e-ISSN: 2582-5208
International Research Journal of Modernization in Engineering Technology and Science
Volume:02/Issue:08/August-2020 Impact Factor- 5.354 www.irjmets.com

PREVALENCE OF AIR-BORNE FUNGI IN INDOOR ENVIRONMENT OF DENTAL CLINIC

Dr. Surendra K. Giri*1

*1Associate Professor, Department of Botany, S.M. Mohota College of Science, Nagpur. (M.S.) INDIA

E-mail-drsk.giri@rediffmail.com

ABSTRACT

The present study was aim to find out the prevalence of airborne fungi in indoor air of dental clinic at Nagpur city. Air sampling was conducted during October, 2013 to March, 2014 by using Hi-Air sampler (Hi-Media-LA002 with RBS-290 and RBS-640 media strips) at monthly intervals. In the past few decades the public health has given more attention to the quality of air and their health impact issue associated with exposure to fungi. There is no uniformity in the suggested guidelines for acceptable levels of fungi in indoor ambient air. Thus, health professionals have no way to determine what levels of fungi may pose a threat to human health. The indoor air concentration of fungal spores was found higher than currently suggested guidance value. The average indoor air concentration was found in dental clinic 952 CFUs/^{3} (colony forming units per cubic meter), whereas in control air (outdoor) levels was averaged 614 CFUs/m^3. Total indoor colony counts ranged from 6 to 133 CFU/m^3, whereas in outdoor air it is ranged from 1 to 92 CFU/m^3 in studied environment. Without intentionally developing a sterile environment, a mold free, indoor environment is not possible to maintain. The most common fungal genera/species isolated from indoor as well as in outdoor environment of dental clinic includes *Aspergillus, Cladosporium, Curvularia, Alternaria, Penicillium, Rhizopus, Mucor, Trichoderma, Nigrospora* and *Candida*. Beside these yeasts and non-sporulated fungi are also isolated. Many health professionals suggest that if the indoor ambient air concentration is more than concentrations observed in outdoor air and if the fungi detected in both are similar, then there is high health risk to patients.

Keywords: Airborne, fungi, indoor, environment, dental clinic.

I. INTRODUCTION

Airborne fungal spores are present in outdoor air all year round, usually in high numbers. These spores can enter indoor environments via natural ventilation (open windows and doors). They are also brought indoors on people's clothing, shoes and pets. Therefore, indoor fungi can be a mixture of fungi from outdoors and fungi from indoor sources. All fungi are eukaryotes and exist in different growth forms such as rusts, mushrooms, mould and yeasts. Filamentous fungi (moulds) consist of long, branching filaments called hyphae and reproduce via formation of spores from sexual or asexual processes. Some fungi can exhibit both growth forms and are known as dimorphic fungi. They are ubiquitous in all environments like indoors and outdoors. Fungi in indoor environments are a problem for a number of reasons like they deteriorate or damage the surfaces, cause unpleasant odors, can cause an allergic response and also be responsible for infections, and other health problems.[15, 21].

Moulds produce millions of spores, which are loosely attached and even slight air currents will disturb the spores making them airborne. Due to their small size (large spores are 10-20mm, average 1-5mm) spores easily stay airborne and may be respirable and breathed deep into the airways. Spores are very tolerant to dryness, changes in temperature, UV light and some chemicals. The spores may carry allergens and toxins, which are stable and may stay active even after the spore has lost its viability. Some fungi do not produce infections but can cause allergic reactions. Fungal spores are generally recognized as important causes of respiratory allergies, in both the lower and upper respiratory tracts[1,6]. Allergic reactions usually occur at the site of allergen deposition. When larger fungal spores are inhaled, they are deposited in the naso-pharynx and are associated with nasal and/or ocular symptoms usually referred to

as hay fever (also known as rhinitis). Spores of <5mm can penetrate the lower airways, where allergic reactions will usually manifest as asthma[5].

Aspergillus flavus is more likely to be recovered from the upper respiratory tract than any other *Aspergillus* species. Allergic fungal sinusitis (AFS) and sinus aspergilloma are caused by *A. fumigatus*[13]. The present study was conducted to find out the prevalence of airborne fungi in indoor environment of dental clinic.

