









### Beach sand quality – a recent concern

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https://orcid.org/0000-0001-7553-227X Email: joao.brandao@insa.min-saude.pt National representative to the European Confederation of Medical Mycology and to the European Microbiology Experts sub-Group (for the European Bathing and Drinking Water Directives) & WHO expert member of the Guidelines development group





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- 1. Information about Sand (9 slides)
- 2. The case study of Porto Pim Beach, Faial, Azores, Portugal (8 slides)
- 3. The Mysosands initiative (6 slides)
- 4. Way forward (1 Slide)
- 5. Acknowledgements (1 slide)



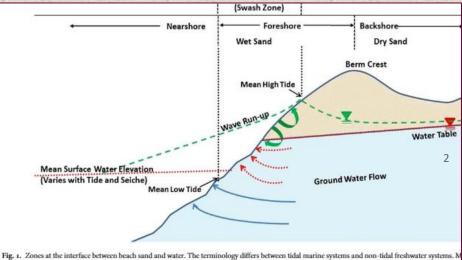
# Beach need-to-knows/concepts

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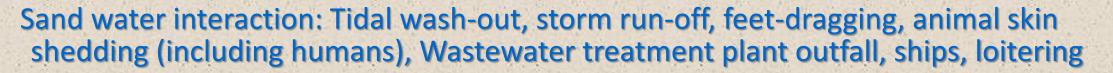
- Coastal sand composition of beaches varies from volcanic to silica-carbonated and other minerals and in granulometry<sup>1</sup>
- Beaches can be natural or man-made/renourished with sand from other locations, possibly carrying pathogens along<sup>2</sup>
- Inland beaches may have sand, sediment, or mixtures of both, in different composition ratios<sup>1</sup>
- Beach sand is divided in two areas: The wet area or swash zone and the dry area or supratidal zone<sup>2</sup>



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Fig. 1. Zones at the interface between beach sand and water. The terminology differs between tidal marine systems and non-tidal freshwater systems. M water elevations for marine systems tends to vary with tides. For freshwater systems, in particular within lakes, the mean surface water elevation tends t seiche. For river systems, mean surface water elevations vary with the seasonal elevation of the groundwater table and waves tend to run parallel to t opposed to the perpendicular direction observed in most marine and lake settings. (Image modified from Whitman *et al.*, 2014).





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Weiskerger, C. J., et al. 2019. Impacts of a changing earth on microbial dynamics and human health risks in the continuum between beach water and sand. Water research, 162, 456–470. <u>https://doi.org/10.1016/j.watres.2019.07.006</u>



# Why does it matter?

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- 1. Sand is a potential source for diffuse pollution in recreational water quality due to loitering, run-off, wild-life and feet-dragging,
  - 2. Sand is where beach users spend most of their time at the beach,

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- 3. On a windy day at the beach, wind will lift sand particles, bringing along viable microbes which will be deposited all of your body, including nostrils, eyes and ears,
- 4. Allergies can potentially originate in highly contaminated beach sand hours before,
- 5. Beach is recommended for minimizing skin problems and to help patients recover from other illnesses, who are often more susceptible to opportunistic microorganisms than the regular beach user.

# "The Micro Monsters Beneath Your Beach Blanket"

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There's a whole complex microbial community living in sand, with predation, decay, biofilms and even more complex organisms. Sand is a entire ecosystem!

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And we lie on it, and love to feel it under our bare feet during a long walk by the sea, etc.



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A Kinorhyncha in Hakai Magazine, Coastal science and societies (text by Adrienne Mason, March 21, 2016) http://www.hakaimagazine.com/videos-visuals/micromonsters-beneath-your-beach-blanket/

