



HAL
open science

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

J Brandão, J P Gangneux, S Arikan-Akdagli, A Barac, a C Bostanaru, S Brito, M Bull, N Çerikçioğlu, B Chapman, M A Efstratiou, et al.

► To cite this version:

J Brandão, J P Gangneux, S Arikan-Akdagli, A Barac, a C Bostanaru, et al.. Mycosands: Fungal diversity and abundance in beach sand and recreational waters - Relevance to human health. *Science of the Total Environment*, 2021, 781, pp.146598. 10.1016/j.scitotenv.2021.146598 . hal-03229103

HAL Id: hal-03229103

<https://hal.science/hal-03229103v1>

Submitted on 24 Apr 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

1 **Mycosands: Fungal diversity and abundance in beach sand and recreational waters - relevance to**
2 **human health**

3

4 J. Brandão^{a,b,*}, J.P. Gangneux^c, S. Arikan-Akdagli^d, A. Barac^e, A.C. Bostanaru^f, S. Brito^a, M. Bull^g, N.
5 Çerikçioğlu^h, B. Chapman^g, M. A. Efstratiouⁱ, Ç. Ergin^j, M. Frenkel^k, A. Gitto^l, C.I. Gonçalves^m, H.
6 Guégan^c, N. Gunde-Cimermanⁿ, M. Güran^o, L. Irinyi^p, E. Jonikaitė^q, M. Kataržytė^q, L. Klingspor^r, M.
7 Mares^f, W.G. Meijer^l, W.J.G. Melchers^s, J. Meletiadis^t, W. Meyer^p, V. Nastasa^f, M. Novak Babičⁿ, D.
8 Ogunc^u, B. Ozhak^u, A. Prigitano^v, S. Ranque^w, R.O. Rusu^f, R. Sabino^x, A. Sampaio^{m,y}, S. Silva^z, J.H.
9 Stephens^l, M. Tehupeiory-Kooreman^s, A.M. Tortorano^v, A. Velegraki^p, C. Veríssimo^x, G.C.
10 Wunderlich^{g,p}, E. Segal^k

11

12 ^aDepartment of Environmental Health, National Institute of Health Dr. Ricardo Jorge, Lisbon, Portugal

13 ^bCentre for Environmental and Marine Studies (CESAM) – Department of Animal Biology, University
14 of Lisbon, Lisbon, Portugal

15 ^cUniv Rennes, CHU Rennes, Inserm, EHESP, Irset (Institut de recherche en santé, environnement et
16 travail) – UMR_S 1085, F-35000 Rennes, France;

17 ^dMycology Laboratory at Department of Medical Microbiology of Hacettepe University Medical
18 School, Ankara, Turkey

19 ^eClinical Centre of Serbia, Clinic for Infectious and Tropical diseases, Faculty of Medicine, University of
20 Belgrade, Serbia

21 ^fIon Ionescu de la Brad University of Agricultural Sciences and Veterinary Medicine of Iasi, Romania

22 ^gQuantal Bioscience, North Parramatta, Australia

23 ^hMycology Laboratory at Department of Medical Microbiology of Marmara University Medical
24 School, Istanbul, Turkey

25 ⁱDepartment of Marine Sciences, University of the Aegean, University Hill, Mytilene, Greece

26 ^jDepartment of Medical Microbiology, Medical Faculty, Pamukkale University, Denizli, Turkey

27 ^kDepartment of Clinical Microbiology and Immunology, Sackler School of Medicine, Tel Aviv
28 University, Tel Aviv, Israel

29 ^lUCD School of Biomolecular and Biomedical Science, UCD Earth Institute, and UCD Conway Institute,
30 University College Dublin, Ireland

31 ^mDepartment of Biology and Environment, University of Trás-os-Montes and Alto Douro (UTAD), Vila
32 Real, Portugal

33 ⁿDepartment of Biology, Biotechnical Faculty, University of Ljubljana, Slovenia

34 ^oFaculty of Medicine, Eastern Mediterranean University, Famagusta, Northern Cyprus, Mersin 10,
35 Turkey

36 ^pMolecular Mycology Research Laboratory, Centre for Infectious Disease and Microbiology, Sydney
37 Medical School, Westmead Clinical School, Westmead Hospital, Marie Bashir Institute for Emerging
38 Infectious Diseases and Biosecurity, Westmead Institute for Medical Research, The University of
39 Sydney, Westmead, Australia

40 ^qMarine Research Institute, Klaipėda University, Klaipėda, Lithuania

41 ^rDivision of Clinical Microbiology, Department of Laboratory Medicine, Karolinska Institutet,
42 Stockholm, Sweden

43 ^sMedical Microbiology, Radboud University Medical Centre (Radboudumc), Nijmegen, The
44 Netherlands

45 ^tClinical Microbiology Laboratory, Attikon University Hospital, Medical School, National and
46 Kapodistrian University of Athens, Athens, Greece

47 ^uDepartment of Medical Microbiology, Akdeniz University Medical School, Antalya, Turkey

48 ^vDepartment of Biomedical Sciences for Health, Università Degli Studi di Milano, Milan, Italy

49 ^wAix Marseille Univ, IHU-Méditerranée Infection, AP-HM, IRD, SSA, VITROME, Marseille, France

50 ^xReference Unit for Parasitic and Fungal Infections, Department of Infectious Diseases, National
51 Institute of Health Dr. Ricardo Jorge, Lisbon,

52 ^YCentre for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB),
53 UTAD, Vila Real, Portugal

54 ^ZDepartment of Epidemiology, National Institute of Health Dr. Ricardo Jorge, Lisbon, Portugal
55 Portugal

56 ^PMycology Research Laboratory and UOA/HCPF Culture Collection, Microbiology Department,
57 Medical School, National and Kapodistrian University of Athens, Athens, Greece and Mycology
58 Laboratory, BIOMEDICINE S.A., Athens, Greece

59 *Corresponding Author (Joao.brandao@insa.min-saude.pt)

60

61 Abstract

62 The goal of most studies published on sand contaminants is to gather and discuss knowledge to avoid
63 faecal contamination of water by run-offs and tide-retractions. Other life forms in the sand, however,
64 are seldom studied but always pointed out as relevant. The Mycosands initiative was created to
65 generate data on fungi in beach sands and waters, of both coastal and freshwater inland bathing
66 sites. A team of medical mycologists and water quality specialists explored the sand culturable
67 mycobiota of 91 bathing sites, and water of 67 of these, spanning from the Atlantic to the Eastern
68 Mediterranean coasts, including the Italian lakes and the Adriatic, Baltic, and Black Seas. Sydney
69 (Australia) was also included in the study. Thirteen countries took part in the initiative. The present
70 study considered several fungal parameters (all fungi, several species of the genus *Aspergillus* and
71 *Candida* and the genera themselves, plus other yeasts, allergenic fungi, dematiaceous fungi and
72 dermatophytes). The study considered four variables that the team expected would influence the
73 results of the analytical parameters, such as coast or inland location, urban and non-urban sites,
74 period of the year, geographical proximity and type of sediment. The genera most frequently found
75 were *Aspergillus* spp, *Candida* spp, *Fusarium* spp and *Cryptococcus* spp both in sand and in water. A
76 site-blind median was found to be 89 Colony-Forming Units (CFU) of fungi per gram of sand in coastal
77 and inland freshwaters, with variability between 0 and 6400 CFU/g. For freshwater sites, that

78 number was 201.7 CFU/g (0, 6400 CFU/g ($p=0.01$) and for coastal sites was 76.7 CFU/g (0, 3497.5
79 CFU/g). For coastal waters and all waters, the median was 0 CFU/ml (0, 1592 CFU/ml) and for
80 freshwaters 6.7 (0, 310.0) CFU/ml ($p<0.001$). The results advocate that beaches should be monitored
81 for fungi for safer use and better management.

82

83 **Keywords:** Beach; Fungi in sand; Bathing; Allergenic fungi; Bathing water; Mycosis

84

85 1. Introduction

86 1.1 Framing fungi in sand and water

87 In 2003, the World Health Organization (WHO) published their Guidelines for safe recreational water
88 environments, recommending that sand be looked into, especially at higher latitudes where due to
89 lower seawater temperatures the population tends to spend less time bathing than in countries with
90 warmer waters; but still uses beaches for all kinds of recreational purposes (WHO, 2003).

91 The Bathing Water Directive (2006/7/EC), which came into effect on the 24th of March 2006, sets
92 tighter standards than the previous directive, although still based on the traditional faecal indicator
93 parameters, *E. coli* and Enterococci, as recommended by the WHO's guidelines. These parameters
94 are directly linked to the probability of a waterborne illness after bathing (WHO). Key features of the
95 new directive include discounting of samples and establishment of a 'bathing water profile'.
96 Microbial water quality classification is based on four-year monitoring data using 95 or 90
97 percentiles, which are based on the probability of gastrointestinal disease due to exposure to bathing
98 waters. Article 6 of the Bathing Water Directive (BWD) ((EU), 2006) calls for the implementation of a
99 'bathing water profile', which results in the "identification and assessment of causes of pollution that
100 might affect bathing waters and impair bathers health".

101 The main cause of faecal pollution in the European Union bathing waters is diffuse or non-point
102 source pollution (Buer et al., 2018; Kataržytė et al., 2018). In contrast to point source pollution, such
103 as combined sewage overflows, diffuse pollution usually has multiple biological and geographical

104 origins, which is therefore difficult to manage. While land run-off due to rainfall is often a major
105 contributor, it is by no means the only contributing source. Other potentially important sources of
106 faecal contamination impacting bathing waters and associated beaches include wildlife, in particular
107 sea birds, dogs as well as local contaminated streams discharging onto a beach.

108 Global warming, human population (over)-growth and climate change is expected to also bring
109 alterations of the native microbiota, due to microbial migration, coast retraction and emerging
110 antimicrobial resistance (Weiskerger et al., 2019). These factors combined probably bring along
111 unexpected illnesses, diagnosed and treated with some degree of difficulty by local clinicians, as
112 addressed by Cooney in 2011 (Cooney, 2011).

113 In terms of bathing water quality research, fungi are an under-investigated biological group, and it is
114 not represented in the BWD. However, invasive fungal infections are associated with a high rate of
115 mortality and other ailments that we are now beginning to understand. For example, many *Candida*
116 species frequently found in the sand are opportunistic pathogens. They are known faecal
117 contaminants that tend to cause mucosal infections of individuals that are susceptible due to
118 underlying medical conditions, such as diabetes or immune suppression. Babies and toddlers with
119 their immune systems still immature, represent another at-risk group.

120 Fungal genera that have been isolated from beach sands include *Aspergillus*, *Chrysosporium*,
121 *Fusarium* (Candan et al., 2020), *Scedosporium*, *Scytalidium*, *Scopulariopsis* (Sabino et al., 2011),
122 *Candida* (Shah et al., 2011), *Penicillium*, *Rhodotorula* (Vogel et al., 2007), *Cladosporium*, *Mucor*,
123 *Stachybotrys* (Bik et al., 2012; Gomes et al., 2008; González et al., 2000; Migahed, 2003),
124 *Phialemonium* (Pong et al., 2014) and many others. *Trichophyton* and *Microsporium*, associated with
125 skin and nail infections, also have been reported from beach sand (Sabino et al., 2011).

126 Fungal levels in beach sands may also be related to weather events (Solo-Gabriele et al., 2016). In the
127 volcanic islands of Madeira and Porto Santo, pathogens in beach sands have been associated with
128 intense rainfall events, flash floods, and debris flows (Marzol et al., 2006B; Romão et al., 2017a).
129 Beach wrack (that consists of organic material) and accumulates along the coast with significant

130 amounts can also be a primary and significant source for higher fungal abundance. *Rhodotorula*,
131 *Alternaria* and *Aspergillus* species, for example, are associated with live or decomposing aquatic or
132 terrestrial plants or algae (Kataržytė et al., 2017; Ogaki et al., 2019).

133 The objective of the Mycosands initiative was to generate data on fungi in beach sands and waters,
134 of both coastal and freshwater inland bathing sites.

135 1.2 The Fungi

136 1.2.1 Yeasts genera *Candida*, *Cryptococcus*, *Trichosporon* and *Geotrichum*

137 The major common human pathogenic *Candida* species include: *Candida albicans*, *Candida glabrata*,
138 *Candida tropicalis*, *Candida parapsilosis* complex, *Pichia kudriavzevii* (formerly *Candida krusei*),
139 *Meyerozyma guilliermondii* (formerly *Candida guilliermondii*) and rare species, such as *Candida*
140 *dublinskiensis*. The recently emerged detected pathogen, *Candida auris*, whose ecological niche is thus
141 far unknown, is of special interest as it shows high levels of resistance to currently available
142 antifungal drugs (Chowdhary et al., 2017; Lockhart et al., 2017). Being an obligate human
143 gastrointestinal tract (GIT) commensal, the presence of *C. albicans* in the environment may almost
144 exclusively be considered as an indicator of human faecal contamination (IFC); and only occasionally
145 from birds (Al-Yasiri et al., 2017; Angebault et al., 2013). The same may apply, partially, also to *C.*
146 *parapsilosis*, as this complex may also be harboured in the human GIT (de Toro et al., 2011; Silva et
147 al., 2012) and on human skin, though it is not an obligate human commensal (Cordeiro et al., 2017).
148 *C. parapsilosis* is a major cause of invasive candidiasis in immunocompromised hosts (Trofa et al.,
149 2008). *Candida* species may also be of environmental origins, such as *M. guilliermondii* (formerly *C.*
150 *guilliermondii*) or *P. kudriavzevii* (formerly *C. krusei*). *M. guilliermondii* is found in polluted areas
151 (Hirayama et al., 2018) and has been reported as causing invasive candidiasis in
152 immunocompromised patients, exhibiting low susceptibility to two major antifungal groups: the
153 polyenes and echinocandins (Marcos-Zambrano et al., 2017).
154 *C. glabrata* (Pfaller et al., 2008) is also a human commensal (Hesstvedt et al., 2017) and *C.*
155 *dublinskiensis* is a closely related species of *C. albicans*.

156 The major human pathogens of the genus *Cryptococcus* are *Cryptococcus neoformans* var.
157 *neoformans*, *Cryptococcus neoformans* var. *grubii*, and *Cryptococcus gattii* (Chang et al., 2018; de
158 Hoog et al., 2000b; Hagen et al., 2015; Kwon-Chung et al., 2014). The pathogenesis of cryptococcal
159 infection involves generally respiratory infections, with a predilection to spread to the central
160 nervous system, causing meningitis or meningoencephalitis (de Hoog et al., 2000a). Bone and
161 cutaneous involvement are also seen. It is estimated that globally about a million cases annually
162 occur (Maziarz and Perfect, 2016). The major susceptible human groups at risk for infection with
163 *Cryptococcus* species are the HIV and AIDS patients, patients with hematologic malignancies and
164 transplant patients. However, *Cryptococcus* species, particularly *C. gattii*, can cause disease also in
165 non-compromised individuals (Rajasingham et al., 2017). *C. neoformans* var. *neoformans* and *C.*
166 *neoformans* var. *grubii* are distributed universally, whereas *C. gattii* was thought to have a more
167 limited geographic distribution. It has recently been reported also from non-endemic areas (Ergin et
168 al., 2019; Rajasingham et al., 2017). In terms of ecology, all three species are environmental fungi. *C.*
169 *neoformans* var. *neoformans* and *C. neoformans* var. *grubii* are mainly found in environments
170 enriched by dry bird excrements, such as pigeon droppings, while *C. gattii* is mainly found in soil
171 associated with certain tree species, such as *Eucalyptus*, and specific animal species, such as *Pteropus*
172 spp. (bats) and *Phascolarctos* spp. (Koalas) (Hagen et al., 2015). *Naganishia albida* has been isolated
173 from air and has been found in water, plants and on animal skin, being reported to cause fungaemia
174 and peritonitis (Chen et al., 2014; Choe et al., 2020; Fonseca et al., 2000; Ragupathi and Reyna, 2015;
175 Rajasingham et al., 2017). *Papiliotrema laurentii* has also been found in soil and water (Ragupathi and
176 Reyna, 2015), with fungaemia and meningitis cases having been reported caused by this species
177 (Banerjee et al., 2013; Vadkertiová and Sláviková, 2006). A recent survey on fungi in the sand of
178 Mediterranean beaches in Israel (Frenkel et al., 2020), revealed the presence of a rare *Cryptococcus*
179 species, *Naganishia uzbekistanensis* (formerly *Cryptococcus uzbekistanensis*, which was reported
180 (Powel et al., 2012) to cause an infection in an immunocompromised patient.

181 *Trichosporon* species are widely distributed in the environment: in soil, water, animals and are part of
182 the human skin flora. Six species are of clinical significance; *Trichosporon asahii*, *Trichosporon*
183 *asteroides*, *Trichosporon cutaneum*, *Trichosporon inkin*, *Trichosporon mucoides* and *Trichosporon*
184 *ovoides* (de Almeida Junior and Hennequin, 2016). *T. inkin* and *T. asahii* cause hair infections of the
185 scalp and groin (White piedra), which are associated with bathing in polluted water (Shivaprakash et
186 al., 2011). *T. asahii*, has also been involved in several systemic infections (Guo et al., 2019).

