

# Exploring fungal contamination in the sand and water around the Mediterranean Sea and other water bodies of Europe

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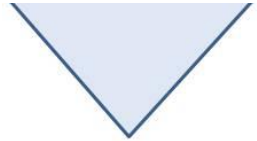
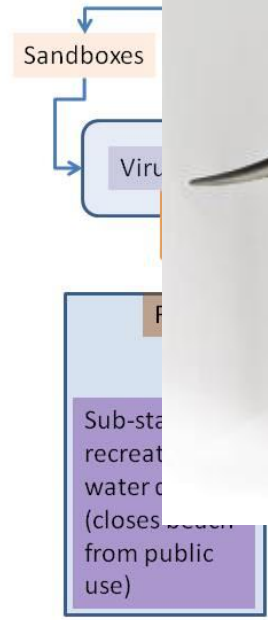
Relevance to human health and wellbeing

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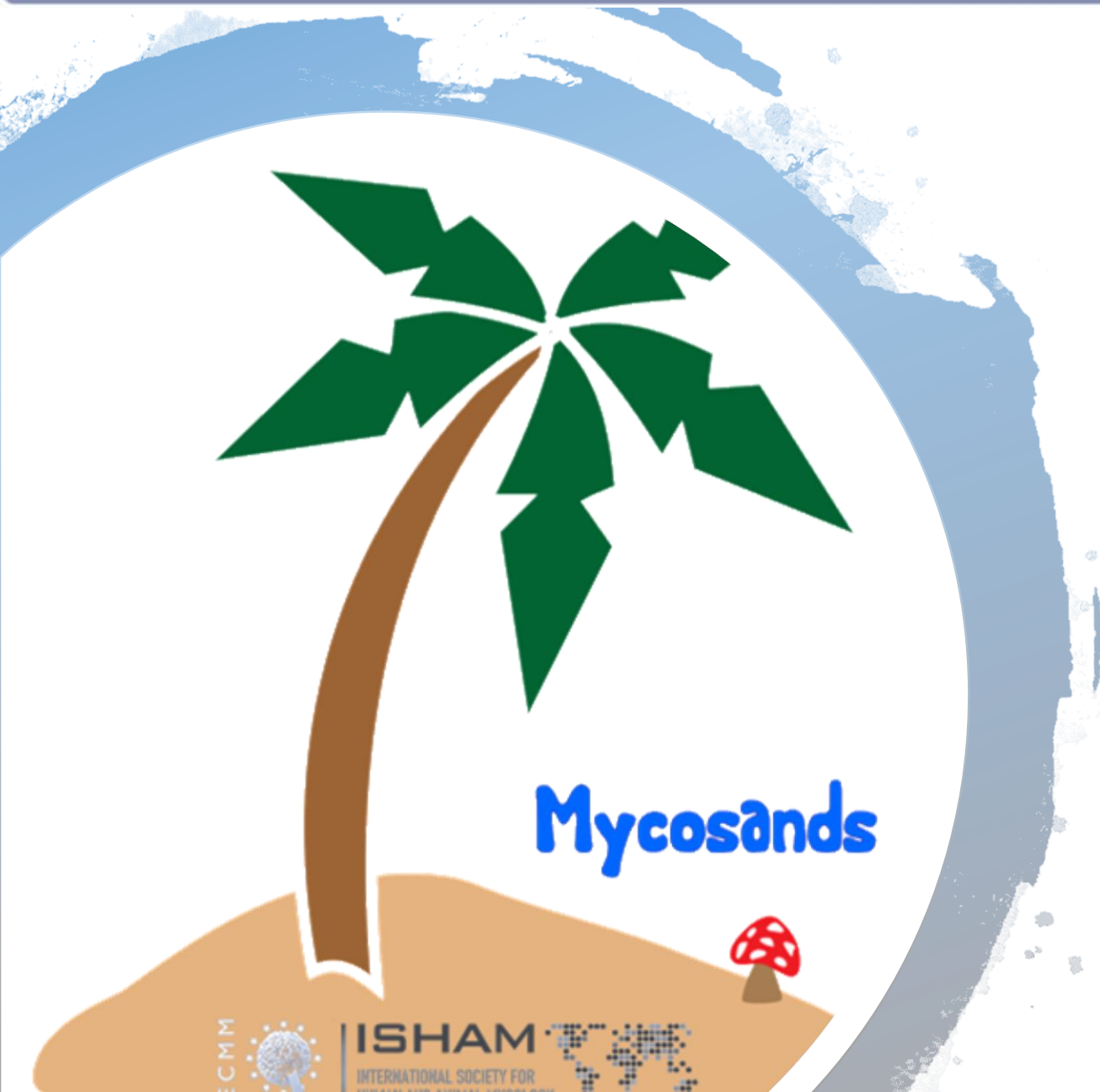
# Fungi.... The elephant in the room!

