

# Advances and practical lessons in fungal contamination exposure assessment

## Integrating field sampling and laboratory analyses

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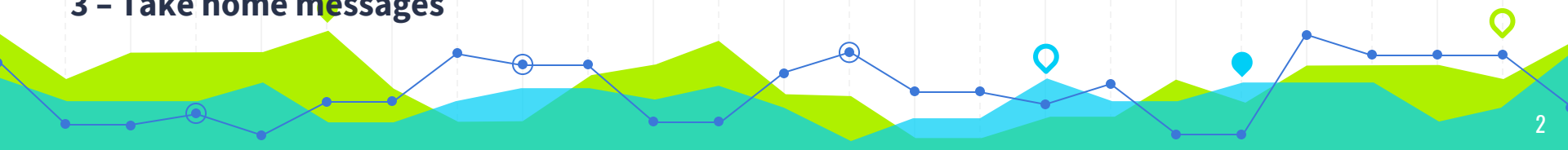
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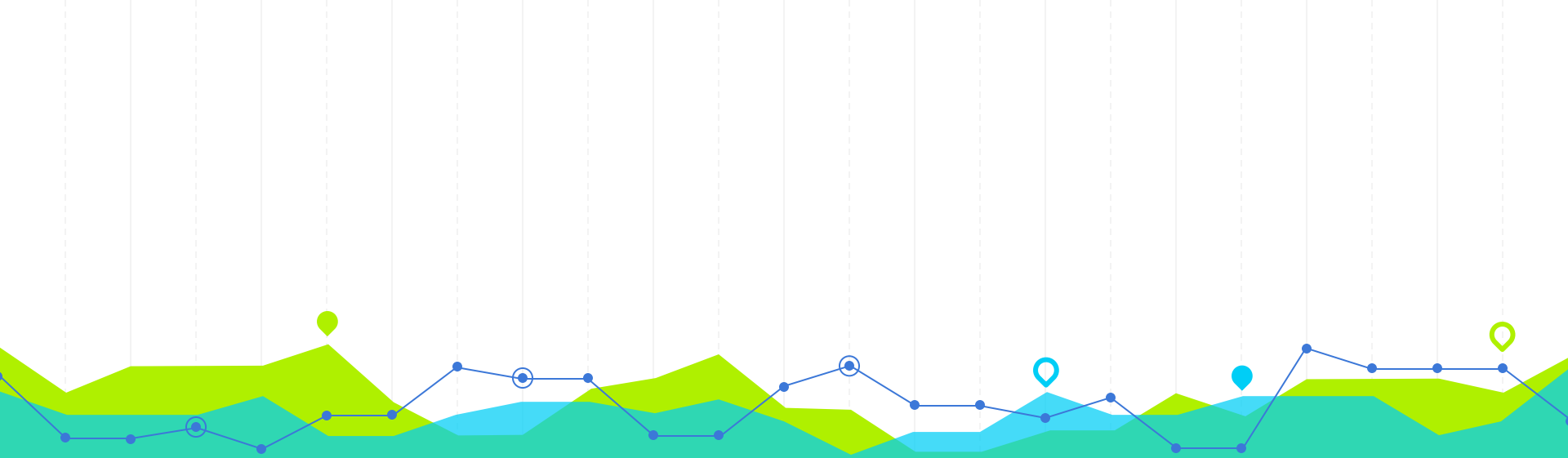
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# Background information

# 1

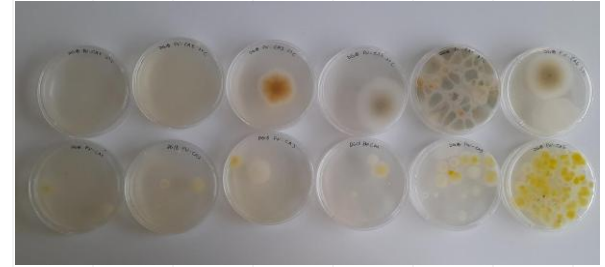
## 1.1 – Introduction

The presence of microorganisms, and more specifically fungal contamination, may result from (Oppliger, 2014):

- Natural colonization of organic material present at the site
- Intentional addition (e.g., in the food industry)



- **Each collected sample is unique:** its composition varies over time and space (in terms of species abundance and diversity) (Oppliger, 2014; Viegas et al., 2021a).
- Assessing exposure to microbiological agents (including fungi) is **a challenging task.**

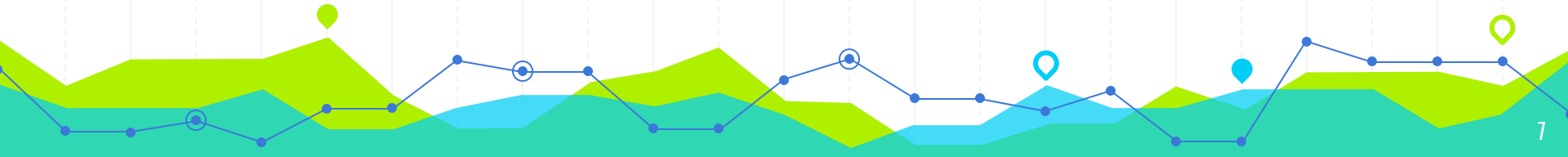


- Exposure can be estimated using a variety of sampling methods; however, **each is unique and requires a specific methodology** (Oppliger, 2014; Viegas et al., 2015a; Viegas, 2018).



## **Sampling is done to** (Viegas and Pinheiro, 2015; Reponen, 2017):

- To verify and quantify the presence of microorganisms in the air or contamination of materials
- To identify sources of contamination
- To monitor the effectiveness of implemented control measures
- To assess human exposure



## Sampling can provide useful information to:

- To characterize exposure conditions
- To determine whether the contamination represents a potential health risk
- To establish the need for control measures

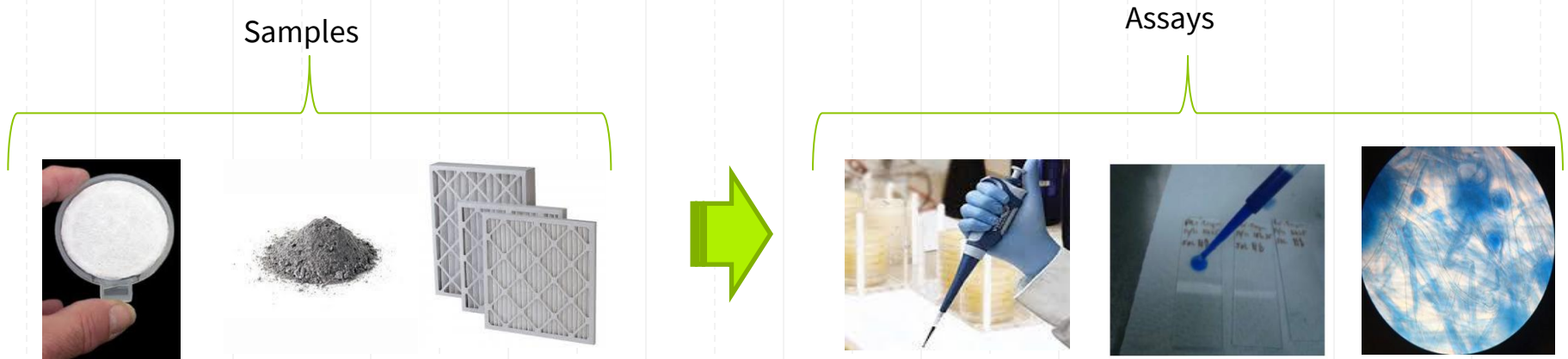
- **Sampling may not be necessary** if there is substantial evidence of **visible contamination** (Viegas e Pinheiro, 2015; Reponen, 2017).



