

Beach sand quality – a recent concern

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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

- Coastal sand composition of beaches varies from volcanic to silica-carbonated and other minerals and in granulometry¹
- Beaches can be natural or man-made/renourished with sand from other locations, possibly carrying pathogens along²
- Inland beaches may have sand, sediment, or mixtures of both, in different composition ratios¹
- Beach sand is divided in two areas: The wet area or swash zone and the dry area or supratidal zone²

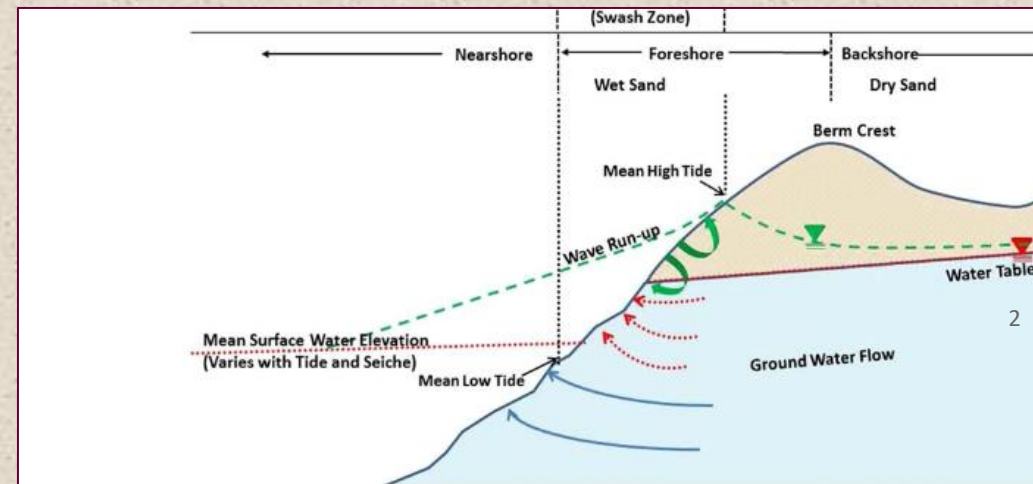
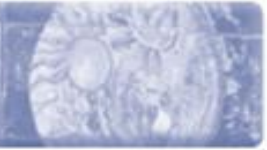


Fig. 1. Zones at the interface between beach sand and water. The terminology differs between tidal marine systems and non-tidal freshwater systems. Mean surface water elevations for marine systems tends to vary with tides. For freshwater systems, in particular within lakes, the mean surface water elevation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

¹Abreu *et al.* 2016 – doi: [10.1016/j.scitotenv.2016.08.160](https://doi.org/10.1016/j.scitotenv.2016.08.160)

²Solo-Gabriele *et al.* 2016 – doi: [10.1017/S0025315415000843](https://doi.org/10.1017/S0025315415000843);



Sand water interaction: Tidal wash-out, storm run-off, feet-dragging, animal skin shedding (including humans), Wastewater treatment plant outfall, ships, loitering





Why does it matter?

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,
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”

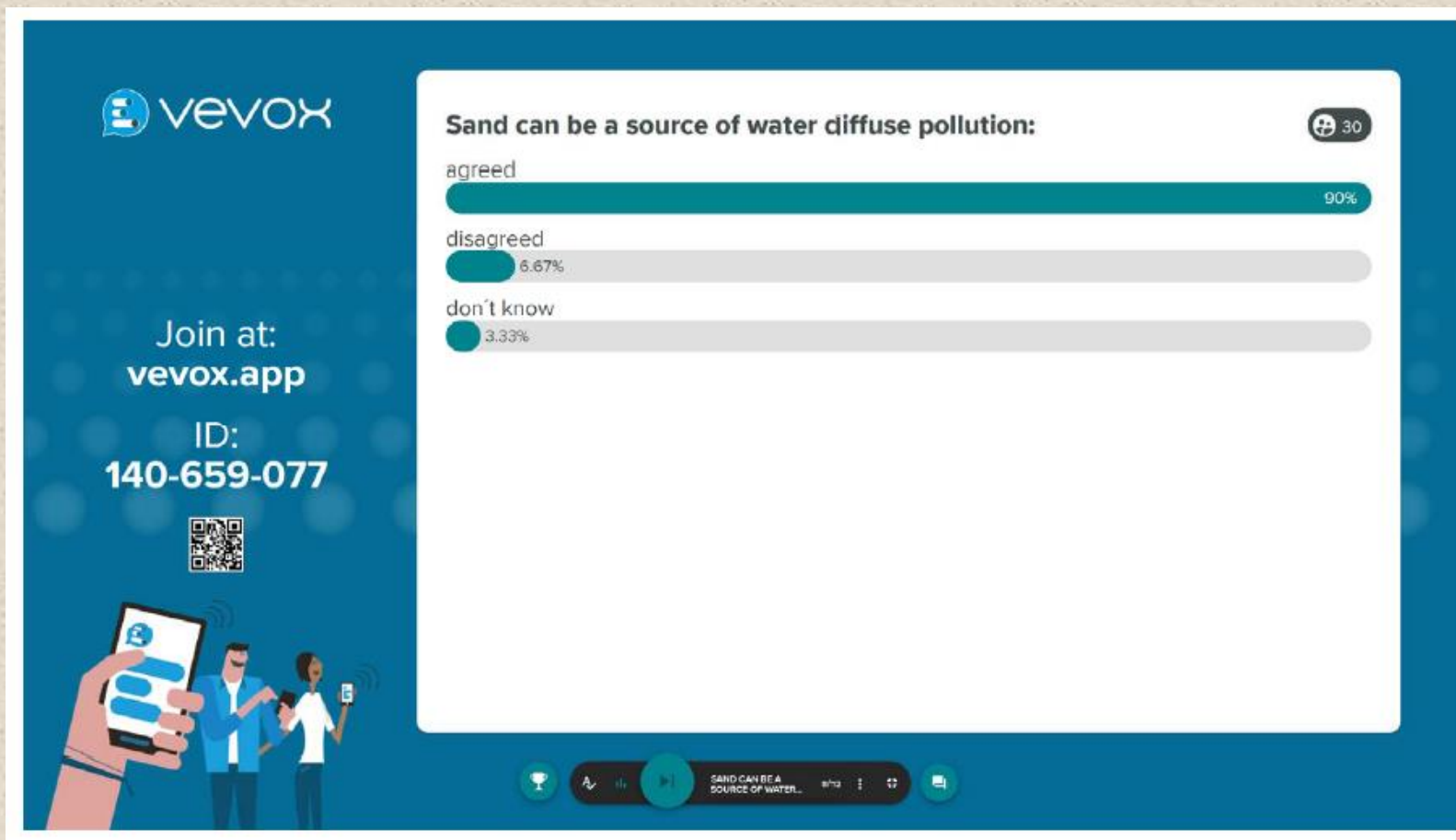
- There's a whole complex microbial community living in sand, with predation, decay, biofilms and even more complex organisms. Sand is a entire ecosystem!
- And we lie on it, and love to feel it under our bare feet during a long walk by the sea, etc.



