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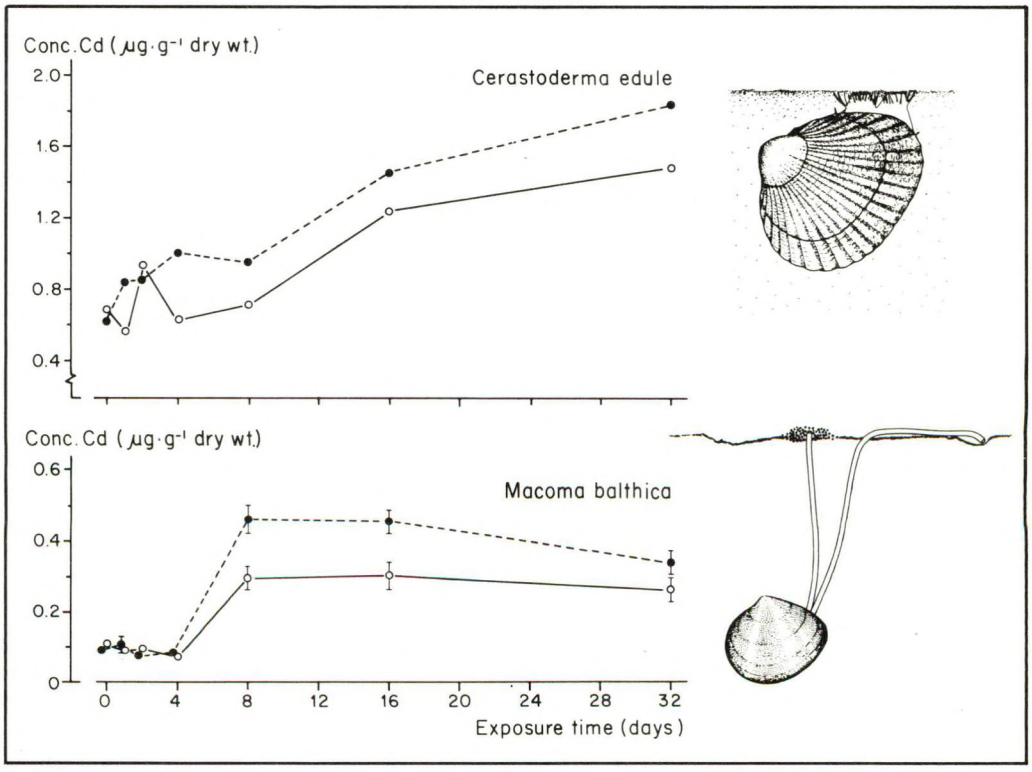

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THE UPTAKE OF CADMIUM AND COPPER IN BIVALVE MOLLUSCS AND SHRIMP EXPOSED TO LOW CONCENTRATIONS

K. Sarala Devi*, J.M. Everaarts

NETHERLANDS INSTITUTE FOR SEA RESEARCH

*Present address: National Institute of Oceanography,
Regional Centre, Cochin-682 018,
Kerala, India

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SUMMARY

The uptake of cadmium and copper at different levels of low ambient concentrations in the bivalve mollusc species Mytilus edulis (blue mussel), Cerastoderma edule (cockle), Macoma balthica (baltic tellin) and in the crustacean species Crangon crangon (brown shrimp) was studied under experimental conditions.

Each species showed a significant difference between control and exposed groups in whole-body tissue concentrations on a dry weight basis, of both metals as well as different concentration levels.

In adult specimens of M. edulis and M. balthica, cadmium was taken up from the seawater after an initial lag period of about 4 days, and accumulated until equilibrium level. In juvenile specimens of C. edule cadmium was taken up linearly until the end of exposure, indicating a first order uptake process, with an uptake-rate of 0.035 µg $Cd.g^{-1}.day^{-1}$. In adult specimens of C. crangon, the cadmium uptake was linearly with an uptake-rate of 0.087 µg $Cd.g^{-1}.day^{-1}$ during the initial exposure period, followed by an equilibrium concentration level.

Copper accumulated linearly in whole-body soft tissues of M. edulis, with an uptake-rate of 0.14 µg $Cu.g^{-1}.day^{-1}$, at a mean ambient copper concentration of 1.8 $\mu g.dm^{-3}$. The whole-body copper concentration in C. crangon varied considerably during exposure and fluctuated around a relatively high concentration level of 60 $\mu g.g^{-1}$ dry wt. Compared to this high copper content present naturally, mainly due to the presence of the copper containing blood pigment haemocyanin, these fluctuation are not significant.

The concentration nominally dosed in the experiments (0.2 $\mu g.dm^{-3}$) was twice as high as the background concentration in open ocean waters for cadmium and even lower than the background level for copper. In both cases the tissue concentration of metals were below the concentration limit considered acceptable for human consumption.

1. INTRODUCTION

In seawater, trace metals can occur dissolved in ionic form, complex-bound to organic substances, associated with humic acids, chelated with colloids, or adsorbed to organic and inorganic particulate material (Stumm & Bilinski, 1972). The exact chemical form of a metal may not only determine its availability and the rate of uptake by aquatic biota but also its toxicity. Aquatic organisms may accumulate metals from the surrounding water by absorption and diffusion across the body surfaces, and by ingestion of food and particulates (Phillips, 1977). For soluble metal species which are the predominant forms of for example Cu, Cd and Zn, there is evidence to suggest that in many marine organisms uptake mainly occurs from the waterphase, and is proportional to the metal ion activity in the water (Engel et al., 1981, 1985; Sanders et al., 1983).

The ability of many molluscs to accumulate metals from the surrounding water in their tissues has made them useful tools in marine environmental research and their soft tissues are generally preferred for such work. The use of mussels in coastal water quality control has received considerable attention following the suggestion of Goldberg (1975) for the Global Mussel Watch. Crustaceans have been widely employed as test animals for bioassays and for uptake experiments in the laboratory, and they are also frequently applied as biological indicators of metal pollution in the field. Both, mussels and shrimps are included in the Joint Monitoring Programme of the International Council for Exploration of the Sea (ICES). On the basis of extensive studies on cadmium toxicity to marine organisms, Eisler (1971) concluded that crustaceans were more sensitive to cadmium than both molluscs and teleosts.

