South Asian Journal of Research in Microbiology

1(1): 1-15, 2018; Article no.SAJRM.40128

Effect of Soil Physicochemical Parameters and Seasonal Variations on the Occurrence of Keratinophilic Fungi of Murtala Amusement Park in Minna, Niger State, Nigeria

U. I. Hamza[1*], M. C. Emere[1] and T. Bulus[2]

[1]Department of Biological Sciences, Nigerian Defence Academy, Kaduna, Nigeria.
[2]Department of Biochemistry, Kaduna State University, Kaduna, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author UIH designed the study, performed the statistical analysis and wrote the protocol. Authors UIH, MCE and TB wrote the first draft of the manuscript. Authors MCE and TB managed the analyses of the study. Authors UIH and TB managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/SAJRM/2018/v1i1730
Editor(s):
(1) Osunsanmi Foluso Oluwagbemiga, Department of Biochemistry and Microbiology, University of Zululand, South Africa.
(2) Lidija Kozacinski, Professor, Department of Hygiene, Technology and Food Safety, Faculty of Veterinary Medicine, University of Zagreb, Croatia.
Reviewers:
(1) S. K. Deshmukh, The Energy and Resources Institute, India.
(2) Ahmed Karmaoui, Morocco.
(3) V. Vasanthabharathi, Annamalai University, India.
Complete Peer review History: http://www.sciencedomain.org/review-history/23834

Original Research Article

Received 11th January 2018
Accepted 20th March 2018
Published 28th March 2018

ABSTRACT

Keratinophilic fungi are the prime cause of various mycotic infections among children. Murtala Amusement Park is one of the preferred recreation grounds in Minna. The aim of this research was to investigate the occurrence of fungal species at Murtala Amusement Park, Minna, with the goal of identifying keratinophilic species among them. A total of 360 soil samples from six different important sites in the Park were collected during dry and rainy seasons. A total of 542 isolates from eleven genera were identified; *Aspergillus, Candida, Fusarium, Paecilomyces, Mucor, Chrysosporium, Alternaria, Penicillium, Trichoderma, Microsporum,* and *Rhizopus.* A total of 142 and 399 fungal isolates were isolated during dry and rainy seasons respectively. Out of the soil physicochemical parameters analyzed, only Ca and Mg showed significant correlation with a number of the fungal colony at 1 % and 5 % levels of significance respectively and it occurred during dry season. There was no association between the numbers of fungal isolates recorded

**Corresponding author: Email: uihamza@yahoo.com;*

across the two seasons. Maximum percentage of contribution was observed with *Aspergillus niger* in both seasons. While the minimum percentage of contribution was observed with *Microsporum gypseum* (1.40 %) and *Paecilomyces variottii* (2.51 %) during the rainy and dry seasons respectively. Simpson's dominance of fungal species showed 10.86 and 9.84 for dry and rainy season respectively. Evenness of fungal species at dry and rainy seasons were 0.0759 and 0.0247 respectively. Diversity index of fungal species were 2.531 and 0.908 for Shannon's Index and Simpson's Index respectively. A very high incidence of keratinophilic fungi demonstrated in the soil of Murtala Amusement Park is a noteworthy finding for public health significance.

Keywords: Dermatophytosis; keratinophilic fungi; Minna; Murtala Amusement Park; physicochemical parameters; seasonal variations, soil.

1. INTRODUCTION

The fungi constitute a large and diverse group of plant kingdom belonging to a large group called thallophyta. According to an estimate, there are about 1.5 to 3.5 million species of fungi in the world but this number is constantly increasing because of the never-ending search for these organisms throughout the world [1]. These organisms are known to about different soil types which are regarded as a reservoir for human infection [2].

Keratin is the most predominant protein found in the epithelial cells of vertebrates which constitute up to 85% of the protein in hair, horn, nail, and feather. The protein chains are packed tightly either in α-helix (α-keratin) or β-sheet (β-keratin) structure, which fold into three dimensional forms [3]. Keratin is dichotomized into hard (feather, hair, hoof and nails) and soft keratin (skin and callus) based on sulphur content [4]. Since keratin is fibrous and insoluble structural protein extensively cross-linked with disulfide, hydrogen, and hydrophobic bonds, it is mechanically stable and resistant to commonly used proteolytic enzymes such as papain, trypsin, and pepsin [5].

Fungi exhibit heterotrophic mode of nutrition and due to an extraordinary plasticity they can colonize various substrates such as keratin, collagen, elastin, lignin, cellulose, hemicellulose and so on using resources which are inaccessible to other species [6].

The biggest group of organisms that can utilize keratin as the sole source of carbon and nitrogen are keratinophilic fungi [4]. Keratinophilic fungi are a small, well-defined and important group of fungi that colonize various keratinous substrates and degrade them to components of low molecular weight [7]. Their presence in an environment largely depends on the presence of human and animal [7].

