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Baseline

A mycological baseline study based on a multidisciplinary approach in a coastal area affected by contaminated torrent input

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ABSTRACT

Fungi include a vast group of eukaryotic organisms able to colonise different natural, anthropised and extreme environments, including marine areas contaminated by metals. The present study aims to give a first multidisciplinary characterisation of marine bottom sediments contaminated by metals (Cd, Co, Cr, Cu, Ni, and Zn), originating in the water leakage from an abandoned Fe-Cu sulphide mine (Libiola, north-western Italy), and evaluate how the chemical and physical parameters of water and sediments may affect the benthic fungal communities. Our preliminary results showed the high mycodiversity of the marine sediments studied (13 genera and 23 species of marine fungi isolated), and the great physiological adaptability that this mycobiota evolved in reaction to the effects of the ecotoxic bottom sediment contamination, and associated changes in the seawater parameters.

Sulphide mine discharges and, in particular, acid mine drainage (AMD) may be considered as sources of high ecotoxic metal concentrations in both river and, consequently, marine environments (Asta et al., 2015; Hierro et al., 2013; Nieto et al., 2007). In the sulphide mine context, pyrite and other associated sulphide minerals are exposed to water and oxygen, creating a series of chemical and biochemical reactions (Nieto et al., 2007). Hence, the production of mine water discharge is characterised by elevated acidity and a high concentration of sulphates and metals (Fe, Cu, Zn, Co, Cr, Mn, Pb, Cd, etc.). Mine-water discharge may flow into streams and rivers, which consequently release a significant contaminant load in estuarine areas, due to the high concentration of dissolved metals and sulphates that they transport. Recent studies have shown that pH, and especially salinity, plays an important role in the bioavailability of these metals to different organisms, including toxicity (Baldó et al., 2005; Riba et al., 2003, 2004). The consequence of an excessive quantity of ecotoxic metals could be a shift in the distribution of living populations, included fungi.

Fungi are a huge group of heterotrophic eukaryotes organisms able to colonise different natural, anthropised and extreme environments (Di Piazza et al., 2016; Khambhaty et al., 2009; Onofri et al., 2008); they play a key role in the decomposition of organic material, such as vegetal tissues (cellulose and lignin) and, to a lesser extent, animal tissues such as keratin and chitin (Bugni and Ireland, 2004).

In the marine environment, fungi could be either obligate or facultative marine-fungi (Cuomo et al., 1988; Kohlmeyer and Kohlmeyer, 1979). In this environment, particularly in coastal waters, they are most common in decomposing wood and plant detritus (Hyde et al., 2000; Kohlmeyer and Kohlmeyer, 1979; Newell, 1996; Raghukumar, 2004; Sridhar, 2005), and are also in calcareous animal shells, algae and corals (Golubic et al., 2005; Zhang et al., 2015). However, it has not been proven whether these species merely survive and endure the prevailing conditions or play an active role in the ecosystem (Mouton et al., 2012). Marine fungi from sediment have been reported to be involved in denitrification processes, and recent studies have demonstrated that several fungi, included marine fungi, have the capacity to reclaim environments contaminated by inorganic substances, such as metals, performing a determinant role in the sector of bioremediation, as in the case of absorption of Ni, Ag, Zn, Pb, Ca, and Cu by the fungal biomass (Cecchi et al., 2017a, 2017b; Mouton et al., 2012; Raghukumar, 2012; Ramalingam et al., 2011; Zotti et al., 2014). However, the marine ecosystem represents a largely unexplored niche for unidentified fungi that could potentially be used in biotechnological processes (Passarini et al., 2011).

The present study, part of a larger research that involves interactions between abiotic and biotic factors, aims to give a first multidisciplinary characterisation of marine bottom sediments in the eastern part of the Gulf of Tigullio (north-western Italy) contaminated by high ecotoxic metal concentrations originating in the AMD of the abandoned Libiola Fe-Cu sulphide mine (Sestri Levante). We also considered how the chemical and physical parameters may affect the biotic composi-

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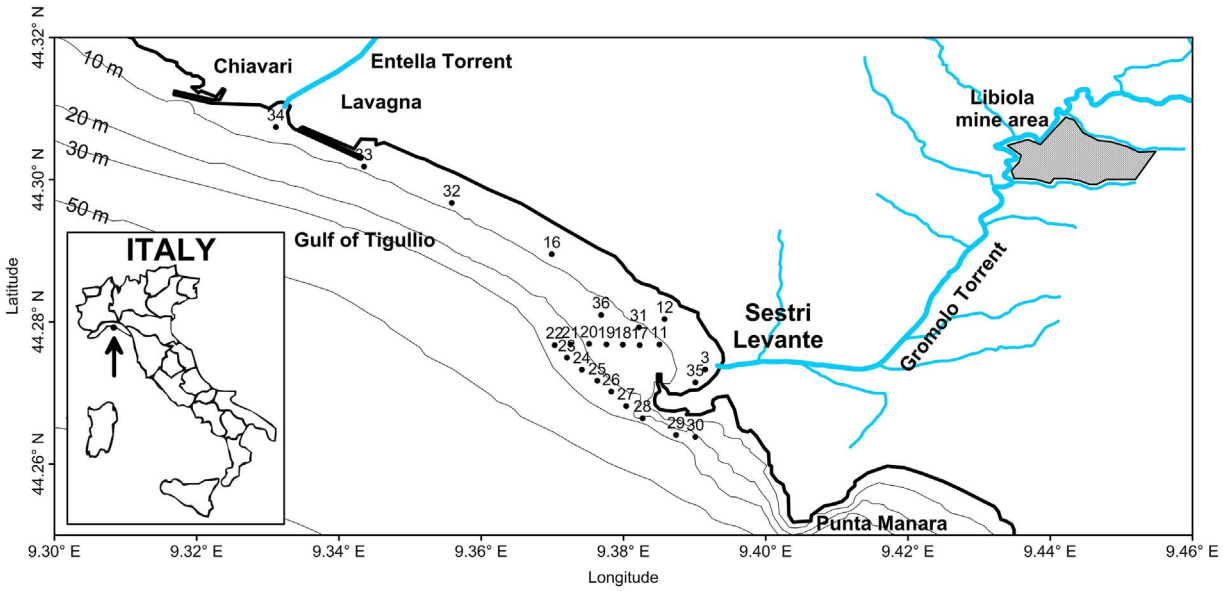

