

**The kinetics of cadmium
accumulation and loss
from *Mytilus trossulus* in
the presence of marine
humic substances***

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KEYWORDS

Mytilus trossulus

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Abstract

The accumulation and loss of cadmium (Cd) by the Baltic blue mussel *Mytilus trossulus* in the presence of marine humic substances (HS) was investigated under laboratory conditions. The organisms were exposed to Baltic Sea water (salinity 7.0 PSU, pH 7.9) containing cadmium ($50 \mu\text{g Cd dm}^{-3}$) or radiocadmium ($9 \text{ kBq } ^{115\text{m}}\text{Cd dm}^{-3}$) and humic substances ($0.0\text{--}7.2 \text{ mg HS dm}^{-3}$). Experiments were carried out in seawater at two different constant temperatures ($6^\circ\text{C} \pm 1^\circ\text{C}$ or $15^\circ\text{C} \pm 1^\circ\text{C}$). The exposure time ranged from 8 to 21 days.

It was found that marine humic substances stimulate cadmium accumulation by the mussels; however, the effect was strongly modified by temperature. At 6°C cadmium was preferably accumulated in the gills while at 15°C the metal was stored mainly in the hepatopancreas. Two pools of cadmium accumulated by *Mytilus trossulus* were detected. Cadmium adsorbed to the shell was desorbed quickly and efficiently after the mussels, previously grown in seawater containing an elevated metal concentration (8 days, $50 \mu\text{g Cd dm}^{-3}$ or $9 \text{ kBq } ^{115\text{m}}\text{Cd dm}^{-3}$), had been

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transferred to natural seawater (60 days, $0.03 \mu\text{g Cd dm}^{-3}$, $0 \text{ kBq } ^{115\text{m}}\text{Cd dm}^{-3}$). The other pool of cadmium, accumulated in the soft tissue of the mussels, remained intact after depuration experiments lasting several weeks. Humic substances had no effect on the depuration of cadmium from either pool.

1. Introduction

The increasing concentrations of heavy metals in the Baltic Sea are thought to constitute a potential threat to marine biota. One of the most toxic of these metals is cadmium (Cd) (George and Coombs, 1977), which in the Baltic Sea is predominantly of anthropogenic origin (Pempkowiak and Szefer, 1992).

Although Cd is readily bioaccumulated by marine organisms, their depuration from the metal is slow (Theede and Jung, 1989). The accumulation of Cd by biota depends on a number of biotic and abiotic factors (Cain and Luoma, 1986; Pelgrom *et al.*, 1994), the most important of the latter being the temperature, salinity, and the concentrations of oxygen and dissolved organic matter in water (Ray, 1984; Shaw, 1994).

Humic substances constitute an organic matter fraction substantially modifying the bioaccumulation of heavy metals, including Cd (Ray, 1984; Kozuch and Pempkowiak, 1992). Laboratory experiments have established that humic substances inhibit Cd accumulation by algae (Gędziorowska *et al.*, 1984), but stimulate its accumulation by some invertebrates and fish (Pempkowiak *et al.*, 1989). The presence of humic substances may increase or decrease the toxicity of heavy metals (Giesy *et al.*, 1977) and persistent organic chemicals (Steinberg *et al.*, 1992, Lee *et al.*, 1993) towards *Daphnia magna*, a zooplankton species utilised in toxicity tests. It has also been reported that the toxicity of copper to juvenile Atlantic salmon (Zitko *et al.*, 1973) and *E. coli* (Milanovich *et al.*, 1975) decreases in the presence of humic substances.

Field studies have revealed a strong correlation between Cd concentration in the soft tissue of *Mytilus edulis* and the concentration of humic substances in the southern Baltic sediments from which the mussels were collected (Pempkowiak and Kozuch, 1993). Detailed laboratory studies have demonstrated that marine humic substances increase the rate of Cd accumulation by the Baltic blue mussel *Mytilus edulis* (Pempkowiak and Kozuch, 1993), currently classified as *Mytilus trossulus* (Väinölä and Hvilisöm, 1991; Gosling, 1992), owing to the complexing properties of these substances towards Cd and the formation of complexes with this metal (Pempkowiak, 1989; Pempkowiak *et al.*, 1994).

