Bioavailability of heavy metals for the deposit feeder *Macoma balthica* with special emphasis on copper

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Proefschrift
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The investigations described in this thesis were carried out at the Centre for Estuarine and Coastal Ecology, Netherlands Institute of Ecology, Yerseke, The Netherlands.

The work presented in Chapters 2, 3 and 4 of this thesis was carried out in cooperation with the Department of Radiochemistry, Interfaculty Reactor Institute, Technical University, Delft.

The experiments described in Chapters 5 and 6 have been carried out at the field station Jacobahaven of the Tidal Water Division, Ministry of Transport and Public Works.

Stellingen

behorende bij het proefschrift "Bioavailability of heavy metals for the deposit feeder *Macoma balthica*, with special emphasis on copper".

1. De bepaling van de koper complexerende capaciteit van water is essentiëel om uitspraken te kunnen doen over dosis-effect relaties van koper.
-Dit proefschrift

2. De incidenteel hoge koperconcentraties in het weefsel van nonnetjes uit anoxische sedimenten, zoals in de Oosterschelde (Stroodorperpolder) en Groot-Brittannië worden niet veroorzaakt door eigenschappen van het sediment.
-Luoma S.N. en Bryan G.W. (1982). Est. Coast. Shelf Sci. 15, 95-108.
-Dit proefschrift

3. In tegenstelling tot wat aangenomen wordt voor mossels, is voor nonnetjes voedsel een belangrijke bron van metaalopname. Deze tegenstelling kan verklaard worden door het verschil in ventilatiesnelheid.
-Riisgård, H.U. Bjørnestad, E. en Møhlenberg, F. (1987). Mar. biol. 96, 349-353.
-Meyhöfer E. (1985). Mar. Biol. 85, 137-142.
-Dit proefschrift.

4. De groei van nonnetjes in de Westerschelde kan alleen verklaard worden als detritus mede als voedselbron wordt beschouwd.
-Dit proefschrift.

5. De ecotoxicologie wordt nog te veel vanuit humaan gezichtspunt beschouwd. De geringe belangstelling voor koper in vergelijking met cadmium getuigt hiervan.

6a. Een causaal verband tussen de fosfaattoename en toegenomen vangsten in de Noordzee tussen 1950 en 1985 hoeft niet te impliceren dat de recente fosfaatdaling in de Noordzee de oorzaak is van verminderde vangsten.
-Boddeke, R. en Hagel, P. (1991). Counc. Meet. of the Int. Counc. for the Exploration of the Sea, La Rochelle 1991 (ICES Copenhagen).

6b. Het ongelijk van R. Boddeke is nog niet bewezen.

7. Edward Goldberg gaat er ten onrechte van uit dat alle metalen die geassociëerd zijn met organische groepen makkelijker worden opgenomen door hun lipofiele eigenschappen.
Goldberg, E. (1992). Mar. Poll. Bull. 25, 45-48.

8. Indien op individueel niveau bij een organisme met (moleculair) biologische technieken een effect van een stof is aangetoond, is het niet nodig te wachten op effecten op populatieniveau, alvorens maatregelen te nemen.

9. Spanjaarden zullen hun recept voor paella moeten aanpassen om instandhouding van de schelpdierpopulaties in de Nederlandse kustwateren te waarborgen.

10. Het is uiterst teleurstellend dat de op handen zijnde Europese richtlijn op het gebied van het Bestrijdingsmiddelenbeleid de per 1 oktober van kracht zijnde *alternatieventoets* te niet doet.

11. Met het oog op het toenemend accent op de presentatie van posters bij wetenschappelijke congressen zou een cursusje posterontwerpen een basisonderdeel van onderzoeksscholen moeten vormen.

12. Om vogels te verdrijven uit het land van een boer is een *verjager* een beter alternatief dan een *jager*.

Voor mijn ouders
en Arnoud

Voorwoord

Dit proefschrift is tot stand kunnen komen dank zij diverse instanties en zeer veel personen. In de eerste plaats richt ik een woord van dank aan Prof. Dr. J.H. Koeman voor zijn belangstelling en het door hem in mij gestelde vertrouwen. Voorts heb ik veel te danken aan de werkgroep estuariene ecofysiologie, waar iedereen mij met raad en daad heeft bijgestaan. Met name dank ik Herman Hummel, door wie ik in de gelegenheid werd gesteld aan dit onderzoek te gaan werken en die steeds bereid was commentaar op mijn manuscripten te geven. Jan Rijstenbil, ook bedankt voor je commentaar en voor je algen-inbreng. Loes Gerringa, jij kwam pas in zicht toen het onderzoek al enige gestalte had gekregen. Toch is je steun voor mij onmisbaar geweest. Door de gesprekken die we hadden en de goede samenwerking (ecotoxicologen kunnen wat koper betreft echt niet om een goede chemicus heen) kon het onderzoek zijn definitieve vorm krijgen. Ab de Zwaan, helaas slechts korte tijd mijn kamergenoot, maar het was toch heel gezellig. Lein de Wolf en Roelof Bogaards stonden altijd klaar als ik hulp in het veld nodig had.
Op deze plaats wil ik ook alle studenten, stagiaires en overige tijdelijke lieden op het CEMO bedanken voor het helpen met nonnetjes verzamelen. In een tijd van studenten-schaarste (hartje zomer) waren de medewerkers van de vakgroep Toxicologie zeer bereid om mij mee te helpen in de Oosterschelde. Dank hiervoor!
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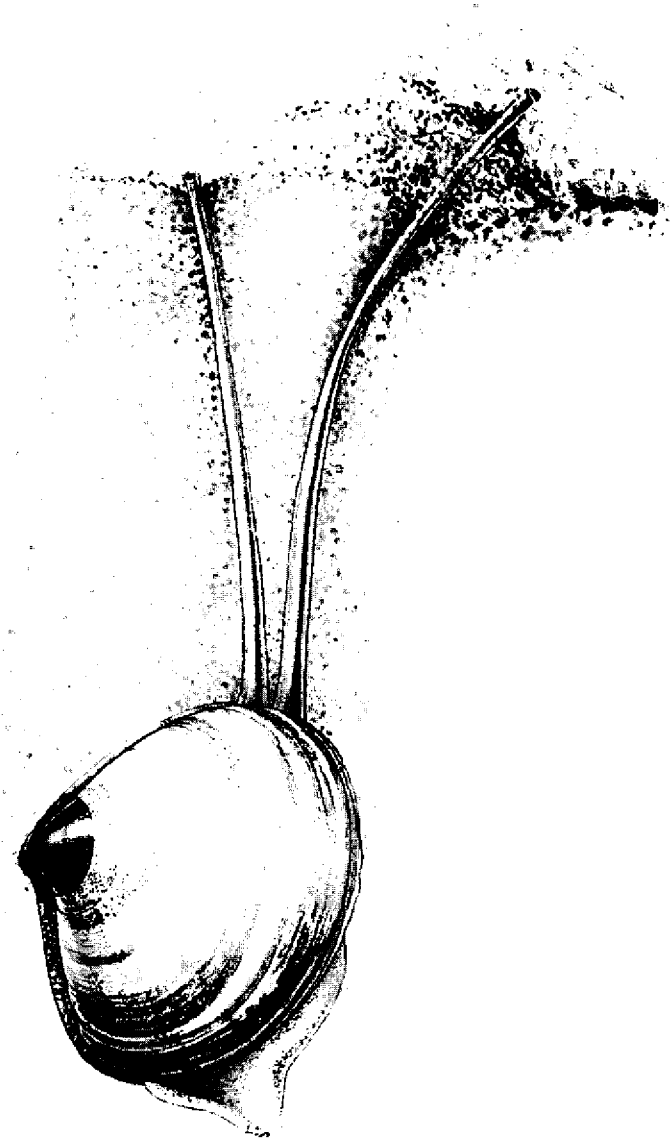
Omdat een 'mesocosmos' alleen nog in de plannen op het CEMO bestond, heb ik dankbaar gebruik kunnen maken van de faciliteiten van de Dienst Getijdewateren op Jacobahaven. In de eerste plaats wil ik André Hannewijk, maar ook Peter van den Hurk, Richard Eertman,

Wies Vonck, Fredt Twisk en Aad Smaal bedanken voor hun meewerken aan het welslagen van mijn experimenten. Vicky Wightman wil ik hartelijk bedanken voor de engelse correctie van een groot gedeelte van dit proefschrift.

Voor velen is het schrijven van een proefschrift een zware en eenzame klus. Dat het een zware klus was zal ik niet ontkennen. Eenzaam was het in elk geval niet. Omdat Arnoud aan eenzelfde klus bezig was is het schrijven (voor ons althans) een erg gezellige en plezierige periode geweest. Helaas heb ik mijn familie en vrienden wat minder aandacht kunnen geven. Last but not least wil ik hier ook Cato ten Hallers bedanken. Cato, je was ooit slechts 'extern' begeleider bij het Maleisië project, toch ben ik met name door jou enthousiast geworden voor ecotoxicologisch onderzoek in de zee en ziehier het resultaat.

Contents



The present study has been carried out in the framework of the research of the group Estuarine Ecophysiology at the Netherlands Institute of Ecology. The aim of the group is to study the relation between the variation in environmental factors (with emphasis on pollution) and the variation in ecophysiology in organisms in estuarine and coastal areas.

Chapter one

Introduction

The large scale pollution in estuarine and coastal areas has been a matter of concern for years. These areas have high biological productivity rates and function as important breeding and nursery areas for juvenile stages of numerous marine species, which also explains why coastal regions have a high value in economic terms as well. Therefore it is understandable that the possible negative effects caused by contaminants are cause for concern.

The concentrations of organic micropollutants and heavy metals in coastal regions are often elevated by the loads brought in by rivers. Moreover, traditionally many industries are located in coastal areas because of harbour facilities and possibilities of waste discharge. A lot of contaminants tend to concentrate in the sediments as a result of geochemical and physical processes in the estuarine turbidity zone that cause a sinking of particles and associated contaminants. Consequently, concentrations of contaminants in the sediment in coastal and estuarine areas are often relatively high compared to the waterphase and thus form a serious threat to animals associated with the sediment.

In order to predict the risks of certain pollutants on the biota it is essential to have information on concentrations as well as susceptibility of the organisms. For the purpose of this risk assessment, several approaches have been followed to set standard criteria for sediment quality (Chapman, 1989). One promising method is the equilibrium partitioning theory (Shea, 1988), where toxicity is correlated with the free concentrations of contaminants in the interstitial water. The main disadvantage of this approach is that it is **not** valid for metals (Chapman, 1989).

Another method to assess sediment quality is the application of short term laboratory bioassays, for example the frequently used assessment of mortality with amphipod species (Long and Chapman, 1985; Chapman, 1989). These tests enable the effects of contaminant mixtures to be assessed. The approach assumes that sediment bioassays performed in the laboratory provide a true measure of in situ biological effects. For metals in particular, this assumption cannot be maintained. Metal availability in the laboratory will be different from the field situation as a result of alteration of the chemical speciation while transporting sediments. For example, oxidation and breakdown of organic matter cause metals like copper and cadmium to leach from the sediment (Gerringa, 1990, 1991). Moreover, oxygen gradients are very difficult to copy in a laboratory situation.

Another disadvantage of standardized bioassays is that indigenous species are likely to react in a different way on contaminated sediments than the standard test species. Other

disadvantages of these tests are that they do not reflect chronic effects. The tests therefore give no information about *in situ* sediment toxicity.

To assess the risks under field conditions, in principle one would like to know the *in situ* biological availability of toxicants from different compartments of the environment. As contaminants can be accumulated through several pathways (sediment, water and food), all these compartments should then be measured regularly, a rather time consuming and expensive option. By using a biological monitor species, the possible exposure pathways are integrated both in time and space. Assessing the accumulation in species that are exposed to the environment (actively or passively) should give an indication of the potential harmfulness of an environmental situation. With heavy metals, uptake is proportional to concentration in invertebrates, which is why they have been suggested and used as environmental monitors. For monitoring of the waterphase, bivalves are considered particularly suitable (Phillips, 1977; Phillips and Segar, 1986). This idea has been worked out in for instance the Mussel Watch Program (Goldberg *et al.*, 1978) where mussels in cages are exposed to different waters for several weeks.

For sediment monitoring, the benthic deposit feeding bivalve *Macoma balthica* is often suggested as an alternative to the mussel *Mytilus edulis*. Several arguments are given for this: deposit and detritus feeders are critical components of the decomposer food web. It is widespread in temperate and coastal regions, probably because of its higher tolerance for lower salinities. It is useful in soft bottom sediments, where there is no suitable substrate for *Mytilus* (Broman and Ganning, 1986) and it responds adequately in short term testing of bioavailability of copper, lead, zinc and cadmium. Moreover, it can be held easily and is easy to collect as it often occurs in high densities (Cain and Luoma, 1985).

A problem with sediment monitoring is that many sediment associated species do not accumulate metals relative to the concentrations in their environment. So far, no consistent relations were found between metals in sediments and in sediment dwelling bivalves (Luoma and Bryan, 1982; Bryan, 1985). Besides the sediment, metals can be accumulated from the overlying or interstitial water and through food. There is still very little known about the relative importance of these uptake rates of metals for *Macoma balthica*. Therefore it will be difficult to interpret field data of accumulated metal levels.

In this thesis, a contribution is made to the understanding of the biological availability of metals from the various uptake routes to *Macoma balthica*. With the experiments, emphasis has been laid on copper.

STUDY AREA: THE OOSTERSCHELDE AND WESTERSCHELDE ESTUARIES

The bioavailability of metals in the field is studied in the Dutch Delta area (SW Netherlands - see Figure 1.1). The rivers Meuse, Rhine and Scheldt used to have a major impact on this estuarine area, but due to major engineering works aimed at protecting the area from flooding, the tidal characteristics of the estuary have been modified. In the northern part, the ecosystem has been changed drastically by the formation of stagnant brackish- and freshwater lakes. Because the Oosterschelde estuary is very important for nature conservation and shellfish culture, it was desirable that the saline tidal character be maintained. Therefore, it is now partially closed off from the North Sea by a storm sturge barrier, which will only be closed under severe storm flood conditions. Riverine input in the Oosterschelde estuary has been largely reduced, so it now has almost a marine character.

In the Delta area, the estuary of the river Scheldt (called the Westerschelde estuary) is the only one left with a true estuarine character. Pollution brought in by the river Scheldt (freshwater input is on average 100 $m^3 s^{-1}$) forms a serious threat to the ecosystem of this estuary. The water is polluted by mostly untreated industrial and municipal waste water from areas such as Brussels and Antwerp. Together with the Rhine and Meuse, the Scheldt is a major source of contamination for the North Sea. In biota of the Westerschelde estuary elevated levels of many contaminants have been found (for a review, see van Eck *et al.*, 1991).

Although the loadings of heavy metals into the Westerschelde have decreased since the early 1980s, dissolved copper levels in the estuary are still high: between 10.7 and 152 nM, (Van Den Berg *et al.*, 1987) which is well within the range that can cause effects in sensitive organisms. In the sediments copper levels vary from 1-207 mg kg^{-1} (Van Eck *et al.*, 1991).

STUDY ORGANISM: *MACOMA BALTHICA*

Macoma balthica is a very common sediment dwelling bivalve. It is found from the Northern latitudes down to France, and on the Atlantic and Pacific coasts of America. The bivalve prefers silty sediments. In Dutch intertidal mudflats, it can be found in the sediment at an average depth of 2 cm in summer, and at a depth of about 5 cm in winter (Zwarts and Wanink, 1989). Through an inhalant and exhalant siphon it feeds on deposited material as well as on suspended particles. It is regarded as a facultative deposit feeder, which means that it can switch between its feeding habits, depending on food availability (Hummel, 1985). *M.*

balthica feeds on benthic and pelagic diatoms, as well as smaller particles like bacteria. The maximum size of the clam is about 18 mm at an age of 7 years. After a pelagic larval stage, the bivalve will settle in the sediment. It is presumed that *M. balthica* will remain at one location for the rest of its life. However, there are reports of postlarval transport, indicated as summer migrations directed to the higher parts of tidal flats, and winter migrations directed towards sublittoral areas (Sörlin, 1988; Günter, 1991). Because of its high densities, the clam plays an important role in the coastal food web: *M. balthica* is an important food source for waders, and the siphon tips are part of the diet of carnivorous bottom feeders (e.g., (De Vlas, 1979).

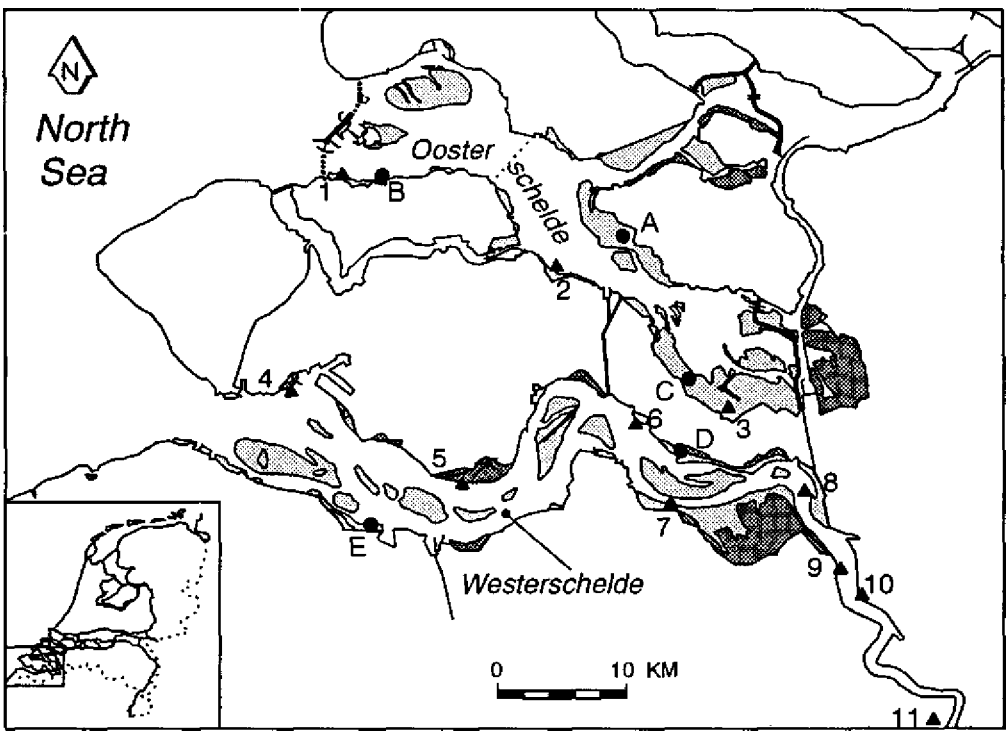


Figure 1.1 Map Delta Area. The marks point at the sampling stations of the different experiments and field work: **In Oosterschelde** *1: Jacobahaven 2: Kattendijke 3: Stroodorpepolder A: Dortsman B: Oesterput C: Krabbendijke.* **In Westerschelde** *4: Rammekes 5: Ellewoutsdijk 6: Kruiningen Veerhaven 7: Baalhoek 8: Appelzak 9: Doel 10: Lillo 11: Burcht D: Waarde E: Paulinapolder.* The shaded areas are saltmarshes (dark) and intertidal mudflats (light).

