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Toxicity decrease in urban wastewaters treated by a new biofiltration process

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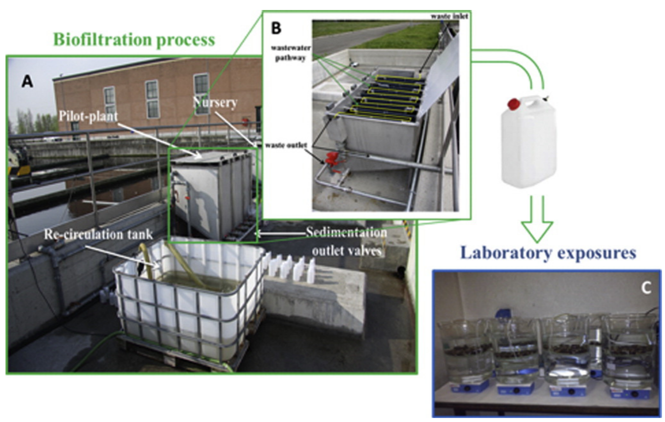
HIGHLIGHTS

- Zebra mussels were used to bio-filter urban wastewaters in a pilot-plant.
- We evaluated the toxicity decrease of two waste mixtures treated by biofiltration.
- Biofiltration reduced the mussel mortality until 29% and 61% in the two mixtures.
- Contradictory results were obtained for the chronic toxicity abatement.

GRAPHICAL ABSTRACT



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ABSTRACT

We carried out a project aimed to evaluate the possible role played by the freshwater zebra mussel (*Dreissena polymorpha*) in the possible decrease of some environmental pollutants recalcitrant to tradition wastewater treatments. By the help of a pilot-plant built in the largest wastewater treatment plant of Milan (Italy), we tested several waste mixtures in order to measure the chemicals' abatement made by mussels' biofiltration. This study represents the last step of the wider project and it aimed to evaluate if the decrease in the concentration of some urban pollutants measured in wastewaters was followed by a corresponding toxicity reduction. Thus, we performed 7-day exposures under laboratory conditions to test the toxicity of the raw wastewaters and those preliminary filtered by zebra mussels, through the measurement of different end-points of acute and chronic toxicity. Results showed a clear positive effect of mussels' biofiltration mainly to decrease the acute toxicity made by the two tested wastewater mixtures, while the biomarkers' suite used to evaluate the chronic toxicity showed contradictory results.

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1. Introduction

The wastewater treatment plants (WWTPs) have been historically focused on the abatement of organic matter and nutrients, as well as the elimination of the faecal contamination only. However, from the beginning of the synthetic chemistry, some man-made chemicals were emerged as dangerous environmental pollutants because of their high persistence and low degradability, the biotic and abiotic transformations in metabolites often more stable and toxic than parent compounds, as well as the high number of molecules discharged in the aquatic ecosystems. In detail, the persistent organic pollutants (POPs) are resistant to biodegradation and are able to pass unchanged through the traditional wastewater treatment processes and consequently they bioaccumulate and exert their toxicity on the freshwater biocoenosis. Along with POPs, another group of pollutants has recently appeared as an emerging environmental problem: the pharmaceuticals and personal care products (PPCPs) and drugs of abuse, whose presence in the aquatic ecosystems worldwide is well documented (Carballa et al., 2008; Kim et al., 2009; Gottschall et al., 2012). The main sources of these pollutants are civil, industrial and hospital wastewaters, but also the livestock discharges (Ellis, 2006). Although many PPCPs and drugs of abuse are polar and hydrophilic compounds, removal rates in WWTPs vary widely depending on several factors, such as the technology used, tertiary treatments, physical and chemical characteristics of the molecules, wastewaters' composition and season (Castiglioni et al., 2006). For instance, the analgesic acetaminophen and the antibiotic amoxicillin have a high removal rate, but clarithromycin (antibiotic), hydrochlorothiazide (diuretic) and diclofenac (non steroidal anti-inflammatory drug; NSAID) are recalcitrant to biodegradation in WWTPs (Riva et al., 2015). Thus, several PPCPs and drugs of abuse have been continuatively discharged in surface waters at concentrations similar to those found for many POPs (Fatta-Kassinos et al., 2011), representing an additional risk for the aquatic fauna, as also demonstrated by previous studies carried out by our research group (Binelli et al., 2009a,b, 2012; Parolini and Binelli, 2012, 2013, 2014; Parolini et al., 2011, 2013, 2014).

