Bull. Environ. Contam. Toxicol. (1989) 43:287–294

Environmental Contamination and Toxicology

Effect of Speciation on Uptake and Toxicity of Cadmium to Shrimp *Crangon Crangon* (L.)

M. L. S. Simões Gonçalves, M. F. C. Vilhena, L. M. V. F. Machado, C. M. R. Pescada, and M. Legrand de Moura

Centre of Structural Chemistry, Complex I, Lisbon Institute of Technology, Technical University of Lisbon, Portugal

A study is presented on speciation of seawater with shrimps Crangon Crangon (L.) contaminated with cadmium in the presence and absence of pyridine - 2,6-dicarboxylic acid (a rough model of a chelate group of humic acids). Ultrafiltration and ion exchange Chelex columns were associated with atomic absorption (AA) and differential pulse anodic stripping voltammetry (DPASV). The uptake and toxicity of cadmium were studied and the organs where this heavy metal accumulated were examined. It was noticed that the cadmium complex with pyridine-2, 6-dicarboxylic was not available to the shrimps and that during the 5 days of the experiment cadmium was preferentially accumulated in the hepatopancreas and carapace.

MATERIALS AND METHODS

All the reagents were of analytical grade. The water was distilled and demineralized and the conductivity value was < 0.1 μS. All the glass material was decontaminated with nitric acid 1:1 for about 14 h and rinsed with deionized water.

For the ultrafiltration experiments the PM10 Amicon 43 mm filter together with the Amicon system model 52 (Ultrafilter Amicon Cooperation - Ireland) were used. In order to avoid adsorption and contamination, the ultrafilters were always rinsed with solutions 0.05 % in nitric acid and 0.10 M in calcium nitrate according to Laxen and Harrison (1981).

In the ion chromatographic experiments a column of Chelex 100 resin (Bio Rad Laboratories, Richmond, California) was used; the solutions were passed through it with a flow rate of 0.5 mL/min, and the elution was done with 2 N nitric acid and 2 N hydrochloric acid. After this the column was regenerated with 2 N hydrochloric acid and washed with water until neutral. The Chelex columns were used in order to distinguish between the complexes that are labile (retained) during the contact time with the resin and those that are inert, i.e., that cannot be dissociated during that time (not retained).

The cadmium in fractions was determined by flame atomic absorption with equipment Perkin-Elmer 403 (Perkin-Elmer, Norwalk USA). The differential pulse anodic stripping voltammetry was carried out with a PAR 174 A polarograph (PAR, Princeton, USA) under potentiostatic control, with a three-electrode system: hanging mercury drop electrode Metrohm EA 290 (Metrohm, Herisau Switzerland) and calomel and platinum electrodes. The differential pulse mode was used with a pulse height of 50 mV, scan rate of 10 mV s^{-1}, deposition potential E_d = -0.8 V, time of electrolysis td = 2 min, followed by 30 s of rest and the supporting electrolyte was the buffer sodium acetate/acetic acid at pH 4.5. We used DPASV as a technique to determine the total concentration of cadmium in solution, after destroying the organic matter with nitric acid, although we could use this technique to determine the relative labile and not labile species and so estimate the rate constants of dissociation of certain fractions of complexes. Indeed in the medium of the biological model, after the experiment the content of organics is quite high, about 100 mg/L of carbon, and so adsorption problems could cause discrepancies in anodic stripping voltammetry, especially in the differential pulse mode.

Two polyethelene containers of 30 L capacity were filled with seawater from Guincho nearshore with a concentration of organic carbon < 1mg/ℓ. Concentrations of cadmium between 2 x 10^{-6} M and 5 x 10^{-6} M were added to one of the aquaria. After the equilibrium was established (about 2 days), which was checked by the constancy of the determined concentration of cadmium, an equal number of shrimps at an identical state of development was added to each container and left in it for 5 days without being fed. Dead shrimps were removed everyday and nitrites, pH and temperature in both aquaria were also daily determined. Before the experiments the shrimps were kept for 24 hrs in another aquarium in order to be acclimatized.

For the analysis of the shrimps the organic matter was decomposed by a mixture of concentrated nitric acid and 30 % hydrogen peroxide.

