Environmental Exposure and Health Impact: Fungal and Antifungal Reservoirs

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- Integrated Researcher at CE3C

Who’s the group?

Started as the Mycosands group/initiative - built to study recreational water and sand microbiota

Lead by Esther Segal, Jean Pierre Gangneux & João Brandão

THE TEAM:

France	
Name	**Area**
Frédéric Roger	Atlantic coast
Hélène Guegan	Atlantic coast
Jean-Pierre Gangneux	Atlantic coast
Laurence Delhaes	Bordeaux
Patrice Le Pape	Mediterranean coast
Sébastien Bertout	Mediterranean coast
Stéphane Ranque	Mediterranean coast

Lithuania	
Name	**Area**
Eglė Jonikaitė	Baltic Sea
Marija Kataržytė	Baltic Sea

Sweden	
Name	**Area**
Lena Klingspor	Atlantic coast

Croatia	
Name	**Area**
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Slaven Josic	Adriatic Sea

Italy	
Name	**Area**
Anna Maria Tortorano	Inland water Basin
Anna Prigitano	Inland water Basin
Antonella De Donno	Adriatic Sea
Florent Morio	Mediterranean coast
Francesca Serio	Adriatic Sea
Laura Trovato	Mediterranean coast
Massimo Cogliati	Adriatic Sea
Salvatore C. Oliveri	Mediterranean coast
Salvatore Rubino	Mediterranean coast

Turkey	
Name	**Area**
Betil Ozhak	Mediterranean coast
Çağrı Ergin	Mediterranean coast
Dilara Ogunc	Mediterranean coast
Gule Cinar	Mediterranean coast
Mümtaz Güran	Mediterranean coast
Nilgün Çerikçioğlu	Mediterranean coast
Sevtap Arikan-Akdagli	Mediterranean coast

Greece	
Name	**Area**
Aristea Velegraki	Mediterranean coast
Emanuel Roilides	Mediterranean coast
Joseph Meletiadis	Mediterranean coast
Maria Efstratiou	Mediterranean coast

USA	
Name	**Area**
Alexis Danielle Guerra	Irvine, Ca, USA
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Larissa Montas	Miami, Fl, USA
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Netherlands	
Name	**Area**
Wieland Meyer & Collaborators	Sidney
Marlou Tehupeiory-Kooreman	Inland water Basin
Paul Verweij	Inland water Basin
Willem Melchers	Atlantic coast

Romania	
Name	**Area**
Mihai Mares & Collaborators	Black Sea

Portugal	
Name	**Area**
Ana Sampaio	Atlantic coast
Cristina Veríssimo	Atlantic coast
Joao Brandao	Atlantic coast
Raquel Sabino	Atlantic coast
Siyu Huang	Atlantic coast
Susana Pereira	Atlantic coast

Ireland	
Name	**Area**
Wim Meijer & Collaborators	Irish Sea

Israel	
Name	**Area**
Esther Segal	Mediterranean coast
Michael Frenkel	Mediterranean coast

Serbia	
Name	**Area**
Aleksandra Barac	Inland water Basin
Valentina Arsić Arsenijević	Inland water Basin

ECMM

ESCMID | Fungal Infection Study Group EFISG

ISHAM International Society for Human and Animal Mycology

The point?

Overview of Environmental Exposure and Health Impact

Identifying Poorly-Studied Fungal Reservoirs in the Environment

Assessing Health Risks of Fungal Exposure

Fine-Tunning methodologies and Translational Research

Expanded Surveillance Scope to Environmental Theaters

Identifying Environmental Pathways of Antifungal Resistance

Climate Change and Globalization Effects On Mycobiomes

Theme #1

Fungal Diversity in Recreational Environments (Sand and water)

Fungal Diversity in Sand and Water

Fungal Diversity and Abundance

Studies reveal significant fungal diversity exists in both beach sand and recreational water environments across regions.

Environmental Influences

Climate, urbanization, and season affect fungal community composition, with hotter climates and cooler seasons favoring fungi.

Health and Regulation

Findings inform WHO guidelines with a 90 CFU/g sand regulation, highlighting overlooked fungal monitoring needs in health safety.