Most toxicological studies with trace metals have been confined to high doses compared to the natural concentration levels. The present study deals with the uptake of cadmium and copper at low ambient concentration levels (only slightly higher than naturally occurring concentrations) by the bivalve molluscs Mytilus edulis, Cerastoderma edule and Macoma balthica and the brown shrimp Crangon crangon.

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2. MATERIAL AND METHODS

Adult specimens of the blue mussel Mytilus edulis and the brown shrimp Crangon crangon were collected with beam trawls of 1 and 3 m, respectively, from the channels of the Balgzand in the westernmost part of the Dutch Wadden Sea. Juvenile (1-year) specimens of the edible cockle Cerastoderma edule and adult specimens of the baltic tellin Macoma balthica were collected by digging from the intertidal mud-flats in the same area.

Length and weight of the bivalves and the weight of the shrimps were measured and animals within a narrow size and/or weight range were selected for use in the experiments (Tables 1-4). The animals were acclimatized for 4 days and were not fed during acclimatization and exposure period; no significant weight loss (cf. Tables) nor indications of starvation were observed.

The experiments were carried out in a continuous flow-through seawater system in a climated room at a constant temperature of 15^{0}C. The animals experienced a night-day regime of 12 h dark - 12 h light and were continuously submerged. Metal contamination of the circulating water was avoided by applying teflon materials. The filtered seawater used in the experiment had a salinity of 30 $^{0}/_{00}$ and was completely oxygen saturated. Rectangular glass aquaria (60/30/30 cm) were used. For each experiment one tank was used as control and the other tank for exposition.

The metal doses were prepared from standard stock solution of cadmium chloride and copper sulphate by dilution with seawater drawn from the system. The flow rate of the seawater in the system was 40 $cm^3.min^{-1}$ and that of the metal solution 0.21 $cm^3.min^{-1}$, so that the water content of the experimental tanks was exchanged at least once in 24 hours. The theoretical concentration of the dose was 0.2 $\mu g.dm^{-3}$, both for cadmium and copper (concentration in standard stock solution 50 $\mu g.dm^{-3}$, dilution factor 191). The exposure time was 24 or 32 days.

Samples of the water (1 dm^3) and animals (10 specimens of M. edulis (Table 1) and C. crangon (Table 4), 20 specimens of C. edule (Table 2) and M. balthica (Table 3)) were taken before starting the experiment and on consecutive days after dosing as indicated in the tables and figures. Pooled wet weights were determined and the samples were freeze-dried to constant weight.

About 400 mg of homogenized dried sample, weighed to the nearest 0.1 mg, was decomposed by an acid destruction-bomb technique (Paus, 1972). For the decomposition of organic material 3 cm^3 of suprapur 65% nitric acid were added and the teflon bombs were kept at 120 ^{0}C for 2 hours. These samples were diluted to 10 cm^3 in polypropylene tubes. Duplicate destructions were carried out for each sample.

For the extraction of water samples, 500 cm^3 of acidified water were used. The dissolved metals in water were complexed with a mixture of 1% ammonium pyrrolidine-dithiocarbonate (APDC) and 1% diethyl-ammonium diethyl-dithiocarbonate (DDDC) and extracted into methyl-isobutyl-ketone (MIBK). After phase separation the complexes in the

MIBK were destroyed by adding concentrated suprapur nitric acid. The metals were back-extracted into double-distilled water and stored in 10 cm^3 teflon tubes for further measurement (Kinrade & van Loon, 1974). Extractions were done in teflon separating-funnels in a laminar flow bench fitted in a clean-laboratory container. To avoid contamination of the samples, all chemicals used were suprapur (Merck). Most materials used were teflon made or teflon coated, and rinsed with 6N HCl and three times with double-distilled water.

Cadmium was measured with a heated graphite-furnace atomizer (HGA 500) coupled to an atomic absorption spectrometer (Perkin Elmer, model 5000). Copper was measured with a flame atomic absorption spectrometer (Perkin Elmer, model 403). The following replication procedure was carried out: single experiment/ single sample of pooled individuals/ duplicate destruction (except for C. edule because of low amount of pooled dry weight)/ each decomposition single analysis of Cd and Cu/ each analysis duplicate AAS-measurement.

3. RESULTS

3.1. Cadmium uptake experiments:

The cadmium concentration in the whole-body soft tissues of the filter feeder M. edulis varied between 0.5 and 0.6 $\mu g.g^{-1}$ dry wt, during the first 4 days of exposure (Fig. 1a). An obvious accumulation of cadmium to a concentration of 0.75 $\mu g.g^{-1}$ was observed on the 8th day and thereafter a plateau was reached.

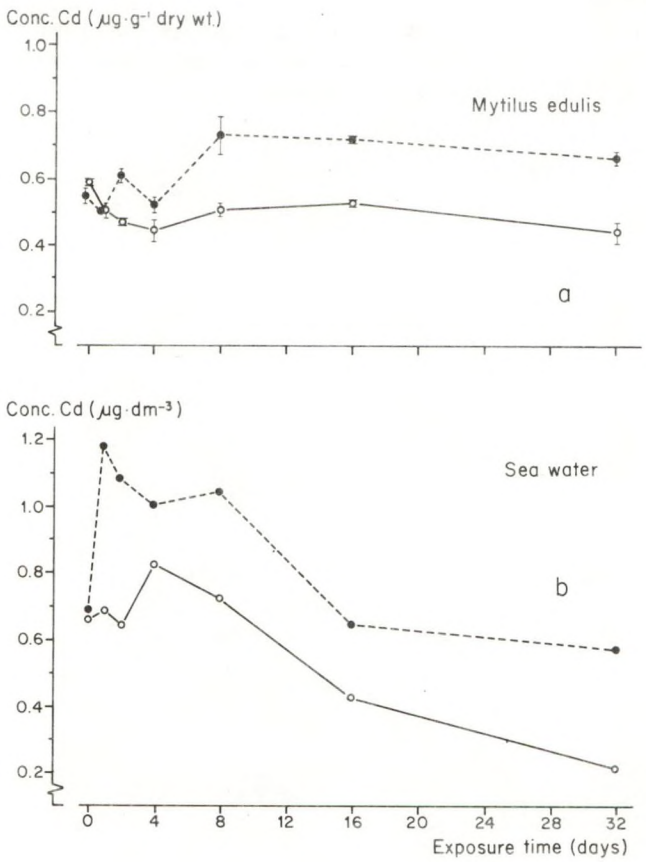

Figure 1. The uptake of cadmium in the common mussel Mytilus edulis. The total cadmium concentration is given in a whole body soft tissues ($\mu g.g^{-1}$ dry weight; mean and duplicate concentration values) and b the waterphase ($\mu g.dm^{-3}$). Exposure time 32 days, with a control (o—o) and a nominal dose of 0.2 $\mu g.dm^{-3}$ (●——●); the actual cadmium concentrations were measured at 15 ^{0}C, with a mean value during the experiment of 0.59 and 0.88 $\mu g.dm^{-3}$ in the control and exposed, respectively.

