EVALUATION OF LOADS AND HUMAN SKIN DISEASE-CAUSING POTENTIAL OF FUNGAL CONTAMINANTS IN WASTES DUMPSITE ATMOSPHERE IN UYO, NIGERIA

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ABSTRACT

The settle plate culture technique was employed to estimate fungal loads of wastes dumpsite atmosphere in Uyo using Saboraud Dextrose Agar (SDA) and Dermatophytic Medium (DM) respectively for moulds/yeasts and dermatophytes respectively. The fungal contaminants included detectable loads of dermatophytic fungi. The estimated loads of fungi per volume of air revealed mean counts of 2.3 log CFU/m^3 and 0.89 log CFU/m^3 for fungi and dermatophytes respectively. Fungal spore loads were higher during the dry season with mean counts of 4.3 log CFU/m^3 although the average density of dermatophytes was slightly lower (0.8 log CFU/m^3). GIS model of spatial distribution of fungal contaminants including dermatophytes in the dumpsite atmosphere revealed a poor spatial distribution pattern. Localized and high number of contaminants were noticed in the South West region of the dumpsite during both seasons. However the numbers of mesophilic fungi obtained by the sedimentation technique were higher than APHA's standard (30 CFU/15 mins or 1.4 log cfu/m^3) for settling technique. A total of 25 fungal species comprising 17 hyphomycetes and 5 yeast species were encountered. Isolates included dermatophytic strains of *Candida albicans, C. pseudotropicalis, Cladosprium* sp, *Penicillium citinum*, *Pichia* sp, *Microsporum* and *Trichophyton*. The presence of dermatophytes in the air portends danger. These isolates elaborated bright red pigments on Dermatophyte Test Medium (DTM), an indication of their ability to cause superficial mycoses or dermatophytoses on susceptible humans. The public health risk is high because airborne fungal cells (yeasts, moulds, spores) can remain viable for much longer periods, even at low relative humidity and high or low temperature extremes.

INTRODUCTION

Air will often contain micro-organisms such as viruses, bacteria, and fungi. None of these actually live in the air, the atmosphere tends to kill off most of them. However, they are frequently transported attached to other particles, such as skin flakes, soil, dust, or dried residues from water droplets. Aggregation of cells into clumps can enhance the survival of airborne flora. Bacterial cells when they become airborne normally rapidly die – within a few seconds, due to evaporation of water associated with the particle. Airborne fungal cells (yeasts, moulds, spores) can remain viable for much longer periods, even at low relative humidity and high or low temperature extremes (Bamba *et al.*,2014). This is attributable to the fact that fungi are better adapted to survive in the air by forming spores. The spores of common air-borne fungi have thick melanized walls which contain complex carbohydrates. These carbohydrates are hydrophobic and waxy which enables the control of water loss. Fang *etal*. (2005) also attributed the high incidence of fungi to the effect of sunlight which causes spore release. Thus, with higher humidity, higher bioaerosol levels can prevail.

A lot of fungal species are involved in biodegradation of MSW. In fact, fungi are better degraders of MSW than bacteria (Eja *et al*., 2010). *Saccharomyces* sp, *Candida pseudotropicalis*, *Candida tropicalis*, *Aspergillus* sp, *Penicillium* sp, *Fusarium* sp,

Cladosporium sp, *Mucor* sp, *Phizopus* sp, *Zygonlynchus* sp, *Neurospora* sp, *Alternavia* sp, *Cephalosporium* sp, *Absidia*sp, *Trichoderma* sp, *Helminthsporium* sp, *Pullaria* sp and *Triammidum* sp are among the fungal species commonly encountered in MSW (Eja*et al*., 2010). Many fungal species including *Saccharomyces* sp., *Candida tropicalis*, *Candida pseudotropicalis*, *Aspergillus niger*, *Aspergillus fumigatus*, *Fusarium* sp, *Penicillium frequentans*, *Penicillium expansun*, *Cladosporium*sp and *Mucor* sp are associated with the degradation of MSW (Essien, *et al.*, 2011).Sharma and Shah (2005) reported thepresence of thermophilic strains of *Geotrichumcandidum*, *Aspergillus fumigatus*, *Mucor pusillus*, *Chazetomiumthermophila*, *Thermoascusauranticans* and *Torulathermophila* in organic wastes dump.

