

Original paper

Trapping of marine-derived fungi on wooden baits to select species potentially usable in mycoremediation

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DOI: [10.6092/issn.2531-7342/10769](https://doi.org/10.6092/issn.2531-7342/10769)**Abstract**

Thanks to their enzymatic activities fungi are used in many studies and applications for remediation purposes in order to degrade organic contaminants such as hydrocarbons. In particular, ligninolytic marine-derived fungi can be applied to biodegradation, since they already play an active role in the biogeochemical cycles of marine substrates. To select potentially usable species in mycoremediation, the occurrence of ligninolytic marine-derived fungi was investigated in the water mass of a commercial port (Port of Genoa, north-western Italy) by exposing baits of different wood-types in the water column. In total, 437 microfungal strains were found belonging to 12 genera and 23 species; the most common fungal species were *Penicillium solitum* and *Galactomyces geotrichum*, and differences in the investigated water column were highlighted.

Keywordswooden baits; marine-derived fungi; fungal diversity; marine port environment

Introduction

Organic pollution is one of the major ecological problems in the world (Revenge et al., 2000; Latimer and Zheng, 2003). Several studies have shown how the organic waste products are extremely persistent in marine environment and can be harmful to both animal and human health (Walker and Livingstone, 1992; Naso et al., 2005; Nfon et al., 2008; Farrington and Takada, 2014; Seltenrich, 2015). Among the organic pollutants, hydrocarbons are well known to alter the immune system, to determine carcinogenic action and to induce genetic mutations (Man et al., 2013). In marine environment, hydrocarbon pollution is mostly due to accidental or intentional spills, or to the fallout and consequent deposition of airborne polluting substances (Singh, 2006; Davis et al., 2018; Davis et al., 2019). Moreover, pollution from polycyclic aromatic hydrocarbons (PAHs) is very common in ports due to industrial activities, ship traffic, street run-off water and accidental spills from fuel distributors.

To solve this ecological problem, bioremediation techniques (such as phytoremediation and microbial remediation) have received during the last two decades wide acclaim becoming promising alternatives to the traditional techniques because they are eco-friendly, they require little energy and may detoxify persistent organic compounds (Singh, 2006). In this context, degradation of PAHs can be induced by the action of extremophile organisms, such as bacteria and fungi (Boonchan et al.,

2000; Cerniglia and Sutherland, 2010; Kostka et al., 2011). In fact, several studies have shown how some fungal species can adapt to adverse marine environmental conditions (for both abiotic factors - e.g. high salinity and hydrostatic pressure - and nutrient deficiency) which would be limiting for most living organisms (Gunde-Cimerman et al., 2005; Gadd, 2007; Gonçalves et al., 2013).

Fungi from marine environments, in particular the ligninolytic marine-derived fungi, play a central and active role in the biogeochemical cycles of hydrocarbon pollutants. These fungal strains were originally terrestrial that have been overtime adapted to the marine environment, developing different genetic makeup and gene expression (Pang et al., 2016). In recent years, many mycological researches have investigated the microfungal communities of environments contaminated by organic substances (Rydin et al., 1997; Schmit and Mueller, 2007; Pautasso and Zotti, 2009), but few studies in marine environment on ligninolytic marine-derived microfungal communities have been carried out. Among the first scientists who dealt with this topic, Montemartini Corte in 1979 laid wooden panels of *Fraxinus ornus* L., *Abies alba* Mill., *Ochroma pyramidale* (Cav. Ex Lam), *Fagus sylvatica* L., *Larix decidua* Mill., *Pinus pinaster* Aiton, *Populus alba* L. and *Olea europaea* L. to catch cellulolytic fungi and investigate the mycodiversity of the Portofino bay (north-western Italy). In the following years, lignicolous marine-derived fungal species have been isolated from driftwood, pilings, wooden boats, mangrove roots and tree trunks (Shearer et al., 2007; D'Souza-Ticlo et al., 2009; Liu et al., 2011; Rämä et al., 2014). These researches ranged from the surface to the depth of 1200 m (Shearer et al., 2007; Dupont et al., 2009) and from the poles to the equator (Booth, 1983; Rämä et al., 2014).

Moreover, wood-inhabited marine-derived fungi produce extracellular enzymes such as peroxidases and laccases capable of degrading or modifying lignin and complex molecules with similar chemical structure, such as hydrocarbon products and aromatic persistent environmental pollutants (Hammel, 1995; Passarini et al., 2011). Hence, thanks to their ecological plasticity and the ability to produce cellulolytic extracellular enzymes, marine-derived fungi may be used in real biotechnological processes (Kumar et al., 2011). With regards to this Cecchi et al. (2019) reported that the exploitation of fungi can prove to be as potential tool to be able to restore port sediments deriving from dredging operations (*ex situ*) or to remediate limited marine areas affected by hydrocarbon spills (*in situ*).

Therefore, starting from the previous studies (Montemartini Corte, 1979; Greco et al., 2018), our study aims i) to deepen the fungal characterisation of the marine environment of the Port of Genoa (north-western Italy), ii) to investigate the lignicolous microfungal component in the water column and iii) to isolate (from wooden panels) lignicolous marine-derived fungi potentially usable for bioremediation purposes.

Materials and methods

Study area

The study area, the Port of Genoa (Fig. 1), is the most important commercial port of the north-western Mediterranean Sea, characterised by several commercial activities such as shipyards, ferry terminals, marinas, industries (e.g. a steel mill). Several terminals are dedicated to receive containers or different materials, such as dry or liquid bulk coal and crude oil transported by oil tankers.

Many different water types finish into the port basin which includes several city streams and all the wastewater from Genova city itself (population of 600,000 units) which is directly overlooking the basin. In addition, the basin is affected daily by freshwater inflows from the mouths of the Bisagno and Polcevera torrents (<http://www.porto.genova.it>). Due to these water and discharge inputs, marine waters inside the port are often rich in nutrients, faecal coliform, chlorophyll α , metals and hydrocarbon compounds (Ruggieri et al., 2011; Cutroneo et al., 2015, 2017).