II. MATERIAL AND METHODS

PHYSIOGRAPHY OF NAGPUR

The city of Nagpur (Latitude: 21^{O} 8'N, Longitude: 79^{O} 9'E) is situated at an approximate centre of India and it is 307.4 meter above the sea level. Nagpur is having population of about twenty eight lakhs which is spread over an area of about 150 sq. kilometer. Nagpur has a tropical wet and dry climate with dry conditions prevailing for most of the year. City receives an annual rainfall of 1,205 mm (47.44 in) from monsoon rains during June to September. Summers are extremely hot lasting from March to June, with maximum temperatures occurring in May. Winter lasts from November to January, during which temperatures can drop below 10°C (50°F).

SAMPLING AND ISOLATION PROCEDURE

Present study was conducted by using a Hi-Air Sampler (LA-002) with RB- PS640 and PS-290 media strips). Air was sampled for 4 minutes in indoor air of dental clinic by using PS-640 strip, and after the indoor sampling, the outdoor sampling was also conducted by using PS-290 strip as a control. These samples were taken at monthly intervals over a period of six months (October, 2013 to March, 2014).

CFU COUNTS AND IDENTIFICATION

Sampled PS-640 and PS-290 media strips were packed in plastic box and return in laboratory for incubation. Exposed media strips were incubated in an inverted position at room temperature; after four to five days of incubation, the colony forming units (CFUs) were visually counted and the total fungal count was expressed as colony forming units per cubic meter of air CFUs/m^3 (Figure. 2) The fungi detected per unit volume of air calculated as under:

$$\text{CFUs/m}^3 = \frac{\text{Colonies on agar strip} \times 25}{\text{Sampling time in minutes (4)}}$$

The identification of fungal colonies was done after sub cultures were prepared from RBS's strips after seven to ten days. Isolated genera/species were identified by macroscopic and microscopic characteristics of fungal genera/species with the help of standard published literature[4,18-20]. Some of the fungal colonies do not form sporulation after prolonged period of incubation, these all are counted as non-sporulating fungi. Maximum and Minimum Temperature, relative humidity were measured by using hygrometer device during the sampling period (Table 1).

III. RESULTS AND DISCUSSION

The present study revealed that total 19 fungal species belonging to 11 genera along with non-sporulating fungi were isolated in indoor air; while in outdoor (control) air recorded 17 species belongs to 10 genera excluding non-sporulating fungi (Table 2). The species of *Aspergillus, Penicillium, Cladosporium, Curvularia, Alternaria, Trichoderma, Mucor, Rhizopus* and *Candida albicans* are the dominant and frequently isolated genera in present study (Figure.1). The majority of fungal genera were found both inside and outside the studied environment. This confirming the ubiquitous nature of these fungi, except the *Aspergillus candidus* and *Candida albicans* are not isolated in outdoor air, where the indoor environment capable of supporting spore survival in the absence of outdoor contributions to the indoor air [1,9]. The indoor environment of dental clinic showed highest colony forming units CFUs than the outdoor (control) air, it was 952 mean CFU/m^3 & 614 mean CFUs/m^3 respectively.

e-ISSN: 2582-5208
International Research Journal of Modernization in Engineering Technology and Science
Volume:02/Issue:08/August-2020 Impact Factor- 5.354 www.irjmets.com

The dominant fungi were isolated from indoor air of clinic are shown in table 2, *Aspergillus flavus* recorded 133 mean CFU/m^3, *Curvularia tetramera* 122 CFU/m3, *Aspergillus niger* 110 CFU/m3, *A. fumigatus* 76 CFU/m3, *Cladosporium herbarum* 71 CFU/m3, *Curvularia geniculata* 48 CFU/m3, *Alternaria spp.* 44 CFU/m3, *Penicillium citrinum* 46 CFU/m^3, Trichoderma 36 CFU/m3, *Rhizopus* 34 CFU/m3, P. chrysogenum *30 CFU/m3,* P. glaucus 25 CFU/m3, *Mucor* 24 CFU/m3 and *Candida albicans* 15 CFU/m3, Non-sporulating fungi 64 CFU/ m^3, followed by other species which are recorded below 10 mean CFU/m^3 (Table 2)

