Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Assessment of Workers' Exposure to Aspergillus in Wastewater Treatment Plant

Wafaa G. Shousha[a], Amal Saad-Hussein[b], Gehan Moubarz[b], Shiamaa Shawky[a], M. M .Shawky[a*]

a Faculty of Science, Helwan University, Cairo, Egypt.

b Environmental & Occupational Medicine Department, National Research Centre, Cairo, Egypt.

Abstract

Workers in wastewater treatment plants (WWTP) are facing an overwhelming threat due to heavy exposure to fungal bioaerosol, mainly *Aspergillus*, released into the surrounding environment for a long time. This results in critical respiratory problems, especially for workers suffering from immunodeficiency. 60 out of 85 WWTP workers were selected for this study. The study's goal was to find out which *Aspergillus* species were prevalent among those plant exposed workers. For the detection of the sputum PCR results and serum specific IgE (sIgE) of three *Aspergillus* species (*A. fumigatus*, *A. niger*, and *A. flavus*) for the aforementioned selected workers, nested PCR and ELISA techniques were utilized. Our study revealed that 76.6% of the workers' sputum specimens were positive for *Aspergillus*. 21.7% of total positive samples tested positive for *A. niger*, 19.6% for *A. flavus*, and 2.2% for *A. fumigatus*. Additionally, 56.5% of workers showed mixed positive results. Furthermore, sIgE of *A. niger* increased significantly among *A. niger* positive workers compared to negative workers. Intriguingly, sIgE for different species of *Aspergillus* exhibited increasing levels among positive workers than among negative ones. Thus, both the significance and non-significance results of sIgE have to be seriously taken into consideration. To summarise, prolonged fungal exposure in WWTPs could be regarded as a potential health hazard. In addition, the results of the *Aspergillus* sIgE test should not be overlooked and thus should be considered a warning sign. For those who have elevated *Aspergillus* sIgE, further investigations were recommended.

Keywords: Aspergillus; wastewater treatment plant; specific IgE; *Aspergillus* exposed workers

1. Introduction

Aspergillus species are considered to be among the most plentiful fungi worldwide [1]. The pivotal evidence for *Aspergillus's* success is indicated by the considerable spread of its numerous species when spores of this genus are believed to be among the most prevalent airborne fungal structures [2]. *Aspergillus spp.* can feed on a variety of substrates, including animal and human tissues and faces. It can spread across a wide scale of temperatures (6°C-55°C) and humidity levels [1].

Particles with a diameter ranging from 1 up to 8 m can penetrate the lung alveoli [3]. As a result, *Aspergillus* may be harmful to many organs, but 90% of affected patients may experience respiratory tract problems [4,5]. According to Stevens et al., [6] airborne *Aspergillus* is considered to have many threats to the lungs in the form of invasive aspergillosis, aspergilloma, and allergic bronchopulmonary aspergillosis (ABPA).

Hedayati et al. [7] demonstrated that several *Aspergillus* species, including *A. fumigatus*, *A. niger*, *A. flavus*, and *A. oryzae*, are allergic and pathogenic. They do not affect healthy individuals, but do conquer immunocompromised patients [8,9]. It was revealed that nearly 50% of confirmed or suspected cases of aspergillosis also had chronic obstructive pulmonary disease (COPD), which is treated mostly with corticosteroid medication that has immunosuppressive impact [10,11].

Recurrent occupational exposure to biological agents, such as fungi, grabs the attention of public health and occupational medicine due to its negative health impacts ranging from simple irritation to allergic reactions, infective diseases, and toxic reactions [12,13]. Wastewater treatment plants (WWTP) have the potential to harm environmental health in a variety of ways. These consequences vary according to the plant's size, technology, and treatment techniques [14]. Domestic and industrial human activities in WWTPs exacerbate the spread of numerous pathogens, such as fungi, bacteria, viruses,

*Corresponding author e-mail: dr_michael_bio@yahoo.com (*M. M. Shawky*)

Receive Date: 17 October 2021, Revise Date: 04 December 2021, Accept Date: 17 January 2022,

DOI: 10.21608/ejchem.2022.101284.4715

and parasites, during the various phases of wastewater treatment, particularly in processes involving mechanical mechanisms and performed aeration of sewage water where the majority of these microorganisms can readily become airborne, referred to as bioaerosols [15,16]. The presence of these airborne bioaerosols in the WWTP working environment deserves attention, and awareness about this biological type of indoor pollution in Egypt seems to be essential.

