



# ENVIRONMENTAL AND BIOCLIMATIC FACTORS INFLUENCING FUNGAL DISTRIBUTION ALONG EUROPEAN SHORES AND HEALTH IMPACT

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Science of the Total Environment

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

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Poor data about mycobiota of beaches

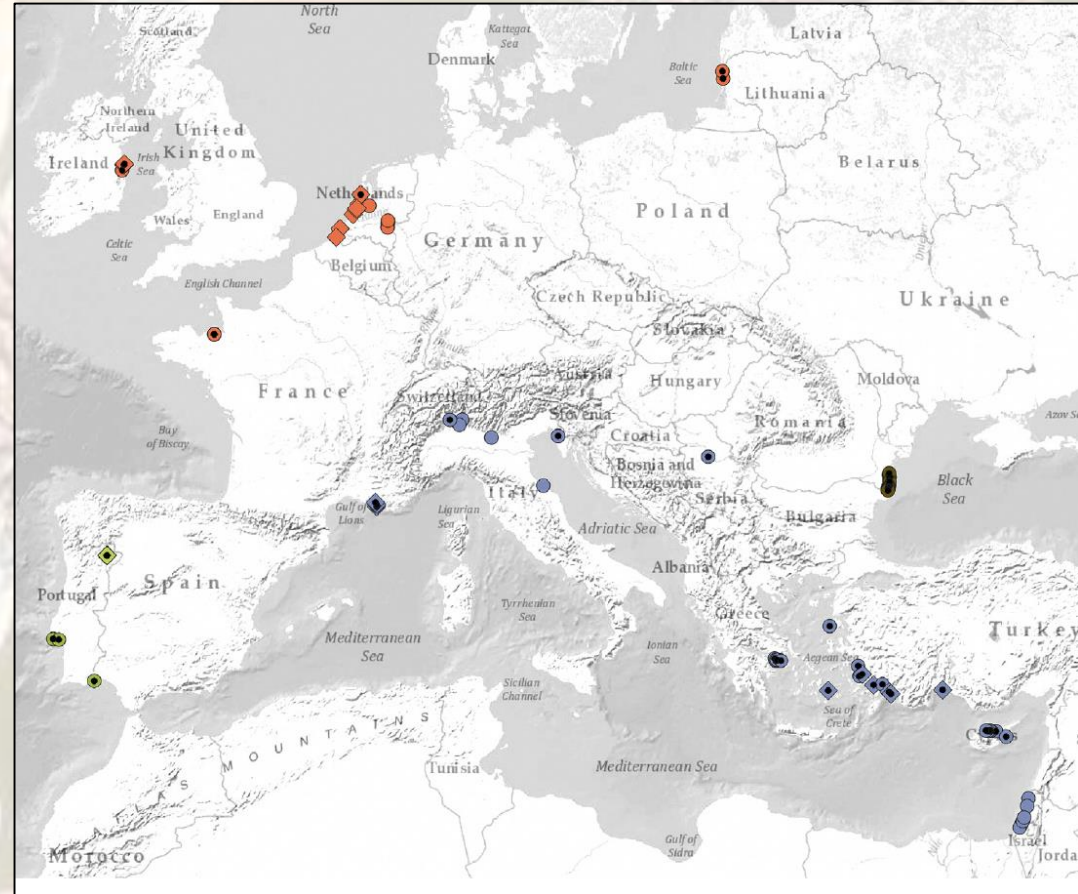
Soil and water samples from European beaches



No parameters and limits for fungi included in the evaluation of beaches quality

fungal pathogens and implication for human health

## Mycosands Study (2019-2021)



Pathogenic or potentially pathogenic fungi were identified

*Aspergillus fumigatus*, *A. niger*, *A. flavus*, *Candida albicans*, *C. tropicalis*, *C. parapsilosis*, *C. glabrata*, *C. dubliniensis*, *Cryptococcus spp.*, *Rhodotorula spp.*, *Dematiaceous fungi*, *Dermatophytes*

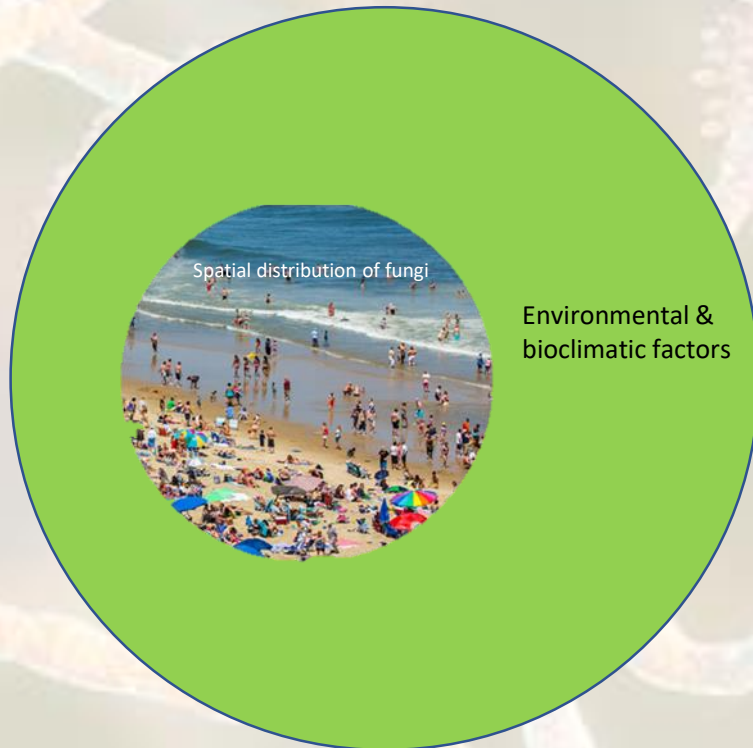




Environmental and bioclimatic factors influencing yeasts and molds distribution along European shores



M. Cogliati <sup>a,\*</sup>, S. Arikan-Akdagli <sup>b</sup>, A. Barac <sup>c</sup>, A.C. Bostanaru <sup>d</sup>, S. Brito <sup>x</sup>, N. Çerikçioğlu <sup>e</sup>, M.A. Efstratiou <sup>f</sup>, Ç. Ergin <sup>g</sup>, M.C. Esposto <sup>a</sup>, M. Frenkel <sup>b</sup>, J.P. Gangneux <sup>1</sup>, A. Gitto <sup>1</sup>, C.I. Gonçalves <sup>k</sup>, H. Guegan <sup>1</sup>, N. Gunde-Cimerman <sup>1</sup>, M. Güran <sup>m</sup>, E. Jonikaitė <sup>n</sup>, M. Kataržytė <sup>n</sup>, L. Klingspor <sup>o</sup>, M. Mares <sup>d</sup>, W.G. Meijer <sup>j</sup>, W.J.G. Melchers <sup>p</sup>, J. Meletiadiš <sup>q</sup>, V. Nastasa <sup>d</sup>, M. Novak Babič <sup>1</sup>, D. Ogunc <sup>r</sup>, B. Ozhak <sup>r</sup>, A. Prigitano <sup>a</sup>, S. Ranque <sup>s</sup>, L. Romanò <sup>a</sup>, R.O. Rusu <sup>d</sup>, R. Sabino <sup>t,u</sup>, A. Sampaio <sup>k,v</sup>, S. Silva <sup>w</sup>, J.H. Stephens <sup>1</sup>, M. Tehupeiory-Kooreman <sup>p</sup>, A. Velegriki <sup>x,aa</sup>, C. Verissimo <sup>1</sup>, E. Segal <sup>h</sup>, J. Brandão <sup>y,z</sup>



# Mycosands spin-off study (2023)

<p><i>Aspergillus spp.</i>  <i>A. fumigatus</i>  <i>A. niger</i>  <i>A. flavus</i></p>	<p><i>Aspergillus spp.</i></p>	<p>Molds</p>
<p><i>Fusarium spp.</i>                      Dematiaceous fungi                      Dermatophytes</p>		
<p><i>Candida spp.</i>  <i>C. albicans</i>  <i>C. parapsilosis</i>  <i>C. tropicalis</i>  <i>C. dubliniensis</i>  <i>C. glabrata</i></p>	<p><i>Candida spp.</i></p>	<p>Yeasts</p>
<p><i>Cryptococcus spp.</i>  <i>Rhodotorula spp.</i></p>		

# Environmental data layers

Type of datasets	Source	Layers	Application
<i>Climatic datasets</i>			
Solar radiation	<a href="https://globalsolaratlas.info">https://globalsolaratlas.info</a>	Horizontal solar radiation	Sand data
Temperature and precipitation	<a href="https://www.worldclim.org">https://www.worldclim.org</a>	Monthly min, max, ave temperature; monthly precipitation	Sand data
<i>Soil datasets</i>			
Physical properties	<a href="https://esdac.jrc.ec.europa.eu">https://esdac.jrc.ec.europa.eu</a>	Dominant soil texture	Sand data
Chemical properties	<a href="https://esdac.jrc.ec.europa.eu">https://esdac.jrc.ec.europa.eu</a>	CEC, BS, CaCO <sub>3</sub> , K, P, N, concentration; CN ratio; pH	Sand data
Haevy metals	<a href="https://esdac.jrc.ec.europa.eu">https://esdac.jrc.ec.europa.eu</a>	As, Cd, Co, Cr, Cu, Hg, Mn, Ni, Pb, Sb concentration	Sand data
<i>Water datasets</i>			
Sea salinity	<a href="https://catalogue.ceda.ac.uk">https://catalogue.ceda.ac.uk</a>	Sea water salinity	Water data
Coastal eutrophication	<a href="https://sedac.ciesin.columbia.edu">https://sedac.ciesin.columbia.edu</a>	Coastal chlorophyill-a concentration	Water data
Water temperature	<a href="https://data.europa.eu">https://data.europa.eu</a>	Monthly water surface temperature	Water data

# Data analysis

## MaxEnt analysis

[http://biodiversityinformatics.amnh.org/open\\_source/maxent/](http://biodiversityinformatics.amnh.org/open_source/maxent/)

Environmental dataset

Occurrence points



Algorithm calculates the probability of presence (suitability) of the analyzed category for each value of the variable in the environmental layer



Outputs:

- Map of suitability distribution of the category
- Response curves of suitability to each variable in the environmental layers
- Contribution of each variable to the final model