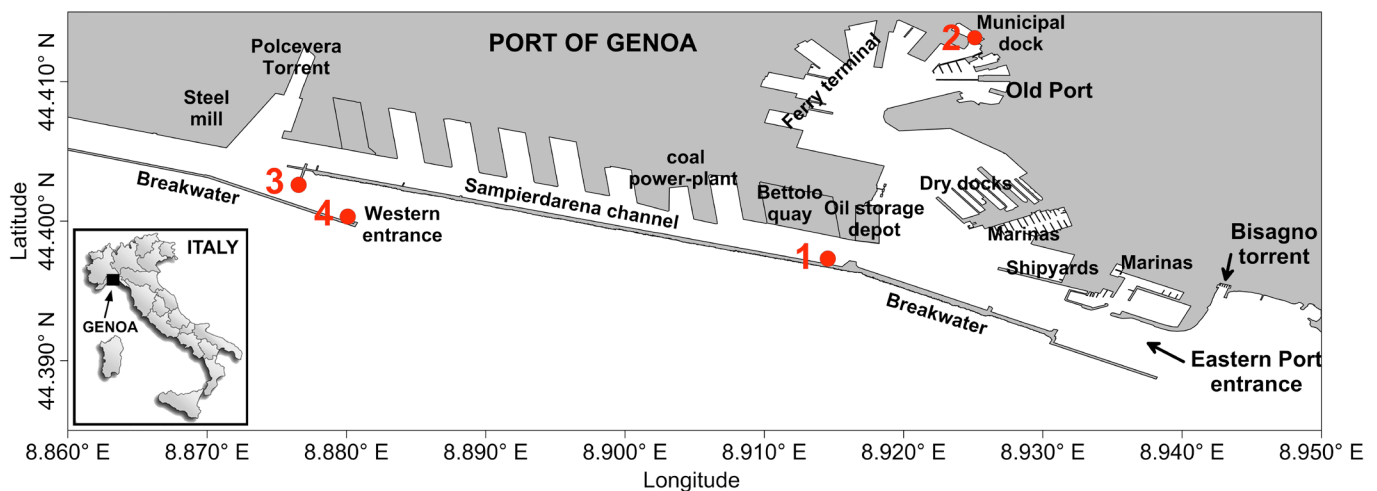


Fig. 1 - Map of the Port of Genoa and position of the four sampling stations (red dots) where ropes with wood baits were moored. Station 1: on the breakwater in the Sampierdarena channel; station 2: in the municipal dock in the inner part of the port; stations 3 and 4: at the western port entrance.

Preliminary test

A preliminary test aimed to assess the presence of marine-derived fungi in the water column was carried out. For this purpose, were employed six panels (12 x 4 x 2 cm) of fir wood (*A. alba*) as an organic substrate, to allow the isolation of lignicolous marine-derived fungi. *Abies alba* panels were sterilised and mounted on a rope, using plastic ties, arranged at 1 m from each other, and moored in the water column at station 1 (Fig. 1) starting from the sea surface for a period of 1 month. The different wooden baits placed in several sampling stations highlight differences in the distribution of marine-derived fungi at varied depth in different areas inside the port. Later was carried out a detailed fungal characterisation once the effectiveness of the method was proven and the presence of the marine-derived fungi along the water column was confirmed.

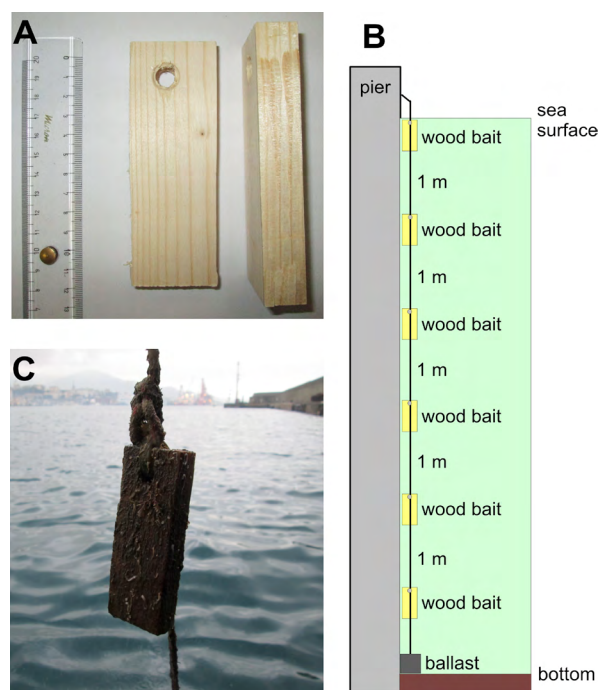
Sampling strategy

The marine-derived fungi were trapped using natural (*A. alba*) and artificial wooden baits (Medium Density Fibreboard) in order to evaluate possible differences in number and type of isolated fungi. The Medium Density Fibreboard (hereinafter MDF) is an engineered wood typically produced starting from wood fibres (82%) combined with urea-formaldehyde resin (9%) and paraffin wax (1%) and treated with high temperature and pressure to produce panels.

Six wooden baits (12 x 4 x 2 cm) were sterilised and then mounted on a rope, using plastic ties, arranged at 1 m from each other forming a bait chain (Fig. 2). Bait ropes were positioned in four different sampling stations in the Port of Genoa (Fig. 1): one rope of fir wood and one rope of MDF were positioned at stations 1 and 2; one rope of fir wood baits was positioned at station 3; one rope of MDF was positioned at station 4. Each station was characterised by different depth, hydrodynamics and rate of potential water contamination due to its position in the inner or external parts of the port. Ropes were tied to the piers starting from the water surface and kept vertical with a weight on the sea bottom. Ropes at stations 1 and 2 were recovered after 269 and 399 days, respectively, whereas, ropes at stations 3 and 4 were not found at the recovery time maybe because of the bad sea conditions. Characteristics of sampling stations and sampling time for each bait chain are reported in Table 1.

Table 1 - Sampling stations positions and sampling time for each bait chain. MDF: Medium Density Fibreboard.

Sampling station	Position in the port basin	Chain of <i>A. alba</i> baits	Chain of MDF baits	Sampling period (dd/mm/yyyy)	Number of sampling days
1	Channel affected by industrial activities and passage of merchant vessels and tugs	YES	YES	16/11/2016 31/01/2017	76
		YES	YES	31/01/2017 27/11/2017	269
2	Inner part of the port impacted by stream waters and city run-off, trawler and tugs traffic	YES	NO	16/12/2016 19/01/2018	399

**Fig. 2** - A) Wood panels of *A. alba*; B) Experimental design of wood bait chain; C) Wood panel at the recovery time.

Laboratory treatment

Wooden panels were recovered and transported in refrigerated bags to the mycological laboratory where they were immediately processed. Later, the panels were scratched and washed with sterile autoclaved seawater to remove the fouling organisms. To isolate marine-derived fungi, 1 mL of homogenized washing water was pipetted in Petri dishes (9 cm diameter) containing a modified culture medium Rose Bengale Agar seawater (RBs; Fig. 3). The plates were incubated at $24 \pm 1^\circ\text{C}$, in the dark, for 7 days.

