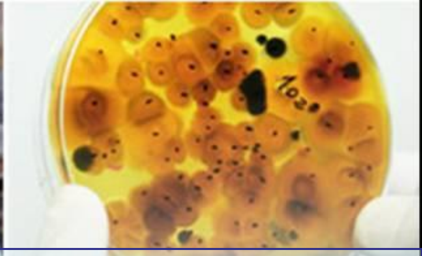

Environmental Water as a Source of Fungal Infections

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WHO fungal priority pathogens list

Table 3. WHO fungal priority pathogens list

Critical group	High group	Medium group
Cryptococcus neoformans	*Nakaseomyces glabrata (Candida glabrata)*	*Scedosporium* spp.
Candida auris	*Histoplasma* spp.	*Lomentospora prolificans*
Aspergillus fumigatus	Eumycetoma causative agents	*Coccidioides* spp.
Candida albicans	Mucorales	*Pichia kudriavzeveii (Candida krusei)*
	Fusarium spp.	*Cryptococcus gattii*
	Candida tropicalis	*Talaromyces marneffei*
	Candida parapsilosis	*Pneumocystis jirovecii*
		Paracoccidioides spp.

World Health Organization (WHO) Report. WHO fungal priority pathogens list to guide research, development and public health action. Oct 25, 2022.

Available at: https://www.who.int/publications/i/item/9789240060241

Specific policies to protect the EU's water resources and ecosystems and to ensure all Europeans have access to clean drinking and bathing water

Bathing water
EU rules to ensure clean and high-quality bathing water across Europe

Drinking water
Improving access to drinking water for all

Floods
EU measures to manage flood risk and the risks floods pose to human health and the environment.

Groundwater
EU action to ensure good quantity and quality of groundwater.

Marine waters
EU action to protect Europe's coasts, seas and oceans.

Nitrates
Protecting waters against pollution caused by nitrates from agricultural sources

Surface water
EU rules protecting surface waters from chemical pollution.

Urban wastewater
EU rules to ensure that urban wastewater is properly dealt with.

Water Reuse
Managing water resources more efficiently and facilitating water reuse in the EU

Water scarcity and droughts
Preventing and mitigating water scarcity and droughts in the EU

EU efforts on the global water agenda
It is essential to sustain the political momentum of the UN 2023 Water Conference, to accelerate actions for clean water and sanitation for all by 2030.

European Commission

by Rousselin, P., 2023, Iberlab meeting, Lisbon, PT

UNDER REVIEW

The 2006/7 Bathing Water Directive

- **Directive 2006/7 EC** covers bathing water (fresh and marine water)
- **Testing** includes enterococci and *E coli*
- **Bathing Beaches:** There are over 22,000 bathing beaches in 27 EU countries.

4.3.2006 EN Official Journal of the European Union L 64/37

DIRECTIVE 2006/7/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL

of 15 February 2006

concerning the management of bathing water quality and repealing Directive 76/160/EEC

By Rousselin, P., 2023, Iberlab meeting, Lisbon, PT

What to expect of the revised bathing water directive

- ✓ The same faecal indicator parameters and values
- ✓ Cyanobacteria and cyanotoxins
- ✓ Predictive modelling
- ✓ Risk assessment for additional parameters
- ✓ Sand quality?

Why sand?

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

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

Current WHO recommendations

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

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

The **Mycosands** working group - what have we been up to?

(Lead by Esther **Segal**, Jean Pierre **Gangneux** & João **Brandão**)

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

THE TEAM:

Bridging Medical Mycology and Recreational Water Quality

France	
Name	**Area**
Frédéric Roger	Atlantic coast
Hélène Guegan	Atlantic coast
Jean-Pierre Gangneux	Atlantic coast
Laurence Delhaes	Bordeaux
Patrice Le Pape	Mediterranean coast
Sébastien Bertout	Mediterranean coast
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Marija Kataržytė	Baltic Sea

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Name	**Area**
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Larissa Montas	Miami, Fl, USA
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Paul Verweij	Inland water Basin
Willem Melchers	Atlantic coast