A. flavus was the most prevalent *Aspergillus* species to be recovered from the air of hospital wards and homes in Iran[12]. In addition, many *A. flavus* isolates produce aflatoxin B1, the most toxic and potent hepatocarcinogenic natural compound[20]. The second largest species was *Curvularia tetramera* isolated and recorded in indoor air. Total mean colony count of *Penicillium spp.* was 90 mean CFU/m^3 in indoor air whereas it was 36 mean CFU/m^3 in outdoor (control) air. Species of *Penicillium* are reported to be allergenic and mycotoxigenic. The role of infections caused by the moulds likes *Mucor* and *Rhizopus* reported[8]. In present investigation *Rhizopus* and *Mucor spp.* isolated and recorded in 34 mean CFU/m3 and 24 mean CFU/m3 respectively in studied environment. However, *Aspergillus, Penicillium* and other fungi also commonly grow in house dust in buildings without obvious moisture problems and can be detected indoors at levels greater than those detected outdoors. In present studied environment there is no moisture problem at all, though these fungal genera were isolated in more numbers in indoor air as compared to outdoor air. Several filamentous fungi such as *Acremonium, Alternaria, Aspergillus spp., A. niger, A. flavus, A. versicolor, Aureobasidium, Botrytis, Cladosporium, Exophiala, Fusarium, Penicillium, Paecilomyces, Scopulariopsis, Stachybotrys, Trichoderma* and *Torula* contribute to deterioration of construction materials made of concrete and stone[11].

Species of *Alternaria and Aspergillus* have been reported highly allergenic and also associated with adverse health effects and some strains produced mycotoxins, including *A. fumigatus, A. niger, A. flavus* and *A. oryzae*. Some species of *Penicillium* also found in indoor environments and are allergenic and produced hypersensitivity pneumonia, allergic alveolitis, keratitis, otomycosis and penicilliosis; *Cladosporium* species are ubiquitous in indoor as well as in outdoor air and are allergenic. Indoor airborne fungi levels are a reflection of outdoor levels and it is estimated that 40% to 80% of outdoor levels with similar rank order species of fungi[6].

Predominant fungi in general hospital are *Cladosporium spp., Penicillium spp., Aspergillus spp*., and *Alternaria spp*. The concentration of fungal counts CFU/m3 recorded in main lobby of hospital was 156 CFU/m3; in ICU it was 65 CFU/m3; in surgical ward it was 96 CFU/m3; and in biomedical laboratory 126 CFU/m3[14]. The airborne fungal flora of two different hospitals in Istanbul (Turkey) were studied and reported dominant species i.e. *Cladosporium* followed by *Alternaria, Penicillium, Aureobasidium, Aspergillus, Mycelia sterilia, Scopulariopsis, Rhizopus, Paecilomyces* and *Ulocladium*[9]. Airborne microbial contamination in Dental practices in Isai, Romania recorded the values ranged from 21 CFU/m3 to 29 CFU/m3 at the beginning of the four hour period and from 52 CFU/m3 to 808 CFU/m3 at the end of the four hour period. The average value was twice as high after clinical activity as compared to before the working sessions i.e. 230.7 CFU/m3 and 109 CFU/m3, respectively. This fungal contamination is involved in respiratory irritations, infections and allergic reactions[16].

The viable and non-viable bacterial and fungal airborne particles in 64 dental clinics were studied and reported the species of *Penicillium notatum, P. chrysogenum, P. digitatum, Aspergillus flavus, A. nidulans, A. parasiticus, A. ochraceous* and *A. niger*. In present study also recorded the same species genera in dominant forms[17]. The species of *Aspergillus* and *Penicillium* in various clinics of dental hospital in Kancheepuram district, Tamilnadu were also reported and isolated the species of *Aspergillus niger, A. fumigatus* and *Penicillium* species 100 CFU/m3/30 minutes. They concluded that, the periodical microbiological survey should be done to examine for the presence of airborne fungal spores[2]. In the previous study we were isolated and recorded 97 fungal genera/species from indoor environment of hospitals of Nagpur city by culture petri-plate method[21].

The bioaerosols in healthcare and the dental environment reported 45 fungal species which are *Absidia, Acremonium, Alternaria, Aspergillus fumigates, A. flavus, A. niger* and *Aspergillus spp. Bipolaris, Candida albicans, Chaetomium, Cladosporium, Curvularia, Fusarium, Mucor, Nigrospora, Penicillium, Phoma, Pithomyces, Rhizopus, Scopulariopsis, Syncephalastrum spp, Sporotrichum, Stemphylium, Trichoderma, Trichosporon, Ulocladium* and *Verticillum* spp. They recorded 233 CFU/m3 colonies before dental procedures, 351 CFU/m3 colonies during patients' procedures and 106 CFU/m3 colonies after the treatment of patients[23]. Present investigation also recorded 19 and 17 species of fungi in indoor and outdoor air respectively; there microscopic structures are shown in Figure 3.

