

Black Soot In Port Harcourt: Incidence of Pathogenic Microbes Associated With Black Soot in Indoor Aerosols of Classrooms In Port Harcourt Nigeria

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Abstract: This research reports the incidence of pathogenic microbes associated with black soot in indoor aerosols of Classrooms in Port Harcourt Nigeria. Six major sources of black soot have been identified; Burning of local “kpo-fire” crude oil for production of diesel and Kerosene, Smoke from refinery and petro-chemical industries, Gas flaring from flow station of oil companies, Smoke from generators both industrial and domestic, Smoke from exhaust of vehicles of all types, Burning of vehicle tyres either at animal slaughter abbatoir or for other purposes. In this study, Students’ classrooms (Junior secondary school JSS1, JSS2, JSS3 and Senior Secondary SSS1, SSS2, SSS3) of Model Girl’s Secondary School Rumueme Port Harcourt was used as the sampling station due to its centrality in Port Harcourt metropolis. Sampling period spans from 16th May to 28th June, 2017. The open plate sedimentation technique of air sampling at exposure period of five (5) minutes was used for the enumeration and isolation of airborne indoor fungi using sabouraud dextrose agar medium, bacteria using nutrient agar and MacConkey agar medium. Percentage frequency of fungi (1% = 0.421CFU/5min) isolated were *Fusarium specie* (18.1%), *Cladosporium resinae* (17.3%), *Aspergillus nidulans* (14.9%), *Aspergillus terreus* (14.3%), *Penicillium spinulosum* (13.3%) *Aspergillus niger* (12.6%), *Aspergillus flavus* (9.5%) while Bacteria [1% = 1.476CFU/5mins]; *Bacillus* (40.5%), *Pseudomonas* (14.2%), *Staphylococcus* (12.5%), *Micrococcus* (10.8%), *Enterobacter* (9.7%), *Escherichia coli* (6.6%), *Streptococcus* (3.7%), *Klebsiella*, (2%). These genera of fungi and bacteria have been shown to be amongst the most common pathogenic microbes often associated with airborne illnesses. More over the incidence of pathogenic organisms such as *Staphylococcus*, *Escherichia*, *Bacillus*, *Streptococcus*, *Aspergillus*, *Fusarium*, *Cladosporium* is of great health concern. These organisms are frequently found in human respiratory tract and minor skin infection, it is a common cause of boil, respiratory disease, food poisoning and scaled skin syndrome. *Aspergillus*, is occasionally involved in incidence of aspergillosis, ear and skin infections. The black soot has long term cancerous effect and often complicates respiratory illnesses.

Keywords: Black soot, Pathogenic microbes, indoor aerosol, classrooms, *Staphylococcus*, *Escherichia*, *Bacillus*, *Streptococcus*, *Aspergillus*, *Fusarium*, *Cladosporium*

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I. Introduction

Indoor air quality is one of the most significant factors affecting the health and well being of people who inhale 10m³ of the air everyday, and spend between 80-95% of their lives indoors (Dacarro, *et al*; (2003). The air inhaled by people is numerously populated with microorganisms which form so-called 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 them viruses, fungal spores and conidia bacteria endospores, plant pollen and fragment of plant tissues (Karwowska, *et al* 2005). Possible sources of microbial aerosol contamination include: people, organic dust, various material, stored in the buildings and the air inflowing from the ventilations and air conditioning system. According to Barbara, *et al* (2006) an aerosol is a colloid fine solid particle or liquid droplets in air. The microorganisms that contaminate the aerosol are the major causes of biological hazards (David, 2006) like chicken pox tuberculosis, small pox, anthrax, etc, majority of these airborne microbes are suggested to be in a fine state while in the atmosphere but research has shown that certain microbes are capable of carrying out basic metabolic activities with cloud water (Nrior and Adiele, 2015). The health and well being of the public are affected by the physical, chemical and biological properties of the indoor environment. The quality of their indoor environment, however, “is not, easily defined or controlled and potentially put human occupants at risk (Jaffal, *et al* 1994). The interest in bio aerosol exposure has increased over the last few decades. This is largely because it is now appropriately recognized that exposures to biological agents in both the occupational and residential indoor environment are associated with a

