# Chromium Accumulation in the Asian Clam, *Corbicula fluminea* (Müller, 1774), as an Indicative of Landfill Leachate Contamination

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**Abstract** The Asian clam *Corbicula fluminea* exposed in situ for periods of 1, 5, 15 and 30 days along a stream which receives landfill leachate effluent showed increased Cr accumulation in gills and digestive gland, although Cr concentrations have been found to be low in sediment. Other metals such as Pb, Ni, Al and Cu were also analyzed but were found to accumulate in clam tissues in lower concentrations or without showing a consistent pattern. Thus, the accumulation of a single contaminant such as Cr is proposed to be used as a tool to assess exposure to a complex mixture such as landfill leachates.

The accumulation of contaminants in bivalve tissues is a parameter commonly used to evaluate the presence of different chemicals in the aquatic environment and can provide valuable information about the quality status of a specific location (Gunther et al. 1999). In this context, the accumulation of a single contaminant in aquatic organisms could be in diagnose and/or monitoring an important indicative of the presence of contaminants even when there is a mixture of them.

Nowadays, landfills or dumps are still a severe environmental problem mainly due to the leachate generation (Kulikouska and Klimiuk 2008), which is a liquid produced during waste decomposition that may have a quite variable composition and contains a mixture of contaminants such as metals (cadmium, chromium, copper, lead, nickel and zinc), xenobiotic organic compounds (aromatic hydrocarbons, phenols, chlorinated aliphatic compounds) and other low-concentration components such as boron, arsenic, barium, selenium, mercury and cobalt (Christensen et al. 2001).

The present work investigated tissue accumulation of some metals often found in landfill leachate in the freshwater bivalve *Corbicula fluminea* confined along a contaminated stream, in order to indicate the use of metal accumulation in monitoring the contamination by leachate effluent.

## Materials and Methods

Periquitos stream is located downstream of the controlled domestic landfill of the municipality of Londrina, Paraná, Brazil and receives the leachate effluent produced there. The landfill is located in the southeast of the city, approximately 7 km from the city centre. This site, whose total area is 135,000 m$^2$, has been receiving waste since 1977 without any specific technical criterion or preparation. Thus, the leachate reaches the stream by punctual and diffuse means, considering that no sealing exists in the landfill and only part of the produced leachate is canalized into a lagoon for aerobic treatment.

Freshwater bivalves of the species *C. fluminea* ($n = 119$), measuring $2.71 \pm 0.02$ cm in length and $2.63 \pm 0.02$ cm in height (mean $\pm$ SE), were collected in an urban lake in the city of Londrina, PR. These fresh-collected specimens formed a group referred herein as "Sampling" ($n = 7$), and the results obtained for this group were compared with those of other sites where in situ

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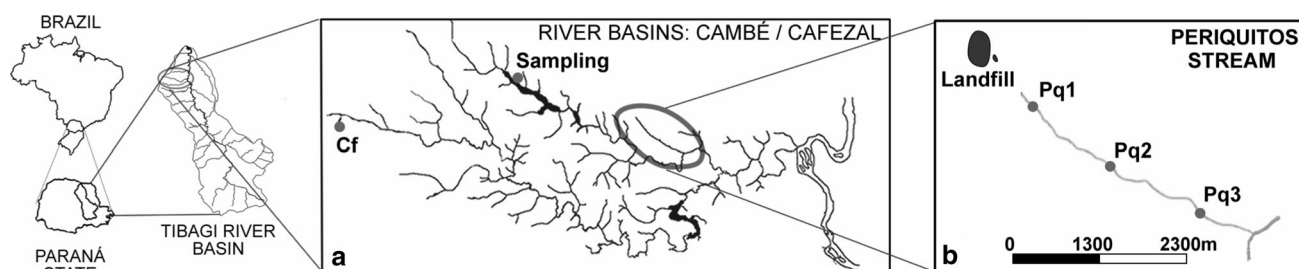e-mail: cbueno@uel.br

