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Assessment of Fungal Propagules in Some Selected Banking Halls of the University of Cape Coast Community, Ghana

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Authors' contributions

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

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ABSTRACT

The objective of this project work was to determine the level of fungal propagule contamination and identify specific species of the fungal propagule in some Banking Halls on the University of Cape Coast campus and its environs. Samples were collected in petri dishes containing Sabouraud dextrose agar (SDA), exposed to the top, middle and bottom parts of the banking halls. The results showed that in all the banks, the bottom part was the most contaminated, followed by the middle and the top being the least contaminated. Banks A, B, C and D recorded fungal loads of 11 cfu/ml, 67 cfu/ml, 58 cfu/ml and 75 cfu/ml respectively, which were in accordance to the sizes of the Banks. In all, six different fungal species were identified with varied percentage frequency of occurrence, which included: *Aspergillus niger* (30.81%), *Aspergillus fumigatus* (27.96%), *Aspergillus flavus* (23.70%), *Fusarium chlamydosporum* (8.06%), *Rhizopus stolonifer* (5.21%) and *Penicillium* spp. (4.27%). *Aspergillus flavus, Aspergillus fumigatus,* and *Aspergillus niger* were present in all the four Banks; *Rhizopus stolonifer* was present in Banks A, B and D; *Fusarium chlamydosporum* was also

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present in Banks B, C and D and *Penicillium* spp. was present in only Banks C and D. Preliminary study showed that out-door fungal concentration was high than all indoor values recorded and this was due to the exo-genal fungal out-door. P-value data was analyzed descriptively using graphic pad prism (version 5), one-way analysis of variance was used to determine significance where ($p \leq 0.05$) is significant whilst ($p \geq 0.05$) is not significant. Only in bank D was there a significant p-value recorded for *Fusarium chlamydosporum* ($p \leq 0.001$). The research concluded that, there was fungal propagule in the Banking Halls, which poses health problems to their workers and patrons over a period of time.

Keywords: Fungi; propagules.

1. INTRODUCTION

Fungi propagules is the trapping, identification and quantification of airborne biological particles such as fungi and plant spores, which are carried by air current from one point to the other Parkin et al. [1]. Airborne fungal spores differ from other microorganisms in their method of obtaining nutrients since they employ adsorptive mode of nutrition Parkin et al. [1]. Studies have shown that this mode of fungal propagule reproduction reveals 12 genera with the majority comprising of *Cladosporium* (33.18%), *Aspergillus* (11.06%), *Saccharomyces* (7.41%), *Penicillium* (3.44%), *Fusarium* (1.73%) and *Alternaria* (1.02%) species respectively. Other genera isolated from previous works, include *Curvularia, Pithomyces, Aureobasidium, Geotrichum, Phoma, Nigrospora, Epicoccum, Rhizopus and Neurospora* (Horner et al. [2]; Chadeganipour et al. [3]; Ogunlana [4]. Fungal propagules are important agents of toxic diseases and allergies. Inhalation of fungal spores can cause nose, throat irritations and upper respiratory infections including bronchitis and pneumonia as well as asthma. Other symptoms may include headache, skin and eye irritations, nausea and allergic reactions especially in immunosuppressed or immune-deficient individuals A.L.A., [5].

Fungi are ubiquitous eukaryotic organisms, comprising an abundance of species. Only about 200 of the thousands of species have been identified as human pathogens, and among these known pathogenic species, fewer than a dozen are responsible for more than 90% of all human fungal infections (Kayser et al*.,* [6]. They may be transported into buildings on the surface of new materials or on clothing and may also penetrate buildings through active or passive ventilation. Once fungi are indoors, their growth can occur only in the presence of moisture. Many fungi grow readily on any surface that becomes wet or moistened; that is, virtually all fungi readily germinate and grow on substrates in equilibrium with a relative humidity below saturation, which is below 100% Morring et al*.* [7].