One of the features distinguishing the fungal pathogens of man from their bacterial counterparts is the fact that unlike the later for whom parasitism is the chief mode of life, the former can for the most part exist as saprophytes in the soil or some organic debris only incidentally – infecting man and/or animals. The most common of these MSW fungal pathogens are dermatophytes (Indira, 1968) Dermatophytes and keratinophytic pathogen fungi such as *Trichophyton ajelloi*, *Microsporun gypseum*, *M. cookei*, *Trichophyton terrestrie*, *Chrysosporium tropicum*, *C. aspertum*, *C. keratinophilum*, *Arthroderma tuberculatum*, *Ctenomyces serratus*, *Nocardia asteroids*, *Histoplasma capsulatum* and *Allescheria boydii* have been isolated from MSW dumps in Ohio, USA (Kurup and Schmitt, 1970), Gugnani *et al*. (2007) also reported that pathogenic fungi of the species *Penicillium marneffei*, *Pseudallescheria boydii*, *Trichosporon asteroids*, *Scytalidium hyalinum*, *Trichophyton mentagrophytes* and *Microsporum gypseum* were isolated in large numbers in the rat burrows associated with MSW in India and Nepal. There no information on the loads and virulence of fungi in MSW dumpsites atmosphere in the Niger Delta of Nigeria. This study was carried out to estimate loads and virulence of fungi in wastes dumpsite atmosphere in Uyo

MATERIALS AND METHODS

Study Area

The dumpsite environ investigated are located within Uyo metropolis in Akwa Ibom State, Nigeria (Fig.1). The site investigated at the time of study was located along the Udo Street end of the ravine, between latitude $05^{o}02^{I}3.33^{II}$ N and longitude $007^{o}56^{I}11.8^{II}$ E at an elevation of 53 meters above sea.

Estimation of Fungal Loads of Dumpsite Atmosphere

The settle plate culture technique was employed to estimate fungal loads (APHA, 1998; Downes and Ito, 2001). The density of fungi (yeast and molds) in was determined using Saboraud dextrose agar (SDA) according to methods proposed by APHA (1992). The mediumwere fortified with 50µg/ml of streptomycin for the selective enumeration and isolation of fungi. Also estimated was the density of fungi that can cause skin infections. The Dermatophytic Medium (DM) was employed to estimate the loads of dermatophytes.

Freshly prepared SDA and DM plates (9 cm diameter) were exposed and distributed at each sample station using 4ft high wooden platforms and exposed for 15 minutes. The samples were obtained from 10 sampling stations. At the end of exposure, the plates were closed, transported to the laboratory and then incubated at 28 ± 2 ºC (room temperature) for 4 days for fungi (yeasts and molds). After incubation the organisms were counted with the aid of a Quebec colony counter and the number of colonies estimated using the Polish standard PN89/Z-04008/8 (Bhatica and Vishwakarma, 2010) and expressed as:

$$CFU/m^3 = a.1000/pt \quad 0.2$$

Where, a = number of colonies on Petri dish
p = Surface diameter of Petri dish
t = Exposure time