The term keratinophilic simply means keratin loving and it is often misunderstood in the sense that all fungi that grow on a natural keratin substrate are regarded as keratinophilic [4]. There are fungi that grow on a natural keratin substrate but they are not interested in the keratin itself [4]. What they are interested in is the non-keratin lipid fraction of the substrate. Hence, only those that can actually degrade keratin should be regarded as keratinophilic fungi [4].

Keratinophilic fungi include a variety of filamentous fungi comprising mainly hyphomycetes and several other taxonomic groups [8]. Hyphomycetes include dermatophytes and a variety of non-dermatophytic keratinophilic fungi that can colonize and invade the keratin of skin, nail, and hair [9]. These dermatophytes are known to cause superficial cutaneous infections (Dermatophytosis) of keratinized tissues (skin, hair, and nails) of humans and animals [10]. The species of keratinophilic fungi have been divided into three categories depending on their natural habitats: anthropophilic when human beings are natural host, zoophilic when animals act as natural host and geophilic when associated with soil [11].

Many researchers have demonstrated the presence of the Keratinophilic fungi from soils of playgrounds around the world. Most recent among the researchers include; Deshmukh et al. [9], Shrivastava et al. [12], Deshmukh and Verekan [13], Min Jung Lee et al. [14], Rizwana et al. [15], Gugnani et al. [16], Maruthi et al. [17], Agu et al. [18], Pakshir et al. [2], and Olajubu and Folorunso [19].

Recreation ground like school field, village square, amusement parks and so on are often invaded by human beings and animals. They deposit organic residues, which may contaminate the soil with keratinaceous debris and possibly

propagules of keratinophilic fungi. Murtala Park is one of the most preferred outdoor recreation grounds in Minna, one of the major cities of Nigeria [20]. It is visited mostly during festive periods and by young children, usually accompanied by adult relatives.

Earlier before now, there were no any single documented data about presence of keratinophilic fungi in this domain. Investigating the presence and occurrence of these fungi will go a long in solving or addressing dermatophytic infections which is known to be common among young children due to constant contact with soil.Therefore, this research to the best of our knowledge is the first efforts to isolate and identify keratinophilic fungi from unexplored Murtala Amusement Park (MAP), Minna. This study, however, is limited to keratin loving fungi found in the soil. Researches of these kinds could provide information which is of public health significance.

2. MATERIALS AND METHODS

2.1 The Study Area

This study was carried out at Murtala Amusement Park which is located in Minna, Niger state capital. Minna lies on Latitude 903′N and Longitude 6033′E on the geological base of undifferentiated basement complex of mainly gneiss and magmatite [20]. Minna comprises most part of Bosso and Chanchaga local government area. The North Eastern part of the city is a more or less continuous step of granite (Paida Hill) which occurs and limits any urban development. The city is both administrative capital and headquarters of Niger State and Chanchaga Local Government. The city with an estimated population of about 889,994 people has land area of over 1,000 ha. The mean annual rainfall of the city is about 1334 mm (53 in) starting from April to September. The peak of the rain is in September (300 mm / 1.7 in). The mean monthly temperature is highest in March (39°C) and lowest in August (30°C). The dry season starts in October through April with North Eastern wind, which is cool, dry and dusty. The people of Minna are mostly migrants; the working age group and male constitute a higher percentage of the population [21].

2.2 Collection of Soil Samples from the Study Area

Soil samples were collected seasonally i.e., dry season (March-May) and rainy season (June-August) from sites of six playing facilities in the park. These sites were mapped out into five regions viz, North, South, West, East, and Central for sample collection. The soil samples were collected from a topmost part of the soils (5 cm depth) with sterile hand trowel and transferred inside sterile polyethylene bags (well labeled). Finally, they were transported to the laboratory for further analysis.

Human hair was obtained. The hair was washed several times in changes of distilled water and defatted by soaking in diethyl ether for twenty-four hours. The hair was thoroughly washed again with distilled water and completely dried in hot air oven at 60°C for 24 hours, and ground into powder with sterilized grinding stone.

2.3 Isolation of Fungi Using the Hair-baiting Method

The modified hair baiting method of Vanbreuseghem [22] was employed for the isolation of keratinophilic fungi. A weight of 50 g of each soil sample was measured and transferred into sterile Petri dishes and some quantities of pulverized defatted human hair were spread over the soil in the dishes. The hair-baited soils in the plates were moistened with sterile distilled water and incubated at room temperature. The soil samples were moistened at regular intervals with small quantities of sterile distilled water to prevent dryness. The soil samples were thereafter examined routinely for fungal growths on the hair baits for two weeks. Portions of any observed growth were aseptically transferred to already prepared Petri dishes of Sabouraud Dextrose Agar (SDA) supplemented with 2 mg/ml gentamycin to check the growth of bacteria. The inoculated plates were incubated at room temperature. The fungi that grew were sub-cultured severally in order to obtain pure cultures.

