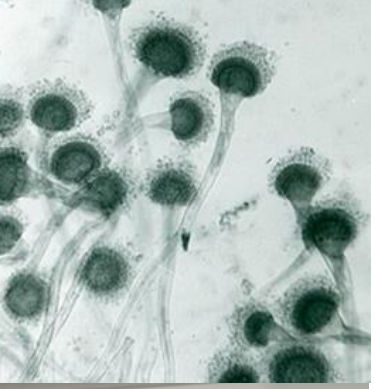
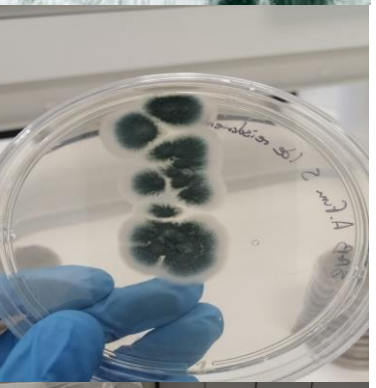




Asociación Española de Higiene Industrial

International Commission on Occupational Health
Founded in 1906 as Permanent Commission

European Platform of Occupational Hygiene

Occupational Exposure to Fungi

Carla Viegas*

*https://orcid.org/0000-0002-1545-6479

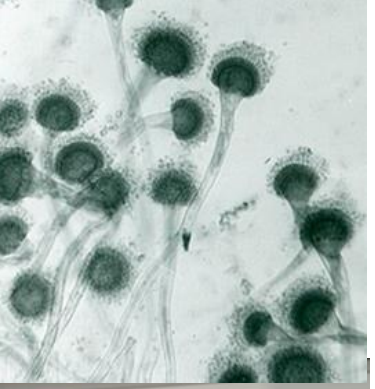
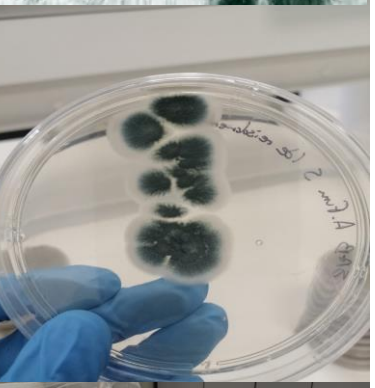
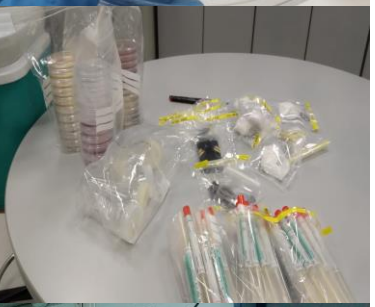
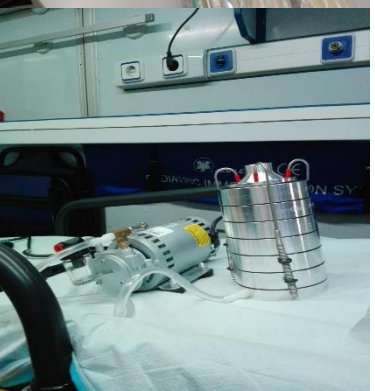
carla.viegas@estesl.ipl.pt

ESCOLA SUPERIOR DE TECNOLOGIA DA SAÚDE DE LISBOA
INSTITUTO POLITÉCNICO DE LISBOA

HTRC Health and Technology Research Center

CISP CENTRO DE INVESTIGAÇÃO EM SAÚDE PÚBLICA

CHRC COMPREHENSIVE HEALTH RESEARCH CENTRE

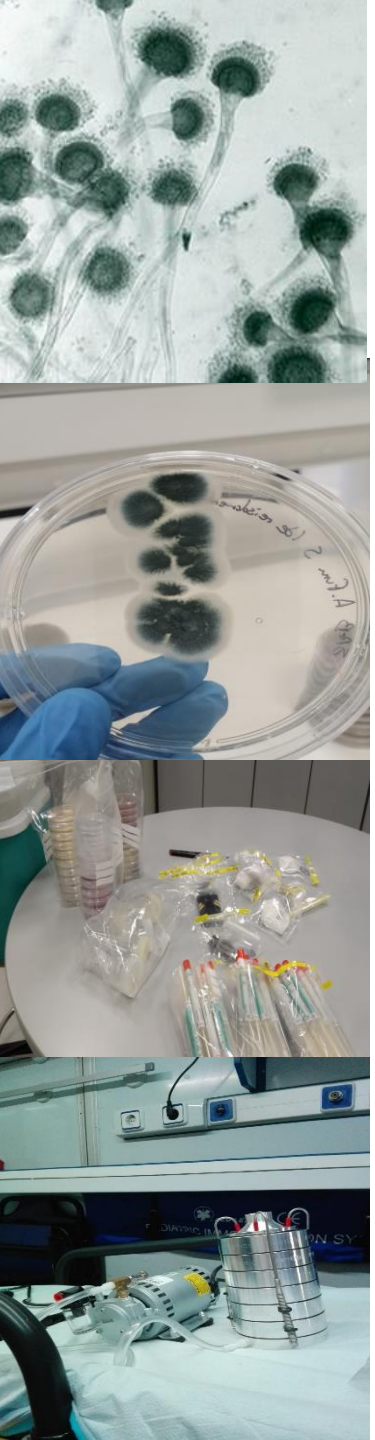





Outline

1 – Background

2 – Sampling and analyses

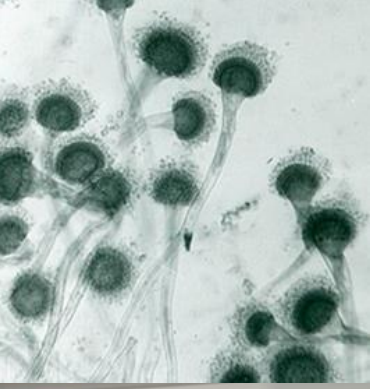
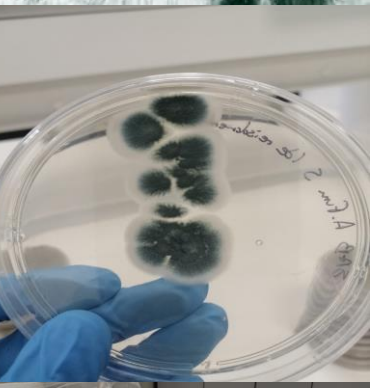
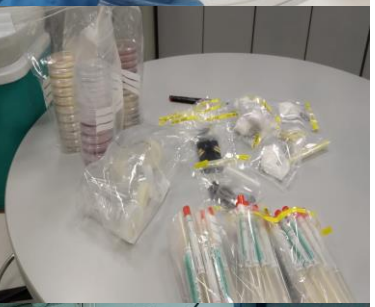
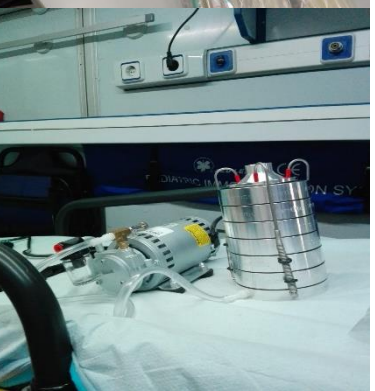
3 – Lessons learned



1 – Background

- The SDGs 3 and 8 highlight the need to guarantee healthy lives and encourage wellbeing and **decent work for all**.
- WHO recognized as **priority settings for action the workplaces** reporting more than 1.2 million deaths annually due to occupational risks.
- **Only a small fraction** of the general workforce has access to **occupational health surveillance**.

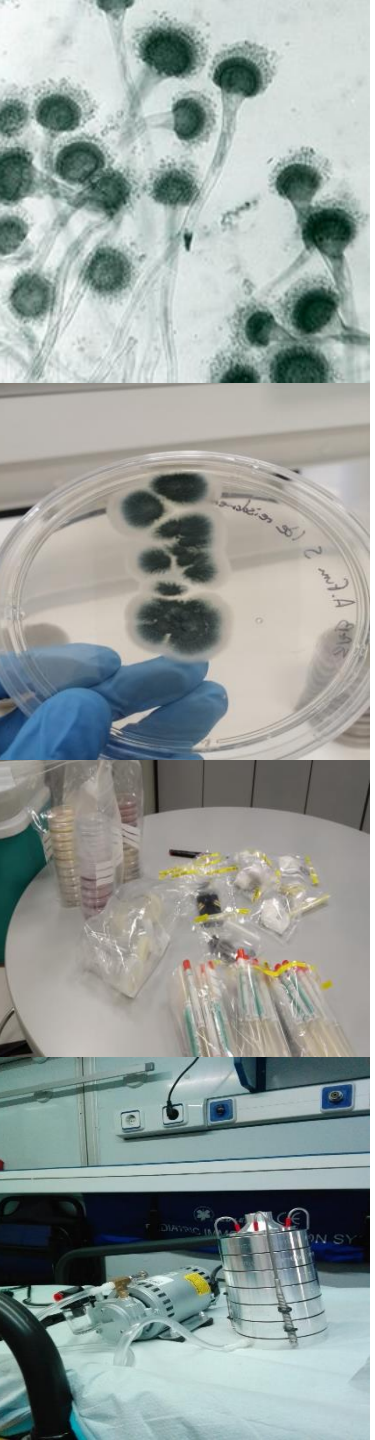
https://www.who.int/tools/compendium-on-health-and-environment/priority-settings-for-action

HERA from EU presented a priority list of **top-3 health threats that require coordination of measures at EU level,** since they have the potential of spreading across Member States:

- **pathogens with high pandemic potential**
- chemical, **biological,** radiological and nuclear threats
- threats resulting from **antimicrobial resistance**.

https://ec.europa.eu/commission/presscorner/detail/en/ip_22_4474



WHO release a list of fungal priority pathogens but without considering the toxicological relevance.