COPPER: SOURCES AND ENVIRONMENTAL RISK

Copper can enter the estuarine environment through various pathways. A large part is brought in by rivers, which contain copper from industrial discharge (mining activities, petrochemical industry) as well as by agricultural runoff. In the Netherlands, emission to surface waters from agricultural areas amounted to about 151 tonnes in 1985. Total emission has not changed considerably since 1977: it amounts to 7000 tonnes per year. However, the contribution of different sources has changed: the supply with the rivers Meuse and Rhine diminished from 1104 in 1977 to 474 tonnes in 1985. However, copper in waste products increased by almost 900 tonnes to 5800 tonnes. In 1985, of the total 258 tonnes discharged in wastewater, 11 tonnes immediately entered the coastal area (Vos, 1987).

For living organisms, copper is an essential metal. For man, daily uptake in the Netherlands is around the advised minimum (1.8 mg), so risks for human health are not to be expected. This might be one of the reasons why copper has not received much attention in risk assessment studies. Attention has been focussed on non-essential metals, e.g., cadmium or mercury, and most of all organic micropollutants like poly-aromatic hydrocarbons (PAH) and polychlorinated biphenyls (PCB), that tend to accumulate through food-chains.

In general, biomagnification is less important in the case of metals, except the methylated forms. It is suggested that higher species like birds will retain only a small portion of heavy metals ingested with their invertebrate diet (Simkiss and Taylor, 1989). In the Westerschelde estuary, cadmium, copper and zinc levels in fish and birds are lower than in sediment dwelling bivalves (Van Eck *et al.*, 1991), indicating that indeed there is no evidence of biomagnification.

Although copper shows relatively little human risk, the ecosystem effects of elevated copper levels are underestimated: for species on the bottom of the food chain like algae and invertebrates, in particular bivalves, copper is extremely toxic. It therefore might have a deleterious effect on marine productivity (Bryan, 1985).

For bivalves, copper is essential for haem-pigments. However, the range between essential and toxic copper concentrations is very narrow (about a factor of 10). Toxicity far exceeds even cadmium toxicity levels (e.g., Watling, 1981). On the other hand, environmental concentrations of copper are generally much higher than cadmium. For copper, concentrations in coastal seawater only need to be one order of magnitude higher, for ecosystem effects to be seen. For cadmium and zinc this factor is 100 and for lead a 1000-fold increase would be needed (Bryan, 1985). The risk of copper to the ecosystem is therefore much more obvious

than for cadmium.

COPPER AVAILABILITY FOR *MACOMA BALTHICA*

Sediment dwelling bivalves are exposed in different ways to copper: copper bount to sediment, copper in the interstitial and overlying water, and copper associated with food particles (Figure 1.2). The relative contribution of the different pathways to uptake by *M. balthica* depends on the partitioning of copper between the various particulate and dissolved metal species: the form in which the metal is present.

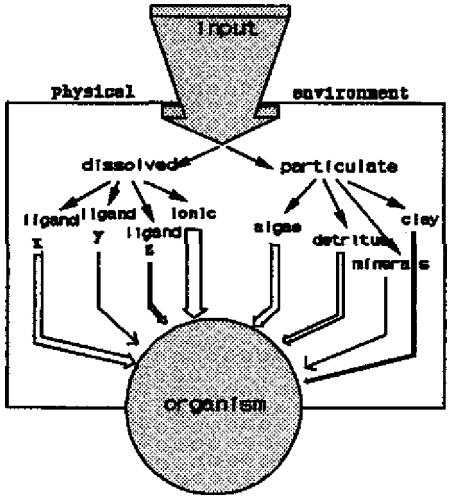


Figure 1.2 Major metal pathways and processes that affect the availability of Cu to aquatic organisms (adapted from Luoma, 1983).

Sediment

Copper concentrations in estuarine sediments are often five orders of magnitude higher than in the overlying water. However, a large part of this copper is firmly associated with sediment compounds like iron oxides, sulphides and organic material. Consequently, it will not be available for uptake. Deposit feeding bivalves like *M. balthica* do filter sediment granules so it might very well be that a certain part of the metals that are associated with the sediment are taken up. Several studies suggest that interstitial water rather than ingested solids is the dominant uptake route of pollutants and the principal source of toxicity for infaunal organisms. However, measurements with dyed water suggested that not more than 4% of the total amount of water ventilated by a clam, was from interstitial origin (Specht and Lee, 1989).

Food

Another source of uptake can be the food particles that are ingested. *M. balthica* mainly feeds on benthic and pelagic diatoms, bacteria and detritus. As this material can be in close contact with high sediment metal concentrations, food metal levels might be high. Unfortunately, data on metal contents in pelagic diatoms are scarce and data on metals in benthic diatoms are not yet available, as far as I know. In general for filter feeding bivalves,

food is considered as a minor source of heavy metal uptake, compared with uptake of dissolved metals from the water (Janssen and Scholz, 1979; Borchardt, 1985; Riisgård *et al.*, 1987; Ettajani *et al.*, 1991). The deposit feeder *Macoma balthica* however has pumping rates that are far inferior to those of suspension feeders and average about 10% of the latter (Hughes, 1969). It is very well possible that for deposit feeding bivalves, metals associated with food particles contribute relatively more to the overall uptake.

Water

If uptake from the waterphase is considered, it is remarkable that with dose-effect studies, limited attention has been paid to the speciation of copper in the dissolved phase. Copper toxicity is generally represented as (total) Cu in $\mu g\ l^{-1}$. However, it is recognized that the free cupric ion (Cu^{2+}) is the most readily available and toxic inorganic species of Cu (Crecelius *et al.*, 1982; Zamuda and Sunda, 1982). This free ion accounts for only a small portion of the total dissolved copper in seawater. The cupric ion can be complexed easily with natural (e.g. humic acids) or artificial (EDTA, NTA) organic ligands, thereby reducing toxicity. Other, more lipophilic organic ligands tend to increase the toxicity of copper: these compounds can easily penetrate the cell membrane (Florence, 1983; Blust *et al.*, 1986; Roesijadi, 1992).

From the above it may be obvious that the operationally defined quantities "dissolved" and "total" metal are of limited use, as in the dissolved (supposedly available) fraction, copper is partitioning between many dissolved organic carbon species that are more or less labile (and therefore differ in biological availability). So far, few field studies have been carried out in which the different chemical species are related to copper accumulation.

COPPER TOXICITY

Because toxicity of a metal is mostly related to the total (nominal) concentration the metal, neglecting the importance of metal speciation, correct information on metal toxicity is hardly available.

Concentrations at which toxic effects are found for bivalve molluscs vary considerably. The differences are to be attributed to variation in metal speciation (see above), but also to inter-species differences and adaptation.

At a concentration of 20 $\mu g.l^{-1}$ (320 nM) above the background copper level, physiological and behavioural responses were observed in *Mytilus edulis* (Manley, 1983). Ventilation rate was affected at 6-7 $\mu g.l^{-1}$ Cu. Valve closure was detected at 10 $\mu g.l^{-1}$ for *Scrobicularia plana*. Lethal concentrations vary depending on life stage and exposure time.

Copper toxicity for *Macoma balthica* is rather variable according to literature data (see also Table 1.1 and 1.2): LC_{50} for 10 days was reported to vary between 210 and 1290 $\mu g.l^{-1}$, depending on the orginal locality of the clams. This indicates that intraspecific tolerance can differ considerably (Luoma *et al.*, 1983). Other reports of LC_{50} between 25 and 70 hours gave concentrations of 5-25 $mg.l^{-1}$ Cu (McLeese and Ray, 1986). As solubility of $CuCl_2$ in seawater decreases above 1 $mg.l^{-1}$, these data are of limited value. The tissue copper levels at which *Macoma balthica* dies vary roughly between 50-150 $\mu g.g^{-1}$, where the higher copper tissue levels are found in clams that were exposed for a prolonged time to low concentrations (personal observations). In Pacific populations (San Francisco Bay) however, tissue copper levels between 50 and 800 $\mu g.g^{-1}$ are common in healthy individuals (Luoma *et al.*, 1985). This can be attributed to interpopulation or maybe even interspecies differences in sensitivity. Based on genetic comparison, it was suggested that populations in the eastern and western North Atlantic were separate species (Meehan, 1985). Considering the geographical isolation, it is very well imaginable that Pacific populations are a different species as well. This hypothesis is supported by the differences in size of the clams: maximum shell lengths of 30 mm with soft tissue dry weight of more than 400 mg are reported for San Fransisco Bay (Cain and Luoma, 1986), while shell length in eastern Atlantic populations generally does not exceed 18 mm, with a dry weight up to 80 mg. In view of possible genetic differences, it should be remarked that in *Macoma balthica* collected from San Fransisco Bay a metal detoxifying mechanism (metallothionein-like proteins) has been detected (Johansson *et al.*, 1986) whereas this was not found in eastern Atlantic populations (Langston and Mingjiang Zhou, 1987).

INFLUENCE OF ENVIRONMENTAL FACTORS ON AVAILABILITY AND TOXICITY

Copper uptake and toxicity is often largely influenced by local environmental conditions. Generally at reduced salinity, toxicity will be increased because competition from Ca and Mg for uptake sites is reduced (Wright and Zamuda, 1987). On the other hand, with an increase in salinity the calcium and magnesium ions will compete with copper for the available binding locations in organic ligands (Mantoura *et al.*, 1978; Langston and Bryan, 1984). An increase in salinity would decrease the available binding sites for copper, with an increased concentration of free ions as a result.

About the influence of temperature, no information for *Macoma balthica* is available. The clam *Mya arenaria* accumulated copper more rapidly at summer temperatures than at winter temperatures, whereas with the oyster *Crassostrea virginica*, copper toxicity was not significantly influenced by temperature (McLusky *et al.*, 1986).

Table 1.1 Summary of dissolved copper toxicity data for various deposit feeding bivalves

Species	Toxicity unit		copper nM	copper ug/l	salinity (g/l)	comments	Reference
Macoma balthica	LC50(h)	144	95240	6000		Exceeding solubility!	McLeese & Ray 1986
M. balthica	body burden 10 (umol/g WW)	68	0	0	5	held in sediment single administration	Kaitala 1988
M. balthica	body burden 10 (umol/g WW)	333	2220	140	5	(sed+water mixed)	
	LC50(d)	10	857	54	5		
M. balthica	100% reduction burrowing activity		31746	2000	6	held in sediment	Eldon et al. 1980
	siphon damage		7940	500			
M. calcarea	LT50(d)	37	476	30	15	semi-static	Neuhoff & Theede 1984
M. balthica	LT50(d)	62	476	30			
						held in sediment:	Marquenie 1985
M. balthica	body burden 10	937	317	20		gravel	
Scrobic. plana	(umol/g ASDW)	-	317	20			
M. balthica		825	317	20		0.1% < 16 mm	
S. plana		3318	317	20			
M. balthica		1667	317	20		8% < 16 mm	
S. plana		1191	317	20			
M. balthica		667	317	20		41% < 16 mm	
S. plana		825	317	20			
M. balthica		444	0	0		41% < 16 mm	
S. plana		667	0	0			
M. balthica	LC50 (d)	10	3333	210	25	Clams from different	Luoma et al. 1983
M. balthica			13968	880		populations	
M. balthica			10794	680		held in sediment	
M. balthica			20475	1260			

Species	toxicity unit		copper nM/g DW	ug/gDW	comments	Reference
M. balthica	body burden 30d (nM/g DW)	251	594	37.4	5.2% organic matter	Ray 1981
M. balthica	body burden 90d (nM/g DW)	240	160	10	2.2% organic matter Waddensea sand	Jenner & Bowmer 1990
		440	1778	112	5.1% organic matter Harbour sludge	
		470	1746	110	2.8% organic matter 100% Pulverized Fuel Ash	
Protothaca staminea	ET50(h) burrowing	15.8-20.4	514	33	sediment enriched with Copper	Phelps 1983
P. Staminea	ET50(h) burrowing	20.3-59.1	478	30	sediment enriched	Phelps 1985
P. Staminea	mortality 48 days	15-25%	478	30	with copper	

Table 1.2 Summary of data on sediment toxicity of copper for two sediment dwelling bivalves.

For benthic organisms, several processes that are related with pH change, might influence copper availability. In field situations with sediments in the reduced state, pH will decrease at the sediment-water interface (microlayer) as a result of oxidation of sulphides. In experimental situations, decreased oxygen pressure causes an increased respiration of organisms, leading to an increase of the CO_2 concentration and, in turn, shifts the balance of the bicarbonate system to a more acidic pH. This explains the increased copper uptake by *M. balthica* at decreased oxygen pressure (Neuhoff, 1983). With decreasing pH the concentration of copper in the free ion form will increase as metal hydroxides, oxides or carbonates will dissolve (Cairns *et al.*, 1984). Also sorption to particulates and complexation with (weak) organic ligands will decrease as a result of proton competition with the binding places.

However, decreasing pH does not necessarily result in increased Cu uptake. With a decreasing pH in chemically defined solutions, the uptake is decreased, in spite of the increase of the free ion form (Blust *et al.*, 1991; Roesijadi, 1992). This phenomena is explained by the increase of the concentration of hydrated metal ions with decreasing pH. At the same time, the concentration of protons which compete with the metals for the carrier molecules in the lipid phase is increased.

METAL ACCUMULATION IN THE FIELD

A country where heavy metal accumulation in estuaries has been studied very thoroughly in the past decade, is Britain (Bryan *et al.*, 1980; Luoma and Bryan, 1981, 1982; Bryan and Gibbs, 1983; Bryan and Langston, 1992). In several estuaries, very high copper and zinc concentrations in the sediment can be found because of past mining activities. However, concentration factors in *Macoma balthica* are generally lower than 1. Although sediment metal levels can be very high, it has been almost impossible to attribute deleterious effects on benthic organisms to *specific* metallic pollutants. In estuaries contaminated with metal-mining wastes, an effect of copper and zinc on species distribution was observed, but it was less obvious than would be expected from experimental data. In Restronguet Creek, the part of the Fal estuary most polluted by copper and zinc, bivalves including the cockle *Cerastoderma edule*, the clam *Macoma balthica* and mussels *Mytilus edulis* were absent. The toxicity of surface sediments containing over 2000 µg g^{-1} of Cu towards juvenile bivalves appears to be the reason (Bryan and Gibbs, 1983).

It was not possible to establish consistent relationships between copper concentrations in the sediment and those in *Scrobicularia plana* and *Macoma balthica* over a wide range of different estuaries. One reason for this are high concentrations of Cu (exceeding 1000 µg g^{-1} DW in *M. balthica*) that were found at relatively uncontaminated sites with anoxic sediments (Luoma and Bryan, 1982). This phenomena has also been observed in the Delta area, although less pronounced: in a mudflat with low sediment copper concentrations (2 mg kg^{-1}, 8% <16 µm and 0.45% organic carbon) in the relatively unpolluted Oosterschelde sea arm, elevated tissue copper levels were found: where the average in the Oosterschelde contained 20 µg g^{-1} DW, in Stroodorpepolder this was more than 40 µg g^{-1} DW (Goossens, 1989). As in the British case, clam shells were blackish in colour as a result of the reduced condition in the sediment. This unexplained phenomena indicates that the redox situation in the sediment could be influencing copper availability for sediment dwelling deposit-feeders.

Another well studied area is the San Fransisco Bay in California.
Concentration factors as high as 5.0 are observed in these populations (Luoma *et al.*, 1985). Tissue concentrations vary from around 50 to more than 400 µg g^{-1} DW, with peak concentrations of more than 1000 µg g^{-1} DW (Thomson *et al.*, 1984). Large fluctuations are observed between stations and years: concentrations could fluctuate up to tenfold at certain stations and as little as two- or threefold in other years (Luoma *et al.*, 1985).

On a mudflat in British Columbia (Canada), *M. balthica* living in sediments with 39.6 µg Cu g^{-1} sed DW contained 314 µg g^{-1} tissue DW copper. Copper bioconcentration factors

ranged from 1.95 to 7.98. In sediments with copper concentrations higher than around 70 µg g^{-1} sed DW (median particle size < 63µm), clams were absent. Heavy metals were considered to be the most controlling factor which affected the settling and survival of larval and juvenile clams. Salinity, substrate grain size and dissolved oxygen did not satisfactorily explain the distribution of *M. balthica* (McGreer, 1982).

In the southern Baltic, copper tissue levels are reported to vary between 26 and 130 µg g^{-1}. Compared with mussels, zinc and copper accumulated strongly in *Macoma balthica*, while cadmium accumulated more efficiently in *Mytilus edulis* (Szefer and Szefer, 1990).

In the Wadden sea and Dutch Delta area, copper levels in clams from relatively unpolluted sites vary between 15 and 30 µg g^{-1} DW. Tissue copper levels in animals from the relatively clean Oosterschelde estuary generally equal those from animals from the more polluted Westerschelde estuary. This is remarkable because dissolved copper concentrations in the Westerschelde are higher than in the Oosterschelde. An explanation can possibly be found in the concentration and character of copper complexing ligands in both waters.

From very polluted areas in the Netherlands, no information is available because clams are mostly absent due to anoxic situations in the water, or a low salinity.

The large variation in concentration factors and tissue levels again indicates that the susceptibility of *Macoma balthica* to copper in the different estuarine areas is extremely variable.