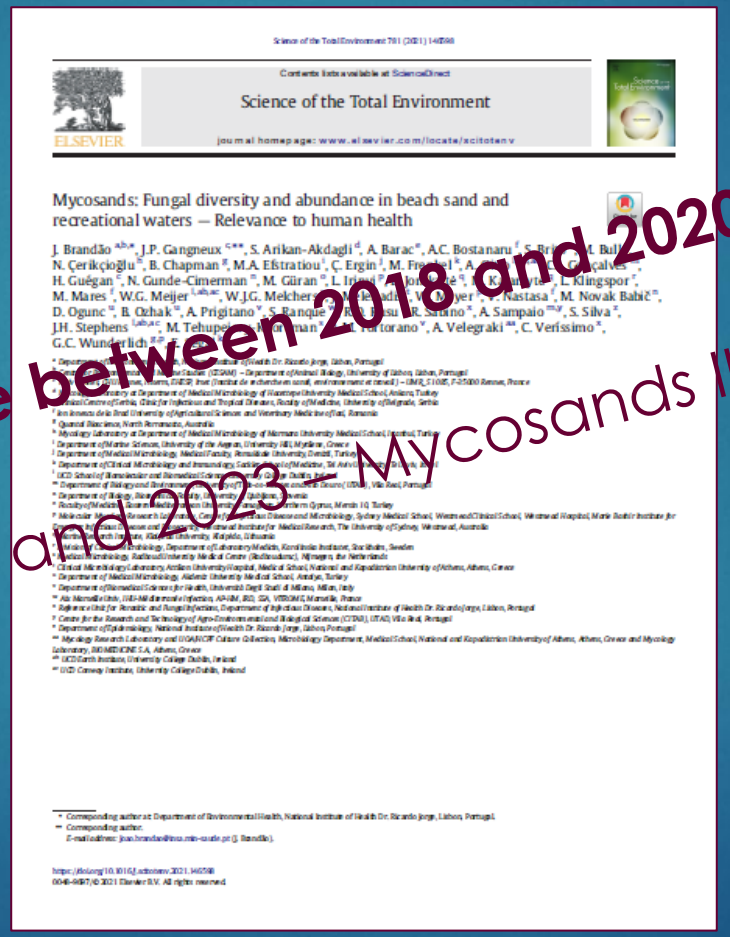
Pathogenic Fungi Presence

Fungi like *Candida albicans* and dermatophytes are present, emphasizing the importance of fungal monitoring.

Fungal Diversity in Sand and Water

Some of our publications	
Gangneux, J. P., Brandao, J., Segal, E., & ECMM/ISHAM MYCOSANDS study group (2024). Knowledge and regulation on fungal contamination of sand and water: Progress report and perspectives. Medical mycology, 62(2), myad137.	https://doi.org/10.1093/mmy/myad137
Steffen, H. C., et al. (2023). Health risk posed by direct ingestion of yeasts from polluted river water. Water research, 231, 119599. https://doi.org/10.1016/j.watres.2023.119599	https://doi.org/10.1016/j.watres.2023.119599
Brandão, J., et al. (2022). Climate Change Impacts on Microbiota in Beach Sand and Water: Looking Ahead. International journal of environmental research and public health, 19(3), 1444.	https://doi.org/10.3390/ijerph19031444
Cogliati, M., et al. (2023). Environmental and bioclimatic factors influencing yeasts and molds distribution along European shores. The Science of the total environment, 859(Pt 1), 160132.	https://doi.org/10.1016/j.scitotenv.2022.160132
Comparative analysis of microbial communities in six urban recreational beach sands and seawater in the United States and Australia	Submitted to Environmental Pollution
Brandão, J., et al. (2021). Mycosands: Fungal diversity and abundance in beach sand and recreational waters - Relevance to human health. The Science of the total environment, 781, 146598.	https://doi.org/10.1016/j.scitotenv.2021.146598
Mycosands II study (incubation at 37ºC and 40ºC to select invasive pathogens)	Manuscript under preparation
Mares et al. The tale of two seas: the Black Sea and the Mediterranean Sea	Submitted to Medical Mycology