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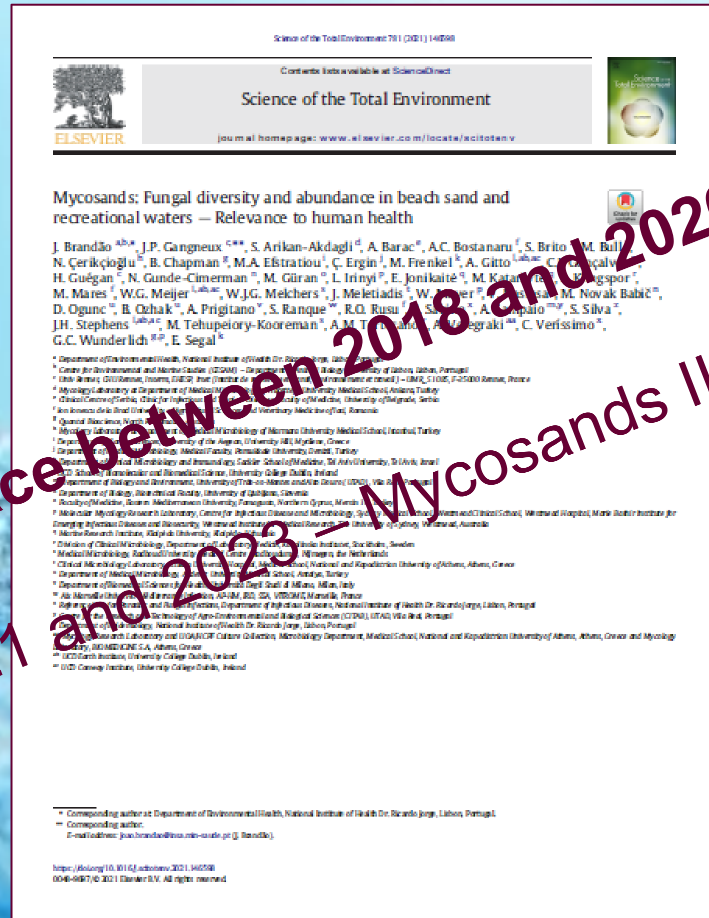
Cristina Veríssimo	Atlantic coast
Joao Brandao	Atlantic coast
Raquel Sabino	Atlantic coast
Siyu Huang	Atlantic coast
Susana Pereira	Atlantic coast

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

Mycosands (1/4) - Fungal diversity and abundance in beach sand and recreational waters - relevance to human health


Science of the Total Environment 781 (2021) 146598

Contents lists available at ScienceDirect

Science of the Total Environment

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

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

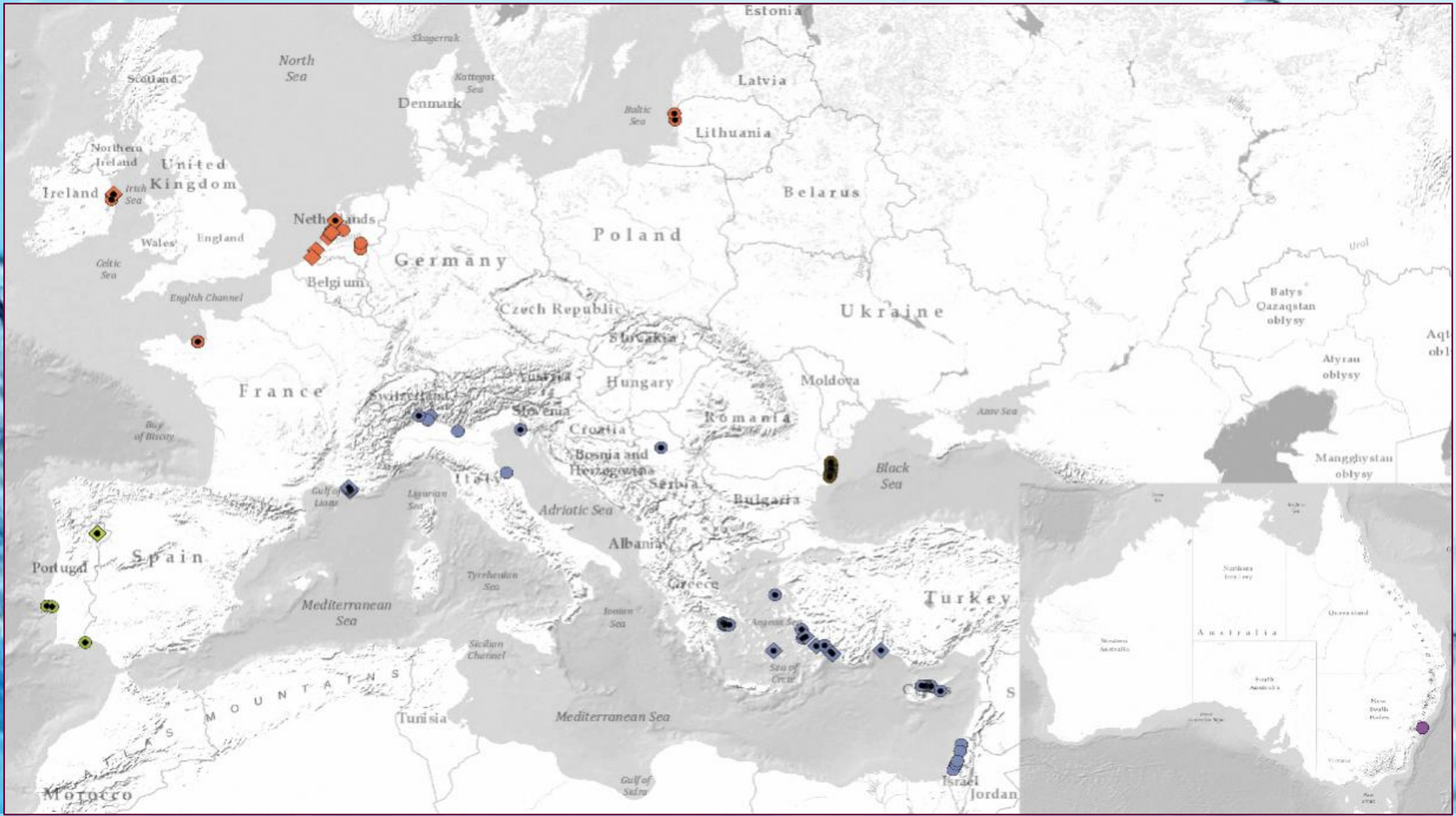
https://doi.org/10.1016/j.scitotenv.2021.146598