To enable the sporulation of the latent fungi, were incubated wooden panels at room temperature for a total of 21 days in Petri dishes in contact with sterilised filter paper (Fig. 3). The panels were checked weekly to be able to isolate marine-derived latent fungal species from wooden substrate.

Later, all the vital fungal strains were isolated in axenic cultures using test tubes containing different media, such as RBs, Malt Extract Agar seawater (MEAs), Czapek Yeast Agar medium (CYA),

Potato Dextrose Agar medium (PDA) and Oatmeal Agar medium (OA), specifically usable based on founded fungal species. The isolated fungal strains were deposited at the Mycological Laboratory of the Department of Earth, Environment and Life Sciences of the University of Genoa.

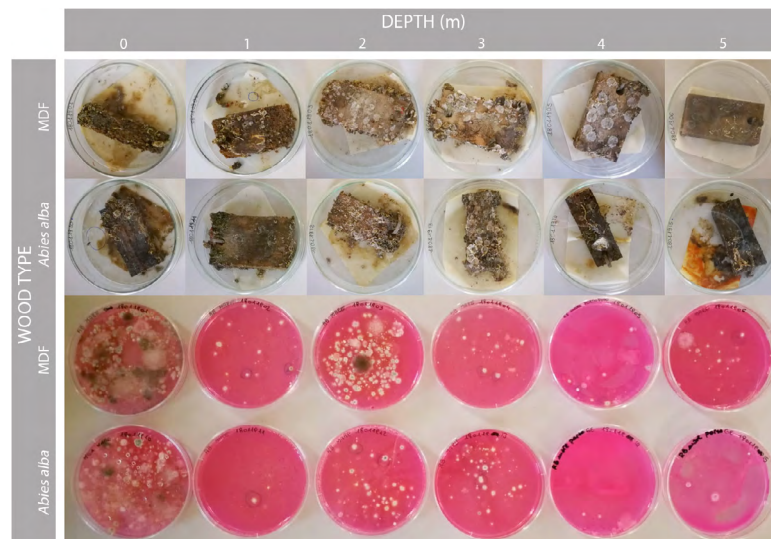


Fig. 3 - The six MDF (Medium Density Fibreboard) and *A. alba* wood panels in Petri dishes with sterilised filter paper (above) and respective Petri dishes containing RBs (below) used for the fungal characterisation in the water column of the station 2.

Strains were identified with an integrated morphological and molecular approach. At first, the micro- and macro-morphological characteristics were evaluated with specific taxonomical keys (e.g., Raper and Fennel, 1977; Pitt, 1979; Klich, 2002; Domsch et al., 2007; Samson and Frisvad, 2004). For this purpose, was employed an optical microscope (10×/0.30 and 40×/0.75). Genomic DNA was extracted from 5-days old cultures by a modified CTAB method (Doyle and Doyle, 1987). The morphological identifications were confirmed by amplification of the ITS region using the universal primers ITS1F/ITS4 (Gardes and Bruns, 1993) and the β -tubulin gene using the primers Bt2a and Bt2b (Glass and Donaldson, 1995). The PCR reaction contained 24 μ l mix (15.875 μ l Milli-Q water, 1.5 μ l 50 mM $MgCl_2$, 5 μ l 5× Green GoTaq® Buffer, 0.5 μ l 10 mM dNTPs, 0.5 μ l 10 μ M of each primer, 0.125 GoTaq® 5 U μ l⁻¹) and 1 μ l of DNA template. The PCR program was: 2 min 95 °C, 35 × (45 sec 95 °C, 45 sec 55 °C, 2 min 72 °C), 5 min 72 °C. PCR products were purified and sequenced using MACROGEN Inc. (Seoul, Republic of Korea). The sequences obtained were compared with the Gen Bank database using the BLASTN algorithm using 97% as threshold for species identification.

Statistical analysis

The biodiversity levels of each sampling series (on MDF and *A. alba* at stations 1 and 2) were evaluated with Shannon's biodiversity index (H') that was calculated in \log_2 (Shannon, 1948) considering the number of fungal strains found for each species in the whole water column.

Results

Preliminary test results

In the preliminary test carried out with *A. alba* panels, a total of 50 microfungi strains were found (Table 2); 5 genera and 9 species have been identified. The most recurrent genera were *Aspergillus*

and *Penicillium*, while among the isolates, the most common fungal species were *Penicillium solitum* Westling (from 2 to 4 m depth), and *Trichoderma longibrachiatum* Rifai (from 2 to 5 m depth; Fig. 4).

No fungal presence was found on wooden panels placed on the surface layer (0 m depth), while the highest fungal abundance was observed at 3 m depth.

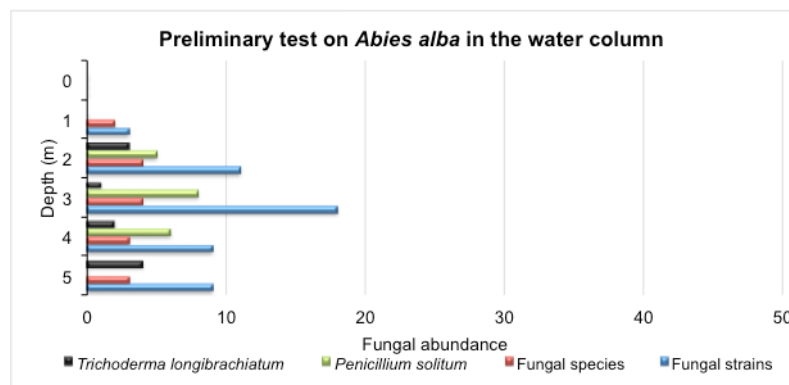


Fig. 4 - Fungal abundance on *A. alba* panels in the water column in the preliminary test.

Table 2 - Fungal distribution in the water columns in the preliminary test. Zero values for the species were not shown to the table.

