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Review
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Knowledge and regulation on fungal contamination of sand and water: Progress report and perspectives

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Abstract

Fungal flora in coastal/inland beach sand and recreational water is a neglected field of study, despite its potential impact on human health. A joint International Society for Human and Animal Mycology/European Confederation for Medical Mycology (ISHAM/ECMM) working group was formed in 2019 with the task to set up a vast international initiative aimed at studying the fungal contamination of beaches and bathing waters. Here we review the importance of the topic, and list the main results and achievements from 12 scientific publications. Fungal contamination exists at different levels, and the genera most frequently found were *Aspergillus* spp., *Candida* spp., *Fusarium* spp., and *Cryptococcus* spp., both in sand and in water. A site-blind median was found to be 89 colony-forming units of fungi per gram of sand in coastal/inland freshwaters. This threshold has been used for the sand quality criterion of the blue flag in Portugal. Additionally, our data were considered pivotal and therefore used for the first inclusion of fungi as a biological taxon of interest in water quality and sand monitoring recommendations of the World Health Organization's new guidelines on recreational water quality (Vol.1–Chap7). The findings of the consortium also suggest how environmental conditions (climate, salinity, soil pH, nitrogen, etc.) influence microbial communities in different regions, and that yeast species like *Candida glabrata*, *Clavispora lusitaniae*, and *Meyerozyma guilliermondii* have been identified as potential fungal indicators of fecal contamination. Climate change and natural disasters may affect fungal populations in different environments, and because this is still a field of study under exploration, we also propose to depict the future challenges of research and unmet needs.

Key words: fungi, sand, water quality, environment, climate change, regulation.

An underexplored field of study

Many fungal ailments are caused by fungi in the environment. In view of climate changes and other physical and chemical factors affecting the environment, it is of significant importance to strengthen studies on fungal flora of various environmental niches and to evaluate its impact on human health. Specifically, a group of experts in medical mycology and water analysis indicated that very limited data are available on two environmental niches to which humans are exposed: beach sand and recreational water. The Bathing Water Directive (2006/7/EC) calls for the implementation of a 'bathing water profile' although based on the identification and enumeration of *Escherichia coli* and enterococci as fecal indicator parameters.[1] Fungi are missing from water, and sand regulatory parameters are not even mentioned. Reference values for fungal parameters still need to be asserted for both matrices, water and sand.

Human exposure to environmental yeasts and filamentous fungi can induce health issues ranging from (i) superficial, minor but highly transmissible diseases such as dermatophytosis and cutaneous/mucosal yeast-associated diseases to (ii) serious, debilitating, and life-threatening infections in susceptible immune-compromised individuals. The latter include, among others, diseases caused by the *Aspergillus*, *Fusarium*, and *Scedosporium* species, Mucorales, or numerous yeasts with the recently famous *Candida auris*, causing infections in hemato-oncological or intensive care patients, patients with organ transplants or intravenous catheters, and other implants. Fungal allergies also affect a high number of people, rendering crucial all knowledge generated, and forwarded, to those of interest.