Fungal contamination and disinfection of dental chairs among private dental clinics in Riyadh, Saudi Arabia was reported *Aspergillus parasiticus, A. fumigates, A. flavus, A. niger, Candida albicans* and *Rhizopus* are the most common contaminant in dental chairs i.e. foot rest found 100% highest level of contamination followed by back rest 83.5%, head rest and seat rest having 66% each[10]. During the clinical activity, dentists performed various treatments to patients need. It includes prophylactic procedures (dental plaque, calculus), drilling with air-driven, root canal and capping. Since it is difficult to discriminate fungi coming from outdoor and indoor sources, it is a challenge to identify the indoor sources by air sampling. Identification of microbial source with direct sampling is also commonly employed. Mold growth is not necessarily visible in large buildings but air sampling may reveal the hidden mold growth. The number of samples or the sampling times needed are important factors if one aims to obtain representative results of viable fungi in indoor air. Sequential duplicate sampling for airborne viable spores has shown that their concentrations vary with time.

IV. CONCLUSION

Concentrations of air fungi in indoor air of dental clinic were found to be lower as the recommended concentration (>700 CFU/m3) and do not have potential to develop adverse health effects on the occupants. Several filamentous fungi such as *Alternaria, Aspergillus spp., A. niger, A. flavus, Cladosporium, Fusarium, Penicillium, Trichoderma, Mucor, Rhizopus* and nonsporulated fungi proved to contribute various health effects on human. *Aspergillus* species was the most common fungi isolated and reported from various indoor environments including hospital wards i.e. ICU, burn ward and Operation theater also. *Aspergillus* is occasionally involved in incidence of aspergillosis, ear and skin infections. It is therefore important to evaluate the quality of the air we breathe in indoor and outdoor environments, especially in hospitals and dental clinic also. The prevalence of airborne fungi and their concentrations in indoor air of dental clinic can be used to determine the degree of cleanliness as well as to determine the source of human discomfort or infection. Infections caused by common indoor environmental moulds, such as *Aspergillus, Penicillium, Fusarium, Mucor, Curvularia, Cladosporium, Rhizopus* and *Alternaria*, are increasing in HIV-infected patients. *Penicillium* and *Aspergillus* are more closely associated with respiratory allergic symptoms and allergic sensitization than the common outdoor moulds *Cladosporium* and *Alternaria*. The importance of this study was that it recommended the usage of the regular environmental microbial monitoring in dental clinics to prevent the transmission of diseases between the healthcare staff, dentists and patients, especially dealing with immunocompromised patients.

Ethical Disclosures:

The author announces that no experiments were performed on voluntaries or animals and no data were collected from patients in this study.

Table-1: Maximum and minimum temperature, relative humidity recorded during the sampling period by using hygrometer**.**

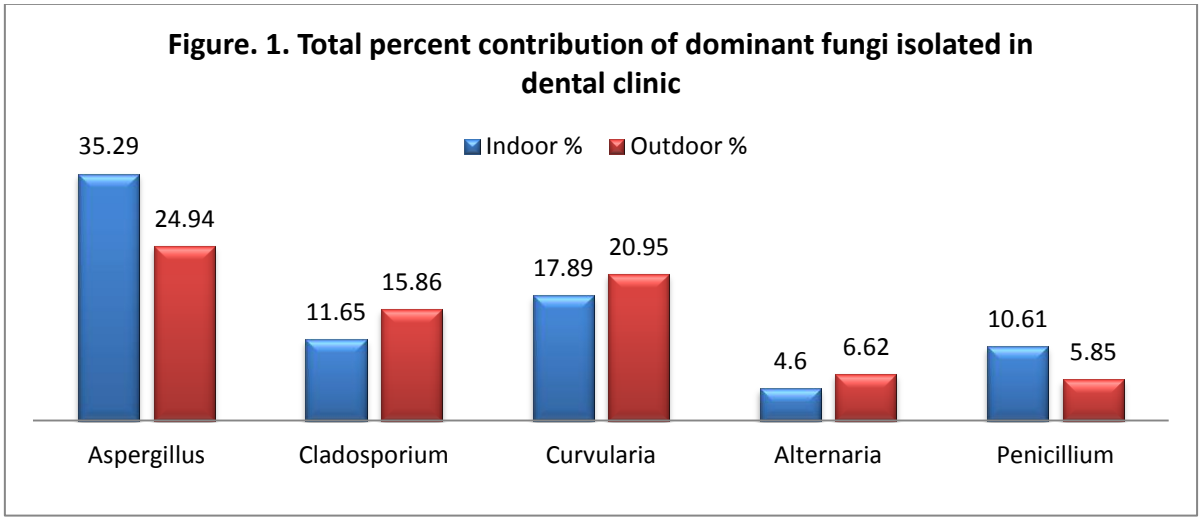
Sampling Months	Indoor Average Temperature (0^C)		Indoor Average Relative humidity
	Minimum	Maximum	
October 2013	27.8	31.8	60

