Marine Environmental Research 91 (2013) 104–108

Contents lists available at SciVerse ScienceDirect

Marine Environmental Research

journal homepage: www.elsevier.com/locate/marenvrev

Short communication

Assessment of toxicity of dissolved and microencapsulated biocides for control of the Golden Mussel *Limnoperna fortunei*

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ARTICLE INFO

Article history:
Received 24 April 2012
Received in revised form
26 December 2012
Accepted 18 February 2013

Keywords:
Amazon
Environmental impact
Environmental protection
Exotic species
Filter feeding
Fouling organisms
Introduced species
LC50
Lethal effects

ABSTRACT

Biological invasions currently pose major threats to ecosystems worldwide. Invasive bivalves such as the Golden Mussel *Limnoperna fortunei* can act as 'environmental engineers', altering biogeochemistry, reducing biodiversity, and literally changing the landscape of aquatic environments. The risk that this mussel will invade the Amazon basin is a great concern for environmental authorities, especially because no efficient control methods presently exist. In this study, we tested new microencapsulated chemicals, along with the traditional dissolved chlorine and KCl, as alternatives to control *L. fortunei* infestation in industrial and water supply plants along rivers. Because these bivalves can close their valves when they sense toxic substances in the water, microencapsulation has improved the effectiveness of the chemicals in controlling *L. fortunei*, reducing variation in the application and increasing toxicity compared to dissolved chemicals. Microencapsulation should be seriously considered as an alternative to replace hazardous chlorine.

1. Introduction

The invasive Golden Mussel, *Limnoperna fortunei*, arrived in South America in 1991, and since then has spread through the La Plata River basin, traveling about 2500 km upstream in less than 10 years (Oliveira et al., 2006). This small mussel has infested Itaipu (Pestana et al., 2008), the largest hydroelectric reservoir in the world, as well as hundreds of other smaller power plants and water-supply reservoirs in the Paraná River watershed, causing ecological and economic losses (Darrigran, 2002). The mussel recently arrived at the northern border of the Pantanal wetland (16° 04′ 14′ S, 57° 40′ 44′ W) in the heart of South America, and is now threatening the Amazon. Within a 500-km^2 area, two major river basins are in dangerous proximity. The infested Cuiabá River in the Paraguay watershed is only 150 km from the Teles Pires River in the Tapajós River basin. This presents a clear opportunity for the mussel to invade the Amazon River, where it will pose a serious threat to the biodiversity of its aquatic communities.

Control of mussel infestation in natural open areas is virtually impossible, and prevention has been the only strategy to avoid dissemination of the pest. Control checkpoints are installed in areas of high fishing activity and the hulls of fishing boats are both painted with antifouling compounds and disinfected before transfer to another river. Contaminated industrial facilities and power plants often use chemical control to minimize infestation damage in pipelines. Chlorine is the most common chemical used in this control strategy, because it is low-cost, easy to acquire, and its use is licensed by the USEPA (2009) and MERCOSUL authorities (CONAMA, 2005). However, the efficiency of chlorine has been strongly questioned and side effects of total residual chlorine (TRC) in non-target species can be more damaging than the infestation itself (see Anderson and Richards, 1966; Rajagopal et al., 1991). Bivalves can detect chlorine concentrations of less than 10 μg L^{-1} in the water (Kramer et al., 1989) and close their shells, isolating themselves from the environment for up to 2 weeks. Long periods

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http://dx.doi.org/10.1016/j.marenvres.2013.02.012

of exposure to high concentrations are necessary to obtain an effect (Turner et al., 1948), making any attempt at chemical control, to say the least, challenging. According to Costa et al. (2008a), we must consider the tripod 'what', 'how' and 'when' to apply the control. In this study, we tested the toxicity of 8 substances, including 2 microencapsulated chemicals, to the Golden Mussel in standard laboratory conditions.

