

Original Research Article

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Microorganisms Isolated from Sawmill and Poultry Farm and their Long Term Health Effects in Human Health

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ABSTRACT

Microorganisms from dusts of organic origin was identified from some saw mill, (Site 1) and a poultry farm (Site 1) in Port Harcourt. The exposure to these organic dusts by people employed in these establishments over a long period of time can lead to occupational health diseases especially in immune compromised persons. Nutrient Agar, Sabaroud Dextrose Agar (SDA), and Mac Conkey Agar (MA) in sedimentation method were used to isolate microorganisms. In Sample Site1, the Total Heterotrophic Bacteria (THB) was greater than the Total Enteric Bacteria (TEB) and the Total Aerobic Fungi (TAF) on a dry day while the THB is greater than TAF > TEB on a wet season. While in station 2, THB> TEB > TAF during the dry season and THB >TEB > TAF during the wet season. This result revealed that heterotrophic bacteria are the most dominant during the rainy and dry season in both sites. Between the two sites, microbial concentration in Site 2 (poultry farm) at 2.115cfu/10min/m² is greater than Station 1(sawmill) at 1.608cfu/10min/m², this might be due to the fact that it is a confined area in which birds are bred and its system of ventilation is poor. These microorganisms identified in various concentrations can cause pulmonary dysfunctions and allergic diseases such as Aspergillosis, Hypersensitivity pneumonitis, chronic bronchitis, rhinitis etc. There is therefore need for workers in these organic dust prone areas to make use of the most practical respirators (nose masks) with the highest assigned protection factor (APF).

Keywords

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Introduction

The risks associated with prolonged exposure to grain dusts were first identified in the early 16th century and its exposure has been a major source of mortality among agricultural workers (Schenker, 2000). Dusts can be referred to as very fine solid particles that are usually suspended in the air and they result from the breakdown of materials in order to

propel fine fragments into a gaseous medium (Laakkonen, 2008). Dusts can have different sizes (ranging from 1-100 µm) and they tend to settle out under gravitational influence (ISO, 1995). Their effects on the human body are to a large extent dependent on their respective sizes and nature, also these factors determine their site of deposition within the respiratory system (Laakkonen, 2008). The dusts are usually either larger sized or smaller

sized, dusts of larger sized particles are referred to as inhalable dusts and most of them are filtered out into the nose, throat and upper respiratory tract (TUC 2011; Laakonen, 2008). Whereas, smaller sized particles when inhaled can go as far as the alveoli and the lungs and they are referred to as respirable dusts, these smaller particles when incessantly inhaled over a long period of time can pose a threat to human health (TUC, 2011).

Based on their sources, dusts can be categorized into two types which are Organic dusts and inorganic dusts (Schenker, 2000).

The word “organic dust” also refers to “bioaerosols” and it is defined as fine particles of biological origin (microbial, plant or animal) that are suspended in the air (Douwess *et al.*, 2003). These particles are usually impregnated with microorganisms and they include include dusts from wood, flour, cotton fibres, paper fibres, fur from animals, hay, grains, animal scales, animal dander, evaporated urine droplets and fecal, household wastes etc (Eduard and Halstensen, 2009). Organic dusts are usually launched into the air by natural forces, such as wind, volcanic eruption, and by mechanical or anthropogenic processes such as crushing, grinding, milling, drilling, demolition, conveying, screening, bagging, and sweeping (ISO, 1995).

Organic dusts occur in a range of occupations including agricultural work; the textile industry, especially cotton processing; flour milling and bakeries; and the wood industry, particularly sawmills, carpentry, and wood processing, the waste management industry and so many others. Many of these occupations, particularly agricultural work, also have the highest potential for concurrent exposure to other substances that affect respiratory health, for example metals, gases, fibres, and chemicals (Omland, 2002).

These bioaerosols are active and they are made up of some components that result in adverse health effects to exposed workers due to prolonged exposure, Some of these agents are bacterial endotoxins, fungi, viruses, high molecular weight allergens, mycotoxins, pollens, moulds, proteins from animal hair, urine and droppings, and enzymes which act as allergens, tannins, plicatic acid etc (Douwess *et al.*, 2003).