November 2014	25.3	29.5	54
December 2013	22.5	26.7	52
January 2014	21.8	25.3	48
February 2014	27.6	31.1	43
March 2014	28.2	32.9	40

Table-2: Fungal species isolated from the indoor and outdoor air of dental clinic and their total mean CFU/m3 and percent contribution.

Sr. No.	Fungal genera/species	Total CFU/m3	Mean	%	Total CFU/m3	Mean	%
1	Aspergillus niger	110		11.54	92		15.01
2	Aspergillus flavus	133		14.01	35		5.68
3	Aspergillus fumigates	76		7.93	22		3.65
4	Aspergillus terrus	10		1.04	4		0.59
5	Aspergillus candidus	7		0.77	00		0.00
6	Cladosporium herbarum	71		7.50	40		6.53
7	Cladosporium spp.	40		4.16	57		9.33
8	Curvularia tetramera	122		12.80	62		10.09
9	Curvularia geniculata	48		5.09	67		10.86
10	Alternaria spp.	44		4.60	41		6.62
11	Mucor spp.	24		2.57	31		5.00
12	Rhizopus spp.	34		3.61	51		8.31
13	Fusarium spp.	10		1.04	22		3.56
14	Trichoderma spp.	36		3.77	8		1.27
15	Nigrospora spp.	6		0.66	1		0.17
16	Candida albicans	15		1.59	00		0.00
17	Penicillium chrysogenum	30		3.12	15		2.46
18	Penicillium citrinum	46		4.87	12		1.95
19	Penicillium glaucus	25		2.63	9		1.44
20	Non-sporulating fungi	64		6.67	45		7.38
Total		**952**		100	**614**		100

e-ISSN: 2582-5208
International Research Journal of Modernization in Engineering Technology and Science
Volume:02/Issue:08/August-2020 Impact Factor- 5.354 www.irjmets.com



