Fungi as tool of polluted port sediment remediation

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Introduction

Contamination of marine sediments related to port activities represents one of the most actual and global scale environmental problems (Akcil *et al.*, 2015; Fathollahzadeh *et al.*, 2014; Tavakoly Sany *et al.*, 2013). In recent decades, anthropogenic sources led to high concentrations of heavy metals and organic material in marine areas (Greco *et al.*, 2017). Polluted sediment represents an important management problem closely related to the need of periodical dredging activities for the maintenance of the navigational depth in ports and waterways, but also of remediation (Akcil *et al.*, 2015). During dredging activities very large volumes of contaminated sediment may be produced and need to be properly handled (Akcil *et al.*, 2015). The contamination is often due to both organic and inorganic pollutants (mostly metals) in the sediment (Capello *et al.*, 2017). Conventional management-remediation strategies of polluted sediment involve sediment washing, electron-chemical separation and thermal treatment (Akcil *et al.*, 2015). Moreover, recently bioremediation strategies have been also considered as a promising answer to the problem of contaminated sediment (Akcil *et al.*, 2015; Tabak *et al.*, 2005). Autochthonous microorganisms, in fact, are both already adapted at the environmental conditions and the most suitable to remediate polluted sediments (Cecchi *et al.*, 2017a). Bioremediation strategies consist basically into stimulate and exploit the microbial function leading to the bioremediation objectives. However, at now the major bioremediation studies of marine sediments were conducted with the employment of bacteria (Johnson, 2013; Tabak *et al.*, 2005), and few researches regarded the role and the bioremediation capability of fungi. These latter, in fact, are pioneer microorganisms able to survive in extreme conditions, and to colonize every kind of environment, including the marine one (Cecchi *et al.*, 2017c; Gadd *et al.*, 2012). Thanks to their metabolic proprieties fungi secrete enzymes and organic acids that may interact with contaminants. Fungi, in fact, are known to bioconcentrate, bioaccumulate and biostabilize heavy metals (Cecchi *et al.*, 2017 a,b; Di Piazza *et al.*, 2016), and to degrade organic pollutants such as heavy hydrocarbons (C>12) and polycyclic aromatic hydrocarbons (PAH) (Capello *et al.*, 2017; Greco *et al.*, 2017). Hence the possibility to employ indigenous fungal strains in order to remediate polluted port sediments.

In the framework of the European Interreg 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, carries on a pilot activity on sediments dredged in the Port of Genoa (Italy), for the mycological characterization, the study, and the treatment with an innovative protocol of mycoremediation in order to evaluate the remediation potential of indigenous fungal strains. This work shows the preliminary results of the mycological pilot activity.

Materials and Methods

30 kg of sediments were collected at the Port of Genoa with a 5-liter steel Van Veen grab, and 3 liters of sea waters were taken with a 10-liters Niskin bottle for the subsequent analyzes and laboratory activities.

1 kg of sediment was immediately sent to the Environmental Protection Agency Laboratories in order to evaluate initial contamination. They investigated the following parameters: PAHs, C>12 Hydrocarbons, PCBs, and Heavy metals (i.e., Al, As, Cd, Cr, Cu, Ni, Pb, Zn, Hg, Mn, Fe).

Moreover, sediment was employed for fungal characterization, using the dilution plate technique (Gams *et al.*, 1987). Marine Rose Bengal (MRB) medium was prepared using sterilized port seawater, and after inoculum plates were incubated in the dark at 24°C, and were checked weekly. Fungal colonies grown on the plates were isolated and maintained in axenic cultures in the cultures collection of the Mycological Laboratory of DISTAV. These strains were studied by a polybasic approach (morphological, physiological and molecular). Five plastic boxes were prepared with 5 kg of port sediment in order to carry out the mycoremediation activities. Each box represents a mesocosm treated with a peculiar protocol in order to select the best mycoremediation practice both for heavy metals bioremediation and organic pollutants degradation. Two boxes tested about fungal metal bioconcentrations were covered with a peculiar membrane allowing the physical (but not chemical) separation between fungus and sediment. The fifth box was used as control (hereinafter C).

Figure 1 showed the experimental preparation. Samples of sediment and colonized membrane were collected from the boxes in order to be analyzed after 15, 30 and 60 days.

Results

Preliminary sediment analyses evidenced they were characterized by high concentrations of contaminants. Among the isolated strains *Aspergillus*, *Penicillium* and *Trichoderma* appeared the most common genera in the original sediments, and 773 colonies forming units (CFU) were counted on MRB plates.

One week after, fungal treatments both sediment and membrane were completely covered by fungi. Sediment and membranes collected during planned samplings (15, 30, 60 days) were analyzed concerning metals, PAH and heavy hydrocarbons content. After 15 days, sediment analyses showed a constant or slightly increased metal concentration, due to the bioaccumulation at the sediment-felt interface, caused by the metabolic and fungal solubilization activities. We are still working on the PAHs and C>12 hydrocarbon concentrations.

Discussion

The results evidenced how contaminated marine sediments were colonized by high number of fungal strains. The majority of these strains were fungi able to live both in terrestrial and marine environment. Typical and closely marine fungi were not found yet. Some of the isolated species were known for their organic pollutants-contaminants degradation and heavy metals bioconcentration capability such as *Fusarium solani* (Mart.) Sacc. and *Penicillium expansum* Link respectively (Di Piazza *et al.*, 2016), and so were selected for the remediation experiment. After 15 days fungi were able to colonize all membranes and sediments, and to biodegrade and bioconcentrate high amounts of organic pollutants and heavy metals.

Conclusion

This work represented the first step in the search of innovative and sustainable remediation techniques of polluted-contaminated marine sediments using pioneer, highly adapted and excellent degradator and accumulator organisms such as fungi. Moreover it showed the preliminary results of the tuning of an efficient fungal – membranes protocol employable in metals mycoremediation processes.

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Figure 1. The five mesocosms used for the pilot activities: the two boxes on the left side are equipped with the membranes while the first one on the right of the photo is the C (control) box.