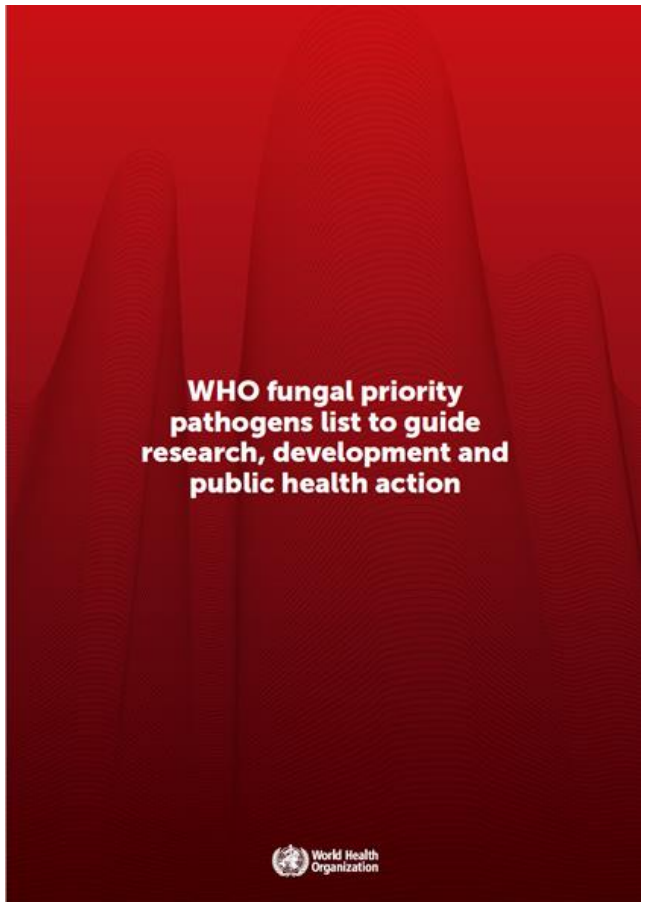
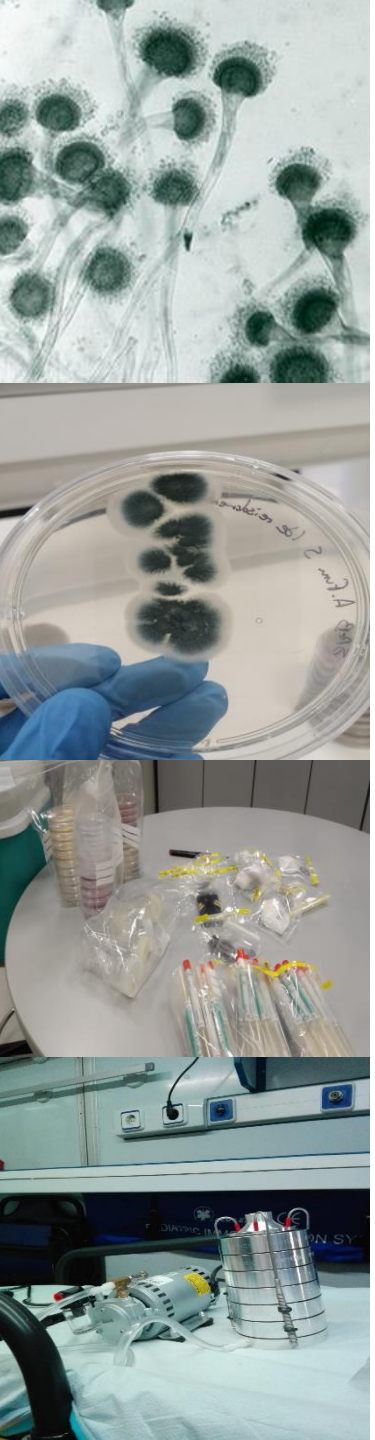


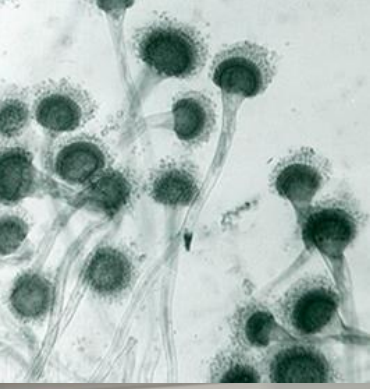
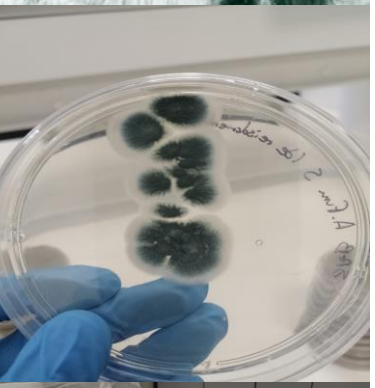
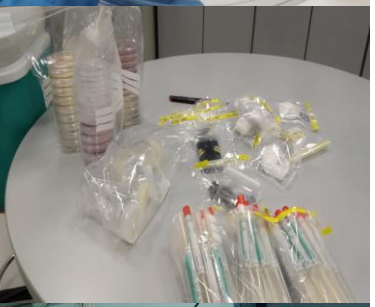
Table 3. WHO fungal priority pathogens list

Critical group	High group	Medium group
Cryptococcus neoformans	*Nakaseomyces glabrata (Candida glabrata)*	*Scedosporium* spp.
Candida auris	*Histoplasma* spp.	*Lomentospora prolificans*
Aspergillus fumigatus	Eumycetoma causative agents	*Coccidioides* spp.
Candida albicans	Mucorales	*Pichia kudriavzeveii (Candida krusei)*
	Fusarium spp.	*Cryptococcus gattii*
	Candida tropicalis	*Talaromyces marneffei*
	Candida parapsilosis	*Pneumocystis jirovecii*
		Paracoccidioides spp.



- In all European countries employers **are obliged by regulation to assess and prevent exposure to occupational risks** (Directive 89/391/EEC) .

- The Directive 2000/54/EC of the European Parliament and the Council of September 18, 2000 **sets the rules regarding risk assessment if exposure to biological agents cannot be avoided (2000/54/EC, 2000).**

- Scientific evidence that associates occupational exposure to bioaerosols (bacteria, fungi and metabolites) to health outcomes.

(Sabino et al. 2019; Mbareche et al. 2019; Schlosser et al. 2020; Madsen & Crook 2021)

ISHAM
INTERNATIONAL SOCIETY FOR HUMAN AND ANIMAL MYCOLOGY

Medical Mycology, 2019, 57, S196–S205
doi: 10.1093/mmy/myy090
Review Article

Review Article

The role of occupational *Aspergillus* exposure in the development of diseases

Raquel Sabino[1,2,*], Cristina Veríssimo[1], Carla Viegas[3,4], Susana Viegas[3,4], João Brandão[5], Magna Alves-Correia[6], Luís-Miguel Borrego[6,7], Karl V. Clemons[8,9], David A. Stevens[8,9] and Malcolm Richardson[10,11]

JOURNAL OF THE AIR & WASTE MANAGEMENT ASSOCIATION
2019, VOL. 69, NO. 7, 789–804
https://doi.org/10.1080/10962247.2019.1587552

Taylor & Francis
Taylor & Francis Group

REVIEW PAPER

Check for updates

On the interpretation of bioaerosol exposure measurements and impacts on health

Hamza Mbareche[a,b], Lidia Morawska[c], and Caroline Duchaine[a,b]

Waste Management 105 (2020) 395–404

Contents lists available at ScienceDirect

Waste Management

journal homepage: www.elsevier.com/locate/wasman

ELSEVIER

Airborne mycotoxins in waste recycling and recovery facilities: Occupational exposure and health risk assessment

