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Increased prevalence of indoor *Aspergillus* and *Penicillium* species is associated with indoor flooding and coastal proximity: a case study of 28 moldy buildings†

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Indoor flooding is a leading contributor to indoor dampness and the associated mold infestations in the coastal United States. Whether the prevalent mold genera that infest the coastal flood-prone buildings are different from those not flood-prone is unknown. In the current case study of 28 mold-infested buildings across the U.S. east coast, we surprisingly noted a trend of higher prevalence of indoor *Aspergillus* and *Penicillium* genera (denoted here as Asp–Pen) in buildings with previous flooding history. Hence, we sought to determine the possibility of a potential statistically significant association between indoor Asp–Pen prevalence and three building-related variables: (i) indoor flooding history, (ii) geographical location, and (iii) the building's use (residential *versus* non-residential). Culturable spores and hyphal fragments in indoor air were collected using the settle-plate method, and corresponding genera were confirmed using phylogenetic analysis of their ITS sequence (the fungal barcode). Analysis of variance (ANOVA) using Generalized linear model procedure (GLM) showed that Asp–Pen prevalence is significantly associated with indoor flooding as well as coastal proximity. To address the small sample size, a multivariate decision tree analysis was conducted, which ranked indoor flooding history as the strongest determinant of Asp–Pen prevalence, followed by geographical location and the building's use.

Environmental significance

Millions of Americans reside in impoverished communities located in flood-prone coastal areas. This case study suggests that residents of coastal homes with flooding history are prone to health risks most frequently associated with exposure to indoor *Aspergillus* and *Penicillium*. Our findings provide the foundation of population health studies that will lead to public health guidance and awareness campaigns for communities at the greatest risk of fungal allergies and infections.

1. Introduction

Indoor molds within the genera *Aspergillus* and *Penicillium* (often referred to as common molds) are the types of fungal spores most frequently associated with an increased risk of adverse health effects to building occupants after flooding.[1–4] Their poisonous toxins (mycotoxins, with examples including aflatoxin, gliotoxin, ochratoxin A, fumagillin, citrinin, cyclopiazonic acid) and hazardous volatile organic compounds contribute to sick building syndrome, suppress immunity, and increase vulnerability to other infections.[1,5] Several species of *Aspergillus* and *Penicillium* are sources of potent allergens.[6–8]

Recent reports document a dramatic increase in the frequency of fall flooding along the Southeastern U.S. coasts[9] and project the odds of extreme coastal flooding to double every five years into the foreseeable future at most locations in the

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U.S.[10] It is predicted that the 'once-in-a-lifetime' coastal floods with a 50 year return period will exceed 90% of coastal U.S. cities.[10] Currently, at least 40 million people reside in such flood-prone zones[11] with a large portion of this population consisting of socio-demographic groups that are more likely to be adversely affected by indoor mold exposures in the upcoming hurricane season.[11] These include the neighborhoods with a large elderly population with chronic illness, economically disadvantaged persons, ethnic and racial minorities, and people who live in poor housing conditions.

Americans generally spend >90% of their time indoors.[12] Early in the response to the COVID-19 pandemic, non-pharmaceutical interventions such as lockdowns and stay-at-home orders forced millions of Americans to stay confined or quarantined in their homes and reduced the time spent in centralized workplaces where air quality must meet regulatory standards. Indoor mold exposures are therefore becoming an increasing public health threat, especially to people living with asthma.[13–15] Many communities experiencing indoor mold problems are located in the Southeastern coastal United States (*e.g.*, South Carolina, Georgia, Florida) that have experienced a drastic increase of hurricanes and repeated flooding events in the past years.[16,17]

In this small-scale study, we investigated a possible association between common indoor molds within the genus *Penicillium* and/or *Aspergillus* (collectively denoted here as Asp–Pen) with any or all of the following factors: (1) whether the building had a history of indoor flooding leading to mold problems or whether the mold problems were associated with other dampness issues; (2) whether the building is in a particular geographic location; (3) whether a building is used for residential purposes or non-residential purposes. Presented here are the results showing all these associations.