Fungal species	Preliminary test on <i>Abies alba</i>					
	0 m	1 m	2 m	3 m	4 m	5 m
<i>Aspergillus tubingensis</i> Mosseray		1				
<i>Aspergillus westerdijkiae</i> Frisvad & Samson			2			
<i>Cladosporium cladosporioides</i> (Fresen.) G.A. de Vries				5		1
<i>Eurotium amstelodami</i> L. Mangin		2				
<i>Galactomyces geotrichum</i> (Butl & Peter.) Redhead & Malloch					1	
<i>Penicillium solitum</i> Westling			5	8	6	
<i>Penicillium sizovae</i> Baghd.			1			
<i>Trichoderma harzianum</i> Rifai				4		4
<i>Trichoderma longibrachiatum</i> Rifai			3	1	2	4
Total fungal strains	0	3	11	18	9	9
Total fungal species	0	2	4	4	3	3

Mycodiversity results

A total of 437 microfungus strains were isolated from the wooden panels, 187 in station 1 (Table 3) and 250 in station 2 (Table 4). All the isolates are well-known marine-derived fungi and some of the microfungus strains belonged to Ascomycota. Twelve genera and 23 species have been identified; among these, 11 genera and 18 species have been isolated from *A. alba* panels, whereas 9 genera and 16 species from MDF. The sequences obtained were deposited in GenBank under the following accession numbers: MT791370-MT791380 for ITS, and MT820427-MT820429, MT820431-MT820435, MT876625 for β -tubulin.

Table 3 - Fungal distribution at different depth (0-5 m) in the water column at station 1 on *A. alba* and MDF panels.

Fungal species	STATION 1 – <i>Abies alba</i>						STATION 1 – MDF					
	0 m	1 m	2 m	3 m	4 m	5 m	0 m	1 m	2 m	3 m	4 m	5 m
<i>Acrostalagmus luteoalbus</i> (Link) Zare, W. Gams & Schroers				1	2	1						
<i>Aspergillus europaeus</i> Hubka, A. Nováková, Samson, Houbraken, Frisvad, M. Kolařík						3						
<i>Aspergillus flavus</i> Link					1					3		
<i>Aspergillus tubingensis</i> Mosseray				8		1						2
<i>Aspergillus terreus</i> Thom				2	3	4		3				
<i>Aspergillus westerdijkiae</i> Frisvad & Samson							2					
<i>Amphichorda felina</i> (DC.) Fr.								1				
<i>Cladosporium</i> sp.					3	3		1	1	3	2	
<i>Epicoccum nigrum</i> Link		1	2		3			1				
<i>Fusarium</i> sp.		1			4							
<i>Galactomyces geotrichum</i> (Butl & Peter.) Redhead & Malloch			17	3	2			2	2	1		
<i>Geotrichum candidum</i> Link		20										
<i>Mucor hiemalis</i> Wehmer		2	2					2				
<i>Penicillium solitum</i> Westling		1	6	6	11	2		5	5	20	8	6
<i>Rhizopus arrhizus</i> A. Fish.											1	
<i>Trichoderma harzianum</i> Rifai						1						
Total fungal strains	0	25	27	20	29	15	2	15	8	27	13	6
Total fungal species	0	5	4	5	8	7	1	7	3	4	4	1

DOI: [10.6092/issn.2531-7342/10769](https://doi.org/10.6092/issn.2531-7342/10769)**Table 4** - Fungal distribution at different depth (0-5 m) in the water columns at station 2 on *A. alba* and MDF panels.

Fungal species	STATION 2 – <i>Abies alba</i>					STATION 2 – MDF						
	0 m	1 m	2 m	3 m	4 m	5 m	0 m	1 m	2 m	3 m	4 m	5 m
<i>Acrostalagmus luteoalbus</i> (Link) Zare, W. Gams & Schroers						2						
<i>Aspergillus flavus</i> Link				1								
<i>Eurotium amstelodami</i> L. Mangin												1
<i>Fusarium</i> sp.												
<i>Galactomyces geotrichum</i> (Butl & Peter.) Redhead & Malloch			6	3	2				5	2	2	
<i>Mucor hiemalis</i> Wehmer							8		2	1	2	
<i>Mucor racemosus</i> Fresen	1											2
<i>Mucor</i> sp.												5
<i>Penicillium camponoti</i> Visagie, David Clark & Seifert						1						
<i>Penicillium solitum</i> Westling	7	10	34	24	2	2	12	23	41	9	7	5
<i>Rhizopus stolonifer</i> (Ehrenb.) Vuill.	2	1				1	19					
<i>Trichoderma koningii</i> Oudem						1						
<i>Trichoderma longibrachiatum</i> Rifai					3		1					
Total fungal strains	10	11	40	28	7	7	40	23	48	12	11	13
Total fungal species	3	2	2	3	3	5	4	1	3	3	3	4

The recurrent genera were *Aspergillus* and *Penicillium*, while the most common fungal species were *P. solitum* and *Galactomyces geotrichum* (Butl & Peter.) Redhead & Malloch (Fig. 5); among the most common fungal species, *P. solitum* was isolated in both sampling stations at different depths in the water column and on both *A. alba* and MDF wooden panels; while *G. geotrichum* was not found on the surface and bottom layers.

Penicillium solitum (from 1 to 5 m depth) and *G. geotrichum* (from 2 to 4 m depth) were the most common isolates in the water column at station 1 on *A. alba* (Fig. 6), while only *P. solitum* was highlighted as the most abundant (from 0 to 5 m depth) on MDF at station 1 (Fig. 6). Instead, in the whole water column (from 0 to 6 m depth) at station 2, *P. solitum* was the most common fungal species both on *A. alba* and MFD (Fig. 6).

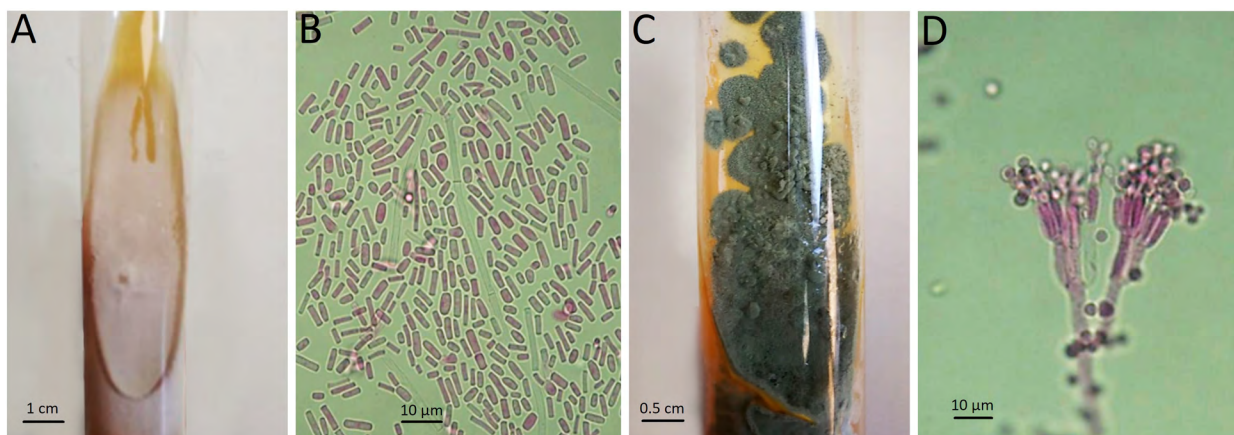


Fig. 5 - Pure culture and micromorphological features of *G. geotrichum* (A and B) and *P. solitum* (C and D).