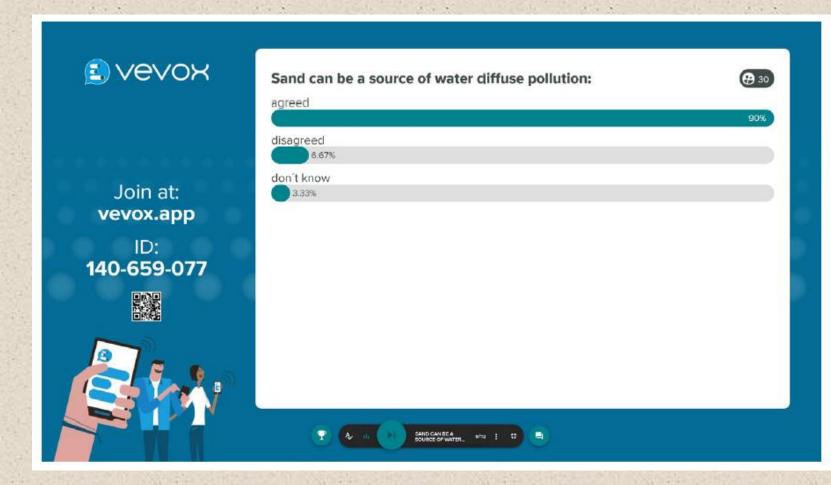








# The international community agrees to the relevance of sand



SG Health Related Water Microbiology and WHO Workshop: Recreational Water Quality - Translating Science to Policy IWA World Water Congress & Exhibition 11 – 15 September 2022 | København, Denmark





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- For Bacteria: 1975 in Hanauma Bay, O'ahu, Hawai'i Fujioka & Oshiro found that wild life and sand quality influenced the water quality on that site (an image will come next),
- For Fungi: 1960 in the Baltic Sea, Germany Schönfeld, Rieth and Thianprasit found geophilic dermatophytes in supratidal sand,
- Also for Fungi: 1973 in the Portuguese, Adriatic and German Baltic coasts -Müller found *Epidermophyton floccosum* (anthropophilic dermatophyte) in supratidal sands.

### Hanauma Bay, O'ahu, Hawai'i (photo by viator.com)



# Where do we stand now

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2017: Argentina included sand inspection for rubbish in its water quality standards,

- 2018: Lithuania added monitoring of helminths in sand to their National regulation,
- 2021: WHO launched its revised guidelines for recreational water quality and recommended sand monitoring,
- 2022: Blue flag in Portugal added sand quality to its awarding system, based on the enumeration per g of sand, of enterococci, *E. coli* and all Fungi.



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# **Current WHO recommendations:**

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 For FIB: 60 MPN/g for enterococci which was calculated through QMRA as the equivalent to 200 CFU/100 mL in water, which represents a risk of illness of less than 5% (H. Solo-Gabriele) - chapter 7 of the guidelines shows the calculations and supporting principles and literature.

 For Fungi: 90 CFU/g of all fungal species, following the Mycosands initiative results on a broad survey of beaches in Europe and Sydney, Australia – see next slides



GUIDELINES ON RECREATIONAL WATER QUALITY Volume 1 Control and First Waters





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"Untreated sewage contamination of beach sand from a leaking underground sewage system - An episode of skin rash was experienced by 29 people at a beach"



#### Contents lists a valiable at ScienceDirect

Science of the Total Environment



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

Untreated sewage contamination of beach sand from a leaking underground sewage system



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- <sup>1</sup> Direção Regional dos Assantas do Mar, Secretaria Regional do Mar, Cância e Tecnologia, Governo Regional das Apares, Harta, Apores, Partugal
- Departmentof (14), An Hundurd, and Brahoom explicit genering. University of Marri, Contl Gabler, FL USA.

#### **NIG NLIGHTS**

- GRAPHICAL ABSTRACT
- An episode of skin rash was experience by 30 people at a beach.
- · Analysis of the sand revealed a substand compatible with NACC on omitant hish levels of fascal indicator OPERATORIES.
- Sodium-hypochiorite was used for
- deaning and disinfection of toilet facili-
- A leakage in the sewage system was found to have been the cause of the outbreak.











