19±0.83
First floor	AEB I	10	22	8	22	3	20	1.15±0.91
	AEB II	4	54	12	19	5	25	1.30±1.27
	AEB III	11	40	19	31	10	6	1.29±1.13
	AEB IV	7	30	6	35	10	8	1.20±1.11
Second floor	PSB I	4	31	6	12	17	19	1.17±0.99
	PSB II	3	7	4	10	15	10	0.91±0.65
	PSB III	4	9	15	15	7	27	1.11±0.91
	PSB IV	7	13	2	8	7	30	1.05±0.99

Key: MCR= Microbiology lecture hall I-IV, AEB= Animal and Environmental Biology lecture hall I-IV, PSB= Plant Science and Biotechnology lecture hall I-IV, RSU = Rivers State University

Table 2: Total Enteric Bacteria in Campus Lecture Halls of three in RSU

Altitude Per 3.5m Height	Dept	7th June		10th June		13th June		Average (Log10Cfu/10mins)
		Morning (Cfu/10mins)	Afternoon (Cfu/10mins)	Morning (Cfu/10mins)	Afternoon (Cfu/10mins)	Morning (Cfu/10mins)	Afternoon (Cfu/10mins)	
Ground floor	MCB I	7	0	4	12	8	13	0.87±0.69
	MCB II	10	4	12	4	12	4	0.88±0.61
	MCB III	1	2	1	1	5	2	0.30±0.19
	MCB IV	19	2	5	3	15	20	1.03±0.92
First floor	AEB I	7	0	4	12	8	13	0.88±0.83
	AEB II	10	4	12	4	12	4	0.83±0.89
	AEB III	1	2	1	1	5	2	0.58±0.70
	AEB IV	19	2	5	3	15	20	0.50±0.36
Second floor	PSB I	5	0	0	8	1	0	0.37±0.53
	PSB II	12	0	0	11	4	5	0.73±0.72
	PSB III	2	1	10	0	0	10	0.58±0.68
	PSB IV	12	0	16	0	2	2	0.73±0.84

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Key: MCR= Microbiology lecture hall I-IV, AEB= Animal and Environmental Biology lecture hall I-IV, PSB= Plant Science and Biotechnology lecture hall I-IV, RSU= Rivers State University

Table 3: Total Heterotrophic Fungi in Campus Lecture Halls of three in RSU

Altitude	Dept	7th June		10th June		13th June		Average
Per 3.5m Height		Morning (Cfu/10mins)	Afternoon (Cfu/10mins)	Morning (Cfu/10mins)	Afternoon (Cfu/10mins)	Morning (Cfu/10mins)	Afternoon (Cfu/10mins)	(Log10Cfu/ 10mins)
Ground floor	MCB I	3	30	20	30	12	15	1.26±1.03
	MCB II	20	10	13	15	6	21	1.15±0.76
	MCB III	5	22	18	25	10	20	1.22±0.88
	MCB IV	7	16	25	20	15	22	1.24±0.80
First floor	AEB I	18	28	7	25	8	23	1.26±0.95
	AEB II	20	15	9	23	10	18	1.20±0.75
	AEB III	9	12	10	20	7	12	1.07±0.65
	AEB IV	4	3	15	30	5	15	1.08±1.02
Second floor	PSB I	6	14	6	10	4	11	0.93±0.58
	PSB II	5	3	4	25	2	1	0.82±0.96
	PSB III	1	8	13	30	6	12	1.07±1.00
	PSB IV	3	4	10	35	3	4	0.99±1.10

Key: MCR= Microbiology lecture hall I-IV, AEB= Animal and Environmental Biology lecture hall I-IV, PSB= Plant Science and Biotechnology lecture hall I-IV, RSU= Rivers State University

