

# 1. Introduction

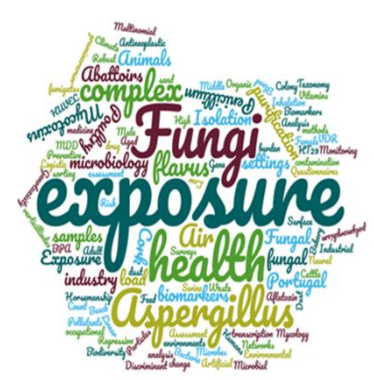
There are more than 3300 bakeries registered in Portugal.

This is a major industrial activity in Portugal and directly relates with the fact that Portuguese bread is a well-known product that is appreciated both nationally and internationally.

*(Guiné et al. 2016; Carbas et al. 2016)*

Although without exact numbers, this implies a considerable work force involving many workers in Portugal.



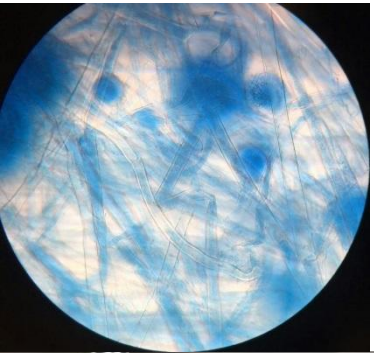


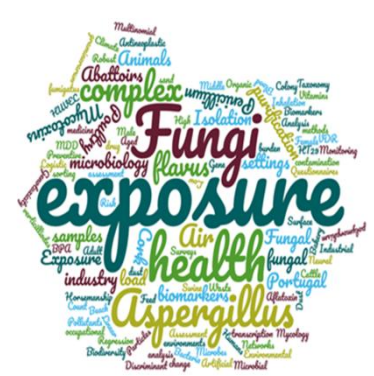
- Flour is a complex organic dust covering one or a mixture of different cereal grains that have been processed or grounded by milling.

*(Meo and AL-Dress 2005)*

- Flour may contain several contaminants, such as fungi and mycotoxins being the raw materials entering the facilities the principal contamination sources for this occupational environment.

*(Milanowski et al. 2002; Karpinski 2003; Viegas et al. 2016, 2018)*

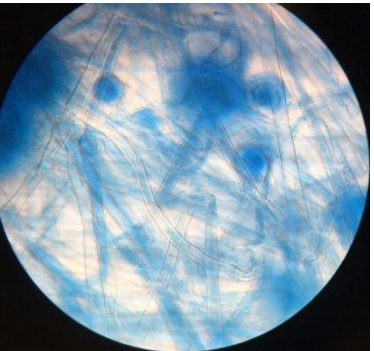
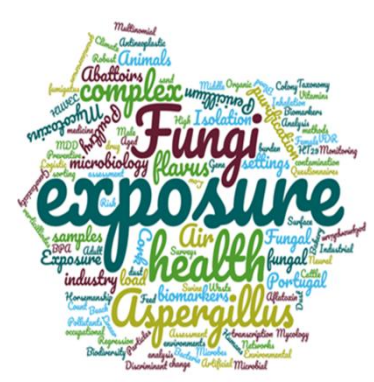




- Several studies report respiratory health effects in workers exposed both in small and large-scale industries.
- Respiratory system symptoms and diseases have frequently been reported to be induced by occupational dust being influenced by the type of dust, dose, duration of exposure and genetic factors.

*(Milanowski et al. 2002; Patouchas et al. 2009; Subbarao et al. 2009).*

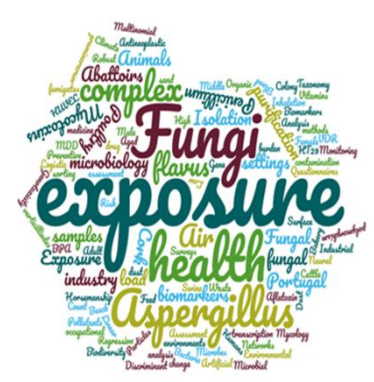




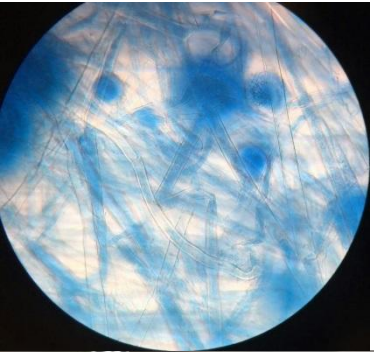
- Different sampling methods should be applied to ensure a more detailed occupational exposure assessment to fungal burden, since each method has unique advantages and disadvantages.
- A multi-approach in the sampling methods will enrich data findings, enabling a more accurate risk characterization.