Not just faecal Indicator or



## Fungal infections transmittable at the beach





# Exploring fungal contamination in the sand and water around the Mediterranean Sea and other water bodies of Europe

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Relevance to human health and wellbeing

## ECMM Participants in Mycosands



Name	Country
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Joao Brandao	Portugal
Joseph Meletiadis	Greece
Laura Trovato	Italia (Sicilia)
Lena Klingspor	Sweden
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Marija Kataržytė	Lithuania
Mihai Mares	Romania
Mümtaz Güran	Turkey
Nilgün Çerikçioğlu	Turkey
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Salvatore Rubino	Italy (Sardinia)
Sevtaç Arikani Akdaglı	Turkey
Stéphane Ranque	France
Valentina Arsić Arsenijević	Serbia
Wieland Meyer	Australia
Willem Melchers	Netherlands
Wim Meijer	Ireland



# Outline of the study - Mycosands



## Fungal Parameters:

- A. Dermatophytes – indicators of human/animal dermal contamination (including the *Arthroderma insingulare* complex, formerly known as *Trichophyton terrestre*)
- B. *Candida albicans* – indicator of human fecal contamination
- C. Allergenic fungi – *Aspergillus*, *Penicillium* and others
- D. Total number of colonies per gram of sand

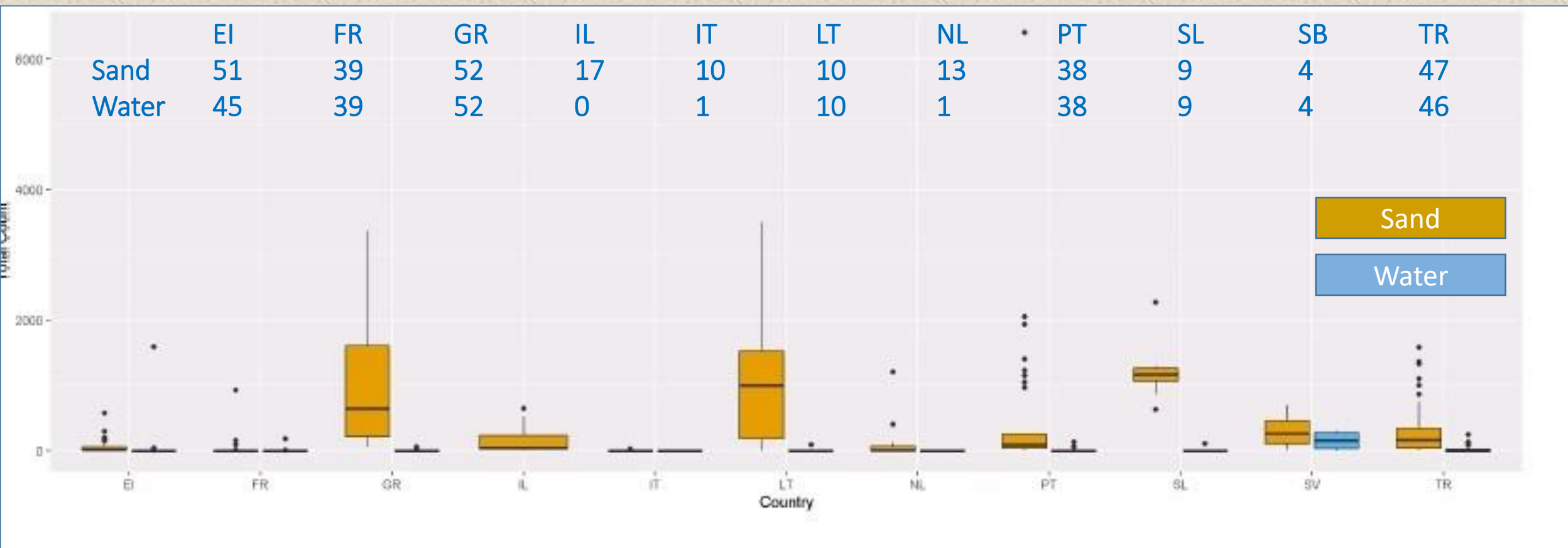
## Methodology:

- A. Culture and quantification
- B. Identification:  
Phenotypic, Mass Spectral (MALDI-TOF) and Molecular (ITS sequencing)

Other parameters include climates (sections), Fresh or sea water, Type of beach (urban or not), Humidity, Hours of sunshine, Date of sampling, Maximum temperature at sampling day, Any extreme weather events during the past 7 days?