Materials and Methods

Study area and sample collection

The study was carried out in two sampling sites, one is the Sawmill located at Timber street by Iloabuchi mile 1, Port-Harcourt (Latitude 4.7893765, N 4⁰47'19.38876'' and Longitude 6.9831649, E6⁰59'18.62376''), and the poultry farm located within Rivers State University, Nkpolu-Oroworukwo, Port-Harcourt (Latitude 4.80234 N4⁰48'8.4096 Longitude 6.97713 E 6⁰58'37.68096''). The Sawmill is a facility where logs of wood are cut into lumber, here wood and wood products are processed, the facility comprises mainly of male workers and the activities that take place in the sawmill involves the transportation of fresh logs of wood from the forest, sawing of the wood, packaging of the lumber, transportation and the export of the cut lumbers.

The Nutrient Agar (NA), Mac Conkey Agar (MA) and Sabouraud Dextrose Agar (SDA) plates were exposed to the organic dusts in sites 1 and 2 for about 10 minutes and the isolates were collected from each source during the wet day and dry day.

The bacterial and fungal isolates were determined using Koch's sedimentation method (settle plate technique). In this technique, microorganisms from the organic dusts get settled directly on the prepared agar

plates exposed on a 4ft high wooden stool for a period of 10 minutes. The exposed Nutrient agar and Mac-conkey agar were incubated at 37⁰c for 24 hours while the Sabouraud dextrose agar plates were incubated at room temperature for 72 hours. The colonies that were formed on the culture plates were recorded as colony forming units per 10 minutes and expressed as cfu/10mins/m² of air using the following formula:

$$\text{Cfu/10min/m}^2 = \frac{\text{No. of colonies} \times 10 \times 3.142r^2}{\text{Time of exposure}}$$

Where,

r = radius of media plate used (in meters)

Isolation of pure cultures

Discrete colonies were all sub-cultured to obtain pure colonies. This was achieved by streaking a loop-full of a particular isolate on an already prepared Nutrient agar plate and incubated at 37⁰c for another 24 hours. The pure cultures were stored accordingly in a nutrient agar slant for further studies.

Characterization of bacterial isolates

This characterization was done firstly by morphological identification of respective colonies, this was followed by using conventional methods which include Gram staining, biochemical tests such as catalase, coagulase, oxidase, urease, motility, methyl-red(MR), Vogues Proskauer (VP), sugar fermentation tests which include mannitol, glucose, maltose, lactose and starch hydrolysis.

Identification was based on comparison of the characteristics of the isolates with those of the taxa. Details of the test procedures are as follows.

Characterization of fungal isolates

The identification of fungal isolates was carried out using standard methods based on macroscopic and microscopic features as described by Ellis (1971), Domsch *et al.*, (1980). In macroscopic identification, the aerial and substratum regions were observed for colour, colony structure, colony number and nature of growth. In microscopic examination, two drops of cotton blue in lacto-phenol is stained in the center of a clean grease-free slide. A small portion of the fungus was picked from the sub-cultured plate using a sterilized inoculating needle and it was placed on the slide and covered with a cover slip. It was examined under the microscope at low power and high power (x10 and x40 respectively).

Results and Discussion

The following fungal features were noted in this test:

- Somatic structure
- Vegetative structure
- Reproductive structure
- Conidial head and vesicle shapes
- Surface appearance
- Colony colour

In station 1, total Heterotrophic Bacteria (THB) 0.804 > Total Enteric Bacteria (TEB) 0.576 > Total Aerobic Fungi (TAF) 0.108 during dry day and TEB 0.204 > THB 0.144 > TAF 0.060 during the wet day. While in station 2, THB 0.846 > TEB 0.732 > TAF 0.192 during the dry day and THB 0.132 > TEB 0.114 > TAF 0.066 during the wet day (Fig. 1–6 and Table 1–6). This result reveals that in both stations, heterotrophic bacteria are the most dominant during the rainy and dry season It also reveals that the concentration of microorganisms decreased in the wet day than during the dry season in both stations and this