187 *Geotrichum* is an ascomycetous yeast genus found worldwide in soil, water, air, sewage, plants,
188 cereals and dairy products. It has also been found in normal human flora, sputum and faeces (Ben
189 Neji et al., 2019). The species of clinical relevance is *Geotrichum candidum* (Durán Graeff et al.,
190 2017). The most important risk factor for invasive infection of *G. candidum* is severe
191 immunosuppression with neutropenia. Mortality associated with *Geotrichum*-related infections is
192 high (Durán Graeff et al., 2017).

193 1.2.2 Allergenic moulds and endemic fungi

194 Fungi can affect human health in other ways as infections, including allergic reactions, irritations and
195 toxic reactions (Fischer and Dott, 2003; Levetin et al., 2016; McGinnis, 2004).

196 Allergic reactions mainly result from sensitization and immune overreaction of the host, as suggested
197 by clinical symptoms of rhinitis and asthma, by skin prick- and provocation tests, and with elevated
198 blood IgE levels as a key surrogate marker of the complex host-allergen interaction (Piarroux et al.,
199 2019).

200 Many fungal cell wall components, including chitin, glucans, mannans, mannoproteins and
201 galactomannan, or fungal metabolites, such as enzymes and toxins, have been reported as having a
202 causative role in allergic reactions (Douwes, 2005; Levetin et al., 2016). Furthermore, exposure to
203 volatile organic compounds (VOC) produced by fungi growing on and degrading substrates may cause
204 nonspecific symptoms, such as eye, nose and throat irritations, headaches and fatigue (Wålinder et
205 al., 2005).

206 Contact allergens are mainly associated with species in two genera: *Malassezia* and *Trichophyton*.
207 Airborne allergens are associated with a much wider variety of fungi, including *Aspergillus*,
208 *Penicillium*, *Fusarium*, Mucorales (mainly *Rhizopus oryzae*), *Cladosporium* and *Alternaria* (for
209 dematiaceous fungi, see below), and even yeasts.

210 Exposure to mould can cause allergic reactions in fungi-sensitized individuals, who account for about
211 10% of the total population and 40% of patients with asthma (Burge, 2001; Mendell et al., 2011).
212 *Aspergillus* is a life-threatening mould in immunosuppressed patient and is also responsible for the
213 most studied and prevalent allergic fungal diseases. Allergic bronchopulmonary aspergillosis (ABPA),
214 severe asthma with fungal sensitisation (SAFS), *Aspergillus* bronchitis and allergic *Aspergillus*
215 rhinosinusitis account for a considerable fungal disease burden (Bongomin et al., 2017). ABPA is a
216 severe form of the disease in atopic patients, particularly in asthmatics with a prevalence estimated
217 from 1% to 3.5% of all asthmatic patients. It displays a higher incidence in patients with poorly
218 controlled asthma, up to 14% (Lalgé and Chamilos, 2019). In cystic fibrosis patients, *Aspergillus*
219 sensitization is also a cause of morbidity. The high prevalence estimated between 7% to 9%
220 underlines the importance of preventive measures in these patients (Guegan et al., 2018; Lalgé and
221 Chamilos, 2019; Michel et al., 2019). In addition to *Aspergillus*, other moulds such as *Penicillium*,
222 *Fusarium*, *Scedosporium*, *Mucor*, *Cephalosporium*, *Verticillium* and *Chrysosporium* have also been
223 shown to induce sensitization (Levetin et al., 2016).

224 Endemic fungi of potential interest to sand exposure are *Histoplasma*, *Coccidioides*, *Paracoccidioides*,
225 *Blastomyces*, *Sporothrix*, *Emergomyces* and *Talaromyces marneffe*. These are soil-dwelling
226 dimorphic fungi that are the cause of endemic fungal diseases (Ashraf et al., 2020). Exposure to an
227 environmental reservoir in an endemic area may result in superficial but also systemic infections, the
228 latter occurs mostly in immunocompromised patients, including AIDS patients, or solid organ
229 transplant recipients.

230 1.2.3 Dematiaceous fungi

231 The ecology of black or melanised fungi is remarkably diverse. They are often described as ubiquitous
232 organisms inhabiting mainly plant material and residing in soil (Revankar and Sutton, 2010;
233 Wijayawardene et al., 2016). Although the majority of genera are related to plants, wood, and
234 decaying plant material (Wijayawardene et al., 2016), several can also be isolated from air and water
235 (Babič et al., 2017; Shelton et al., 2002), or extremophilic niches, like hypersaline environments (Zalar
236 et al., 2019), rocks and marble (De Leo et al., 2019; Sterflinger, 2006), and also outdoor or indoor
237 places polluted with BTEX compounds (Novak Babič et al., 2020; Prenafeta-Boldu et al., 2006). In the
238 past, many dematiaceous fungi have been detected also in beach sand (Abdallaoui et al., 2007; de
239 Moura Sarquis and de Oliveira, 1996; Dunn and Baker, 1984; Gomes et al., 2008; Londono et al.,
240 2018; Migahed, 2003; Sabino et al., 2014; Salleh et al., 2018; Salvo and Fabiano, 2007; Solo-Gabriele
241 et al., 2016; Ulfig et al., 1997; Vezzulli et al., 2009; Yee et al., 2016). Fungi, associated with beach
242 sand belong to the plant-related genera *Alternaria*, *Chaetomium*, *Cladosporium*, *Curvularia*, *Phoma*,
243 and *Scopulariopsis*. Other, yeast-like dematiaceous fungi like *Aureobasidium*, *Exophiala*, and
244 *Phialophora* have rarely been isolated, the likely reason being their slow growth (Dunn and Baker,
245 1984; Efstratiou and Velegraki, 2010; Solo-Gabriele et al., 2016). Beach sand is a specific environment
246 that may promote the growth of melanised fungi via different factors - exposure to sun radiation,
247 elevated humidity, strong wind, presence of salts, plants, animals, and humans (Brandão et al.,
248 2020b; Solo-Gabriele et al., 2016). The coastline, and therefore also the sand, are from time to time
249 exposed to adverse events, like oil or gas spills, which may additionally contribute to the presence of
250 melanised fungi (Bik et al., 2012; Solo-Gabriele et al., 2016).

251 Although linked mainly to plants and saprophytic material, their presence in beach sand may affect
252 the health of beach users (Brandão et al., 2020b; Solo-Gabriele et al., 2016). The most common
253 diseases associated with sand include those of the respiratory tract, keratitis, and cutaneous and
254 subcutaneous infections. However, also other diseases may occur from exposure to sand combined
255 with a traumatic event. The severity depends on the extension of the trauma and host immune
256 response (de Hoog et al., 2019; Revankar and Sutton, 2010).

257 1.2.4 Dermatophytes

258 Dermatophytosis is currently a disease of global importance and a considerable public health burden,
259 as it is estimated that 10% to 15% of the population being infected by a dermatophyte at some point
260 during their lives (Pires et al., 2014).

261 Anthropophilic species naturally colonize humans, being transmitted between humans and causing
262 chronic, mild, non-inflammatory infections, often reaching epidemic proportions. Geophilic
263 dermatophytes have their reservoir in the soil around burrows of specific terrestrial mammals,
264 feeding on keratinous debris. All animals naturally shed skin and hair, including humans. The
265 presence of skin fragments and hairs in the soil enables the survival of these fungi (Kushwaha and
266 Guarro, 2000). When transmitted to humans, both zoo- and geophilic species cause acute,
267 inflammatory mycoses. Occasionally, humans infected by zoophiles remain contagious, leading to
268 small, self-limiting outbreaks by *Microsporum canis*, *Trichophyton mentagrophytes*, *Trichophyton*
269 *benhamiae*, *Trichophyton verrucosum*, for example, while most infections by geophilic species are
270 quickly resolved. Due to the effectivity of human-to-human transmission, an increasing trend is
271 observed from geophilic via zoophilic to anthropophilic (de Hoog et al., 2017).

272 The occurrence of fungi in soil can be influenced by non-biological factors such as soil temperature,
273 humidity, rainfall, the chemical composition of the soil (including organic matter and pH) and sunlight
274 irradiation (Coulibaly et al., 2016). Dermatophytes are no exception to this (Pontes et al., 2013).

275 Dermatophytes are important microorganisms of the soil microbiota, with cosmopolitan species and
276 others presenting a restricted geographic distribution (Pontes et al., 2013).

277 The high prevalence of anthropophilic dermatophytes derived from dense urbanisation and
278 increasing access to sports areas and bathing facilities (Dolenc-Voljč, 2015). Living close to animals
279 but also living in crowded spaces or with frequent contact with soil provides multiple opportunities
280 for disease transmission. The rise of the number of recreational areas where people may lie down
281 (on the sand or other soils) or walking barefoot has led to an increasing concern with the possible

282 presence of dermatophytes, reported in densely populated beach sands (Efstratiou et al., 2011;
283 Kishimoto and Baker, 1969; Müller, 1973; Sabino et al., 2011).

284 2. Materials and methods

285 2.1 Sampling sites

286 The Mycosands initiative came to be by a call for partners to join voluntarily and thus with no
287 financial support. The sites chosen by each partner were based on selection criteria and should
288 include: water and sand; from a coastal beach and an inland beach; of both urban and not urban
289 areas, sampled as often and as long as possible. All partners and sites were approved by the
290 executive committee (J. Brandão, J.P.Gagneaux and E. Segal) before enrolment.

291 The following beaches were sampled for this project by **Regions**, as shown in Figure1 (Country codes
292 follow ISO 3166-1 definition of AU=Australia (Sydney), IE=Ireland, FR=France, GR=Greece, IL=Israel,
293 IT=Italy, LT=Lithuania, NL=Netherlands, PT=Portugal, RO=Romania, SL=Slovenia, RS=Serbia,
294 TR=Turkey):

295 **Black Sea** - 20 beaches - (RO): Mamaia North, Mamaia South, Constanta Modern, Constanta 3
296 Papuci, Eforie Nord 1, Eforie Nord 2, Eforie Sud, Navodari, Costinesti 1, Costinesti 2, Venus, Neptun,
297 Olimp, Jupiter, Cap Aurora, Mangalia 1, Mangalia 2, Saturn, 2 Mai, Vama Veche; **Mediterranean** -40
298 beaches - (RS): River Dunav;(GR): Amorgos (Kato Akrotiri), Phaneromeni, Perani, Aeantio, Selinia,
299 Bikini beach Alimos, Eressos;(IT):Desenzano (Garda Lake), Menaggio (Como Lake), Carate Urio (Como
300 Lake), Pesaro (Adriatic sea), Cannero Riviera (Maggiore Lake); (TR): Belek/Kadriye, Güzelçamlı, Didim,
301 Akbük, Ören, Akyaka, İztuzu, Sarıgerme, Alagadi, Kervansaray, Yavuz Çıkarma, Lapta, Karşıyaka,
302 Kayalar, Devran; (IL): Ashdod, Ashkelon, Haifa, Kesaria, Palmachim, Tel Aviv; (SL): Portorož; (FR):
303 Plage de la Lave, Plage des Catalans, Plage de l'Huveaune, Plage de la Pointé Rouge, and Plage des
304 Goudes; **Southwest Europe** - 7 beaches - (PT): Praia da Fraga da Pegada 1 to 4, Alburrica, Carcavelos
305 Praia Verde; **Northwest Europe** - 21 beaches - (FR): Saint-Malo (Môle beach sites 1 to 4); (IE):
306 Sandymount, Donabate, Portrane; (LT): Melnragė, Palanga; (NL): Zandvoort, Bergen beach, River
307 Waal (Nijmegen), Scheveningen, Kraayenbergse plassen (Cuijk), Noordwijk aan zee, Strand Blijburg,

308 Rijkerswoerdse Plas, Beach Noordwijkerhout, Nieuw-Haamstede, Beach Renesse, Beach Dishoek;
309 **Australia** (Sydney): 3 beaches - (AU): Bondi Beach, Manly Beach, Murray Rose Pool.

310 The coordinates for each site were recorded and then mapped on Figure 1 with QGIS (Ver 3.10.0-A
311 Coruña) which is a free licensed GIS application (GNU General Public License CC BY-SA).

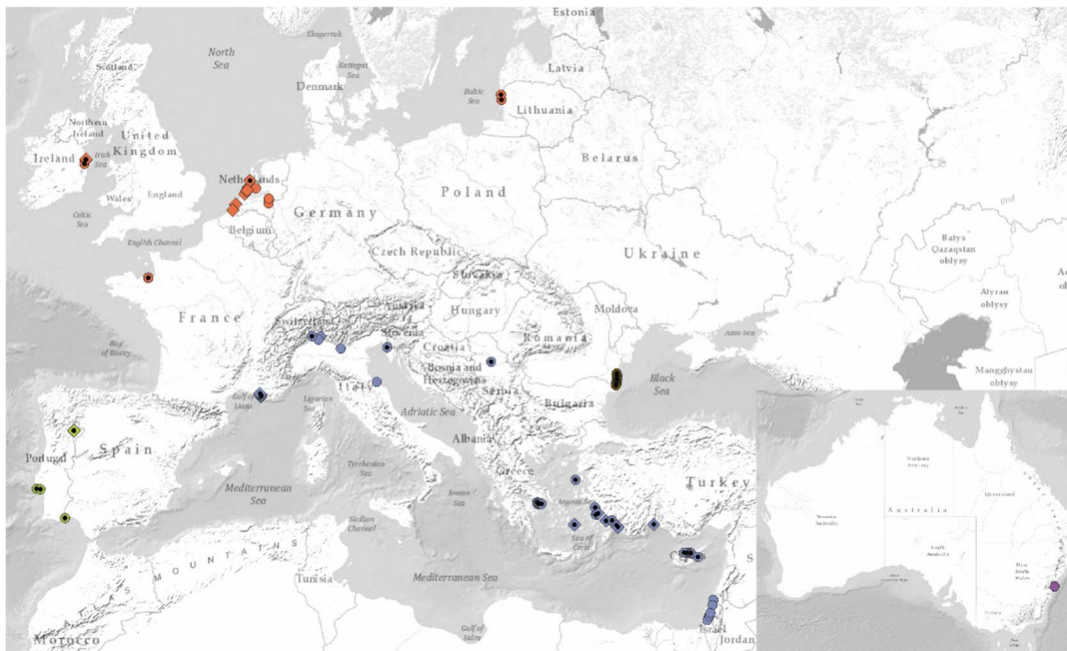
312

313 **Figure 1:** Geographical distribution of the sampling points using mapping with QGIS (Version 3.10.0-A

314 Coruña). Circles correspond to urban beaches and diamonds to non-urban beaches. Dots within the

315 shapes indicate water-sampling sites. Red=Northwest Europe, Green=Southwest Europe,

316 Blue=Mediterranean, Brown=Black Sea and Purple=Sydney (Australia)



317

318

319 2.2 Analytical procedures

320 2.2.1 Sample preparation and incubation

321 Sand: Dry (supratidal) sand samples (between 100 and 200g, between 5 and 10 cm deep) were

322 collected aseptically (between 8 am and 12 pm) into a sterile plastic bag, labelled, and transported in

323 a cooler to the lab as described in (Sabino et al., 2011). Official air temperatures were recorded for

324 comparison purposes.

325 Forty grams of crude sand (not dry weight) were extracted with 40 ml of sterile distilled water by
326 orbital shaking for 30 minutes at 100 rpm and the extract was then plated (0.2 ml) in triplicates per
327 media (Sabouraud's Dextrose agar (SDA) and Mycosel agar (Cycloheximide, Chloramphenicol agars)).
328 Plates were incubated for 5 days in SDA and 21 days in Mycosel agar, both at 27.5°C +/- 0.5°C.
329 Identical colonies were counted and identified to match the different study parameters and the
330 results were given in colony-forming units (CFU) per gram of crude sand (equivalent), as mean
331 numbers of each triplicate.

332 Water: water samples (about 400 ml) were collected aseptically underwater (20 cm deep in 1 m deep
333 water column between 8 a.m. and 12 a.m.), into a sterile vessel, and transported cooled (less than
334 20°C) to the laboratory for direct plating of 0.2 ml in triplicates, after gentle shaking and as described
335 previously, for sand.

336 2.2.2 Colony counting on plates after incubation.

337 The team performed a quality control assessment based on ISO 13528:2015 to determine the
338 consensus value of colony counting with a collective result of 95% accuracy; deemed acceptable for
339 the group's performance.

340 2.2.3 Taxonomic identification of the colonies.

341 Colonies were identified either by comparing macroscopic and microscopic features of the fungal
342 colonies with the morphology shown in the Atlas of Clinical Fungi (de Hoog et al., 2019), and/or as
343 described in (Frenkel et al., 2020), by the use of MALDI-ToF MS (matrix-assisted laser desorption
344 ionization-time of flight mass spectrometry) following the manufacturer's instructions, comparing
345 the obtained spectra against the fungal reference spectral libraries, or via molecular identification,
346 amplifying either the primary (the Internal Transcribed Spacers (ITS 1/2) regions of the ribosomal
347 DNA) or the secondary (the elongation factor 1 α (*TEF1 α*) or the RNA polymerase II gene (*RPB2*))
348 fungal DNA barcodes further detailed per participating laboratory in the supplementary material,
349 followed by the sequencing of the amplification products using the same primers. Consensus
350 sequences were uploaded onto BLAST for taxonomic assignment. Taxonomy was only assigned for

351 sequence matches with >98 similarity and >99% query cover. All identifications followed the
352 requisites of point 7.7 (Ensuring the validity of the results) of ISO 17025 (extended to ISO 15189 for
353 laboratories servicing in clinical analysis), of competence of testing and calibration laboratories,
354 which enforces many controls to ensure the validity of the results, including fungal identifications
355 (through inter-laboratory quality assessment schemes). A table detailing the identification methods
356 and respective quality assessment certifications is provided in the supplementary material.

