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Removal of enteric viruses and *Escherichia coli* from municipal treated effluent by zebra mussels

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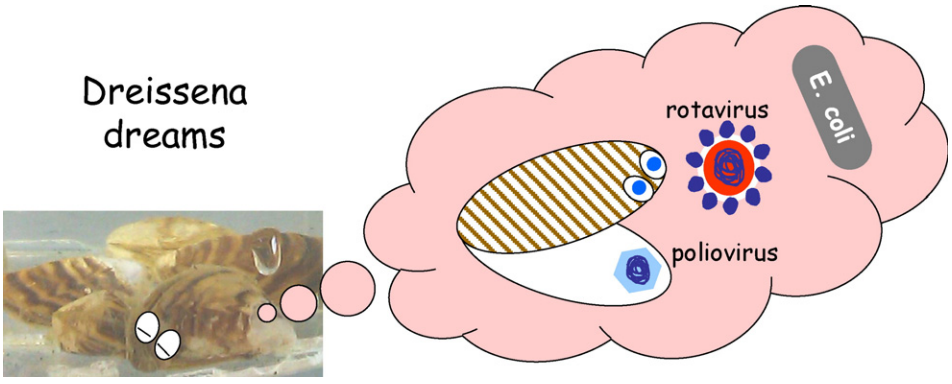
HIGHLIGHTS

- Polio and rotavirus titers are significantly reduced by zebra mussel biofiltration.
- *E. coli* counts are almost completely reduced by zebra mussels.
- A bioremediation strategy by zebra mussel biofiltration.
- Zebra mussel ability to filter/inactivate pathogens may control human health risks.

GRAPHICAL ABSTRACT



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ABSTRACT

Dreissena polymorpha is a widespread filter-feeder species, resistant to a broad range of environmental conditions and different types of pollutants, which has recently colonized Italian freshwaters. Although widely used to monitor pollution in freshwater environments, this species is also an important food source for some fish and water birds. It can also be used to concentrate or remove particulate organic matter to interrupt avian-to-human transmission of pollutants and control health risks for animals and humans. In this study, the accumulation/inactivation in *D. polymorpha* of human health-related spiked enteric viruses was described. The removal of endogenous *Escherichia coli*, the classical indicator of fecal contamination, was tested as well. Our preliminary lab-scale results demonstrate that zebra mussels can reduce significantly poliovirus titer after 24 h and rotavirus titer after 8 h. *E. coli* counts were also reduced in the presence of zebra mussels by about 1.5 log after 4 h and nearly completely after 24 h. The fate of the two enteric viruses after concentration by zebra mussels was also investigated after

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mechanical disruption of the tissues. To our knowledge, the accumulation from water and inactivation of human health-related enteric viruses by zebra mussels has never been reported.

1. Introduction

The zebra mussel *Dreissena polymorpha* (*D. polymorpha*) is a Ponto-Caspian zebra mussel bivalve species, that has invaded and colonized Italian freshwaters during the late 1980s (Schloesser and Schmuckal, 2013). Zebra mussels are small, sessile organisms, widespread filter feeders, resistant to a broad range of environmental conditions (Claudi and Mackie, 1993) and to different types of pollutants (Bervoets et al., 2005). They can concentrate particulate organic matter and indigestible components from water with a clearance rate ranging between 5 and 400 mL/individual/h (Ackerman, 1999; Baldwin et al., 2002). *D. polymorpha* have their most suitable habitat in stable riverbeds under high flows, a velocity below 1.2 m/s, and a depth of less than 5 m under regular flows (Sanz-Ronda et al., 2014). They have been extensively used to monitor pollution in freshwaters, especially in bioaccumulation studies, by determining the level of pollutants in their soft tissues (Voets et al., 2006). The impact of river colonization by these mussels has been considered one of the most important ecological changes in freshwater systems, both for the drop in biodiversity and for the socio-economic problems they can cause (Sanz-Ronda et al., 2014; Stankovic and Jovic, 2013). The ability of zebra mussels to attach and foul structures has also caused problems in the withdrawal of drinking water and electric-power plants (Schloesser and Nalepa, 1994) and their ability to colonize the surfaces of all solid structures in the water have caused nuisances to fishermen (Schloesser and Nalepa, 1994). On the other hand, they have also modified the aquatic environment, making the habitat more suitable for themselves and other organisms (Stankovic and Jovic, 2013). When studying their influence on physical and chemical characteristics of the Zhrebchevo water reservoir (Bulgaria) during the periods before (1977–1980) and after (2009–2011) their invasion, water quality improved with a statistically significant effect on turbidity, pH, concentrations of dissolved oxygen, and NH_4–N, NO_2–N, NO_3–N (Kalchev et al., 2013). In particular, two different studies showed zebra mussels efficiency in nutrient (Piesik, 1983) and algae (Richter, 1986) removal.