MYCOSANDS - FUNGAL DIVERSITY AND ABUNDANCE IN BEACH SAND AND RECREATIONAL WATERS - RELEVANCE TO HUMAN HEALTH



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Mycosands: Fungal diversity and abundance in beach sand and recreational waters — Relevance to human health

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https://doi.org/10.1016/j.scitotenv.2021.146598
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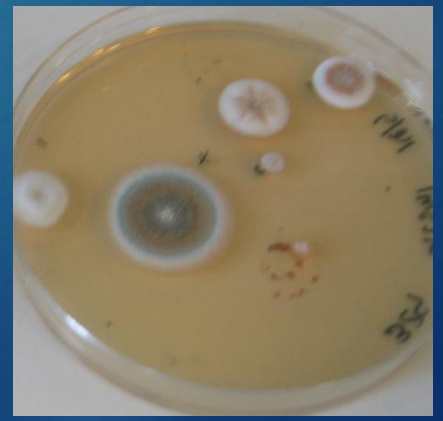
Sampling took place between 2018 and 2020 – Mycosands I

and between 2021 and 2023 – Mycosands II (being processed)



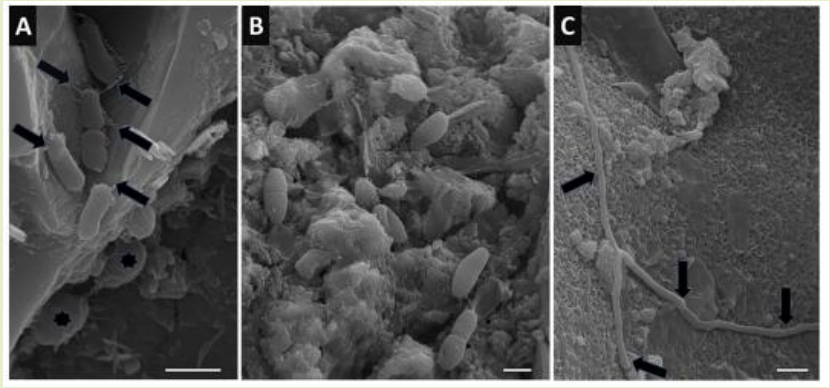
Brandão *et al.* 2019 - doi.org/10.1016/j.scitotenv.2021.146598

Fungal Diversity in Sand and Water

Mycosands: Fungal diversity and abundance in beach sand and recreational waters - relevance to human health https://doi.org/10.1016/j.scitotenv.2021.146598

Median number of **all fungi in any beach** sand ('All Fungi') is 89.2 CFU/g*
Inland beaches have **higher** counts **than coastal** beaches (2017.0 vs 76.7 CFU/g)
Composition of mycoflora differs between coastal and inland beaches.
Hotter climates **favour** presence of fungi in sand.
Fungi and Yeasts **correlate negatively** to the hours of **sunshine**
Fall/Winter present **higher** counts of fungi in sand **than Spring/Summer.**
Urban and **non-urban** beaches have **different mycoflora** composition
***Integrated (rounded to 90 CFU/g) in the new WHO guidelines – chapter 7**

"Fig. 3. Scanning electron micrographs of sand samples A (3.A), B (3.B) and 2 (3.C) showing colonisation by bacteria and/or fungi. Yeast (asterisks in A), hyphae (arrows in C) and bacterial fimbriae (arrows in A) are highlighted in the micrographs. Scale bar = 1 μm."