357

358 2.3 Statistical analyses

359 Descriptive analysis was performed, using means, standard deviation, median and range (minimum
360 and maximum) for continuous variables. The distributions of parameters were compared between
361 urbanisation type (urban and non-urban); beach type (freshwater shores and coastal beaches); type
362 of sand (mix and pure sand); for regions of countries aggregated by geographical and climatological
363 proximity (Black Sea, Mediterranean, Northwest Europe, Southwest Europe and Sydney (Australia);
364 seasons (Fall/Winter and Spring/Summer); using non-parametric tests (Wilcoxon and Kruskal-Wallis
365 tests with Bonferroni correction for multiple comparisons). Pearson correlation coefficient was used
366 to measure the association between parameters. Values of $p < 0.05$ were accepted as statistically
367 significant. All statistical analyses were performed using R version 4.0.2.

368 2.4 General Notes and categorisation

369 2.4.1 Samples

370 Sand data correspond to 372 samples from 13 different countries, collected between 2018-01-08 and
371 2020-07-24. 38 are inland freshwater shores and 334 are coastal beaches sites; Water data
372 correspond to 315 samples from 11 different countries, collected between 2018-01-08 and 2020-02-
373 23. 14 are inland freshwater and 301 are coastal beach water samples. Sydney (Australia) and Israel
374 only reported results for the sand sample and Romania did not provide detailed identification of its

375 filamentous fungi (other than for dermatophytes). Australia sampled lagged compared to the
376 northern hemisphere for comparability purposes.

377 2.4.2 Parameters

378 Besides the independent analytical parameters corresponding mainly to representative species of
379 clinical relevance found in the study, a few (**non-independent**) parameters were built to represent
380 the less prevalent species of the study and when only the genera were identified, as follows:

381 **Yeasts** – contains all species belonging to the genera *Candida*, *Cryptococcus*, *Rhodotorula*,
382 *Saccharomyces*, *Trichosporon* and all unidentified reports with yeast in the name (e.g. “unidentified
383 yeast”); ***Candida* spp** – contains all *Candida* species, including all species processed independently;
384 **‘Dermatophytes’** – contains all identifications as *Microsporum*, *Trichophyton*, *Arthroderma*,
385 *Epidermophyton* and (unidentified) Dermatophytes; **‘Allergenic fungi’** – contains all species of fungi
386 excluding Yeasts and Dermatophytes; **‘Dematiaceous fungi’**: contains all fungi with melanin-like
387 pigments in the cell walls as described above, described by Brandt and Warnock (Brandt and
388 Warnock, 2003).

389 The following independent parameters are defined hereafter: ***Aspergillus* section *Fumigati***
390 represents *Aspergillus fumigatus sensu stricto* and all unidentified cryptic species of its section
391 (*Fumigati*). The same applies to ***Aspergillus* section *Nigri***, ***Aspergillus* section *Flavi*** and ***Candida***
392 ***parapsilosis*** which includes in this study represents the *sensu stricto* and its cryptic species *C.*
393 *ortopsilosis* and *C. metapsilosis*.

394 2.4.3 Quantification

395 Quantification values for each fungus are displayed in CFU/g for crude sand and per millilitre for
396 water (CFU/ml).

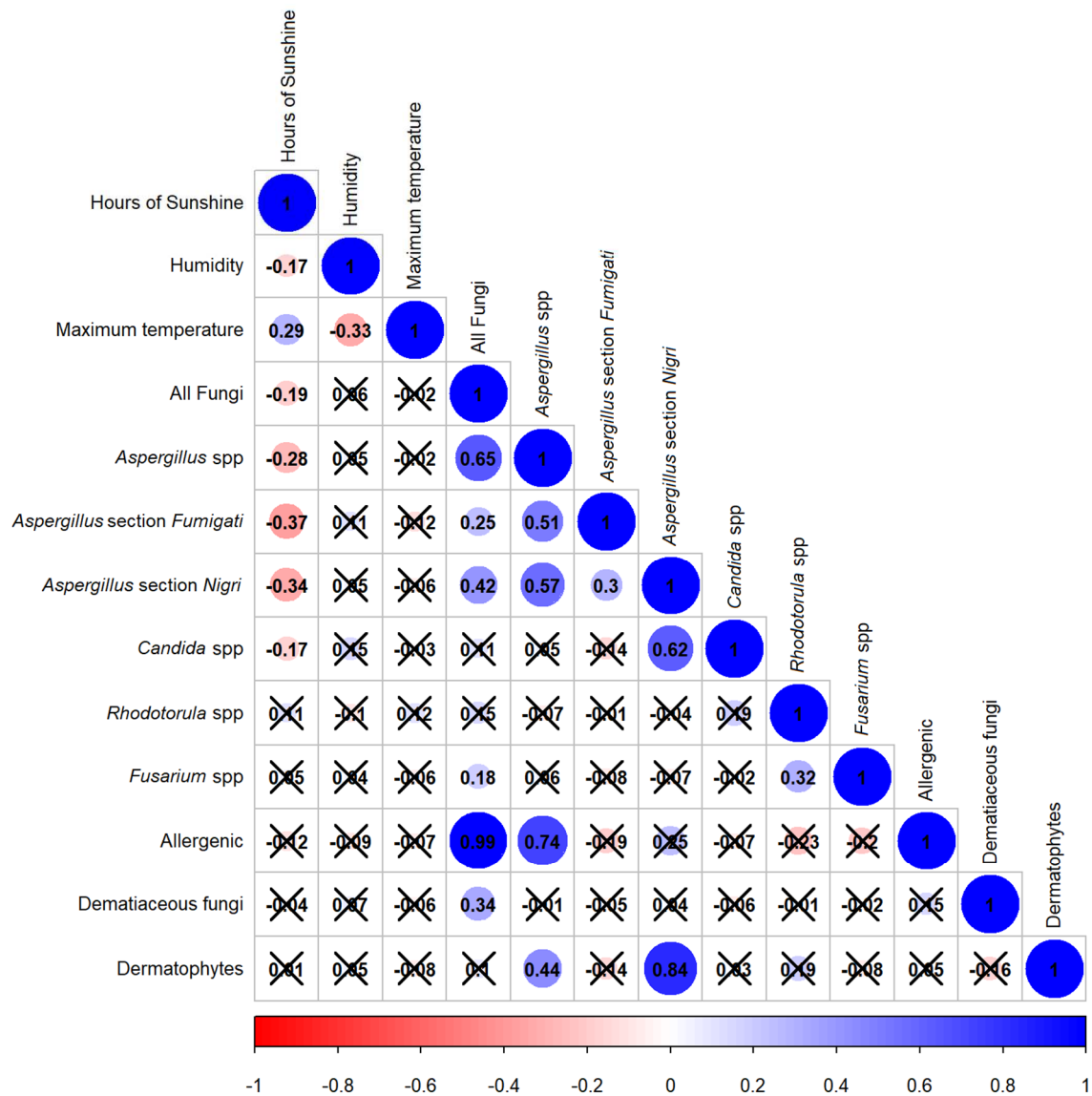
397 3. Results

398 3.1 Sand

399 3.1.1 Pearson’s correlation

400 Figure 2 presents Pearson's statistically significant correlations between fungal parameters,
401 maximum temperatures, humidity and hours of sunshine during sampling of sand. For humidity, the
402 parameters 'All Fungi', *Aspergillus* spp., *A. section Fumigati*, *A. section Nigri* and *Candida* spp.
403 correlate negatively to the hours of sunshine at the sampling day (-0.17, -0.19, -0.28, -0.37, and -0.34,
404 respectively). Conversely, the maximum temperature correlates positively (0.29). Positive
405 correlations were found between the following independent parameters: *Aspergillus* section
406 *Fumigati*, *Candida* spp. and 'Dermatophytes' with *A. section Nigri* (0.3, 0.62 and 0.84 respectively)
407 and *Fusarium* spp. with *Rhodotorula* spp. There were not enough pairs of data with *A. section Flavi*,
408 *Candida albicans*, *C. parapsilosis*, *C. glabrata*, *C. tropicalis*, *C. dubliniensis* and *Cryptococcus* spp. to
409 estimate correlations.

410 **Figure 2:** Sand correlations between fungal parameters, maximum temperatures during sampling and
411 hours of sunshine (statistically significant correlations do not have an X on top of the value).



412

413

414 3.1.2 Culturable mycobiota

415 The median number of fungal CFU/g in beach sand (of any kind, place, or period – All Fungi) was 89.2

416 CFU/g, with a range between 0.0 and 6400.0 CFU/g. By coastal or inland freshwater beaches, that

417 number is split into a median of 76.7 (0.0, 3497.5) CFU/g for 330 coastal beach sands and 201.7 (0.0,

418 6400.0) CFU/g for 42 inland freshwater beach sands (p= 0.010 - Table 1). Table 1 shows also that the

419 distribution of fungi between them may be quite different. The parameters *C. albicans*, *Cryptococcus*

420 spp., ‘Allergenic fungi’ and ‘Dematiaceous fungi’ appear with lower medians in coastal beach sands

421 than in freshwater beach sands, respectively 0.0 (0.0, 20.0) CFU/g to 29.2 (0.0, 50.0) CFU/g (p=0.037),
 422 0.0 (0.0, 500.0) CFU/g to 63.3 (0.0, 110.0) CFU/g (p=0.013), 10.0 (0.0, 350.0) CFU/g to 252.5 (50.0,
 423 6400.0) CFU/g (p<0.001) and 0.0 (0.0, 2545.0) CFU/g to 19.2 (0.0, 600.0) CFU/g (p<0.001), which
 424 suggests a difference in the typical composition of sand culturable mycobiota. Even ‘Dermatophytes’
 425 shows a difference, these fungi are more present in coastal beach sands ((1.7 (0.0, 150.0) CFU/g)
 426 than in freshwater beach sands (0.0 (0.0, 166.7) CFU/g, p=0.005). The other parameters showed no
 427 statistical significance for this comparison. *A. section Flavi* (not shown in Table 1), *C. parapsilosis*, *C.*
 428 *tropicalis* and *C. dubliniensis* were reported only in coastal beaches.

429 **Table 1** – Comparison of results by beach types and period of the year for sand samples

| Median (range) in CFU/g | | | | | | | | | |
|--|-------------------------|----------------------------|-------------------|--------------------------|-----------------------|-------------------|---------------------|-----------------------|--------------|
| Sand | | | | | | | | | |
| Variable | Coastal beaches (N=330) | Fresh water beaches (N=42) | P value | Non-Urban beaches (N=86) | Urban beaches (N=286) | P value | Fall/Winter (N=128) | Spring/Summer (N=244) | P value |
| All Fungi | 76.7 (0.0, 3497.5) | 201.7 (0.0, 6400.0) | 0.010 | 70.8 (0.0, 6400.0) | 94.2 (0.0, 3497.5) | 0.344 | 127.5 (0.0, 6400.0) | 76.7 (0.0, 3497.5) | 0.016 |
| <i>Aspergillus</i> spp | 5.0 (0.0, 2930.0) | 13.3 (0.0, 943.3) | 0.291 | 16.7 (0.0, 900.0) | 3.3 (0.0, 2930.0) | 0.176 | 16.7 (0.0, 2357.5) | 3.3 (0.0, 2930.0) | 0.071 |
| <i>Aspergillus</i> section <i>Fumigati</i> | 0.0 (0.0, 425.0) | 0.0 (0.0, 66.7) | 0.459 | 0.0 (0.0, 166.7) | 0.0 (0.0, 425.0) | 0.340 | 0.0 (0.0, 425.0) | 0.0 (0.0, 91.7) | 0.479 |
| <i>Aspergillus</i> section <i>Nigri</i> | 0.0 (0.0, 950.0) | 0.0 (0.0, 943.3) | 0.996 | 8.3 (0.0, 833.3) | 0.0 (0.0, 950.0) | 0.778 | 0.0 (0.0, 950.0) | 0.0 (0.0, 943.3) | 0.381 |
| <i>Candida</i> spp | 0.0 (0.0, 555.0) | 0.0 (0.0, 50.0) | 0.500 | 4.2 (0.0, 555.0) | 0.0 (0.0, 250.0) | < 0.001 | 0.0 (0.0, 555.0) | 0.0 (0.0, 168.3) | 0.389 |
| <i>Candida albicans</i> | 0.0 (0.0, 20.0) | 29.2 (0.0, 50.0) | 0.037 | NA | 0.0 (0.0, 50.0) | | 0.0 (0.0, 50.0) | 0.0 (0.0, 50.0) | 0.388 |
| <i>Candida parapsilosis</i> | 0.0 (0.0, 123.3) | NA | | NA | 0.0 (0.0, 123.3) | | 0.0 (0.0, 10.0) | 2.5 (0.0, 123.3) | 0.205 |
| <i>Candida tropicalis</i> | 0.0 (0.0, 41.7) | NA | | 0.0 (0.0, 41.7) | 0.0 (0.0, 11.7) | 0.189 | 0.0 (0.0, 35.0) | 0.0 (0.0, 41.7) | 0.156 |
| <i>Candida dubliniensis</i> | 1.7 (0.0, 516.7) | NA | | 3.3 (0.0, 516.7) | 0.0 (0.0, 2.5) | 0.052 | 33.3 (0.0, 516.7) | 1.7 (0.0, 126.7) | 0.017 |
| <i>Rhodotorula</i> spp | 0.0 (0.0, 1333.3) | 0.0 (0.0, 60.0) | 0.227 | 0.0 (0.0, 666.7) | 0.0 (0.0, 1333.3) | 0.082 | 0.0 (0.0, 833.3) | 0.0 (0.0, 1333.3) | 0.443 |
| <i>Cryptococcus</i> spp | 0.0 (0.0, 500.0) | 63.3 (0.0, 110.0) | 0.013 | NA | 0.0 (0.0, 500.0) | | 0.0 (0.0, 83.3) | 0.8 (0.0, 500.0) | 0.090 |
| <i>Fusarium</i> spp | 2.5 (0.0, 428.3) | 0.8 (0.0, 123.3) | 0.628 | 0.0 (0.0, 400.0) | 7.5 (0.0, 428.3) | 0.068 | 0.0 (0.0, 400.0) | 8.8 (0.0, 428.3) | 0.176 |
| Yeasts | 0.0 (0.0, 1333.3) | 0.0 (0.0, 246.7) | 0.420 | 1.7 (0.0, 666.7) | 0.0 (0.0, 1333.3) | 0.097 | 0.0 (0.0, 833.3) | 0.0 (0.0, 1333.3) | 0.510 |
| Allergenic fungi | 10.0 (0.0, 350.0) | 252.5 (50.0, 6400.0) | < 0.001 | 29.2 (0.0, 6400.0) | 15.0 (0.0, 350.0) | 0.070 | 68.3 (0.0, 6400.0) | 14.2 (0.0, 340.0) | 0.006 |
| Dematiaceous fungi | 0.0 (0.0, 2545.0) | 19.2 (0.0, 600.0) | < 0.001 | 0.0 (0.0, 600.0) | 0.0 (0.0, 2545.0) | 0.125 | 0.0 (0.0, 600.0) | 0.0 (0.0, 2545.0) | 0.083 |
| Dermatophytes | 1.7 (0.0, 150.0) | 0.0 (0.0, 166.7) | 0.005 | 0.0 (0.0, 166.7) | 0.0 (0.0, 150.0) | 0.820 | 1.7 (0.0, 166.7) | 0.0 (0.0, 83.3) | 0.066 |

430
 431 **3.1.3 Sand composition**

432 The composition of the sand influences the culturable mycobiota. Pure sand has lower (All Fungi)
 433 presence of fungi (16.7 (0.0, 300.0) CFU/g) than non-sandy shores (sediment and/or gravel) which
 434 amounted to 90.0 (0.0, 6400.0) CFU/g (p=0.026). The other parameters, *Candida* spp., *C. albicans*, *C.*
 435 *parapsilosis*, *C. tropicalis*, *C. dubliniensis*, *Rhodotorula* spp., *Cryptococcus* spp., Yeasts, ‘Allergenic
 436 fungi’ and Dermatophytes, were not detected in non-sandy beaches; only ‘Dematiaceous fungi’,
 437 though without statistical significance (0.0 (0.0, 2545.0) CFU/g for sandy beaches and 0.0 (0.0, 183.3)
 438 CFU/g for non-sandy beaches (p=0.251). However, since these beaches were much less represented
 439 than the sandy ones, the results of the latter parameters should not be considered as either robust
 440 or very relevant.

441 3.1.4 Geography

442 The grouping of countries into regions (Table 2) was statistically significant only for some parameters
 443 of sand with Sydney (Australia) showing the highest concentration of several parameters of all
 444 regions: All Fungi (p<0.001), *A. section Nigri* (<0.001), *Candida* spp. (p=0.027), *Rhodotorula* spp.
 445 (p<0.001), *Cryptococcus* spp. (0.0191), ‘Dematiaceous fungi’ (<0.0011), *Fusarium* spp. (< 0.001) and
 446 the dependent parameters Yeasts (p<0.001). Nonetheless, somewhat surprisingly the hotter climates
 447 are not necessarily the least (myco-) populated ones at the beach.

