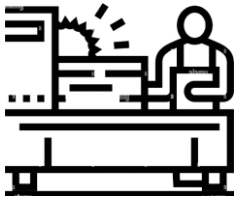
Sampling protocol to assess *Aspergillus* section *Fumigati* in woodworking environments

Marta Dias; Bianca Gomes; Pedro Pena; Renata Cervantes; Margarida Rodriguez; Bruna Riesenberger; Liliana Marques; Edna Ribeiro; Carla Viegas

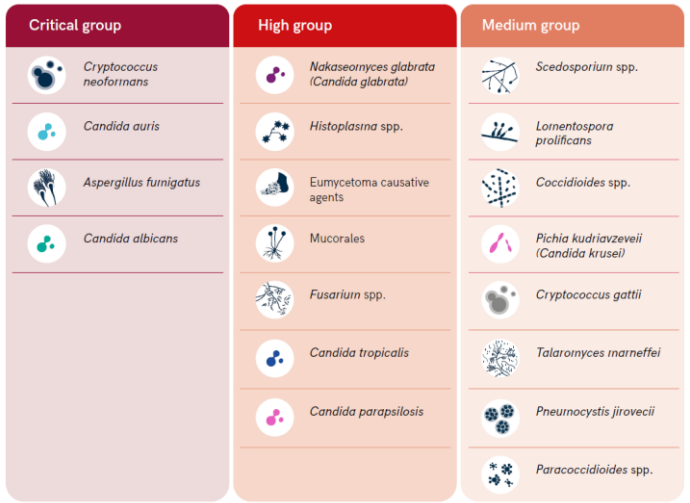
Introduction

- Portugal’s wood industry employs over 80,000 people and comprises about 16,600 enterprises in 2021 [1]
- Sawmill workers are exposed to several occupational hazards, including microorganisms [2,3]
- Health problems have been documented by workers exposed to wood dust infected with fungi [2,3]



