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Accumulation, Elimination, and Speciation of Cadmium and Zinc in Mussels, *Mytilus edulis*, in the Natural Environment

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Accumulation of trace metals like cadmium and zinc by the common mussel (*Mytilus edulis*) has been often studied.
Most of these studies have been carried out under laboratory conditions. It has been shown that the accumulation of cadmium by the common mussel is affected by abiotic factors like salinity, temperature and the presence of complexing agents and biotic factors like animal size, sex and maturity (Ray 1984).
Elimination studies of cadmium from the common mussel are scarce (Scholz 1980; Gutiérrez-Galindo 1980; Nolan and Duke 1983a).
Detoxification of cadmium by the common mussel takes place by binding to low (metallothionein) and high molecular weight proteins (Noël-Lambot 1976; Talbot and Magee 1978; George et al 1979; Frankenne et al 1980; Scholz 1980; Köhler and Riisgård 1982; Nolan and Duke 1983 a, b).

The objective of this study was to investigate accumulation and elimination of cadmium and zinc by the common mussel in the natural environment in combination with a study about the speciation of cadmium and zinc in the common mussel.

MATERIALS AND METHODS

In the period november-december 1984 approx. 60 kg of mussels (*Mytilus edulis*) with a length of 30 - 70 mm was collected from the Eastern and Western Scheldt respectively. The Western Scheldt can be considered as a rather polluted estuary while the Eastern Scheldt is an unpolluted area where mussels for human consumption are cultured.
Subsamples of approx. 3 kg were placed in nylon baskets.
The mussels from the Eastern Scheldt were subsequently exposed for 70 days to the environment of the Western Scheldt for the accumulation experiment. The mussels from the Western Scheldt were for a period of 77 days exposed to the Eastern Scheldt environment for the elimination experiment.
At regular intervals subsamples from the two locations were collected. All samples were deepfrozen and stored at -35°C for later analysis.
At least 75 individual animals were carefully opened by cutting the aductor muscle. The shell cavity liquor was discarded and the

entire remaining shell content was collected and homogenized with an Ultra Turrax.
Mussels, exposed during 40 days to 200 µg Cd/l under laboratory conditions, were used as reference.

Cadmium and zinc were determined by graphite furnace atomic absorption spectrometry (GFAAS) respectively flame atomic absorption spectrometry (FAAS). One gram portions of the samples were decomposed by 5.0 ml of 70% nitric acid in a teflon-lined stainless steel decomposition vessel for at least 3 hours at 150°C. After destruction the nitric acid solution was evaporated until dry. Then 0.5 ml of 37% hydrochloric acid and 1 ml bidistilled water were added. The solution was transferred to a volumetric flask and made up to 25 ml with bidistilled water. The cadmium and zinc concentrations were determined by standard addition method with an Instrumentation Laboratory atomic absorption spectrophotometer (IL 551).

About 25 grams of the mussel sample were homogenized with 50 ml of 0.01 M tris/1% NaCl solution (pH 8.2) during 30 seconds at 4°C. The homogenate was centrifuged at 42000 g during 60 minutes at 0°C. The cadmium and zinc contents in the mussel supernatant (cytosol) were determined as described above.

The high performance gel filtration chromatography (HPGFC) procedure, based on the method of Suzuki (1980) and Pfannkoch et al (1980), was carried out on a high pressure liquid chromatograph (Perkin Elmer Series 4) equipped with gel filtration column (Bio-Sil TSK-400, 600 x 7.5 mm) with a precolumn (100 x 7.5 mm). Proteins were detected with a variable UV-wavelength detector (Perkin Elmer LC-85 B) at 280 nm.
The mussel supernatant was applied in a 200 µl portion and eluted with 50 mM tris-HCl buffer (pH = 6.8) containing 0.2 M KH_2PO_4 and 0.1% NaN_3 with a flow rate of 1.0 ml/min.
Eluate fractions (1 - 2 ml) were collected for a direct analysis of cadmium and zinc by GFAAS and FAAS. The column was calibrated for molecular weight estimations with a protein mixture of bovine γ-globuline (MW = 160,000), ovalbumine (MW = 45,000), bovine myoglobine (MW = 17,000) and cyanobalamine (MW = 1355).
A standard of metallothionein isolated from rabbit liver (Sigma, USA) according to the procedure of Nordberg et al (1972) was used as a reference compound.