The species that grow on a given substrate depends largely on the water activity of the substrate. The minimum water activity required for fungal growth on building surfaces varies from less than 0.80 and greater than 0.98, Cheong et al., [8]. On the basis of their water requirements, indoor fungi can be divided into: [1] primary colonizers, which can grow at a water activity less than or equal to 0.80; [2] secondary colonizers, which grow at a water activity level of 0.80–0.90; and [3] tertiary colonizers, which require a water activity greater than 0.90 to germinate and start mycelial growth. The amount of moisture varies from each species. Some are *xerophilic* (colonize under very dry conditions), some are *xerotolerant* (colonize under a wide range of moisture levels) and some are *hydrophilic* (colonize at high moisture levels). Humidity or moisture content of substrate can often be sufficient, but relative humidity at 50% is problematic in many indoor environments. Although high levels of humidity and some surface interstitial condensation may be sufficient for most primary and secondary colonizers, tertiary colonizers generally require serious condensation problems. These problems may be due to construction faults, including inadequate insulation, in combination with poor ventilation, or water damage from leaks, flooding and groundwater intrusion, Cheong et al., [8].

Fungal spores can be transported from outdoor sources into indoor environments through a variety of means such as, adherence to human skin, clothes, and shoes which can be directly conveyed into the indoor environment by means of ventilation or open doors and windows. Suitable niches for growth and sporulation of fungal spores include stored food, house plants, air conditioners, humidifiers, cool air vaporizers, books and papers, carpets and damp areas Morring et al. [7]. The use of air conditioners provides conditions which are perfect for fungal

sporulation or growth. Also, since the air in most indoor environments (especially banking halls) is stagnant as a result of absence of extraction, it is easy for fungal spores to settle in the air current. Studies have shown that proper cleaning and maintenance can reduce the various components of dust (fungi inclusive) and reduce the concentration on surfaces including floors and air conditioners if the cleaning is of a high standard, Flannigan [9]. Therefore, it becomes imperative to undertake this study, so as to enumerate and identify the airborne fungi in selected banking halls on the University of Cape Coast campus and its environs.

Previous studies have shown that there are high levels of airborne fungi in both the indoor and outdoor environments (Chadeganipour et al. [3]; Perner [10]; Ogunlana (4). The airborne fungi, isolated by Chadeganipour et al. [3] revealed that fungi have a significant role in infecting immunocompromised hosts, after they obtained positive results from test rabbits with the fungi extract through a skin prick test. Codina et al. [11] also determined the types and levels of airborne fungal spores in air-conditioned homes with moisture problems, and their findings revealed high levels of fungal air spore in such homes. These findings makes it imperative to undertake this study in some of the banking halls, since from the above mentioned works, the banking halls also serve as potential niches for fungal growth, especially with the conditions provided by the air conditioners. Also judging from the health complications such as upper respiratory tract infections, headache, nausea, allergic reactions as well as asthma especially in immunosuppressed individuals that arise from inhalation of these fungal air spore, Cheesbrough [12], it of great importance to undertake this study. The aim of the research is to assess the levels of fungal spore contamination in selected banking halls on the University of Cape Coast campus and its environs.

2. MATERIALS AND METHODS

All materials and equipment's used were obtained from the Department of Chemistry, Molecular Biology and Laboratory Technology of the University of Cape Coast.

2.1 Study Area

This study was carried out in four banking halls on the University of Cape Coast campus and its environs.

2.2 Sampling Technique

The settling plate method was used for the sampling as employed by Chadeganipour et al. [3] to trap, enumerate and identify fungal spores using purposive sampling. Samples were collected on Wednesdays and Fridays between the hours of 1-2 pm for a period of two weeks. A total of twelve samples were taken in all from the four banks, with three samples from each bank, thus top, middle and bottom parts of each bank. The various banks were represented with the alphabets A, B, C and D.

2.3 Microbial Analysis

Sabouraud dextrose agar (SDA) was prepared according to the manufacturer's specification and was inoculated with chloramphenicol (250 mg) as an antibiotic to prevent bacterial growth. The prepared media was mixed well to ensure uniformity and then sterilized by autoclaving for 15 minutes at 121ºC to get rid of any spores or microorganism that may have been present. The petri dishes containing the solidified media were then carried in a canister to the various banks for sampling. The plates were placed at varying heights thus the bottom, middle and top of the study areas for a period of ten minutes each. The exposed plates were sent to the laboratory and incubated in a canister at temperature between 25 to 28ºC for a period of seven days. Between these periods frequent observations were made to see if there was growth and how well the growth was.