448 **Table 2** – Fungal parameters results for the sand samples by region

| Median (range) in CFU/g | | | | | | |
|--|--------------------|-----------------------|-------------------------|-------------------------|-------------------------|---------|
| Sand | | | | | | |
| Variable | Black Sea (N=80) | Mediterranean (N=159) | Northwest Europe (N=90) | Southwest Europe (N=34) | Sydney, Australia (N=9) | P value |
| All Fungi | 72.5 (0.5, 2170.0) | 150.0 (0.0, 3365.0) | 20.0 (0.0, 3497.5) | 90.8 (1.7, 6400.0) | 366.7 (83.3,1533.3) | < 0.001 |
| <i>Aspergillus</i> spp | NA | 33.3 (0.0, 2930.0) | 1.7 (0.0, 156.7) | 1.7 (0.0, 900.0) | 48.3 (0.0, 943.3) | < 0.001 |
| <i>Aspergillus</i> section <i>Fumigati</i> | NA | 0.0 (0.0, 425.0) | 0.0 (0.0, 91.7) | 0.0 (0.0, 16.7) | NA | |
| <i>Aspergillus</i> section <i>Nigri</i> | NA | 0.0 (0.0, 950.0) | 26.2 (2.5, 50.0) | 0.0 (0.0, 833.3) | 723.3 (40.0, 943.3) | 0.005 |
| <i>Aspergillus</i> section <i>Flavi</i> | NA | 0.0 (0.0, 2000.0) | NA | NA | NA | |
| <i>Candida</i> spp | 0.0 (0.0, 40.0) | 0.0 (0.0, 250.0) | 1.7 (0.0, 555.0) | 0.0 (0.0, 21.7) | 0.0 (0.0, 123.3) | 0.027 |
| <i>Candida albicans</i> | 0.0 (0.0, 20.0) | 0.0 (0.0, 50.0) | NA | NA | NA | |
| <i>Candida parapsilosis</i> | 0.0 (0.0, 30.0) | 0.0 (0.0, 30.0) | NA | NA | 0.0 (0.0, 123.3) | |
| <i>Candida tropicalis</i> | NA | 0.0 (0.0, 10.0) | 0.0 (0.0, 41.7) | NA | NA | |
| <i>Candida dubliniensis</i> | NA | NA | 1.7 (0.0, 516.7) | NA | NA | |
| <i>Rhodotorula</i> spp | 0.0 (0.0, 106.0) | 0.0 (0.0, 1333.3) | 0.0 (0.0, 130.0) | 0.0 (0.0, 3.3) | 40.0 (0.0, 166.7) | < 0.001 |
| <i>Cryptococcus</i> spp | NA | 5.0 (0.0, 83.3) | 0.0 (0.0, 500.0) | 0.0 (0.0, 1.7) | 13.3 (0.0, 110.0) | 0.019 |
| <i>Fusarium</i> spp | NA | 16.7 (0.0, 428.3) | 0.0 (0.0, 77.5) | 0.0 (0.0, 68.3) | 33.3 (6.7, 123.3) | < 0.001 |
| Yeasts | 0.0 (0.0, 106.0) | 0.0 (0.0, 1333.3) | 1.7 (0.0, 1265.0) | 0.0 (0.0, 21.7) | 123.3 (16.7, 190.0) | < 0.001 |
| Allergenic fungi | NA | 0.0 (0.0, 0.0) | 15.0 (0.0, 170.0) | 252.5 (50.0, 6400.0) | NA | |
| Dematiaceous fungi | NA | 0.0 (0.0, 416.7) | 0.0 (0.0, 2545.0) | 15.0 (0.0, 600.0) | 3.3 (0.0, 36.7) | < 0.001 |
| Dermatophytes | NA | 20.8 (0.0, 83.3) | 1.7 (0.0, 53.3) | 0.0 (0.0, 166.7) | NA | |

449 Note: N=Number of samples

450
 451 During this project, the median relative humidity is highest in the Northwest Europe region (78.0
 452 (60.0, 97.0) %) and lowest in Sydney (Australia) (60.0 (60.0, 67.0) %). Sydney (Australia) is where the
 453 highest median of CFU/g of All Fungi can be found (366.7 (83.3, 1533.3)) CFU/g; compared to 150.0
 454 (0.0, 3365.0) CFU/g in the Mediterranean region (64.0 (19.0, 98.0) % humidity), 20.0 (0.0, 3497.5)
 455 CFU/g, in the Northwest Europe region (78.0 (60.0, 97.0) % humidity) and 90.8 (1.7, 6400.0) in the
 456 Southwest Europe region (70.0 (25.0, 96.0) % humidity) (Table 3). *A. section Flavi* was only reported
 457 in the Mediterranean region, *C. dubliniensis* in the Northwest Europe region and *C. albicans* in the
 458 Mediterranean and the Black Sea regions.

460 3.1.5 Period of the year

461 There were statistically significant differences found between the period Fall/Winter and
462 Spring/Summer (Table 1). The parameter All Fungi shows higher counts in the Fall and Winter (127.5
463 (0.0, 6400.0) CFU/g) compared to the Spring and Summer (76.7 (0.0, 3497.5) CFU/g), $p=0.016$. The
464 'Allergenic fungi' parameter makes up for a large fraction of the All Fungi parameter, with the
465 following data: 68.3 (0.0, 6400.0) CFU/g for Fall and Winter and 14.2 (0.0, 340.0) CFU/g for Spring
466 and Summer ($p=0.006$). Although not statistically significant, *Aspergillus* spp. also differ between
467 period, with 16.7 (0.0, 2357.5) CFU/g and 3.3 (0.0, 2930.0) CFU/g respectively ($p= 0.071$). *C.*
468 *dublinskiensis* also contributes to the difference in periods for All Fungi with 33.3 (0.0, 516.7) CFU/g in
469 the Fall/Winter and 1.7 (0.0, 126.7) CFU/g in the Spring/Summer (0.017).

470 3.1.6 Urban versus non-urban

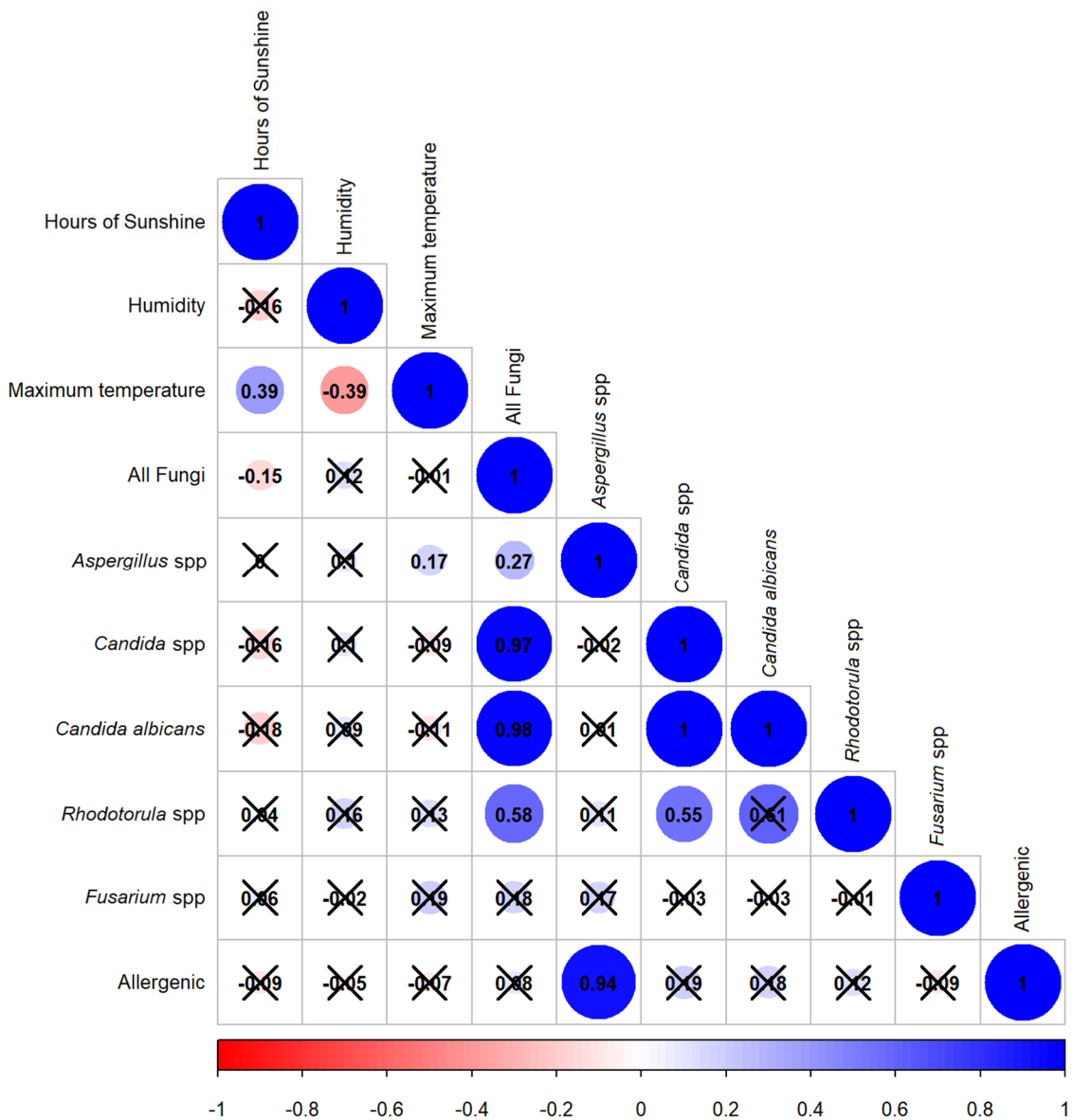
471 *Candida* spp. and *C. dublinskiensis* are more prone to non-urban environments, as shown in (Table 1),
472 with respectively, 4.2 (0.0, 555.0) CFU/g compared to 0.0 (0.0, 250.0) CFU/g in urban environments
473 ($p<0.001$) and 3.3 (0.0, 516.7) CFU/g to 0.0 (0.0, 2.5) CFU/g ($p=0.052$) in non-urban environments.
474 *Rhodotorula* spp., *Cryptococcus* spp., *C. albicans* and *C. parapsilosis* were only detected in urban
475 beaches. The *Rhodotorula* spp. results were 0.0 (0.0, 666.7) CFU/g in non-urban beaches, compared
476 to 0.0 (0.0, 1333.3) CFU/g in urban beaches ($p=0.082$), as medians. However, with means of 47.2
477 CFU/g for non-urban beaches and 76.6 CFU/g for urban beaches, the very high Standard Deviations
478 (SD) take away any strength to the original behaviour expectation (SD=160.5CFU/g and 213.7CFU/g,
479 respectively). 'Dematiaceous fungi', for which the median provides no relevant information or p -
480 value either when comparing non-urban and urban beaches (0.0 (0.0, 600.0) CFU/g and 0.0 (0.0,
481 2545.0) CFU/g, respectively, $p=0.125$), the means and standard deviations are respectively 53.3
482 (133.3) and 27.2 (191.7), granting some expectation that maybe with more isolations 'Dematiaceous
483 fungi' would possibly confirm a non-urban preference. Unlike *Fusarium* spp. which also has no
484 statistically relevant difference (0.0 (0.0, 400.0) CFU/g for non-urban and 7.5 (0.0, 428.3) CFU/g for
485 urban, but almost ($p= 0.068$), that shows means of 28.7 (SD= 79.2) and 47.8 (SD=98.1) respectively.

486 3.2 Water

487 3.2.1 Pearson's correlation

488 Figure 3 presents statistically significant correlations between fungal parameters, maximum
489 temperatures, humidity and hours of sunshine during sampling for water. All Fungi and Yeasts
490 correlate negatively to the hours of sunshine on the sampling day (-0.15, -0.16, respectively). The
491 maximum temperature correlates positively with hours of sunshine with a Pearson's correlation
492 factor of 0.39 but negatively with humidity with a factor of -0.39. Positive correlations were found
493 between the following independent parameters: *Rhodotorula* spp. and *Candida* spp. with a factor of
494 0.55. There were not enough pairs of data with *A.* section *Fumigati*, *A.* section *Nigri*, *A.* section *Flavi*,
495 *C. parapsilosis*, *C. glabrata*, *C. tropicalis*, *C. dubliniensis*, *Cryptococcus* spp. and 'Dermatophytes' to
496 estimate correlations.

497 **Figure 3:** Water correlations between fungal parameters, maximum temperatures during sampling
498 and hours of sunshine (statistically significant correlations do not have an X on top of the value).



499

500

501 3.2.2 Culturable mycobiota

502 The median number of fungal CFU/ml of water (of any kind, place, or period – All fungi) was 0.0
 503 CFU/g, with a range between 0.0 and 1591.7 CFU/g. The reason for this is the number of samples
 504 with 0 CFU/ml in the pool of analyses done. Yet, comparing freshwaters and coastal waters, there is a
 505 median number of 6.7 (0.0, 310.0) CFU/ml for freshwaters and 0.0 (0.0, 1591.7), $p < 0.001$ for coastal
 506 waters. Since the number of coastal waters (293) is considerably higher than freshwaters (23), and

507 mainly with low to zero CFU/ml, they pull down to 0 CFU/ml the overall medians of all beaches and
 508 of coastal beaches (Table 3).

509 **Table 3** – Comparison of results by beach types and period of the year for water samples

| Median (range) in CFU/ml | | | | | | | | | |
|--|-------------------------|----------------------------|---------|--------------------------|-----------------------|---------|---------------------|-----------------------|---------|
| Water | | | | | | | | | |
| Variable | Coastal beaches (N=293) | Fresh water beaches (N=23) | P value | Non-Urban beaches (N=86) | Urban beaches (N=286) | P value | Fall/Winter (N=113) | Spring/Summer (N=202) | P value |
| All Fungi | 0.0 (0.0, 1591.7) | 6.7 (0.0, 310.0) | < 0.001 | 3.3 (0.0, 1591.7) | 0.0 (0.0, 310.0) | 0.005 | 0.0 (0.0, 1591.7) | 0.0 (0.0, 310.0) | 0.894 |
| <i>Aspergillus</i> spp | 0.0 (0.0, 181.7) | 0.0 (0.0, 201.7) | 0.153 | 0.0 (0.0, 83.3) | 0.0 (0.0, 201.7) | 0.014 | 0.0 (0.0, 153.3) | 0.0 (0.0, 201.7) | 0.406 |
| <i>Aspergillus</i> section <i>Fumigati</i> | 0.0 (0.0, 90.0) | NA | | 0.0 (0.0, 20.0) | 0.0 (0.0, 90.0) | 0.008 | 0.0 (0.0, 20.0) | 0.0 (0.0, 90.0) | 0.387 |
| <i>Aspergillus</i> section <i>Nigri</i> | 0.0 (0.0, 41.7) | 0.0 (0.0, 83.3) | 0.865 | 0.0 (0.0, 83.3) | 0.0 (0.0, 41.7) | 0.087 | 0.0 (0.0, 83.3) | 0.0 (0.0, 41.7) | 0.440 |
| <i>Candida</i> spp | 0.0 (0.0, 1585.0) | 3.3 (0.0, 8.3) | 0.057 | 0.0 (0.0, 1585.0) | 0.0 (0.0, 15.0) | 0.321 | 0.0 (0.0, 1585.0) | 0.0 (0.0, 35.0) | 0.995 |
| <i>Candida albicans</i> | 0.0 (0.0, 170.0) | 3.3 (0.0, 8.3) | < 0.001 | 0.0 (0.0, 170.0) | 0.0 (0.0, 8.3) | 0.348 | 0.0 (0.0, 170.0) | 0.0 (0.0, 8.3) | 0.831 |
| <i>Candida parapsilosis</i> | 6.0 (0.0, 12.0) | NA | | NA | 6.0 (0.0, 12.0) | | 12.0 (12.0, 12.0) | 0.0 (0.0, 0.0) | 1.000 |
| <i>Candida tropicalis</i> | 0.0 (0.0, 90.0) | NA | | 0.0 (0.0, 90.0) | 0.0 (0.0, 1.0) | 0.339 | 0.0 (0.0, 90.0) | 0.0 (0.0, 16.7) | 0.813 |
| <i>Candida dubliniensis</i> | 0.0 (0.0, 1325.0) | NA | | 0.0 (0.0, 1325.0) | 0.0 (0.0, 2.5) | 0.919 | 0.0 (0.0, 1325.0) | 0.0 (0.0, 28.3) | 0.571 |
| <i>Rhodotorula</i> spp | 0.0 (0.0, 250.0) | 0.0 (0.0, 40.0) | 0.280 | 0.0 (0.0, 25.0) | 0.0 (0.0, 250.0) | 0.932 | 0.0 (0.0, 100.0) | 0.0 (0.0, 250.0) | 0.932 |
| <i>Cryptococcus</i> spp | NA | NA | | NA | NA | | NA | NA | |
| <i>Fusarium</i> spp | 0.0 (0.0, 33.3) | 0.0 (0.0, 10.0) | 0.161 | 0.0 (0.0, 0.0) | 0.0 (0.0, 33.3) | 0.072 | 0.0 (0.0, 8.3) | 0.0 (0.0, 33.3) | 0.239 |
| Yeasts | 0.0 (0.0, 1585.0) | 0.0 (0.0, 98.3) | 0.188 | 0.0 (0.0, 1585.0) | 0.0 (0.0, 250.0) | 0.513 | 0.0 (0.0, 1585.0) | 0.0 (0.0, 250.0) | 0.809 |
| Allergenic fungi | 1.7 (0.0, 13.3) | 5.8 (0.0, 131.7) | 0.001 | 1.7 (0.0, 131.7) | 1.7 (0.0, 26.7) | 0.150 | 3.3 (0.0, 131.7) | 1.7 (0.0, 13.3) | 0.015 |
| Dematiaceous fungi | 0.0 (0.0, 111.7) | 0.0 (0.0, 16.7) | < 0.001 | 0.0 (0.0, 33.3) | 0.0 (0.0, 111.7) | 0.878 | 0.0 (0.0, 33.3) | 0.0 (0.0, 111.7) | 0.006 |
| Dermatophytes | 0.0 (0.0, 1.7) | NA | | 0.0 (0.0, 1.7) | 0.0 (0.0, 0.3) | 0.661 | 0.0 (0.0, 1.7) | 0.0 (0.0, 0.3) | 0.450 |

510
 511 The following parameters distribute themselves significantly between coastal and freshwaters in the
 512 following way, respectively: *C. albicans* - 0.0 (0.0, 170.0) CFU/ml to 3.3 (0.0, 8.3) CFU/ml, p<0.001,
 513 'Allergenic fungi' -1.7 (0.0, 13.3) CFU/ml to 5.8 (0.0, 131.7) CFU/ml, p=0.001 and 'Dematiaceous
 514 fungi'- 0.0 (0.0, 111.7) CFU/ml to 0.0 (0.0, 16.7) CFU/ml, p<0.001. Additionally, *A. section Fumigati*, *C.*
 515 *parapsilosis*, *C. tropicalis*, and *C. dubliniensis* and 'Dermatophytes' were only reported in coastal
 516 beaches (Table 3).