wide range of adverse health effects with major public health impact including contagious infections disease, acute toxic effects, allergies and cancer. Several new industrial activities have emerged in recent times in which exposures to biological agents can be abundant. One example is the waste recycling industry. Workers in this industry are often exposed to very high levels of microorganisms (Donwes et al; 2009; Nrior and Adiele, 2015). In the dust and air of institutions and hospital wards or the rooms of person suffering from infectious disease, microbes such as *Tubercie bacilli*, *Streptococci*, *Pneumococci* and *Staphylococci* have been demonstrated. These respiratory bacteria are dispersed in air in the droplet of saliva and mucus produced by coughing, sneezing, talking and laughing; viruses of respiratory tract and some enteric tract are also transmitted by dust and air. Droplets are usually formed by sneezing, coughing and talking, pathogens in dust are primarily derived from the objects contaminated with infectious secretions that after drying become infectious dust. The amount of pathogenic microorganisms is higher in indoor compared to out door air (Dacarro *et al*, 2003). Microbial damage is caused most frequently by bacteria and molds, these microorganisms can enter indoor area either by means of ventilation systems used indoor or by means of passive ventilation, many bacterial genera are also emitted by indoor sources like flower pots, food, animals and waste baskets. In most cases normal flora, are not harmful.

The importance of the evaluation of the quality and types of air borne microorganism are that these values can be used as an index for the cleanliness of the environment as well as an-index they bear in relation to human health and as a source of hospital acquired infection (Splendlore, *et al*; 1983; Nrior and Adiele, 2015). This research is aimed at evaluating the types and frequency of microorganisms associated with black shoot in indoor aerosols of Classrooms in Port Harcourt Nigeria.

II. Material And Method

2.1 Study Area

Government Girls Secondary School Rumueme now Model Girls Secondary School Rumueme Port Harcourt was founded in 1986. It is sited along Ikwere Road opposite Rivers State School of Health Science in Obio-Akpor Local Government area in the heart of Port Harcourt, Port Harcourt being one of the major centers of economic activities in Nigeria. The Secondary School is populated with over 1500 estimated students and over hundred staffs. It is located between latitudes 4^o81N, longitude 6^o98 E, and attitude 14^oE.

2.2 Characteristics of Classrooms

Prior to air sampling, classrooms characteristics viz. type of ventilation, no of installed fans and exhaust system, floor area of selected classrooms, ceiling height, volume, furniture status, walls cleanliness, number of students, average occupancy daily were evaluated as shown in Table 1. Students age bracket ranges from 11 to 14years in the junior secondary and 14 to 18 years in the senior secondary session.

2.3Quality assessment of School Building

The school building was constructed over 31 years ago and rehabilitated in the year 2009 which change the name to Model Girls Secondary School Rumueme from Government Girls Secondary School Rumueme, visible symptoms of small black mold patches on the walls and enormous deposition of dust on the various, surfaces were noticed during investigation. Terrible damp smell was also experienced there in the classrooms.

Table 1 Characteristics of Classroom of Model Girls Secondary School Rumueme

<i>Characteristics</i>	<i>JSS1</i>	<i>JSS2</i>	<i>JSS3</i>	<i>SS1</i>	<i>SS2</i>	<i>SS3</i>
Location	Rumueme Obi/Akpor LGA	Rumueme Obi/Akpor LGA	Rumueme Obi/Akpor LGA	Rumueme Obi/Akpor LGA	Rumueme Obi/Akpor LGA	Rumueme Obi/Akpor LGA
Ventilation	Natural	Natural	Natural	Natural	Natural	Natural
Sun light	Too dim to moderate	Too dim to moderate	Too dim to moderate	Too dim to moderate	Too dim to moderate	Too dim to moderate
Walls	Dirty	Dirty	Dirty	Dirty	too Dirty	Dirty
Dampness	-	-	-	-	-	-
Odor	Yes	Yes	Yes	Yes	Yes	Yes
Cleaning	Moderately	Daily	Daily	Inadequately	Moderately	Moderately
Sweeping	Rarely	Rarely	Sometimes	Rarely	Rarely	Sometimes
Moping	No	No	No	No	No	No
Moping with pesticide	No	No	No	No	No	No
Dust bins	Not frequently covered	Not frequently covered	Not frequently covered	Not frequently covered	Not frequently covered	Not frequently covered
Black soot deposition on surface	Yes	Yes	Yes	Yes	Yes	Yes
• Horizontal	Yes	Yes	Yes	Yes	Yes	Yes
• Vertical						