Check for updates

Olivier Schlosser *, Samuel Robert, Naike Noyon

SUEZ, CIRSEE, 38 rue du Président Wilson, 78230 Le Pecq, France

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

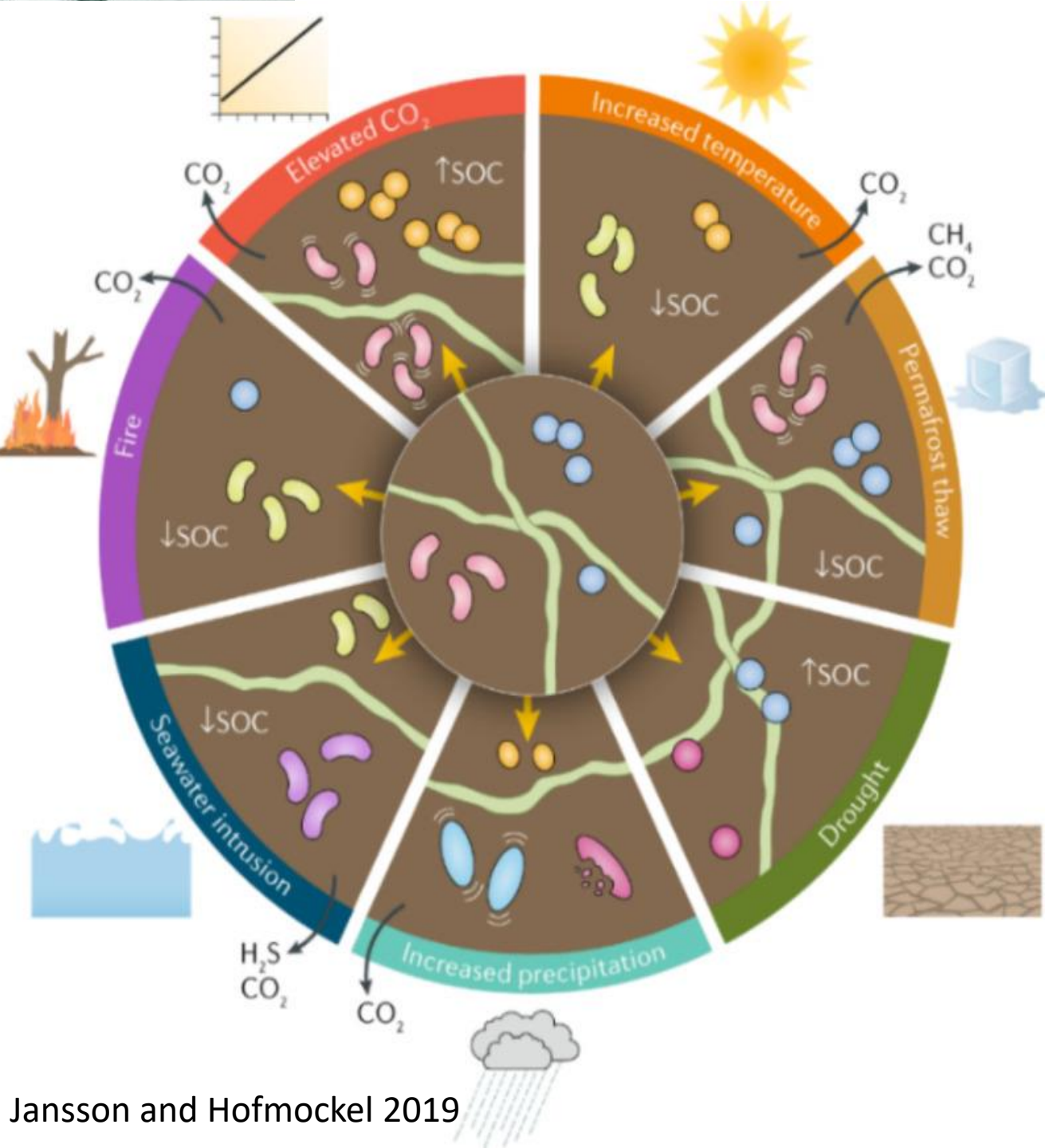
ELSEVIER

Review

Occupational exposure to fungi on recyclable paper pots and growing media and associated health effects – A review of the literature

Check for updates

Anne Mette Madsen [a,*], Brian Crook [b]



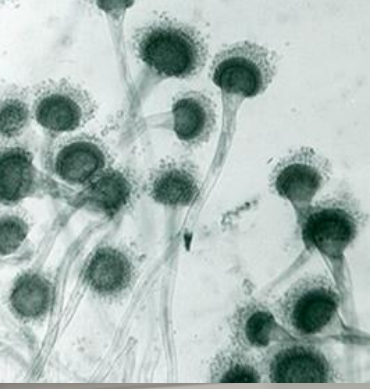
Jansson and Hofmockel 2019

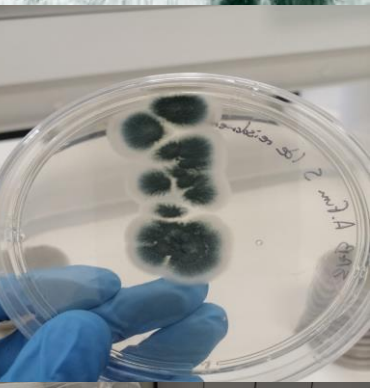
The relative scarcity of fungal diseases among mammals has been hypothesized to be associated with the inability of many fungal species to survive at temperatures >37°C

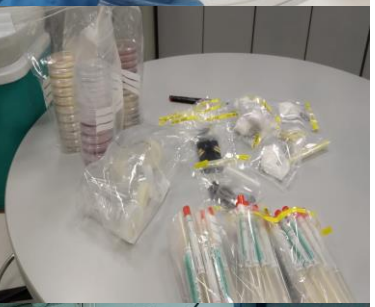
Warmer ambient temperatures may enable nonpathogenic fungi to acquire the ability to infect warm-blooded hosts.

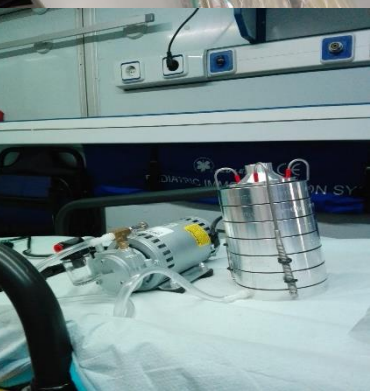
https://wwwnc.cdc.gov/eid/article/20/3/13-1230_article

Floods may increase exposure to fungi and warmer temperatures may increase their ability to infect.









ESCOLA SUPERIOR DE TECNOLOGIA DA SAÚDE DE LISBOA
INSTITUTO POLITÉCNICO DE LISBOA

H&TRC
Health and Technology Research Center

CISP
CENTRO DE INVESTIGAÇÃO EM SAÚDE PÚBLICA

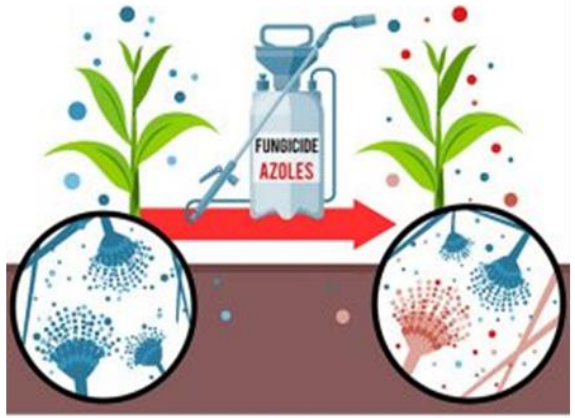
CHRC
COMPREHENSIVE HEALTH RESEARCH CENTRE

Climate change

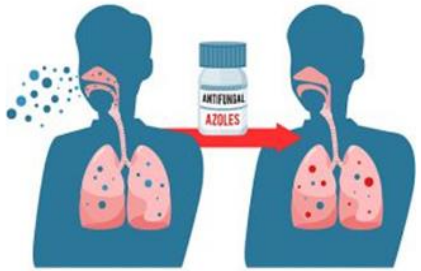
Increase of fungi and mycotoxins contamination

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.