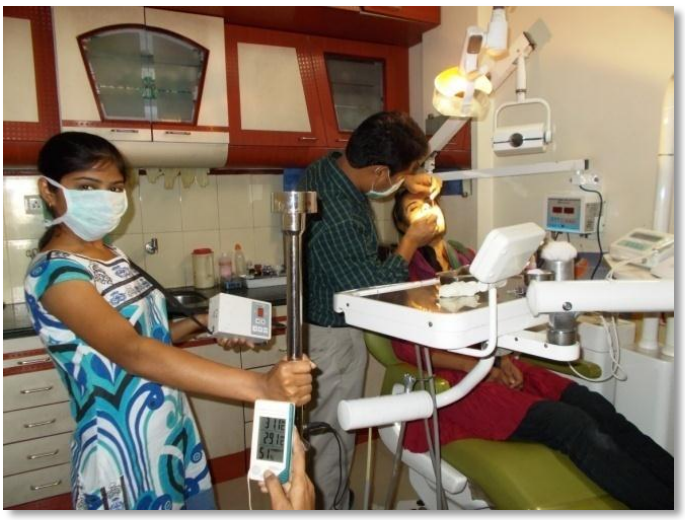
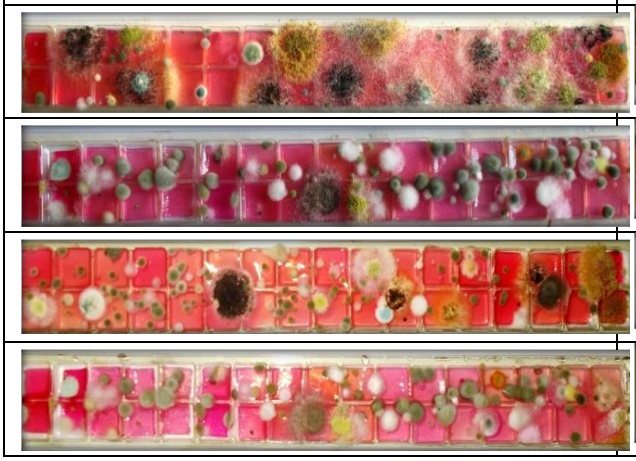
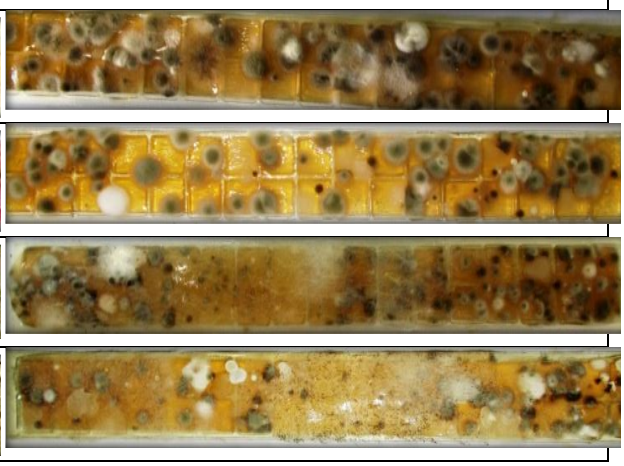
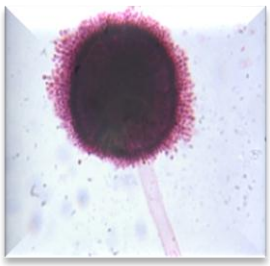
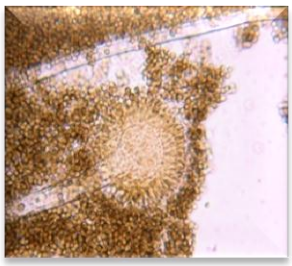
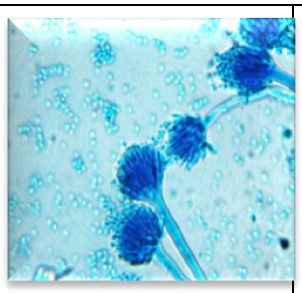
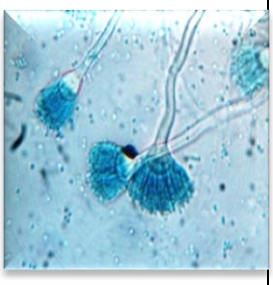
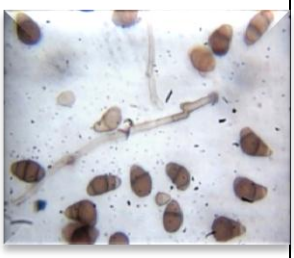
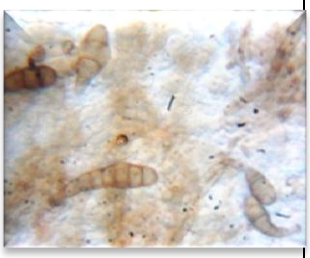
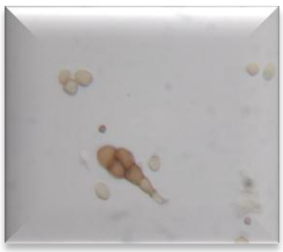
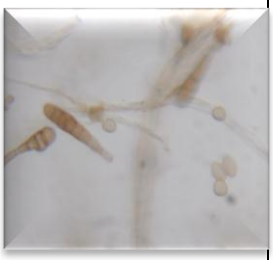
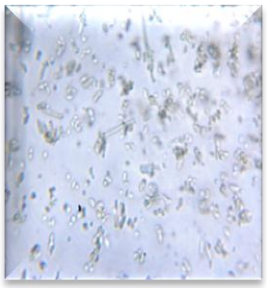
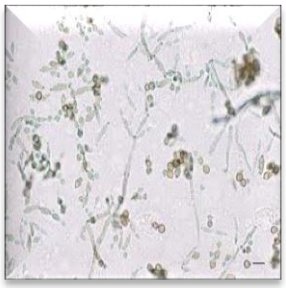
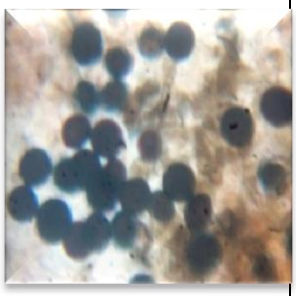
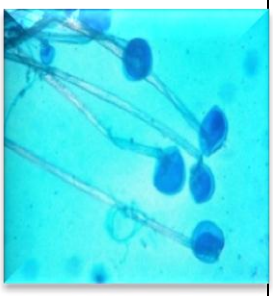
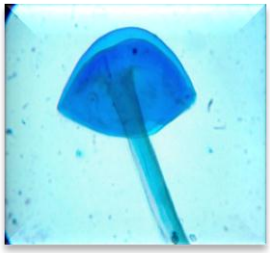
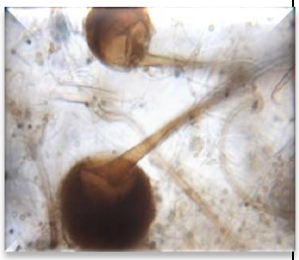
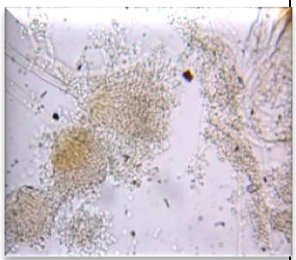
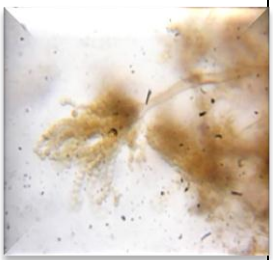