- Exposure assessment of microorganisms can be carried out using **qualitative or quantitative methods**, or a combination of both.
- **Qualitative assessment includes visual and olfactory observations** resulting from damage in indoor environments, such as damage caused by water infiltration (Mendell e Kumagai, 2016).

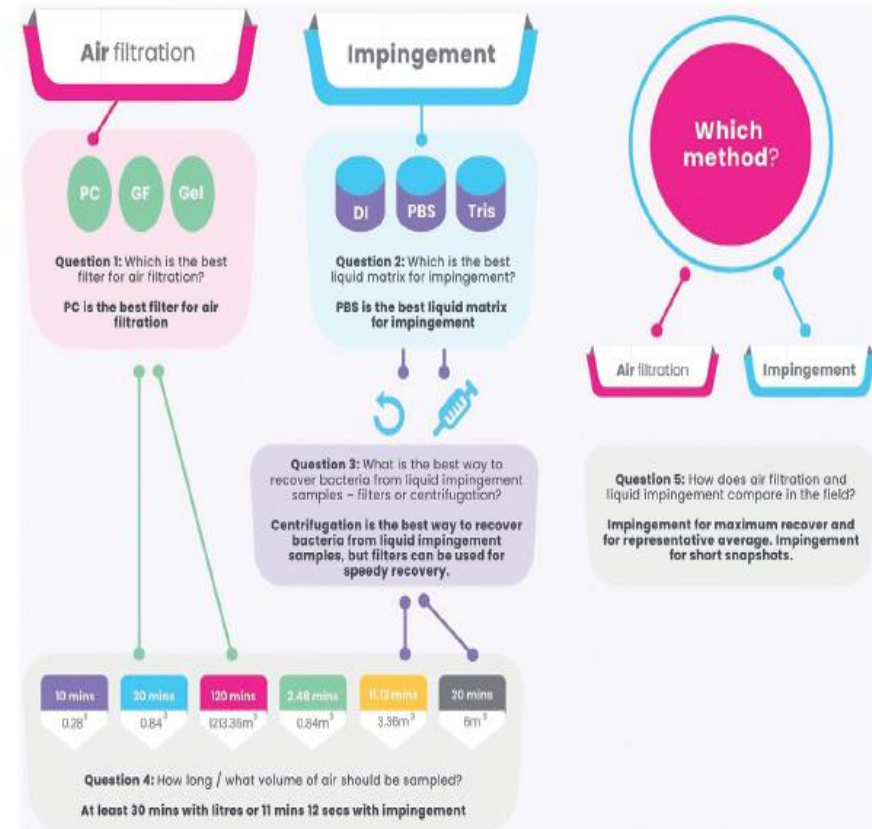


- Quantitative assessment requires two distinct steps: **sample collection** and **analysis**.



## 1.2 – Sampling strategy

- There is still a **lack of consensus** on the sampling methods to be used in a specific context or environment.
- Difficulty in comparing results and in establishing exposure limit values by regulators** (Ferguson et al. 2019; Whitby et al., 2022).



# What is the assessment goal?

What is the sampling method?

What is the kind of environment?

What is the agent to target?

Is there a need of results in real time?

What are the assays to be employed?

It is necessary to know the health effects?

What is the type of exposure?

What level of detail do we need?

Adopted from Whitby et al., 2022.

## BOX 1 Key questions to consider before air sampling

1. What are the research, regulatory or health impact questions to be addressed?
2. What are the objective and goals of monitoring (e.g. emission control efficacy, compliance monitoring, exploratory, exposure conditions)?
3. What information is needed (e.g. concentration, identification, worst-case concentration, particle size distribution)?
4. What is the relevance and relationship of average and worst-case concentrations?
5. Is personal or occupational exposure important?
6. What is the environment being sampled?—is it expected to have a high concentration of microorganisms or is this information unknown?
7. What is the anticipated nature and variability of the environment being sampled in time and space?
8. Are there logistical challenges to the sampling location (e.g. limited/no access to electricity supply)?
9. What human activity is occurring at the environment being sampled (e.g. office workers, hospital surgeries etc)?
10. What are the climatic conditions when the sample is being collected?
11. What are the optimal locations and time to collect samples (e.g. background, baseline, worst-case scenario, outdoors, indoors)?
12. Are microbiological taxa the target, or is it their specific components or products?
13. Are specific microbial groups or species (e.g. bacterial pathogens, viruses) important?
14. Are specific genes or biomarkers the target (e.g. pathogenicity genes, antimicrobial resistance genes) or are metagenomes needed?
15. What is the source of biological agent of interest?
16. Are viable microorganisms needed- for example for downstream culturing?
17. How will the collected air sample be analysed- for example culture-based or non-culture-based (molecular) methods?
18. What is the suitability, cost, availability, constraints, and requirements of sampling and downstream analysis methods?
19. What is the required sampling volume, the collection period (duration, frequency/interval) and the time pattern (e.g. continuous, periodic, random), including the levels of sensitivity for detection for the sampling/analysis method to be used?
20. Is real-time data important and/or high sample throughput required?

- Should sampling focus on **individual exposure or on the workplace/indoor environment?**
- **What are the research targets?** (Microorganisms, their metabolites, or specific genes related to pathogenicity or resistance)
- **Which analytical methods should be applied?** (Culture-based methods and/or molecular tools)
- **What constraints exist?** (Access to the site, costs, sampling methods, and analytical methods)
- **What sample volume/quantity is needed?** (To apply the analytical methods we intend to use)

(Whitby et al., 2022)



## Persistent challenges influence the selection of sampling methods:

- **Low microbial contamination** in some environments (clinical settings and viral contamination)
- The **sampling methods** to be applied are **dependent the analytical methods**
- **Legal framework** in place only considers results obtained from **culture based-methods** (CFU·m<sup>-3</sup>)



(Viegas et al. 2017; Viegas 2018; Whitby et al., 2022)