2.4 Identification of Fungi

A drop of lacto phenol cotton blue was placed on a microscopic slide and an inoculum from fungus culture representing all fungal structures was transferred on the slide. The fungal inoculum was separated with teasing needle and mixed with stain. Cover slip was placed on the slide to avoid air entrapment and finally examined under microscope. The structures seen were sketched and morphology of each was described and identified based on the characteristics.

Micrographs of Watanabe [23]; David et al. [24] were used as atlas for comparison.

2.5 Analyses of Physicochemical Parameters of the Soil Samples

2.5.1 Determination of soil pH

The *pH* of the soil samples was determined using a modified method of Steffi and Josephine [25] using a Jenway digital pH meter. Soil samples were suspended in a beaker using distilled water in the ratio of 1:5. The mixture was stirred and was allowed to stand for 30 minutes. A buffer solution was used to zero the *pH* meter then the electrode of the *pH* meter was inserted into the mixture and the *pH* readings were taken and recorded in triplicates.

2.5.2 Determination of organic matter

A weight of thirty gram of each soil samples was measured in porcelain crucible and placed on an oven to dry the soil. The crucible was heated in a muffle furnace at 400°C for three hours. It was stirred occasionally for complete oxidation of organic matter. The oxidation was completed when the soil became light tan. The samples were cooled and reweighed. The loss in weight was determined and percent organic matter was calculated using the formula below:

$$\% \text{ organic matter} = \frac{loss\ in\ Weight}{Weight\ of\ Soil\ used} \times 100 \text{ [26].}$$

2.5.3 Determination of organic carbon

Walkley-Black wet oxidation method [27] was employed to determine organic carbon. Soil samples were pulverized to pass through 0.5 mm sieve. Thirty gram of each soil sample was weighed in duplicate and transferred to 250 ml Erlenmeyer flask. Then, 10 ml of 1N $K_2Cr_2O_7$ solution was pipetted accurately into each flask and swirled gently to disperse the soil. A 20 ml of concentrated H_2SO_4 was added using pipette directing the stream into the suspension. At once the flask was swirled gently until the soil and the reagents were mixed. It was swirled more vigorously for another one minute. The flask was rotated and allowed to stand on a sheet of asbestos for about thirty minutes. A 100 ml of distilled water was added. Some drops of the indicator were added and titrated with 0.5 N ferrous sulphate solutions. As the end point was approached, the solution took a greenish cast and then changed to dark green. At this point, ferrous sulphate was added drop by drop until the colour changed sharply from blue to red (maroon colour in reflected light against a white background). A blank titration was made, but without soil to standardize the dichromate.

$$\% \text{ organic carbon} = \frac{N(V1-V2)0.3f}{W}$$

Where,

N = Normality of ferrous sulphate solution
V1 = ml ferrous sulphate solution required for the blank
V2 = ml ferrous sulphate solution required for the sample ferrous sulphate solution
W = mass of sample in gram
F = correction factor = 1.33

2.5.4 Determination of nitrogen

Nitrogen in the soil samples was determined by regular macro-Kjeldahl method as described by Udo et al [26]. Ten gram of each soil samples were measured accurately, air- dried and ground to pass through 0.5 mm sieve into a dry 500 ml macro- kjeldahl flask and 200 ml of distilled water was added. The flask was swirled for a few minutes and allowed to stand for thirty minutes. A tablet of mercury catalyst was added and ten gram of K_2SO_4. Then 30 ml of concentrated H_2SO_4 was added through a pipette. The flask was heated cautiously at low heat on the digestion stand. After water had been removed and frothing had ceased, the heat was increased until the digestion is cleared. The mixture was boiled for five hours. The heat was regulated and H_2SO_4 condensed about the middle of the way up the neck of the flask. The flask was allowed to cool and 100 ml of water was added slowly to the flask. The digest was carefully transferred into another clean macro-kjeldahl flask. All sand particles in the original digestion flask were retained (because sand can cause severe bumping during kjeldahl distillation). The sand residue was washed with 50 ml of distilled water several times and the aliquot was transferred into the same flask. 50 ml of H_3BO_3- indicator solution was poured into a 500 ml Erlenmeyer flask that was placed under the condenser of the distillation apparatus. The end of condenser was 3 cm above the surface of the H_3BO_3 solution. 750 ml of kjeldahl flask was attached to the distillation apparatus. 150 ml of 10 ml NaOH solution was poured through the distillation flask by opening the funnel stopcock. Distillation was commenced immediately. Condenser was kept

cool by allowing sufficient air to flow through, and heat was regulated to minimizing frothing and prevent suck back. 150 ml of distillate was collected and the distillation was stop. The NH_4-N in the distillate was determined by titrating with 0.01M standard HCl using a 25 ml burette graduated at 0.1 ml intervals. The colour changed at the end point from green to pink.

$$\% N = \frac{T \times M \times 14 \times 100}{\text{Weight of Soil used}}$$

Where T = titre value, M = molarity of HCl

2.5.5 Determination of available Fe, Mn, Cu, and Zn in the soil samples

Available Fe, Mn, Cu, and Zn were extracted by Hydrochloric Acid Extraction as described by Udo et al*.,* [26]. A weight of five gram of each soil samples was measured into a 100 ml plastic bottle. 50 ml of 0.1M HCl was added and shook for thirty minutes. The samples were then filtered using Whatman No. 42 filter paper.