Figure-2: Air Sampling conducted (Hi-Air sampler) during patient's procedure in dental clinic and also recorded the maximum, minimum temperature and relative humidity by using hygrometer.

Figure-3: Fungal colonies grown on RBS- agar strip (PS-640) exposed in indoor air	**Figure-3:** Fungal colonies grown on agar strip (PS-290) exposed in outdoor air
	

e-ISSN: 2582-5208
International Research Journal of Modernization in Engineering Technology and Science
Volume:02/Issue:08/August-2020 Impact Factor- 5.354 www.irjmets.com

Microphotographs of Isolated Fungal species

			
Aspergillus niger (15x40)	Aspergillus flavus (15 x40)	Aspergillus terrus (15x40)	Aspergillus fumigatus (15x40)
			
Curvularia geniculata (15x40) magnification	Curvularia tetramera (15x40) magnification	Alternaria spp. (15x40) magnification	Alternaria spp. (15x40) magnification
			
Cladosporium herbarum (15x40)	Trichoderma spp, (15x40)	Nigrospora spp. (15x40)	Mucor spp. (10x40)
			
Mucor spp (15x40)	Rhizopus spp. (10x40)	Penicillium chrysogenum (15x40)	Penicillium citrinum (15x40)

ACKNOWLEDGEMENT

Author is thankful to Dr. Ashish Matey (Dentist) for allowed me to take air sampling indoor as well as from outdoor of their dental clinic. I am very much thankful to Mrs. Priyanka Matey faculty member of

environmental studies and some students helped me to sample the air and preparing slides for identifications of fungi. I also extend my sincere thanks to Principal and Head Department of Botany.

V. REFERENCES

[1] Burge, H.: Health effects of biological contaminants. In Gammage and B.A. Berven, 1996. Indoor Air and Human Health, 2nd ed., pp. 171-178 Boca Raton, Fla.: Lewis Publishers.

[2] Cecilia, Lakshya, Divyarani and Chandran et. al., 2017. Analysis of airborne fungal spores in the various dental clinics of a dental hospital in Kancheepuram district, Tamilnadu, India. Int. J. of Medical, Clinical and Applied Microbiology. Vol. 1 Issue. 1 pp. 15-20.