Number of occurrence points for each analyzed category of fungi

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

Categories including less than 8 occurrence points were not analyzed

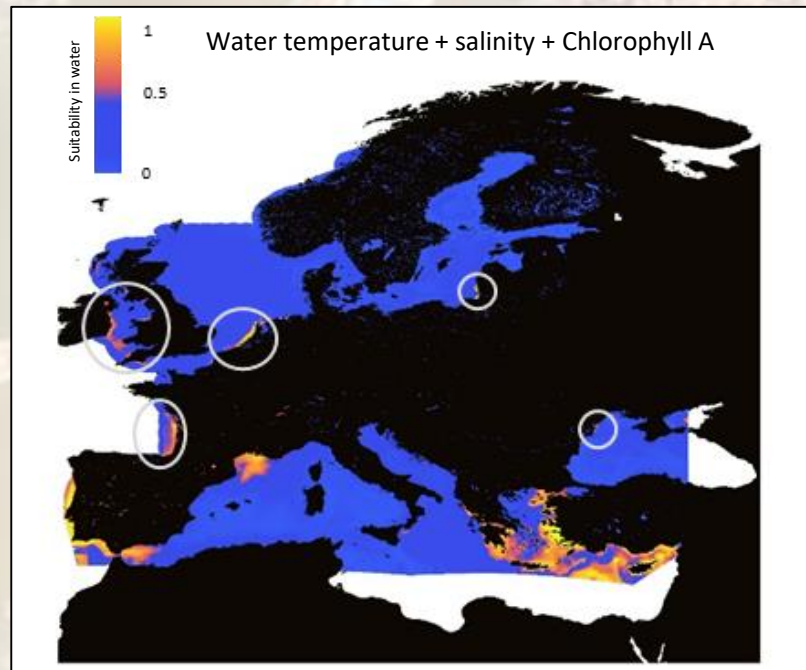
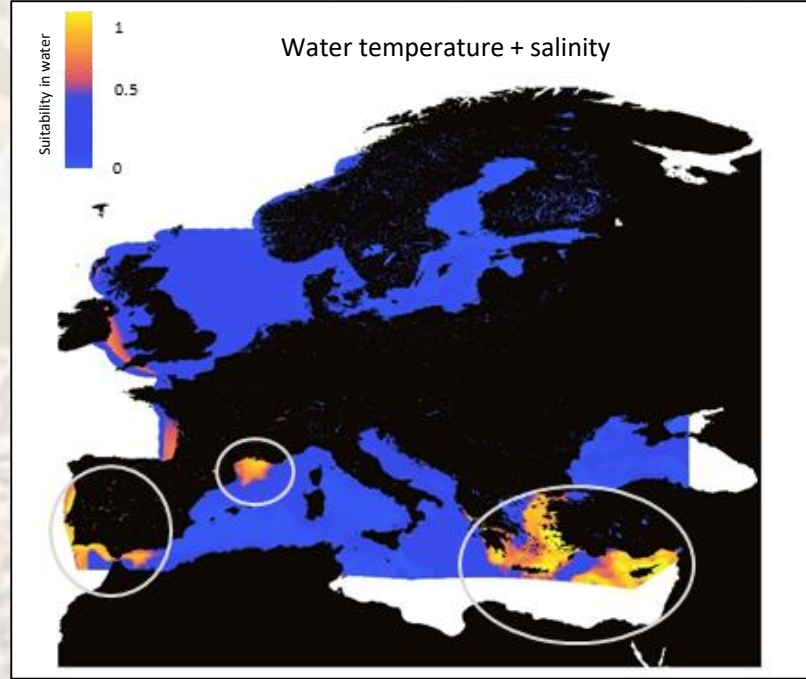
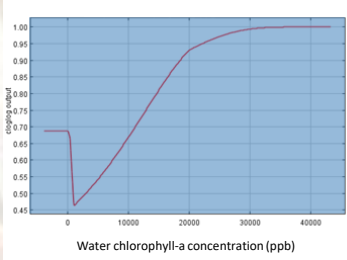
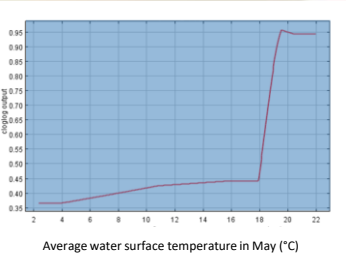
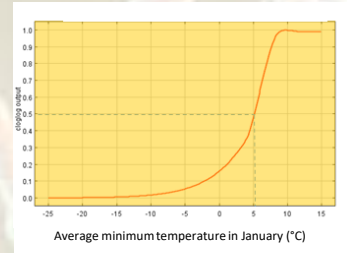
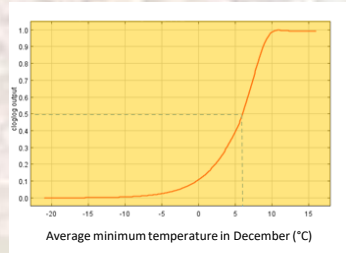
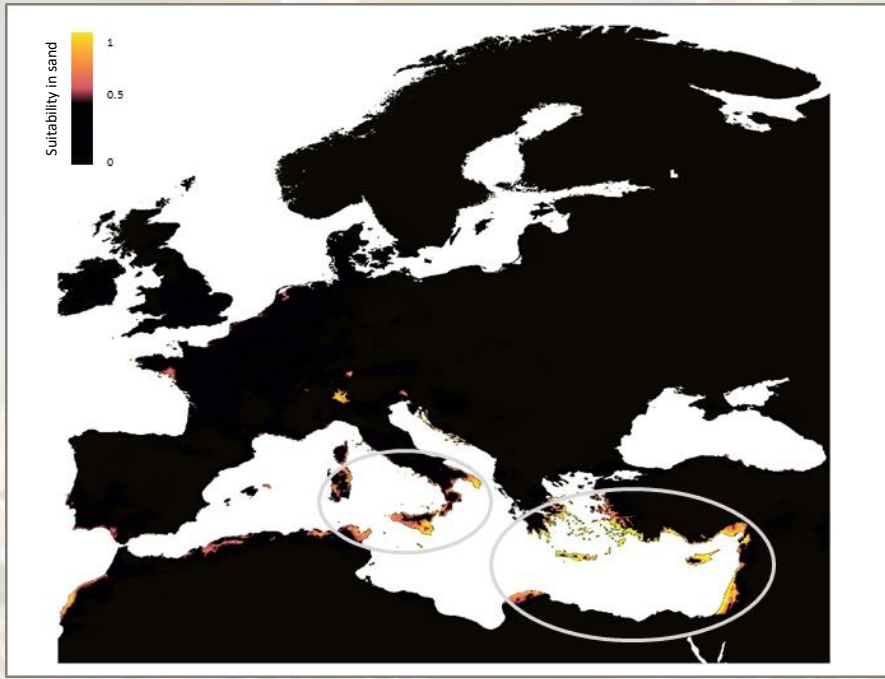
51 different analyses

44 analyses using sand occurrence points  
(11 categories x 4 dataset groups)

7 analyses using water occurrence points  
(7 categories x 1 dataset group)

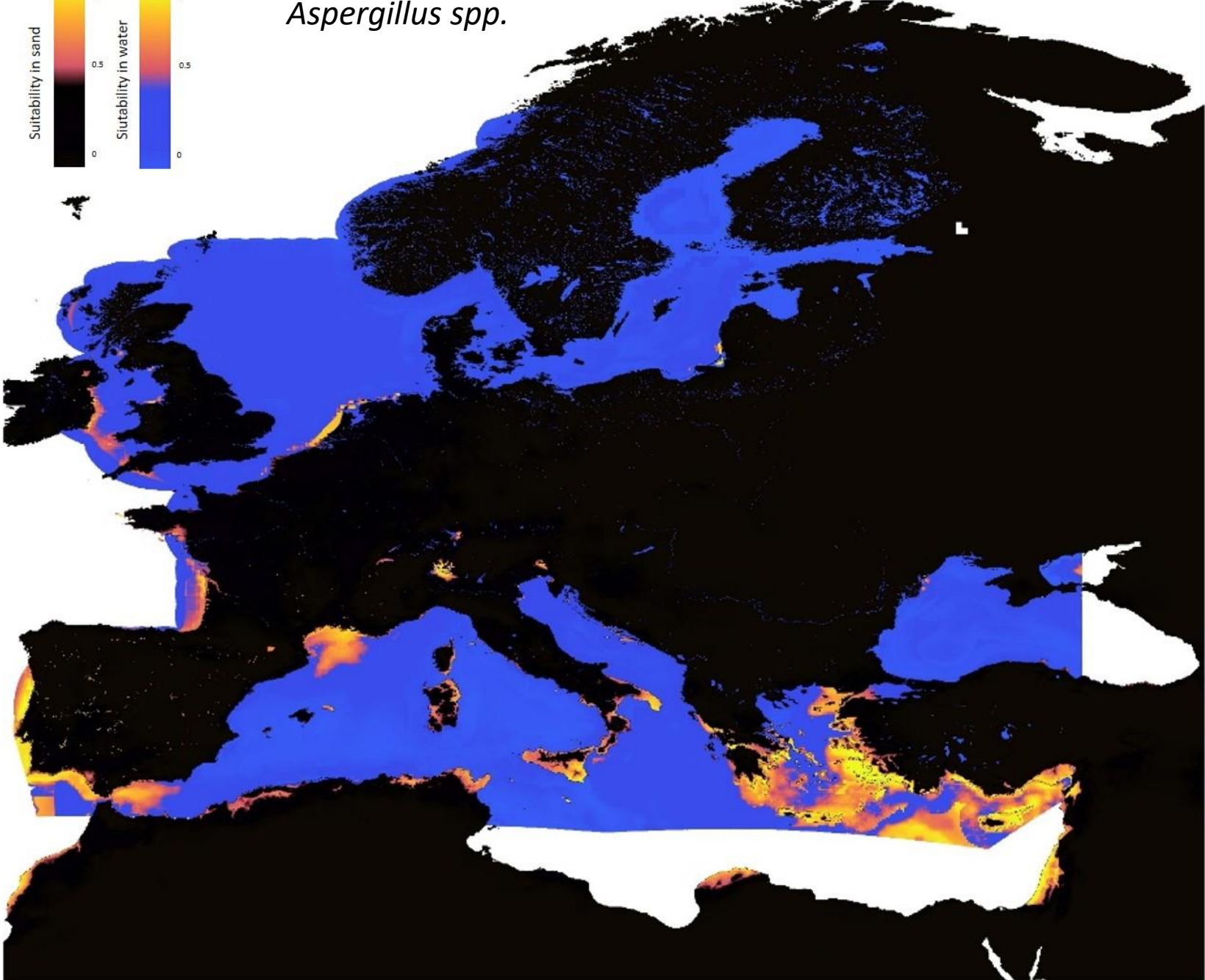
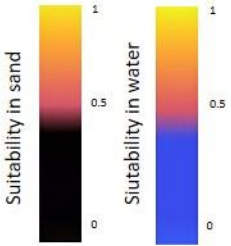


*Aspergillus spp.* in sand

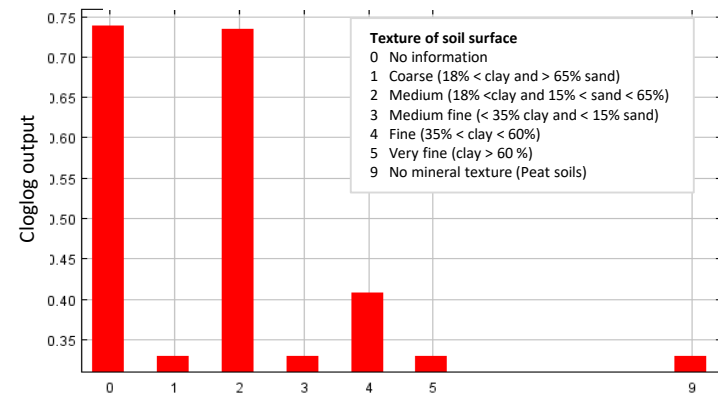
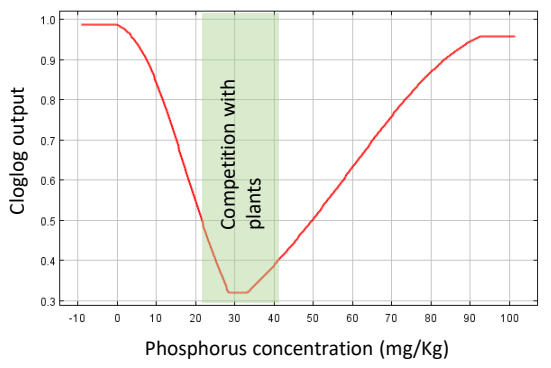
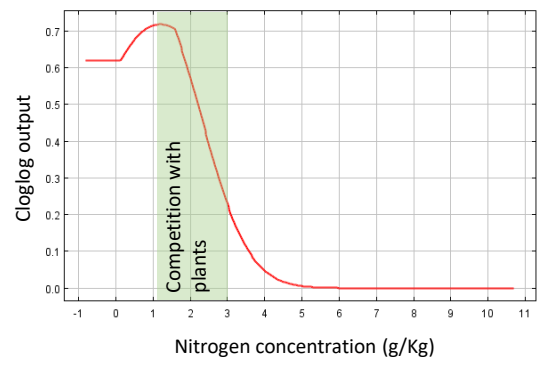
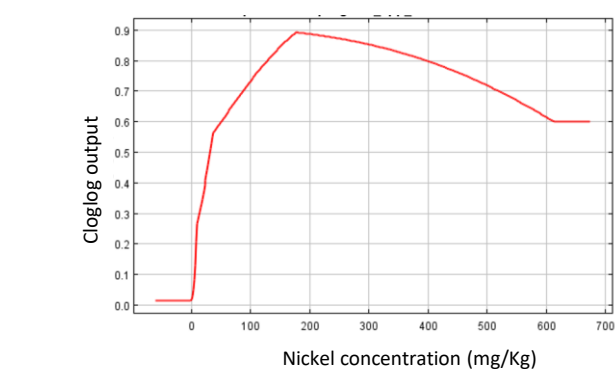
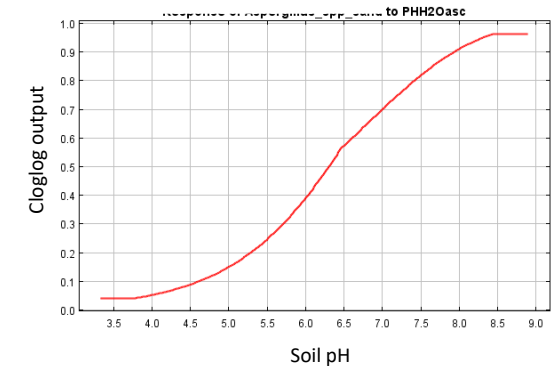
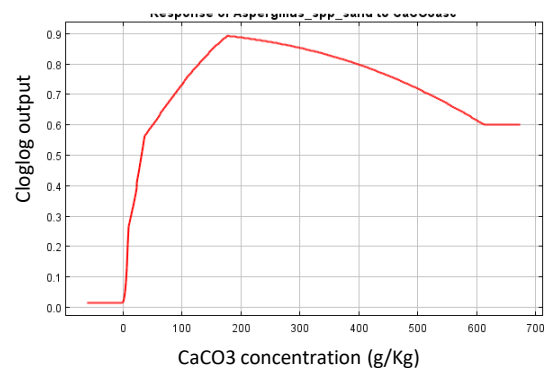
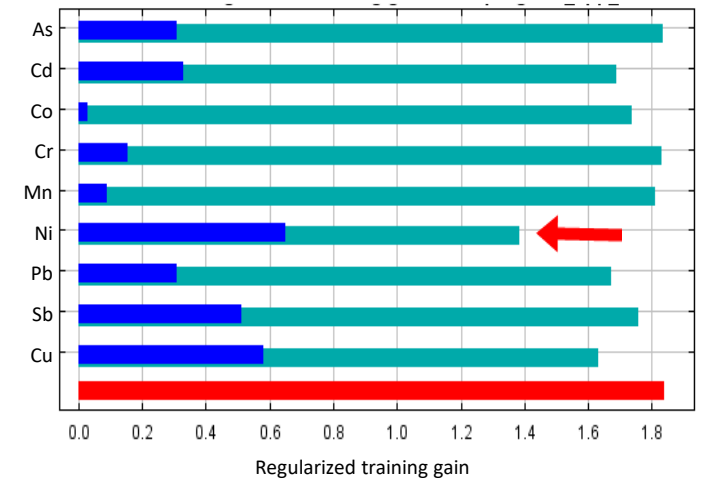
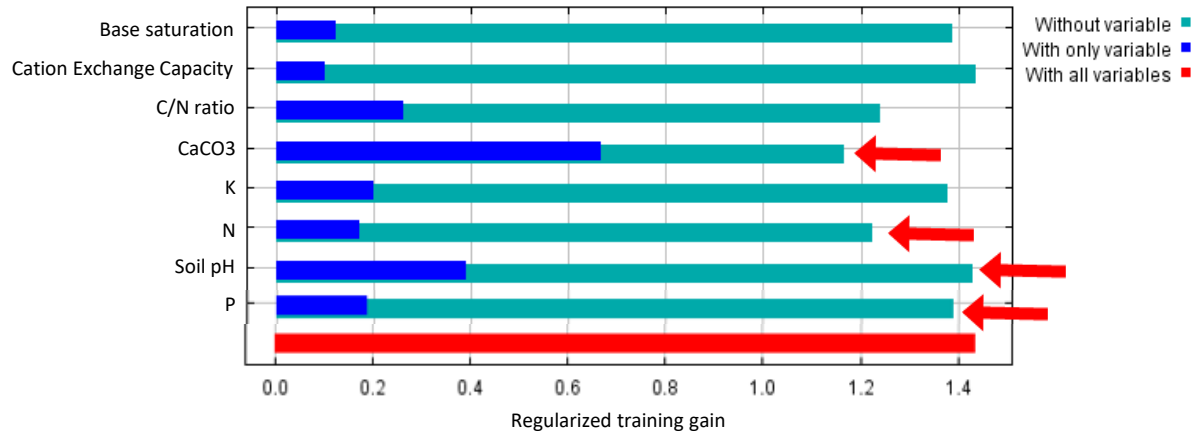