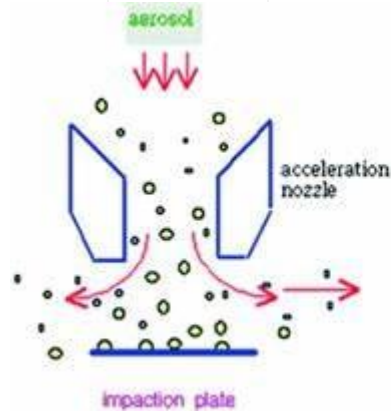
## 1.3 – Sampling methods

### Two different sampling methods: active and passive

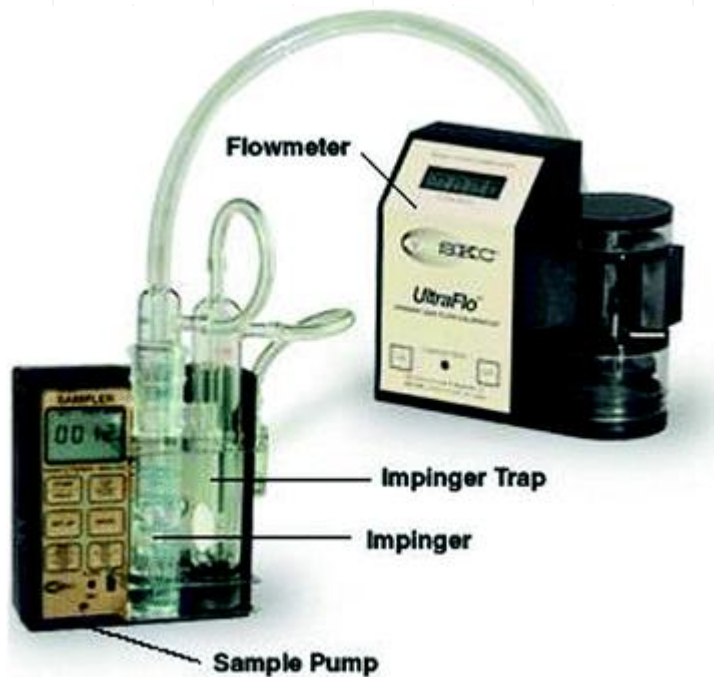
- **Active samplers** collect and draw a volume of air into a particle collection device
- The air inlet should be designed so as not to disturb the airflow (Delort e Amato 2018).
- The results are generally expressed in  $\text{CFU}\cdot\text{m}^{-3}$ .
- These methods allow the determination of the load of the targeted microorganisms (Mainelis 2020).

The general principles of **sampling using active methods** are the same as those used for other airborne particles (Viegas e Pinheiro, 2015; Reponen, 2017):

- impactation
- impingement
- filtration



Impactation mechanism and active sampling device (Andersen one stage)



Impinger device (SKC)



Filtration device (SKC Button Sampler)

# Features and constrains of active methods

## Features (Cox et al. 2020; Whitby et al., 2022)

- They can be used in combination with **classical microbiology and molecular tools**.
- They can collect **large volumes of air in a short time**.
- They can operate **for hours or days**.
- They are more suitable for **low-contamination environments (e.g., hospitals)**.

## Constrains

- The sampling is dependent on the **selectivity of each device (particle size)**
- **Expensive**



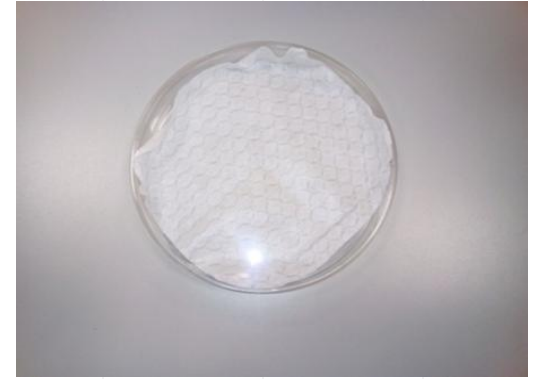
- **Passive methods** are the easiest to use, more cost-effective, and do not cause disturbances during sampling (Haig et al. 2016).
- They rely on the deposition of particles, due to gravity, onto a surface or medium (Haig et al. 2016).
- They provide quantifiable results (CFU/m<sup>2</sup>/h) (Haig et al. 2016).
- The deposition velocity of particles depends on their size and density (Hinds 1998).
- These methods allow the assessment of contamination by the targeted microorganisms (Mainelis 2020).
- Examples: Rodac plates, surface swabs and Electrostatic Dust cloths.



*Rodac plates*



Surfaces swabs



Electrostatic dust cloth

# Advantages and disadvantages of passive methods

## Features (Cox et al. 2020; Whitby et al., 2022)

- They can be used in combination with **classical microbiology and molecular tools**.
- They can be used over **extended sampling periods**.
- They **do not disturb** ongoing activities.
- They are **cost-effective**.
- They **do not require a power source**.

## Constrains




- They may cause **loss of microbial viability due to desiccation of the medium**.
- When settle plates **with a selective medium are used, the investigation is limited**.
- The particle **deposition rate is low (< 5 m<sup>3</sup>/day)**.

The sampling campaign is carried out through air sampling (**active methods**) and **passive methods**, as well as through the **collection of materials** that allow the direct assessment of indoor contamination sources (Viegas et al., 2018; 2019c; 2020a,b; 2022b).



Article

## A Novel Multi-Approach Protocol for the Characterization of Occupational Exposure to Organic Dust—Swine Production Case Study

Carla Viegas <sup>1,2,\*</sup> , Tiago Faria <sup>1,3</sup>, Ana Monteiro <sup>1</sup>, Liliana Aranha Caetano <sup>1,4</sup> ,  
Elisabete Carolino <sup>1</sup>, Anita Quintal Gomes <sup>1,5</sup> and Susana Viegas <sup>1,2</sup> 



Microbial contamination in firefighter Headquarters': A neglected occupational exposure scenario

Carla Viegas <sup>a,b,c,\*</sup>, Bianca Gomes <sup>a</sup>, Raquel Pimenta <sup>a</sup>, Marta Dias <sup>a,c</sup>, Renata Cervantes <sup>a</sup>, Liliana Aranha Caetano <sup>a,d</sup>, Elisabete Carolino <sup>a</sup>, Magdalena Twarużek <sup>e</sup>, Ewelina Soszczyńska <sup>e</sup>, Robert Kosicki <sup>e</sup>, Susana Viegas <sup>a,b,c</sup>



Article

# 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>

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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>



Waste Management 102 (2020) 856–867

**ELSEVIER**Contents lists available at [ScienceDirect](https://www.sciencedirect.com)**Waste Management**journal homepage: [www.elsevier.com/locate/wasman](https://www.elsevier.com/locate/wasman)

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>

- *Aspergillus* spp. case...

Table 2. Frequency distribution of the detected *Aspergillus* by sections, sampling method, and indoor environment.

<i>Aspergillus</i> Sections	Media		Sampling Approach	n (%)	Indoor Environment	n (%)
	MEA	DG18				
	n (%)	n (%)				
<i>Candidi</i>	36 (5.8%)	79 (14.7%)	EDC	167 (14.5%)	Bakeries	91 (7.9%)
<i>Circumdati</i>	40 (6.5%)	117 (21.8%)	Air impaction	325 (28.2%)	Dwellings	99 (17.3%)
<i>Nigri</i>	246 (39.9%)	49 (9.1%)	Settled dust	48 (4.2%)	Air	8 (0.7%)
<i>Fumigati</i>	163 (26.4%)	95 (17.7%)	HVAC filter	31 (2.7%)	Taxi	17 (1.5%)
<i>Versicolores</i>	55 (8.9%)	62 (11.6%)	Surface swab	42 (3.6%)	College indoors	17 (1.5%)
<i>Aspergilli</i>	22 (3.6%)	80 (14.9%)	Air impingement	6 (0.5%)	Swine	26 (2.3%)
<i>Terrei</i>	2 (0.2%)	0 (0.0%)	Filter air sampler	90 (7.8%)	Dairies	29 (2.5%)
<i>Flavi</i>	35 (5.7)	48 (9.0%)	MPG	125 (10.8%)	Veterinary clinics	6 (0.5%)
<i>Nidulantes</i>	5 (0.8%)	1 (0.2%)	FRPD	280 (24.3%)	Elementary school	2 (0.2%)
<i>Clavati</i>	3 (0.5%)	2 (0.4%)	Ventilation grid—swab	39 (3.4%)	Healthcare facility	31 (20.0%)
<i>Usti</i>	2 (0.3%)	2 (0.4%)			Elderly care center	11 (1.0%)
<i>Cremeri</i>	1 (0.2%)	0 (0.0%)			Waste sorting	83 (41.9%)
<i>Restricti</i>	7 (1.1%)	1 (0.2%)			Thermal baths	17 (1.5%)
					Firefighters' ambulances	16 (1.4%)
Total	617	536	Total	1153	Total	1153

MEA, malt extract agar; DG18, dichloran-glycerol agar.

