



## RESEARCH ARTICLE

# Fungal Community Associated with Sawdust of Direct Evaporative Cooler and their Health Impact in Erbil City

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## ABSTRACT

Evaporative coolers (ECs) are widely used in semi-arid regions. During operation, mashies pass from outdoors to indoors through wet straws or wood shavings, and these materials act as filters of bio-aerosolized particles. The present work was conducted in Erbil City to screen the fungal community associated with sawdust in ECs and explain the health impact of the predominant genera. Three hundred fifty fungal colonies were counted belonging to 17 identified isolates besides the sterile mycelia. The highest occurrence and frequency belong to *Aspergillus* spp. (O = 98% and F = 33.7%), followed by *Cladosporium* spp. (O = 50% and F = 20.6%). *Aspergillus* spp. has the highest importance value index. Three genera had a moderate incidence, namely, *Mucor* spp., *Alternaria* spp., and *Penicillium* spp. The predominant genera that were isolated from sawdust are common outdoor air mycobiota in the study area and are considered the most effective allergens. Indeed, all recorded genera have a potentially significant effect on human health causing several types of pulmonary disorders and involve in opportunistic infections, especially for immunocompromised patients. We concluded that ECs may act as a source and transmitter of indoor airborne fungi pollution.

**Keywords:** Sawdust, water air cooler, evaporative cooler, airborne-fungi

## INTRODUCTION

An evaporative cooler (EC), also known as a swamp cooler, is a device that cools the air by passing it through a wet cellulosic material, such as wood shavings or straw, causing water to evaporate; its low energy consumption and ability to add moisture to the atmosphere make it particularly suitable for dry and warm environments.<sup>[1]</sup>

The EC has two types, direct ECs (DEC) and indirect ECs, and could be either fixed or movable.<sup>[2]</sup> The DEC type draws the outside air, water droplets, and aerobiological particles into occupied space. Several workers have mentioned that DEC microbial water contamination diffuses into the air and causes microbial pollution in the cooling device.<sup>[3]</sup> The relationship between the microbial contamination of water tanks and indoor air was reported.<sup>[4]</sup> A specific fungal isolate "*Micrococcus luteus*" was used as a tracer to determine the number of microorganisms detected in the flowed air based on the microbial air volume.<sup>[5]</sup> Previous studies suggested that EC increases personal exposure to particulate matter along with the increase in respiratory illnesses.<sup>[6,7]</sup> These particles contribute to many respiratory disorders such as asthma, allergic disorders, and hypersensitivity pneumonitis.<sup>[8-10]</sup> A comparison study between air conditioners (AC) and EC showed that the EC increased the indoor environment humidity and resulted in the growth of fungi as well as the diversity of indoor airborne fungi.<sup>[3,7]</sup>

The wide use of EC in Iraq and a lack of studies on their relationship with indoor air bioaerosols pollution gave the present study specific importance as the first study for isolation and identification of airborne fungi associated with DEC and to analysis the fungal community as well as highlight health impact of predominant genera.

## MATERIALS AND METHODS

### Sawdust Samples Collection and Culturing

A cross-sectional study was conducted by collecting 50 sawdust samples from 50 ECs during July 2023 in Erbil City. Samples were collected from several locations, including minimarkets, houses, and baker shops at the last week of July. Samples were

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collected from the air ECs 2 months after its initial operation. The pieces of wood straw were taken by forceps and kept in sterilized vials. Sabouraud's dextrose agar and the lab-made medium – Typha pollen agar <sup>[11]</sup> were prepared for primary culturing through the direct plating method.

Three pieces (2 cm length) were put on the culture media surface and then incubated in a laboratory environment. The cultures were checked daily from the 3<sup>rd</sup> day over a period of 4 weeks. The observed developing fungal growths were directly transferred to Sabouraud's dextrose agar to prepare a pure culture.

## Fungal Identification

The isolated fungi were identified based on the macro and micro morphological characteristics as fully described in.<sup>[12,13]</sup>

## Fungal Community Analysis

The occurrence% and frequency% were calculated as follows in:<sup>[14]</sup>

$$\text{Occurrence (O\%)} = \frac{\text{No. of samples in which the genus occurred}}{\text{No. of total samples}} \times 100$$

$$\text{Frequency (F\%)} = \frac{\text{No. of (species / genus) colonies}}{\text{No. of total (species / genus) colonies}} \times 100$$

$$\text{Importance value index} = (O+T)/2$$

## RESULTS

### Analysis of Fungal Community

Fungal growth appeared on 96% of the sawdust (48/50) samples in the dishes. A total of 350 colonies were enumerated, which included sterile mycelia and 17 distinct species [Table 1]. The highest frequency (33.7%) and occurrence (98%) were shown by *Aspergillus* spp., which were followed by *Cladosporium* spp. (50% occurrence and 20.6% frequency). *Alternaria* spp., *Penicillium* spp., and *Mucor* spp. had a moderate occurrence. The frequency of the remaining isolates was 5.1% or less, and their occurrence was 8% or less [Table 1].

*Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus flavus*, and *Aspergillus ochraceous* were the four species identified. The 14 isolates are all filamentous and belong to hyphomycetous fungi accounting for 77.7%, zygomycetes for 16.6%, and one strain of coelomycetes for 5.5% [Table 1].

The importance value index IVI [Figure 1] showed that *Aspergillus* spp. with the highest level (65.85) followed by *Cladosporium* spp., 12.85 and sterile mycelium 35.3, 12.85 and 9.3, respectively.

## DISCUSSION

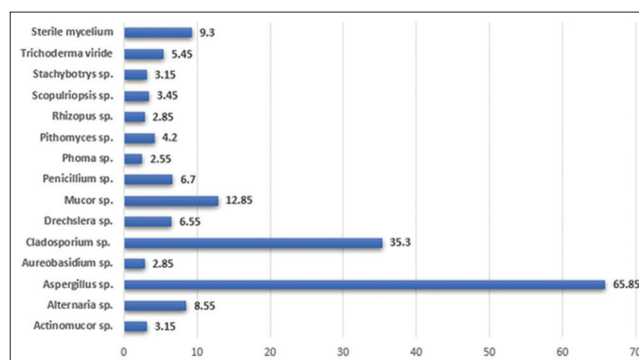
### Fungal Community

The present study revealed that the predominant fungal genera isolated from the ECs sawdust were *Aspergillus* spp. and *Cladosporium* spp., followed by *Mucor* spp., *Alternaria* spp.,

**Table 1:** The isolated fungi from sawdust samples of direct evaporative coolers

S. No.	Fungi	O%	F%	T.G.
1	<i>Actinomuor</i> spp.	4	2.3	Z
2	<i>Alternaria</i> spp.	12	5.1	H
3	<i>Aspergillus flavus</i>	98	3.4	H
4	<i>Aspergillus fumigatus</i>		9.7	H
5	<i>Aspergillus niger</i>		17.7	H
6	<i>A.ochraceous</i>		2.9	H
7	<i>Aureobasidium</i> spp.	4	1.7	H
8	<i>Cladosporium</i> spp.	50	20.6	H
9	<i>Drechslera</i> spp.	8	5.1	H
10	<i>Mucor</i> spp.	20	5.7	Z
11	<i>Penicillium</i> spp.	10	3.4	H
12	<i>Phoma</i> spp.	4	1.1	C
13	<i>Pithomyces</i> spp.	6	2.4	H
14	<i>Rhizopus</i> spp.	4	1.7	Z
15	<i>Scopulriopsis</i> spp.	4	2.9	H
16	<i>Stachybotrys</i> spp.	4	2.3	H
17	<i>Trichoderma viride</i>	8	2.9	H
18	Sterile mycelium	10	8.6	H

O%: Occurrence%, F%: Frequency%, T.G: Taxonomic group, Z: Zygomycetes, H: Hyphomycetes, C: Coelomycetes



**Figure 1:** The importance value index for the isolated fungi

and *Penicillium* spp. These genera are commonly recognized as major components of the aeromycobiota in the study region. *Aspergillus* spp., *Penicillium* spp., *Cladosporium* spp., and *Alternaria* spp. were the predominant isolates during the storm and calm climates in Erbil city, while *Mucor* spp. showed high occurrence during storm climate only.<sup>[15]</sup> It is worth mentioning that the isolated genera in the present study were also isolated in high incidence from AC systems of hospitals, classrooms, as well as automobiles.<sup>[16-18]</sup>

In general, fungi possessing dry and hydrophobic spores, dark pigmentation (melanin), and thick cell walls are well-adapted for airborne dispersal and tend to predominate in warm, dry geographical regions. In addition, fungal species producing small-sized spores (typically 2–10 μm) are more likely to remain suspended in the air for extended periods. In such environments, the predominant fungal genera commonly

isolated from aerosols include *Alternaria solani*, *Aspergillus candidus*, *A. flavus*, *A. fumigatus*, *A. niger*, *Aspergillus terreus*, *Aspergillus wentii*, *Curvularia clavata*, *Drechslera dematioidea*, and *Penicillium* species. The major myco-airspora of the atmospheric in Karachi-Pakistan.<sup>[19]</sup>

The study's leading genera were noted as the most significant air fungal allergens worldwide.<sup>[20]</sup> The report of the World Health Organization<sup>[21]</sup> emphasizes *Aspergillus* spp., *Cladosporium* spp., *Mucor* spp., *Alternaria* spp., and *Penicillium* spp. as harmful airborne fungi that ability to infect humans, cause allergies and create mycotoxins.