RESULTS AND DISCUSSION

The shrimps Crangon Crangon (L.) were used because they are quite resistant and can survive for 5 days without being apparently affected by the absence of food and because we can have a significant number of individuals. They are crustaceans that can accumulate heavy metals in high percentages without suffering any apparent damage (Sunda et al.1978), although they are not in the beginning of the food chain. As they can accumulate the heavy metals, not only due to the contamination of water but also to the contamination of food, we have to bear in mind that our results express only the influence of one of the causes of contamination.

In Tables 1 and 2 one can see the results of speciation of the seawater used in the biological models after contamination with

Table 1. Percentages of cadmium (within an error ⩽ 10%) in the several fractions of unfiltered seawater after contamination with 2 x 10^{-6}M of cadmium and in the presence of shrimps Crangon Crangon (L.) during 5 days. Values determined by flame atomic absorption (not saline fraction) and differential pulse anodic stripping voltammetry (saline fraction).

Chelex 100 column % retained	Chelex 100 column % not retained	UV+Chelex 100 column % retained	UV+Chelex 100 column % not retained	PM 10 % not retained
45.4	54.5	45.6 (a)	54.5 (a)	-
54.8	45.0	66.6 (b)	33.0 (b)	31.0

(a) Irradiation of the sample for 4 h before passage through the column.

(b) Irradiation of the sample for 6 h before passage through the column

Table 2. Percentages of cadmium (within an error of ⩽ 10%) in the several fractions of seawater, filtered through 0.45 μm membrane after contamination with cadmium 3 x 10^{-6} M and in the presence of shrimps Crangon Crangon (L.) during 5 days. Values determined by differential pulse anodic stripping voltammetry.

Chelex 100 column % retained	Chelex 100 column % not retained	UV+Chelex 100 column % retained	UV+Chelex 100 column % not retained	PM 10 % not retained
73.5	26.4	79.5 (a)	20.6 (a)	37.0

(a) Irradiation of the sample for 8 h before passage through the column.

cadmium, respectively 2 x 10^{-6} M and 3 x 10^{-6} M. The sample used in Table 2 was previously passed through a 0.45 μ filter and so the analysed fractions are considered to be the percentages of the total soluble cadmium. Contrarily to this, the sample used in Table 1 expresses the results of several fractions of total cadmium in seawater, cadmium adsorbed on particles and colloids having also been included. The total cadmium in each fraction

was determined in Table 1 by A.A. for the samples without the macroconstituents of seawater (retained fraction of Chelex 100 column) and by differential pulse anodic stripping voltammetry (DPASV) for the saline samples. In Table 2 all the cadmium analyses have been done by DPASV.

In the third and fourth columns of both tables the sample was previously irradiated with ultraviolet radiation of 400 watts at 254 nm in a quartz container, and it has been noticed that even an 8 h period is not enough to destroy all the soluble organic complexes (Table 2). It would be important, in the future, to notice what is the nature of the organic matter that remained in solution and whether the totality would be destroyed by the previous addition of hydrogen peroxide or sodium persulfate.

From the results of both tables it can be noticed that the fraction retained by Chelex 100 column is about twice the fraction that passed through the PM 10 ultrafilter. This means that the complexes that are labile in terms of ion exchange chromatography are not only the compounds smaller than 1.8 nm, but also a fraction of the species larger than this value. Moreover, it is important to emphasize that small molecules may also be trapped in the pores of the resin. As in our experimental conditions the flow rate is of the order of 0.5 $\pm$ 0.1 mL/min., the contact time is about 12 s. This means that one can derive the expression $\ln \frac{1}{1-x} = k_d t$ where x is the fraction of the complex ML dissociated in time t = 12 s and k_d is the rate constant of dissociation. Since all the complexes smaller than 1.8 nm are retained in the Chelex 100 column, we may conclude that the complexes are 100% dissociated. Let us then assume two types of complexes: one with f - the initial fractions of ML - equal to 0.3, totally dissociated, and another with f = 0.7 and x $\simeq$ 0.4. Using equation $x/f = 1 - \exp(-k_d t)$, rate constants of dissociation higher than 0.6 s^{-1} can be estimated for the first group, and of $\simeq$ 0.07 s^{-1} for the second (Figura and McDuffie 1980).

Comparing the results of both tables, one can estimate that about 28% of cadmium is associated with particles, 22% are complexes not labile during the time scale of ion exchange experiment, which corresponds to complexes with large molecules, and 50% is in the form of hydrated ion and inorganic and organic complexes, labile during the time of contact with Chelex 100 column.