2. Materials and methods

Mussels were collected from floating structures in the Jucuí River (southern Brazil; 29°58′12″ S; 51°16′56″ W) in January 2007 and maintained in 500-L tanks in the laboratory (25 °C; pH 7 ± 0.2; 48 mg L^{-1} $CaCO_3$ and oxygen saturation) until testing. Mussels 15–25 mm long were sorted and then were acclimatized to the test conditions (2 L; 10 org L^{-1}) for 24 h prior to exposure. Mussels were exposed to sodium dichloroisocyanurate (NaDCC – 32% Cl – 1, 5, 10, 50, 100, 500, 1000, 1500, 2000 mg L^{-1}); trichloroisocyanuric acid (TCCA – 65% Cl – 10, 100, 500, 1000, 1500, 2000 mg L^{-1}); chlorine dioxide (ClO_2 – 53% Cl – 1, 10, 50, 75, 100, 150, 200, 250, 300, 400, 500, 600, 700, 800 mg L^{-1}); sodium hypochlorite (NaOCl – 48% Cl – 10, 100, 500, 1000 mg L^{-1}); potassium chloride (KCl – 10, 100, 1000, 2000, 4000, 6000, 8000, 10,000 mg L^{-1}) and sodium chloride (NaCl – 1000, 2000, 3000, 4000, 5000, 10,000, 15,000, 20,000 mg L^{-1}). Two substances were tested in microencapsulated form, as an alternative means of delivering the toxicants (Aldridge et al., 2006): potassium chloride (mKCl 12, 25, 125, 250, 500, 1500, 3000, 6000 mg L^{-1}) and poly-diallyldimethyl ammonium chloride (polyDADMAC – 12, 25, 50, 100, 125, 200, 250, 400, 500, 800, 1000 mg L^{-1}). All concentrations were tested in triplicate. Semi-static 48 h-LC50 tests were performed according to standard methods (EPA-821-R-02-012). Test solution was replaced after 24 h, mortality was checked every 8 h and dead mussels were removed to prevent water spoiling. Mussels were considered dead if failed to close their valves in response to a blunt probe. The microcapsules were kept in suspension in the test vessels by aeration. Mortality was checked every 8 h, and dead mussels were removed to prevent the water from spoiling. Mussels were allowed to recover for 48 h in clean water before they were considered dead (if they failed to close their valves in response to a blunt probe). Results were rejected if the control mortality exceeded 10%. Experiments were repeated whenever a refinement in the effective concentrations range was necessary. Sometimes, up to 6 independent replications were necessary to establish ranges. To simulate the pipeline environment were mussels are found in industrial installations, we performed flow-through toxicity test in 2 m-long flumes (10 cm wide, 8 cm depth; 11 L capacity; 3.5 mL s^{-1} flow) containing 100 mussels (1 cm) each for microencapsulated KCl (90, 250, 500, 1000 mg L^{-1}) and polyDADMAC (90 mg L^{-1}). Microencapsulated biocides were mixed in water immediately before they were administered to the flow-through system, allowing the mussels to filter the particles while carried way by the water flow on the flume. The microencapsulate dose were administrated were every other 30 min for 6 h. Concentrations in the flow-through system were tested in triplicate in 3 independent experiments. The 48 h-LC50 values were predicted by Probit (goodness of fit measured by chi-square test with $p < 0.05$) using an *R* function (see Pacheco and Rebelo, 2013). The high variability inherent to bivalve ecotoxicological test, made impossible to reliably calculate NOEC and LOEC and supported by the criticism of the parameter made by Warne and van Dam (2008), we have decided no to present it. Instead, due to the mussel population control aim of this study, we presented two other values that we considered more relevant: the Highest control mortality concentrations (HCMC), which is the observed concentration at which in at least one of the replicates all animals survived like in the control; and least full mortality concentration (LFMC), which is the observed concentration at which in at least one of the replicates, all animals died, like in the most toxic concentration.

3. Results and discussion

The results from replicates and independent experiments were grouped for LC50 calculation and box-plot graphic analysis. Table 1 shows the 48 h-LC50 values and Fig. 1 shows the dose–response relationships for all substances tested.

The microencapsulated polyDADMAC showed the highest toxicity of all substances tested, with a 48 h-LC50 of 270.9 mg L^{-1} (Fig. 1a). The toxicity is even higher if we consider that only 20% of the total microencapsulated particle is the active product, with an estimated 48 h-LC50 of 54.18 mg L^{-1}. Despite the wide variability in mortality at exposure concentrations of 25–200 mg L^{-1}, microencapsulated polyDADMAC was 9.3-fold more toxic than microencapsulated KCl. Specific literature on the mechanism of polyDADMAC toxicity in mussels is unavailable, but generally, surfactants tend to adsorb on the plasma membranes, including gill tissues, disrupting transport processes between cells and the environment (e.g., Post et al., 1996). The LC50 value is comparable to other anionic surfactants (linear dodecylbenzene sulphonate, tetrapropylene benzene sulphonate, lauryl ether sulphate and tallow alcohol ethoxylate) that Swedmark et al. (1971) tested in bivalves (*Mytilus edulis*, *Cardium edule* and *Mya arenaria*) obtaining

Table 1
48 h-LC50 values predicted with the Probits regression with 95% confidence intervals, highest control mortality concentration (HCMC) and least full mortality concentration (LFMC) for each chemical substance[a] tested (in dissolved or microencapsulated – m – form) against *Limnoperna fortunei* in the semi-static and flow-through (ft) toxicity test. Refer to Fig. 1 for dose–response relationships.

Chemical	48 h-LC50 (mg L^{-1})	−95.0%CL	+95.0%CL	*N* [*n*][b]	Df	Resid. dev	Pr(>Chi)	HCMC	LFMC
polyDADMAC-m	270.9	253.6	288.2	8 [13]	139	668.44	2.20E-16	25	500
TCCA	368.2	287.6	448.7	1 [6]	19	123.02	2.20E-16	10	500
NaDCC	376.0	328.1	423.9	3 [9]	49	423.92	2.20E-16	10	500
ClO_2	427.6	282.4	572.9	6 [21]	57	488.86	7.05E-03	1	10
NaOCl	663.6	594.8	732.4	2 [4]	28	77.68	2.20E-16	5	1000
KCl	1439.0	1235.9	1642.2	2 [9]	34	192.38	2.20E-16	10	2000
KCl-m[c]	2536.9	2350.6	2723.2	6 [10]	94	411.39	2.20E-16	250	6000
NaCl	8336.7	7075.1	9598.3	3 [8]	25	68.961	2.20E-16	2000	20,000
Chemical	6 h-LC50 (mgL-1)	−95.0%CL	+95.0%CL	*N* [*n*]b	Df	Resid. Dev	Pr(>Chi)	HCMC	LFMC
polyDADMAC-m-ft	1313.3	1037.0	91589.6	3 [1]	16	183.82	3.57E-11	90	90
KCl-m-ft	8303.1	7728.4	8877.	3 [4]	43	979.66	2.20E-16	90	1000

[a] Chemicals are PolyDADMAC – poly-diallyldimethyl ammonium chloride; ClO_2 – chlorine dioxide; TCCA – trichloroisocyanuric acid; NaDCC – sodium dichloroisocyanurate; NaOCl – sodium hypochlorite; KCl – potassium chloride and NaCl – sodium chloride.
[b] '*N*' indicates the number of independent experiments and '*n*' the number of concentrations tested.
[c] The estimated LC50 is based on the total weight of the microcapsule, which was 30% and 20% active product for PolyDADMAC-m and KCl-m respectively.