

ESCOLA SUPERIOR DE  
TECNOLOGIA DA SAÚDE  
DE LISBOA



# A new approach to assess fungal burden and mycotoxins occupational exposure in waste trucks workers

**Carla Viegas**

[carla.viegas@estesl.ipl.pt](mailto:carla.viegas@estesl.ipl.pt)

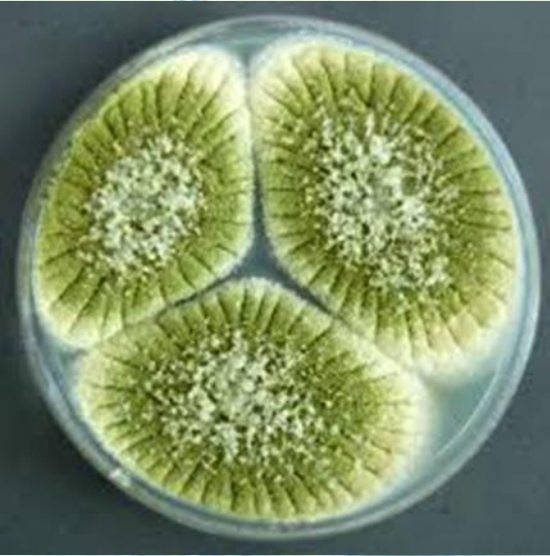
Tiago Faria; Ana Cebola de Oliveira; Liliana Aranha Caetano; Anita Quintal-Gomes; Magdalena Twarużek; Robert Kosicki, Ewelina Soszczyńska and Susana Viegas



## BACKGROUND

**Waste application facilities are considered critical regarding occupational exposure to fungal burden since provide optimal conditions for fungal growth (moisture and decaying matter).**

Heinonen-Tanski et al. 2009; Thirumala et al. 2012; Abdel Hameed and El Gendy 2013; Viegas et al. 2014 – 2016;



www.alamy.com - EN0THF







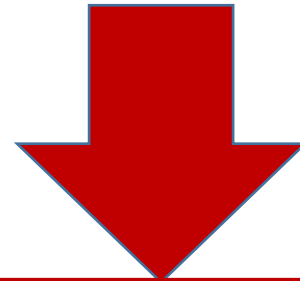
***Aspergillus* genera dominance was already reported in Portuguese waste industry**

(Viegas et al. 2014 – 2016)



**Waste-sorting the most critical setting, presenting not only the highest load, but also the wider diversity of *Aspergillus* sections.**

(Viegas et al. 2017 *in press*)



**It is important to consider the co-occurrence of mycotoxins in this occupational environment.**

(Viegas et al. 2014)





**In waste management industry it is common to use forklifts for waste transportation and workers can spend all their working hours in a forklift cab.**

Madsen et al. 2016; Viegas et al. 2017;



www.alamy.com - EN0THK



www.alamy.com - EN0THF







In cars, the bioburden filtered from the air stream by the automobile air conditioner filter could proliferate under high humidity conditions and, consequently, presenting a potential exposure source to bioaerosols.





**When the air conditioning system is turned on, the air stream passing through the vehicle filtration system could reaerosolize the bioburden and subsequently carry them into the vehicle cabinet increasing the exposure.**

Li et al. 2013

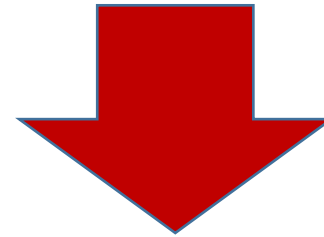






## Additional information from the company engaged in the study:

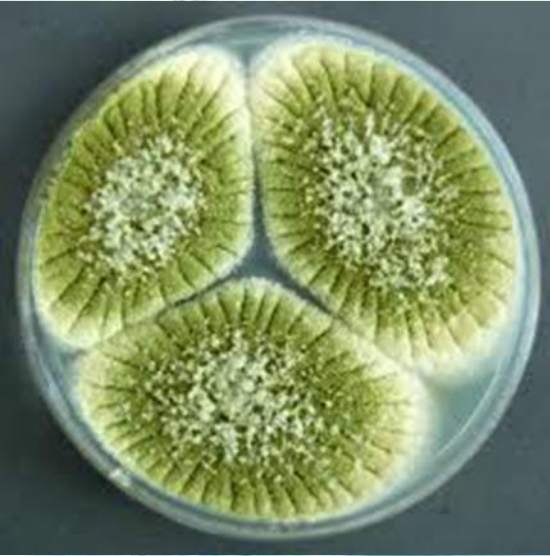
- Impossible to use the number of working hours suggested by the filter fabricant due to **filter blocking and odour in the cabinet**
- **Visual inspection** is the criteria to replace the filter



