

## Predominance of Keratinophilic Fungi and Dermatohytes Species in Dumpsite Locations in Ogun State, Nigeria

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### ABSTRACT

This study determined the predominance of keratinophilic fungi and dermatophytes species in dumpsites located within four geopolitical zones of Ogun State, Nigeria using the traditional hair baiting technique. The physiological condition of the soil samples was analyzed to understand the role of ecophysiological parameters on the growth and survival of the inherent organisms of the studied sites while the identification of isolated organisms was carried out based on both micro and macro morphological parameters. A total of ninety four (94) strains of filamentous fungi belonging to thirteen(13) different species namely *Lichtheimia corymbifera* 6.3 (3.19%), *Aspergillus carbonarius* 1 (1.06%), *Aspergillus flavus* 1 (1.06%), *Aspergillus fumigatus* 21(22.34%), *Aspergillus niger* 11(11.70%), *Aspergillus terreus* 9(9.58%), *Aspergillus versicolori* 1(1.06%), *Epidermophyton floccosum* 6(6.38%), *Microsporum gypseum* 15(15.96%), *Penicillium chrysogenum* 4(4.26%), *Trichoderma longibrachiatum*1(1.06%), *Trichophyton mentagrophytes* 16(17.02%) and *Trichophyton rubrum* 5(5.33%) were identified from the sampled dumpsites. All the isolated organisms grew in pH value ranging between 5.8 and 9.1. The Shannon index of diversity was found to be approximately 1.98 while both specie's richness and percentage of specie dominance were 13 and 22.3%, respectively. In conclusion, the dumpsites sampled showed evidence of keratinophilic fungi prone environment and should be given appropriate health attention to avoid this public health nuisance being a vehicle for the transmission of infectious diseases.

**Keywords:** Dermatophytes, Dumpsites, Keratinophilic fungi, Soil, Ecophysiology.

### INTRODUCTION

Soil is the uppermost layer of the earth that provides a heterogeneous and complex environment for all soil inhabitants. They are also known as the main reservoir of different types of microorganisms including fungi among which are keratinophilic fungi (Marchisio, 2000). These keratinophilic fungi are a highly specialized group of fungi that have the ability to degrade native keratin (Malek *et al.*,2013), while their presence in soil is dependent on the nature of such soil and its composition (Irum *et al.*,2007; Kumar *et al.*, 2013).

Deshmukh and Verekar (2006) stated that understanding the epidemiology of soil microorganisms especially dermatophytes and other keratinophilic fungi is important as changes in factors such as ecology, socio-economic, therapeutic and migration processes affect their distribution (Hay,2003; Havlickova *et al.*,2008;Jain *et al.*, 2008). Some of these

keratinophilic fungi especially the dermatophytes group are known for their role in causing dermatophytosis (Ali-Shtayeh and Jamous, 2000) and it occurs predominantly in rural areas and commonly associated with poor personal hygiene, overcrowding and low socio-economic level (Rebollo *et al.*, 2008).

The ability of these organisms to adhere to combs, hats, pillows and theatre seats have also been documented (Arenas, 2002). Many scholars have also documented the ubiquitous presence of these organisms in various environments (Anbu *et al.*, 2004; Mahmoudabadi and Zarrin, 2008; Deshmukh *et al.*, 2010) and their involvement in several superficial and cutaneous infections of keratinized tissues of humans and animals (Bindu and Pavithran, 2002).

In the recent decade, the prevalence of dermatophytosis has reduced significantly in many developed nations unlike in the developing

world due to improved social, economic, healthcare and hygiene practices (Havlickova *et al.*, 2008;). Nigeria as a developing country located in the tropics with a wet humid climate falls into that category with a high prevalence of dermatophytosis especially among primary school children of rural, suburban and urban extract (Gugnani and Njoku-Obi, 1995; Rudy, 1999). This country also has a very high proportion of dumpsites due to indiscriminate refuse disposal by the populace causing contamination of the environment and subsequently upsurge in the abundance of keratinophilic microorganisms. This study was therefore aimed at determining the distribution of keratinophilic fungi and other dermatophytes in dump sites from the four geopolitical zones of Ogun State, Nigeria.

