



Aeromycoflora of the Lake of Futala area, Nagpur (M.S.) India

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ABSTRACT

The spores of fungal origin are allergic and capable of causing allergenic responses in susceptible individuals. More than 80 per cent microfungi genera have been associated with the respiratory disorders. Epidemiological studies revealed that higher concentration of the fungal spores in the air proved allergic, however even very low concentration can cause serious diseases. In the present study, aeromycoflora from various locations of the Lake of Futala area was reported for a month. A population of 3187 fungal colonies falls under 18 genera and 37 species have been confined by culture plate exposure method. Members of Deuteromycota and Ascomycota were reported predominant contributing 39.8 and 38.9 per cent colony count respectively, followed by Zygomycota and sterile mycelia while Oomycota had least count of colonies. Members of Basidiomycota did not appear. *Aspergillus* was reported dominant in terms of count of colonies and number of species while *Trichoderma viride*, *Cladosporium cladosporioides* and *Rhizopus stolonifer* were encountered sub-dominant. Altogether eleven species of single genus, *Aspergillus* have been reported from surrounding area of the Lake, followed by *Curvularia* with three species. *Rhizopus*, *Phytophthora*, *Penicillium*, *Mucor*, *Fusarium*, *Cladosporium* and *Alternaria* had two species each and remaining had single species. The concentration of spores of *Aspergillus niger*, *A. flavus*, *Cladosporium cladosporioides*, *Curvularia lunata*, *Rhizopus stolonifer*, *R. microsporus* and *Trichoderma viride* were reported most significant in the extramural environment of the Lake area under study.

Keywords: Aeromycoflora, outdoor, saprobic, microbes, allergy, asthma, extramural.

INTRODUCTION

Earth's atmosphere contains propagules of diverse group of microbes and other particles of biological origin (Rajendran et al., 2017). Among these, fungal spores play a significant role in causing disorders such as childhood

asthma, allergies, mycotoxicity, biodeterioration and infections in human and animals (Aimanianda et al., 2010). Airborne fungal spores are ubiquitous in nature and can survive in both wet and dry environment (Verma et al., 2013). The quantity of aeromycoflora is affected by the parameters like temperature, humidity, wind speed, rainfall, light, sand storm, and available organic matter. High temperature; storms and wind help in quite dispersal of the fungal propagules along with the dust particles to large distance whereas rainfall and humidity reduces the quality as well as the quantity of fungal propagules in the air (Manzelat, 2017).

Micro-propagules of fungal origin of extramural environment are considered to act as indicator of the level of atmospheric bio-pollution (Kakde et al., 2001). Majority of them are reported to be pathogenic to human beings causing disorders including allergy and asthma due to differentiated deposition in the respiratory system. Literature survey revealed that more than 80 per cent microfungi genera have been associated with the respiratory disorders (Ghosh et al., 2011). The prevalence of respiratory allergy by micro-propagules of fungi is estimated at 20 to 30 per cent among atopic individuals and up to 6 per cent in general population. It is of the most importance that allergens, viable microbes and other noxious agents that prevail in any particular environment are induced by changes in meteorological conditions. These measures help to monitor the levels of these agents in environment and aid clinicians in advising and treating patients as well as those at risk before they are exposed and sensitized (Ianovici and Tudorica, 2009).

The Lakes are visualized as uniform mass of water, evenly mixed from top to bottom with shallow depth and are extremely heterogeneous or patchy having physical, chemical and biological characteristics (Wikipedia, 2018). The Lake located near Futala, in the west Nagpur, spread over 60 acres and surrounded by wetlands, farmlands, forests, gardens, and many shops and a landscaped cowpat on one side. Several civilian visits the place in early morning to benefit in term of good health while others can spend a couple of hours in late evening for the peace, relaxation and enjoyment. It is famous place for immersion of idols of God Ganesh from Nagpur during *Ganesh Chaturthi Utsav*. During these days, tons of garbage is deposited on the shore line of Lake, composing of biodegradable and non-biodegradable wastes. South-east region of the Lake is covered by slums and used for dumping garbage, poly

bags, waste papers and food waste near the edges of Lake. Moreover, drain water is directly allowed to release into the Lake. Due to these, shore line area is intensely polluted.