2. Methods for data collection and statistical analyses

2.1. Sample collection

Buildings used in this study were randomly selected based on accessibility (consent from owners to enter the building) and the presence of visible mold contamination before restoration. Samples were collected from 28 buildings across three U.S. states (South Carolina, North Carolina, and New Jersey) and one office building in Washington, D.C. Other than state-wise categorization, the mold samples were categorized as (1) indoor flooded (mold growth associated with a history of indoor flooding within buildings), (2) non-flooded (mold growth associated with indoor dampness issues other than indoor flooding), (3) coastal (samples collected from buildings in coastal locations as defined previously as a zone within 100 km from the shoreline[18]), (4) non-coastal (samples collected from buildings in non-coastal locations), (5) residential (samples collected from residential buildings) and (6) non-residential (samples collected from non-residential buildings).

Viable molds in the buildings' indoor air were estimated using the settle-plate method, an established post-flood fungal bioaerosol sampling method comparable to the microbial air-sampler methodology under resource-restricted situations.[19] Although settle-plate gravitational or depositional sampling is considered a non-quantitative sampling method, this method is more practical and relevant in flood-affected homes where environmental conditions may be hazardous, access of researchers is limited, power supply is frequently missing, and this is the only method to qualitatively assess the indoor airborne fungal contamination over prolonged exposure times. A total of 10 plates were installed per site spread across different building locations to ensure that our observations of mold prevalence most closely represent that of the site's indoor air. Fungal spores and hyphal fragments settled by gravity on potato dextrose agar (PDA) plates were securely sealed, labeled with time, dates of collection, and transported to the laboratory for analysis.

2.2. Identification of fungal colonies by PCR amplification of ITS regions and sequence analyses

Plates were incubated at a temperature of 25 ± 2 °C in the dark for about 4 to 5 days. The visible colonies were sub-cultured into pure colonies using single-spore isolation.[20] Genomic DNA extraction and sequencing of the ITS2 region was performed by Accugenix, Inc., following standard operating procedures for the AccuGENX-ID® service. Briefly, DNA was extracted using standard alkaline lysis methods, and the extract was used for PCR amplification using standard M13 tailed ITS2 primers ITS3F and ITS4R.[21] The ABI 3130XL capillary sequencer was used to run the sequencing reactions, which were done according to the manufacturer's instructions and using BigDye Terminator v1.1 cycle sequencing kits (Life Technologies, Carlsbad, CA). After confirmation of acceptable raw data quality, the ITS2 sequences were searched against the Accugenix fungal library database using the BLAST algorithm and proprietary software to determine the closest library reference matches to the unknown sequence. These reference library entries entered in the phylogenetic analysis pipeline with a pairwise alignment, percent difference and genetic distance calculations. Once the evolutionary distance measurements were calculated, the Neighbor Joining tree was constructed using proprietary software and the identification of the isolates from the phylogenetic tree were inferred to the genus level (for all isolates) and to the species level (for most isolates). The Accugenix phylogenetic analysis pipeline was adopted based on a previously conducted validation in which we found that the Accugenix phylograms were exactly in consensus with our bootstrap method;[21] the NJ phylograms generated by computing evolutionary distances using maximum likelihood analysis (with MEGA6; from 1000 replicates) matched the Accugenix phylograms.