The target of this study was to detect whether *Aspergillus* species were prevalent among WWTP workers who were occupationally exposed to environmental *Aspergillus* on a daily basis.

2. Subjects and Methods

2.1. Subjects

This study was cross-sectional. It includes 85 sewage workers out of 114 workers at Abu-Rawash WWTP station, Giza, Egypt, occupationally exposed to airborne *Aspergillus spp.* that were: *A. niger*, *A. flavus*, and *A. fumigatus* as demonstrated by Saad-Hussein et al. (under review). The current study was thought to be a continuation of the work done by Saad-Hussein et al. (under review). All of the workers participated in this study worked an eight-hour shift six days a week. After obtaining written consents from all the included workers, they were asked to bring morning sputum. Workers who could not bring morning sputum samples were excluded from this study (29 workers). However, only 60 out of 85 samples were used for DNA extraction and amplification due to the exclusion of saliva samples.

2.2. Sputum sampling and DNA isolation

Sputum samples were collected from exposed workers in sterile plastic cups, liquefied using the NALC-NAOH procedure, and then refrigerated at -20^{0}C until needed for DNA extraction. All previously liquefied specimens were submitted to DNA extraction using the EZ-10 Spin column genomic DNA (Biobasic, Ontario, Canada) according to the manufacturer's instructions. DNA has to be precipitated and concentrated using the Sambrook and Russell technique to get the ideal DNA concentration required for accurate PCR results [17].

2.3. DNA amplification and set of primers

Depending on the conserved sequences of internal transcribed spacer 1 (TSI1) ribosomal DNA and its flanking regions saved in the Gene Bank database, a set of primers (ASAP) was designed in this study for selective amplification of the *Aspergillus* genus. These primers were ASAP1:5'-CAGCGAGTACATCACCTTGG-3' and ASAP2:5' CCATTGTTGAAACTTTTAACTGATT-3'.
Moreover, three further primer sets, termed as species-specific primers, were used for PCR and subsequent identification of *Aspergillus* species, reckoning on specific regions within amplicons of ASAP1 and ASAP2. Specimens were denaturated for 4 minutes at 94^{0}C, then subjected to 30 cycles of (denaturation at 94^{0}C for 1 minute, primer annealing at 55^{0}C for 2 minutes, and extension at 72^{0}C for 1.5 minutes) before being polymerized for 10 minutes at 72^{0}C. *The Aspergillus* species planned to be defined by nested PCR technique were *A. niger*, *A. flavus*, and *A. fumigatus*, as they are the most common pathogenic species. Thus, the primer sets for *A. fumigatus* were ASPU (5'-ACTACCGATTGAATGGCTCG-3') and Af3r (5'-CATACTTTCAGAACAGCGTTCA-3'), ASPU and Ni1r (5'-ACGCTTTCAGACAGTGTTCG-3') for *A. niger*, and ASPU and Fl2r (5'-TTCACTAGATCAGACAGAGT-3') for *A. flavus*. For this second amplification step, 1 µl of the first PCR products, diluted 1/5, was used as a template. Each mixture was denaturated for 4 minutes at 94^{0}C, followed by 25 cycles of denaturation at 94^{0}C for 1 minute, primer annealing (60^{0}C) for 15 seconds, and extension (72^{0}C) for 15 seconds. The polymerization of the primers was then completed at 72^{0}C for 10 minutes [18].

2.4. Agarose gel electrophoresis

5 µl of the amplified PCR product was electrophoresed against both positive and negative controls in a 2-3% agarose gel stained with ethidium bromide in the presence of a marker with a range of 200-1000 bps to be visualised in the gel documentation system as bright bands.

2.5. Collection of blood specimens

Blood samples were collected in 5 ml sterile tubes and centrifuged at 4000 rpm for 5 min after leaving them to clot for 30 min at 37^{0}C. Serum was separated, aliquoted and kept frozen at -80^{0}C until needed for the detection of specific IgE against different *Aspergillus* species.

2.6. Detection of specific IgE

Specific IgE levels for different *Aspergillus* species were determined using an ELISA kit (RIDASCREEN® Spec. IgE) in the presence of specific allergen discs for *A. niger*, *A. flavus*, and *A. fumigatus*, and worker sera. The technique steps were carried out in accordance with the kit protocol provided by the manufacturer.

