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SEDIMENTS AS A DYNAMIC NATURAL RESOURCE – FROM CATCHMENT TO OPEN SEA

From waste to resource: mycoremediation of contaminated marine sediments in the SEDITERRA Project

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Abstract

Purpose Port-dredging activities produce large volumes of contaminated sediments. Dredged sediments are considered a waste by national laws, but recently the desire to consider them a resource has become widespread and remedies for their contamination are being searched to allow their reuse. In this work, we studied, developed, and tested a method for remediate marine-dredged sediments contaminated by heavy metals using native fungi and a microporous membrane, in order to achieve the sediment quality and allow their reuse.

Materials and methods Activity was carried out on port sediments from Genoa, Leghorn, Pisa, and Cagliari (Italy). Autochthonous fungi were isolated from each sediment and employed in mycoremediation tests. Two plastic boxes were prepared (for each Port) with 5 kg of sediment in each box, employed for metal bioaccumulation using a sterile polyester membrane inoculated with fungi. Membranes were analyzed at 15, 30, and 60 days after inoculums, and sediments were analyzed after 60 days at the end of the experiment to verify metal contamination degree. Recovery efficiency (RE%) and difference recovery efficiency (DRE%) were calculated for each experiment: the first shows the absorption capability of the membrane-fungi consortium; the second evidences only the fungal contribution to the metal absorption. To assess sediment contamination before and after the mycoremediation treatment, we considered chemical levels of reference L1 (the lowest chemical level of reference) and L2 (the highest chemical level of reference), and the evaluation of chemical hazard (HQ) for the chemical contaminants defined by the Italian Ministerial Decree 173/2016.

Results and discussion Fungi from Genoa sediments increase the membrane absorption of Cu and Zn. Regarding Leghorn results, RE (%) increases and reaches the maximum value after 60 days of treatment for each considered metal. Cr tot, Ni, and Mn appear to be hyper-bioaccumulated. DRE values of Pisa sediments show that Mn is excluded by fungi and it does not bioaccumulate, while other metals and in particular Cd, Cr tot, Zn, and Sb are bioaccumulated. Cagliari DREs show that fungi are not able to bioaccumulate Cr tot, Ni, and Mn and their accumulation is due to the membrane, while As and Cd are bioaccumulated.

Conclusions Our work evidenced that selected fungi are able to grow on a microporous support and actively reduce metal concentrations in the sediments, achieving their quality. This biomembrane system may represent an important instrument for the remediation of the residual metal contamination of port sediments.

Keywords Autochthonous fungi · Dredged sediments · Heavy metals · Mycoremediation

1 Introduction

Port-dredging activities every year produce large volumes of contaminated sediments (OSPAR 2014; Akcil et al. 2015). Dredging consists in the removal of sediments from the aquatic environment (i.e., port and harbor navigation channels), berthing areas, and marinas (Harrington et al. 2016). However, recently, dredged sediments have been considered not only as waste but also as a resource. Sustainable dredged sediment management, in fact, represents the new border of

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dredging projects: a wide range of management options can be considered involving disposal, treatment, and/or beneficial use (Akcil et al. 2015; Harrington et al. 2016). Disposal options can include the onshore disposal or the offshore disposal; the latter can be confined or carried out in an open water facility (Harrington et al. 2016).

Beneficial use options can be categorized under the following: engineering uses, where the dredged sediment is often a substitute of the traditional land-based sources; environmental enhancement on terrestrial or aquatic environments; and agricultural and/or product uses, where useful and potentially marketable products are developed (Harrington et al. 2016). The choice of the sediment management technique required can be influenced by many factors, including characteristics of the sediment, whether it is contaminated or not; the dredged volume involved; local site conditions including site accessibility; and current local, national and international practice, and laws (Akcil et al. 2015; Harrington et al. 2016). Dredged material often does not have appropriate physical and/or chemical properties for reuse in certain application. Contamination of dredged sediments represents one of the actual environmental problems (Fathollahzadeh et al. 2014; OSPAR 2014; Ammami et al. 2015). Indeed, organic and inorganic pollutants, derived from different sources (Akcil et al. 2015), often contaminate port sediments.

Treatment of dredged material to create a higher value product is possible (Ammami et al. 2015). In situations where relocation becomes extremely costly, treatment may be a cost-effective option, but such conditions also increase dredging costs (Walker et al. 2013; Brils et al. 2014). Unfortunately, dredged material is characterized by a negative image due to not only its unattractive physical properties for construction, but also from potential contaminants and the impact of dredging on many environmental compartments (Brils et al. 2014; Akcil et al. 2015). Since 2009, the European Sediment network SedNet (www.sednet.org) has been emphasizing that sediment is an essential and dynamic part of river, delta, and coastal systems (Brils et al. 2014). In order to do this, it is indispensable to decontaminate sediments. In the framework of a few scientific researches, traditional and innovative techniques, which allow sediments decontamination, have been tested (Ammami et al. 2015). In terms of bioremediation strategies, microorganisms (mainly bacteria and fungi) have been recently exploited and stimulated in order to decontaminate various substrata (e.g., sediments; Fonti et al. 2015; Li and Yu 2015; Zhang et al. 2015). In particular, mycoremediation consists in the exploiting of fungal organisms naturally able to synthesize enzymes and organic acids, which interact with contaminants (Cecchi et al. 2019b). Fungi are able to bioconcentrate, bioaccumulate, and biostabilize heavy metals and to degrade organic pollutants (Cecchi et al. 2017a; Liu et al. 2017; Cecchi et al. 2018a, b; Spina et al. 2018; Cecchi et al. 2019b). In the framework of the European Interreg Italy-France 2014–2020 Maritime Project SEDITERRA "Guidelines for the sustainable treatment of dredged sediments in the Marittimo area,", the Department of Environmental, Earth and Life Sciences (DISTAV) of the University of Genoa, Partner of the Project, developed a protocol for mycoremediating marine-dredged sediments affected by heavy metals. The aim of this study was to evaluate the efficiency of the mycoremediation protocol using selected autochthonous fungal strains from four port sediments (Genoa, Leghorn, Pisa, and Cagliari—Italy) and compare results obtained in each treatment.

2 Materials and methods

Mycoremediation pilot activity was carried out on different kinds of sediments from the Ports of Genoa, Leghorn, and Cagliari and the Navicelli Canal of Pisa (Fig. 1). Bottom superficial sediments were collected inside the Port of Genoa using a Van Veen grab, whereas sediments of the Ports of Leghorn and Cagliari were collected using a steel shovel from the reclamation areas located in these ports, where sediments were positioned during recent dredging. In these last two cases, sediments were in dry conditions. Finally, Pisa sediments were sampled using a Van Veen grab from the Navicelli Canal that was built in the sixteenth century to connect Pisa to the Port of Leghorn and it is characterized by brackish water. A total of 30 kg of sediment was collected from each area and stored in closed plastic barrels in a cool environment and away from light. At laboratory, 1 kg was used for chemical characterization (metals), 215 g for physical characterization (definition of organic and inorganic fraction and grain size analysis), 5 g for fungal characterization, and 25 kg for the mycoremediation activity. Sediment aliquot destined to the analysis of fungal community was taken immediately upon arrival in the laboratory; while to sample the aliquots destined to the other analyses and treatment, sediments were poured alternatively in small quantities into the treatment plastic boxes, further homogenized and then sub-sampled.