Classroom measurement						
• Length	30ft	30ft	32ft	23ft	32ft	64ft
• Width	21ft	21ft	23ft	19ft	23ft	23ft
Cooling systems	Fans	Fans	Fans	Fans	Fans	Fans
No of students	300	280	270	200	240	300

2.3 Air sampling and microbiological examination

Air samples were analyzed from all the classrooms by exposing the Petri dishes containing sterile Sabouraud dextrose agar, Nutrient agar and MacConkey agar respectively for a period of (5mins). After exposure, the plates were covered and aseptically transported to the laboratory and incubated at 28⁰c for 2-3 day for fungi and 24hours for bacteria. After the incubation, the plates were examined and colonies that developed were counted and recorded; results expressed in CfU/5min.

2.4 Enumeration and Identification of bacterial and fungal isolates

The isolates were obtained and subjected to various characterization procedures. Pure isolates of bacteria were identified on the basis of their, cultural, morphological and physiological characteristics (Buchanan and Gibbons, 1974; Cowan, 1974). The following standard characterization tests were performed, Grams staining reaction motility test, oxidase test, catalase test, coagulase test, starch hydrolysis, methyl red and indole test, carbohydrate fermentation test. Pure cultures of fungi were obtained by subculturing discrete colonies unto prepared sterile Sabouraud dextrose agar plates and incubated at 28⁰c for 2-3 days. The isolates were characterized based on macroscopic features such as colonial morphology, colour of colony, texture, shape, and surface appearance. The microscopic examination was done by using the wet prep (needle mount) as described by Barnet and Hunter (1972) for observing cultural characteristic like asexual and sexual reproductive structure like sporangia, conidia head, the vegetative mycelia, septate or non-septate hyphae

III. Result and Discussion

The result in Fig. 1-3 shows the count of total fungal, total heterotrophic bacteria, and total enteric bacteria from direct sedimentation sampling method (open plate techniques) using nutrient agar for total heterotrophic bacteria, Sabouraud dextrose agar for fungi species, and MacConkey agar for enteric bacteria species. Average microbial load from Fig 1-3 shows; Total Heterotrophic Bacteria (CFU/5min) were: JSS1 (32.0±21.28) > SSS3 (31.33±12.06) > SSS2 (25.0±15.39) > JSS2 (23.0±6.93) > SSS1 (19.33±11.06) > JSS3 (17.0±9.64); Total Fungi (CFU/5min): JSS2 (10.0±4.0) > JSS3 (8.67±4.51) > JSS1 (8.33±4.04) > SSS1 (5.67±1.53) > SSS2 (5.33±2.52) > SSS3 (4.0±1.0). Enteric bacteria (CFU/5min) were; SSS1 (2.67±3.79) > SSS2 (2.33±2.52) > JSS2 (2.0±1.73) > JSS1 1.67± 1.53) > JSS3 (1.33±1.15) > SSS3 (0.33±0.58). This could be as a result of human activities such as talking, walking, laughing and sweeping, sneezing, coughing and the no of students, teachers, or visitors entering and exiting the classrooms as at the time of the study. These result agreed with the report of (Dugid et al., 1946) that in poor hygienic quality and crowded rooms, the higher number of people confined to a small space result in the build up of air borne microbes shed by the human body.