After an initial increase during the first four days of the experiment, the cadmium levels in the water progressively decreased slightly (Fig. 1b).

The mean cadmium concentration in the water during the experiment was 0.59 $\mu g.dm^{-3}$ in the control and 0.88 $\mu g.dm^{-3}$ in the exposed aquarium. A comparison between the difference in the cadmium concentration in whole-body soft tissues of exposed and control animals (ΔC_{Cd} = C_{Cd}(exposed) - C_{Cd}(control); cf. Fig. 1a) and their whole-body soft tissues pooled dry weight (ΔD.W. = D.W.(exposed) - D.W.(control); cf. Table 1) at a certain exposure time showed no evidence for a relationship (Fig. 2a). Also no correlation exists between the concentration of cadmium in whole-body soft tissues and the mean wet weight.

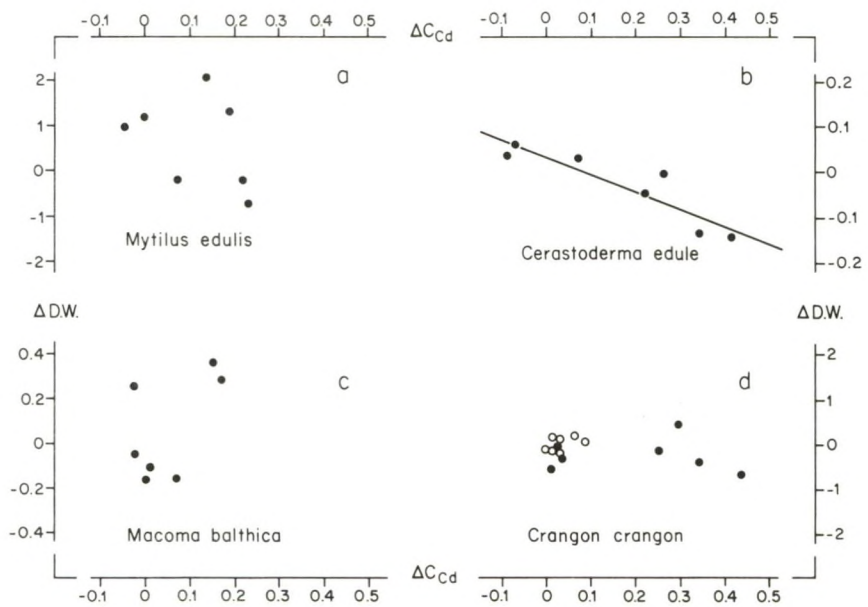

Figure 2. The relationship between the cadmium concentration in whole-body (ΔC_{Cd} = C_{Cd}(exposed) - C_{Cd}(control)) and body dry weight (ΔD.W. = D.W.(exposed) - D.W.(control)) in adult mussel Mytilus edulis (a), in juvenile cockle Cerastoderma edule (b), in adult baltic tellin Macoma balthica (c) and in adult brown shrimp Crangon crangon (d), where o and • refer to two different experiments (cf.: p. 9; Fig. 10).

The results on the uptake of cadmium by the bivalves C. edule and M. balthica are given in Fig. 3. In C. edule, cadmium was taken up linearly, according to a normal first order accumulation pattern, with an uptake rate of 0.035 µg Cd.g^{-1}.day^{-1} (linear regression: Y = 0.771 + 0.035 * X; N = 7; r = 0.968; p < 0.001), in the exposed specimens.

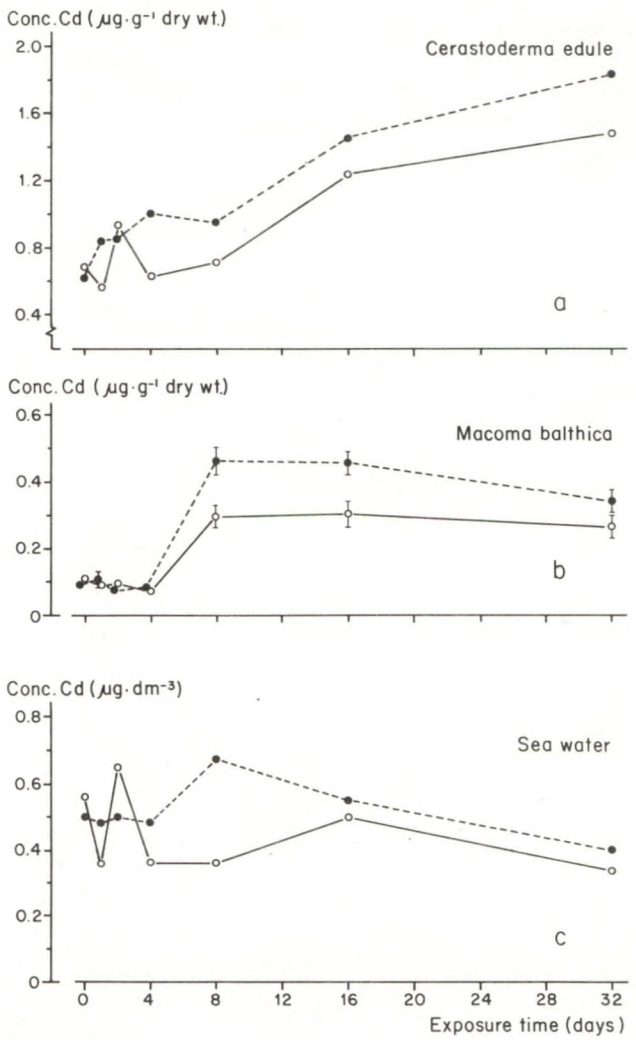

Figure 3. The uptake of cadmium in the edible cockle Cerastoderma edule (a) and the baltic tellin Macoma balthica (b). The total cadmium concentration is given in a and b whole body soft tissues (µg.g^{-1} dry weight; mean and duplicate concentration values) and c the waterphase (µg.dm^{-3}). Exposure time 32 days, with a control (o—o) and a nominal dose of 0.2 µg.dm^{-3} (●—●) the actual cadmium concentrations were measured at 15 ^{0}C, with a mean value during the experiment of 0.45 and 0.51 µg.dm^{-3} in the control and exposed, respectively.