A Kinorhyncha in Hakai Magazine, Coastal science and societies
(text by Adrienne Mason, March 21, 2016)

<http://www.hakaimagazine.com/videos-visuals/micro-monsters-beneath-your-beach-blanket/>

The international community agrees to the relevance of sand





How it all began

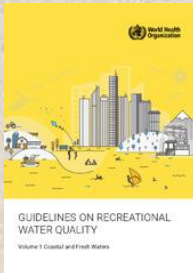
- For Bacteria: **1975** in Hanauma Bay, O'ahu, Hawai'i - Fujioka & Oshiro found that wild life and sand quality influenced the water quality on that site (an image will come next),
- For Fungi: **1960** in the Baltic Sea, Germany - Schönfeld, Rieth and Thianprasit found geophilic dermatophytes in supratidal 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

- 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.



Current WHO recommendations:

- 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





What happened in this case?

- 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

Areal views of the beach Porto Pim



Analytical results (Microbiology)

	Coliforms (100)*	E. coli (20)*	Enterococci (20)*	Filamentous fungi (560)*	Yeast (60)*	Dermatophytes (15)*
1st campaign (10th July 2019)						
Sample A	>201	>201	>201	100 (50 <i>Fusarium</i> sp)	<1.0	<1.0
Sample B	29	<1.0	9	109	90 (88 <i>M. guilliermondii</i> + 2 <i>C. tropicalis</i>)	<1.0
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 <i>Rhodotorula</i> 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 <i>Fusarium</i> sp.)	<1.0	<1.0
Sample 7	13	<1.0	1	183 (165 <i>Aspergillus</i> section <i>Circundati</i>)	2	<1.0
Sample 8	9	<1.0	<1.0	55	<1.0	<1.0
Sample 9	2	<1.0	13	5	2 (<i>Rhodotorula</i> 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.