## An association was observed between the *Fumigati* section and:

- **Certain workplaces** (waste sorting stations, veterinary clinics, and dairy farms)
- **Respiratory protective masks (FRPD)** as a sampling method

- **MEA**



Article

**Culture Media and Sampling Collection Method for *Aspergillus* spp. Assessment: Tackling the Gap between Recommendations and the Scientific Evidence**

Carla Viegas <sup>1,2,3,4</sup>, Marta Dias <sup>1</sup>, Elisabete Carolino <sup>1</sup> and Raquel Sabino <sup>4,5</sup>



- Results obtained from active and passive methods **represent different types of microbial exposure, and it may be advantageous to use a combination of both** (Viegas et al., 2021b).
- **Sampling strategy needs to consider** (Reponen, 2017; Whitby et al., 2022):
  - The key decision-making aspects and the 20 key questions
  - The microorganisms to be investigated
  - The variables that may contribute to their proliferation

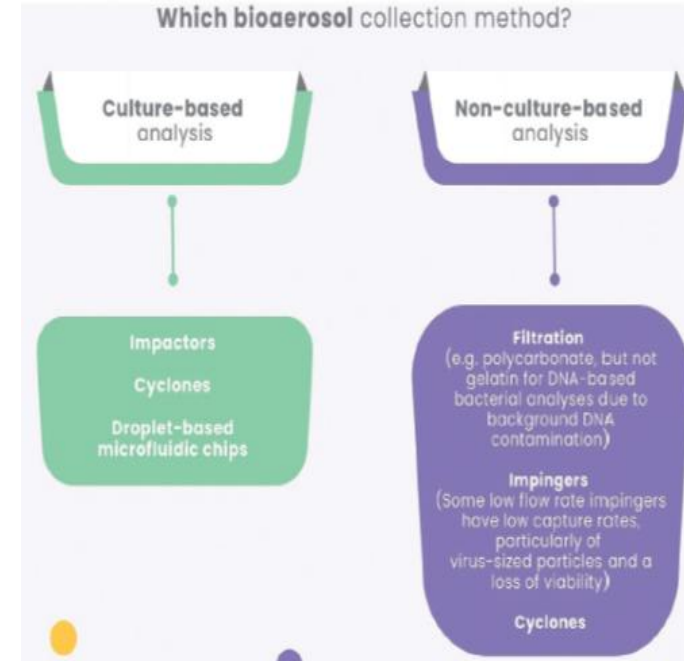


**Sampling strategy to applied**

# 1.4 – From sampling to analysis: Key considerations

## Assays to employ

- **Active methods** (Impaction, Filtration and Impingement) **can be applied for culture-based and molecular methods** (Ferguson et al., 2019).
- **Impingers and impaction devices** are commonly used with **culture based-methods** (Lindsey et al., 2017).
- Filters have been widely used for both types of analysis, **but the type of filter may favour either culture-based methods or molecular analyses** (Whitby et al., 2022).



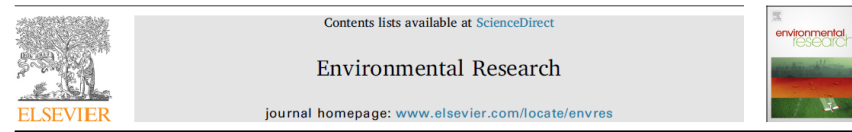
Whitby et al., 2022.

## Representativeness of the sample (Ferguson et al., 2019)

- Sampling duration and air volume depend on the **type of study or assessment**.
- Microbial counts obtained **using an impinger are not real**.
- Sampling 300 L over 10 minutes is not = to 300 L over 2 hours (**different microbial communities**).
- **Less abundant microorganisms may not be detected** in high air-volume samples
- **Molecular tools:** Filters (3.36 m<sup>3</sup> of air); impingers (6 m<sup>3</sup> of air).

## Other influencing factors

- Microorganism concentrations **vary over time and space** (Lindsley et al., 2010)
- Contamination **sources are seasonal** (Emerson et al. 2017)
- For long-term exposure assessment, **ventilation and air conditioning system filters can be used** (Hurley et al., 2019; Viegas et al., 2018)
- Impingers are suitable for snapshot sampling ( $100 \text{ L}\cdot\text{min}^{-1}$ ), **but they do not allow assessment of potential effects related to exposure duration** (Ferguson et al. 2019; Cox et al. 2020)



Filters from taxis air conditioning system: A tool to characterize driver's occupational exposure to bioburden?

Carla Viegas<sup>a,b,\*,</sup>, Ana Monteiro<sup>a</sup>, Mateus dos Santos<sup>a</sup>, Tiago Faria<sup>a,c</sup>, Liliana Aranha Caetano<sup>a,d</sup>, Elisabete Carolino<sup>a</sup>, Anita Quintal Gomes<sup>a,e</sup>, Geneviève Marchand<sup>f</sup>, Nancy Lacombe<sup>f</sup>, Susana Viegas<sup>a,b</sup>



## 1.5 – Protocols to assess exposure

**For each study or assessment, there is an appropriate protocol, which depends on**

(Whitby et al., 2022):

- the environment (indoor or outdoor)
- the relevant legal frameworks (e.g. hospital settings or poultry farms)
- the questions we aim to answer (quantification, characterization through identification, or detection of pathogens)

## In conclusion (Whitby et al., 2022):

- The sample should be representative of the environment and of what is intended to be investigated.
- It should also ensure sample suitability for the respective analysis.



## Combine different appropriate sampling and assays methods

- to the questions we aim to answer
  - to each type of environment

## Contextual information

- Ventilation conditions
- Emission sources
- Occupancy and activities occurring
- Temperature and relative humidity
- Carbon dioxide levels
- Occupants health surveillance data

## Aims

- Fast screening in case of an outbreak
- Risk assessment
- Testing the efficacy of any exposure control measures
- Scientific studies

## Sampling campaign

- Environmental vs personnel
- Particle size
- Sample size and number
- Emission sources characteristics (patient, machine, activity,...)
- Viability need (all samples vs samples subset)
- Safety for operators
- Technical/ethical requirements needed (power sources to plug the sampling devices, devices supervision, workers aware for noise or any other disturbance during sampling)



**Transport and storage conditions**  
Viability need (all samples vs samples subset)

## Assays to employ

- Elution conditions (if needed)
- Positive control
- Targets per setting
- Specificities for each virus to target
- Viability need (all samples vs samples subset)

Fig. 2. Virus exposure assessment considerations.