However, it was also seen that in untreated animals accumulation also occurs, though to a lesser extent, with an uptake rate of 0.027 µg $Cd.g^{-1}.day^{-1}$ (linear regression: $Y = 0.649 + 0.027 * X$; $N = 7$; $r = 0.905$; $p < 0.001$). After a lag-time of four days, the cadmium concentration in M. balthica increased to values of about 0.45 $\mu g.g^{-1}$ dry wt. Within exposure time C. edule accumulated cadmium to a higher concentration than M. balthica.

The cadmium concentration in the water of both the exposed and the control aquarium varied considerably during the experiment (Fig. 2c). The exposed animals experienced only slightly higher cadmium concentrations; the mean concentration of cadmium in the water was 0.45 $\mu g.dm^{-3}$ in the control and 0.51 $\mu g.dm^{-3}$ in the exposed.

When correlating the relatively augmented cadmium concentration ($\Delta C_{Cd} = C_{Cd}(exposed) - C_{Cd}(control)$) with the difference in body weight of exposed and control animals (Fig. 2b), it can be concluded that an increase in the cadmium concentration in whole-body soft tissues of C. edule was correlated with a significant ($p < 0.01$) decrease in body weight (whole-body soft tissues dry weight). In M. balthica, on the contrary, no such a relationship between these parameters was found (Fig. 2c).

In one experiment, the cadmium concentration in whole-body of C. crangon increased linearly during the first four days of exposure, with an uptake rate of 0.087 µg $Cd.g^{-1}.day^{-1}$ (linear regression: $Y = 0.077 + 0.087 * X$; $N = 4$; $r = 0.958$; $p < 0.05$), indicating a normal first order accumulation pattern (Fig. 4a). This was followed by a relatively constant concentration level towards the end of exposure.

The mean cadmium concentration in the water differed 0.17 $\mu g.dm^{-3}$ between control (0.69 $\mu g.dm^{-3}$) and exposed (0.86 $\mu g.dm^{-3}$) (Fig. 4b). The data of the second exposure experiment, with a very slight difference (0.07 $\mu g.dm^{-3}$) between the mean cadmium concentration in the water of control (0.39 $\mu g.dm^{-3}$) and exposed (0.46 $\mu g.dm^{-3}$) (Fig. 4d), did not show any significant chance in whole-body cadmium concentration, both in exposed and control animals.

In both experiments, no correlation existed between whole-body cadmium concentration, expressed as $\Delta C_{Cd} = C_{Cd}(exposed) - C_{Cd}(control)$ and body weight, expressed as $\Delta D.W. = D.W.(exposed) - D.W.(control)$ on dry weight basis (Fig 2d). Moreover, neither the concentration in the water nor the tissue concentration showed any relation to the body weight (cf. Table 4, Fig. 4).

3.2. Copper uptake experiments:

In Mytilus edulis the tissue concentration of copper increased linearly during exposure, with an uptake rate of 0.14 µg $Cu.g^{-1}.day^{-1}$ (Fig 5a). The copper concentration in the control specimens varied only slightly and showed a mean value of 6.4 $\mu g.g^{-1}$ (S.D. $\pm$ 0.3), with maximum and minimum values of 6.7 and 6.0, respectively.

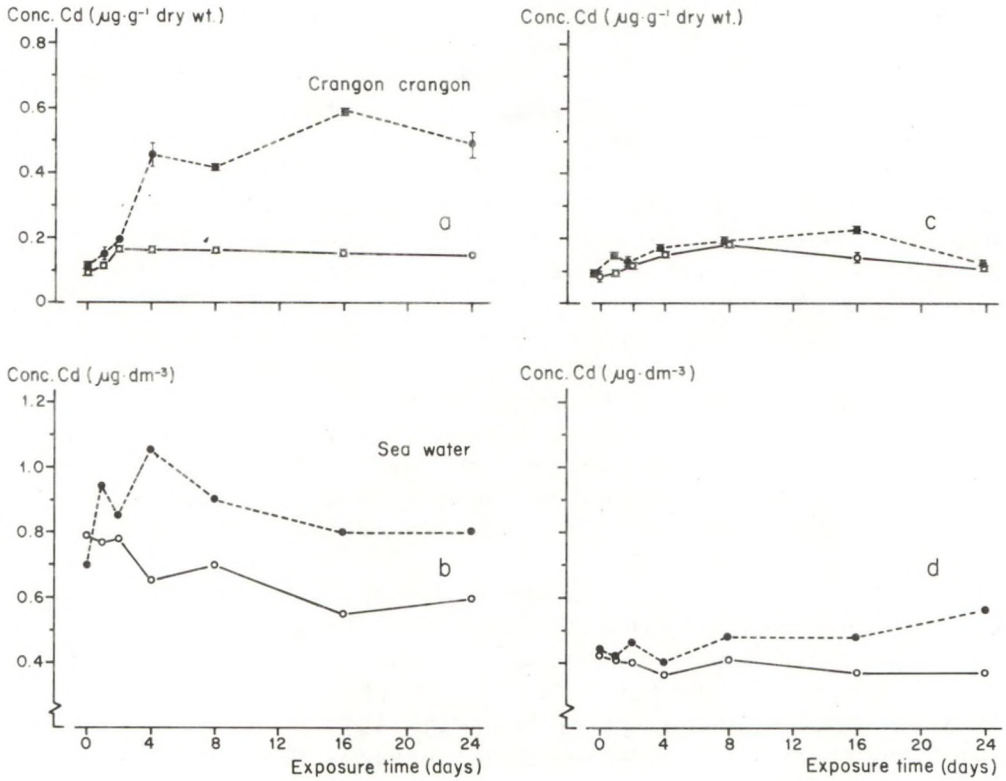

Figure 4. The uptake of cadmium in the brown shrimp Crangon crangon. The total cadmium concentration is given in a and c whole body (µg.g^{-1} dry weight; mean and duplicate concentration values) and b and d the waterphase (µg.dm^{-3}). Exposure time 24 days, with a control (o—o) and a nominal dose of 0.2 µg.dm^{-3} (●——●); the actual cadmium concentrations were measured at 15 ^{0}C, with a mean value during the first experiment (b) of 0.69 and 0.86 µg.dm^{-3} and the second experiment (d) of 0.39 and 0.46 µg.dm^{-3} in the control and exposed, respectively.