2.7. Statistical analysis

The Statistical analysis was carried out using Minitab software version 20.1.3. An independent t-test was used for the comparison of the two groups. The quantitative results were expressed as means ±

standard deviation (SD). The qualitative results were expressed as numbers (No.) and percentages (%).When the P-value was less than 0.05, statistical analysis was regarded significantly different.

3. Results

The first PCR amplification showed that 46 (76.6%) of the sputum samples of sewage workers showed positive results for *Aspergillus* as they were approved to be in partitions polluted with *Aspergillus* in bioaerosol (Fig.1.).

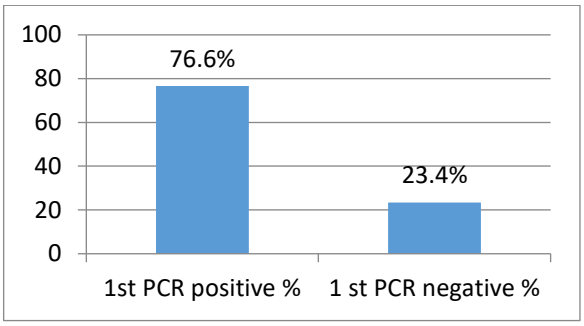


Fig.1. Distribution of sewage workers depending on the first amplification results of polymerase chain reaction (PCR) for *Aspergillus* in sputum specimens

In the second PCR amplification, 10 (21.7%) of the 46 PCR-positive samples tested positive for *A. niger*, 9 (19.6%) tested positive for *A. flavus*, and only one (2.2%) tested positive for *A. fumigatus*. In terms of mixed positivity, 12 (26.1%) were positive for all three species; 11 (23.9%) were positive for *A. niger* and *A. flavus*; and 3 (6.5%) were positive for *A. niger* and *A. fumigatus* (Fig.2.).

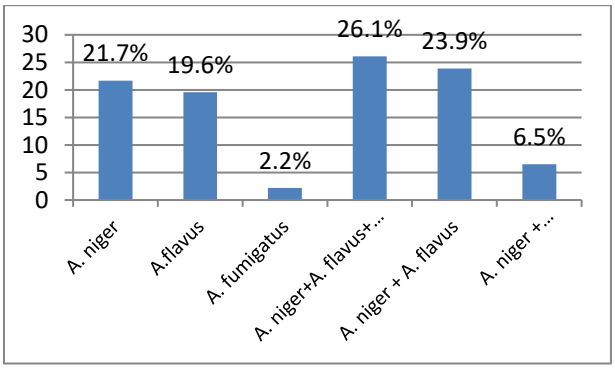
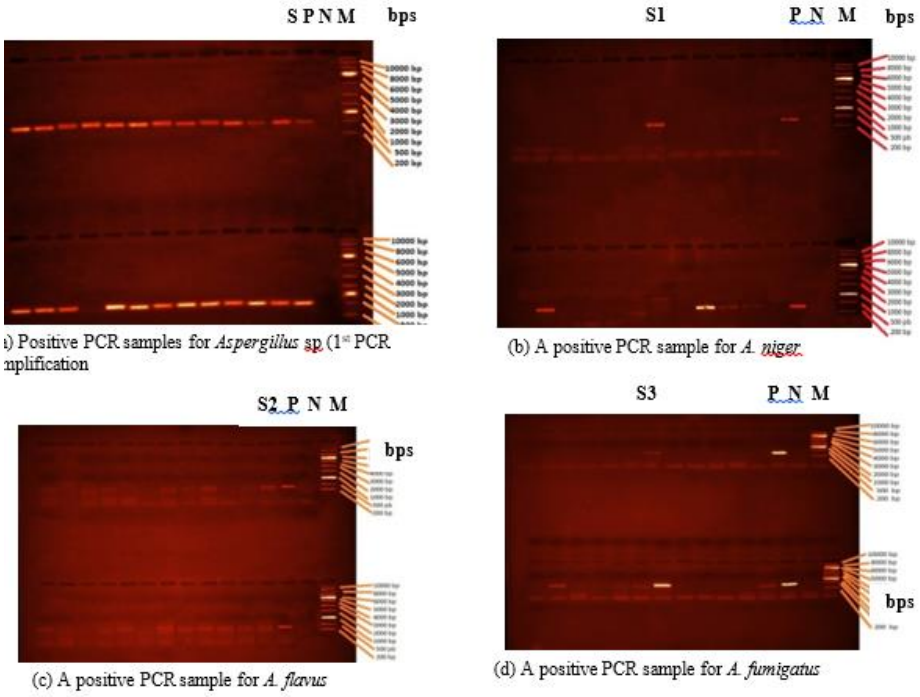