result correlates with that of (Achudume *et al.*, 2009) which states that dusts and microbial proliferation are much higher in dry seasons than in wet seasons. In station 1, THB are of 12 species which include *Paenibacillus lautus*, *Bacillus badius*, *Bacillus carboniphilus*, *Staphylococcus saccharolyticus*, *Brevibacillus laterosporus*, *Staphylococcus aureus*, *Lactobacillus kitasatonis*, *Macrococcus brunensis*, *Bacillus smithii*, *Staphylococcus massiliensis*, and *Streptococcus parasuis* with *staphylococcus* species forming about 40.4% of the total heterotrophic bacteria. The TEB include *Bacillus badius*, *Erwiniaspp*, *Shigelladysenteriae*, *Escherichia coli*, *Klebsiella pneumonia*, *Lactobacillus kitasatonis* *Corynebacteriumafermentans* with *Klebsiella pneumonia* being the most dominant forming 26.67% of the total enteric bacteria. The TAF include *Apergillus Flavus*, *MucorSpp*, *Rhizopus stolonifer*, *Aspergillus Niger*, *Aspergillus Fumigatus*, *Rhizopus arrhizus*, and *Epicoccum nigrum* with *Aspergillus Niger* being the most dominant with about 21.97% of the total aerobic fungi.

Whereas in station 2, the THB are of 9 species which include *Staphylococcus saccharolyticus*, *Pseudomonas spp*, *Bacillus badius*, *Staphylococcus aureus*, *Lactobacillus kitasatonis*, *Macrococcus brunensis*, *Bacillus smithii*, *Streptococcus parasuis*, and *Staphylococcus massiliensis* out of which *Staphylococcus aureus* was the most dominant constituting 34.66% of the total heterotrophic bacteria. Seven (7) species of enteric bacteria were identified and they include *Serratia* species, *Escherichia coli*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterobacter cloacae* and *Hafnia alvei* out of which *Escherichia coli* dominated most constituting 25.42% of TEB. Eight (8) species of aerobic fungi were identified and they include *Apergillus flavus*, *Mucor spp*, *Aspergillus*

niger, *Aspergillus fumigatus*, *Rhizopus arrhizus*, *Epicoccum nigrum*, *Saccharomyces spp*, *Penicillium spp* with *Apergillus flavus* being the most dominant constituting about 24.47% of the total aerobic fungi. Between the two stations, microbial concentration in station 2 (poultry farm) 2.115cfu/10min/m² >station 1(sawmill) 1.608cfu/10min/m²), this might be due to the fact that it is a confined area in which birds are bred and its system of ventilation is poor. Bacterial and fungal concentration in organic dust and their harmful effect on human health depends on different environmental factors including source materials, climatic condition and the level of ventilation in the place of study (Dutkiewicz *et al.*, 2000).

Among the microorganisms occurring in organic dust three (3) groups were identified from the major groups that could be identified. These groups include gram-negative bacteria (producing endotoxin, which are mostly epiphytic species developing abundantly on plant surfaces as saprobionts), gram-positive bacteria (which are predominant organisms in dusts of animal origin and may be also very common in dusts from stored plant materials) and fungi (comprising multicellular filamentous fungi described as moulds and unicellular yeasts, are common in organic dusts). These microorganisms may penetrate into deeper parts of the lungs causing undesirable harmful effects on human health. Bacteria and fungi occurring in organic dusts are mainly non-infectious but may however exert adverse effects on respiratory tract of exposed persons causing mucous membrane irritation (MMI), immunotoxic diseases such as organic dust toxic syndrome (ODTS), inhalation fever, grain fever, toxic pneumonitis, byssinosis, humidifier syndrome, mycotoxicoses and allergic diseases such as allergic alveolitis (hypersensitivity pneumonitis) chronic bronchitis, granulomatous pneumonitis,

asthma and allergic rhinitis. Though over 180 *Aspergillus* spp are known, only four are associated with invasive infections in humans, these species include *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus flavus* and *Aspergillus terreus* out of which the first three were isolated. These species cause chronic infections especially in immune-compromised individuals, the infections include fungus ball

(Aspergilloma) allergic broncho-pulmonary aspergillosis (ABPA), chronic pulmonary aspergillosis (CPA) Invasive pulmonary aspergillosis (IPA). (Jorge, 2004). Gram negative bacteria such as *E.coli* as well as other pathogenic microbes which include *Yersinia* sp and *Pseudomonas* sp release endotoxins which cause byssinosis.