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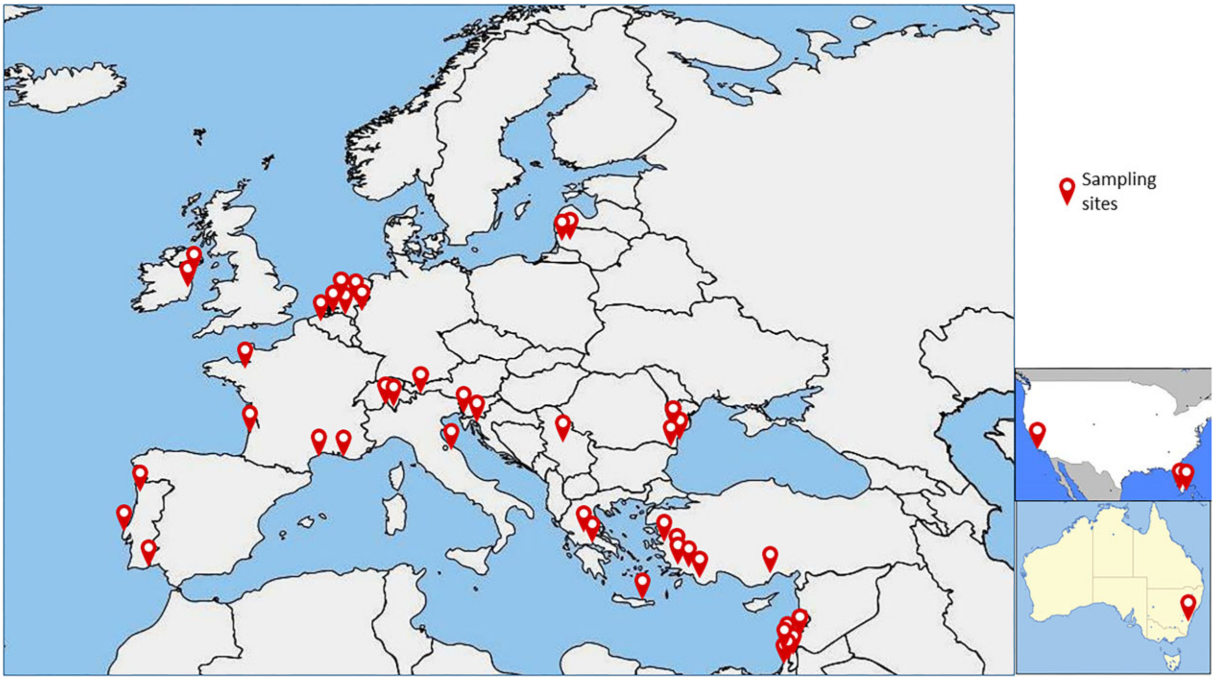

Figure 1. Sampling sites of the MYCOSANDS project in Europe, North America and Australia.

Establishment of a task force with medical and environmental mycologists

A joint ISHAM/ECMM working group was formed in 2019 with a first task to set up a vast international study aimed at studying the fungal contamination of beaches and bathing waters (Fig. 1). An extensive 2-year study, the MYCOSANDS project, which screened fungal flora in beach sand and water of various coastal and inland water bodies in Europe, revealed a great diversity of yeast and mould species, primarily in sand, and to a lesser extent in water. Many of the yeast and mould species found possess the potential to cause human disease, particularly in immunocompromised or debilitated individuals.

The ISHAM/ECMM MYCOSANDS study group was composed of 14 countries, as shown in Fig. 1, and was recently extended to have 53 members from 16 countries to run a new version of studies focused strictly on known pathogenic and opportunistic fungi and their antimicrobial resistance. The new version of the study started in 2022 with two new countries joining the team—the USA (California and Florida) and Croatia.

Results obtained and main achievements

A real dynamic activity within the MYCOSANDS study group has been created, primarily due to several pivotal studies that have been published, as well as the recognition of the results by the World Health Organization (WHO). The main contributions of the working group concern both the improvement of knowledge and regulatory system, as presented in Table 1.

What challenges for tomorrow?

All these results taken together emphasize the importance of studies focusing on environmental fungal population in relation to human health and wellbeing. It is expected that awareness of the health risk of environmental fungi in water bodies and their surrounding sand beaches will lead to regulatory measures in order to lower that risk, as it happens with drinking water sources in few places in the world.

However, many challenges still need to be met. The significance of ecological environmental niches in the era of climate changes, occurring currently globally, is an additional aspect to consider. Furthermore, the climate changes may possibly affect the fungal population, and change internal balance of the microbial community.

