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

Stefano Magni [a,*], Marco Parolini [a], Carlo Soave [a], Francesca Marazzi [b], Valeria Mezzanotte [b], Andrea Binelli [a,**]

[a] Department of Biosciences, University of Milan, Via Celoria 26, 20133 Milan, Italy
[b] Department of Environmental and Territory Science, University of Milan-Bicocca, Piazza della Scienza 1, 20126 Milan, Italy

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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 μg/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.

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 μg/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.

* Corresponding author. Tel.: +39 0250314729; fax: +39 0250314713.
** Co-corresponding author. Tel.: +39 0250314714; fax: +39 0250314713.
E-mail addresses: stefano.magni@unimi.it (S. Magni), andrea.binelli@unimi.it (A. Binelli).

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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^2 [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 areal; 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.

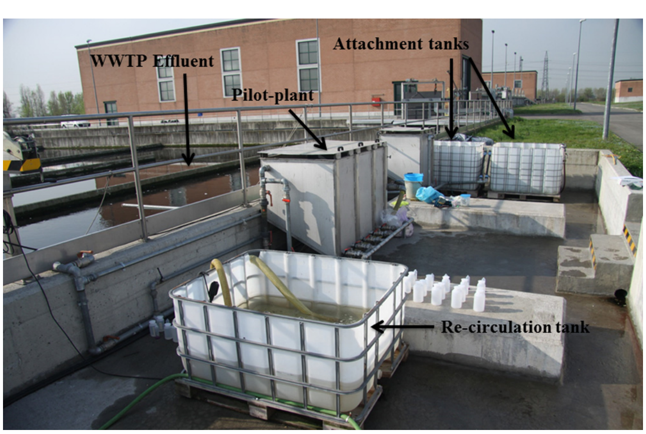

Fig. 1. Structure of the pilot-plant located at the Milano-Nosedo WWTP.

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® 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 alga *Spirulina* spp. The Plexiglas® 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

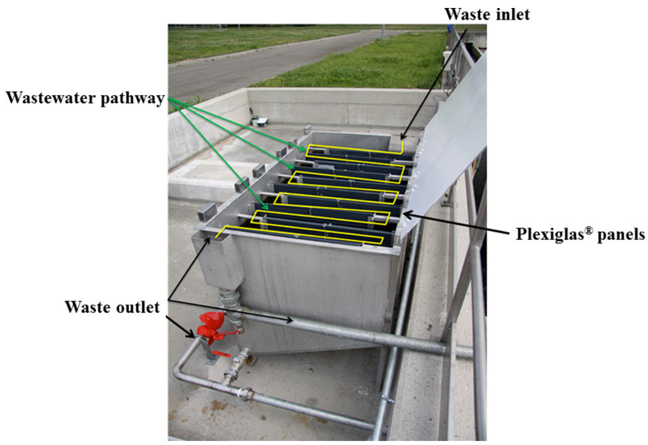

Fig. 2. Plexiglas® panels placed into the pilot-plant. The yellow line indicates the zig-zag flow pathway of wastewater within the pilot-plant.

environmental pollutants (pharmaceuticals and personal care products and illicit drugs).

Experimental design and samples collection

As previously described, an important point was the necessity to evaluate the removal efficiency of *D. polymorpha* independently from any other settling process, which would remove the metals adsorbed on suspended solids. The filtering action of *D. polymorpha* was first evaluated on the effluent outflowing secondary settling. However, since this effluent had a very low COD (≈10 mg/L) and, consequently, a low suspended solid concentration (on which a relevant amount of MEs is normally adsorbed) [39,40], the following tests were performed with three other different wastewater mixtures, previously filtered through a 1 mm mesh bag filter to remove coarse matter. This allowed us to evaluate the filtration efficiency of *D. polymorpha* on wastewater with polluting load and different amounts of suspended particulate, also taking into account that this bivalve selects particles for food with a diameter ranging between 15 and 40 μm [41]. The mixtures used in the tests, in addition to 100% outlet, are the following: 25% inlet/75% outlet, 50% inlet/50% outlet and 100% inlet (wastewater incoming at WWTP). The ME removal evidence from wastewater were carried out through the measurement of their concentrations in the water samples taken from the pilot-plant with bivalves inside; at the same time, control tests were conducted into the pilot-plant without adhering animals. All tests were performed in triplicate. The ME removal progress was monitored for 4 h, by sampling the wastewaters every 30 min, which enabled to obtain the removal slope for each ME. We chose to evaluate the ME removal within 4 h, taking into account that the treated wastewaters remain in the Milano-Nosedo WWTP for about 24 h; thus, the selected time seemed to be a fair compromise in view of integrating the conventional treatment with limited dimensional requirements. To check the practicability of such assumptions, we carried out further final tests in single for a period of 24 h, taking only two samples, one at the beginning and one at the end of the tests. The tests were conducted with an initial flow rate corresponding to 3500 L/h, which would imply 18 min contact time, recirculating the effluent in the pilot-plant 84 times to obtain an overall 24 h contact time. After each test, the entire pilot-plant was washed with tap water, to avoid memory-effects related to the

