



# Recent advances on BioPM sampling techniques and characterisation

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03/02/2021



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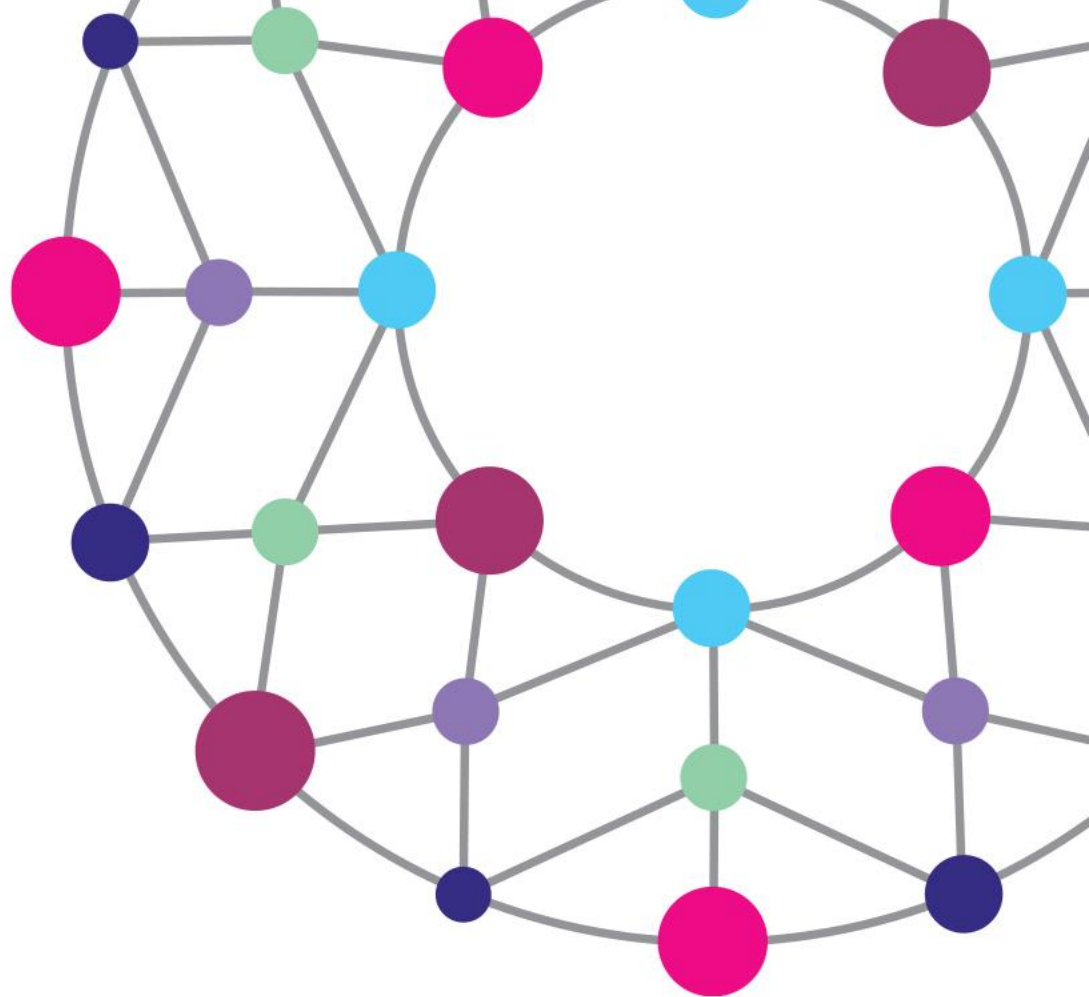
# Outline

1 - Background

2 - Sampling methods multi-approach

3 - Assays applied

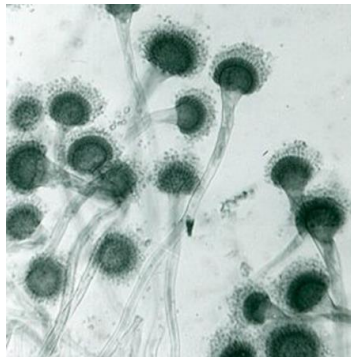
4 - Main findings



03/02/2021

# 1 - Background

## 1.1 – Challenging environment – waste sorting



- There is scientific evidence that associates occupational exposure to bioaerosols (bacteria and fungi) in the waste industry to health outcomes.

(Domingo and Nadal 2009; Viegas et al. 2019)

- Among fungi, *Aspergillus* is the most prevalent genera on waste-sorting, incineration and composting.

(Viegas et al. 2016, 2017)

## ■ Occupational exposure to mycotoxins

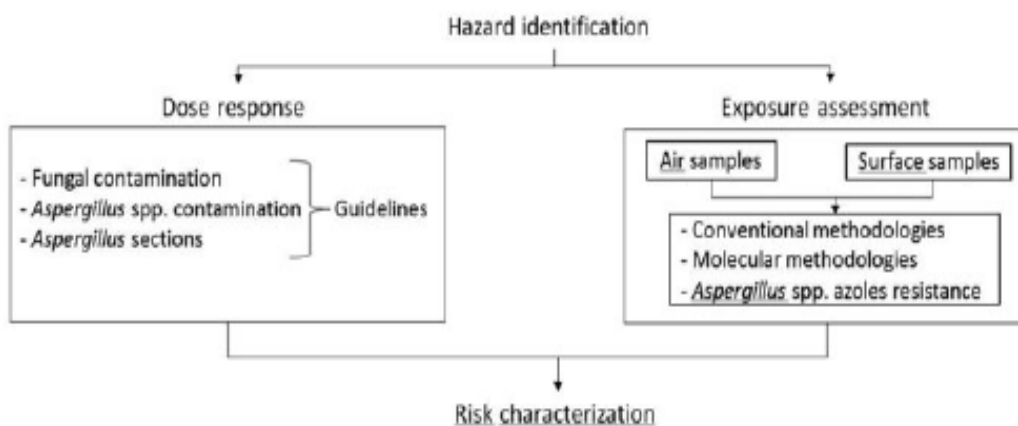
JOURNAL OF OCCUPATIONAL AND ENVIRONMENTAL HYGIENE  
2017, VOL. 14, NO. 10, 771-785  
<https://doi.org/10.1080/15459624.2017.1334901>



Check for updates

*Aspergillus* spp. prevalence in different Portuguese occupational environments:  
What is the real scenario in high load settings?