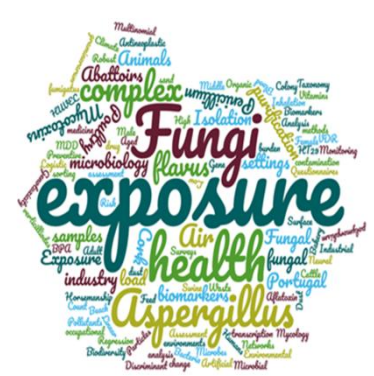
*(Reponen 2017; Viegas et al. 2018).*





**Assess the exposure to fungal burden (fungi and mycotoxins) on 13 Portuguese artisanal bakeries applying active and passive methods as sampling strategy.**





## 2. Materials and methods

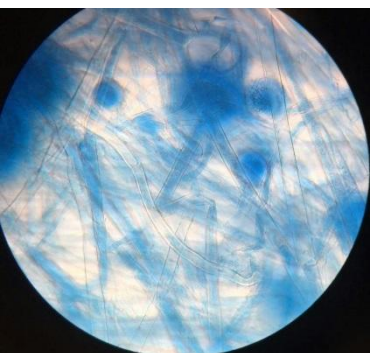
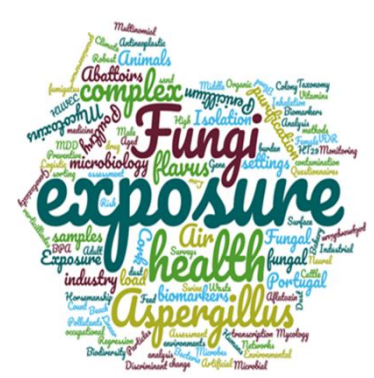
13 Portuguese bakeries located in the Lisbon district

Financial support from the Portuguese Authority for Working Conditions

### Three different areas:

- Production—where kneading machines and ovens were located and where dough shaping was performed;
- Raw material warehouse—where workers have to go several times to collect the raw materials for dough preparation;
- Store—where final product is sold (bread or pastry).





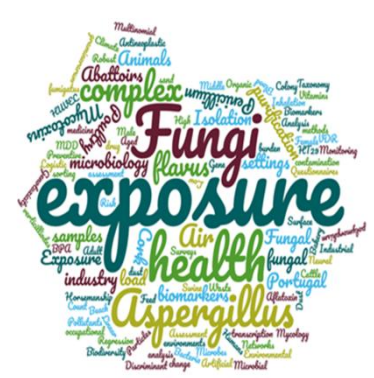
Bakery	Facilities	Sampling approaches (samples number)				
		Indoor air sampling impaction MEA and DG18	Indoors air sampling impinger	Surfaces swabs	Settled Dust #	EDC*
1 (22-11)	Enlarged company	3	3	3	-	2
2 (6-12)	Enlarged company	5	5	5	-	3
3 (10-01)	Enlarged company	4	4	4	1	2
4 (19-01)	Enlarged company	4	4	4	1	2
5 (24-01)	Enlarged company	5	5	5	1	3
6 (31-01)	Enlarged company	4 <sup>+</sup>	4	4	1	3
7 (8-2)	Enlarged company	5	5	5	1	3
8 (15-2)	Enlarged company	4	4	4	1	3
9(12-4)	Supermarket	4	4	4	1	3
10(26-4)	Supermarket	4	4	5	1	3
11(18-5)	Supermarket	4	4	4	1	3
12(23-5)	Supermarket	3	3	3	1	3
13 (7-6)	Supermarket	4	4	4	1	3
<b>Total</b>		<b>53</b>	<b>53</b>	<b>58</b>	<b>11</b>	<b>36</b>

- **8 bakeries** - enlarged company producing for selling in their own stores and also for different school canteens and vending machines.
- **5 bakeries** - supermarket facilities and belonged to the supermarket holder