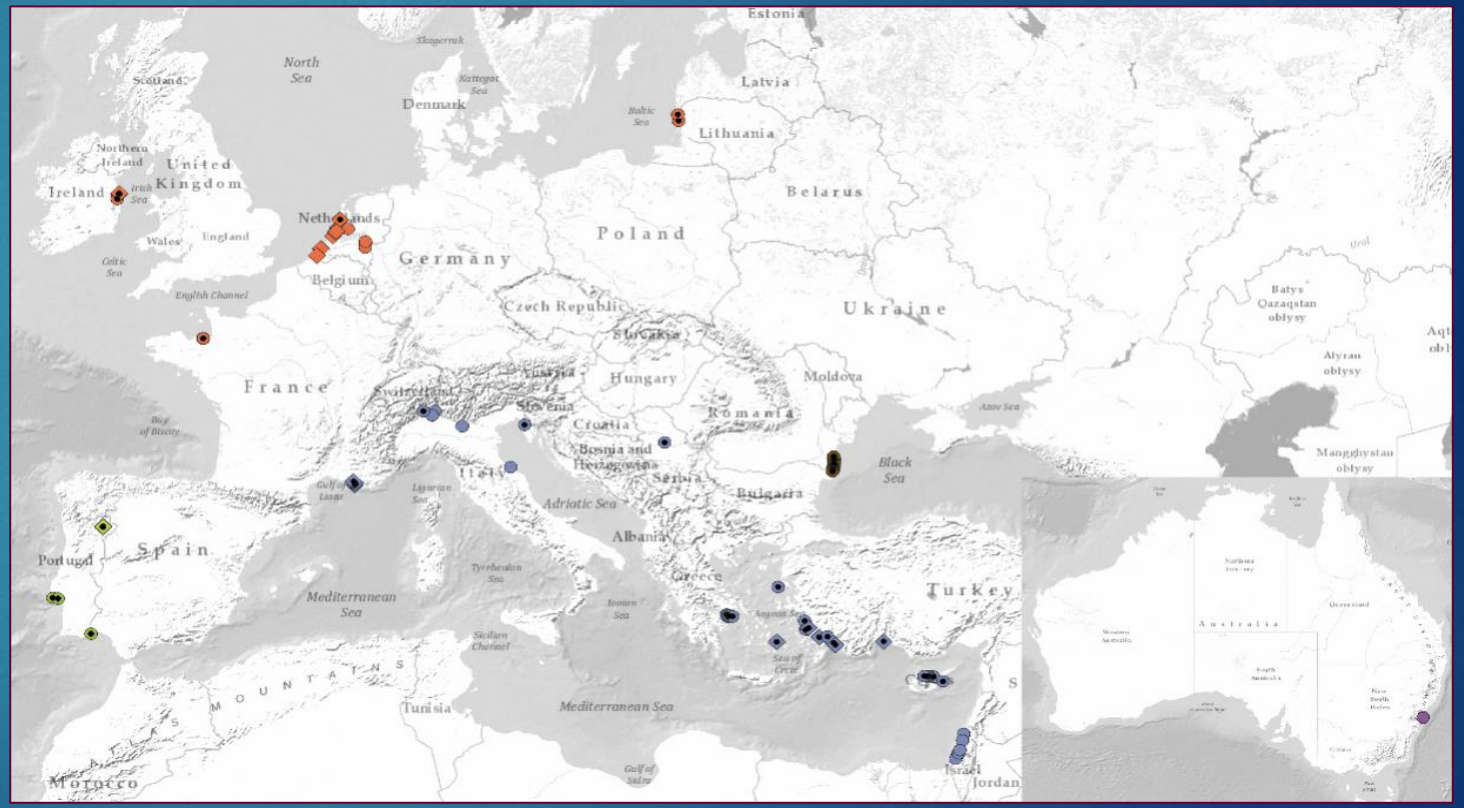


In Brandão J, et al. (2020). Untreated sewage contamination of beach sand from a leaking underground sewage system. Sci Total Environ. Oct 20;740:140237. https://doi.org/10.1016/j.scitotenv.2020.140237

Mycosands - Sampling sites

Geographical distribution of the sampling points using mapping with QGIS (Version 3.10.0-A Coruña). Circles correspond to urban beaches and diamonds to non-urban beaches. Dots within the shapes indicate water-sampling sites.

- Red=Northwest Europe,
- Green=Southwest Europe,
- Blue=Mediterranean,
- Brown=Black Sea
- Purple=Sydney (Australia)



Theme #2

Antifungal Resistance and Environmental Surveillance

Wastewater and Compost as Reservoirs

Reservoirs of Antifungal Resistance

Wastewater and compost serve as critical reservoirs for pathogens like *Candidozyma auris* and resistant *Aspergillus fumigatus* sensu stricto.

Challenges in Wastewater Treatment

Wastewater treatment plants often fail to remove antifungal compounds, contributing to resistance spread in natural ecosystems.

Surveillance Methods

Monitoring uses culture-based methods and quantitative PCR to detect antifungal pathogens in environmental samples.