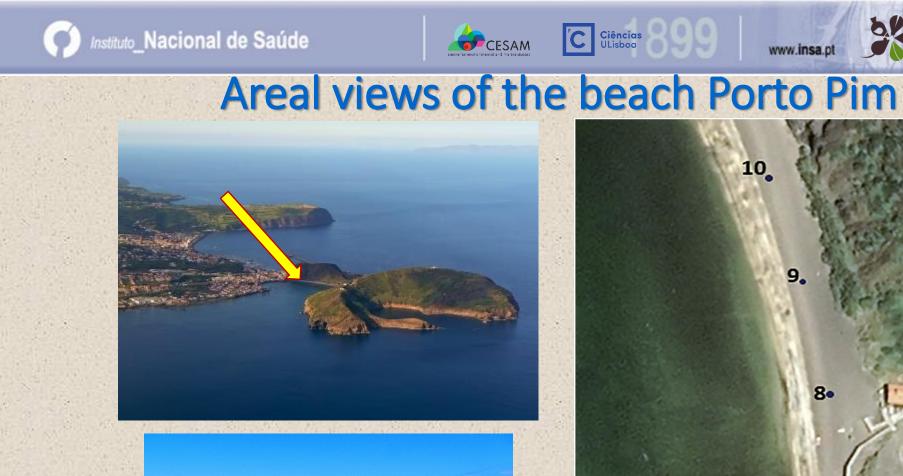
# What happened in this case?

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The chemical analysis of the sand revealed a substance compatible with Sodium-hypochlorite which was concomitant with high levels of viable faecal indicator organisms.

This chemical was used for a major cleaning and disinfection operation of the toilet facilities, due to the start of the bathing season.

A leakage in the sewage system was, in fact, the cause of the outbreak





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# Analytical results (Microbiology)

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	Coliforms	E. coli	Enterococci	Filamentous fungi	Yeast	Dermatophytes
	(100)*	(20)*	(20)*	(560)*	(60)*	(15)*
1st campaign (10th July 2019)						
Sample A	>201	>201	>201	100 (50 Fusarium sp)	<1.0	<1.0
Sample B	29	<1.0	9	109	90 (88 M. guilliermondii + 2	<1.0
					C. tropicalis)	
2nd campaign (23rd July 2019)						
Sample 1	2	2	4	84	<1.0	<1.0
Sample 2	>201	<1.0	>201	385	<1.0	<1.0
Sample 3	>201	<1.0	6	85	65 (64 Rhodotorula sp.)	<1.0
Sample 4	33	<1.0	145	125	<1.0	<1.0
Sample 5	23	<1.0	<1.0	107	<1.0	<1.0
Sample 6	<1.0	<1.0	<1.0	190 (152 Fusarium sp.)	<1.0	<1.0
Sample 7	13	<1.0	1	183 (165 Aspergillus	2	<1.0
				section Circundati)		
Sample 8	9	<1.0	<1.0	55	<1.0	<1.0
Sample 9	2	<1.0	13	5	2 (Rhodotorula sp.)	<1.0
Sample 10	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0

'MPN' = Maximum Probable Number, 'CFU' = Colony Forming Unit. \*Maximum reference values per gram of sand.



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(A) - Lid of the distribution box.

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(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 (80m3 in total). Point 3 had the highest levels of contamination (>201 MPN of Coliforms, of *E. coli*, and *Enterococci*)

Brandão et al. 2020 'Untreated sewage contamination of beach sand from a leaking underground sewage system' Science of The Total Environment 740:140237



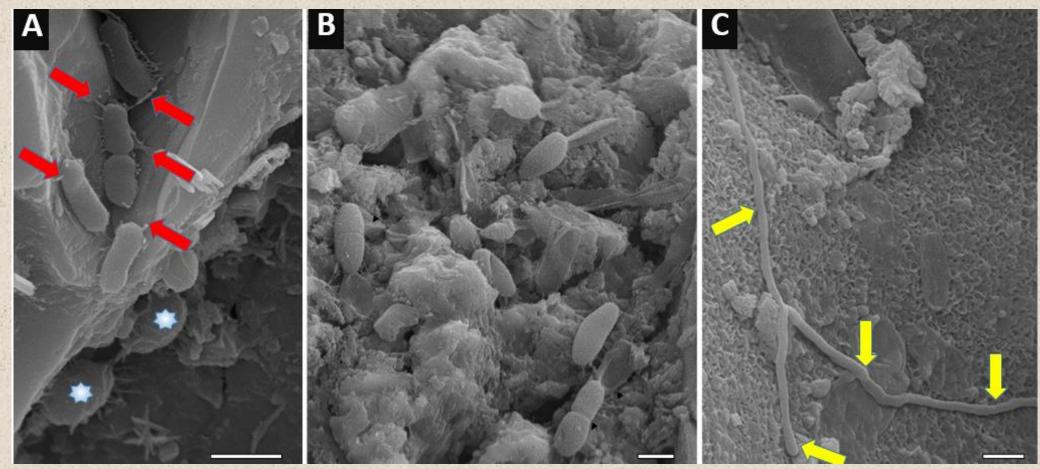




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# 'Little monsters' on the surface of grains of sand

