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Uptake and Release of Cadmium in Various Organs of the Common Mussel, *Mytilus edulis* (L.)

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Bivalve molluscs are widely used as biological indicators of water quality in the marine environment. The use of mussels (*Mytilus edulis* and similar species) for the purpose of coastal water quality control has received considerable attention in the 'Mussel Watch' program for global marine monitoring (Goldberg 1975), providing a method of assessing the degree of contamination of marine systems. The 'mussel watch' approach to coastal water quality control is based on the chemical analysis of contaminant residues in mainly whole-body soft tissues. It should be established, however, how closely body burdens in the mussel are related to chemical concentrations in its surrounding environmental compartments. This relationship is controlled by uptake and elimination kinetics of chemicals in the mussel.

Numerous data on the internal distribution of trace metals in *Mytilus edulis* have been published. Highest concentrations and main body burden of cadmium are reported for the mid-gut gland (Janssen and Scholz 1979; Scholz 1980) and in the kidney, where the element is localized predominantly in membrane-limited granules (George and Pirie 1979). No common pattern for subcellular distribution and for the binding of various heavy metals, including cadmium, could be seen within the tissues of the kidney or the digestive system (Julshamn and Andersen 1983).

The present study describes the rate of uptake of cadmium in, and its release rate from, various organs and tissues of the common mussel. This provides further understanding on how internal distribution of a physiologically non-essential trace metal takes place. These fluxes have been studied at two temperatures (15 and 20^0C) commonly found in the natural habitat of mussels from the western most part of the Dutch Wadden Sea. Besides which, it can also be estimated which organs may play a role in the biological elimination of cadmium. The present study differs from others for two reasons: the experiments were carried out at a relatively low concentration level of cadmium in the waterphase and it focuses on the effect of temperature on the rate of cadmium uptake.

MATERIALS AND METHODS

The common or blue mussel, *Mytilus edulis* (L.), was collected from a continuously submerged estuarine area in the most western part of the Dutch Wadden Sea by means of a 3 m beam-trawl. The animals were selected at a shell-length of 5.5-6.5 cm, and transferred to the laboratory for 2 weeks acclimatization. Mussels were placed in glass-tanks (0.32x0.48x1.81 m) in which oxygen-saturated seawater with a salinity of about 27 ‰ was continuously supplied at a flow rate of 290 $ml \cdot min^{-1}$. From a standard stock solution, cadmium chloride was added to the seawater (pumping rate 0.15 $ml \cdot min^{-1}$) resulting in a nominal cadmium concentration of 50 $\mu g \cdot l^{-1}$. In the first experiment 60 specimens were exposed for 40 days at 15°C. In the second experiment 200 specimens were exposed for 51 days at 20°C, of which 40 were removed from exposure after 43 days and placed in clean seawater for 17 days to measure the depuration of cadmium. Together with the exposed animals, controls were running with the same number of animals for each experiment. Experiments continued until all specimens were sacrificed. One experimental sample consisting of 10 specimens was taken from both the control and exposed animals at the beginning of exposure and at subsequent days (Exp. 1: day 1, 3, 5, 20, 40; Exp. 2: day 2, 3, 4, 6, 8, 12, 14, 16, 18, 22, 24, 36, 46, 51). Animals were not fed during the course of the experiment; no significant wet- or dry-weight loss was measured and no indication of starvation was observed.

The organs used for analysis were carefully dissected from each specimen: the kidney, the gills, the hepatopancreas (mid-gut gland), the mantle, muscular tissue of the musculus adductor and the rest of the soft tissue, mainly the foot (henceforth indicated as 'foot'). Organs of ten mussels were pooled for each sample and dried at 110°C, till constant dry weight. About 400 mg, weighed to the nearest 0.1 mg, were decomposed by an acid-destruction bomb technique (Paus 1972). For the decomposition of the organic material, 3 ml of 65% nitric acid was added and the bombs were kept at 120°C for 2 hours. The samples were then transferred quantitatively to polypropylene tubes and diluted with double-distilled water to 10 ml.