2.1 Chemical and physical characterization of sediments

Metal concentration was determined using the method EPA3050B 1996 + EPA6010D 2014 at the Eurochem Italia laboratory in Genoa. Concerning physical characterization, 15 g of sediment was analyzed in order to evaluate the percentage of the organic fraction (OF) and inorganic fraction (IF). The sediment (with a known dry weight, S) was burnt in an ISCO muffle (ISM320 mod.) for 3 h at 550 °C to remove the OF. The unburned fraction (IF) was weighed, whereas the OF was determined by difference between S and IF. Furthermore, 200 g of sediment was analyzed for the grain-size characteristics. Sediment was first dried at 60 °C in a

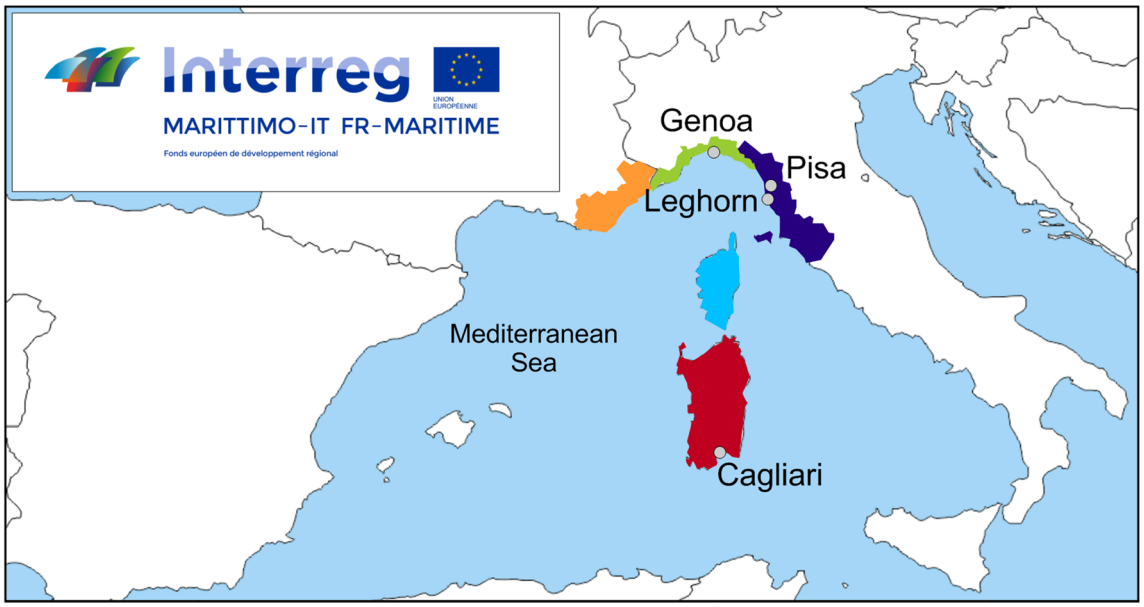

Fig. 1 Map of the Interreg Maritime areas of cooperation: Corse (entire region; France) in light blue; Liguria (entire region; Italy) in green; PACA (Maritime Alps and Var; France) in orange, Sardinia (entire region; Italy) in red; Tuscany (city of Grosseto, Lucca, Leghorn, Massa Carrara, and Pisa; Italy) in violet. Gray dots show the cities in which the sediments treated in the present study were collected: Genoa, Pisa, Leghorn, and Cagliari

thermostatically controlled oven and weighed. Subsequently, the fine fraction (particle diameter Ø < 63 μm) was divided from the coarse fraction (particle diameter Ø > 63 μm) using a wet sieve (63-μm mesh sieve). Coarse fraction was dried at 60 °C and its grain size analysis was carried out by dry sieving, considering the follow dimensional classes as indicated by Capello et al. (2016): 63–125 μm, 125–250 μm, 250–500 μm, 500–1000 μm, 1000–2000 μm, and > 2000 μm. The analysis of the fine fraction was carried out using a Coulter Counter® Multisizer 3 (Beckman Coulter, Inc.). The values of OF, IF, and grain size classes were expressed in percentage (%).

2.2 Quality assessment of the sediments

To evaluate the chemical quality of studied sediments and to quantify the effect of the mycoremediation treatment on them, the evaluation procedure reported by The Italian Ministerial Decree (I.M.D.) 173/2016 "Technical procedures and criteria for authorizing the disposal of dredged sediments into the sea" was considered. This decree considers the first Weight of Evidence (WOE) approach that combines various lines of evidence (LOE), such as chemical analysis, toxicity testing, and the in situ benthic community structure, to evaluate the contamination degree of sediments and their dangerousness for the marine environment (Regoli et al. 2019). In order to protect the marine environment, the Italian decree determines the following: (i) the homogeneous criteria for the whole national territory for the use of dredged materials for nourishment purposes; (ii) the management of materials dredged from port areas and coastal marine areas not included in sites of national interest; (iii) the management of materials from national interest sites resulting from dredging operations in the port and coastal marine areas, outside the sites of national interest. Moreover, the decree demands the characterization and classification of the dredged sediments: it asks the quality assessment. Therefore, following the decree rules, as showed also in Surricchio et al. (2019), to assess sediment contamination before and after the mycoremediation treatment, we considered the chemical analysis LOE. We did not consider the ecotoxicological evaluation part because it was outside the scope of our study. Therefore, we applied the chemical levels of reference L1 (the lowest chemical level of reference) and L2 (the highest chemical level of reference) (Table 1) and the evaluation of chemical hazard (HQ) for the chemical contaminants defined by HQ based on the international classification (according to Annex II of Directive 2008/105/EC) of "not priority," "priority," or "dangerous and priority" substances. It assigns the substances a weight based on their priority classification: "not priority" substances have weight = 1; "priority" substances have weight = 1.1; and "dangerous and priority" substances have weight = 1.3. Starting from each contaminant concentration (C) found in sediments, the "ratio to references L1 or L2" (RTR) and the "weighted ratio to reference" (RTR_w) are defined for each contaminant and then HQ of sediment is found:

$$RTR(i) = C(i)/\text{chemical level of reference L1 or L2}(i)$$

$$RTR_w = RTR(i) \times \text{weight}(i)$$

Table 1 Contaminants analyzed in sediments; analysis methods; detection limits; Italian chemical levels of reference L1 and L2 (Italian Ministerial Decree 173/2016); "weight" attributed to the contaminant by the Italian Ministerial Decree 173/2016 based on its international classification of "not priority" substances (weight = 1), "priority" substances (weight = 1.1), and "dangerous and priority" substances (weight = 1.3); initial chemical characterization of sediments; initial chemical metals characterization of the membrane (M0). *n.a.* not available