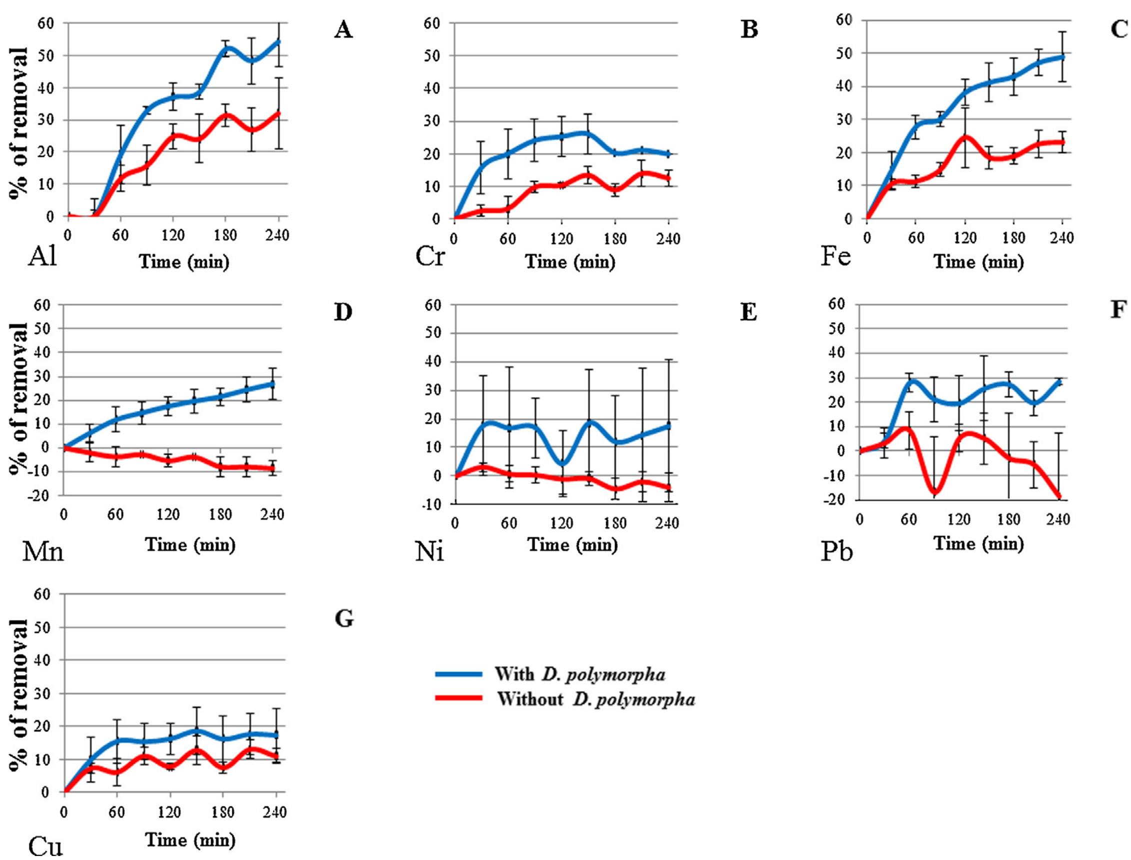

Fig. 3. Mean trends (±SEM) of metallic element removal during the first 4 h (240 min; aluminum, A; chromium, B; iron, C; manganese, D; nickel, E; lead, F; copper, G) with *D. polymorpha* (blue curve) and without bivalves (red curve) inside the pilot-plant for the 25% inlet/75% outlet mixture. The differences between controls and treated, with the exception of chromium, were statistically significant (two-way ANOVA).

previous tests. For this reason, to minimize this problem, as well as to decrease the bivalve stress, the test schedule started with the most diluted waste (100% outlet) and gradually increased its concentration until 100% inlet. We monitored the wastewater temperature both at the beginning and at the end of each test in order to take into account its possible interference with the filtration activity of zebra mussels. The wastewater temperature within the pilot-plant during the spring season ranged from 14 to 24 °C, comparable with the optimal values for *D. polymorpha* filtration activity (10–20 °C) [42]; we can thus exclude any negative interference of temperature on the filtration-removal process. Samples were taken from the pilot-plant at the selected times by the use of a 250 mL plastic bottles, acidified with 1% of HNO_3 and stored at 4 °C at dark until analysis.