The ISHAM/ECMM MYCOSANDS study group therefore wishes to continue in order to address different questions:

- How representative are the results obtained thus far? and how increasing the number of sampling sites might change the perspective?
- Are ocean/sea water and lake/river waters, which are physically and chemically different, loaded with comparable fungal burden?
- How to assess the importance of yeast and filamentous fungi resistance to antifungal drugs in these ecological niches, particularly regarding *Candida* such as *Candida auris* and *Aspergillus* notably azole-resistant *Aspergillus fumigatus*?
- What are the missing data to ensure the value and pertinence to include fungi in the regulatory system?
- What is the impact of natural disasters on the rapid and violent modification of the microbiological flora and, therefore, on human health?
- How to amplify the significance of these ongoing changes to the next generation of the medical community in order to advance in near future the implementation of measures for control of environmental hazards to the health and wellbeing of humans?
- Now that basic fungal parameters have been recommended in the latest WHO, how do they perform in a

Table 1. Advances in knowledge on the epidemiology and methodology to characterize the fungal burden in sand and recreational water and the regulatory achievements.

Objectives and methods	Main results and conclusions	References
Major advances in knowledge Objective: Exploring the fungal flora in beach sand and water of coastal and inland bathing sites. Methods: Sand culturable mycobiota of 91 bathing sites, and water of 67 of these, spanning from the Atlantic to the Eastern Mediterranean coasts, including the Italian lakes and the Adriatic, Baltic, Black Sea, and Sydney (Australia).	1. Qualitative results:The genera most frequently found were *Aspergillus* spp., *Candida* spp., *Fusarium* spp., and *Cryptococcus* spp. both in sand and in water. 2. Quantitative results: • A site-blind median was found to be 89 colony-forming units (CFU) of fungi per gram of sand in coastal and inland freshwaters, with variability between 0 and 6400 CFU/g. • For freshwater sites, that number was 201.7 (0, 6400) CFU/g ($P = 0.01$)) and for coastal sites was 76.7 (0, 3497.5) CFU/g. • For coastal waters and all waters, the median was 0 CFU/mL (0, 1592 CFU/mL) and for freshwaters 6.7 (0, 310.0) CFU/mL ($P < 0.001$). Conclusions: Fungal contamination exists at different levels depending on the niches. This suggests a need for regulatory measures to protect public health.	[2]Brandão, 2021; doi: 10.1016/j.scitotenv.2021.146 598
Objective: This study investigated fungal contamination on Israeli Mediterranean Sea beaches, focusing on its impact on human health, including: 1. Fecal contamination: detected through human enteric fungi presence. 2. Dermal infections: assessing potential skin infection risks. 3. Respiratory health: Identifying moulds that could cause allergies and infections. Collected sand samples from six urban beaches and used water extraction for analysis. Identified fungi using various techniques.	Results: About 80% were moulds (e.g., *Aspergillus fumigatus, Fusarium*) and 20% yeasts (e.g., *Candida*). Some are opportunistic pathogens or allergens. Conclusions: Fungal contamination exists on Israeli Mediterranean Sea beaches. No regulatory measures to protect public health exist regarding fungi.	[3]Frenkel, 2020; doi: 10.1111/myc.13 144
Objective: This study aimed to investigate the presence of fungi with potential health implications in the sand and water of Israeli Mediterranean Sea coast beaches. Methods: The research involved screening six urban beaches along the Israeli Mediterranean coast, from north to south. Sand samples were collected using water extraction, and both sand and water were cultured and quantified. Fungal identification was performed through MALDI–TOF mass spectrometry analysis and Internal Transcribed Spacer (ITS) sequencing.	Results: The study assessed various parameters: • Presence of fecal-contamination-related fungi • Presence of dermal-infection-related fungi • Presence of allergy-related fungi • Presence of fungi posing risks for immunocompromised individuals Approximately 80% of the isolates were moulds, while the remaining 20% were yeasts. The mould species included opportunistic pathogens and potential allergens like *Aspergillus fumigatus*, other *Aspergillus* species, *Fusarium, Penicillium*, and Mucorales species. Yeast isolates comprised *Candida* species, including *Candida albicans* and *Candida tropicalis, Cryptococcus,* and *Rhodotorula* species. Conclusions: The findings suggest the importance of monitoring fungi on beaches to enhance safety, management, and public health benefits.	[4]Frenkel, 2022; doi: 10.3390/jof8090950
Objective: This study focused on the limitations of current beach safety regulations that primarily rely on fecal indicators in water, neglecting sand and fungi, despite reports of their presence in both environments. Methods: The research investigated the abundance, diversity, and seasonal variations of mycobiota (fungi) in sand and seawater at Portorož beach in Slovenia over 1 year.	Results: • Sand and seawater diversity: The study identified 64 fungal species from 43 genera in sand and 29 species from 18 genera in seawater. • Environmental influences: Machine learning analysis revealed that changes in environmental factors like air and water temperature, sunshine hours, humidity, precipitation, air pressure, and wind speed affected the mycobiota. • Stable fungal community: Certain genera, including *Aphanoascus, Aspergillus, Fusarium, Bisifusarium, Penicillium, Talaromyces,* and *Rhizopus*, formed a consistent community within the sand. However, their presence and abundance fluctuated with weather changes.	[5]Novak Babič, 2022; doi: 10.3390/jof8080860