Sampling took place between 2018 and 2020 – Mycosands I

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

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

Mycosands - 2/4 (Sampling sites)



Geographical distribution of the sampling points using mapping with QGIS (Version 3.10.0-A Coruña). Circles correspond to urban beaches and diamonds to non-urban beaches. Dots within the shapes indicate water-sampling sites. Red=Northwest Europe, Green=Southwest Europe, Blue=Mediterranean, Brown=Black Sea and Purple=Sydney (Australia)

Mycosands - 3/4 (Results of sand)

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

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

Mycosands - 4/4 (Conclusions)

- ✓ Fungi are missing from water and sand health protection regulatory parameters.
- ✓ Both sand and water should be monitored for fungi
- ✓ The median value of 89 CFU/g of all fungi may serve as a reference for sand regulation
- ✓ *Candida albicans*, dermatophytes, endemic fungi and other fungi should be considered
- ✓ **Fungal analysis of water needs more data before reference values can be established** (**comment:** dispersive nature of water produces very low counts of fungi per 100ml which is a problem in statistical analyses)

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

Recast of the Drinking Water Directive 2020/2184

- ✓The recast Drinking Water Directive is the EU’s main law on drinking water. It concerns the access to, and the quality of water intended for human consumption to protect human health.
- ✓There are no changes in *E. coli* parameter and microbiological method specifications
- ✓According to DWD 2020/2184, the monitoring frequency of intestinal enterococci increases (inclusion as a Group A parameter).

By Rousselin, P., 2023, Iberlab meeting, Lisbon, PT

Recast of the Drinking Water Directive 2020/2184

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

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

*** or any alternative method equivalente according to ISO 17994**

By Rousselin, P., 2023, Iberlab meeting, Lisbon, PT

Exposure paths

Urban water supply

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

In-hospital water use (aerossols and streaming water)

Hospital Wastewater pre-treatment plant

Urban(community Wastewater treatment plant

Most common waterborne fungi in Europe causing opportunistic infections and other health problems (Novak Babič, M, *et al*. 2017)

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

(**Comment:** *C. auris* is thought to be an aquatic organisms by many but is not (yet) a waterborne problem. It's currently mainly a nosocomial problem**)**

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

Fungal Contaminants in Drinking Water Regulation? A Tale of Ecology, Exposure, Purification and Clinical Relevance by Novak Babič, M, *et al*. 2017 (1/2)

Recommendations

- ✓ Monitor drinking water in **relevant contexts**.
- ✓ Adopt the current Swedish legislation, **updating the fungal parameters** to levels compatible with current knowledge.
- ✓ **Pay special attention to hospitals and other public buildings** where immunocompromised people are present for extended periods of time and **molecular typing may be required to track sources or link infections together**.

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

Fungal Contaminants in Drinking Water Regulation? A Tale of Ecology, Exposure, Purification and Clinical Relevance by Novak Babič, M, *et al*. 2017 (2/2)

Future Scientific Research Needs

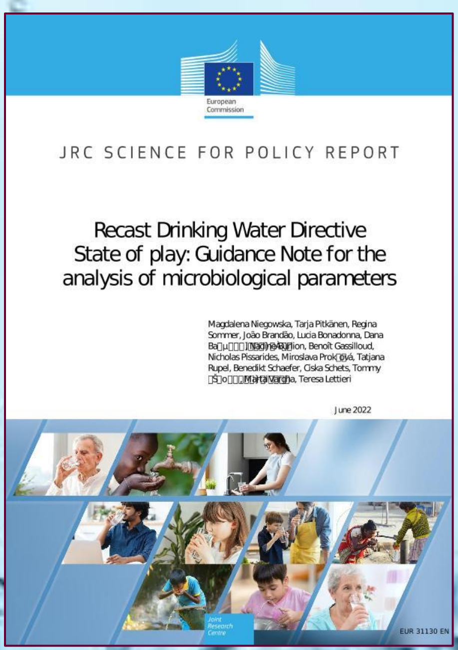
- ✓ **Develop a standard procedure** for assessing fungal contaminants in drinking water.
- ✓ Create a global report on fungal contaminants in water, using a standardized procedure.
- ✓ **Develop sampling techniques to detect sporadic particles released by biofilms**.
- ✓ **Assess the presence and quantity of mycotoxins** and Microbial Volatile Organic Compounds in drinking water on a large scale.
- ✓ Conduct **agent-specific epidemiological assessments** of the health effects of drinking-waterborne fungi.