*Aspergillus spp.* in water

*Aspergillus spp.*



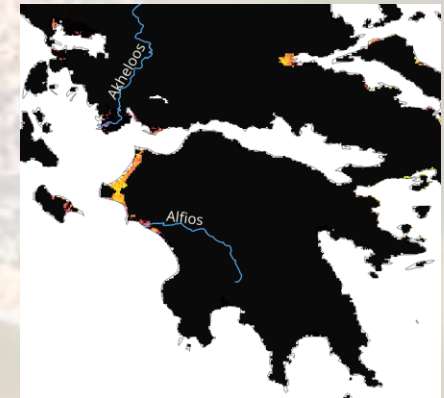
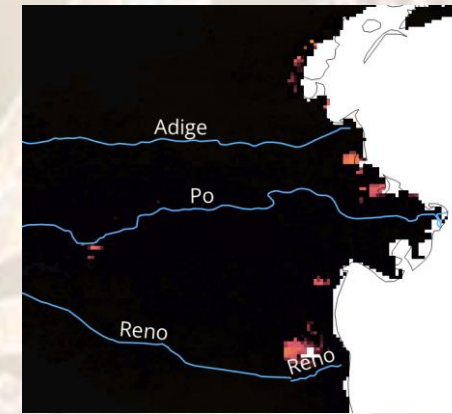
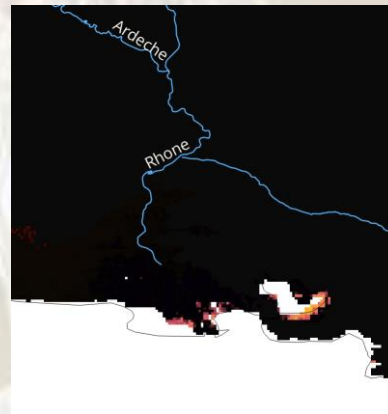
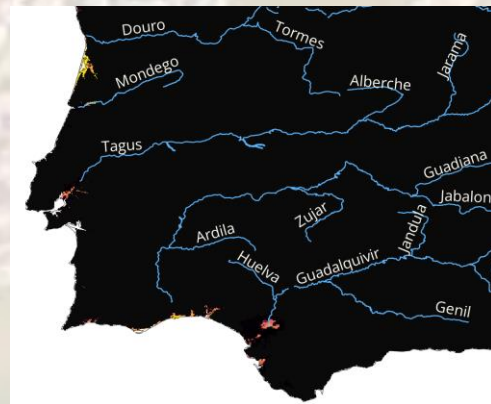
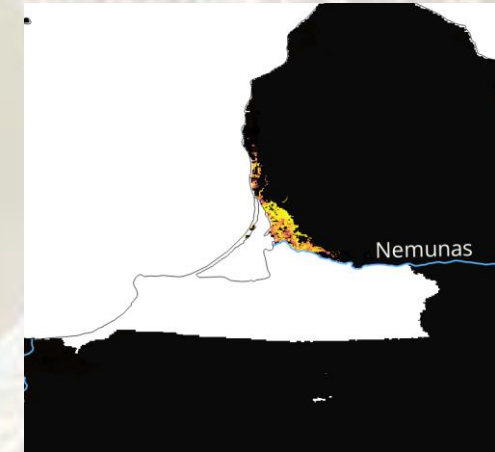
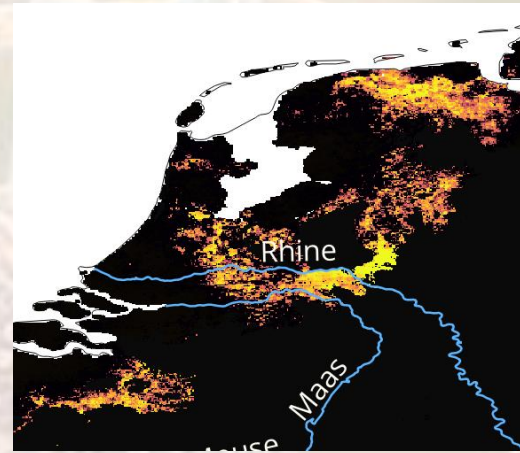
Aspergillus spp. in sand vs. soil properties



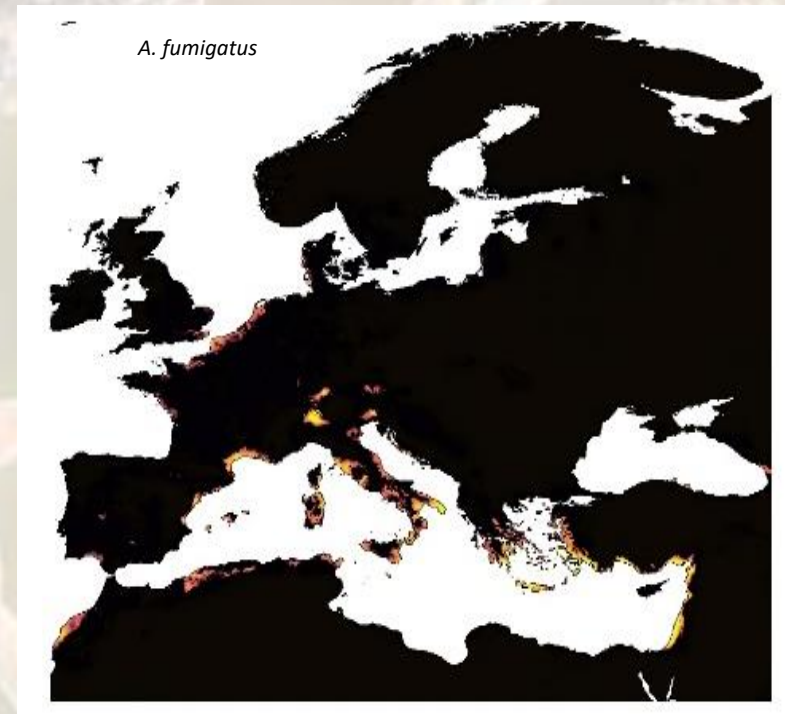
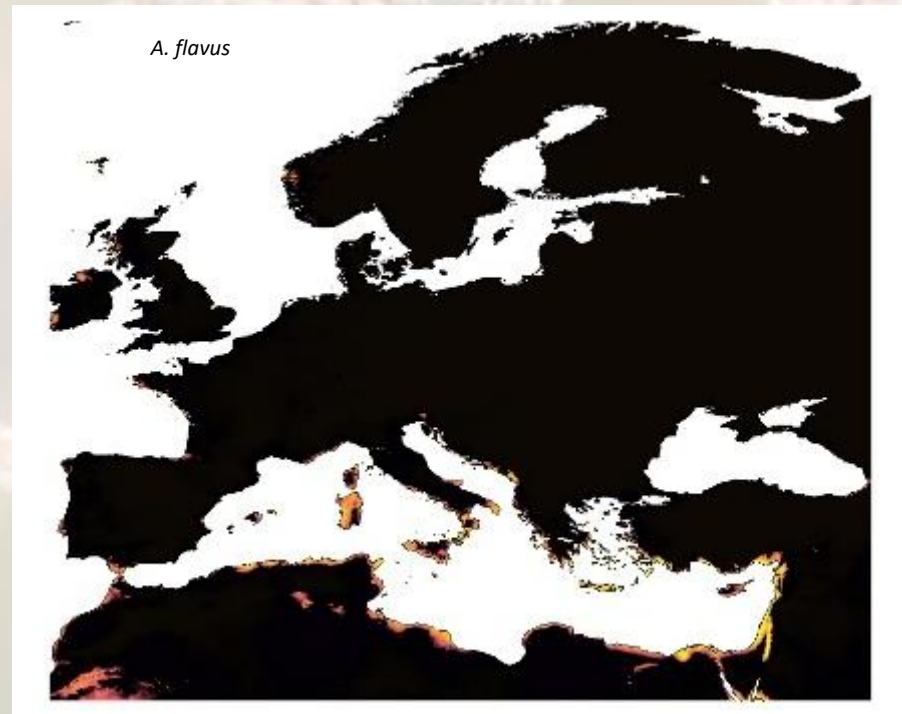
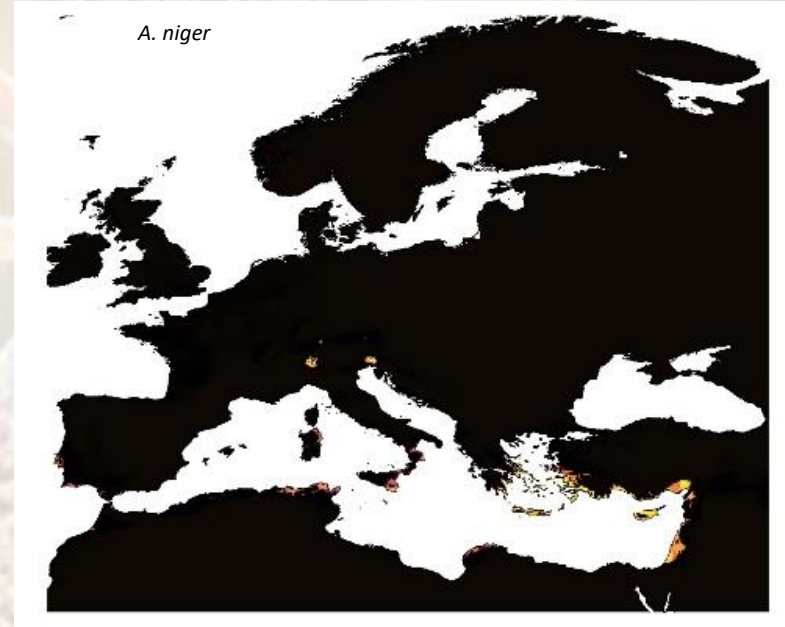
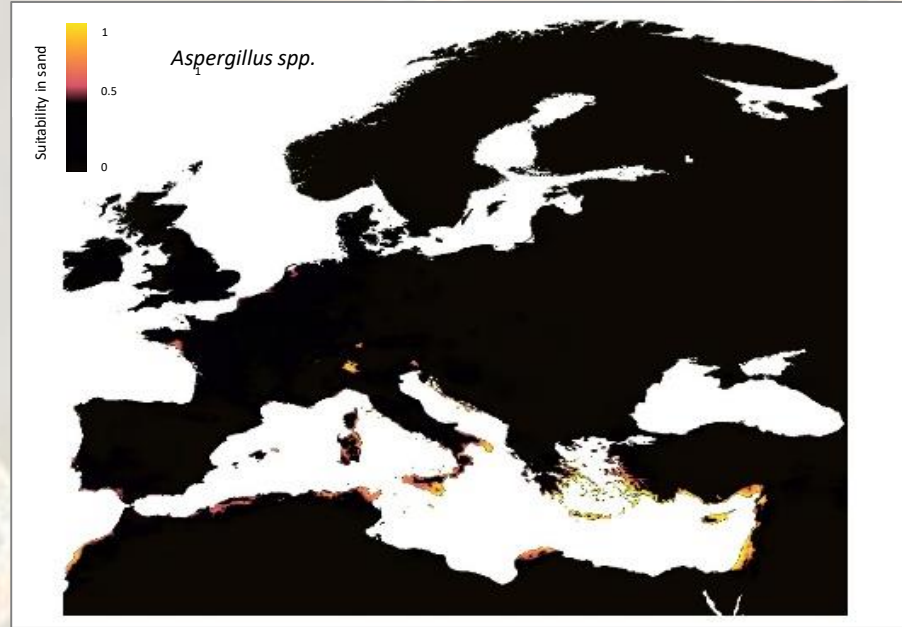