## MATERIALS AND METHODS

### Study Area

The study was carried out in Ogun State, South West Nigeria. The climate is tropical with the rainy season from March to November, followed by the dry season. Average annual rainfall varies from 128 mm in the southern parts of the state to 105 mm in the northern areas, while the average monthly temperature ranges from 23°C in July to 32°C in February. The northern, central and southern parts are mainly of derived savannah, rain forest belt and mangrove swamp, respectively. The greater part of the state however lies in the tropical rain forest zone. The relative humidity ranges between 76% and 95%, coinciding with the dry and wet seasons, respectively.

### Collection of Samples

Sixty soil samples were randomly collected from sixty dumping sites (a sample per site) across Egba, Remo, Ijebu and Yewa regions of Ogun State. Before collection of the soil samples, superficial debris and other vegetative materials were removed from the soil surface using a sterilized iron rod. The samples were collected from the superficial layer of soil at a depth not exceeding 3–5 cm with a pre-sterilized

iron spoon in sterile universal bottles and then transported to the Microbiology Laboratory of the Olabisi Onabanjo University, Ago Iwoye, Ogun State.

### Determination of Soil pH

pH of each soil sample was measured after preparation of soil suspension (one gram of soil to five milliliters (mL) deionized water) using a pH meter (Kachuei *et al.*, 2012)

### Isolation and Identification of Keratinophilic Fungi

#### Cultural Technique

Keratinophilic fungi were isolated using the hair baiting technique as described by Vanbreuseghem (1952). Sterile Petri dishes were half-filled with soil samples, moistened with sterile distilled water and baited with pre-sterilized hair samples buried inside the soil. These dishes were incubated at room temperature and examined daily from the third day for fungal growth over three weeks. Baits with mycelial growth were subcultured on Potato Dextrose Agar (Oxoid, UK) medium and Dermasel Agar Base (Oxoid, UK) and then characterized using macroscopic characteristics as described by Roberts and Friedlander (2005) and microscopic features used for the identification of keratinophilic fungi and other dermatophytes by McDonald, (2000) and Ellis *et al.* (2007).

#### Statistical Analysis

The prevalence of the isolated organisms was scored using the formula  $P = n/N$ , where  $n$  = number of specific organisms,  $N$  = total number of isolated organisms while Shannon index of diversity was calculated for the isolated organisms using the formula  $S = P \cdot \ln P$ , where  $P$  = Prevalence,  $\ln$  = natural logarithm.

## RESULTS

The longitudinal and latitudinal distribution of the isolated keratinophilic and other dermatophytic fungi are represented in Table 1. All the sites sampled harbored at least one fungal isolate. Areas labeled X had no visible fungal growth.

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The Shannon index of diversity for the isolated organisms connotes significant disparities as shown by differences in their level of abundance (Table 2). The percentage of dominant species *Aspergillus fumigatus* was found to be 22.3% while the least dominant species *Aspergillus carbonarius*, *Aspergillus flavus*, *Aspergillus versicolor* and *Trichoderma longibrachiatum* represented 1.06 % of the population identified.

The prevalence of the ninety four (94) strains of filamentous fungi belonging to thirteen (13) different species are as follows; *Lichtheimia corymbifera* 3 (3.19%), *Aspergillus carbonarius* 1 (1.06%), *Aspergillus flavus* 1 (1.06%), *Aspergillus fumigatus* 21 (22.34%), *Aspergillus niger* 11 (11.70%), *Aspergillus terreus* 9 (9.58%), *Aspergillus versicolor* 1 (1.06%), *Epidermophyton floccosum* 6 (6.38%), *Microsporum gypseum* 15 (15.96%), *Penicillium chrysogenum* 4 (4.26%), *Trichoderma longibrachiatum* 1 (1.06%), *Trichophyton mentagrophytes* 16 (17.02%) and *Trichophyton rubrum* 5 (5.33%).

The distribution of the keratinophilic fungi was related to the pH level of the sampled dumpsites. Except for a pH value of above 9, where no keratinophilic fungi were found growing, the isolates grew in a pH range of 5.8 – 9. In general, pH value of 7 – 8 favoured the isolated organism's growth than the other pH values, This was followed closely by pH 5.8 to 7.0 (Table 3).