517 3.2.3 Geography

518 Regarding the parameters distributed by country and by region (Table 4), there are no noticeable
 519 regional differences in fungal parameters in bathing water, besides for *Aspergillus* spp., which show a
 520 p-value of 0.041 for the comparison between the Mediterranean, Northwest Europe and Southwest
 521 Europe regions only. The medians (and ranges) of this comparison are respectively 0.0 (0.0, 201.7)
 522 CFU/ml, 0 (0.0, 181.7) CFU/ml and 0.0 (0.0, 83.3) CFU/ml and mean values (and standard deviations)
 523 being 11.4 (31.9) CFU/ml, 3.1 (21.6) CFU/ml and 3.4 (14.9) CFU/ml. Excluding Romania, the same test
 524 adds statistical significance for 'Dematiaceous fungi' (0.0 (0.0, 111.7) CFU/ml for the Mediterranean,

525 0.0 (0.0, 2.5) CFU/ml for the Northwest Europe and 0.0 (0.0, 21.7) CFU/ml for the Southwest Europe,
 526 with a $p < 0.0011$.

527 **Table 4** – Fungal parameters results for water samples by region

| Median (range) in CFU/ml | | | | | |
|--|------------------|-----------------------|-------------------------|-------------------------|-------------------|
| Water | | | | | |
| Variable | Black Sea (N=80) | Mediterranean (N=159) | Northwest Europe (N=90) | Southwest Europe (N=34) | P value |
| All Fungi | 0.0 (0.0, 55.0) | 0.0 (0.0, 310.0) | 0.0 (0.0, 1591.7) | 4.2 (0.0, 131.7) | 0.083 |
| <i>Aspergillus</i> spp | NA | 0.0 (0.0, 201.7) | 0.0 (0.0, 181.7) | 0.0 (0.0, 83.3) | 0.0041 |
| <i>Aspergillus</i> section <i>Fumigati</i> | NA | 0.0 (0.0, 20.0) | 0.0 (0.0, 90.0) | NA | |
| <i>Aspergillus</i> section <i>Nigri</i> | NA | 0.0 (0.0, 41.7) | NA | 0.0 (0.0, 83.3) | |
| <i>Aspergillus</i> section <i>Flavi</i> | NA | 0.0 (0.0, 0.7) | NA | NA | |
| <i>Candida</i> spp | 0.0 (0.0, 2.0) | 0.0 (0.0, 12.0) | 0.0 (0.0, 1585.0) | NA | |
| <i>Candida albicans</i> | NA | 3.3 (0.0, 8.3) | 0.0 (0.0, 170.0) | NA | |
| <i>Candida parapsilosis</i> | NA | 6.0 (0.0, 12.0) | NA | NA | |
| <i>Candida glabrata</i> | NA | 0.0 (0.0, 2.5) | 0.0 (0.0, 1.7) | NA | |
| <i>Candida tropicalis</i> | 0.0 (0.0, 1.0) | NA | 0.0 (0.0, 90.0) | NA | |
| <i>Candida dubliniensis</i> | NA | NA | 0.0 (0.0, 1325.0) | NA | |
| <i>Rhodotorula</i> spp | 0.0 (0.0, 5.0) | 0.0 (0.0, 250.0) | 0.0 (0.0, 7.5) | 0.0 (0.0, 25.0) | 0.989 |
| <i>Fusarium</i> spp | NA | 0.0 (0.0, 33.3) | NA | 0.0 (0.0, 1.7) | |
| Yeasts | 0.0 (0.0, 10.0) | 0.0 (0.0, 250.0) | 0.0 (0.0, 1585.0) | 0.0 (0.0, 25.0) | 0.519 |
| Allergenic fungi | NA | NA | 1.7 (0.0, 13.3) | 5.8 (0.0, 131.7) | |
| Dematiaceous fungi | NA | 0.0 (0.0, 111.7) | 0.0 (0.0, 2.5) | 0.0 (0.0, 21.7) | < 0.001 |
| Dermatophytes | NA | NA | 0.0 (0.0, 1.7) | NA | |

528
 529 Note: N=Number of samples

530 3.2.4 Period of the year

531 As with sand, 'Allergenic fungi' are more prevalent in the Fall/Winter (3.3 (0.0, 131.7) CFU/ml) than in
 532 the Spring/Summer period (1.7 (0.0, 13.3) CFU/ml) with a p-value of 0.015 (Table 3).

533 3.2.5 Urban versus non-urban

534 We can find differences in the distributions of fungi in water samples by urbanisation type in the
 535 parameters 'All Fungi' with 3.3 (0.0, 1591.7) CFU/ml for non-urban beaches and 0.0 (0.0, 310.0)
 536 CFU/ml for urban beaches ($p=0.005$); in *Aspergillus* spp. with 0.0 (0.0, 83.3) CFU/ml for non-urban
 537 beaches and 0.0 (0.0, 201.7) CFU/ml for urban beaches ($p=0.014$); and in *A. section Fumigati* with 0.0
 538 (0.0, 20.0) CFU/ml for non-urban beaches and 0.0 (0.0, 90.0) CFU/ml for urban beaches ($p=0.008$).

539 4. Discussion

540 Environmental fungal exposure is recognized as being associated with a range of adverse health
 541 effects, including infectious or allergic diseases and toxic reactions. Efforts have been made in

542 standardizing methods to assess indoor fungal contamination (Méheust et al., 2013). Only limited
543 data on sand and water contamination are available, other than the ASTM D4249-83(2005) (ASTM
544 D4249-83(2005), 2005). *Candida* Standard, withdrawn in 2013 due to its limited use by the industry.
545 The major strengths of the Mycosands initiative are providing a large amount of original data and the
546 standardization of methods between thirteen countries worldwide.

547 4.1 Sand

548 The results of this study, reporting a great diversity of fungi in the sand of bathing beaches, reinforce
549 the findings of previous research (Abdallaoui et al., 2007; de Moura Sarquis and de Oliveira, 1996;
550 Dunn and Baker, 1984; Echevarría, 2019; Efstratiou and Velegraki, 2010; Frenkel et al., 2020; Gomes
551 et al., 2008; Kishimoto and Baker, 1969; Müller, 1973; Papadakis et al., 1997; Romão et al., 2017a;
552 Romão et al., 2017b; Salleh et al., 2018; Tokura, 1984; Ulfing et al., 1997; Vezzulli et al., 2009; Yee et
553 al., 2016). A variety of fungi have been identified in coastal and inland recreational beaches by other
554 research teams (Arvanitidou et al., 2002; Di Piazza et al., 2017; Efstratiou et al., 1998;
555 Muntañolacvetković and Ristanović, 1980; Oliveira et al., 2011; Papadakis et al., 1997; Romão et al.,
556 2017b; Rudenko et al., 2011; Sherry et al., 1979; Vezzulli et al., 2009). We identified over 300 taxa,
557 which is, to the best of our knowledge, and this is the largest number of taxa ever reported from
558 beach sand and water up to now.

559 During our study, we isolated a median which is lower than what Stevens *et al.* (Stevens et al., 2012)
560 determined, with mean populations of 109.36 CFU/g in scarcely used coastal beaches, 140.49 CFU/g
561 in averagely used beaches and 472.29 CFU/g in heavily used beaches. Dunn & Baker (Dunn and
562 Baker, 1984) reported from 1 - 15,900 CFU/g fungi from beach sand of Hawaii. Much smaller
563 numbers were observed by Echevarría (Echevarría, 2019) from the sand of beaches of Costa Rica (6
564 to 17 CFU/g), but they were isolating only filamentous fungi. Salvo & Fabiano (Salvo and Fabiano,
565 2007) isolated 0 to 11.470 CFU/g of yeasts and 58 - 1.778 CFU/g of filamentous fungi in the
566 northwestern Italian beaches. Migahed (Migahed, 2003) in Egypt, reported that the total count of
567 fungi in marine sand beaches ranges between 49.7 CFU/mg to 149.93 CFU/mg. It is not easy to

568 compare and/or draw concrete conclusions concerning the abundance of fungi on beach sand, as this
569 depends heavily on the overall pollution of the beach, the time of the year, environmental conditions
570 such as temperature, UV radiation, precipitation events, the numbers of bathers, the grain size and
571 the chemical nature of the sand, organic load, the presence of animals and birds. What is undeniably
572 established by our work and that of other teams, is that the sand of bathing beaches is seeded with
573 fungi.

574 Regarding the research published on the fungal load of sand in inland, freshwater beaches, we only
575 identified one research article (Zatoń and Błaszczak, 2015) that isolated fungi in the sand of an urban
576 lake beach in Poland, their numbers ranging between 1700 CFU/g and 2800 CFU/g. Considerably
577 higher than the median abundance of all fungi observed in our project. In this area, we isolated
578 significantly more fungi (as All Fungi numbers) from freshwater beaches than from marine beaches
579 ($p=0.010$). This could be attributed to the more favourable environmental conditions (organic load,
580 lack of salinity, vegetation nearby). Additionally, freshwater beaches harboured in the sand
581 significantly more *C. albicans* ($p=0.037$) as well as 'Allergenic fungi' ($p<0.001$), 'Dematiaceous fungi'
582 ($p<0.001$) and 'Dermatophytes' ($p=0.005$). The inland beaches examined in this project were few
583 compared to the marine beaches (little above 13%). This result may hence be seen as an indication of
584 a trend, but more investigation would be needed to give a clearer picture.

585 Our findings on the influence of the presence of bathers on overall fungal numbers/diversity in sand
586 suggest more isolates in urban beaches. However, the difference between urban and non-urban was
587 not statistically significant ($p=0.344$). *C. albicans*, *C. parapsilosis* and *Cryptococcus* spp. were only
588 detected in the sand of urban beaches. Our results reinforce the findings of several research groups
589 claiming that the presence of humans influences the numbers and species of fungi on beach sand
590 (Bergen and Wagner-Merner, 1977; Elmanama et al., 2005; Gomes et al., 2008; Kishimoto and Baker,
591 1969; Londono et al., 2018; Muntañolacvetković and Ristanović, 1980; Müller, 1973; Papadakis et al.,
592 1997; Rudenko et al., 2011; Salvo and Fabiano, 2007; Stevens et al., 2012; Vezzulli et al., 2009; Vogel
593 et al., 2007). This can be attributed to the fact that many isolates are opportunistic human

594 pathogens, easily shed into the environment. Statistically significant differences between urban and
595 non-urban beaches were observed in the concentrations of *Candida* spp. ($p < 0.001$) and *C.*
596 *dublinsiensis* ($p = 0.052$), which appeared in higher concentrations in non-urban beach sand. This may
597 be explained by *C. dublinsiensis* having been reported to be associated with non-urban wild-life (Nunn
598 et al., 2007).

599 Differences exist between our observations on the presence of *Rhodotorula* and the isolations of
600 Stevens et al. (Stevens et al., 2012) who, investigating the correlation of human beach use to the
601 abundance of fungi in the sand, describe *Rhodotorula mucilaginosa* and *R. slooffiae* only in marine
602 beaches heavily used by humans, whilst our results indicate the presence of *Rhodotorula* spp in both
603 urban and non-urban beach sand, the latter being significantly less used by people.

604 *Rhodotorula* spp. and *Cryptococcus* spp. were expected to show a statistically significant presence in
605 urban compared to non-urban beaches, under the premise that urban beaches may sustain some
606 level of pollution directly deriving from the urban run-off, pigeons and fossil energy hydrocarbons.
607 Yet, only *Cryptococcus* spp. displayed that behaviour, to the extreme of not even having been
608 detected in non-urban beaches.

609 Dermatophytes were isolated from several samples and locations, confirming their possible presence
610 in any beach sand. Yet, no *Epidermophyton floccosum* was isolated when the bathing season ceased
611 or from sands untouched by human foot. These data corroborate Müller (Müller, 1973), one of the
612 earliest investigations published on pathogenic fungi in beaches, who reported isolating the
613 dermatophyte from beach sand of the South and the North of Europe only during the bathing season
614 (June to September). However, the epidemiology of dermatophytoses shows that this species is
615 increasingly less present as an infectious agent (Zhan and Liu, 2017).

616 We recorded statistically significant higher counts of All fungi in the Fall/Winter period, compared to
617 the samples from the same sampling spots in the Spring/Summer period ($p = 0.016$). Significant was
618 the differences for *C. dublinsiensis* ($p = 0.017$) and 'Allergenic fungi' ($p = 0.006$) also. *Aspergillus* spp.,
619 *Cryptococcus* spp., 'Dematiaceous fungi' and 'Dermatophytes' followed this trend, although not

620 statistically significant. Previously published research results support our findings, as it has been
621 reported that weather considerations can be important for the fungal load of sand beach. Sarquis &
622 Oliveira (de Moura Sarquis and de Oliveira, 1996) and Assami Doi *et al.* (Doi et al., 2018) reported
623 that in the sands of Brazilian beaches the largest number of filamentous fungi was isolated during the
624 winter and the smallest number the summer. The contradictory results of Stevens *et al.* (Stevens et
625 al., 2012) and Salvo & Fabiano (Salvo and Fabiano, 2007), who recorded larger numbers of yeasts in
626 May/June/July than in September can be attributed to the fact that they did not sample during the
627 winter. Dunn & Baker (Dunn and Baker, 1984) reported from Hawaii that in sands of comparatively
628 high temperature (51°C) the numbers of isolated fungi were significantly fewer, than at beaches with
629 a sand temperature of 30-35°C. Cases, where fungal populations peaked outside the bathing period
630 have been attributed to pollution events, like a rupture of a sewage pipe (Salvo and Fabiano, 2007).
631 In fact, that was also visible during our study with one sampling site in the Southwest region of
632 Europe at the end of 2018, which suffered a rupture in the sewage piping facilities, resulting in the
633 high fungal presence (September 2018) in the sand (200 CFU/g) and in the water (65 CFU/ml). The
634 water cleared promptly upon reparation of the facilities but the sand remained contaminated for at
635 least 3 months (105 CFU/ml and 3.3 CFU/ml in March 2019), compared to 55 CFU/g and 3.3 CFU/ml
636 in July 2019).

637 Our results are explained by the fact that although fungi can survive a wide range of environmental
638 conditions e.g. light, temperature and salinity (Anderson, 1979), some of these parameters can
639 eventually become detrimental to survival for microorganisms on sand particles. Anderson
640 (Anderson, 1979) reported that elevated seawater temperatures, as high as 35°C are considered
641 stress conditions for the fungi and inhibit the growth of *C. albicans*, *T. mentagrophytes* and *T.*
642 *cutaneum*. Only *Microsporium gypseum* responded positively to increased temperature. The sand
643 habitat is severely influenced by environmental changes, particularly temperature and UV light
644 exposure. Sand particles exposed to the sun can become very hot. When the ambient temperature is
645 32°C, the sand temperature can be over 49°C (Cohen, 2019). Furthermore, exposure of

646 microorganisms to Ultra-violet radiation (UV) causes denaturation of proteins and damage to their
647 genetic material. High temperatures of the sand in the summer can inhibit the growth or destroy
648 fungi, and this combined with prolonged UV exposure in the long summer days can account for our
649 findings.

650 Additionally, stormwater runoff, more frequent in winter than in summer, has been identified as the
651 most frequent source of beach pollution (Dorfman et al., 2009).