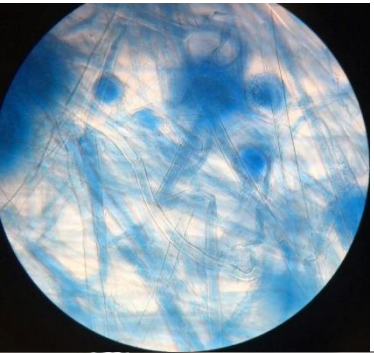






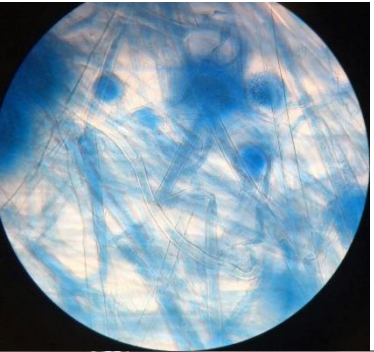
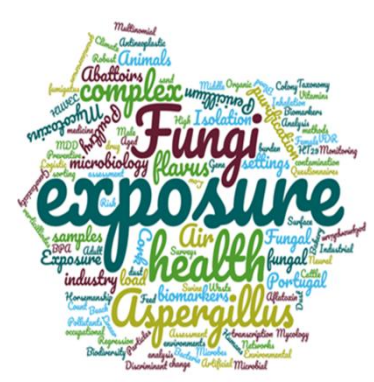


## Multi-approach analyses strategy

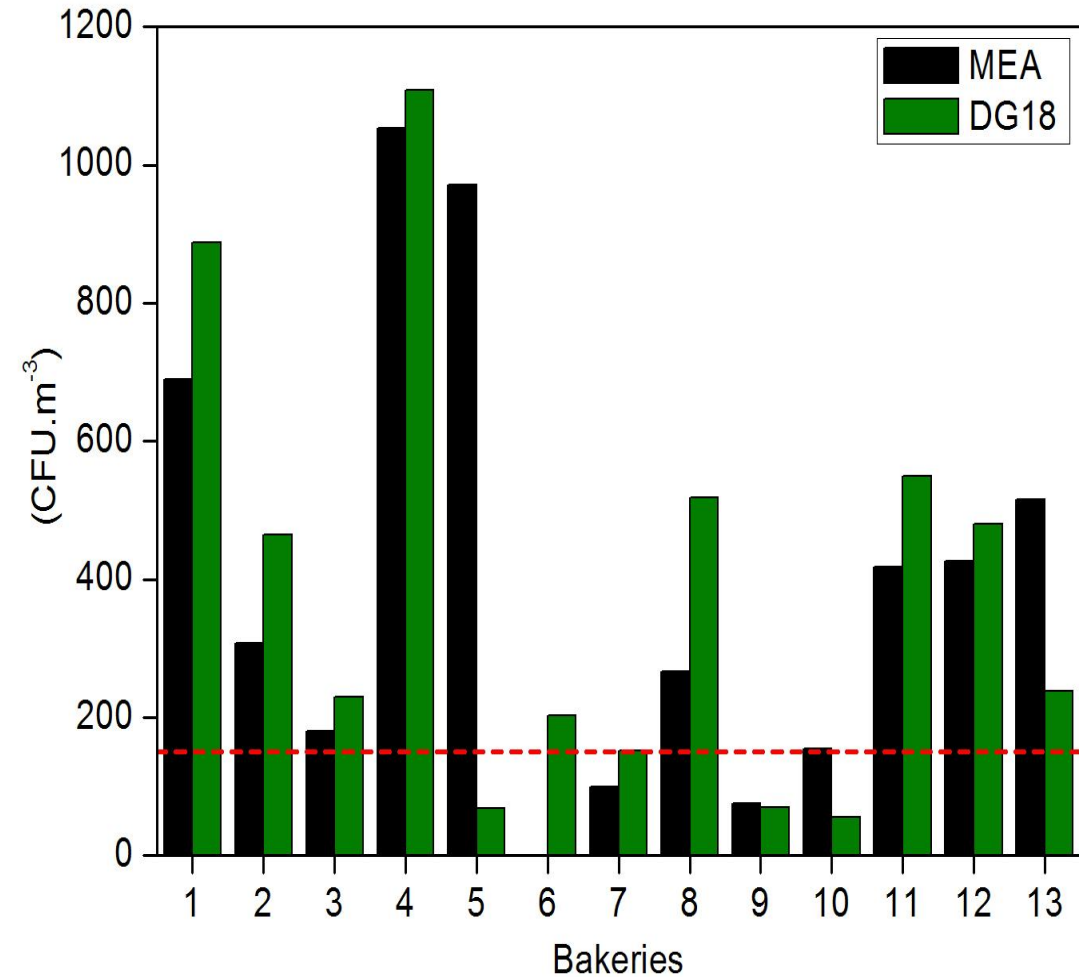


- Quantification and morphological identification by culture-based methods
- MEA and DG18
- Molecular detection of the toxigenic *Aspergillus* sections *Flavi*, *Fumigati*, *Circumdati* and *Versicolores*.
- 36 Mycotoxins in the air and settled dust samples were analyzed by LC-MS/MS system.