### DISCUSSION

Keratinophilic fungi are ecologically important organisms that have attracted attention throughout the world due to their significant role

in the natural degradation of keratinized residues (Fillipello, 2000). These organisms are known to share some characteristics with dermatophytes and are known for causing human and animal infections (Alli-shatayeh *et al.*, 1989). In this study, the traditional microbiological method was used for identifying keratinophilic fungi and other dermatophytes of dumpsites in Ogun State. Keratinophilic fungi isolated in this study have also been quoted in other literatures (Shokohi *et al.*, 2005; Zarei and Zarrin, 2008; Agu *et al.*, 2013; Pakshir *et al.*, 2013), but *Lichtheimia corymbifera* and *Trichoderma longibrachiatum* were not reported in those studies probably due to differences in samples collected and a number of samples analyzed on one side and favourable micro-environmental conditions of the studied dumpsites on the other side. This is because these isolates have earlier been reported to be more favoured in the environment with pH ranging between 3 and 8 (Asahi *et al.*, 1985; Pakshir *et al.*, 2013) which incidentally still fall within the observed pH of the studied dumpsites. The documentation of *Aspergillus fumigatus* as the most prevalent in this study is contrary to that reported by Agu *et al.* (2013) where *Aspergillus flavus* was the most frequently isolated and also to that of Mini *et al.* (2012) who reported *Aspergillus niger* as the most prevalent in their study. The reason for this observation may be attributed to differences in the study site as well as the study region in addition to favourable micro-environmental conditions

**Table 1:** Occurrence of keratinophilic fungi and other dermatophytes in soil samples collected from dumpsites in Ogun State

| Sample Code | Collection sites | ABS | AC | AF | AFU | AN | AT | AW | EF | MS | PC | TC | TM | TR | NG |
|-------------|------------------|-----|----|----|-----|----|----|----|----|----|----|----|----|----|----|
| AB1         | Obantoko         | -   | -  | -  | +   | +  | -  | -  | -  | -  | -  | -  | -  | -  |    |
| AB2         | OGD              | -   | -  | -  | -   | -  | -  | -  | -  | -  | -  | -  | -  | -  | X  |
| AB3         | Asero            | -   | -  | -  | -   | -  | -  | -  | -  | -  | -  | -  | -  | -  | X  |
| AB4         | Ita-aka          | -   | -  | -  | +   | +  | -  | -  | +  | +  | +  | -  | -  | -  |    |
| AB5         | Kugba            | -   | -  | -  | -   | -  | -  | -  | -  | -  | -  | -  | -  | -  | X  |
| AB6         | Adatan           | -   | -  | -  | +   | -  | -  | -  | -  | -  | -  | -  | -  | -  |    |
| AB7         | Aregba           | -   | -  | -  | -   | -  | -  | -  | -  | +  | -  | -  | -  | -  |    |
| AB8         | Shaje            | -   | -  | -  | -   | -  | -  | -  | -  | -  | -  | -  | -  | -  | X  |
| AB9         | Lafiaji          | -   | -  | -  | -   | -  | -  | -  | -  | -  | -  | -  | +  | -  |    |
| AB10        | Elega            | -   | -  | -  | -   | -  | -  | -  | -  | -  | -  | -  | -  | -  | X  |
| AB11        | Sapon            | -   | -  | -  | -   | -  | -  | -  | -  | -  | -  | -  | -  | -  | X  |
| AB12        | Itoku            | -   | -  | -  | +   | +  | +  | -  | -  | +  | -  | -  | -  | -  |    |
| AB13        | Ijaiye           | -   | -  | -  | +   | -  | +  | -  | -  | +  | -  | -  | +  | -  |    |
| AB14        | Isabo            | -   | -  | -  | +   | -  | -  | -  | -  | +  | -  | -  | +  | -  |    |
| AB15        | Kuto             | -   | -  | -  | +   | -  | -  | -  | -  | +  | -  | -  |    | -  |    |
| AG1         | Igan road        | +   | -  | -  | -   | -  | -  | -  | -  | -  | -  | -  | +  | -  |    |
| AG2         | Ago market       | -   | -  | -  | -   | -  | -  | -  | -  | +  | -  | -  | -  | -  |    |
| AG3         | Koroko           | -   | -  | -  | +   | -  | -  | -  | +  | +  | -  | -  | -  | -  |    |