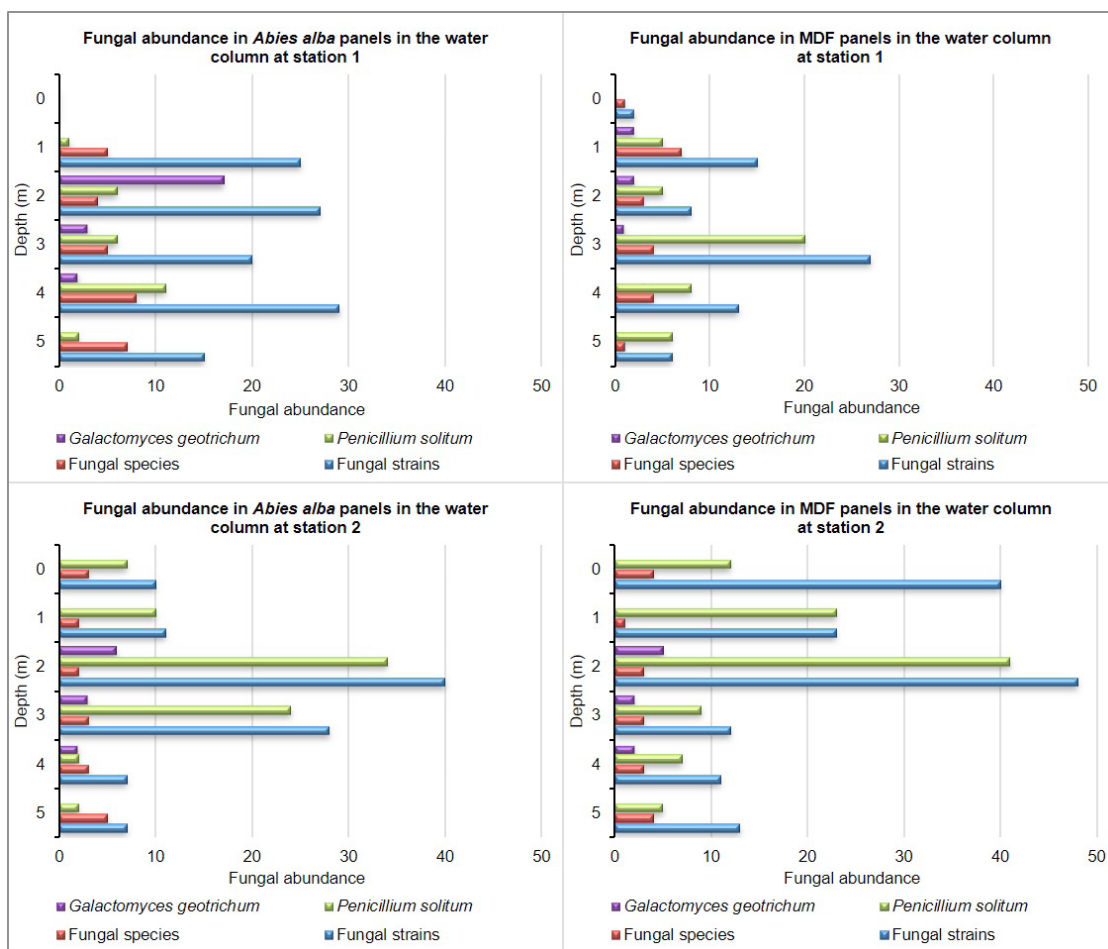


Fig. 6 - Fungal abundance in the water column at stations 1 (above) and 2 (below) on *A. alba* (left) and MDF (right) panels.

On the surface layer (0 m depth) at station 1, there was not fungal presence on *A. alba*, while only one species (*Aspergillus westerdijkiae* Frisvad & Samson) was isolated from the MDF panels. At this station, on both *A. alba* and MDF, the highest fungal abundance was observed from 1 to 4 m depth. Otherwise, at station 2, the highest abundance was found from 0 to 3 m depth (from 0 to 3 m depth on *A. alba*, and from 0 to 2 m depth on MDF). At 5 m depth, fungal abundance on *A. alba* and MDF was significantly reduced at both station 1 and station 2. The highest H' values, as shown in table 5, were observed at station 1 on both the wooden panels, confirming the higher fungal diversity present in this station than in station 2.

Table 5 - Shannon' index (H') results.

Station	Wood panel	H'
1	<i>A. alba</i>	3.18
1	MDF	2.11
2	<i>A. alba</i>	1.34
2	MDF	1.68

Discussion

All the fungal colonies isolated belong to well-known marine-derived *Taxa*; this information support the hypothesis that fungal spores/conidia may be transported in seawater by the inputs from the waterways and/or by the wastewater discharge from treatment plants surrounding the site. Moreover, results highlight a significant presence of microfungal communities in the water columns at stations 1 and 2 on both *A. alba* and MDF panels which confirm a high level of adaptability to the environmental contamination. In this case, wax and resin binders composing MDF do not influence the fungal presence.

Fungal absence was observed in the surface layer in the preliminary test, while only one species (*A. westerdijkiae*) was isolated in the surface layer at station 1. These results are probably due to the high hydrodynamics and wave-effect that characterised this port section, which is exposed to strong winds from the North and to the traffic of merchant vessels and tugboats (Cutroneo et al., 2017), these conditions prevent the microfungal colonization of the wooden substrates.

At 5-m depth at stations 1 and 2, on both *A. alba* and MDF, fungal abundance was significantly reduced; these results are due to the variations of physical parameters in relation to depth (e.g. light, temperature and dissolved oxygen) that often strongly impact on the composition of microfungal communities, as reported by Li et al. (2016).

