Bull. Environ. Contam. Toxicol. (2000) 65:638–645

DOI: 10.1007/s001280000171

Bulletin of Environmental Contamination and Toxicology

Cadmium Uptake by *Corbicula fluminea* and *Dreissena polymorpha:* Effects of pH and Temperature

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Received: 20 March 2000/Accepted: 25 July 2000

Cadmium is a well-known environmental contaminant that affects aquatic environments. To monitor this pollutant and its bioavailability in freshwater ecosystems, the use of bioindicators, such as bivalves, is particularly well suited. However, prior experimental studies are required in order to determine the characteristics (transfer kinetics and level) of the contamination to the organisms. Two species were selected, the Asiatic clam (*Corbicula fluminea*) and the zebra mussel (*Dreissena polymorpha*). By reason of theirs ecological and physiological features (particularly a high filtration rate), these two species are very suitable as heavy metal bioindicators as shown by ecotoxicological studies (Graney *et al.* 1983; Kraak *et al.* 1991; Merch, 1993; Claudi and Mackie 1994; Inza 1996). In addition, for monitoring the aquatic environment these two species are complementary, since they differ in habitat requirements. *C fluminea* is a benthic infaunal species, whereas *D. polymorpha* is an epilithic species. The species also differ in optimal temperature for growth and reproduction, 14-22 °C for *C. fluminea* and 8-16 °C for *D. polymorpha* (Claudi and Mackie 1994). One of the particularity of *C.* fluminea is to feed off by the suspended mater presents in the water column, as *D. polymorpha*, and also by the sediment particles by pedal feeding (Way and Hornbach 1990).

The aim of the present study was to determine the effects of pH and temperature on the accumulation and distribution of cadmium in the soft tissues of the two molluscs.

MATERIALS AND METHODS

Corbicula fluminea (20 ± 2 mm length) were reaped in Sanguinet Lake (France), and *Dreissena polymorpha* (24 ± 2 mm length) came from Garonne River (France). Molluscs were introduced into glass tanks (Experimental Units – EU-, 24x24x30 cm) lined with plastic bag (Plastiluz,

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polycarbonate, alimentary standard) to limit the adsorption of cadmium. The tanks contained 11L dechlorinated tap water (Table 1) and 4 Kg of substrate (5 cm thick) made up of 50% of pure sand (98% silica, granulometry 0.8 to 1.4 mm) and 50% natural sediment, collected from the bank of the Garonne River, upstream from Bordeaux (France). This sediment was an homogeneous silt, rich in clay (75-80 %) with a low total organic carbon content (2% on average). Such a substrate (sand + sediment) is suitable for easy burying of the Asiatic clam (Graney *et al.*, 1984). Each EU is fitted with a plastic horizontal grid, placed 20 cm from the bottom of the EU, to maintain the zebra mussels in the water column. Oxygen saturation of water was maintained by permanent air bubbling with diffusers and the daily period of light was fixed at 12hr. The molluscs were introduced into the tanks 10 days after the water and the sediment to allow the physical and chemical conditions to stabilize.

Table 1. Chemical composition of the dechlorinated tap water

cations (mg L^{-1})			anions (mg L^{-1})					
Ca^{++}	Mg^{++}	NH_4^+	HCO_3^-	SO_4^{--}	Cl^-	NO_2^-	NO_3^{--}	PO_4^{3-}
53.5	12.2	<0.01	231.8	37.5	16.0	0.09	1.8	<0.05

Cadmium accumulation by the two species was investigated for two temperatures and pH conditions : 16±0.2 and 24±0.2°C, pH 6±0.2 (regulated pH) and 8.2±0.1 (unregulated pH). In the EUs at pH 6, regulation was carried out automatically by measurement on a scale of milliseconds and the injection of a controlled volume of a dilute acid solution (HNO_3). In the other case, and for both temperatures, the pH remained stable at 8.2±0.1, during the 14 days of the experiment. For all four conditions and both of the two sampling times (7 and 14 days) two replicates were prepared (16 EUs in all). The nominal concentration of cadmium in water was maintained at 1 μg L^{-1} and metal was added daily in order to compensate for the decrease of its concentration due to permanent aeration of the water column, bioaccumulation in the organisms and adsorption on the tank walls and the sediment. Cd concentration was measured before and after the metal addition, each 24 hours. The addition of metal represented twofold the decrease between the nominal value and the concentration recorded before the addition. During the course of the experiment, three physical and chemical parameters were monitored in the water : pH, temperature and dissolved oxygen.