Available Fe, Mn, Cu, and Zn were extracted by Hydrochloric Acid Extraction as described by Udo et al*.,* [26].The concentration of Fe, Mn, Cu, and Zn in the extract was determined by atomic absorption spectrophotometer (AAS).

2.5.6 Determination of phosphorus

A weight of three gram of each soil samples was measured, air-dried and ground to pass through 2 mm sieve into a 500 ml centrifuge tube. 20 ml of extracting solution was added. Then it was shaken for 1 minute on a mechanical shaker and the suspension was centrifuged on a mechanical shaker at 2000 rpm for 15 minutes. It was decanted and the extract was ready for phosphorus determination [28].

Exactly 5 ml of the extract was pipetted into 50 ml volumetric flask. Distilled water was added to bring up the volume to approximately 40 ml. Exactly 8 ml of dissolved 1.056 g of ascorbic acid in 200 ml and 12 g of ammonium molybdate in 250 ml in distilled water were added and mixed thoroughly. The absorbance or optical density of the coloured solution was matched against reagent blank at 882 nm after 30 minutes [29].

2.5.7 Soluble and exchangeable potassium

A weight of 12.5 gram of each soil sample was measured into a 250 ml beaker. 100 ml of 1M NH_4Cl was added (1M= 100 ml of dissolved 53.5 gram of ammonium chloride in distilled water and make up to 1 litre). It was left overnight. The soil was filtered and leached to a total volume of 250 ml with the 1M NH_4Cl [26]. Potassium was determined from the extract using a flame photometre.

2.5.8 Determination of total Ca, Mg, and Na in the soil sample

2.5.8.1 Extraction of total Ca, Mg and Na

Total Ca, Mg, and Na were extracted using Perchloric Acid/ Nitric Acid Digestion as described by Udo et al. [26]. A weight of 2 gram of air-dried soil from each soil samples was measured into 150 ml beaker. A 20 ml of concentrated HNO_3 was added and the mixture was allowed to stand for an hour. A 15 ml of concentrated HCO_4 was carefully added. The mixture was digested on a hot plate at about 225°C until it turned yellow. The digest was dissolved in 0.1M HCl and filtered into a 250 ml volumetric flask.

Atomic absorption spectrophotometre was used to determine the concentration of the elements

2.6 Data Analyses

2.6.1 Correlation analysis

Pearson's correlation analysis was used to assess the relationship between the physicochemical parameters and total fungal colonies.

2.6.2 Chi- square

Chi- square was used to determine the association between fungal colonies recorded in the two seasons (dry and rainy).

2.6.3 Diversity index

Diversity index was determined using Shannon's index and Simpson's index
Shannon's index = $-\Sigma P_i \ln (P_i)$ [30].

Where P_i is the proportion of individuals found in species *i*. For a well-sample community, we can estimate this proportion as $P_i = ni/N$, where n*i* is the number of individuals in species *I*, N is the total number of individuals in the community **ln** is the natural log and Σ is the sum of the calculations.

Simpson's index (D1) = $1- \Sigma P_i^2$ [31].

2.6.4 Dominance and evenness

Species dominance and evenness were determined using Simpson's equations:

Dominance (D2) = $1/ \Sigma P_i^2$

In the Simpson equations, **p** is the proportion (n/N) of individuals of one particular species found (n) divided by the total number of individuals found (N), Σ is still the sum of the calculations, and **s** is the number of species.

Evenness = D2/S

Where D2 is the dominance and S is the number of species.

2.6.5 Community similarities

Sorenson's coefficient and Jaccard's index were used to determine what the seasons have in common using the following formulas;

Sorenson's coefficient = $\frac{2C}{S1+S2}$

Where C is the number of species the two communities have in common, S1 is the total number of species found in community 1, and S2 is the total number of species found in community 2.

Jaccard's index = $\frac{Sc}{Sa+Sb+Sc}$

Where *Sa* and *Sb* are the numbers of species unique to samples a and b, respectively, and
Sc is the number of species common to the two samples.

2.6.6 Percentage of contribution and percentage of frequency

Percentage of contribution and percentage of frequency of fungal isolates were calculated by using the following formulas.

% contribution =

$$\frac{\text{Number of colonies of fungus in a sample}}{total\ number\ of\ all\ colonies\ of\ all\ species\ in\ a\ sample} \times 100$$

% frequency =

$$\frac{number\ of\ samples\ in\ which\ a\ particular\ fungus\ occurred}{total\ number\ of\ samples\ examined} \times 100$$

Based on the frequency occurrences the fungi were grouped as: rare, R= (0-25% frequency), occasional, O = (26-50% frequency), frequent, F= (51-75% frequency) and common, C= (76-100% frequency) species.