This paper presents data indicating that, in the presence of marine humic substances, the rate of Cd accumulation by blue mussels and the

target organ in which Cd is accumulated is temperature-dependent. Cd accumulation by these mussels depends strongly on the concentration of humic substances in seawater. Depuration proceeds according to two different mechanisms: rapid desorption from the shell and slow release from the soft tissue.

2. Materials and methods

Baltic blue mussels *Mytilus trossulus*, collected from Puck Bay and selected according to size (25–35 mm in length) were used in the accumulation and depuration experiments.

Laboratory tests on Cd accumulation from seawater by the mussels were performed in glass aquaria filled with 15 dm⁻³ of continuously aerated Baltic seawater (salinity 7.0 PSU, pH 7.9) at constant temperature (6°C ± 1°C or 15°C ± 1°C), containing cadmium Cd (50 µg Cd dm⁻³ concentration) or radiocadmium ^{115m}Cd (T_{1/2} = 44.6 days; specific activity 9 kBq ^{115m}Cd dm⁻³ at the time of addition; in the experiments with radiocadmium the cadmium concentration in seawater was 9 µg Cd dm⁻³, since the addition of 1 kBq ^{115m}Cd dm⁻³ introduces 1 µg Cd dm⁻³), and different concentrations of humic substances HS (0.0, 2.1 and 7.2 mg dm⁻³). Seawater depleted of humic substances (0.0 mg dm⁻³) and without added metal (Cd concentration 0.03 µg Cd dm⁻³, ^{115m}Cd specific activity 0 kBq ^{115m}Cd dm⁻³) served as a control. The exposure time varied from 8 to 21 days.

The humic substances used in the experiments were isolated from seawater collected from the western part of the Gulf of Gdańsk. Water was filtered through Whatman GF/F filters, acidified to pH 2.0 with concentrated HCl and passed through a glass column (3 × 35 cm) filled with Amberlite XAD-2. Humic substances adsorbed onto the Amberlite were eluted with 0.5 M NH₄OH, concentrated in a rotary evaporator and stored in a refrigerator at 4°C.

During a three-week-long exposure of mussels to cadmium (Cd) or radiocadmium (^{115m}Cd) Cd concentrations or the specific activity of ^{115m}Cd in whole organisms, soft tissue, inner organs (gills, hepatopancreas, the rest of the soft tissue) and shells were measured.

In the depuration experiments two different approaches were taken. The mussels were first exposed to Cd in seawater at a concentration of 50 µg Cd dm⁻³ or to radiocadmium ^{115m}Cd at a specific activity in seawater of 9 kBq ^{115m}Cd dm⁻³. After an 8 day-long exposure, the mussels containing cadmium or radiocadmium were transferred to natural Baltic seawater (0.03 µg Cd dm⁻³, 0 kBq ^{115m}Cd dm⁻³) and concentrations of cadmium or specific activities of radiocadmium, either in the whole organisms or in sectioned organs, were determined. The depuration time varied from 56 to 60 days.

Cd concentrations in the soft tissue and organs of *Mytilus trossulus* were measured in a Video 11E atomic absorption spectrophotometer (Thermo Jarrell Ash) after samples had been wet-digested with $\text{HNO}_3/\text{HClO}_4$. The activity of the radiocadmium was determined with a SSU Scintillation Counter (30% yield).

3. Results and discussion

The accumulation rates of Cd by various organs of *Mytilus trossulus* at different seawater temperatures and concentrations of humic substances are listed in Tab. 1. It follows from these data that Cd accumulation rates range from 6.01 to 51.71 $\mu\text{g Cd g}^{-1} \text{ day}^{-1}$, depending on the organ in question, the experimental temperature and the concentration of humic substances.

