

Studies on the Mycoflora of the Outdoor Air Environment of Delta State University Site III, Abraka, Nigeria

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Abstract

Fungal spores are potential source of different types of environmental diseases, causing harm to humans, animals, and plants. This study is aimed at isolation and identification of fungal flora of site III outdoor environment of Delta State University, Abraka. This was determined by using open plat technique method at five different locations for the period of six (6) months (November 2014 to April 2015). Potato Dextrose Agar (PDA) supplemented with chlorophenicol to inhibit the growth of bacteria was used for enumeration of fungal concentrations in the environment. Media- filled petri dishes were exposed to the air for five (5) minutes at human breathing level of 1.5m. Exposed plates were incubated in the laboratory and observed fungal growth were subcultured and identified microscopically. A total of nineteen fungal genera with one thousand eight hundred and forty colonies were Isolated and identified with varying frequencies of occurrence. *Aspergillus flavus*, *Cercospora* sp. *Fusarium saloni*, *Alternaria alternata*, *Aspergillus Niger*, and *Spizellomyces* sp. were found to be frequently occurring in all the five locations studied. High concentrations of fungi were observed in December which may be due to cold, dryness, and harmattan wind. Majority of the identified fungal species are characterized as allergenic, hence exposure to their spores has great implication to environment and human health.

Keywords: Isolation, identification, mycoflora, outdoor environment, Delta State University

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1. Introduction

Fungi are multicellular, achlorophyllous, heterotrophic, and eukaryotic and spore bearing organisms surrounded by a well-defined cell wall made up of chitin with or without fungal cellulose, along with many other complex organic molecule.

Fungi are unique organisms due to their morphological, physiological and genetic features. They are able to colonize all matrices (soil, water and air) in natural environment in which they play key role in maintaining the ecosystem equilibrium (Hussain *et al.*, 2014). Fungi secrete enzymes which breakdown solid materials to soluble compounds for absorption through their outer walls. They vary greatly in their ability to utilize different types of substrates although some species that are obligate parasites are only able to utilize substrates from living host tissue (Culling *et al.*, 2010).

The fungi constitute a major eukaryotic lineage equal in numbers to animals and exceeding plants. The group includes molds, yeast, mushrooms, polypores, plant parasitic rusts and smuts and *Penicillium chrysogenum*, *Neurospora crassa*, *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. Fungal infection is one major cause of environmental diseases, both animals and plants diseases causing significant economic loses in the commercialization phase which are rendered unfit for human consumption (Chiejina and Ukeh, 2013).

Air pollution is the addition of any harmful substance to the atmosphere. Due to industrialization and urbanization, air pollution has become a major threat to human and environment. Pollutants can be classified as particulate and gaseous matter. The composition of particulate matter depends on the source, aerolization mechanism and environmental condition prevailing at the site (makut *et al.*, 2014).

Several sources are found to be responsible for emission of these bioaerosols in air which include natural sources such as soil, water, plants and animals and human as well as anthropogenic sources like agricultural practices, healthcare unit, industrial operation (Cullinan *et al.*, 2001).

Exposure to airborne pathogens is a common denominator of human life. The improvement of research methods for studying airborne pathogens has become necessary from the evidence that microorganisms (e.g. fungi and other microorganism) from an infectious source may disperse over very great distance by air currents and ultimately be inhaled, digested or come into contact with individuals who have had no contact with the infectious source. Exposure to bioaerosols, including the fungal spores has been linked to a range of detrimental health effect (Douwes *et al.*, 2003). For instance, molds are known to be associated with the onset of asthma in both infants (Jaakkola *et al.*, 2010) and adults (Kurvula *et al.*, 2010).

Air quality is one of the most significant factor affecting the health and well-being of people. It has been reported that a single person inhales an average of approximately 10m³ of air every day (Dacarro *et al.*, 2003). However, the air inhaled by people is abundantly loaded with fungi and other microorganism like virus, bacteria, plant pollen and fragments of plants tissues (Karwowska, 2005) which form part of bioaerosol (Makut *et al.*, 2014).

Biological contamination of air is mostly caused by fungi and other microorganism. They can be dangerous as pathogenic living cells but they also secrete some substances harmful to human health (Makut *et al.*, 2014). Airborne microorganisms are usually derived from various natural environments such as soil, animals and human (Makut *et al.*, 2014).