2.3. Statistical and computational methods

Boxplots on medians and two-way analysis of variance, including interactions [General Linear Model (GLM) and type III sums of squares], were conducted using statistical software SAS (SAS Institute, Inc., Cary, NC, USA) on mean numbers to assess the influence of Asp–Pen prevalence as a function of four

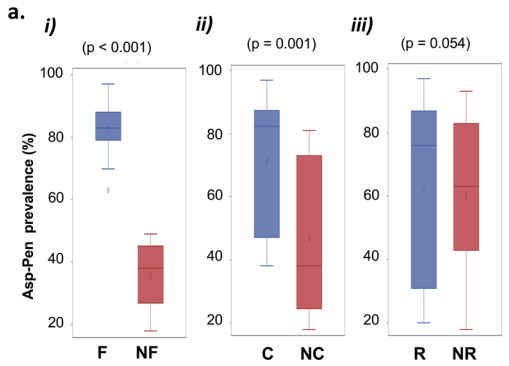
different categorical variables: indoor flooding, coastal proximity and residential *versus* non-residential use on Asp–Pen prevalence. Individual and Moving Range (I and MR charts) were conducted using the statistical software JMP 14 Pro (SAS Institute, Inc., Cary, NC, USA) to illustrate the variability within the examined groups' sample population. Comparison of Asp–Pen prevalence (the percentage of Asp–Pen in the total colonies identified) was performed using F-test (with $\alpha = 0.05$).

Small sample size is a limitation of this study. However, a multivariate decision tree analysis similar to a method described previously for a small sample size[21] was used to identify the factors that have the strongest relationship with Asp–Pen percentage prevalence. To account for the heterogeneity in building parameters that, in turn, influence mold population densities, it was assumed that this model would perform differently in mold communities with various mold population densities. Maximum depth and features of the decision tree were applied to stop excessive splitting, improve the predictive accuracy, and control overfitting. The K-fold cross-validation approach was also used to account for the small sample size.[22] For each split, LogWorth ($\log_{10}$ of adjusted *p*-value) log was used to determine the best cut point. The accuracy of the predicted Asp–Pen prevalence within different mold population densities was tested using the coefficient of determination (R^2), the root mean squared error (RMSE), and the corrected Akaike information criterion (AICc) to estimate the relative amount of information lost by the statistical model and avoid potential overfitting typical for small sample size.[23]

3. Results

The raw data for Asp–Pen prevalence in each building is provided in ESI Table 1† and the phylograms of all fungi identified in this study are provided in ESI Fig. 1.† As the first step in this study, we examined whether the prevalence of Asp–Pen in a moldy building was influenced by any or all of the following factors: (i) indoor flooding, (ii) its relative geographic location such as coastal proximity (whether a building was within 100 km from the coast), and (iii) whether it was being used as for residential or non-residential purpose. As shown in Fig. 1 and the individual and moving range (I and MR) charts (ESI Fig. 2†), indoor flooding and coastal proximity emerged in our analysis as the most significant factors associated with Asp–Pen prevalence in the mold-damaged buildings. The summary of the results of ANOVA on Asp–Pen for these parameters is provided in Table 1. Both indoor flooding and coastal proximity (whether a building is within 100 km from the shoreline) showed strong association (with high statistical significance) with an increased prevalence of Asp–Pen. While we did observe a trend of increased Asp–Pen prevalence in residential buildings compared to non-residential buildings, the association was not statistically significant.

The hierarchy of the influencing factors on Asp–Pen prevalence for the facilitation of classification and prediction (underlying decision-making) was next examined using the decision-tree method. The regression tree presented in Fig. 2 was obtained after 4 splits ($R^2 = 0.95$). Column contributions



b.

Parameter	Response	Mean estimated Asp-Pen	SEM	95% CI
Indoor Flooding	Yes (F)	79.3	2.32	(74.47, 84.15)
	No (NF)	33.97	2.18	(29.44, 38.50)
Coastal Zone (within 100 Km from shoreline?)	Yes (C)	64.84	3.08	(58.44, 71.24)
	No (NC)	48.44	2.49	(43.25, 53.62)
Residential (R) *vs.* Non-residential (NR) use of a building	R	60.25	2.73	(54.58, 65.93)
	NR	53.03	2.32	(48.21, 57.85)

Fig. 1 Association of indoor Asp–Pen prevalence with indoor flooding, coastal proximity and residential *versus* non-residential use. (a) Box plots showing comparisons of Asp–Pen prevalence in (i) buildings with indoor flooding (F) as primary issue of dampness *versus* buildings with non-flood dampness issues (NF) (ii) buildings located in a coastal zone (within 100 km from shoreline) and non-coastal zones and (iii) residential and non-residential buildings ($n = 28$), (b) summary of estimated mean Asp–Pen prevalence for these parameters (the actual counts corresponding to each parameter is indicated in ESI Table 1†).

