



## Removal of metallic elements from real wastewater using zebra mussel bio-filtration process



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### ABSTRACT

The metallic element pollution is a serious environmental problem but still unsolved since these contaminants are released mainly by human activity, reaching all the environmental compartments. Traditional wastewater treatment plants are very efficient in removing metallic elements only when their concentration is in the order of mg/L, but are not able to remove them until  $\mu\text{g/L}$ , as it would be needed to cope with the water quality standards in low flow receptors. Therefore, the aim of our study was to evaluate the potential removal of some recalcitrant metallic elements to the classical treatments, by the natural process of bio-filtration performed by the invasive zebra mussel (*Dreissena polymorpha*). For this purpose we built a pilot-plant at the Milano-Nosedo wastewater treatment plant, where we placed about 40,000 *D. polymorpha* specimens appointed to the wastewater bio-filtration. The metallic element removal due to zebra mussel activity was evaluated in the treated wastewater with a plasma optical emission spectrometry (ICP-OES). Data obtained in these experiments showed an encouraging metallic element removal due to *D. polymorpha* activity; in particular, the total abatement (100%) of Cr after one day of bio-filtration exposure is remarkable. Therefore, this study encourages further research related with the use of bivalves as a new tool for the wastewater depuration process; in this regard, the contaminated mollusks used in the bio-filtration could be incinerated or stored in special landfills, as is also the case of traditional sewage sludge.

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### Introduction

Metallic element (ME) pollution is a major global concern since these inorganic contaminants are continuously released into the environment by human activities [1,2]. The ability of these compounds to be accumulated in the organisms and to trig the onset of diseases and disorders makes MEs very dangerous for many organisms, including humans, at very low concentrations [3]. In particular, the water pollution due to MEs is a serious and partially unsolved issue because the removal needed to reach acceptable concentrations in the receiving waters (in the order of  $\mu\text{g/L}$ ) is well over the efficiency of wastewater treatment plants (WWTPs), normally reported as between 40 and 90% [4]. Because

of this reason, alternative methods for the ME abatement have been identified in order to be complementarily applied to traditional wastewater treatment processes. However, most of these techniques, such as precipitation/neutralization, ion exchange, membrane separation, reverse osmosis, electrodialysis and activated carbon adsorption [5–7] have high costs for the regeneration of resins or activated carbons and/or for the disposal of chemical sludge or concentrates [8]. Therefore, the attention of the scientific community need to be focused on the development of natural methods which were more eco-sustainable and, possibly, less expensive. In this regard, biosorption is a possible natural method for ME elimination; this term defines the passive pollutant uptake from an aqueous solution by a dead or non-growing microbial biomass [9,10]. Although this treatment has the advantage to not undergo inhibition due to the pollutants' toxicity, the early biomass saturation by adsorbing contaminants represents an important limitation for further exploitation of this process [7]. In addition to the biosorption, the bioaccumulation process of many organic and inorganic contaminants by different aquatic microorganisms such as fungi, algae, bacteria and yeast

Abbreviations: MEs, metallic elements; WWTPs, wastewater treatment plants.

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[11,12] may be considered. In particular, bioaccumulation due to microorganisms living on aquatic macrophyte tissues is correlated with ME removal in constructed wetlands. This methodology is certainly the most used natural system of wastewater treatment, which couples accumulation in microbial biomass and in macrophytes such as *Phragmites australis*, *Eichhornia crassipes* and *Lemna* spp. [13–16]. This alternative method, in addition to the removal of MEs, also reduces organic matter and nutrients from wastewater [16]. Despite the existence of these eco-friendly methodologies, in recent years, further studies have been conducted in order to identify new methods for natural purification of waters from some recalcitrant pollutants. In this regard, it is of great interest the research carried out by Ledda et al. [17] aimed at assessing how small breeding of Mediterranean sponges *Ircinia variabilis* and *Agelas oroides* could remove some contaminants from marine waters. In the same way, the use of other filtering organisms can be interesting for the improvement of waters quality. In this context, the freshwater bivalve *Dreissena polymorpha* has some characteristics that would make it suitable for the above mentioned purpose: an enormous filtering capacity, ranging from 5 to 400 mL/bivalve/h [18,19], a high population density, with more than 700,000 individuals/m<sup>2</sup> [20], and the ability to produce feces and pseudofaeces where many contaminants are adsorbed. In fact, these two *D. polymorpha* waste products, being settleable [21], could easily remove from the water column the bounded pollutants (as MEs). Moreover, taking into account the indirect ability of bivalves to bioaccumulate many environmental contaminants, including MEs [22], we can point out the potential of *D. polymorpha* to this purpose [23–26]. In this regard, a study conducted in 1983 by Piesik [27] highlighted how *D. polymorpha* is able to remove nutrients from eutrophic waters and a subsequent research confirmed the potential of *D. polymorpha* in the reduction of algal density [28]. In the last two decades, several other studies have demonstrated the filtering capacity of this bivalve, whose breeding could be developed for an alternative treatment of polluted freshwaters [25,29–31]. In this regard, a recent study conducted by Binelli et al. [21] showed the ability of this mollusk to remove different types of emerging contaminants, such as pharmaceuticals and drugs of abuse, from wastewaters. Nevertheless, it is important to take into account that *D. polymorpha* is considered an invasive alien species all over Europe and the United States, even if this mollusk was present in Europe before the last glaciation [32] and was then bounded in some basins of Eastern Europe in the post-glacial period until the 18th century [33]. The human activity has then favored the distribution of *D. polymorpha* all over its original European area; in Italy, for example, this bivalve has first been found in 1973 [34] and its presence in the Italian inland waters has been confirmed by subsequent studies