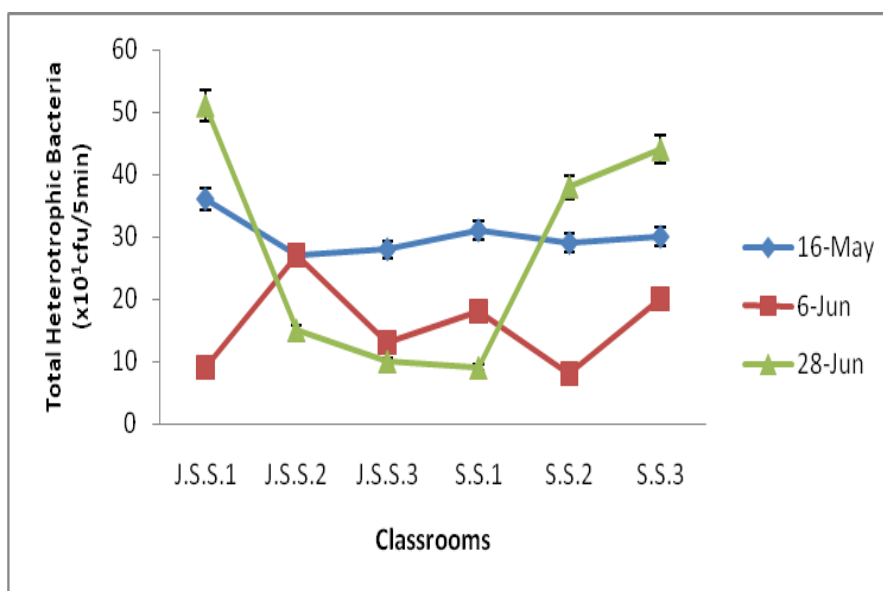


Fig. 1: Total Heterotrophic Bacteria (x10¹cfu/5mins) associated with black soot from indoor aerosol in classrooms

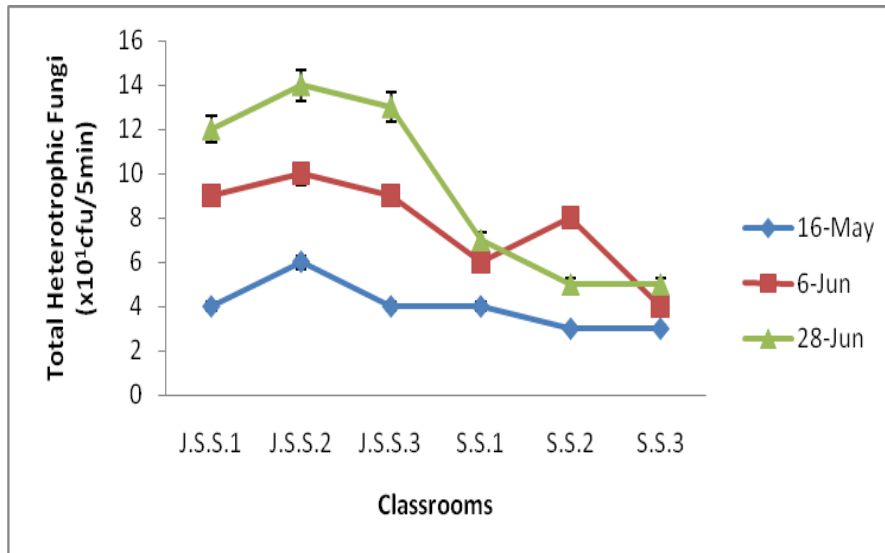


Fig. 2: Total Heterotrophic fungi (x10¹cfu/5mins) associated with black soot from indoor aerosol in classrooms

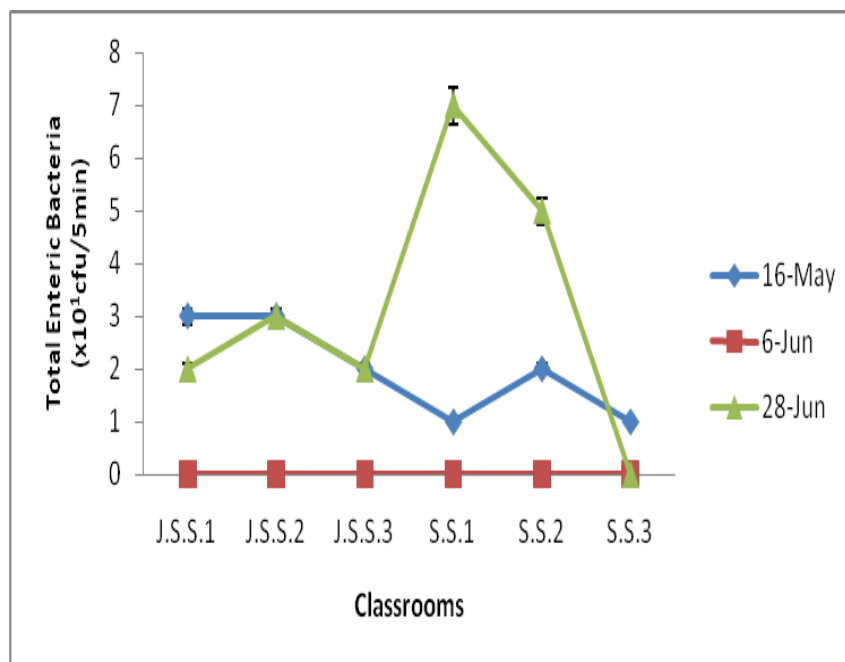


Fig. 3: Total Enteric Bacteria (x10¹cfu/5mins) associated with black soot from indoor aerosol in classrooms