Location of the degraded distribution box



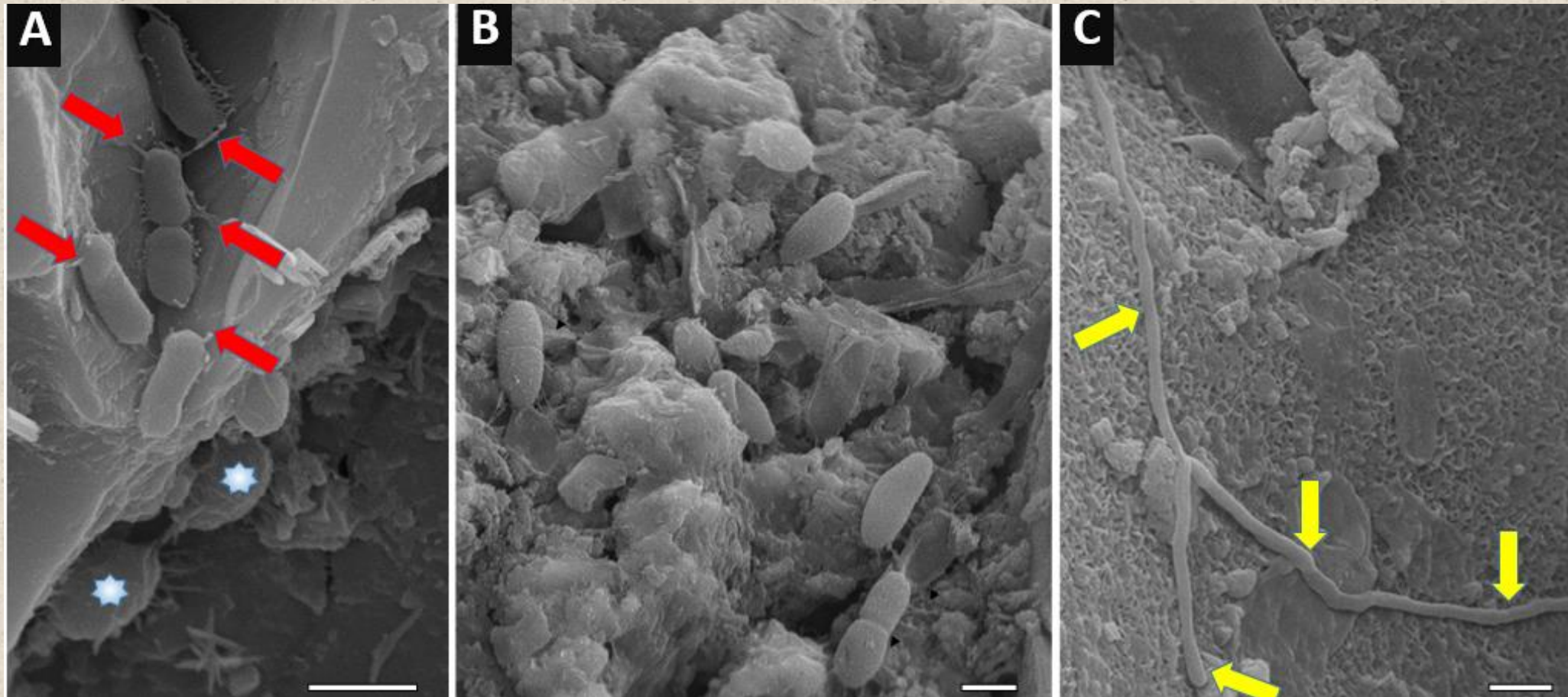
(A) – Lid of the distribution box.

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

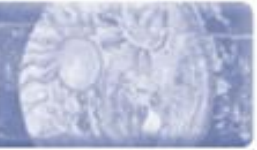
(C1) – representation of the distribution box's position and beach access.

(C2) – Mechanic removal of all of the contaminated sand, as delineated by the analytical results on FIB until 50cm deep (80m³ in total). Point 3 had the highest levels of contamination (>201 MPN of Coliforms, of *E. coli*, and *Enterococci*)

'Little monsters' on the surface of grains of sand



Scanning electron microscopy of sand particles



Fungi can be informative as to types of contamination

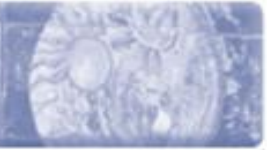
Fungi found are typical plant pathogens and saprophytes(*) and common fecal pollution(**) presences

**Aspergillus section circundati*

**Fusarium* sp

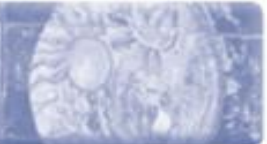
***Meyerozyma guilliermondi*

***Rhodotorula* sp



How did it end?

- 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.



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*



Article

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

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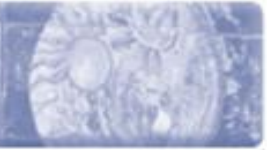
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Abstract: Beach sand may act as a reservoir for numerous microorganisms, including enteric pathogens. Several of these pathogens originate in human or animal feces, which may pose a public health risk. In August 2019, high levels of fecal indicator 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)



What happened?

- In August 2019, high levels of fecal indicator bacteria (FIB) were detected in the sand of the Azorean beach Praínha, 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 dog-walking at the beach, which local inhabitants often did in the evenings