The electrochemical analysis and characterization of the mussel supernatant was carried out with the Polarographic Analyzer 384 B (EG&G/PAR) and a Model 303 static mercury drop electrode. Differential pulse anodic stripping voltammetry (DPASV) was performed by adding 2 ml of the mussel supernatant to 10 ml 10%/10% sodiumacetate/acetic acid buffer. The pH was adjusted to 3,0 ± 0.2 by adding hydrochloric acid (37%).
Prior to the deposition procedure the solution was purged during 5 minutes with nitrogen. During the deposition procedure the solution was stirred.

The DPASV conditions were as follows:

initial potential	: -1.00 V (vs Ag/AgCl)
final potential	: -0.35 V (vs Ag/AgCl)
pulse height	: 50 mV
deposition time	: 300 seconds
equilibrium time	: 30 seconds
drop time	: 0.3 seconds
scan increment	: 2 mV
drop mode	: large.

Standard addition measurements were used for the determination of cadmium.

RESULTS AND DISCUSSION

The results of the accumulation of cadmium and zinc by the common mussel exposed at the Western Scheldt are presented in figures 1 and 2. From figure 1 it can be seen that there is a linear uptake of cadmium (0.029 mg Cd/kg dry matter/day) during the exposure period. A linear uptake of cadmium by the soft parts of the common mussel during an exposure (20 - 70 days) to 10 - 100 µg Cd/l under laboratory conditions has been observed by Köhler and Riisgård (1982) and Scholz (1980).

The final cadmium content in the mussels (2.5 mg/kg dry matter) is about 30% of the cadmium content in mussels originally derived from the Western Scheldt area.

The accumulation of zinc by the mussels (0.47 mg/kg dry matter/day) during the exposure at the Western Scheldt is linear with the time although the correlation coefficient is somewhat lower than in the case of cadmium. Application of an experimental accumulation model for zinc did not give better results. At the end of the exposure period the zinc content in mussels (106 mg/kg dry matter) is about 40% of the zinc content in mussels originally derived from the Western Scheldt.

Data on the cadmium and zinc content in mussels from the Western Scheldt exposed at the Eastern Scheldt, given in figure 3 and 4, show that cadmium and zinc are not eliminated under these circumstances. The average cadmium and zinc content over the whole exposure period is 8.9 respectively 248 mg/kg dry matter. The cadmium content in the cytosol of mussels exposed at the Western Scheldt increase also linearly with the exposure time. This increase of 0.017 mg Cd/kg dry matter/day represents approx. 60% of the total cadmium increase in the mussels. These results show that at the beginning of the accumulation experiment the soluble cadmium fraction is 32% of the total cadmium content and at the end of the exposure period 52%.

The cadmium content in the cytosol of mussels exposed at the Eastern Scheldt varies from 2.0 - 3.9 mg/kg dry matter. The average content is 3.0 mg/kg dry matter, being 34% of the total average cadmium content of the mussels.

The zinc content in the cytosol of mussels from the accumulation experiment does not increase significantly with the exposure time. The average zinc content in the cytosol of these mussels was 21 mg/kg dry matter. Due to the linear increase of the total zinc content in the mussels during the accumulation experiment the

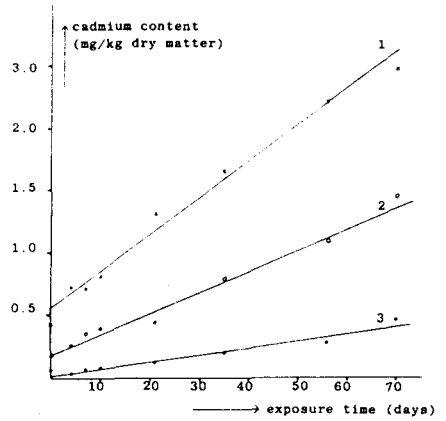

Figure 1. The accumulation of cadmium in mussels during exposure at the Western Scheldt: 1= total cadmium; 2= cadmium in cytosol; 3= DPASV-active cadmium