Parameters	Analysis method	Detection limit	Italian chemical levels of reference L1–L2 (D.M. 173/2016)	Weight of parameter (D.M. 173/2016)	Genoa sediments	Leghorn sediments	Pisa sediments	Cagliari sediments	M0
Al (mg kg^{-1})	EPA3050B 1996 + EPA6010D 2014	5	n.a.	n.a.	6800 ± 579	4800 ± 429	29,000 ± 1976	4100 ± 375	710 ± 85
Sb (mg kg^{-1})	EPA3050B 1996 + EPA6010D 2014	1	n.a.	n.a.	1.2 ± 0.4	< 1	6.7 ± 1.6	2.7 ± 0.7	2 ± 0.6
As (mg kg^{-1})	EPA3050B 1996 + EPA6010D 2014	5	12–20	1	8 ± 1.9	< 5	< 5	12 ± 3	< 1
Cd (mg kg^{-1})	EPA3050B 1996+ EPA6010D 2014	0.3	0.3–0.8	1.3	0.42 ± 0.15	< 0.3	22 ± 4	1.9 ± 0.6	< 1
Cr tot (mg kg^{-1})	EPA3050B 1996 + EPA6010D 2014	1	50–150	1	55 ± 10	8.7 ± 2.0	320 ± 43	8.2 ± 1.9	8.9 ± 2
Fe (mg kg^{-1})	EPA3050B 1996 + EPA6010D 2014	5	n.a.	n.a.	15,000 ± 1129	6900 ± 584	23,000 ± 1623	7200 ± 205	1700 ± 178
Mn (mg kg^{-1})	EPA3050B 1996 + EPA6010D 2014	1	n.a.	n.a.	650 ± 78	100 ± 16	500 ± 63	89 ± 14	82 ± 14
Hg (mg kg^{-1})	EPA3050B 1996 + EPA6010D 2014	0.3	0.3–0.8	1.3	0.32 ± 0.12	< 0.3	0.59 ± 0.20	< 0.3	< 1
Ni (mg kg^{-1})	EPA3050B 1996 + EPA6010D 2014	1	30–75	1.1	31 ± 6	8.4 ± 2.0	57 ± 10	5.6 ± 1.4	5.4 ± 1.3
Pb (mg kg^{-1})	EPA3050B 1996 + EPA6010D 2014	3	30–70	1.1	59 ± 10	< 3	60 ± 10	97 ± 16	4.7 ± 1.2
Cu (mg kg^{-1})	EPA3050B 1996 + EPA6010D 2014	10	40–52	1	15 ± 3	< 10	110 ± 17	40 ± 7	3.7 ± 1
Zn (mg kg^{-1})	EPA3050B 1996 + EPA6010D 2014	15	100–150	1	62 ± 11	< 15	590 ± 72	210 ± 30	12 ± 3

$$HQ = [\Sigma\, RTR_w(j) \text{ if } RTR(j) \leq 1]/N + \Sigma\, RTR_w(k) \text{ if } TRT_w(k) > 1$$

Finally, sediments are classified according to the following HQ classes: "absent" contamination if HQ < 0.7; "negligible" if 0.7 ≤ HQ < 1.3; low if 1.3 ≤ HQ < 2.6; medium if 2.6 ≤ HQ < 6.5; high if 6.5 ≤ HQ < 13; and very high if HQ ≥ 13.

2.3 Fungi isolation and identification

Fungi were isolated by the dilution plate technique as described in Cecchi et al. (2019a) using a dilution factor of 10. In order to favor fungal growth, Rose Bengal Agar medium (SIGMA-ALDRICH®) was prepared. In each plate (12 cm Ø), 1 ml of solution was inoculated and then spatulated to increase the fungal isolation possibility. After inoculums, the plates were incubated in the dark at 24 °C and monitored weekly. After being grown, fungal strains were isolated in axenic cultures in test tubes, thanks to the method of subsequent isolations. Fungal identification was carried out by a polybasic approach (morphological and molecular; Cecchi et al. 2019a). Morphological identification was carried out using stereoscope and microscope, analyzing macro- and micro-morphological features of fungi. Genus and species identification was possible by the consultation of specific monographs (Zycha et al. 1969 for Mucorales order; Domsch et al. 1980 for soil fungi; Nelson et al. 1983 for *Fusarium* genus; Pitt et al. 2000 for *Penicillium* genus). Genomic DNA was extracted from 100 mg of fresh fungal culture using a modified CTAB method (Doyle and Doyle 1987). The PCR amplification of β-tubulin gene was performed using Bt2a and Bt2b primers (Glass and Donaldson 1995), and in ITS region amplification, universal primers ITS1F and ITS4 (White et al. 1990; Gardes and Bruns 1993) were used to identify the most critical strains (Di Piazza et al. 2018). The PCR protocol was as follows: one cycle of 5 min at 95 °C; 40 s at 94 °C; 45 s at 55 °C; 35 1-min cycles at 72 °C; one 10-min cycle at 72 °C. Later, PCR products were purified and sequenced using Macrogen Inc. (Seoul, Republic of Korea). The sequence assembly and editing were performed using Sequencher® (Gene Codes Corporation, version 5.2). The taxonomic assignment of the sequenced samples was carried out using the BLASTN algorithm to compare the sequences obtained in the present study against the GenBank database. We took a conservative approach to species-level assignment (identity ≥ 97%) and we verified the accuracy of the results by also studying the macro- and micro-morphological features of the colonies. The isolated fungal strains were conserved at 4 ± 1 °C and cryopreserved at − 20 °C in the culture collection of the Laboratory of Mycology of DISTAV of the University of Genoa.

2.4 Mycoremediation test

Two plastic boxes were prepared with 5 kg of port sediment for pilot mycological activity for each port. Box 1 and 2 were used for testing fungi efficiency in bioaccumulating metals. Sterile polyester membranes were positioned on sediments and inoculated with 0.5 L of selected fungal strain inocula (Cecchi et al. 2019a). Membranes allow physical (not chemical) mycelia separation from sediments. Membrane samples were collected from the boxes after 15 (1), 30 (2), and 60 (3) days after inoculum in order to evaluate their metal content. The recovery efficiency (RE%) was calculated as the percentage of metal recovery between metal initial and final concentrations (Rashid et al. 2016; Aftab et al. 2017; Di Piazza et al. 2017; Cecchi et al. 2019a) for each membrane sample. Moreover, the difference recovery efficiencies (DREs%) between the REs of the membrane inoculated with fungi and the natural RE membrane (without fungi) employed in this study were calculated for the boxes at each experimental time (1DRE, 2DRE, 3DRE) in order to better evidence the fungal contribution in the membrane metal uptake (Cecchi et al. 2019a).

3 Results

3.1 Chemical and physical characteristics of sediments

Chemical characterization results show that Pisa sediments are the most contaminated in terms of inorganic contaminants in particular for Cr, Cd, Ni, Cu, and Zn (Table 1). In each port Al, Fe, Mn, and Pb appear the most abundant metals (Al > 4000 mg kg^{-1}; Fe > 6000 mg kg^{-1}; Mn > 80 mg kg^{-1}; Pb > 50 mg kg^{-1}), while Hg is characterized by the lowest concentrations (< 0.6 mg kg^{-1}). However, elements such as Fe and Al are main constituents of the earth crust and consequently they are abundant; while as concern trace metals Pb, Cd, Cr, and Hg reach high concentration mainly in Pisa sediments (60, 22, 320, and 0.59 mg kg^{-1}, respectively), but less markedly in Genoa (59, 0.42, 55, 0.32 mg kg^{-1}, respectively). All characterized sediments show dominance of the inorganic fraction (Table 2); the organic fraction varied from 3 to 12% and is mostly represented in Pisa sediments (Table 2).

Regarding the grain size analysis, sediments of Genoa and Cagliari are mainly composed by the coarse fraction (Ø > 63 μm) and in particular by sand between mean and very fine (83.9 and 67.7%, respectively; Table 2). Sediments of Leghorn present high values of gravel (22.6%), consisting in gravel and shells, and fine silt (20.0%). Fine fraction (Ø < 63 μm) is dominant in Pisa sediments (72.6%; Table 2) with 30.8% of fine silt and 9.5% of clay.