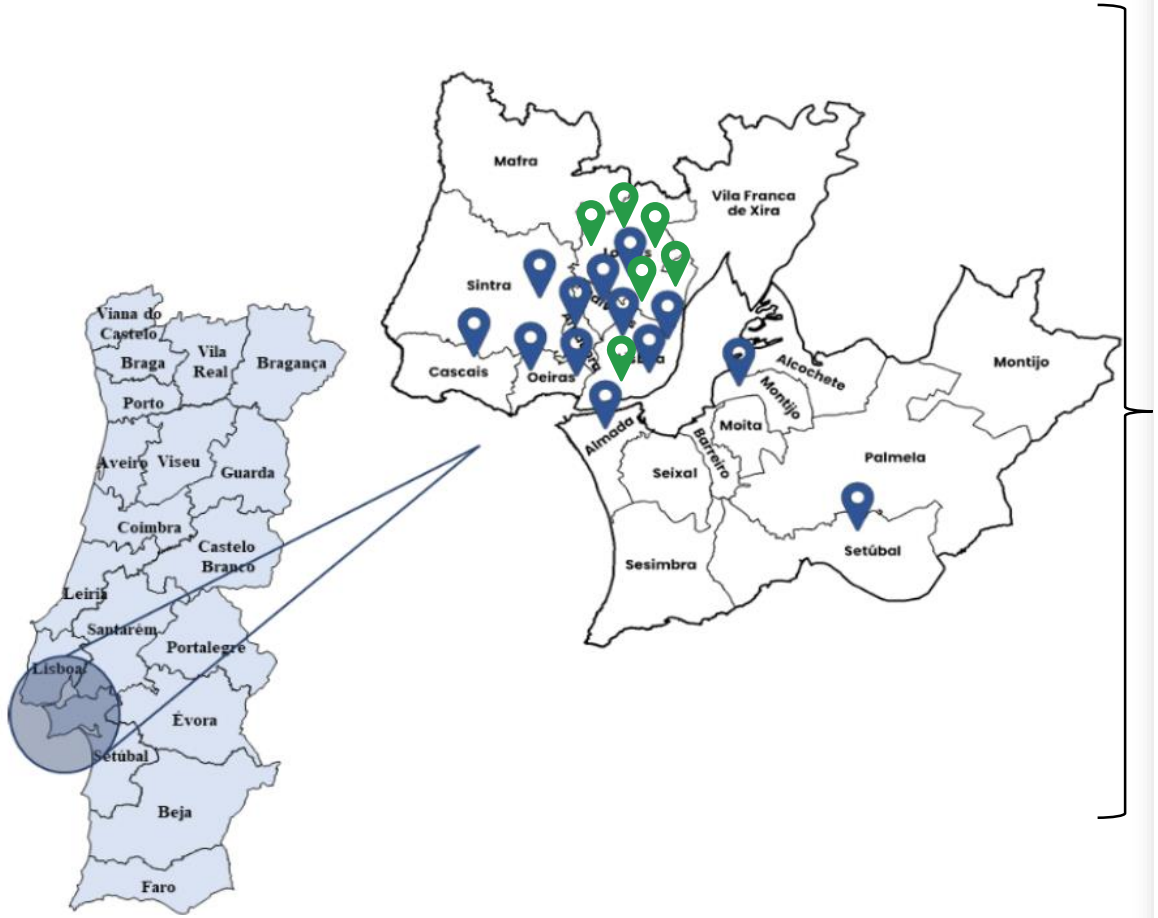


- A fungal priority pathogens list (WHO FPPL) was recently released by the World Health Organization (WHO) [4]
- It prioritizes fungal pathogens based on their perceived public health importance and unmet research and development needs [4]
- Some of the species on this list are frequently found in sawmills [5,6]
- Azole fungicides are commonly used by sawmills to protect wood [5]
- *Aspergillus* section *Fumigati* azole antifungal resistance has previously been observed in this environment [2,5]



The aim of this study was to establish a sampling protocol to effectively isolate *Aspergillus* section *Fumigati* in woodworking environments