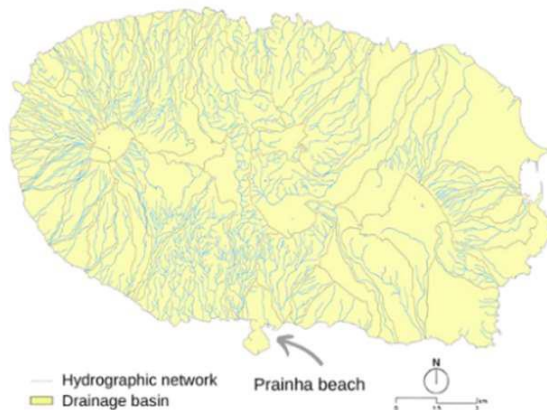
Praínha and the pollution

Satellite image



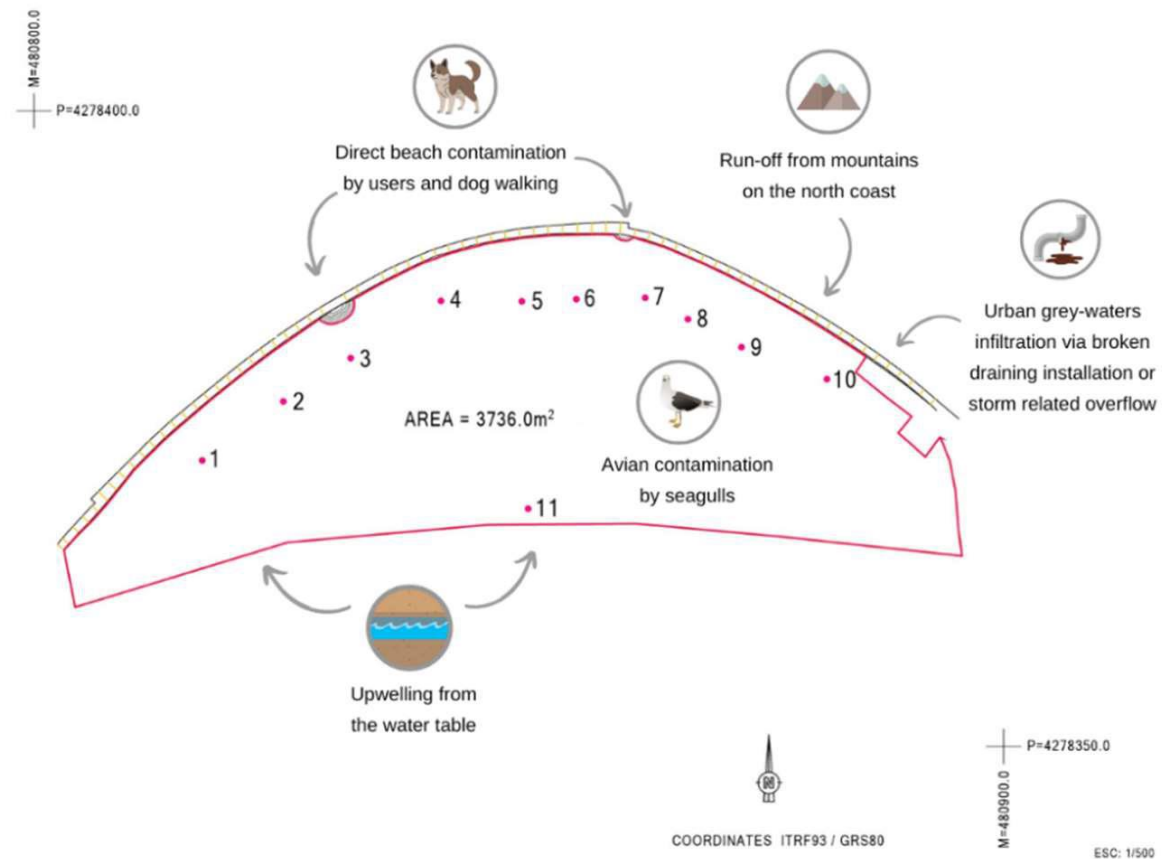
Google Earth, ©2019 CNES / Airbus [48]

Hydrographic map



Simbiente Açores - Engenharia e Gestão Ambiental [49]

Beach topography & Sampling points