**Assess the fungal contamination and mycotoxin levels in filters from the air conditioning system of forklift cabinets, as an indicator to assess occupational exposure of the drivers working in one waste sorting industry.**

# MATERIALS AND METHODS

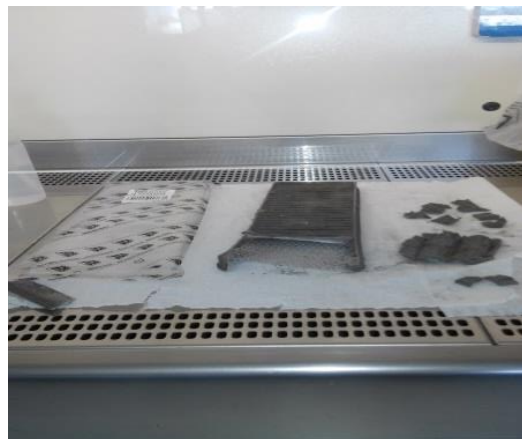
Filters from eleven vehicles were collected and subject to fungal contamination characterization and mycotoxins assessment.







Two filters pieces (2cm<sup>2</sup> )



10 mL NaCl 0.9% with 0.1% Tween 80  
30' at 250 rpm will be used.

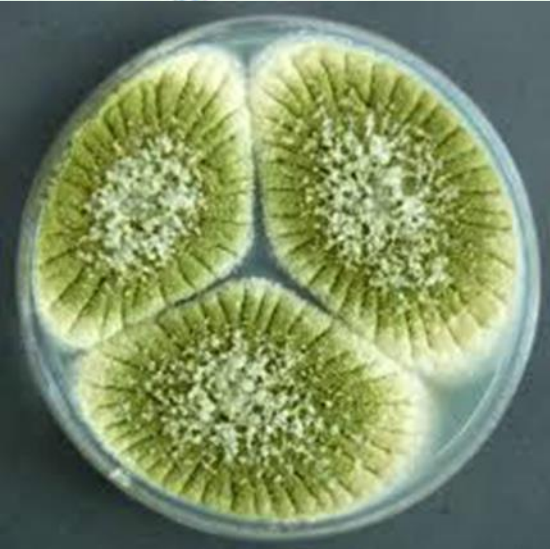
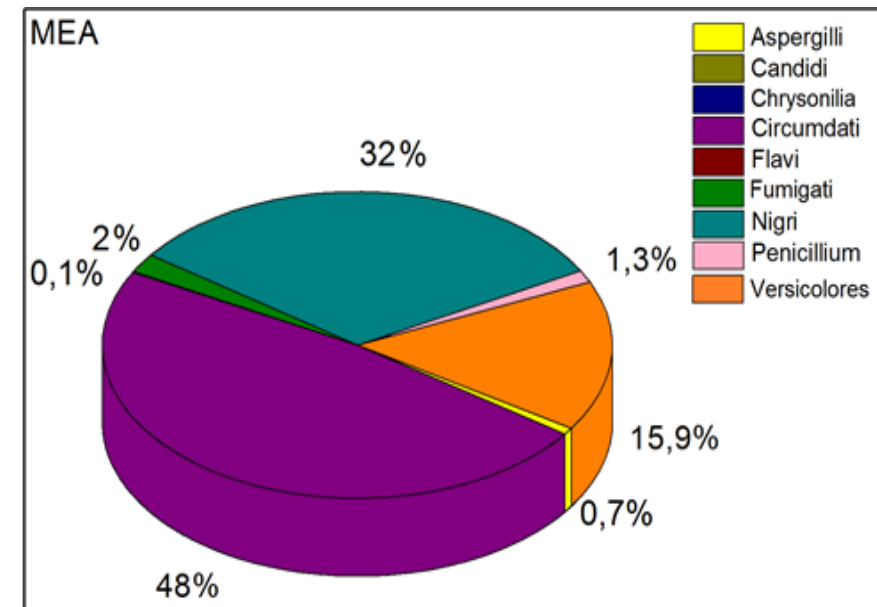
Mycotoxins assessment  
LC-MS/MS system

