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Assessment of domestic landfill leachate toxicity to the Asian clam *Corbicula fluminea* via biomarkers

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ABSTRACT

In order to evaluate the effects of domestic landfill leachate to bivalves *Corbicula fluminea*, clams were exposed to different leachate concentrations (v/v): 2, 3, 6 and 10 percent, corresponding to dilutions observed along a stream that receives this effluent, or only to clean water for comparisons. After 5 and 15 days of exposure the activity of the biotransformation enzymes 7-ethoxyresorufin-*O*-deethylase (EROD) and glutathione S-transferase (GST), the multixenobiotic resistance mechanism (MXR) and lipid peroxidation (LPO) in gills and digestive gland and metallothionein (MT) content in gills were evaluated. Differences in biomarkers responses were observed between gills and digestive gland, except for MXR that decreased in both tissues of clams exposed to 6 percent for 5 days. EROD activity in gills was reduced in all leachate concentrations after 5 days and only in 2 percent after 15 days exposure, while an EROD increase was observed in digestive gland after 15 days exposure to 6 percent. GST activity increased only in the gills of clams exposed to 10 percent for 5 days. LPO varied between tissues and different conditions. A significant increase in LPO was observed in the gills, after 5 days exposure to 2 and 6 percent, and in digestive gland after 5 and 15 days exposure to 2 and 3 percent. MT content in the gills increased after 15 days exposure to 2 percent. In conclusion, different leachate concentrations tested here caused biochemical changes in *C. fluminea*, but due to the observed variability in biomarkers responses among leachate concentrations, it was difficult to determine patterns or thresholds concentrations.

1. Introduction

Leachate is a complex mixture produced by the decomposition of wastes and can contain dissolved organic matter, inorganic macrocomponents, metals and xenobiotic organic compounds (Christensen et al., 2001). Risk assessment of this mixture is often difficult due to its complex and highly variable composition and should be based not only on chemical analysis but the interactions among its chemicals and/or toxic degradation products with the biota as well (Tsarpali et al., 2012).

Bioassays have already been successfully performed for the evaluation of landfill leachate toxicity (Thomas et al., 2009; Ribé et al., 2012), but biomarkers application is not frequent. Biomarkers are assessment and monitoring tools able to detect effects of chemical exposure in aquatic organisms before they become significant in conservation or ecological terms (Long et al., 2004), however there are only few studies regarding biomarkers application to assess landfill leachate effects on bivalves (Tsarpali and Dailianis, 2012; Toufexi et al., 2013). These animals have been considered as good bioindicators (O'Connor, 2002) as they exhibit alterations in response to contaminant exposure present in the leachate, like metals (Marie et al., 2006; Zhang et al., 2010) and hydrocarbons (Large et al., 2002). In particular, the Asian clam *Corbicula fluminea* has been the focus of several toxicological studies, including biomarkers approach (Santos and Martinez, 2014).

Several biomarkers can be used to evaluate leachate toxicity in aquatic organisms, such as the mechanisms related to the biotransformation of xenobiotics, which usually consist of three phases including numerous different enzyme systems and several types of substrates. Phase I reactions involve oxidation, reduction and hydrolysis, catalyzed mainly by cytochrome P450, that facilitate the excretion of compounds, transforming them into more water-soluble compounds and also serving as a substrate for phase II reactions that increase the excretion rate (Stegeman et al., 1992). As well stablished for fishes, several studies showed that the

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activity of 7-ethoxyresorufin-*O*-deethylase (EROD) should be used in bivalves as an indirect measurement of the catalytic activity of one P450 family, CYP1A (Binelli et al., 2006; Faria et al., 2009). Glutathione S-transferase (GST) is an enzyme of phase II responsible for conjugation reactions which is frequently used as biomarker (Hodgson et al., 2008). Related to phase III, the multixenobiotic resistance mechanism (MXR) is responsible for the elimination of xenobiotics or its metabolites through ATP-dependant proteins and could be alterated after exposure to contaminants (Bard, 2000). Osaki et al. (2006) showed that in the medaka fish, *Oryzias latipes*, the exposure to landfill leachate induced EROD activity, but the inhibition of the same biomarker was already been observed in the fish cell line RTG-2 by Pablos et al. (2011). This evidenced that variable composition of landfill leachates can promote different alterations in biomarkers.

In addition, contaminants could stimulate reactive oxygen species (ROS) production and the resultant oxidative stress has been indicated as a mechanism of toxicity in aquatic organisms exposed to a broad range of contaminants (Livingstone, 2003). Damage caused by ROS can be considered proportional to the levels of tissue lipid peroxidation (Almeida et al., 2005), which makes this an important tool to assess generic toxicity of mixtures such as landfill leachates. Tsarpali and Dailianis (2012) and Toufexi et al. (2013) recently showed that hemocytes of *Mytilus galloprovincialis* treated with relevant concentrations of leachate showed a significant enhancement of lipid peroxidation products (malondialdehyde (MDA)).

Metals within landfill leachate are also of concern due to their high toxicity (Thomas et al., 2009) and metallothionein (MT) is a commonly used biomarker usually related to the presence of metals (Amiard et al., 2006). Other chemical compounds, particularly oxidizing agents, also induce MT, since it also works as an antioxidant (Viarengo et al., 1999). It was previously observed that landfill leachate increases MT content in gills and digestive glands of *M. galloprovincialis* (Tsarpali and Dailianis, 2012).

Thus, the purpose of this work was to evaluate the effects promoted by leachate from domestic waste landfill on the freshwater clam *C. fluminea* measuring biochemical biomarkers such as biotransformation enzymes, MXR mechanism, lipid peroxidation and metallothionein content in gills and digestive glands. These organs were chosen for biomarkers analyses considering that digestive gland is a primary organ for bioaccumulation and is involved in pollutant detoxification and homeostasis maintenance (Cappello et al., 2013), while the gills are the main entrance of contaminants present in the environment (Rocha and Souza, 2012).