Annals of Work Exposures and Health, 2018, 1–19
doi: 10.1093/annweh/wxy070
Review

Review

Occupational Exposure to Mycotoxins: Current Knowledge and Prospects

Susana Viegas,[1,2]* Carla Viegas[1,2] and Anne Oppliger[3]

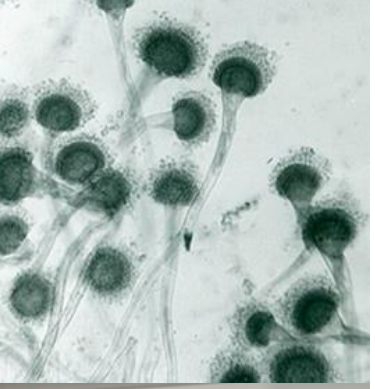
toxins

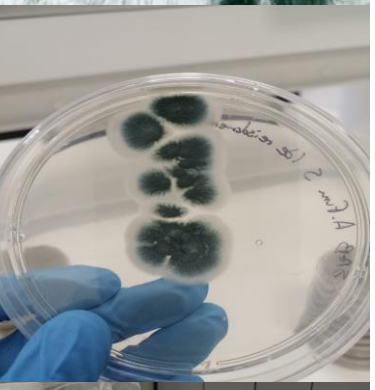
MDPI

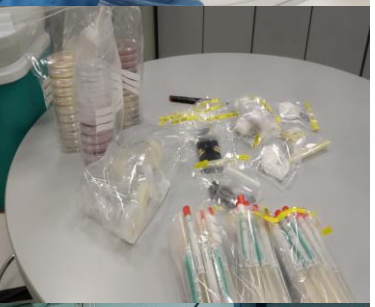
Review

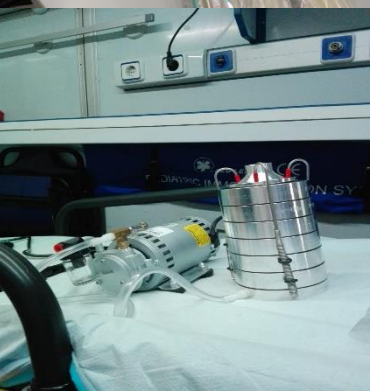
Occupational Exposure to Mycotoxins—Different Sampling Strategies Telling a Common Story Regarding Occupational Studies Performed in Portugal (2012–2020)

Susana Viegas [1,2,3,*], Carla Viegas [1,2,3], Carla Martins [1,2,4,5] and Ricardo Assunção [1,2,4,5]







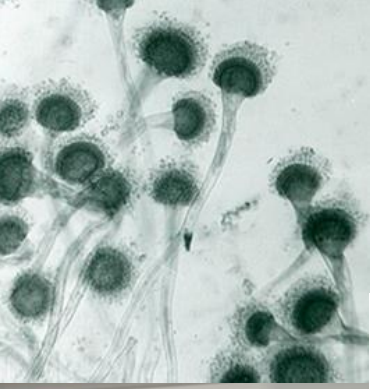


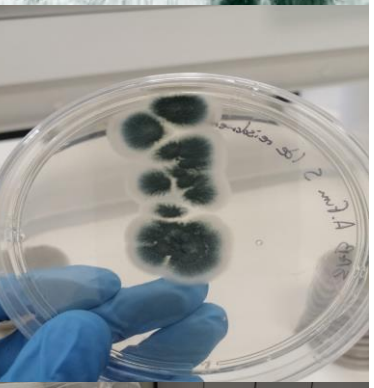
To sum up:

- Awareness from **WHO and EU**
- International and national **legal framework**
- Occupational exposure leads to **health outcomes**
- **Climate changes** will **increase** microbial contamination and **AMR** (fungal resistance)
- **Circular economy** will **boost exposure** (waste industry increased workforce)
- **Emergent risks** due to microbial contamination exposure (Mycotoxins,…)

Main drawback is the lack of exposure limits due to:

- The heterogeneity of the sampling and assays employed
- Limited new analysis methods that identify the risk potential





We need to:

BIOAEROSOL PUBLIC DATABASE

STUDY DESIGN

STANDARDIZED PROTOCOLS

- sampling strategy
- sample processing
- analytical methods - (bacteria, fungi, viruses, endotoxins, mycotox-ins, (1-3)-β-d glucan & MVOCs)

BIOAEROSOL CONTENT

BIOLOGICAL

- endotoxins
- mycotoxins
- (1-3)-β-d glucan
- MVOCs
- bacteria
- fungi
- viruses

bioaerosol core microbiome & nasopharynx microbiome

CHEMICAL

- pH
- organic & inorganic chemical compounds

HEALTH MEASUREMENTS

- blood assays (immunological profile)
- respiratory functions (FEV1/FVC)
- questionnaires (self-reported health info by exposed subjects)

MARKERS

TYPE OF ENVIRONMENT | SHARED MICROBIOME BETWEEN BIOAEROSOLS & NASOPHARYNX | QUANTIFICATION DATA OF BIOLOGICAL CONTENT OF BIOAEROSOLS | HEALTH MEASUREMENTS

Create a bioaerosol public database to gather information for long-term association studies and help determine specific environmental markers of exposure.

JOURNAL OF THE AIR & WASTE MANAGEMENT ASSOCIATION
2019, VOL. 69, NO. 7, 789–804
https://doi.org/10.1080/10962247.2019.1587552

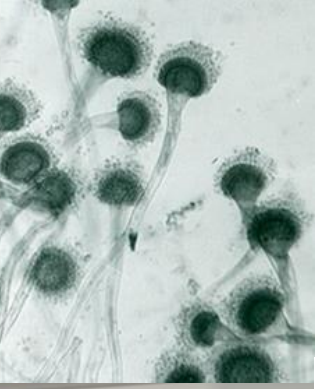
Taylor & Francis
Taylor & Francis Group

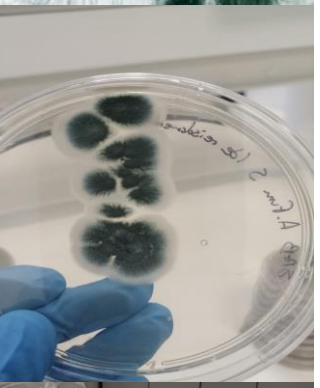
REVIEW PAPER

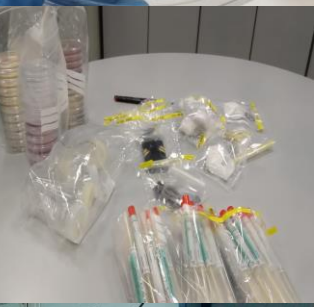
Check for updates

On the interpretation of bioaerosol exposure measurements and impacts on health

Hamza Mbareche[a,b], Lidia Morawska[c], and Caroline Duchaine[a,b]







2– Sampling and analyses

Standard protocols from the field to the lab

3.4 Mops/Cloth

3.4.1 Materials required for the collection of Mop samples

Below is a list with the different materials needed for performing the samples.

- Sterilized scissors
- Sterilized bags

3.4.2 Sampling procedure

The samples should be performed by a trained technician according to the location. Cut approximately 6 cm of a mop strip/piece of cloth into a sterilized bag and store the sample according to the instructions below (see sketch below, figure 4).

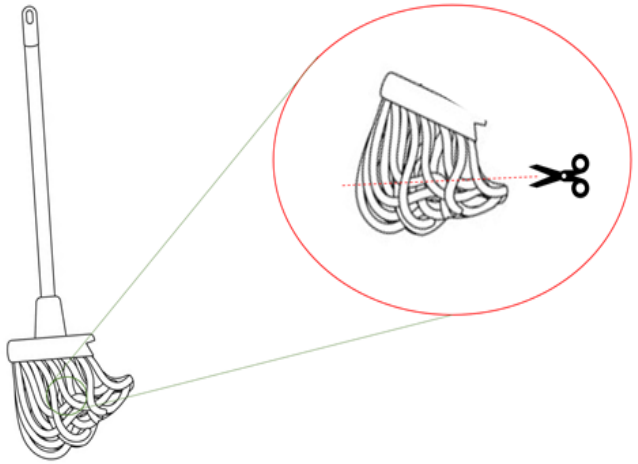


Figure 4 – Mops Sampling

4 Inoculation procedure (microbial contamination and azole resistance screening)

4.1 Microbial contamination

- For assessment of microbial contamination inoculate each of the chosen media (TSA, VRBA, MAC, MEA and DG18) with 150 µL of the liquid sample.
- Keep the plates for fungal contamination assessment (MEA and DG18) at 27ºC. For fungal pathogenic potential assessment keep one plate of DG18 at 37ºC. After incubation for 5 to 7 days, count the different colonies and identify them microscopically using lactophenol cotton blue mount procedures.
- Keep the plates for total bacteria contamination assessment (TSA) at 30ºC, the plates for Gram-negative bacteria contamination assessment: VRBA at 37ºC for 7 days and MAC at 35ºC+2ºC for 18 to 24h. Count the colonies.