**Fungal contamination characterization**  
*Culture-based methods and qPCR for  
detection of Aspergillus sections Fumigati,  
Flavi, Circumdati and Versicolores*

*Aflatoxins, agroclavin, deoxynivalenol, deoxynivalenol-3-glucoside, nivalenol, fusarenon X, deepoxy-deoxynivalenol, 3-acetyldeoxynivalenol, neosolaniol, monoacetoxyscirpenol, diacetoxyscirpenol, HT-2 toxin, T-2 toxin, beauvericin, enniatins (B, B1, A1 and A) hydrolyzed fumonisin B1, ....*

# RESULTS – FUNGAL CONTAMINATION

- Was not possible to count colonies in 5 of the 11 filters analyzed due to fungal overgrowth.
- Fungal contamination ranged from  $5 \times 10^2$  to  $501 \times 10^3$  CFU/m<sup>2</sup>.
- *Aspergillus* genus sections *Circumdati* (48%) and *Nigri* (32%) were the most frequent, followed by *Versicolores* (15.9%), *Fumigati* (2%), *Aspergilli* (0.7%) and *Flavi* (0.03%).
- *Penicillium* sp. was found in only one filter in high amounts (1.3%).

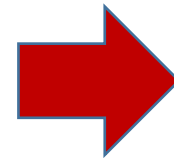




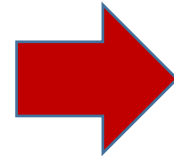


## RESULTS – FUNGAL DETECTION

- *Aspergillus* section *Fumigati* was the only one detected.
- *Aspergillus* section *Fumigati* was detected by qPCR in all the assessed filters, whereas it was identified only in 6 filters when assessed by culture based methods.



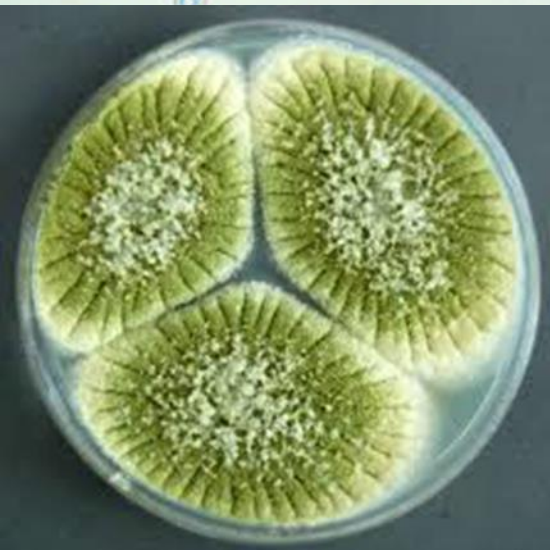
11 filters by qPCR



6 filters by culture based-methods

Complementary methods to assess fungal contamination

Viegas et al., 2012 - 2017



- No significant differences were observed between fungal load and filter usage time.

- **Although high fungal contamination was observed no mycotoxins were detected in the analyzed filters.**



Filter	Working hours
1	4237
2	4087
3	4329
4	4329
5	4237
6	3961
7	5938
8	856
9	794
10	22249
11	*



# DISCUSSION/MAIN FINDINGS

## Assessment strategy

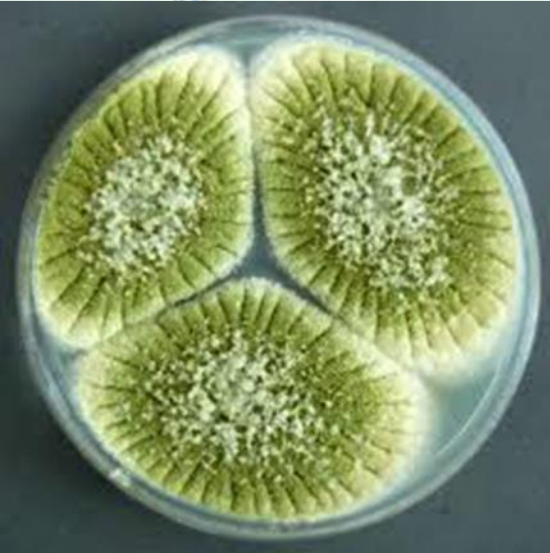
- The collection and analysis of filter samples provides a time-integrated sample of the bioburden ranging from days to months or even longer.

