

## DOCTORAL THESIS

### Responses of indoor moulds to water dynamics: the transient water conditions rendered by non-24 h air-conditioning

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## **Abstract**

Due to rapid urbanisation, people in metropolises spend the majority of their time indoors. Indoor mould contamination, as one of the most pungent biohazards in built environments, can ubiquitously present in humid areas and potentially compromise the health of occupants. Governmental institutions like the World Health Organisation and United States Environmental Protection Agency have put forward guidelines for indoor mould prevention. However, these guidelines normally require occupants to maintain a low indoor humidity (<75% or even 40%), and thus, in tropical and subtropical areas, one of the most widely used approaches to prevent indoor mould contamination is to continuously operate air-conditioners or dehumidifiers (AC/D). The 24 h operation of AC/D, however, conflicts with the requirement of energy sustainability, and hence posits a trade-off between sustainability and indoor mould hygiene. The aim of this study was to facilitate the development of sustainable and effective mould prevention strategies for indoor environments.

The literature on currently adopted mould prevention strategies including that target moisture (24 h AC/D), temperature (air-conditioning system and cool wall paint) and nutrient (dust removal) elements as well as new nanoparticles technology (Ag, TiO<sub>2</sub> and MgO nanoparticles), was reviewed and the main limitations of these strategies were discussed. It was found that none of these current mould prevention measures has addressed both sustainability and mould hygiene on balance, urging further investigations.

Therefore, the objective of the first phase investigation was to develop sustainable cause-specific mould control measures in built environments. A case study of a mould contaminated site was conducted to illustrate the micro-environments that contribute to mould contamination in buildings. The currently used 24-h AC/D approach was compared with and ranked against other sustainable alternatives. The results of this case study suggest that determining an effective non-24 h AC/D management regime tends to be a sustainable and user-friendly solution.

To develop such a regime, understanding the critical mechanisms regulating indoor mould responses to water dynamics is essential. Thus, the objective of the second phase was to characterise the critical mechanism regulating the growth of common indoor moulds under water dynamics. It was hypothesised that oxidative stress is associated with the growth of indoor moulds under water dynamics. Using *Cladosporium cladosporioides* as a model, both its pre-germination and germinated spores were exposed to daily wet-dry cycles. Afterwards, the growth was assessed and cellular H<sub>2</sub>O<sub>2</sub> concentration and catalase activity were measured. It was found that under water dynamics, the longer growth delay in *C. cladosporioides* was associated with a higher encountered oxidative stress, with 12-12 wet-dry cycle (12 h wet, 12 h dry) showing the longest delay and highest oxidative stress. Pearson correlation and linear regression analysis suggest a positive correlation between growth delay and oxidative stress under water dynamics ( $R^2=0.85$ ,  $P<0.0001$ ). Moreover, pre-germination spores generally exhibited shorter growth delay, lower cellular H<sub>2</sub>O<sub>2</sub> concentration and higher

catalase activity. Collectively, these results suggest that the growth of *C. cladosporioides* is associated with oxidative stress under water dynamics.

After revealing the association between the growth of *C. cladosporioides* and oxidative stress under water dynamics, at the third phase, this finding was extrapolated to different mould species (*C. cladosporioides*, *Aspergillus niger* and *Aspergillus penicillioides*), water activity ( $a_w$ ) (0.4  $a_w$ , 0.6  $a_w$  and 0.8  $a_w$ ) and temperature levels (19 °C and 28 °C). In addition, the antioxidant responses of treated moulds, including antioxidant enzymes (superoxide dismutase, catalase, glutathione reductase and glutathione peroxidase) were monitored. The results showed that lower water activity levels imposed higher oxidative stress to moulds, and *A. penicillioides* exhibited the highest tolerance which displayed the highest antioxidant activities and encountered lowest oxidative damage under water dynamics. Moreover, no significant difference was measured in terms of the survival, oxidative stress and antioxidant responses between these two temperature levels. The third phase of the study, for the first time discovered the reason contributing to the different resistance towards water dynamics among common indoor moulds, and further confirmed the important role of oxidative stress adaptation in withstanding transient water supply.

In conclusion, this study reveals the critical role of oxidative stress adaptation in helping moulds to cope with changing water conditions, which may shed light on a new perspective for the future development of indoor mould prevention strategies. It also indicates that longer operation time of AC/D each day may not necessarily lead to better prevention of mould

contamination, suggesting that in order to sustainably prevent mould contamination, one should operate reasonable non-24 h AC/D each day (12 h/day according to the examined species in this study) to yield a more stressful wet-dry cycle to moulds. The outcomes of this study foster the development of novel and sustainable indoor mould prevention strategies.

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