Cadmium was measured with a heated graphite furnace atomizer (HGA 500) coupled to an atomic absorption spectrophotometer (Perkin Elmer 5000). The AAS measurements were carried out in duplicate. To calculate the cadmium concentration, the standard addition method was applied. To avoid contamination of the samples, all chemicals used were suprapure grade (Merck). All materials used were rinsed for 12 hours with 5 N HCl and subsequently rinsed three times with double-distilled water.

RESULTS AND DISCUSSION

During 40 days of exposure to 50 $\mu g \cdot l^{-1}$ at 15°C, cadmium accumulated linearly in mussel kidney to 300 $\mu g \cdot g^{-1}$ dry weight, in the gills to 130 $\mu g \cdot g^{-1}$ dry wt and in the hepatopancreas to 170 $\mu g \cdot g^{-1}$ dry wt (Fig. 1). The concentration of cadmium in the foot increased linearly to about 110 $\mu g \cdot g^{-1}$ dry wt, whereas in the

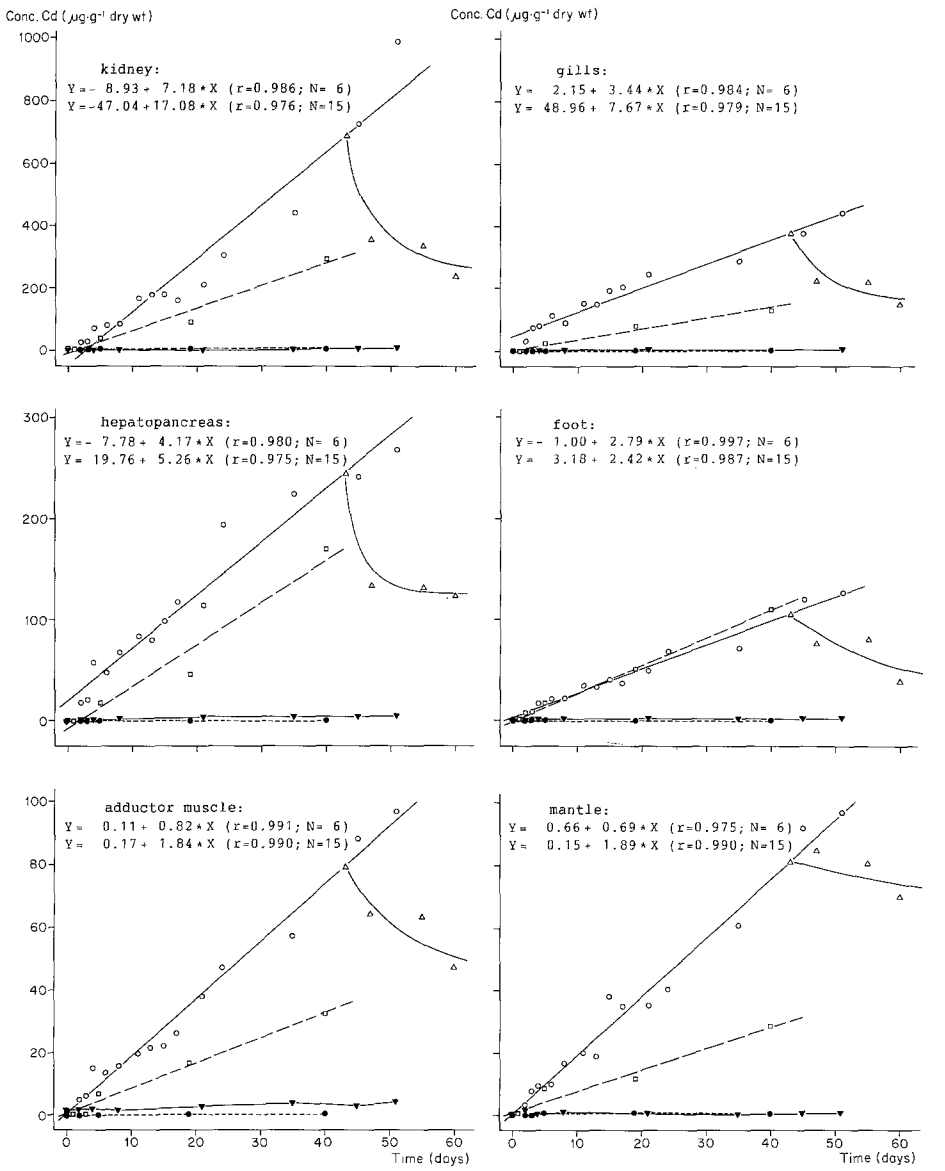

Figure 1. The accumulation of cadmium in various organs of the common mussel *Mytilus edulis* (L.), after exposure for 40 days at 15^0C (□ : exposed; ● : control) and after exposure during 51 days at 20^0C (○: exposed; ▼: control) to a cadmium concentration of 50 $\mu g \cdot l^{-1}$ in filtered seawater with a salinity of 27 ‰. The rate of uptake of cadmium in the various organs is expressed as the slope of the regression line. All regressions are highly significant ($p < 0.005$). Cadmium release from the various organs during 17 days of exposure to clean seawater is given (△), subsequent to 43 days of exposure to a cadmium concentration of 50 $\mu g \cdot l^{-1}$ in the seawater at 20^0C.

Table 1. The rates of uptake of cadmium for various organs.

Organ	Rate of uptake ($\mu g \cdot g^{-1} \cdot day^{-1}$) 15^0C	20^0C
Kidney	7.18	17.08
Hepatopancreas	4.17	5.26
Gill	3.44	7.67
Foot	2.79	2.42
Adductor muscle	0.82	1.84
Mantle	0.69	1.89