Methodology



13 DIY Stores

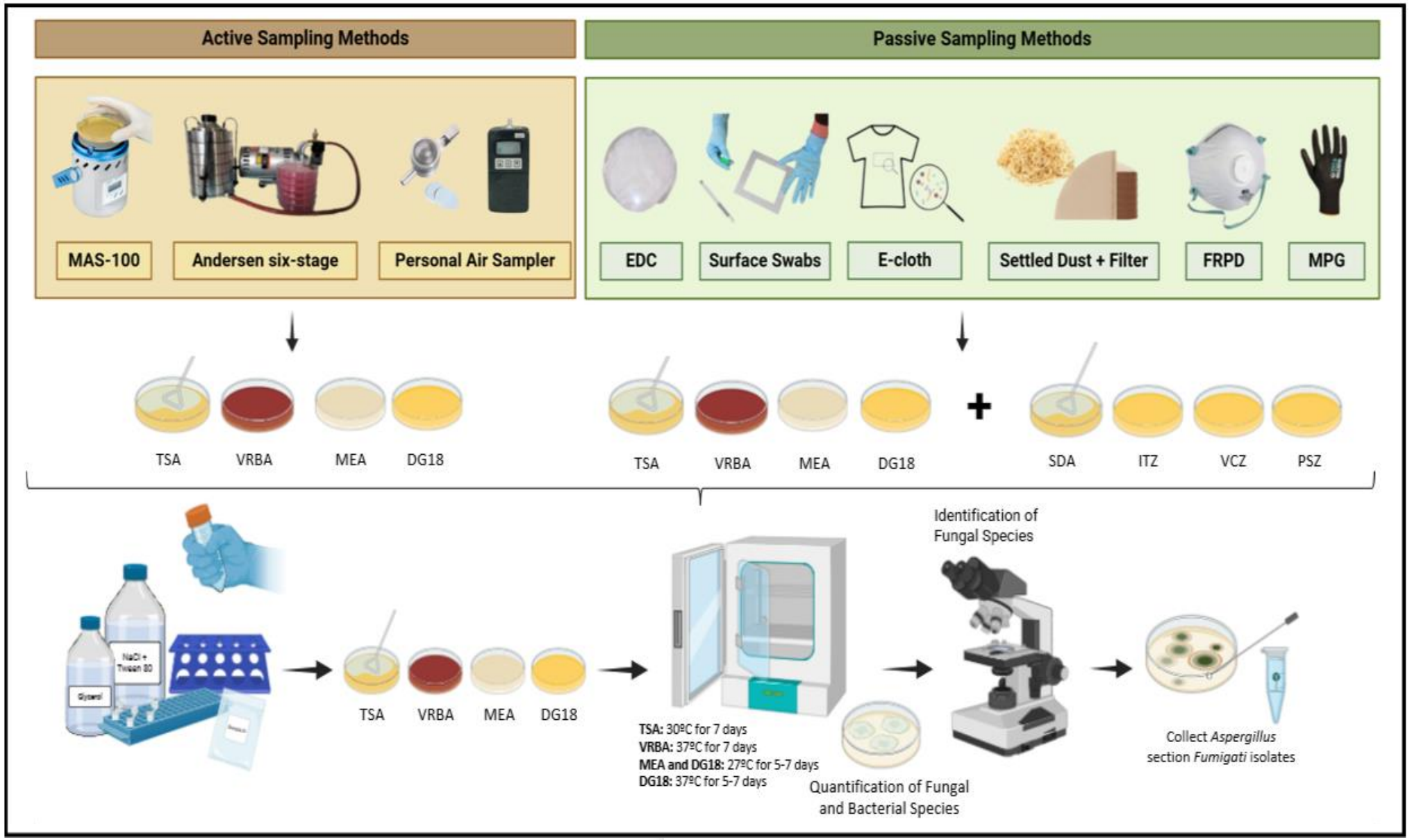
6 Sawmills

Active Sampling Methods

MAS-100 | Andersen six-stage | Personal Air Sampler

Passive Sampling Methods

EDC | Surface Swabs | E-cloth | Settled Dust + Filter | FRPD | MPG

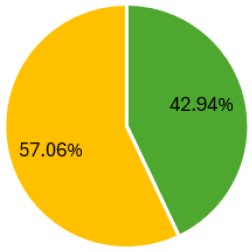

Active Sampling Methods
Passive Sampling Methods
MAS-100
Andersen six-stage
Personal Air Sampler
EDC
Surface Swabs
E-cloth
Settled Dust + Filter
FRPD
MPG
TSA
VRBA
MEA
DG18
TSA
VRBA
MEA
DG18
SDA
ITZ
VCZ
PSZ
NaCl + Tween 80
Glycerol
TSA
VRBA
MEA
DG18
TSA: 30ºC for 7 days
VRBA: 37ºC for 7 days
MEA and DG18: 27ºC for 5-7 days
DG18: 37ºC for 5-7 days
Identification of Fungal Species
Quantification of Fungal and Bacterial Species
Collect Aspergillus section Fumigati isolates

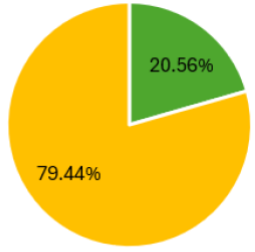
Results and Discussion

1153 *Aspergillus* sp. isolates were recovered from these woodworking environments

656 isolates from DIY stores

497 isolates from Sawmills





Aspergillus section Fumigati

Other Aspergillus sections

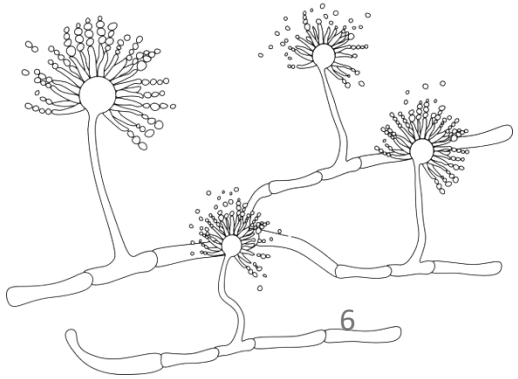
***Aspergillus* section *Fumigati* isolates:**

- 383 recovered from both woodworking environments
- 280 isolates (73.1%) from DIY stores
- 103 isolates (26.9%) from Sawmills

DIY stores:

- About twice the number of stores assessed
- About twice the number of samples (793 DIY stores > 484 sawmills)
- About twice the % of *Aspergillus* section *Fumigati* isolates

Possible to predict a similar number of *Aspergillus* section *Fumigati* isolates in both environments if we had similar sample characteristics.