Fig. 1. The study area of Sestri Levante (north-western Italy) is localised between the mouth of the Entella Torrent (West) and Punta Manara (East); Entella and Gromolo (9 km-long) torrents are highlighted by a light blue line and the localisation of the Libiola mine is shown by a grey polygon; sampling stations are represented by black circles; the thin black line shows the bathymetry (in m). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

tion, in particular that of the fungal communities.

The study area (Fig. 1) lies in north-western Italy, along the Ligurian coast, between the mouth of the Entella Torrent and the Punta Manara headland, and comprises the headland of Sestri Levante. The western bay of Sestri Levante hosts the Gromolo Torrent mouth (Fig. 1) that is affected by the metal contamination originating in the AMD of the abandoned Libiola Fe-Cu sulphide mine (period of exploitation 1864–1962; Carbone et al., 2005). The torrent contamination also affects the marine-bottom sediments off the torrent mouth, with enrichment mainly in Cu, Zn, Cr and Ni (Capello et al., 2016).

Bottom sediments were collected along the coast between Chiavari and Punta Manara at 24 sampling stations (Fig. 1) on the 21st July 2015, in weather condition of stable high pressure, calm seas and a light breeze. The bottom sediments were sampled using a 5-L Van Veen grab. A volume of 100 mL of superficial bottom sediments (upper 2 cm of the sediments) was collected at each study station and stored in wide-necked plastic jars previously treated with HCl to eliminate any organic substance. Another portion of sediment was collected to determine the grain-size, as well as for mineralogical and chemical analyses according with the methodology reported in Capello et al. (2016). Twelve metal and trace element concentrations (As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, V, and Zn) were determined in the sediments: the metal composition was investigated with an Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-ES) on 1 g of samples digested in hot aqua-regia, while trace element concentrations were determined by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) on 0.5 g of sample after modified aqua-regia digestion at Bureau Veritas Mineral Laboratories (Canada; ISO9001Quality Management Systems). The chemical-physical characteristics of the seawater near the bottom were obtained using a conductivity–temperature–depth (CTD) multiparametric probe (Idromar Ambiente) equipped with a turbidimeter (values in Formazin Turbidity Units, FTU; range 0 to 100 FTU), Photosynthetically Active Radiation (PAR; light in the 400 to 700 nm wavelength range, expressed in μmol m^{-2} s^{-1}), and dissolved oxygen (in %) sensors. The salinity was determined using the Practical Salinity Scale. The pH values were determined from the water of the sediment samples with a portable pH meter (ExStick®ph Meter, Extech Instruments Corporation). Current velocity and direction data (expressed in E and N components) were measured with an over-the-side-mounted current meter (Vertical Acoustic Doppler Current Profile - V-ADCP, Teledyne RDI 600-kHz Workhorse®) with bottom-track function.

Due to limited data on the fungal community in this kind of environment, and in order to collect useful data to plan the subsequent survey, the isolation of the vital fungal strains was performed on three non-specific culture media prepared with sea water (collected in the study area): MEA (Malt Extract Agar), MEA + C (MEA with the addition of Chloramphenicol), and RB (Rose Bengal). 1 mL of each contaminated sediment sample was placed in a Petri dish (diameter 9 cm). A total of 72 Petri dishes were inoculated. Later, they were incubated at 20 °C in the dark for 28 days. The plates were checked weekly and the number of fungal colonies grown was counted after 4 weeks. Then, vital fungal strains were isolated from the plates by repetitive culturing, and preserved in axenic cultures using test tubes containing MEA medium. Moreover, the isolated microfungal strains were stored at 4 °C and cryopreserved at − 20 °C in the Mycological collection of the Laboratory of Mycology at the Department of Earth, Environment and Life Sciences of the University of Genoa. The isolated fungal strains were later identified with a polybasic approach which determined the micro- and macromorphological characteristics through the use of specific taxonomical keys (Klich, 2002; Korneup and Wansher, 1978; Malloch, 1981; Pitt, 1979; Raper and Fennel, 1977; Samson and Frisvad, 2004; Samson and Pitt, 1985) and then by optical microscopy (10 ×/0.30 to 40 ×/0.75). The identities of the isolated fungal strains were confirmed using nuclear DNA extraction, PCR amplification and DNA sequencing.

The genomic DNA was extracted from 100 mg of fresh fungal culture using a modified CTAB method (Doyle and Doyle, 1987). The morphological identifications were confirmed by amplification of the β-tubulin gene using Bt2a and Bt2b primers (Glass and Donaldson, 1995) and the ITS region amplification using universal primers ITS1F/ITS4 (Gardes and Bruns, 1993).The PCR products were purified and sequenced using MACROGEN Inc. (Seoul, Republic of Korea). Sequence assembly and editing were performed using Sequencher® version 5.2 (sequence analysis software, Gene Codes Corporation, Ann Arbor, MI USA).

Taxonomic assignment of the sequenced samples was carried out using the BLASTN algorithm to compare sequences obtained in the present study with the GenBank database (https://www.ncbi.nlm.nih.gov/genbank/).

All the samplings, measurements and analyses carried out on the