In recent years, the crucial challenge in the wastewater treatment is the abatement of this new class of emerging contaminants by using well-known or pioneering methodologies, such as the activated carbon adsorption, membrane filtration, reverse osmosis and biofiltration (Yu et al., 2008; Kim et al., 2008; Snyder et al., 2007; Lee et al., 2012; Reungoat et al., 2011). In this context, we have exploited the natural characteristics of the freshwater filter-feeder zebra mussel (*Dreissena polymorpha*) to investigate its potential capability to reduce the content of some PPCPs and drugs of abuse (Binelli et al., 2014), as well as trace metals (Magni et al., 2015) from the wastewaters. In brief, we built a pilot-plant in the largest WWTP of Milan (Milano-Nosedo, Italy) in which about 40,000 mussels were inserted and attached to 20 Plexiglas® panels. We hypothesized that the very high filtration rate (from 5 to 400 mL bivalve^{-1} h^{-1}; Baldwin et al., 2002) due to hundreds of thousands of zebra mussel specimens should be able to decrease the concentration of PPCPs and drugs of abuse in wastewater by the increase of suspended particulate matter deposition on the bottom of the pilot-plant by faeces and pseudo-faeces. Moreover, the high filtration rate can allow a fast intake of lipophilic environmental pollutants in the mussel soft tissues, increasing their elimination from the wastewater. After many tests carried out with different wastewater mixtures, we obtained encouraging results since the biofiltration by *D. polymorpha* remarkably reduced the concentration of several drugs (Binelli et al., 2014) and trace metals (Magni et al., 2015).

After these promising outcomes, in this study we established to check if the effective content abatement of some environmental pollutants corresponded also to a decrease in the toxic effect of the wastewaters treated by biofiltration. Thus, we circulated again the two mixtures for which we obtained the best abatement performances, collecting wastewaters at the start of treatment and after 4 and 24 h, respectively. Then, the possible decrease of toxicity due to mussels' biofiltration was evaluated under laboratory conditions by means of other zebra mussel specimens exposed to these treated mixtures for 7 days. We measured the bivalve mortality and hemocytes' viability as acute toxicity endpoints, while we used a suite of nine biomarkers to evaluate some chronic effects. In detail, we measured the destabilization of the lysosomal membranes (Neutral Red Retention Assay, NRRA), a typical indicator of general cellular stress in bivalves, the activity of three antioxidant enzymes, namely superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), and the phase II detoxifying enzyme glutathione S-transferase (GST). Furthermore, the lipid peroxidation (LPO) and protein carbonyl content (PCC) were measured as oxidative damage indices, while primary (DNA strand breaks) and fixed (micronucleated cell frequency) genetic damage were investigated on zebra mussel hemocytes by the single cell gel electrophoresis (SCGE) assay and the micronucleus test (MN test), respectively.

2. Materials and methods

2.1. The pilot-plant and tests in situ

Since the pilot-plant and the other facilities built at the WWTP of Milano-Nosedo have been well described by Binelli et al. (2014) and Magni et al. (2015), we only highlight its main characteristics. It was a stainless steel tank with a volume of about 1000 L ($l = 154.0$ cm, $h = 102.0$ cm, $w = 80.5$ cm), in which 20 removable Plexiglas® panels can be inserted, allowing a zig-zag pathway to wastewater. Hundreds of thousand zebra mussels were collected in different times from Lake Lugano, considered a pristine site for PPCPs and drugs of abuse (Zuccato et al., 2008), in order to carry out firstly many preliminary assays and then definitive tests. We added about 2000 specimens for each Plexiglas® panel for a total of 40,000 mussels for each test and we finally tested four different wastewater mixtures: 100% of the WWTP inlet (100% IN), 100% of the WWTP outlet from sedimentation tanks (100% OUT), 25% of the inlet and 75% of the outlet (25% IN) and 50% of the inlet and 50% of the outlet (50% IN). We conducted every test under recirculation conditions to increase the time of mussel filtration; a submerged pump (3500 L h^{-1}) allowed the circulation of the mixture into the pilot-plant, which was flanked by a 200 L recirculation tank. In these conditions, the residence time of the wastewater in the pilot-plant was 15 min and the total time for a complete recirculation of the wastewater was 18 min, considering the additional volume of recirculation tank. This experimental design enabled a longer contact of the wastewater with the mussels because the wastewater recirculates approximately 14 times in 4 h and 84 times in 24 h.

Since the best abatement performances for drugs and trace metals have been obtained with the mixtures 25% IN and 50% IN (Binelli et al., 2014; Magni et al., 2015), we analysed the possible reduction in toxicity only for these ones. For our purposes, two other tests were then carried out with these mixtures for 24 h with 40,000 mussels in the pilot-plant, sampling 60 L of each mixture at the start of exposures ($t = 0$ h) and after 4 and 24 h of recirculation. Contemporarily, we collected at the same times also the outlet from the WWTP sedimentation tanks (canal). Samples were put in plastic tanks (60 L) opportunely washed, transported to laboratory and maintained at 4 °C in a cold room.

2.2. Exposures at laboratory conditions

Several hundreds of mussels collected in the Lake Lugano were inserted in aquaria (12 L) filled with tap water and deionized water (1:1) for two weeks to allow the mussel acclimatization and eliminate eventual other pollutants bioaccumulated in their soft tissues. The laboratory conditions were the follow: temperature of 20 ± 1 °C, photoperiod of 16:8 light/night hours and oxygen concentration >90%. Water