(Hoisington et al. 2014)

- **The sampling collection time can be considered the working hours from each assessed filter (usage time).**

- **Fungal burden filtered through the air conditioner filter can be carried into the cabinet and potentiate workers exposure to bioaerosols.**

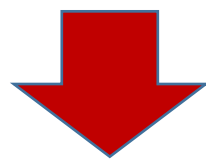
(Li et al., 2013)





# Fungal contamination

- All filters presented high fungal load
- Potential toxigenic fungal species were identified and detected
- *Aspergillus* was the dominant genera



## Co-exposure to fungi and mycotoxins

From a **occupational health perspective** mycotoxins occur in occupational environments **whenever toxigenic fungi are present**  
(Mayer et al., 2008)

*Journal of Toxicology and Environmental Health, Part A*, 77:57–68, 2014  
Copyright © Taylor & Francis Group, LLC  
ISSN: 1528-7394 print / 1087-2620 online  
DOI: 10.1080/15287394.2014.865583



*Ann. Occup. Hyg.*, 2014, 1–9  
doi:10.1093/annhyg/meu082



ASSESSMENT OF FUNGAL CONTAMINATION IN WASTE SORTING AND INCINERATION—CASE STUDY IN PORTUGAL

Carla Viegas<sup>1</sup>, Anita Q. Gomes<sup>1,2</sup>, João Abegão<sup>3</sup>, Raquel Sabino<sup>1,4</sup>, Tiago Graça<sup>1</sup>, Susana Viegas<sup>1,5</sup>

Assessment of Workers' Exposure to Aflatoxin B1 in a Portuguese Waste Industry

Susana Viegas<sup>1,2\*</sup>, Luisa Veiga<sup>3</sup>, Paula Figueiredo<sup>3</sup>, Ana Almeida<sup>3</sup>, Elisabete Carolino<sup>1</sup> and Carla Viegas<sup>1</sup>



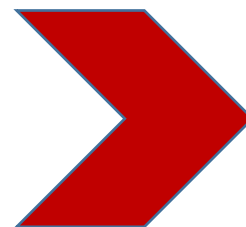


## None of the targeted mycotoxins were found in the filters

- Constant air movement in the filter that moves the particles of smaller diameter (carrying mycotoxins) into the vehicle cabinet not allowing mycotoxins retention in the filter.
- To retain and detect airborne mycotoxins it is required the use of filters with very small pore sizes ( $0.2\mu\text{m}$ ) and the forklifters filters had  $3.0\mu\text{m}$  or higher pores dimension (Level 2 of protection).

Johanning et al., 2002; Kildesø et al., 2000; Pasanen et al., 1993; Nielsen, 2003 Capacci and Rondelli, 2014

**Mycotoxins are not being retained in the filter and are probably being send to the forklift cabinet.**



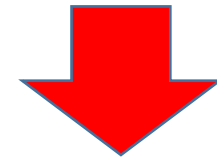
**Already reported occupational exposure to AFB1 in the forklifts drivers from the same unit**

(Viegas et al., 2014)