3. RESULTS

The pH (water) was weakly acidic throughout. **SWC** had the highest Ca content in the soil (4.462 cmol/ kg) and the lowest Mn content in the soil (5.882 mg/kg). **Swing** had the lowest percentage of organic carbon (%) O/C (0.579), % O/M (0.998), %N (0.070), Na (0.208 cmol/kg), K (0.624 cmol/kg), and Zn (6.254 mg/kg) but had the highest Cu (12.501 mg/kg), AP (19.946mg/kg) and Fe (12.216 mg/kg) content in the soil sampled. **SWOC** had the lowest Ca (2.986 cmol/kg), Mg (1.032 cmol/kg), and Fe (8.824 mg/kg) content in the soil sampled. **M-G-R** had the lowest Cu (7.787 mg/kg) but the highest Zn (10.200 mg/kg) content in the soil sampled. **RS** had the highest % O/C (0.911), % O/M (1.575) but had the highest Mg (3.180 cmol/kg). % O/C is directly proportional to % O/M, that is, when % O/C increased % O/M also increased and vice versa. Concentrations of Ca and Mg were relatively high throughout the sites. This is presented in Table 1.

The **SWC** had the lowest Mg (1.331 cmol/kg), Zn (6.357 mg/kg) and Fe (8.670 mg/kg) content in the soil. **Swing** had the highest pH (6.850), % O/C (0.760), % O/M (1.314), Na (0.230 cmol/kg), K (0.780 cmol/kg), and Fe (11.558 mg/kg) content in the soil. **SWOC** had the highest Mg (2.241cmol/kg) and Zn (11.558 mg/kg) but had the lowest percentage of Organic Carbon (0.345) and O/M (0.597) content in the soil sampled. **Seesaw** had the highest AP (17.593 mg/kg) and Cu (14.583 mg/kg) but had the lowest pH (6.559), percentage of N (0.070), Ca (3.224 mg/kg), Na (0.717 cmol/kg), and K (0.513 cmol/kg). The percentage of Organic Carbon is directly proportional to the percentage of Organic Matter that is, when percentage of Organic Carbon increased percentage of Organic Matter also increased and vice versa. Concentrations of Ca and Mg were relatively low throughout the sites. This is shown in Table 2.

The pH showed a high positive correlation across the two seasons. The percentage of Organic Carbon and percentage of Organic Matter showed a weak positive correlation. The percentage of N, K and Na showed a weak negative correlation. The Ca and Mg were significant at 1 % and 5 % respectively. The AP showed a weak negative correlation during the

dry season but high negative correlation in the rainy season. The Cu and Mn showed weak positive correlation at the dry season but high negative correlation in the rainy season. The Zn and Fe showed a weak negative correlation in the dry season but weak positive correlation in the rainy season. This is shown in Table 3.

In the dry season, the highest and lowest numbers of fungal isolates were recorded in **RS** and **SWOC** which were 28 and 18 respectively. While in the rainy season, **M-G-R** and **RS** had the highest and lowest numbers of fungal isolates which were 188 and 30 respectively. In the rainy season, the highest number of fungal isolates was recorded. However, the difference was statistically significant; in other words, there was no association between numbers of fungal isolates recorded during dry and rainy seasons. (Chi-square = **61.067,** table value at 5 Degree of freedom and 0.001 value of p is **20.51).** This is presented in Table 4.

Aspergillus oryzae occurred only in dry season. The highest percentage of contribution at the two seasons was observed with *A. niger.* There was variation in terms of the species with the lowest percent contribution in the two seasons. The lowest percentage of contribution was observed with *Microsporum gypseum* and *Paecilomyces variottii* during dry and rainy seasons respectively as shown in Table 5.

Great variations were observed. *Trichoderma species* were frequent in both seasons while *Fusarium oxysporium* was occasional in both seasons as shown in Table 6.

According to Simpson's dominance, the species of dry season had the highest dominance value. In addition, species of dry season had the highest evenness value. This is presented in Table 7.

According to Sorenson's Coefficient, samples of RS had the highest value (0.241) while those of SWC had the lowest value (0.095). According to Jaccard's Index, samples of RS had the highest value (0.108) while those of SWC and MGR had the lowest value (0.046). This is shown in Table 8.

Shannon's diversity and Simpson's diversity Index were both higher in the dry season with the value 2.531 and 0.908 respectively. While in the rainy season, the values were lower (2.287 and 0.898 respectively) as shown in Table 9.