Table 1 Analysis of variance (ANOVA) results of Asp–Pen prevalence with respect to factors of indoor flooding, coastal proximity and residential use (Y/N)[e]

Parameter	Df[a]	SS[b]	MS[c]	*F*-Statistic	*P* value
Indoor flooding (Y/N)	1	9854.64	9854.64	282.71	<0.0001
Coastal zone location (Y/N)	1	512.6	512.6	14.71	0.0010
Residential use (Y/N)	1	144.6	144.6	4.15	0.0545[d]

[a] Df: degrees of freedom. [b] SS: sum of squares. [c] MS: mean squares. [d] Not significant. [e] R^2 value obtained for this model: 0.96.

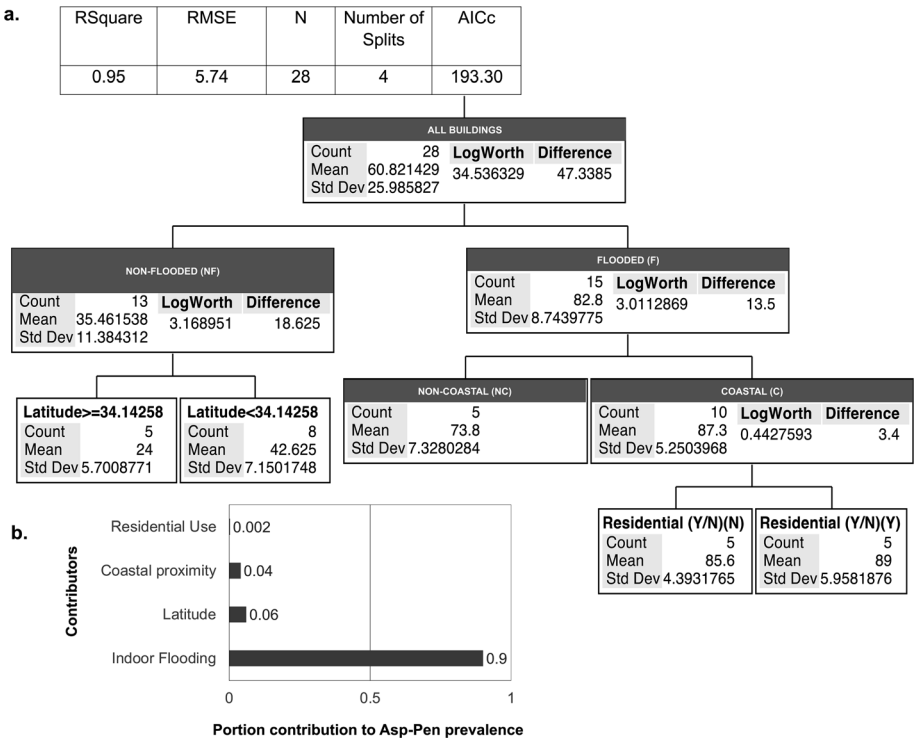


Fig. 2 Results from a multivariate decision tree analysis for the association between predictors of Asp–Pen prevalence in mold-infested buildings. (a) Leaf report, (b) column contribution report showing relative contributions of the predictors to Asp–Pen prevalence.