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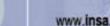


### Scanning electron microscopy of sand particles

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# Fungi can be informative as to types of contamination

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

### \*Aspergillus section circundati \*Fusarium sp

\*\*Meyerozyma guilliermondi \*\*Rhodotorula sp

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# How did it end?

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- The island's health protection office interdicted the use of the beach until the pollution source was fully resolved and analytically proved.
- The beach was closed for more than a month.
- There were no reported follow-up cases of GI illness in the rash patients, despite the high levels of viable faecal indicator bacteria.
- A tropical storm destroyed the entire beach access shortly after the necessary corrections were made but soon became fully functional once again.





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# Can Microbial Source Tracking (MST) be used for sand?

"supratidal sand samples were collected from several sites along the beach, followed by microbial source tracking (MST) analyses of Bacteroides marker genes for five animal species, including humans."



International Journal of Environmental Research and Public Health

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Article

### Microbial Source Tracking as a Method of Determination of **Beach Sand Contamination**

Elisabete Valério 1,2,\*,†, Maria Leonor Santos 1,†, Pedro Teixeira 1,2,3, Ricardo Matias 4, João Mendonca 5, Warish Ahmed 6 and João Brandão 1,2

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- † These authors contributed equally to this work.

Citation: Valério, E.: Santos, M.L.: Teixeira, P.: Matias, R.: Mendonca, I.: Ahmed, W.; Brandão, J. Microbial Source Tracking as a Method of Determination of Beach Sand Contamination. Int. I. Environ. Res

Abstract: Beach sand may act as a reservoir for numerous microorganisms, including enteric pathogens. Several of these pathogens originate in human or animal feces, which may pose a public health risk. In August 2019, high levels of fecal indicator bacteria (FIB) were detected in the sand of the Azorean beach Prainha, Terceira Island, Portugal. Remediation measures were promptly implemented, including sand removal and the spraying of chlorine to restore the sand quality. To determine the source of the fecal contamination, during the first campaign, supratidal sand samples were collected from several sites along the beach, followed by microbial source tracking (MST)





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In August 2019, high levels of fecal indicator bacteria (FIB) were detected in the sand of the Azorean beach Prainha, Terceira Island, Portugal

 Remediation measures were promptly implemented, including sand removal and the spraying of chlorine to restore the sand quality, followed by microbial source tracking (MST)

 Some of the sampling sites revealed the presence of marker genes from dogs, seagulls, and ruminants. The municipality enforced restrictive measures for dogwalking at the beach, which local inhabitants often did in the evenings





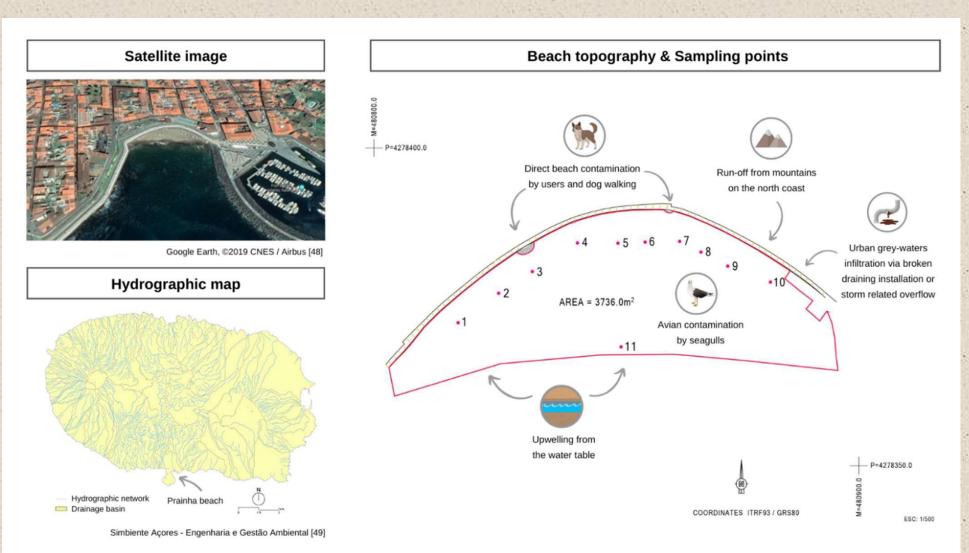
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# Prainha and the pollution