In earlier laboratory experiments it was found that the accumulation of Cd by the organisms tested was time-dependent and proportional to the Cd concentration in seawater (Kożuch and Pempkowiak, 1992). The dependence between the Cd concentration in the soft tissue and/or inner organs of mussels and the exposure time in water enriched with the metal can be best approximated by the linear equation $C_t = C_0 + at$, where $C_{t,0}$ denotes the concentration at time t and at the beginning of the experiment respectively, t is the time, and a is the rate of Cd accumulation (Kożuch, 1995). When t is given in days and C in $\mu\text{g Cd g}^{-1}$ dry weight, a is expressed in $\mu\text{g Cd g}^{-1} \text{ day}^{-1}$.

It has also been reported that cadmium is stored primarily in the hepatopancreas (Kożuch and Pempkowiak, 1992), most probably in the form of complexes with metal-bonding protein-thionine (Coombs, 1979; Nolan and Duke, 1983; Kożuch *et al.*, 1992). The data presented in Tab. 1 and Fig. 1 confirm this in the case of Cd distribution in mussels grown at a temperature of $15^\circ\text{C} \pm 1^\circ\text{C}$. When mussels were grown at $6^\circ\text{C} \pm 1^\circ\text{C}$, most of the Cd accumulated in the gills. This is very probably caused by the slow rate of Cd transfer from the gills, where the metal is taken up, to the hepatopancreas, where it usually accumulates. In addition, the increase in the rate of Cd accumulation at higher temperatures may contribute to the phenomenon (O'Hara, 1973; Fisher, 1986). A limited rate of Cd transfer to the liver at low temperatures was suggested for mussels by Coombs (1979). Cd distribution in various organs of the oyster *Saccostrea echinatea* also differed depending on the ambient temperature (viscera > gills at 30°C , gills > viscera at 20°C (Denton, 1981)).

The stimulating effect of humic substances on Cd accumulation by marine organisms has been reported for soil and marine humic substances (George and Coombs, 1977; Pempkowiak *et al.*, 1989). The data in Tab. 1

Table 1. Coefficients a (accumulation rates) of the function $C_t = C_0 + at$ relating the cadmium concentration in *M. trossulus* soft tissue (C_t) to time (t) in experiments with varying concentrations of humic substances (HS) at two different temperatures

Cadmium conc. in seawater	Seawater tempera- ture	Tissue of <i>M. trossulus</i> analysed	Rate of accumulation a [$\mu\text{g g}^{-1}$ (d.w.) day^{-1}]	
			HS = 0.0 mg dm^{-3}	HS = 7.2 mg dm^{-3}
50 $\mu\text{g dm}^{-3}$	15°C	hepatopancreas	29.01 \pm 0.91	39.81 \pm 1.11
		gills	11.77 \pm 0.66	17.96 \pm 0.62
		mantle and muscles	6.01 \pm 0.15	10.11 \pm 0.34
		total	10.76 \pm 0.32	20.06 \pm 0.88
	6°C	hepatopancreas	22.62 \pm 2.37	24.70 \pm 2.04
		gills	23.00 \pm 2.04	24.69 \pm 2.18
		mantle and muscles	12.57 \pm 1.18	14.52 \pm 0.76
		total	16.96 \pm 1.30	18.49 \pm 1.07