The copper concentration in water showed a slightly decreasing trend towards the 8th day and remained almost constant till the 32nd day (Fig. 5b). Concentrations were only slightly higher (about 0.3 µg.dm^{-3}) in exposed than in control aquaria, with mean values during the experiment of 1.79 and 1.50 µg.dm^{-3}, respectively.

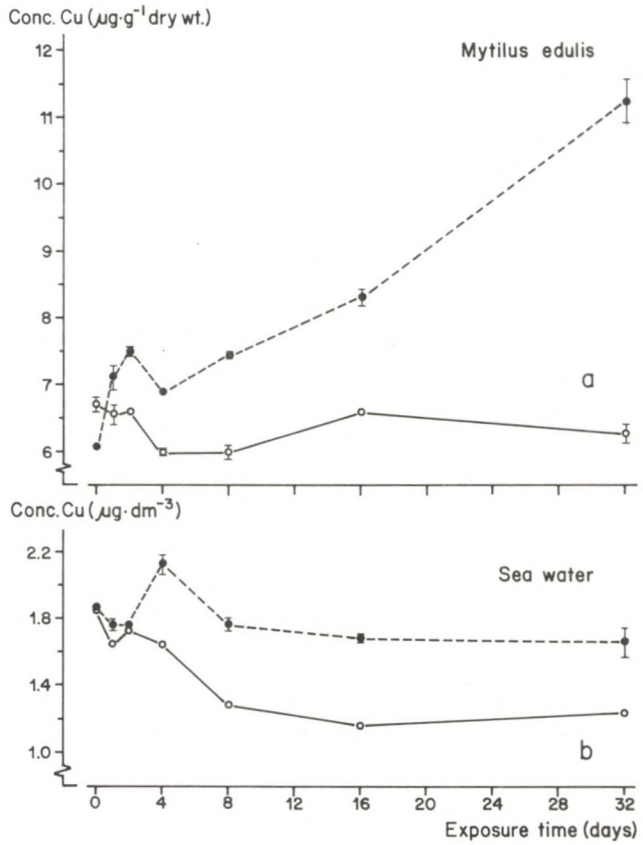

Figure 5. The uptake of copper in the common mussel Mytilus edulis. The total copper concentration is given in a whole body soft tissues ($\mu g.g^{-1}$ dry weight; mean and duplicate concentration values) and b the waterphase ($\mu g.dm^{-3}$). Exposure time 32 days, with a control (o—o) and a nominal dose of 0.2 $\mu g.dm^{-3}$ (●—●) the actual copper concentrations were measured at 15 ^{0}C, with a mean value during the experiment of 1.50 and 1.79 $\mu g.dm^{-3}$ in the control and exposed, respectively.

After an initial 'first day' increase, the whole-body concentration of copper in C. crangon decreased during the subsequent 7 days, followed by a linear increase again (Fig. 6a). Remarkably, the same pattern in the course of the copper concentration was observed in the control specimens, at only slightly lower ambient concentrations.

The copper levels in the water varied considerably, with mean values during the experiment of 0.75 and 1.05 $\mu g.dm^{-3}$ in the control and exposed groups, respectively (Fig. 6b).

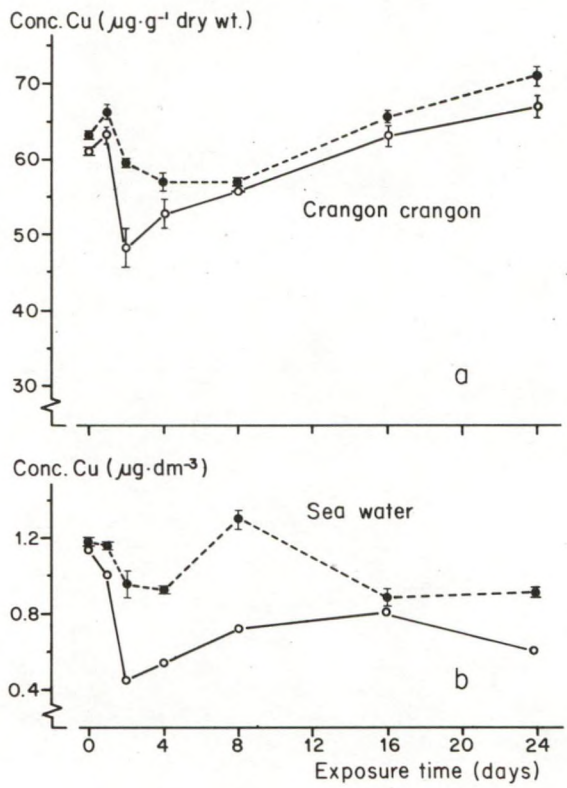

Figure 6. The uptake of copper in the brown shrimp Crangon crangon. The total copper concentration is given in a whole body ($\mu g.g^{-1}$ dry weight; mean and duplicate concentration values) and b the waterphase ($\mu g.dm^{-3}$). Exposure time 32 days, with a control (o—o) and a nominal dose of 0.2 $\mu g.dm^{-3}$ (●——●); the actual copper concentrations were measured at 15 ^{0}C, with a mean value during the experiment of 0.75 and 1.05 $\mu g.dm^{-3}$ in the control and exposed, respectively.

No correlation was found between the relatively augmented copper concentration (ΔC_{Cd} = C_{Cd}(exposed) - C_{Cd}(control)) and the difference in body weight of exposed and control animals (ΔD.W.), neither in M. edulis (Fig. 7a) nor in C. crangon (Fig. 7b).