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Science of the Total Environment

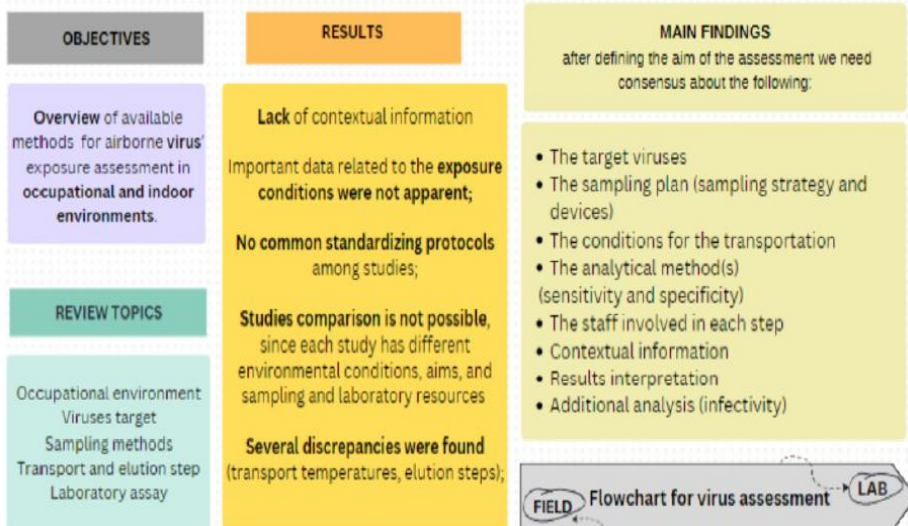
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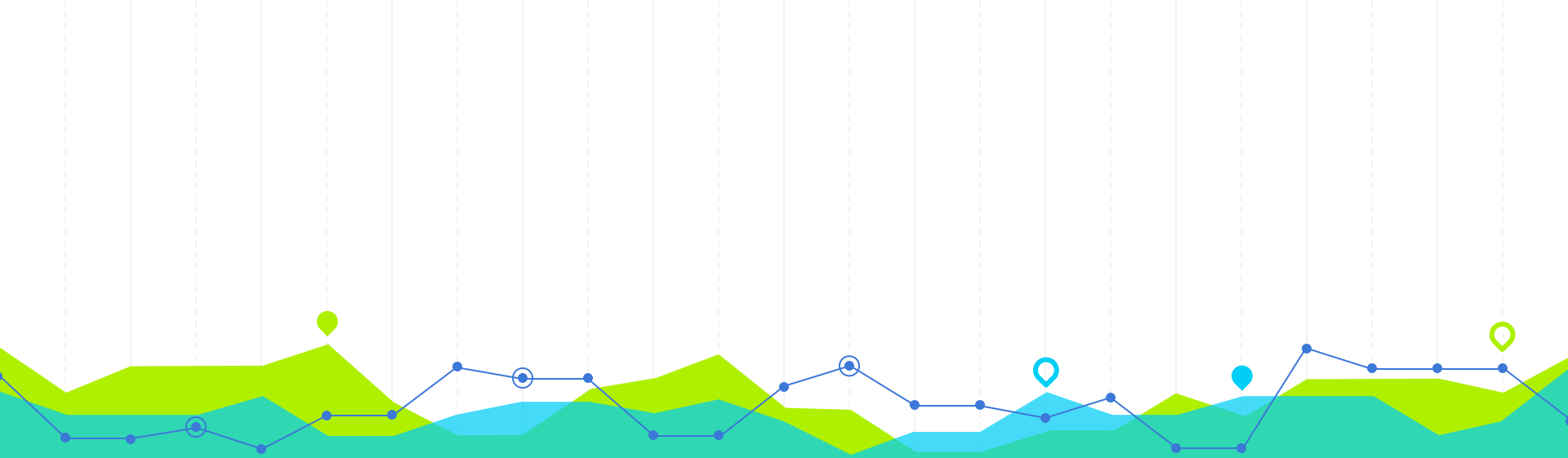


Review

Filling the knowledge gap: Scoping review regarding sampling methods, assays, and further requirements to assess airborne viruses

Marta Dias<sup>a,b</sup>, Bianca Gomes<sup>a,c</sup>, Pedro Pena<sup>a,b</sup>, Renata Cervantes<sup>a,b</sup>, Alan Beswick<sup>d</sup>, Caroline Duchaine<sup>e</sup>, Annette Kolk<sup>f</sup>, Anne Mette Madsen<sup>g</sup>, Anne Opplinger<sup>h</sup>, Clara Pogner<sup>i</sup>, Philippe Duquenne<sup>j</sup>, Inge M. Wouters<sup>k</sup>, Brian Crook<sup>d</sup>, Carla Viegas<sup>a,b</sup>





# Practical cases **2**

# 2.1 – Waste collection - Canada

Environmental Research 212 (2022) 113597

Contents lists available at ScienceDirect

Environmental Research

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ELSEVIER



Microbial contamination and metabolite exposure assessment during waste and recyclable material collection

Fabiola R.D. Salambanga<sup>a,b</sup>, Loïc Wingert<sup>b</sup>, Isabelle Valois<sup>a</sup>, Nancy Lacombe<sup>a</sup>, François Gouin<sup>a</sup>, Julien Trépanier<sup>b</sup>, Maximilien Debia<sup>a</sup>, Ewelina Soszczyńska<sup>c</sup>, Magdalena Twarużek<sup>c</sup>, Robert Kosicki<sup>c</sup>, Marta Dias<sup>d,e</sup>, Susana Viegas<sup>d,e,f</sup>, Liliana Caetano<sup>d,g</sup>, Carla Viegas<sup>d,e,f</sup>, Geneviève Marchand<sup>a,b,\*</sup>



Sampling



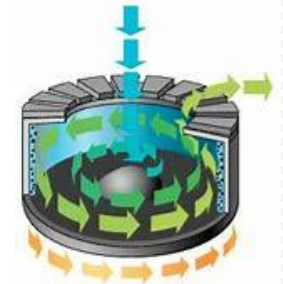
ESCOLA SUPERIOR DE SAÚDE DE LISBOA



Analyses



SASS air 3100 air sampler



CIP10-M

**Table 2**  
Analysis performed per type of sample

Analysis	Microbial target	Culture media	Sample type	
			Personal	Ambient air
Culture	Bacteria	TSA	X	X
	Gram-negative	VRBA		X
	Fungi	MEA	X	X
	Fungi	DG18		X
	Fungi	SAB		X
	Fungi-Azole	ITRA		X
	Fungi-Azole	VORI		X
	Fungi-Azole	POSA		X
Endotoxin			X	X
Mycotoxin				X
Cytotoxicity				X
dd-PCR	Fungal Biomass		X	X
	Asp/Pen		X	X
	A.section Fumigati		X	X
	A.section Flavi		X	X
	E.coli		X	X
	Enterococcus		X	X
	Legionella		X	X

## Publication highlights

- **Waste collection activities influence workers exposure to biological agents.**
- **Garbage collector's exposure to bioaerosols may represent a health risk.**
- *Microbial exposure assessment is improved by the DD-PCR absolute quantification.*
- *Cytotoxic testing enables the overall toxicity of the air inhaled by workers.*

# Waste collection - Portugal

Journal of Environmental Management 314 (2022) 115086



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Contents lists available at ScienceDirect

Journal of Environmental Management

journal homepage: [www.elsevier.com/locate/jenvman](http://www.elsevier.com/locate/jenvman)



Research article

## Microbial contamination in waste collection: Unveiling this Portuguese occupational exposure scenario

Carla Viegas<sup>a,b,c,\*</sup>, Pedro Pena<sup>a</sup>, Marta Dias<sup>a,b,c</sup>, Bianca Gomes<sup>a</sup>, Renata Cervantes<sup>a</sup>, Elisabete Carolino<sup>a</sup>, Magdalena Twarużek<sup>d</sup>, Ewelina Soszczyńska<sup>d</sup>, Robert Kosicki<sup>d</sup>, Liliana Aranha Caetano<sup>a,e</sup>, Susana Viegas<sup>a,b,c</sup>