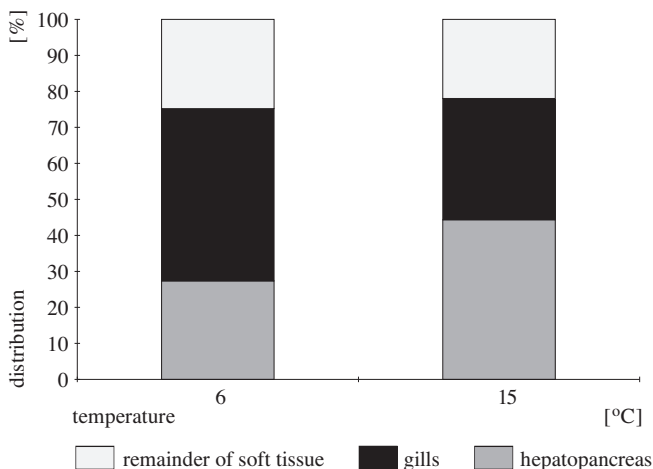


Fig. 1. Average percentage distributions of cadmium in organs of *M. trossulus* exposed for 20 days to cadmium in seawater ($\text{Cd} = 50 \mu\text{g dm}^{-3}$; $\text{HS} = 2.1 \text{ mg dm}^{-3}$) at two different temperatures

confirm the stimulatory influence of marine humic substances on Cd accumulation by mussels. Cd accumulation rates in mussels grown in water containing humic substances ($\text{HS} = 2.1 \text{ mg dm}^{-3}$ and $\text{HS} = 7.2 \text{ mg dm}^{-3}$) clearly exceed those in mussels grown in water depleted of these substances ($\text{HS} = 0.0 \text{ mg dm}^{-3}$), regardless of the experimental temperature of the seawater. These data also indicate that the accumulation rate of cadmium depends on the concentration of humic substances in the water. In our earlier study the accumulation rate were found to increase more rapidly in the lower concentration range of humic substances (Kożuch and Pempkowiak, 1992).

The rates listed in Tab. 1 are substantial, thus confirming the field data indicating the preference of *Mytilus trossulus* for Cd as expressed by the highest coefficients of Cd accumulation from seawater among a variety of zoobenthic species (Pempkowiak and Szefer, 1992). Because of their selective accumulation of Cd, mussels of the genus *Mytilus* are believed to be good indicators of metal pollution in the marine environment (Gosling, 1992). However, the concentrations of metals in seawater vary over a broad range. A depuration experiment was therefore performed to evaluate whether and to what extent mussels contaminated with Cd can 'purify' themselves from the metal when transferred to aquaria containing seawater.

Fig. 2 shows changes in the Cd concentration in the whole organism, which had accumulated substantial quantities of Cd and was transferred to natural seawater. The depuration experiment lasted 60 days. Cd concentrations in the mussel soft tissue and shells were assessed at the beginning, and after 16, 39 and 60 days. The data in Fig. 2 indicate that Cd loss

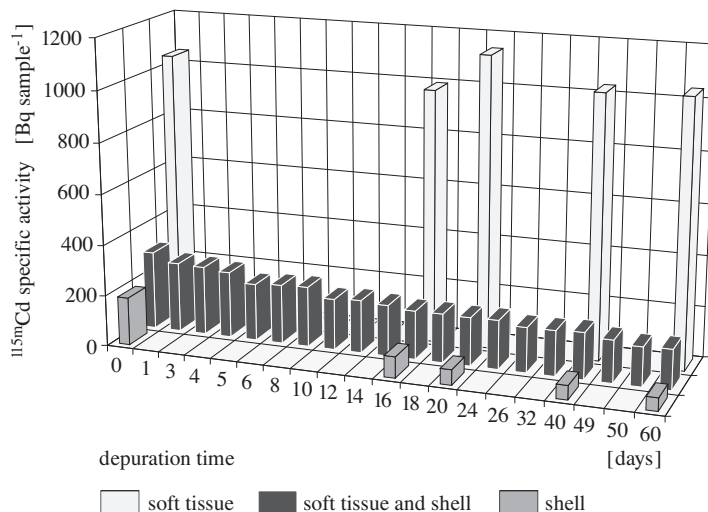


Fig. 2. Changes in specific activity of cadmium in the soft tissue and shell of *M. trossulus* ($Cd_{M.t.}$) in the course of depuration; prior to depuration, the mussels had been contaminated with cadmium during an 8-day-long exposure to radiocadmium ^{115m}Cd (9 kBq dm^{-3}) in seawater