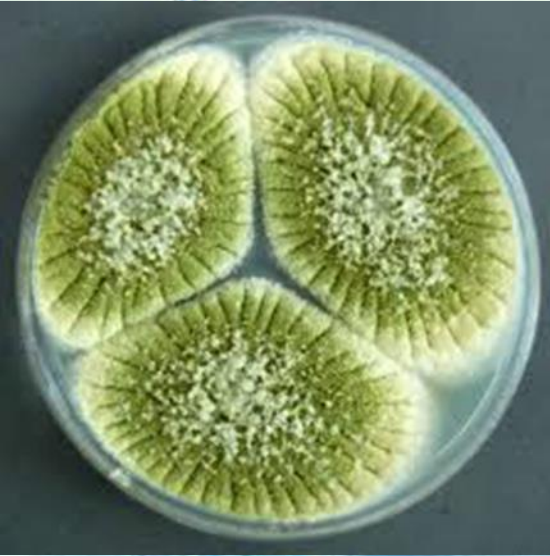


**Results showed that being inside a cabin in these occupational settings do not imply being protected from exposure to the fungal burden.**

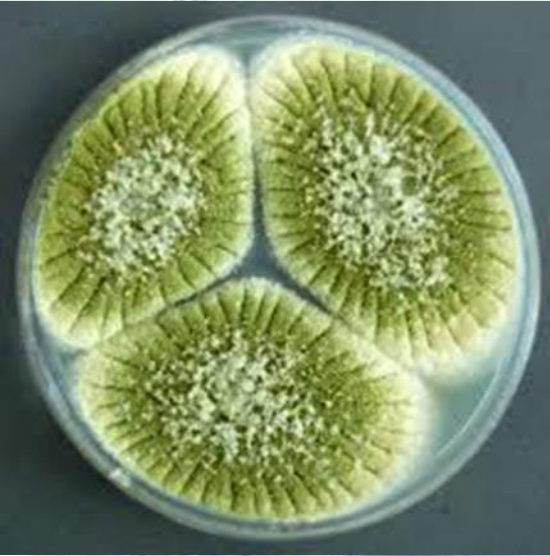
## **Ventilation filters may be a source of fungal contamination and mycotoxins**



- Different filters should be employed in air conditioning system (pore sizes  $< 0.2\mu\text{m}$ ) to guarantee workers protection to mycotoxins.
- Filters replacement should be more regular to avoid workers exposure to fungal burden due to fungi re-aerosolization to the forklift cabinet.
- Further studies should be developed to assess if the filter conditions allow the production of mycotoxins and to understand how mycotoxins are disseminated in the cabinet during the normal use of the vehicles.