Human activities such as sewage treatment, plant and animal rendering, fermentation processes and agricultural activities emit microorganism into the air. Exposure to outdoor air microorganism like fungi has been associated with allergic respiratory symptoms, asthma exacerbation, asthma related deaths and infections (Hussain *et al.*, 2014).

Based on the above implications, this study was therefore aimed at isolation and identification of the mycoflora of outdoor air environment of Site III of Delta State University, Abraka. It is hope that this study will provide baseline information for effective diagnostic measures against allergic reactions in humans and other environmental matters.

Materials and Methods

Study Area

The study was carried out at Site III of Delta State University, Abraka. Delta State University is located in Abraka which lies between latitude $5^{\circ} 45'$ and $5^{\circ} 50'$ North of the equator and longitude 6° and $6^{\circ} 15'$ East of Greenwich meridian. It is bounded on the north by Edo State's Orhionmwon Local Government Area. Abraka is bounded by Ukwuani and Ughelli North Local Government Areas to the east and south respectively. It is bounded on the west by Agbor plain. Abraka is in Ethiope East Local Government Area of Delta State Nigeria (latitude of $5^{\circ} 47'N$ and longitude of $6^{\circ} 60' E$) (Figure 1).

Samples were collected from five (5) locations in site III environment which include Pre-Degree (PD), Basic Medical Sciences (BMS), Faculty of Social Sciences (FSS), Faculty of Pharmacy (PHM) and New Halls (NH).

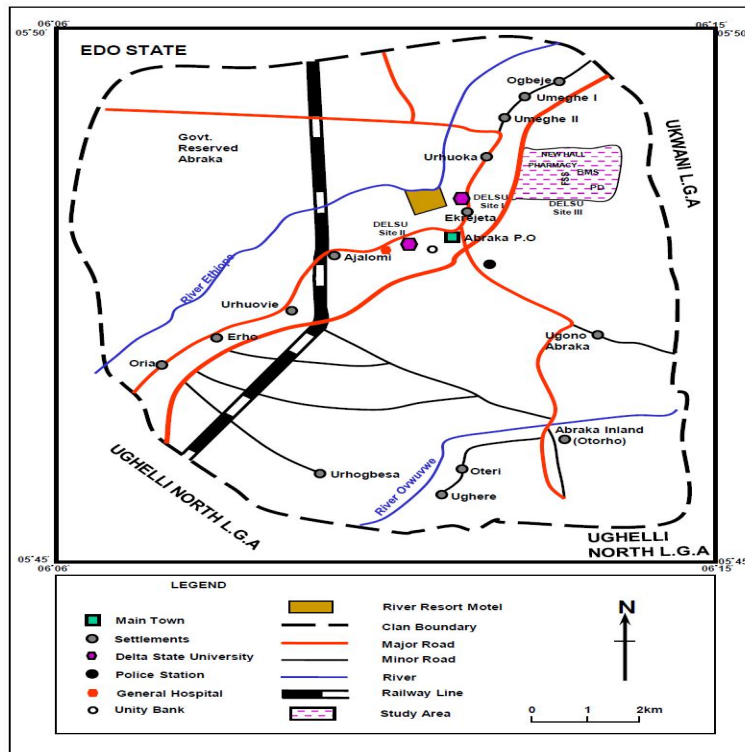


Figure 1: Map of Abraka showing Site III, Delta State University, Abraka

Culture Medium

Potato Dextrose Agar (PDA) supplemented with chlorophenicol to inhibit the growth of bacteria was used for enumeration of fungal spores in the environment.

Air Sample Collection and Laboratory Studies

Method of sample collection was by open plate technique and exposure was for 5minutes. The sampling was done once a week for the period of six (6) months. The Petri dishes were covered, labeled, and taken to the Department of Botany laboratory.