Following the sediment quality assessment strategy reported in Italian Ministerial Decree 173/2016, considering L1 (the lowest chemical level of reference), sediments of Leghorn have no contamination. Genoa is characterized by a high contamination, while sediments of Pisa and Cagliari have a very high contamination (Table 3). Instead, considering L2 (the highest chemical level of reference), sediments of Genoa and Leghorn have absent contamination; sediments of Cagliari have medium contamination, while Pisa sediments have a very high contamination. After the mycoremediation treatment, except for Leghorn, there was an improvement of sediment quality, but only for Genoa the improvement corresponds to a decrease in the sediment contamination class for L1. Leghorn sediments, in fact, show a slight deterioration of their quality class for L1 from 0.24 HQ to 3.92 HQ (Table 3). Genoa before the treatment was characterized by an HQ (L1) of 8.16 (high contamination class) and after the mycoremediation, this value has decreased up to 4.59 changing the sediment contamination class for L1 (medium contamination class) (Table 3). Pisa shows a decrease in particular of the HQ (L1) values (from 117.23 to 80.27), but the contamination class is the same (very high contamination). Cagliari results show small changes both for L1 and L2 (Table 3).

3.2 Fungal isolation results

Macro- and micro-morphological characterizations of fungi show that the dominant fungal genera in the sediments analyzed are *Penicillium*, *Fusarium*, and *Mucor* (Genoa results were published in Cecchi et al. 2019a). In particular, from Leghorn, sediments counted, isolated, and conserved were two morphotypes referable to the genera *Penicillium* and *Mucor*. In Pisa sediments, the recognized morphotypes belong to the genera *Cunninghamella* and *Penicillium*; while in Cagliari sediments, strains belong to the genera *Fusarium* and *Cladosporium*. DNA analyses allowed the specific identification of these strains: *Penicillium brevicompactum* Dierckx and *Mucor racemosus* Fresen from Leghorn sediments; *Cunninghamella elegans* Lendn. and *Penicillium citrinum* Thom from Pisa sediments; *Fusarium oxysporum* complex Schltdl. and *C. cladosporioides* from Cagliari sediments. These DNA results showed an identity ≥ 97%. Moreover, they were verified with the morphological studies. *Penicillium* strain, isolated from Leghorn sediments and resulted by molecular analysis belonging to the *brevicompactum* species, is characterized by slow growth and formation of dull green colonies (12–22 mm diameter) after 7 days at 25 °C. It is a terverticillate *Penicillium*; conidia are ellipsoidal (2.5–3.5 μm long). *Mucor racemosus* strain (from Leghorn sediments), belonging to the Zygomycota Phylum, forms dark gray-light gray colonies and produces sporangiophores with an apical swelling enclosed by large sporangium filled with ellipsoidal, single celled, smooth-walled, sporangiospores. *Cunninghamella elegans* (from Pisa sediments) also belong to the Zygomycota Phylum and rapidly grows (4 days)

Table 2 Physical characterization of the sediments analyzed in the present study: inorganic and organic fractions (%), results of the grain size analysis (%)

Samples	Genoa	Leghorn	Pisa	Cagliari
Inorganic fraction	97	97	88	95
Organic fraction	3	3	12	5
Total coarse fraction (Ø > 63 μm)	86.7	60.4	27.4	76.6
Total fine fraction (Ø < 63 μm)	13.3	39.6	72.6	23.4
Gravel(Ø > 2000 μm)	0.3	22.6	3.4	2.1
Very coarse sand (1000 μm < Ø < 2000 μm)	0.7	6.2	2.9	1.9
Coarse sand (500 μm < Ø < 1000 μm)	1.7	6.3	4.4	5.0
Mean sand (250 μm < Ø < 500 μm)	3.7	12.1	4.5	23.2
Fine sand (125 μm < Ø < 250 μm)	65.5	7.2	5.9	35.7
Very fine sand (63 μm < Ø < 125 μm)	14.7	6.0	6.3	8.8
Coarse silt (30 μm < Ø < 63 μm)	7.2	4.9	16.5	5.0
Mean silt (16 μm < Ø < 30 μm)	2.3	8.4	15.8	5.6
Fine silt (4 μm < Ø < 16 μm)	3.2	20.0	30.8	10.4
Clay (Ø < 4 μm)	0.7	6.3	9.5	2.3

forming cottony and gray colonies. Here, the vesicles have spine-like denticles on their surfaces. Sporangiospores are one-celled, solitary, and globose. *P. citrinum* (from Pisa sediments) forms green-gray-blue colonies (18–25 mm diameter). It is characterized by smooth-walled and globose conidia (2–2.5 × 1.8–2.5 μm). The strain belonging to the *F. oxysporum* complex (from Cagliari sediments) is characterized by rapid colony growth (4.5 cm in 4 days) and by a white initial aerial mycelium, becoming purple after some days. Conidiophores are short and single. Macroconidia are fusiform and slightly curved, mostly triseptate (23–24 × 3–4.5 μm). Microconidia are abundant, ellipsoidal to cylindrical (5–12 × 2.3–3.5 μm). *C. cladosporioides* strain (from Cagliari sediments) is characterized by olive-green velvety colonies. It produces brown to olive-brown, small, single-celled, and lemon-shaped conidia.

3.3 Mycoremediation results

As described in Cecchi et al. (2019a), Genoa Port sediments were treated regarding heavy metal contamination with a mixed inoculum of *Penicillium expansum* and *Paecilomyces formosus.* As for Leghorn sediments, they were treated with mixed inoculum of *Penicillium brevicompactum* and *Mucor racemosus.* Fungal strains employed in the bioremediation test on Pisa sediments were *Cunninghamella elegans* and *Penicillium citrinum*. Finally, Cagliari sediments were treated with *Fusarium oxysporum* and *C. cladosporioides*.

As described in Cecchi et al. (2019a), selected fungi from Genoa sediments increase the membrane absorption of metals (in particular of Zn and Cu). Figure 2 shows Leghorn results and evidences that the recovery efficiency increases and reaches the maximum value after 60 days of treatment for each considered metal. Cr tot, Ni, and Mn appear to be hyper-bioaccumulated (percentage of recovery ≥ 100%). However, DRE values show that fungi start active bioaccumulating Cr tot after 15 days and Ni after 30 days: initial accumulation is due to the membrane (negative values). For Pisa sediments, RE data demonstrate that all metals are immediately bioaccumulated, but the accumulation values after the first 15 days is quite constant and does not exceed 20%; only Sb reaches the peak of accumulation after

Table 3 Results of sediment quality (HQ) assessment before and after mycoremediation treatment. Green: absent or negligible contamination; yellow: medium contamination; red: high contamination; and black: very high contamination

Initial characterization	Genoa sediments	Leghorn sediments	Pisa sediments	Cagliari sediments
HQ(L1)	8.16	0.24	117.23	16.08
HQ(L2)	0.51	0.09	44.84	6.39
After treatment	Genoa sediments	Leghorn sediments	Pisa sediments	Cagliari sediments
HQ(L1)	4.59	3.92	80.27	14.86
HQ(L2)	0.46	0.46	30.76	5.88