adductor muscle and the mantle the concentration increased to a relatively low level of about 30 $\mu g \cdot g^{-1}$ dry wt (Fig. 1). The rates of uptake, given in $\mu g \cdot g^{-1}$ dry $wt \cdot day^{-1}$, for the various organs are summarized in Table 1; at 15^0C the sequence of decreasing uptake rates is as follows: kidney (7.2) > hepatopancreas (4.2) > gill (3.4) > foot (2.8) > muscle (0.8) ~ mantle (0.7).

After 40 days of exposure at 20^0C, cadmium accumulation in the kidney, gill, mantle and adductor muscle was a factor 2.2 to 2.7 higher than at 15^0C (Table 1). The hepatopancreas and the foot showed a considerably lower temperature effect (a factor of 1.3 and 0.9, respectively). This can be related possibly to the significantly higher cadmium uptake rates at 20^0C in the various other organs considered. The uptake rate in the hepatopancreas increased only slightly, whereas in the foot about the same rate of uptake was found at both temperatures. The sequence in the rates of uptake at 20^0C for the various organs is as follows: kidney (17.1) > gill (7.7) > hepatopancreas (5.3)> foot(2.4)> mantle (1.9) ~ adductor muscle (1.8).

The depuration of cadmium was measured only at 20^0C. Concentrations decreased according to a first order reaction: a rapid release during the first four days of exposure to clean seawater and a more gradual loss thereafter until a level of equilibrium was reached (Fig. 1 and Table 2). Cadmium release, in terms of the absolute decrease of the cadmium concentration, from the kidney was very fast during the first four days (300 $\mu g.g^{-1}$) in clean seawater; this loss was about twice that in gills (150 $\mu g.g^{-1}$) and three times higher compared to the hepatopancreas (100 $\mu g.g^{-1}$). However, in terms of loss-rates, cadmium loss in kidney, hepatopancreas and gill were almost the same. After 17 days in clean seawater, cadmium loss from the various organs, expressed as the % of total cadmium concentration in the organs after 43 days exposure to 50 $\mu g\ Cd \cdot l^{-1}$, shows the following sequence: kidney (65.1%) > foot (62.4%) > gill (60.3%) > hepatopancreas (49.2%) > adductor muscle (40.1%) > mantle (14.1%).