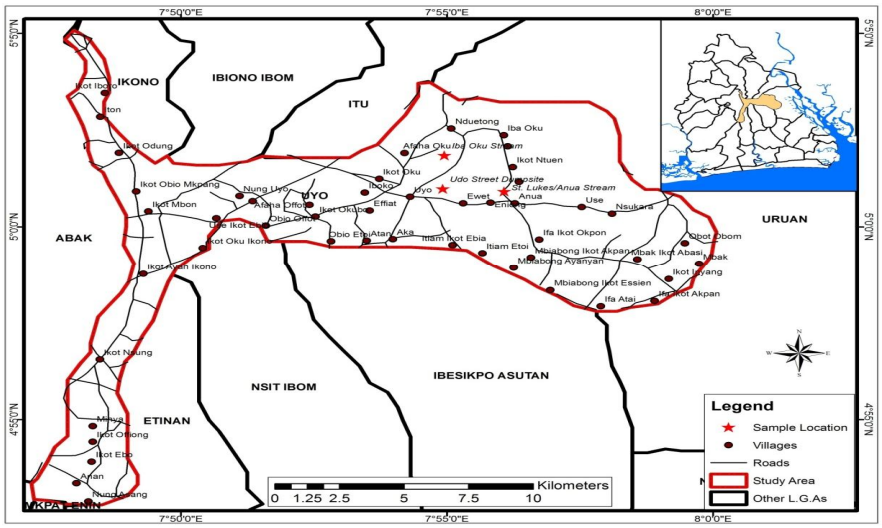


Figure 1: Uyo Metropolis showing the locations of dumpsites investigated

Determination of Spatial Variations in the Fungal Loads of the Dumpsites Atmosphere
Geographic information system (GIS) was adopted to perform dynamic modeling of the fungal spores distribution pattern in the dumpsite atmosphere. This involves establishing the spatial variations through a period of time. To achieve the goal, the GIS-based pollution mapping which uses interpolation techniques such as distance weighting and kriging was employed (Kunzli *et al*., 2015).

Characterization of Fungal Isolates
The characterization of fungal isolates was carried out according to the taxonomic scheme of Domsch *et.al.* (1980), Samson *et. al.*, (1984) and, Barnett and Hunter (1987). Their identification were based mainly on their cultural and morphological characteristic. Dermatophytes and Yeast isolates were also subjected to series of biochemical tests as described by Larone (1995) and Agarwal *et al.,* (2011).

Evaluation of Skin Disease Causing Potential of the Fungal Isolates
This test was done to identify fungi that commonly cause skin diseases in humans and animals especially fungi of the genera: -*Microsporum*, *Epidermophyton* and *Trichophyton*. Suspected isolates were streaked on to plates of Dermatophyte Test Medium (DTM) which had been autoclaved at 121^{o}C for 15 minutes, then cooled to 45^{o}C. Plates were brought to room temperature, and the agar surface dried prior to inoculation. The specimen was incubated directly onto the medium by pressing the specimen lightly onto the surface of the agar, and then incubated at room temperature (20-30^{o}C) for up to 10-14 days. The plate was daily examined for colour change. Development of red colour change in the medium, with white aerial hyphae indicated positive reaction. Most pathogenic dermatophytes will produce a colour change in 3 to 6 days. Negative results will be without colour change to red (Campbell and Stewart, 1980).

RESULTS AND DISCUSSION

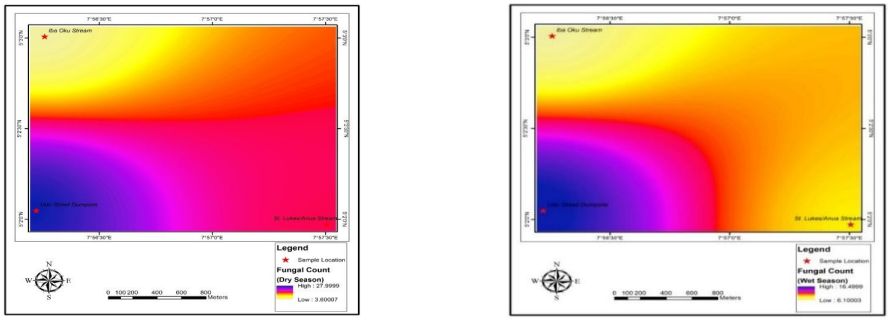
The estimated loads of fungi per volume of air revealed mean counts of 2.3 log CFU/m^3 and 0.89 log CFU/m^3 for fungi and dermatophytes respectively. Fungal spores load were higher during the dry season with mean counts of 4.3 log CFU/m^3 although the average density of dermatophytes was slightly lower with mean count of 0.8 log CFU/m^3 during the season. (Table 1) However, the airborne microbial standards as recommended by Polish Standard, PN89/JZ – 0400818 are as follows: For fungi :> 10.0 log CFU/m^3 polluted air, posing a hazard for human environment; 5.0-10.0 log CFU/m^3 polluted air – with a potential negative effect on human environment and 3.0 – 5.0 log CFU/m^3 – approximately clean atmospheric air. When these limits are compared with the fungal loads obtained in the present study, it is suggestive that the

MSW dumpsite atmosphere investigated is almost clean of fungal concentrations although the presence of dermatophytes in the air portends danger.