## Health Impact of Predominant Isolates

*Aspergillus* is a very large, saprophytic, globally distributed genus. It is common in soil, decaying vegetation, seeds, and keratinous tissues. Some species, such as *A. fumigatus*, *A. niger*, and *Aspergillus nidulans* are considered opportunistic pathogens in humans,<sup>[22]</sup> and are associated with respiratory disorders including allergic asthma, allergic bronchopulmonary, and hypersensitivity.<sup>[23]</sup> The incidence of disseminated aspergillosis, similar to other opportunistic fungal infections, has increased significantly over the past two decades. This rise is strongly associated with the growing population of immunocompromised patients, including those with cancer, organ transplants, human immunodeficiency virus/acquired immunodeficiency syndrome, and individuals receiving immunosuppressive therapies.<sup>[24]</sup> The high production of dry, lightweight, and easily airborne conidia by *Aspergillus* species contributes to their increased prevalence in airborne bioaerosols. Therefore, strict preventive measures are essential, particularly for immunocompromised individuals such as hospitalized patients, those with burn wounds, and individuals exposed to highly contaminated environments, including poultry farms, grain storage facilities (silos), and municipal waste management sites.<sup>[25-27]</sup>

*Cladosporium* is common in many areas of the world, the fungus is cosmopolitan and inhabited different substrates. Their spores have variable shapes and sizes and can be isolated from air, soil, accommodation, and food products. Some species are phytopathogens, others are mycotoxin producers.<sup>[28]</sup> From an allergological perspective, *Cladosporium* species are well-known as common airborne allergens, with *Cladosporium herbarum* and *Cladosporium cladosporioides* identified as the primary species responsible for inducing allergic reactions in sensitized individuals.<sup>[29]</sup> *Cladosporium* spp. can cause opportunistic infections, these cases are related mainly to people with compromised immune systems. Infections in healthy people were also reported, appearing as lesions of chromoblastomycosis.<sup>[23]</sup>

*Alternaria* is one of the predominant isolates from sawdust in the present study; the genus has about 275 spp. according to morphological diagnosis.<sup>[30]</sup> The spores of *Alternaria* are considered one of the most abundant and main sources of airborne allergens.<sup>[31]</sup> Spores of *Alternaria* are more allergenic than other fungi, even though they occur in low concentration (CFU/m<sup>3</sup>), this may be related to the ratio of viable spores in a cubic meter of air. One of the most widely distributed species is *Alternaria alternata*, which has large, dark, and easily separated conidia. The species is implicated in types of

pulmonary infections, including hypersensitivity pneumonitis, bronchial asthma, and allergic sinusitis and rhinitis as well as keratitis.<sup>[32]</sup> It is well known as a mycotoxin producer, such as alternariol (AOH); alternariol monomethyl ether (AME), altenuene (AE), and altertoxins.<sup>[33]</sup>

The genus *Mucor*, which had the third level of incidence in sawdust samples, consists of approximately 50 species, and it has a widespread distribution. Depending on spatial and temporal factors, *Mucor* spp. appeared as the second most commonly isolated airborne fungus in Lithuania.<sup>[34]</sup> It was also predominant in the hospital environment and office ventilation systems.<sup>[35]</sup> Indeed, certain pathogenic *Mucor* species are a threat to animal and human health and are identified more frequently as mucormycotic causative agents, especially in immunocompromised patients.<sup>[36]</sup> *Mucor circinelloides* is the species that is most frequently isolated from clinical sources, it causes specific pulmonary infections in humans. The primary infection occurred after fungal particles were inhaled, the lung infection may develop in the sinuses. A skin infection may also follow contamination of the wound.<sup>[37]</sup>

*Penicillium* is frequently isolated from indoor and outdoor environments.<sup>[38]</sup> The genus associates with a higher rate of incident wheezing and persistent cough.<sup>[39]</sup> The rate of pulmonary infections is of higher relevance to adult asthma than childhood asthma.<sup>[40]</sup> *Penicillium* spp. is predominant in hospitals' indoor aero mycobiota and in their ventilation system. Moreover, *Penicillium* with *Cladosporium* is the most common agent that causes allergies and asthma in healthcare facilities.<sup>[41]</sup>

The remaining isolated fungi, including *Actinomucor* spp., *Aurobasidium* spp., *Drechslera* spp., *Phoma* spp., *Pithomyces* spp., *Rhizopus* spp., *Scopulariopsis* spp., *Stachybotrys* spp., and *Trichoderma* spp., are known allergens or could act as opportunistic pathogens causing various human diseases.<sup>[13]</sup> These fungi have been reported to pose health hazards and are associated with several infections.<sup>[42,43]</sup> They show a significant relationship with immunocompromised individuals and are sometimes linked to specific environments that promote the release of their aerosolized particles.<sup>[44]</sup> Moreover, these airborne particles have been regarded as air pollutants and are suggested to cause adverse health effects in humans.<sup>[45]</sup>

## CONCLUSION

According to the results of this study, sawdust appears to be a rich and appropriate habitat for a high number and variety of airborne fungi. Wet sawdust increases the density of the listed genera, which are common outdoor airborne fungus.

The sawdust fungi are abundant outdoor airborne fungi, alive, highly diverse, and include common fungal allergens, namely, *Aspergillus*, *Alternaria*, *Cladosporium*, and *Penicillium*.

During operation, fungal propagules gather on sawdust, which appears to be a source of indoor air bioaerosols. These propagules are especially transferred from the dry sawdust in the initial minutes of operation.

The high fungal contamination detected in sawdust highlights a potential health risk, particularly for atopic

individuals susceptible to allergies and asthma. Therefore, regular replacement of sawdust pads is essential to minimize exposure and reduce the associated health hazards.

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