Carla Viegas<sup>a,b</sup>, Tiago Faria<sup>a</sup>, Liliana Aranha Caetano<sup>a,c</sup>, Elisabete Carolino<sup>a</sup>, Anita Quintal Gomes<sup>a,d</sup>,  
and Susana Viegas<sup>a,b</sup>



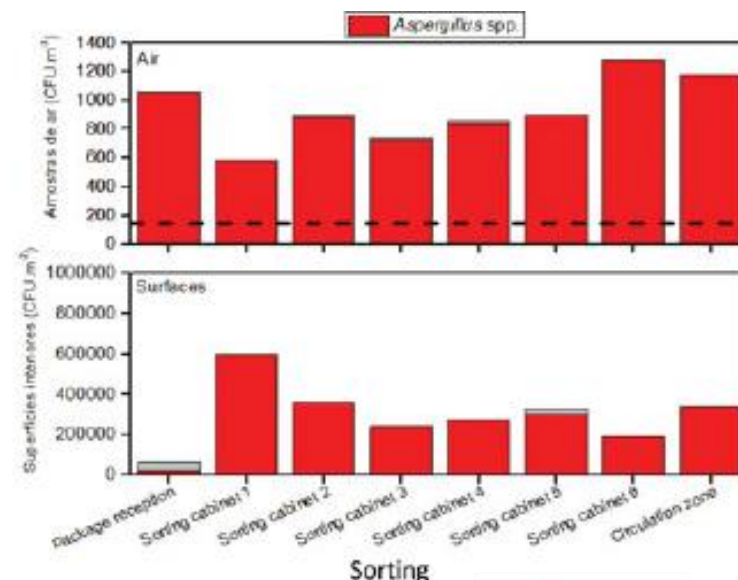
*Ann. Occup. Hyg.*, 2014, 1–9  
doi:10.1093/annhyg/meu082

**BOHS**  
The Chartered Society for  
Worker Health Protection



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

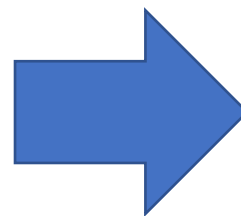
Susana Viegas<sup>1,2\*</sup>, Luisa Veiga<sup>3</sup>, Paula Figueiredo<sup>3</sup>, Ana Almeida<sup>3</sup>,  
Elisabete Carolino<sup>1</sup> and Carla Viegas<sup>1</sup>





- **Azole resistance**

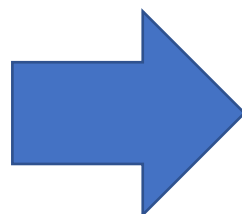
**Climate change**



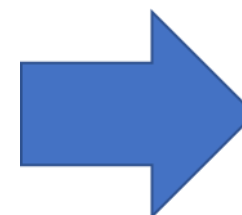
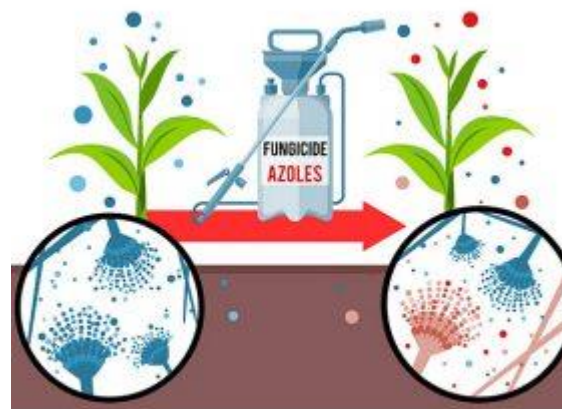
**Increase of fungi and mycotoxins contamination**



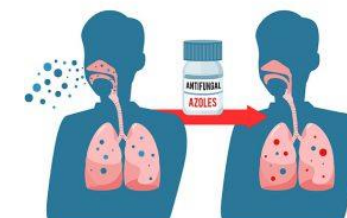
**Intensive use of azoles in crops to avoid toxigenic species**



**Resistant species can multiply and survive (*A. fumigatus*)**



**Infections are hard to treat and life-threatening.**



## 2 – Sampling methods multi-approach

### ■ Previous work – waste sorting

*Journal of Toxicology and Environmental Health, Part A*, 77:57–68, 2014

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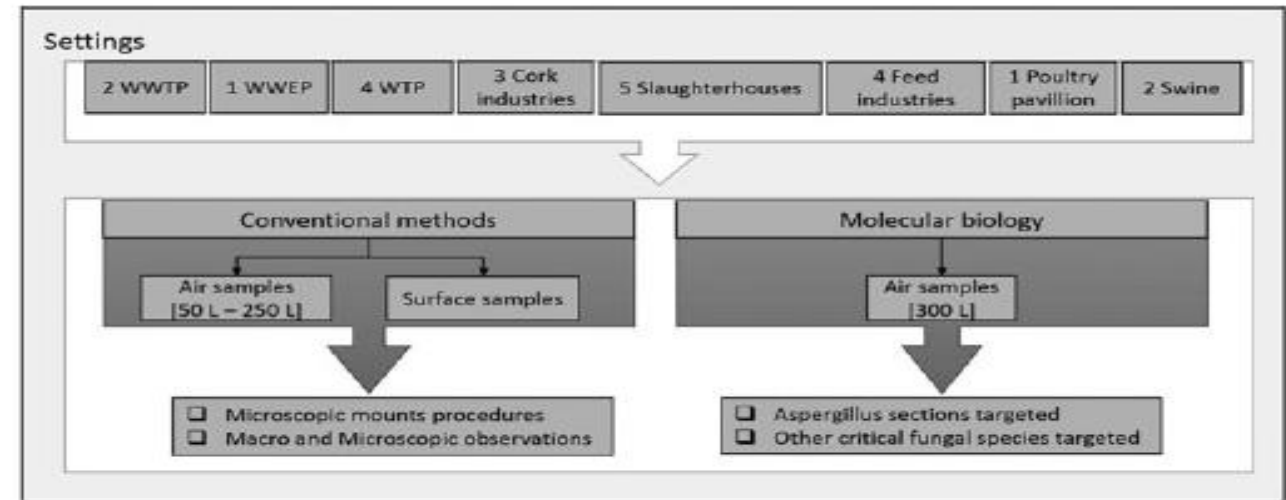
ISSN: 1528-7394 print / 1087-2620 online

DOI: 10.1080/15287394.2014.865583



#### 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>



WWTP – Waste Water Treatment Plants, WWEP – Waste Water Elevation, Plant, WTP – Waste Treatment Plants

# A new approach to assess occupational exposure to airborne fungal contamination and mycotoxins of forklift drivers in waste sorting facilities