Variations in Total fungi (TF) for morning and afternoon by direct sampling using open plate technique showed high values in afternoon than morning (Table 3). The highest TF value (35 cfu/10min) was obtained in the afternoon at 10m height Second floor PSB IV. This shows that fungal spores can attain great heights more than bacteria bio aerosol whose highest count were obtained in first floor 7m height probably due to their buoyant adaptive structures (Nrior and Chioma, 2017). Microbial Percentage (%) frequency associated with black soot at different altitude Ground floor (GF), First floor (FF) and Second floor (SF) were; Total Heterotrophic Bacteria: FF (38.7) > GF (34.1) > SF (27.1); Enteric Bacteria: GF (44.9) > FF (27.9) > SF (27.2); Total Fungi: GF (38.8) > FF (36.6) > SF (27.5) (Fig. 1). The overall percentage (%) microbial load revealed that Ground floor sampled at a height of 2m form the ground has the highest load 39% (117.8), followed by First floor 34% (110.3) while Second floor had the least 27% (81.8) (Fig. 2)

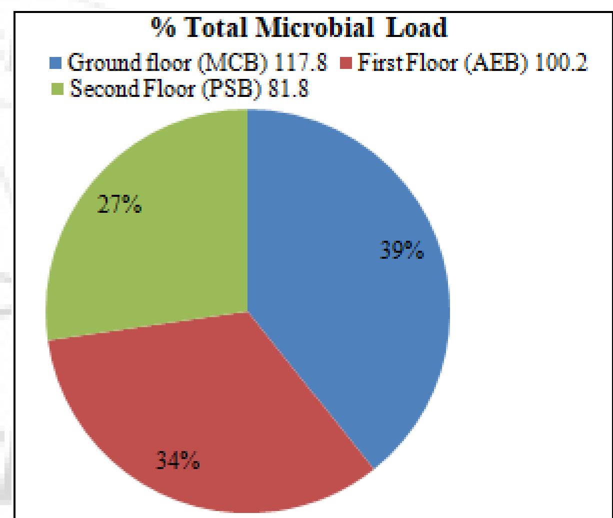

Figure 2: Percentage (%) Frequency of Total Microbial Load at different altitude

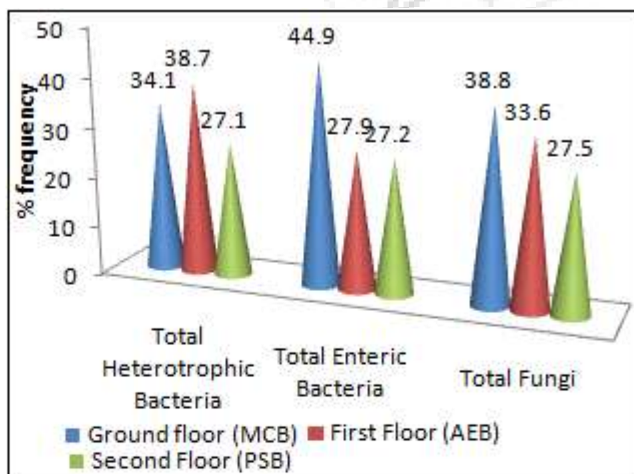

Figure 1: Percentage (%) Frequency of Total Heterotrophic bacteria, Enteric Bacteria and Total fungi at different altitude

The result in Table 3 showed first day 7th June, 2018 sampling for Total Heterotrophic Bacteria (THB – cfu/10min) (16.92) having the highest, followed by 10th June, 2017 (13.84), least on 13th June, 2018 (13.58). Enteric bacteria (EB – cfu/10min) showed a reverse pattern with last day 13th June, 2018 having the highest load (7.59) > 10th June, 2018 (5.38) > 7th June, 2018 (5.09). Total fungi result revealed an alternate range; Day 2 10th June, 2018 having the highest fungal load (18.25) > 7th June, 2018 (11.92) > 13th June, 2018 (11.09). This result shows that different types of microbes may have varying population in the atmosphere at the same place at different time which could be due to varied anthropogenic activities per day and source of emission in relation to air flow direction/wind speed and climatic conditions (Nrior and Adiele, 2015; Nrior and Chioma, 2017).