Table 1. Continued

Objectives and methods	Main results and conclusions	References
	• Drug resistance: *Aspergillus* species, particularly *Aspergillus niger* and *A. welwitschiae*, were the most abundant fungi in the sand and showed resistance to amphotericin B, an antifungal drug. • Human pollution indicators: Four potential indicators of human pollution, including *Meyerozyma*, were isolated during the bathing season, suggesting their use in beach microbial regulation. Conclusions: The findings emphasize the need for broader research on sand and seawater mycobiota and highlight the potential impact of global warming and extreme weather events on fungi in these environments. Additionally, the study suggests the importance of including fungi in beach safety regulations to enhance public health protection.	
Objective: The study used data from the Mycosands survey to analyze yeast and mould distribution along European shores.	Results: • Environmental factors: Occurrence data were compared to various environmental datasets, including climate (temperature, precipitation, and solar radiation), soil (pH, nitrogen concentration), and water (temperature, salinity, chlorophyll-a concentration). • Different distributions: Yeasts and moulds showed distinct distribution patterns. Yeasts tolerated low temperatures better, making them more suitable for Northern European coasts. • Soil pH influence: Soil pH played a significant role, with acidic soils favoring bacterial growth, impacting fungal distribution. • Nitrogen concentration: High soil nitrogen concentrations were unfavorable for fungal growth but conducive to plant growth. • Metal affinity: Moulds preferred soils rich in nickel, while yeasts were associated with cadmium-rich soils, leading to their distribution near river mouths and lagoons. Conclusions: • Understanding these distribution patterns is vital for managing fungal populations in coastal ecosystems. • The study contributes to our knowledge of how environmental conditions influence microbial communities in different regions.	[6]Cogliati, 2023; doi: 10.1016/j.scitotenv.2022.160 132
Objective: This study, based on the MYCOSANDS survey data, examines the environmental factors impacting the distribution of yeasts and moulds along European shores. Methods: They utilized species distribution modeling and compared occurrence data to various environmental datasets, including climate, soil, and water parameters.	Results: • Distinct distributions: Yeasts and moulds exhibit different distribution patterns along European shores. • Temperature tolerance: Yeasts are more resilient to low winter temperatures, making them prevalent in Northern European coasts, particularly in aquatic environments. • Soil pH influence: Both yeasts and moulds' distribution is influenced by soil pH, with acidic soils favoring bacterial growth and affecting fungal prevalence. • Nitrogen concentration: High soil nitrogen concentrations hinder fungal growth but promote plant growth, creating an environment less conducive to fungi.	[7]Prigitano, 2023; doi: 10.1016/j.scitotenv.2022.160 417