Carla Viegas<sup>1,2</sup> • Tiago Faria<sup>1</sup> • Ana Cebola de Oliveira<sup>1</sup> • Liliana Aranha Caetano<sup>1,3</sup> •  
Elisabete Carolino<sup>1</sup> • Anita Quintal-Gomes<sup>1,4</sup> • Magdalena Twarużek<sup>5</sup> •  
Robert Kosicki<sup>5</sup> • Ewelina Soszczyńska<sup>5</sup> • Susana Viegas<sup>1,2</sup>

**Table 3** Fungal distribution on MEA and DG18 media

Fungal species	MEA (%; n)	DG18 (%; n)
<i>Aspergillus</i> section <i>Circumdati</i>	47.97; 780,500	41.02; 74,250
<i>Aspergillus</i> section <i>Nigri</i>	32.02; 521,000	17.27; 31,250
<i>Aspergillus</i> section <i>Versicolores</i>	15.86; 258,000	0.00; 0
<i>Aspergillus</i> section <i>Fumigati</i>	1.97; 32,000	0.00; 0
<i>Penicillium</i> sp.	1.32; 21,500	15.19; 27,500
<i>Aspergillus</i> section <i>Aspergilli</i>	0.68; 11,000	15.19; 27,500
<i>Aspergillus</i> section <i>Flavi</i>	0.12; 2000	0.28; 500
<i>Aspergillus</i> section <i>Candidi</i>	0.03; 500	4.97; 9000
<i>Chrysosporium</i> sp.	0.03; 500	0.00; 0
<i>Mucor</i> sp.	0.00; 0	5.80; 10,500
<i>Rhizopus</i> sp.	0.00; 0	0.28; 500







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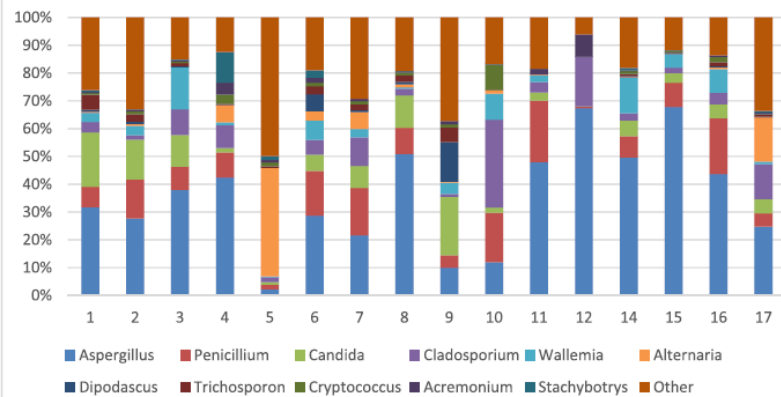


## The effects of waste sorting in environmental microbiome, THP-1 cell viability and inflammatory responses

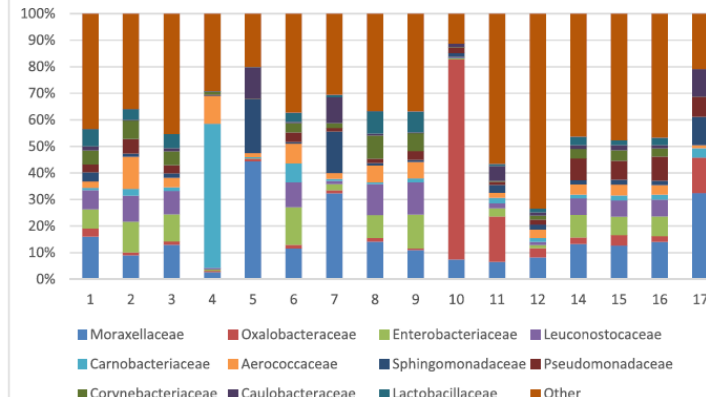
C. Viegas<sup>a,b,c,\*</sup>, L.A. Caetano<sup>a,d</sup>, J. Cox<sup>e</sup>, M. Korkalainen<sup>f</sup>, S.R. Haines<sup>g,h,i</sup>, K.C. Dannemiller<sup>g,h</sup>, S. Viegas<sup>a,b,c</sup>, T. Reponen<sup>e</sup>



Relative abundance of the most dominant fungal genera



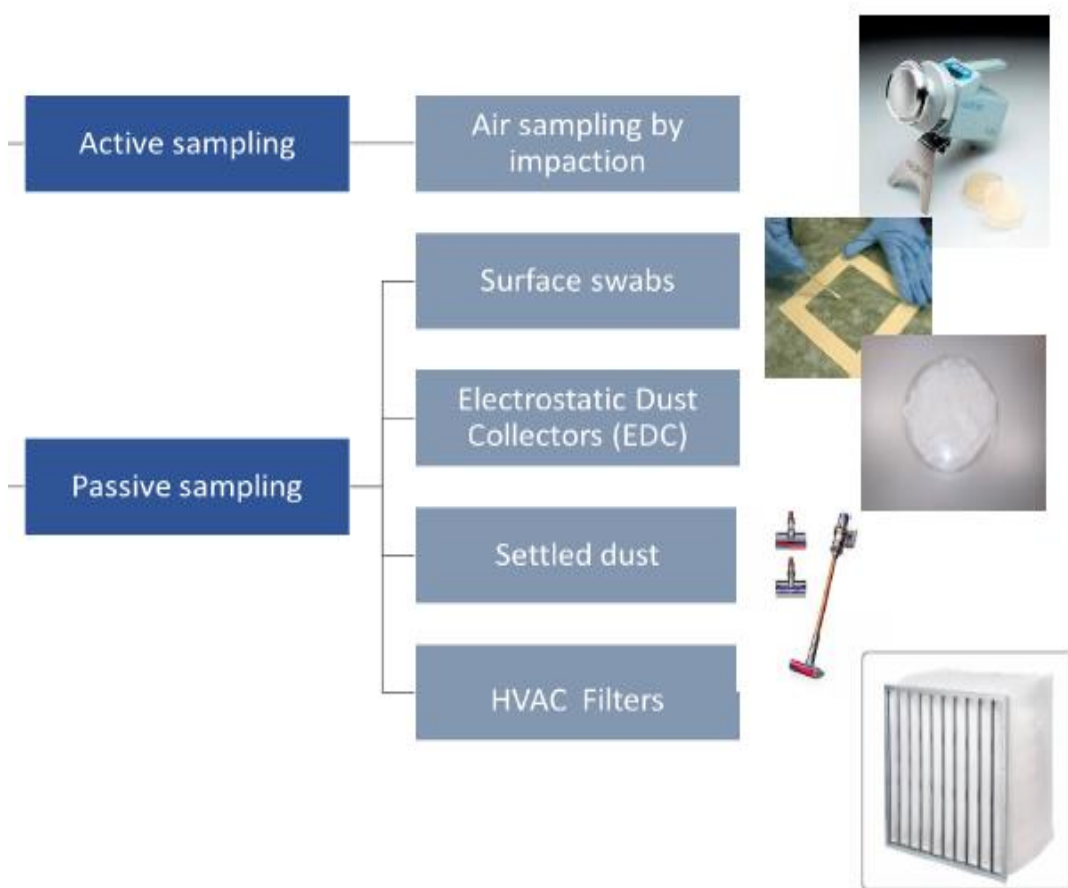
Relative abundance of the most dominant bacterial families