Figura and McDuffie (1980) divided the species into groups according to the lability in terms of ASV Chelex 100 column chromatography, Chelex 100 batch, non-labile to all of these techniques and adsorbed on colloids. From these experiments these authors noticed that in river water the percentage of cadmium adsorbed in colloids and particles is within 0%-19%, that retained by Chelex column is between 65% and 83%, and that not retained between 0% and 15%. So the fact that our results are of the same order of magnitude means that most of cadmium is in the form of free cadmium and chlorocomplexes, especially $CdCl_2$ (Simões Gonçalves et al. 1981).

In terms of uptake and toxicity of cadmium to the shrimps, for a total concentration of cadmium higher than 3×10^{-6} M there is 30% of dead shrimps in the contaminated aquarium versus 13% for the non contaminated one. The percentage of dead shrimps in the first aquarium is of 27% in four days. For a total concentration of cadmium 5×10^{-6} M, the percentage of dead shrimps at the end of the experiment is of about 84% and of 72% in the first four days. This means that $^{96}L\ C_{50}$ is roughly 4.1×10^{-6} M or 0.46 ppm of total cadmium, it being also noticed that the surviving shrimps were affected in terms of mobility. This value agrees with the value of $^{96}L\ C_{50}$ = 0.32 ppm for shrimps Crangon Septemspinosa (Ferreira 1979).

In these experiments it is not possible to distinguish if the toxic species are the inorganic complexes or the free cadmium only, because total cadmium is proportional to free cadmium. In order to check this, the concentration of chloride had to be changed, as has been done by Sunda et al. (1978) for the grass shrimps Palaemonetes pugio, who concluded that the toxic species is free cadmium.

However, the cadmium chlorocomplexes and the cadmium bound to the organics may not be available to the organisms, as they would have to pass through the biological membrane and cannot be dissociated. Indeed the transport through the biological membrane is very slow, as in this context this membrane is very similar to an interface in potentiometry (Turner and Whitfield 1980).

The results of speciation of seawater in the biological model show that the value of free cadmium necessary to reduce the number of shrimps to half during four days is not smaller than 1.3×10^{-7} M. The stability constant of $CdCl_2$, the dominant complex in seawater (Simões Gonçalves et al. 1981), has been taken into account.

On the other hand the free cadmium will be 0.9×10^{-7} M and 1.5×10^{-7} M, respectively, for a total concentration of cadmium 3×10^{-6} M and 5×10^{-6} M.

Even if cadmium were assimilated like iron(III) where sometimes the organisms seggregate certain siderophores to complex the cation in order to be assimilated, the rate of the process would be controlled by the free ion that could act as the toxic species.

When EDTA and cadmium were added to the container in a concentration of 5×10^{-6} M each, only 25% of the shrimps died during the 5 days of the experiments. The percentage is of 46% when instead of EDTA the acid pyridine - 2,6-carboxylic is added in a concentration of 4.8×10^{-4} M.

Considering the stability constants of the complexes valid for seawater conditions (Smith and Martell 1976) and the correction by the activity coefficient assuming Davies equation, the con-

centration of inorganic and organic cadmium complexes is 7×10^{-7} M and 4.3×10^{-6} M respectively, in the presence of EDTA. For the system with pyridine-2,6-dicarboxylic acid the fractions are respectively 3.1×10^{-6} M and 1.7×10^{-6} M. This corresponds to a concentration of about 2.1×10^{-8} M of free cadmium in the first system, and of about 0.9×10^{-7} in the second.

These values agree reasonably with the former results without the presence of added ligands and seem to indicate that the cadmium complex with EDTA and pyridine-2,6-dicarboxylic does not pass through the biological membrane, although the second complex is labile in terms of the ion exchange experiment. The lability in terms of Chelex 100 ion chromatography should be expected since these complexes are labile in terms of ASV according to Davison (1978) and the measurement time is higher for the former technique.

Table 3. Percentage of cadmium within an error of $\leqslant$ 15 % in shrimps and in the several organs after 5 days in not contaminated (NC) and contaminated (C) container with a total cadmium concentration (C_{Cd}) of 2×10^{-6} M and 5×10^{-6} M determined by A.A.