Penicillium solitum has been commonly observed on *A. abies* and MDF panels in the whole water column at both station 1 (except in the surface layer) and station 2, showing high levels of adaptability to the environmental pollution that characterised the area. At both sampling stations with *P. solitum*, also *G. geotrichum* and some species belonged to *Trichoderma* genera (except in MDF panels of station 1) were frequently isolated from the two different panel types. *P. solitum* was reported as the most recurrent fungal species also in the previous survey carried out by Greco et al. (2018). *Penicillium solitum* has a wide geographical spread and in fact, it was also isolated from glacial ice in the extremely cold Polar Regions, and it is also known to grow at low temperatures and at high salinity levels (Sonjak et al., 2006). It was considered an important pan-global contaminant of foods

such as refrigerated dry meat, cheese, apples, pears or nuts (Pitt, 1979; Samson and Frisvad, 2004; Domsch et al., 2007). Moreover, *P. solitum* can degrade various polymeric substrates (Gonçalves et al., 2013) and it is a well-known degrader of cellulose, which has a structure very similar to many hydrocarbon compounds; that's why it could be used effectively in marine environments for bioremediation purposes. Furthermore, its hydrocarbon-degradation capacity is not well-known because no preliminary tests have been carried out.

Along with *P. solitum*, also *G. geotrichum* is known for its ecological plasticity; it grows at a range of temperatures between 25-30 °C and at a pH between 3 and 11 with an optimum around 5-7 (Grygier et al., 2017). It was isolated from different environments including freshwater (Nagahama, 2006) and marine sediments (Sutani et al., 2015). As indicated by Grygier et al. (2017), *G. geotrichum* is very important in the dairy industry and has been isolated from milk and derivatives and alcoholic beverages. This species plays an important role in ecology because it can be used to improve environmental quality being often used in the flocculation processes of wastewater treatment. Moreover, Grygier et al. (2017) indicates *G. geotrichum* as able to degrade hazardous substances such as DDT (Dichlorodiphenyltrichloroethane), and Khan et al. (2015) showed that it is an effective petroleum-degrading fungus.

As highlighted also by H' values, the highest level of mycodiversity and fungal strains was reached on the wooden panels placed at station 1 (Fig. 1), in front of the area dedicated to receiving coal and crude oil transported by oil tankers. In this station the water column is affected by PAH contamination, as reported by Cutroneo et al. (2015). The area is also affected by strong hydrodynamics due to its exposure to strong winds from N (Cutroneo et al., 2017). Hence, achieved data suggest that the high hydrocarbon concentration can produce a considerable increase of fungal species adapted to organic polluted environments. Station 2 presents a lower level of mycodiversity this may be due to the high level of organic contamination of the water column (ammonia–nitrogen and fecal coliform), low value of oxygenation (40-50%) and low hydrodynamics, with strong parameter variation due to the direct input of a city stream especially during rainy autumn days (Ruggieri et al., 2011).

Trichoderma longibrachiatum and *T. harzianum*, even if found in very small numbers, are cosmopolitan filamentous fungi, known as successful colonizers of common habitats (Zafra and Cortés Espinosa, 2015). Their enzymatic activity due also to cellulases, chitinases, glucanases, and proteases production, enable the decomposition and utilization of substrates present in soils, but in particular, the degradation of lignocellulosic material (Jaklitsch, 2009). These cosmopolitan species were studied in relation to diverse biotechnological applications and they are associated with the ability to metabolise a variety of both high and low molecular weight polycyclic aromatic hydrocarbons (PAHs) such as naphthalene, phenanthrene, chrysene, pyrene, and benzo[a]pyrene (Zafra and Cortés Espinosa, 2015).

Trichoderma longibrachiatum and *T. harzianum*, together with *A. flavus*, *A. tubingensis*, *A. terreus*, *Fusarium* sp., *G. geotrichum*, *M. racemosus*, *P. solitum*, *R. arrhizus*, and *R. stolonifer* were also found in the previous study of Greco et al. (2018) in the Port of Genoa, that reported them as species adapted to contaminated marine environment.

Even though the TRL (Technology Readiness Level) of these techniques has yet to rise, bioremediation with fungi is undoubtedly a cost-effective technology. Microfungi may grow easily and fast in bioreactors, so they can be directly used *in situ* during sediment dredging, to remediate hydrocarbon-polluted waters. Another option is to inoculate solutions containing conidia of the selected microfungi and periodically turning the soil to enable oxygenation and therefore ease the colonization of soil by selected strains that make the sediments ready for the colonization of other less tolerant organisms.

Conclusions

This research shows that a quite diverse microfungal community is present in the water of the Port of Genoa and that the isolated lignicolous marine-derived fungi are perfectly adapted to the surrounding organic-polluted environments.

Among the isolated, *P. solitum*, *G. geotrichum*, and the species belonged to *Trichoderma* genus are the most common microfungi founded in the investigated port. Thanks to their ability to adapt to extreme environments, such as those of the port in question, these marine-derived fungi may be employed in *in situ* and *ex situ* sustainable remediation techniques with the aim to degrade persistent organic contaminants (e.g. hydrocarbon products). However, the degradative potential of the above-mentioned fungal species, not yet fully investigated, should be studied thoroughly, in order to draw up protocols aimed to allow the mycoremediation of marine polluted port matrices.