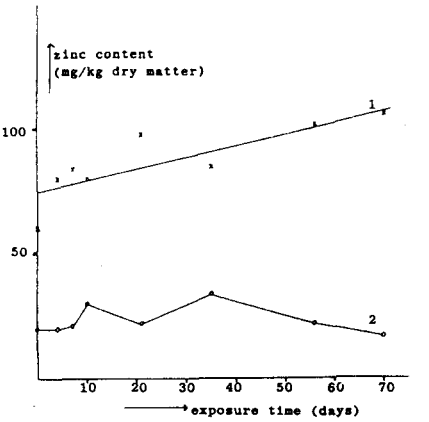

Figure 2. The accumulation of zinc in mussels during exposure at the Western Scheldt: 1= total zinc; 2= zinc in cytosol

soluble zinc fraction decreases linearly from 28% at the beginning to 20% of the total zinc content after 70 days of exposure.
The zinc content in the cytosol of mussels exposed at the Eastern Scheldt varies from 31 - 52 mg/kg dry matter. The average content is 39 mg/kg dry matter, being 16% of the total average zinc content of the mussels.
Figure 5 shows a typical DPASV scan of the cytosol from mussels exposed at the Western Scheldt. The small peak at -0.55 V is due to the oxidation of cadmium. The addition of cadmium nitrate to the cytosol results in a linear increase of the peak current at this potential. The content of this so-called DPASV-active cadmium in the mussel supernatant is given in figure 1 and 3 as a function of the exposure time. Figure 1 shows a linear increase with exposure time at the Western Scheldt. Ten percent of the cadmium in the cytosol of mussels is DPASV-active at the beginning of the exposure period and approx. 30% after an exposure of 70 days.
The content of DPASV-active cadmium in the cytosol of mussels exposed at the Eastern Scheldt (figure 3) does not alter with the exposure time and varies from 0.96 - 1.46 mg/kg dry matter. The average content of DPASV-active cadmium (1.23 mg/kg dry matter) is approx. 40% of the cadmium present in the cytosol.
The peak potential of cadmium in a DPASV-scan of metallothionein isolated from rabbit liver ($E_{\frac{1}{2}}$ = -0.65 V) is significantly different from the potential obtained in the cytosol of mussels ($E_{\frac{1}{2}}$ = -0.55 V). This difference might be due to a difference of the binding of cadmium in the cytosol of mussels and in metallothionein from rabbit liver.
The effect of varying amounts of the cytosol on the peak current of a cadmium nitrate solution is shown in figure 6.
Very small amounts of the cytosol added to a cadmium nitrate solution cause a sharp reduction of the peak current of cadmium.

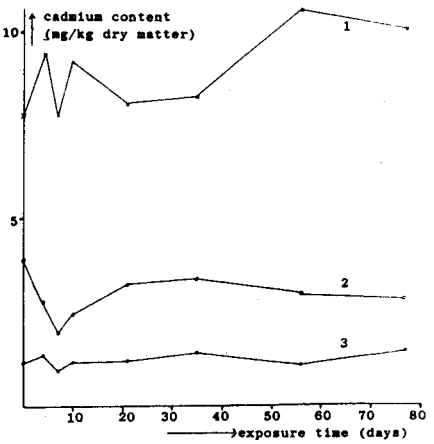

Figure 3. The elimination of cadmium from mussels during exposure at the Eastern Scheldt: 1= total cadmium; 2= cadmium in cytosol; 3= DPASV-active cadmium

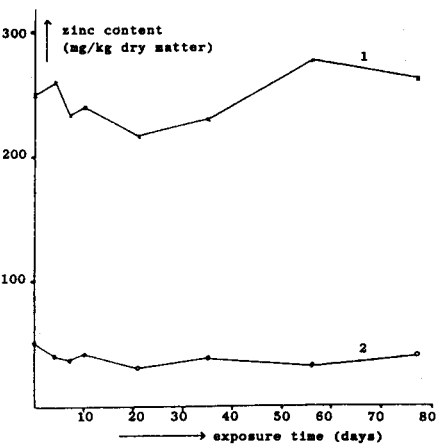

Figure 4. The elimination of zinc from mussels during exposure at the Eastern Scheldt:
1 = total zinc
2 = zinc in cytosol