652 Not all examined beaches harbour the same fungal genera/species. This holds not only for pathogens
653 (attributed to the presence of affected humans, animals or anthropogenic interference), but also to
654 saprophytic species. We observed the greatest variety of fungi in the Mediterranean region.
655 Northwest European beach sands exhibited a slightly larger diversity of fungal populations than
656 Southwestern European regions (Table 2). These observations are supported by Dunn & Baker (Dunn
657 and Baker, 1984); Gomes *et al.* (Gomes et al., 2008); Salvo & Fabiano (Salvo and Fabiano, 2007), who
658 noted that although the beaches they examined shared several species, each beach had species
659 unique to it.

660 Considering the expectations surrounding the ecological niches of dematiaceous fungi, Sydney
661 (Australia) shows similarities with inland water catchments because of being located in a river mouth
662 and thus inevitably populated with vegetable matter originating upstream in the river. The site
663 Murray Rose Pool, being situated inland compared to the other two sites, has twice as many genera
664 of black moulds; this might explain the contrast of their results with other urban sampling sites.
665 Urban or not urban might thus not be as much of an influencing variable on 'Dematiaceous fungi's as
666 initially thought. In fact, the following taxa were isolated during this study: *Alternaria*, *Arthrinium*,
667 *Aureobasidium*, *Bipolaris*, *Chaetomium*, *Cladophialophora*, *Cladosporium*, *Coniochaeta*, *Curvularia*,
668 *Didymella*, *Epicoccum*, *Exophiala*, *Exserohilum*, *Fonsecaea*, *Hortaea*, *Madurella*, *Microsphaeropsis*,
669 *Neopyrenochaeta*, *Paraphoma*, *Phaeoacremonium*, *Phialophora*, *Phoma*, *Pseudallescheria*,
670 *Pyrenochaeta*, *Robillarda*, *Scopulariopsis*, and *Sporothrix*. Their presence was more noticeable in

671 freshwater settings given the proximity of vegetation, which provides a carbon source for their
672 growth.

673 Despite the relatively large scale of this study, the presence and composition of fungal mycobiota
674 may be site-dependent, since many sites exhibit very low numbers of fungal presence and others,
675 quite the opposite. This renders modelling of sand culturable mycobiota relatively difficult for any
676 site without supporting historical data. Values presented in this study should thus serve only as a
677 location-blind starting point for more geographically focused studies with similar aims and scopes.

678 4.2 Water

679 The results of the water analysis indicate an interest in determining fungi in water, especially in
680 freshwater, given the opportunistic and almost exclusive human faecal natures of *C. albicans* and the
681 intimate contact with bathers, as mentioned in the introduction. Under this premise, faecal indicator
682 bacteria (FIB) currently used in bathing water quality regulation - *E. coli* and *enterococci* ((EU), 2006)
683 may not be used in this study, which focuses on fungi only, but our results show potential human
684 faecal pollution in 45 of the 302 coastal bathing waters (15%) and 4 of the 14 bathing freshwaters
685 (28.6%).

686 Our results reveal a great diversity of fungi in the water of the beaches examined throughout Europe.
687 These findings are in agreement with previous research, reporting a variety of filamentous fungi and
688 yeasts from marine beaches (Arvanitidou et al., 2002; Aulicino et al., 2001; Gomes et al., 2008;
689 Loureiro et al., 2005; Maciel et al., 2019; Mates, 1994; Oliveira et al., 2020; Oliveira et al., 2011; Roth
690 Jr et al., 1962; Velegraki-Abel et al., 1987; Vezzulli et al., 2009). From freshwater beaches, there are
691 also reports of considerable diversity in fungi isolations (Biedunkiewicz and Góralaska, 2016; Brandão
692 et al., 2010; Falcão et al., 1993; Góralaska et al., 2020; Kiziewicz et al., 2004; Sherry et al., 1979; Wójcik
693 et al., 2003).

694 Whilst several research projects have dealt qualitatively with the issue of fungi in recreational
695 waters, publishing on the diversity and the taxa detected, little was published on the abundance of
696 isolated fungi. We isolated significantly more fungi (measured as 'All fungi') from freshwater than

697 from coastal waters ($p < 0.001$). This could be attributed (as in the case of sand) to environmental
698 conditions being more favourable to their survival and growth in freshwater bodies than the sea
699 (organic nutrients, lack of salinity, vegetation). The freshwaters of the inland beaches harboured
700 significantly more *C. albicans* ($p < 0.001$), 'Allergenic fungi' ($p = 0.001$) and 'Dematiaceous fungi'
701 ($p < 0.001$). More specifically we detected from 0 to 1592 CFU/ml of All fungi from coastal waters
702 (mean 0 CFU/ml). Velegraki-Abel *et al.* (Velegraki-Abel *et al.*, 1987) isolated 30-1020 CFU/50ml yeasts
703 from marine beach water, Aulicino *et al.* (Aulicino *et al.*, 2001) from $1 - 10^3$ /100ml yeasts. From
704 inland beach waters, we isolated from 0 to 310 CFU/ml of All fungi (mean 6.7 CFU/ml). Similar
705 findings were published by Wójcik *et al.* (Wójcik *et al.*, 2003) who isolated from 8 to 32800
706 CFU/100ml yeasts from reservoir water used for recreational bathing. Goralska *et al.* (Góralaska *et al.*,
707 2020) detected an average of 40 to 460 CFU/100ml in recreational freshwater ponds in Poland.

708 The presence of bathers appears to affect the abundance of fungi isolated from beach water. Urban
709 beaches have significantly more fungi (measured as All fungi) than non-urban beach water ($p = 0.005$).
710 *Aspergillus* spp. and *Aspergillus* section *Fumigati* are the two fungal groups that show significantly
711 higher numbers in urban beaches ($p = 0.014$ and $p = 0.008$ respectively). Similar results have been
712 reported for yeasts. Papadakis *et al.* (Papadakis *et al.*, 1997) claim that yeasts of human origin
713 correlated with the numbers of swimmers in seawater. Velegraki-Abel *et al.* (Velegraki-Abel *et al.*,
714 1987), also investigating in Greek coastal bathing waters, reported that yeast counts increased during
715 the summer. Sherry *et al.* (Sherry *et al.*, 1979) published that maximum numbers of *C. albicans* were
716 observed in association with peak bather loads at the beaches of a lake in Canada.

717 Different seasons appeared to affect significantly the numbers of fungi we isolated from water only
718 in the case of 'Allergenic fungi' ($p = 0.015$) and 'Dematiaceous fungi' ($p = 0.006$), who were more
719 abundant in the Fall/Winter period as compared to the Spring/Summer period. In all other
720 categories, the differences were not statistically significant. Data discussing differences in fungal
721 isolation from bathing waters have not been published, to the best of our knowledge, except for
722 Goralska *et al.* (Góralaska *et al.*, 2020) who reported, in inland bathing ponds, the highest diversity and

723 abundance of filamentous fungi in June, just before the bathing season, compared to the period July
724 – September.

725 The geographical profile of fungal taxa we isolated from recreational waters is similar to that of fungi
726 isolated from the sand: the greatest variety appeared in the Mediterranean region, the smallest in
727 the Black Sea bathing waters. Northwest European beach waters presented a slightly larger diversity
728 of fungal populations than the Southwest (Table 4).

729 The statistical analysis of the water samples suggests that the amount of inoculum used in this study
730 (less than 1 ml per sample) was not enough to produce a robust overview of fungal contaminants in
731 water. Filtering 100 ml, as for drinking water, leads to too many colonies in a small filter (personal
732 experience of one of the authors with coastal bathing waters - J. Brandão). Extending this study with
733 more sampling events and replicas might resolve this problem. Alternatively, filtering up to 5 ml (25x
734 more sample than for each replica of this study) might also help produce more data to generate
735 more data.

736 4.3 GENERAL COMMENTS TO BOTH WATER AND SAND

737 *A. section Fumigati* and *A. niger sensu lato* constituted a major portion of the isolates in the
738 Mediterranean (present in 10 samples) whereas *Penicillium* spp. dominated (present in 9 samples) in
739 the Southwest region. *Rhodotorula* and *Candida* species were the truly ubiquitous ones isolated from
740 every region. *C. albicans* is the yeast most often isolated from marine and freshwater beaches by
741 other researchers (Arvanitidou et al., 2002; Aulicino et al., 2001; Biedunkiewicz and Góralaska, 2016;
742 Gomes et al., 2008; Loureiro et al., 2005). *Rhodotorula* was reported in higher numbers in two cases
743 (Velegraki-Abel et al., 1987; Wójcik et al., 2003), but these research groups reported *C. albicans*
744 isolations amongst the most common species, too.

745 Valdes-Collazo *et al.* (Valdes-Collazo et al., 1987) found that *C. albicans* survives well in freshwater
746 and marine water, so bathing in its presence necessarily represents an exposure setting to this
747 organism were intimate contact with the bather's skin and mucosae take place. Conversely, this
748 species does not respond well to dehydration, which renders it to be a possible indicator of recent

749 human faecal pollution in the sand and in water; its decay is similar to that of *E. coli*, as noted by
750 Kashbur *et al.* (Kashbur *et al.*, 1980).

751 Given their allergenic nature, *Penicillia* were considered in this study solely as part of the parameter
752 'Allergenic fungi' statistical strength, although the authors recognize that with high counts, aerosols
753 might trigger allergic reactions in susceptible patients.

754 The prediction of pathogen risks is essential to beach management. The increase of chances of an
755 introduction of fungal pathogens into the population should be avoided, because of the increased
756 numbers of immunocompromised people, the advances in chemotherapeutic treatments that allow
757 patients to move around and visit recreational spots, as well as the ageing of the human population.
758 Identifying the most important routes of fungal transmission in the sand would allow beach
759 management officials to improve services and reduce risks of exposure to pathogenic fungi. Little
760 research has been published on within-one-beach differences in fungal populations (Brandão *et al.*,
761 2020a; Velegriaki *et al.*, 2012) providing evidence that sand around showers exhibits the highest
762 numbers of keratinophilic isolates, followed by sand from children's playground areas and from
763 sports activities areas. Should such findings be supported by further research it would be possible for
764 beach managers to improve the management of shower run-offs or the treatment of shower
765 effluents or relocate showers in order to avoid spread/unnecessary contact.

766

767 5. Conclusions

768 Traditionally, microbial safety regulation of beaches is based on the exposure results of a general
769 population (WHO). However, fungi need to be addressed differently: many of the fungi found in this
770 study are the cause of fungal ailments in susceptible beach users; both in the sand and in water.
771 Regulation should thus enforce their detection in order to advise these users of the probable
772 exposure.

773 Fungi are nature's organic matter recycling machines. Different species may thrive mainly with
774 specific substrates, rendering them good indicators for specific situations. The presence of *Fusarium*

775 species in the sand, for example, may indicate remains of vegetable debris, as these species are plant
776 pathogens and colonisers. Another group highly associated with densely vegetated areas are the
777 Melanised fungi. These include species that cause deep and often lethal infections, as well as
778 allergies. A list of the genera found in this study can be found in the supplementary material.

779 The current absence of a dose-response on fungal ailments, quantitative mycological risk assessment
780 or any epidemiological study, hinders possible regulatory plans based on the health implications of
781 exposure to fungi at the beach. The current study, involving the assessment of culturable mycobiota
782 in a variety of geographic areas and climatic conditions, might contribute to the formation of a broad
783 view on its relevance to human health. A median of site-blinded total fungal of 89 CFU/g was found
784 in this study and could be used as a reference for beaches with no historical analytical data (in
785 Europe and possibly in other geographical areas).

786 It is the opinion of the authors that the monitoring of fungi in beach sand and water is relevant,
787 particularly for the most susceptible beach users. The authors of this study also consider that at least
788 *C. albicans* and dermatophytes should also be monitored as additional health-oriented parameters
789 (besides total fungal colony count). Other possible fungal parameters, species or genera should be
790 considered on a need-to-do basis, according to persistent pollution or specific health endemic
791 requirements.

792 More work needs to be done on water. Further research will more comprehensively characterise the
793 water fungal contaminants better and allow a better assessment of possible future regulatory
794 parameters.

795

796 Highlights

- 797 - Fungi are missing from water and sand health protection regulatory parameters.
- 798 - Both sand and water should be monitored for fungi
- 799 - The median value of 89 CFU/g of all fungi may serve as a reference for sand regulation
- 800 - *Candida albicans*, dermatophytes, endemic fungi and other fungi should be considered

801 - Fungal analysis of water needs more data before reference values can be established

802

803 Acknowledgements

804 A. Abdilllah, M. C. Esposto, J. Kabtani, K. Sarioglou, P. E. Verweij and F. Vieira for their collaboration in
805 this study;

806 The European Confederation of Medical Mycology for financially supporting the team meeting in
807 2019, in Nice, France;

808 International Society for Human and Animal Mycology for some financial support

809 Financial support from CESAM (UID/AMB/50017-POCI-01-0145-FEDER-007638) and CITAB
810 (UID/AGR/04033/2019), via FCT/MCTES, from national funds (PIDDAC), cofounded by FEDER,
811 (PT2020 Partnership Agreement and Compete 2020) and an NHMRC APP1121936 to W. Meyer;

812 National and Kapodistrian University of Athens, Greece, Special Accounts for Research Grants (SARG
813 K.A. 70/3/6915).

814

815 Declaration of interests

816 The authors declare that they have no known competing financial interests or personal relationships
817 that could have appeared to influence the work reported in this paper.

818

819 Author contributions (CRediT author statement)

820 **List of authors and their abbreviations:** J. Brandão (JB), J.P. Gangneux (JPG), S. Arian-Akdagli (SA-A),
821 A. Barac (AB), A.C. Bostanaru (ACB), S. Brito (SB), M. Bull (MB), N. Çerikçioğlu (NÇ), B. Chapman (BC),
822 M. A. Efstratiou (MAE), Ç. Ergin (ÇE), M. Frenkel (MF), A. Gitto (AG), C.I. Gonçalves (CIG), H. Guégan
823 (HG), N. Gunde-Cimerman (NG-C), M. Güran (MG), L. Irinyi (LI), E. Jonikaitė (EJ), M. Kataržytė (MK), L.
824 Klingspor (LK), M. Mares (MM), W.G. Meijer (WGM), W.J.G. Melchers (WJGM), J. Meletiadis (JM), W.
825 Meyer (WM), V. Nastasa (VN), M. Novak Babič (MNB), D. Ogunc (DO), B. Ozhak (BO), A. Prigitano
826 (AP), S. Ranque (SR), R.O. Rusu (ROR), R. Sabino (RS), A. Sampaio (AS), S. Silva (SS), J.H. Stephans

827 (JHS), M. Tehupeiori-Kooreman (MTK), A.M. Tortorano (AMT), A. Velegraki (AV), C. Veríssimo (CV),
828 G.C.Wunderlich (GCW), E. Segal (ES)

829 **Author contributions: Conceptualization:** JB, JPG and ES; **Methodology:** JB, JPG, MB, NÇ, BC, AG,
830 WGM, WM, SR, RS, SS, JHS, AMT, CV, GCW and ES; **Software:** SS; **Validation:** JB, MB, BC, AG, LI,
831 WJGM, WM, AP, SS, JHS, AMT, AV, GCW and ES; **Local data analysis:** JB, JPG, AB, ACB, SB, MB, NÇ,
832 BC, MAE, ÇE, MF, AG, CIG, HG, NG-C, MG, LI, MK, LK, MM, WGM, WJGM, JM, WM, VN, MNB, DG, BO,
833 SR, ROR, RS, AS, JHS, MTK, AV, CV, GCW and ES; **Final formal analysis:** JB, JPG, ÇE, MNB, SS and ES;
834 **Investigation:** JB, JPG, AB, ACB, SB, NÇ, MAE, ÇE, MF, AG, CIG, HG, NG-C, MG, EJ, MK, LK, MM, WGM,
835 WJGM, JM, VN, MNB, DO, BO, AP, SR, ROR, RS, AS, JHS, MTK, AV, CV, GCW and ES; **Resources:** JB,
836 ACB, SB, MB, NÇ, BC, ÇE, MF, CIG, HG, MG, LI, MK, LK, WGM, JM, WM, VN, MNB, AP, SR; ROR, AS,
837 AMT, AV, GCW and ES; **Data Curation:** JB, MF, AG, LI, WGM, WJGM, MNB, AP, SR, RS, SS, JHS and
838 GCW; **Writing - Original Draft:** JB, JPG, MAE, ÇE, NG-C, WGM, MNB, SR, RS, SS, CV and ES; **Writing -**
839 **Review & Editing:** JB, JPG, SA-A, AB, ACB, SB, MB, NÇ, BC, MAE, ÇE, MF, AG, CIG, HG, NG-C, MG, LI,
840 EJ, MK, LK, MM, WGM, WJGM, JM, WM, VN, MNB, DO, BO, AP, SR, ROR, RS, AS, SS, JHS, MTK, AMT,
841 AV, CV, GCW and ES; **Visualization:** JB, JPG, ÇE, WM, MNB, SS and ES ; **Supervision:** JB, JPG MB, BC,
842 LI, WM and ES; **Central project administration:** JB, JPG and ES; **Local Project administration:** JB, JPG,
843 SA-A, BC, NG-C, MK, MM, WGM, WJGM, WM, SR, AS, AMT, AV and ES; **Funding acquisition:** JB, JPG,
844 BC, MM, WM, AS, AV and ES.