The mixed variations in the microbial load of the different classes between junior and senior could be traced not only to human activities such as talking, walking, laughing and sweeping, sneezing, coughing and the number of students, teachers, or visitors entering and exiting the classrooms as at the time of the study but also to the homes sanitation conditions, microbial status of school bags and uniform (all clothing worn to school) including cleanliness and parental habits. Many studies on microbial containment in indoor air have been reported by several investigators in different environments, such as hospital, residential building (Jaffal et al, 1997; Ayanru 1981; Nrior and Adiele, 2015). The Variation of Bacterial and fungal isolates count (x10¹cfu/5mins) at sampling interval in model secondary school indoor aerosol from 16th May to 6th June, 2017 were shown in Fig 4-5. Eight genera of bacteria species were identified; *Bacillus*, *Pseudomonas*, *Micrococcus*; *Staphylococcus*, *Escherichia coli*, *Enterobacter*, *Klebsiella*, and *Streptococcus*. The fungal species isolated were *Aspergillus niger*, *Aspergillus flavus* *Aspergillus tereus*, *Aspergillus nidulans*, *Fusarium specie*, *Penicillium spinulosum* and *Cladosporium resinae* (Fig. 4-7). These genera of fungi have been shown to be amongst the most common fungal species often associated with airborne illnesses (Jaffal, et al 1997; Nrior and Adiele, 2015)

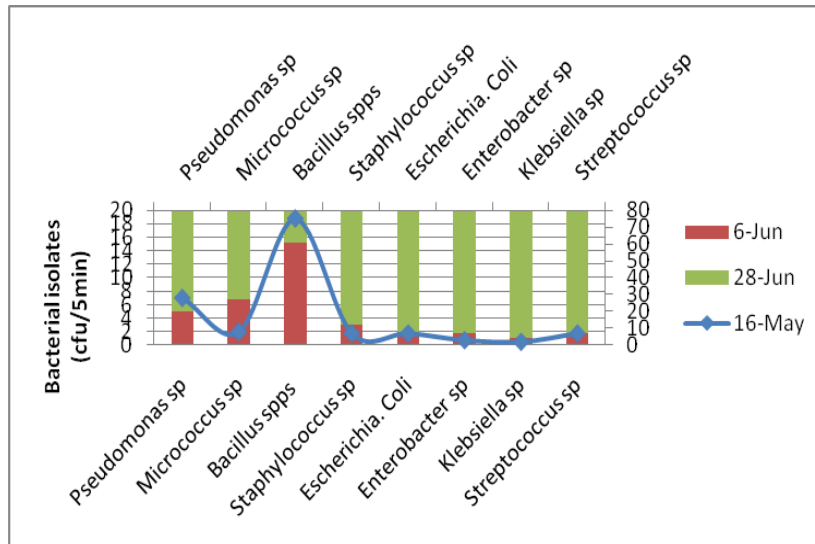


Fig. 4: Variation Bacterial isolates count ($\times 10^1$ cfu/5mins) at sampling interval associated with black soot from indoor aerosol in classrooms