OBJECTIVES AND APPROACH OF THE PRESENT STUDY

The aim of the research was to assess the contribution of the major uptake routes of trace metals to the body burdens in *Macoma balthica*. These routes include the sediment, the overlying water and food. The importance of these pathways was assessed using a multi-level approach: short-term laboratory experiments with the radiotracer ^{64}Cu (Chapters Two to Four); flow-through systems for long term accumulation studies (Chapters Five and Six), and a monitoring program in the field, for assessment of the actual situation (Chapters Seven and Eight). In the uptake experiments, emphasis has been laid on the study of copper. In all experiments, environmentally realistic concentrations of metals were used.

In Chapter Two, a method for the effective separation of different metal uptake pathways is described. For this method, the radiotracer ^{64}Cu is used in combination with the chelating agent EDTA.

In Chapter Three, the availability of copper from phytoplankton and water is studied, based on the method described in Chapter two.

The influence of salinity and organic ligand content of natural waters on copper uptake is described in Chapter Four. The use of the radiotracer ^{64}Cu in these experiments was essential because due to the lability of the natural ligands, uptake experiments had to be performed within a short time period.

Accumulation and effects of copper, cadmium and zinc from spiked unaged and aged sediments in a long term study is described in Chapter Five. The aged sediments with an oxygen gradient with depth give a more realistic representation of the field situation than do freshly spiked, oxidized sediments.

The results from long term kinetic experiments under ambient conditions with continuous copper administration through water or food are given in Chapter Six.

As it was obvious that food might play a significant role in the overall metal uptake by *Macoma balthica*, contents of copper, cadmium, zinc and lead in benthic diatoms from the Westerschelde and Oosterschelde were assessed. A description of the collection method and the results of the survey are presented in Chapter Seven.

Largely based on the results of the laboratory uptake studies, a dynamic simulation model of growth and uptake of heavy metals by *Macoma balthica* has been developed. With the concept of this model, special attention is given to the feeding behaviour in different environments. The growth submodel is calibrated with data from monitoring program, where sediment, water and *Macoma balthica* tissue metal levels from an intertidal mudflat in the Westerschelde estuary were followed for two consecutive years. The metal uptake submodel is validated with the data from the field monitoring program. The model and simulation results are presented in Chapter Eight.

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Chapter Two

Separation of ^{64}Cu uptake via water and via food by *Macoma balthica.*

ABSTRACT

To study the role of food in Cu accumulation by bivalves, algae spiked with Cu can be used. With spiked algae however, redistribution of Cu between the dissolved and the particulate phase hampers the assessment of the contribution of food. This also occurred in our efforts to label algae with the radiotracer ^{64}Cu.

A method was designed to overcome this problem of redistribution. By adding excess EDTA to the seawater, the biological availability of dissolved Cu was minimized. The effectiveness of complexation by EDTA was controlled through adsorption on *Macoma balthica* shells and uptake in *Macoma balthica* tissue.

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INTRODUCTION

The assessment of the contribution of different uptake pathways to the overall metal accumulation in aquatic organisms has been proven to be relatively difficult. Because of their feeding behaviour, they can be directly exposed to metals in the waterphase, as well as to food-associated metals. For bivalves, some information is available on Cd accumulation via food (Borchardt, 1985; Riisgård *et al.*, 1987). On Cu accumulation however, very little is known. An obvious reason for the scarcity on Cu data is the problem of assessment of the bioavailability of different Cu species: when Cu-contaminated particles are placed in clean water, a solute-solid equilibrium will be establised. The distribution of Cu between the dissolved and particulate phase depends among other things on the ligand concentration in solution (Cu complexing capacity) (Zamuda and Sunda, 1982; Gerringa *et al.*, 1991). From the dissolved species, the free ionic form is considered the best biologically available (Crecelius *et al.*, 1982; Zamuda and Sunda, 1982; Cross and Sunda, 1985). The complex chemistry of Cu makes it difficult to assess the contribution of a particular species to the overall accumulation. The objective of this study was to design a method that would make it possible to study the dissolved and particulate Cu uptake route separately. To this end, we labelled algae with the radiotracer ^{64}Cu and used ethylenediaminetetraacetate (EDTA) to minimize the biological availability of dissolved ^{64}Cu. The effectiveness of EDTA complexation was assessed in adsorption and uptake experiments with the bivalve *Macoma balthica*.

MATERIALS AND METHODS

^{64}Cu preparation and measurement

^{64}Cu was obtained by irradiating 3 mg copper wire (99.99%; Ventron, Karlsruhe) for 24 hours in the nuclear reactor of the Interfaculty Reactor Institute of the University of Technology, Delft, The Netherlands (the so-called Hoger Onderwijs Reactor (HOR); neutron flux = 10^{12}-10^{13}/cm^2/sec). Radioactivity of the wire after 24 hours was 125 MBq/mg. The irradiated wire was dissolved in 25 µl concentrated nitric acid, and diluted in 50 mM Na-acetate buffer (pH 5.6). The final Cu^{2+} concentration in the stock solution was 1 mg/ml. The isotope has a half-life of 12.8 hours. By preparing this stock solution just before starting the experiment, we were able to perform measurements for at least 4 days. The starting activity of a 30 nM Cu solution, spiked with ^{64}Cu, was about 900 cpm/ml. As a simplification, Cu solutions spiked with ^{64}Cu are further referred to as ^{64}Cu solutions.

Separation of uptake pathways

^{64}Cu accumulation in *Macoma balthica* was followed by measuring radioactivity of dissected shells and tissue, or living individuals (whole bodies). To facilitate accurate dissecting after exposure, the individuals were quickly frozen and subsequently separated into shells and tissue. Samples (water, labelled algae, or *M. balthica*) were counted with the help of a NaI detector. Counting time was maximally 10 minutes; counting error was ≤ 5%. Corrections were made for background radioactivity, ^{64}Cu decay, and shell size.

^{64}Cu uptake by phytoplankton (experiment 1).

The diatom *Phaeodactylum tricornutum* was spiked with ^{64}Cu. This Cu-tolerant diatom species is often used as food source for bivalves in experimental situations. It can continue to grow at Cu concentrations up to 8 µM. Other reports also mention a relatively high survival and growth of *P. tricornutum* under Cu stress, compared with other phytoplankton species (Bentley-Mowat and Reid, 1977). Strains from a batch culture in the late exponential phase were concentrated using a tangential flow membrane filtration system (Millipore). Further, they were resuspended in 2-ltr polyethylene beakers in 0.45 µm membrane filtered sea water (FSW, salinity 32 ‰). The water contained 0.79 or 7.9 µM ^{64}Cu. The algae were allowed to grow for 24-48 hours at room temperature under continuous tube light (Philips, 40 W, colour nr. 33). The algal culture was stirred gently with a magnetic stirrer.

After the uptake period, the ^{64}Cu-labelled algae were concentrated by tangential flow flux and additional centrifuging for 10 minutes at 2000 g. The algae were resuspended in a 5 µM EDTA solution in FSW in 50-ml centrifuge tubes to remove the loosely bound ^{64}Cu. After a rinsing period in the EDTA solution, varying between 1 and 24 hours, the algae were centrifuged and rinsed for 30 minutes in FSW. Concentrations of ^{64}Cu in the water and in the algae were measured by pipetting 5 ml suspension on a 0.45 µm filter (Nuclepore). Four ml of the filtrate was counted simultaneously with a 5 ml unfiltered sample. After volume correction of the samples, the difference between filtered and unfiltered sample, was considered to be adsorbed on the algae. A comparable method to assess radionuclide adsorption to algae was described by Fisher *et al.* (1983). A correction of 2% was made for ^{64}Cu retention by the filter from spiked water without algae. After centrifuging, the concentrated algae were resuspended in 1.5-L volumes.

These spiking experiments were carried out to study the uptake and adsorption by *P. tricornutum*. The duration of the spiking period and the intensity of rinsing were varied in order to obtain the highest possible ^{64}Cu load and as little loss as possible of ^{64}Cu from the algae during resuspension in clean water.

Complexation of ^{64}Cu by EDTA (experiment 2).

As the labelled algae were leaking ^{64}Cu when resuspended in ^{64}Cu-free FSW, this could seriously influence the outcome of uptake experiments with bivalves. By adding EDTA to the seawater, the biological availability of dissolved ^{64}Cu should be minimized. In several studies, EDTA has been shown to reduce the accumulation and toxicity of Cu, indicating that the EDTA-Cu complex is less biologically available than the free Cu (Stephenson and Taylor, 1975; Cheng, 1979).

The effectiveness of EDTA-complexation was examined by measuring adsorption processes on separate *M. balthica* shells. Adsorption to shells was used as a measure for the amount of "free" Cu. The theoretical concentrations of free and complexed Cu and EDTA were calculated with the chemical equilibrium program SOILCHEM (Sposito and Cobes, 1988).

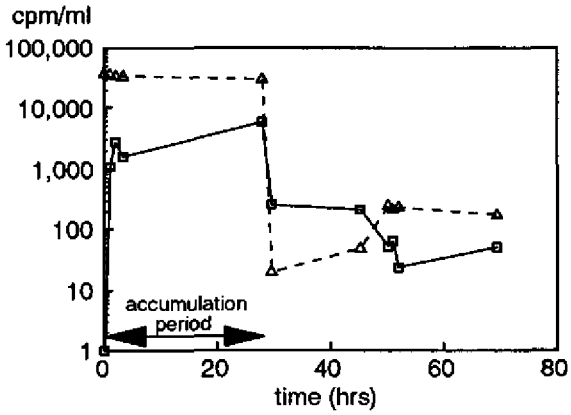


Figure 2.1a Accumulation and elimination of ^{64}Cu by *P. tricornutum*. Accumulation period was 28 hours. At t=28 hours, the algae were concentrated and resuspended in ^{64}Cu-free filtered seawater. (△): ^{64}Cu in seawater. (□): ^{64}Cu in algae.

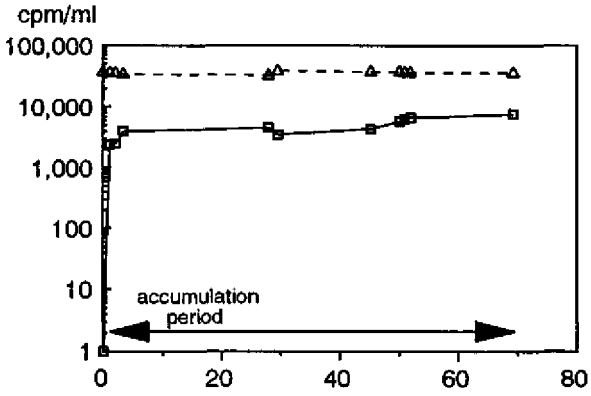


Figure 2.1b Accumulation of ^{64}Cu by *P. tricornutum*. Accumulation period was 72 hours. At t=28 hours, the algae were concentrated and resuspended in a fresh ^{64}Cu solution. (△): ^{64}Cu in seawater. (□): ^{64}Cu in algae.

In a 1.5 L volume of filtered seawater with ^{64}Cu and EDTA (Merck, Titriplex III p.a.), shells were allowed to adsorb ^{64}Cu for 48 hours. At regular time intervals, the shells were taken from the medium and rinsed in FSW for a few seconds. The shells were transferred to glass vials and immediately counted. Adsorption kinetics were followed by measuring ^{64}Cu activity in four different situations: in the first, ^{64}Cu (31 nM) and EDTA (2.7 µM) were introduced together with *M. balthica* shells in the seawater. In the second, the shells were introduced 20 hours after the introduction of ^{64}Cu (31 nM) and EDTA (2.7 µM). In the third, adsorption of 31 nM ^{64}Cu without EDTA was measured. In the fourth situation, ^{64}Cu (310 nM) and EDTA (2.7 µM) were mixed together before they were introduced with the shells in the seawater.

In a further experiment, ^{64}Cu adsorption on shells and accumulation in tissue of living *Macoma balthica* in the presence of excess EDTA (270 µM) was studied. For this, clams (11-14 mm) were taken from a stock which was held in coarse dune sand, receiving unfiltered flowing seawater at the Oosterschelde field station (Tidal Water Division, Middelburg). For the experiment, the individuals were held in polyethylene acid washed beakers, 7 cm from the bottom on a net, tightened between a polyvinyl chloride ring. Water was gently stirred with a magnetic stirrer. The individuals were allowed to acclimatize to the laboratory conditions for three days. Temperature was held constant at 6 °C.

RESULTS

^{64}Cu uptake by phytoplankton (experiment 1).

In Figure 2.1a a typical example of the adsorption of ^{64}Cu on *P. tricornutum* is given. The high concentration on the algae in the first measurement (after a few minutes) showed that very fast uptake occurred immediately after the introduction of ^{64}Cu. After this initial high uptake, the concentration on the algae increased slowly. Within 3.45 hours, average adsorption to the algae was 3270 ± 870 cpm/ml. During the following 24 hours, adsorption increased to 6290 ± 960. Initial concentration in the water was 35990 ± 870 cpm/ml. After 28 hours, the concentration was 29900 ± 1710 cpm. At this time, 17.5% of the initial activity in the water was adsorbed to the algae. Refreshing the seawater after one day (Fig. 2.1b) did not result in an increased concentration in the algae, although the dissolved ^{64}Cu concentration in the water was elevated. The reason for this was a considerable loss of algae during the refreshing procedure (note the log-scale!).

The algae, as treated in Figure 2.1a, were centrifuged after 28 hours, washed with EDTA

and FSW and resuspended in ^{64}Cu-free FSW. The decrease of total ^{64}Cu associated with algae in Figure 1a was due to the loss of algae during centrifugation and the loss of loosely adsorbed ^{64}Cu. After the resuspension in ^{64}Cu-free FSW, the algae lost ^{64}Cu immediately, with a resulting higher ^{64}Cu concentration in the water, than in the algae. This effect was shown in all spiking treatments, irrespective of the duration of the washing periods with EDTA and seawater. In our experiments however, leaking of tracer to the dissolved phase was not desirable, as the ultimate goal was to separate the two pathways of tracer uptake. Because redistribution of ^{64}Cu in the system could not be prevented, EDTA was used as a complexing agent to prevent leached ^{64}Cu from being taken up by bivalves, feeding on the algae.

ADDED			CALCULATED		
[Cu] ug/l	[Cu] uM	[EDTA] uM	[Cu free] uM	[EDTA free] uM	[Ca free] M
1	0.016	27	3.3E-09	2.5E-07	0.394
2	0.032		0.0041		0.394
2	0.032	2.7	4.38E-07	3.46E-10	0.394
20	0.32	2.7	4.92E-06	3.07E-10	0.394
5	0.079	0.027	0.0069	1.8E-14	0.394
5	0.079	0.27	2.1E-05	1.8E-11	0.394
5	0.079	2.7	1.1E-06	3.4E-10	0.394
5	0.079	270	9.9E-09	3.8E-08	0.385

Table 2.1 Added and theoretical concentrations of dissolved and complexed copper and EDTA. The theoretical concentrations were calculated with help of the computer program SOILCHEM.

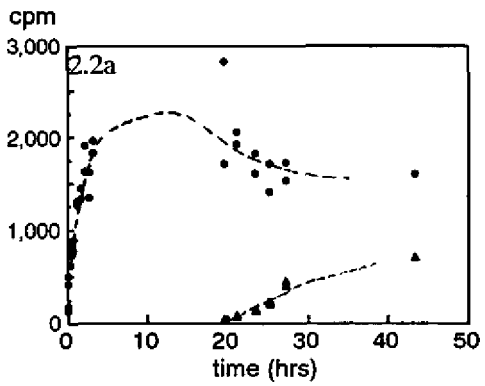
Complexation of ^{64}Cu by EDTA (experiment 2).

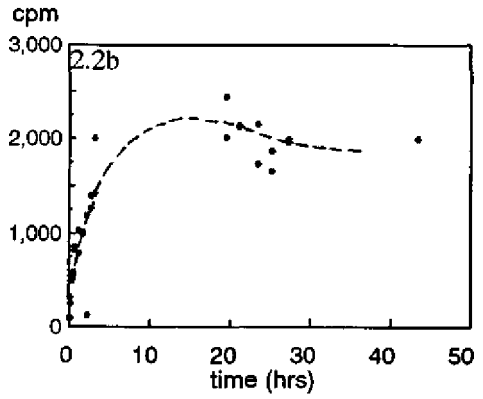
In Figure 2.2a, the adsorption of ^{64}Cu onto shells introduced together with ^{64}Cu and EDTA addition, is presented. The sorption process on the shell was comparable with a situation without EDTA (Fig. 2.2b). Measurements after 20 hours indicated no further adsorption on the shell, suggesting an equilibrium situation. The ^{64}Cu concentration in the water had decreased considerably as a result of adsorption to the wall and the shells. The decrease was described best with an exponential curve and amounted to 55% of the original concentration after 24 hours.

If shells were introduced after equilibration of the seawater with ^{64}Cu and EDTA for at least

20 hours, sorption occurred at a very low rate. This indicated that in this situation, ^{64}Cu was largely complexed by EDTA (Fig. 2.2a).

Mixing ^{64}Cu (310 nM) and EDTA together before introduction in the seawater resulted in a lower sorption rate (Fig. 2.2c). This effect was demonstrated at a 31 as well as at a 310 nM ^{64}Cu solution. Shells that were introduced after 24 hours now showed a low adsorption rate, indicating that the concentration of uncomplexed ^{64}Cu had remained relatively constant during this period.





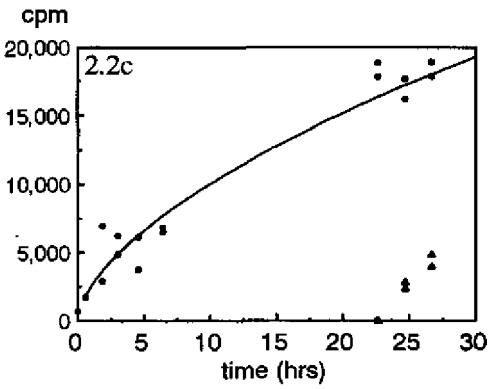


Figure 2.2a Adsorption on shells: 31 nM ^{64}Cu + 2.7 µM EDTA. (●) immediate adsorption (EDTA and ^{64}Cu not mixed in advance). (▲): adsorption in the case that the shells are introduced in the seawater after a 20 hours equilibration time. 2.2b: 31 nM ^{64}Cu without EDTA. 2.2c: 310 nM ^{64}Cu + 2.7 µM EDTA are mixed together before introduction in the seawater. (●) adsorption on direct introduced shells. (▲) adsorption in the case that the shells are introduced after 23 hours equilibration time.