**Fig. 1** Studied sites location in Tibagi river basin, Paraná, Brazil. **a** Cambé river basin, where the Sampling site and the Periquitos stream are located, and Cafezal river basin, where is located the Cf site. **b** Periquitos stream in detail, indicating the Londrina' domestic waste landfill and the site where in situ tests were performed (*Pq1*, *Pq2* and *Pq3*)

tests were performed. Animals ($n = 7$ for each site, for each period of time) were submitted to in situ tests over a 1, 5, 15 and 30 days period in three distinct sites within the Periquitos stream (Pq1, Pq2 and Pq3), distant approximately 100, 1,300 and 2,500 m from effluent release, respectively (Fig. 1b). In situ tests were also performed in the Cafezal stream (Cf), showed in Fig. 1a, which was chosen due to its proximity to the stream headwaters, in a non-urbanized area, with a well-preserved riparian zone and no evidence or reports indicating the presence of leachate in that area.

Throughout the in situ toxicity tests, the bivalve maintenance was made using bags made of net with a mesh size of 1 cm, which allows water flow. These bags were placed near the bank in contact with the surface sediment layer, and weights were added at the base to prevent flotation. After the experimental periods, all specimens were placed in plastic containers filled with stream-water from each site and transported to the laboratory, where sampling took place.

Water physical and chemical parameters such as conductivity, pH, turbidity, dissolved oxygen and temperature, were measured in the field using a multi-parameter meter, Horiba 5000, at the Sampling site and at the initial and final phases of all in situ tests.

Samples of water and superficial sediment layer from each site in Periquitos stream, as well as the Sampling and Cf sites, were collected and stored in acid treated plastic tubes. Water samples were fixed in 1 % nitric acid and stored at 4°C while sediment samples were digested according to Almeida et al. (2005). To avoid contamination, all material used for metal determination was soaked in 10 % nitric acid for 24 h and rinsed with ultrapure water. In order to determine metals concentrations in tissues, gills and digestive glands of bivalves were removed, washed in a saline solution (26 mM NaCl; 4.3 mM sucrose; pH 7.4), transferred into tubes and kept at 60°C to obtain completely dry tissues. After that, dry tissues were weighed and digested with a suprapure nitric acid 65 % (Merck) at 60°C until complete evaporation of the acid. After this step,

1 mL of 0.2 % suprapure nitric acid 65 % (Merck) was added to the sample and the digested material was centrifuged ($2,000g$; 10 min). The concentrations of Pb, Ni, Al, Cu and Cr were determined by electro thermal ionization in a graphite furnace attached to an atomic absorption spectrophotometer (Perkin Elmer AAnalyst 700). In order to assure the quality of metal analysis, a standard reference material (tissue) of the bivalve *Mytilus edulis* (ERM-CE728—IRMM) was used and resulted in a recovery rate of 105 % for the Cr and 104 % for Cu.

The results were expressed as mean ± standard error. Physical and chemical parameters of the water, as well as metals concentrations in sediment and water samples were compared among the different sites analyzed (Sampling site, Cf, Pq1, Pq2 and Pq3), without taking into account different experimental periods. Mean values obtained from each metal concentration in tissues were used for comparisons among: (1) all sites where in situ tests were performed (Cf, Pq1, Pq2 and Pq3) and the Sampling site, within the same experimental period and; (2) different experimental periods (1, 5, 15 and 30 days) of the same site. A parametric analysis of variance (ANOVA) was applied on these analyses, followed by a multiple comparisons test (Student–Newman–Keuls test) when necessary. Statistical significance was defined at $p \leq 0.05$.