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|      |               |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
|------|---------------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AG4  | Ololo         | - | - | - | - | - | - | - | - | - | - | - | - | - | X |
| AG5  | Ayegbami      | - | - | - | + | - | - | - | - | + | + | - | - | - |   |
| AG6  | Fibigbade     | - | - | - | - | - | - | - | - | - | - | - | - | - | X |
| AG7  | Ojore         | - | - | - | - | - | - | - | - | - | - | - | - | - | X |
| AG8  | Ijesha road   | - | - | - | - | - | - | - | - | - | - | + | - | - |   |
| AG9  | Pepsi         | - | - | - | - | + | - | - | - | - | + | - | - | - |   |
| AG10 | Okodo         | - | - | - | - | - | - | - | - | - | - | - | - | - | X |
| AG11 | Ibipe         | - | - | - | - | - | - | - | - | + | - | + | - | - |   |
| AG12 | Oladipupo     | - | - | - | - | - | - | - | - | - | - | - | - | - | X |
| AG13 | Imoro         | - | - | - | + | - | - | - | - | - | - | - | - | - |   |
| AG14 | Imere road    | - | - | - | - | - | - | - | - | - | - | - | - | - | X |
| AG15 | Mariam        | - | - | - | - | - | - | - | - | - | - | - | - | - | X |
| SS1  | Isale-oko     | - | - | - | - | - | - | - | - | - | - | - | - | - | X |
| SS2  | Sabo          | - | - | - | - | - | - | - | - | - | - | - | - | - | X |
| SS3  | Mogbonran     | - | - | - | - | - | - | - | - | - | - | - | - | - | X |
| SS4  | Hospital road | - | + | - | + | - | - | - | - | - | - | - | - | - |   |
| SS5  | Inside OOUTH  | - | - | - | - | + | - | - | - | - | - | - | + | - |   |
| SS6  | Adiewocoat    | - | - | - | - | - | - | - | - | - | - | - | - | - | X |
| SS7  | Ajaka         | - | - | - | + | - | - | - | - | + | + | - | - | + |   |
| SS8  | Ajebo         | + | - | - | - | - | - | - | - | - | - | - | - | - | X |
| SS9  | Kafaru        | - | - | + | + | - | + | - | - | + | - | - | + | - |   |
| SS10 | Awosanya str. | - | - | - | - | - | - | - | - | - | - | - | - | - | X |
| SS11 | Mosumola      | - | - | - | - | - | - | - | - | - | - | - | - | - | X |

|      | str.              |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
|------|-------------------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SS12 | Awolesi rd.       | - | - | - | - | - | - | - | - | - | - | - | - | - | X |
| SS13 | Express rd.       | - | - | - | + | + | + | + | - | - | - | - | + | - |   |
| SS14 | Makun             | - | - | - | - | + | + | - | - | - | - | - | + | + |   |
| SS15 | Ijagba            | - | - | - | - | - | - | - | - | - | - | - | - | - | X |
| YE1  | Oja Odan          | + | - | - | + | + | - | - | - | + | - | - | - | - |   |
| YE2  | Oja Odan          | - | - | - | + | + | - | - | - | - | - | - | + | + |   |
| YE3  | Oja Odan          | - | - | - | - | - | - | - | - | - | - | - | - | - | X |
| YE4  | Oja Odan          | - | - | - | + | - | - | - | - | - | - | - | - | - |   |
| YE5  | Oja Odan          | - | - | - | - | + | - | - | + | + | - | - | + | + |   |
| YE6  | Oja Odan          | - | - | - | + | + | + | - | - | - | - | - | + | - |   |
| YE7  | Oja Odan          | - | - | - | - | - | + | - | - | + | - | - | + | + |   |
| YE8  | Jubilee,<br>Ilaro | - | - | - | - | - | - | - | - | - | - | - | - | - | X |
| YE9  | Jubilee,<br>Ilaro | - | - | - | + | - | - | - | + | - | - | - | + | - |   |
| YE10 | Barrack,<br>Ilaro | - | - | - | - | - | - | - | - | - | - | - | - | - | X |
| YE11 | Oke-Obese         | - | - | - | - | - | - | - | - | - | - | - | - | - | X |
| YE12 | Oke-Obese         | - | - | - | - | - | - | - | - | - | - | - | - | - | X |
| YE13 | Oke-Obese         | - | - | - | - | - | - | - | - | - | - | - | - | - | X |
| YE14 | Park, Ilaro       | - | - | - | + | - | - | - | - | - | - | - | - | - |   |
| YE15 | Garage,<br>Ilaro  | - | - | - | - | - | - | - | - | - | - | - | - | - | X |