From these experiments it could be concluded that adding EDTA was effective, but only after at least 20 hours equilibration time, or when EDTA and ^{64}Cu were mixed before being added. However, in a feeding experiment, 20 hours equilibration would take too long. Moreover, mixing of ^{64}Cu and EDTA in advance was not possible, as the ^{64}Cu was being released from the algae during the experiment. Immediate complexation by EDTA was desirable. Therefore, the sorption kinetics experiments were repeated with an EDTA concentration 100 times higher (Fig. 2.3a). In this situation, sorption onto shells was reduced to less than 0.5% compared with the situation without EDTA. Uptake by *M. balthica* appeared to be reduced to about 1% of the uptake without EDTA (Fig. 2.3b). In the situation without EDTA, shell adsorption was

at least 3 times higher than tissue uptake.

DISCUSSION AND CONCLUSION

With the algal spiking procedure, the problem of ^{64}Cu leaking from the labelled algae occured, irrespective of any algal washing period (experiment 1). Gutknecht (1963) observed a silimar loss with ^{65}Zn from benthic marine algae and Rice & Willis (1959) observed this with ^{144}Ce from marine planktonic algae. Because the redistribution of ^{64}Cu in feeding experiments is undesirable, we used EDTA to prevent dissolved ^{64}Cu from being accumulated. In experiment 2, it was noticed that although sufficient EDTA was available in theory (see Table 2.1), the results indicated that EDTA was not totally efficient in complexing Cu immediately. This can be explained by the fact that in seawater, competition with other cations that are available in much higher concentrations (mainly calcium and magnesium) is interfering with the Cu-EDTA complexation. As the conditional equilibrium constant of the Cu-EDTA complex is higher than the other complexes, Cu will ultimately displace the other cations. The displacement by Cu takes hours (Morel *et al.*, 1979), while the figures, computed with SOILCHEM, have an equilibrium situation as a starting point. Fortunately, an excess amount of EDTA came up to our expecations. No direct harmful effects of this excess EDTA on the algae were expected, as the free concentration of major ions like calcium was hardly influenced (Table 2.1). However, it remains doubtful whether algae are able to grow further with this EDTA amount, because essential metals like Zn, Fe and Mn are complexed for more than 99%.

In conclusion, the aim of this study was to design an experimental setup that would make it possible to measure uptake of particulate Cu without interference of dissolved Cu. In conventional experiments with spiked algae (or any other food source), redistribution of Cu takes place, complicating the assessment of a separate route to the overall Cu uptake. The tendency of dissolved Cu to form strong EDTA complexes that are hardly biologically available, made it possible to separate the uptake routes. Because the complexation of Cu by EDTA is a slow process in sea water, it was necessary to use an excess amount of EDTA. By minimizing the uptake of dissolved ^{64}Cu through complexation, it is possible to study the uptake of ^{64}Cu associated with algae, by aquatic organisms that feed on the algae. Details on uptake experiments with ^{64}Cu labelled algae are described in Chapter Three.

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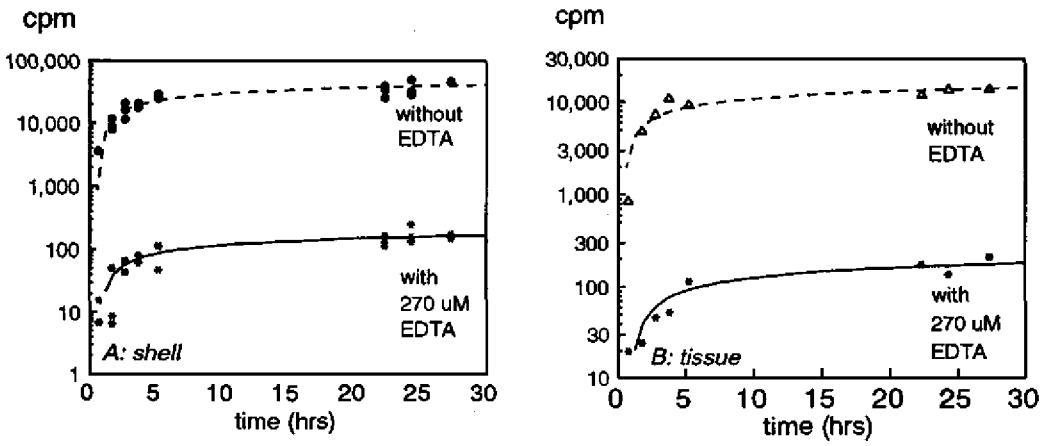

Figure 2.3 Adsorption onto shells (A) and accumulation in tissue (B) of *Macoma balthica* with and without 270 µM EDTA. ^{64}Cu concentration was 310 nM.

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Chapter Three

Availability of ^{64}Cu from phytoplankton and water for the bivalve *Macoma balthica.*

ABSTRACT

The amount of copper taken up via algae and water by *M. balthica* was established using the radioisotope ^{64}Cu. As far as we know, this isotope has never been used before in marine food chain studies. As a model food source, the marine diatom *Phaeodactylum tricornutum* was allowed to accumulate ^{64}Cu for one day. These labelled algae were fed to the clams in the presence of the complexing agent EDTA (0.27 mM). EDTA was added to prevent uptake of dissolved ^{64}Cu that could be leaking from the labelled diatoms. In control experiments, unlabelled diatoms were fed to *M. balthica* in the presence of dissolved ^{64}Cu (with and without EDTA) to assure a similar filtration activity. In repeated experiments with varying particulate/dissolved copper ratios, uptake through food always turned out to be at least as efficient as uptake from the water. It was concluded that Cu, associated with food, is well available for uptake by *Macoma balthica.*

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INTRODUCTION

It is often suggested to use filter feeding bivalves as a biological monitor in estuarine areas. However, bivalves are exposed to various dissolved as well as particulate metal species in the waterphase. In addition to this, sediment dwelling bivalves are also directly exposed to sediment-associated metals. Little information is available on the relative contribution of food as a particulate metal species to the overall metal accumulation. The sediment dwelling deposit feeder *Macoma balthica* is feeding by taking in algae, bacteria and detrital material through its siphons (Brafield and Newell, 1961; Gilbert, 1977). Like many bivalve mollusc species, *M. balthica* can ingest preferentially nutritious particles and reject non-nutritious particles like sediment grains as pseudofaeces (Morton, 1973; Levinton, 1989). The metal concentration in food particles is much higher than in the surrounding water (Luoma and Bryan, 1982; Bryan, 1985). As food material is digested, it seems logical to assume that uptake of metals through food is an important pathway for marine bivalves. However, the total quantity of metal to which a filter feeder is exposed via the water can be very large, considering the large water volumes with which they have contact during respiration. So far no conclusive evidence for the importance of either of the pathways is available.

For cadmium, various studies indicated that the contribution of food to the overall metal accumulation is low: Janssen and Scholz (1979) assessed a food contribution of only 10% and Borchardt (1983) concluded that food contributed for not more than 0.2-0.5% to the Cd body burden of *Mytilus edulis*. Uptake of bacterially-associated ^{109}Cd by *Macoma balthica* resulted in 39% of a 14-day total uptake. Although it is an essential metal, ^{65}Zn from bacteria resulted in only 23% of the total uptake. ^{57}Co on the other hand accounted for 81.6% of the total uptake (Harvey and Luoma, 1985). Data on Pb are contradicting: *Mytilus edulis* accumulated Pb from water and food in equal amounts (Schulz-Baldes, 1974), whereas with oysters (*Crassostrea gigas*) direct uptake from water led to body burdens approximately 100 times higher than those reached after contaminated food ingestion (Amiard-Triquet *et al.*, 1988). Accumulation of ^{75}Se by the clam *Puditapes philippnarum* was mainly from Se-labelled phytoplankton (Zhang *et al.*, 1990). Particulate organo-Se was assimilated with 86% efficiency by *Macoma balthica* when the clam was fed ^{75}Se labeled diatoms.

Cu has not been studied before in sufficient detail. A reason for the scarcity on uptake data is the lack of a suitable tracer. Radioactive tracers enable the use of low, ecologically realistic concentrations, and often give the opportunity to separate different routes of metal uptake. Cu radioisotopes however are either not easy to prepare (^{67}Cu) or have relatively short half lives (^{64}Cu).

In spite of the limitations described above, we have tried to assess the role of Cu uptake through food by a radioactive tracer study using ^{64}Cu. The objective of this study was to

measure ^{64}Cu uptake by *Macoma balthica* via food and water by separation of uptake pathways.

MATERIALS AND METHODS

The experimental conditions of the clams, the preparation of ^{64}Cu and ^{64}Cu-labelled algae are described in Chapter Two. The experiments were carried out in the dark because in this situation, gut evacuation time is comparable with gut evacuation time of clams burrowed in sediment (Hummel, 1985). After acclimatizing, the individuals were introduced in the experiment and received filtered sea water (FSW) with equal amounts of labelled or unlabelled algae. The algal density was kept similar to ensure a comparable filtration activity for the different experiments.

Experimental setup

To assess the contribution of food-associated copper to the total copper uptake by *Macoma balthica*, four different experiments were carried out. With these experiments, *M. balthica* individuals were allowed to feed on (tracer labelled) algae for a short period. After the feeding period, the retention of the tracer was measured during the depuration of the ingested food. This so called 'pulse-chase' experimental setup has several advantages, compared with other types of uptake experiments (Luoma *et al.*, 1992). The major advantages in this case were a minimization of recycling of tracer in the experiment and fewer problems, caused by altered animal behaviour in long term experiments, e.g., decreased activity as a result of a deteriorated condition.

In experiment 1, clams were allowed to feed on algae (*P. tricornutum*), labelled with ^{64}Cu. 0.27 mM ethylenediaminetetraacetate (EDTA) was added to prevent the uptake of ^{64}Cu that could have been leaking from the labelled algae. This excess amount of EDTA has proved to be very efficient in minimizing the uptake of dissolved ^{64}Cu. In experiment 2, clams were allowed to feed on ^{64}Cu-labelled algae without EDTA. If differences between experiments 1 and 2 occurred, this would indicate that indeed the algae were leaking. The presence of dissolved ^{64}Cu would also be indicated by increased adsorption on the shell. In experiment 3, ^{64}Cu was dissolved in the water in the presence of 0.27 mM EDTA. Unlabelled (control) algae were added to this experiment to ensure a comparable filtration activity. This experiment functioned as a control: if EDTA were shown to be an effective inhibitor of Cu uptake, accumulation by *M. balthica* and adsorption to the shell should be negligible in this

experiment. In experiment 4, again ^{64}Cu was dissolved in the water, but no EDTA was added. Here also fresh, unlabelled algae were added to stimulate the filtration activity. As dissolved Cu without EDTA is supposed to be readily available for uptake, considerable accumulation and adsorption were expected. The difference between uptake from ^{64}Cu labelled algae in experiments 1 and 2 on the one hand and ^{64}Cu labelled water in experiments 3 and 4 on the other hand should give more information on the relative importance of the different sources of Cu uptake.

Experiments 1 through 4 were repeated three times, with different ratios between particulate and dissolved ^{64}Cu. Also the accumulation and elimination periods were varied. The experimental scheme is summarized in Figure 3.1.

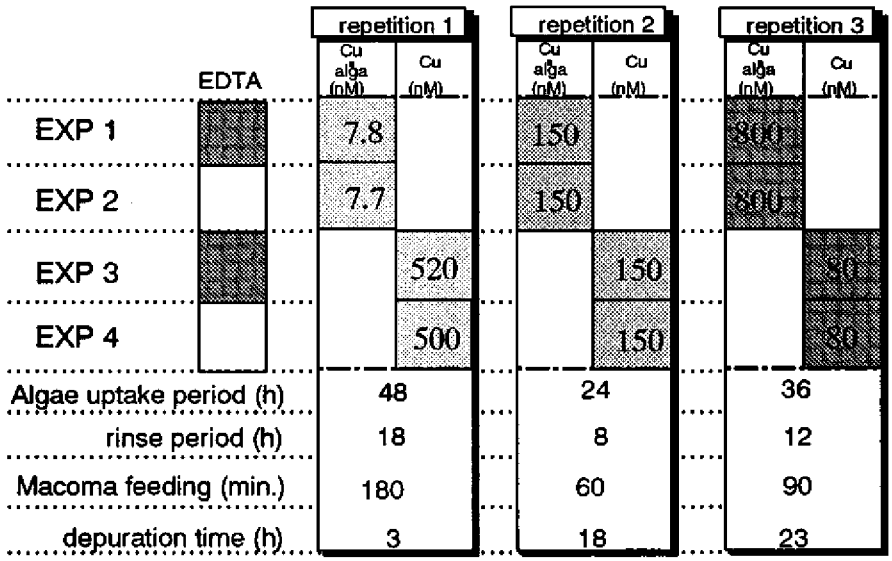

Figure 3.1 Experimental setup of feeding experiments 1 to 4 with 3 repetitions. The ^{64}Cu concentrations (in nM) in the experiments are shown in the shaded boxes.

In the first series of experiments 1 through 4 (repetition 1), the algae were exposed to 790 nM ^{64}Cu for 48 hours and rinsed with 5 µM EDTA for 18 hours (for more details see Chapter two). After a three-hour feeding period in 2-L beakers, the individuals were put in FSW for another three hours to clean their guts. This time was considered to be sufficient, as the average gut passage time for *M. balthica* was reported to be approximately 90 minutes, irrespective of the temperature (range 5.5-21°C) (Hummel, 1985). After a short freezing period, the individuals were dissected and the radioactivity of the shells and tissue was

measured immediately. For each measurement, three individuals were taken. The measurements were carried out in triplicate.

Water samples were taken regularly during the feeding period to follow the decrease in algal density. For algal counts, the samples were fixated with 2% formalin. Algal densities were measured with a particle counter (Coulter Multisizer). To follow further filtration behaviour, nine individuals per experiment were allowed to continue filtering after the three-hour accumulation period. The accumulation results were corrected for the possible differences in pumping rate: the metal accumulation rate is supposed to be directly dependent on the amount of water that has passed the gills. This depends on the pumping activity of *M. balthica*. The amount of water that had passed the gills was assessed by measuring the decrease in algal density, relative to the number of individuals during the experiment.

In the second run of experiments 1 through 4 (Figure 3.1), four *M. balthica* individuals were kept per 100 ml beaker. Total ^{64}Cu concentration on the labelled algae was similar to the dissolved concentration with unlabelled algae: 150 nM. Because of the short half-life of ^{64}Cu, the loading procedure of the algae was shortened to 24 hours with 7.9 µM ^{64}Cu spiking and 8 hours rinsing to be able to measure elimination as long as possible. The clams were allowed to feed on labelled algae and on unlabelled algae with dissolved ^{64}Cu for 60 minutes. After the feeding period, the clams were transferred to glass scintillation vials, each containing 20 ml FSW and unlabelled algae. The algae were added to continue digestive activity. While digesting, the total ingested material was determined by whole body counts on the living individuals. After 90 minutes, the individuals were transferred to other vials with FSW and unlabelled algae and allowed to depurate for another 90 minutes. The first series of vials with defecated material was measured for radioactivity. After a second transfer, the individuals were left overnight. By this method, elimination from the individual *M. balthica* could be measured without losses. This series was carried out in duplicate, revealing 2x4 individuals for each experiment.

The third run of the experiments was comparable with the second, but now the transfers to a fresh vial were more frequent, in order to detect any pattern in the elimination. The transfers were carried out every 60 minutes for the first three hours, every 90 minutes for the following 10 hours and finally after two hours. The transfers were continued until the radioactivity of the depuration products was near the detection limit (caused by a decrease of the elimination and the decay of ^{64}Cu). The individuals were subsequently dissected and the remaining radioactivity of the shells and tissue were counted. This series was carried out in triplicate, revealing 3x3 individuals for each experiment.

With the elimination data of the second and third runs, the half-life of ^{64}Cu in the ingested food was calculated. The elimination of ^{64}Cu can be described by the exponential function:

$$A_t = A_o e^{-\lambda t}$$

Where A_o = ^{64}Cu at time o
A_t= ^{64}Cu at time t
t= time since feeding
λ= loss rate constant

The halftime for the ingested material is:

$$T_{1/2} = \frac{\ln 2}{\lambda}$$

As a measure for the part of the radionuclide from the food that is retained by *M. balthica*, the absorption efficiency is calculated by comparing the total ingested and total eliminated radioactivity:

$$F = \frac{I - E}{I} * 100$$

Where F= apparent absorption efficiency
I=ingested material
E=eliminated material

RESULTS

In the first run of experiments 1 and 2, the concentration of ^{64}Cu, associated with the algae was 7.8 and 7.7 nM (see Figure 3.2). The ^{64}Cu concentration in the water with unlabelled algae (experiments 1 and 2) was 520 and 500 nM. Because the initial ^{64}Cu concentrations in the media were very different, the accumulation results in Figure 2 were related to the ^{64}Cu concentration in the water. Surprisingly, the uptake of ^{64}Cu by *M. balthica* from labelled algae was considerable (3.2b). The low amount of ^{64}Cu on the shell indicates that if any Cu had been lost from the algae, this was effectively complexed by EDTA (3.2a). Without EDTA in the water, the sorption on the shells was a little higher, indicating that a small amount of ^{64}Cu had been leaking from the labelled algae. The difference in accumulation between experiments 1 and 2 was not significant. In experiments 3 and 4 with unlabelled algae and dissolved ^{64}Cu, overall sorption on shells and uptake in tissue was very low in the presence of EDTA. This indicates again that dissolved ^{64}Cu was effectively complexed by EDTA. It was also clearly demonstrated in experiment 3 that Cu-EDTA was

not accumulated. Without EDTA, uptake was considerable (as expected). Sorption on shells was even higher than uptake. This pattern is also seen in uptake experiments without algae (Chapter Two). The total decrease of algae in experiment 3 was comparable with experiment 1 and 2 (Fig. 3.3). The filtration rate in experiment 4 was distinctly lower than in the other test beakers (Fig. 3.3).