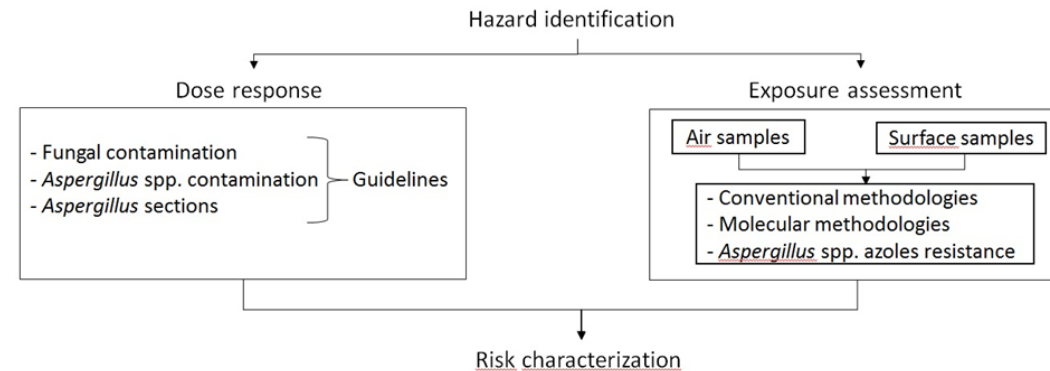


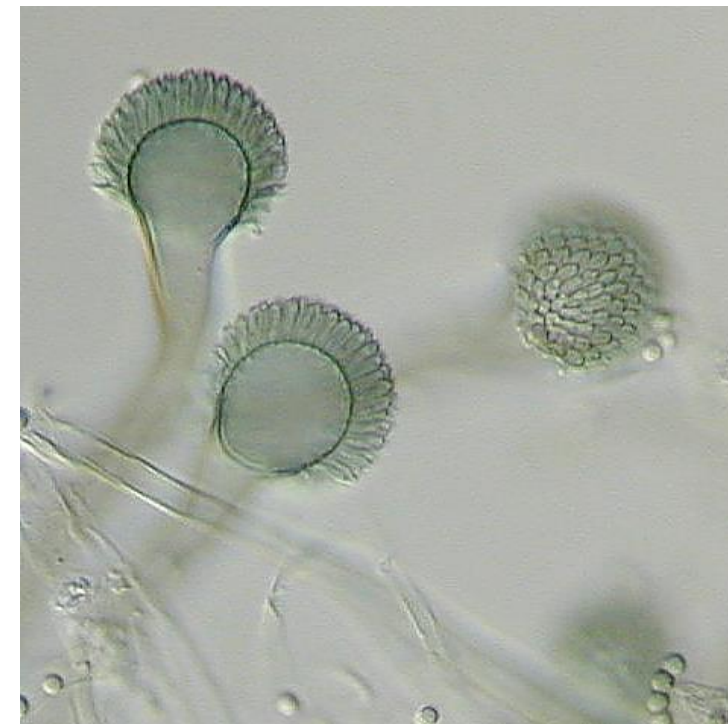
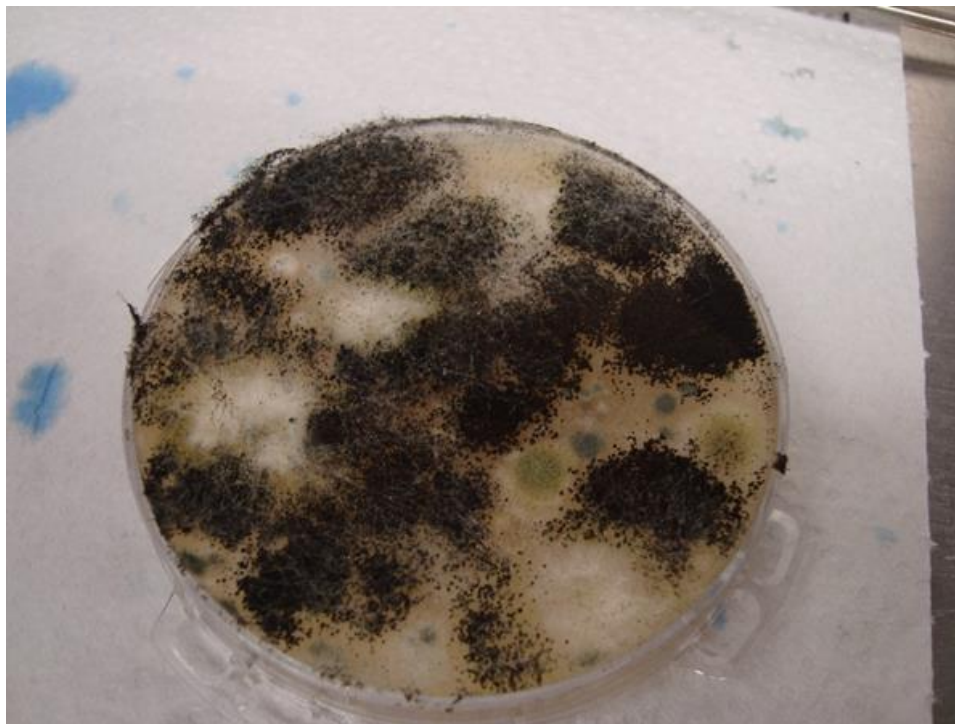
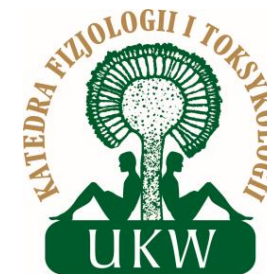




## Next steps...

- Next sequencing genome assay ➡ Greater diversity?
- Bacteria and endotoxins assessment ➡ co-exposure to multiple risk factors.
- *Aspergillus* clinical azoles resistance screening.
- Azoles fungicide resistance screening (propiconazole, tebuconazole, difenoconazole, epoxiconazole and bromuconazole) also to agriculture and animal production settings.





**Thank you for your attention!**

*The authors are grateful to Instituto Politécnico de Lisboa, Lisbon, Portugal for funding the Project "Waste Workers' Exposure to Bioburden in the Truck Cab during Waste Management - W2E Bioburden" (IPL/2016/W2E\_ESTeSL)*