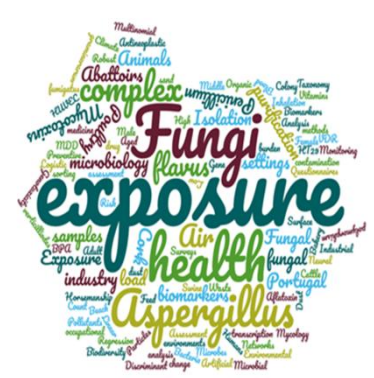




- Fungal load ranging from 0 to 2620 CFU.m<sup>-3</sup> on **DG18**
- **58.5%** with fungal load exceeding the WHO limit.
- **43.4%** presented higher indoor fungal load when compared to the outdoor sampling.



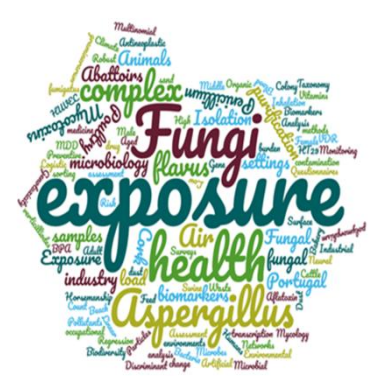




- *Cladosporium* sp. was the most prevalent species in indoor air samples in both media (29.7% MEA; 48.7% DG18), followed by *Penicillium* sp. (22.3% MEA; 30.5% DG18).
- *Aspergillus* spp. was observed on air samples on MEA and DG18 (0.3 and 1.2%, respectively).
- Among *Aspergillus* genera, section *Candidi* was the most prevalent (62.5%) on MEA followed by *Nigri* (25%), whereas sections *Candidi* and *Circumdati* (37.9%) were more prevalent on DG18.
- *Aspergillus* section *Fumigati* was possible to detect in 22.4% on air, 27.8% on surface swabs and in 7.4% on EDC samples; section *Versicolores* was detected in one air sample through molecular tools.
- **Increased *Aspergillus* species identification on DG18**







## 4. Main findings discussion

- **76.9% (10 out of 13) of the assessed bakeries surpassed the WHO guideline**

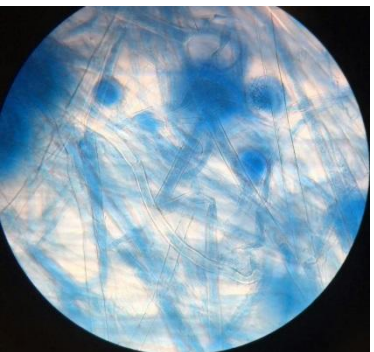
Additionally, fungal species with toxigenic potential were identified in all bakeries in the different environmental matrices collected, such as *Penicillium* sp., *Fusarium* sp. and *Aspergillus* sections.

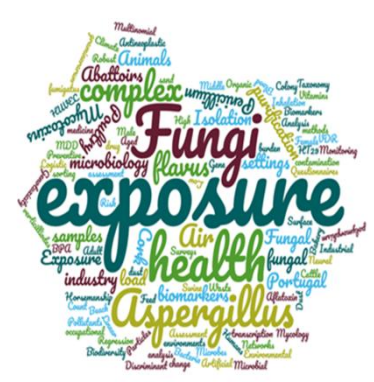
(Varga et al. 2015)

- **Mycotoxins detection only on settled dust**

Assessing mycotoxins through passive sampling methods, such as settled dust or other environmental samples that collect contamination instead of load, seems to be the trend for the mycotoxins contamination assessment in occupational environments .

(Kryszka-Traczyk et al. 2001; Nordby et al. 2004; Halstensen et al. 2006; Mo et al. 2014; Lai et al. 2014; Straumfors et al. 2014).

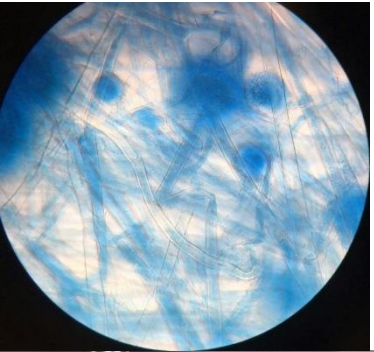


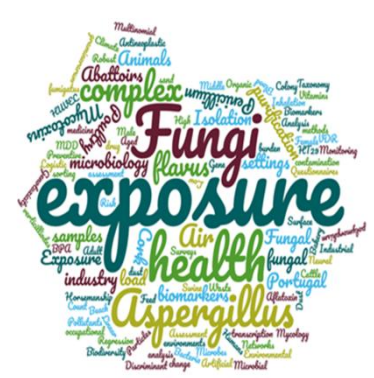


- It was not possible to observe or detect fungal growth in settled dust but several mycotoxins were present.