Evaluation of ME abatement

We evaluated the removal of some MEs relatively abundant in civil wastewaters: Aluminum (Al), Chromium (Cr), Copper (Cu), Iron (Fe), Manganese (Mn), Nickel (Ni) and Lead (Pb). The samples, taken from the pilot-plant, were treated according to the CNR IRSA 3010 method. Briefly, an aliquot of each sample was transferred into a flask and heated up to 100 °C to remove turbidity. After cooling, samples were brought back to the starting volume with distilled water. Samples were analyzed in a plasma optical emission spectrometer (ICP-OES; OPTIMA 2100 DV, PerkinElmer; detection limits for each ME: Al 0.5 μg/L; Fe 0.2 μg/L; Mn 0.1 μg/L; Ni 0.5 μg/L; Pb 1.0 μg/L; Cu 0.5 μg/L; Cr 0.2 μg/L) equipped with ultrasonic nebulizer (CETAC Ultrasonic Nebulizer, model U5000AT +). The ME concentrations were quantified by a calibration curve at two points, starting from appropriate dilutions of mixed certificate standard (AccuStandard MES 16-1).

Statistical analyses

Data normality and homoscedasticity were verified using the Shapiro–Wilk and Levene's tests, respectively. We performed a statistical comparison (SPSS 21 IBM software package) between tests carried out with and without mussels in the pilot-plant, where the dependent variable is the ME concentration in the wastewater and the fixed factors are the treatment and the exposure time. For all these cases, we conducted the comparison using the two-way analysis of variance (two-way ANOVA; $^{*}p < 0.05$; $^{**}p < 0.01$).

Results and discussion

Evaluation of D. polymorpha *filtering ability in the ME removal*

The results obtained from the tests carried out with a 25% inlet/75% outlet mixture (Fig. 3A–G) showed a good removal performance by *D. polymorpha* due to the bio-filtration effect, probably because of the suitable concentration of the suspended matter. The removals obtained for each ME tested through the filtering activity of bivalves were always greater than those related to the natural sedimentation evaluated in controls. In fact, for the majority of the analyzed MEs, the contribution of the zebra mussel filtration was evident, since the differences between the removal percentage