Coriolis Air sampler



Swab surface

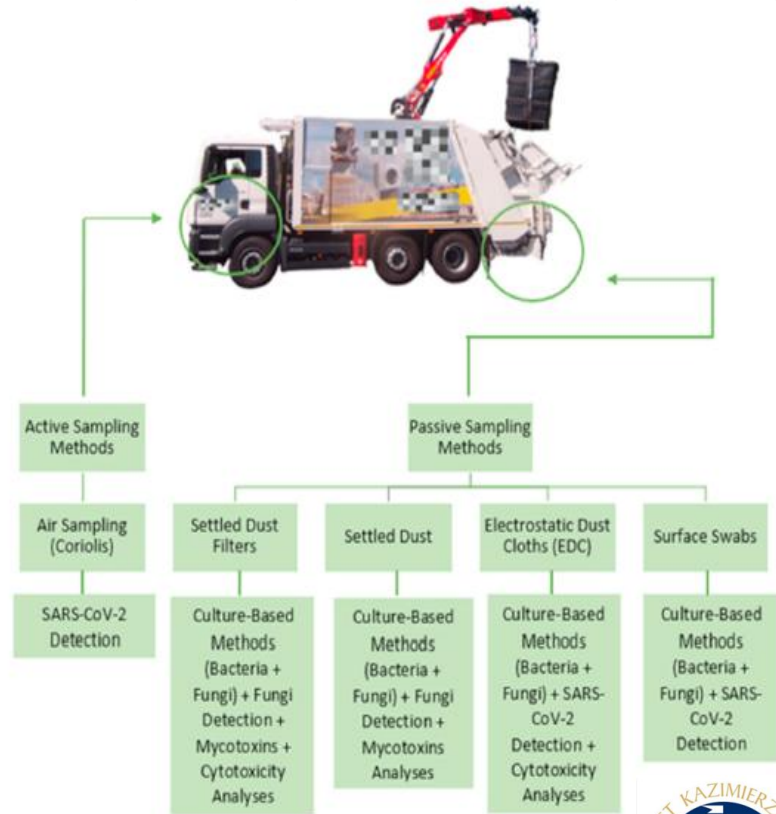


Fig. 1. WCT sampling and analysis performed.



## Publication highlights

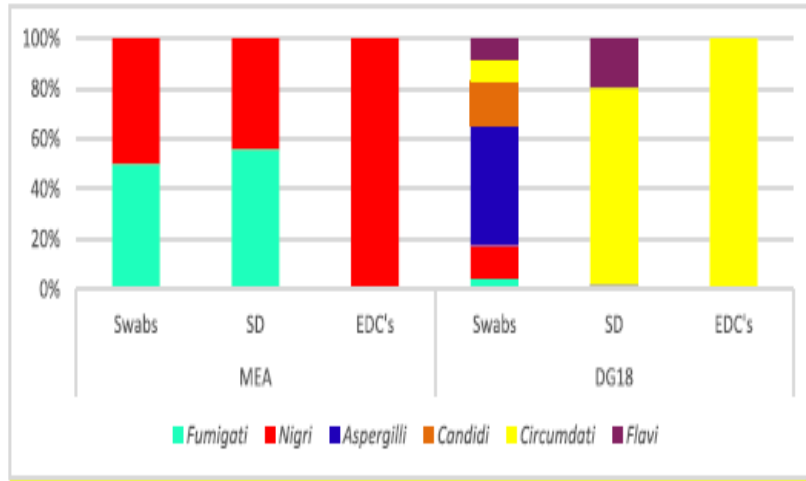


Fig. 6. *Aspergillus* sections in both culture media (MEA and DG18) from WCT.

- ***Comprehensive sampling and analyses approach is needed for microbial exposure assessments***
- ***Aspergillus section Fumigati was suggested an indicator of occupational health risk.***
- ***Waste collection might be a hotspot for the development of azole resistance.***
- ***Mycotoxins were detected in the waste collection trucks***

# Waste sorting - Portugal



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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>

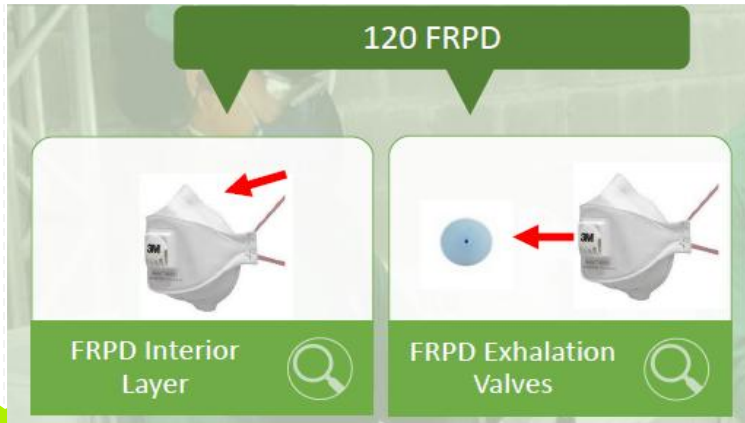


Table 1 Number of FFR collected by each workstation

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*	–	8
Total		120

- **Bacteria quantification**
- **Fungal quantification and identification**
- **Azole resistance profile**
- **Fungal biomass and toxigenic strains detection**
- **Mycotoxins detection**
- **Cytotoxicity analyses**



**Aspergillus spp. burden on filtering respiratory protective devices. Is there an occupational health concern?**



Carla Viegas<sup>1,2,3</sup> · Marta Dias<sup>1</sup> · Beatriz Almeida<sup>1</sup> · Elisabete Carolino<sup>1</sup> · Anita Quintal Gomes<sup>1,4</sup> · Susana Viegas<sup>1,2,3</sup>



microorganisms



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 Verissimo<sup>1</sup>, Carla Viegas<sup>4,6,7</sup> and Raquel Sabino<sup>1,8,\*</sup>

Contents lists available at [ScienceDirect](https://www.sciencedirect.com)

Environment International

journal homepage: [www.elsevier.com/locate/envint](http://www.elsevier.com/locate/envint)



**Cytotoxicity of filtering respiratory protective devices from the waste sorting industry: A comparative study between interior layer and exhalation valve**

Carla Viegas<sup>a,b,c,1,\*</sup>, Magdalena Twarużek<sup>d,1</sup>, Marta Dias<sup>a</sup>, Beatriz Almeida<sup>a</sup>, Elisabete Carolino<sup>a</sup>, Ewelina Soszczyńska<sup>d</sup>, Susana Viegas<sup>a,b,c</sup>, Liliana Aranha Caetano<sup>a,e</sup>

## Publication highlights

- **Filtering Respiratory Protective Devices (FRPD) presented high levels of microbial contamination.**
- **FRPD can be used as passive sampling methods to assess occupational exposure.**
- **Waste sorting is a hot spot for azole resistance**
- **Interior layers exhibited higher cytotoxicity effect than exhalation valves.**