Diverse group of fungal species constitute the major components of airborne fungal flora and they are the major cause of respiratory ailments of human beings, causing allergies, asthma, pathogenic infections of the respiratory tract, plant diseases and as well as important agents of degradation of cellulosic and non-cellulosic material in outdoor environment (Turkel and Bhajbhujje, 2017). Thus there is great need of understanding aerobiological studies of extramural environment from various locations of Lake of Futala area. Presently, prevalence of aeromycoflora from outdoor environment has so far not been reported earlier from this place, hence it seemed to be worthwhile to undertake a more comprehensive and systematic study of the diversity of aeromycoflora of Lake of Futala during winter season.

MATERIALS AND METHODS

1. Selection of Sampling Site:

The Lake of Futala area, near Telankhedi Garden, Nagpur (M.S.), India has been selected as sampling site as it is one of the picnic spot spread over 60 acres and several civilian visits the place in the morning and evening time for walking exercise. One can spend a couple of hours but it is not maintained and cleaned property. Disposal of poly bags, papers and waste near the edges of Lake make the spot unhygienic for human beings and domestic animals. Moreover, it is famous spot for immersion of idols of God Ganesh during Ganesh Chaturthi Utsav.

2. Media Preparation:

The culture plate exposure method was adopted for trapping the atmospheric fungal spores. Potato Dextrose Agar composed of 200 gm peeled potato, 20 gm dextrose and 20 gm agar in a liter of distilled sterile water was used for the isolation of aeromycoflora. After sterilization at 15 psi for 20 minutes, 1 ml aqueous solution of streptomycin sulphate (25mg/l) was added to medium preventing the growth of bacteria. About 10 ml PDA medium was poured to each sterile petri plate under aseptic condition. After solidification of medium at room temperature, the petri plates were sealed by sellotape.

3. Spore sampling:

The spore sampling was carried out for a month of September (2017) at an interval of week. The sealed petri plates containing agar jelly were exposed for 10 minutes in triplicates at each of the location of the Lake of Futala area. The exposed petri plates were again sealed by sellotape and incubated for 3-5 days at room temperature.

4. Identification of fungal flora:

The fungal colonies appeared on agar jelly were counted based on their macro-morphological properties. The species were identified on the basis of micro- and macro-morphology, reverse and surface coloration of colonies grown in Czapek's medium. The colonies were recorded for number, species and their distribution on petri plates. The isolates are authenticated by authority. The per cent distribution and incidence was calculated employing formula (Kayarkar and Bhajbhujje, 2014).

RESULTS AND DISCUSSION

The airborne fungal spores are linked to a range of detrimental health effects including asthma and allergy (Adams et al., 2013). Apart from these, their dispersal from the various sources brings about biodegradation of various perishable commodities in the market, environment, transport and storage (Kakde et al., 2001).

Aeromycological survey of the Lake, Futala area, near Telankhedi Garden, Nagpur, has been conducted for a month (Sep. 2017) employing the culture plate exposure method which proved to be more appropriate over others recording fungal diversity (Kayarkar and Bhajbhujje, 2014). This is in agreement with the findings of Nayak (2015); Turkel & Bhajbhujje (2017) who reported the significant count of fungal isolates as well as greater colony count of aeromycoflora by culture plate exposure method.

In the present survey, a population of altogether 3187 fungal colonies classified under 18 genera and 37 species have been recorded by culture exposure method (Table 1). Of these, Deuteromycota contributed 39.8 per cent colonies exhibiting greatest count followed by Ascomycota contributing 38.9 per cent colonies. The colony count was recorded declining to 10.6 and 9.6 per cent for Zygomycota and *sterile mycelia* respectively. Oomycota had least colony count (Fig. 1). Fungal spores from Basidiomycota did not appear on agar jelly.

Deuteromycota dominated with 10 genera and 15 species exhibiting comparative greater fungal flora followed by Ascomycota and Zygomycota. Least count was confined to Oomycota and *Sterile mycelia* (Fig. 2).