- This study highlights the need of thorough exposure assessment in waste-sorting industry.
- High-throughput sequencing can be used, in parallel with other molecular tools and culture based-methods, for a deeper understanding of the exposure data.
- The broad spectrum of microbial contamination detected in this study demonstrates that adequate monitoring of bioaerosol exposure is necessary to evaluate and minimize health risks.



## ■ Previous work – different occupational environments



Building and Environment 160 (2019) 106226



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Building and Environment

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Bioburden in health care centers: Is the compliance with Portuguese legislation enough to prevent and control infection?

Carla Viegas<sup>a,b,\*</sup>, Beatriz Almeida<sup>a</sup>, Ana Monteiro<sup>a,c</sup>, Liliana Aranha Caetano<sup>a,d</sup>, Elisabete Carolino<sup>a</sup>, Anita Quintal Gomes<sup>a,e</sup>, Magdalena Twarużek<sup>f</sup>, Robert Kosicki<sup>f</sup>, Geneviève Marchand<sup>g</sup>, Susana Viegas<sup>a,b</sup>



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Exposure assessment in one central hospital: A multi-approach protocol to achieve an accurate risk characterization

Carla Viegas<sup>a,b,c,\*</sup>, Beatriz Almeida<sup>a</sup>, Ana Monteiro<sup>a,d</sup>, Inês Paciência<sup>e,f,g</sup>, João Rufo<sup>e,f</sup>, Livia Aguiar<sup>h</sup>, Bruna Lage<sup>h</sup>, Lídia Maria Diogo Gonçalves<sup>l</sup>, Liliana Aranha Caetano<sup>a,i</sup>, Elisabete Carolino<sup>a</sup>, Anita Quintal Gomes<sup>a,j</sup>, Magdalena Twarużek<sup>k</sup>, Robert Kosicki<sup>k</sup>, Jan Grajewski<sup>k</sup>, João Paulo Teixeira<sup>f,h</sup>, Susana Viegas<sup>a,b,c</sup>, Cristiana Pereira<sup>f,h</sup>

Article

# Bioburden Assessment by Passive Methods on a Clinical Pathology Service in One Central Hospital from Lisbon: What Can it Tell Us Regarding Patients and Staff Exposure?

Carla Viegas <sup>1,2,3,\*</sup> , Magdalena Twarużek <sup>4,\*</sup> , Raquel Lourenço <sup>1</sup> , Marta Dias <sup>1</sup> , Beatriz Almeida <sup>1</sup> , Liliana Aranha Caetano <sup>1,5</sup> , Elisabete Carolino <sup>1</sup> , Anita Quintal Gomes <sup>1,6</sup>, Robert Kosicki <sup>4</sup> , Ewelina Soszczyńska <sup>4</sup> and Susana Viegas <sup>1,2,3</sup>



Swabs from ventilation grids

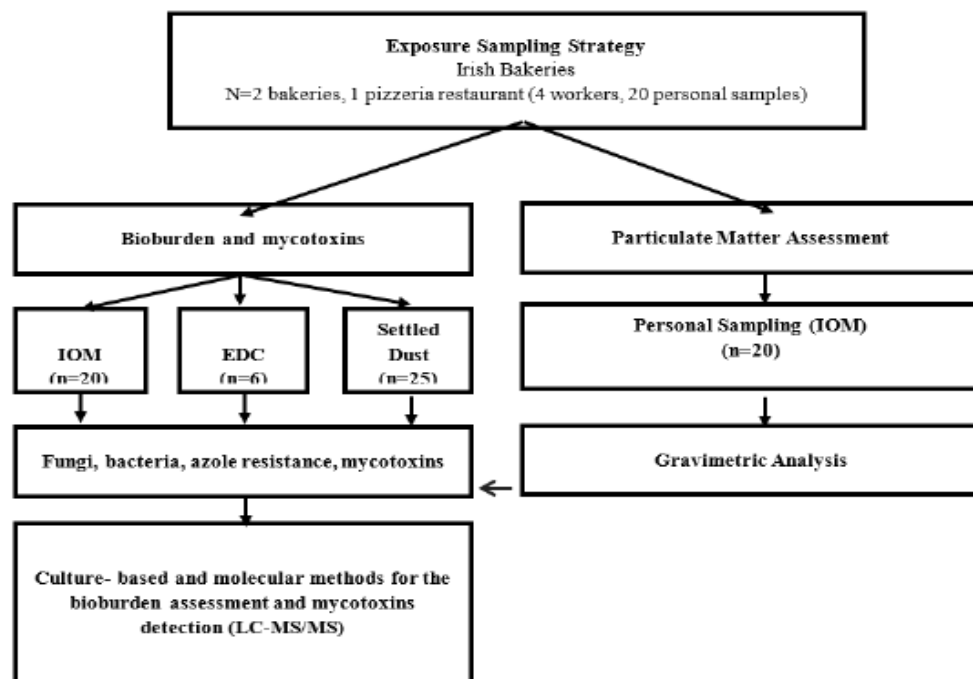


EDC






- The two sampling methods used in this study unveiled a more complete characterization of the bioburden permitting to obtain information regarding workers and patient's potential exposure.
- Culture based methods and molecular tools used in parallel should be the tendency to follow.
- Critical need of analyzing the azole resistance profile for fungal species that might represent some health risk.
- An intervention including the use of UVC-emitting device and an increased maintenance and cleaning of the HVAC was proposed, to promote the reduction of microbial contamination.