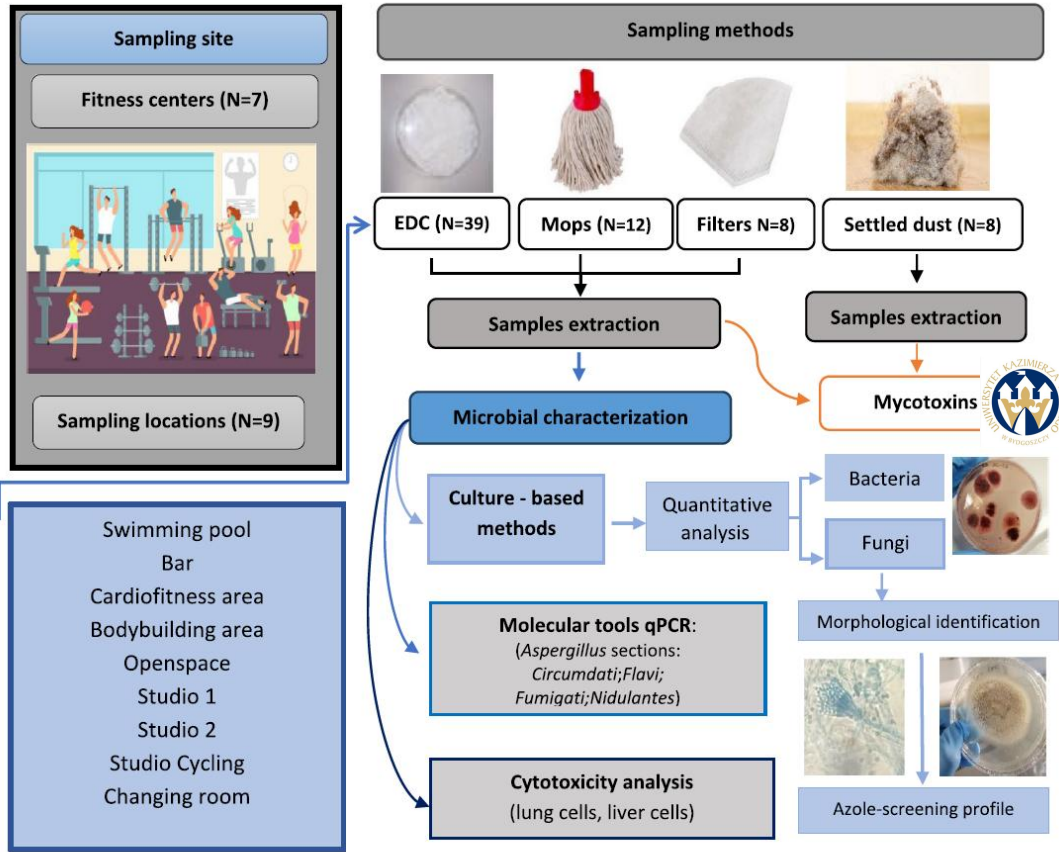


Fig. 1. Sampling methods and assays employed.

Assessment of Portuguese fitness centers: Bridging the knowledge gap on harmful microbial contamination with focus on fungi\*

Carla Viegas<sup>a,b,\*</sup>, Cátia Peixoto<sup>c,d</sup>, Bianca Gomes<sup>a,e</sup>, Marta Dias<sup>a,b</sup>, Renata Cervantes<sup>a,b</sup>, Pedro Pena<sup>a,b</sup>, Klara Slezakova<sup>d</sup>, Maria do Carmo Pereira<sup>d</sup>, Simone Morais<sup>c</sup>, Elisabete Carolina<sup>a</sup>, Magdalena Twarużek<sup>f</sup>, Susana Viegas<sup>a,b</sup>, Liliانا Aranha Caetano<sup>a,g</sup>

## Publication highlights

- **Passive sampling can be useful in identifying the most contaminated areas.**
- **Having a swimming pool in the facilities increases microbial contamination.**
- **Widespread of *Aspergillus* section *Fumigati* and species with toxigenic potential was observed.**
- **Mops may boost cross-contamination including mycotoxins dissemination.**
- **Frequent and effective cleaning procedures should be implemented.**

# Clinical environment - Ambulances

Environmental Research 197 (2021) 111125

Contents lists available at ScienceDirect

Environmental Research

journal homepage: [www.elsevier.com/locate/envres](http://www.elsevier.com/locate/envres)



ELSEVIER



Bioburden contamination and *Staphylococcus aureus* colonization associated with firefighter's ambulances

Carla Viegas<sup>a,b,c,\*</sup>, Pedro Sousa<sup>a</sup>, Marta Dias<sup>a</sup>, Liliana Aranha Caetano<sup>a,d</sup>, Edna Ribeiro<sup>a</sup>, Elisabete Carolino<sup>a</sup>, Magdalena Twarużek<sup>e</sup>, Robert Kosicki<sup>e</sup>, Susana Viegas<sup>a,b,c</sup>



**Table 1**

Number of samples taken in each Firefighters Headquarters (FFH) by each sampling method.

		Vehicles (n)	Air sampler Andersen six-stage air sampler	Air sampler Millipore	Swabs (Ambulances)	Swabs (Locker Rooms)	EDC	Mop and Cleaning Cloth	Uniform Ranks	Settled Dust
FFH1	EMS	4	96	16	65 <sup>a</sup>	2	6	2	3	4
	NEPT	1	24	4	7		2			1
FFH2	EMS	5	120	20	40	2	5	5	0	6
	NEPT	2	48	8	16		1			2
	<b>Total</b>	<b>12</b>	<b>288</b>	<b>48</b>	<b>128</b>	<b>4</b>	<b>14</b>	<b>7</b>	<b>3</b>	<b>13</b>

EMS - Emergency Medical Services.

NEPT - Non-Emergency Patient Transport.



## Publication highlights



- ***Toxigenic fungi with clinical relevance were found on ambulance's air.***
- ***Surfaces contamination increased after cleaning.***
- ***Mycotoxins were detected in the mops and EDC***
- ***MRSA and fungi azole resistant were detected in the ambulances.***
- ***Ambulances crew presented colonization with MSSA and MRSA.***

# Clinical environment – Health care centers

Building and Environment 160 (2019) 106226



Contents lists available at ScienceDirect

Building and Environment

journal homepage: [www.elsevier.com/locate/buildenv](http://www.elsevier.com/locate/buildenv)



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>



Millipore Air sampler



Coriolis Air sampler



HVAC filters



Surface swabs



EDC



Settled dust

## Publication highlights

Table 13  
Fungal air load mean, outdoor and I/O values.

PHCC	Mean (CFU.m <sup>-3</sup> )	Outdoor (CFU.m <sup>-3</sup> )	I/O	Toxigenic species
1	28.57	144	0.20	
2	65	20	3.25	<i>Aspergillus</i> section <i>Circumdati</i> ; <i>Aspergillus</i> section <i>Versicolores</i>
3	479	612	0.78	<i>Aspergillus</i> section <i>Versicolores</i>
4	402	260	1.55	
5	291	96	3.03	<i>Aspergillus</i> section <i>Fumigati</i>
6	155.5	228	0.68	<i>Aspergillus</i> section <i>Versicolores</i>
7	474.91	140	3.39	
8	526	384	1.37	
9	505.71	508	1.00	
10	520	208	2.50	

- **Portuguese legal criteria for bacteriota was not complied in all PHCC and for fungal load 60% did not comply.**
- **The presence of mycotoxins was found both in air and HVAC filter samples.**
- **Fungal biomass results have shown to be good predictors for bacterial and fungal counts in HVAC filters.**
- **The multi-approach sampling protocol used allowed to unveil a more real scenario regarding exposure to bioburden.**



HVAC filters

# Clinical environment – Central hospital



ELSEVIER

Contents lists available at ScienceDirect

Environmental Research

journal homepage: [www.elsevier.com/locate/envres](http://www.elsevier.com/locate/envres)



## 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>a</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>

Table 1  
Environmental parameters measurements and bioburden sampling performed in each sampling location.