Moreover, *Aspergillus* was encountered with greater count of colonies on agar jelly. These results are confirmed with the findings of Bhajbhujje (2015); Manzelat (2017) who reported greater colony count of *Aspergilli* in extramural environment. The genera, *Trichoderma*, *Cladosporium*, *Curvularia*, *Mucor* and *Rhizopus* were recorded most significant. Among the species, *Aspergillus niger*, *Cladosporium cladosporioides* and *Trichoderma viride* were reported most dominant followed by *Aspergillus flavus*, *Curvularia lunata* and two species of *Rhizopus*, viz., *R. stolonifer* and *R. microsporus*. Other isolates, *Blackeslea trispora*, *Mucor pusillus*, *Mucor mucedo*, *Aspergillus fumigatus*, *A. ochraceus*, *A. versicolor*, *Alternaria alternata*, *Torula herbarum*, *Helminthosporium tetramera* and *Penicillium notatum* contributed 2.5-1.0 per cent fungal airspora. *Sterile black* and *white mycelia* contributed 4.6 and 5.0 per cent fungal airspora respectively (Fig. 3).

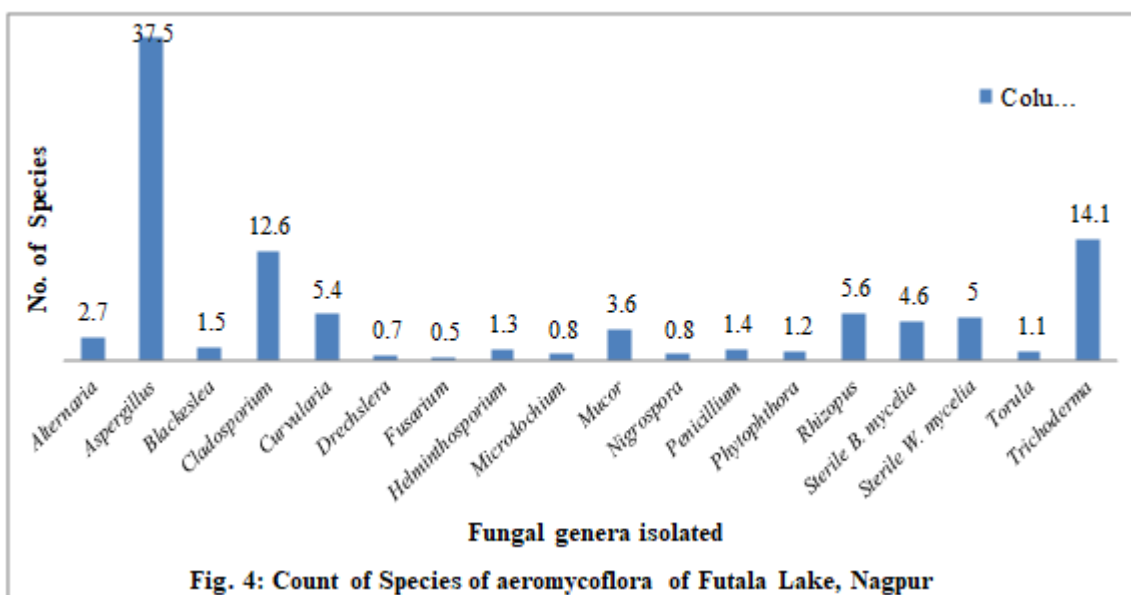
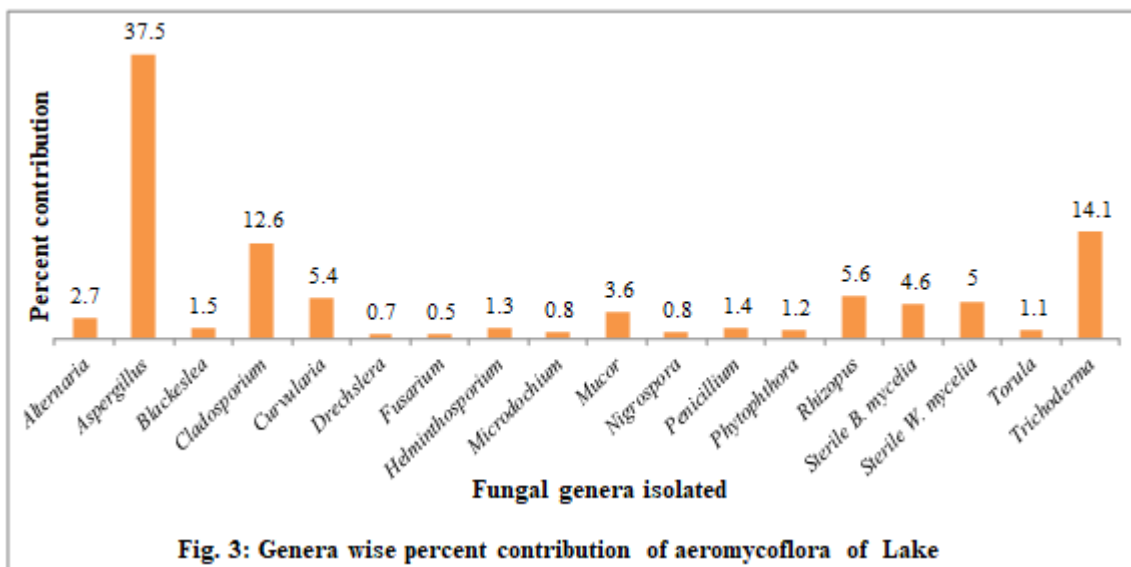
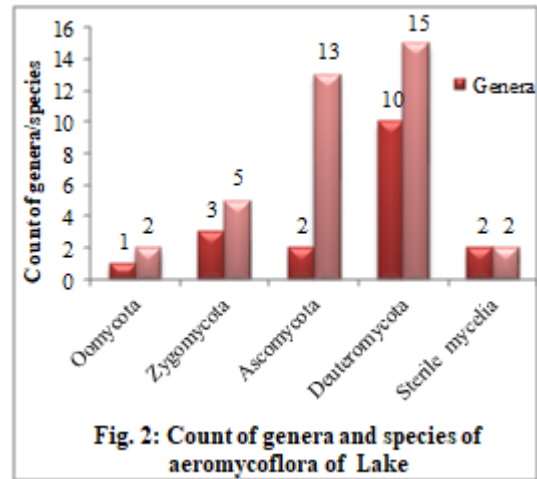
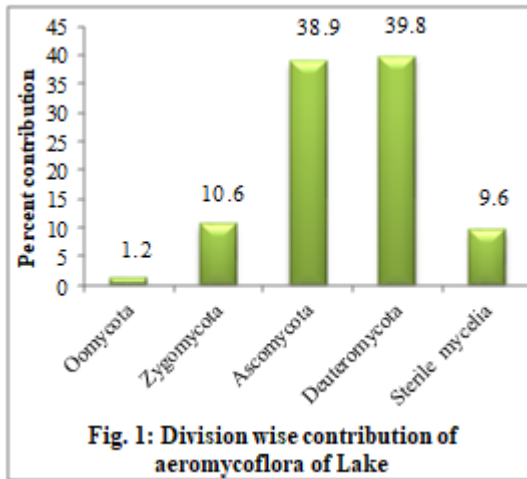
Aspergillus was dominated with greater number of species against other genera. This is in agreement with the findings of Katre (2016) who reported the greater count of species of *Aspergilli* from open air of Railway station, Nagpur. Kaur (2017) reported significant count of *Aspergilli* from open air of Bus Stand of Nagpur. *Curvularia* had 3 species while *Rhizopus*, *Phytophthora*, *Penicillium*, *Mucor*, *Fusarium*, *Cladosporium* and *Alternaria* had 2 species each. Single species was detected for *Trichoderma*, *Torula*, *Nigrospora*, *Microdochium*, *Helminthosporium*, *Drechslera*, *Blackeslea*, *sterile black* and *white mycelia* (Fig. 4).

Deuteromycota had comparatively greater count of isolates and contributed highest concentration of airspora (Fig. 1 & 2). Members of this group are well known saprobes involved in the biodegradation of organic substrate of cellulosic nature (Verma et al., 2013). It was interesting to record that members of Basidiomycota did not appear in environment of Lake under study, may be possibly attributed to mode of nutrition, as majority of fungal organisms of this group are obligate parasites of crop plants (Bhajbhujje, 2015).