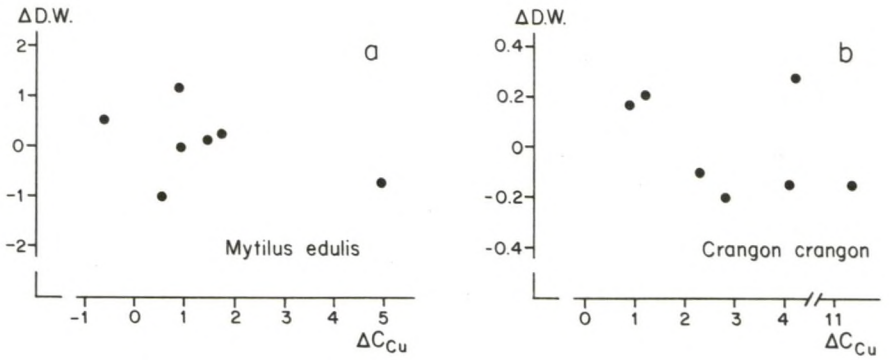

Figure 7. The relationship between the copper concentration in whole-body (ΔC_{Cd} = C_{Cd}(exposed) - C_{Cd}(control)) and body dry weight (ΔD.W. = D.W.(exposed) - D.W.(control)) in adult mussel Mytilus edulis (a) and in adult brown shrimp Crangon crangon (b).

4. DISCUSSION

The concentration of total cadmium and copper in the waterphase varied considerably in the various experiments, especially during the initial period of exposure. Such fluctuations of cadmium and copper in the waterphase are mainly due to changing circumstances in the aquaria, such as adsorption to the glass wall, physiological activity of the organisms and the decreasing biomass during exposure. Another source for variations in the metal concentration dosed, might be irregularities in the flow-rate of the peristaltic pump, especially at the low pumping rates as applied in the present experiments in order to obtain a low dose. Moreover, the analytical procedure for metal analysis in water, especially at low concentrations, is very susceptible of contamination.

In the present study, the concentration of cadmium in the whole body soft tissues of M. edulis showed no significant correlation with the concentration of cadmium in water. No relationship between the accumulation of cadmium and its average levels in the environment could either be described in a study by Coleman and coworkers (1986). However, those data did reveal a significant interspecific difference in the cadmium concentration and content between specimens which received the same average dose. Although uptake rates may be related to external concentrations, there is no evidence that the concentration in the organism will reflect the environmental

conditions (Bryan, 1976). Some species are able to excrete a higher proportion of the metal intake under contaminated conditions and may thereby keep their tissue concentration at fairly normal levels. In the present study, the limit for further accumulation after 8 days of exposure may be due to excretion. During two weeks at background levels, mussels are able to eliminate about 5% of the cadmium, accumulated in the previous weeks (George & Coombs, 1977; Coleman et al., 1986). M. edulis exposed to ionic cadmium also shows an initial lag period before accumulation occurs, possibly because cadmium must be complexed before uptake can occur (George & Coombs, 1977). Also Ritz et al. (1982) and Coleman et al. (1986) observed a higher accumulation rate of cadmium in M. edulis previously exposed to higher concentrations, even at low concentration ranges. This pattern of uptake is consistent with the suggestion of George & Coombs (1977) that cadmium must be complexed eg. to organic matter of low molecular weight, before possible uptake. The low and fluctuating values during the initial period and the subsequent increase in uptake noticed during the present experiments agree well with these data; marked differences in concentration were noticed between control and exposed animals. Amiard-Triquet et al. (1986) also observed a significant difference in concentrations between exposed and control mussels, even at lower experimental concentrations (2.5 $\mu g.dm^{-3}$) and lowest periods of exposure time. Like in the present experiments, previous studies have also failed to demonstrate a direct relation between the concentration in the water and cadmium uptake (Fowler & Benayoun, 1974; von Westernhagen et al., 1978, Ritz et al., 1982). A linear accumulation at shorter period of exposure was recorded on mussels exposed to concentrations as high as 50-200 $\mu g.dm^{-3}$ (George & Coombs, 1977).

In Mytilus from Port Phillip Bay, contaminated with water with elevated cadmium levels which circulate from Corio Bay, a value ranging between 8.7 and 21.6 $\mu g.g^{-1}$ was found for the different size groups between 10 and 90 mm (Talbot, 1985). The mean value of the cadmium concentration in mussels with a size of 50-60 mm, calculated from those data, was 12.6 $\mu g.g^{-1}$ at a cadmium concentration in the seawater of 0.2 $\mu g.dm^{-3}$. These data showed no relation between the body size of the organisms and the body concentration, at exposure to ambient concentration levels of 0.2 $\mu g.dm^{-3}$ in filtered seawater. This agrees with the present data, showing no relationship between the tissue concentration of cadmium and body weight, both on wet- and dry weight basis (cf. Fig. 2a). Moreover, the cadmium concentration measured in whole-body soft tissues of M. edulis (40-50 mm) exposed to 0.29 $\mu g.dm^{-3}$ was lower than the reported values of Talbot (1985), irrespective of a progressive increase in uptake towards the end of exposure.

In the cockle C. edule the cadmium concentration increased gradually until the end of the exposure time, both in the control and exposed specimens. A marked difference in the concentration level between control and exposed animals was induced. Accumulation of cadmium occurred according to a normal first order proces, both at

mean ambient concentrations of 0.51 µg. dm^{-3} (exposed) and 0.45 µg.dm^{-3} (control). Thus a concentration difference of only 0.06 µg.dm^{-3} results in a difference of 0.3 µg.g^{-1} in the cadmium concentration in the tissues, mainly due to the initial respons towards slightly augmented ambient concentrations resulting in a higher uptake-rate in exposed animals. The concentration levels of cadmium in water did not show any pattern, though a reduction in concentration was noticed towards the end of exposure. From a study on the biological availability of sediment-bound cadmium to the cockle, it was concluded that cadmium bound to biogenic calcium carbonate was readily available to C. edule, whereas cadmium bound to precipitated calcium carbonate was of considerably lower bio-availability (Cooke et al., 1979). However, this availability was achieved by means of marked desorption of cadmium from the sediment. Thus actual uptake in the organisms occurred from the waterphase. No cadmium uptake occurred from sediments, of which the cadmium content had not decreased after eight days in seawater. Unfortunately, the concentration of cadmium in the waterphase was not measured, but at a concentration of 24.4 µg.g^{-1} dry weight cadmium in biogenic calcium carbonate, an increase from an initial value of 0.55 µg.g^{-1} dry wt. to 7.3 µg.g^{-1} dry wt. was observed in C. edule, during four days of exposure (Cooke et al., 1979). In the present study, it has been shown that even at very low augmented ambient cadmium concentrations in the waterphase a significant bioaccumulation took place (cf. Fig. 2a).