Mycotoxins can persist in an occupational environment even in fungi absence since they resist more to adverse abiotic factors, such as temperature and humidity and this is the reason why the absence of fungal growth cannot be a surrogate for the absence of mycotoxins' contamination.

*(Alborch et al. 2011; Halstensen 2008; Viegas et al. 2015; Mayer 2015; Viegas et al. 2016).*



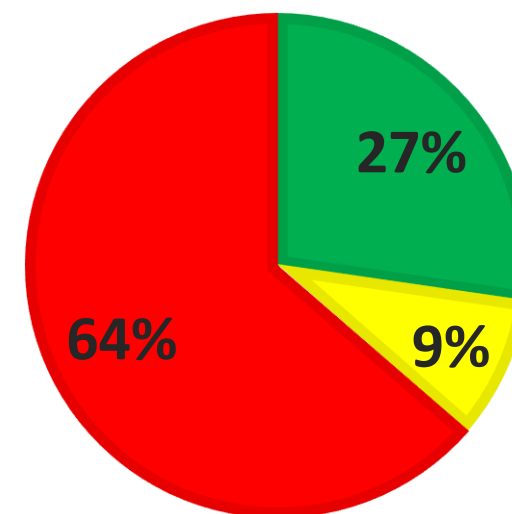


## ■ Raw materials can be contaminated with fungi and multiple mycotoxins

- In 5 bakeries from the 13 assessed was collected bread raw material (N= 39)
- In **4 bakeries** was verified fungal growth in different raw materials.
- In **22 samples (56.4%)** of raw material was observed quantifiable results of DON and was clearly the mycotoxin measured in higher amounts followed by OTA and FB1 and FB2.

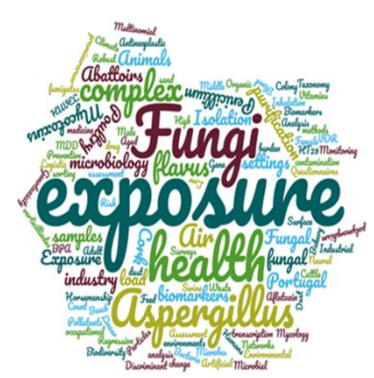
Fungal distribution from the raw material assessed

■ Aspergillus sp. ■ Mucorales sp. ■ Penicillium sp.



(Viegas et al. data not published)





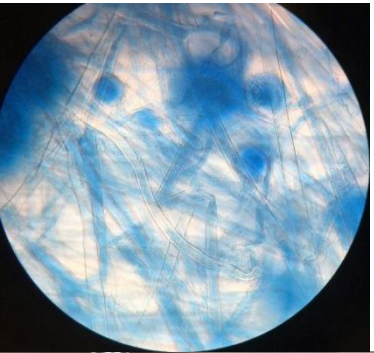
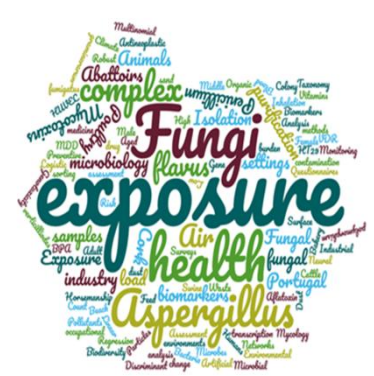
## ■ Particulate matter can boost exposure to mycotoxins

Mycotoxins are commonly present in airborne dust and in fungal spores or fragments of microbial growth and both can act as mycotoxin carriers to the workers respiratory system, since in this occupational setting exposure to organic dust is commonly observed enhancing exposure to mycotoxins by inhalation.

*(Croft et al. 1986; Flannigan 1987; Burstyn et al. 1997; Roberge et al. 2012; Viegas et al. 2017; Huttunen and Korkalainen, 2017).*







**Thank you for your attention**

The authors are grateful to Portuguese Authority for Working Conditions for funding the Project "*Occupational exposure assessment to particulate matter and fungi and health effects of workers from Portuguese Bakeries*" (005DBB/12) and also to Occupational Health Services from the industries.