Use the appropriate formulas for the samples to obtain the CFU per measuring unit:

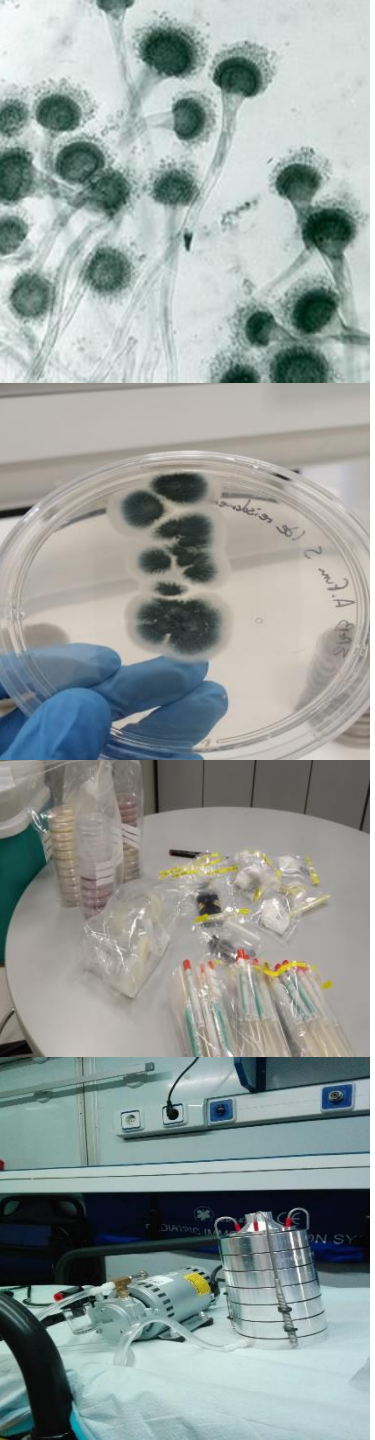
- For **EDC** samples the calculation formula is: CFU.m-2 / day-1
  - Example (x=colonies counted)

    $CFU.m^{-2}\ day = \left(1\frac{x}{3.14 * r2}\right)$ /days sampling - if a petri dish is used

    $CFU.m^{-2}\ day = \left(1\frac{x}{h * w}\right)$ /days sampling - if a swiffer in an A4 paper is used
- For **EDCT** samples the calculation formula is: CFU.m-2 / day-1
  - Example (x=colonies counted)

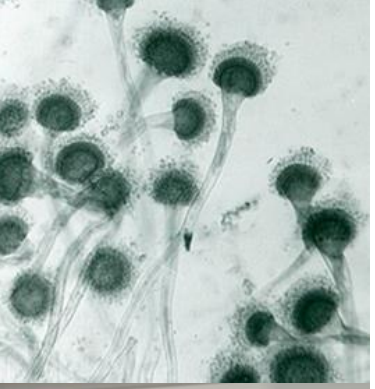
    $CFU.m^{-2}\ day = \left(1\frac{x}{h * w}\right)$ /days sampling
- For **filter** samples the calculation formula is:
  - Example (x=colonies counted)

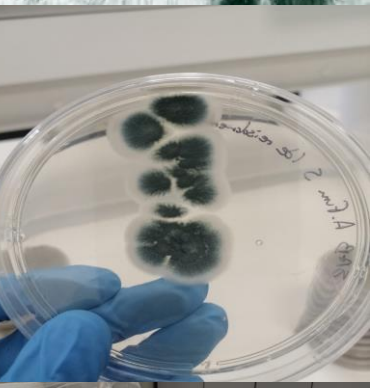
    $CFU.m^{-2} = \left(0.1\frac{x}{2 * 10\ \hat{}\ -4}\right)$

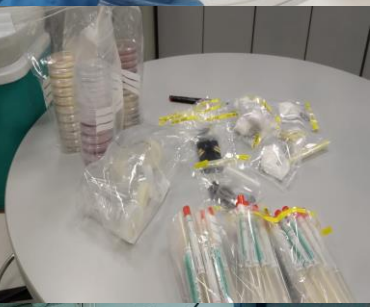


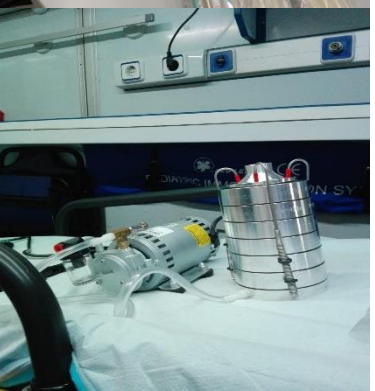
Sampling

The active methods devices used to sample airborne fungi mainly rely on **impaction, impingment and filtration**. (Mandal & Brandl 2011; Viegas et al. 2015a)



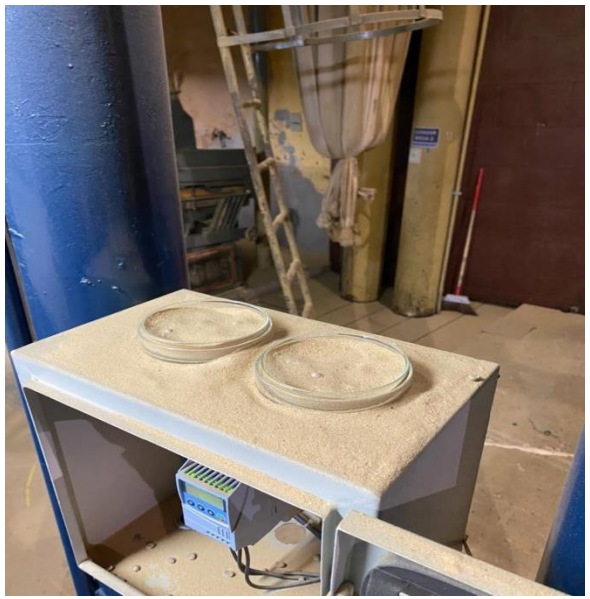


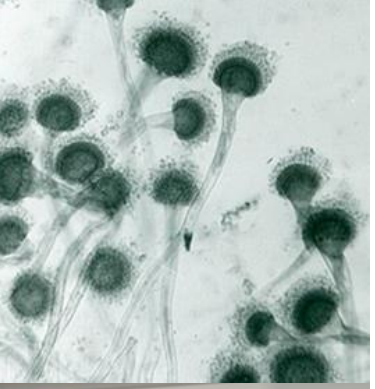


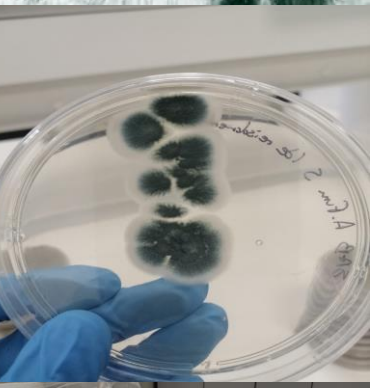


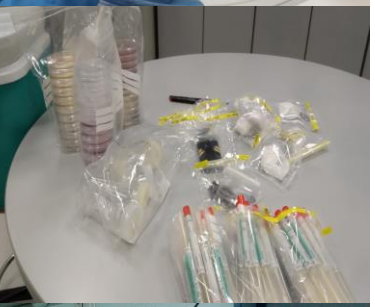
Passive sampling provides a valid risk assessment as it measures the harmful part of the airborne population which falls onto a critical surface.

(French et al. 1980)