Fig.2. Distribution of sewage workers based on the second amplification results of polymerase chain reaction (PCR) for *A. niger*, *A. flavus*, *A. fumigatus*, *and mixed Aspergillus* species in the sputum specimens



ı) Positive PCR samples for *Aspergillus* sp (1st PCR mplification

(b) A positive PCR sample for *A. niger*

(c) A positive PCR sample for *A. flavus*

(d) A positive PCR sample for *A. fumigatus*

Fig.3(a, b, c, d). Polymerase chain reaction (PCR) product utilizes a specific *Aspergillus* primer set designated ASAP (0.5 kbps) and subsequent various *Aspergillus* species based on secondary amplification in the presence of species-specific primer sets in comparison to species-specific positive controls where M, N, and P denote DNA marker, the negative control product, and the positive control product, respectively. S denotes the first amplification product, S1 the second amplification product using *A. niger* primer sets, S2 the second amplification product using *A. flavus* primer sets, and S3 the second amplification product using *A. fumigatus* primer sets.

The bands of both the 1st and 2nd PCR amplifications are shown in Fig.3. (a, b, c, and d) in the presence of negative and positive PCR products and a DNA ladder (200-1000 bp).

Table 1 illustrates that there was a difference and significant relationship between the results of serum specific IgE in positive and negative workers in the second PCR amplification step for *A. niger* species where P = 0.04. However, the relationship was not significant as P value was > 0.05 for both *A. flavus* and *A. fumigatus* species, but these results should be taken into consideration due to elevated sIgE levels of these aforementioned species in WWTP workers.

4. Discussion

In wastewater treatment plants (WWTPs), sewage and unstable sludge are considered to be the main source of a variety of pathogens such as bacteria, fungi, viruses, and parasites. These microorganisms can be carried through the surrounding air in wastewater droplets released during aeration or mechanical agitation of the sewage water [19]. Owing to the pathogenicity of these bioaerosols, they may play a critical role in infection of workers in the WWTPs and nearby inhabitants, mainly by inhalation, causing serious health consequences depending on their immunity [20].

Aspergillus species, such as *A. niger, A. flavus and A. fumigatus*, are crucially predominant in a lot of occupational environments as airborne pathogens, especially in WWTPs [21]**.** This was consistent with the results of this current study regarding the prevalence of *Aspergillus* and its previously mentioned species in the sputum specimens of sewage workers that were occupationally exposed to airborne *Aspergillus* at the Abu-Rawash WWTP.

According to Viegas et al. [21], *A. niger* was the most abundant airborne *Aspergillus* species in the assessed WWTP environment, which may explain why it had the highest positive results for selected plant workers' sputum. Despite the limited human pathogenicity of *A. niger*, it cannot be neglected when several cases of invasive pulmonary aspergillosis and ABPA in immunocompromised patients have been detected [22**,**23] . In spite of their tiny size (6-7 mm), *A. niger* spores' penetration into the lower respiratory tract is hampered by the production of strong interspore bridges, but they are nevertheless easily caught by inhalation and removed by the host's mucociliary system [24].

A. flavus is known to be the second most frequent pathogenic species causing either invasive pulmonary aspergillosis or ABPA, after *A. fumigatus* [25, 26]. However, *A. flavus* was assessed to be more virulent than *A. fumigatus* [7]. This may elucidate the results of the current study, where there were a significant number of workers in Abu Rawash WWTP whose sputum samples were PCR positive for *A. flavus*. It's worth noting that the number of workers who tested positive for *A. flavus* was significantly greater than the number of workers who tested positive for *A. fumigatus*. This may be attributed to contamination of the sputum from their postnasal discharge. Furthermore, both meteorological and geographic circumstances were found to have a substantial impact on the abundance and dispersion of *Aspergillus* species in the air. For instance, Adhikari et al. [27] demonstrated that *A. flavus* was the most common airborne *Aspergillus* in India. However, in Madrid (Spain), *A. fumigatus* was found to be the most predominant species (54%) [28].