Table.1 Frequency of occurrence and CFU/10mins of fungal isolates from sample site 1 (Saw Mill)

Organisms	Dry Day		Rainy Day	
	Mean Frequency	CFU/10mins /m ²	Mean Frequency	CFU/10min /m ²
<i>Apergillus flavus</i>	3	0.018	1	0.006
<i>Mucor spp</i>	2	0.012	1	0.006
<i>Rhizopus stolonifer</i>	2	0.012	1	0.006
<i>Aspergillus niger</i>	4	0.024	1	0.006
<i>Aspergillus fumigatus</i>	2	0.012	3	0.018
<i>Rhizopus arrhizus</i>	2	0.012	1	0.006
<i>Epicoccum nigrum</i>	3	0.018	2	0.012

Table.2 Frequency count and CFU of Total Heterotrophic Bacteria from Saw mill

Organisms	Dry Day		Rainy Day	
	Mean frequency	CFU/10mins /m ²	Mean frequency	CFU/10mins /m ²
<i>Paenibacillus lautus</i>	11	0.066	1	0.006
<i>Bacillus badius</i>	17	0.102	2	0.012
<i>Bacillus carboniphilus</i>	12	0.072	1	0.006
<i>S. saccharolyticus</i>	12	0.072	2	0.012
<i>Brevibacillus laterosporus</i>	10	0.060	2	0.012
<i>Staphylococcus aureus</i>	26	0.156	1	0.006
<i>Lactobacillus kitasatonis</i>	7	0.042	4	0.024
<i>Macroccoccus brunensis</i>	4	0.024	3	0.018
<i>Bacillus smithii</i>	12	0.072	6	0.036
<i>Staphylococcus massiliensis</i>	13	0.078	0	0
<i>Streptococcus parasuis</i>	10	0.060	2	0.012

Table.3 CFU and frequency for enteric bacteria from sample site 1(saw mill)

Organisms	Dry day		Rainy Day	
	Frequency	CFU/10mins /m ²	Frequency	CFU/10min /m ²
<i>Bacillus smithii</i>	8	0.048	3	0.018
<i>Bacillus badius</i>	15	0.09	7	0.042
<i>Erwinia spp</i>	18	0.108	4	0.024
<i>Shigella dysenteriae</i>	12	0.072	5	0.030
<i>Escherichia coli</i>	8	0.048	4	0.024
<i>K. pneumoniae</i>	23	0.138	3	0.018
<i>L. kitasatonis</i>	4	0.024	4	0.024
<i>Corynebacterium afermentans</i>	8	0.048	4	0.024

Table.4 Frequency count and CFU of aerobic fungi isolated from sample site 2 (RSU poultry farm)

Organisms	Dry day		Rainy day	
	Mean frequency	CFU/10mins/m ²	Mean frequency	CFU/10mins/m ²
<i>Apergillus flavus</i>	4	0.024	1	0.006
<i>Aspergillus fumigatus</i>	3	0.018	0	0
<i>Rhizopus arrhizus</i>	4	0.024	1	0.006
<i>Epicoccum nigrum</i>	3	0.018	2	0.012
<i>Saccharomyces spp</i>	3	0.018	0	0
<i>Penicillium spp</i>	7	0.042	3	0.018

Table.5 Frequency count for Heterotrophic Bacteria from sample site 2 (RSU poultry farm)

Organisms	Dry Day		Rainy Day	
	Mean frequency	CFU/10mins /m ²	Mean frequency	CFU/10mins /m ²
<i>Pseudomonas spp</i>	4	0.024	5	0.030
<i>Bacillus badius</i>	5	0.030	0	0
<i>S. saccharolyticus</i>	13	0.078	5	0.030
<i>Staphylococcus aureus</i>	48	0.288	3	0.018
<i>Lactobacillus kitasatonis</i>	12	0.072	2	0.012
<i>Macrocooccus brunensis</i>	9	0.054	1	0.006
<i>Bacillus smithii</i>	20	0.120	2	0.012
<i>Staphylococcus massiliensis</i>	15	0.090	1	0.006
<i>Streptococcus parasuis</i>	15	0.090	3	0.018

Table.6 Mean frequency and CFU of Enteric Bacteria from sample site 2 (RSU poultry farm)

Organisms	Dry Day		Rainy Day	
	Mean frequency	CFU/10mins/m ²	Mean frequency	CFU/10mins/m ²
<i>Escherichia coli</i>	31	0.186	3	0.018
<i>Serratia marcescens</i>	4	0.024	5	0.030
<i>Enterococcus faecalis</i>	5	0.030	0	0
<i>Klebsiella pneumoniae</i>	13	0.078	5	0.030
<i>Proteus mirabilis</i>	48	0.288	3	0.018
<i>Enterobacter cloacae</i>	12	0.072	2	0.012
<i>Hafnia alvei</i>	9	0.054	1	0.006