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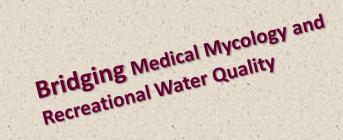
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THE TEAM:

### The Mycosands working group (Lead by Esther Segal, Jean Pierre Gangneux & João Brandão)

Aim: Fungal diversity and abundance in beach sand and recreational waters - relevance to human health



Keywords:	Environmental
Beach Sand	Health
<b>Recreational water</b>	<b>Inland and Coastal</b>
quality	Beaches
Anti-Microbial	Urban Beaches
Resistance	Moulds
Core taxa	Yeasts
Ecology	

Fra	ince	lta	aly	Greece		
Name	Area	Name	Area	Name	Area	
		Anna Maria	Inland water	Aristea	Mediterranea	
Frédéric Roger	Atlantic coast	Tortorano	Basin	Velegraki	coast	
		Anna Prigitano	Inland water	Emanuel	Mediterranea	
Hélène Guegan	Atlantic coast		Basin	Roilides	coast	
Jean-Pierre		Antonella De		Joseph	Mediterranea	
	Atlantic coast	Donno	Adriatic Sea Mediterranean	Meletiadis	coast	
Gangneux		Donno		Meretiaurs	Mediterranea	
Laurence	Bordeaux	Florent Morio		Maria Efstratiou		
Delhaes	Dorucaux		coast		coast	
Patrice Le Pape	Mediterranean	Francesca Serio	Adriatic Sea	USA		
Fatrice Le Fape	coast			Name	Area	
Sébastien	Mediterranean	Laura Trovato	Mediterranean coast	Alexis Danielle	Aica	
Bertout	coast			-	Irvine, Ca, USA	
Stéphane	Mediterranean	Massimo Cogliati	Adriatic Sea Mediterranean coast	Guerra	Miami, Fl, USA	
Ranque	coast	Wassinio Cognati		Helena Solo-		
		Salvatore C.		gabriele & Co		
Lith	uania	Oliveri		8		
Name	Area		Mediterranean	Larissa Montas	Miami El LIS/	
		Salvatore Rubino	coast	Larissa ivioritas	Miami, Fl, USA	
Eglė Jonikaitė	Baltic Sea	coust		Lunny linna		
	Baltic Sea	Turkey		Sunny Jiang	Irvine, Ca, USA	
Marija Kataržytė		Name	Area	Nethe	erlands	
Sweden		Betil Ozhak	Mediterranean	Name	Area	
		Beth Ozhak	coast	Wieland Meyer	Alea	
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		Co You Fundin	Mediterranean	& Collaborators	Sidney	
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# Fungal diversity and abundance in beach sand and recreational waters - relevance to human health

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- "91 bathing sites, 372 samples of sand, 13 countries"
- "315 water samples,11 countries"