from mussel soft tissue is a prolonged process. This is in agreement with the results of experiments aimed at removing copper and cadmium from the Pacific oyster *Crassostrea gigas*, which indicate that about 2 years are necessary for the organisms to lose previously accumulated metals (Ikuta and Morikawa, 1991). The noticeable decrease in Cd during the first week of clean-water treatment is caused by the rapid decrease in the Cd adsorbed on the shell. Cd sorbed onto the shell is lost quickly and efficiently after moving to a Cd-free environment, whereas Cd accumulated in the soft tissue of mussels is lost slowly. The time needed for the Cd concentration in *Mytilus trossulus* to decrease by half is estimated to be between 150 and 180 days, which is of the same order of magnitude as that in oysters and shellfish (Ikuta and Morikawa, 1991).

Humic substances, which effectively stimulate Cd accumulation by *Mytilus*, have no influence on Cd loss, as can be concluded from the results of a 56-day-long Cd depuration experiment (Fig. 3). Mussels were grown in seawater depleted of humic substances or enriched with them ($HS = 7.2 \text{ mg dm}^{-3}$). Prior to the experiment they were contaminated with Cd during an 8-day-long exposure to the metal in seawater at a concentration of $50 \mu\text{g Cd dm}^{-3}$. As can be seen from Fig. 3, no statistically significant differences between Cd concentrations in the hepatopancreas (likewise in the gills and mantle) were found in mussels grown in water differing in the concentration of humic substances.

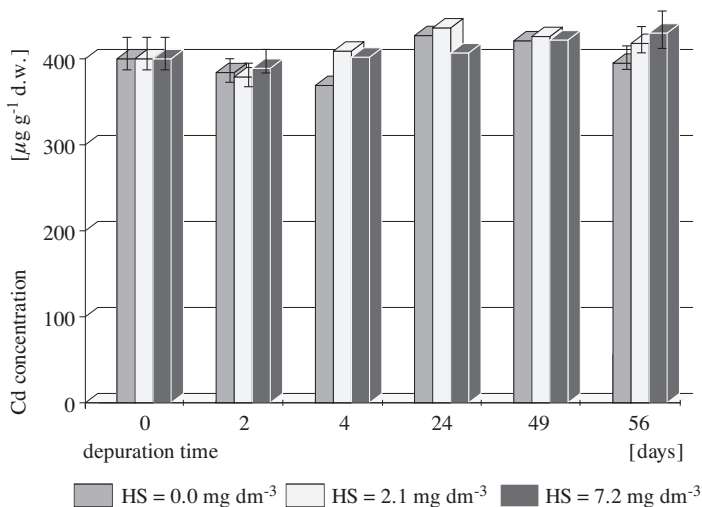


Fig. 3. The relationship of cadmium concentration in *M. trossulus* ($\text{Cd}_{M.t.}$) hepatopancreas to depuration time in seawater containing different concentrations of humic substances (HS); prior to depuration, the mussels had been contaminated with cadmium during an 8-day-long exposure to Cd ($50 \mu\text{g dm}^{-3}$) in seawater

4. Conclusions

Humic substances from the Baltic Sea are an important environmental factor stimulating Cd accumulation by the mussel *Mytilus trossulus*. The Cd taken up is then transferred to and stored in various organs of the mussels at a rate depending on the seawater temperature. At 15°C it is stored predominantly in the hepatopancreas, while at 6°C the metal is accumulated mainly in the gills.

Depuration of the mussels from Cd is a two-step process. The half-life of the metal in the soft tissue is some six months, while that in the shell is just three days. Humic substances have no influence on the rate of the soft tissue depuration from Cd.

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