In each experimental unit, 6 Asiatic clams were placed on the sediment, in which they buried themselves within twenty or thirty minutes, and 6 zebra mussels were placed on the grid. At point zero of the experiment, a sample of 8 specimens of each species was taken in order to determine the background Cd concentration in soft body and organs of the mollusks. The first addition of cadmium was made 2 hr after introduction of the organisms into the tanks. Assessment of the accumulation of the cadmium after 7 and 14 days was based on measurements taken on all the molluscs of the two replicates corresponding to the four experimental conditions. Four organisms of each replicate were used for metal determination in the mantle, gills, visceral and muscular mass. The soft bodies were dissected while still frozen. Two samples of each organ were pooled in glass tubes used for the digestion step before Cd determination. Metal concentration in soft body was obtained from the sum of the burden in the four organs divided by the body mass. Organ samples were digested in 3mL HNO_3 at 95 °C in a pressurized medium (borosilicate glass tubes) and diluted up to 20 ml with ultra-pure distilled water. Total cadmium, from water and mineralized samples, was determined with a Varian AA 20 spectrophotometer equipped with a model GTA 96 graphite atomizer and autosampler.

The 16 EUs prepared represented a complete experimental design including combinations of the two temperature and pH conditions. Data obtained were analyzed by ANOVA. These analyses were made using Excel and SYSTAT. The values indicated in the text and figures are mean values with the associated 95 % confidence interval.

RESULTS AND DISCUSSION

For the whole range of conditions, average cadmium concentration in water column was maintained at a value of 0.96 ± 0.11 μg L^{-1}. A strong decrease in metal concentration was noticed between daily additions. During the first 7 days, this daily decrease was of 40% and 60% in the EUs at pH 6 / 16°C and pH 8.2 / 24°C, respectively. Subsequently, this difference tended to become less marked, and the decrease in the Cd concentration in the water settled at 30 %, regardless of the conditions.

With regard to the background values (wet wt) measured in the two species (114 ± 17 ngCd g^{-1} in *C. fluminea* and 78 ± 9 ngCd g^{-1} in *D. polymorpha*), the accumulation of cadmium during the experiment was lower overall in the Asiatic clam than in the zebra mussel. In the soft body of *Corbicula fluminea* (Figure 1), the concentration remained relatively low even after 14 days of exposure, its maximum was only 2.5 times the initial value. For the muscles and mantle, no accumulation of cadmium was observed, the concentration