Table (1). Comparison of serum-specific IgE for several *Aspergillus* species in workers with negative and positive PCR (second amplification) for various *Aspergillus* species in sputum.

Type of species	Mean	±SD	Independent t-test	P-value
***A. niger* in sputum**				
Negative	0.262	0.019	-2.09	0.04
Positive	1.43	0.56		
A. flavus in sputum				
Negative	0.614	0.088	-1.15	0.257
Positive	2.9	1.9		
A. fumigatus in sputum				
Negative	0.384	0.059	-1.27	0.212
Positive	2.9	1.9		

Although mixed infections of various *Aspergillus* species are not common in the literature, however they have been previously reported in immunosuppressed and immunocompetent patients, which is in line with the results of this study [29,30]. The possible cause of immunocompetent subjects' infection may be due to long-term exposure to fungal toxins released by living or dead particles, e.g., aflatoxins, gliotoxin, trichothecenes, and ochratoxin, which in turn results in suppression or modulation of their immune response [31]

Aspergillus Specific IgE (sIgE) can be used as a necessary and critical test in demonstrating serum sensitivity for subsequent diagnosis of ABPA. For example, an elevated level of serum *A. fumigatus* specific IgE (>0.35 kUA/l) is currently the most accurate test in the diagnosis of ABPA with sensitivity and specificity of 100% and 70%, respectively. Hence, sIgE test results in early diagnosis and treatment of ABPA that could contribute to noticeable progress in the quality of life and prognosis of patients, thereby preventing the condition from being negatively shifted and reducing the medical burden [32–34]. Additionally, Greenberger [35] stated that IgE levels were shown to be significantly raised during the early stages of *Aspergillus* infection and returned to normal by the end of lung damage. In the present study, the results of sIgE for *A. niger* were significantly elevated in workers with positive PCR than in those with negative PCR. The clear significance of sIgE of *A. niger* in the previous results confirms the precision and accuracy of this diagnostic test. Although the results of sIgE for both *A. flavus* and *A. fumigatus* were not significantly different, they have to be taken into consideration. Hence, further investigations were suggested, especially with the increased levels in sIgE of different *Aspergillus* species.

5. Conclusion

Long-lasting fungal exposure in WWTPs should be regarded as a significant health risk, particularly for those with low immunity, as nearly three-fourths of the WWTP workers' sputum samples tested positive for *Aspergillus*. The most common species among the positive workers were *A. niger* and *A. flavus*. The significant and non-significant increase in sIgE for the three *Aspergillus* species among WWTP workers with positive PCR results should not be overlooked and should be considered as a warning sign. Further investigations were also recommended, particularly for WWTP workers with elevated sIgE to several *Aspergillus* species.

6. Ethical Approval

Approval of the Ethical committee of the National Research Centre was taken prior to the study (Registration number 17085).

7. Conflict of interest

The authors declare that they have no conflict of interest.

8. Acknowledgement

The authors express their sincere thanks to Academy of Scientific Research and Technology (ASRT) for funding this work (2017–2020). Our thanks also go to the managers of Abu-Rawash WWTP as well as the workers in it for their cooperation to finish this work.

9. References

1. Krijgsheld P., Bleichrodt R.V., Van Veluw G.J., Wang F., Müller W.H., Dijksterhuis J., and Wösten H.A. Development in aspergillus. *Stud. Mycol.*, **74**, 1-29, doi: 10.3114/sim0006 (2013).
2. Bennett J.W. An overview of the genus Aspergillus, p 1-17. *Aspergillus Mol. Biol. genomics. Caister Acad. Press. Norfolk, United Kingdom* (2010).
3. Herrick R.F., Dement J.M., Baker E.L., and Matte T.P. Principles of industrial hygiene. *In Textbook of Clinical Occupational and Environmental Medicine*, Elsevier, 45-82 (2005).
4. Segal B.H. Aspergillosis. *N. Engl. J. Med.*, **360**(18), 1870-1884, doi:10.1056/NEJMra0808853 (2009).
5. Saad-Hussein A., Taha M.M., Fadl N.N., Awad AH, Mahdy-Abdallah H., Moubarz G., Aziz H., and El-Shamy K.A. Effects of airborne Aspergillus on serum aflatoxin B1 and liver enzymes in workers handling wheat flour. *Hum. Exp. Toxicol*, **35**(1), 3-9, doi: 10.1177/0960327115573596 (2016).
6. Stevens D.A., Kan V.L., Judson M.A., Morrison V.A., Dummer S., Denning D.W., Bennett J.E., Walsh T.J., Patterson T.F., and Pankey G.A. Practice guidelines for diseases caused by Aspergillus. *Clin. Infect. Dis.*, **30**(4), 696–709, doi: 10.1086/313756 (2000).
7. Hedayati M.T., Pasqualotto A.C., Warn P.A., Bowyer P., and Denning D.W. Aspergillus flavus: Human pathogen, allergen and mycotoxin producer. *Microbiology*, **153**(6), 1677-1692, doi: 10.1099/mic.0.2007/007641-0 (2007).
8. Pitt J.I. The Current role of aspergillus and penicillium in human and animal health. *Med. Mycol.*, **32**(sup1), 17-32, doi: 10.1080/02681219480000701 (1994).
9. Brakhage A.A. Systemic fungal infections caused by Aspergillus species: epidemiology, infection process and virulence determinants. *Curr. Drug Targets*, **6**(8), 875-886, doi:10.2174/138945005774912717 (2005).