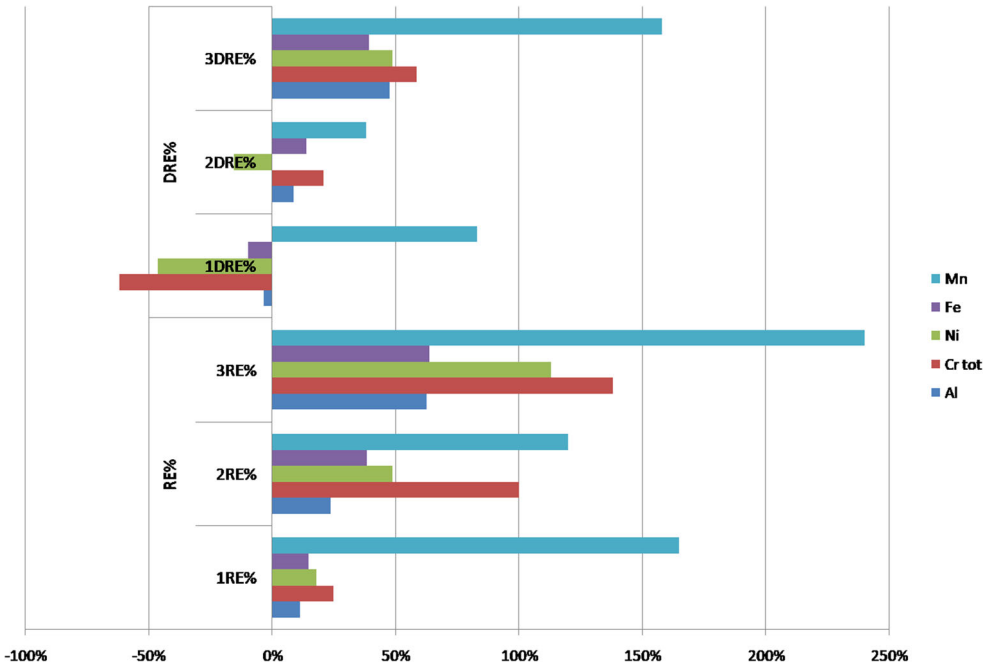

Fig. 2 The histogram shows the heavy metal recovery efficiency (RE %) and difference recovery efficiency (DRE %) of the membrane inoculated with autochthonous fungi on Leghorn sediments at 15, 30, and 60 days

60 days (> 70%; Fig. 3). DRE values show that Mn is excluded by selected fungi and it does not bioaccumulate, while other metals and in particular Cd, Cr tot, Zn, and Sb are actively bioaccumulated by fungi.

Cagliari RE results are shown in Fig. 4 and provide evidence that the main accumulated metals are as follows: Cr tot and Ni up to 30 days of treatment; Mn, Sb, and As up to the end of the treatment (60 days); Cd only at 60 days. However, DRE data show that selected fungal species are not able to bioaccumulate Cr tot, Ni, and Mn, and their accumulation is due to the membrane (negative values), while As and Cd are effectively bioaccumulated by fungi.

4 Discussion

The isolates belong to typically terrestrial and saprotroph fungi. Many studies have reported that some terrestrial fungi are able to adapt to marine environmental conditions playing a central role in the carbon cycle as biodegradators of organic

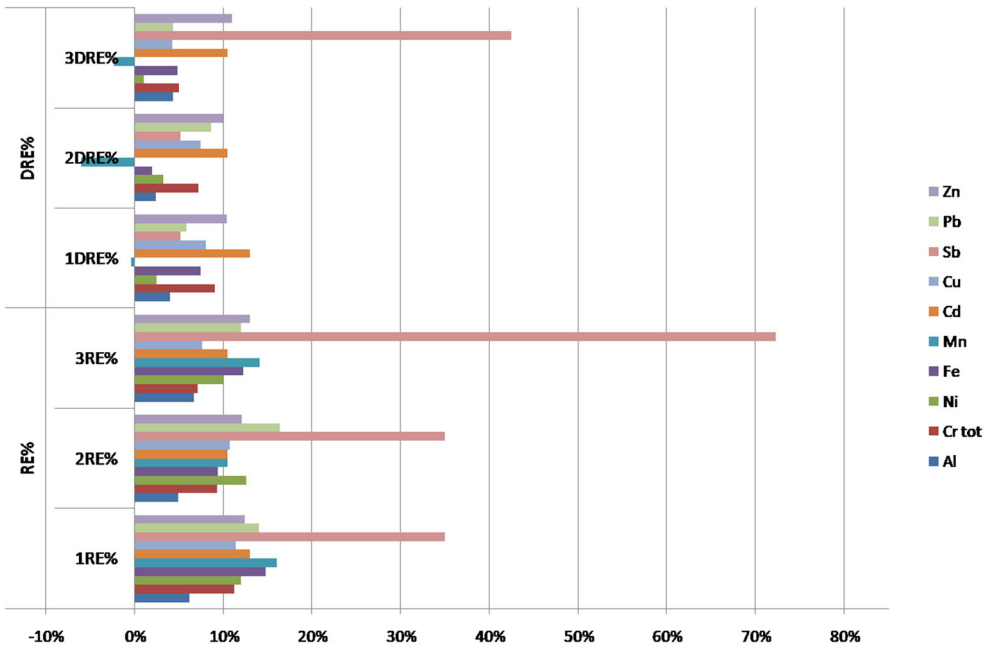

Fig. 3 The histogram shows the heavy metal recovery efficiency (RE %) and difference recovery efficiency (DRE %) of the membrane inoculated with autochthonous fungi on Pisa sediments at 15, 30, and 60 days

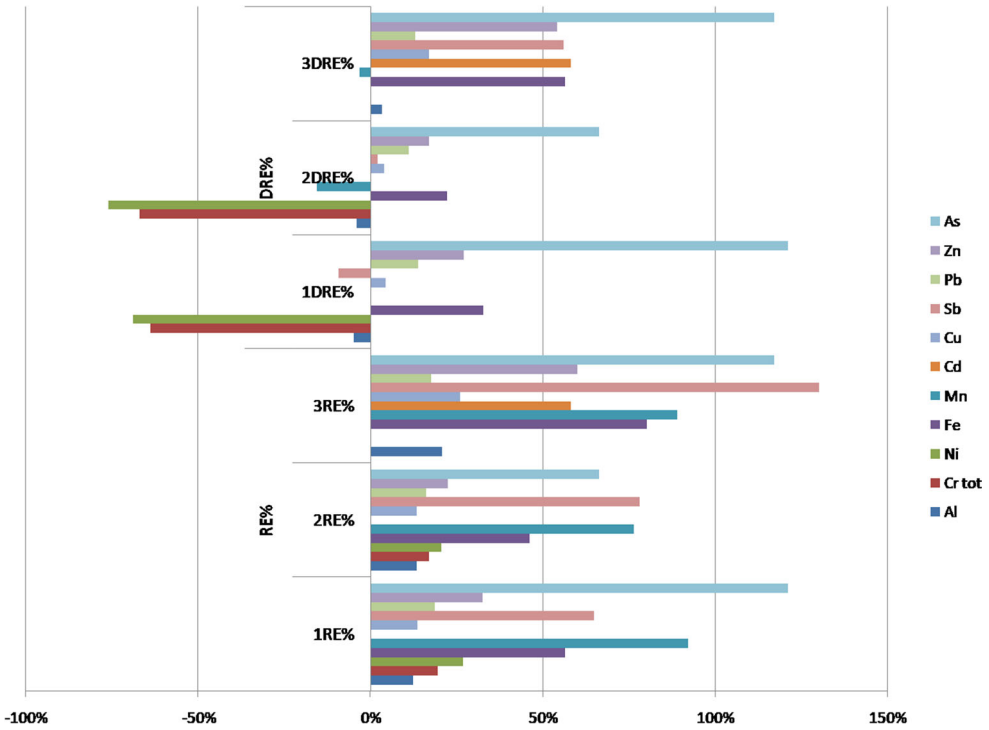

Fig. 4 The histogram shows the heavy metal recovery efficiency (RE %) and difference recovery efficiency (DRE %) of the membrane inoculated with autochthonous fungi on Cagliari sediments at 15, 30, and 60 days