[35–37]. Therefore, the idea of using this invasive species for anthropic purposes (bio-filtration, human food, animal feed, fertilizer and biogas) [29] would be of huge interest, especially in the economic sphere. On the basis of these above mentioned studies on *D. polymorpha*, we assessed the efficiency of this bivalve as a new biological method as the last step of wastewater treatment in a conventional WWTP. For this purpose, we built at the Milano-Nosedo WWTP (Northern Italy) a pilot-plant in which 40,000 *D. polymorpha* specimens were added in order to filtrate some types of wastewaters and we subsequently evaluated the abatement of some MEs, such as Aluminum (Al), Chromium (Cr), Copper (Cu), Iron (Fe), Manganese (Mn), Nickel (Ni) and Lead (Pb). This study is particularly innovative because, according to our knowledge, for the first time, *D. polymorpha* has been used in a real civil WWTP for the removal of some micropollutants. In fact, the few studies conducted using *D. polymorpha* as bio-filtering agent mostly evaluated algal or organic matter removal, but not the abatement of emerging contaminants (as previously reported in Binelli et al. [21] or potentially toxic metals.

## Materials and methods

### Pilot-plant construction and placement at the Milano-Nosedo WWTP

A scuba diver collected the bivalves from the Lake Maggiore and Lake Lugano, both located close to the Italy–Switzerland border. Since it is well-known that *D. polymorpha* is a biofouling organism [38], we placed approximately 40,000 specimens in an attachment tank in order to let them naturally re-adhered to twenty Plexiglas<sup>®</sup> panels (size: 70 × 40 cm; Fig. 1) via their byssus over a period of 2 weeks. During this acclimatization period, the bivalves were kept in tap water and fed with the blue–green alga *Spirulina* spp. The Plexiglas<sup>®</sup> panels were then placed into the pilot-plant (Fig. 1), a stainless steel tank with a volume of about 1000 L (L = 154.0 cm, h = 102.0 cm, w = 80.5 cm), where were disposed following a zig-zag pathway (yellow line, Fig. 2), in order to increase both the surface and the contact time between the wastewater and each bivalve. In addition to the steel tank, we installed a recirculation tank (Fig. 1) with a volume of 200 L with a submerged pump to allow a constant wastewater flow (3500 L/h) into the pilot-plant. The recirculation tank further increases the contact time between the wastewater and the filter-feeding bivalves placed into the pilot-plant, as well as limits the efficiency of settling which would remove part of the contaminants adsorbed on suspended solids. The pilot-plant can directly collect the effluent from the canal placed between the sedimentation tanks and the sand filters of the Nosedo WWTP using a submersible pump (0–5000 L/h). The installation site of the pilot-plant allows to test a clarified effluent and to avoid the risk that suspended solids cannot only compromise the filtration capability of bivalves but also cause the animal death due to gill occlusion. Moreover, the pilot-plant position into the Nosedo WWTP guaranteed the lack of any possible accidental release of *D. polymorpha* specimens into the surrounding environment because the sand filters and the following process of disinfection with peracetic acid stop and kill any possible leaked organism.

### Evaluation of *D. polymorpha* filtration ability

The preliminary tests designed to evaluate the filtering and purifying performance of *D. polymorpha* have been described in detail by Binelli et al. [21]. In that study, the following issues have been discussed: (1) the adaptation of *D. polymorpha* to wastewater; (2) the estimation of *D. polymorpha* filtering efficiency; and (3) the analysis of *D. polymorpha* capacity in the removal of new classes of



Fig. 1. Structure of the pilot-plant located at the Milano-Nosedo WWTP.

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