4. DISCUSSION

Soil properties such as macro- and microelements affect or influence occurrence and distribution of keratinophilic fungi in the soil. Thus, it was important to study the relationship between physicochemical properties of the soil and the distribution of keratinophilic fungi. In the present study, all the keratinophilic fungi isolated during the dry and rainy season were from soil samples with pH range between 6.559 to 6.960. This finding has confirmed the studies of Pakshir et al [2] who in their study isolated keratinophilic fungi from soils with pH between six and nine. Many researchers had stated that keratinophilic fungi could not be found in the soils with low pH levels 3–4.5 [2]. However, some researchers such as Kornikowicz-Kowalska and Bohacz [32] isolated keratinophilic fungi from samples of strongly acidic soils (pH KCl 3.36; 4.06; 4.19; 4.29). In contrast, Ashok et al [33] isolated fungi from alkaline soils with pH range from 8.16-8.76. In this study, there was no significant difference in the seasonal variation of pH. Olajubu and Folorunso [19], Neetu and Sharma [34] and Ezikael et al [35] had found similar results.

Among all the physicochemical parameters analyzed, only Ca and Mg showed significant correlation with numbers of fungal isolates at 1 % and 5 % levels of significance respectively, and this occurred in the dry season. Anitha and Nayak [36] reported that there was no significant relationship between physicochemical parameters and fungal population in the soils of Tamil Nadu coast. Bohacz and Kornikowicz-Kowalska [37] investigated the species diversity of keratinophilic fungi in various soil types and revealed that none of the soil properties analyzed correlated significantly with the total frequency of keratinophilic fungi. The uneven distribution of keratinophilic fungi in soils with different physicochemical properties could be attributed to the differences in their physiology and their ability to tolerate changes in the abiotic factors.

Association between numbers of fungal isolates recorded during the dry and rainy seasons was investigated. The difference was statistically significant, which implied that there was no relationship between numbers of fungal isolates recorded across the two seasons. The highest number of fungal isolates was recorded during the rainy season. This could be attributed to high keratinous substrate deposited because of biological activities that took place in the park. This result contradicts Olajubu and Folorunso

[19] who isolated the highest number of keratinophilic fungi during the dry season. However, Saravanakumar and Kaviyarasan [38] demonstrated that there was no significant difference in their abundance and fungal distribution of both seasons. The small number of fungi colony recorded in the dry season could be because the fungal spores were dormant waiting for conducive environmental conditions.

In this study, the maximum **percentage of contribution** was observed with *Aspergillus niger* in both seasons. This result contradicts those of Ashok et al [33] who in their study found the maximum percentage of contribution with *Rhizopus stolonifer*, *Alternaria fasciculata, Fusarium moniliforme*, and *Curvularia spp*.from Palayakayal, Punnakayal and Tuticorin stations respectively and Saravanakumar and Kaviyarasan [38] who found *Aspergillus terreus* to occurred in the highest percent contribution.

In this study, there was variation in the percentage of frequency across the seasons. During the dry season, *Aspergillus niger, Mucor species, Chrysosporium tropicum,* and *Penicillium notatum* were the common fungi while during the rainy season, *Aspergillus flavus and Fusarium solani* were the common fungi. This result is contrary to Ashok et al [33] who in their study found *Acrocylindrium oryzae, Aspergillus conicus, A. fuscus, Penicillium granulatum* and *Rhizopus stolonifer* as the common species.

Table 1. Physicochemical parameters of the soil samples collected from Murtala Amusement Park, Minna, Niger State during dry season

Parameter	SWC	Swing	SWOC	Seesaw	M-G-R	RC
P^H (water)	6.617	6.621	6.585	6.882	6.725	6.955
% O/C	0.731	0.579	0.678	0.815	0.781	0.911
% O/M	1.260	0.998	1.169	1.409	1.350	1.575
% N	0.145	0.070	0.105	0.210	0.305	0.108
Ca (cmol/kg)	4.462	4.331	2.986	3.897	3.900	4.120
Mg(cmol/kg)	2.835	2.084	1.032	2.710	2.010	3.180
Na(cmol/kg)	0.245	0.208	0.223	0.336	0.290	0.210
K (cmol/kg)	0.736	0.624	0.669	0.810	0.690	0.570
AP (mg/kg)	10.260	19.946	11.559	8.260	9.870	10.900
Cu (mg/kg)	10.417	12.501	11.765	9.930	7.787	11.930
Zn (mg/kg)	8.333	6.254	9.375	9.800	10.200	8.620
Fe(mg/kg)	10.147	12.216	8.824	9.470	11.700	8.971
Mn (mg/kg)	5.882	8.824	7.294	6.870	7.250	7.790

SWC=slide with canopy, SWOC=slide without canopy, M-G-R=Merry-Go-Round, RC=Relaxation shed, O/C = organic carbon, O/M = organic matter, and AP= Available phosphorus

Table 2. Physicochemical properties of the soil samples collected from Murtala Amusement Park, Minna, Niger state during rainy season