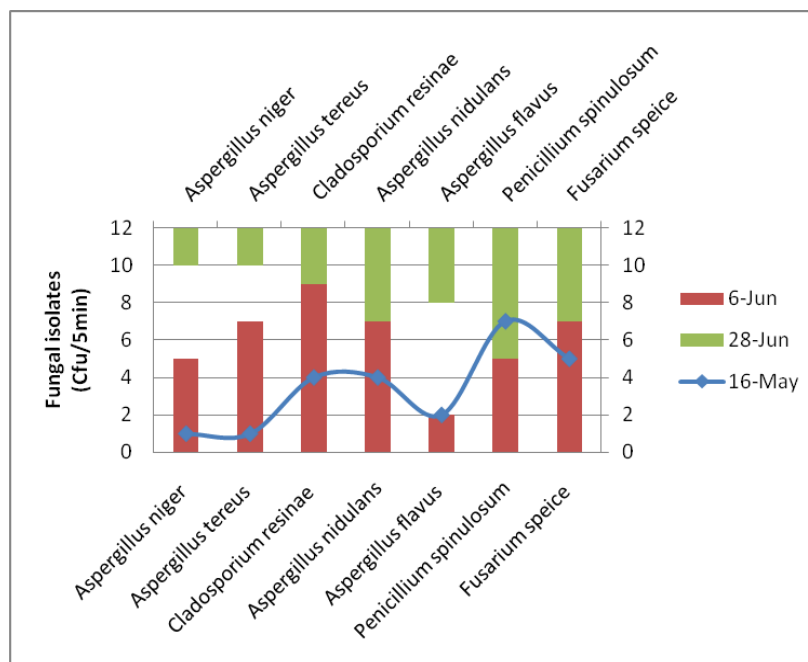


Fig. 5: Variation of fungal isolates count ($\times 10^1$ cfu/5mins) at sampling interval associated with black soot from indoor aerosol in classrooms

The mean percentage (%) frequency of bacteria species identified; Bacillus, Pseudomonas, Micrococcus; Staphylococcus, Escherichia coli, Enterobacter, Klebsiella, and Streptococcus and that of fungal species Aspergillus niger, Aspergillus flavus Aspergillus tereus, Aspergillus nidulans, Fusarium specie, Penicillium spinulosum and Cladosporium resinae were shown in Fig. 6-7. Percentage frequency of fungi (1% = 0.421CFU/5min) isolated were Fusarium specie (18.1%), Cladosporium resinae (17.3%), Aspergillus nidulans (14.9%), Aspergillus terreus (14.3%), Penicillium spinulosum (13.3%) Aspergillus niger (12.6%), Aspergillus flavus (9.5%) while Bacteria [1% = 1.476CFU/5mins];

Bacillus (40.5%), Pseudomonas (14.2%), Staphylococcus (12.5%), Micrococcus (10.8%), Enterobacter (9.7%), Escherichia coli (6.6%), Streptococcus (3.7%), Klebsiella, (2%). These genera of fungi and bacteria have been shown to be amongst the most common pathogenic microbes often associated with airborne illnesses. More over the incidence of pathogenic organisms such as Staphylococcus, Escherichia, Bacillus, Streptococcus, Fusarium, Cladosporium is of great health concern.

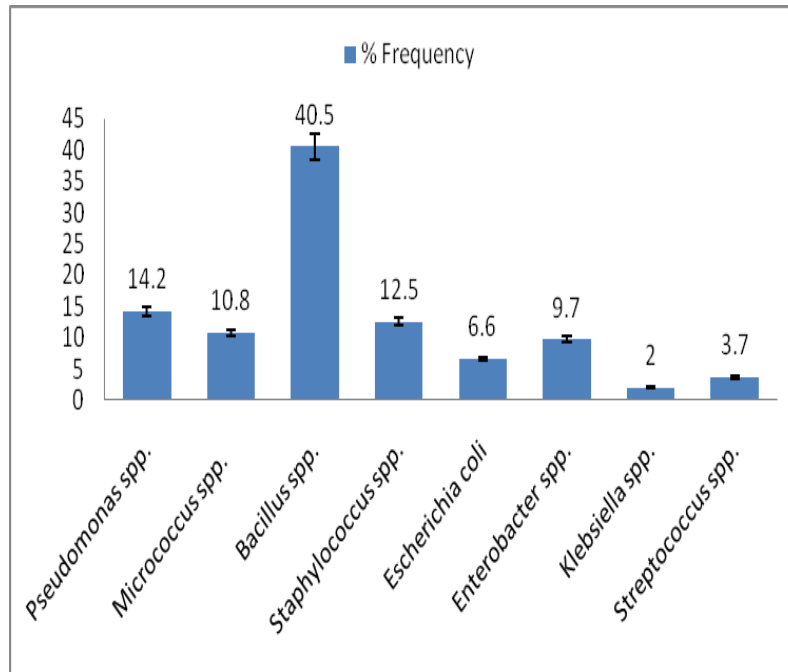


Fig. 6: Mean Percentage (%) frequency of bacterial isolates associated with black soot from indoor aerosol in classrooms