## Occupational Exposures to Organic Dust in Irish Bakeries and a Pizzeria Restaurant

Carla Viegas <sup>1,2,3,\*</sup>, Gerard T. A. Fleming <sup>4</sup>, Abdul Kadir <sup>5</sup>, Beatriz Almeida <sup>1</sup>, Liliana Aranha Caetano <sup>1,6</sup>, Anita Quintal Gomes <sup>1,7</sup>, Magdalena Twarużek <sup>8</sup>, Robert Kosicki <sup>8</sup>, Susana Viegas <sup>1,2,3</sup> and Ann Marie Coggins <sup>5,\*</sup>



## Characterization of Occupational Exposure To Fungal Burden in Portuguese Bakeries

Carla Viegas <sup>1,2</sup>, Tiago Faria <sup>3</sup>, Liliana Aranha Caetano <sup>1,4</sup>, Elisabete Carolino <sup>1</sup>, Anita Quintal-Gomes <sup>1,5</sup>, Magdalena Twarużek <sup>6</sup>, Robert Kosicki <sup>6</sup> and Susana Viegas <sup>2,1</sup>

- Toxigenic fungal species were observed and detected by qPCR and species with clinical relevance were observed on the azole resistance screening.
- The results also point to the possible exposure to mycotoxins, with flour the probable contamination source.
- Both studies showed the benefit of using a multi-approach regarding sampling methods and assays applied.

## ■ Protection devices as passive sampling methods

Waste Management 102 (2020) 856–867



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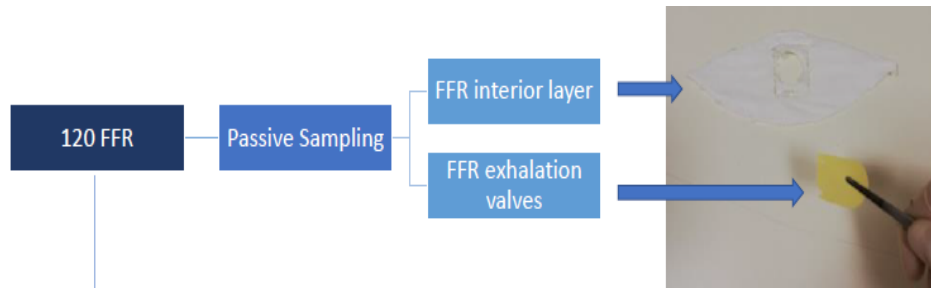
Waste Management

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Are workers from waste sorting industry really protected by wearing Filtering Respiratory Protective Devices? The gap between the myth and reality

Carla Viegas<sup>a,b,\*</sup>, Marta Dias<sup>a</sup>, Beatriz Almeida<sup>a</sup>, Liliana Aranha Caetano<sup>a,c</sup>, Elisabete Carolino<sup>a</sup>, Anita Quintal Gomes<sup>a,d</sup>, Magdalena Twarużek<sup>e</sup>, Robert Kosicki<sup>e</sup>, Jan Grajewski<sup>e</sup>, Geneviève Marchand<sup>f</sup>, Susana Viegas<sup>a,b</sup>



Workstations	Tasks	FFR number
FMW	Feeding machines with waste	33
SW	Sorting waste	54
MI	Machines inspection	12
MSVO	Machines and special vehicles operator	13
Not specified (without information)		8
Total		120



- Filtering Respiratory Protective Devices (FRPD) presented high levels of bioburden.
- Several *Aspergillus* sections exhibited reduced susceptibility to the tested azoles.
- Toxigenic fungal strains were detected on FRPD.
- **Workers with more waste contact showed increased bioburden on FRPD.**
- FRPD can be used as passive sampling methods to assess occupational exposure.



## ■ Protection devices as passive sampling methods

Environmental Research 189 (2020) 109881



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Environmental Research

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Assessment of the microbial contamination of mechanical protection gloves used on waste sorting industry: A contribution for the risk characterization

Carla Viegas<sup>a,b,c,\*</sup>, Magdalena Twarużek<sup>d,\*\*</sup>, Marta Dias<sup>a</sup>, Beatriz Almeida<sup>a</sup>,  
Elisabete Carolino<sup>a</sup>, Robert Kosicki<sup>d</sup>, Ewelina Soszczyńska<sup>d</sup>, Jan Grajewski<sup>d</sup>,  
Liliana Aranha Caetano<sup>a,e</sup>, Susana Viegas<sup>a,b,c</sup>



JOURNAL OF OCCUPATIONAL AND ENVIRONMENTAL HYGIENE  
<https://doi.org/10.1080/15459624.2020.1834113>



SHORT REPORT



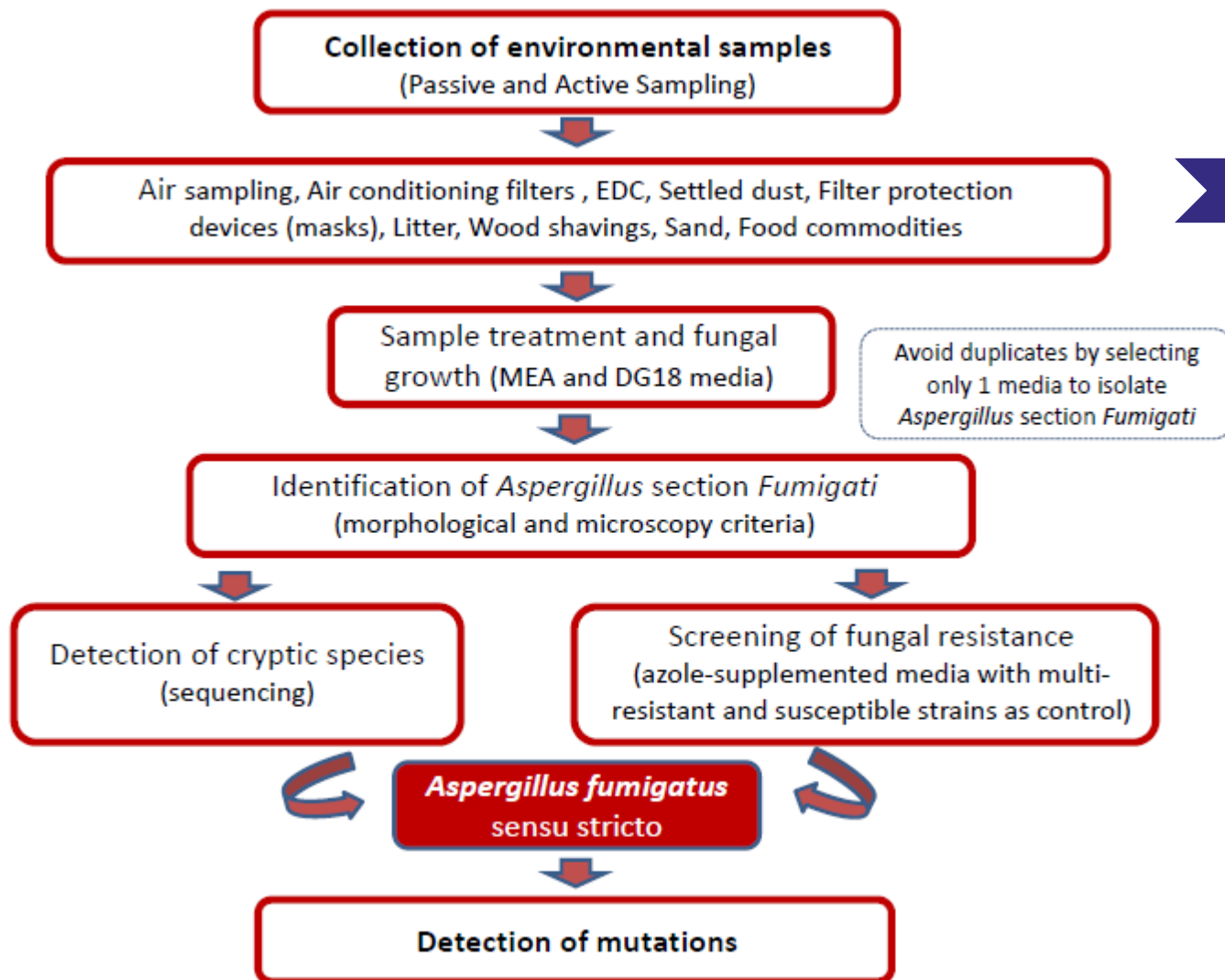
*Aspergillus* spp. presence on mechanical protection gloves from the waste sorting industry