# Boxplot of Inland and Coastal Samples analysed (by Country and type)





## First results 1/7 – Sand: Associations with type of beach (urban and non-urban)

### No associations:

- Black fungi (because  $p=0.242$ )
- Allergenic fungi (because  $p=0.615$ )

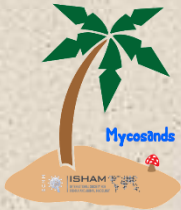
### Associations:

- *Cryptococcus* spp ( $p=0.019$ )
- *Rhodotorula* spp ( $p < 0.001$ )
- *Fusarium* spp ( $p=0.003$ )
- Unidentified fungi ( $p=0.002$ ), and mainly in Coastal beaches ( $p=0.032$ )



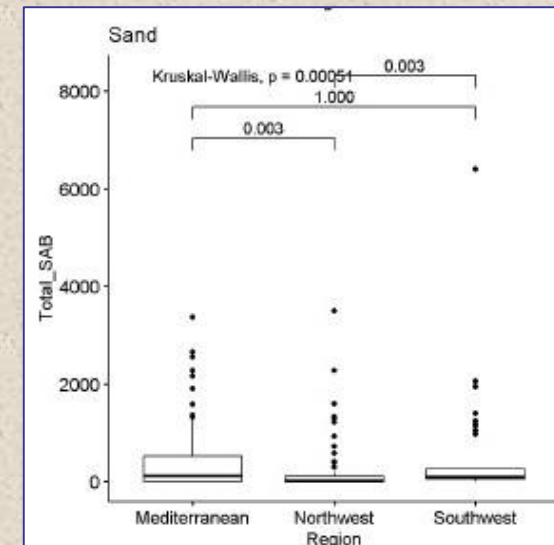
# First results 2/7 – Sand: Boxplot of total count of fungus by regions (geographical data)

Nr of samples	Mediterranean (N=154)	Northwest (N=98)	Southwest (N=38)
Mean (SD) of colonies isolated	410.28 (629.77)	193.54 (517.97)	542.16 (1182.07)
Median (Q1, Q3) of colonies isolated	116.67 (5.00, 525.00)	18.33 (2.71, 100.83)	90.83 (45.00, 256.25)



Fungi as a group prefer warmer climates (p < 0.001)

Descriptive statistics - Kruskal-Wallis rank sum test (multiple comparisons with bonferroni correction)