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References

- Boonchan S, Britz ML, Stanley GA (2000) Degradation and mineralization of high-molecular-weight polycyclic aromatic hydrocarbons by defined fungal-bacterial cocultures. *Applied and Environmental Microbiology* 66(3):1007-1019. <https://dx.doi.org/10.1128/AEM.66.3.1007-1019.2000>
- Booth T (1983) Lignicolous marine fungi from Sao Paulo, Brazil. *Canadian Journal of Botany*. 61(2):488-506. <https://doi.org/10.1139/b83-057>
- Cecchi G, Vagge G, Cutroneo L, Greco G, Di Piazza S, Faga M, Zotti M, Capello M (2019) Fungi as potential tool for polluted port sediment remediation. *Environmental Science and Pollution Research* 26:35602-35609. <https://doi.org/10.1007/s11356-019-04844-5>
- Cerniglia CE, Sutherland JB (2010) Degradation of polycyclic aromatic hydrocarbons by fungi. In: Timmis K.N. (eds) *Handbook of Hydrocarbon and Lipid Microbiology*. Springer, Berlin, Heidelberg, pp. 2079-2110. https://dx.doi.org/10.1007/978-3-540-77587-4_151
- Cutroneo L, Carbone C, Consani S, Vagge G, Canepa G, Capello M (2017) Environmental complexity of a port: Evidence from circulation of the water masses, and composition and contamination of bottom sediments. *Marine Pollution Bulletin* 119(1):184-194. <https://doi.org/10.1016/j.marpolbul.2017.03.058>
- Cutroneo L, Castellano M, Carbone C, Consani S, Gaino F, Tucci S, Magri S, Povero P, Bertolotto RM, Canepa G, Capello M (2015) Evaluation of the boundary condition influence on PAH concentrations in the water column during the sediment dredging of a port. *Marine Pollution Bulletin* 101(2):583-593. <https://doi.org/10.1016/j.marpolbul.2015.10.055>
- Davis E, Walker TR, Adams M, Willis R (2018) Characterization of polycyclic aromatic hydrocarbons (PAHs) in small craft harbour (SCH) sediments in Nova Scotia, Canada. *Marine Pollution Bulletin* 137:285-294. <https://doi.org/10.1016/j.marpolbul.2018.10.043>
- Davis E, Walker TR, Adams M, Willis R, Norris GA, Henry RC (2019) Source apportionment of polycyclic aromatic hydrocarbons (PAHs) in small craft harbor (SCH) surficial sediments in Nova Scotia, Canada. *Science of the Total Environment* 691:528-537. <https://doi.org/10.1016/j.scitotenv.2019.07.114>

- D'Souza-Ticlo D, Garg S, Raghukumar C (2009) Effects and interactions of medium components on laccase from a marine-derived fungus using response surface methodology. *Marine Drugs* 7:672-688. <https://doi.org/10.3390/md7040672>
- Domsch KH, Gams W, Anderson T (2007) *Compendium of Soil Fungi*, second ed. IHW Verlag, Eching, Germany, pp. 1-672.
- Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19(1):11-15.
- Dupont J, Magnin S, Rousseau F, Zbinden M, Frebourg G, Samadi S, De Forges BR, Jones EG (2009) Molecular and ultrastructural characterization of two ascomycetes found on sunken wood off Vanuatu Islands in the deep Pacific Ocean. *Mycological Research* 113(12):1351-1364. <https://doi.org/10.1016/j.mycres.2009.08.015>
- Farrington JW, Takada H (2014) Persistent organic pollutants (POPs), polycyclic aromatic hydrocarbons (PAHs), and plastics: Examples of the status, trend, and cycling of organic chemicals of environmental concern in the ocean. *Oceanography* 27(1):196-213. <http://dx.doi.org/10.5670/oceanog.2014.23>
- Gadd GM (2007) Geomycology: biogeochemical transformations of rocks, minerals, metals and radionuclides by fungi, bioweathering and bioremediation. *Mycological Research* 111(1):3-49. <https://doi.org/10.1016/j.mycres.2006.12.001>
- Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes - application to the identification of mycorrhizae and rusts. *Molecular Ecology* 2(2):113-118. <https://doi.org/10.1111/j.1365-294X.1993.tb00005.x>
- Glass NL, Donaldson C (1995) Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. *Applied and Environmental Microbiology* 61(4):1323-1330.
- Gonçalves VN, Campos LS, Melo IS, Pellizari VH, Rosa CA, Rosa LH (2013) *Penicillium solitum*: a mesophilic, psychrotolerant fungus present in marine sediments from Antarctica. *Polar Biology* 36:1823-1831. <https://dx.doi.org/10.1007/s00300-013-1403-8>
- Greco G, Cecchi G, Di Piazza S, Cutroneo L, Capello M, Zotti M (2018) Fungal characterisation of a contaminated marine environment: the case of the Port of Genoa (North-Western Italy). *Webbia* 73(1):97-106. <https://doi.org/10.1080/00837792.2017.1417964>
- Grygier A, Myszkka K, Rudzińska M (2017) *Galactomyces geotrichum*-moulds from dairy products with high biotechnological potential. *Acta scientiarum polonorum. Technologia Alimentaria* 16(1):5-16. <https://dx.doi.org/10.17306/J.AFS.2017.2017.0445>
- Gunde-Cimerman N, Oren A, Plemenitaš A (2005) Introduction. In: Gunde-Cimerman N, Oren A, Plemenitaš A (Eds.) *Adaptation to Life at High Salt Concentrations in Archaea, Bacteria, and Eukarya* Springer, Dordrecht, pp. 1-6. <https://doi.org/10.1007/1-4020-3633-7>
- Hammel KE (1995) Mechanisms for polycyclic aromatic hydrocarbon degradation by ligninolytic fungi. *Environmental Health Perspectives* 103:41-43. <https://doi.org/10.1289/ehp.95103s441>
- Jaklitsch WM (2009) European species of *Hypocrea* Part I. The green-spored species. *Studies in Mycology* 63:1-91. <https://doi.org/10.3114/sim.2009.63.01>
- Khan SR, Kumar NJI, Kumar RN, Patel JG (2015) In vitro study on assessment of petrol, kerosene and diesel degrading potential of indigenous fungal isolates from different petroleum product effected soils. *International Journal of Recent Research and Review* 8(1):8-15.
- Klich MA (2002) Biogeography of *Aspergillus* species in soil and litter. *Mycologia* 94(1):21-27. <https://doi.org/10.1080/15572536.2003.11833245>