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

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

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

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


JRC SCIENCE FOR POLICY REPORT

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

Magdalena Niegowska, Tarja Pitkänen, Regina Sommer, João Brandão, Lucia Bonadonna, Dana ... Benoît Gassilloud, Nicholas Pissarides, Miroslava ... Tatjana Rupel, Benedikt Schaefer, Ciska Schets, Tommy ... Márta Vargha, Teresa Lettieri

June 2022

EUR 31130 EN

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

Examples of relevant studies about microfungi in drinking water supplying hospitals

- ✓ **Caggiano, G, *et al.*, 2020.** Occurrence of Fungi in the Potable Water of Hospitals: A Public Health Threat. Pathogens (Basel, Switzerland), 9(10), 783. https://doi.org/10.3390/pathogens9100783:

"This study evaluated the occurrence of fungi in potable water distribution systems in hospitals. The frequency of **samples positive for the presence of fungi was 56.9%;** among them, filamentous fungi and yeasts were isolated from 94.2% and 9.2% of the samples, respectively. **The intensive care unit (87.1%) had the highest frequency of positive samples**."

- ✓ **Warris, A., *et al*., 2003.** Molecular epidemiology of Aspergillus fumigatus isolates recovered from water, air, and patients shows two clusters of genetically distinct strains. J. Clin. Microbiol. 2003, 41, 4101–4106:

"The genotypic relatedness between clinical and environmental isolates suggests that **patients with invasive aspergillosis can be infected by strains originating from water or from air**"
&
"The sampling was performed at the pediatric oncology ward at the Rikshospitalet University Hospital in **Oslo, Norway**. This ward, containing two bone marrow transplantation (BMT) units, is situated in a relatively new wing of the department of pediatrics built in 1990."

Clinically relevant fungi in water and on surfaces in an indoor swimming pool facility

- ✓ The highest fungal counts in water and on surfaces were in the recreational pool (17 CFU/100 mL) and on a flexibeam (5.8 CFU/cm2), respectively as compared with low counts (<0.1 CFU/cm2) on the diving platform, bench tops and walls.
- ✓ 357 isolates belonged to **79 species** and species complexes, 42 of which known as clinically relevant. ***Phialophora oxyspora*** (13.7%) and ***Phoma*** spp. (12.3%) were the most frequently identified groups.
- ✓ Including ***Candida auris*** and *Trichophyton interdigitale* !
- ✓ WHO guidelines address (only) dermatophytes

Ekawati et al. 2017 www.doi.org/10.1016/j.ijheh.2017.07.002

The revised Urban Wastewater Directive

- At the European level Council Directive (EU) 2024/3019 concerning urban wastewater treatment (recast):
  - –Includes Urban wastewater surveillance

Article 17

Urban wastewater surveillance

1. Member States shall set up a national system for cooperation and coordination between competent authorities responsible for public health and competent authorities responsible for urban wastewater treatment with regard to:

(a) the identification of relevant public health parameters that are to be monitored at least in the inlet of urban wastewater treatment plants, taking into account available recommendations by, inter alia, the European Centre for Disease Prevention and Control (ECDC), the Health Emergency Preparedness and Response Authority (HERA) and the World Health Organization (WHO), such as:

(i) SARS-CoV-2 virus and its variants;

(ii) poliovirus;

(iii) influenza virus;

(iv) emerging pathogens;

(v) any other public health parameters that are considered relevant by the competent authorities for monitoring;

(b) the clear allocation of roles, responsibilities and costs among operators and relevant competent authorities, including where related to sampling and analysis;

Fungi that we are most worried about in Waste Water Surveillance and why

- *Candida* spp, especially *Candida auris* – To monitor its presence in a community

- Azole resistance in *Aspergillus fumigatus* sensu stricto

https://wastewater-observatory.jrc.ec.europa.eu/#/content/glowacon

AgIR – Action plan to manage industrial wastewaters of wider- and West-Lisbon

- **Technical support to industries to optimize their wastewater pretreatment processes.**
- **Characterization of hospital wastewater to improve epidemiological surveillance and optimize treatment processes.**
- **Training of technicians in industrial effluent management.**
- **Recognition of industries with "Evolving Industry" Quality.**

So far: *Candida auris:* The majority of samples were negative, 4 samples were positive in 4 different hospitals, detected solely by Real-Time PCR (not detected in culture)