matter (e.g., mineralization; Hyde et al. 1998; Capello et al. 2017). The use of autochthonous fungi and, in particular, of marine-derived fungi in the bioremediation of polluted saline environments is facilitated by their tolerance to saline conditions (Vala et al. 2018). In this study, autochthonous fungal strains are employed. *P. expansum* and *P. formosus* are selected by Cecchi et al. (2019a) because of their known capability of bioconcentrating metals (Di Piazza et al. 2017). *P. brevicompactum* and *Mucor racemosus*, isolated from Leghorn sediments, were also previously isolated from extreme environments contaminated by heavy metals and they exhibited the capability to bioaccumulate metals (Zhu et al. 2015; Cecchi et al. 2018b, 2019b). Moreover, *P. citrinum* together with *Cunninghamella elegans* are isolated from Pisa sediments and are known to bioaccumulate heavy metals such as Cu and Cd (de Lima et al. 2013; Verma et al. 2013). Abdullahi and Ibrahim (2018) together with Nakkeeran et al. (2018) highlighted the potential use of *C. cladosporioides* and *F. oxysporum* (isolated from Cagliari sediments) in the bioaccumulation of toxic metals.

Cecchi et al. (2019a) evidenced the high capability of fungal strains selected from Genoa sediments to increase the membrane absorption of metals. Treatment of Leghorn sediments showed the increase of metal bioaccumulation of the membrane by the selected fungal species during experimental time. It is interesting that the fungal uptake of Cr tot and Ni starts after 15 and 30 days, respectively. These metals are indifferent, without biological function, and they are often contaminants and toxic not only for the environments but also for organisms. Cr is highly toxic, mutagenic, and carcinogenic, and it spreads widely beyond the site of initial contamination because of its mobility. Filamentous fungi, such as *Aspergillus* sp., *Penicillium* sp., and *Trichoderma* sp., are known to reduce Cr(VI) to Cr(III) by exploiting the reducing power generated by carbon metabolism as mechanism of Cr(VI) detoxification, and are known for the ability to both biotransform Cr(VI) and accumulate it in the biomass (Viti et al. 2014). Ni naturally occurs in the environment, often combined with iron and sulfur, and it also has many industrial uses and anthropogenic sources that may contaminate the environment, representing a threat for human health. Cecchi et al. (2017b) selected a *Trichoderma harzianum* Rifai strain able to hyperaccumulate Ni.

For Pisa sediments, the DREs of metals show the Cd, Cr tot, Zn, and Sb bioaccumulation in fungal cells. Cd represents an indifferent metal without any biological function but its biomagnification in nature and migration through drinking water, food, and air to live organisms can cause severe health effects (Fazli et al. 2015). However, fungal biomass can act as a metal sink, either by Cd biosorption to biomass (cell walls, pigments and extracellular polysaccharides), intracellular accumulation and sequestration, or precipitation of Cd compounds onto and/or around hyphae (Fazli et al. 2015). On the contrary, Zn represents essential metal for the fungal cell metabolism and so it is actively accumulated, thanks to small molecular weight proteins such metallothioneins and phytochelatins (Lerch 1980; Cecchi et al. 2019a). Antimony (Sb) is considered a high priority global contaminant and there

are various studies elucidating antimony removal from polluted sites highlighting, among other approaches (e.g., coagulation) and reverse osmosis, methods based on the adsorption by Mn oxides (Milová-Žiaková et al. 2016). However, it is known that fungi play a central role in antimony mobility (Milová-Žiaková et al. 2016).

Cagliari results show that selected species exclude Cr tot and Ni as survival strategy in contaminated environments. Fungi, in fact, can adopt three possible strategies against toxic elements: (1) active metal bioaccumulation in fungal cell and storage in vacuoles and/or passive metals bioabsorption on fungal wall; (2) metal mobilization/transformation/immobilization in the external environments, thanks to metabolites and secondary organic acids production; (3) metal exclusion (Gadd 2007). The main accumulated metals, thanks fungal contribute, are Cd and As. Srivastava et al. (2011) isolated fifteen fungal strains from arsenic-contaminated soils. Out of fifteen, only five fungal strains were found resistant and survived with tolerance index pattern as 0.956: the most effective removal of arsenic was observed in the *Trichoderma* sp., *Neocosmospora* sp., and *Rhizopus* sp. (Srivastava et al. 2011).

The different degrees of metal bioaccumulation by fungi observed in the tests can be due to the following: environmental conditions, which can affect in situ mycoremediation of sediments (e.g., pH, temperature, low molecular weight organic acids and humic acids); season in which pilot activities were conducted; physical characteristics of sediments; and the different fungal species employed, altering transformation, transportation, valance state of heavy metals, and the bioavailability of heavy metals (Fonti et al. 2015). At the end of the experiment, the quality evaluation shows an improvement in sediments after mycoremediation, even if not such as to lower the sediment hazard class (Table 3). This could mean that for high levels of metal contamination, as in the case of Pisa sediments, the positive effect of mycoremediation is not enough for a possible re-use of sediments. This could be due to environmental and external factors which can negatively influence and slow down the metal uptake process (as mentioned above), for example, the physical characteristics of sediments, in the case of Pisa very fine and rich in clay and silt sediments (9.5% and 63.1%, respectively). Clay and silt sediments, in fact, as gravel do not positively influence fungal growth: the first does not allow fungal colonization due to the low oxygen values; the second favors the loss of the waters and nutrients by the leaching processes which affect fungi (Sinclair and Ghiorse 1989). On the other hand, other factors such as organic fraction (OF) can positively influence fungi. It increases fungal growth, because saprotrophic fungi degrade organic matter in their nutrition and speed up their metabolism favoring metal uptake (Yanardağ et al. 2017). Probably, a longer exposure of the sediments to the mycoremediation process could lead to the achievement of the targets in terms of hazard class. Surely, the chemical-physical-mycological characterization of the sediment becomes very important to well define the contamination degree, the potential limits of the sediments to the mycoremediation treatment, and the fungal species to employ. Fungi, in fact, can be generalist or selective in the metal bioaccumulation and the previous study of their behavior is essential. For example in Pisa Port sediments, Cd, Cr tot, Zn, and Sb are actively bioaccumulated by fungi (from 22, 320, 590, 6.7 mg kg^{-1} to 15.50, 210, 345, 5.25 mg kg^{-1}, respectively), while Mn is excluded, but the bioaccumulation process is significantly reduced after first 15 days of treatment. This fact underlines that some external factors (probably high amount of clay fraction) have affected fungal performance for the rest of experimental time not allowing the sediment transition to a lower hazard class. Regarding Cagliari, the scenario is totally different: selected fungi exclude the major metals and only Cd were actively bioaccumulated and removed from sediments (from 1.9 to 1.45 mg kg^{-1}), and the target has not been reached. Therefore, it will be necessary to deepen this aspect in the treatment process and found a possible solution. In this context, in the last decade, some researchers have started to evidence that integrated soil remediation technologies which combine physical/chemical/biological techniques appear to be promising approaches in this technical field allowing improved organic and inorganic pollutant removal efficiencies to be achieved (Gan et al. 2009). Fungi can be inhibited in their metabolic activities by too high concentrations of inorganic contaminants, so the mycoremediation