The fungal organisms require more than 65 per cent humidity for their growth providing nutrient rich substrates (Kayarkar and Bhajbhujje, 2014).

Table 1: Distribution of Aeromycoflora of Lake of Futala area, Nagpur for a month

S.N.	Fungal organism	Total count	Per cent Contribution	
			Species	Genus
A.	Oomycota	38	1.20	1.20
1	<i>Phytophthora infestans</i> (Mont.) de Bary	17	0.53	1.20
2	<i>Phytophthora</i> sp.	21	0.66	
	<i>Genera/(species)</i>	1(2)		
B.	Zygomycota	338	10.6	10.6
3	<i>Blackeslea trisporea</i> Thaxter	47	1.47	1.47
4	<i>Mucor mucedo</i> de Bary & Woron	58	1.82	
5	<i>M. pusillus</i> Lindt	56	1.76	3.58
6	<i>Rhizopus microsporus</i> Tiegh	67	2.10	
7	<i>R. stolonifer</i> (Eh.Ex.Rr.) Lind	110	3.45	5.55
	<i>Genera/(species)</i>	3(5)		
C.	Ascomycota	1238	38.9	38.9
8	<i>Aspergillus amstelodami</i> (Mang) Thom & Church	22	0.70	37.53
9	<i>A. candidus</i> Link	19	0.60	
10	<i>A. carneus</i> (Tieghem) Blochwitz	11	0.35	
11	<i>A. flavus</i> Link.	260	8.16	
12	<i>A. fumigatus</i> Fres.	57	1.80	
13	<i>A. nidulans</i> G Winter	14	0.44	
14	<i>A. niger</i> Van Tieghem	662	20.8	
15	<i>A. ochraceus</i> Wilh	69	2.17	
16	<i>A. sulphureus</i> (Fres.) T&C	6	0.20	
17	<i>A. terreus</i> Thom.	9	0.28	
18	<i>A. versicolor</i> Tiraboschi	66	2.07	
19	<i>Penicillium citrinum</i> (C & S) Pitt.	9	0.30	
20	<i>P. notatum</i> Crulina	34	1.07	
	<i>Genera/(species)</i>	2(13)		
D.	Basidiomycota	-	-	
E.	Deuteromycota	1268	39.8	39.8
21	<i>Alternaria alternata</i> Keissler	81	2.54	2.67
22	<i>A. trititica</i> Prasada & Prabhu	4	0.13	
23	<i>Cladosporium cladosporioides</i> (Fresen.) de Vries	373	11.7	12.55
24	<i>C. herbarum</i> (Pers.) Link	27	0.85	
25	<i>Curvularia lunata</i> (Wakker) Boedijn.	152	4.77	5.43
26	<i>C. ovoide</i> (Hiroe & N.Vatan) MuntCvetk	7	0.22	
27	<i>C. brachyspora</i> Boedijn	14	0.44	
28	<i>Drechslera rostrata</i> (Drechsler) Richardson & Fraser	23	0.72	0.72
29	<i>Fusarium oxysporum</i> Schlecht.	13	0.40	0.53
30	<i>F. semitectum</i> Berk & Ravenel	4	0.13	
31	<i>Helminthosporium tetramera</i> McKinney	41	1.30	1.30
32	<i>Microdochium dimerum</i> (Penz.) Arx	25	0.78	0.78
33	<i>Nigrospora oryzae</i> (Berk & Broome) Petch	24	0.75	0.75
34	<i>Torula herbarum</i> (Pers.) Link	33	1.04	1.04
35	<i>Trichoderma viride</i> Pers.	447	14.0	14.0
	<i>Genera/(species)</i>	10(15)		
F.	Other types	305	9.60	9.60
36	<i>Sterile white mycelium</i>	160	5.00	5.00
37	<i>Sterile black mycelium</i>	145	4.60	4.60
	<i>Genera/(species)</i>	2(2)		
	Sum of total colonies	3187	100.1	100.1