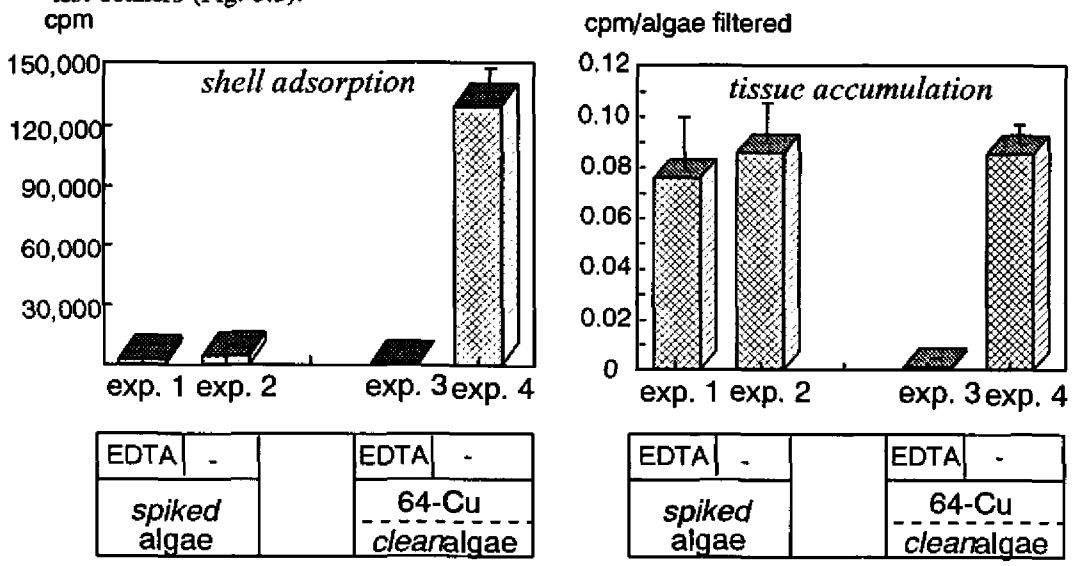

Figure 3.2: Shell adsorption (A) and tissue accumulation (B) after 3 hours feeding on ^{64}Cu-labelled and unlabelled algae in experiment 1 to 4. Accumulation is plotted in relation to the administered ^{64}Cu concentration: Y-axis label = cpm clam.cpm^{-1}.ml.10^{2}. Each bar represents the average of 9 clams.

In the second run, the particulate ^{64}Cu concentration (associated with the algae) was similar to the dissolved concentration: 150 nM. Because in the first run, the limited depuration time could have masked real ^{64}Cu accumulation through a large portion of undigested algae, depuration time was extended. Although the total ^{64}Cu concentration in all four experiments was similar, the uptake results were very different (Fig. 3.4). The difference in ingestion between individual clams in the same treatment could be more than 100% because of the different feeding activities of the individual clams. However, in spite of this large variation, differences between the treatments were always significant (Anova: R^2>0.75 and P<0.001). The ingestion of ^{64}Cu by *M. balthica* through the labelled algae was high and the presence of EDTA did not cause a decreased uptake. In contrast, accumulation of ^{64}Cu from the water (in presence of unlabelled algae) in experiment 4 was much lower, although algal density and total ^{64}Cu present, were comparable. The presence of EDTA reduced uptake of dissolved ^{64}Cu to amounts near the detection limit (experiment 3). The biological half-life of the ingested ^{64}Cu-algae was 49.3 ±22.6 hrs in experiment 1 and 54.6 ±16.9 hrs in experiment 2 (without

EDTA). The half-life of the ingested dissolved ^{64}Cu in experiment 4 (without EDTA) was 6.3 ±2.4 hrs. In the situation with dissolved ^{64}Cu and EDTA (experiment 3), no reliable half-lifes could be calculated. The elimination from the clams that accumulated ^{64}Cu from the water was difficult to measure: counts were near background measurements. Counting error was more than 20% in this case. In spite of the difficult elimination measurement, it can be concluded that based on the large differences in accumulation results from experiments 1 through 4, ^{64}Cu uptake from labelled algae was more efficient than uptake of dissolved ^{64}Cu from seawater.

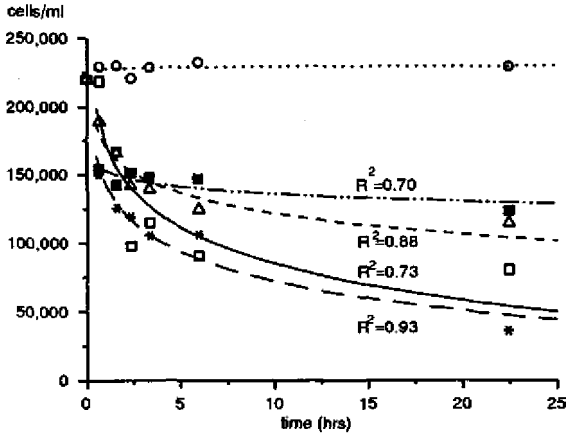

Figure 3.3: Decrease of algal density as a result of feeding by *Macoma balthica* in repetition 1 of experiment 1 to 4. (□) exp. 1; (▵) exp. 2; (○) control (no clams); (*) exp. 3; (■) exp. 4.

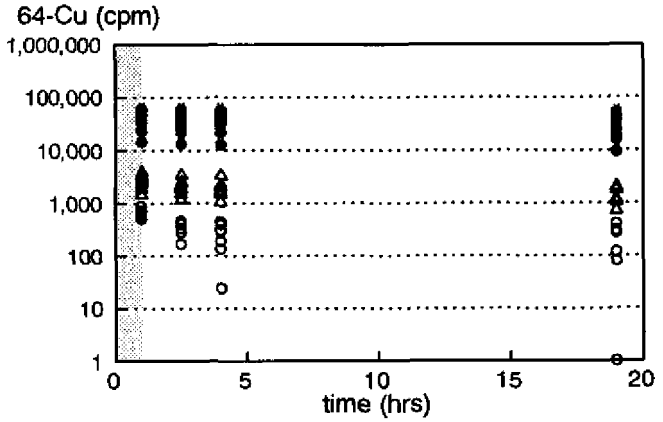

Figure 3.4: Ingestion and depuration of ^{64}Cu in repetition 2 of experiment 1 to 4. The total (dissolved or particulate) ^{64}Cu concentration was equal in all test situations. (*) exp. 1; (●) exp. 2; (○) exp. 3; (▵) exp. 4. The shaded part indicates the feeding period. The marks at t=1 hour indicate the ingested ^{64}Cu. The marks at t=2.5, t=4 and t=19 hours indicate the ingested ^{64}Cu minus the sum of depurated material at that time.

In the third run of the feeding experiments, elimination was followed in more detail. Total ^{64}Cu concentration on the labelled algae in experiments 1 and 2 was 800 nM. Total dissolved ^{64}Cu in the water in experiments 3 and 4 was 80 nM. In Figure 3.5a, a typical example of the elimination in experiment 1 is given. The three individuals had the same treatment, but differed in ^{64}Cu accumulation (as in the second run). However, the elimination pattern was comparable. The ^{64}Cu concentration in the faeces was high initially, but declined progressively with time. The clams were dissected after 25 hours' depuration time. In Figure 3.5b, elimination is compared with the ingested concentration (after the feeding period) in the clams (as in Figure 3.4). The total ^{64}Cu content in shells and tissue of the dissected clams (bars) was the same as the initial ingested amount minus the sum of the elimination products at t=25h. Very little ^{64}Cu was adsorbed to the shell, compared with the situation when ^{64}Cu was accumulated from the water (see Table 3.1). As in the first and second run, the difference in accumulation between experiments 1 and 2 on the one hand and experiments 3 and 4 on the other hand was remarkable.

The biological half-life of ^{64}Cu, accumulated from labelled algae in experiment 1 was 94.5 ± 20 (n=7) (Table 3.1). The counts in individuals 1.3 and 2.5 were very low, compared with other individuals in the same treatment. Considering that these individuals were probably in bad condition, they were not used in the calculation of the average half-life. In experiment 2, the half-life was 126.2 ±50.8. The half-life of accumulated ^{64}Cu from water (experiment 4) was 61.5 ± 11.2. Because accumulation and elimination in experiment 3 were near background values, they were not used in Table 3.1.

Dissolved ^{64}Cu concentrations in the water in experiments 3 and 4 were kept very low, to prevent toxic effects, and thus to achieve a maximal uptake efficiency from the dissolved phase. In Table 1 it can be seen that the relationship between ingested and available ^{64}Cu was comparable for experiment 1 and 4. The relationship was slightly better in experiment 2. Possibly in this repeat of experiment 2, more ^{64}Cu had been leaking from the algae than in the first and second runs. In experiment 1, this leaked ^{64}Cu was immediately complexed by EDTA. In experiment 2 also a slightly higher percentage of ^{64}Cu was found in the shell, compared with experiment 1 (Table 3.1).

Experiment 3 (unlabelled algae with ^{64}Cu and EDTA) resulted in a very low accumulation. The elimination data were around the detection limit, so they were not used for further calculations. The shorter biological half-life of dissolved ^{64}Cu (experiment 4) could be due to the fact that the major part of the Cu was reversibly bound to the shell. Absorption efficiency (F) was 87.9% ± 3.0 (n=7) in experiment 1. In experiment 2 it was 89.6 % ± 3.7 (n=9). In experiment 4 it was 80.1 % ± 3.8 (n=9). From the accumulated ^{64}Cu, a major part will be adsorbed on the shell.

treatment	clam no.	I (cpm)	I-E (cpm)	F (%)	tissue (cpm)	tiss+shell (cpm)	in shell (%)	ingested/ available	t1/2 (hrs)
EXP. 1	1.1	22400	17330	77	11820	13640	13.3	28.0	67
	1.2	13260	11380	86	9710	10660	8.9	16.6	119
800 nM	1.3	520	270	52	n.m.	n.m.		0.6	
Cu*algae	1.4	8110	6660	82	4200	5250	20.1	10.1	95
+EDTA	1.5	550	250	45	n.m.	n.m.		0.7	
	1.6	22740	19190	84	17630	18600	5.2	28.4	89
	1.7	6320	5310	84	2850	4060	29.7	7.9	100
	1.8	26040	22730	87	21380	22440	4.7	32.5	122
	1.9	9770	7720	79	7270	7680	5.3	12.2	70
EXP. 2	2.1	44230	38480	87	19910	25440	21.8	55.3	115
	2.2	11470	10310	90	8440	10040	15.9	14.3	231
800 nM	2.3	10310	8950	87	6870	8230	16.6	12.9	128
Cu*algae	2.4	13900	11590	83	6530	9310	29.8	17.4	90
-EDTA	2.5	55710	49960	90	44670	48430	7.8	69.6	197
	2.6	19630	16790	86	12680	16400	22.7	24.5	105
	2.7	9880	7740	78	3720	5760	35.4	12.4	75
	2.8	34560	29360	85	24830	27760	10.5	43.2	119
	2.9	3690	2920	79	1460	2530	42.3	4.6	75
EXP. 4	4.1	1274	993	78	74	1137	53.3	15.9	86
	4.2	306	267	87				3.8	
80 nM	4.3	862	648	75	373	646	42.2	10.8	60
in water	4.4	295	208	71		229	114	3.7	
-EDTA	4.5	1020	735	72	307	645	52.2	12.8	50
	4.6	1124	810	72	442	559	19.7	14.1	53
	4.7	301	236	78	78	318	75.4	3.8	68
	4.8	862	627	73	342	423	19.2	10.8	58
	4.9	284	208	73	36	220	84	3.6	56

Table 3.1. Ingestion (I), elimination (E) and apparent absorption efficiency (F) of ^{64}Cu from labelled algae and water in repetition 3 of experiment 1, 3 and 4.

DISCUSSION AND CONCLUSION

Food density or even food absence might influence metal uptake from solute as well as from particulate sources for the following reasons: firstly, because metals are possibly accumulated from the food particles; secondly, because food quantity influences filtration activity and consequently the amount of dissolved metal that passes the gills (Janssen and Scholz, 1979; Riisgård *et al.*, 1987); thirdly, food availability will influence the physiological condition of organisms, which also determines the uptake rate of metals (Luoma, 1983).

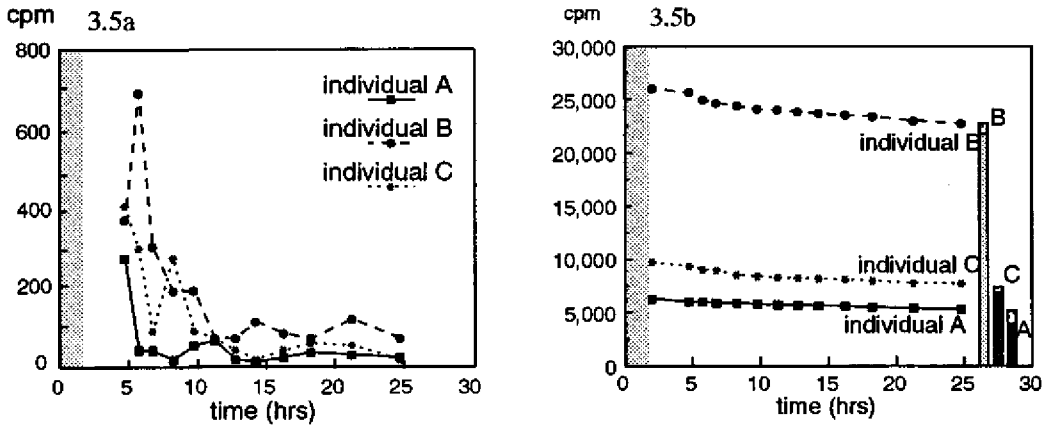

Figure 3.5a. Typical elimination patterns of ingested ^{64}Cu labelled algae in 3 clams (repetition 3 of experiment 1). 3.5b. Ingestion and depuration of ^{64}Cu in the individual clams (see 3.5a). The shaded part indicates the feeding period. The first mark indicates the ingested ^{64}Cu. The following markes indicate the ingested ^{64}Cu minus the sum of depurated material at that time. The bars represent the ^{64}Cu content of the dissected clam at the end of the depuration time. The crossed part stands for the proportion adsorbed on the shell.

To compare uptake via food and uptake from the water a similar pumping (or filtration) rate is required. This was achieved by adding the same amount of (unlabelled) algae to ^{64}Cu-labelled sea water in experiments 1 to 4. However, dissolved Cu has a tendency to adsorb to particulates, including algae. Adding unlabelled algae might reduce the dissolved Cu concentration and increase the particulate concentration, with unknown effects on the experimental results. Spiking experiments (Chapter Two) showed that only a minor fraction of dissolved ^{64}Cu could become adsorbed on the algae during the time period of the feeding experiment.

The filtration rate in experiment 3 was distinctly lower than in the other experiments (Fig. 3.3). Possibly, dissolved ^{64}Cu concentrations were high enough to inhibit filtration rate in these treatments. If Figure 3.2b was corrected for the difference in filtration rate, still a large uptake from the labelled algae series would be seen. As in the following runs of the experiments, dissolved ^{64}Cu concentrations were much lower, filtration activity was expected to be unaffected.

The calculated half-life of the ingested ^{64}Cu was rather different between the second and third run. An explanation for the noticed differences might be the manipulation in the third run. The frequent transfer of the clams to fresh vials could have caused certain stress, that retarded the digestive process. Another explanation can be found in the time of the year that the experiments were carried out: the second run took place in June, whereas the third run

was carried out in February. The animals used here were kept in a field station under ambient seawater temperatures. The clams are likely to have adapted their feeding activity to the seasonal food availability (Hummel, 1985). Difference in feeding activity could therefore have influenced the half-life of the ingested material. Nevertheless, both runs of exp. 1 through 4 indicated that uptake of ^{64}Cu, associated with algae, might be at least as efficient as uptake of dissolved ^{64}Cu.

It can be argued that the conditions in these experiments are never met with in natural waters, because the algae were exposed to much higher Cu concentrations for the accumulation than were the bivalves in the feeding experiments. However, because the spiking period was limited, a high concentration had to be used to obtain sufficiently labelled algae. The total Cu concentrations and the ratio between dissolved ^{64}Cu and food-associated ^{64}Cu in this experiments were realistic for estuarine and coastal environments. Although the distribution of Cu over the dissolved and particulate phase in estuarine waters is varying considerably, generally dissolved and particulate fractions are of the same magnitude with a K_D between 1 and 2 (Valenta *et al.*, 1986; Baeyens *et al.*, 1987; Golimowski *et al.*, 1990).

In a fjord with dissolved Cu concentrations varying from 0.3 to 4.0 µg/l (4.8-64 nM), the Cu concentration in *Phaeodactylum tricornutum* (exposed in dialysis bags) varied between 6 and 54 * 10^{-9} µg/cell (Eide and Jensen, 1979). Considering a moderate algal bloom with 2 * 10^7 cells/l, the amount of Cu associated with the algae would be 0.12-1.08 µg/l. In that particular situation, a *M. balthica* individual would have received the major part of its Cu through the food.

The results from this study cannot be compared with other aquatic accumulation studies using ^{64}Cu, because as far as we know, this isotope has not been used so far. The only data on the contribution of food-associated Cu are known for young oysters (*Crassostrea gigas*): Cu contaminated algae induced poor growth and high mortalities in grazing larvae (Wikfors and Ukeles, 1982). Amiard-Triquet et al (1988) found a retention rate of phytoplankton-associated Cu of around 42%. Body burdens induced by exposure to Cu-contaminated seawater or contaminated water plus food were at least ten times higher than those registered in oysters exposed via phytoplankton. These results cannot be compared directly with our observations because no mention was made of the actual amount of copper that was associated with the algae. In our experiments it was shown that even with relatively low particulate Cu concentrations, accumulation from food was considerable.

It is recognized, that food particles will increase metal accumulation, because the filtration activity is stimulated (Borchardt, 1983; Martincic *et al.*, 1987). On top of this effect, we

measured considerable Cu uptake from algae. A possible explanation can be found in the difference between deposit and suspension feeding. Suspension feeders have to concentrate a very dilute food source, while deposit feeders select from a concentrated source (Gilbert, 1977). For several *M. balthica* species it is known that their pumping rates are far inferior to those of suspension feeders and average about 10% of the latter (Hughes, 1969; Meyhöfer, 1985). If less water per unit of time is passing the gills, metal accumulation from the water phase is probably less important for deposit feeding bivalves when compared with suspension feeding bivalves. This could explain the discrepancy between the data on Cd for the filter feeding *Mytilus edulis* and the deposit feeding *Macoma balthica* (see introduction).