Parameter	SWC	Swing	SWOC	Seesaw	M-G-R	RC
P^H (water)	6.850	6.960	6.723	6.559	6.934	6.680
% O/C	0.429	0.760	0.345	0.618	0.638	0.599
% O/M	1.741	1.314	0.597	1.065	1.099	1.033
% N	0.169	0.120	0.110	0.070	0.070	0.105
Ca (cmol/kg)	4.260	3.840	4.290	3.224	3.243	3.453
Mg(cmol/kg)	1.331	1.722	2.241	1.628	1.526	1.824
Na(cmol/kg)	0.210	0.230	0.190	0.171	0.186	0.215
K (cmol/kg)	0.597	0.780	0.687	0.513	0.557	0.647
AP (mg/kg)	12.550	11.880	15.383	17.593	10.211	3.889
Cu (mg/kg)	11.130	10.880	12.100	14.583	10.243	13.251
Zn (mg/kg)	6.357	8.770	11.550	7.353	10.292	9.728
Fe(mg/kg)	8.670	11.558	9.991	9.862	11.028	10.637
Mn (mg/kg)	11.212	10.112	6.813	9.334	6.386	11.117

SWC=slide with canopy, SWOC=slide without canopy, M-G-R=Merry-Go-Round, RC=Relaxation shed, O/C = organic carbon, O/M = organic matter, and AP= Available phosphorus

Table 3. Comparative correlation analysis between the numbers of fungal isolates and chemical properties of the soil collected from Murtala Amusement Park, Minna, Niger state during dry and rainy season

Season	P^H	% O/C	% O/M	% N	Ca (cmol/kg)	Mg (cmol/kg)	K (cmol/kg)	Na (mg/kg)	AP (mg/kg)	Cu (mg/kg)	Zn (mg/kg)	Fe (mg/kg)	Mn (mg/kg)
Dry	0.6056	0.3759	0.3756	-0.3604	0.7469*	0.8735**	-0.2996	-0.2062	-0.2297	0.3871	-0.4905	-0.02094	0.2170
Rainy	0.5927	0.3379	0.3354	-0.4739	-0.3570	-0.2602	-0.2100	-0.2158	-0.6890	-0.6297	0.3483	0.4801	-0.6588

Table 4. Numbers of fungal isolates of Murtala Amusement Park, Minna, Niger State from soil samples during the dry and rainy seasons

Season	Seesaw	SWC	Swing	RC	M-G-R	SWOC	Total	Percent
Dry	24	28	25	28	20	18	143	26.58
Rainy	36	35	67	30	188	43	399	73.62
Total	60	63	92	58	208	61	542	100.00
Percent	11.07	11.62	16.97	10.70	38.38	11.25	100.00	

Chi-square = 61.067, table value at 5 Degree of freedom and 0.001 value of p is 20.51
SWC=slide with canopy, SWOC=slide without canopy, M-G-R=Merry-Go-Round, RC=Relaxation shed.

Table 5. Percentage of contribution of fungal isolates found in the soil samples of Murtala Amusement Park, Minna, Niger State during the dry and rainy seasons

Fungal species	Dry season number of colony	Dry season percent contribution	Rainy season number of colony	Rainy season percent contribution
Aspergillus oryzae	3	2.10	-	-
A. flavus	8	5.59	50	12.53
A. fumigatus	9	6.29	32	8.02
A. niger	28	19.58	79	19.80
Candida albicans	7	4.90	48	12.03
Fusarium solani	5	3.50	25	6.27
F. oxysporum	7	4.90	36	9.02
Paecilomyces variottii	7	4.90	10	2.51
Rhyzopus stolonifer	12	8.39	11	2.76
Mucor species	10	6.99	11	2.76
Chrysosporium tropicum	13	9.09	20	5.01
Alternaria alternata	10	6.99	17	4.26
Penicillium notatum	14	9.79	19	4.76
Trichoderma species	8	5.59	21	5.26
Microsporium gypseum	2	1.40	20	5.01
Total	143	100	399	100

Table 6. Percentage of frequency of fungal isolates found soil samples of Murtala Amusement Park, Minna, Niger State during dry and rainy seasons

Fungal Species	Dry season number of Sample	Dry season percent frequency	Rainy season number of sample	Rainy season percent frequency
Aspergillus oryzae	3	O	-	-
A. flavus	3	O	5	C
A. fumigatus	4	F	3	O
A. niger	6	C	4	F
Candida albicans	4	F	3	O
Fusarium solani	3	O	5	C
F. oxysporum	4	O	3	O
Paecilomyces variottii	2	O	4	F
Rhyzopus stolonifer	4	F	3	O
Mucor species	5	C	4	F
Chrysosporium tropicum	6	C	4	F
Alternaria alternata	4	F	2	O
Penicillium notatum	6	C	3	O
Trichoderma species	4	F	4	F
Microsporium gypseum	2	O	4	F

Common (C) =76-100%, Frequent (F) = 51-75%, Occasional (O) = 26-50%, Rare (R) = 0-25%

Table 7. Simpson's Dominance and Evenness of fungal isolates from soil samples of Murtala Amusement Park, Minna, Niger State during the dry and rainy seasons

Season	Simpson's Dominance	Simpson's Evennness
Dry	10.86	0.0759
Rainy	9.84	0.0247