## Results and Discussion

The physical and chemical parameters did not show significant variations with the exception of the electric conductivity ($\mu S\ cm^{-1}$) that decreased as the distance from the contamination source increased (Pq1: $974 \pm 88$; Pq2: $590 \pm 47$; Pq3: $436 \pm 40$; mean ± SE), and were significantly higher than those measured in Sampling ($133 \pm 21$) and Cf ($42 \pm 1$) sites. The temperature (18.6–23.0°C; interval), dissolved oxygen (7.57–9.82 mg L$^{-1}$), pH (6.75–7.35) and turbidity (8.25–17.07 NTU) did not vary significantly among sites.

**Table 1** Metals concentrations ($\mu$g g$^{-1}$) in sediment from studied sites (mean $\pm$ SEM, $n$ = 3–4)

| Site | Cr | Cu | Pb | Al | Ni |
|------|------|------|------|------|------|
| Sampling | 2.45 $\pm$ 0.67 | 32.00 $\pm$ 3.26 | 4.72 $\pm$ 0.39 | 818.25 $\pm$ 112.21 | 1.07 $\pm$ 0.06 |
| Cf | 1.56 $\pm$ 0.53 | 47.44 $\pm$ 13.93 | 1.82 $\pm$ 0.96* | 685.92 $\pm$ 158.79 | 1.34 $\pm$ 0.30 |
| Pq1 | 3.95 $\pm$ 0.59[#] | 60.86 $\pm$ 4.91 | 3.96 $\pm$ 0.50 | 1074.20 $\pm$ 125.86 | 2.61 $\pm$ 0.17*,[#] |
| Pq2 | 4.44 $\pm$ 0.19[#] | 55.86 $\pm$ 4.59 | 2.55 $\pm$ 0.13 | 1080.80 $\pm$ 61.45 | 2.61 $\pm$ 0.15*,[#] |
| Pq3 | 3.82 $\pm$ 0.45[#] | 61.94 $\pm$ 1.75 | 2.26 $\pm$ 0.44 | 904.03 $\pm$ 27.77 | 2.62 $\pm$ 0.16*,[#] |

* Significant difference from sampling site; [#] significant difference from Cf ($p \leq 0.05$). *n.d.* not detected. Detection limits: Al 0.1 $\mu$g L$^{-1}$; Cr 0.004 $\mu$g L$^{-1}$; Cu 0.014 $\mu$g L$^{-1}$; Ni 0.07 $\mu$g L$^{-1}$; Pb 0.05 $\mu$g L$^{-1}$

Animal mortality was observed during the experiments, especially in those sites located in the leachate-contaminated stream. Animals were considered dead when their shells gaped. At site Pq1, specimens' mortality reached 100 % after 15 days of experiment, 27 % in 5 days and 3 % in 1 day. At Pq2 there was 100 % mortality in the 30-day test, 15 % in 15 days and 3 % in other experimental periods. Finally, 19 % mortality was observed at site Pq3 in the 30-day test, and only 3 % in the remaining experimental periods. Therefore, a decreasing pattern was observed in the mortality rate of animals held in Periquitos stream as the distance from the leachate discharge source increased. At the Cf site, the observed mortality was always lower than 6 %. Thus, due to the mortality data from Pq1 in 15 and 30 days and Pq2 in 30 days were not obtained.

Metals showed low concentrations in water. The concentrations ($\mu$g L$^{-1}$) of Cr ranged from 0.70 (Cf) to 3.58 (Pq2), Cu from 1.53 (Pq2) to 4.84 (Cf), Pb from 0.11 (Sampling) to 1.15 (Pq3) and Ni from 0.53 (Cf) to 3.05 (Pq2). The concentrations of metals measured in the sediment collected from the studied sites are displayed in Table 1. Significantly higher concentrations of Cr and Ni were detected in sediment samples from all sites in the leachate contaminated stream, nevertheless, these values were below the limits set to dredged materials by the Brazilian Guidelines (CONAMA 2004) (Cr = 37.3 $\mu$g g$^{-1}$; Ni = 18 $\mu$g g$^{-1}$) and also when compared with other contaminated sites in previous studies (Cossu et al. 2000). It should be noted that even though the concentrations found were lower than the risk-based limits, metals that were in sediment could be transferred to water through several chemical processes, such as elevation in salinity, changes in sediment redox status, reduced pH and the presence of organic complexing compounds (Bjerregaard and Andersen 2007). As a consequence metals can accumulate in *C. fluminea* tissues. The relation between sediment concentration and tissue accumulation was observed herein for Cr.