Many works have also been published on the ability of zebra mussel to remove and accumulate chemical pollutants even when present at very low concentrations (De Jonge et al., 2012; Peck et al., 2007; Voets et al., 2006). Very recently, it was also demonstrated the role played by zebra mussel biofiltration in decreasing the concentration of many pharmaceuticals and traces of metals from wastewaters (Binelli et al., 2014; Magni et al., 2015).

However, in spite of the numerous data on the accumulation of chemical pollutants, only a few studies are available on the ability of *D. polymorpha* to remove microorganisms. Frischer et al. (2000) demonstrated that zebra mussels use bacteria as a food source and a recent study (Winters et al., 2011) analyzed the composition of the bacterial community in zebra mussels from three locations of the Great Lakes basin waters (Michigan, USA). Bacteria potentially pathogenic for aquatic and terrestrial animals, such as *Aeromonas* spp., *Flavobacterium* spp., *Pseudomonas fluorescens*, *Shewanella putrefaciens* and *Shigella* spp., were also detected after disrupting mussel tissues. *Cryptosporidium parvum*, *Giardia duodenalis* and *Toxoplasma gondii* oocysts were also found in tissues of zebra mussels exposed for one week to different protozoan concentrations, showing that their bioaccumulation was proportional to the environment contamination (Palos Ladeiro et al., 2014). *Escherichia coli* removal by oysters and hard shell clams (Love et al., 2010) as well as by common mussels (de Mesquita et al., 1991) has also been studied, and oysters or hard shell clams were also used for norovirus (Flannery et al., 2013), poliovirus, hepatitis A (Love et al., 2010) or Norvalk virus removal (Schwab et al., 1998). However, to our knowledge, the accumulation of human health-related enteric viruses by zebra mussels from water was never described, the only one case referring to avian influenza virus (Faust et al., 2009).

The aim of this study was to evaluate whether zebra mussels could remove the residual load of fecal bacteria and enteric viruses from treated effluents of a municipal wastewater treatment plant (WWTP) to decrease public health risks, especially in effluent dominated streams. Therefore, a series of lab-scale experiments have been carried out to verify the ability of *D. polymorpha* to remove endogenous *E. coli* and two enteric viruses (poliovirus and rotavirus) experimentally spiked into the samples. The fate of the two enteric viruses after concentration by zebra mussels was also investigated.

2. Materials and methods

2.1. Water sampling

Samples were collected from the Nosedo (Milan, Italy) wastewater treatment plant (WWTP) that receives wastewater from 1,250,000 IE, with a negligible industrial contribution. It performs a conventional physical-biological-chemical treatment sequence that includes pre-treatments, primary settling, biological treatment by activated sludge, secondary settling, and filtration. This treatment sequence is followed by disinfection with peracetic acid. Sampling was performed after the secondary settling of the effluent, to better mimic the conditions of many WWTPs not provided with filtration and disinfection. These WWTPs discharge suspended solids that can feed zebra mussels, thus creating favorable conditions to their growth. Five samplings were performed and samples were refrigerated at 4 °C until the microbiological analyses were carried out, within 24 h.

2.2. D. polymorpha sampling

Zebra mussels were collected during Spring 2013 by scuba divers from Lake Lugano, located at the Italy-Switzerland border. Acclimation of mussels was performed by keeping the bivalves in a large-mesh nylon net immersed in a beaker containing 300-mL of the effluent sample, and slowly stirred. Ten mussels (around 2-cm long) were used for each experiment. The net was laid over a stainless-steel grid at around 2 cm from the bottom (Fig. 1) and the beakers were kept at room temperature (around 23 °C).

2.3. Cells

Green monkey kidney cells (Vero) were grown in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% heat-inactivated newborn calf serum (CS; Gibco Life Technologies, Grand Island, NY, USA), 100 U/mL penicillin and 100 mg/mL streptomycin (P/S). *Macaca mulatta* fetal kidney cells (MA104) were grown in the same medium except that fetal calf serum (FCS; Gibco Life Technologies) was used.

2.4. Viruses

The poliovirus type I (attenuated Sabin strain from our lab) and the simian SA11 rotavirus were used. Every sample of the poliovirus- or the rotavirus-inoculated water was filtered through a 0.22-μm membrane before titration. Titration was performed in triplicate on Vero and