Measurements and sampling Locations	Room	Active		Sampling methods		
		Air impaction	Impinger	Passive		
				Settled dust	EDC <sup>o</sup>	Surface swabs
Emergency Room	Nursing room	8	1	1	1	3
	Medical office	8	1		1	3
Day hospital	Medical office	8	1	1	1	3
	Endocrinology nursing room	8	1		1	3
	Oncology – treatments room	8	1		1	3
	Oncology – medical office	8	1		1	3
Internment ward ( <i>Medicina A</i> )	Medical office	8	1	1	1	3
	Nursing room	8	1		1	3
Operating Room	Operating theatre	8	1	1	0	3
	Antechamber of the operating theatre	8	1		2	3
Outpatient	Room 48 – Echography	8	1	1	1	3
	Room 210 – Nursing room	8	1		1	3
	Service desk – K4	8	1		1	3
	Service desk – K0	8	1		1	3
	Back office	8	1		1	3



MAS-100 air sampler



Coriolis Air sampler



Button aerosol sampler

## Publication highlights

- **Portuguese legislation was not effective to assess IAQ.**
- **Toxigenic fungal species were identified by culture based-methods and detected by qPCR.**
- **Low levels of endotoxins were observed in the environmental matrices.**

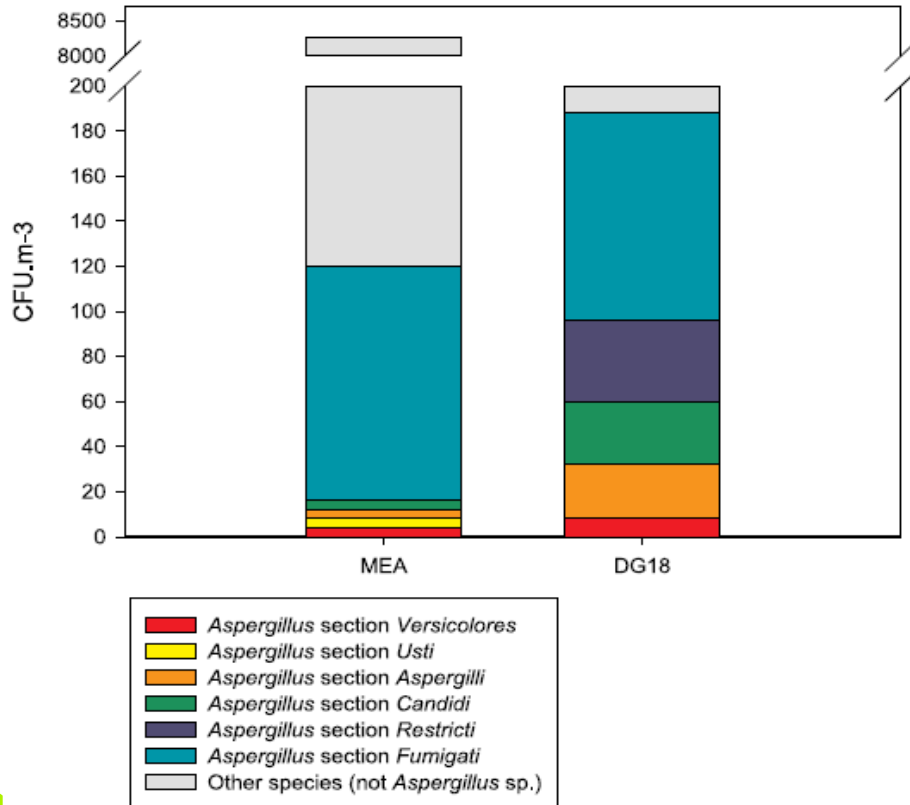
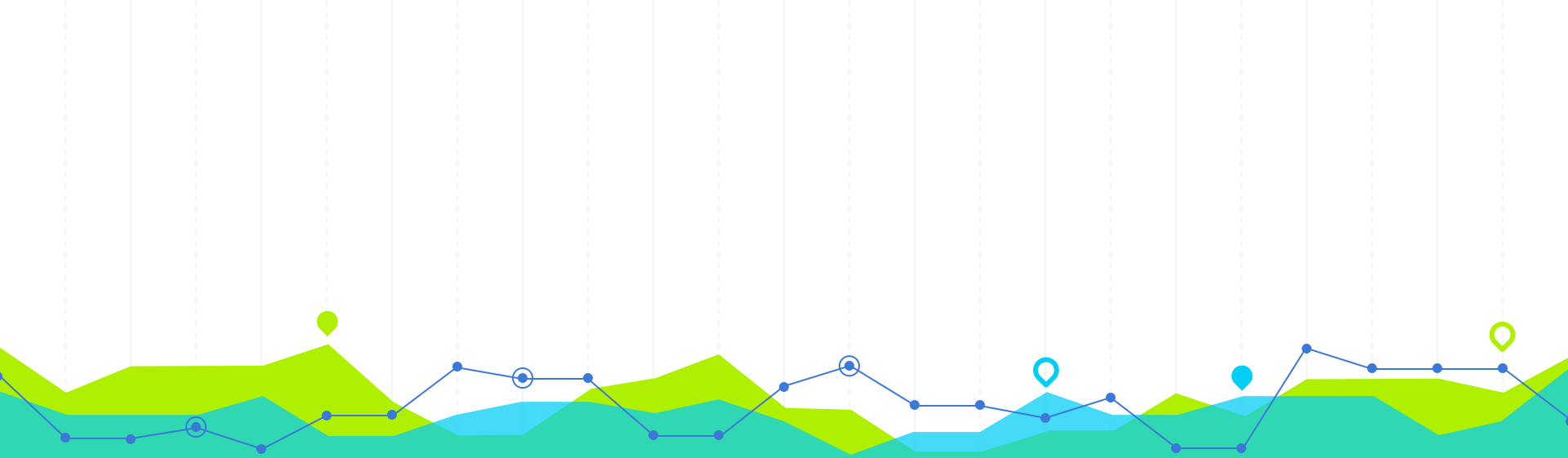


Fig. 1. *Aspergillus* sp. contamination in air impaction samples.



# Take home messages

# 3

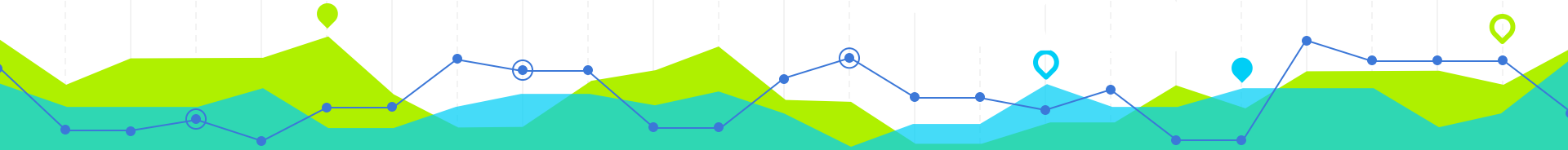
- Fast, reliable, and cost-effective methods are needed **to characterize microbiological contamination, identify occupational risk factors, and, in the future, establish exposure limit values.**
- Both **sampling methods** (active and passive) and the associated **assays have advantages and disadvantages.**
- It is necessary to understand the **limitations underlying each method in order to properly interpret the results.**



- There is an urgent need **to establish a consensus** to be applied in each specific situation, in order to contribute **to accurate exposure assessment and to the characterization and risk control.**
- **Sampling methods and assays** play a decisive role in the field of **Public Health** and, more specifically, in **Occupational Health.**



# Aknowledgements



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