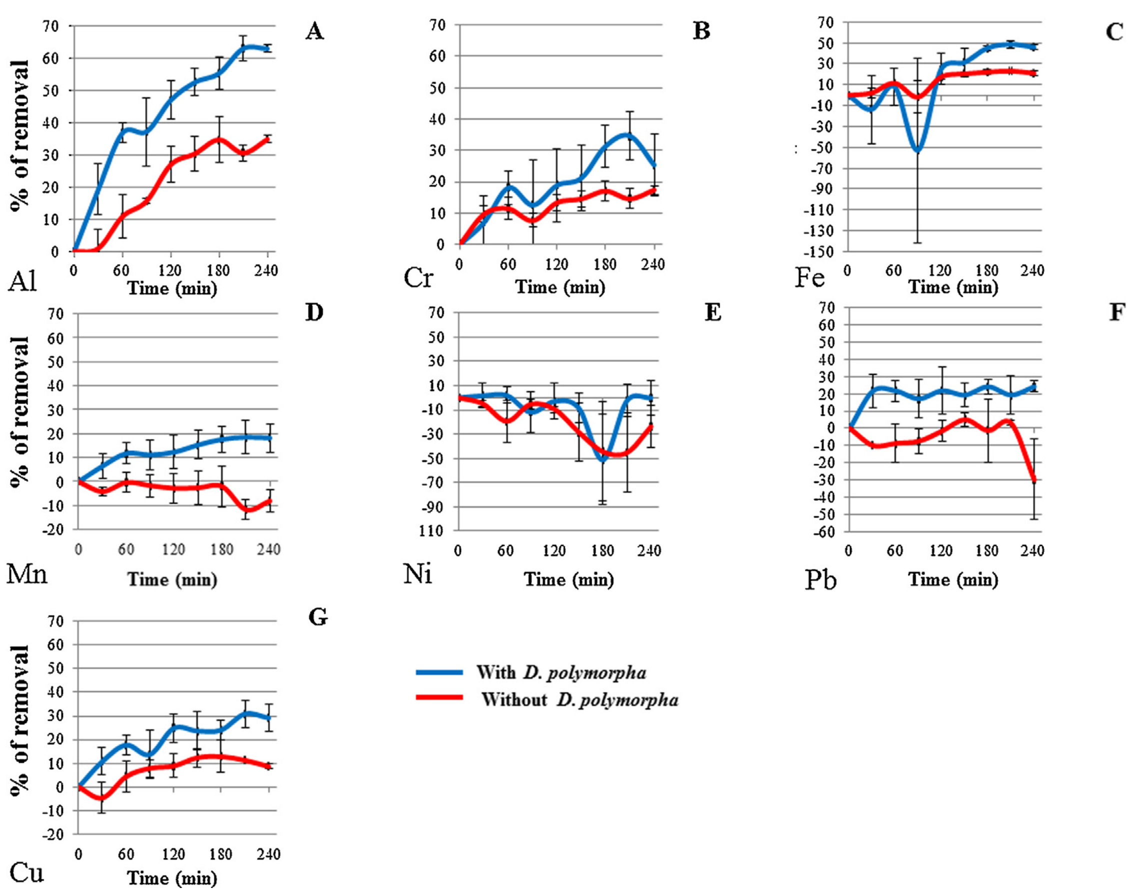

Fig. 4. Mean trends (±SEM) of metallic element removal during the first 4 h (240 min; aluminum, A; chromium, B; iron, C; manganese, D; nickel, E; lead, F; copper, G) with *D. polymorpha* (blue curve) and without bivalves (red curve) inside the pilot-plant for the 50% inlet/50% outlet mixture. The differences between controls and treated, with the exception of nickel and iron, were statistically significant (two-way ANOVA).

with and without bivalves in the pilot-plant were statistically significant: Al (F = 36.809, $p < 0.01$); Fe (F = 62.686, $p < 0.01$); Mn (F = 125.452, $p < 0.01$); Ni (F = 5.695, $p < 0.05$); Pb (F = 16.645, $p < 0.01$); Cu (F = 6.220, $p < 0.05$). In detail, observing the trends reported in Fig. 3, it has to be highlighted that the differences between the removal percentages measured at the end of the tests reached the 30% for Fe and Pb, while for Al, Ni and Mn the removal was about 20–25% higher than controls. Thus, in only 4 h, zebra mussels have been able to significantly decrease levels of most of the tested MEs, even if the removal of Cu was only 8% higher than natural sedimentation. On the other hand, the time selection to conduct the tests is crucial for the possible engineering of the process that cannot be longer than few hours, since the entire cycle of the wastewater treatment ends in about 24 h. Tests carried out by adding 50% of inlet to the WWTP outlet (Fig. 4A–G) showed a lower difference compared to control than the previous tests, probably due to an excessive presence of suspended particulate matter that determined a stress condition to the animals, which may require a longer time than 4 h to acclimate and begin the filtering process. Moreover, we cannot exclude the possible presence of toxic compounds into the inlet of WWTP that could have led to a further decrease in the filtration activity. Despite these possible interfering processes, we found statistically significant difference between tests carried out with bivalves in the pilot-plant and their respective controls for Al (F = 68.587 $p < 0.01$), Mn (F = 38.710, $p < 0.01$), Pb (F = 26.183, $p < 0.01$), Cu (F = 22.861, $p < 0.01$) and Cr (F = 4.729, $p < 0.01$). In this regard, at the end of the test the removal was around 20–25%, comparable to the results obtained for the mixture 25% inlet/75% outlet for Al, Mn, Pb and Cu, while for the other tested metals it decreased dramatically. The fluctuating values obtained for Ni could be due to the low concentration of this metal in the analyzed wastewater (<10 μg/L), taking into account the huge variability of pollutant load in the inlet wastewaters. The role of the initial concentrations of metallic elements into the considered mixtures (Table 1), which depends on the WWTP inlet, must always be considered when drawing conclusions in terms of percent removal: if these are very low, small variations (which could also be partly due to analytical reasons) assume relevant percent weight. In both the considered tests (25% inlet/75% outlet and 50% inlet/50% outlet mixtures) negative values of sedimentation, comprised in a range of −5 and −10%, are observable; these values are likely to be related to the coefficient of variation of the method used to perform the wastewater ME quantification. These data do not appear to be random, because, except for the fluctuating values of Ni (Fig. 4E), Mn and Pb showed null sedimentation values in both tests performed (Fig. 3D,F and Fig. 4D,F). This result can be reasonably related to the chemical speciation phenomenon because these metals can probably be dissolved in water and not bounded to the particulate. Therefore, the observed Mn and Pb removal process carried out by *D. polymorpha* could mainly be related to bioaccumulation. Further studies are needed in order to deepen the knowledge about some of the above-mentioned aspects, as also suggested by Camusso et al. [43]. In this regard, the wastewater pH value, which influences the metal speciation, is kept constant in WWTPs and, therefore, should not compromise the *D. polymorpha* purification activity. Finally, with regard to the test with 100% inlet, there has been a serious decline in the bivalves' performance related to a high mortality of the animals (data not shown). This result further confirms how an excessive suspended particulate matter amount and the possible presence of toxic substances into the WWTP can decrease the bivalves filtering capacity and even compromise their health status. However, this aspect does not limit the possible engineering of this method, since it would be sufficient to control the particulate matter of the wastewater, as suggested by Binelli et al. [21]. Moreover, despite the suspended matter concentration represents a limiting factor of *D. polymorpha* filtering capacity, it should be noted that the specimens used in this study are the same used in the pharmaceuticals and illicit drugs removal process, described by Binelli et al. [21]. Despite an exposure to multiple pollutants, the bivalves' purifying ability is stable during the whole experimental trial, representing a sure advantage in the use of this very resistant organism. Furthermore, the data shown refer to the ME removal within the first 4 h of wastewaters exposure to *D. polymorpha*, and that the bivalves' performance can be improved with increasing contact time between mollusks and wastewater, as described below.