Besides the fact that metals do not constitute the major problem in leachate contamination, mainly because of their low concentrations within this mixture (Christensen et al. 2001), the use of their accumulation in aquatic organisms' tissues could be a potential indicator of mixtures contamination, such as landfill leachate. Metal concentration in animal tissues is affected by several factors, such as its presence and concentration in food, sediment and water, and therefore represents the local environmental condition through time, rather than just at the sampling time (Peltier et al. 2008).

Bivalves had higher metal accumulation in gills than in digestive glands. In fact, metal concentrations were two to five times greater in gills. Among the analyzed metals, Cr was the only one that clearly presented an increasing pattern in the tissues of *C. fluminea* kept at all three sites along Periquitos stream when compared to those of animals from Sampling and Cf sites (Tables 2, 3). Furthermore, after 1 day of exposure an increased accumulation of this metal was already detected followed by a continuous increase over time, which was up to three times higher in gills and four times higher in digestive glands of animals caged in the contaminated stream. It seems that when Cr concentration reached certain level (approximately 35 $\mu$g g$^{-1}$) in *C. fluminea* gills landfill leachate was lethal, suggesting a relation between this metal accumulation and leachate toxicity. Cu also increased in both tissues, but only in animals confined at Pq2 (Tables 2, 3).

The accumulation of most of the other evaluated metals (Al, Ni and Pb) showed a less consistent pattern when one compares animals caged along Periquitos stream with Sampling and Cf sites (Tables 2, 3). The concentrations of these metals in *C. fluminea* tissues were similar to those found in other studies with the same species under control conditions, in which only Cr and Cu were detected at higher levels (Angelo et al. 2007; Peltier et al. 2009; Shoults-Wilson et al. 2009). With regard to the Cr, Shoults-Wilson et al. (2009) observed that concentration of this metal in control individuals varied from 1 to 8 $\mu$g g$^{-1}$ dry weight (considering the entire animal), values somewhat similar to those obtained herein in specimens from the Sampling and Cf sites. On the other hand, Cr concentrations determined in gills samples from the Periquitos stream ranged up to 34 $\mu$g g$^{-1}$ dry weight, and up to 18 $\mu$g g$^{-1}$ dry weight in the digestive gland.

**Table 2** Metal accumulation (µg g$^{-1}$ dry tissue) in gills of *C. fluminea* from sampling site and submitted to in situ tests for 1, 5, 15 and 30 days in Cafezal stream (Cf) and at the three sites along the leachate contaminated stream (Pq1, Pq2 and Pq3)