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Table 4: Result of Total Heterotrophic bacteria, Enteric Bacteria and Total fungi during morning and afternoon sampling at different days/ different altitude

		7th June, 2017		*10th June, 2017*		*13th June, 2017*	
		Morning	Afternoon	Morning	Afternoon	Morning	Afternoon
Total Heterotrophic Bacteria (cfu/10min)	Ground floor	14.5	23	6	21	10.25	16.5
	First floor	8	36.5	11.25	26.75	7	14.75
	Second floor	4.5	15	6.75	11.25	11.5	21.5
	AVE	9±5.07	24.83±10.87	8±2.84	19.67±7.84	9.58±2.32	17.58±3.50
	THB/day	16.92		13.84		13.58	
Enteric Bacteria (cfu/10min)	Ground floor	9.25	2	5.5	5	10	9.75
	First floor	9.25	2	5.5	5	10	9.75
	Second floor	7.75	0.25	6.5	4.75	1.75	4.25
	AVE	8.75±0.87	1.42±1.01	5.83±0.58	4.92±0.14	7.25±4.76	7.92±3.18
	EB/day	5.09		5.38		7.59	
Total fungi (cfu/10min)	Ground floor	8.75	19.5	19	22.5	10.75	19.5
	First floor	12.75	14.5	10.25	24.5	7.5	17
	Second floor	3.75	7.25	8.25	25	3.75	7
	AVE	8.42±4.51	13.75±6.16	12.5±5.72	24±1.32	7.33±3.50	14.5±6.61
	TF/day	11.09		18.25		10.92	

The studies also showed that microbes are of higher concentration in the afternoon than in the morning as seen in Table 4 above. The microbial isolates identified include *Bacillus* sp (39.6%) > *Staphylococcus* sp (21.2%) > *Pseudomonas* sp (14.9%) > *Streptococcus* sp (14.4%) > *Klebsiella* sp (6%) > *Micrococcus* sp (4.5%). The fungal isolates include *Candida* sp (34.9%) > *Penicillium* sp (21.4%) > *Mucor* sp (21.4%) > *Aspergillus niger* (11.3%) *Aspergillus flavus* (10.4%). The enteric organisms are *Klebsiella* sp (34.3%) > *Escherichia coli* (32.2%) > *Enterobacter* sp (25.5%) > *Shigella* sp (8%) (Fig. 3-5)

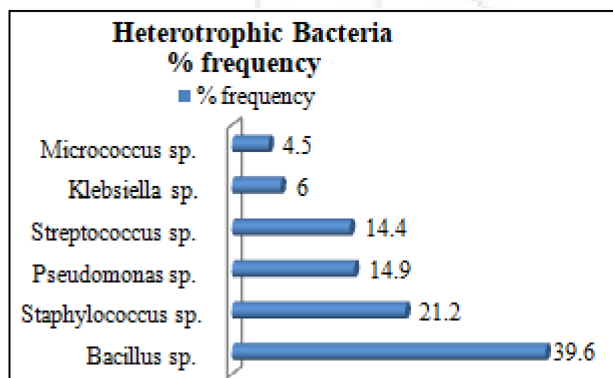

Figure 3: Percentage (%) frequency of Total Heterotrophic Bacterial isolates associated with black soot

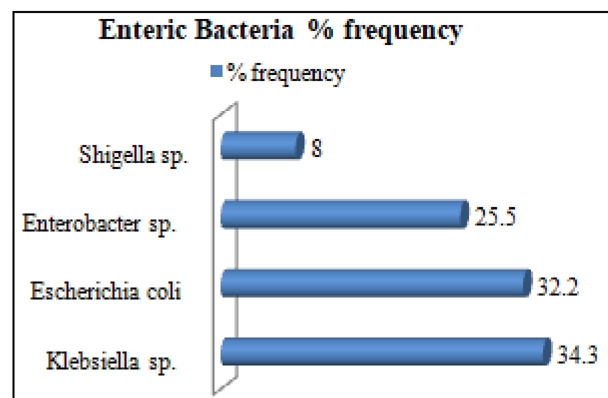

Figure 4: Percentage (%) frequency of Enteric Bacterial isolates associated with black soot