Carla Viegas<sup>a,b,c</sup> , Marta Dias<sup>a</sup>, Beatriz Almeida<sup>a</sup> , Elisabete Carolino<sup>a</sup>, and Susana Viegas<sup>a,b,c</sup>



- Gram-negative bacteria were found in all the Mechanic Protection Gloves (MPG).
- *Aspergillus* sections with toxigenic and azole resistance potential were observed.
- Mycotoxins exposure through ingestion route by hand-mouth contact is possible.
- Cytotoxic potential affecting hepatic cells more than renal cells was observed.
- Workers can have health effects due to the contamination found on MPG.
- MPG can be an adequate passive sampling method to estimate workers exposure.

### 3 – Assays applied

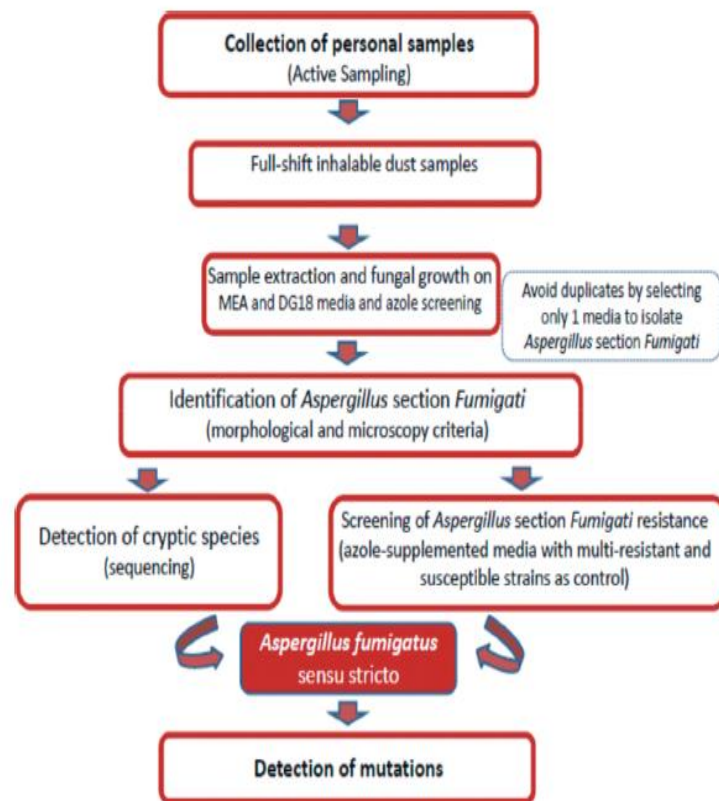


- CFU.m<sup>3</sup>/m<sup>2</sup>/g
- Detection of harmful fungal species
- Mycotoxins detection

- Azole resistance profile
- Mutations detection on *Aspergillus fumigatus* sensu stricto

## Algorithm to assess the presence of *Aspergillus fumigatus* resistant strains: The case of Norwegian sawmills

Carla Viegas<sup>a,b,c</sup>, Beatriz Almeida<sup>a</sup>, Liliana Aranha Caetano<sup>a,d</sup>, Anani Afanou<sup>e</sup>, Anne Straumfors<sup>e</sup>, Cristina Veríssimo<sup>b,f</sup>, Paulo Gonçalves<sup>f,g</sup> and Raquel Sabino<sup>f,h</sup>



### Article

## Azole-Resistant *Aspergillus fumigatus* Harboring the TR<sub>34</sub>/L98H Mutation: First Report in Portugal in Environmental Samples

Paulo Gonçalves<sup>1,2</sup>, Aryse Melo<sup>1,3</sup>, Marta Dias<sup>4</sup>, Beatriz Almeida<sup>4</sup>, Liliana Aranha Caetano<sup>4,5</sup>, Cristina Veríssimo<sup>1</sup>, Carla Viegas<sup>4,6,7</sup> and Raquel Sabino<sup>1,8,\*</sup>

Isolate Number	Source	Azole Screening Media			Minimal Inhibitory Concentration (mg/L)			<i>cyp51A</i> Mutations
		ICZ	VCZ	PCZ	ICZ	VCZ	PCZ	
VA299CP	Dairy air	+	+	+	4	4	2	TR <sub>34</sub> /L98H
VA610CP	Hospital air	±	–	–	2	0.5	0.5	No mutation detected
VA873CP	Waste sorting plant FRPD	+	+	+	4	2	1	TR <sub>34</sub> /L98H
VA978CP	Waste sorting plant FRPD	–	+	–	1	0.25	0.25	No mutation detected
V1207CP	Waste sorting plant FRPD	–	–	+	1	0.5	0.5	No mutation detected
VA1209CP	Waste sorting plant FRPD	+	–	+	8	4	1	TR <sub>34</sub> /L98H
VA1215CP	Waste sorting plant FRPD	–	+	+	1	0.25	0.125	N248K
VA1216CP	Waste sorting plant FRPD	–	+	–	1	0.25	0.25	N248K

ICZ: itraconazole; VCZ: voriconazole; PCZ: posaconazole; –: negative (no growth); ±: residual growth (growth of only one or few small colonies); +: relevant growth (growth similar to positive control).