| | | Sampling | Cf | Pq1 | Pq2 | Pq3 |
|---|---|---|---|---|---|---|
| Cr | 1 | 13.35 ± 0.11 | 11.43 ± 0.69[a] | 8.24 ± 2.44[a,#] | 16.95 ± 1.16[a,#] | 22.59 ± 2.16[a,*,#] |
| | 5 | | 11.04 ± 1.53[a] | 31.44 ± 4.39[b,*,#] | 20.04 ± 1.51[ab,#] | 17.51 ± 1.48[a,#] |
| | 15 | | 13.74 ± 0.99[a] | n.a. | 29.83 ± 1.87[b,*,#] | 34.32 ± 1.70[b,*,#] |
| | 30 | | 10.85 ± 1.16[a] | n.a. | n.a. | 22.75 ± 1.36[a,*,#] |
| Cu | 1 | 96.98 ± 2.84 | 65.95 ± 8.61[a] | 99.08 ± 12.34[a] | 78.14 ± 5.38[a,*] | 93.24 ± 12.87[a] |
| | 5 | | 58.74 ± 7.82[a,*] | 112.73 ± 12.33[a,#] | 69.99 ± 4.75[a,*] | 91.99 ± 13.56[a] |
| | 15 | | 115.94 ± 13.12[b] | n.a. | 133.17 ± 3.54[b,*] | 93.98 ± 3.92[a] |
| | 30 | | 71.88 ± 5.48[a] | n.a. | n.a. | 100.04 ± 6.01[a,#] |
| Pb | 1 | 0.34 ± 0.11 | 0.51 ± 0.11[a] | 0.22 ± 0.00[a] | 0.20 ± 0.04[a,#] | 0.23 ± 0.06[a] |
| | 5 | | 0.76 ± 0.08[b,*] | 0.34 ± 0.05[a] | 0.29 ± 0.06[a,#] | 0.60 ± 0.14[a] |
| | 15 | | 0.41 ± 0.05[a] | n.a. | 0.51 ± 0.07[a] | 0.21 ± 0.17[a] |
| | 30 | | 0.26 ± 0.02[a] | n.a. | n.a. | 0.54 ± 0.12[a,#] |
| Al | 1 | 172.04 ± 20.29 | 80.51 ± 7.44[a,*] | 143.39 ± 39.88[a] | 93.81 ± 17.94[a,*] | 189.71 ± 17.58[a,#] |
| | 5 | | 50.31 ± 6.23[ab,*] | 62.84 ± 26.25[a] | 51.28 ± 11.59[a,*] | 73.36 ± 19.84[b,] |
| | 15 | | 30.71 ± 3.04[b,*] | n.a. | 62.05 ± 5.96[a,*] | 86.54 ± 30.48[b,*] |
| | 30 | | 22.96 ± 4.05[b,*] | n.a. | n.a. | 82.63 ± 9.00[b,*,#] |
| Ni | 1 | 2.79 ± 0.41 | 2.41 ± 0.31[a] | 2.01 ± 0.30[a] | 1.39 ± 0.07[ab,*] | 1.83 ± 0.36[a,*] |
| | 5 | | 1.29 ± 0.22[a] | 1.09 ± 0.12[a,*] | 0.92 ± 0.11[a,*] | 1.37 ± 0.20[a,*] |
| | 15 | | 2.55 ± 0.43[a] | n.a. | 1.93 ± 0.22[b,*] | 1.31 ± 0.25[a,*] |
| | 30 | | 3.92 ± 1.15[a] | n.a. | n.a. | 1.25 ± 0.04[a,*,#] |

Values are mean ± SEM (n = 4–7)

* Significant difference from Sampling site; # significant difference from Cf in a same test-period; Different letters indicate significant difference between the test-periods on a same site (p ≤ 0.05). n.a.: not analyzed because the animals died

**Table 3** Metal accumulation (µg g$^{-1}$ dry tissue) in digestive gland of *C. fluminea* from sampling site and submitted to in situ tests for 1, 5, 15 and 30 days in Cafezal stream (Cf) and at the three sites along the leachate contaminated stream (Pq1, Pq2 and Pq3)