AgIR

PARCEIROS TÉCNICO-CIENTÍFICOS

ÁGUAS DO TEJO ATLÂNTICO Grupo Águas de Portugal

LISBOA CÂMARA MUNICIPAL

EPAL Grupo Águas de Portugal

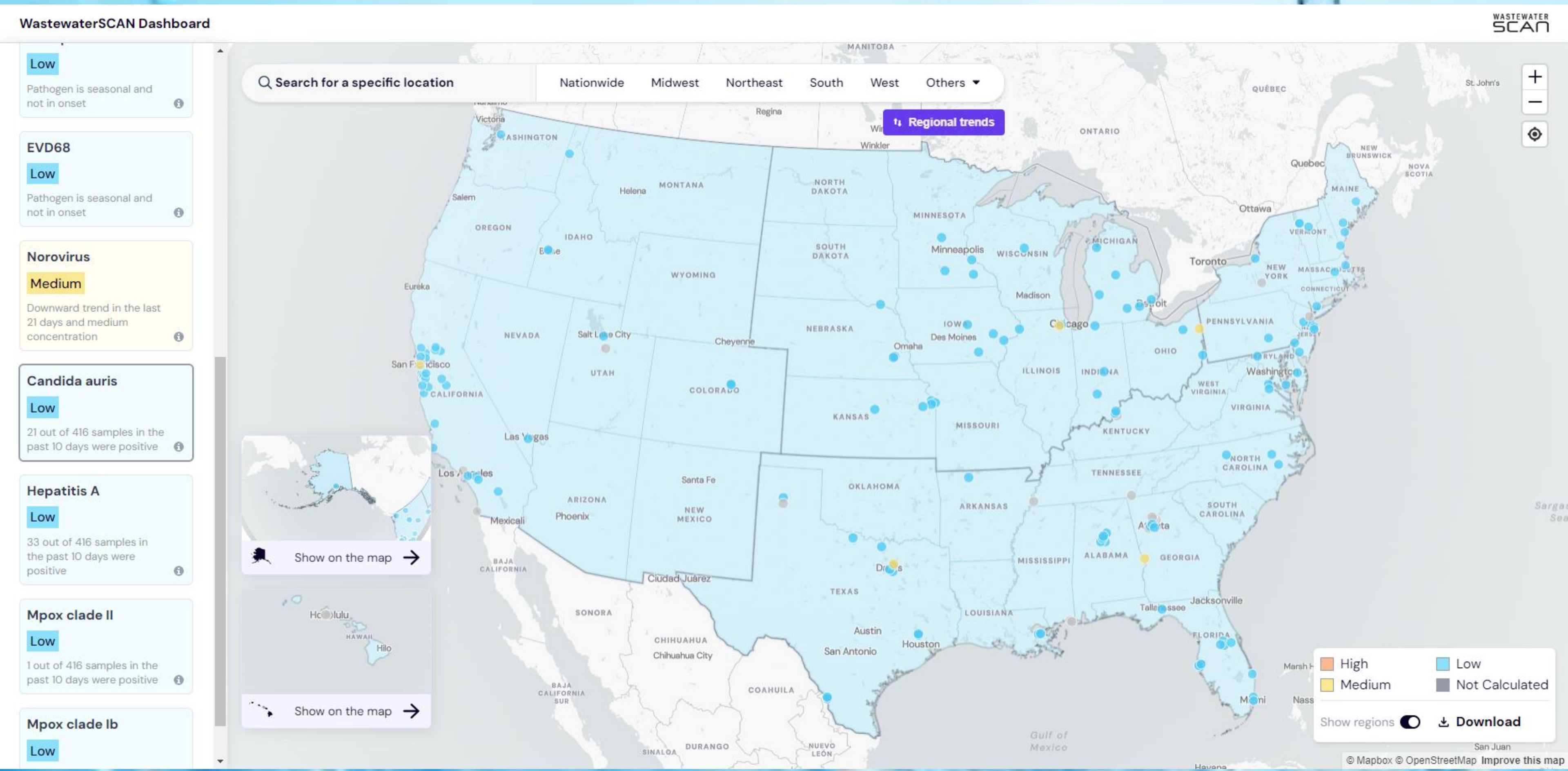
TÉCNICO LISBOA

Instituto_Nacional de Saúde Doutor Ricardo Jorge

FUNDO AMBIENTAL

AgIR COM OS MUNICÍPIOS

USA - WastewaterSCAN Dashboard. It's happening!



https://data.wastewaterscan.org/?charts=CjMQASABSABaB0NfYXVyaXNyCjIwMjQtMDgtMjdyCjIwMjQtMTAtMDiKAQY5NGY5MzDAAQE%3D&selectedChartId=94f930

So….

- ✓ Wastewaters of non-endemic areas should be monitored the presence and determination of genetic clade/AMR of *C. auris* as community and hospital early detection warning system
- ✓ PCR and culture can be used to perform this job, but PCR is more sensitive than culture
- ✓ Monitoring wastewaters for *C. auris* can help controlling and eliminating nosocomial infections and help health authorities to assess the presence of the yeast in the population and design health protection policies

The Water Reuse Regulation 2020/741 - No fungi – think aerosols

- The quality of reclaimed water is divided into four minimum quality classes rated A, B, C and D. Each class determines the use (crop categories) and method of agricultural irrigation permitted.
- **For each class, quality criteria are established for *E. coli***
- The operator must carry out routine checks at frequencies ranging from once a week to twice a month depending on the water class and parameter.

Table 2 – Reclaimed water quality requirements for agricultural irrigation

Reclaimed water quality class	Indicative technology target	Quality requirements				
		E. coli (number/100 ml)	BOD_5 (mg/l)	TSS (mg/l)	Turbidity (NTU)	Other
A	Secondary treatment, filtration, and disinfection	≤ 10	≤ 10	≤ 10	≤ 5	*Legionella* spp.: < 1 000 cfu/l where there is a risk of aerosolisation Intestinal nematodes (helminth eggs): ≤ 1 egg/l for irrigation of pastures or forage
B	Secondary treatment, and disinfection	≤ 100	In accordance with Directive 91/271/EEC (Annex I, Table 1)	In accordance with Directive 91/271/EEC (Annex I, Table 1)	-	
C	Secondary treatment, and disinfection	≤ 1 000			-	
D	Secondary treatment, and disinfection	≤ 10 000			-	

The regulation on water reuse has applied since 26 June 2023.

