Aspergillus genera fungal contamination in a Portuguese composting plant

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ABSTRACT
Fungal contamination in composting facilities has been associated with increased respiratory and skin pathologies among compost workers. In this study we aim to characterize the fungal contamination caused by Aspergillus genera within a totally indoor composting plant located in Portugal. Air samples of 50L were collected from 6 sampling sites through an impaction method. Samples surfaces were collected by swabbing the surfaces of the same indoor sites. Pretreatment and waste screw were the sampling sites of the analyzed composting plant with the highest Aspergillus load in the air. Globally, the genus Aspergillus presented the highest prevalence both in the air from (90.6%), and surfaces from the same sampling sites (60.8%). The results obtained in this study claim the attention to the need of further research regarding to fungal contamination due to Aspergillus genus in composting plants.

Keywords: Fungal contamination; Aspergillus genus; composting plant

1. INTRODUCTION
Composting is a natural self-heating process involving the biological degradation of organic matter under aerobic conditions. Installations for composting vary greatly in size (from domestic to large-scale facilities), degree of enclosure (open, partially enclosed, enclosed facilities), design (static window systems, aerated static piles, bioreactors, etc.) and the type of wastes composted (Swan et al., 2003). The handling of waste and compost that occurs frequently in the process (compost turning, shredding, and screening) has shown to be responsible for the release of dust and airborne microorganisms and their compounds in the air of the composting facilities (Duquenne et al., 2012). Consequently, several microorganisms, such as species from Aspergillus genera have been reported (Swan et al., 2003; Duquenne et al., 2012). Furthermore, this kind of contamination in composting facilities has been associated with the increase of respiratory and skin pathologies among compost workers (Bünger et al., 2007). In this study we aim to characterize the fungal contamination caused by Aspergillus genera within a totally indoor composting plant located in Portugal.

2. MATERIALS AND METHODS
Air samples of 50L were collected from 6 indoor sampling sites (Maintenance workshop, Centrifuges, Maturation Park, Pre-treatment, Control room, Waste screw). Samples were collected through an impaction method with a flow rate of 140 L/min onto malt extract agar (MEA) supplemented with chloramphenicol (0.05%), using the Millipore air Tester (Millipore). An outdoor sample was also collected since this was the place regarded as reference. Surfaces samples were collected by swabbing the surfaces of the same indoor sites, using a 10 by 10 cm square stencil disinfected with 70% alcohol solution between samples according to the International Standard ISO 18593 (2004). The obtained swabs were then plated onto MEA. All the collected samples were incubated at 27°C for 5 to 7 days. After laboratory processing and incubation of the collected samples, quantitative (colony-forming units - CFU/m² and CFU/m²) and qualitative results were obtained with identification of the isolated fungal species. For species identification, microscopic mounts were performed using tease mount or Scotch tape mount and lactophenol cotton blue mount procedures. Morphological Identification was achieved through macro and microscopic characteristics as noted by Hoog et al. (2002).

3. RESULTS AND DISCUSSION
In this kind of industry it is impossible to eradicate all fungi, not only because the permanent bioaerosol generating operations carried out inside the facilities (Marchand et al., 1995), but also due to the high amounts of waste that is the perfect substrate for fungal growth and development in suitable levels of temperature and humidity (Wouters et al., 2000). When waste is handled in indoor environments, the bioaerosols' exposure is higher (Wouters et al., 2006), increasing the potential health effects due to fungal exposure. Pre-treatment and Waste screw were the sampling sites with the highest air fungal load from Aspergillus genera (Figure 1).
In a global analysis, *Aspergillus* genus presents the highest prevalence in air from the analyzed composting plant (90.6%). *Aspergillus niger* (32.6%), *A. fumigatus* (26.5%) and *A. flavus* (16.3%) were the most prevalent species found in the air, but *A. sydowii*, *A. versicolor*, *Neosartorya fumigata* and *A. glaucus* were also isolated. Besides those *Aspergillus* species, the genera *Mucor* sp. and *Penicillium* sp. were also identified (Table 1).

Four different species were isolated from surfaces samples with a total of 1810000 isolates. *Aspergillus* genus showed the highest prevalence in surfaces from the same sampling sites (60.8%). *Mucor* sp. (39.2%), *Aspergillus niger* (30.9%) and *A. fumigatus* (28.7%) were the most frequently found, but *A. flavus* was also isolated (Table 1).

**Table 1 – Most common fungi identified in the composting plant**

<table>
<thead>
<tr>
<th>AIR</th>
<th>FREQUENCY (n*:;%)</th>
<th>SURFACES</th>
<th>FREQUENCY (n*:;%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. niger</em></td>
<td>320; 32.6</td>
<td><em>Mucor</em> sp.</td>
<td>710000; 39.2</td>
</tr>
<tr>
<td><em>A. fumigatus</em></td>
<td>260; 26.5</td>
<td><em>A. niger</em></td>
<td>560000; 30.9</td>
</tr>
<tr>
<td><em>A. flavus</em></td>
<td>160; 16.3</td>
<td><em>A. fumigatus</em></td>
<td>520000; 28.7</td>
</tr>
<tr>
<td>Other fungal genera</td>
<td>242; 24.6</td>
<td>Other fungal genera</td>
<td>20000; 1.2</td>
</tr>
</tbody>
</table>

* Total number of counted colonies

Besides the quantitative assessment, it is crucial to analyze the fungal species present, since adverse health effects depended on fungal species (Rao et al., 1996; Hoog et al., 2000). According to the American Industrial Hygiene Association (AIHA 1996) in the Field Guide for the Determination of Biological Contaminants in Environmental Samples, the identification of the species *A. flavus* and *A. fumigatus*, both of them identified in the analyzed plant, requires implementation of corrective measures.

4. CONCLUSIONS

The results obtained in this study claim the attention to the need of further research regarding occupational exposure to fungi, and specifically to *Aspergillus* genus, in composting plants. The toxigenic potential of the species belonging to this genus suggests the need to apply molecular biology to assess toxigenic strains.

5. REFERENCES

American Industrial Hygiene Association, 1996. Field Guide for the Determination of Biological Contaminants in Environmental Samples. AIHA.