In comparison, the baltic tellin M. balthica, evidently reacted in a different way upon such a slight increase in the ambient cadmium concentration (cf. Fig. 2b), showing a cadmium equilibrium level after 8 days of exposure. An inverse relationship between the size of M. balthica and ambient cadmium concentration was observed by McLeese & Ray (1984). When exposed to a relatively high cadmium concentration of 45 µg.dm^{-3} for 14 days, small, medium and large clams showed cadmium levels of 21.8, 12.5 and 8.5 µg.g^{-1} dry wt., respectively. However, at a much lower concentration of 1.4 µg.dm^{-3} no net-uptake was found. The data of present study did show a net-uptake, even at slightly augmented ambient concentrations of cadmium.

Extensive studies on cadmium and copper toxicity to marine organisms demonstrated that crustaceans are more sensitive to cadmium than are both molluscs and teleosts (Eisler, 1971). However, major problems encountered by many authors are cannibalism observed in control as well as exposed animals, and the stress of captivity leading to moulting of the animals (Ahsanullah, 1976). A higher incidence of moulting will affect the results of toxicity and uptake experiments since during moulting a large amount of the metal accumulated during the bioassay is eliminated (Fowler & Benayoun, 1974). This phenomenon may cause up to 30% loss of the animals. In the present experiments with brown shrimp C. crangon only a low incidence of mortality was observed, not significantly inluencing the number of experimental animals. Dethlefsen (1977) observed minimum concentrations during initial exposure (day 1 and 3) in C. crangon exposed to high concentgraions of cadmium (10 and 20 µg.dm^{-3}).

However, in specimens exposed to 1.5 $\mu g.dm^{-3}$ under constant flow conditions after 30 days of exposure, the whole-body concentration of cadmium had not increased significantly. On the contrary, specimens exposed to a concentration of 2.5 $\mu g.dm^{-3}$ for 30 days, showed a linear increase in whole-body cadmium concentration (Dethlefsen, 1977). Thus, the results of the present study (cf. Fig. 4a,b) agree with these data with respect to a rapid short-term accumulation characterized by the building of an early plateau in concentration level. Due to the low ambient cadmium concentrations applied in the present study, no long term accumulation characterized by an increase of whole-body load was found. Also in another shrimp species, Crangon septemspinosa, a steep rise in the cadmium concentration was found within the first period of exposure (Ray et al., 1981).

An increase of 0.3 $\mu g.dm^{-3}$ in the total copper concentration in the waterphase, induces a significant accumulation of copper in M. edulis, according to a linear first order proces. Thus exposure to even slightly augmented copper concentrations will already be reflected in total body burdens. Amiard-Triquet et al. (1986) found that at a 330-times higher external copper concentration of 100 $\mu g.dm^{-3}$, the concentration level in the visceral mass of mussels did not vary significantly during the first four days; after 8 and 16 days of exposure an increase in copper concentration attained a higher constant level. The ability of mussels to regulate the bioaccumulation of copper varies from organ to organ and decreases with higher levels of contamination and increasing period of exposure (Amiard-Triquet et al., 1986). Although copper is an essential trace element in biological processes, even low concentrations of copper may be lethal for several marine species (Bryan, 1976). Mussels show a physiological reaction to copper concentrations in seawater in the range of 0.5 to 10 $mg.dm^{-3}$, in closing their shells for several hours (Scott & Mayor, 1972; Davenport, 1977). These concentrations were several orders of magnitude higher than the concentrations applied in the present study, where the individuals did not show any valve-closing reaction upon starting the exposure. So accumulation of copper started immediately and continued gradually as a linear proces till the end of the experiment, even at the only slightly augmented ambient copper concentration.

The copper concentration in whole-body of C. crangon is high compared to the bivalve molluscs species involved in present experiments. These high baseline body burdens varying between 50 and 65 $\mu g.g^{-1}$ dry wt, are due to the very presence of the copper containing bloodpigment haemocyanin in the body fluid of the shrimps. An increase of 0.3 $\mu g\ Cu.dm^{-3}$ in the waterphase apperently does not initiate significant uptake of copper. Though the copper concentration showed considerable variations, yet it is remarkable that whole-body copper concentrations in exposed animals are consistenly higher than in control animals. However, taking into account the high concentration level (around 60 $\mu g.g^{-1}$ dry wt), this increase is negligible and will not exert any physiological effect. In a study of Ray et al. (1981), the copper concentration in C. septemspinosa

exposed to two sets of highly contaminated sediments showed no significant changes from the initial value, even though the concentration in the overlying water appeared to be high (60 $\mu g.dm^{-3}$) compared to the present study.

During the experiments described in the present study, the animals were not fed. The role of 'nutritive stress', which alters for example the condition and metabolism of M. edulis, may not be excluded in affecting the accumulation of metals (eg. cadmium; Bayne & Thomson, 1970; Thomson & Bayne, 1972). However, Coleman et al. (1986) did not find evidence of a decline in accumulation rates of cadmium in mussels which were subjected to nutritive stress. It is not very likely that nutrition stress would have influenced uptake of metals in the organisms significantly, because of the relatively short exposure period.

The data of the present study gave no evidence for a relationship between the whole-body soft tissues dry weight and the concentration of both cadmium and copper in adult specimens of M. edulis, M. balthica and C. crangon. Not being fed during the course of the experiment apparently did not affect the body weight (cf. Tables 1-4). No correlations were found between the relatively augmented cadmium and copper concentration in whole-body tissues and its dry weight (Fig. 2 and 7). However, in juvenile (1-year) specimens of the cockle C. edule, a significant correlation was found. Higher whole-body cadmium concentrations were related to lower body weights (Fig. 2b).

The concentration of 0.3 $\mu g\ Cu.dm^{-3}$ dosed additional to control seawater concentration during the present experiment is less than the background level of about 1.5 $\mu g.dm^{-3}$ for copper in the coastal waters of the Southern Bight of the North Sea (Duinker & Nolting, 1977). It is of the same level as the values from the Central North Sea (0.2 - 0.45 $\mu g.dm^{-3}$; Kremling & Hydes, 1988) and only 10 times higher than the lowest values measured in open ocean surface water (Bruland, 1980) The nominal concentrations dosed in the present experiment thus reflect only very slightly augmented contaminant levels and are only twice as high as the background concentrations in open ocean waters for cadmium and even lower than the background level for copper.