2.4 Total Plate Count

The plates were examined and enumerated for fungal growth on the seventh day of incubation. The fungal colonies were enumerated using a colony counter to find the total colonies of fungi growing on each plate. Individual colonies were identified macroscopically and their percentage occurrences calculated for.

2.5 Isolation of Organisms

Pure cultures of the individual colonies were isolated using the streaking method. Freshly prepared SDA inoculated with chloramphenicol was sterilized by autoclaving at 121ºC for 15 minutes, after which it was allowed to cool to about 40ºC.

Strains of the individual colonies from the mixed cultures were picked with the sterilized

inoculating pin and streaked on the surfaces of the solidified media in the petri dishes. The inoculating pin was flamed after each streaking to prevent contamination. The streaked plates were then incubated at 25 to 28ºC for seven days in the canister to allow for microbial growth. The plates were continually observed within the seven days to see if there was growth and how well the growth was.

2.6 Microscopy

Microscopic identification of the fungi was done with the aid of a microscope, where the fungal isolates from the subculture were prepared on a slide using the wet mount method.

A drop of distilled water was placed on the slide and a sterilized inoculating pin was used to pick a tiny strain from the isolate and emulsified in the distilled water on the slide. A coverslip was placed on it perfectly to avoid trapping of bubbles between the coverslip and the slide and then viewed under the microscope (Olympus-CH20i) set at × 40 magnifications to enable easy visualization. Strains that were difficult to identify were made easier using Lactophenol cotton blue as the wet mount, Astrid [13]. The features observed under the microscope from the individual isolates were compared to a chart of fungal species so they could be identified into their various genera, M.S.H., [14].

The procedures were performed aseptically to avoid contamination and to provide accurate results.

2.7 Statistical Analysis

The results of the analysis were subjected to statistical analysis using the Graphic Pad Prism (version 5) program to analyze the variation of fungal airspora within the four banks under study and their varying heights (top, middle and bottom), using the p-value and determined their percentage frequency of occurrence. The analysed data were then presented in both tabular and graphical forms.

3. RESULTS AND DISCUSSION

The investigation as seen in table 1 revealed the presence of fungi spores in the banks. There were variations in the levels of fungal spore at different parts (top, middle and bottom) of the banking halls with the bottom recording the highest fungal spore of 6 cfu/ml, 50 cfu/ml, 47 cfu/ml and 54 cfu/ml for Banks A, B, C and D respectively; followed by the middle, 4 cfu/ml, 15 cfu/ml, 10 cfu/ml and 18 cfu/ml for Banks A, B, C and D respectively; with the top recording the least fungal spore of 1 cfu/ml, 2 cfu/ml, 1 cfu/ml and 3 cfu/ml for Banks A, B, C and D respectively. There was no significant difference in the fungal population except for *Fusarium clamydosporium* in Bank D, which was highly significant ($P<0.001$).

From the bar graph illustrated in Fig. 1, Bank A had most of its isolated species to be *Aspergillus fumigatus* (45.45%) and its lowest isolate to be *Aspergillus niger* (9.09%). The other isolates were *Aspergillus flavus* (27.27%) and*Rhizopus stolonifer* (18.18%). Bank B had its highest isolated species to be *Aspergillus niger* (32.84%) and its lowest isolate to be *Rhizopus stolonifer* (5.97%). The other isolates included *Aspergillus flavus* (26.86%), which was much closer to that of *Aspergillus fumigatus* (23.88%) and then followed by *Fusarium chlamydosporum* (10.45%). Bank C also had its highest isolated species to be *Aspergillus fumigatus* (32.0%) and its lowest isolate being *Penicillium* spp. (5.17%). Others included *Aspergillus niger* (26.87%), followed closely by *Aspergillus flavus* (20.69%) and *Fusarium chlamydosporum* (10.34%). Bank D had its highest isolated species to be *Aspergillus niger* (32.0%) and *Fusarium chlamydosporum* being its lowest isolate (5.33%). The other isolates were *Aspergillus fumigatus* (25.33%), followed by *Aspergillus flavus* (22.67%) with *Penicillium* spp. and *Rhizopus stolonifer* having a low of 8.0 and 6.67% respectively.