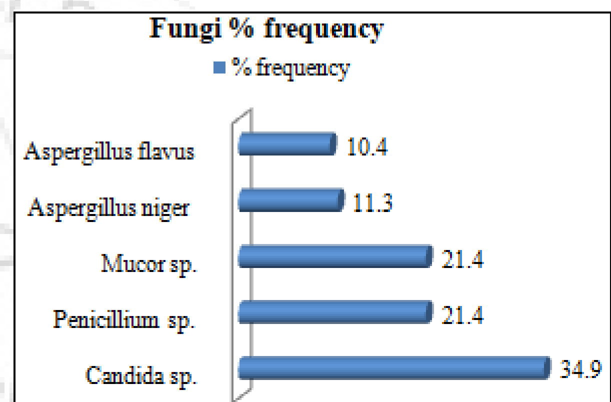

Figure 5: Percentage (%) frequency of Total Fungal isolates associated with black soot

Increased exposure to fungal spores can lead to skin irritation and even breathing irritations that can dangerous. Increased exposure to fungal spores can lead to skin irritation and even breathing irritations that can dangerous. The information on the indoor microbial concentrations of airborne bacteria and fungi is necessary to estimate the health hazard (Fung and Hughson, 2003). Candida species was the most occurring fungi. Studies have shown that it is a normal flora of the mouth so can be easily disseminated in the air, thus, an opportunist. *Aspergillus* are also potential threat to health of students causing pathogenic infections like allergic broncho-pulmonary aspergillosis.

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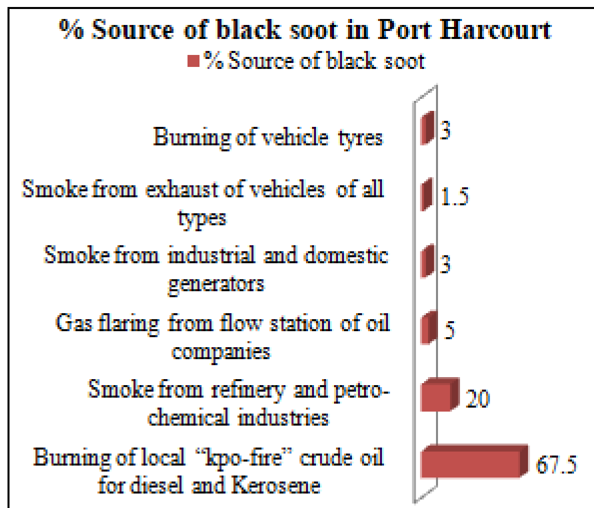

Figure 6: Percentage (%) sources of black soot in Port Harcourt

Black soot and its associated bio-aerosols in the Niger Delta, Nigeria especially Port Harcourt is attracting global concern. Figure 6 revealed percentage (%) of six major sources of black soot identified; Burning of local "kpo-fire" crude oil for production of diesel and Kerosene 67.5%, Smoke from refinery and petro-chemical industries 20%, Gas flaring from flow station of oil companies 5%, Smoke from generators both industrial and domestic 3%, Smoke from exhaust of vehicles of all types 1.5%, Burning of vehicle tyres either at animal slaughter abbatoir or for other purposes 3%, similar report have observed by Nrior and Chioma, 2017, Gaurdian Newspaper report, 2017.

4. Conclusion and Recommendation

Aspergillus species are potential threat to health causing pathogenic infections like allergic broncho-pulmonary aspergillosis. The occurrence of pathogenic bacteria such as *Escherichia coli, Staphylococcus* and *Bacillus* mixed with black soot is of great health importance. Also, long exposure to black soot could cause cancer. It is therefore recommended that urgent proactive action should be taken by individual and government/environmental authorities to curb these issues.

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