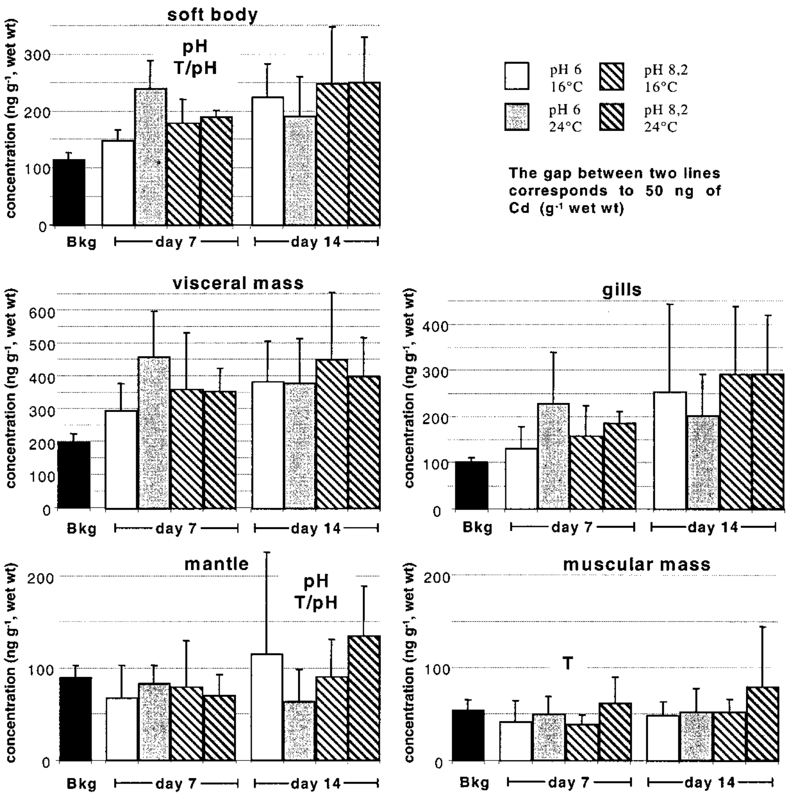

Figure 1. Cadmium concentration in soft body and tissues *of Corbicula fluminea* (mean value with the associated 95% confidence interval). The bold letters indicates the condition effect detected by ANOVA for each sampling day - T : temperature ; pH ; T/pH : interaction between temperature and pH ; Bkg : background.

values after 7 and 14 days of exposure showing no significant difference from the background. The low cadmium accumulation recorded for the soft body as a whole thus stems exclusively from an increase in the concentration in the gills and the visceral mass (Figure 1). The latter tissue group contributes about 65% of the cadmium total burden of the soft body after 7 and 14 days of contamination (Table 2). With regards to the condition effects, at the 7th day of exposure, the impact of the temperature is observed for pH 6, the cadmium concentration being 239 ± 41 μg g^{-1} (wet wt) for 24 °C and 148 ± 16 μg g^{-1} (wet wt) for 16°C. In addition, after 14 days, vs 7, the cadmium concentration is generally higher, as it is for pH 8.2 vs 6 (Figure 1). Quite consistently, these observations also apply to the visceral mass and gills, but with characteristics more or less accentuated compared to those applying to the soft body.

Overall, the impact of the three parameters considered, exposure time, temperature and pH, on the level of accumulation of cadmium in the soft tissues of *Corbicula fluminea* is slight. This conclusion is consistent with the results obtained by Graney *et al.* (1983) and with the conclusions of Inza *et al.* (1998), who showed that these abiotic factors played no role in the elimination of this metal by *C. fluminea*.

Table 2. Mass balance of Cd (%) in each tissue types for both bivalve species at day 14 (± 95% confidence interval).

Species	Visceral mass	Gills	Mantle	Muscular mass
C. fluminea	63 ± 11	21 ± 2	11 ± 1	5 ± 1
D. polymorpha	57 ± 3	20 ± 2	12 ± 2	11 ± 2

In contrast to *Corbicula fluminea*, the contamination of *Dreissena polymorpha* is characterized by a significant accumulation of cadmium by all the tissue groups. In the soft body, after 14 days of exposure and depending on the conditions of contamination, the concentration was from 5 to 12 times higher than that of the background (Figure 2). Although the mantle and muscular mass accumulated fairly high levels of cadmium (5 to 15 times the background values), as in the case of the Asiatic clam, the gills and the visceral mass exhibited the highest levels of contamination and accounted for the contamination of the soft body as a whole, for 57 and 20 % after 14 days exposure respectively (Table 2). As regards the impact of the three parameters considered, the cadmium content of all the tissues increased with the exposure time for mussels maintained at 24 °C. After 7 days, the effect of the temperature is barely or not at all apparent, whereas that of the pH is quite different. Thus, the cadmium concentration is much higher in the tissues of molluscs maintained in water with a pH of 8.2 vs pH 6 (Figure 2). After 14 days of contamination the pH value has little or no impact on the level of accumulation of cadmium, whereas the cadmium concentration in the tissues of the zebra mussel was about twice as high at 24°C as at 16°C. All these observations apply equally to the muscular mass and mantle, but the impact of the two parameters cited above is much less distinct than for the other tissues and for the soft body as a whole.

D. polymorpha would appear to be a species that is sensitive to variations in pH. For example, the incoming fluxes of calcium and sodium are markedly reduced when the pH is lower than 7, and the ion deficit occurs when the pH