# *Aspergillus* spp. vs. heavy metals

(Ni, Co, Sb, Cu, Pb, Cd, Cr, Mn, As)



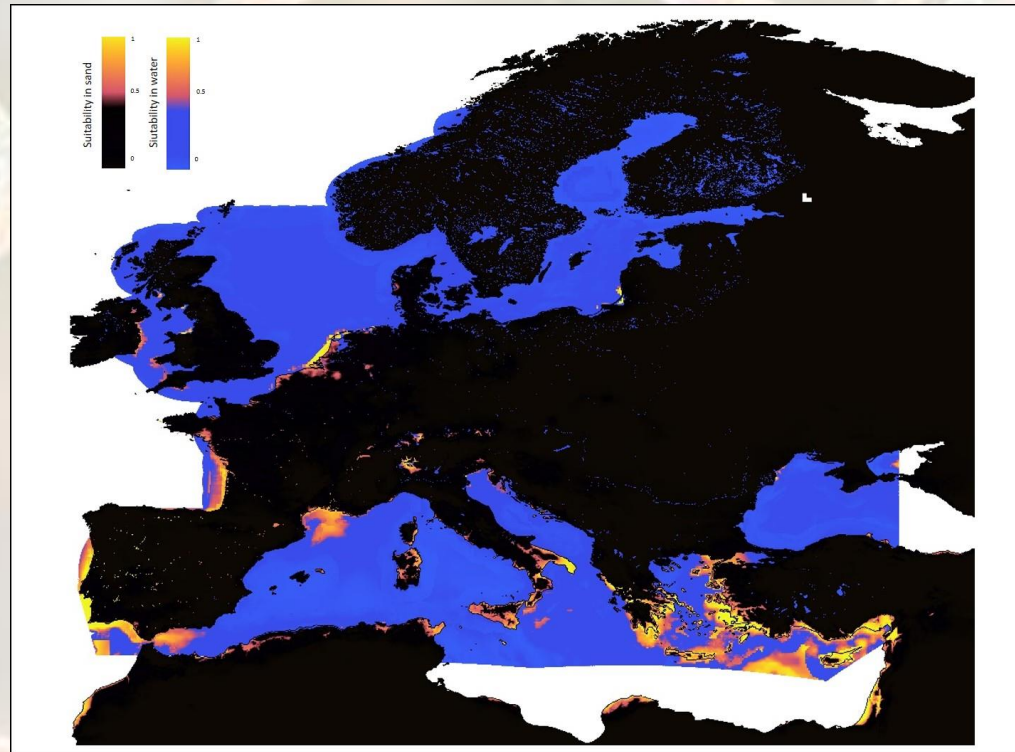
Distribution at the mouths of the main European rivers



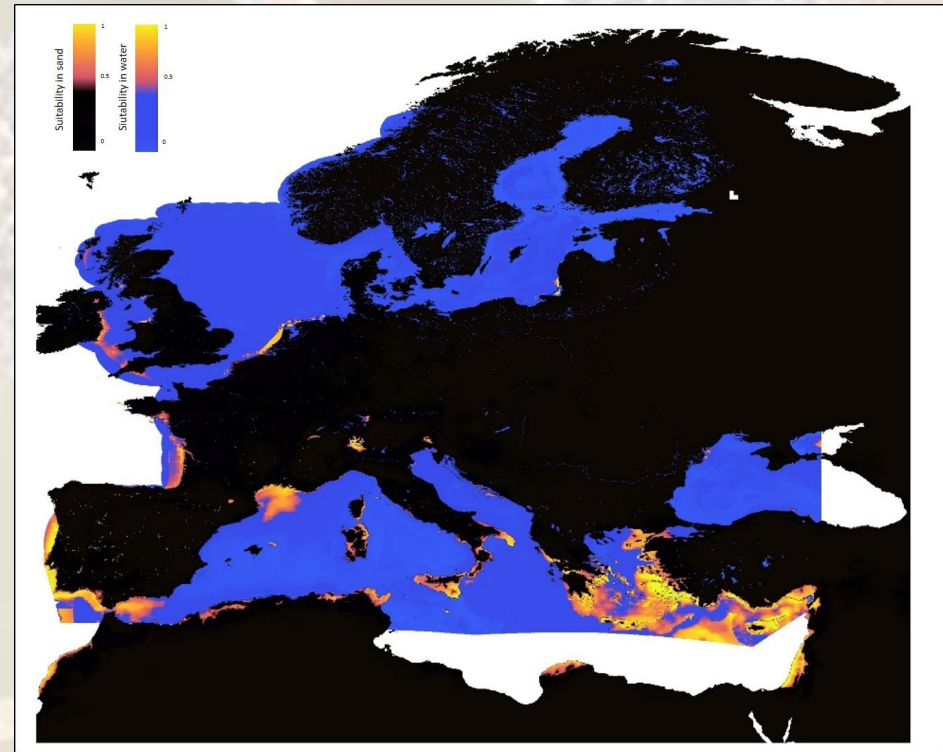
*Aspergillus spp.* model vs. species-specific models



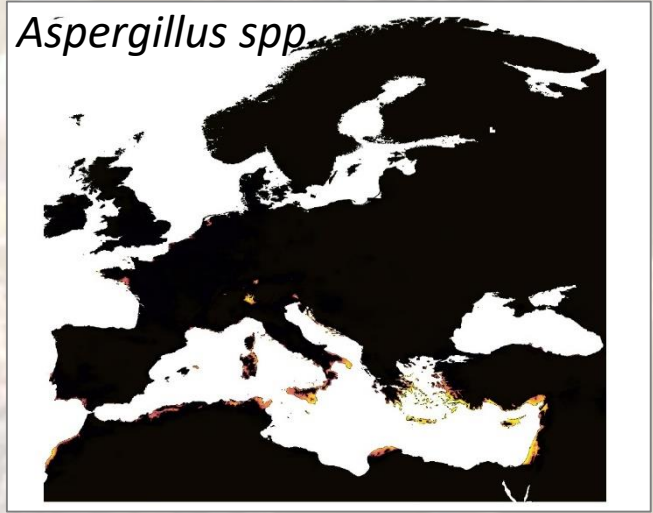
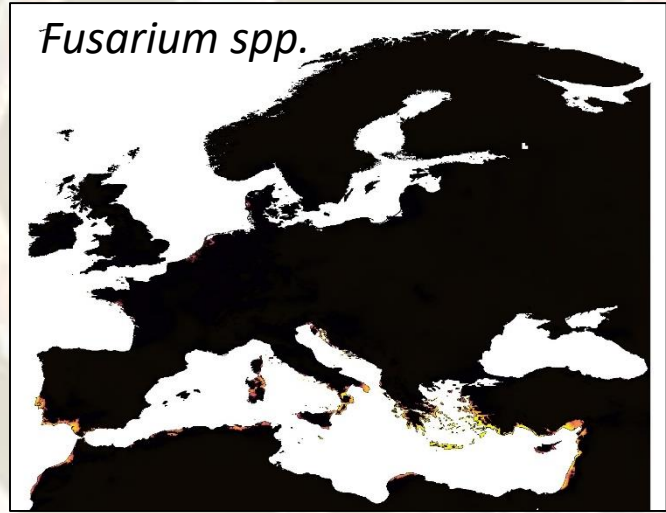
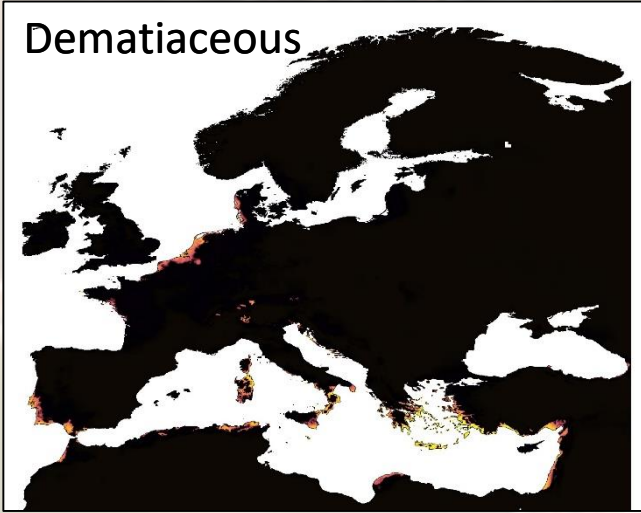
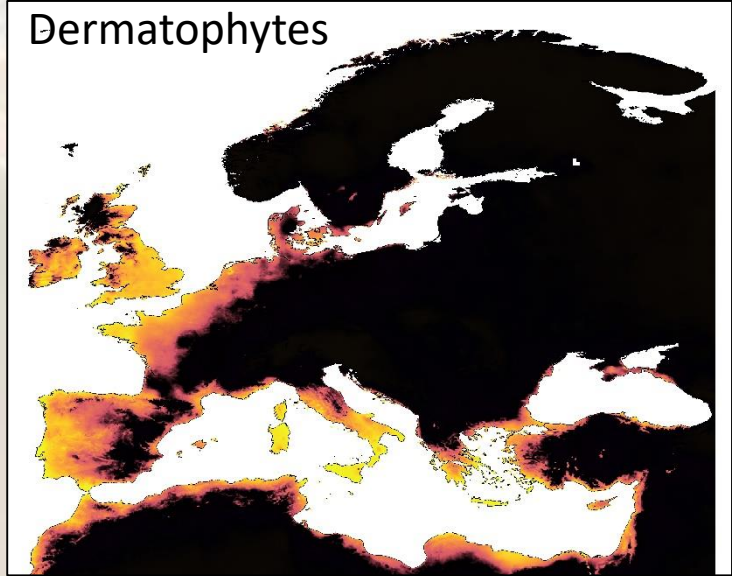
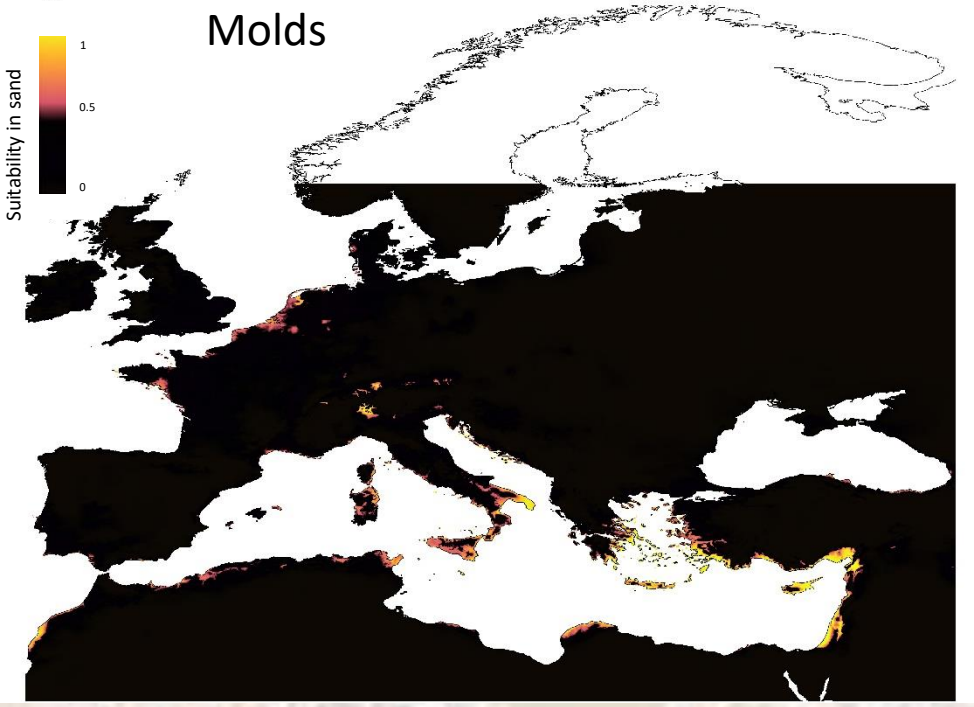
All molds