Within 50 days, neither regulation nor limitation of Cd-uptake was observed in the various tissues of *Mytilus edulis* exposed to 50 $\mu g.l^{-1}$. Cadmium accumulation in tissues was not completely reversible. Depuration of cadmium is described to be mediated principally by an active mechanism, possibly involving the accumulation in lysosomes, with subsequent excretion of metal-rich

Table 2. Depuration of cadmium from various organs of the common mussel *Mytilus edulis* after placement in clear water.

Organ	% loss after 4 days	% loss after 17 days	% (loss over 4 days/ loss over 17 days)
Kidney	48.3	65.1	74.2
Hepatopancreas	45.4	49.2	92.3
Gill	40.7	60.3	67.5
Foot	27.2	62.4	43.6
Adductor muscle	18.6	40.1	46.4
Mantle	-3.6	14.1	N/A

residual bodies (George and Viarengo 1985). The uptake of soluble forms of metals such as Zn, Cu, Hg and Cd seems to be mainly a passive process (Simkiss 1983). In gills of *M. edulis*, the cadmium uptake was found to occur by diffusion, and both uptake and accumulation were facilitated by intracellular binding and sequestration (Carpene and George 1981). A field study on the accumulation of cadmium from seawater by *M. edulis* indicated a significant equilibrium relationship between total recoverable cadmium in seawater and its concentration in the mussel (Talbot 1985). Linear cadmium accumulation in *M. edulis* as found in the present study has previously been demonstrated, both in whole-body soft tissues and various organs separately (e.g., Scholz 1980; Kohler and Riisgard 1982; Poulsen et al. 1982). However, with respect to the degree of accumulation and uptake rate for various organs and tissues, no consistency exists between different data. Janssen and Scholz (1979) found that the cadmium concentration in various organs of mussels after 21 days of exposure to 100 µg $Cd \cdot l^{-1}$, at 10^0C and a salinity of 25 ‰ decreased in the order of hepatopancreas > gill > kidney > adductor muscle > mantle > foot. Quantifying the rates of uptake found in the hepatopancreas, gills and kidney from data published by Scholz (1980), it is noted that at 10^0C and a salinity of 17 ‰, the uptake rates decrease in the order hepatopancreas (4.9) > gill (3.2) > kidney (1.4 $\mu g \cdot g^{-1}$ dry $wt \cdot day^{-1}$). This sequence appeared not to be dependent on the nominal concentration of cadmium dosed. From these data it can also be concluded that differences in salinity apparently do not cause changes in the relative capacity of cadmium accumulation of various organs.

Uptake of cadmium from seawater into various tissues may involve a number of different processes, both of physical (e.g., diffusion, adsorption) and biochemical origin. The uptake process can be considered as a sequence of events with a certain reaction velocity. The temperature influence on the overall reaction velocity (in the present study the Cd-uptake rate) can be described by the Arrhenius equation:

$$F = A * e^{-E/RT}$$ (cf. Glasstone and Lewis 1965),

where F is the specific rate (Cd uptake in present study), A is a constant, E is the critical incremental energy of activation ($Joule \cdot mol^{-1}$), R is the gas constant (8.314 $Joule \cdot mol^{-1} \cdot {}^0K^{-1}$) and T is the absolute temperature (^0K). Thus, the influence of