Sampling Media:

- DG18 incubated at 37ºC presented a higher number of isolates in both environments (DIY stores: 71.5%; Sawmills: 71.9%)
- Followed by:
  - DG18 incubated at 27ºC in DIY stores (17.6%)
  - MEA in Sawmills (19.4%)

Sampling Method:

- Active Sampling: 184 isolates (49.7%)
- Passive Sampling: 186 isolates (50.3%)

DIY stores: Highest prevalence on active sampling methods (150 isolates (56.2%))
Sawmills: Highest prevalence on passive sampling methods (69 isolates (67%)

Sampling Method		DIY Stores			Sawmills			Total per Sampling Method
		MEA	DG18	DG18 incubated at 37ºC	MEA	DG18	DG18 incubated at 37ºC	
Active Sampling	Andersen six-stage	1	24	88	0	1	25	184
	MAS-100	2	2	0	0	2	0	
	Button Sampler	11	6	16	4	1	1	
Passive Sampling	EDC*	4	1	22	0	1	3	186
	EDCP*	3	4	9	4	0	7	
	Filter*	6	1	23	6	2	13	
	Surface Swab	2	4	19	3	2	8	
	Settled Dust	0	0	12	2	0	17	
	MPG*	0	0	2	1	0	0	
	FRPD*	0	5	0	0	0	0	
Total per Culture Media		29	47	191	20	9	74	370

*EDC - Electrostatic Dust Cloth | EDCP - EDC from t-shirt (e-cloth) | Filter - Filter from vacuum cleaner | MPG - Mechanical protective gloves | FRPD - Filtering respiratory protective device

Ventilation, building design, environmental features, water infiltrations, damage, geographical location, and the type of task developed affect fungi found indoors [2,7-10]

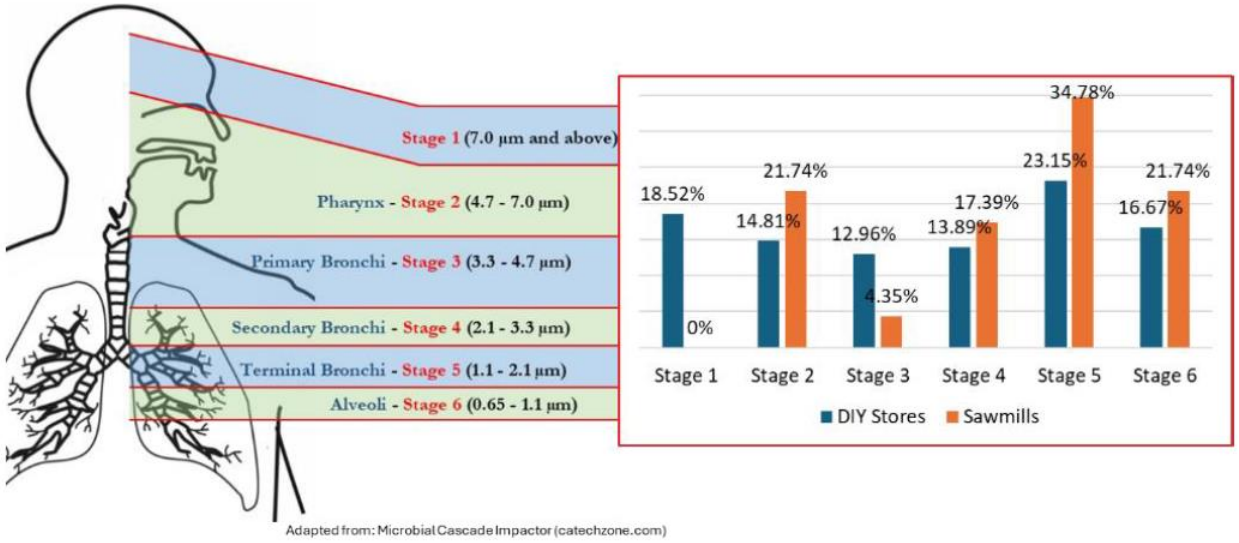
Differences in the characteristics and the number of isolates highlight the need to use both sampling methods in parallel.

Each environment had prevalence in different culture media and incubation temperature.

DG18 incubated at 37ºC had the highest number of isolates considering all sampling methods, culture media, and environment.