| | | Sampling | Cf | Pq1 | Pq2 | Pq3 |
|---|---|---|---|---|---|---|
| Cr | 1 | 5.09 ± 0.89 | 4.05 ± 0.72[a] | 8.75 ± 1.55[a,#] | 7.61 ± 1.06[a] | 9.05 ± 1.27[a,*,#] |
| | 5 | | 3.28 ± 0.24[a] | 13.46 ± 1.69[b,*,#] | 14.81 ± 2.29[b,*,#] | 10.45 ± 2.03[a,*,#] |
| | 15 | | 4.80 ± 0.67[a] | n.a. | 17.31 ± 1.25[b,*,#] | 17.03 ± 1.32[b,*,#] |
| | 30 | | 3.51 ± 0.45[a] | n.a. | n.a. | 18.22 ± 1.36[b,*,#] |
| Cu | 1 | 70.18 ± 8.51 | 48.46 ± 5.10[a,*] | 66.94 ± 2.91[a,#] | 66.76 ± 4.72[a,#] | 57.91 ± 3.17[a] |
| | 5 | | 48.69 ± 1.12[a,*] | 86.18 ± 13.07[a,#] | 90.91 ± 2.53[b,*,#] | 69.18 ± 4.36[a] |
| | 15 | | 31.53 ± 4.70[a,*] | n.a. | 101.28 ± 5.00[b,*,#] | 79.58 ± 5.50[a,#] |
| | 30 | | 29.13 ± 5.33[a,*] | n.a. | n.a. | 80.32 ± 6.01[a,#] |
| Pb | 1 | 0.10 ± 0.03 | 0.67 ± 0.22[a,*] | 0.06 ± 0.00[a,#] | 0.11 ± 0.02[a,#] | 0.13 ± 0.03[a,#] |
| | 5 | | 0.37 ± 0.07[ab] | 0.10 ± 0.03[a,#] | 0.14 ± 0.05[a,#] | 0.15 ± 0.02[a,#] |
| | 15 | | 0.12 ± 0.02[b] | n.a. | 0.09 ± 0.01[a] | 0.24 ± 0.05[a,#] |
| | 30 | | 0.09 ± 0.01[b] | n.a. | n.a. | 0.15 ± 0.08[a] |
| Al | 1 | 42.96 ± 14.49 | 84.80 ± 25.45[a] | 27.04 ± 9.43[a,#] | 18.84 ± 4.76[a,*,#] | 21.11 ± 4.87[a,#] |
| | 5 | | 48.08 ± 6.67[a] | 47.79 ± 10.98[a] | 12.40 ± 2.04[a,*,#] | 30.70 ± 11.43[a] |
| | 15 | | 60.44 ± 9.94[a] | n.a. | 11.86 ± 2.82[a,*,#] | 7.65 ± 1.70[a,*,#] |
| | 30 | | 67.90 ± 19.37[a] | n.a. | n.a. | 6.81 ± 0.96[a,*,#] |
| Ni | 1 | 0.71 ± 0.04 | 0.42 ± 0.05[a,*] | 0.44 ± 0.06[a] | 0.45 ± 0.05[a,*] | 0.75 ± 0.16[a] |
| | 5 | | 0.28 ± 0.03[b,*] | 0.67 ± 0.12[a,#] | 0.51 ± 0.05[a,*] | 0.43 ± 0.07[a] |
| | 15 | | 0.54 ± 0.06[a] | n.a. | 0.51 ± 0.02[a,*] | 0.40 ± 0.04[a] |
| | 30 | | 0.53 ± 0.07[a] | n.a. | n.a. | 0.43 ± 0.04[a] |

Values are mean ± SEM (n = 3–7)

* Significant difference from Sampling site; # significant difference from Cf in a same test-period; Different letters indicate significant difference between the test-periods on a same site (p ≤ 0.05). n.a.: not analyzed because the animals died

Considering what was mentioned above, the metal Cr can be considered as a leachate potential contaminant and its accumulation in *C. fluminea*'s tissue may be used as a biomarker of chemical exposure to leachate, taking into account that a biomarker is generally defined as changes induced by toxic stressors at a molecular or cellular level, affecting processes, structural or functional components (Gagnon et al. 2006), that can be measured within an organism or in its products and not in the intact, i.e. untouched, specimen (Depledge et al. 1995).

In conclusion, chromium was detected in water and sediment, but only its accumulation in clam's tissues evidenced this metal as an important contaminant in the mixture. Thus, in order to monitor leachate contaminated sites Cr accumulation should be considered as a suitable biomarker.

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