From the percentage frequency of the fungal airspora isolated in from the Banking halls (Total) as shown in Fig. 2, it revealed that out of the six species isolated, *Aspergillus niger* was the most frequent (30.81%), followed closely by *Aspergillus fumigatus* (27.96%) and *Aspergillus flavus* (23.70%) with *Fusarium chlamydosporum, Rhizopus stolonifer* and *Penicillium* spp. recording lower percentage frequencies of 8.06%, 5.21%, and 4.27% respectively.

The results obtained from this study revealed that there were fungal airspora in the banks with variations in the level of fungal population from one bank to the other and from one height to the other (bottom, middle and top). Among the four banks, only six different species were identified microscopically which included, *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger,*

Rhizopus stolonifer, Fusarium clamydosporium and *Penicillium* spp. These microorganisms found in the banking halls can be attributed to their variable sizes, poor ventilation and the exo-genal fungi which finds it way in when banking doors are opened. These findings were partially in agreement with work done by Chadeganipour et al. [3] since a few of the fungi isolated, (*Rhizopus* spp*, Penicillium* spp. and *Fusarium* spp.) fell in line with those investigated by them, in addition to *Cladosporium* spp, *Yeast* spp*, Alternaria* spp*, Ulocladium* spp*, Curvularia* spp*, Acremonium* sppand *Helminthosporium* spp. from an indoor environment. The study also compared favorably with recent study conducted revealing indoor relation with outdoor.