845 **Additional information:** The original draft introduction was written in sections: ‘Framing fungi in
846 sand’ by JB and WGM; The yeasts genera *Candida*, *Cryptococcus*, *Trichosporon*, *Geotrichum* by ES;
847 ‘Allergenic fungi’ moulds and endemic fungi by JPG and SR; Black mould by MNB and NG-C; and
848 Dermatophytes by RS and CV. Statistical analysis was performed by SS, supported by JB, ES, JPG and
849 MNB and reviewed by ÇE. The original draft “Conclusions” were written by ES and JB and the original
850 draft “discussion”, by MAE and JB. ÇE generated the map of all sampling *loci*.

851

852 References

853 (EU) EEU. Directive 2006/7/EC of the European Parliament and of the Council of 15 February
854 2006 concerning the management of bathing water quality and repealing
855 directive 76/160/EEC. Official Journal of the European Union 64 2006: 37-51.

856 Abdallaoui MS, Boutayeb H, Guessous-Idrissi N. Fungal flora from the sand of two beaches of
857 Casablanca (Morocco) Analysis and epidemiological corollary. Journal De Mycologie
858 Medicale 2007; 17: 58-62.

859 Al-Yasiri MH, Normand AC, Piarroux R, Ranque S, Mauffrey JF. Gut yeast communities in
860 *Larus michahellis* from various breeding colonies. Med Mycol 2017; 55: 436-444.

861 Anderson JH. In vitro survival of human pathogenic fungi in seawater. Sabouraudia 1979; 17:
862 1-12.

863 Angebault C, Djossou F, Abelanet S, Permal E, Ben Soltana M, Diancourt L, et al. *Candida*
864 *albicans* is not always the preferential yeast colonizing humans: a study in Wayampi
865 Amerindians. J Infect Dis 2013; 208: 1705-1716.

866 Arvanitidou M, Kanellou K, Katsouyannopoulos V, Tsakris A. Occurrence and densities of
867 fungi from northern Greek coastal bathing waters and their relation with faecal
868 pollution indicators. Water Res 2002; 36: 5127-5131.

869 Ashraf N, Kubat RC, Poplin V, Adenis AA, Denning DW, Wright L, et al. Re-drawing the maps
870 for endemic mycoses. Mycopathologia 2020; 185: 843-865.

871 ASTM D4249-83(2005). Standard Test Method for Enumeration of *Candida albicans* in Water
872 (Withdrawn 2013), ASTM International, West Conshohocken, PA, 2005,
873 (www.astm.org). 2005.

874 Aulicino FA, Orsini P, Carere M, Mastrantonio A. Bacteriological and virological quality of
875 seawater bathing areas along the Tyrrhenian coast. Int J Environ Health Res 2001; 11:
876 5-11.

877 Babič MN, Gunde-Cimerman N, Vargha M, Tischner Z, Magyar D, Veríssimo C, et al. Fungal
878 contaminants in drinking water regulation? A tale of ecology, exposure, purification
879 and clinical relevance. Int J Environ Res Public Health 2017; 14: 636.

880 Banerjee P, Haider M, Trehan V, Mishra B, Thakur A, Dogra V, et al. *Cryptococcus laurentii*
881 fungemia. Indian J Med Microbiol 2013; 31: 75-77.

882 Ben Neji H, Bchir M, Hamdoun M, Kallel A, Kallel K, Bahri O, et al. *Geotrichum capitatum*
883 fungemia in patients treated for acute leukemia. Med Mal Infect 2019; 49: 284-286.

884 Bergen L, Wagner-Merner DT. Comparative survey of fungi and potential pathogenic fungi
885 from selected beaches in the Tampa Bay area. Mycologia 1977; 69: 299-308.

886 Biedunkiewicz A, Góralaska K. Microfungi potentially pathogenic for humans reported in
887 surface waters utilized for recreation. Clean-Soil Air Water 2016; 44: 599-609.

888 Bik HM, Halanych KM, Sharma J, Thomas WK. Dramatic shifts in benthic microbial eukaryote
889 communities following the Deepwater Horizon oil spill. PLoS One 2012; 7: e38550.

890 Bongomin F, Gago S, Oladele RO, Denning DW. Global and multi-national prevalence of
891 fungal diseases-estimate precision. J Fungi (Basel) 2017; 3: 57.

892 Brandão J, Albergaria I, Albuquerque J, José S, Grossinho J, Ferreira FC, et al. Untreated
893 sewage contamination of beach sand from a leaking underground sewage system. Sci
894 Total Environ 2020a; 740: 140237.

895 Brandão J, Weiskerger C, Novak Babič M. Fungal exposure and relevant recreational settings:
896 Elsevier, 2020b.

897 Brandão LR, Medeiros AO, Duarte MC, Barbosa AC, Rosa CA. Diversity and antifungal
898 susceptibility of yeasts isolated by multiple-tube fermentation from three freshwater
899 lakes in Brazil. J Water Health 2010; 8: 279-289.

900 Brandt ME, Warnock DW. Epidemiology, clinical manifestations, and therapy of infections
901 caused by dematiaceous fungi. J Chemother 2003; 15 Suppl 2: 36-47.

902 Buer AL, Gyraite G, Wegener P, Lange X, Kataržytė M, Hauk G, et al. Long term development
903 of Bathing Water Quality at the German Baltic coast: spatial patterns, problems and
904 model simulations. Mar Pollut Bull 2018; 135: 1055-1066.

905 Burge HA. Fungi: toxic killers or unavoidable nuisances? Ann Allergy Asthma Immunol 2001;
906 87: 52-56.

907 Candan AY, Katilmis Y, Ergin C. First report of *Fusarium* species occurrence in loggerhead sea
908 turtle (*Caretta caretta*) nests and hatchling success in Iztuzu Beach, Turkey. Biologia
909 2020.

910 Chang EY, Fatima S, Balan S, Bhyravabhotla K, Erickson M, Chan A, et al. *Candida dubliniensis*
911 abscess: A clinical case and a review of the literature. Med Mycol Case Rep 2018; 21:
912 41-43.

913 Chen SC, Meyer W, Sorrell TC. *Cryptococcus gattii* infections. Clin Microbiol Rev 2014; 27:
914 980-1024.

915 Choe YJ, Blatt DB, Yalcindag A, Geffert SF, Bobenchik AM, Michelow IC. *Cryptococcus albidus*
916 fungemia in an immunosuppressed child: Case report and systematic literature
917 review. J Pediatric Infect Dis Soc 2020; 9: 100-105.

918 Chowdhary A, Sharma C, Meis JF. *Candida auris*: A rapidly emerging cause of hospital-
919 acquired multidrug-resistant fungal infections globally. PLoS Pathog 2017; 13:
920 e1006290.

921 Cohen PR. Beach feet: A sand-associated thermal injury to the soles of the feet and the
922 plantar aspect of the toes. Cureus 2019; 11: e6429.

923 Cooney CM. Climate change & infectious disease: is the future here? Environ Health Perspect
924 2011; 119: a394-7.

925 Cordeiro RA, Sales JA, Castelo-Branco D, Brilhante RSN, Ponte YB, Dos Santos Araujo G, et al.
926 *Candida parapsilosis* complex in veterinary practice: A historical overview, biology,
927 virulence attributes and antifungal susceptibility traits. Vet Microbiol 2017; 212: 22-
928 30.

929 Coulibaly O, Kone AK, Niare-Doumbo S, Goita S, Gaudart J, Djimde AA, et al.
930 Dermatophytosis among schoolchildren in three eco-climatic zones of Mali. PLoS
931 Negl Trop Dis 2016; 10: e0004675.

932 de Almeida Junior JN, Hennequin C. Invasive *Trichosporon* infection: a systematic review on a
933 re-emerging fungal pathogen. Front Microbiol 2016; 7: 1629.

934 de Hoog GS, Dukik K, Monod M, Packeu A, Stubbe D, Hendrickx M, et al. Toward a novel
935 multilocus phylogenetic taxonomy for the dermatophytes. Mycopathologia 2017;
936 182: 5-31.

937 de Hoog GS, Guarro J, Gené J, Ahmed S, Al-Hatmi AMS, Figueras MJ, et al. Atlas of Clinical
938 Fungi. Utrecht / Reus, 2019.

939 de Hoog GS, Guarro J, Gene J, Figueras MJ. Basidiomycetous yeasts. Genus *Cryptococcus*. In:
940 de Hoog GS, Guarro J, Gene J, Figueras MJ, editors. Atlas of Clinical Fungi. CBS,
941 Utrecht, The Netherlands, 2000a, pp. 131-143.

942 de Hoog GS, Guarro J, Gene J, Figueras MJ. Classification. In: de Hoog GS, Guarro J, Gene J,
943 Figueras MJ, editors. Atlas of Clinical Fungi. CBS, Utrecht, The Netherlands 2000b, pp.
944 1-10.

945 De Leo F, Antonelli F, Pietrini AM, Ricci S, Urzi C. Study of the euendolithic activity of black
946 meristematic fungi isolated from a marble statue in the Quirinale Palace's Gardens in
947 Rome, Italy. *Facies* 2019; 65.

948 de Moura Sarquis MI, de Oliveira PC. Diversity of microfungi in the sandy soil of Ipanema
949 Beach, Rio de Janeiro, Brazil. *J Basic Microbiol* 1996; 36: 51-58.

950 de Toro M, Torres MJ, Maite R, Aznar J. Characterization of *Candida parapsilosis* complex
951 isolates. *Clin Microbiol Infect* 2011; 17: 418-424.

952 Di Piazza S, Baiardo S, Cecchi G, Ambrosio E, Paoli C, Vassallo P, et al. Microfungal diversity in
953 the swash zone interstitial water (SZIW) of three Ligurian urban beaches (NW, Italy).
954 *Ital J Mycol* 2017; 46: 8-20.

955 Doi SA, Pinto AB, Canali MC, Polezel DR, Chinellato RAM, de Oliveira AJFC. Density and
956 diversity of filamentous fungi in the water and sediment of Araca bay in São
957 Sebastião, São Paulo, Brazil. *Biota Neotropica* 2018; 18: e20170416.

958 Dolenc-Voljč M. Dermatophyte infections in humans: Current trends and future prospects.
959 In: Razzaghi-Abyaneh M, Shams-Ghahfarokhi M, Rai M, editors. *Medical Mycology:
960 Current Trends and Future Prospects*. CRC Press, Bato Racon, 2015, pp. 2-27.

961 Dorfman M, Rosselot KS, Stoner N. Testing the waters: A guide to water quality at vacation
962 beaches. National Resources Defence Council. 2009; 19th edn.

963 Douwes J. (1-->3)-Beta-D-glucans and respiratory health: a review of the scientific evidence.
964 *Indoor Air* 2005; 15: 160-169.

965 Dunn PH, Baker GE. Filamentous fungal populations of Hawaiian beaches. *Pacific Science*
966 1984; 38: 232-248.

967 Durán Graeff L, Seidel D, Vehreschild MJ, Hamprecht A, Kindo A, Racil Z, et al. Invasive
968 infections due to *Saprochaete* and *Geotrichum* species: Report of 23 cases from the
969 FungiScope Registry. *Mycoses* 2017; 60: 273-279.

970 Echevarría L. Molecular identification of filamentous fungi diversity in North Coast beaches
971 sands of Puerto Rico. *Int J Mol Microbiol* 2019; 2: 51-61.

972 Efstratiou MA, Kitsiou D, Vintenko A, Velegraki A. Quantitative estimation and sampling
973 optimisation of fungi in beach sand with the use of GIS and geostatistical analysis. 4th
974 Congress, Federation of European Microbiological Societies (FEMS), Geneva, 2011.

975 Efstratiou MA, Mavridou A, Richardson SC, Papadakis JA. Correlation of bacterial indicator
976 organisms with *Salmonella* spp., *Staphylococcus aureus* and *Candida albicans* in sea
977 water. *Lett Appl Microbiol* 1998; 26: 342-346.

978 Efstratiou MA, Velegraki A. Recovery of melanized yeasts from Eastern Mediterranean beach
979 sand associated with the prevailing geochemical and marine flora patterns. *Med
980 Mycol* 2010; 48: 413-415.

981 Elmanama AA, Fahd MI, Afifi S, Abdallah S, Bahr S. Microbiological beach sand quality in
982 Gaza Strip in comparison to seawater quality. *Environ Res* 2005; 99: 1-10.

983 Ergin C, Sengul M, Aksoy L, Dogen A, Sun S, Averette AF, et al. *Cryptococcus neoformans*
984 recovered from olive trees (*Olea europaea*) in Turkey reveal allopatry with African
985 and South American lineages. *Front Cell Infect Microbiol* 2019; 9: 384.

986 Falcão DP, Leite CQ, Simões MJ, Giannini MJ, Valentini SR. Microbiological quality of
987 recreational waters in Araraquara, SP, Brazil. *Sci Total Environ* 1993; 128: 37-49.

988 Fischer G, Dott W. Relevance of airborne fungi and their secondary metabolites for
989 environmental, occupational and indoor hygiene. *Arch Microbiol* 2003; 179: 75-82.

990 Fonseca A, Scorzetti G, Fell JW. Diversity in the yeast *Cryptococcus albidus* and related
991 species as revealed by ribosomal DNA sequence analysis. *Can J Microbiol* 2000; 46: 7-
992 27.

993 Frenkel M, Yunik Y, Fleker M, Blum SE, Sionov E, Elad D, et al. Fungi in sands of
994 Mediterranean Sea beaches of Israel-Potential relevance to human health and well-
995 being. *Mycoses* 2020: (In press).

996 Gomes DN, Cavalcanti MA, Fernandes MJ, Lima DM, Passavante JZ. Filamentous fungi
997 isolated from sand and water of "Bairro Novo" and "Casa Caiada" beaches, Olinda,
998 Pernambuco, Brazil. *Braz J Biol* 2008; 68: 577-582.

999 González MC, Hanlin RT, Herrera T, Ulloa M. Fungi colonizing hair-baits from three coastal
1000 beaches of Mexico. *Mycoscience* 2000; 41: 259-262.

1001 Góralaska K, Blaszkowska J, Dzikowiec M. The occurrence of potentially pathogenic
1002 filamentous fungi in recreational surface water as a public health risk. *J Water Health*
1003 2020; 18: 127-144.

1004 Guegan H, Chevrier S, Belleguic C, Deneuille E, Robert-Gangneux F, Gangneux JP.
1005 Performance of molecular approaches for *Aspergillus* detection and azole resistance
1006 surveillance in cystic fibrosis. *Front Microbiol* 2018; 9: 531.

1007 Guo LN, Yu SY, Hsueh PR, Al-Hatmi AMS, Meis JF, Hagen F, et al. Invasive infections due to
1008 *Trichosporon*: Species distribution, genotyping, and antifungal susceptibilities from a
1009 multicenter study in China. *J Clin Microbiol* 2019; 57: e01505-18.

1010 Hagen F, Khayhan K, Theelen B, Kolecka A, Polacheck I, Sionov E, et al. Recognition of seven
1011 species in the *Cryptococcus gattii/Cryptococcus neoformans* species complex. *Fungal*
1012 *Genet Biol* 2015; 78: 16-48.

1013 Hirayama T, Miyazaki T, Yamagishi Y, Mikamo H, Ueda T, Nakajima K, et al. Clinical and
1014 microbiological characteristics of *Candida guilliermondii* and *Candida fermentati*.
1015 *Antimicrob Agents Chemother* 2018; 62: e02528-17.

1016 Kashbur IM, Ayliffe GA, George RH. The survival of *Candida albicans* in moist and dry
1017 environments. *J Hosp Infect* 1980; 1: 349-356.

1018 Kataržytė M, Mėžinė J, Vaičiūtė D, Liaugaudaitė S, Mukauskaitė K, Umgiesser G, et al. Fecal
1019 contamination in shallow temperate estuarine lagoon: Source of the pollution and
1020 environmental factors. *Mar Pollut Bull* 2018; 133: 762-772.

1021 Kataržytė M, Vaičiūtė D, Bučas M, Gyraitė G, Petkuvienė J. Microorganisms associated with
1022 charophytes under different salinity conditions. *Oceanologia* 2017; 59: 177-186.

1023 Kishimoto RA, Baker GE. Pathogenic and potentially pathogenic fungi isolated from beach
1024 sands and selected soils of Oahu, Hawaii. *Mycologia* 1969; 61: 537-548.

1025 Kiziewicz B, Kozłowska M, Godlewska A, Muszynvska E, Mazalska B. Water fungi occurrence in
1026 the River Suprasl-bath Jurówce near Białystok. *Wiad Parazytol* 2004; 50: 143-150.

1027 Kushwaha RKS, Guarro J. Biology of dermatophytes and other keratinophilic fungi. Bilbao:
1028 Berekintza, 2000.

1029 Kwon-Chung KJ, Fraser JA, Doering TL, Wang Z, Janbon G, Idnurm A, et al. *Cryptococcus*
1030 *neoformans* and *Cryptococcus gattii*, the etiologic agents of cryptococcosis. *Cold*
1031 *Spring Harb Perspect Med* 2014; 4: a019760.

1032 Latgé JP, Chamilos G. *Aspergillus fumigatus* and Aspergillosis in 2019. *Clin Microbiol Rev*
1033 2019; 33: e00140-18.