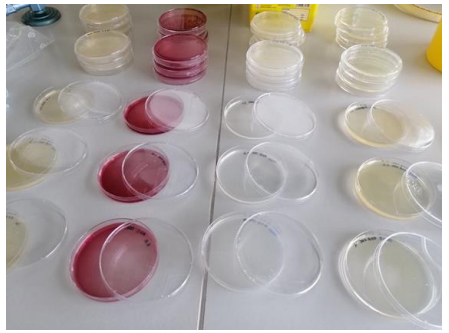




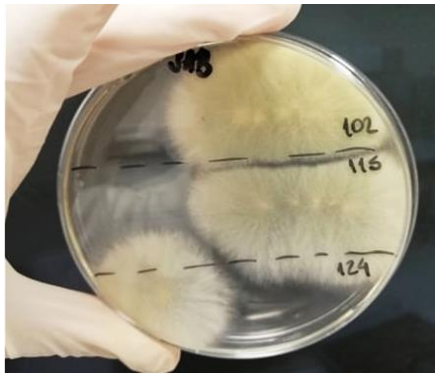


Analyses

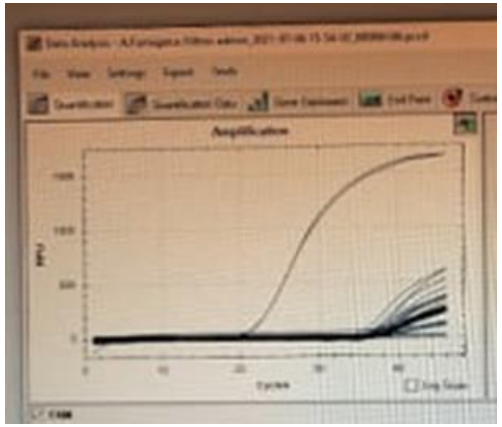
- Microbial contamination



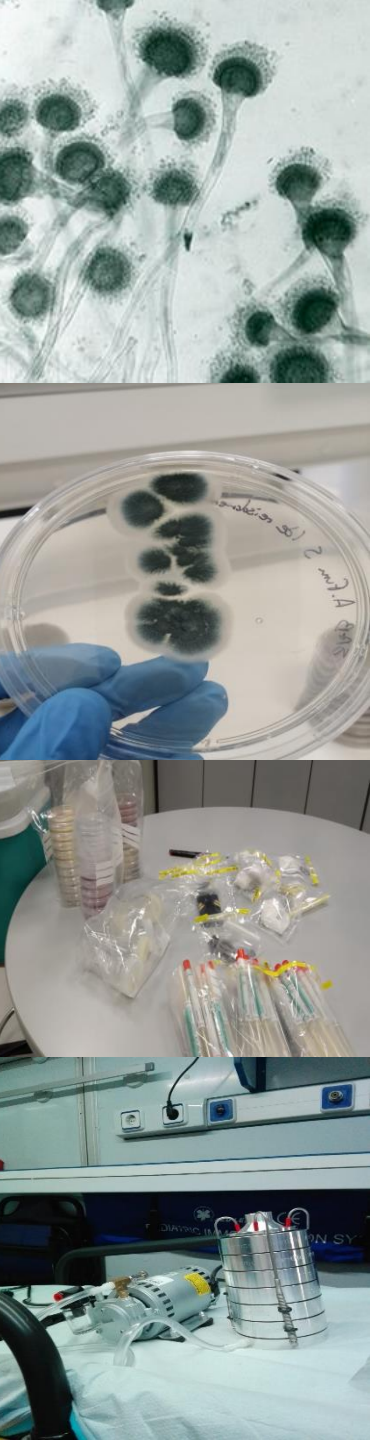
- Azole resistance screening



- Molecular detection of *Aspergillus* sections

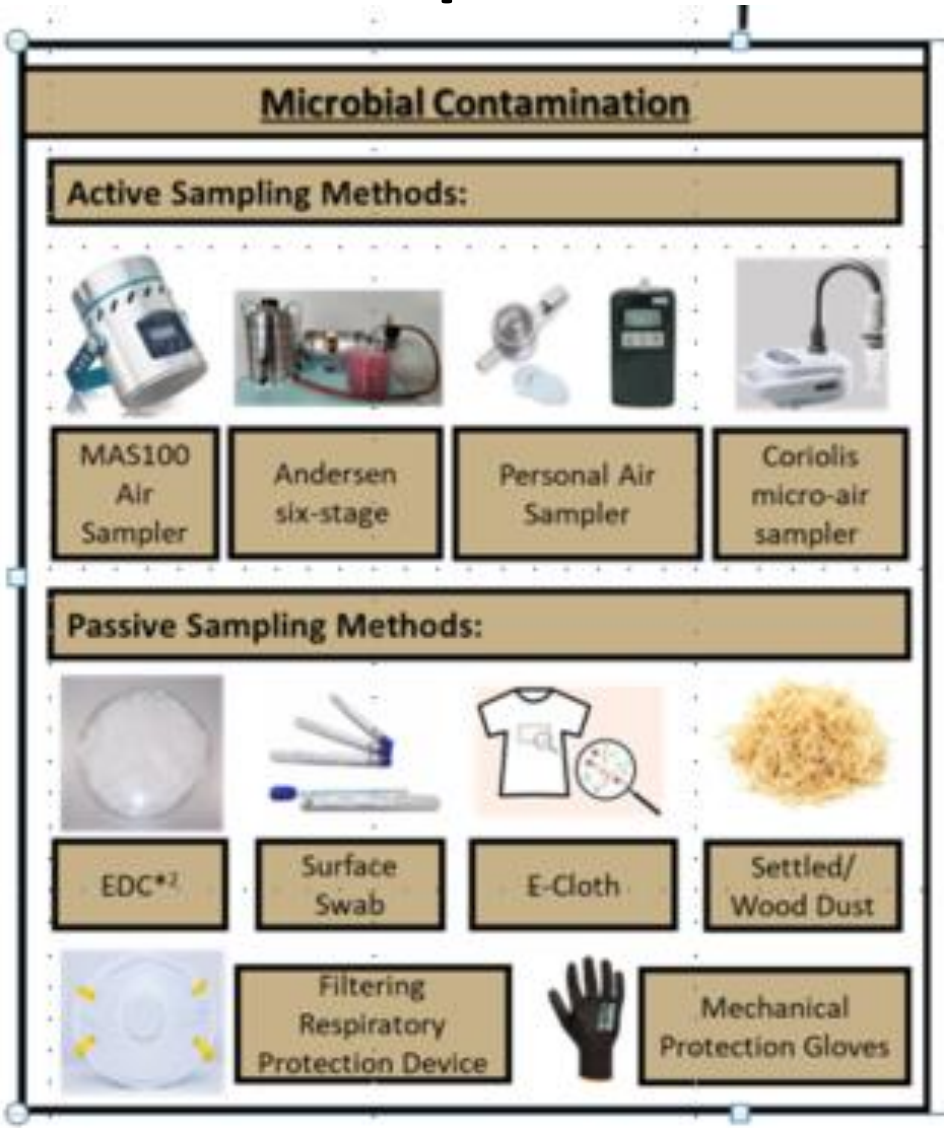


- 38 Mycotoxins detection



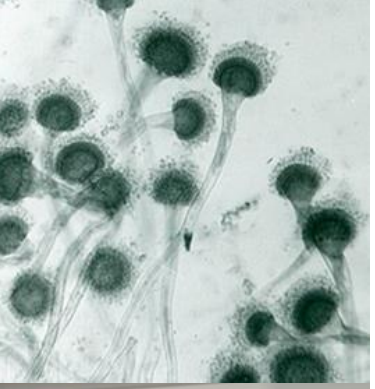
3 – Lessons learned

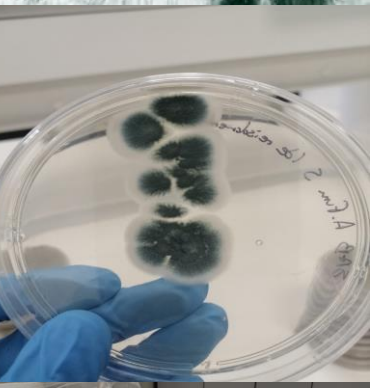
- **Use a comprehensive sampling approach**

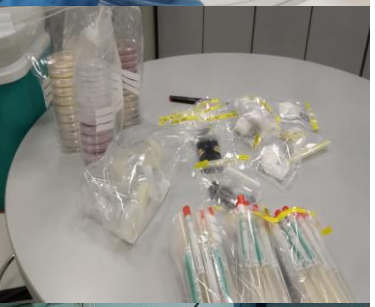


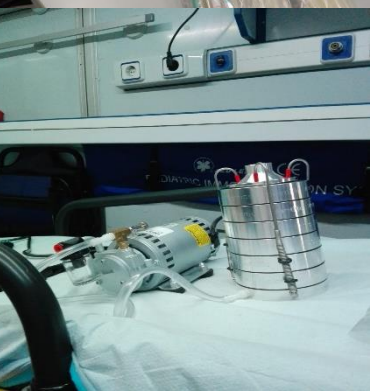
Passive sampling methods, as well as material collection, should be included in the sampling campaigns, and should **be adjusted to:**

- **the identified contamination sources**
- **contextual information**
- **and variability of the exposure.**









- **Apply more than one culture media (and temperatures)**

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.