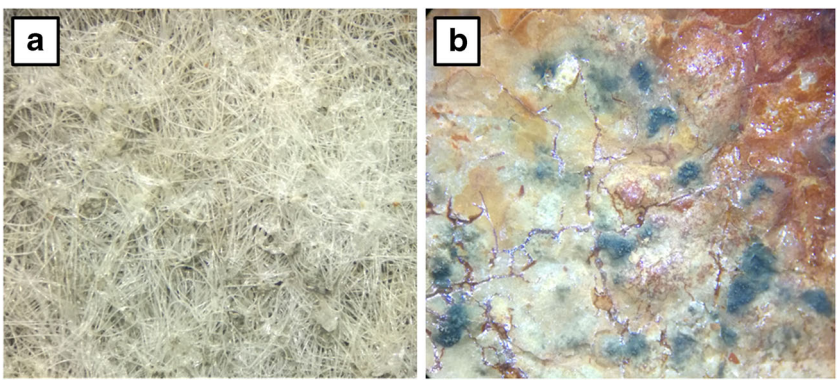

Fig. 5 The images were taken by the stereomicroscope and show the polyester fibrous membrane structure without fungal biofilm (**a**) and the membrane aspect after 60 days from the fungal inoculum of Leghorn sediment (**b**) (end of the experiment)

treatment can be longer and cheaper. On the other hand, fungi can be successfully employed in bioaccumulation of the remaining metal contamination in the sediments (Gan et al. 2009). This integrated biochemical approach can represent the new border of the remediation combining a shorter treatment time and sustainable methods able not only to remove the residual contamination but also to restore natural conditions. Moreover, a large number of studies highlighted the benefit of not only bioremediation integrated to traditional techniques but also of bioremediation of a combination of contaminants, because it is economic, environmental compatible, and of high disposal ability for combined contaminants of organics and inorganics (Zhang et al. 2007; Chen et al. 2015; Ma et al. 2016). The employed adsorbent membrane enriched with fungal inocula allows mycelium colonization (Fig. 5) and increases the metal accumulation not only on the membrane absorbent fibers but also in the fungal cells, representing a green and sustainable solution to the problem of the metal residual contamination of dredged sediments.

5 Conclusions

Our work evidenced that these fungal species are able to grow and root on a microporous support and are metabolically active. In particular, selected native species actively bioaccumulate metals into their cells, improving the membrane metal absorption. Only in the Cagliari case that fungi excluded metals as survival strategy. The application of a biomembrane system potentially employable in situ, in the remediation of the residual inorganic contamination, could represent useful and important approach. However, further studies should be conducted to asses this, in particular regarding the metal tolerance and bioaccumulation potential of fungi in different contaminated sediments. Mycotreatments may potentially offer low-impact chemical/physical remedial options for highly contaminated port sediments, allowing them to become a useful resource for future applications.

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References

Abdullahi M, Ibrahim AD (2018) Bioaccumulation of lead (Pb), chromium (Cr) and cadmium (Cd) by *Aspergillus flavus* and *Fusarium oxysporum* isolated from tannery wastewater. J Toxicol Environ Health 3:18–24

Aftab K, Akhtar K, Kausar A, Khaliq S, Nisar N, Umbreen H, Iqbal M (2017) Fungal strains isolation, identification and application for the recovery of Zn (II) ions. J Photochem Photobiol B 175:282–290

Akcil A, Erust C, Ozdemiroglu S, Fonti V, Beolchini F (2015) A review of approaches and techniques used in aquatic contaminated sediments: metal removal and stabilization by chemical and biotechnological processes. J Clean Prod 86:24–36

Ammami MT, Portet-Koltalo F, Benamar A, Duclairoir-Poc C, Wang H, Le Derf F (2015) Application of biosurfactants and periodic voltage gradient for enhanced electrokinetic remediation of metals and PAHs in dredged marine sediments. Chemosphere 125:1–8

Brils J, de Boer P, Mulder J, de Boer E (2014) Reuse of dredged material as a way to tackle societal challenges. J Soils Sediments 14:1638–1641

Capello M, Cutroneo L, Consani S, Dinelli E, Vagge G, Carbone C (2016) Marine sediment contamination and dynamics at the mouth of a contaminated torrent: the case of Gromolo Torrent (Sestri Levante, north-westernItaly). Mar Pollut Bull 109:128–141

Capello M, Carbone C, Cecchi G, Consani S, Cutroneo L, Di Piazza S, Greco G, Tolotti R, Vagge G, Zotti M (2017) A mycological baseline study based on a multidisciplinary approach in a coastal area affected by contaminated torrent input. Mar Pollut Bull 119(1):446–453

Cecchi G, Marescotti P, Di Piazza S, Zotti M (2017a) Native fungi as metal remediators: silver mycoaccumulation from metal contaminated waste rock dumps (Libiola Mine, Italy). J Environ Sci Health B 52(3):191–195

Cecchi G, Roccotiello E, Di Piazza S, Riggi A, Mariotti MG, Zotti M (2017b) Assessment of Ni accumulation capability by fungi for a possible approach to remove metals from soils and waters. J Environ Sci Health B 52(3):166–170

Cecchi G, Ceci A, Marescotti P, Persiani AM, Di Piazza S, Ballirano P, Mariotti MG, Zotti M (2018a) The geological roles played by microfungi in interaction with sulfide minerals from libiola mine, Liguria, Italy. Geomicrobiol J 35(7):564–569

Cecchi G, Marescotti P, Di Piazza S, Lucchetti G, Mariotti MG, Zotti M (2018b) Gypsum biomineralization in sulphide-rich hardpans by a native *Trichoderma harzianum* Rifai strain. Geomicrobiol J 35(3):209–214

Cecchi G, Vagge G, Cutroneo L, Greco G, Di Piazza S, Faga M, Zotti M, Capello M (2019a) Fungi as potential tool for pollute port sediment remediation. Environ Sci Pollut Res:1–8. https://doi.org/10.1007/s11356-019-04844-5

Cecchi G, Ceci A, Marescotti P, Persiani AM, Di Piazza S, Zotti M (2019b) Interactions among microfungi and pyrite-chalcopyrite mineralizations: tolerance, mineral bioleaching, and metal bioaccumulation. Mycol Prog 18(3):415–423

Chen M, Xu P, Zeng G, Yang C, Huang D, Zhang J (2015) Bioremediation of soils contaminated with polycyclic aromatic hydrocarbons, petroleum, pesticides, chlorophenols and heavy metals by composting: applications, microbes and future research needs. Biotechnol Adv 33:745–755

de Lima M, Franco L, de Souza P, do Nascimento A, da Silva C, Maia R, Rolim H, Takaki G (2013) Cadmium tolerance and removal from *Cunninghamella elegans* related to the polyphosphate metabolism. Int J Mol Sci 14(4):7180–7192

Di Piazza S, Cecchi G, Cardinale AM, Carbone C, Mariotti MG, Giovine M, Zotti M (2017) *Penicillium expansum* link strain for a biometallurgical method to recover REEs from WEEE. Waste Manag 60:596–600

Di Piazza S, Zotti M, Barranco R, Cecchi G, Greco G, Ventura F (2018) Post-mortem fungal colonization pattern during 6 weeks: two case studies. Forensic Sci Int 289:18–23