- Kostka JE, Prakash O, Overholt WA, Green SJ, Freyer G, Canion A, Delgardio J, Norton N, Hazen TC, Huettel M (2011) Hydrocarbon-degrading bacteria and the bacterial community response in Gulf of Mexico beach sands impacted by the Deepwater Horizon oil spill. *Applied and Environmental Microbiology* 77(22):7962-7974. <https://dx.doi.org/10.1128/AEM.05402-11>
- Kumar A, Bisht BS, Joshi VD, Dhewa T (2011) Review on bioremediation of polluted environment: A management tool. *International Journal of Environmental Science* 1:1079-1093.
- Latimer JS, Zheng J (2003) The sources, transport and fate of PAHs in the marine environment. In: Douben PET (Ed.) *PAHs: an ecotoxicological perspective*, Wiley, Sharnbrook, Bedford, UK, pp. 9-33. <https://doi.org/10.1002/0470867132.ch2>
- Li W, Wang M, Bian X, Guo J, Cai L (2016) A high-level fungal diversity in the intertidal sediment of Chinese seas presents the spatial variation of community composition. *Frontiers Microbiology* 7:2098. <https://doi.org/10.3389/fmicb.2016.02098>
- Liu D, Li XM, Meng L, Li CS, Gao SS, Shang Z, Proksch P, Huang CG, Wang BG (2011) Nigerapyrones A–H, α -pyrone derivatives from the marine mangrove-derived endophytic fungus *Aspergillus niger* MA-132. *Journal of Natural Product* 74(8):1787-1791. <https://doi.org/10.1021/np200381u>
- Man YB, Kang Y, Wang HS, Lau W, Li H, Sun XL, Giesy JP, Chow KL, Wong MH (2013) Cancer risk assessments of Hong Kong soils contaminated by polycyclic aromatic hydrocarbons. *Journal of Hazardous Material* 261:770-776. <https://doi.org/10.1016/j.jhazmat.2012.11.067>
- Montemartini Corte A (1979) La micoflora marina della baia di Portofino. *Società Botanica Italiana* 113(4):297-325. <https://dx.doi.org/10.1080/11263507909426650>
- Nagahama T (2006) Yeast biodiversity in freshwater, marine and deep-sea environments. In: Péter, G., Rosa, C. (Eds) *Biodiversity and Ecophysiology of Yeasts. The Yeast Handbook*. Springer, Berlin, Heidelberg, pp. 241-262. https://doi.org/10.1007/3-540-30985-3_12
- Naso B, Perrone D, Ferrante MC, Bilancione M, Lucisano A (2005) Persistent organic pollutants in edible marine species from the Gulf of Naples, Southern Italy. *Science of the Total Environment* 343(1-3):83-95. <https://doi.org/10.1016/j.scitotenv.2004.10.007>
- Nfon E, Cousins IT, Broman D (2008). Biomagnification of organic pollutants in benthic and pelagic marine food chains from the Baltic Sea. *Science of the Total Environment* 397(1-3):190-204. <https://doi.org/10.1016/j.scitotenv.2008.02.029>
- Pang KL, Overy DP, Jones EG, da Luz Calado M, Burgaud G, Walker AK, Johnson JA, Kerr R G, Cha H, Bills GF (2016) ‘Marine fungi’ and ‘marine-derived fungi’ in natural product chemistry research: toward a new consensual definition. *Fungal Biology Reviews* 30(4):163-175. <https://doi.org/10.1016/j.fbr.2016.08.001>
- Passarini MR, Rodrigues MV, da Silva M, Sette LD (2011) Marine-derived filamentous fungi and their potential application for polycyclic aromatic hydrocarbon bioremediation. *Marine Pollution Bulletin* 62(2):364-370. <https://doi.org/10.1016/j.marpolbul.2010.10.003>
- Pautasso M, Zotti M (2009) Macrofungal taxa and human population in Italy’s regions. *Biodiversity and Conservation* 18:473-485. <https://doi.org/10.1007/s10531-008-9511-4>
- Pitt JI (1979) The genus *Penicillium* and its teleomorphic states *Eupenicillium* and *Talaromyces*. *Mycologia* 73(3):582-584.
- Rämä T, Nordén J, Davey ML, Mathiassen GH, Spatafora JW, Kauserud H (2014) Fungi ahoy! Diversity on marine wooden substrata in the high North. *Fungal Ecology* 8:46-58. <https://doi.org/10.1016/j.funeco.2013.12.002>
- Raper KB, Fennell DI (1977) *The Genus Aspergillus*. Reprint RE Krieger Publication Company Malabar, FL, p. 686.
- Revinga C, Brunner J, Henninger N, Kassem K, Payne R (2000) Pilot analysis of global ecosystems: Freshwater Systems. World Resources Institute, Washington, DC. https://wriorg.s3.amazonaws.com/s3fs-public/pdf/page_freshwater.pdf

- Ruggieri N, Castellano M, Capello M, Maggi S, Povero P (2011) Seasonal and spatial variability of water quality parameters in the Port of Genoa, Italy, from 2000 to 2007. *Marine Pollution Bulletin* 62(2):340-349. <https://doi.org/10.1016/j.marpolbul.2010.10.006>
- Rydin H, Diekmann M, Hallingback T (1997) Biological characteristics, habitat associations, and distribution of macrofungi in Sweden. *Conservation Biology* 11(3):628-640. <https://doi.org/10.1046/j.1523-1739.1997.96437.x>
- Samson RA, Frisvad JC (2004) *Penicillium* subgenus *Penicillium*: new taxonomic schemes and mycotoxins and other extrolites. *Studies in Mycology* 49:1-251.
- Schmit JP, Mueller GM (2007) An estimate of the lower limit of global fungal diversity. *Biodiversity and Conservation* 16:99-111. <https://doi.org/10.1007/s10531-006-9129-3>
- Seltenrich N (2015) New link in the food chain? Marine plastic pollution and seafood safety. *Environmental Health Perspectives* 123:A34-A41. <https://doi.org/10.1289/ehp.123-A34>
- Shannon CE (1948) A mathematical theory of communication. *Bell System Technical Journal* 27: 379-423.
- Shearer CA, Descals E, Kohlmeyer B, Kohlmeyer J, Marvanová L, Padgett D, Porter D, Raja HA Schmit, JP Thorton, HA Voglymayr H (2007) Fungal biodiversity in aquatic habitats. *Biodiversity and Conservation* 16:49-67. <https://dx.doi.org/10.1007/s10531-006-9120-z>
- Singh H (2006) *Mycoremediation: fungal bioremediation*. John Wiley & Sons, pp. 592.
- Sonjak S, Frisvad JC, Gunde-Cimerman N (2006) *Penicillium* mycobiota in Arctic subglacial ice. *Microbial Ecology* 52:207-216. <https://dx.doi.org/10.1007/s00248-006-9086-0>
- Sutani A, Ueno M, Nakagawa S, Sawayama S (2015). Melon aroma-producing yeast isolated from coastal marine sediment in Maizuru Bay, Japan. *Fisheries Science* 81:929-936. <https://dx.doi.org/10.1007/s12562-015-0912-5>
- Walker CH, Livingstone DR (1992) *Persistent pollutants in marine ecosystems*. Elsevier. <https://doi.org/10.1016/C2009-0-07980-X>
- Zafra G, Cortés-Espinosa DV (2015) Biodegradation of polycyclic aromatic hydrocarbons by *Trichoderma* species: a mini review. *Environmental Science and Pollution Research* 22:19426-19433. <https://dx.doi.org/10.1007/s11356-015-5602-4>