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

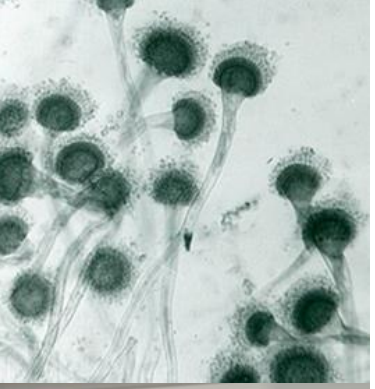
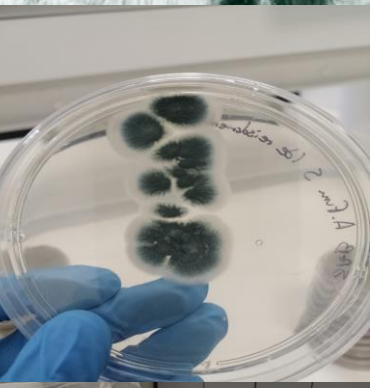
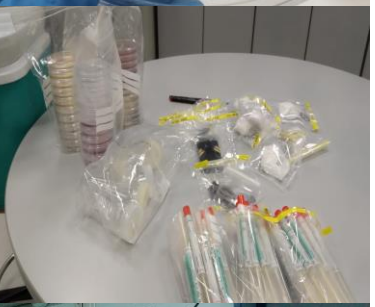
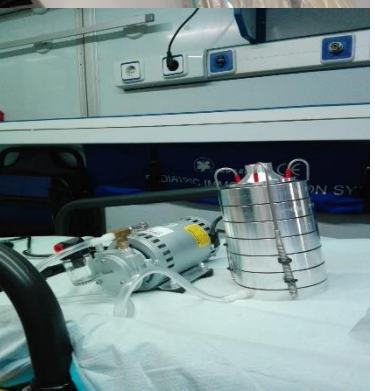
Aspergillus Sections	Media		Sampling Approach	n (%)	Indoor Environment	n (%)
	MEA	DG18				
	n (%)	n (%)				
Candidi	36 (5.8%)	79 (14.7%)	EDC	167 (14.5%)	Bakeries	91 (7.9%)
Circumdati	40 (6.5%)	117 (21.8%)	Air impaction	325 (28.2%)	Dwellings	199 (17.3%)
Nigri	246 (39.9%)	49 (9.1%)	Settled dust	48 (4.2%)	Air	8 (0.7%)
Fumigati	163 (26.4%)	95 (17.7%)	HVAC filter	31 (2.7%)	Taxi	17 (1.5%)
Versicolores	55 (8.9%)	62 (11.6%)	Surface swab	42 (3.6%)	College indoors	17 (1.5%)
Aspergilli	22 (3.6%)	80 (14.9%)	Air impingement	6 (0.5%)	Swine	26 (2.3%)
Terrei	2 (0.2%)	0 (0.0%)	Filter air sampler	90 (7.8%)	Dairies	29 (2.5%)
Flavi	35 (5.7)	48 (9.0%)	MPG	125 (10.8%)	Veterinary clinics	6 (0.5%)
Nidulantes	5 (0.8%)	1 (0.2%)	FRPD	280 (24.3%)	Elementary school	2 (0.2%)
Clavati	3 (0.5%)	2 (0.4%)	Ventilation grid—swab	39 (3.4%)	Healthcare facility	231 (20.0%)
Usti	2 (0.3%)	2 (0.4%)			Elderly care center	11 (1.0%)
Cremei	1 (0.2%)	0 (0.0%)			Waste sorting	483 (41.9%)
Restricti	7 (1.1%)	1 (0.2%)			Thermal baths	17 (1.5%)
					Firefighters' ambulances	16 (1.4%)
Total	617	536	Total	1153	Total	1153

atmosphere MDPI

Article

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

Carla Viegas [1,2,3,*], Marta Dias [1], Elisabete Carolino [1] and Raquel Sabino [4,5]

Combine the use of culture based-methods and molecular tools

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 (specific questions to address).**

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

Environmental Research 189 (2020) 109881

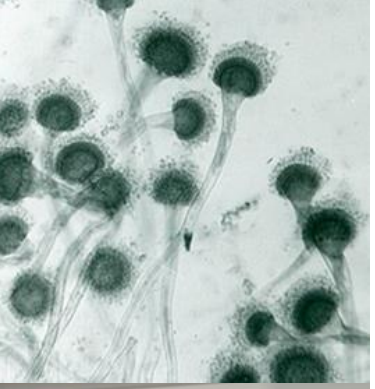
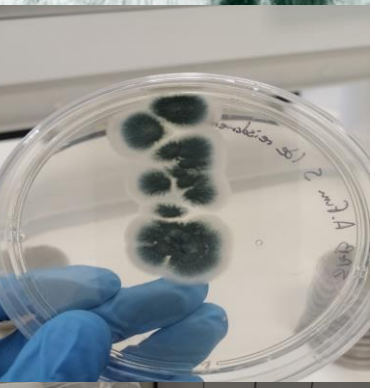
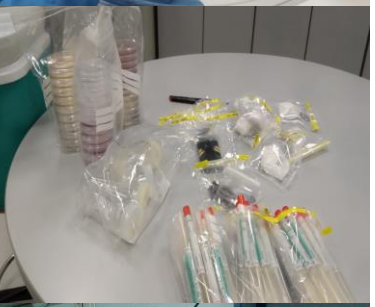
Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

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

Carla Viegas [a,b,c,*], Magdalena Twarużek [d,**], Marta Dias [a], Beatriz Almeida [a], Elisabete Carolino [a], Robert Kosicki [d], Ewelina Soszczyńska [d], Jan Grajewski [d], Liliana Aranha Caetano [a,e], Susana Viegas [a,b,c]

- **Azole resistance should be always screened**

Different settings were identified **as potential hotspots for the development of azole resistance.**

Isolate Number	Source	Azole Screening Media			Minimal Inhibitory Concentration (mg/L)			*cyp51A* Mutations
		ICZ	VCZ	PCZ	ICZ	VCZ	PCZ	
VA299CP	Dairy air	+	+	+	4	4	2	TR_{34}/L98H
VA610CP	Hospital air	±	–	–	2	0.5	0.5	No mutation detected
VA873CP	Waste sorting plant FRPD	+	+	+	4	2	1	TR_{34}/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_{34}/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).

INTERNATIONAL JOURNAL OF ENVIRONMENTAL HEALTH RESEARCH
https://doi.org/10.1080/09603123.2020.1810210
Taylor & Francis
Taylor & Francis Group
ARTICLE
Check for updates

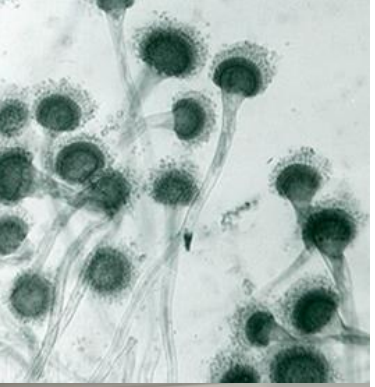
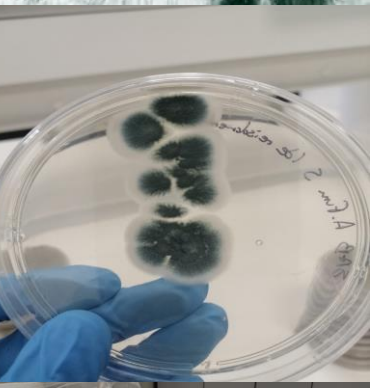
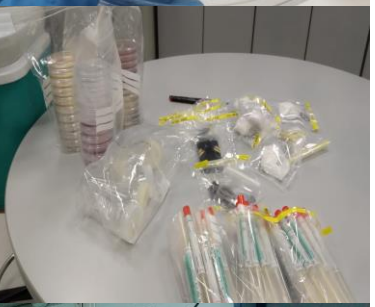
Algorithm to assess the presence of *Aspergillus fumigatus* resistant strains: The case of Norwegian sawmills

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

microorganisms
MDPI
Article

Azole-Resistant *Aspergillus fumigatus* Harboring the TR_{34}/L98H Mutation: First Report in Portugal in Environmental Samples

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


Mycotoxins contamination should be always assessed

Mycotoxins were detected in different settings unveiling this occupational health threat.

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

Environmental Research 189 (2020) 109881

Contents lists available at ScienceDirect

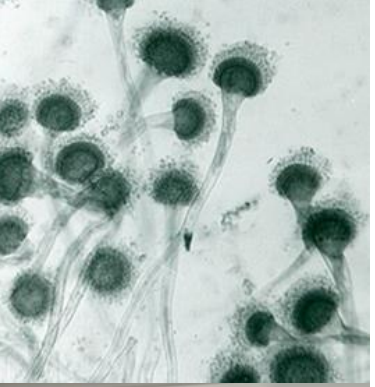
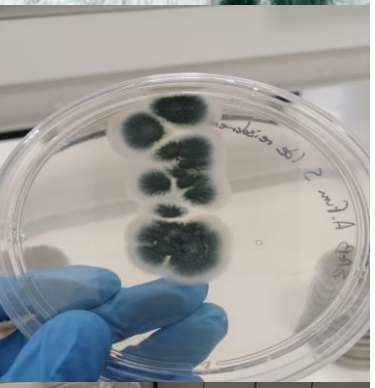
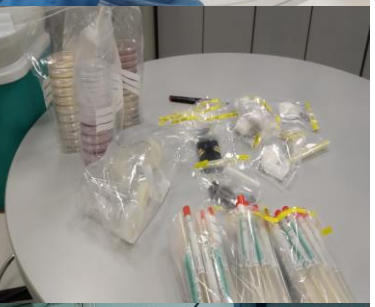
Environmental Research

journal homepage: www.elsevier.com/locate/envres

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

Carla Viegas [a,b,c,*], Magdalena Twarużek [d,**], Marta Dias [a], Beatriz Almeida [a], Elisabete Carolino [a], Robert Kosicki [d], Ewelina Soszczyńska [d], Jan Grajewski [d], Liliana Aranha Caetano [a,e], Susana Viegas [a,b,c]