Table 1. Continued

Objectives and methods	Main results and conclusions	References
	• Metal affinity: Moulds are associated with nickel-rich soils, while yeasts thrive in cadmium-rich soils. This leads to their prevalence near European river mouths and lagoons, where these metals accumulate. Conclusions: These findings shed light on the complex relationship between environmental factors and fungal distribution along European shores, with potential implications for understanding microbial communities and public health.	
Objectives and methods: The 2021 WHO guidelines suggest monitoring sand quality alongside water quality at recreational beaches. This review provides essential background information on beach types, sand characteristics, and the microbiological aspects to measure. It outlines analytical methods for quantifying fungi and fecal indicator bacteria (FIB) in beach sand.	Results and conclusions: The review discusses strategies for assessing beach sand quality, monitoring protocols, sand remediation, and the future of beach sand monitoring programs. It proposes recommendations for acceptable levels of fungi, considering their distribution in the environment. Furthermore, it suggests evaluating FIB distributions at beaches worldwide to establish acceptable FIB levels, similar to the proposed standards for fungi. This approach aims to enhance beach safety and public health.	[8]Brandão, 2023; doi: 10.3390/ijerph20095710
Recent knowledge generated by members of the group		
Objective: Discussing the significance of beach sand and water microbiology for human health and the evolving challenges in monitoring and safeguarding beachgoers.	Discussion: • Microbiology importance: Beach sand and water have long been studied due to their relevance to human health. • WHO recommendation: The WHO now recommends including recreational beach sands in monitoring for enterococci and fungi. • Climate change impact: Global climate change is altering beach microbial contamination. Factors like water temperature, sea level, precipitation, and waves are changing. • Human mobility: People's movement and relocation introduce new microbiota to coastal areas, especially in regions affected by desertification. • Need for adaptation: A warmer future will likely demand rethinking traditional water quality indicators to ensure the safety of waterways for bathing and recreation. • Complex sand matrix: Sand is a complex medium, requiring new microbial standards to protect beachgoers from both sand and water contaminants. • Adapting regulations: To ensure safer beach use in a changing climate, regulations need adaptation. In essence, this text emphasizes the need to update regulations and monitoring practices to safeguard human health at beaches in a world undergoing climate change and shifting demographics.	[9]Brandão, 2022; doi: 10.3390/ijerph19031444
Objective: Beach sand can harbor various microorganisms, including potentially harmful enteric pathogens originating from human and animal feces, posing a public health risk. In August 2019, high levels of fecal indicator bacteria (FIB) were detected in the sand of Prainha Beach on Terceira Island, Portugal.	Results and conclusions: Using this information, the municipality enforced restrictions on dog-walking at the beach. Immediate remediation measures were taken, including sand removal and chlorine spraying. Subsequent sampling showed reduced FIB contamination due to the mitigation efforts. This study is the first to use MST to determine contamination sources in supratidal beach sand. It underscores the value of MST as a tool for identifying fecal contamination sources in beach sand and emphasizes the need for holistic beach management beyond water quality monitoring for FIB.	[10]Valério, 2022; doi: 10.3390/ijerph19137934