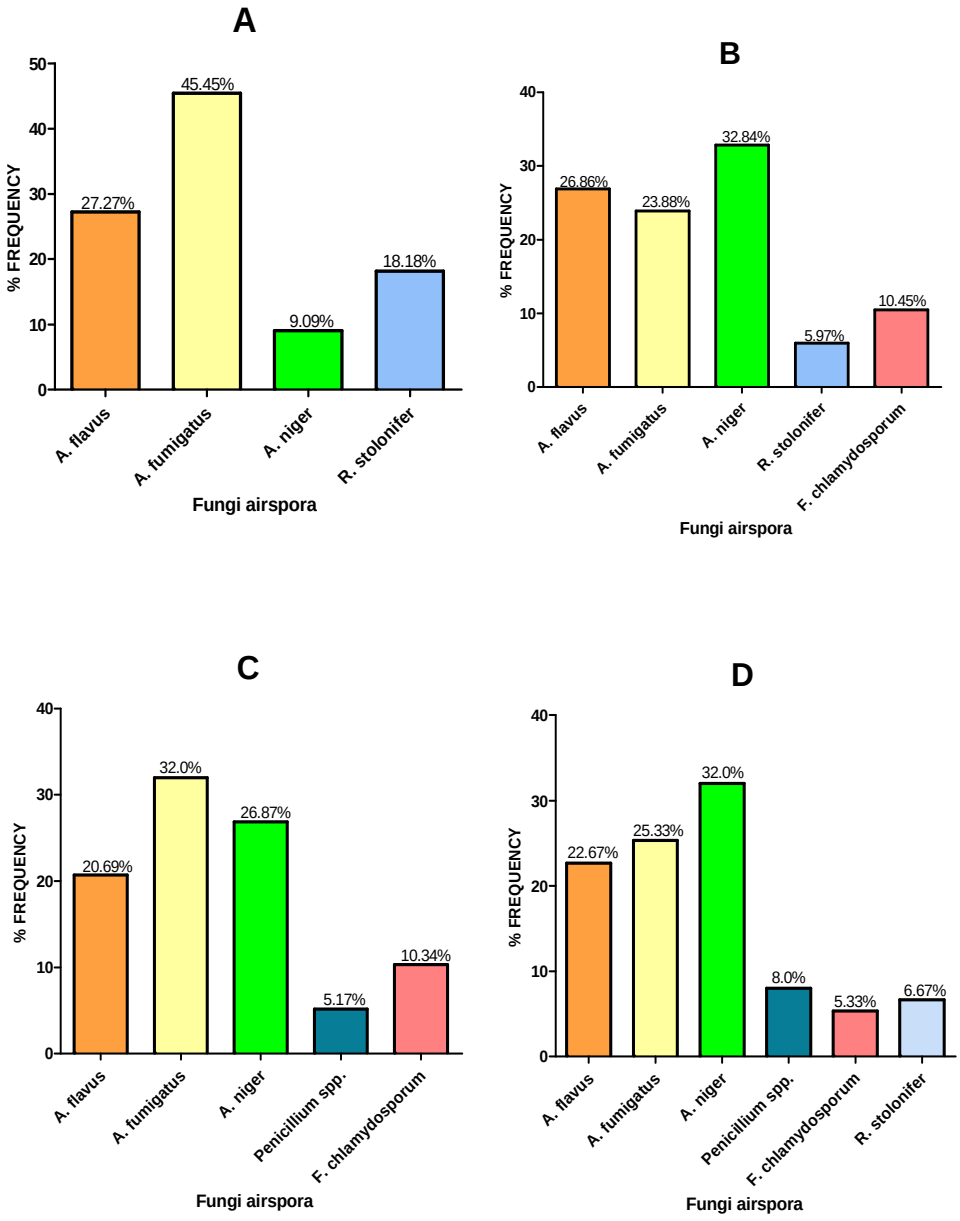

Fig. 1. Occurrence of fungal spore isolated from different Banking Halls (A, B, C and D) on the University of Cape Coast campus and its environs

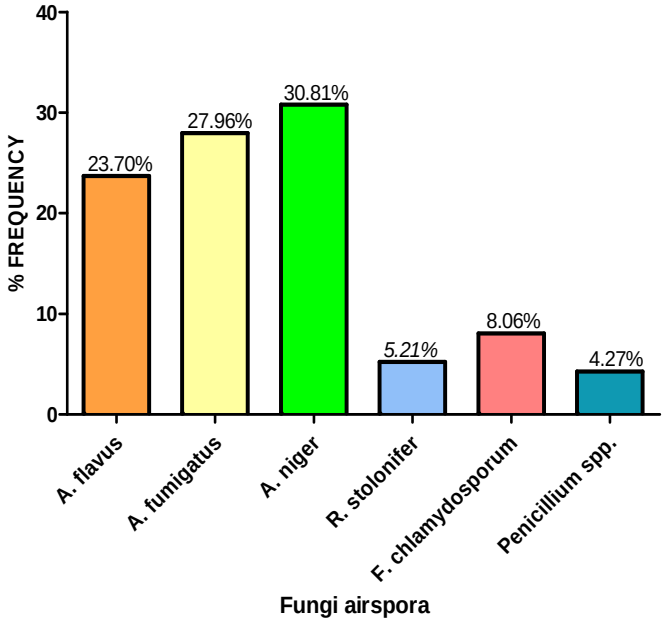

Fig. 2. Occurrence of fungal spore isolated from different Banking Halls (total) on the University of Cape Coast campus and its environs

As seen in the Table 1 and Fig. 1, there were variations in the number of fungal airspora enumerated from the various banks and from the various heights. This could be attributed to the size of the sampling areas (banks) and how often people patronized them. According to Morring et al. [7], fungal airspora is known to be transported from outdoor sources into indoor environments through a variety of means including, adherence to human skin, clothes, shoes and directly conveyed into the indoor environment by means of ventilation or open doors and windows, it confirms the higher levels of fungal air spora in the bigger and busier banking halls thus B, C and D, unlike bank A which is much smaller with fewer patronage. With bank A, its doors are not often opened since it is not highly patronized like banks B, C and D, hence air spore which may have been transported through opening of doors by customers was low. High levels of fungal air spore in some of the banks could also be attributed to the conditions provided by the air-conditioners within the banking halls. This confirms study done by Codina et al. [11], which indicated high levels of fungi in air-conditioned rooms with high moisture problems. The bottom sections of the banks showed more fungal growth followed by the middle and the least shown at the top. These findings were eminent to the fact that the spores carried by the air current settled more on the floor (bottom) than remaining in the air Parkin [1].