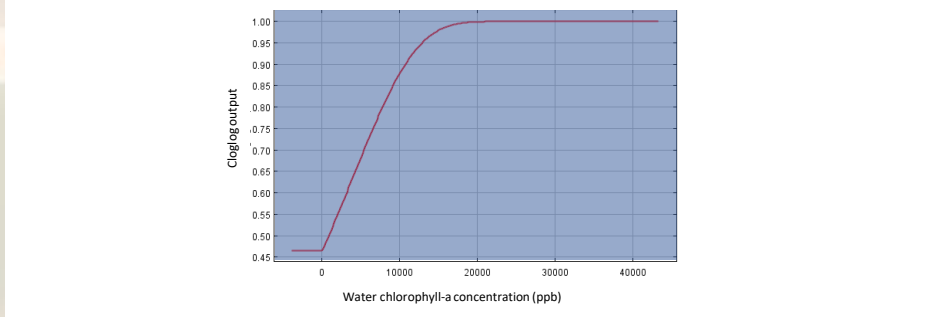
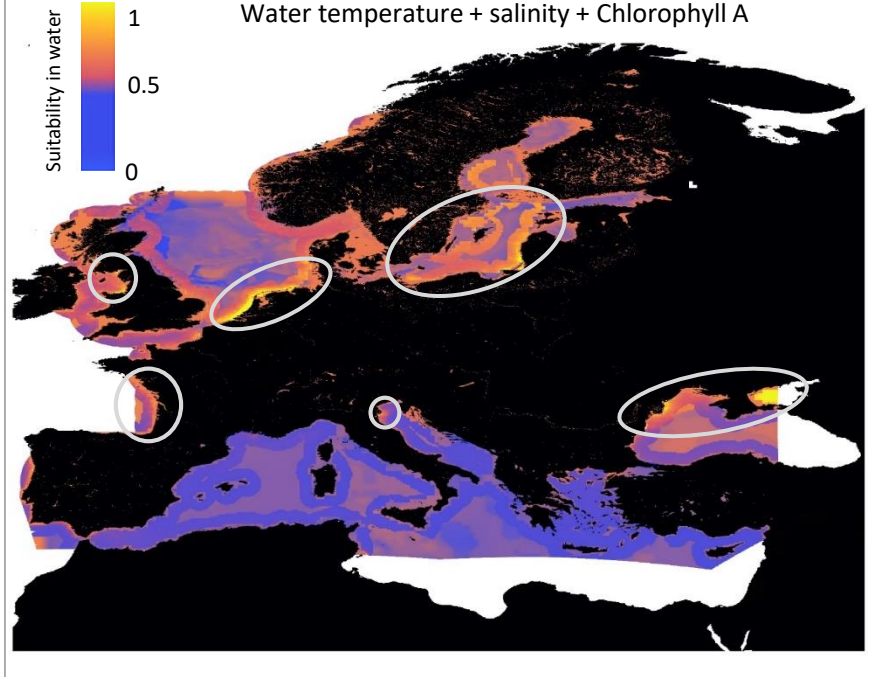
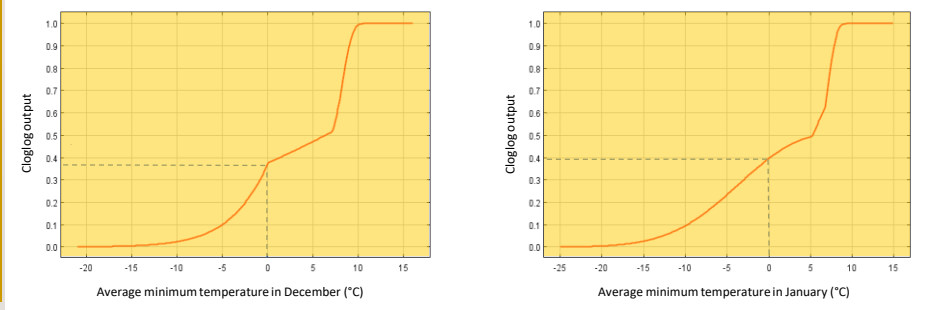
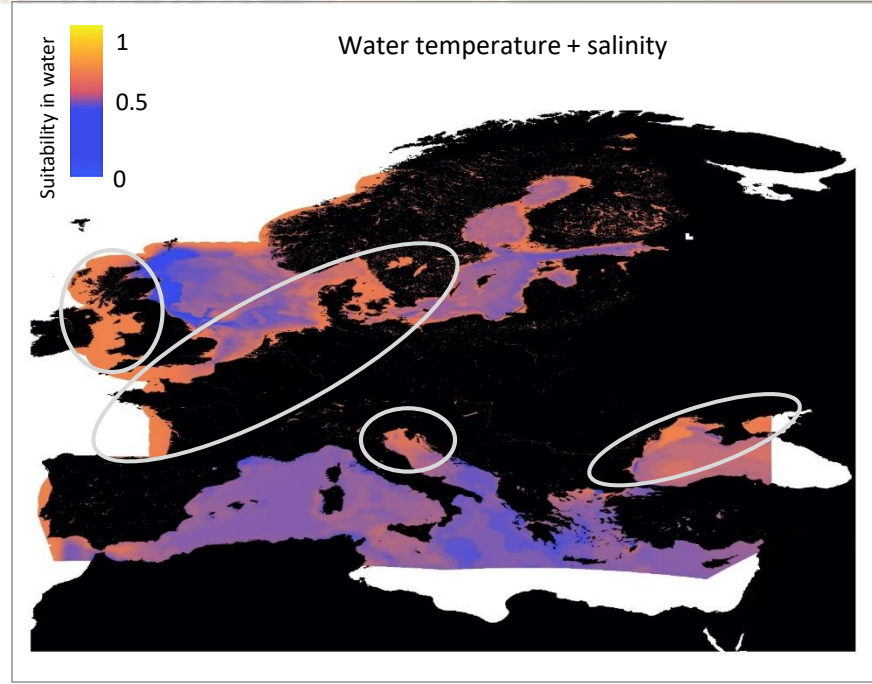
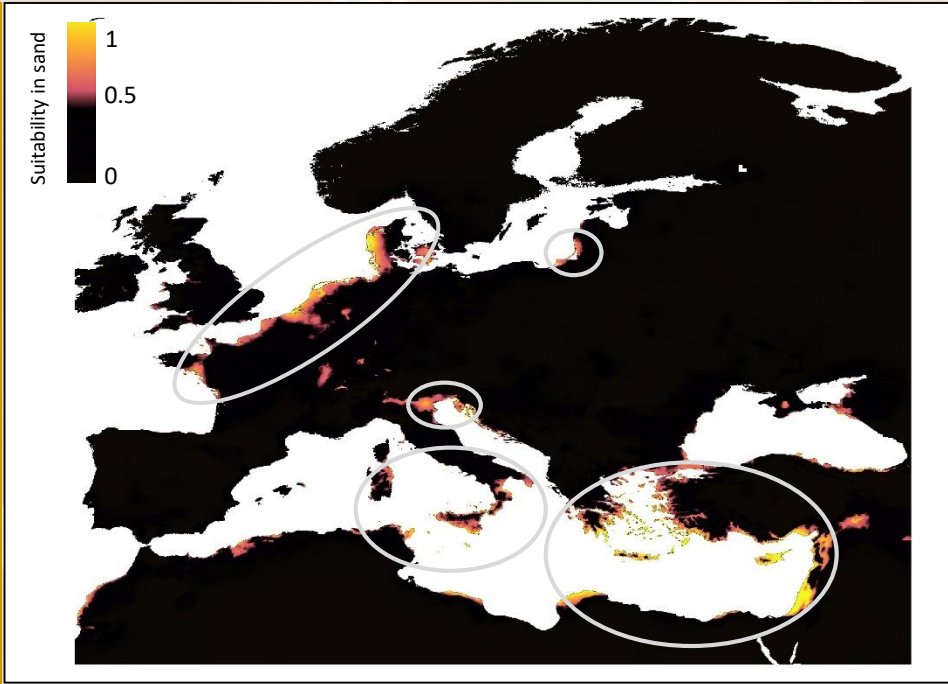
*Aspergillus* spp.







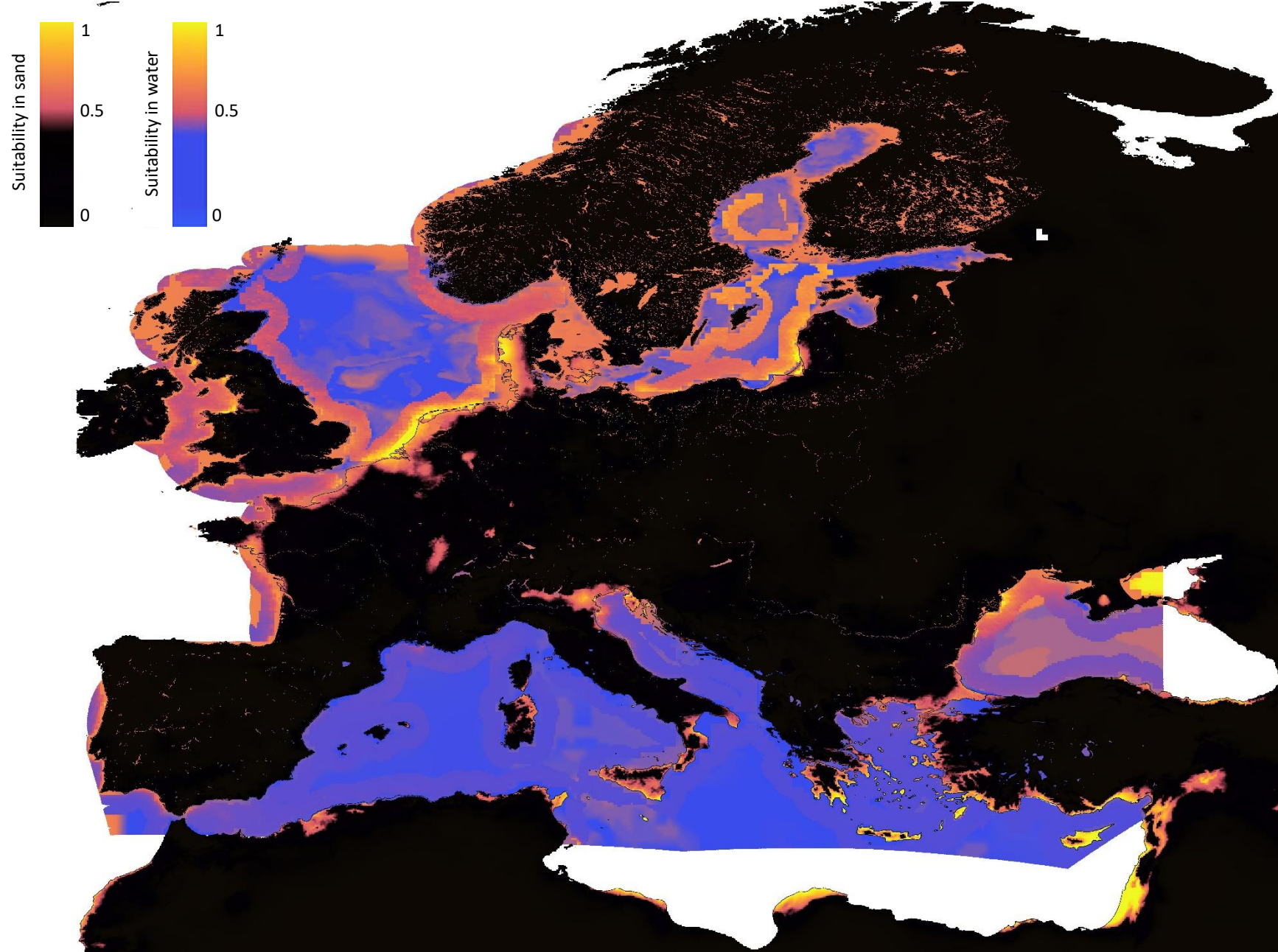
*Candida* spp. in sand



*Candida* spp. In water



*Candida* spp.