Public Health Implications

Routine environmental monitoring aids early detection and informs strategies to control antifungal resistance spread.

Fungal Diversity in Sand and Water

Publications	
Gil *et al*. Predicted No Effect Concentrations (PNECs) of antifungals for wastewater management and agricultural use	Submitted to Medical Mycology
To be continued….	

Gil *et al* (submitted):

The study shows that fungi are incubated with antifungals during the wastewater treatment process and then released viable in the environment together with the non-removed antifungals

The study also provides predicted no-effect concentrations (PNECs) to guide regulation and establish safe discharge levels.

This has not been considered in the recent Urban Wastewater Directive Directive (EU) 2024/3019, concerning urban wastewater treatment.

Preprint

Theme #3

Fungi in drinking water quality

Recast of the Drinking Water Directive 2020/2184

Annex III: Specifications for the analysis of parameters
Part A : Microbiological parameters for which methods of analysis are specified

Parameter	Parametric value	Unit	Standard *
Escherichia coli (E. coli)	0	Number/100 ml (250 mL for bottled water)	EN ISO 9308-1 or **EN ISO 9308-2**
Coliform bacteria	0	Number/100 ml	EN ISO 9308-1or **EN ISO 9308-2**
Intestinal Enterococci	0	Number/100 ml (250 mL for bottled water)	EN ISO 7899-2
Colony count or heterotrophic plate counts at 22°C	No abnormal change		EN ISO 6222
Clostridium perfringens including spores	0	Number/100 ml	EN ISO 14189
Somatic coliphages	50 (for raw water)	Plaque Forming Units (PfU) /100 ml	For operational monitoring EN ISO 10705-2 or EN ISO 10705-3
*** or any alternative method equivalente according to ISO 17994**			

Exposure paths

Urban water supply

Internal Hospital plumbing
(biofilms may be presente and seed the water before its use)

In-hospital water use
(aerossols and streaming water)

Hospital Wastewater pre-treatment plant

Urban (community Wastewater treatment plant

Most common waterborne fungi in Europe causing opportunistic infections and other health problems

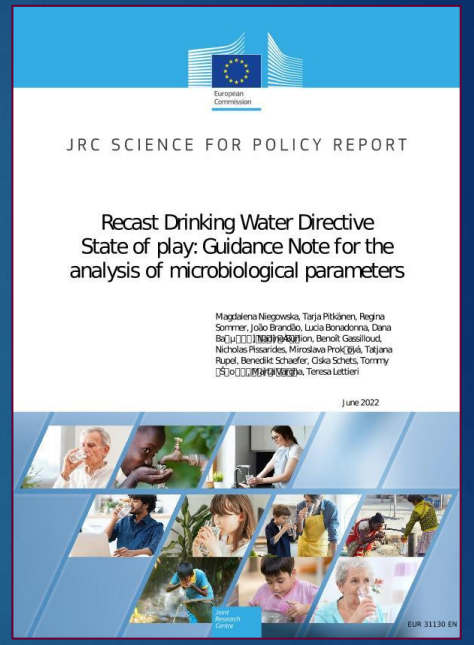
Alternaria:
A. alternata
Aspergillus:
A. flavus
A. fumigatus
A. niger
A. terreus
A. ustus
A. versicolor
Aureobasidium:
A. pullulans
A. melanogenum
Beauveria:
B. bassiana
Botrytis:
B. cinerea
Candida:
C. albicans
C. parapsilosis
species complex
Chaetomium:
C. globosum
Cladosporium:
C. cladosporioides
C. herbarum
C. sphaerospermum
Epicoccum:
E. nigrum
Exophiala:
E. dermatitidis
E. jeanselmei
Fusarium:
F. oxysporum
F. solani
Paecilomyces:
P. variotii
Penicillium:
P. brevicompactum
P. chrysogenum
P. citrinum
P. expansum
P. glabrum
P. simplicissimum
Purpureocillium:
P. lilacinum
Sarocladium:
S. kiliense
S. strictum
Scopulariopsis:
S. brevicaulis
Stachybotrys:
S. chartarum
Trichoderma:
T. harzianum
T. viride
Rhodotorula:
R. mucilaginosa
Mucor:
M. circinelloides
M. hiemalis
M. racemosus
Rhizopus:
R. arrhizus
R. stolonifer

Novak Babič, M., 2017 https://doi.org/10.3390/ijerph14060636

Recast Drinking Water Directive - State of play: Guidance note for the analysis of microbiological parameters

"The Guidance Note presents an overview of current and novel methods for testing the new microbiological parameters and provides recommendation on harmonising the choice of alternative methods at EU level."