- Mycotoxins were detected in 89.6% (60 out of 67)
- Seven mycotoxins were detected and the most reported was mycophenolic acid (89.6%), roquefortine C (43.3%) and griseofulvin (43.3%).
- Most samples presented only one type of mycotoxin (mycophenolic acid), with the maximum number of mycotoxins per sample being four mycotoxins


We need fungal targets for each occupational environment

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

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

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

Contents lists available at ScienceDirect

Journal of Environmental Management

journal homepage: www.elsevier.com/locate/jenvman

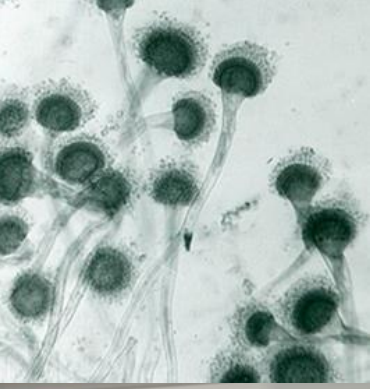
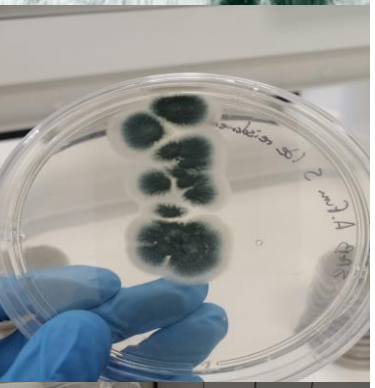
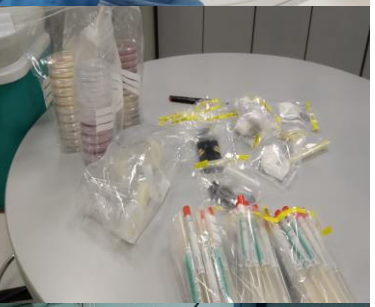
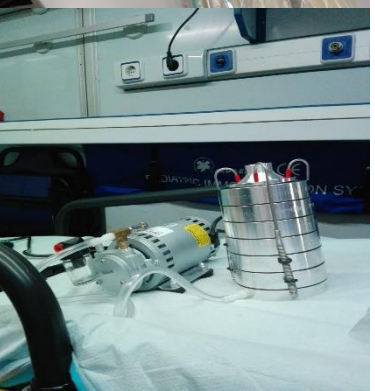
Research article

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

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

Aspergillus fumigatus (section *Fumigati*) as indicator of harmful fungal contamination in waste collection.

+ **Toxicological potential**

- All the studies allowed to **support preventive and corrective measures** regarding fungal and mycotoxins contamination.

Environmental Research 194 (2021) 110674

Contents lists available at ScienceDirect

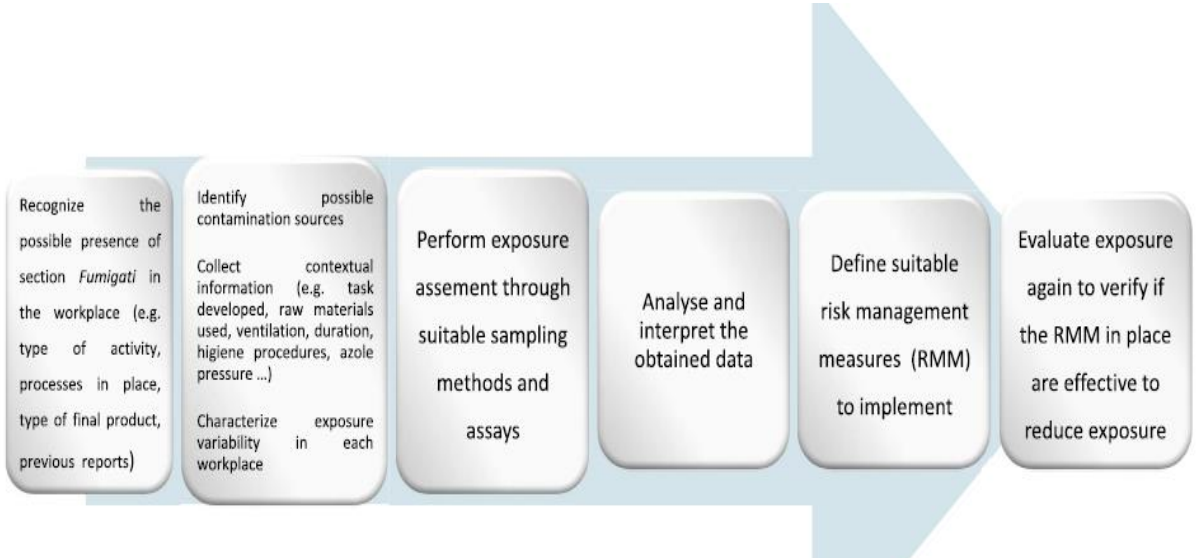
Environmental Research

journal homepage: www.elsevier.com/locate/envres

Review article

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

Carla Viegas [a,b,c,*], Liliana Aranha Caetano [a,d], Susana Viegas [a,b,c]



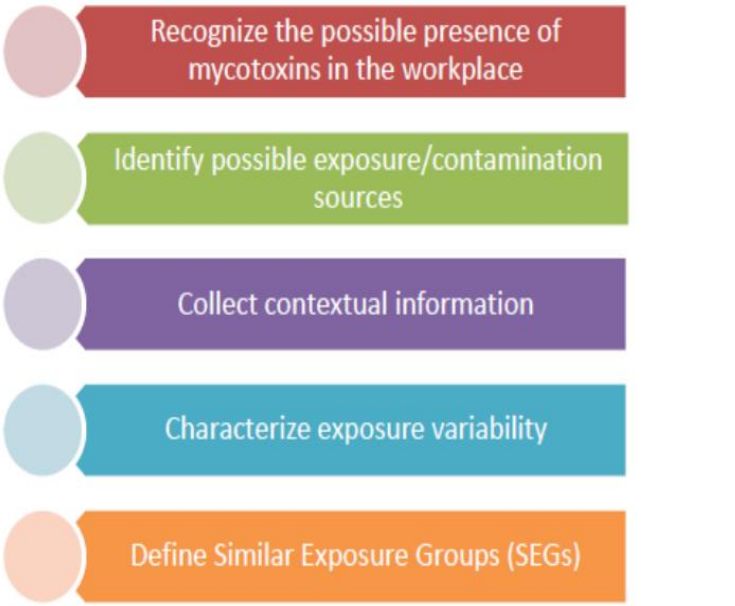
toxins

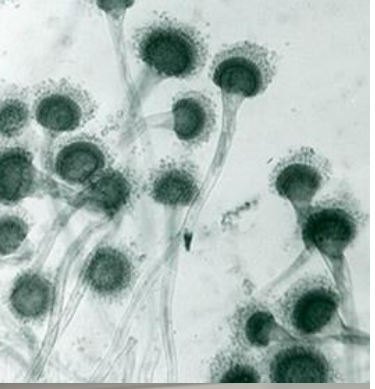
MDPI

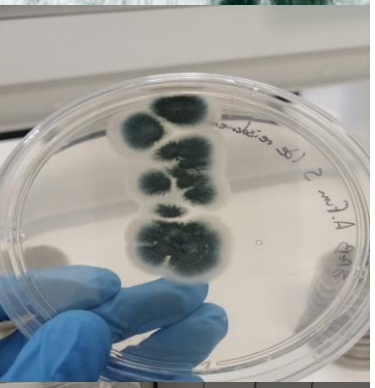
Review

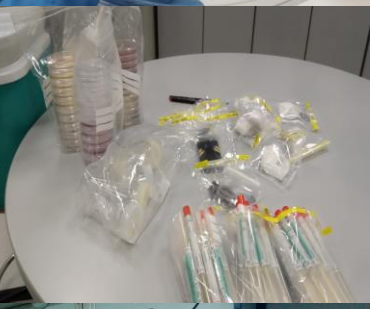
Occupational Exposure to Mycotoxins—Different Sampling Strategies Telling a Common Story Regarding Occupational Studies Performed in Portugal (2012–2020)

Susana Viegas [1,2,3,*], Carla Viegas [1,2,3], Carla Martins [1,2,4,5] and Ricardo Assunção [1,2,4,5]









Asociación Española de Higiene Industrial

International Commission on Occupational Health
Founded in 1906 as Permanent Commission

European Platform of Occupational Hygiene

Thank you for your attention!!

H&TRC author gratefully acknowledge the FCT/MCTES national support through the UIDB/05608/2020 and UIDP/05608/2020.

ESCOLA SUPERIOR DE TECNOLOGIA DA SAÚDE DE LISBOA
INSTITUTO POLITÉCNICO DE LISBOA

H&TRC Health and Technology Research Center

CISP CENTRO DE INVESTIGAÇÃO EM SAÚDE PÚBLICA

CHRC COMPREHENSIVE HEALTH RESEARCH CENTRE