Table 8. Species similarity between soil samples collected from different spots in Murtala Amusement Park, Minna, Niger State during dry and rainy seasons

Index	Seesaw	SWC	SWOC	RC	M-G-R	Swing
Sorenson	0.100	0.095	0.197	0.241	0.096	0.109
Jaccard	0.048	0.046	0.090	0.108	0.046	0.052

SWC=slide with canopy, SWOC=slide without canopy, M-G-R=Merry-Go-Round, RC=Relaxation shed

Table 9. Diversity index of fungal isolates found in the soil samples of Murtala Amusement Park, Minna, Niger State during dry and rainy seasons

Season	Shannon's index	Simpson's index
Dry	2.531	0.908
Rainy	2.287	0.898

In the present study, *Aspergillus* was the common genus in both seasons. *Aspergillus niger* was the species that frequently occurred in the two seasons. This result is in line with Mini et al. [14]; Olajubu and Folurunso [19]; Ashok et al. [33] but disagrees with the study of Agu et al. [18] who in their study isolated *A.flavu*s as the most predominant fungi. The dominance of the genus *Aspergillus* in the soil may be due to their greater rate of spore production, dispersal, their extreme resistance to environmental conditions, and their suitability to grow in different soil pH concentration. Furthermore, the genus *Aspergillus* is known to produce some toxins such as aflotoxins, achrotoxins. These toxins, if secreted may inhibit the growth of other fungi.

Some species of *Aspergillus, Fusarium solani,* and *Candida albicans* are important causative organisms of mycosis-mycotic keratitis [39]. Nevertheless, several investigations in different regions have identified different species of *Chrysosporium* dominanting [40,41,42]. There are several reports on various types of infections caused by *Chrysosporium* species in man and animals particularly in immuno-compromised patients [43]. *Chrysosporium zonatum* was reported to cause disseminated infection in a patient with chronic granulomatous disease [44]. While Pakshir et al [2] isolated *Fusarium* genus to be the most common.

Dominance gives more weight to common or dominant species. In this case, a few rare species with only a few representatives will not affect the diversity. Evenness represents the degree to which individuals are split among species with low values indicating that one or a few species dominate, and high values indicate that relatively equal numbers of individuals belong to each species [45]. According to Simpson's dominance, the species of dry season had the highest dominance value. In addition, species of dry season had the the highest evenness value. However, there was the close margin between the values. Thus, Kerkhoff [46] said, ''evenness and dominance are two sides of the coin, their measures are complimentary.'' Evenness is not calculated independently but rather is derived from compound diversity measures such as Shannon's diversity (H'), Simpsons's diversity (D1), and Simpson's dominance (D2) as they inherently contain richness and evenness components [45]. However, evenness as calculated from D2 is mathematically independent of D1 [47] and therefore a more useful measure of evenness in many contexts [45].

Generally, both seasons showed no similarity of fungal species in the sample sites. This is because their species similarity values are far less than one. According to Sorenson the closer the species similarity is to one, the more the communities (seasons) have in common. Among all the sites, fungi species isolated from RC showed the highest similarity between the dry and rainy season with a value of 0.241 and 0.108 for Sorenson and Jaccard coefficients respectively. It was then followed by SWOC, Swing, and Seesaw respectively. However, SWC and MGR showed the least species similarity with 0.048 for Jaccard's Index.

Both Shannon Index and Simpson Index values were higher in dry season. The higher value is an indication of higher diversity. Hence, it could be said that diversity is higher in dry season compared to rainy season. However, the values were almost similar in the two seasons. The similarities in values may be because same study area was used to isolate the fungal species. The Shannon index is an information

Fig. 1. Sites where soil samples were collected

A= Swing, B=Slide without Canopy (SWOC), C=Slide with Canopy (SWC), D=Relaxation Shed (RC), E=Merry-Go-Round (MGR), F= Seesaw

statistic index, which means it assumes all species that are represented in a sample and that they are randomly sampled while Simpson's diversity represents the probability that two randomly chosen individuals belong to different species [48]. Reported values of fungal diversity are often attributed to the method employed to isolate the fungi. Identification of fungi is so complicated that researchers find it difficult to identify fungi up to species level. Many researchers stopped at generic level. This is because fungi exhibit different life cycle in the soil and in the laboratory. In addition, no single one medium is ideal for fungi culture.

5. CONCLUSION

This research shows that the soil of Murtala Amusement Park was contaminated with keratinophilic fungi: *Aspergillus niger, Aspergillus fumigatus, Aspergillus flavus, Aspergillus oryzae, Candida albicans, Penicillium notatum, Fusarium solani, Fusarium oxysporum, Trichoderma species, Rhizopus stolonifer, Mucor species, Chrysosporium tropicum, Alternaria alternata, Microsporum gypseum,* and *Paecilomyces variottii*. The physicochemical parameters have influenced the distribution of keratinophilic fungi. The presence of these organisms calls for close monitoring of children

playing in the park because they are an important group at a high risk of being exposed to these fungi.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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