Table 1: Moulds and Dermatophytic Fungal loads (log cfu/m^3) of the atmosphere at the MSW dumpsites and the environ during the wet season

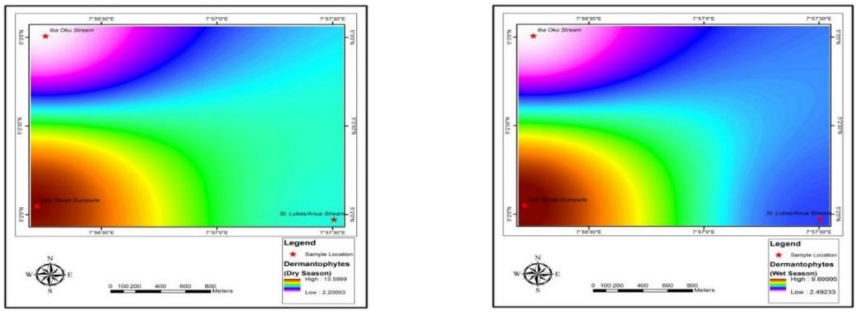
Sample Location	Sample ID	Fungal count	Dermatophytes
	WET		
Udo St. Dumpsite	BAS-1	2.51	0.60
	BAS-2	2.65	0
	BAS-3	2.52	3.34
	BAS-4	0.25	0.54
	BAS-5	3.57	0
Mean		2.3	0.89
	DRY		
	BAS-1	3.74	0.38
	BAS-2	4.92	0.38
	BAS-3	2.86	0.50
	BAS-4	4.40	0.64
	BAS-5	5.64	2.08
Mean		4.3	0.8

Special distribution analysis using GIS modelling has shown that fungal counts were high in the South West region both in the wet and dry seasons (Fig. 1). For dermatophytes, it was the same observation, where more was found in the South West zone (brown band) in the main dumpsite for both wet and dry seasons (Figure 2). From the GIS spatial distribution outlay, it is apparent that those people living around the Udo Street dumpsite are more prone to being infected by the myriads of pathogenic ailments associated with the virulent strains of fungi isolated from MSW dump environ.



(a) dry season (b) wet season

Figure 1: Spatial Distribution of Fungal Count in the dumpsite impacted atmosphere during the (a) dry and (b) wet seasons



(a) dry season (b) wet season

Figure 2:: Spatial Distribution of Dermatophytes in the dumpsite impacted atmosphere

The taxonomic attributes of the fungal isolates have shown that a total of 25 fungal species were encountered (Table 2).

Table 2: Occurrence and Virulence of fungi isolated from MSW impacted atmosphere

Isolate	Atmosphere (n=12)	Growth on DTM
A. *flavus*	+ (8)	-
A. *fumigatus*	+ (7)	-
A. clavatus	+ (2)	-
A. *carbonarius*	+ (3)	-
A. *glaucus*	+ (5)	-
A. *terreus*	+ (8)	-
A. *restrictus*	+ (1)	-
Aspergllusniger	+ (8)	-
A. *ochraceous*	+ (2)	-
A. *parasiticus*	+ (6)	-
Candida albicans	+ (7)	+ (5)
C. tropicalis	+ (6)	+ (2)
Cephalosporium sp	+ (3)	-
Cladosporium sp	+ (7)	+ (4)
Fusarium sp	+ (7)	-
Geotrichumcandidum	+ (8)	+ (3)
Microsporium sp	+ (2)	+ (13)
Mucor sp	+ (7)	-
P. citrinum	+ (4)	+ (4)
Penicilliumitalicum	+ (8)	-
*Pichia*sp	+ (5)	+(2)
Rhizopusstolonifer	1(-)	-
Rhodotorula sp	+ (4)	-
Sclerotium sp	+ (-)	-
Trichophyton sp	+ (5)	+ (10)
Species Richness	25	7

Note: Values in parenthesis are number of isolates obtained from the samples analyzed.
+ = isolate; - = not isolated.