1034 Levetin E, Horner WE, Scott JA, Environmental Allergens W. Taxonomy of allergenic fungi. *J*
1035 *Allergy Clin Immunol Pract* 2016; 4: 375-385 e1.

1036 Lockhart SR, Etienne KA, Vallabhaneni S, Farooqi J, Chowdhary A, Govender NP, et al.
1037 Simultaneous emergence of multidrug-resistant *Candida auris* on 3 continents
1038 confirmed by whole-genome sequencing and epidemiological analyses. Clin Infect Dis
1039 2017; 64: 134-140.

1040 Londono CO, Fernandez RR, Gullosi ERM. Dermatophytes in the coastal area of district of
1041 Riohacha, La Guajira. Contemp Eng Sci 2018; 11: 4691-4699.

1042 Loureiro STA, Cavalcanti MAD, Neves RP, Passavante JZD. Yeasts isolated from sand and sea
1043 water in beaches of Olinda, Pernambuco state, Brazil. Braz J Microbiol 2005; 36: 333-
1044 337.

1045 Maciel NO, Johann S, Brandão LR, Kucharikova S, Morais CG, Oliveira AP, et al. Occurrence,
1046 antifungal susceptibility, and virulence factors of opportunistic yeasts isolated from
1047 Brazilian beaches. Mem Inst Oswaldo Cruz 2019; 114: e180566.

1048 Marcos-Zambrano LJ, Puig-Asensio M, Perez-Garcia F, Escribano P, Sanchez-Carrillo C,
1049 Zaragoza O, et al. *Candida guilliermondii* complex is characterized by high antifungal
1050 resistance but low mortality in 22 cases of candidemia. Antimicrob Agents
1051 Chemother 2017; 61: e00099-17.

1052 Marzol MV, Yanes A, Romero C, Brito de Azevedo E, Prada S, Martins A. Caractéristiques des
1053 précipitations dans les îles de la Macaronesia [Açores, Madère, Canaries et Cap Vert].
1054 Les Risques Liés au Temps et au climat. XIX Colloque de l'Association International de
1055 Climatologie [AIC] 2006B; B: 415-420.

1056 Mates A. Quantitative determination of *Candida albicans* in sea water. Microbios 1994; 79:
1057 27-30.

1058 Maziarz EK, Perfect JR. Cryptococcosis. Infect Dis Clin North Am 2016; 30: 179-206.

1059 McGinnis MR. Pathogenesis of indoor fungal diseases. Med Mycol 2004; 42: 107-117.

1060 Méheust D, Le Cann P, Gangneux JP. Rapid quantification of viable fungi in hospital
1061 environments: analysis of air and surface samples using solid-phase cytometry. J
1062 Hosp Infect 2013; 83: 122-126.

1063 Mendell MJ, Mirer AG, Cheung K, Tong M, Douwes J. Respiratory and allergic health effects
1064 of dampness, mold, and dampness-related agents: a review of the epidemiologic
1065 evidence. Environ Health Perspect 2011; 119: 748-756.

1066 Michel M, Gomez C, Sereme Y, Gouitaa M, Chartier C, Blanchard P, et al. Evaluation of
1067 cellular responses for the diagnosis of allergic bronchopulmonary mycosis: A
1068 preliminary study in cystic fibrosis patients. Front Immunol 2019; 10: 3149.

1069 Migahed FF. Distribution of fungi in the sandy soil of Egyptian beaches. Pak J Biol Sci 2003; 6:
1070 860-866.

1071 Muntañolacvetković M, Ristanović B. A Mycological Survey of the South Adriatic Sea. Journal
1072 of Experimental Marine Biology and Ecology 1980; 43: 193-206.

1073 Müller G. Occurrence of dermatophytes in the sands of European beaches. Sci Total Environ
1074 1973; 2: 116-118.

1075 Novak Babič M, Gostinčar C, Gunde-Cimerman N. Microorganisms populating the water-
1076 related indoor biome. Appl Microbiol Biotechnol 2020; 104: 6443-6462.

1077 Nunn MA, Schäfer SM, Petrou MA, Brown JR. Environmental source of *Candida dubliniensis*.
1078 Emerg Infect Dis 2007; 13: 747-750.

1079 Ogaki MB, de Paula MT, Ruas D, Pellizzari FM, García-Laviña CX, Rosa LH. Marine fungi
1080 associated with antarctic macroalgae. In: Castro-Sowinski S, editor. The Ecological
1081 Role of Micro-organisms in the Antarctic Environment. Springer, Cham, 2019, pp.
1082 239-255.

1083 Oliveira JO, Santos IMR, Silva DP, Calumby RJN, Moreira RTF, Araujo MAS. Occurrence of
1084 fungi in the water and sand of urban beaches. *Divers J* 2020; 5: 2779-2791.

1085 Oliveira LG, Cavalcanti M, Passavante JZ, Fernandes MJ, Lima DM. Filamentous fungi isolated
1086 from Candeias Beach, Pernambuco, Brazil. *Hoehnea* 2011; 38: 215-220.

1087 Papadakis JA, Mavridou A, Richardson SC, Lampiri M, Marcelou U. Bather-related microbial
1088 and yeast populations in sand and seawater. *Water Research* 1997; 31: 799-804.

1089 Pfaller MA, Diekema DJ, Gibbs DL, Newell VA, Nagy E, Dobiásova S, et al. *Candida krusei*, a
1090 multidrug-resistant opportunistic fungal pathogen: geographic and temporal trends
1091 from the ARTEMIS DISK Antifungal Surveillance Program, 2001 to 2005. *J Clin*
1092 *Microbiol* 2008; 46: 515-521.

1093 Piarroux RP, Dubus JC, Reynaud-Gaubert M, Gouitaa M, Ranque S, Vitte J. A new IgE Western
1094 blot identifies *Aspergillus fumigatus* sensitization and may discriminate allergic
1095 bronchopulmonary aspergillosis. *Allergy* 2019; 74: 1808-1810.

1096 Pires CA, Cruz NF, Lobato AM, Sousa PO, Carneiro FR, Mendes AM. Clinical, epidemiological,
1097 and therapeutic profile of dermatophytosis. *An Bras Dermatol* 2014; 89: 259-264.

1098 Pong DL, Marom T, Makishima T. *Phialemonium* infection complicating chronic suppurative
1099 otitis media. *Med Mycol Case Rep* 2014; 4: 5-7.

1100 Pontes ZB, Oliveira AC, Guerra FQ, Pontes LR, Santos JP. Distribution of dermatophytes from
1101 soils of urban and rural areas of cities of Paraíba State, Brazil. *Rev Inst Med Trop Sao*
1102 *Paulo* 2013; 55: 377-383.

1103 Powel MS, Alizadeh AA, Budvytiene I, Schaenman JM, Banaei N. First isolation of
1104 *Cryptococcus uzbekistanensis* from an immunocompromised patient with lymphoma.
1105 *J Clin Microbiol* 2012; 50: 1125-1127.

1106 Prenafeta-Boldu FX, Summerbell R, Sybren de Hoog G. Fungi growing on aromatic
1107 hydrocarbons: biotechnology's unexpected encounter with biohazard? *FEMS*
1108 *Microbiol Rev* 2006; 30: 109-130.

1109 Ragupathi L, Reyna M. Case report of *Cryptococcus albidus* peritonitis in a peritoneal dialysis
1110 patient and a review of the literature. *Perit Dial Int* 2015; 35: 421-427.

1111 Rajasingham R, Smith RM, Park BJ, Jarvis JN, Govender NP, Chiller TM, et al. Global burden of
1112 disease of HIV-associated cryptococcal meningitis: an updated analysis. *Lancet Infect*
1113 *Dis* 2017; 17: 873-881.

1114 Revankar SG, Sutton DA. Melanized fungi in human disease. *Clin Microbiol Rev* 2010; 23:
1115 884-928.

1116 Romão D, Abreu R, Calado G, Freitas F, Rodrigues P, Ferreira C, et al. Madeira 2010:
1117 aftermath of flashfloods and mudslides on bathing water quality indicators and on
1118 sand microbial levels. *PanEuropean Symposium Water and Sanitation Safety Planning*
1119 *and Extreme Weather Events, Bilthoven, Netherlands, 2017a.*

1120 Romão D, Staley C, Ferreira F, Rodrigues R, Sabino R, Verissimo C, et al. Next-generation
1121 sequencing and culture-based techniques offer complementary insights into fungi
1122 and prokaryotes in beach sands. *Mar Pollut Bull* 2017b; 119: 351-358.

1123 Roth Jr FJ, Ahearn DG, Fell JW, Meyers SP, Meyer SA. Ecology and taxonomy of yeasts
1124 isolated from various marine substrates. *Limnol Oceanogr* 1962; 7: 178-185.

1125 Rudenko AV, Savluk OS, Saprykina MN, Yastremskaya AV, Goncharuk VV. Microscopic fungi
1126 in water of the Dnieper river. *Journal of Water Chemistry and Technology* 2011; 33:
1127 323-327.

- 1128 Sabino R, Rodrigues R, Costa I, Carneiro C, Cunha M, Duarte A, et al. Routine screening of
1129 harmful microorganisms in beach sands: implications to public health. *Sci Total*
1130 *Environ* 2014; 472: 1062-1069.
- 1131 Sabino R, Verissimo C, Cunha MA, Wergikoski B, Ferreira FC, Rodrigues R, et al. Pathogenic
1132 fungi: an unacknowledged risk at coastal resorts? New insights on microbiological
1133 sand quality in Portugal. *Mar Pollut Bull* 2011; 62: 1506-1511.
- 1134 Salleh SL, Raup R, Azman N, Mohd Zainudin NAI. Microfungal community in sandy beaches
1135 located in Kedah, Pahang and Sabah, Malaysia. *Stud Mycol* 2018; 3: 321-332.
- 1136 Salvo VS, Fabiano M. Mycological assessment of sediments in Ligurian beaches in the
1137 Northwestern Mediterranean: pathogens and opportunistic pathogens. *J Environ*
1138 *Manage* 2007; 83: 365-369.
- 1139 Shah AH, Abdelzaher AM, Phillips M, Hernandez R, Solo-Gabriele HM, Kish J, et al. Indicator
1140 microbes correlate with pathogenic bacteria, yeasts and helminthes in sand at a
1141 subtropical recreational beach site. *J Appl Microbiol* 2011; 110: 1571-1583.
- 1142 Shelton BG, Kirkland KH, Flanders WD, Morris GK. Profiles of airborne fungi in buildings and
1143 outdoor environments in the United States. *Appl Environ Microbiol* 2002; 68: 1743-
1144 1753.
- 1145 Sherry JP, Kuchma SR, Dutka BJ. The occurrence of *Candida albicans* in Lake Ontario bathing
1146 beaches. *Can J Microbiol* 1979; 25: 1036-1044.
- 1147 Shivaprakash MR, Singh G, Gupta P, Dhaliwal M, Kanwar AJ, Chakrabarti A. Extensive white
1148 piedra of the scalp caused by *Trichosporon inkin*: A case report and review of
1149 literature. *Mycopathologia* 2011; 172: 481-486.
- 1150 Silva S, Negri M, Henriques M, Oliveira R, Williams DW, Azeredo J. *Candida glabrata*, *Candida*
1151 *parapsilosis* and *Candida tropicalis*: biology, epidemiology, pathogenicity and
1152 antifungal resistance. *FEMS Microbiol Rev* 2012; 36: 288-305.
- 1153 Solo-Gabriele HM, Harwood VJ, Kay D, Fujioka RS, Sadowsky MJ, Whitman RL, et al. Beach
1154 sand and the potential for infectious disease transmission: observations and
1155 recommendations. *Journal of the Marine Biological Association of the United*
1156 *Kingdom* 2016; 96: 101-120.
- 1157 Sterflinger K. Black yeasts and meristemetic fungi: Ecology, diversity and identification. In:
1158 Péter G, Rosa C, editors. *Biodiversity and Ecophysiology of Yeasts*. Springer, Berlin,
1159 Heidelberg, 2006, pp. 501-514.
- 1160 Stevens JL, Evans GE, Aguirre KM. Human beach use affects abundance and identity of fungi
1161 present in sand. *Journal of Coastal Research* 2012; 28: 787-792.
- 1162 Tokura R. Sand-inhabiting marine fungi from Japanese beaches. *Botanica Marina* 1984; 27:
1163 567-569.
- 1164 Trofa D, Gácsér A, Nosanchuk JD. *Candida parapsilosis*, an emerging fungal pathogen. *Clin*
1165 *Microbiol Rev* 2008; 21: 606-625.
- 1166 Ulfig K, Guarro J, Cano J, Gene J, Vidal P, Figueras MJ. General assessment of the occurrence
1167 of keratinolytic fungi in river and marine beach sediments of Catalonian waters
1168 (Spain). *Water Air and Soil Pollution* 1997; 94: 275-287.
- 1169 Vadkertiová R, Sláviková E. Metal tolerance of yeasts isolated from water, soil and plant
1170 environments. *J Basic Microbiol* 2006; 46: 145-152.
- 1171 Valdes-Collazo L, Schultz AJ, Hazen TC. Survival of *Candida albicans* in tropical marine and
1172 fresh waters. *Appl Environ Microbiol* 1987; 53: 1762-1767.
- 1173 Velegraki-Abel A, Marseloukinti U, Richardson C. Incidence of yeasts in coastal sea-water of
1174 the Attica Peninsula, Greece. *Water Res* 1987; 21: 1363-1369.

1175 Velegraki A, Efstratiou MA, Arabatzis M, Prodromou P. A survey on the occurrence of
1176 dermatophytes in recreational beach sand. International Society for Microbial
1177 Ecology (ISME), Copenhagen, Denmark, 2012.

1178 Vezzulli, L., Zotti M, Marin V, Moreno M, Pezzati E, Fabiano M. Swash zone interstitial water
1179 is a reservoir of fungal micro-organisms on a Mediterranean beach (Genoa City,
1180 Italy). *Mar Biodivers Rec* 2009; 2: E19.

1181 Vogel C, Rogerson A, Schatz S, Laubach H, Tallman A, Fell J. Prevalence of yeasts in beach
1182 sand at three bathing beaches in South Florida. *Water Res* 2007; 41: 1915-1920.

1183 Wålinder R, Ernstgård L, Johanson G, Norbäck D, Venge P, Wieslander G. Acute effects of a
1184 fungal volatile compound. *Environ Health Perspect* 2005; 113: 1775-1778.

1185 Weiskerger CJ, Brandão J, Harwood VJ, Aslan A, Avolio L, Badgley BD, et al. Microbial
1186 dynamics and human health risks in the continuum between beach water and sand,
1187 and possible effects of a changing earth. *Water Res* 2019; 162: 456-470.

1188 WHO. Guidelines for safe recreational water environments. Volume 1: Coastal and fresh
1189 waters. Geneva: World Health Organization 2003.

1190 Wijayawardene NN, Hyde KD, Wanasinghe DN, Papizadeh M, Goonasekara ID, Camporesi E,
1191 et al. Taxonomy and phylogeny of dematiaceous coelomycetes. *Fungal Diversity*
1192 2016; 77: 1-316.

1193 Wójcik A, Rózga A, Kurnatowski P. Prevalence of potentially pathogenic fungi in the bathing
1194 sites of the Sulejow Reservoir. *Wiad Parazytol* 2003; 49: 173-185.

1195 Yee TL, Tajuddin R, Nor NMIM, Mohd MH, Zakaria L. Filamentous ascomycete and
1196 basidiomycete fungi from beach sand. *Rendiconti Lincei-Scienze Fisiche E Naturali*
1197 2016; 27: 603-607.

1198 Zalar P, Zupančič J, Gostinčar C, Zajc J, de Hoog GS, De Leo F, et al. The extremely
1199 halotolerant black yeast *Hortaea werneckii* - a model for intraspecific hybridization in
1200 clonal fungi. *IMA Fungus* 2019; 10: 10.

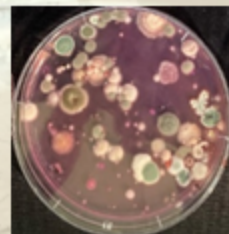
1201 Zatoń K, Błaszak M. The microbiological and sanitary state of sand in the municipal bathing
1202 beach in Szczecin. *Journal of Ecological Engineering* 2015; 16: 40-45.

1203 Zhan P, Liu W. The changing face of dermatophytic infections worldwide. *Mycopathologia*
1204 2017; 182: 77-86.

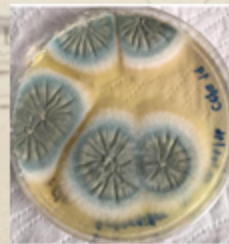
1205

1206

Sand Sample



Primary Isolation



Single Colony

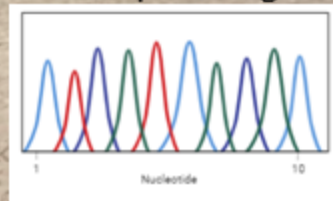
MALDI-TOF

Macroscopic and
Microscopic ID

Fungal DNA Barcode Amplification
(ITS1/2, *TEF1α* and/or *RPB2*) and
other *taxa*-specific sequences

Comparison to Reference
Spectral Database for ID

Sequencing



Fungal characterisation of beach sand and recreational
waters (species and CFU/g & CFU/ml)

BLAST to Reference
Sequence Databases for ID