Most of the spores of fungal origin remain existed predominantly in the environment during the rainy season (June-Sept.) when temperature ranges between 20-30°C and relative humidity remains 75 per cent or above (Mishra and Deshmukh, 2009). The environment of the Lake provides maximum humidity and favourable medium for the growth of airborne fungal spores. Dust level in an open air is relatively high, especially when the human and other activities are significant. Greater bioaerosol levels occur when dust is stirred up during cleaning, playing and dumping waste materials. Similarly, these activities are responsible creating health problems in human beings (Vijayalakshmi and Jeyachandran, 2010) and other domesticated animals. Majority of the researchers proved that optimum temperature, high moisture content, nutritive substrate creates favorable microclimates for a profuse growth, proliferation and sporulation of airspora leading to higher population of fungal species (Kayarkar and Bhajbhujje, 2014). Variation in these factors, particularly temperature resulted increase dormancy and inhibition of fungal growth (Bhajbhujje, 2015).

Nagpur has tropical savannah climate with dry conditions prevailing for most of the year. The rainfall decreases from September to October and winter lasts from November to January, during which temperature can drop below 10°C, is known for very pleasant weather with average minimum temperature and high humidity. This moderately cold season is expected ideal for rapid multiplication and enhancement of the growth rate of biotic community including fungal organism (Wikipedia, 2018).

Many fungal species bring about food spoilage and also associated with unpleasant odors, discoloring and degradation of cellulosic and non-cellulosic substrate (Jyoti and Malik, 2013). Many fungal spores are allergic capable of causing allergenic responses in susceptible individuals. Epidemiological studies showed that greater concentration of microorganisms in the air can be allergic, however very low concentration of microorganism can also cause serious diseases (Chandel, 2002).

The liberation of spores follow dispersion mechanism; both are interrelated and related to wind velocity, weather and other existing environmental conditions. The liberation of spores of *Aspergilli* and *Penicilli* were favored by high air humidity and while those of *Alternaria*, *Cladosporium* and *Curvularia*, were liberated

mechanically by the action of wind (Ianovici, 2008; Kayarkar and Bhajbhujje, 2014).

Total three genera belongs to Mucorales were confined on agar jelly can cause a mucormycosis in human population. Metabolic products of *Rhizopus* induced significant inhibition was proportional with the increase in concentrations of the metabolic products. *Rhizopus stolonifer* is an opportunistic agent of disease and hence causes infection in people with a weakened immunity. Zygomycosis is the main disease that might be caused by this fungus in humans and while it is not entirely understood yet, this disease is very dangerous and can be fatal. The action of smelling spoiled food may be a source of inhalation exposure to the mold (Wikipedia, 2018). *Mucor* has been linked with zygomycosis, allergies, and mold sensitivity. Inhalation of its spores caused septic arthritis, renal infections, gastritis and severe pulmonary infection, difficulty in breathing, mucocutaneous and rhinocerebral infections (Adams et al., 2013). Detailed investigations on epidemiology and clinical presentations of these unusual infections may improve early diagnosis and treatment.

The report of present study revealed that the micro-fungal organisms from extramural environment reported under Ascomycota appeared to be dominated. Among these most prevalent fungi, *Aspergillus* was the most abundant and widely distributed microfungus organism. Members of the genus *Aspergillus* are known obligate saprophyte and survive in the indoor and outdoor environment provided favorable climatic condition and nutritive substrate (Adams et al., 2013). *Aspergillus flavus*, *A. niger*, and *A. fumigatus* had highest count of occurrence. Some strains of *A. niger* have been reported to produce potent mycotoxins called ochratoxins other sources disagree, claiming this report is based upon misidentification of the fungal species. Recent evidence suggested that some true *A. niger* strains did produce ochratoxin A isoflavone orobol. Fungal organisms are known to produce B1, B2, G1, and G2 as major aflatoxins. Aflatoxin B1 is the most toxic and potent hepatocarcinogenic natural compound characterized. *A. flavus* also produces other toxic compounds including sterigmatocystin, kojic acid, β -nitropropionic acid, aspertoxin, aflatrem, gliotoxin and aspergillilic acid. *A. fumigatus* grown on certain building materials can produce genotoxic and cytotoxic mycotoxins such as gliotoxin (Wikipedia, 2018).