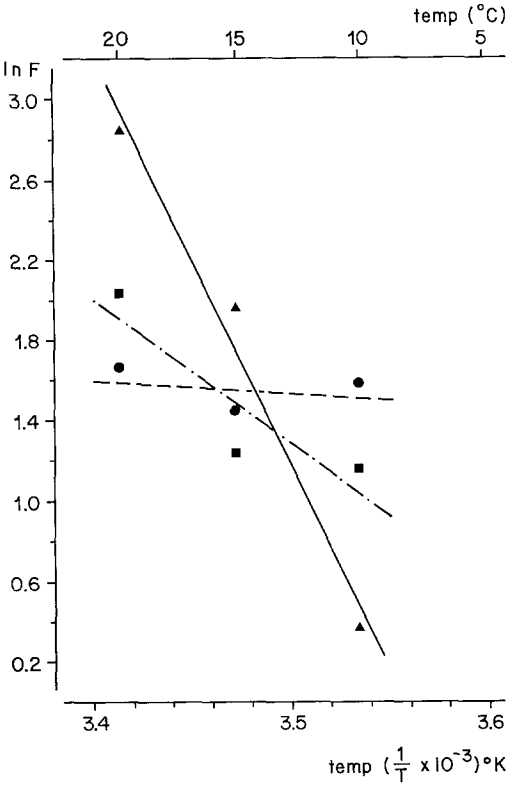

Figure 2. The influence of temperature on the cadmium-uptake-rate (F), expressed as µg $Cd \cdot g^{-1}$ dry weight $\cdot day^{-1}$, of the kidney (▲; regression equation: Y = 72.78 - 20.46 * X; r=-0.998; N=3), the gills (■ ; regression equation: Y = 26.56 - 7.22 * X; r=-0.898; N=3) and the hepatopancreas (● ; regression equation: Y = 3.48 - 0.55 * X; r=-0.283; N=3); the values at 10^0C were calculated from data of Scholz (1980).

temperature on the Cd-uptake rate can be derived directly by plotting the logarithm of the specific rate against the reciprocal of the absolute temperature (Fig. 2). The slope of the regression line expresses the activation energy E for the rate limiting process. The definite value of E ('slope' times -R) characterizes the temperature influence on cadmium uptake. The present data on the three organs accumulating cadmium at the highest rates (kidney, gills and hepatopancreas) demonstrate different rates of uptake of the various organs. From these uptake rates and including the rates calculated from the data of Scholz (1980) at 10^0C, it can be concluded that temperature exerts a considerable enhancing effect on the uptake of cadmium in the kidney (Fig. 2), whereas accumulation in the hepatopancreas is apparently insensitive to temperature changes. At 20^0C, the uptake of cadmium in the gills tends to be higher than at lower temperatures. The low temperature sensitivity of the Cd-uptake rate in the hepatopancreas (E = 4.6 $J \cdot mol^{-1}$) and the intermediate temperature sensitivity of Cd-uptake in the gills (E = 60 $J \cdot mol^{-1}$) indicate that mainly physical processes are involved in Cd-uptake in these

Table 3. Accumulation in three organs of *Mytilus edulis* during the first period of exposure.

Cadmium concentration ($\mu g \cdot g^{-1}$ dry wt)	Temp. (^0C)	Exposure time Day 0	1	2	3	4	5
Gill	15	2.4	2.0	-	2.1	-	25.1
	20	3.1	-	32.5	75.8	83.2	-
Kidney	15	3.1	3.0	-	3.1	-	38.4
	20	5.0	-	28.9	29.4	71.3	-
Hepatopancreas	15	0.7	0.2	-	0.8	-	18.2
	20	1.1	-	18.1	20.9	58.0	-

organs. The highly temperature dependent uptake rate of the kidney ($E = 170\ J \cdot mol^{-1}$) suggests that biochemical (enzymatic) controlled uptake processes are involved. Thus, an increase of metabolic processes at increased temperatures appears to affect the potential for cadmium uptake in the kidney.

Considering only the first period of exposure, it was observed that at 15^0C significant uptake actually starts after 4 days, whereas at 20^0C accumulation starts after the second day of exposure (Table 3). Calculating the rates of uptake during the first five days of exposure, it is noted that the gills accumulate cadmium at the rate of 21.4 $\mu g \cdot g^{-1} \cdot day^{-1}$, which is a much higher rate than those of the kidney (14.7) and hepatopancreas (12.6); thus, the gills are the primary sites of cadmium uptake. In the other organs the uptake appears to be retarded during the very first period of exposure to cadmium.