Time influence on the ME removal by D. polymorpha

Data obtained by the above-mentioned tests suggested that the contact time between wastewater and the filter-feeding bivalves was probably one of the most crucial parameters, affecting the extent of ME removal from wastewater. As previously mentioned, although the increase of contact time could be almost impossible at full scale, we decided to carry out tests 24 h long. On the basis of the results obtained at 4 h, the 24 h tests were performed only on 25% inlet/75% outlet and 50% inlet/50% outlet mixtures. For most of the MEs, the removal due to mussel filtration was about 70% with the 25% inlet/75% outlet mixture (Fig. 5A). The natural sedimentation, at the same time, removed 50% of Cr and Fe and, surprisingly, only 10–25% of Cu, Mn and Pb (Fig. 5A). Thus, zebra mussels' filtration is able to increase the removal of Pb and Mn by about 60% with respect to the settling effect in blanks. Notably, Cr removal appeared very interesting because of its high toxicity for aquatic organisms [44,45]; in fact, *D. polymorpha* completely removed it in 24 h, while the blank removal was only 50%. Therefore, contact time seems to affect significantly the extent of ME removal by the filter-feeding bivalves, considering that at the end of the first 4 h the mean removal was 20% higher with *D. polymorpha* than in the blank tests. This was also confirmed in the test performed with the 50% inlet/50% outlet mixture (Fig. 5B), where the ME removal due to *D. polymorpha* was always over 70%. In particular, for Cu, Mn and Pb the net removal due to *D. polymorpha* (calculated as the difference from the blank removal) was 50%, 70% and 60%,

Table 1
Initial concentrations (μg/L) of metallic elements detected in 25% inlet/75% outlet and 50% inlet/50% outlet mixtures into the pilot-plant at the beginning of the removal tests without and with *D. polymorpha*. The data related to the initial concentration of COD and total suspended solids into the two considered mixtures are shown in Binelli et al. [21].