The exposed plates were incubated at room temperature ($30\pm 2^{\circ}\text{C}$) for 2-3 days (and are left for further incubation for the development of slow-growing organisms). Observed colonies were subcultured aseptically to obtain pure cultures, isolates were identified by observation of the cultural morphology and microscopic examination at mag X40 using the mycological manual by Barnett and Hunter (1999). Frequency of occurrence of all isolates was calculated from the formula:

$$\frac{\text{No. of times a fungus is isolated}}{\text{Total no. of times all fungi were isolated}} \times \frac{100}{1}$$

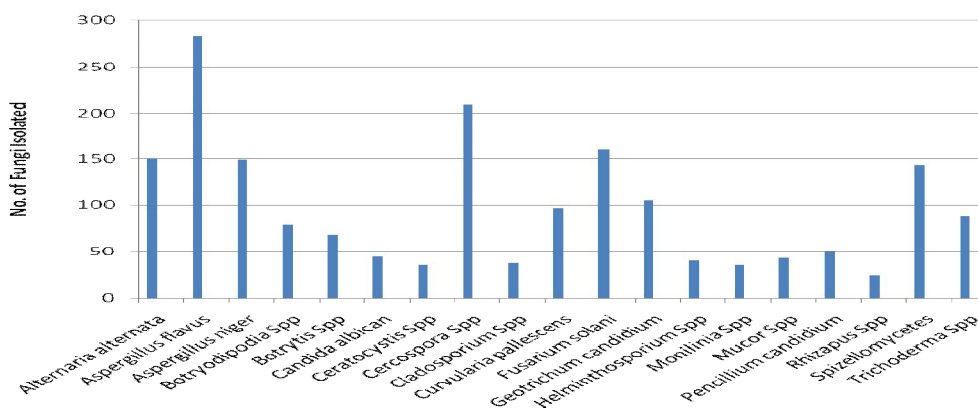
Results

A total of nineteen fungal genera with one thousand eight hundred and forty colonies were observed. These fungi include *Aspergillus*, *Alternaria alternata*, *Trichoderma* Sp., *Curvularia pallescens*, *Spizellomyces* sp., *Cercospora* sp. among others. All the fungi isolated with their frequency of occurrence in the Site III environment is presented in Table 1. Table 2 presents the isolated fungi in their various classes. The summary of all the fungi isolated and identified in the study is represented in figure 1. Fungi isolates at each location of study are represented in figures 2 - 6. The percentage of fungi isolated at each months of study is represented in Figure 7.

Table1: Fungi isolated in the study locations with their frequency of occurrence

Fungi Isolated	Number of times Isolated					Total	Frequency of Occurrence (%)
	BMS	FSS	PHM	NH	PD		
<i>Alternaria alternata</i>	40	50	17	15	29	151	8.21
<i>Aspergillus flavus</i>	50	57	82	56	38	283	15.38
<i>Aspergillus niger</i>	38	20	31	40	20	149	8.09
<i>Botryodipodia</i> sp.	12	6	40	8	13	79	4.29
<i>Botrytis</i> sp.	5	9	7	17	30	68	3.70
<i>Candida albican</i>	9	0	10	14	11	44	2.39
<i>Ceratocystis</i> sp.	0	9	11	0	15	35	1.90
<i>Cercospora</i> sp.	38	60	40	50	21	209	11.36
<i>Cladosporium</i> sp	8	7	10	12	0	37	2.01
<i>Curvularia pallescens</i>	22	21	7	34	13	97	5.27
<i>Fusarium solani</i>	32	18	70	19	21	160	8.70
<i>Geotrichum candidum</i>	0	31	20	35	16	105	5.71
<i>Helminthosporium</i> sp.	5	10	0	7	18	40	2.17
<i>Monilinia</i> sp.	14	0	3	8	10	35	1.90
<i>Mucor</i> sp.	7	7	4	5	20	43	2.34
<i>Penicillium candidum</i>	9	12	8	20	0	49	2.66
<i>Rhizopus</i> sp.	5	3	2	22	3	24	1.30
<i>Spizellomycete</i> sp.	50	10	29	40	15	144	7.83
<i>Trichoderma</i> sp.	7	9	17	27	28	88	4.78