## First results 3/7 - Geographically relevant associations

### No associations:

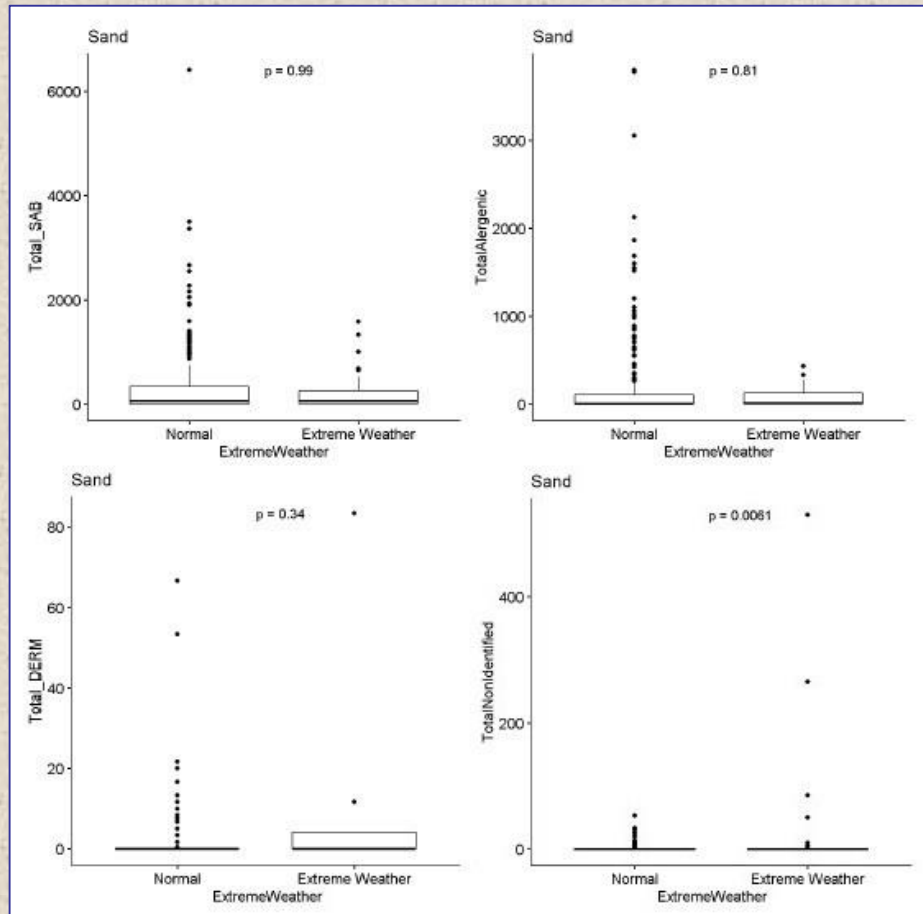
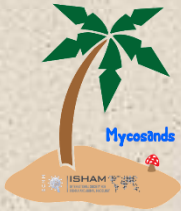
- *Aspergillus* section *Fumigati* (because  $p=0.0357$ )
- *Penicillium* (because  $p=0.197$ )

### Associations:

- *Aspergillus* section *Nigri* ( $p=0.019$  - ear infections)
- All species of *Aspergilli* ( $p=0.013$ )
- *Candida* species ( $p < 0.001$  – mucosal infections)
- Total colony count ( $p < 0.001$ ) and high humidity ( $p < 0.001$ )
- *Rhodotorula* spp ( $p=0.044$ )
- *Fusarium* spp ( $p=0.019$ )
- Allergenic fungi and unidentified fungi ( $p < 0.001$  for both)



# First results 4/7 - Boxplots of mould and total dermatophytes in sand samples by extreme weather (maxT >= 30°C).



Total fungal count in temperatures over and under 30°C (statistical significance not achieved for all but the unidentified fungi)

	Under 30°C Median (Q1, Q3)	Over 30°C Median (Q1, Q3)°C	p-value
Total colony count (upper left)	60.00 (5.00, 341.25)	60.00 (6.67, 260.00)	0.987
Total allergen count (upper right)	5.00 (0.00, 105.00)	10.00 (0.00, 125.00)	0.812
Total dermatophytes count (lower left)	0.00 (0.00, 0.00)	0.00 (0.00, 4.17)	0.333
Total undefined count (lower right)	0.00 (0.00, 0.00)	0.00 (0.00, 0.00)	0.006

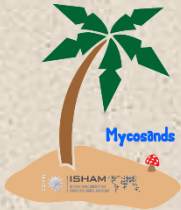
## First results 5/7 - Water bound - Inland Vs Coastal

### No associations:

- Black fungi (because  $p=0.129$ )

### Associations:

- *Rhodotorula* spp ( $p < 0.001$ )
- *Fusarium* spp ( $p=0.005$ )
- allergenic fungi ( $p=0.004$ )
- dermatophytes ( $p=0.026$ )



## First results 6/7 - Water bound - Geography

### No associations:

- *Aspergilli* & water of specific region (because  $p=0.171$ )
- *Rhodotorula* spp and run-off due to temperature preferences ( $p=0.204$ )