Sample	$C_{Cd} = 2 \times 10^{-6}$ M (a)		$C_{Cd} = 5 \times 10^{-6}$ M (b)	
	% N.C. (dry weight)	% C. (dry weight)	% N.C. (dry weight)	% C. (dry weight)
Muscle	4×10^{-4}	4×10^{-4}	3×10^{-4}	4×10^{-4}
Muscle (dead shrimps)	-	-	5×10^{-4}	6×10^{-4}
Stomach	5×10^{-3}	6×10^{-3}	-	-
Eggs	1×10^{-2}	1×10^{-2}	-	-
Hepatopancreas	2×10^{-3}	8×10^{-3}	2×10^{-3}	8×10^{-3}
Head	7×10^{-4}	2×10^{-3}	5×10^{-4}	3×10^{-3}
Head (dead shrimps)	-	-	4×10^{-4}	7×10^{-3}
Carapace	6×10^{-4}	1×10^{-3}	6×10^{-4}	4×10^{-3}

(a) The shrimp did not die significantly in the contaminated aquarium

(b) The shrimp died 84% in the contaminated aquarium

Table 3 shows the results of the analyses carried out. The content of cadmium in dead and live shrimps at the end of the

experiment was determined by AA (after destroying the organic matter), a more adequate technique than DPASV because it is less sensitive to organic matter. The latter technique, on the other hand, is more adequate in saline samples which already contain the supporting electrolyte, whereas in AA the salts can cause dispersion of the radiation.

From this Table it can be concluded that cadmium has a tendency to be accumulated in the hepatopancreas (an organ that acts as liver and pancreas) and in the outside carapace. This agrees with the tendency of living organisms to accumulate the heavy metals in the liver and sometimes in the shell.

When it was possible to analyze the eggs of the shrimps, the level of cadmium was found to be higher in the beginning of the experiment than in other organs, but there was no significant increase during the stay in the contaminated container. This also seems reasonable in biological terms, since the embryos have a tendency to accumulate heavy metals during gestation.

Table 3 shows that when the total cadmium concentration increases from 2×10^{-6} M to 5×10^{-6} M there is a slight increase of cadmium absorbed by the shrimps which is responsible for the increase of toxicity.

Because the levels of cadmium are generally much higher than in the natural environment, the results obtained with biological models (bioassays) cannot be easily extrapolated to the natural medium. However, although experiments depend not only on the biological species but also on their age, degree of acclimatization, stress, temperature, etc., it seems possible to relate acute toxicity to cadmium speciation in seawater and to determine the organs where the heavy metal is preferentially accumulated.

Finally, although only acute toxicity has been considered, there are other ways in which heavy metals may affect living organisms, e.g., chronic or sublethal toxicity which affects growth, movement, time of attainment to the adult state, capacity to select food and to self-protection. Movement and time of response to external stimulus were affected in the shrimps contaminated with total concentration of cadmium 5×10^{-6} M.

Acknowledgments. This work was partially supported by Junta Nacional de Investigação Científica e Tecnológica under research contract nº 315.81.57.
We are indebted to Dr. Maria Alice Ramos of Instituto Nacional de Investigação das Pescas (INIP) for the help provided on the biological plane.

REFERENCES

Davison W (1978) Defining the electroanalytically measured species in a natural water sample. J Electroanal Chem 87:395

Ferreira MJR (1979) Produtos Químicos e Ambiente - o Cádmio. Comissão Nacional do Ambiente, Lisboa
Figura P, McDuffie B (1980) Determination of labilities of soluble trace metal species in aqueous environmental samples by anodic stripping voltammetry, Chelex column and batch methods. Anal Chem 52:1433
Laxen DPH, Harrison RM (1981) The Physicochemical speciation of Cd, Pb, Cu, Fe and Mn in the final effluent of a seawage treatment work and its impact on speciation in the receiving river. Wat Res 15:1053
Simões Gonçalves MLS, Vaz MCTA, Fraústo da Silva JJR (1981) Stability constants of chlorocomplexes of cadmium(II) in seawater medium. Talanta 28:237
Smith RM, Martell AE (1976) Critical stability constants. Plenum Press, New York
Sunda WG, Engel DW, Thuotte RM (1978) Effect of chemical speciation on toxicity of cadmium to grass shrimp Palaemonetes pugio: importance of free cadmium ion. Environ Sci Technol 12:409
Turner DR, Whitfield M (1980) Chemical definition of the biologically available fraction of trace metals in natural waters. Thal Yugosl 16:231

Received January 25, 1988; accepted March 2, 1989.