Although the cytosol behaves as a strong complexing agent for cadmium, a significant amount of cadmium is still available for DPASV.
The results of HPGFC of the cytosol of mussels from the Western Scheldt exposed at the Eastern Scheldt are given in figure 7-9. A few peaks were observed with UV-detection at 280 nm. It can be seen that cadmium and zinc are present in only one peak. The maximum cadmium concentration is present in the eluate after a retention volume of 30 - 34 ml. Similar chromatograms were obtained for mussels exposed at the Western Scheldt during 77 days. Mussels with a cadmium content 97 mg/kg dry matter due to a cadmium exposure under laboratory conditions show a similar cadmium distribution in the cytosol after HPGFC. The maximum concentration in the eluate of these mussels was observed after a retention volume of 29 - 31 ml.
The elution profile of cadmium from the rabbit liver metallothionein standard after HPGFC is given in figure 10. This figure shows that a distinct cadmium peak is present at a retention volume of approx. 26 - 27 ml corresponding with a molecular weight of approx. 12,000 Dalton. HPGFC of a solution of cadmium nitrate showed that cadmium was eluted with a maximum concentration at a retention volume of 36 - 38 ml.
Molecular weight estimations, based on the retention volume of several proteins with known molecular weight, showed that the cadmium in the mussels are associated with proteins with a molecular weight smaller than 2,000 Dalton.
It seems therefore that cadmium present in the cytosol of mussels from the natural environment is bound to a protein smaller than metallothionein.

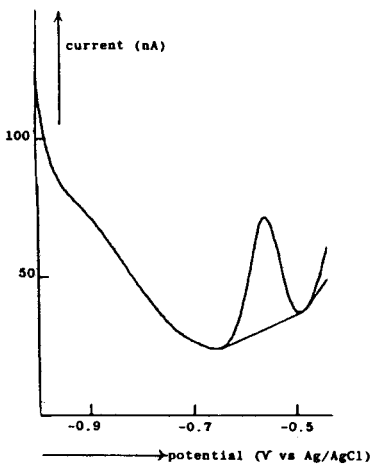

Figure 5. A DPASV-scan of cytosol of mussels exposed at the Western Scheldt

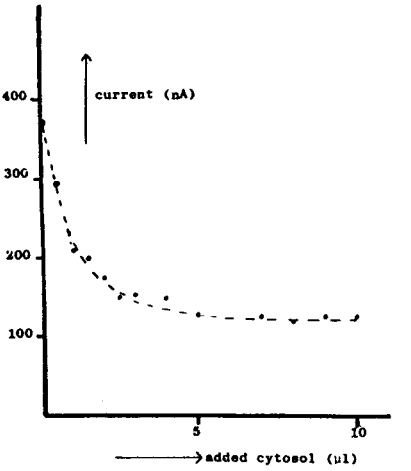

Figure 6. The peak current of cadmium during a DPASV-scan of a cadmium nitrate solution (20 µg Cd/l as a function of the added cytosol of mussels

So far very few investigators show the presence of cadmium bound to a small protein in mussels. Talbot and Magee (1978) showed that cadmium in the gills and viscera of mussels from a cadmium polluted bay was present in three distinct fractions. The first fraction correspondends with unidentified high molecular weight proteins, the second fraction with low molecular weight proteins (metallothionein, MW $\approx$ 10,000 Dalton) and the third fraction consists probably of low molecular weight amino acids. Köhler and Riisgård (1982) showed that cadmium metallothionein was not present in the total soft parts of mussels (82 mg Cd/kg dry matter) after an exposure to cadmium (50 µg/l) during 40 days. Cadmium was present in a high and low molecular weight protein fraction.
The results of this study show that accumulation of cadmium and zinc in mussels under environmental conditions takes place as a linear function of the exposure time. Elimination of cadmium and zinc from natural exposed mussels does not seem to take place. Therefore it seems to be unlikely that cleansing of mussels will lower the content of these elements in mussels. Electrochemical characterization as well as high performance gel filtration chromatography indicate that cadmium in mussels from the natural environment is bound to a protein smaller than metallothionein.

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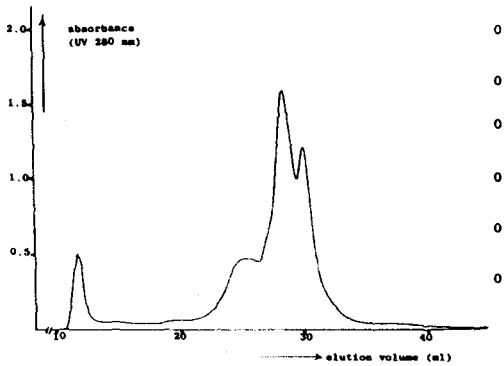

Figure 7. High performance gel filtration chromatography of cytosol of mussels from the Western Scheldt exposed at the Eastern Scheldt