Science of the Total Environment 781 (2021) 140299

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

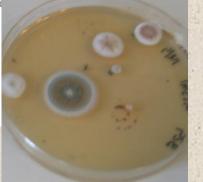


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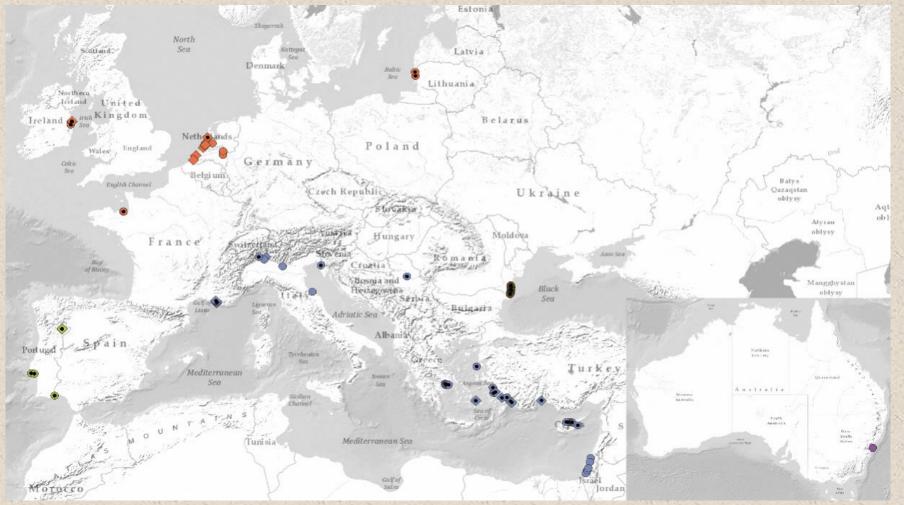


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# **Mycosands Sampling sites**

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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 and Purple=Sydney (Australia)









# Mycosands conclusions 1/2

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- 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)
- Species composition of mycoflora differs between coastal and inland beaches.
- Hotter climates favour the presence of fungi in sand.
- Fungi and Yeasts correlate negatively to the hours of sunshine

\*Integrated (rounded to 90 CFU/g) in the WHO guidelines









# Mycosands conclusions 2/2

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- Fall/Winter present higher counts of fungi in sand than Spring/Summer.
- Urban and non-urban beaches have different mycoflora composition
- Both sand and water should be monitored for fungi
- Candida albicans, dermatophytes, endemic fungi and other fungi should be considered in the future
- Fungal analysis of water needs more data before reference values can be established









# Mycosands II (To be concluded during 2024)

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- Focus on fungi that can grow at 37°C, the ones able to cause invasive fungal infections (IFI).
- Rerun looking for dermatophytes in both sand and water
- Look for Candida auris
- Test anti fungal resistances of all Candida spp and A. fumigatus sensu stricto strains isolated from sand and from water
- Generate more data on fungi in water (37<sup>a</sup>C) to complement data from the first version of the Mycosands initiative, including resistance to antimicrobials





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### "The present study employed data collected during the Mycosands survey to investigate the environmental factors influencing yeasts and molds distribution along European shores applying a species distribution modelling approach."



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Environmental and bioclimatic factors influencing yeasts and molds distribution along European shores



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# What was studied

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Data were compared to climatic
datasets (temperature, precipitation, and solar radiation), soil datasets
(chemical and physical properties), and water datasets (temperature, salinity, and chlorophyll-a
concentration) downloaded from web
databases (analyses were performed
by MaxEnt software).

#### Table 1

Number of locations where filamentous fungi and yeast-like fungi were isolated from sand or water samples.

Filamentous fungi			Yeast-like fungi		
Fungal species/category	Sand	Water	Fungal species/category	Sand	Water
Aspergillus flavus	8	1	Candida albicans	5	2
Aspergillus fumigatus	20	8	Candida dubliniensis	3	2
Aspergillus niger	29	11	Candida. glabrata	3	3
Aspergillus spp.	45	27	Candida parapsilosis s.l.	4	1
Fusarium spp.	20	4	Candida tropicalis	4	2
Dematiaceous	27	11	Candida spp.	21	8
Dermatophytes	10	2	Cryptococcus spp.	7	0
Molds	51	31	Rhodotorula spp.	21	7
			Yeasts	32	14

Red numbers indicate that the number of occurrence points was not sufficient to perform MaxEnt analysis.



# What was found

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- Yeasts seem to tolerate low temperatures better during winter than molds and this reflects a higher suitability for the Northern European coasts. This difference is more evident considering suitability in waters.
- Both distributions of molds and yeasts are influenced by basic soil pH, probably because acidic soils are more favorable to bacterial growth.
- Soils with high nitrogen concentrations are not suitable for fungal growth, which, in contrast, are optimal for plant growth, favored by this environment.
- Finally, molds show affinity with soil rich in nickel and yeasts with soils rich in cadmium resulting in a distribution mainly at the mouths of European rivers or lagoons, where these metals accumulate in river sediments.