Adapted from By Rousselin, P., 2023, Iberlab meeting, Lisbon, PT

And… what about antifungals in treated wastewaters being released into the environment

(and acting on resistance emergence due to selective pressure?)

Predicted No Effect Concentrations (PNECs) of antifungals for wastewater management and agricultural use – a comparison to levels documented in waters within the environment

D. Gil[1 ‡], S. José[1 ‡], A. Ascenso[1], M. Novak Babič[2], E. Segal[3], J. Meletiadis[4], J.P. Gangneux[5,6], C. J. Weiskerger[7], H. M. Solo-Gabriele[8], E. Valério[1,9*], J. Brandão[1,9*]

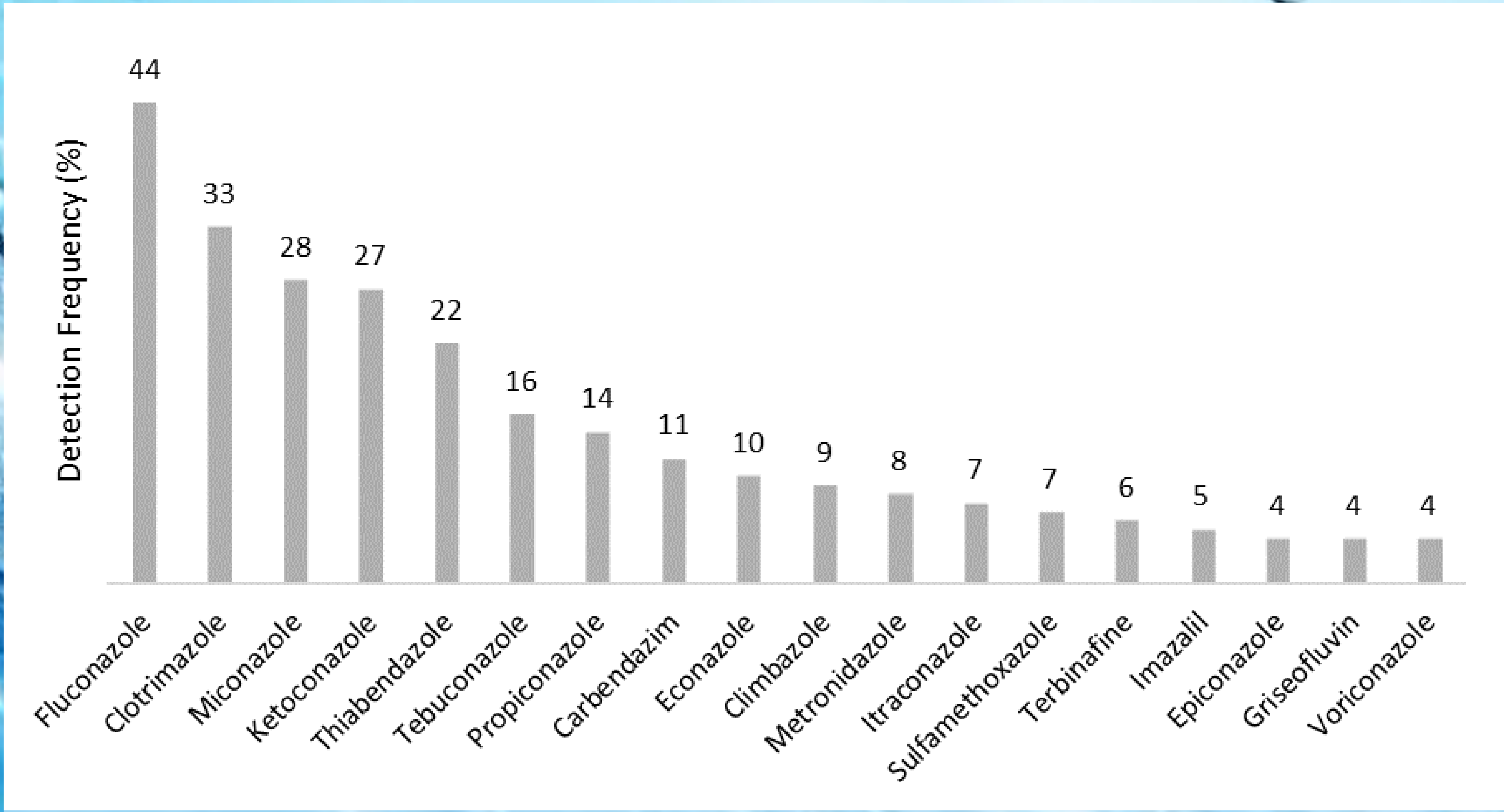
Review and opinion (PRISMA-SLR) – submitted for publication