All species used in the experiments showed marked differences in concentration of cadmium and copper between control and exposed groups. Moreover, each species investigated showed different tissue concentration levels on a dry weight basis. These results indicate the importance of species specific physiological processes in the bioaccumulation of cadmium and copper from the environment. Sublethal studies are now becoming increasingly important and sensitive tools for the evaluation of the impact of contaminants on aquatic life and the determination of lower "no effect" concentrations of contaminants can be used as a method of environmental assessment.

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Table 1.
Cadmium and copper exposure experiment Mytilus edulis: the range and the average shell length (cm) and the pooled wet- and dry weights (g) of the whole- body soft tissues of 10 specimens.

Exp. time (days)	Length (cm) Control Average (range)	Length (cm) Exposed Average (range)	Pooled whole-body soft-tissues wet weight (g) Control	wet weight (g) Exposed	dry weight (g) Control	dry weight (g) Exposed
			Cd exposure experiment			
0	5.0 4.8-5.6	5.0 4.6-6.1	48.1	50.4	8.28	9.21
1	5.0 4.5-6.0	5.1 4.4-5.7	42.3	45.8	7.30	8.49
2	4.9 4.5-5.9	5.2 4.5-6.1	42.9	53.7	7.41	9.45
4	5.1 4.6-5.9	5.2 4.6-5.9	44.0	46.6	7.77	7.58
8	5.1 4.7-5.7	5.2 4.5-6.0	48.4	47.3	8.75	8.00
16	5.0 4.4-5.8	5.1 4.4-5.8	43.1	46.8	7.13	8.43
32	4.9 4.5-5.7	5.0 4.5-5.9	49.2	47.6	8.46	8.23
			Cu exposure experiment			
0	4.2 3.8-4.5	4.7 3.8-4.8	39.0	42.8	4.50	5.04
1	4.8 4.6-5.0	4.7 4.3-5.1	48.5	41.6	5.43	4.44
2	4.8 4.4-5.3	5.0 4.5-5.5	44.7	47.5	4.20	5.37
4	4.9 4.6-5.3	4.7 4.4-5.4	39.4	40.4	4.32	4.31
8	4.9 4.6-5.2	4.9 4.3-5.4	34.1	33.9	3.86	3.98
16	4.9 4.6-5.1	5.1 4.3-5.3	34.1	35.1	3.58	3.79
32	4.6 4.1-5.4	4.5 4.0-5.1	37.4	39.3	3.61	3.90

Table 2.
Cadmium exposure experiment Cerastoderma edule: the range and the average shell length (cm) and the pooled wet- and dry weights (g) of whole-body soft tissues of 20 specimens.

Exp. time	Length (cm)		Pooled whole-body soft-tissues wet weight (g)		dry weight (g)	
	Control Average (range)	Exposed Average (range)	Control	Exposed	Control	Exposed
0	1.6 1.2-1.9	1.6 1.3-2.2	7.32	7.79	0.57	0.63
1	1.6 1.3-2.0	1.5 1.3-2.2	6.37	5.72	0.49	0.52
2	1.5 1.3-1.7	1.5 1.3-2.0	6.84	7.46	0.45	0.49
4	1.6 1.4-1.9	1.4 1.2-1.6	6.34	8.11	0.54	0.39
8	1.4 1.1-1.7	1.4 1.1-1.7	6.48	7.17	0.35	0.34
16	1.5 1.2-1.7	1.5 1.2-1.8	6.29	5.05	0.34	0.29
32	1.4 1.2-1.8	1.4 1.1-1.8	6.21	4.91	0.35	0.21

Table 3.
Cadmium exposure experiment Macoma balthica: the range and the average length (cm) and the pooled wet- and dry weights of whole-body soft tissues of 20 specimens.

Exp. time	Length (cm)		Pooled whole-body soft-tissues			
			wet weight (g)		dry weight (g)	
	Control Average (range)	Exposed Average (range)	Control	Exposed	Control	Exposed
0	2.1 (1.9-2.5)	2.1 (1.7-2.3)	14.5	14.7	2.27	2.22
1	2.0 (1.6-2.4)	2.0 (1.6-2.4)	13.2	12.7	1.86	1.75
2	2.0 (1.6-2.4)	2.0 (1.8-2.2)	14.0	14.2	1.96	2.21
4	2.0 (1.7-2.3)	2.0 (1.8-2.2)	13.4	12.6	1.81	1.64
8	2.0 (1.6-2.2)	1.9 (1.6-2.3)	14.1	14.8	1.85	2.13
16	2.0 (1.7-2.3)	2.0 (1.6-2.5)	13.3	14.0	1.65	2.01
32	2.1 (1.7-2.2)	1.9 (1.6-2.2)	14.3	13.0	1.88	1.72

Table 4.
Cadmium and copper exposure experiment Crangon crangon: the wet- and dry weights (g) of the whole-body tissues of 10 specimens pooled; I and II refer to two separate cadmium exposure experiments.

Exp. time (days)	Pooled whole-body wet weight (g) Control	Exposed	dry weight (g) Control	Exposed
	Cd exposure experiment			
	I / II	I / II	I / II	I / II
0	25.3 --	21.5 --	5.82 / 2.23	5.28 / 2.08
1	24.3 --	22.0 --	5.25 / 1.96	4.96 / 2.17
2	24.6 --	23.5 --	5.43 / 2.14	5.39 / 2.30
4	24.8 --	23.6 --	4.99 / 2.02	5.47 / 1.93
8	26.2 --	25.1 --	5.58 / 2.30	5.46 / 2.21
16	24.2 --	23.9 --	5.97 / 2.08	5.32 / 2.12
24	19.9 --	12.9 --	4.19 / 1.89	3.80 / 1.99
	Cu exposure experiment			
0	6.62	6.66	2.45	2.17
1	6.09	7.32	2.02	2.23
2	5.85	6.43	2.14	1.99
4	5.87	6.53	1.84	2.12
8	5.76	7.08	2.00	2.17
16	6.66	6.35	2.40	2.30
24	5.74	4.48	1.95	1.80

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