10. Denning D.W. Invasive aspergillosis. *Clin. Infect. Dis.*, **26**(4), 781-805, doi: 10.1086/513943 (1998).
11. Samarakoon P., and Soubani A.O. Invasive pulmonary aspergillosis in patients with COPD: a report of five cases and systematic review of the literature. *Chron. Respir. Dis.*, **5**(1), 19-27, doi:10.1177/1479972307085637 (2008).
12. Dutkiewicz J. Occupational bio hazards: Current issues. *Med. Pr.*, **55**(1), 31-40 (2004).
13. Górny R. Hazardous Biological Agents: Standards, Recommendations and Proposals of the Limit Values. *Princ. Meth. Assess. Work. Env.*, **3**(41), 17-39 (2004).
14. Sánchez-Monedero M.A., Aguilar M.I., Fenoll R., and Roig A. Effect of the aeration system on the levels of airborne microorganisms generated at wastewater treatment plants. *Water Res.*, **42**(14), 3739-3744, doi:10.1016/j.watres.2008.06.028 (2008).
15. Carducci A., Tozzi E., Rubulotta E., Casini B., Cantiani L., Rovini E., Muscillo M., and Pacini R. Assessing airborne biological hazard from urban wastewater treatment. *Water Res.*, **34**(4), 1173-1178, doi:10.1016/s0043-1354(99)00264-x (2000).
16. Pascual L., Pérez-Luz S., Yáñez M.A., Santamaría A., Gibert K., Salgot M., Apraiz D., and Catalán V. Bioaerosol emission from wastewater treatment plants. *Aerobiologia*, **19**(3), 261-270, doi:10.1023/b:aero.0000006598.45757.7f (2003).
17. Sambrook J., and Russell D.W. Standard ethanol precipitation of DNA in microcentrifuge tubes. *Cold Spring Harb. Protoc.*, **2006**(1), pdb-prot4456, doi:10.1101/pdb.prot093377 (2006).
18. Sugita C., Makimura K., Uchida K., Yamaguchi H., and Nagai A. PCR identification system for the genus Aspergillus and three major pathogenic species: Aspergillus fumigatus, Aspergillus flavus and Aspergillus niger. *Med. Mycol.*, **42**(5), 433-437, doi: 10.1080/13693780310001656786 (2004).
19. Korzeniewska E. Emission of bacteria and fungi in the air from wastewater treatment plants–a review. *Front biosci* (Schol ed), **3**, 393-407 (2011).
20. Han Y., Li L., Wang Y., Ma J., Li P., Han C., and Liu J. Composition, dispersion, and health risks of bioaerosols in wastewater treatment plants: a review. Front. *Environ. Sci. Eng*., **15**(3), 1-16, doi: 10.1007/s11783-020-1330-1 (2020).
21. Viegas C., Faria T., Caetano L.A., Carolino E., Gomes A.Q., and Viegas S. Aspergillus spp. prevalence in different Portuguese occupational environments: What is the real scenario in high load settings?. *J. Occup. Environ. Hyg.*, **14**(10), 771-785, doi: 10.1080/15459624.2017.1334901 (2017).
22. Atchade E., Jean-Baptiste S., Houzé S., Chabut C., Massias L., Castier Y., Brugière O., Mal H., and Montravers P. Fatal invasive aspergillosis caused by Aspergillus niger after bilateral lung transplantation. *Med. Mycol. Case Rep.*, **17**, 4-7, doi: 10.1016/j.mmcr.2017.05.002 (2017).
23. Person A.K., Chudgar S.M., Norton B.L., Tong B.C., and Stout J.E. Aspergillus niger: An unusual cause of invasive pulmonary aspergillosis. *J. Med. Microbiol.*, **59**(7), 834-838, doi: 10.1099/jmm.0.018309-0 (2010).
24. Saad-Hussein A., Morcos N.Y., Rizk S.A., Ibrahim K.S., El-Zaher N.A., and Moubarz G. Aspergillus hazardous problem in ceramic workers. *Toxicol. Ind. Health*, **28**(10), 886-893, doi: 10.1177/0748233711425077 (2012).
25. Rudramurthy S.M., Paul R.A., Chakrabarti A., Mouton J.W., and Meis J.F. Invasive aspergillosis by Aspergillus flavus: epidemiology, diagnosis, antifungal resistance, and management. *J. Fungi*, **5**(3), 55, doi:10.3390/jof5030055 (2019).
26. Krishnan S., Manavathu E.K., and Chandrasekar P.H. Aspergillus flavus: An emerging non-fumigatus Aspergillus species of significance. *Mycoses*, **52**(3), 206-222, doi: 10.1111/j.1439-0507.2008.01642.x (2009).
27. Adhikari A., Sen M.M., Gupta-Bhattacharya S., and Chanda S. Airborne viable, non-viable, and allergenic fungi in a rural agricultural area of India: A 2-year study at five outdoor sampling stations. *Sci. Total Environ.*, **326**(1-3), 123-141, doi: 10.1016/j.scitotenv.2003.12.007 (2004).
28. Guinea J., Peláez T., Alcalá L., and Bouza E. Outdoor environmental levels of Aspergillus spp. conidia over a wide geographical area. *Med. Mycol.*, **44**(4), 349-356, doi: 10.1080/13693780500488939 (2006).
29. Xavier M.O., Pasqualotto A.C., Sales M.D., Severo C.B., Camargo J.J., and Severo L.C. Invasive pulmonary aspergillosis due to a mixed infection caused by Aspergillus flavus and Aspergillus fumigatus. *Rev. Iberoam. Micol.*, **25**(3), 176-178, doi: 10.1016/S1130-1406(08)70041-X (2008).
30. Moreno-González G., Ricart De Mesones A., Tazi-Mezalek R., Marron-Moya M.T., Rosell A., and Mañez R. Invasive Pulmonary Aspergillosis with Disseminated Infection in Immunocompetent Patient. *Can. Respir. J.*, **2016**, 7984032, doi: 10.1155/2016/7984032 (2016).
31. Fischer G., and Dott W. Relevance of airborne fungi and their secondary metabolites for