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

Bridging Medical Mycology and Recreational Water Quality

Keywords:

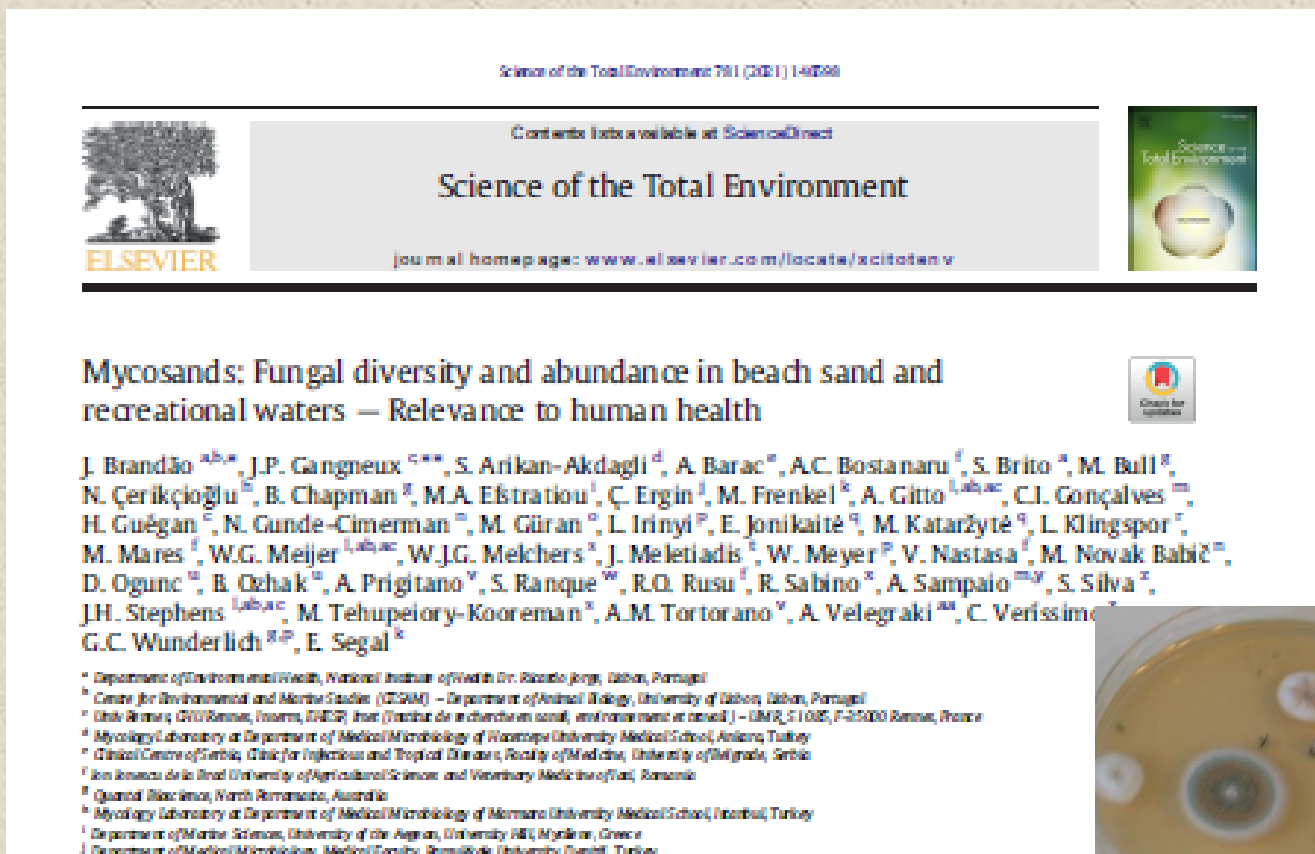
Beach Sand	Environmental Health
Recreational water quality	Inland and Coastal Beaches
Anti-Microbial Resistance	Urban Beaches
Core taxa	Moulds
Ecology	Yeasts