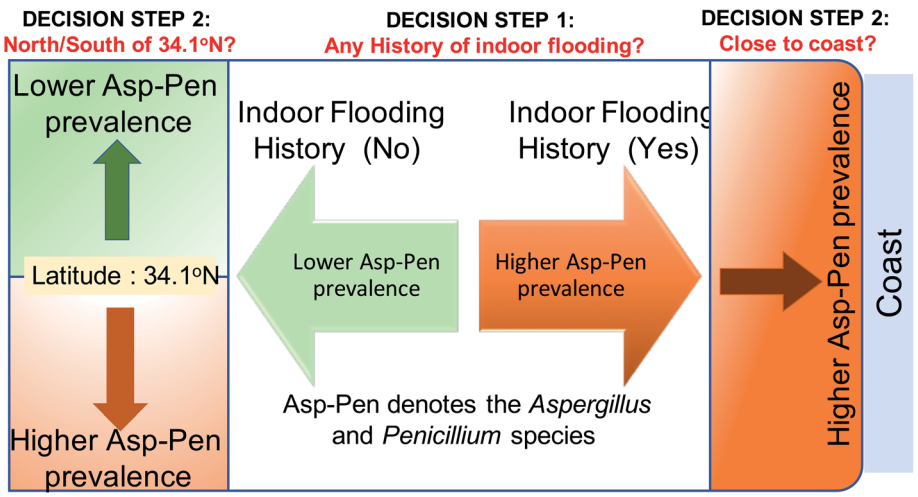


Fig. 3 Graphical illustration of the potential factors that contribute to the prevalence of indoor molds within the genera *Aspergillus* and *Penicillium* (Asp–Pen) in moldy homes as suggested by the current study.

indicate that indoor flooding, latitude, coastal and residential status all collectively influence the Asp–Pen percentage prevalence. However, in concurrence with GLM and I–MR charts, the regression tree identified indoor flooding as the highest-ranked factor influencing Asp–Pen prevalence showing a mean increase of 47.3 percentage Asp–Pen upon indoor flooding. Non-flooded and non-coastal buildings with zip codes that are south of 34.1°N (*e.g.*, in the state of SC) are predicted to show a higher Asp–Pen prevalence (by ~18.6) than those located north of 34.1°N (*e.g.*, in the state of NC). For flooded homes, Asp–Pen prevalence is predicted to increase in a coastal location by ~13.5%. The use of buildings for residential *versus* non-residential purposes appeared to be the least influential factor in determining Asp–Pen prevalence in buildings.

4. Conclusions

This pilot study supports the general idea illustrated in Fig. 3 which suggests that millions of residents in the eastern coastal flood-prone zones of the United States are potentially at risk of

health hazards caused by indoor *Aspergillus* and *Penicillium* exposures. A large population among these residents are underrepresented groups and minorities in impoverished communities. Previous studies on indoor air microbiomes in damp buildings indicate similarities in microbial ecology,[24,25] including fungal ecology[21,26] and even the prevalence of indoor molds within the genera *Aspergillus* and *Penicillium*.[27] However, little is known about how increased indoor floods influence the fungal ecology of indoor air. Our findings support the possibility that floodwaters may serve as sources of new and enriched nutrients within buildings and select for robust and fast-growing common indoor molds, mostly within the genus *Aspergillus* and *Penicillium*. A previous study found that these molds are most prevalent in damp buildings. Hence, we reasoned that the prevalence of Asp–Pen would likely increase with indoor flooding. Our findings underscore the need for further studies that improve our understanding of how (a) intensifying hurricane seasons impact indoor flooding events in the eastern U.S., (b) how such flood events relate to the prevalence of Asp–Pen mold contamination in affected buildings, and (c) what are the best practices (*e.g.*, targeted humidification, *etc.*) that can prevent Asp–Pen growth and the risks of secondary fungal infections in populations already impacted by the recent COVID-19 pandemic. This is critical in light of the documented causal linkages between indoor dampness and health effects that include aggravation of existing upper respiratory tract symptoms including cough, wheeze, and asthma, as well as incident asthma among children.[28]