Will the ingested ^{64}Cu actually be accumulated? This depends on the digestive process. After ingestion, the first step in particle selection involves the gills: 'quality' particles are passed to the labial palps. Unwanted particles are removed by ciliae and ejected as pseudofaeces via the inhalant opening. At the labial palps, further sorting takes place. Finally, particles of a suitable size are passed to the mouth and stomach (Gilbert, 1977). In the stomach, extracellular digestion takes place through enzymes, released by the crystalline style. Finer particles are send to the tubules of the digestive gland. Digestive cells phagocytize the particles and digest them intracellularly (Morton, 1973). Food particles of suitable size can enter the stomach. In the gut of deposit feeders, pH is around 6-7. This level is lower than the surrounding seawater and it can be expected that some weakly bound Cu species will be stripped from the algae. At this moderate pH level, carrier molecules will still be efficient in complexing the metal for transport (Luoma, 1983). It is assumed that with microalgae, the majority of Cu is in a readily exchangeable form, namely associated with carboxyl groups on the cell wall.

For our experiments, it was necessary to have an idea about the duration of the different stages of digestion. Decho and Luoma (1991) assessed the time courses for ingestion, retention and release of microbial food and associated ^{51}Cr. Our experimental setup was comparable in some ways, but had the drawback of the limited time for measuring elimination. With the microbial food, Decho and Luoma found a gut passage time of 9.6 hours. This was rather different from the 1.5 hours gut passage time when feeding on diatoms, mentioned by Hummel (1985). The large difference between these figures can possibly be caused by the food source: a food specific gut passage time is reported by several authors (Calow, 1975; Taghon *et al.*, 1978; Bricelj *et al.*, 1984). As our food source more resembled the case with 1.5 hours gut passage time, this was assumed to be a more realistic figure for our experiment.

The elimination pattern of the clams (Fig. 3.5a) showed a peak in the first 2 hours of elimination and a steady decline of tracer radioactivity in the subsequent fecal products. No distinction was made between faeces or pseudofaeces production, but the elimination in the first two hours of the depuration time was considered to be pseudofaeces, as the gut passage time was supposed to be 1.5 hours. Although we could not make a distinction between intestinal or glandular digestion (Decho and Luoma, 1991), we considered the ingested material that remained after the gut passage time, to be subjected to intracellular digestion and available for uptake. If the elimination pattern with a 9.6 hours gut passage time was chosen, still a considerable portion of the ingested ^{64}Cu would be adsorbed from the food particles.

From the above it can be concluded that although the radiotracer ^{64}Cu has a rather short half-life, assessment of the accumulation of Cu via labelled algae or water was possible. A main advantage of ^{64}Cu in comparison with 'cold' Cu (^{63}Cu) is the possibility to assess accumulation in short term experiments at environmentally realistic concentrations. From the results with feeding experiments it was obvious that Cu, associated with food particles was very available for accumulation by *M. balthica*. The actual contribution of food-associated Cu to the overall Cu accumulation by *M. balthica* will depend on factors like Cu content in the food, feeding behaviour (suspension or deposit), food availability and nutritive value of the ingested material.

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Chapter Four

The relation between salinity and copper complexing capacity of natural estuarine waters and the uptake of dissolved ^{64}Cu by *Macoma balthica.*

ABSTRACT

The radiotracer ^{64}Cu was used to assess the influence of natural organic ligands on the bioavailability of copper. Biological availability of the ^{64}Cu-complexes was measured by accumulation in the bivalve *Macoma balthica.* The experiments were carried out in April as well as in February with water from the relatively clean Oosterschelde Sea arm and the relatively polluted Westerschelde estuary. Adsorption onto shells as well as uptake in tissues was assessed at salinities of 10 ‰ and 30 ‰. Simultaneously with the exposure experiments, ligand characteristics of the natural waters were assessed. High ligand concentrations, as occurring in the Westerschelde around February, reduced ^{64}Cu (320 nM) uptake by more than 50%, in spite of the much lower salinity in the Westerschelde water. At the low salinity, uptake was increased slightly in Westerschelde water, but considerably in Oosterschelde water. This implies that at low ambient ligand concentrations (during the whole year in Oosterschelde water and in the summer period also in Westerschelde water) the influence of salinity on ^{64}Cu uptake is more pronounced.

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INTRODUCTION

Copper occurs in various dissolved forms in natural waters. The biological availability, and related toxicity, varies among these forms. Numerous laboratory studies, mostly employing artificial ligands, have demonstrated the importance of the free ionic Cu as the form determining toxic effects in organisms (Florence *et al.*, 1983; Blust *et al.*, 1986; McLeese and Ray, 1986; Martell, 1989; Daly *et al.*, 1990). However, these artificial ligands do not represent the mixture of dissolved organic ligands, present in natural waters.

Laboratory studies on the effects of natural ligands in estuarine waters are rather difficult to carry out, because a considerable fraction of the (unidentified) complexing ligands can be rather labile with regard to degradation processes. Consequently, the results of a long-term accumulation experiment, using natural water from a storage tank are not representative of the natural situation, because the copper complexing capacity (CC_{Cu}) of stored natural waters progressively changes, compared with the in situ situation. On the other hand, introduction of organisms in the experiment will influence the concentration of dissolved organic ligands.

One method for assessing the influence of natural organic ligands on the biological availability of copper is to correlate in situ measurements of CC_{Cu} with copper accumulated in organisms. In practice, environmental factors (salinity, temperature, food availability) and physiological factors (growth and reproduction) will influence copper accumulation and toxicity as well in the uncontrolled field situation (Luoma, 1983; McLusky *et al.*, 1986). For these reasons, little direct evidence for the reduction of copper toxicity due to complexation by natural organic ligands has been given so far.

This research was undertaken to establish the relationship between the concentration and the nature of the different organic ligands in natural estuarine waters, and the amount of copper, accumulated from these waters by the bivalve *Macoma balthica*. To minimize the drawback of breakdown of part of the organic ligands during the accumulation period, ^{64}Cu was used. With this radiotracer, accumulation studies at environmentally relevant concentrations could be carried out within three days.

Two different natural waters were compared: the first location was in the Westerschelde estuary (see Figure 1.1). The water quality in the estuary is mainly determined by the input from the river Scheldt, which is often considered the most polluted in Western Europe. The second location was in the relatively clean Oosterschelde Sea arm, a nearly marine embayment (salinity 28-32 ‰) with a negligible freshwater input. Based on the considerable differences between concentrations of dissolved organic carbon (DOC) in the two water bodies, it was expected that the copper complexing capacity of Westerschelde water (as

assessed with voltammetric methods) would be much higher, compared with Oosterschelde water. Consequently, less copper should be accumulated by *Macoma balthica* when exposed in Westerschelde water, compared with Oosterschelde water.

Experiments were carried out in April, when DOC concentrations are average, and in February, as in the winter period DOC concentrations in the Westerschelde estuary are elevated due to increased riverine influence.

MATERIALS AND METHODS

Experimental setup

Macoma balthica were exposed to ^{64}Cu in freshly collected Oosterschelde and Westerschelde water. For this, the water was taken in the Oosterschelde near Krabbendijke and in the Westerschelde near Waarde (see Figure 1.1). The water was collected from the edge in 25-l polyethylene cans during high tide. Within 4 hours after sampling, the water was filtered (0.45 µm) to remove particulate material. The water was stored at 5 °C until further use in the experiment (within 24 hours).

The April experiment was carried out in 1992. For this experiment, *M. balthica* individuals (11.5-14 mm) were taken from a stock which was held in coarse dune sand, receiving unfiltered flowing seawater at the Oosterschelde field station (Tidal Water Division, Ministry of Transport and Public Works, Middelburg). They were acclimatized for 3 days to the experimental conditions and a further 48 hours to the exposure water (Oosterschelde or Westerschelde). This time was considered to be sufficient to recover from changes in salinity (Akberali, 1978). For the experiments, acid-cleaned polyethylene beakers were filled with 1500 ml 0.45 µm filtered water from the Oosterschelde Sea arm (OS, salinity=30 ‰) or from the Westerschelde estuary (WS, local salinity 10-22 ‰). 80 or 400 nM Cu, spiked with ^{64}Cu, were added to the WS and OS water. As a simplification, the spiked Cu is further called ^{64}Cu. Some of the media also received 1000 nM ethylenediaminetetraacetate (EDTA) to study the influence of a strong chelator on the bioavailability of ^{64}Cu.
After 20 hours equilibration of the added ^{64}Cu with the natural ligands and EDTA, the animals were added to the beakers. Adsorption and uptake were measured during 2 days. Water was not refreshed during the experiment. Each exposure was carried out in triplicate. Water samples were taken regularly to follow the total dissolved ^{64}Cu concentration.

For the experiment in February (1993), *M. balthica* individuals were collected from intertidal mudflats in the Oosterschelde at Dortsman and in the Westerschelde at Baalhoek

(see Figure 1.1). The salinity of the water at these locations was comparable with the water used in the experiments. For the experiment, animals from both locations were exposed to 320 nM ^{64}Cu in Oosterschelde (30 ‰) as well as in Westerschelde water (10 ‰). To compensate for differences in uptake caused by the variation in salinity, a second series of animals was exposed in Westerschelde water with a salinity of 30 ‰ (through addition of the major sea salts according to Kester (1967)) or in Oosterschelde water diluted to 10 ‰. The animals were acclimatized to the laboratory conditions and the different salinities for 28 days. During this period, a concentrated algal suspension was added regularly as a food supply.

Animals originating from different locations probably have different ventilation rates, due to other local food conditions. Differences in ventilation rate might influence heavy metal uptake rates (Riisgård *et al.*, 1987). To control the ventilation activity of the animals from the different locations, the clearance rate was measured after field collection and 5 days before the exposure experiment. The clearance rate was assessed by allowing 10 individuals in 1500 ml filtered water to feed for two hours on added algae (*Isochrysis albana*) with an initial concentration of 10^4 cells/ml. This measurement was carried out in triplicate. The clearance rate was calculated with the following formula (Coughlan, 1969). A correction was made for the decrease in particle concentration caused by sinking.

$$m = \frac{M}{nt} \ln \frac{C_0}{C_t}$$

Where

m = filtration rate in ml/ind./h
M = volume of test solution (ml)
n = number of animals/aquarium
t = duration of the experiment (h)
C_0= algal concentration at the beginning of the determination of the filtration rate
C_t= algal concentration at time t.

Water was collected and treated as in the April experiment. ^{64}Cu was allowed to equilibrate for 24 hours before introduction of the animals (5 OS and 5 WS animals in 1500 ml). Each series (OS 10 ‰, OS 30 ‰, WS 10 ‰ and WS 30 ‰) was carried out in triplicate. A control series with animals in OS 30 ‰ and WS 10 ‰ without ^{64}Cu was carried out simultaneously to assess the influence of the clams on metal and ligand concentration of the water. The exposed animals were dissected and measured after 48 hours. Because the dissolved ^{64}Cu concentrations during the exposure differed from the initial concentrations (see results),

accumulation in each series was corrected for the actual ^{64}Cu concentration.

Measurement of ^{64}Cu, Cu and organic ligand characteristics

^{64}Cu was obtained as described in Chapter Two. The stock solution of 1 mg/l Cu, spiked with ^{64}Cu, was always prepared just before starting the experiment. Samples (water, *M. balthica* shells or tissue) were counted in glass vials in a gamma counter with a NaI detector. Counting time was 10 minutes. Counting error was ≤ 5%. Corrections were made for ^{64}Cu decay. To minimize counting differences as a result of size variation, results were calculated per mm shell-length and per mg dry-weight.

Simultaneous with the collection of water for the exposure experiments, samples were taken for copper analysis and ligand characterization. All material used was acid-washed polyethylene. The samples were filtered under a low nitrogen pressure through an 0.45 µM cellulose nitrate filter. The filters were destroyed in a low temperature asher and redissolved in HCl/HNO_3. Particulate Cu was measured with graphite furnace atomic absorption spectroscopy (GFAAS) furnished with a Zeeman background correction using graphite tubes with L'vov platforms.

Part of the sample of the dissolved phase was acidified to pH=2 for the determination of total dissolved Cu and Zn. Dissolved organic ligands were destroyed by 4 hours of UV irradiation in the presence of H_2O_2 (Mart, 1979). Total dissolved Cu was measured by differential pulse anodic stripping voltammetry (DPASV), using a hanging drop mercury electrode and a collection potential of -0.6 V (PAR-EEG 303 or 303A electrode stand with a 384B analyzer).

Samples for DOC analysis were taken by filtering through pretreated glass fibre filters using a glass syringe with filter set. $HgCl_2$ was added to these samples to overcome bacterial activity. Dissolved organic matter was destroyed by UV irradiation and persulfate and determined as CO_2 colorimetrically (Schreurs, 1978).

Speciation of Cu was assessed by DPASV (differential pulse anodic stripping voltammetry), DPCSV (differential pulse cathodic stripping voltammetry) and by reversed phase chromatography (Sep-pak C_{18} cartridges) (Mills *et al.*, 1982; Mills and Quinn, 1984). DPASV gives us the complexation characteristics (total ligand concentration L_t in nanoequivalents of Cu/l=neq/l, and the conditional stability constant K' of the metal-ligand complex) of a group of ligands with moderately strong binding strengths with respect to Cu (log K' around 9.5) (Van den Berg and Donat, 1992). This ligand fraction is defined as those complexes that remain intact during the collection time (240 s. in this case) at the conditioning potential (-0.6

V in this case). With DPCSV the complexation characteristics of ligand groups with a relatively strong binding strength with respect to Cu (log K' around 14) are determined. This ligand group is defined by the complexation characteristics and concentration of an added ligand (Van den Berg and Donat, 1992). The added ligand was salicylaldoxime (SA) (Campos and Van den Berg, 1993). DPCSV was only applied to the samples from February 1993. A collection time of 60 s. was used at -0.1 V with an SA concentration of 10^{-5} M. The additions of Cu used to determine complexation with DPASV and DPCSV for the samples from the Westerschelde were respectively: 0, 32, 60, 80, 100, 140, 180, 220, 350, 425, 500, 750, 1000, 1300, 1700 and 2100 nM (the last five additions were only applied for DPASV). The additions for the samples from the Oosterschelde were respectively 0, 4, 8, 12, 16, 24, 32, 60, 80, 100, 140, 180, 260, 320, 400, 460 and 500 nM Cu (the last five additions were only used for DPASV). A nonlinear transformation of the Langmuir equation was used to estimate the conditional stability constant K' and the ligand concentration L_{asv} or L_{csv} (Gerringa *et al.*, 1991). pH was 7.8.

Sep-pak C_{18} cartridges retain relatively hydrophobic organic material. This method thus gives us the concentration of copper complexed with relatively hydrophobic organic material.

Calculation of copper species

The measured total dissolved copper and zinc concentrations were used in combination with the measured ligand concentrations and binding strength to calculate the concentrations of the different copper species in the experiments. For this calculation, an updated version of the chemical speciation program MINEQL (Westall *et al.*, 1976) was used (MINEQL$^+$, version 2.1) with pH fixed at 7.8.

RESULTS

Uptake and adsorption of ^{64}Cu

In the April experiment (Figure 4.1), accumulation in tissue was much more efficient in WS water than in OS water for both ^{64}Cu concentrations. There was less difference in shell adsorption between the two waters. The addition of EDTA caused a decreased tissue uptake in WS water with 400 nM ^{64}Cu, but at 80 nM ^{64}Cu did not have a significant effect. At 80 nM ^{64}Cu, uptake in OS water was increased by the addition of EDTA. Adsorption on shells was not influenced by EDTA addition.

In all experiments, part of the radiotracer disappeared from the solution through adsorption on surfaces. The dissolved ^{64}Cu concentration in the February experiment is shown in Figure

4.2. In the series with WS water, removal was less than in OS water, indicating that a larger part of the dissolved ^{64}Cu was kept in solution by complexing ligands. Because the actual ^{64}Cu concentration to which the animals were exposed was different from the initial concentration, uptake and adsorption were normalized to the actual ^{64}Cu concentration.

In Figure 4.3, the uptake of ^{64}Cu after 48 hours' exposure in WS and OS water in February is represented (expressed as cpm.mg DW^{-1} animal per cpm ml^{-1} water). In Figure 4.4, adsorption onto shells is given (expressed as cpm.mm^{-1} per cpm ml^{-1} water).
In contrast to the April experiment, uptake and adsorption in WS water was much lower than in OS water. The difference in uptake was very significant (Table 4.2). Uptake in WS water for OS animals was only 41 and 58% (for 10 and 30 ‰) of the values in OS water and only 23 and 48% for WS animals (Table 4.1).

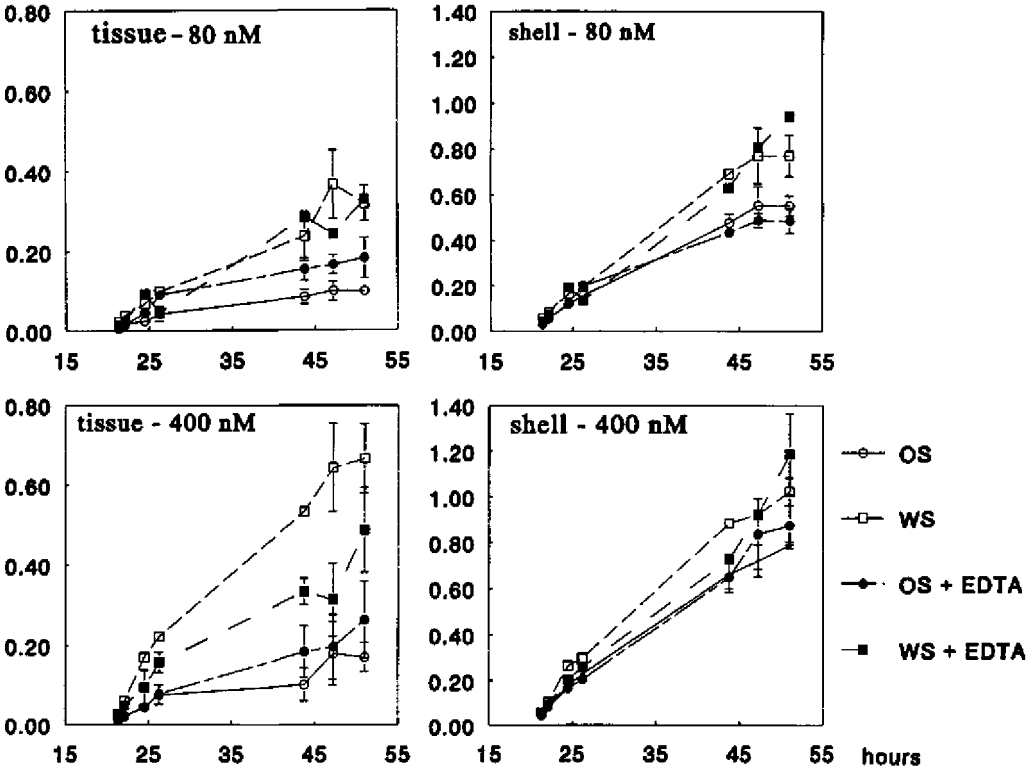

Figure 4.1 Tissue uptake and shell adsorption at 80 and 400 nM in April, expressed as cpm.mm^{-1} shell per cpm.ml^{-1} water.