Key: ABS -*Lichtheimia corymbifera*, AC - *Aspergillus carbonarius*, AF - *Aspergillus flavus*, AFU - *Aspergillus fumigatus*, AN - *Aspergillus niger*, AT - *Aspergillus terreus*, AW - *Aspergillus versicolori*, EF - *Epidermophyton floccosum*, PC - *Penicillium chrysogenum*, MS - *Microsporium gypseum*, TC - *Trichoderma longibrachiatum*, TM - *Trichophyton mentagrophytes*, TR - *Trichophyton rubrum*, + Present, - Absent, X= dumpsites where no visible growth of fungi was found, NG = no growth

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**Table 2:** shannon index of diversity of keratinophilic fungi and other dermatophytes isolated from selected dumpsites in Ogun State

| <b>ORGANISMS INDEX</b>             | <b>N</b> | <b>P (N / N)</b> | <b>- INP</b> | <b>SHANNON P – IN (P)</b> |
|------------------------------------|----------|------------------|--------------|---------------------------|
| <i>Lichtheimia corymbifera</i>     | 3        | 0.0319           | 3.45         | 0.11                      |
| <i>Aspergillus carbonarius</i>     | 1        | 0.0106           | 4.55         | 0.05                      |
| <i>Aspergillus flavus</i>          | 1        | 0.0106           | 4.55         | 0.05                      |
| <i>Aspergillus fumigatus</i>       | 21       | 0.223            | 1.5          | 0.33                      |
| <i>Aspergillus niger</i>           | 11       | 0.1170           | 2.15         | 0.25                      |
| <i>Aspergillus terreus</i>         | 9        | 0.9574           | 0.044        | 0.042                     |
| <i>Aspergillus versicolor</i>      | 1        | 0.0106           | 4.55         | 0.05                      |
| <i>Epidermophyton floccosum</i>    | 6        | 0.0638           | 2.75         | 0.17                      |
| <i>Microsporum gypseum</i>         | 15       | 0.1596           | 1.84         | 0.29                      |
| <i>Penicillium chrysogenum</i>     | 4        | 0.0426           | 3.16         | 0.13                      |
| <i>Trichoderma longibrachiatum</i> | 1        | 0.0106           | 4.55         | 0.05                      |
| <i>Trichophyton mentagrophyte</i>  | 16       | 0.1702           | 1.77         | 0.30                      |
| <i>Trichophyton rubrum</i>         | 5        | 0.0532           | 2.93         | 0.156                     |
| Total                              | 94       | 1.8611           | 37.794       | 1.978                     |

Key:  $P = n/N$

Where n= number of specific organisms, N= total number of fungal isolates, ln= natural logarithms