Fig.1 Percentage frequency of occurrence of fungal isolates from sample site 1 (Saw Mill)

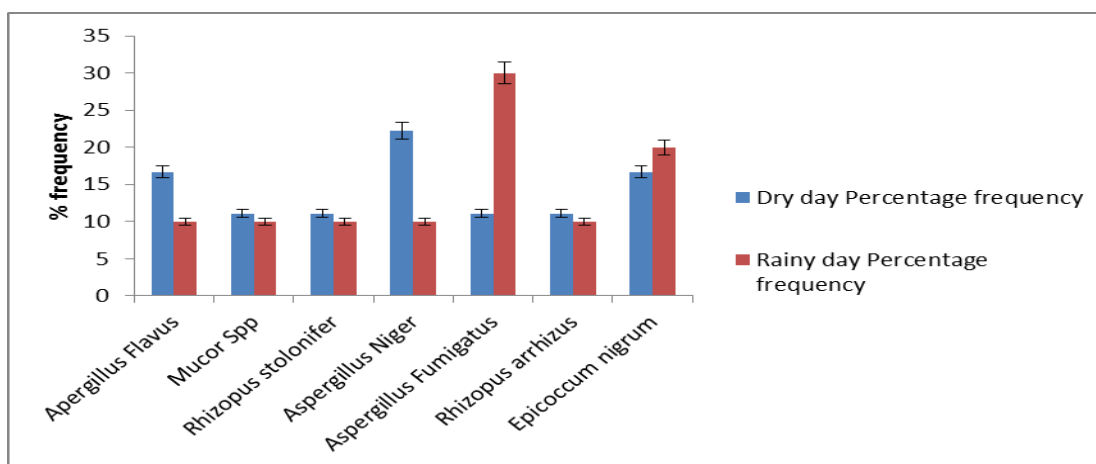


Fig.2 Chart showing percentage frequency of heterotrophic bacteria isolates from sample site 1 (saw mill)