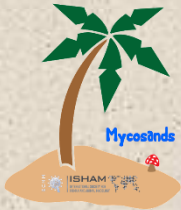
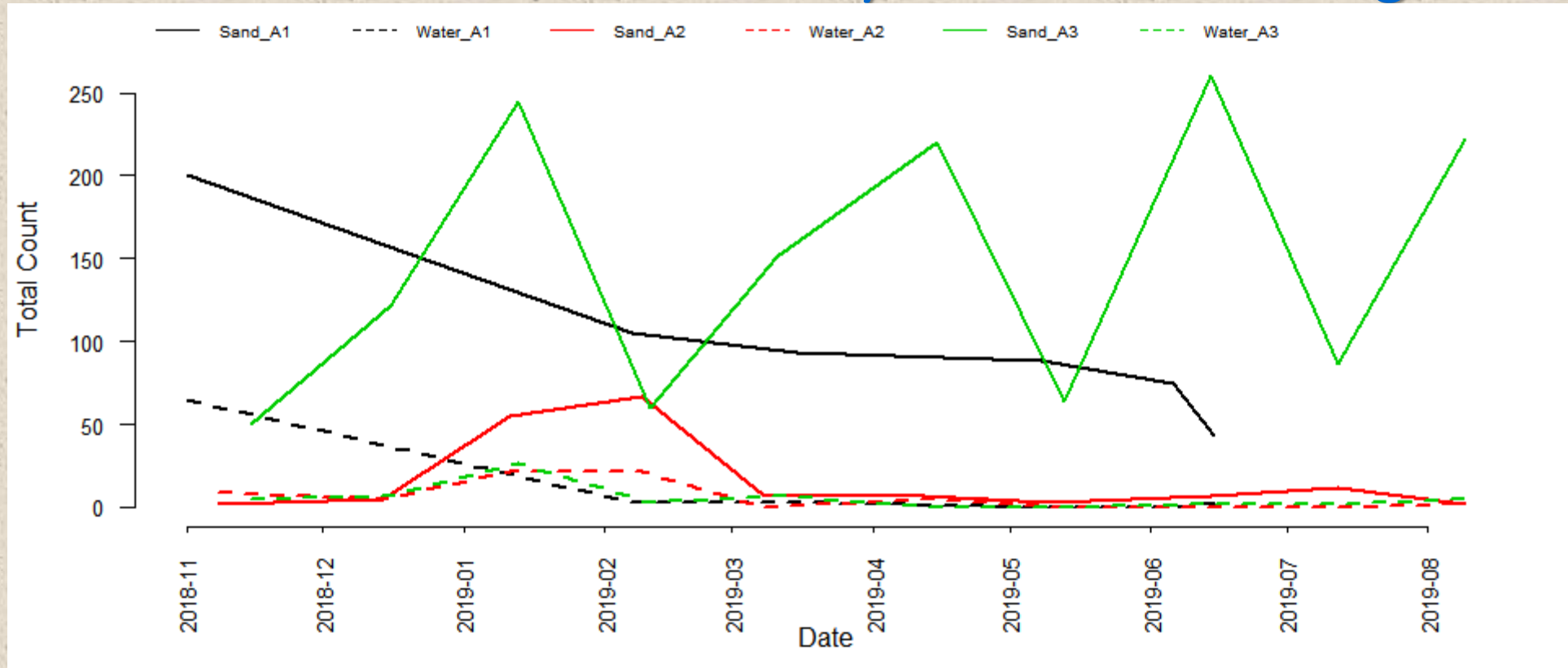
### Associations:

- Borderline association between *Aspergillus* section *Fumigati* and wastewater ( $p=0.050$ ) - species in this section are biofilm composing microbes. Associations with sand match only partially so it's very likely that they are partially associated with biofilms, common in water and sewage distribution systems, and treatment facilities.
- Partial association between *Candida* species & run-off ( $p=0.011$ )
- Allergenic fungi and black mould with warmer climate ( $p<0.001$ )

NOTE: we have very few data so these results must be taken carefully.



## First results 7/7 – Monthly variation in Portugal



This is the series of data from Portuguese Coastal Beaches. The first one to be checked for sand and for water. The green lines represents a beach from transition waters. The other two lines represent coastal beaches. The black line represents a beach in Algarve and the red line one in Lisbon area – erratic distribution of fungal colonies in the sand of the transition beach, almost trimestral which is the typical survival period of microbial communities in sand after a contamination event

## Fungi as pollution type indicators 1/2

“Raw sewage contamination of beach sand from a leaking underground sewage system – 30 cases of skin rash”

Fungi found are typical plant pathogens and saprophytes(\*) and common fecal pollution(\*\*) presences

\**Aspergillus section circumdati*

\**Fusarium sp*

\*\**Meyerozyma guilliermondi*

\*\**Rhodotorula sp*



## Fungi as pollution type indicators 2/2



(A) – Lid of the distribution box.

(B) – Inside of the distribution box after partial recovery (bottom) and before full sealing of the sidewalls.

(C1) – representation of the distribution box's position and beach access.