Table 3: Distribution of keratinophilic fungi by genera and in relation to pH

| FUNGAL GENERA         | pH        |      |           |      |           |      |          |   | TOTAL     |      |
|-----------------------|-----------|------|-----------|------|-----------|------|----------|---|-----------|------|
|                       | 5.8 - 7   |      | 7 - 8     |      | 8 - 9     |      | >9       |   |           |      |
|                       | n         | %    | n         | %    | n         | %    | n        | % | n         | %    |
| <i>Lichtheimia</i>    | 2         | 5.7  | 1         | 2.1  | 0         | 0    | 0        | 0 | 3         | 3.2  |
| <i>Aspergillus</i>    | 19        | 54.3 | 22        | 46.8 | 3         | 25   | 0        | 0 | 44        | 46.8 |
| <i>Epidermophyton</i> | 2         | 5.7  | 3         | 6.4  | 1         | 8.3  | 0        | 0 | 6         | 6.4  |
| <i>Microsporium</i>   | 4         | 11.4 | 8         | 17.0 | 3         | 25   | 0        | 0 | 15        | 16.0 |
| <i>Penicillium</i>    | 1         | 2.9  | 2         | 4.3  | 1         | 8.3  | 0        | 0 | 4         | 4.3  |
| <i>Trichoderma</i>    | 0         | 0    | 1         | 2.1  | 0         | 0    | 0        | 0 | 1         | 1.1  |
| <i>Trichophyton</i>   | 7         | 20   | 10        | 21.3 | 4         | 33.3 | 0        | 0 | 21        | 22.3 |
| <b>Total</b>          | <b>35</b> |      | <b>47</b> |      | <b>12</b> |      | <b>0</b> |   | <b>94</b> |      |

n= number of organisms, %= percentage

Irrespective of the types of keratinophilic fungi and other dermatophytes isolated, their presence represents a large variety of diseases that can affect glabrous skin, nails and hair (Borman *et al.*, 2007). From the public health perspective, the growth of some of these organisms especially the Aspergilli suggest an imminent danger because of their ability to produce secondary metabolites known as mycotoxins (Zimmerli and Dick, 1996). These mycotoxins are secondary metabolites produced by some fungal isolates known not to be toxic to the producer but the consumer of the substrate containing them (Kuiper – Goodman, 2004).

The documentation of *Aspergillus* spp as the most prevalent genera in the sampled dumpsites is not unexpected as this organism has been reported as the most abundant soil microflora (Bennet and Klich, 2003). Some of these *Aspergilli* and *Fusarium* have been implicated in mycotic keratitis. Other isolated organisms especially the *Penicillium* spp have also been reported in different disseminated diseases. *Microsporium gypseum* which also show good representation especially among the dermatophyte is a frequent geophilic

dermatophyte commonly distributed in soil worldwide but their presence in the soil signifies their potential high risk for transmission of dermatophytes. A relatively high incidence of *M. gypseum* observed in this study is a noteworthy finding of public health significance that is similar to that recorded in the soil of Nevisand Brazil (Da Silva Pontes and Oliveira, 2008; Giudice *et al.*, 2012; Gugnani *et al.*, 2012). The little disparity observed in our study and those studies in terms of incidence rate may also be attributed to the difference in study location and samples. The growth of these organisms was more favoured at pH range of 5.8 to 9.1 which negates the findings of Garg *et al.* (1985) who reported that acidic soil with pH 5.9 is free of keratinophilic fungi but is similar to Asahi *et al.* (1985) and Pakshir *et al.* (2013) who reported that pH value between 6 and 9 is favourable for keratinophilic fungi. Our findings of pH range of 7 to 8 that best favoured the isolated organism corroborated the findings of Da Silva Pontes and Oliveira (2008) who reported that the majority of the isolates of *M. gypseum* were recovered from the soil with almost neutral pH but is in parallel to that of Muhammed and Laji (1978) who reported that majority of the isolates of dermatophytes



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including *M. gypseum* were recovered from soils with acidic pH. Generally, 51.7% of the dumpsites were contaminated with keratinophilic fungi and other dermatophytes.

### CONCLUSION

Results of this study have shown that the soil of the dumpsites analyzed harbours different keratinophilic fungi that grow best at pH range of 7-8 and thus labeling the sampled dumpsites as cutaneous fungal and dermatophytoses prone areas

### ACKNOWLEDGEMENTS

I sincerely acknowledge the technical support rendered by Dr (Mrs) R.M. Kolawole of the Department of Cell Biology and Genetics, University of Lagos during this study. I also appreciate the laboratory space provided by the technical head of the Microbiology Laboratory, Olabisi Onabanjo University, Ago Iwoye, Ogun State, Nigeria.

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