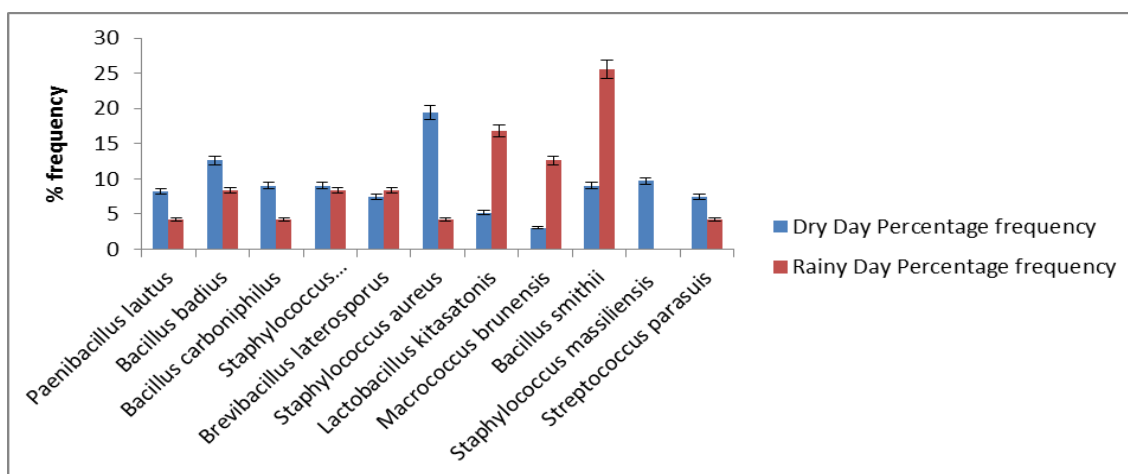


Fig.3 Percentage frequency of enteric bacteria isolates from saw mill

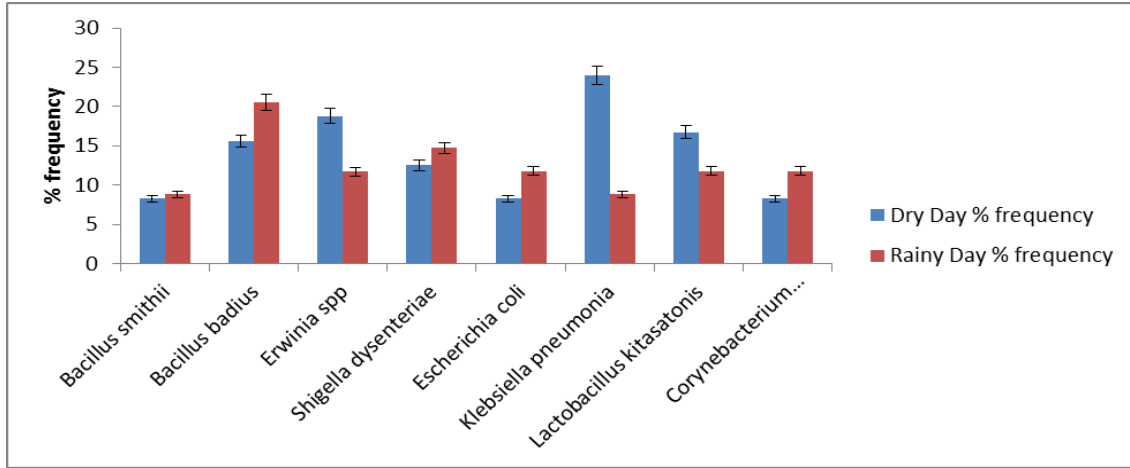


Fig.4 Percentage frequency of aerobic fungi from sample site 2

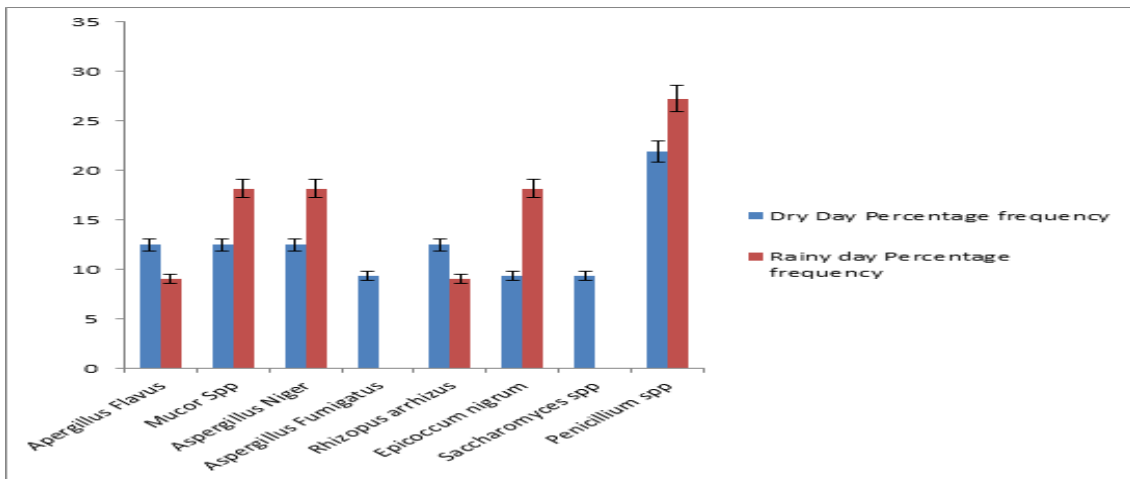


Fig.5 Percentage frequency for heterotrophic bacteria from sample site 2 (RSU poultry farm)