Test without *D. polymorpha*							Test with *D. polymorpha*						
25% inlet/75% outlet													
Al	Cr	Fe	Mn	Ni	Pb	Cu	Al	Cr	Fe	Mn	Ni	Pb	Cu
56.6	2.5	802.4	41.9	5.2	2.7	11.7	67.5	1.5	249.2	15.1	3.3	3.0	11.7
50% inlet/50% outlet													
Al	Cr	Fe	Mn	Ni	Pb	Cu	Al	Cr	Fe	Mn	Ni	Pb	Cu
88.2	3.4	469.3	25.8	4.5	3.4	11.6	103.2	2.1	272.7	17.4	2.2	3.4	13.6

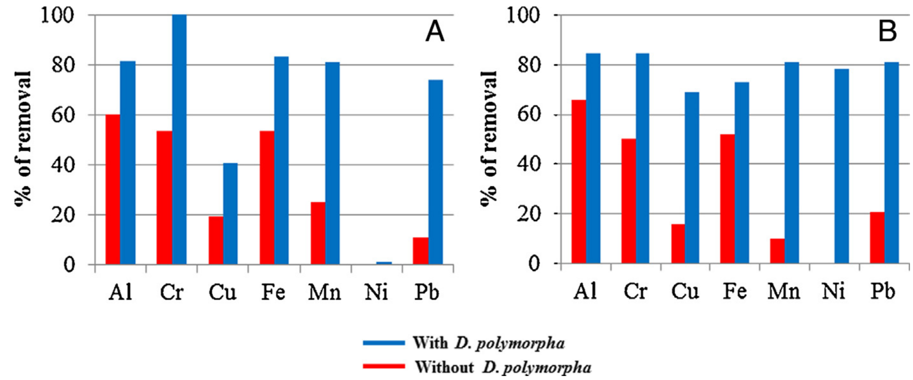

Fig. 5. Removal percentage of metallic elements from wastewater after 24 h in the 25% inlet/75% outlet (A) and 50% inlet/50% outlet (B) mixtures.

respectively. At the same time, the high removal observed for Ni contradicts the results obtained in the experimental data set. The 24 h tests, although only performed in single and therefore needing further confirmation, provide first evidence that better ME removal performances may be obtained by increasing the contact time between the bivalves and the feed. Further, the obtained data may indicate that the bivalve could need a period of acclimation to the wastewater, especially if characterized by a considerable amount of suspended particulate material, before starting the filtration process.

Future perspectives

Due to the scarcity of scientific data regarding the use of *D. polymorpha* in the wastewater treatment context, we faced many technical and logistical problems during our research, not foreseeable during the experimental design drafting; in fact, the best performances of bio-filtration were obtained with prolonged exposure times (24 h) and with moderate amounts of particulates. Therefore, the ability of *D. polymorpha* to remove certain types of pollutants from pretreated wastewater could suggest, in a possible future research or in an engineered scenario, the placement of this filter-feeding bivalve as the last step of conventional WWTPs or to include it in other natural systems, such as constructed wetlands or lagooning, where the hydraulic retention time is of one or more days, and thus a longer contact time between wastewater and the bivalves is allowed. Furthermore, in future studies, it would be interesting to investigate the ME removal mechanisms and to monitorate the fate and presence of MEs in the bivalve soft tissues, shells, feces and pseudofaeces.

Conclusions

This work, according to our knowledge, represents one of the very few studies concerning the possibility to use bivalves in the wastewater treatment processes. The results appear to be very encouraging, considering that the use of non-native species, such as *D. polymorpha*, for anthropogenic purposes, could have interesting economic implications and represents an important starting point for the alien species exploitation. In this regard, the prevention strategies regarding the non-native and invasive species introduction determine complex social and ethical implications; furthermore, while the procedures on how to respond to invasions have been delineated, their application is still severely limited. Therefore, in the exclusive case of *D. polymorpha*, it may be advantageous to exploit the potential of this bivalve, now almost present in all the Europe inland waters. This will not certainly be an easy process; in fact, being *D. polymorpha* considered a serious threat for the aquatic environment and a dangerous fouling agent of many industrial structures [46,47], is poorly perceived by the scientific community as a valid filtering factor, despite the presence of encouraging results in the depuration context [28,48,25,49,30,21]. In this regard, the construction of appropriate facilities for bio-filtration, followed by further downstream treatment aimed to contain bivalves accidentally leaked from the plant (such as the peracetic acid treatment and sand filters) would avoid the problem related to fouling. The ideal condition would be to use native bivalves, such as unionids; however these mollusks, besides being affected by a serious population decline [50], have a parasite larval stage that would be disadvantageous for the engineering of the bio-filtration process. Once contaminated by the filtration process, the bivalves may then be dehydrated and stored in dedicated landfills or incinerated, as it is currently the case for sewage sludges.

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