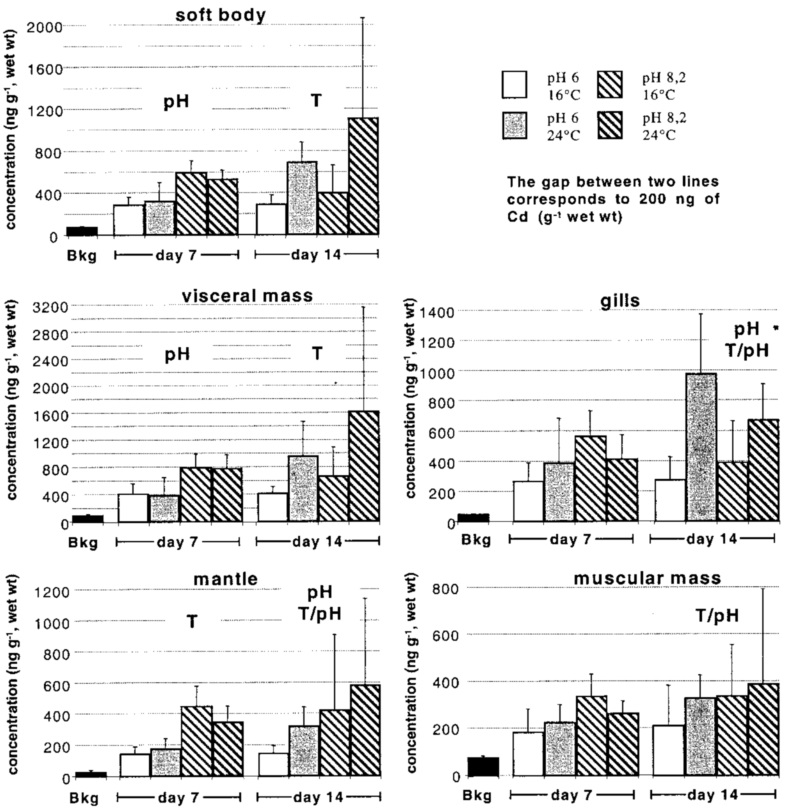

Figure 2. Cadmium concentration in soft body and tissues *of Dreissena polymorpha* (mean value with the associated 95% confidence interval). The bold letters indicates the condition effect detected by ANOVA for each sampling day - T : temperature ; pH ; T/pH : interaction between temperature and pH ; Bkg : background.

is from 6.8 to 6.9 (Vinogradov *et al.* 1993). Nevertheless, according to these authors, the ion balance is restored within a few days to reach a new state of equilibrium. In living organisms, notably in the molluscs, cadmium is a competitor of calcium (Markich and Jeffree 1994; Sidoumou *et al.* 1997). Consequently, the results obtained after 7 days of contamination may be attributed to the ion imbalance resulting from a pH of 6 which would limit the uptake of cadmium in the zebra mussel. On the 4^{th} day, the disappearance of the effect of pH 6 on the level of accumulation of cadmium may be explained by the reestablishment of the ion balance, as shown by Vinogradov *et al.* (1993). In addition, the differences in the level of accumulation between 16°C and 24°C may be attributed to the impact of the temperature on the metabolic activity. Thus at 24°C, the upper limit of the temperature preference range of *D. polymorpha,* the demand for oxygen, and consequently the filtration rate, increases, which might logically induce an increase in the cadmium uptake (Mouabad *et al.* 1988).

For the two species studied, a difference in pH (6-8.2) or temperature (16-24°C) during exposure to cadmium results in two different behavior patterns. Either it is compensated by homeostatic process as in *Cobicula fluminea* (Byrne and Dietz 1997), or it causes severe disturbances in the ion balance, as is the case in *Dreissena polymorpha* (Vinogradov *et al.* 1993). These two species, of invasive character, have only recently colonized the freshwater environment (Pleistocene). Despite this parallel in their evolution, *C. fluminea* has developed a more efficient osmoregulation system than *D. polymorpha* (Dietz *et al.* 1994). This physiological difference might, for example, make *C. fluminea* much less sensitive to non-oxidizing molluscides, in particular, than *D. polymorpha* (Bidwell *et al.* 1995). pH and temperature have effects on metal speciation and on biological activities. For bivalves exposed to low pH, Alabaster and Loyd (1980) observed a reduction of filtration rate and a production of mucus on gills and mantle. High temperature globally accelerates chemical and metabolic reactions and oxygen needs. From the point of view of the biomonitoring of aquatic ecosystems for pollution by cadmium and heavy metals in general, the choice of bioindicator species should mainly be based on two criteria : the predictable level of concentration of the pollutant in the medium and the stability of the abiotic factors. Thus, in a weakly contaminated system, *D. polymorpha* would be favored for its higher accumulation capacity, whereas if the medium were subject to strong variations of pH and temperature, *C. fluminea* would be more suitable.

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