From Fig. 2, it was noted that an individual is likely to be highly exposed to *Aspergillus niger* the most at a frequency of 30.81% when he/she patronizes any of the banking halls under study over a period of time. *Aspergillus niger,* which happens to the most abundant species of *Aspergillus* in nature has health effects such as*otomycosis* (fungal ear infections), which can cause pain, temporary hearing loss and in severe cases, damage to the ear canal and tympanic membrane. It can also cause serious lung diseases (*aspergillosis*) when inhaled frequently (Samson et al*.,* 2001). The other species included *Aspergillus fumigatus* (27.96%), *Aspergillus flavus* (23.70%), *Fusarium chlamydosporum* (8.06%), *Rhizopus stolonifer* (5.21%) and *Penicillium* spp*.* (4.27%). *Aspergillus fumigatus* causes hypersensitivity to it so that people develop severe allergic reactions to the mold likewise *Aspergillus flavus* which produces the carcinogenic mycotoxin (aflatoxin) which contaminates food Samson et al. [15]. Though the other species were not isolated in larger quantities, continuous exposure to them could lead to health effects in an immune-compromised patient over a period of time Kavita et al. [16].

Table 1. Occurrence of fungal Air spora from different levels of selected banking halls on the UCC campus and its environs expressed incolony forming units

Banks	Fungal airspora	Levels (CFU/ml)			P-value
		Top (n = 1)	Middle (n = 4)	Bottom (n = 6)	
A	*A. flavus*	1 (100)	1 (25.0)	1 (16.67)	0.221
	A. fumigatus	0 (0.0)	2 (50.0)	3 (50.0)	0.632
	A. niger	0 (0.0)	1 (25.0)	0 (0.0)	0.466
	R. stolonifer	0 (0.0)	0 (0.0)	2 (33.33)	0.361
		(n = 2)	**(n = 15)**	**(n = 50)**	
B	*A. flavus*	2 (100)	4 (26.67)	12 (24.0)	0.059
	A. fumigatus	0 (0.0)	3 (20.0)	13 (26.0)	0.646
	A. niger	0 (0.0)	6 (40.0)	16 (32.0)	0.511
	R. stolonifer	0 (0.0)	0 (0.0)	4 (8.0)	0.485
	F. chlamydosporum	0 (0.0)	2 (13.33)	5 (10.0)	0.828
		(n = 1)	**(n = 10)**	**(n = 47)**	
C	*A. flavus*	1 (100)	2 (20.0)	9 (19.15)	3.904
	A. fumigatus	0 (0.0)	3 (30.0)	16 (34.04)	0.757
	A. niger	0 (0.0)	4 (40.0)	14 (29.79)	0.651
	Penicillium spp.	0 (0.0)	0 (0.0)	3 (6.38)	0.691
	F. chlamydosporum	0 (0.0)	1 (10.0)	5 (10.64)	0.941
		(n = 3)	**(n = 18)**	**(n = 54)**	
D	*A. flavus*	2 (66.67)	5 (27.78)	10 (18.52)	0.128
	A. fumigatus	1 (33.33)	3 (16.67)	15 (27.78)	0.611
	A. niger	0 (0.0)	6 (33.33)	18 (33.33)	0.479
	Penicillium spp.	0 (0.0)	0 (0.0)	6 (11.11)	0.281
	F. chlamydosporum	0 (0.0)	4 (22.22)	0 (0.0)	**0.001**
	R. stolonifer	0 (0.0)	0 (0.0)	5 (9.26)	0.353

Values in parenthesis are percentage frequency, n= total numbers of isolates; A, B, C & D are different banking Halls, CFU= Colony Forming Unit, P< 0.05

4. CONCLUSION

It can be concluded that there was fungal airspora in the banking hall under study with six different fungal species isolated from them in all. The bigger and much busier banks (B, C and D) with poor ventilation had more fungal load than the smaller and less busy one (A) with a good ventilation. The most frequent fungal species isolated was *Aspergillus niger* (30.81%) followed closely by *Aspergillus fumigatus* (27.96%), and *Aspergillus flavus* (23.70%). *Fusarium clamydosporium*, *Rhizopus stolonifer*and *Penicillium* spp. were the least frequent thus: 8.06%, 5.21% and 4.27% respectively.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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