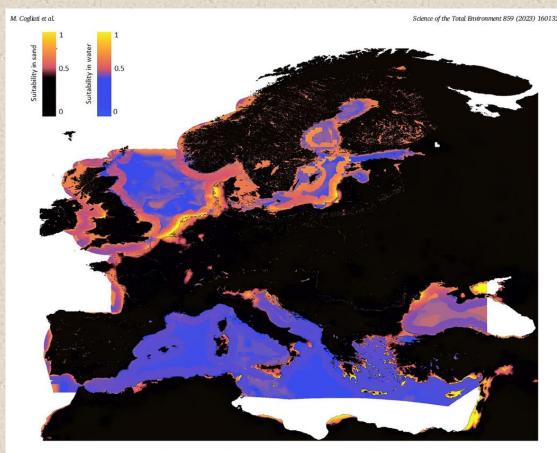




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- On the basis of physical properties of soil, the model did not identify neither a specific geographical area nor a specific type of soil associated to *Candida* spp.).
- On the contrary, most of soil textures seem to be suitable for Candida spp. survival.
- Furthermore, considering heavy metal concentrations in soil, the analysis of contributing variables showed a correlation with soils containing high cadmium concentrations which are spotted on the distribution map in some specific locations.
- Selvarajan et al. 2024 also studied metals and fungi for further reference (Beach sand mycobiome: The silent threat of pathogenic fungi and toxic metal contamination for beachgoers - <u>https://doi.org/10.1016/j.marpolbul.2023.115895</u>



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Fig. 6. Merging image showing shore and water distribution models for Candida spp.





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## What about Candida auris?

- "We sampled coastal wetlands, including rocky shores, sandy beaches, tidal marshes, and mangrove swamps, around the Andaman group of the Andaman & Nicobar Islands, Union Territory, in India."
- "Forty-eight samples of sediment soil and seawater were collected from eight sampling sites representing the heterogeneity of intertidal habitats across the east and west coast of South Andaman district."
- "C. auris was isolated from two of the eight sampling sites, a salt marsh and a sandy beach."

#### doi.org/10.1128/mbio.03181-20)







Environmental Isolation of *Candida auris* from the Coastal Wetlands of Andaman Islands, India

Parth Arora,\*\* Prema Singh,\* Yue Wang,<sup>c</sup> Anamika Yadav,\* Kalpana Pawar,\* Ashutosh Singh,\* Gadi Padmavati,\* ©Jianping Xu,<sup>c</sup> ©Anuradha Chowdhary\*

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ABSTRACT Candida auris is a multidrug resistant pathogen that presents a serious global threat to human health. As C. auris is a newly emerged pathogen, several guestions regarding its ecological niche remain unexplored. While species closely related to C. auris have been detected in different environmental habitats, little is known about the natural habitat(s) of C. auris. Here, we explored the virgin habitats around the very isolated Andaman Islands in the Indian Ocean for evidence of C quris. We sampled coastal wetlands, including rocky shores, sandy beaches, tidal marshes, and mangrove swamps, around the Andaman group of the Andaman & Nicobar Islands. Union Territory, in India, Forty-eight samples of sediment soil and seawater were colected from eight sampling sites representing the heterogeneity of intertidal habitats across the east and west coast of South Andaman district. C. auris was isolated from two of the eight sampling sites, a salt marsh and a sandy beach. Interestingly, both multidrug-susceptible and multidrug-resistant C. auris isolates were found in the sample. Whole-genome sequencing analysis clustered the C auris isolates into clade I, showing close similarity to other isolates from South Asia. Isolation of C. auris from the tropical coastal environment suggests its association with the marine ecosystem. The fact that viable C. auris was detected in the marine habitat confirms C. auris survival in harsh wetlands. However, the ecological significance of C. auris in salt marsh wetland and sandy beaches to human infections remains to be explored

**IMPORTANCE** Candida autis is a recently emerged multidrug-resistant fungal pathogen capable of causing severe infections in hospitalized patients. Despite its recognition as a human pathogen a decade ago, so far the natural ecological nichels;) of C. autis remains enigmatic. A previous hypothesis suggested that C. autis might be native to wetlands, that its emergence as a human pathogen might have been linked to global warming effects on wetlands, and that its enrichment in that ecological niche was favored by the ability of C. autis for thermal tolerance and salnity tolerance. To understand the mystery of environmental niches of C. autis we explored the coastal wetland habitat around the very isolated Andaman Islands in the Indian Ocean. C. autis was isolated from the virgin habitats of salt marsh area with no human activity and from a sandy beach. C. autis isolation from the marine wetlands suggests that prior to its recognition as a human pathogen, it existed as an environmental fungus.