THE TEAM:

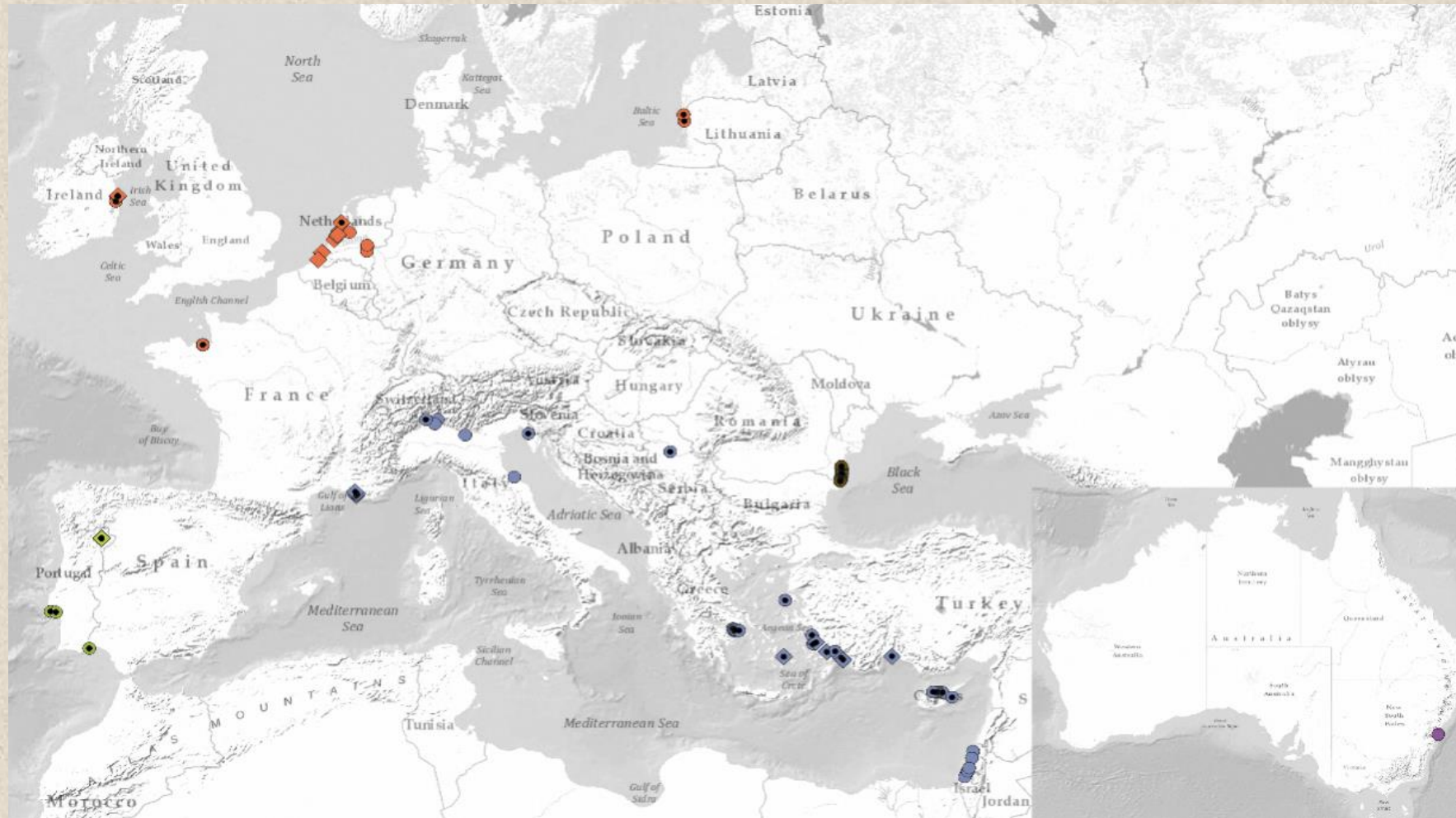
France		Italy		Greece	
Name	Area	Name	Area	Name	Area
Frédéric Roger	Atlantic coast	Anna Maria Tortorano	Inland water Basin	Aristea	Mediterranean coast
Hélène Guegan	Atlantic coast	Anna Prigitano	Inland water Basin	Velegaki	Mediterranean coast
Jean-Pierre Gangneux	Atlantic coast	Antonella De Donno	Adriatic Sea	Emanuel	Mediterranean coast
Laurence Delhaes	Bordeaux	Florent Morio	Mediterranean coast	Roilides	Mediterranean coast
Patrice Le Pape	Mediterranean coast	Francesca Serio	Adriatic Sea	Joseph Meletiadis	Mediterranean coast
Sébastien Bertout	Mediterranean coast	Laura Trovato	Mediterranean coast	Maria Efstratiou	Mediterranean coast
Stéphane Ranque	Mediterranean coast	Massimo Cogliati	Adriatic Sea	USA	
Lithuania		Salvatore C. Oliveri	Mediterranean coast	Name	Area
Name	Area	Salvatore Rubino	Mediterranean coast	Alexis Danielle Guerra	Irvine, Ca, USA
Eglė Jonikaitė	Baltic Sea	Turkey		Helena Sologabriele & Co	Miami, FL, USA
Marija Kataržytė	Baltic Sea	Name	Area	Larissa Montas	Miami, FL, USA
Sweden		Betil Ozhak	Mediterranean coast	Sunny Jiang	Irvine, Ca, USA
Name	Area	Çağrı Ergin	Mediterranean coast	Netherlands	
Lena Klingspor	Atlantic coast	Dilara Ogunc	Mediterranean coast	Name	Area
Croatia		Gule Cinar	Mediterranean coast	Wieland Meyer & Collaborators	Sidney
Name	Area	Mümtaz Güran	Mediterranean coast	Marlou Tehupeiory-Kooreman	Inland water Basin
Darija Vukić Lušić	Adriatic Sea	Nilgün Çerikçioğlu	Mediterranean coast	Paul Verweij	Inland water Basin
Slaven Josic	Adriatic Sea	Sevtap Arikon-Akdagli	Mediterranean coast	Willem Melchers	Atlantic coast
Serbia		Ireland		Portugal	
Name	Area	Name	Area	Name	Area
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Valentina Arsić Arsenijević	Inland water Basin	Israel		Cristina Veríssimo	Atlantic coast
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Name	Area	Esther Segal	Mediterranean coast	Raquel Sabino	Atlantic coast
Mihai Mares & Collaborators	Black Sea	Michael Frenkel	Mediterranean coast	Siyu Huang	Atlantic coast
				Susana Pereira	Atlantic coast