Andersen six-stage air sampler

- Stage 5 had the highest number of isolates (DIY stores: 23.15%; Sawmills: 34.78%)
- Followed by:
  - Stage 1 in DIY stores (18.52%)
  - Stages 2 and 6 in Sawmills (both with 21.74%)

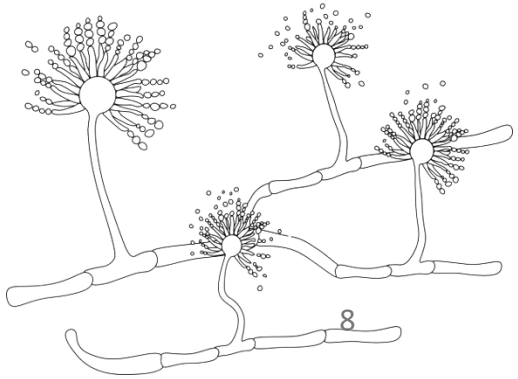


Adapted from: Microbial Cascade Impactor (catechzone.com)

High number of isolates highlight some of the characteristics of this sampling device

Highest number of isolates on stage 5 and 6 demonstrates that wood dust can penetrate until lung's gas exchange zone

High number of isolates in stages 1 and 2 may aggravate health conditions [11]

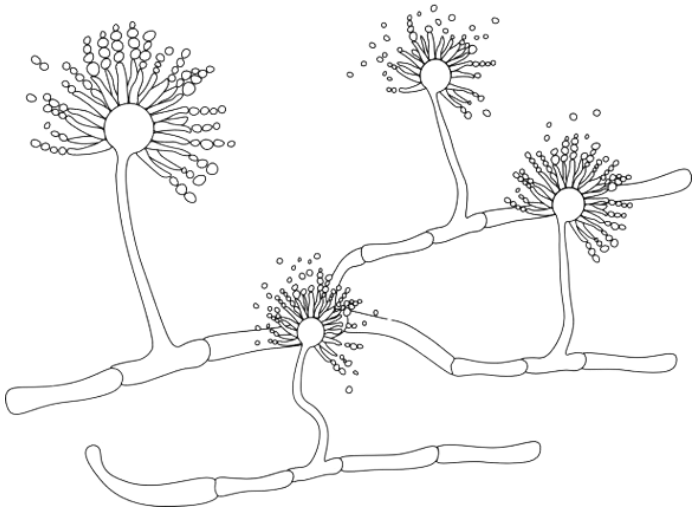


Conclusions

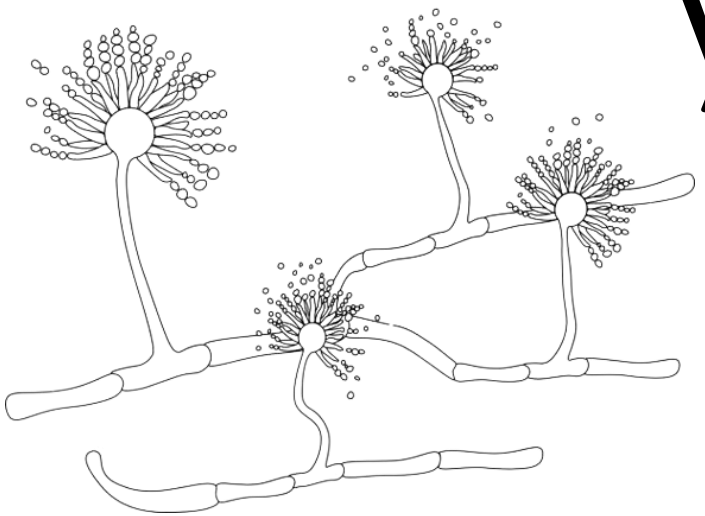
It was possible to identify:

- ✓ Differences between woodworking environments and how they can affect the growth of *Aspergillus* section *Fumigati*
- ✓ Which sampling methods, culture media and incubation temperature allows us to recover *Aspergillus* section *Fumigati* isolates more efficiently
- ✓ Both active and passive sampling methods are effective depending on the specific wood environment
- ✓ Incubation of DG18 at 37ºC is more effective for the recovery of *Aspergillus* section *Fumigati* isolates (thermotolerance).

This study validated the used protocol to assess *Aspergillus* section *Fumigati* in woodworking environments to enable Occupational Health Services to prioritize interventions in these specific occupational environments aiming to perform accurate exposure assessment and detailed risk assessment and management.



Thank you for your attention



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