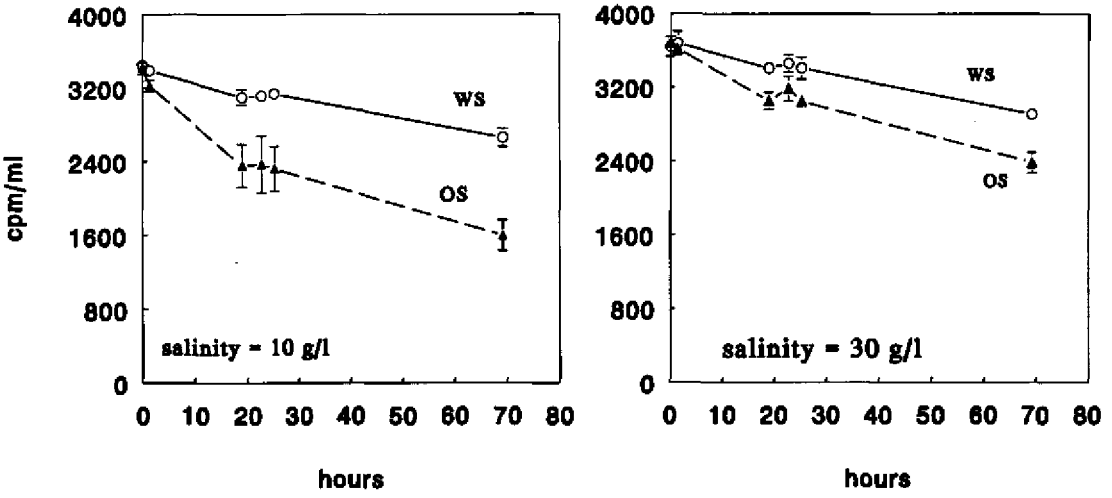

Figure 4.2 ^{64}Cu concentration (expressed as cpm.ml^{-1}) in Westerschelde (WS) and Oosterschelde (OS) water at different salinities in the February experiment.

The origin of the animals also influenced uptake results: in comparable exposure situations, uptake in animals originating from the Westerschelde was always lower than in animals originating from the Oosterschelde (Table 4.1).

Salinity had a distinct influence on ^{64}Cu uptake. In all conditions, uptake was increased at the lower salinity. The factors origin of the animal, kind of water and salinity were used as dummies in a regression analysis.

$$Cu_{tiss} = -0.22*SAL*OS\ -0.04*SAL*(1-OS)+\ 0.05*ORIG\ +\ 0.05*WA$$

(-4.1) (-0.7) (1.39) (4.81)

df=19 R^2=0.67

Where	$^{64}Cu_{tiss}$	^{64}Cu accumulated in *M. balthica*
	SAL	Salinity 10 ‰ or 30 ‰
	OS	Oosterschelde
	ORIG	*M. Balthica* from Oosterschelde or Westerschelde
	WA	water from Oosterschelde or Westerschelde
	( )	t-values are given between brackets

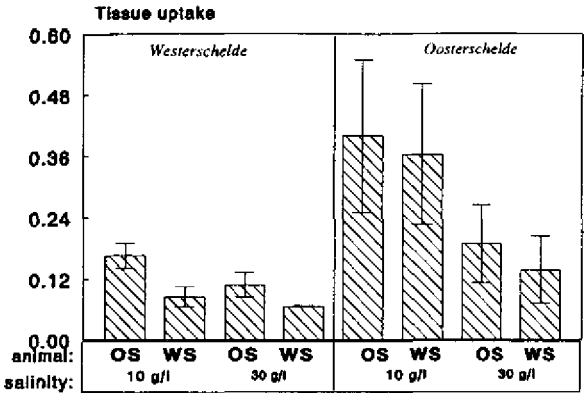

Figure 4.3 ^{64}Cu uptake in *Macoma balthica* after exposure in Westerschelde and in Oosterschelde water in February. Expressed as cpm.mg^{-1} DW per cpm.ml^{-1} water.

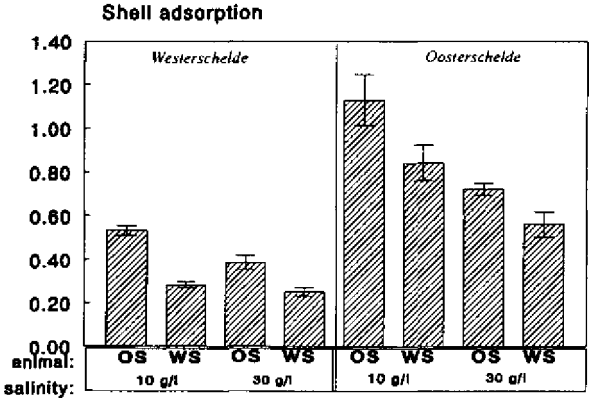

Figure 4.4 ^{64}Cu adsorption on the shells after exposure in Westerschelde and in Oosterschelde water in February. Expressed as cpm.mm^{-1} shell per cpm.ml^{-1} water.

WATER	ANIMAL	TISSUE	SHELL
10	OS	41	47
10	WS	23	34
30	OS	58	53
30	WS	48	44

Table 4.1a Accumulation in WS water relative to OS water (in %)

WATER	TISSUE	SHELL
WS-10	52	53
WS-30	60	65
OS-10	91	75
OS-30	73	78

Table 4.1b Accumulation by WS animals compared to OS animals (in %)

The salinity effect was very significant in Oosterschelde water (sal*OS), as was demonstrated with the regression analysis. Also the type of water (OS or WS) contributed significantly to the difference in ^{64}Cu uptake (see also Table 4.2).

WATER	ANIMAL	TISSUE	SHELL
WS	OS	66	72
WS	WS	76	88
OS	OS	47	64
OS	WS	38	67

Table 4.2 Uptake in 30 g/l salinity in relation to uptake in 10 g/l salinity (expressed in %)

Immediately after collection in the field, the water ventilation rate (expressed as clearance rate, CR) was lower for WS animals than for OS animals. After 23 days acclimatization to WS water (10 ‰), the clearance rate of OS animals had increased, whereas WS animals remained the same (Table 4.3). On the other hand, clearance rate of WS animals in OS water (30 ‰) had increased, but was still lower than the clearance rate in OS animals.

WATER	ANIMAL	CR start	sd	CR 23 days	sd
WS-10	WS	9.4	(3.6)	9.2	(4.9)
WS-10	OS			12.8	(5.5)
OS-30	WS			16.9	(5.4)
OS-30	OS	16.7	(3.6)	27.6	(4.4)

Table 4.3 Water ventilation rates during the acclimatization period, expressed as clearance rate (CR in $ml.ind^{-1}.h^{-1}$). Standard deviation is given between brackets.

Ligand characteristics

In the April experiment, DOC concentrations were around 4.25 mg/l in OS as well as WS water. L_{asv} was slightly higher in WS water. The conditional stability constant (log K'_{asv}) did not differ significantly between OS and WS water. As expected, total dissolved organic carbon (DOC) was not influenced by the EDTA addition (Table 4.4).

The relatively hydrophobic ligand-Cu complexes that were retained by Sep-pak C_{18} cartridges (Table 4.5) increased with added copper. Addition of EDTA caused a decrease in the amount of Sep-pak retained material. Except the control situation without added ^{64}Cu, in OS water always more Cu was retained by the Sep-pak C_{18} columns than in WS water.

In the February experiment, ligand characteristics were determined with ASV (L_{asv} and K'_{asv}) as well as with CSV (L_{csv} and K'_{csv}). Both L_{asv} and L_{csv} concentrations were much higher

in WS water (Table 4.6). Log K'_{csv} was difficult to assess in OS water, considering the large standard deviation with the starting measurement. At the end, determination of log K'_{csv} was not possible at all. In general, measurement of ligands was more subject to disturbance after the introduction of the clams as this caused an increase in surface active material.
During the experiment, the concentration of L_{csv} increased, whereas L_{asv} decreased during the 4 days between the collection of the water and the end of the experiment. L_{asv} in OS water was comparable with April 1992. In WS water, L_{asv} was more than twice the concentration of April 1992. Log K'_{asv} was significantly different between OS and WS water.

	start				end			
	OS	(sd)	WS	(sd)	OS	(sd)	WS	(sd)
total diss. Cu (nM)	7.72		20.7					
total part. Cu (nM)	14.8		67					
Lasv (neq/l)	191	(38)	237	(24)				
log K' asv	9.38	(0.37)	9.3	(0.3)				
DOC	2.75		-		4.25	(0.22)	4.38	(0.07)
DOC +EDTA					4.12	(0.22)	4.35	(0.14)

Table 4.4 Characterization of Westerschelde and Oosterschelde water in April 1992. Standard deviation is given between brackets.

	OS	WS
80 nM Cu addition	14.7	11.4
400 nM Cu addition	26.2	24.2
80 nM Cu + EDTA	10.4	7.6
400 nM Cu + EDTA	21.1	18.2
no addition	1.2	3.3

Table 4.5 Cu retained by Sep-pak C_{18} columns. The figures are the average of two measurements.

Calculated copper concentrations

Without added ^{64}Cu in Oosterschelde water as well as in Westerschelde water, L_t with a high log K' (K'_{csv} =14) is not saturated and therefore rules the concentration of Cu^{2+} and the distribution over the organic (L_{asv}) and inorganic species (e.g., OH and CO_3, see Table 4.7). This means that more than 99% of the total dissolved Cu is present as CuL_{csv} and that the ionic Cu concentration is extremely low (± 10^{-14} M). The ionic Cu concentration in OS water is slightly higher than in WS water, since the concentration L_{csv} OSis lower than the concentration L_{csv} WS (Table 4.7). After addition of ^{64}Cu, L_{csv} in WS water is saturated, but L_{asv} is still half filled. Now L_{asv} rules the free Cu^{2+} concentration and the inorganic species by

its low K'_{asv}. The free Cu^{2+} concentration is around 10^{-9} M (1 nM). In Oosterschelde water however, both L_{csv} and L_{asv} are saturated. The excess Cu is governed by the inorganic Cu species. As a consequence, the free Cu^{2+} concentration is very high, as are the inorganic species. According to the MINEQL calculations, the Cu^{2+} concentration would even result in precipitation of CuO. Because this actually did not happen, the CuO concentration was added up with the Cu^{2+} concentration.

	OS start	(sd)	OS end	(sd)	Ws start	(sd)	WS end	(sd)
DOC (mg/l)	1.9		3.4		15.5			
Sep-pak Cu (nM)	1.9				5.5			
total diss. Cu (nM)	10.2		50.2		31.3		80.7	
total diss. Zn (nM)	44		559		215.5		1301	
L asv (neq/l)	211	(16)	126	(39)	573	(105)	520	(258)
Log K' asv	9.48	(0.13)	8.93	(0.36)	9.4	(0.46)	8.77	(0.72)
L csv (neq/l)	19.6	(1.7)	58.9		116.8	(6.7)	137.6	(14.1)
Log K' csv	15.52	(3.5)	-		13.59	(0.17)	13.77	(0.33)

Table 4.6 Cu speciation of the control series with Westerschelde and Oosterschelde water in February 1993. Standard deviation is given between brackets.

DISCUSSION

The results of the April 1992 experiment were not as expected. In a preliminary experiment, similar unexpected results were obtained. Although the ligand concentration in WS water was predicted to be higher, uptake was much higher in WS water. Adsorption on the shell was comparable for WS and OS water. Addition of EDTA did not have any influence on ^{64}Cu uptake. This can be due to the influence of iron, which could have occupied the major part of EDTA binding sites. The ligand concentrations in OS as well as in WS water turned out to be relatively low, so differences in uptake could not be explained by copper complexation.

Another factor that could influence metal uptake, was salinity. The salinity of the estuarine WS water is lower than the salinity in OS water. In a low salinity environment copper may replace either calcium or magnesium in ion transport. In addition, the negative potential difference of the inner body is increased, so ion transport into the organism

	INPUT		WESTERSCHELDE				OUTPUT	WESTERSCHELDE			
	nM [Zn-t]	nM [Cu-t]	neq [L asv]	log K' asv	neq [L csv]	log K' csv	-log [Cu2+]	-log [CuL asv]	-log [CuL csv]	-log Cu(OH)2	-log CuCO3
Control start (15/2)	215	31.3	573	9.4	117	13.59	14.02	10.87	7.51	13.89	13.38
Control end (19/2)	1301	80.7	520	8.77	138	13.77	13.61	11.13	7.09	13.48	12.98
salin. 10 start	1072	351.3	573	9.4	117	13.59	9.57	6.63	6.93	9.44	8.93
salin. 10 end	955	400.7	520	8.77	138	13.77	8.8	6.6	6.86	8.67	8.17
salin. 30 start	440.6	326.3	573	9.4	117	13.59	9.65	6.68	6.93	9.57	9.6
salin. 30 end	347	375.7	520	8.77	138	13.77	8.87	6.63	6.86	8.79	8.82
salin. 10' start	440.6	426.3	573	9.4	117	13.59					
salin. 10' end	347	475.7	520	8.77	138	13.77					

	INPUT		OOSTERSCHELDE				OUTPUT	OOSTERSCHELDE			
	nM [Zn-t]	nM [Cu-t]	neq [L asv]	log K' asv	neq [L csv]	log K' csv	-log [Cu2+]	-log [CuL asv]	-log [CuL csv]	-log Cu(OH)2	-log CuCO3
Control start (15/2)	44	10.2	211	9.48	19.5	14	13.97	11.17	7.99	13.89	13.92
Control end (19/2)	559	50.2	126	9.48	58.9	14	13.25	11.21	7.3	13.17	13.2
salin. 10 start	64.4	250	211	9.48	19.5	14	8.34	6.71	7.71	8.21	7.7
salin. 10 end	102.5	290	126	9.48	58.9	14	7.84	6.91	7.23	7.71	7.2
salin. 30 start	152	280	211	9.48	19.5	14	7.88	6.69	7.71	7.8	7.83
salin. 30 end	254	320	126	9.48	58.9	14	6.87*	6.91	7.23	7.58	7.61
salin. 10' start	64.4	280	70	9.48	6.5	14	6.88*	7.16	7.19	7.58	7.07
salin. 10' end	102.5	320	42	9.48	20	14	7.37*	7.38	7.7	7.58	7.07

Table 4.7 Concentrations of Cu species in Oosterschelde and Westerschelde water as calculated by the speciation program MINEQL$^+$. The input values for added copper are derived from the ^{64}Cu measurements in the experiment (see Figure 4.2). * Indicates precipitation of CuO.

consequently increases (Phillips, 1976; McLusky *et al.*, 1986). This salinity effect was demonstrated in the February experiment. The effect could possibly explain the results of the April experiment, in particular if the acclimatization time of the clams had not been sufficient. Similar results were reported by Wright and Zamuda (1987). At fixed cupric ion activities together with varying salinities, they found that both oysters and soft shell clams accumulated significantly more copper at progressively lower salinities.

The April results could also be explained if the ligands in WS water had a rather hydrophobic (or lipophilic) nature. It is generally accepted that copper complexation by organic ligands decreases the biological availability of the metal, as the ionic form is transported across the cellular membrane. However, for some artificial metal chelators, it is reported that copper toxicity is greatly increased, instead of decreased (Florence, 1983). This is due to the lipophilic character of the ligand, which facilitates the transport of the Cu-ligand complex across the lipid-bilayer. The unidentified ligands in WS water could be partly more biologically available because of a lipophilic character. Whether this was the case was checked by the application of Sep-pak C_{18} cartridges, which are able to retain a relatively hydrophobic fraction (see methods). The amount of Cu retained by the Seppak cartridges in the April experiment (Table 4.6) in WS water was less than in OS water. This implies an absolute difference of 2-3 nM Cu. Considering the uptake results, a substantial contribution of hydrophobic ligands was not likely.

In the February experiment, WS water contained a considerable amount of Sep-pak retained ^{64}Cu, compared with OS water. However in this situation, ^{64}Cu uptake from WS water was reduced, which implies that these relatively hydrophobic Cu-species are not particularly bioavailable or toxic. The hypothesis of a high bioavailability of these lipophilic ligands can then be rejected. Also in literature, reports on positive correlations between complexed copper concentrations and copper levels in tissue are scarce. Only for oysters, has this been shown (Martincic *et al.*, 1986, 1987). However, tissue levels in mussels from the same area correlated better with 'ionic' Cu. In tests with *Daphnia magna* it was shown that complexed copper in natural waters was more toxic than Tris-Cu. However, it was less toxic than free copper (Borgmann and Charlton, 1984).

In the February experiment, DOC concentrations of the WS water were very high (>12 mg/l, compared to ± 5 mg/l during the rest of the year). The elevated ligand concentrations in the Westerschelde estuary are typical for the months December untill March (Gerringa, pers. comm.). The origin of this increase is most probably dissolved organic material, released through ground water from inland soils. Considering the low salinity (10 ‰), the riverine influence at the time of water collection has been extra large.

The total ligand concentration of WS water was very high, compared with OS water. The decrease of L_{ASV} during the experiment was most probably due to degradation of ligands. The increase of L_{CSV}, ligand groups with a relatively strong binding strength with respect to Cu, could be caused by a conversion of L_{ASV} to L_{CSV}, or by excretion of DOC by the animals. This would imply that biological availability of copper in steady state experiments will decrease not only through sorption but also by complexation as a result of the presence of organisms.