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Review article

## Occupational exposure to *Aspergillus* section *Fumigati*: Tackling the knowledge gap in Portugal

Carla Viegas<sup>a,b,c,\*</sup>, Liliana Aranha Caetano<sup>a,d</sup>, Susana Viegas<sup>a,b,c</sup>



Recognize the possible presence of section *Fumigati* in the workplace (e.g. type of activity, processes in place, type of final product, previous reports)

Identify possible contamination sources  
  
Collect contextual information (e.g. task developed, raw materials used, ventilation, duration, hygiene procedures, azole pressure ...)  
  
Characterize exposure variability in each workplace

Perform exposure assessment through suitable sampling methods and assays

Analyse and interpret the obtained data

Define suitable risk management measures (RMM) to implement

Evaluate exposure again to verify if the RMM in place are effective to reduce exposure

- *Aspergillus* section *Fumigati* should be assessed by using active and passive sampling methods.
- Culture-based methods and molecular tools should be apply in parallel.
- Mycobiota azole resistance profile should be assessed.
- Mycotoxins should be considered when assessing workers occupational exposure.



## 4 – Main findings

### ■ What we should not do

#### The use of active sampling methods as stand-alone method

Passive sampling methods should be included in the assessment campaigns, and should be adjusted to the identified contamination sources, contextual information and variability of the exposure.



## The use of only one culture media.

The use of DG18 in addition to MEA allows for a broader characterization of contamination of the *Aspergillus* genus in the workplace, as it restricts the size of fungal colonies with higher growth rates as well as the identification of fungi with clinical relevance within the identified fungi.

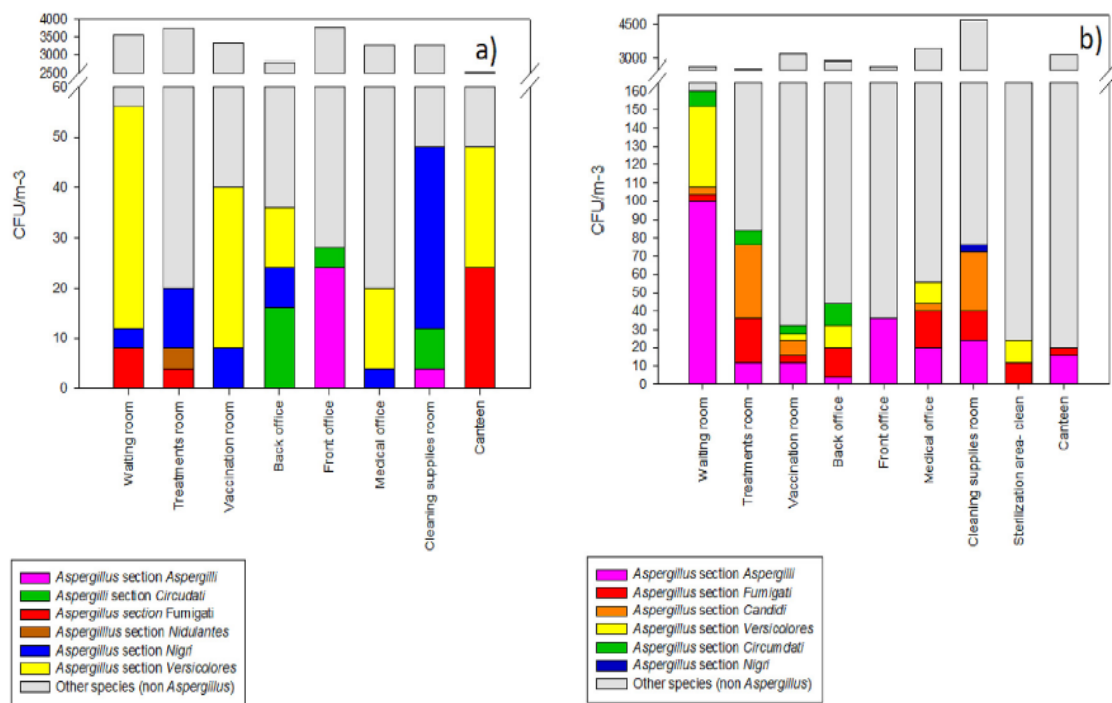


Fig. 2. *Aspergillus* sections distribution on air samples collected by impaction method in a) MEA and b) DG18.

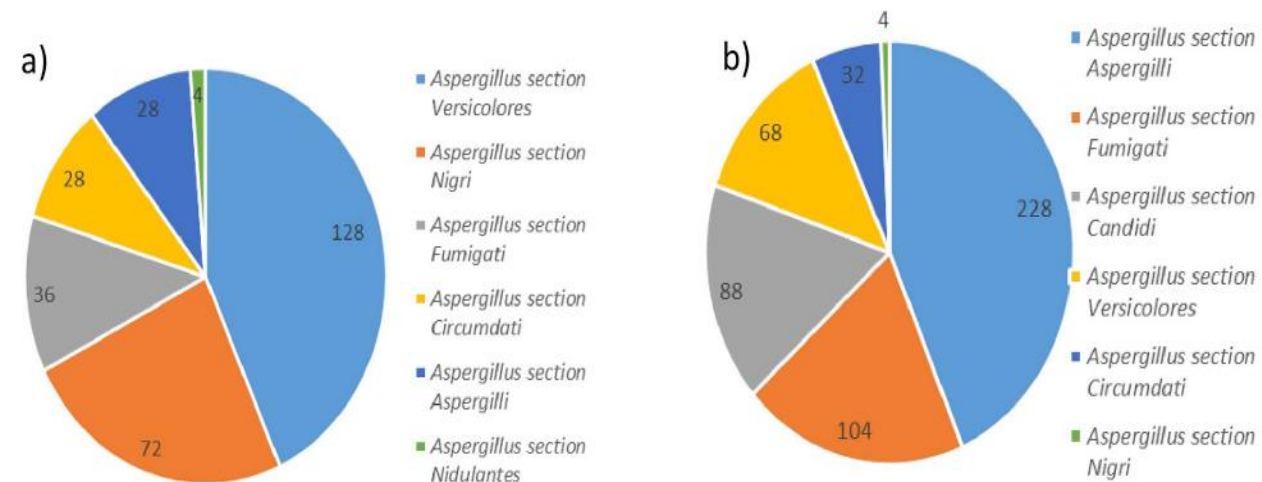


Fig. 3. *Aspergillus* sections and respective total load (CFU.m<sup>-3</sup>) found in air samples in a) MEA and b) DG18.