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

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

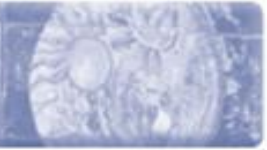


Mycosands Sampling sites



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 and Purple=Sydney (Australia)



Mycosands conclusions 1/2

- 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

- 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

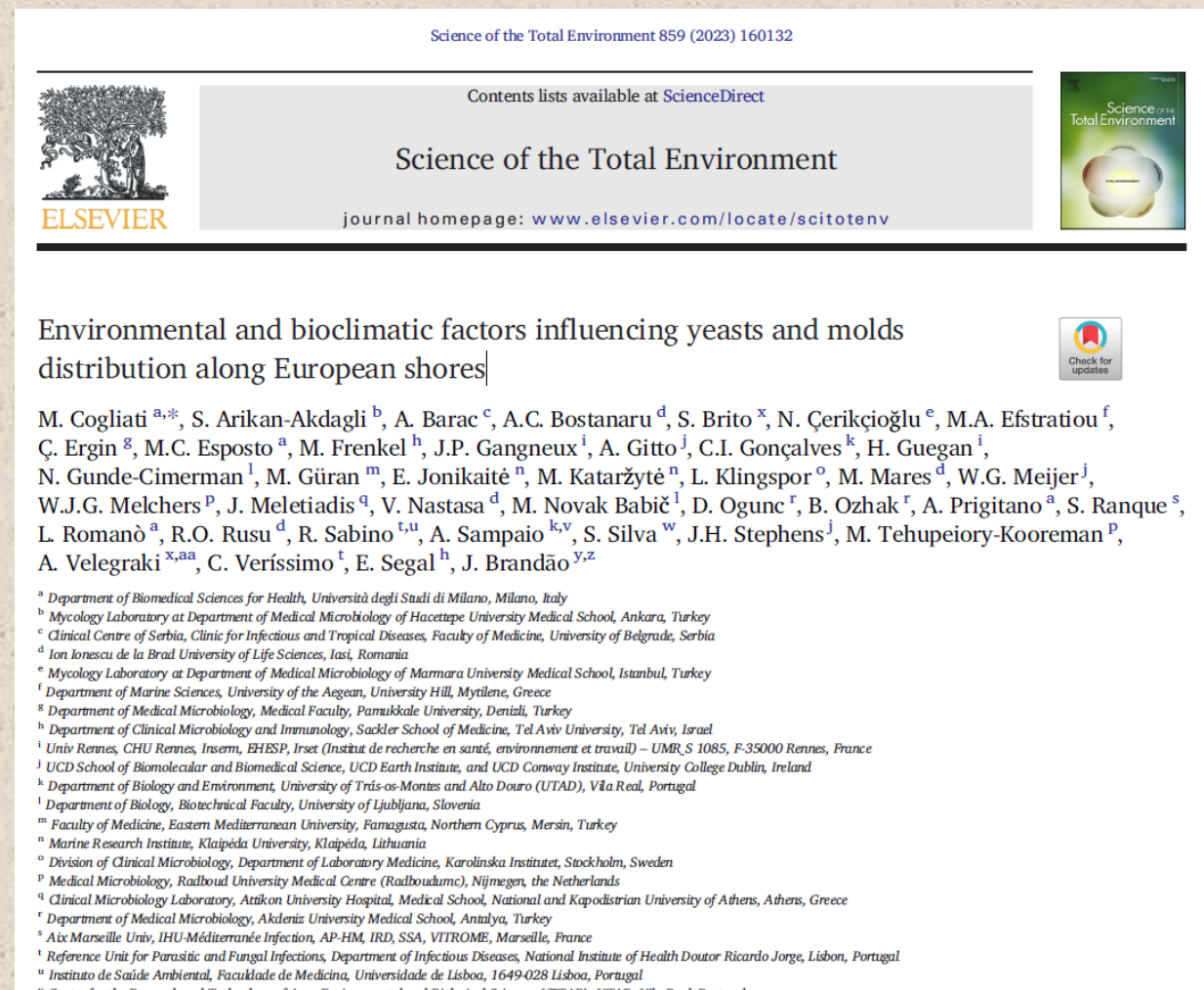


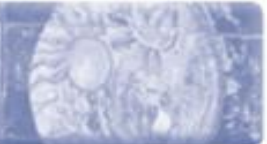
Mycosands II (To be concluded during 2024)

- 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°C) to complement data from the first version of the Mycosands initiative, including resistance to antimicrobials

Mould and yeasts: what to expect (Spin-off of Mycosands)

- “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.”





What was studied

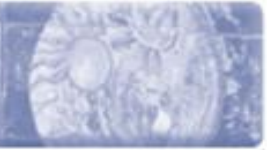
- Data were compared to climatic datasets (temperature, precipitation, and solar radiation), soil datasets (chemical and physical properties), and water datasets (temperature, salinity, and chlorophyll-a concentration) downloaded from web databases (analyses were performed by MaxEnt software).

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
<i>Aspergillus flavus</i>	8	1	<i>Candida albicans</i>	5	2
<i>Aspergillus fumigatus</i>	20	8	<i>Candida dubliniensis</i>	3	2
<i>Aspergillus niger</i>	29	11	<i>Candida glabrata</i>	3	3
<i>Aspergillus</i> spp.	45	27	<i>Candida parapsilosis</i> s.l.	4	1
<i>Fusarium</i> spp.	20	4	<i>Candida tropicalis</i>	4	2
Dematiaceous	27	11	<i>Candida</i> spp.	21	8
Dermatophytes	10	2	<i>Cryptococcus</i> spp.	7	0
Molds	51	31	<i>Rhodotorula</i> spp.	21	7
			Yeasts	32	14