Key: BMS= Basic Medical Science, FSS= Faculty of Social Science, PHM= Pharmacy



NH= New Hall and PD= Pre-degree

Figure 2: Number of Fungi Isolated from all the locations of study

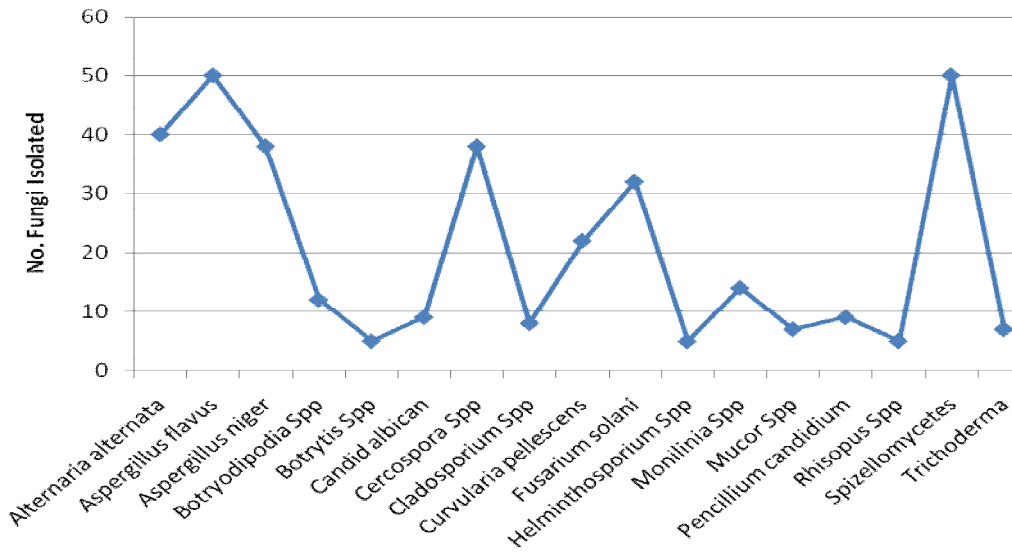


Figure 3: Fungi isolated from location BMS

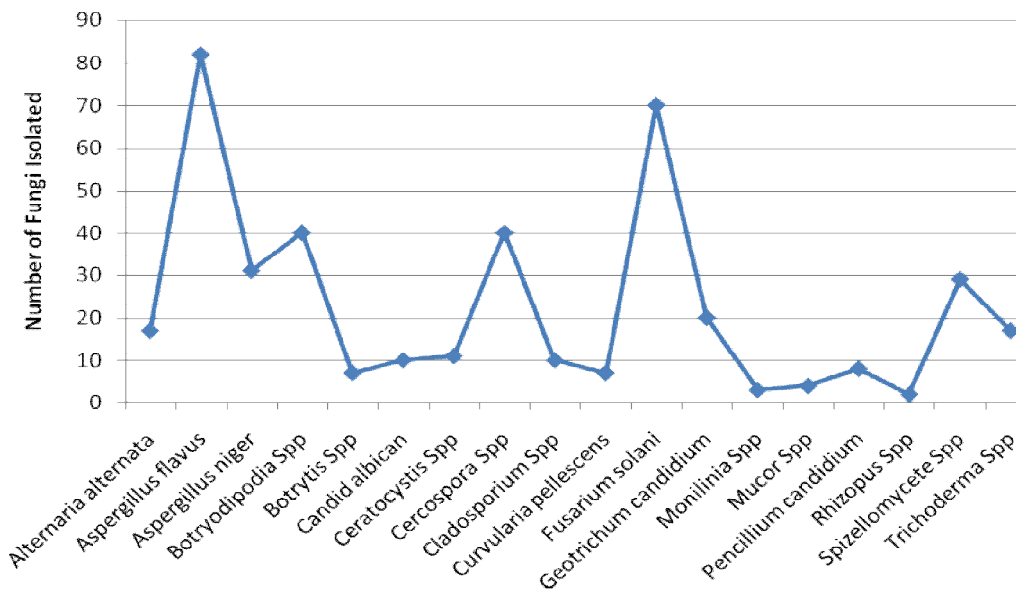


Figure 4: Fungi isolated from FSS

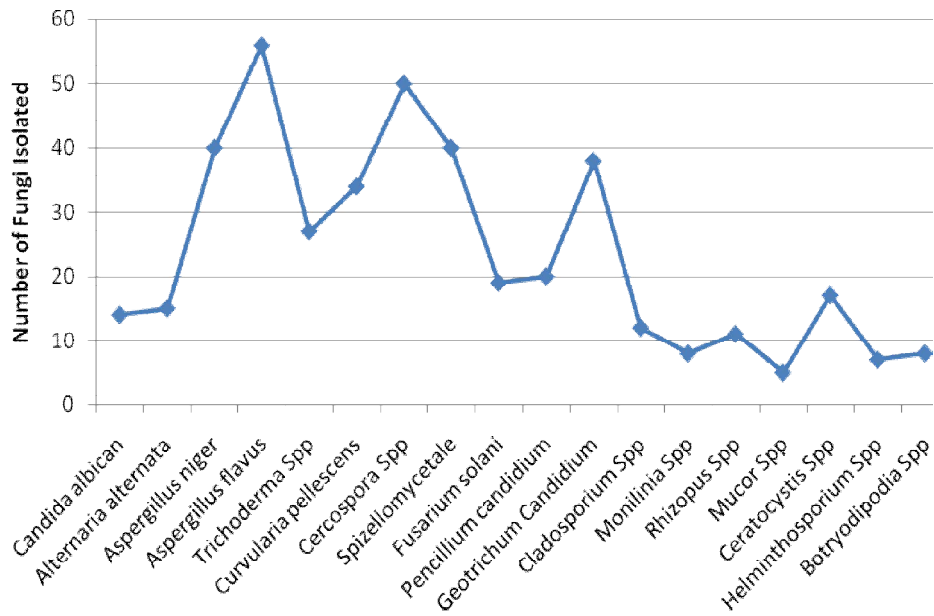


Figure 5: Fungi isolated from PHM

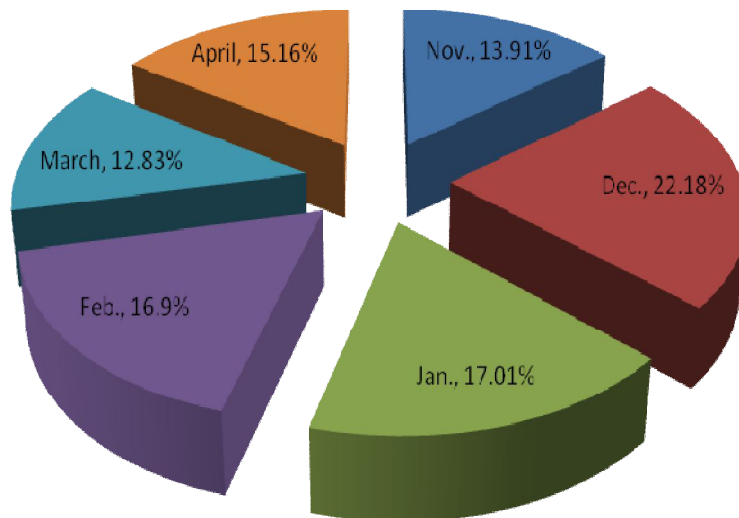


Figure 6: Fungi isolated from NH

Table 2: Class of isolated fungi

Fungi Isolated	Class
<i>Alternaria alternata</i>	Dothideomycetes
<i>Aspergillus flavus</i>	Eurotiomycetes
<i>Aspergillus niger</i>	Eurotiomycetes
<i>Botryodiplodia</i> sp.	Sordariomycetes
<i>Botrytis</i> sp.	Leotiomycetes
<i>Candida albican</i>	Saccharomycetes
<i>Ceratocystis</i> sp.	Sordariomycetes
<i>Cercospora</i> sp.	Dothideomycetes
<i>Cladosporium</i> sp.	Dothideomycetes
<i>Curvularia pallescens</i>	Euascomycetes
<i>Fusarium solani</i>	Sordariomycetes
<i>Geotrichum candidum</i>	Sacchromycetes
<i>Helminthosporium</i> sp.	Dothedeomycetes
<i>Monilinia</i> sp.	Leotiomycetes
<i>Mucor</i> sp.	Mucormycotina
<i>Penicillium candidum</i>	Eurotriomycetes
<i>Rhizopus</i> sp.	Mucormycotina
<i>Spizellomycete</i> sp.	Chytriodiomycetes
<i>Trichoderma</i> sp.	Sordariomycetes