A limitation of this study is its relatively small sample size, and thus our conclusions have been drawn accordingly. The sample size was sufficient for the GLM procedure to determine the co-influence of indoor flooding and coastal proximity on Asp–Pen prevalence with high statistical significance. However, the sample size was not sufficient to test by GLM whether residential use of a building or the location of a building in a U.S. state had a statistically significant influence on Asp–Pen prevalence. While the interaction of two parameters (indoor flooding and coastal proximity) was statistically significant, the data did not permit the development of an extended interactive predictive model by GLM. To address the pitfall of a small dataset, we used a decision tree analysis validated with a generalized regression model with Lasso estimation. The integrative computational analyses collectively identified indoor flooding as the most influential determinant of increased Asp–Pen prevalence, followed by geographical location and residential *versus* non-residential use of a building.

The strength of this study was the use of molecular methods (ITS2 sequencing and subsequent phylogenetic analysis) for confirmation of the Asp–Pen strains. The method was particularly useful to confirm the *Penicillium* strains as these are difficult to identify using microscopic characteristics and phenotypic identification often requires a number of different media and need several weeks until distinguishable morphological characters appear.[8,29,30] On this note it is essential to emphasize here that our method was only able to identify viable, culturable fungi and may not be representative of the broader fungal diversity of the environments we analyzed. Despite these limitations our study was still able to identify the increased prevalence of fungi other than Asp–Pen species (see ESI Fig. 1†). *Cladosporium* was detected with highest prevalence among the non-Asp–Pen fungi followed by *Epicoccum*, *Bipolaris*, *Chaetomium*, and *Trichoderma* species.

In conclusion, we emphasize here that high humidity in buildings that have a history of indoor flooding can lead to an increased indoor Asp–Pen prevalence. To minimize risks of such exposures, it is critical to adhere to ASHRAE's recommended indoor environment parameters of 75 °F (24 °C), 50% RH, which is a 55 °F (12.8 °C) dew-point temperature.[31,32]

This study has successfully brought together a team with complementary expertise from multiple research and teaching institutions along with the small and big businesses that share a common goal to build a cost-effective, community science-based framework for establishing a mold awareness program that can determine the resiliency and remediation needs for existing and future flooded homes as the country prepares to come back to normalcy. At this point, there are very limited data on secondary mold infections associated with indoor molds, and this study successfully provides a scientific premise for a global surveillance of fungal diseases associated with indoor molds. As a follow-up to this study, the current team is already identifying the communities that are at greatest risk of mold exposures and infections through collaborations and partnerships with local organizations, schools, businesses, and residents. In parallel, this team is also exploring the possible cost-effective intervention strategies such as public awareness campaigns, de-humidifiers, and teleworking that can reduce risks of indoor mold exposures. Results from these projects will be presented in our future publications.

Author contributions

MO: data curation, formal analysis, investigation, methodology, writing – original draft, writing – -review and editing; AADE: formal analysis, investigation, methodology; CM: investigation, methodology, writing – review and editing; PC: formal analysis, methodology writing – original draft, writing – review and editing; GMG: resources, writing – review and editing; NP: resources, writing – review and editing; BV: resources, software, supervision, validation, formal analysis, writing – review and editing; CF: resources, software, supervision, validation, formal analysis, writing – review and editing; MH: validation, writing – review and editing; PB: validation, writing – review and editing; KL: validation, writing – review and editing; AADH: methodology, validation, writing – review and editing; BA: conceptualization, methodology, resources, validation, writing – original draft, writing – review and editing; JAH: writing – Review and editing; AC: conceptualization, methodology, project administration, resources, supervision, validation, writing – original draft, writing – review and editing.

Conflicts of interest

There are no conflicts of interest to declare.