https://papers.ssrn.com/sol3/papers.cfm?abstract_id=5148819

Methodology

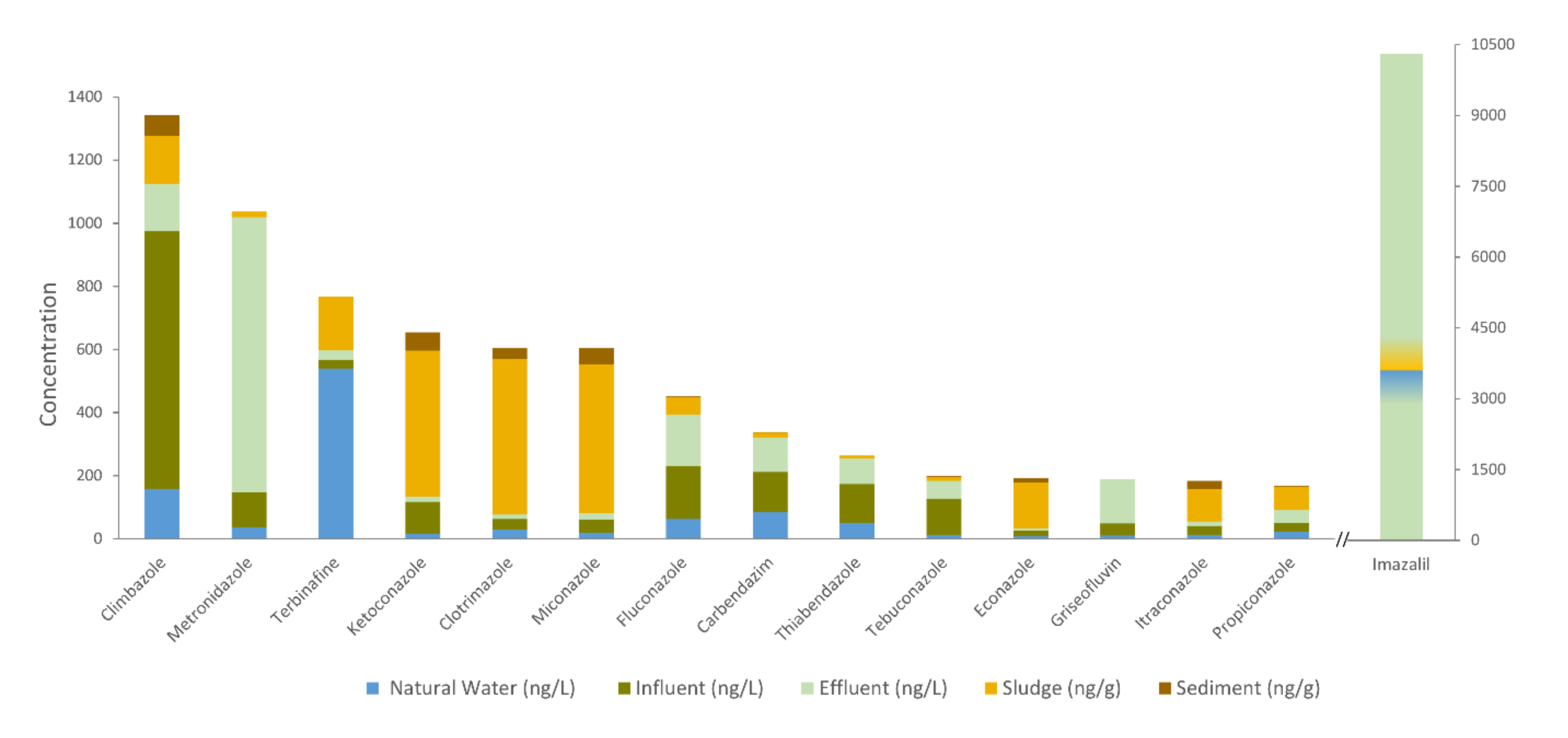
- ✓ The initially search retrieved 13,527 results across the selected databases and registers, including scientific 126 articles, books, and master's theses but a PNEC could no be determined. Therefore,
- ✓ PNEC-MICs were calculated as MIC1%/10 to account for the differences between MICs and the lowest concentration of an antibiotic that results in selection of resistance and competitive advantage based on growth rate (Andersson & Hughes, 2014) of *Candida albicans* (next slide)

Detection frequency of antifungal compounds in environmental samples.



The detection frequency (%) was calculated as the number of studies in which a given antifungal was reported divided by the total number of studies analyzed (122), multiplied by 100

Median concentration of antifungals found in different environmental matrices retrieved by the systematic literature review performed.



Calculated PNECs

Drug	Modal MIC (mg/L)	Observed lowest MIC (mg/L)	R^2 of Normal distribution fit	$MIC_{1\%}$ (mg/L)	PNEC-MIC (ng/L)
Amphotericin B	0.25	0.008	0.976	0.016	1,600
Flucytosine	0.25	0.06	0.986	0.032	3,200
Micafungin	0.008	≤0.004	0.994	0.0005	50
Anidulafungin	0.004	≤0.002	0.998	0.0003	30
Caspofungin[a]	0.5	0.06	0.969	0.063	6,300
Terbinafine[b]	0.008	≤0.008	0.724	0.001	100
Rezafungin	0.002	≤0.002	0.999	0.001	100
Fluconazole	0.25	0.03	0.999	0.032	3200
Voriconazole	0.016	≤0.002	0.994	0.002	200
Posaconazole	0.016	≤0.008	0.954	0.004	400
Itraconazole	0.016	≤0.016	0.983	0.002	200
Isavuconazole	0.004	≤0.002	0.999	0.001	100
Miconazole[c]	0.016	≤0.004	0.933	0.002	200
Econazole[c]	0.016	0.008	0.970	0.002	200
Clotrimazole[c]	0.008	≤0.004	0.995	0.002	200
Ketoconazole[c]	0.008	≤0.004	0.986	0.002	200
Fenticonazole[c]	0.5	0.125	0.986	0.063	6300