(C2) – Mechanic removal of all of the contaminated sand, as delineated by the analytical results on FIB until 50cm deep (80m<sup>3</sup> in total). Point 3 had the highest levels of contamination (>201 MPN of Coliforms, of *E. coli*, and *Enterococci*)

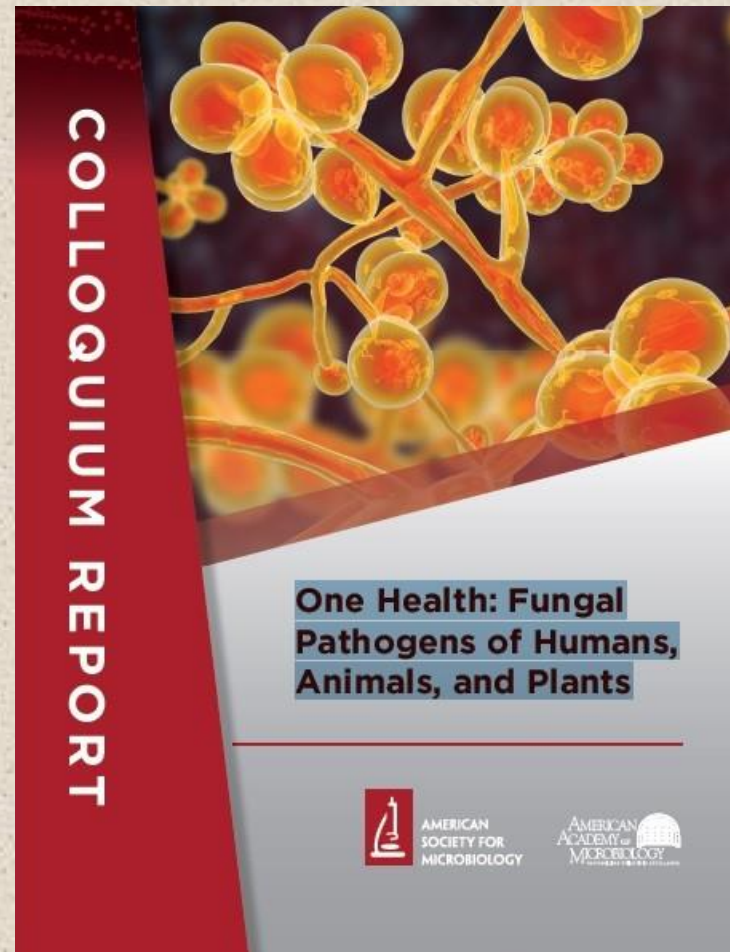
## Highlights and management actions 1/2 – General public

1. When you visit the beach, leave nothing behind but your footprints. You may even help clean up if you see a loose item that belongs in the trashcan
2. Shower thoroughly when you get home but make also use of showers at the beach, when available. Don't forget to get rid of any sand in your ears
3. If you have open wounds, dress them properly before you go to the beach and avoid exposure to water
4. Don't scratch your eyes if you have sand in them
5. Avoid overcrowded beaches and pets don't belong there. Take them to non-designated bathing areas instead. They will love the day at the beach in your company and you will see no evil eyes in your direction

## Highlights and management actions 2/2 – Beach managers

1. Keep an eye on your sand quality, not just the water!
2. Every beach has its unique microbiota. Some of the fungal species may help you determine possible kinds of exposure to pathogens, allergens and opportunists.
3. Preventive management actions means keep the litter contained and gone at the end of the day.
4. Sanitary inspections may help you avoid nasty media reported outbreaks that will affect visitors, professionals and local economies.
5. Don't comb the sand. Consider sifting instead. You never know what is lurking in the moist and deeper layers. If need be, contact sand cleaning professionals. They will help.
6. Expose data with the water quality but also the kind of fungal contaminants and respective levels, so chronically ill patients can determine if it's safe to be at your beach.
7. Your visitors want to have a good time and your professionals want to go home in good health after a day's work at the beach. Helping them do that is your biggest accomplishment.

# One Health: Fungal Pathogens of Humans, Animals, and Plants



## Acknowledgments

The entire team of the Mycosands project for generating new data on fungal contaminants of beach sand,

The European Confederation of Medical Mycology and The International Society of Human and Animal Mycology for financing Mycosands' meetings, calibration actions and publication fees,

All those involved in the episode in Azores, authors and otherwise,

The organisers for allowing me to show this presentation,



Thank you! Questions?