[3] Ekhaise, FO, Ighosewe, OU, and Ajakpovi, OD. 2008. Hospital indoor airborne microflora in private and government owned Hospitals in Benin City, Nigeria. World Journal of Medical Sciences 3(1): 19-23.

[4] Ellis, M. B. Dematiaceous Hyphomycetes. Kew; Commonwealth Mycological Institute, pp. 608, 1971.

[5] Falvey, DG, Streifel, AJ. 2007. Ten year air sample analysis of Aspergillus prevalence in a university hospital. Journal of Hospital Infection. Doi:10.1016: pp. 1-7.

[6] Garrett MH, Rayment PR, Hooper MA, Abramson MJ, Hooper BM. 1998. Indoor airborne fungal spores, house dampness and associations with environmental factors and respiratory health in children. Clin Exp Allergy. 28:459-67.

[7] Gniadek A and Macura AB 2007. Intensive care unit environment contamination with fungi. Advances in Medical Sciences. 52: 283-287.

[8] Gunay Colakoglu, Iskender Karlati. 2011. The determination of airborne fungal flora of two different hospitals in Istanbul (Turkey). Advances in Environmental Biology, 5(11): 3645-3652.

[9] Haifa Abdulaziz Binhuwaishel, Almutairi, Saad Aldusari et. al., 2019. Fungal contamination and disinfection of dental chairs among private dental clinics in Riyadh, Saudi Arabia. EC Dental Science. Vol. 18. Issue (12): 01-08.

[10] Haverinen U. 2002. Modeling moisture damage observations and their association with health symptoms. Ph.D. Thesis. Publications of the National Public Health Institute A10, Kuopio, Finland. Pp. 106.

[11] Hedayati, M.T. Pasqualotto, A.C., Warn, P.A., Bowyer, P. and Denning, D.W. 2007. Aspergillus flavus: human pathogen, allergen and mycotoxins producer. Microbiology, 153, 1677-1692.

[12] Kennedy HF, Michie JR, Richardson MD. 1996. Air sampling for Aspergillus spp. During building activity in a pediatric hospital ward. J. Hosp. Infect. 32:322-324.

[13] Ki Youn Kim, Yoon Shin Kim, and Daekeun Kim. 2010. Distribution characteristics of airborne bacteria and fungi in the general hospitals of Korea. Industrial Health. 48: 236-243.

[14] Lacey, J., 1991. Aerobiology and Health: The Role of Airborne Fungal Spores in Respiratory Disease. In: D.L. Hawks worth Ed., Frontiers in Mycology. C.A.B. International, Kew, UK. pp. 157-185.

[15] Lucia Barlean, Luminita Smaranda Iancu, Manuela Luminita Minea, Ioan Danila, Dana Baciu. 2010. Airborne microbial contamination in Dental Practices in Isai, Romania. OHDMBSC – Vol. IX-No.1 – March 2010. Pp. 16-20.

[16] Maher YA, Jastania RA, Beyari MM. et. al., 2017. Variability of airborne bacterial and fungal population in educational dental hospital, Umm Al-qura University. Int. J. of Health Science & Research. Vol. 7, issue 2. Pp-165-175.

[17] Nagamani, I. K. Kunwar and C. Manoharachary. 2006. Hand book of Soil Fungi. I. K. International Pvt. Ltd.

[18] Raper, K.B. and Fennell, D.I., 1977. Aspergillus ustus group. - In: The genus Aspergillus. Repr. R.E. Krieger Publ. Co. pp. 543-557. - Malabar, FL.

[19] Raper, K.B. and Thom, C., 1949. A Manual of Penicillia. The williams and Wilkins Co. Baltimore.

[20] Ronald E Gots; Nancy J. Layton and Suellen W. Pirages. 2003. Indoor health: Background levels of fungi. American Industrial Hygiene Association Journal 64:427-438.

[21] Saoji, A.A. and Giri, S.K., 1997. Concentration of Aero allergenic fungal spores in Intramural environments of Nagpur City - Hospital ward and Library. Proceeding 5th International Conference on Aerobiology, (Ed. S.N. Agashe), pp. 211-218. Oxford & IPH Publishing Co. Ltd., New Delhi.

[22] Zemouric C, de Soet H, Crielaard W, Laheij A. 2017. A scoping review on bio-aerosols in healthcare and the dental environment. PLOS ONE 12(5) : e0178007.