Conclusions

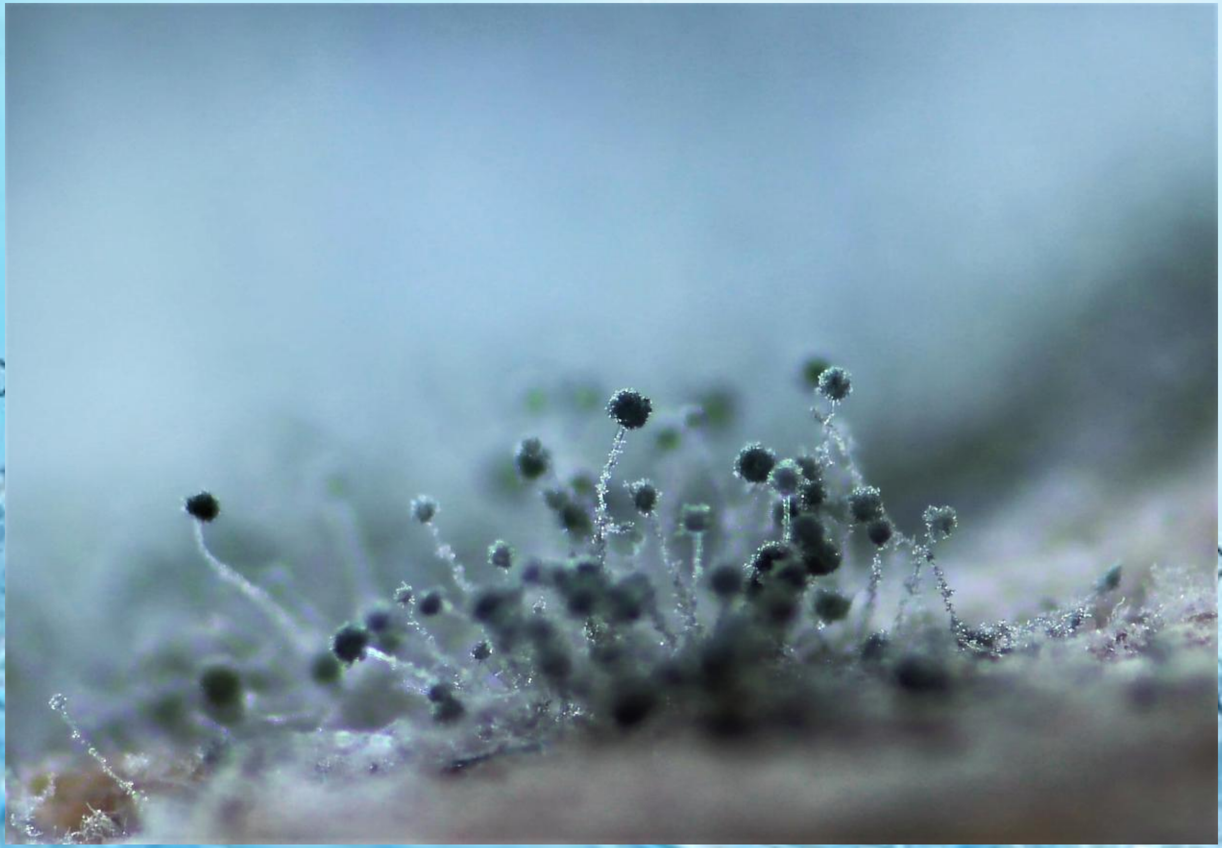
- ✓ The most frequently studied antifungals are not necessarily the ones found at the highest concentrations in the environment.
- ✓ This discrepancy suggests that research focus may not always align with the compounds posing the greatest environmental occurrence.
- ✓ We found higher concentrations for some antifungals than the PNECs calculated
- ✓ The PNECs can be used to prioritize antifungals for regulations and to determine acceptable levels in wastewater effluents.

Concluding remarks

- ✓ The bathing water under revision may include sand quality but, for now, it will only incorporate a safety value for Enterococci, as recommended by the new WHO guidelines for recreational water quality. For fungal species we only have a guiding value based on the Mycosands 2018-2020 sampling, not safety values yet because…
- ✓ The WHO fungal priority watch list makes a difference for incorporating fungi regulation of water environments but…
- ✓ We still need to determine infectious doses of opportunistic and pathogenic fungi to use for quantitative microbial risk assessment (QMRA) – calculations based on all variables influencing or determining the exposure risk.
- ✓ Water environments are most definitely means of propagating antifungals and fungi.

Brandão *et al.* 2020 - doi.org/10.1016/j.scitotenv.2020.140237

Thank you for your time



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