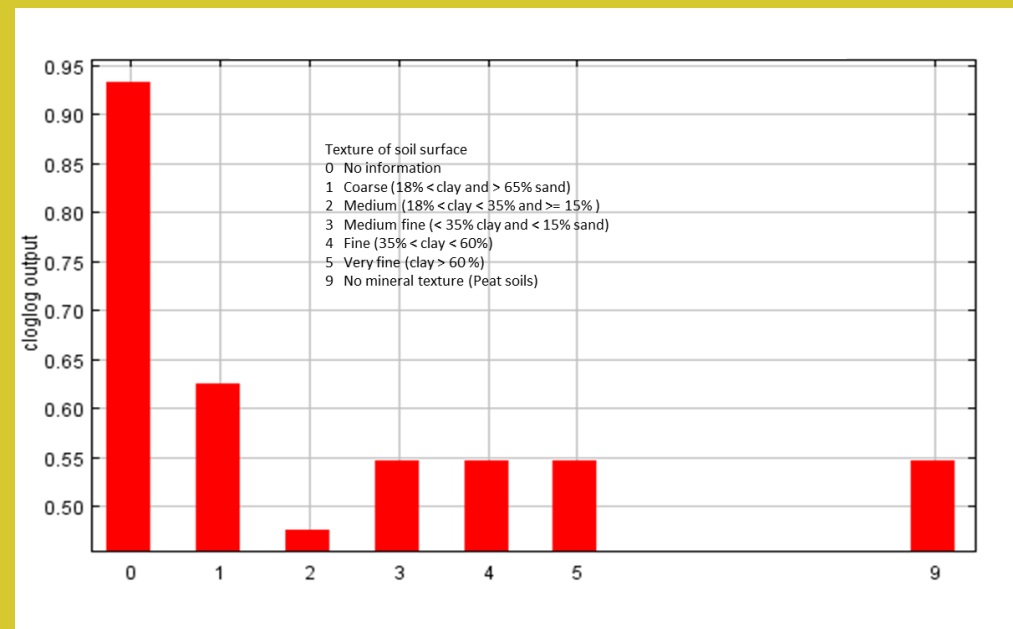


## *Candida spp. vs. soil properties*

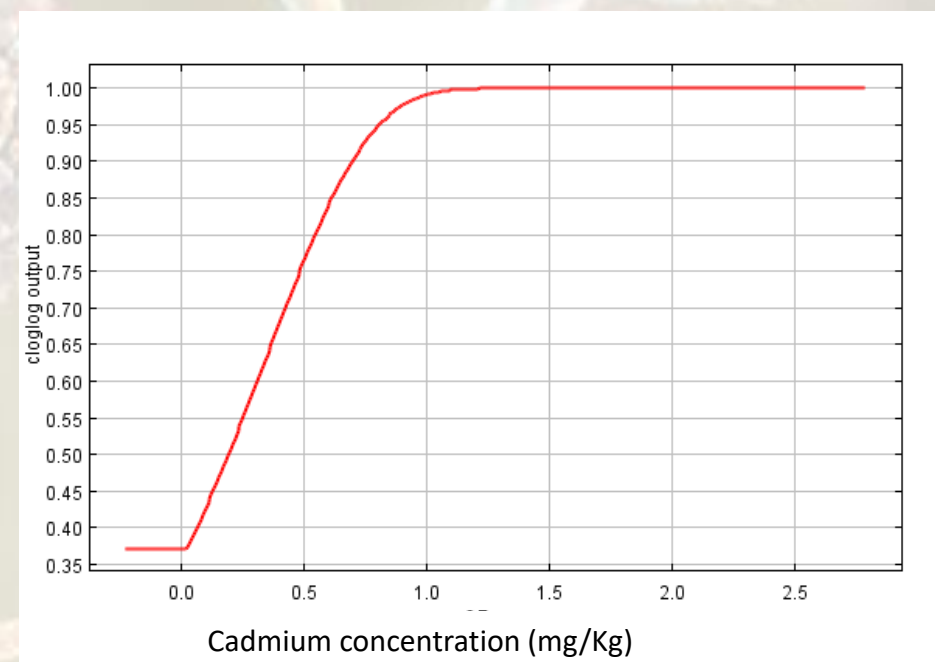
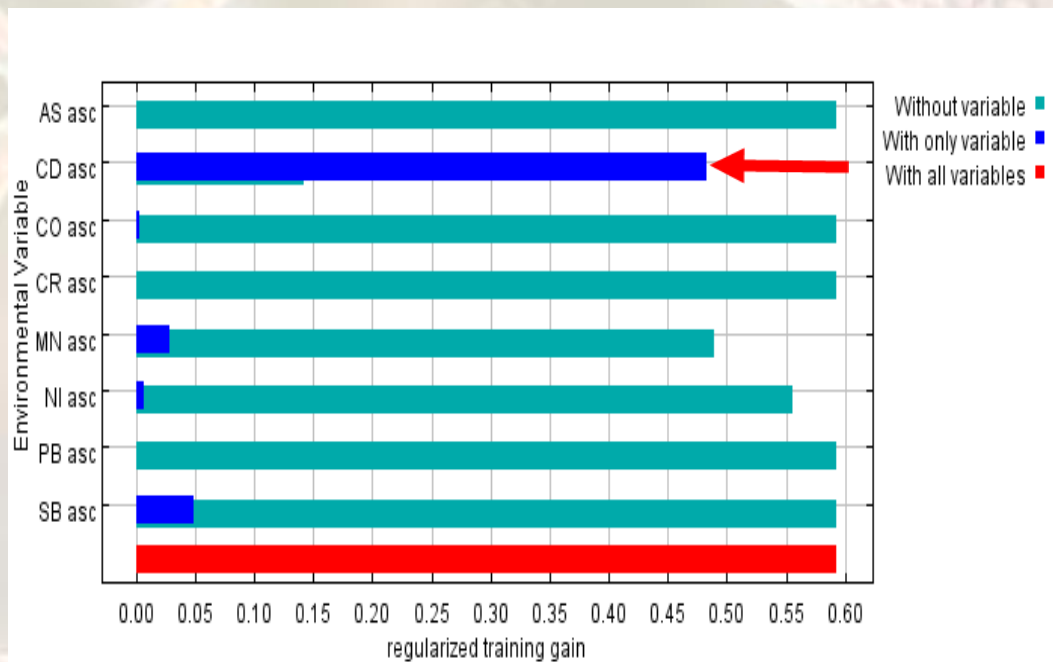
### *Soil chemical properties*

Analysis using layers with soil chemical properties was not possible due to the scarce number of occurrence points falling in the raster area