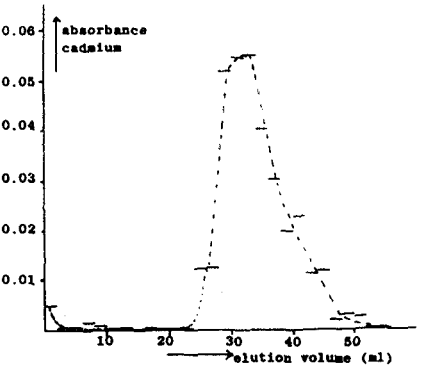

Figure 8. The cadmium elution profile of cytosol from mussels from the Western Scheldt exposed at the Eastern Scheldt after HPGFC

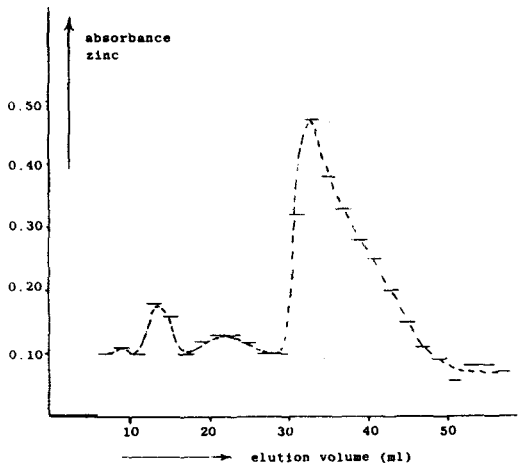

Figure 9. The zinc elution profile of cytosol from mussels from the Western Scheldt exposed at the Eastern Scheldt after HPGFC

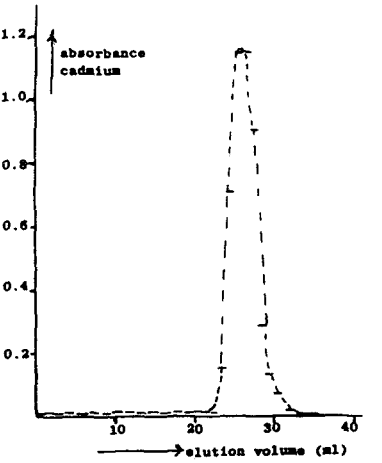

Figure 10. The cadmium elution profile of rabbit liver cadmium metallothionein after HPGFC

REFERENCES

Frankenne F, Noël-Lambot F, Disteche A (1980) Isolation and characterization of metallothioneins from cadmium-loaded mussel Mytilus edulis. Comp Biochem Physiol 66: 179

George GE, Carpene E, Coombs TL, Overnell J, Youngson A, (1979) Characterization of cadmium-binding proteins from mussels, Mytilus edulis (L), exposed to cadmium. Biochimica et Biophysica Acta 580: 225

Gutiérrez-Galinda EA (1980) Etude de l'elimination du cadmium par Mytilus edulis en présence d'EDTA et de phosphate. Chemosphere 9: 495

Köhler K, Riisgård HU (1982) Formation of metallothioneins in relation to accumulation of cadmium in the common mussel Mytilus edulis. Mar Biol 66: 53

Noël-Lambot F (1976) Distribution of cadmium, zinc and copper in the mussel Mytilus edulis: Existence of cadmium binding proteins similar to metallothioneins. Experientia 32: 324

Nolan CV, Duke EJ (1983a) Cadmium-binding proteins in Mytilus edulis: relation to mode of administration and significance in tissue retention of cadmium. Chemosphere 12: 65

Nolan CV, Duke EJ (1983b) Cadmium accumulation and toxicity in Mytilus edulis involvement of metallothioneins and heavy-molecular weight protein. Aquatic Toxicology 4: 153

Nordberg GF, Nordberg M, Piscator M, Vesterberg O (1972) Separation of two forms of rabbit metallothionein by isoelectric focusing. Biochem J 126: 491

Pfannkoch E, Lu KC, Regnier FE, Barth HG (1980) Characterization of some commercial high performance size-exclusion chromatography columns for water-soluble polymers. J Chrom Science 18: 430

Ray S (1984) Bioaccumulation of cadmium in marine organisms. Experientia 40: 14

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

Suzuki KT (1980) Direct connection of high-speed liquid chromatograph (equipped with gel permeation column) to atomic absorption spectrophotometer for metalloprotein analysis: metallothionein. Anal Biochem 102: 31

Talbot V, Magee RJ (1978) Naturally-occurring heavy metal binding proteins in invertebrates. Arch Environ Contam Toxicol 7: 73

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