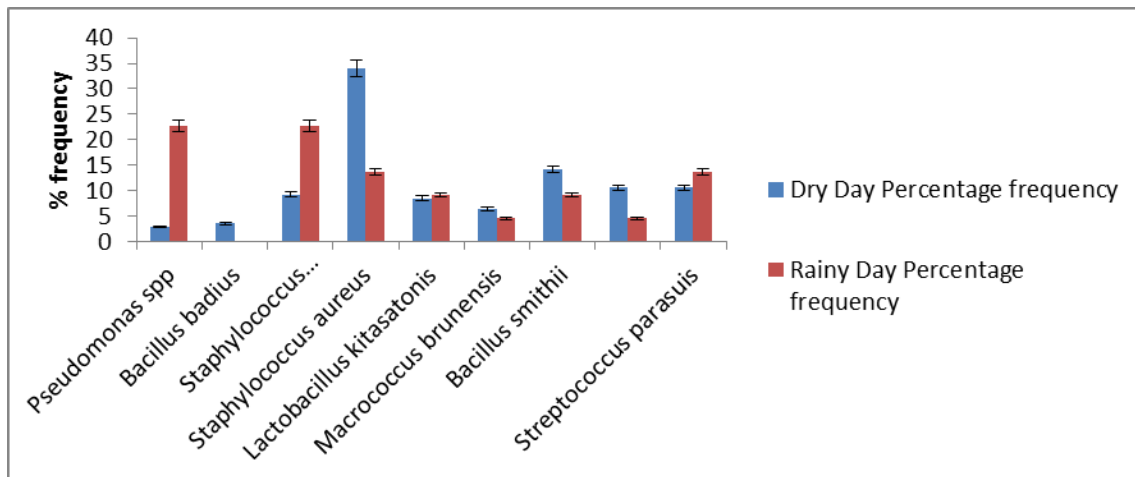
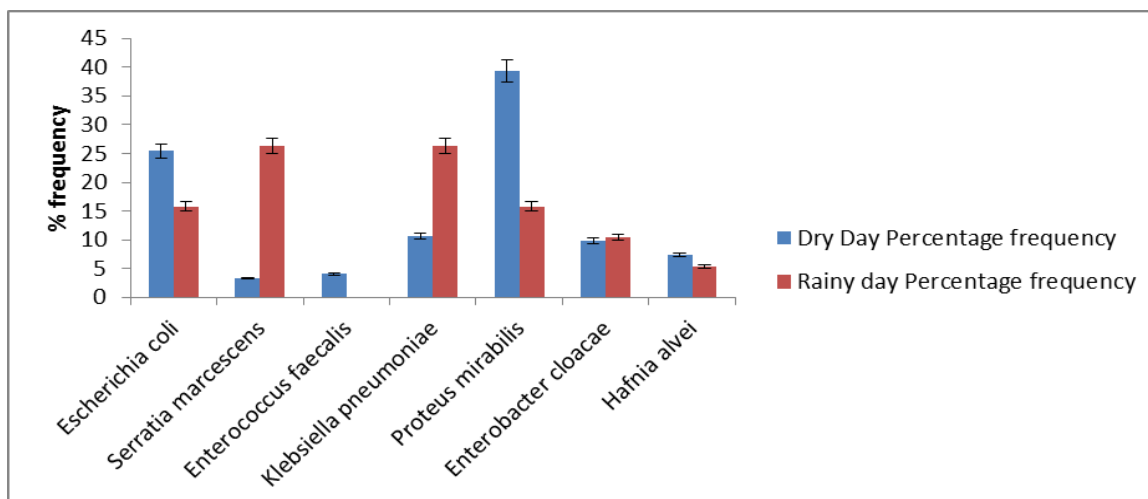


Fig.6 Percentage frequency of enteric bacteria from sample site 2 (RSU poultry farm)



Based on this study, it was concluded that the anthropogenic activities of man, such as the Sawmill and poultry farm give rise to organic dusts and organic dust inhalation results in many acute and chronic diseases of the pulmonary tract especially in immune-compromised individuals. This work revealed the microorganisms associated with organic dusts and discovered some pathogenic bacteria and fungi that can cause serious infections and inflammation of the respiratory tract. The first and fundamental step in the control of organic dust hazards is their recognition, but recognition requires a clear understanding of the nature, origin, mechanism of generation and release of the particles, as well as knowledge on the conditions, of exposure and possible associated side effects.

Recommendation

It is recommended that exposed workers wear the most practical respirators with the high assigned protection factor (APF).

The poultry farm should be well ventilated so as to reduce dusts.

Health education and periodic medical examination of individuals exposed to

organic dust should be practised.

Regular cleaning of poultry and saw-mill environments should be observed.

Proper personal hygiene should be encouraged amongst personnel working in the poultry and saw-mill.

The use of protective gears such nose mask, helmets, and safety boots should be encouraged amongst workers and visitors within the facilities.

Immuno-compromised individuals should avoid exposure to organic dust prone areas and affected individuals should consult a physician for medical check-up.

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