"Note 2: **The DWD 2020/2184 does not yet include microfungi**, although there are currently MS which perform the analysis of this parameter and there is scientific evidence and recommendation to do so (Caggiano et al. 2020; Novak Babič et al. 2017;). At this stage, **they should be regarded in risk assessment and recommended to be monitored at end-points in hospitals and buildings where immune-compromised users may congregate.**"



Niegowska, M.Z., Pitkänen, T., Sommer, R., **Brandão, J.**, Bonadonna, L., Budišová, D., Burlion, N., Gassilloud, B., Pissarides, N., Proksova, M., Rupel, T., Schaefer, B., Schets, C., Šlapokas, T., Vargha, M. and Lettieri, T., Recast Drinking Water Directive - State of play: Guidance note for the analysis of microbiological parameters, EUR 31130 EN, **Publications Office of the European Union**, Luxembourg, 2022, ISBN 978-92-76-53688-8, http://doi.org/10.2760/14494, JRC129755.

Fungal Diversity in Sand and Water

Publications	
Babič, M. N., et al. (2017). Fungal Contaminants in Drinking Water Regulation? A Tale of Ecology, Exposure, Purification and Clinical Relevance. International Journal of Environmental Research and Public Health, 14(6), 636.	https://doi.org/10.3390/ijerph14060636
To be continued….	

Regulatory and Public Health Implications

Policy Recommendations and Future Directions

Expanding Water Quality Regulations

Current water directives focus on bacteria but should include fungi to better protect public health.

Fungal Risk Assessment Importance

Incorporating microfungi in risk assessments is critical, especially for immunocompromised individuals.

Future Research Priorities

Research should focus on infectious doses and standardized fungal monitoring procedures.

Regulatory Guidelines and Wastewater

Use PNECs for antifungal compounds to guide regulatory limits and manage wastewater effectively.

REGULATORY ADVANCES USING OUTPUTS OF THE GROUP

WHO, 2021 – Guidelines on recreational water quality (Chapter 7 – Sand)
European Commission - Recast Drinking Water Directive - State of play: Guidance note for the analysis of microbiological parameters (2022)
Blue Flag Portugal (https://bandeiraazul.abae.pt/wp-content/uploads/sites/2/2023/02/ABAE-sand-criterion_2023-1.pdf)

Conclusion

Summary and Call to Action

Environmental Reservoirs

Water, sand, wastewater, and compost are key environmental reservoirs that harbor fungi and spread antifungal resistance.

Multidisciplinary Insights

The ECMM group's multidisciplinary approach reveals fungal biodiversity and resistance linked to human activity.

Surveillance and Strategies

Integrated surveillance is vital for public health, requiring updated regulations including fungal parameters.

Call to Action

Ongoing research, policy development, and awareness are essential to tackle fungal reservoirs and safeguard health.

Future subjects – One health is the way to go!

A collaborative, multisectoral approach - interconnectedness of **human**, **animal**, **plant**, and **environmental** health to achieve optimal health outcomes for all

Next subjects of study
TIMM 12, Bilbao, 2025 : - Symposium 9: ECMM study group on water and sand: fungal and antifungals reservoirs (Joao Brandao) - Antifungal susceptibility and resistance assessment of mycobiota from the maritime ecosystem (Poster 80)
Exposure to *Aspergillus fumigatus* sensu stricto and AMR via compost
Quantification of risk of exposure based on infectious doses/potential – **please check:** Weiskerger, C. J., & Brandão, J. (2020). Fungal contaminants in water and sand: A new frontier for quantitative microbial risk assessment. *Current Opinion in Environmental Science & Health, 16*, 15–20. https://doi.org/10.1016/j.coesh.2020.03.001