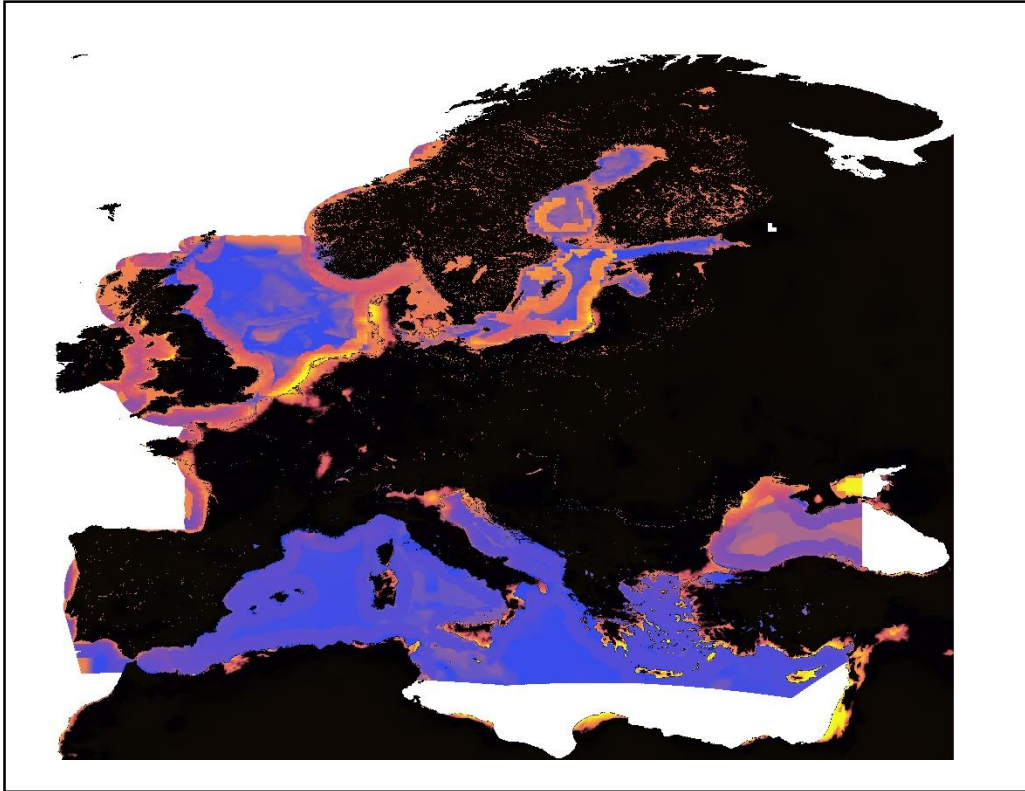
### *Soil physical properties*



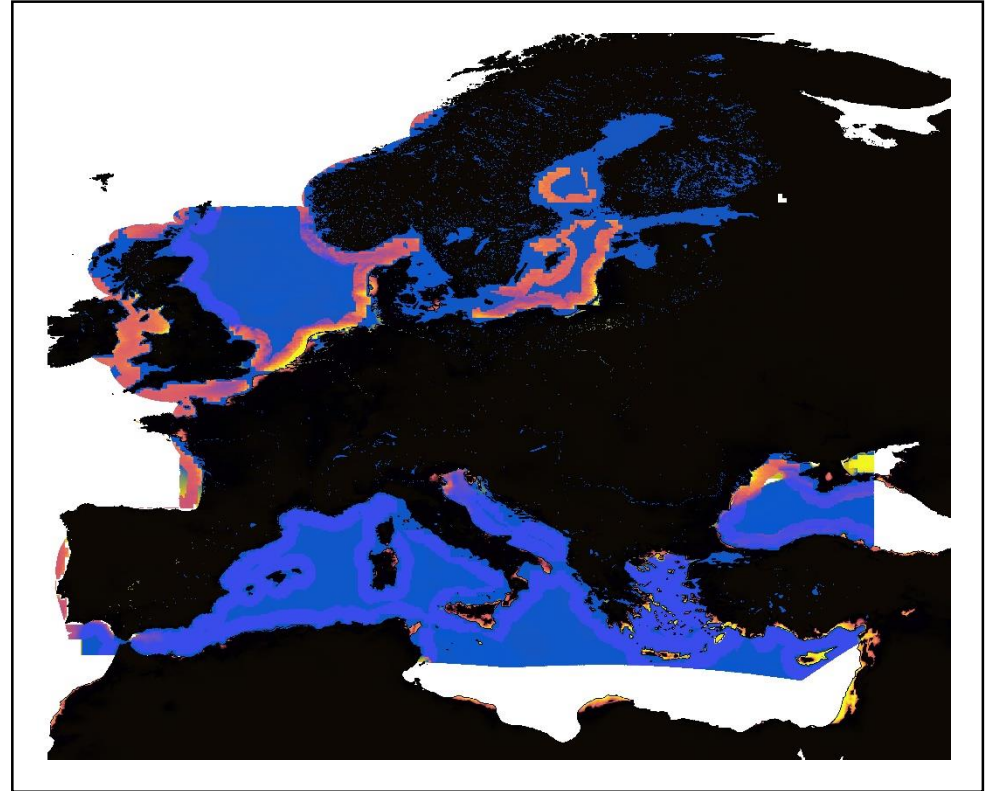
# *Candida* spp. affinity to cadmium



***Candida* spp. vs. climatic and water features**

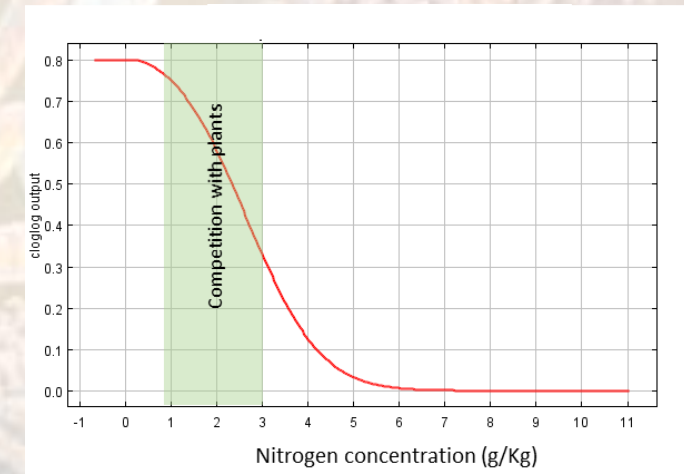
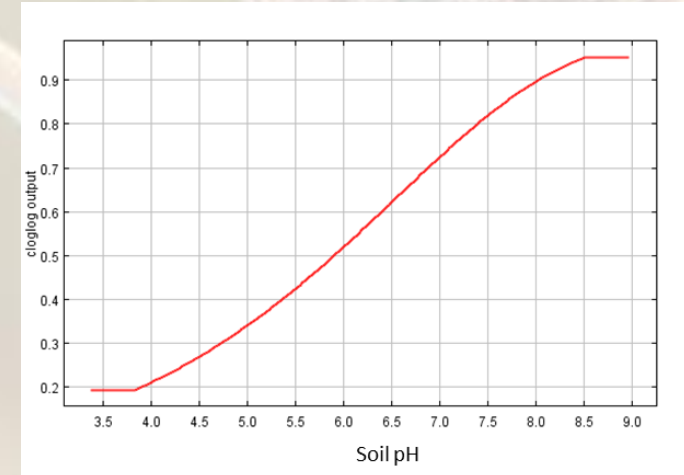
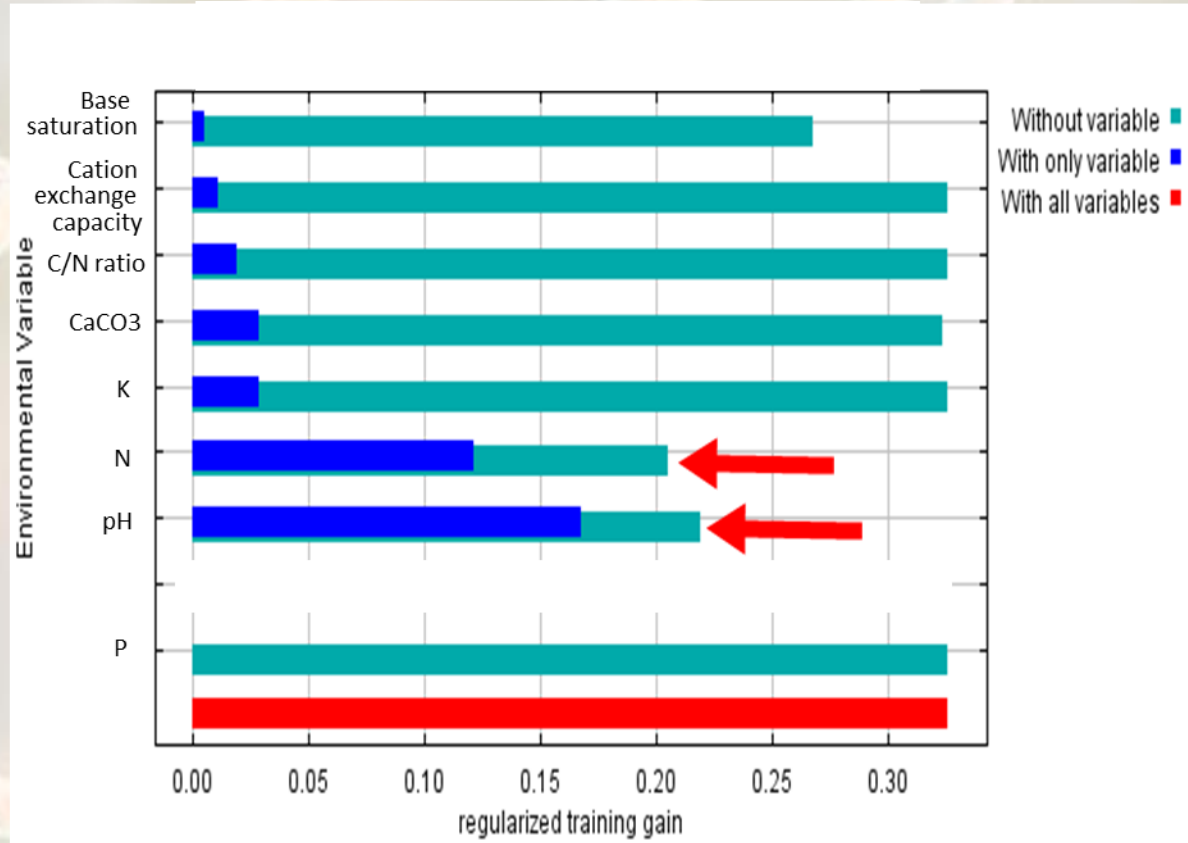