This comprises 17 hyphomycetes and 5 yeast species. Isolates included dermatophytic strains of *Candidaalbicans*, *C. pseudotropicalis*, *Cladospium*sp, *Penicillium citinum*, *Pichia*sp, *Microsporum* and *Trichophyton*. Some isolates of *Cladosporium* and *Penicillium* sp also elaborated bright red pigments on Dermatophyte Test Medium (DTM), an indication of their ability to cause superficial mycoses or dermatophytoses. Their occurrences varied with the sample station and season.

Moulds including *Aspergillus fumigatus* were found in the MSW dumpsite atmosphere. They are commonly encountered on manure, compost, wood, other organic material. Allergic reactions, infections and irritation, toxic syndrome through exposure to organic dusts (ODTS) are commonly associated with moulds in the air. The nature of the dose-response relationship is not known, nor is the existence of a safe exposure threshold. However, concentrations of up to 10^5 CFU/m^3 of *Aspergillus fumigatus* would not be considered a risk for healthy individuals but one spore could be infectious for immunocompromised persons (Forcier, 2002). Dermatophyticmoulds such as *Cladosporium* sp, *Microsporon*sp*Microsporium*sp and *Epidermophyton*sp were also isolated from the waste- impacted atmosphere.

Certain pathogenic micro-organisms present in municipal biosolids, municipal solid wastes, waste from slaughterhouses, manure and other fecal or animal waste matter may end up as bioaerosols. The ones that receive the most attention in terms of risk to human health are the enteric bacteria, viruses and dermatophytes.This confirms the work of Kurup and Schmutt (1970) and Gugnani *et al.* (2007) of the presence of dermatophytes and other keratinophytic pathogenic fungi in MSW dumps in Ohio, USA. It is also known that microbial metabolites or toxins such as endotoxins and mycotoxins are commonly found in contaminated atmosphere. Endotoxins are complexes that are integral parts of the outer membranes of Gram negative bacteria. Their presence is often associated with organic dust while mycotoxins (spores and propagules) are released by moulds. The effects of endotoxins and their role as a bioaerosol are not well known (Olenchock, 1994). Symptoms are cough, shortness of breath, fever, obstruction and inflammation of the lungs, and gastro-intestinal problems. The effects of mycotoxins are not well known. However, symptoms such as skin and mucous membrane irritations, dizziness, immuno-supression, headache, nausea and cognitive effects are commonly associated with the presence of mycotoxins in the atmosphere (Forcier, 2002).Diseases caused by dermatophytes (dermatophytosis) involving the hair, skin or nails include *Tinea capitis, Tinea pedis* and onychomycosis (Midgley *et al.*, 1994; Ghannouum and Perfect, 2009).

CONCLUSION AND RECOMMENDATION

This study has clearly brought to our knowledge the presence of harmful or virulent strains of fungal contaminants in the bio-aerosols of MSW dumpsites. These isolates especially the virulent strains of ringworm-causing fungi (dermatophytes) such as *Candida albicans, Microsporum* sp *Trichophyton* sp elaborated bright red pigments on Dermatophyte Test Medium (DTM), an indication of their ability to cause superficial mycoses or dermatophytoses on susceptible humans. The public health risk is high because airborne fungal cells (yeasts, moulds, spores) can remain viable for much longer periods, even at low relative humidity and high or low temperature extremes. Avoid living close to dumpsites otherwise routine monitoring of MSW dumpsite atmosphere is recommended to address possible outbreak of skin infections amongst inhabitants of dumpsite environ.

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