An Integrated Study On Mytilus Galloprovincialis in Large-Scale Mesocosms

This work presents the effect of a soil washing procedure on a biological system (Mytilus galloprovincialis) maintained in a mesocosm system designed to maintain the resuspension of the sediments, as it can happen in open water in case of storms or turbulence induced by the wave motion or by boats. The set up included:

1- a mesocosm, consisting of uncontaminated seawater;

2- a mesocosm consisting of contaminated sediment;

3- a mesocosm conceived to test the soil washing process efficiency on the biological environment.

The multidisciplinary approach included: the evaluation of metal uptake by the organisms, the study on DNA damages, morphological and ultrastructural observations and mRNA expression of those genes involved in cellular stress response, with particular emphasis to that caused by heavy metal exposure. All together our results support the concept that is important to evaluate the remediation method not only in terms of reduction of toxic components but also on a biological system since the effectiveness of the treatment may be, even though temporary, adverse effects on biological systems.

Introduction

Marine sediments may contain several hazardous compounds including petroleum hydrocarbons, organochlorines and heavy metals. Although the delivery of the perilous elements from the sediments can be very low, there is no doubt that, after the release, they are gradually taken into human body through the bio-concentration in aquatic animals (Masaharu et al. 1997). Due to its crucial geographical position, the Augusta bay (eastern Sicily, Italy) hosts one of the largest and most complex petrochemical industry sites in Europe which consists of oil refineries, chemical plants, mineral deposits, and a military base (Rossi et al. 2016; Cappello et al. 2013; Sprovieri et al., 2011). Nowadays, Augusta bay is an area of high environmental risk due to the uncontrolled discharges that occurred before the Water Pollution Control Law as well as frequent industrial accidents (Ausili et al., 2008); consequently, sediments resulted contaminated by several pollutants, included mercury (Hg), derived from a chloralkali plants since the 60s. The chloralkali plants carry out industrial process for the electrolysis of sodium chloride, a technology used to produce chlorine and sodium hydroxide (Castner-Keller method, or “Solvay method”) which are commodity chemicals required by industry.

The electrolytic cell consists of a graphite anode and metallic Hg cathode, therefore each ton of sodium hydroxide produced release small quantities of Hg in the air and more massive amount in the process water. It is estimated that about 500 tons of Hg have been spilled to the sea between 1958 and 1979. Furthermore, as reported in recent studies on sediments collected from the coastal zone of Augusta (Di Leonardo et al., 2014), the levels of aromatic hydrocarbons, dioxins, and metals, exceed national and international regulatory guidelines, thus the removal of contaminants from marine ecosystem is one of the biggest environmental concerns that is of interest for both researchers and industries (Abbasian et al. 2016). It is also known that marine sediments are key contributors of methyl-Hg, the organic formulation of Hg, which shows a greater toxicity compared to inorganic Hg-compounds and is the main cause of Hg bioaccumulation and biomagnification in fish. Indeed in the last decade, several studies provided detailed information on the pollution levels and risks for human health of resident population of Augusta Bay (ICRAM, 2005; Ausili et al., 2008; Di Leonardo et al., 2007, 2008; ENVIRON International Team, 2008; Ficco et al., 2009; Sprovieri et al., 2011). Data recently collected by ICRAM (2008), ENVIRON International Team (2008) Ausili et al. (2008), demonstrated the Hg transfer from the abiotic system (sediments and seawater) to fishes (top predators and filter-feeders) and documented a significant health risks associated with the consumption of fish caught in the area.

This scenario suggests a need to apply methods for the identification, evaluation and intervention to reduce the risks for the environment and inhabitants. In this respect several strategies, particularly focused to metal removal, have been set up, tested and then adopted (Randall, 2006).

The countermeasures adopted for the remediation of marine sediments include physical, chemical, thermal, and biological processes (Ndimele et al., 2018). Among several technologies, that of soil washing, which employs a chemical water-based process and physical particle size separation, can be applied to reduce contaminant concentration in soil and sediments (USEPA 1997, USEPA, 2007; Dermont, 2008). This process is based on the concept that most contaminants tend to bind to the finer soil particles (clay and silt) rather than the larger particles (sand and gravel). Physical methods are used to concentrate contaminants by exploiting differences in characteristics between the metal particles and soil particles (Sierra, 2010, 2011). Sodium thiosulfate, thiosulfate, iodide and nitric acid, hydrochloric acid (HCl), sodium hydroxide (NaOH) and potassium iodide (KI) are the most suitable reagents to remove Hg from soil and sediments (Wang, 2012). Stronger extracting agents, such as a mixture of 100 mM KI + 50 mM HCl (pH 1.5), can achieve up to 90% Hg extraction efficiency from soil (Wasay, 1995) but significantly alter the soil structure; conversely, weaker reagents, showing lower detrimental effects on the matrix, have revealed limited extraction efficiency (Evangelou, 2007). Subirés-Munoz et al. (2011) studied the effectiveness of different chelant agents (thiosulfate, EDTA, iodide and HNO3) for Hg removal from a soil highly contaminated (1000 mg kg?1) finding that the most effective chelants were iodide, EDTA, and thiosulfate.

Usually, the effectiveness of the recovery processes in the contaminant removal is assessed by comparing contaminant level and chemical-physic parameters “before” and “after” the treatment. However, with this approach, the evaluation of possible biological effects on organisms are overlooked and the residual risks for ecosystems and human health after the mitigation treatment may not be assessed. With this study we sought to evaluate the effectiveness of the “soil washing” treatment determining the effects of washed-sediments directly on biological system using the mussel Mytilus galloprovincialis as animal model, maintained in a mesocosm system. Mesocosm can reproduce, in a controlled laboratory scale, coastal marine environment conditions. In particular the system is able to simulate the action of marine turbulence favouring the contact between sediment and biological organisms (Pirrone et al., 2018). For this purpose, the system was set up as follow:

i) uncontaminated seawater (W),

ii) contaminated sediment (B), and

iii) a system in which the contaminated sediment that constitutes mesocosm B has been previously treated through the soil washing (G).

The multidisciplinary approach adopted to achieve our purpose included the evaluation of morphological organization, mRNA expression, metals accumulation and genotoxicity studies. The mRNAs considered in this work are those generally involved in physicochemical and oxidative stress response (Superoxide dismutase 1, Catalase, Cytochrome P450, Glutathione S-transferase, Heat Shock Protein 70, Heat Shock Protein 90, and Metallothionein 10) and can be used as early warning signals of environmental disturbance (Rossi et al., 2009; Gornati et al., 2004). However, mRNA changes may be transient, because of the adaptive processes developed by the mussels, then they are often not sustained by modification of the protein content. Conversely, genotoxicity biomarkers, such as the comet assay and micronucleus, are powerful methods to evaluate DNA and chromosomal damages respectively, furthermore they can discriminate among different classes of genotoxic compounds; this is why they are widely used both in field and laboratory studies (Bolognesi and Cirillo, 2014; Bolognesi and Hayashi, 2011; Jha, 2008). Also the evaluation of bioaccumulation of toxic metals, such as Hg, Cd and Pb, is a good integrative indication of the animal exposure to polluted environments (Rainbow 1993; Phillips and Rainbow 1994), which can help to better understand the effects of metal released from sediments on the organisms.

Overall we think that our approach has provided clear indications on the effect of the contaminated sediments on the sea life and on the effectiveness of the recovery process implemented.