Within 4 days the hepatopancreas loses 92.3% of the total cadmium lost over 17 days (Table 2). These loss-rates during the first depuration period are also considerable in the kidney and in the gills (74.2% and 67.5%, respectively, of the total cadmium released). Of the other organs analyzed, the foot and adductor muscle still show within the first period substantial loss of the total cadmium lost over 17 days, whereas the mantle does not (Table 3). The present data on the loss of cadmium in *M. edulis* agree with the results of studies on the uptake and depuration of trace metals in the oysters *Crassostrea gigas* and *C. virginica* (Okazaki and Panietz, 1981; Zaroogian and Johnson, 1983).

In the present study, release of cadmium did not continue until the original control level was reached. This was possibly due to cadmium binding to metal binding proteins, hampering the excretion of the metal-rich residual bodies as described by George and Viarengo (1985). After exposure during 9 days to an estimated dosage of 180 $\mu g\ Cd \cdot l^{-1}$, and a subsequent recovery period of 28 days in clean seawater, the amount of cadmium bound to thionein in the digestive gland of *Mytilus galloprovincialis* at the end of the recovery period had increased by approximately 250% (Viarengo et al. 1987). These data demonstrate the importance of metallothionein induction in the reduction of the cytotoxic effects exerted by high levels of cadmium accumulation.

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REFERENCES

Carpene E and George SG (1981) Absorption of cadmium by gills of *Mytilus edulis* (L.). Mol Physiol 1: 23-34

George SG and Pirie BJS (1979) The occurrence of cadmium in subcellular particles in the kidney of the marine mussel, *Mytilus edulis*, exposed to cadmium. Biochim Biophys Acta 580: 234-244

George SG and Viarengo A (1985) An integration of current knowledge of the uptake, metabolism and intracellular control of heavy metal in mussels. In: Vernberg FJ, Thurberg FP, Calabrese A and Vernberg WB (eds) Marine Pollution and Physiology: Recent Advances, University of South Carolina Press, Columbia p 125-144

Glasstone S and Lewis D (1965) Elements of physical chemistry. MacMillan and Comp. Ltd., London, p 627-628

Goldberg ED (1975) The Mussel Watch - A first step in global marine monitoring. Mar Pollut Bull 6: 111

Janssen HH and Scholz N (1979) Uptake and cellular distribution of cadmium in *Mytilus edulis*. Mar Biol 55: 133-141

Julshamn K and Andersen K-J (1983) Subcellular distribution of major and minor elements in unexposed molluscs in western Norway II. The distribution and binding of cadmium, zinc, copper, magnesium, manganese and iron in the kidney and the digestive system of the common mussel *Mytilus edulis*. Comp Biochem Physiol 75A: 13-16

Köhler K and Riisgard HU (1982) Formation of metallothioneins in relation to accumulation of cadmium in the common mussel *Mytilus edulis*. Mar Biol 66: 53-58

Okazaki RK and Panietz MH (1981) Depuration of twelve trace metals in tissues of the oysters *Crassostrea gigas* and *C. virginica*. Mar Biol 63: 113-120

Paus PE (1972) Bomb decomposition of biological materials. Atomic Absorption Newsletter 11: 129-130

Poulsen E, Riisgard HU and Mohlenberg F (1982) Accumulation of cadmium and bioenergetics in the mussel *Mytilus edulis*. Mar Biol 68: 25-29

Scholz N (1980) Accumulation, loss and molecular distribution of cadmium in *Mytilus edulis*. Helgoländer Meeresunters 33: 68-78

Simkiss K (1983) Lipid solubility of heavy metals in saline solutions. J mar biol Ass UK 63: 1-7

Talbot V (1985) Relationship between cadmium concentrations in seawater and those in the mussel *Mytilus edulis*. Mar Biol 85: 51-54

Viarengo A, Moore MN, Mancinelli G, Mazzucotelli A, Pipe RK and Farrar SV (1987) Metallothioneins and lysosomes in metal toxicity and accumulation in marine mussels: the effect of cadmium in the presence and absence of phenanthrene. Mar Biol 94: 251-257

Zaroogian GE and Johnson M (1983) Chromium uptake and loss in the bivalves *Crassostrea* virginica and *Mytilus edulis*. Mar Ecol Prog Ser 12: 167-173

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