environmental, occupational and indoor hygiene. *Arch. Microbiol.*, **179**(2), 75-82, doi: 10.1007/s00203-002-0495-2n(2003).

32. Lou B., Xu Z., Yang G., Guo C., Zheng S., Lou H., Ma G., Sadhukhan A., Lin S., and Chen Y. Role of Aspergillus fumigatus-specific IgE in the diagnosis of allergic bronchopulmonary aspergillosis. *Int. Arch. Allergy Immunol.*, **178**(4), 338-344, doi: 10.1159/000495365 (2019).
33. Selvaggi T.A., Walco J.P., Parikh S., and Walco G.A. Hypersensitivity testing for aspergillus fumigatus IgE is significantly more sensitive than testing for aspergillus niger IgE. *Am. J. Clin. Pathol.*, **137**(2), 203-206, doi: 10.1309/AJCPZYX6SJ8FCLRN (2012).
34. Agarwal R., Maskey D., Aggarwal A.N., Saikia B., Garg M., Gupta D., and Chakrabarti A. Diagnostic performance of various tests and criteria employed in allergic bronchopulmonary aspergillosis: a latent class analysis. *PLoS One*, **8**(4), e61105, doi: 10.1371/journal.pone.0061105 (2013).
35. Greenberger P.A. Allergic bronchopulmonary aspergillosis. *J. Allergy Clin. Immunol.*, **110**(5), 685-692, doi: 10.1067/mai.2002.130179 (2002).