2. Material and methods

2.1. Experimental design

Adult bivalves of the species *C. fluminea* ($n=120$), measuring 3.9 ± 0.06 cm in length and 2.74 ± 0.06 cm in height (mean $\pm$ SEM), were collected from an urban lake in the municipality of Londrina (PR, Brazil) and acclimated in the laboratory for 7 days before the start of toxicity tests. During acclimation the specimens were maintained under 12 h:12 h photoperiod in 70 L glass aquaria containing 30 L of clean aerated and dechlorinated water with the following characteristics (mean $\pm$ SE, $n=4$): temperature: 16.65 ± 0.37 °C, conductivity: 72.25 ± 1.98 μS cm^{-1}, dissolved oxygen: 6.83 ± 0.15 mg L^{-1} O_2, pH: 7.08 ± 0.21. After this period, animals were exposed to different concentrations of leachate for a period of 5 and 15 days in 5 L glass containers, with 12 animals in each, filled with 1 L of clean water (control group or CTR) or with 1 L of different leachate concentrations (2, 3, 6 and 10 percent v/v). The longer tests (15 days) were performed in semi-static conditions with water renewal after 7 days. For each period of exposure (5 and 15 days) toxicity tests were run independently, not simultaneously.

The raw leachate used in the present study was obtained from the controlled landfill of the municipality of Londrina before the aerobic treatment and was maintained in plastic container protected from the light at room temperature.

Table 1
Physical and chemical characteristics of raw leachate from the controlled landfill of the municipality of Londrina collected before the aerobic treatment, analyzed in accordance to APHA, AWWA and WEF (2005).

Characteristics	Values (units)
Electrical conductivity	8865 μS cm^{-1}
Biochemical oxygen demand (BOD)	593 mg O_2 L^{-1}
Dissolved organic carbon (DOC)	3025 mg O_2 L^{-1}
Ammonia	957 mg L^{-1}
Total Kjeldahl nitrogen (NKT)	1052 mg L^{-1}
Chlorides	1751 mg L^{-1}
Total phosphorus	2 mg L^{-1}
Total aluminum	0.435 mg L^{-1}
Total cooper	0.081 mg L^{-1}
Total chromium	0.06 mg L^{-1}
Total iron	8.74 mg L^{-1}
Total manganese	1.68 mg L^{-1}
Total nickel	0.017 mg L^{-1}
Total silver	0.401 mg L^{-1}

Some physical and chemical analyses of the leachate were made in accordance to APHA, AWWA and WEF (2005) and the results are presented in Table 1.

For the experiments the landfill leachate was diluted in different proportions with declorinated water to obtain the following concentrations (v/v): 2, 3, 6 and 10 percent. The conductivity of these concentrations corresponds to the conductivity observed along the stream that receives the leachate from the controlled landfill of the municipality of Londrina, determined in a previous study performed in our laboratory (data not published).

2.2. Sampling

After the experimental periods (5 and 15 days) the anterior adductor muscles of the clams were cut allowing the valves to open and the gills and digestive glands were removed. The tissues were frozen at −72 °C for subsequent analysis of the biomarkers.

2.3. Biomarkers

The gills and digestive gland removed from the clams ($n=6$–8) were individually homogenized in potassium phosphate buffer (0.1 M, pH 7) and then centrifuged (14,000*g*; 20 min, 4 °C) for the determination of 7-ethoxyresorufin-*O*-deethylase (EROD), glutathione S-transferase (GST) and lipid peroxidation (LPO) in the supernatant.

2.3.1. Ethoxyresorufin-O-deethylase (EROD)

The CYP1A activity was determined by analyzing the EROD activity, which was estimated by the rate of conversion of 7-ethoxyresorufin to resorufin, according to the protocol of Eggens and Galgani (1992), with modifications. The reaction was initiated by adding the sample to the reactive mixture (0.1 M potassium phosphate buffer, pH 7.6; 2 mM NADPH and 0.1 mM 7-ethoxyresorufin). The progressive increase in fluorescence resulting from the formation of resorufin was measured at 1-min intervals for 30 min (ex/em: 530/590 nm). The EROD activity was expressed in pmol resorufin min^{-1} mg of $protein^{-1}$.

2.3.2. Glutathione S-transferase (GST)

The GST activity was determined using the method described by Keen et al. (1976). This method is based on the GST catalyzed conjugation of reduced glutathione (GSH) with 1-chloro-2,4-dinitrobenzene (CDNB). The increase in CDNB conjugate was monitored for 1 min in a spectrophotometer at 340 nm and the enzyme activity was expressed in nmol CDNB conjugate min^{-1} mg of $protein^{-1}$.

2.3.3. Multixenobiotic resistance mechanism (MXR)

The test for rhodamine B fluorescence accumulation (substrate of P-gp) was performed according to Kurelec et al. (2000) to evaluate the multixenobiotic resistance mechanism (MXR). In this assay, an increase in accumulated fluorescence represents a reduction in MXR. The gills and digestive glands were kept *ex vivo* in a solution of 1 μM of rhodamine B fluorescent dye for a period of 2 h. After this period, the tissues were washed in saline (26 mM NaCl; 4.3 mM sucrose; pH 7.4), homogenized in distilled water (1:7–w/v) and centrifuged (1700*g*; 7 min; 4 °C). The fluorescence corresponding to accumulated rhodamine was measured (ex/em: 544/590 nm) and the concentrations were determined by a rhodamine standard curve, with the data expressed in μM rhodamine mg of wet $tissue^{-1}$.