## The use of culture-based methods only to assess fungal contamination.

Culture-based methods and molecular tools (such as qPCR) should be used in parallel. The use of high-throughput sequencing, in parallel with other molecular tools and culture-based methods, can also be evaluated depending on specific information regarding the key aspects of the occupational exposure assessment.

Species Detected	Culture-based		Molecular	
	Number of samples	%	Number of samples	%
<i>Aspergillus</i> section <i>Circumdati</i>	9	13.43	22	32.84
<i>Aspergillus</i> section <i>Flavi</i>	22	32.84	➡ 6	8.96
<i>Aspergillus</i> section <i>Fumigati</i>	19	28.36	59	88.06
<i>Aspergillus</i> section <i>Nidulantes</i>	1	1.49	61	91.05

## Neglecting azole-resistance in the workplace.

The results obtained support the theory that azole pressure is present in the environment, while there is evidence that exposure of *Aspergillus* section *Fumigati* to azoles in the environment can cause cross-resistance to medical triazoles and hamper fungal therapy.

Isolate Number	Source	Azole Screening Media			Minimal Inhibitory Concentration (mg/L)			<i>cyp51A</i> Mutations
		ICZ	VCZ	PCZ	ICZ	VCZ	PCZ	
VA299CP	Dairy air	+	+	+	4	4	2	<u>TR<sub>34</sub>/L98H</u>
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VA873CP	Waste sorting plant FRPD	+	+	+	4	2	1	<u>TR<sub>34</sub>/L98H</u>
VA978CP	Waste sorting plant FRPD	–	+	–	1	0.25	0.25	No mutation detected
V1207CP	Waste sorting plant FRPD	–	–	+	1	0.5	0.5	No mutation detected
VA1209CP	Waste sorting plant FRPD	+	–	+	8	4	1	<u>TR<sub>34</sub>/L98H</u>
VA1215CP	Waste sorting plant FRPD	–	+	+	1	0.25	0.125	N248K
VA1216CP	Waste sorting plant FRPD	–	+	–	1	0.25	0.25	N248K

ICZ: itraconazole; VCZ: voriconazole; PCZ: posaconazole; –: negative (no growth); ±: residual growth (growth of only one or few small colonies); +: relevant growth (growth similar to positive control).



## Disregard occupational exposure to mycotoxins

Mycotoxins should be recognized as real occupational risk factors in certain specific occupational environments and in the presence of *Aspergillus* genera. It is therefore really important to accurately characterize mycotoxin exposure (which mycotoxins, at which concentrations, for which duration) and to understand which factors can influence that exposure.

Exposure to mixtures of different mycotoxins, as well as the simultaneously exposure to fungi, such as section *Fumigati*, should be considered in exposure assessments.



Article

### Occupational Exposures to Organic Dust in Irish Bakeries and a Pizzeria Restaurant

Carla Viegas <sup>1,2,3,\*</sup>, Gerard T. A. Fleming <sup>4</sup>, Abdul Kadir <sup>5</sup>, Beatriz Almeida <sup>1</sup>, Liliana Aranha Caetano <sup>1,6</sup>, Anita Quintal Gomes <sup>1,7</sup>, Magdalena Twarużek <sup>8</sup>, Robert Kosicki <sup>8</sup>, Susana Viegas <sup>1,2,3</sup> and Ann Marie Coggins <sup>5,\*</sup>

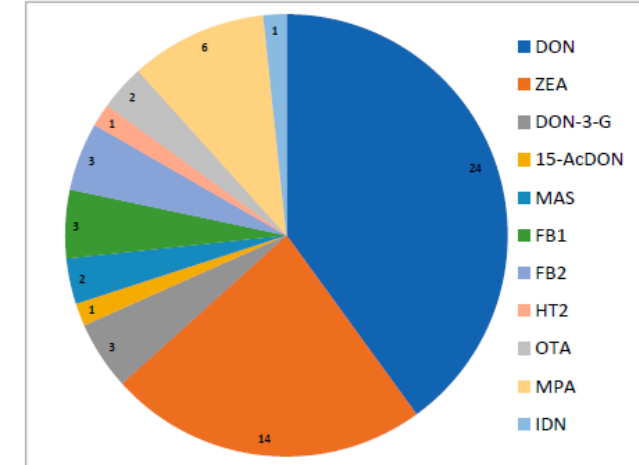
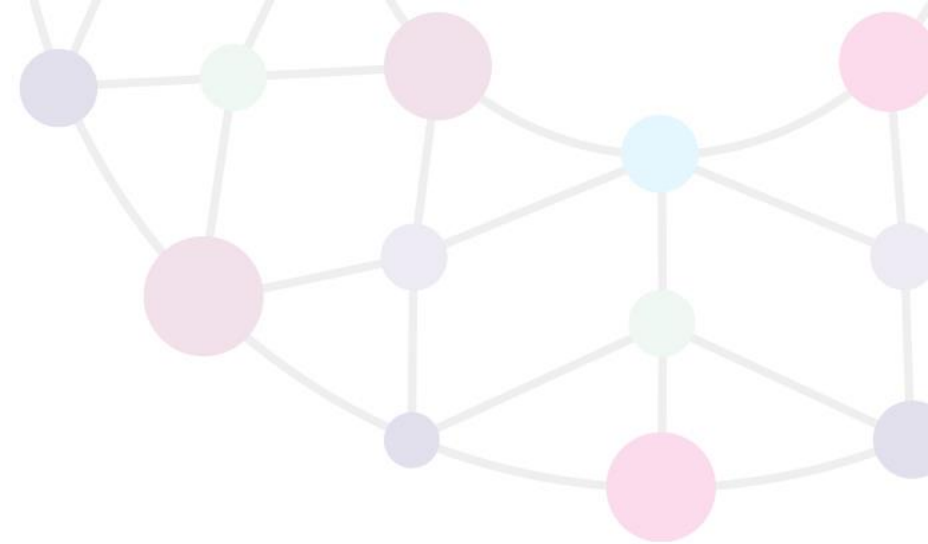


Figure 5. Reported frequency of mycotoxins. DON—deoxynivalenol; MAS—monoacetoxyscirpenol; OTA—ochratoxin A; MPA—mycophenolic acid; IDN—indomethacin.



**Thank you for your attention!!**

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