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References

1 A. W. Campbell, J. D. Thrasher, M. R. Gray and A. Vojdani, Mold and mycotoxins: effects on the neurological and immune systems in humans, *Adv. Appl. Microbiol.*, 2004, **55**, 375–408.
2 J. A. Holme, E. Øya, A. K. Afanou, J. Øvrevik and W. Eduard, Characterization and pro-inflammatory potential of indoor mold particles, *Indoor Air*, 2020, **30**(4), 662–681.
3 W. A. Żukiewicz-Sobczak, The role of fungi in allergic diseases, *Adv. Dermatol. Allergol.*, 2013, **30**, 42.
4 Z. Chauhan, H. Asif, U. Deonarine and G. Ferrer, "Common Mold" pneumonia in an immunocompetent host, *J. Precis. Respir. Med.*, 2018, **1**, 6.
5 G. S. Shephard, Impact of mycotoxins on human health in developing countries, *Food Addit. Contam.*, 2008, **25**, 146–151.
6 K. J. Goh, A. C. A. Yii, T. S. Lapperre, A. K. Chan, F. T. Chew, S. H. Chotirmall and M. S. Koh, Sensitization to *Aspergillus* species is associated with frequent exacerbations in severe asthma, *J. Asthma Allergy*, 2017, **10**, 131.
7 K. Watai, Y. Fukutomi, H. Hayashi, M. Iwata, K. Nagayama, Y. Nakamura, J. Tanaka, Y. Hamada, K. Ryu and Y. Tomita, New sensitization to *Aspergillus fumigatus* in adult asthma over a 10-year observation period, *Eur. Respir. J.*, 2018, **52**, 3911.
8 G. Reboux, S. Rocchi, M. Vacheyrou and L. Millon, Identifying indoor air *Penicillium* species: a challenge for allergic patients, *J. Med. Microbiol.*, 2019, **68**, 812–821.
9 T. Ezer, Analysis of the changing patterns of seasonal flooding along the US East Coast, *Ocean Dyn.*, 2020, **70**, 241–255.
10 M. Taherkhani, S. Vitousek, P. L. Barnard, N. Frazer, T. R. Anderson and C. H. Fletcher, Sea-level rise exponentially increases coastal flood frequency, *Sci. Rep.*, 2020, **10**, 1–17.
11 W. Shao, N. P. Jackson, H. Ha and T. Winemiller, Assessing community vulnerability to floods and hurricanes along the Gulf Coast of the United States, *Disasters*, 2020, **44**, 518–547.
12 J. C. Nwanaji-Enwerem, J. G. Allen and P. I. Beamer, Another invisible enemy indoors: COVID-19, human health, the home, and United States indoor air policy, *J. Exposure Sci. Environ. Epidemiol.*, 2020, **30**, 773–775.
13 J. P. Gangneux, M. Bouvrais, S. Frain, H. Morel, S. Deguen, S. Chevrier and P. Le Cann, Asthma and Indoor Environment: Usefulness of a Global Allergen Avoidance Method on Asthma Control and Exposure to Molds, *Mycopathologia*, 2020, **185**, 367–371.
14 W. Liu, J. Cai, C. Sun, Z. Zou, J. Zhang and C. Huang, Associations between household airborne culturable fungi and allergies and airway illnesses in childhood in Shanghai, China, *Environ. Sci. Pollut. Res. Int.*, 2020, **27**, 36570–36578.
15 A. I. Tiotiu, P. Novakova, D. Nedeva, H. J. Chong-Neto, S. Novakova, P. Steiropoulos and K. Kowal, Impact of Air Pollution on Asthma Outcomes, *Int. J. Environ. Res. Public Health*, 2020, **17**, 6212–6241.
16 W. Botzen, J. M. Mol, P. J. Robinson, J. Zhang and J. Czajkowski, Individual Hurricane Preparedness During the COVID-19 Pandemic: Insights for Risk Communication and Emergency Management Policies, *SSRN*, 2020, 3699277.