Domsch KH, Gams W, Anderson TH (1980) Compendium of soil fungi. Academic Press, London, p 860

Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissues. Phytochem Bull 19:11–15

Fathollahzadeh H, Kaczala F, Bhatnagar A, Hogland W (2014) Speciation of metals in contaminated sediments from Oskarshamn

Harbor, Oskarshamn, Sweden. Environ Sci Pollut Res 21(4):2455–2464
Fazli MM, Soleimani N, Mehrasbi M, Darabian S, Mohammadi J, Ramazani A (2015) Highly cadmium tolerant fungi: their tolerance and removal potential. J Environ Health Sci Eng 13:19
Fonti V, Beolchini F, Rocchetti L, Dell'Anno A (2015) Bioremediation of contaminated marine sediments can enhance metal mobility due to changes of bacterial diversity. Water Res 68:637–650
Gadd GM (2007) Geomycology: biogeochemical transformations of rocks, minerals, metals and radionuclides by fungi, bioweathering and bioremediation. Mycol Res 111(1):3–49
Gan S, Lau EV, Ng HK (2009) Remediation of soils contaminated with polycyclic aromatic hydrocarbons (PAHs). J Hazard Mater 172(2–3):532–549
Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes application to the identification of mycorrhizae and rusts. Mol Ecol 2(2):113–118
Glass NL, Donaldson GC (1995) Development of primer sets designed for used with the PCR to amplify conserved genes from filamentous ascomycetes. Appl Environ Microbiol 61(4):1323–1330
Harrington JR, Murphy J, Coleman M, Jordan D, Debuigne T, Szacsuri G (2016) Economic modelling of the management of dredged marine sediments. Geol Geophys Environ 42(3):311–324
Hyde KD, Jones EG, Leaño E, Pointing SB, Poonyth AD, Vrijmoed LL (1998) Role of fungi in marine ecosystems. Biodivers Conserv 7(9):1147–1161
Lerch K (1980) Copper metallothionein, a copper-binding protein from *Neurospora crassa*. Nature 284:368–370
Li WW, Yu HQ (2015) Stimulating sediment bioremediation with benthic microbial fuel cells. Biotechnol Adv 33:1–12
Liu SH, Zeng GM, Niu QY, Liu Y, Zhou L, Jiang LH, Tan X, Xu P, Zhang C, Cheng M (2017) Bioremediation mechanisms of combined pollution of PAHs and heavy metals by bacteria and fungi: a mini review. Bioresour Technol 224:25–33
Ma XK, Ding N, Peterson EC, Daugulis AJ (2016) Heavy metals species affect fungal-bacterial synergism during the bioremediation of fluoranthene. Appl Microbiol Biotechnol 100:7741–7750
Milová-Žiaková BM, Uríka M, Boriova K, Bujdosa M, Kolencík M, Mikusova P, Takacova A, Matúsa P (2016) Fungal solubilization of manganese oxide and its significance for antimony mobility. Int Biodeterior Biodegradation 114:157–163
Nakkeeran E, Rathna R, Viveka R (2018) Mechanism and action of A*aureobasidium pullulans* on biosorption of metals. In: Waste bioremediation. Springer, Singapore, pp 215–231
Nelson PE, Toussoun TA, Marassas WFO (1983) *Fusarium* species, an illustrated manual for identification. Pennsylvania State University Press, University Park
OSPAR Commission (2014) OSPAR Guidelines for the management of dredged material at sea. Draft Summary Record - EIHA, Annex 7. https://dredging.org/media/ceda/org/documents/guidance/ospar/ospar-dredged-material, pp 34. Accessed 31 March - 4 April 2014
Pitt JI, Samson RA, Frisvad JC (2000) List of accepted species and their synonyms in the family *Trichocomaceae*. In: Samson RA, Pitt JI (eds) Integreation of modern taxonomic methods for *Penicillium* and *Aspergillus* classification. Harwood Academic Publishers, Amsterdam, pp 9–79
Rashid A, Bhatti HN, Iqbal M, Noreen S (2016) Fungal biomass composite with bentonite efficiency for nickel and zinc adsorption: a mechanistic study. Ecol Eng 91:459–471
Regoli F, d'Errico G, Nardi A, Mezzelani M, Fattorini D, Benedetti M, Di Carlo M, Pellegrini D, Gorbi S (2019) Application of a weight of evidence approach for monitoring complex environmental scenarios: the case-study of off-shore platforms. Front Mar Sci 6:377
Sinclair JL, Ghiorse WC (1989) Distribution of aerobic bacteria, protozoa, algae, and fungi in deep subsurface sediments. Geomicrobiol J 7(1–2):15–31
Spina F, Cecchi G, Landinez-Torres A, Pecoraro L, Russo F, Wu B, Cai L, Liu XZ, Tosi S, Varese GC, Zotti M, Persiani AM (2018) Fungi as a toolbox for sustainable bioremediation of pesticides in soil and water. Plant Biosyst 152(3):474–488
Srivastava PK, Vaish A, Dwivedi S, Chakrabarty D, Singh N, Deo Tripathi R (2011) Biological removal of arsenic pollution by soil fungi. Sci Total Environ 409:2430–2442
Surricchio G, Pompilio L, Novelli AA, Scamosci E, Marinangeli L, Tonucci L, d'Alessandro N, Tangari AC (2019) Evaluation of heavy metals background in the Adriatic Sea sediments of Abruzzo region, Italy. Sci Total Environ 684:445–457
Vala AK, Sachaniya B, Dave BP (2018) Marine-derived fungi: promising candidates for enhanced bioremediation. In: Prasad R, Aranda E (eds) Approaches in bioremediation. Nanotechnology in the life sciences. Springer, Cham
Verma A, Singh A, Bishnoi NR, Gupta A (2013) Biosorption of Cu (II) using free and immobilized biomass of *Penicillium citrinum*. Ecol Eng 61:486–490
Viti C, Marchi E, Decorosi F, Giovannetti L (2014) Molecular mechanisms of Cr(VI) resistance in bacteria and fungi. FEMS Microbiol Rev 38:633–659
Walker TR, MacLean B, Appleton R, McMillan S, Miles M (2013) Cost-effective sediment dredge disposal options for small craft harbours in Canada. Remed J23(4):123–140
White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR – Protocols and applications – a laboratory manual. Academic Press, pp 315–322
Yanardağ IH, Zornoza R, Bastida F, Büyükkiliç-Yanardağ A, García C, Faz A, Mermut AR (2017) Native soil organic matter conditions the response of microbial communities to organic inputs with different stability. Geoderma 295:1–9
Zhang Y, Huang DL, Jiang XY, Chen YN (2007) A hydroquinone biosensor based on immobilizing laccase to modified core-shell magnetic nanoparticles supported on carbon paste electrode. Biosens Bioelectron 22:2121–2126
Zhang Z, Lo I, Yan D (2015) An integrated bioremediation process for petroleum hydrocarbons removal and odor mitigation from contaminated marine sediment. Water Res 83:21–30
Zhu S, Tang J, Zeng X, Wei B, Yang S, Huang B (2015) Isolation of *Mucor circinelloides* Z4 and *Mucor racemosus* Z8 from heavy metal-contaminated soil and their potential in promoting phytoextraction with Guizhou oil seed rap. J Cent South Univ 22(1):88–94
Zycha H, Siepmann R, Linnemann G (1969). Mucorales, eine Beschreibung alter Gattungen und Arten dieser Pilzgruppe. Verlag Lehre, J. Cramer, 355 pp