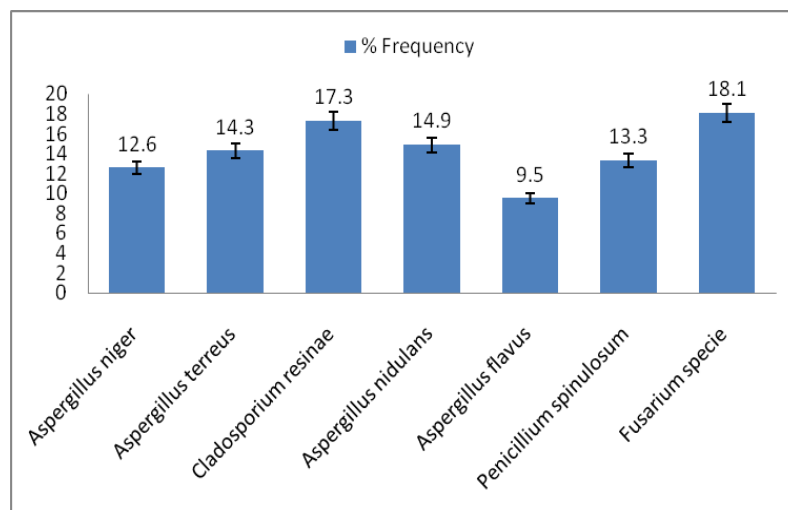


Fig. 7: Mean Percentage (%) frequency of fungal isolates associated with black soot from indoor aerosol in classrooms

Pathogenic bacteria accounted for 30% of all isolate. It is also noted in this study that *Bacillus species* has the highest frequency of occurrence (40.5%) followed by *Pseudomonas specie* with the frequency occurrence of (14.2%) while *klebsiella specie* has the lowest frequency occurrence (2.0%) followed by *Streptococculi* (3.7%) in bacteria isolates. In fungal isolates, *Aspergillus spp* occurred most frequently than other fungal specie, followed by *Fusarium specie*. It was also noted in this study that the amount of materials brought from outside the classrooms which include personal belongings, food and fruits were more common in the hall. The number of dust bins was also not frequently covered and these are seen as source of indoor air contamination. *Staphylococcus aureus* are major pathogenic microorganisms which can create a lot of health problem to humans associated with air, *Staphylococcus auerus* are frequently found in human respiratory tract and minor skin infection, it is a common cause of boil, respiratory disease food poisoning and scaled skin syndrome. According to Curran et al., (1980), it is estimated that 20% of the human population are long term carriers of *Staphylococcus aureus* which can be found as part of the normal skin flora. *Aspergillus spp* was the most frequent fungi isolated. (Jaffal et al., 1997) reported that *Aspergillus*, *chaetomium* and *Alternaria* were the most common genera frequently isolated from indoor environments and air conditioned systems. The most common of them, *Aspergillus*, is occasionally involved in incidence of aspergillosis, ear and skin infections.

IV. Conclusion

This study showed the microbiological quality of indoor air associated with black soot in classrooms can create a potential danger with regard to public health. Because of the need for systematic and universally applicable approach to indoor air safety, personal cleanliness and adoption of proper sanitation habit is very important. It can be concluded that the fungal organisms: *Aspergillus spp*, *Penicillium spp*, *Fusarium spp*, *Cladosporium spp*, *Mucor spp*. were the fungal isolates most frequently associated with black soot in the sites studied and *Staphylococcus spp*, *Bacillus spp*, *Pseudomonas spp*, *Micrococcus spp*, *Escherichia coli*, *Klebsiella spp* and *Streptococcus spp* are the most common bacterial genera frequently associated with black soot. These contaminants do not only pose health hazard to indigenes and occupants of the school community but also to visitors exposed to inhalation of such contaminated air and the entire dwellers in Port Harcourt and beyond. Six major sources of black soot have been identified; Burning of local "kpo-fire" crude oil for production of diesel and Kerosene, Smoke from refinery and petro-chemical industries, Gas flaring from flow station of oil companies, Smoke from generators both industrial and domestic, Smoke from exhaust of vehicles of all types, Burning of vehicle tyres either at animal slaughter abattoir or for other purposes.

It is therefore important to evaluate the quality of the air we breathe whether indoor or outdoor, especially in the school and hospital environments. The number and type of air borne microorganisms can be used to determine the degree of cleanliness as well as to determine the source of human discomfort. It is recommended that proactive measures should be adopted by Government, Industries, individuals including teachers and students in reducing the level of air borne contaminant by educating the entire public of the danger of burning of fossil fuel, also workable implementation of regulatory measures for companies involved.

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