17 S. Pei, K. A. Dahl, T. K. Yamana, R. Licker and J. Shaman, Compound risks of hurricane evacuation amid the COVID-19 pandemic in the United States, *GeoHealth*, 2020, **4**, e2020GH000319.
18 C. Small and R. J. Nicholls, A global analysis of human settlement in coastal zones, *J. Coastal Res.*, 2003, 584–599.
19 T. Khawcharoenporn, A. Apisarnthanarak, K. Thongphubeth, C. Yuekyen, S. Damnin, M. K. Hayden and R. A. Weinstein, Post-flood measurement of fungal bio-aerosol in a resource-limited hospital: can the settle plate method be used?, *J. Hosp. Infect.*, 2013, **83**, 150–152.
20 Y.-W. Choi, K. D. Hyde and W. Ho, Single spore isolation of fungi, *Fungal Divers.*, 1999, **3**, 29–38.
21 V. E. Hopkins, A Study of an Uncharacterized Penicillium Species Isolated from a Hypersaline Pond in San Salvador, Bahamas, Master's thesis, University of South Carolina, 2014.
22 R. I. Adams, I. Sylvain, M. P. Spilak, J. W. Taylor, M. S. Waring and M. J. Mendell, Fungal signature of moisture damage in buildings: identification by targeted and untargeted approaches with mycobiome data 86.17 (2020): e01047-20., *Appl. Environ. Microbiol.*, 2020, **86**(17), e01047-20.
23 S. Gholinejad, A. A. Naeini and A. Amiri-Simkooei, Robust particle swarm optimization of RFMs for high-resolution satellite images based on k-fold cross-validation, *IEEE J. Sel. Top. Appl. Earth Obs. Remote Sens.*, 2018, **12**, 2594–2599.
24 K. P. Burnham and D. R. Anderson, Multimodel inference: understanding AIC and BIC in model selection, *Sociol. Methods Res.*, 2004, **33**, 261–304.
25 B. Jayaprakash, R. I. Adams, P. Kirjavainen, A. Karvonen, A. Vepsalainen, M. Valkonen, K. Jarvi, M. Sulyok, J. Pekkanen, A. Hyvarinen and M. Taubel, Indoor microbiota in severely moisture damaged homes and the impact of interventions, *Microbiome*, 2017, **5**, 138.
26 Y. Shan, W. Wu, W. Fan, T. Haahtela and G. Zhang, House dust microbiome and human health risks, *Int. Microbiol.*, 2019, **22**, 297–304.
27 B. Hegarty, K. C. Dannemiller and J. Peccia, Gene expression of indoor fungal communities under damp building conditions: Implications for human health, *Indoor Air*, 2018, **28**, 548–558.
28 B. Andersen, J. C. Frisvad, I. Sondergaard, I. S. Rasmussen and L. S. Larsen, Associations between fungal species and water-damaged building materials, *Appl. Environ. Microbiol.*, 2011, **77**, 4180–4188.

29 G. M. Solomon, M. Hjelmroos-Koski, M. Rotkin-Ellman and S. K. Hammond, Airborne mold and endotoxin concentrations in New Orleans, Louisiana, after flooding, October through November 2005, *Environ. Health Perspect.*, 2006, **114**, 1381–1386.
30 R. A. Samson, J. Houbraken, U. Thrane, J. C. Frisvad and B. Andersen, *Food and Indoor Fungi*, Westerdijk Fungal Biodiversity Institute, 2019.
31 C. Visagie, J. Houbraken, J. C. Frisvad, S.-B. Hong, C. Klaassen, G. Perrone, K. Seifert, J. Varga, T. Yaguchi and R. Samson, Identification and nomenclature of the genus *Penicillium*, *Stud. Mycol.*, 2014, **78**, 343–371.
32 L. Harriman, Damp Buildings, Human Health and Ventilation Design, *ASHRAE J.*, 2020, 50–52.