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

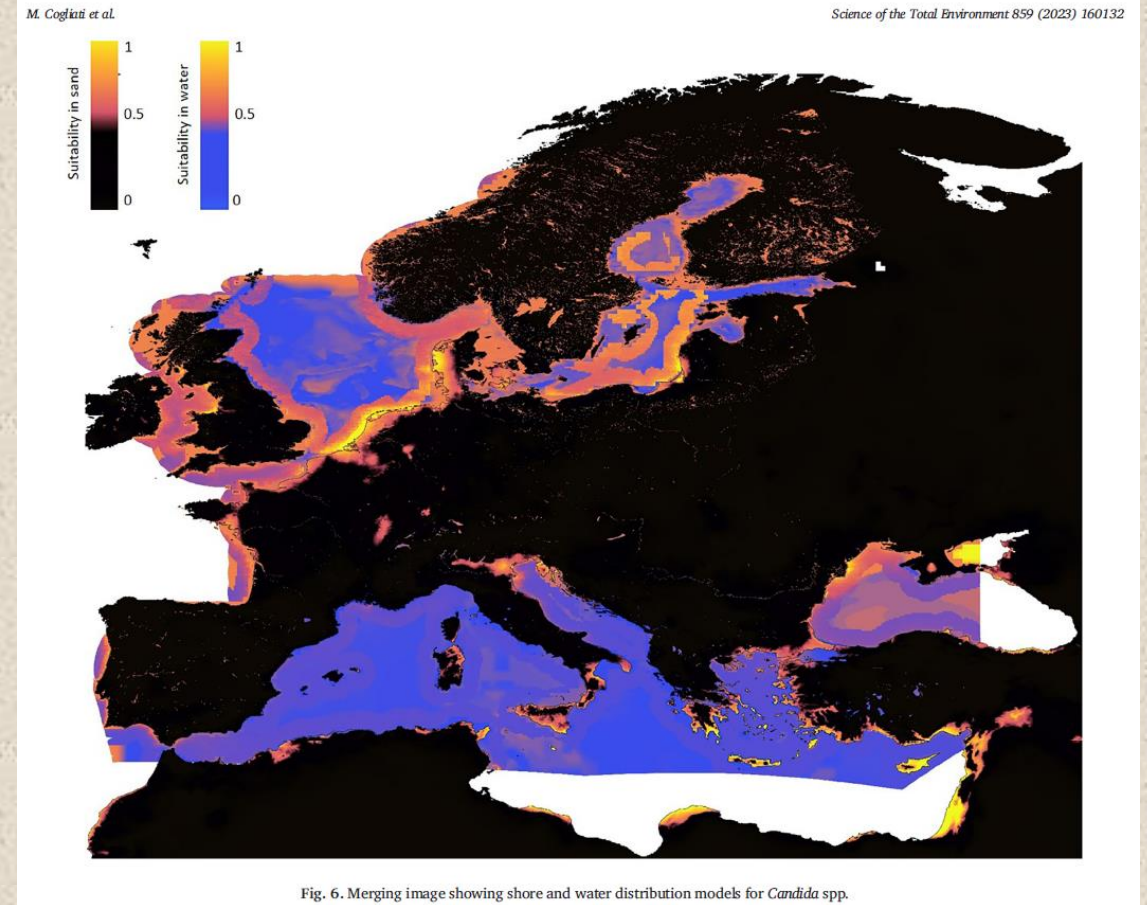


What was found

- 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.

Finding *Candida* spp at the beach


- 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 - <https://doi.org/10.1016/j.marpolbul.2023.115895>



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

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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 questions 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. 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. 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 auris* 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 niche(s) of *C. auris* remains enigmatic. A previous hypothesis suggested that *C. auris* 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. auris* for thermal tolerance and salinity tolerance. To understand the mystery of environmental niches of *C. auris*, we explored the coastal wetland habitat around the very isolated Andaman islands in the Indian Ocean. *C. auris* was isolated from the virgin habitats of salt marsh area with no human activity and from a sandy beach. *C. auris* isolation from the marine wetlands suggests that prior to its recognition as a human pathogen, it existed as an environmental fungus.

KEYWORDS *Candida auris*, natural habitat, marine environment, ecology, wetlands, Andaman Islands, India

Citation Arora P, Singh P, Wang Y, Yadav A, Pawar K, Singh A, Padmavati G, Xu J, Chowdhary A. 2021. Environmental isolation of *Candida auris* from the coastal wetlands of Andaman Islands, India. mBio 12:e03181-20. <https://doi.org/10.1128/mbio.03181-20>.

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Detection of enteric viruses in beach sand

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

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HIGHLIGHTS

- 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

GRAPHICAL ABSTRACT

The graphical abstract illustrates the spatial distribution of enteric viruses in beach sand across three zones: Supratidal, Intertidal, and Subtidal. The Supratidal zone (yellow) shows an overall viral positivity of 89%. The Intertidal zone (light blue) shows 100% positivity, while the Subtidal zone (dark blue) shows 50% positivity. The diagram also indicates the High tide and Low tide levels.

Findings

- 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

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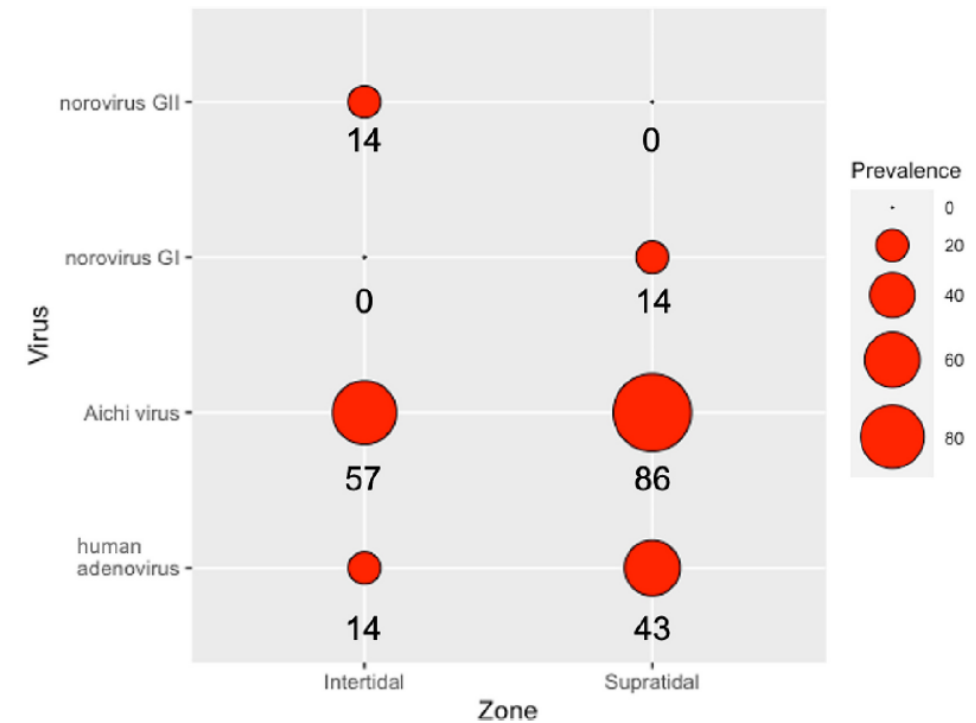
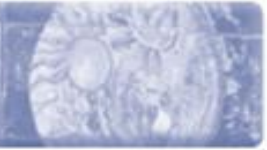


Fig. 2. Detection of viruses in beach samples from the supratidal and intertidal zones. The size of the circle is related to the relative percentage of detection of the viruses in each zone.



Way forward

- Beach classification based on sand monitoring, as currently happens with water, is a relevant issue requiring international strategies,
- 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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Thank you! Questions?