Table 1. Continued

Objectives and methods	Main results and conclusions	References
Methods: To identify the source of contamination, supratidal sand samples were collected from different beach locations. Microbial source tracking (MST) analysis of bacteroides marker genes for five animal species, including humans, revealed the presence of markers from dogs, seagulls, and ruminants at some sampling sites.		
Objectives and methods: In June 2019, an incident of skin rash affected 30 individuals, primarily children, following a sand sifting operation at Porto Pim Beach in Faial, Azores. An investigation was conducted to determine the cause of this outbreak.	Results: • Some affected individuals had not entered the beach water, suggesting a different exposure route. • Water quality parameters (fecal indicator bacteria levels) remained within regulatory limits during the outbreak. • Sand contact was considered the likely primary exposure route. • Sand microbiological analysis and electron microscopy indicated fecal contamination. • Chemical analysis of the sand revealed the presence of a substance compatible with sodium hypochlorite, confirmed through gas chromatography and free chlorine analysis. • Inspection of beach toilet facilities and sewage systems uncovered a leaking sewage distribution box located 40 m from the beach. • The outbreak was attributed to this sewage distribution box, which serviced the beach toilets and used sodium hypochlorite for cleaning. The sewage contamination affected the surface sands where beachgoers were exposed. • Chlorine, known to be irritating, was likely responsible for the skin rashes, given the abrupt onset and resolution of symptoms. • No gastrointestinal illnesses were reported during or after the incident. Conclusions: This study highlights the importance of monitoring beach sand quality, particularly in areas with aging infrastructure, to ensure the safety of beachgoers and prevent incidents like this one.	[11]Brandão, 2020; doi.org: 10.1016/j.scitotenv.2020.140 237
Objectives and methods: During the summer of 2021, a comprehensive survey collected samples from 67 stations located in 32 cities along the Caspian Sea coastline. These samples, consisting of dry/wet sand and shoreline water, were primarily analyzed for fungal characteristics through macro/microscopic morphological features. Additionally, identification through polymerase chain reaction with fragments restriction (PCR-RFLP) was performed for yeasts, dermatophytes, and *Aspergillus* sp. strains. Antifungal susceptibility tests were conducted on suspected *Aspergillus* and *Candida* spp.	Results: • A total of 268 samples were collected, resulting in 181 (67.54%) isolates. • Yeast-like fungi and potentially pathogenic black fungi were found in 12 (6.6%) and 20 (11%) of the sand samples (dry/wet), respectively. • Potential pathogenic hyaline fungi were prevalent and identified in 136 (75.1%) samples. *Aspergillus* sp. was the dominant genus, comprising 76/136 (47.8%) samples with various sections, including Flavi, Nigri, Nidulantes, and Fumigati. • The most effective azole antifungal agent varied by *Aspergillus* section: PSZ for A. section Fumigati, ITZ and ISZ for A. section Nigri, EFZ for A. section Flavi, and ISZ for A. section Nidulantes. • Candida isolates showed susceptibility to the tested antifungals. Conclusions: This study provides a comprehensive analysis of fungal populations along the Caspian Sea coastline, revealing the presence of various potentially pathogenic fungi and their susceptibility to antifungal agents, with implications for public health and environmental management.	[12]Moazeni, 2022; doi: 10.3390/ijerph20010459

Table 1. Continued

Objectives and methods	Main results and conclusions	References
Objectives and methods: This paper addresses the potential health risk of yeast infection through exposure to river water, recognizing that river water can be contaminated with microorganisms. The study explores fungal communities in less polluted (LP) and highly polluted (HP) river water, emphasizing potentially opportunistic yeast species.	Results: • Pollution impact: Pollution levels significantly alter fungal communities, with opportunistic and pathogenic genera being more abundant in highly polluted waters. • Indicator yeasts: Yeast species like *Candida glabrata* and *Clavispora lusitaniae* are identified as indicators of contamination. • Quantitative risk assessment: A quantitative microbial risk assessment (QMRA) framework is proposed to evaluate the potential risk of yeast infection, taking into account water contamination levels, yeast species pathogenicity, and antifungal resistance. • Seasonal Variability: *Meyerozyma guilliermondii* blooms during the wet season, suggesting that environmental factors such as dissolved oxygen levels and water turbulence influence yeast growth characteristics and, consequently, annual infection risk distribution. • Implications: Antifungal-resistant yeasts are observed, further contributing to the variation in infection risk. Research on yeast ecophysiology in these environments is essential to refine the QMRA model. Conclusions: This study provides insights into the presence of opportunistic and pathogenic yeasts in river water, their association with pollution, and proposes a QMRA framework to assess yeast infection risks. It emphasizes the importance of understanding yeast behavior in natural environments and its potential impact on public health.	[13]Steffen, 2023; doi: 10.1016/j.watres.2023.119599.
Regulatory advances		
First inclusion of fungi as an indicator of water quality (Chapter 7). This section emphasizes the management and communication strategies for beach sand contamination.	Management actions: 1. Animal excreta: Animal excreta, including dogs, birds, and other local animals, can increase fecal indicator organism (FIO) levels and introduce pathogens to beach sands. Measures include avoiding dog exercise in beach areas, designating sections for this purpose, and managing feral animals humanely. Management plans for birds, both native and non-native, should be in place. 2. Garbage disposal: Proper solid waste disposal facilities should be designed to minimize access by animals. Toilet facilities at the beach encourage hygiene practices. Appropriate drainage systems should be in place to avoid contamination. 3. Beach cleaning: While beach cleaning is often done for aesthetic reasons, it may also remove animal excreta and litter. Care should be taken to minimize impacts on sand quality and ecology. 4. Beach renourishment: Sand renourishment involves adding sand from external sources to restore or build beaches. The quality of the imported sand should be considered to preserve native ecosystems and avoid importing non-endemic species. 5. Sewage management: Sewage should not be dumped near recreational areas. Communication: • Educational campaigns should include signage about policies regarding dogs, wildlife feeding, and trash disposal. • Promote good hygiene practices among beachgoers, such as using clean towels, washing hands, and covering wounds. • Encourage the use of shoes to prevent injuries while walking on the beach.	[14]WHO, 2021