**Yeasts vs. climatic and water features**

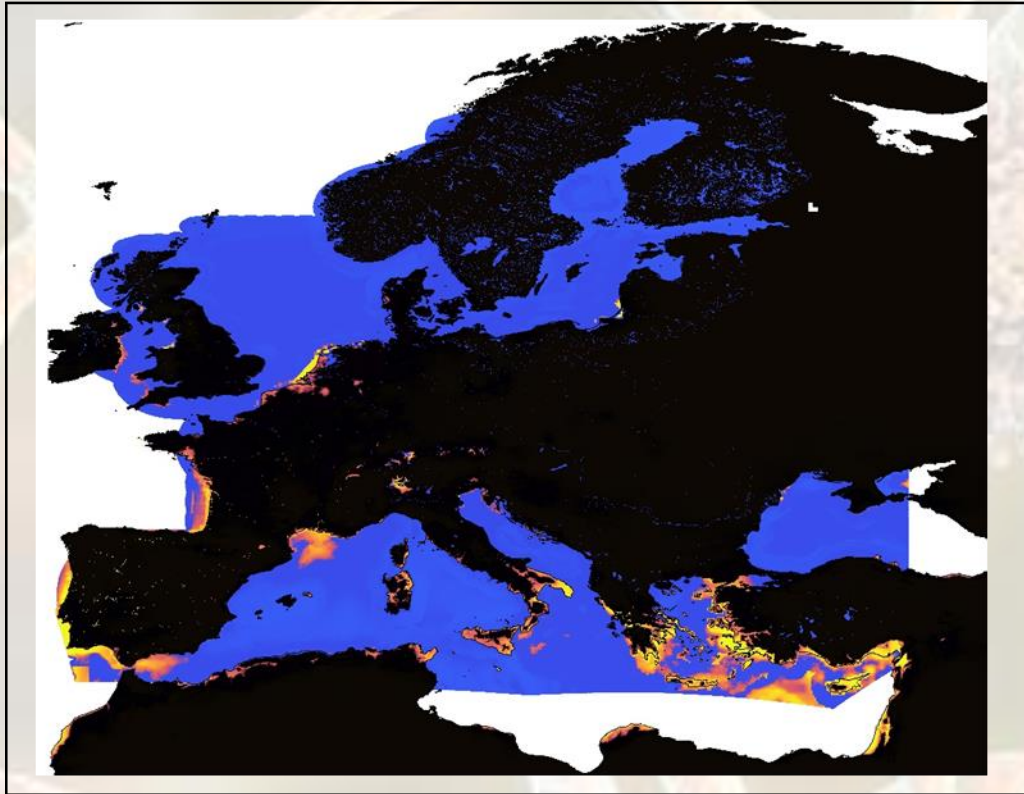




# Yeasts vs. soil properties

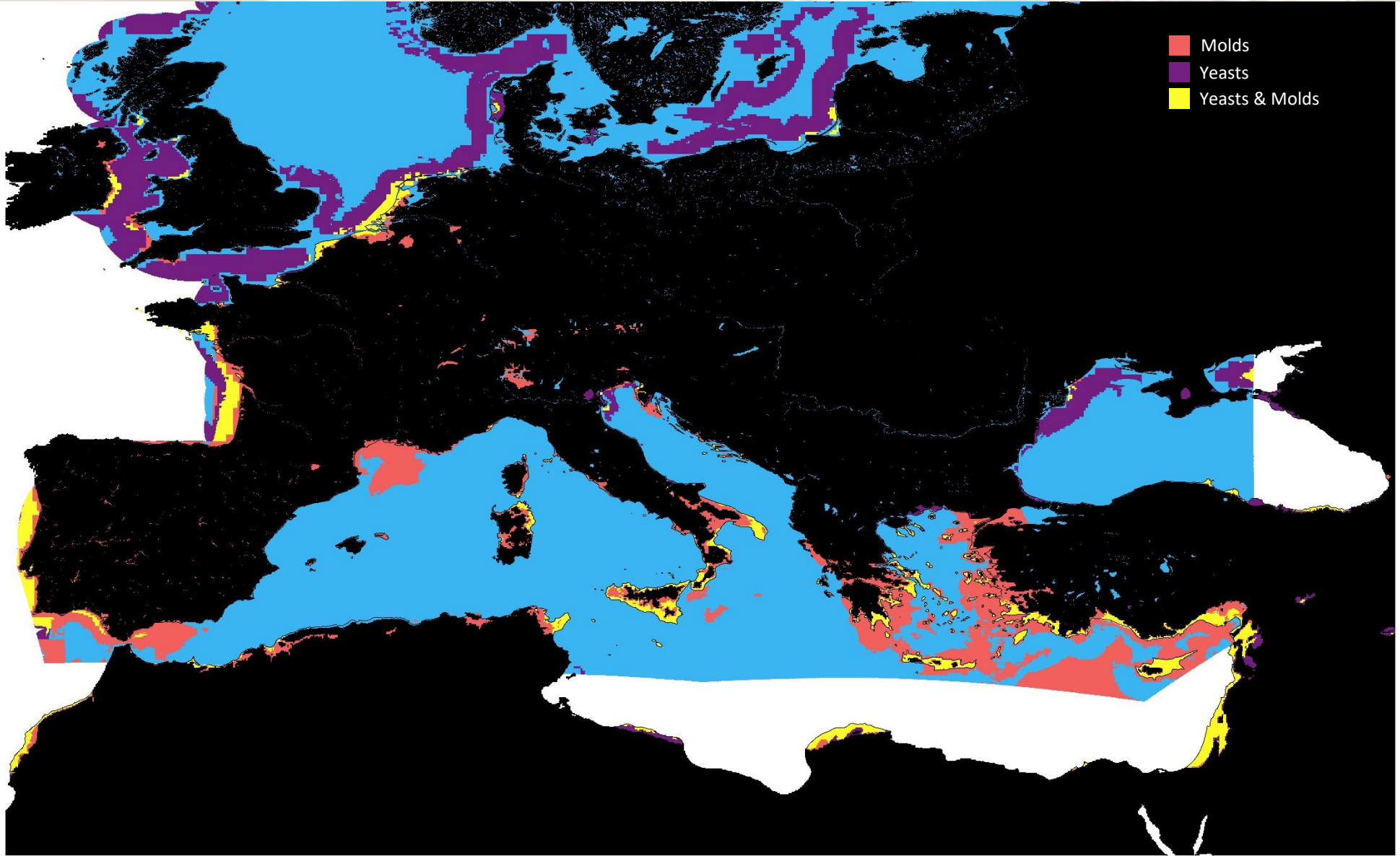


Molds vs. climatic and water features

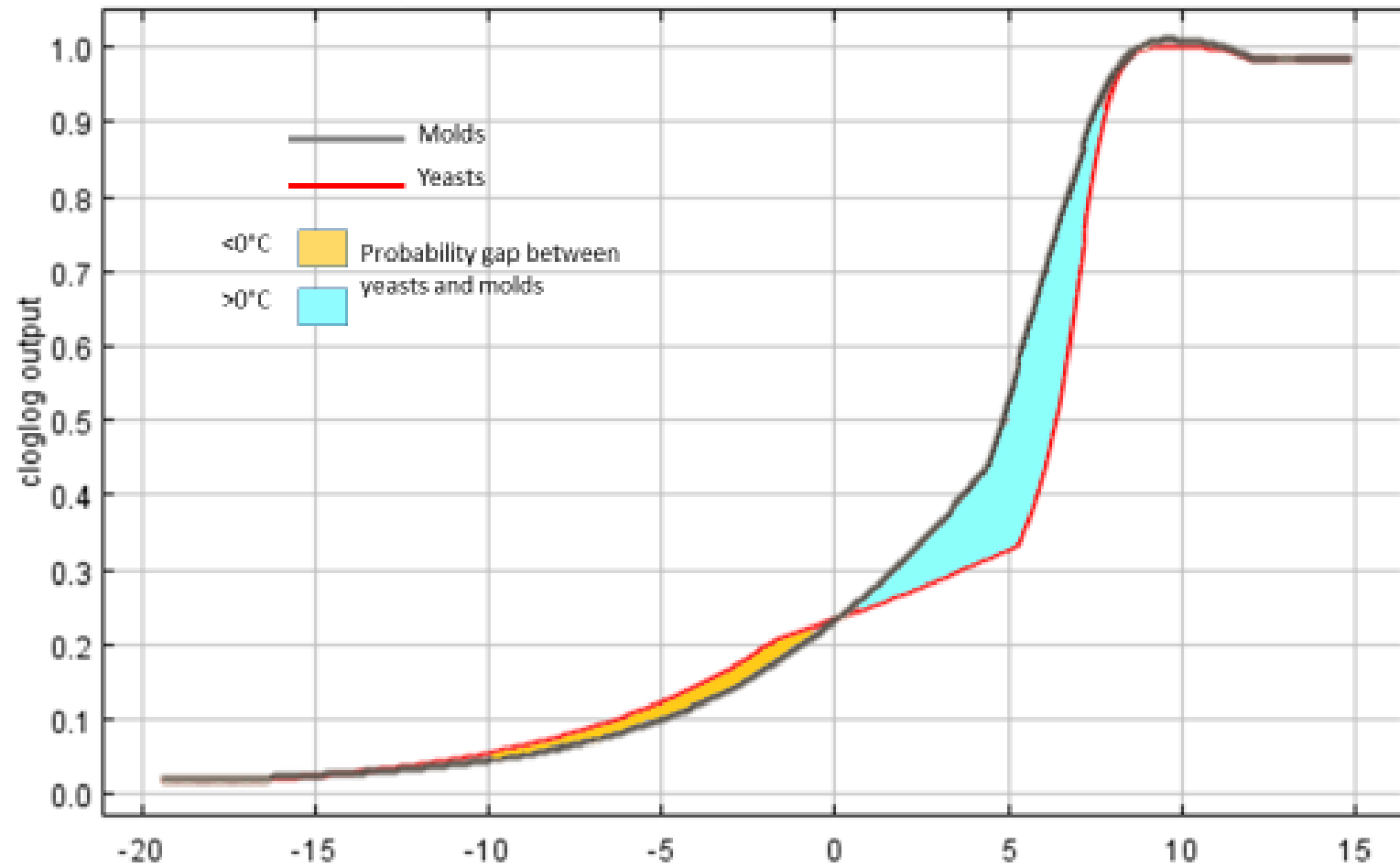


Yeasts vs. climatic and water features









Average minimum temperature (°C) in January (2011-2018)

# Climatic conditions determining spatial distribution of molds and yeasts in sand

## MOLDS

### *Mild winters*

Minimum temperature above 0 °C

Spores germination inhibited at low temperatures



Wide surface exposed to environment

Hyphal sensitivity to freezing

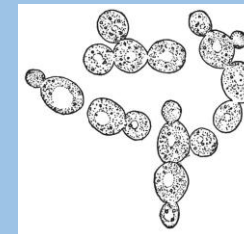
Hyphal growth inhibited at low temperatures but rapid at warm temperatures

## YEASTS

### *Mild winters*

Tolerant to minimum temperature below 0 °C

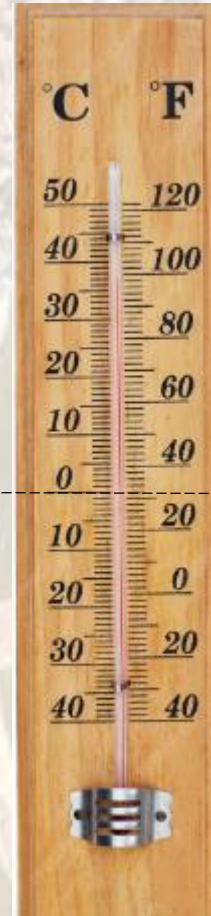
Limited surface exposed to environment



A lot of yeasts are psychrophile

Reproduction by budding

Colonies with internal yeasts cells protected by the environment



# Water features determining spatial distribution of molds and yeasts in water

## MOLDS

### *Warm waters*

Average water temperature above 18 °C

Spores germination inhibited at low temperatures



Rapid loss of heat due to a wide surface exposed to water environment

Hyphal growth rapid at warm temperatures

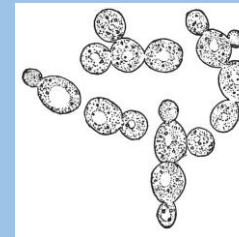
Superficial growth

## YEASTS

### *All waters*

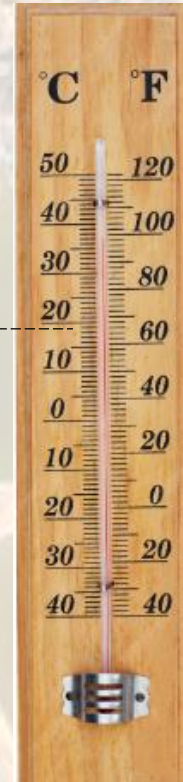
No correlation to water temperature

Limited surface exposed to water environment



Reproduction by budding

Underwater growth





# Chemical properties of soil determining spatial distribution of molds and yeasts

## MOLDS

High  $\text{CaCO}_3$  concentration, basic pH, low concentration of nitrogen, low or high concentration of phosphorus

High efficiency of hyphae in the uptake of low concentration nutrients



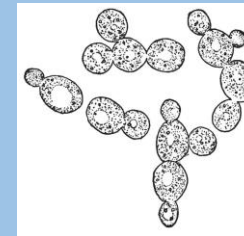
High basic pH advantages molds against bacteria

Low concentration of nutrients advantages molds against plants

Calcium uptake is important for hyphal growth process

## YEASTS

Basic pH, low concentration of nitrogen



High basic pH advantages yeasts against bacteria

Low concentration of nutrients advantages yeasts against plants

# Physical properties of soil determining spatial distribution of molds and yeasts

## MOLDS

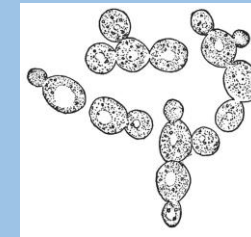
Medium or medium-fine soil texture

Medium soil texture advantages molds which are very efficient to uptake water with hyphae



## YEASTS

All soil textures



Yeasts are able to grow on all surfaces often forming biofilms

# Heavy metals concentration in soil determining spatial distribution of molds and yeasts

## MOLDS

High concentration of nickel

Ability to precipitate nickel



Appl Microbiol Biotechnol (2002) 59:382–388  
DOI 10.1007/s00253-002-1020-x

ORIGINAL PAPER

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D.S. Clark · J.D. Keasling

### Nickel accumulation and nickel oxalate precipitation by *Aspergillus niger*

Received: 3 October 2001 / Revised: 18 March 2002 / Accepted: 2 April 2002 / Published online: 8 May 2002  
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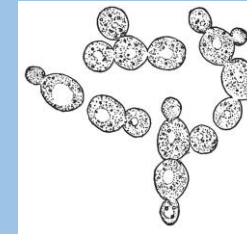
**Abstract** A strain of *Aspergillus niger* isolated from a metal-contaminated soil was able to grow in the presence of cadmium, chromium, cobalt, copper, and unusually high levels of nickel on solid (8.0 mM) and in liquid (6.5 mM) media. This fungus removed >98% of the nickel from liquid medium after 100 h of growth but did not remove the other metals, as determined by inductively coupled plasma spectroscopy. Experiments with non-growing, live fungal biomass showed that nickel removal was not due to biosorption alone, as little nickel was bound to the biomass at the pH values tested. Further-

Once released to the environment nickel readily forms complexes with many ligands, making it more mobile than most heavy metals. While nickel is an essential element at low concentrations for many organisms, it is toxic at higher concentrations. As little as 0.34 μM Ni inhibits the growth of *Escherichia coli* (Abelson and Aldous 1950), whereas concentrations between 0.1 and 1.6 mM have been shown to inhibit the growth of various fungi (Adiga et al. 1961; Gadd 1993; Kumar et al. 1992; Mohan and Sastry 1984). Because of its toxicity, drinking water standards mandate nickel concentrations below

## YEASTS

High concentration of cadmium

Efficient uptake for cadmium



### Cadmium Uptake by Yeast, *Candida tropicalis*, Isolated from Industrial Effluents and Its Potential Use in Wastewater Clean-Up Operations

Abdul Rehman · Muhammad Sohail Anjum

Received: 6 November 2008 / Accepted: 23 March 2009 / Published online: 4 April 2009  
© Springer Science + Business Media B.V. 2009

**Abstract** This study is aimed at assessing the ability of metal-resistant yeast, *Candida tropicalis*, to uptake cadmium from the liquid medium. The minimum inhibitory concentration of Cd<sup>2+</sup> against *C. tropicalis* was 2,800 mg L<sup>-1</sup>. The yeast also showed tolerance towards Zn<sup>2+</sup> (3,100 mg L<sup>-1</sup>), Ni<sup>2+</sup> (3,000 mg L<sup>-1</sup>), Hg<sup>2+</sup> (2,400 mg L<sup>-1</sup>), Cu<sup>2+</sup> (2,300 mg L<sup>-1</sup>), Cd<sup>2+</sup> (2,000 mg L<sup>-1</sup>), and Pb<sup>2+</sup> (1,200 mg L<sup>-1</sup>). The yeast isolate showed typical growth curves, but low specific rate of growth was observed in the presence of cadmium. The yeast isolate showed optimum growth at 30°C and pH 7. The metal processing ability of the isolate was determined in a medium containing 100 mg L<sup>-1</sup> of Cd<sup>2+</sup>. *C. tropicalis* could decline Cd<sup>2+</sup> 57%, 69%, and 80% from the medium after 48, 96, and 144 h, respectively. *C. tropicalis* was also able to remove Cd<sup>2+</sup> 56% and 72% from the wastewater after 6 and 12 days, respectively. Cd produced an increase in glutathione (GSH) and non-protein thiol levels by 146.15% and 59.67% at 100 mg L<sup>-1</sup> concentration, respectively. Metal tolerance and accumulation together with changes in the GSH status and non-protein thiols under Cd exposure were studied in *C. tropicalis*.

**Keywords** Cadmium · Glutathione · Metabolic inhibitors · *Candida tropicalis* · Bioremediation

#### 1 Introduction

Heavy metal contamination due to natural and anthropogenic sources is a global environmental concern. Release of heavy metal without proper treatment poses a serious threat to public health because of its persistence, biomagnification, and accumulation in the food chain. Most of the heavy metals like chromium, cadmium, lead, mercury, and copper are highly toxic for almost all the living organisms. The health of people living near the dumping grounds is also being constantly affected by the metal contamination of food and drinking water. A number of studies have elaborated the effects of heavy metals on animals, plants, and human health (Unger and Roesijadi 1996; Chipasa 2003; Chisti 2004; Coeurdassier et al. 2004).

Cadmium (Cd) is a heavy metal contaminant in the environment. It is extensively used in the industry for a number of applications, including electroplating, production of pigments, and stabilizing plastic



## Conclusions

On the basis of the species distribution analysis carried out using the data collected during the Mycosands study, the major differences were observed between *Aspergillus* spp. and *Candida* spp. groups, that corresponded to the same differences observed between molds and yeasts. Therefore the observed differences in distribution seem to be determined by characteristics that distinguish molds from yeasts such as morphology and ways of reproduction and dispersion.

This study provides indications about parameters and limits useful to determine the quality of beaches and recreational areas along shores

This study provides indications about the geographical areas where beaches could be potentially exposed to the presence of fungal pathogens

# THANKS!

Science of the Total Environment 859 (2023) 160132



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



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