Discussion

This study evaluates the mycoflora of Site III, Delta State University, Abraka. The fungi isolated from various locations during the period of study include *Aspergillus flavus* (15.38%), *Alternaria alternata* (8.21%), *Aspergillus niger* (8.09%), *Spizellomycete* (7.83%), *Trichoderma* sp. (4.78%), *Penicillium candidum* (2.66%) and *Candida albican* (2.39%). *Curvularia pallescens* (5.27%), *Cercospora* sp. (11.36%), *Fusarium solani* (8.70%), *Geotrichum candidum* (5.71%), *Cladosporium* sp (2.01%), *Monilinia* sp (1.90%), *Rhizopus* sp (1.30%), *Mucor* sp (2.34%), *Ceratocystis* sp (1.90%), *Botrytis* sp (3.70%), *Helmnithosporium* sp (2.17%), and *Botryodipodia* sp (4.29%).

Most of these fungi have been reported locally in Nigeria and elsewhere in the world. They include works by Dongo and Ayodele, (1997), Makut *et al.* (2014), Syed and Sarangi, (2013), Swapna *et al.* (2012). Some of these fungi recorded low incidence while others high incidence which may be due to time of survey. Of all the fungi isolated, *Aspergillus flavus*, *Cercospora* sp., *Fusarium solani*, *Alternaria alternata*, *Aspergillus niger* and *Spizellomyces* were the most prevalent in all the locations studied. *Aspergillus*, *Penicillium* and *Fusarium* are common contaminants of agricultural commodities. Aflatoxins are known to be produced by *Aspergillus*, *Fusarium* and other fungi as reported by Dutta *et al.* (2010).

Frequent and high level of exposure to spores of *Aspergillus* sp during dry season could lead to pulmonary Aspergillosis when the spores are inhaled. Individuals with low immunity are vulnerable to infection from *Aspergillus* sp. (Shiaka and Yakubu, 2013). Fungi infection is one of the most significant factor affecting the health and well-being of people. Exposure to *Candida albicans* can cause skin reaction (Haleen and Mohan, 2012). Most Fungi are known to be associated with asthma both in infants (Jaakkola *et al.*, 2010) and adults (Karvula *et al.*, 2010).

Most allergenic fungi which include *Cladosporium*, *Penicillium*, *Alternaria*, *Fusarium* and *Curvularia* have been reported (El-Gali and Abdullrahman, 2014). Similarly, various species of *Alternaria*, *Botrydiplodia*, *Cercospora*, *Culvalaria* and *Helminthosporium* have been implicated as leafspot pathogens of various crops (Agrios, 2005).

Various fungi were not encountered in some locations of study such as *Ceratocystis* sp. (BMS and NH), *Candida albicans* and *Monilinia* sp. (FSS), *Helminthosporium* (PHM), *Cladosporium* sp. and *Penicillium candidum* (PD).

The result is comparable to those from previous studies by Ayanbimpe *et al.* (2010), Dutta *et al.* (2010), El-Gali and Abdullrahman, (2014) and Njokuocha and Agwu, (2007). In this study, the highest percentages of fungi were isolated in the month of December which may be due to cold, dryness, and harmattan wind. This finding was similar to the work of El-gali and Abdullrahman (2014), Hussain *et al.* (2014) and Karita *et al.* (2014).

Conclusion

Good air quality is source of life for all living souls and the source of this essential commodity is the environment. Over the years, due to man's quest for financial freedom, development and civilization, man's activities in his environment had lead to environmental pollution in different forms.

Biological contamination of air is one of the most serious issues of the environment because of the health problems associated with it. On this note, it is important to evaluate the quality of air human breathe whether indoor or outdoor especially the urban area where there are high vehicular traffic, human activities involving rapid movements. The number and types of air microorganism can also be used to determine the degree of cleanliness as a means of determining source of human discomfort and certain air microbial infections.

Recommendation

The consequences of air contamination with fungi indicate that much is to be done in identifying and managing air quality deficiencies and seeing that our environment, food items and even human skin are contaminated by fungal spores, and owing to health challenges of these fungal spores.

It is therefore recommended that the environment should be sanitized and fumigated periodically, food items be properly washed before eating, individuals should make sure that snacks are in enclosed medium and human skin bathed regularly to reduce fungal infections. To further reduce fungal growth, the environment must be kept clean.

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