The European Interreg Maritime Project "SEDITERRA". From waste to resource: mycoremediation of contaminated marine sediments

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Introduction: Contamination of dredged sediments represents one of the most actual environmental problems [1]. Indeed, port sediments are often contaminated by organic and inorganic pollutants derived from different sources. Moreover, port dredging activities produce large volumes of contaminated sediments [1]. In recent decades, dredge sediments were considered no longer as waste but as a resource employable for example in building routes. In order to do this, it is indispensible to decontaminate sediments. In the framework of a few scientific researches, traditional and innovative techniques, which allow sediments decontamination, have been tested. In terms of bioremediation strategies, microorganisms are stimulated and exploited to decontaminated media (e.g. sediments). In particular, mycoremediation consists in the exploiting of fungal organisms naturally able to synthesize enzymes and organic acids which interact with contaminants. Fungi are able to bioconcentrate, bioaccumulate, and biostabilize heavy metals and to degrade organic pollutants (e.g. PAHs, hydrocarbons C>12 and PCB) [2; 3]. 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 mycoremediate marine dredge sediments. The aim of this study was to characterise fungal communities of 4 port sediments, evaluate the efficiency of the mycoremediation protocol and compare results obtained in each treatment.

Methods: Mycoremediation pilot activity was carried out on port sediments from Genoa, Leghorn, Pisa and Cagliari. 30 Kg of sediment were collected in each port: 5 g was used for fungal characterization, 1 kg for chemical characterization (metals, PAHs, PCB, and hydrocarbons C>12), 215 g for physical characterization (definition of organic and inorganic fraction and grain size analysis), 25 kg for mycoremediation activity. Fungi were isolated by the dilution plate technique and identified by a polybasic approach (morphological, physiological and molecular). 5 plastic boxes were prepared with 5 kg port sediment for pilot mycological activity. Box 1 and 2 were used for testing fungi efficiency in bio-accumulating metals. Sterile membranes were positioned on sediments and inoculated with selected fungal strains. Membranes allow physical (not chemical) mycelia separation from sediments. Box 3 and 4 were treated with selected fungi for testing specifically PAH and PCB degradation. Box 5 was used as control: it was maintained at the same condition of the other boxes and a control membrane was positioned on sediment without fungal inoculum. Sediment samples were collected from the boxes after 15, 30, and 60 days after inoculum in order to evaluate their metals, PAH, PCB, hydrocarbons C>12 content. Moreover, colonized and control membranes were collected at the same time in order to evaluate their metals content.

Results: Characterization of the fungal flora shows that the predominant fungal genera were *Penicillium*, *Trichoderma* and *Aspergillus*. All ports sediments were predominantly composed by inorganic material and, except Pisa sediments, they were characterized by the coarse grain size (>63 μm). Results of chemical analysis show differences in the 4 port sediments treated: metals and organic pollutants were respectively absorbed and degraded in varying degrees.

Discussion: Differences in mycoremediation efficiency may be due to the different contamination degree, chemical and physical sediment characteristics, season in which pilot activities were conducted and, in particular, the different fungal species employed. In fact, indigenous fungal species were used for each sediment in order to avoid introduction of new species in environments in view of the possible application of this protocol directly *in situ*.

Acknowledgment: This study was funded by the European Interreg Italy-France 2014-2020 Maritime Project SEDITERRA "Guidelines for the sustainable treatment of dredged sediments in the Marittimo area" (CUP I42F17000010006).

References: [1] OSPAR Commission (2014) *Draft Summary Record - EIHA*, Annex **7**, pp. 34; [2] Cecchi et al. (2017) *J. Environ. Sci. Heal. B* **52**: 191–195; [3] Spina et al. (2018) *Plant Biosyst* **152**:474-488.