Alternariol monomethyl ether, tenuazoic acid and altertoxins were secreted by *Alternaria alternata*, can affect respiratory system, skin, and nails in humans and also induced reduction in seed germination and seedling emergence with chromosomal abnormalities in plants (Bhajibhuje, 2013; Kayarkar and Bhajibhuje, 2014). *Cladosporium* was most correlated with meteorological parameters. This may be attributed dry conidia in chains easily carried through air. Therefore dispersion of *Cladosporium* spores is more influenced by meteorological parameters than *Alternaria* spores (Ianovici, 2008). The fungus, *Cladosporium cladosporioides* predominated and it is the most important genus in terms of defining variations in total count. This genus is reported to be a major constituent of fungal bioaerosol (Kayarkar and Bhajibhuje, 2014; Turkel and Bhajibhuje, 2017). *Cladosporium cladosporioides* rarely causes infections in humans, although superficial infections have been reported. It can occasionally cause pulmonary and cutaneous phaeohyphomycosis and it has been isolated from cerebrospinal fluid in an immunocompromised patient. *Cladosporium cladosporioides* can also induce respiratory inflammation due to the up-regulation of macrophage inflammatory protein (MIP)-2 and keratinocyte chemoattractant (KC), which are cytokines involved in the mediation of inflammation (Wikipedia, 2018).

Curvularia lunata is one of the main causative agents of phaeohyphomycosis. Initial infection via breaks to the epidermal barrier or the inhalation of spores can lead to disseminated infections, which are often associated with a poor prognosis. *C. lunata* is an opportunistic pathogen infecting immunocompromised patients and those on rigorous steroid drug regimens such as solid organ transplant recipients, advanced AIDS patients and cancer patients. Dematiaceous fungus *Curvularia lunata* may facilitate foreign body infections of catheters, heart valves and pacemakers. Allergic fungal manifestations include asthma, rhinitis, sinusitis and broncho-pulmonary mycoses caused by a variety of etiological fungal agents including *Curvularia lunata*. Mycotic keratitis and conjunctivitis are more commonly reported in tropical climates (Wikipedia, 2018).

Majority of the airborne fungal propagules including spores are potential allergen. Hence, outdoor aeromycological surveys help considerably to locate the sources of spores, their identification, concentration and seasonal variation. Thus, such information provides basic data for the treatment of sensitive individuals

suffering from an allergy. Data obtained from such a survey help to obtain spore calendar for the allergens, their avoidance and management strategies (Mishra and Deshmukh, 2009).

CONCLUSIONS

The results of aeromycoflora during entire sampling period revealed prevalence of heavy load of several fungal spores and other contaminants in the atmosphere. Main sources of fungal spores are the surrounding dirty area of Lake of Futala, where there is the dumping of waste food material, vegetable matter and other garbage by human population of nearby locality and by the visitors. Different environmental factors such as humidity, temperature fluctuation changes the physical and chemical properties of the Lake are conducive for the growth of the fungal organisms accelerating the deterioration process. Deuteromycota contributed highest count of isolates followed by Ascomycota. Zygomycota had moderate count while Oomycota and sterile mycelia had least count of isolates. The dominant microfungus genera include *Aspergillus* and Deuteromycetous isolates that are *Alternaria*, *Cladosporium*, *Curvularia* and *Trichoderma* in the environment of the Lake.

Study of this kind of aeromycoflora near the Lake is highly interdisciplinary and has tremendous scope to find the significant application in human health. The spores in air are the representatives of the members of microorganisms growing in that habitat. In this context, this study will certainly enlighten the scientists and planners to make a better environment. Exposure to outdoor airborne inhalant mould allergens develops respiratory symptoms, airways disorders and allergies. Thus cleanliness is most importantly required for maintenance of good health, it is therefore, necessary to regularly monitor the prevalence of fungal air spora by modern technologies. It may be convenient in the prevention of fungal allergic disorders. Effective disposal of solid waste to other area may improve the air quantity of this Lake area. The air current plays an important role in sweeping the aeromycoflora; therefore it is necessary to clean the area of Lake at regular interval.

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