Table 1. Continued

Objectives and methods	Main results and conclusions	References
	• Communication for beach managers includes sanitary inspections to identify contamination sources, litter containment, management plans for animals, and signage for beachgoers about health risks and appropriate beach use. • Dissemination of educational materials is essential for public awareness and beach safety. This section emphasizes the importance of proactive management and communication to safeguard beachgoers' health and maintain beach sand quality.	
Blue Flag award implements sand quality criteria in 2023 in Portugal, Chapter 12. (G) Monitoring the quality of the sands, according to the defined parameters and methodologies and with the percentage of beaches established annually.	Starting from 2023, the Blue Flag Sand quality criterion extends to include beach sand and soil of coastal and inland beaches. The sampling involves compositing sand samples from various locations along the supratidal area of the beach into one sample. Sampling occurs before the bathing season and monthly during the season, coinciding with water sampling. Analytical methods for fungi involve extracting 40 g of crude sand with sterile distilled water and plating on malt agar/potato dextrose agar with chloramphenicol. Results are given in CFU per gram of crude sand. For bacteria (enterococci and *E. coli*), 10 g of sand is extracted with distilled water and analyzed using Quanti-Tray® systems or membrane filtration methods. Quality control through participation in a sand microbial analysis quality assessment scheme is recommended. Classification of beach sand quality involves classifying beaches as compliant or not compliant, allowing a certain number of results to fail due to microbiota fluctuations. For fungi, a rejection rate of 20% is suggested, resulting in a guidance value of 89 CFU/g of total fungi in sand and a rejection limit of the 80% percentile. For enterococci, the provisional value is 60 CFU/g or NMP/g of sand, reflecting the health effect of water thresholds, and exceeding this value should be carefully considered. *Escherichia coli* serves as an extra fecal indicator with a reference value of 25 CFU/g. References include Sabino et al. (2011) and Brandão et al. (2021), providing insights into sand quality assessment and microbial analysis methods. This updated criterion emphasizes regular monitoring of beach sand quality to ensure public health and safety.	Blue Flag Portugal (https://bandeiraazul.abae.pt/wp-content/uploads/sites/2/2023/02/ABAE-sand-criterion_2023-1.pdf)

broader geography? Should there be local added parameters as a risk based approach as recommended in the guidelines? How shall these be defined?

Conclusion

It is the authors' belief that initiatives like MYCOSANDS should help contribute to the promotion of public health. The first reports on the presence in sand of the multidrug-resistant bug *C. auris*, which is a fearsome and dreaded agent of nosocomial epidemics, demonstrate the significance of doing research in this field.[15] Moreover, as it is an example of a 'bug' whose environmental behavior and perhaps emergence may be linked to global warming (*Candida auris*).[16]

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Author contributions

Jean-Pierre Gangneux (Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing), Joao Brandao (Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing), Ester Segal (Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing).

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Conflict of interest

None to declare with the submitted work.

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