KEYW ORD S Candida auris, natural habitat, marine environment, ecology, wetlands, Andaman Islands, India

Candida auris is a multidrug-resistant fungal pathogen that presents a serious threat to patients and health care facilities worldwide (1). Due to the widespread clinical and economic impact of difficult-to-treat C *auris* infections, the U.S. Centers for

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Gtation Arora P, Singh P, Wang Y, Yadav A

andida auris from the coastal wetlands of

Andaman Islands, India, mBio 12:e03181-20.

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Pawar K, Singh A, Padmavati G, Xu J, Chowdhary A. 2021. Environmental isolation of Detection of enteric viruses in beach sand

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"In this study, the first objective was to evaluate the presence of seven viruses (Aichi virus, enterovirus, hepatitis A virus, human adenovirus, norovirus, rotavirus, and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)) in sands collected at public beaches. The second objective was to assess the spatial distribution of enteric viruses in beach sand."

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Science of the Total Environment

Science of the Total Environment 901 (2023) 165836

#### Detection of enteric viruses and SARS-CoV-2 in beach sand

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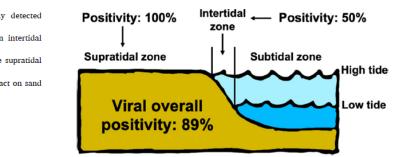
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#### HIGHLIGHTS

#### GRAPHICAL ABSTRACT

- · Enteric viruses detected in high prevalence (89 %) in beach sand
- Aichi virus most frequently detected virus (74 %)
- · Distinct viral distribution in intertidal and supratidal beach sand
- · Higher viral diversity in the supratidal 20116
- · Beach events with high impact on sand quality













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- Enteric viruses detected in high prevalence (89 %) in beach sand
- Aichi virus most frequently detected virus (74 %)
- Distinct viral distribution in intertidal and supratidal beach sand
- Higher viral diversity in the supratidal zone
- Beach events with high impact on sand quality

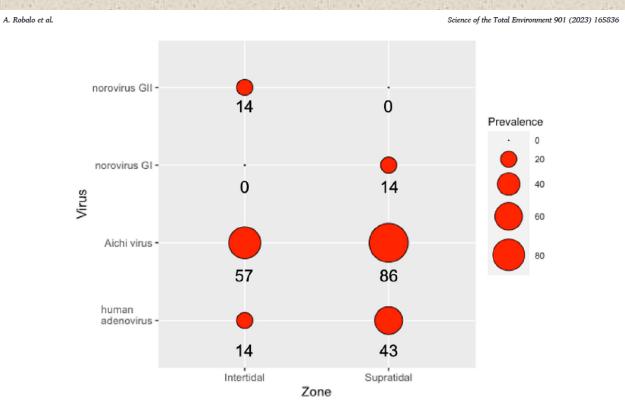


Fig. 2. Detection of viruses in beach samples from the supratidal and intertidal zones. The size of the circle is related to the relative percentage of detection of the viruses in each zone.



# Way forward

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 Beach classification based on sand monitoring, as currently happens with water, is a relevant issue requiring international strategies,

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- Epidemiological studies should be run to confirm the validity of the new WHO 60 MPN/g limit recommendation for enterococci,
- Antimicrobial resistance and other microbes need to be investigated in sand and water, some of which on a risk-based approach, keeping in mind that climate change will have implications on what currently know,
- More fungal parameters need to be designed for endemic areas for sand and water, as recommended by the new WHO guidelines.



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

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- All those involved in the episode in Azores, authors and otherwise,
  - To Dr. Kirs and Dr Yan for organizing this presentation







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# Thank you! Questions?