The results from the uptake and adsorption experiment of February showed that the availability of dissolved copper was largely reduced in WS water, compared with OS water (see Figure 4.3). In spite of the extended acclimatization time, the salinity effect was still present. This effect was less obvious in WS water (see regression equation), which can be explained by its high organic complexation: with a high ligand concentration in WS water, a considerable part of the ions is complexed. With decreasing salinity, copper would normally replace magnesium or calcium in ion transport. However, copper complexation prevents the increased uptake. This can explain the small difference between uptake in 10 ‰ and 30 ‰ salinity. The increased uptake in OS 10 ‰ water can also be caused by a lower concentration of dissolved organic ligands as a result of the dilution from 30 to 10 ‰. Regarding the increased $[Cu^{2+}]$ when calculated with 1/3 of the initial ligand concentrations (Table 4.7), this is an obvious possibility.

With the calculation of Cu species in OS water, precipitation of $Cu(OH)_2$ occurred. Cu^{2+} concentrations were apparently high enough to exceed the solubility product.

Another salinity effect is also interfering with complexation: at lower salinities, fewer ions compete for binding with organic ligands. As a consequence, copper complexation will be more effective and uptake would be decreased at lower salinities. However, this effect seems to have played a minor role in the final experiment. With the MINEQL calculations, this effect was incorporated in K' (which is salinity specific).

In February, uptake in animals origining from the Westerschelde was always lower than uptake in animals from the Oosterschelde. The difference in water ventilation rates (Table 4.4) is the most obvious explanation for this. WS animals had a consistently lower ventilation rate, compared with OS animals.

How important are the factors salinity and ligand concentration for the determination of copper uptake in the Westerschelde estuary? *Macoma balthica* is tolerant of low salinities (Broman and Ganning, 1986), so it will adapt to a large extent to the salinity range in its

environment. It is not certain how a sudden salinity change will influence metal uptake. However, the decreased ion competition at the membrane surface at lower salinities is a physico-chemical process that will in any case increase the biological availability of trace metals. On the other hand, high ligand concentrations (in estuarine areas often co-occurring with low salinities) tend to reduce the increased bioavailability, caused by the lower salinity.

To conclude, experiments carried out in April, showed an unexpectedly high uptake of ^{64}Cu by *Macoma balthica* in natural water with a theoretically higher proportion of complexed copper. This could be explained by either the nature of the natural ligands or by salinity effects. To unravel the relative importance of these mechanisms, comparable experiments was carried out in February, when DOC concentrations were known to show a peak in WS water. The animals were allowed to get accustomed to an increased (or decreased) salinity for an extended period. In addition, degradation of ligands and the water ventilation rates of the animals were controlled. From the results of these experiments it was obvious that complexation by natural ligands, present in the Westerschelde, caused a large reduction of biologically available ^{64}Cu. Total dissolved Cu concentrations did not have any relation with the bioavailable fraction, while the free ionic Cu did.
The higher ^{64}Cu uptake in WS water in the April experiments could be explained by a salinity effect. This salinity effect was masked in the February experiments by high ligand concentrations in the Westerschelde. In the Westerschelde estuary, dissolved copper uptake by *Macoma balthica* will be determined mainly by the ligand concentration in the overlying water. At low ligand concentrations (as occurred in April), salinity will have a major influence.

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Chapter Five

Accumulation and effects of copper, cadmium and zinc from spiked aged sediments on *Macoma balthica* (L).

ABSTRACT

In semi-field experiments, the accumulation and behavioural effect of heavy metals from different sediment types on the benthic bivalve *Macoma balthica*. Sediments with different grain size composition and organic carbon content were spiked with cadmium, copper and zinc and aged, in order to reach equilibrium conditions that would be comparable to the field situation. The maximum metal concentrations in the spiked sediments were comparable with the worst case harbour sludge from Dutch estuarine regions. During the exposure, clean filtered seawater was running continuously over the sediment.

The observed effects on burrowing behaviour, mortality and bioaccumulation were to a large extent related to sediment characteristics. The strongest effects and the highest bioaccumulation were observed in sediments with the lowest silt and clay fractions. In sediments with more than 50 % < 20 µm no effects on burrowing behaviour were observed, not even in the highest dosage. In this most polluted sediment tissue body burdens of metals did not reach lethal concentrations. Cu and Zn accumulation was related to sediment type as well as pollution level. Cd accumulation was only related to the pollution level. In our experiments, spiked aged sediments were much less toxic than freshly spiked sediments. From the results the conclusion may be drawn that metal availability was very low in aged silty organic rich sediments. It was demonstrated that it is important to pay close attention to the experimental setup, so the achieved data can be extrapolated to the natural situation in the field.

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INTRODUCTION

Estuaries are generally sinks for pollutants, brought in by rivers. Dissolved pollutants will adsorb onto suspended matter and in the turbidity zone they will sink to the sediment. The result is that estuarine sediments contain high concentrations of pollutants. Metals will partition among different sediment components, so that they will be distributed among several physico-chemical forms. Trace metals are adsorbed onto iron or manganese oxides, clays, associated with organic matter such as bacteria and humic substances, precipitated in sulphides (in reduced sediments) or in lattice positions in secondary minerals. The different metal fractions in the sediment are more or less labile and therefore differ in biological availability (Förstner, 1990).

To assess biologically available trace metals, efforts have been made to relate chemical stability -as assessed by sequential extraction procedures- to accumulation in benthic invertebrates (Luoma and Bryan, 1978; Langston and Bryan, 1984; Tessier *et al.*, 1984; Tessier and Campbell, 1987). In both freshwater and marine systems, generally there is a higher correlation between metal levels in benthic invertebrates and the relatively easily extracted fractions than total metal concentrations (Diks and Allen, 1983).

As a supplement to these geochemical estimates of metal bioavailability, metal accumulation by organisms can provide a measure of metal bioavailability. Because most aquatic organisms, and benthic invertebrates in particular, tend to accumulate toxicants from the environment, they can be used as a monitor for the assessment of pollution (Bryan, 1985). However, uptake from either the sediment or the water will be influenced by physico-chemical factors in the aqueous and particulate phases. Although the biological and geochemical processes affecting metal bioavailability have been reviewed extensively (Bryan, 1985; Campbell *et al.*, 1988; Luoma, 1989), most processes are still not adequately understood.

To establish relations between bioaccumulation and various sediment characteristics, spiking procedures are a useful tool in assessing effects and accumulation in organisms. However, it is difficult to compare the results from spiked sediment assays with natural sediments, because as a result of manipulation of the sediment, biological availability can be different from the natural situation. Also, one must be sure that an equilibrium situation has been reached with the toxicant in the sediment (Giesy and Hoke, 1990).

In an attempt to approach 'estuarine' conditions with spiked sediments, an experimental setup was designed in which the deposit feeding tellinid bivalve *Macoma balthica* (Baltic tellin) was exposed to different degrees of metal pollution in four sediments with different grain size distribution and organic matter content. After preparing the spiked and control sediments, they were left for five months to age with regular water refreshment. During the

experiment the water was refreshed continuously, as it is in naturally occurring estuarine conditions.

The aims of the experiment were threefold: Firstly, the behavioural response of *Macoma balthica* in aged spiked sediments in relation to sediment quality was measured. A burrowing behaviour test can be a very useful tool in the first assessment of toxicity of a certain sediment (McGreer, 1979; Chapman *et al.*, 1987; Phelps, 1989). Secondly, growth and bioaccumulation in *Macoma balthica* were assessed in relation to metal concentrations in the sediment and to sediment characteristics in aged as well as in freshly spiked sediments. Finally, bioaccumulation in *Macoma* was related to different metal fractions in aged spiked sediments: total metal content, metals in the grain size fraction <63 µm and 1 M HCl extracted metals from the fraction <63 µm.

MATERIALS AND METHODS

Location

The experiments were carried out at a field laboratory situated near the storm surge barrier at the mouth of the Oosterschelde estuary in the Netherlands. At the laboratory, seawater (salinity 32 ‰) is pumped at a rate of 20 m^3 h^{-1} from an inlet 200 meters from the shore, close to the storm surge barrier. The water used in this experiment is filtered through a 2 m^3 sand filter.

Experimental design

-Unaged sediment experiment (Experiment 1)

As a preliminary experiment, *Macoma balthica* was exposed to a sandy low organic and a silty high organic sediment, spiked with Cu in a stagnant water system. The clams were introduced immediately after spiking and allowed to accumulate during ten days. At regular times, sediment boxes were collected and Cu accumulation in *Macoma* was assessed.

-Aged sediment experiment (Experiment 2)

A sandy low organic sediment and a silty organic rich sediment were mixed in different ratios, to obtain a range of four degrees of organic enrichment and four degrees of grain size composition in the sediment: *A)* 100% sand and 0% silt; *B)* 65% sand and 35% silt; *C)* 35% sand and 65% silt; *D)* 0% sand and 100% silt. The silty sediments in experiment 1 and 2 were similar. The sandy sediments were from a different location. The composition of the

natural sediments and the mixtures of experiment 2 is described in Table 5.1. The grain size distributions of the natural sediments and the mixtures are given in Figure 5.1.

sed. mixture	A	B	C	D
% <63 um	8.2	29.4	58.9	83.8
% <19 um	2.6	18.2	36.8	53.8
% CaCO3	2.3	6.6	10.9	16.4
% organic carbon	0.09	0.69	0.81	1.41
% nitrogen	0.02	0.06	0.11	0.19
Cd (mg/kg)	0.05	0.24	0.57	0.82
Cu	1.3	3.3	7.5	14
Zn	11	31	66	122
Pb	4.6	15	30.8	45
Mn	93	125	185	260
Fe (g/kg)	6.6	9.3	13.8	19.2

Table 5.1 Composition of the natural sediments and the mixtures of experiment 2 before spiking.

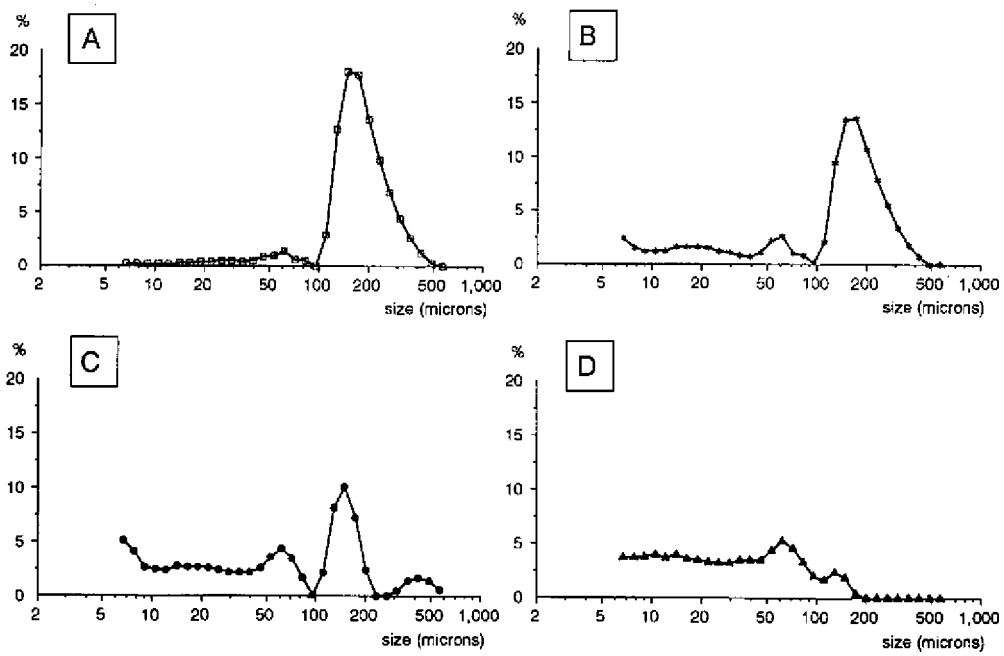

Figure 5.1 Grain size distribution of the natural sediments and the mixtures of experiment 2.

Poll. level	0 (control)	1	2	3	
Cadmium	1.2	10	20	50	mg/kg
Copper	12	100	200	500	mg/kg
Zinc	100	500	1000	2000	mg/kg

Table 5.2 Nominal values (in case of 100% sorption) of cadmium, copper and zinc in the spiked sediments in experiment 2.

The sediment mixtures *A* to *D* were spiked with a mixture of Cd, Cu and Zn to obtain four different degrees of contamination (three spiked and one control sediment). The nomimal values (in case of 100% sorption) are described in Table 5.2. The concentration range was representative for the different degrees of metal contamination that can be found on polluted locations in Dutch estuaries. The highest contamination level in the 100 % sand was not used in the experiments, because the adsorption capacity of the sediment was apparently exceeded: after spiking, metal precipitates were visible on the sand surface. The 15 experimental groups will be indicated as A_0 to A_2, B_0 to B_3, C_0 to C_3 and D_0 to D_3.

To reach equilibrium conditions and to be able to compare the biological availability of the spiked sediments with natural sediment, an aging time of five months was taken into account. *Macoma balthica* was introduced in the sediment after five months' aging. With this introduction, a burrowing test was carried out. After burrowing, the clams were left in the sediment for the accumulation experiment. At the beginning and at the end of the 54 days exposure time, the clams were analysed for condition and tissue metal content.

Collection of sediment and Macoma

For both experiments, the sediments were collected from the surface during low tide at two locations in the Delta area in the Netherlands (see Figure 1.1). At *Oesterput*, an old abandoned fishing harbour in the relatively unpolluted Oosterschelde Sea arm (salinity 32 ‰), silty sediment of high organic content was collected. This silty sediment was used in experiment 1 and 2. For experiment 1, sand, low in organic matter was collected in the Oosterschelde (salinity 32 ‰). Because on this location no natural population of *M. balthica* existed, this sand was not used for experiment 2.

For experiment 2, sand was collected 160 m from the dike at the mudflat *Baalhoek* in the more polluted Westerschelde Sea arm (salinity 19 ‰). On this location, a healthy natural population of *Macoma* exists. For experiment 1, the sediments were frozen to kill indigenous

organisms. For experiment 2, the mud and sand were filtered through a 4 mm polyethylene sieve to remove the larger invertebrates. The smaller invertebrates were allowed to remain in the sediment, because a basic level of bioturbation was desired to approximate natural conditions. After settling for 12 hours, the sediments were decanted and stirred roughly. The sediments were sealed in 1-l polyethylene bags and stored for one week at 4 °C to minimize degradation of organic matter before further use.

Three- and four-year-old *Macoma balthica* (12-15 mm) were collected at *Paulinapolder* (see Figure 1.1), an intertidal mudflat in the Westerschelde estuary west of Terneuzen. Although this estuary is relatively polluted, *Macoma balthica* background tissue-metal levels are similar to, or even lower than Oosterschelde *Macoma*. To acclimatize, the *Macoma* were kept for two weeks in clean sandy sediment in unfiltered flowing Oosterschelde water (50 l/hour). In experiment 1, the clams were not fed during the 10-day experiment. In experiment 2, the animals were fed once a week with 1 l of an outdoor batch culture of the algae *Phaeodactylum tricornutum* (average density 1.7 x 10^6 cells/ml). With the food addition, water flow was stopped during one hour to prevent the algae from being washed out too quickly.

Preparation of test material

For the spiking procedure, the sediments were mixed (1:4 wet sediment: filtered seawater) in 20-l Plexiglass circular tanks. Stock solutions of the metals were added at the start of the mixing period. Mixing was carried out with stainless steel stirrers for two hours. The control sediments were mixed as well, but received deionized water instead of metal stock solutions. After mixing and settling of the sediment particles, the overlying water was drained. Fresh filtered seawater was added and after another hour of mixing, the sediment was left to settle again.

For experiment 1, the spiked and control sediments were divided among Plexiglass beakers and placed with five together in Plexiglass circular tanks. Twenty *M. balthica* were allowed to burrow in each sediment beaker. Water was not refreshed during the ten days' experiment, but kept in circulation to provide sufficient oxygen. If a sediment beaker was removed for analysis, the water level in the tank was adjusted to the orgininal with filtered sea water. The experimental series were carried out in duplicate.

For experiment 2, the spiked sediments were divided equally among polyethylene exposure chambers (0.7 dm^3), yielding seven replicates. The chambers were placed in large white polyethylene water baths (42b x 60l x 30h cm). The sediments were left to age from January until June 1991. The sediments were left to age for five months while being kept in stagnant water and dim light. From January to June (the aging period), the ambient temperature at the field laboratory was varying between 5 and 15 °C. The water was refreshed weekly. During

the experiment, the water temperature varied between 13 and 17 °C. Three weeks before the introduction of the animals a continuous laminar water flow was set on the water baths. This flow was achieved by peristaltic pumps that pumped water into an overflow gutter from which water entered over the whole width of the bath. At the opposite side of the bath, a row of outflow holes provided the water discharge. The pumps provided a flow of 30 dm^3 per hour of filtered seawater over the exposure chambers (refreshment rate in the baths was approximately three times per hour). Sediments with the same pollution level were kept together in one water bath. The sediments varied in organic matter, so possible differences in leaching by the overlying water were to be expected. The sediments with the least adsorption capacities (the sandiest sediments) were placed near the outflow. In this way, *Macoma* could not accumulate metals from other sediments (via the overlying water), only from the one they were inhabiting.

After the aging period (at the start of the sediment exposure), one exposure chamber was taken for analysis of the sediment. The upper 5 mm layer (where *Macoma* feeds) and the bottom (4-7 cm) layer of the sediment (where *Macoma* dwells) were separately collected. Further, a burrowing test was started by distributing 45 randomly chosen *Macoma* from the stock in each exposure chamber. In four out of the six remaining replicates, the burrowing time was assessed. The number of *Macoma* that were not 100 % buried (no longer visible) was recorded every five minutes for the first hour and every ten minutes for the next hour. Difference in burrowing behaviour was assessed at T=60 minutes: data were tested in an Analysis of Variance after arcsin transformation of the data. Calculations were carried out with the software package SYSTAT. After burrowing, the clams were left in the sediment for the accumulation experiment. During the experiment, number of clams that appeared on the surface (moribund) and the number that died afterwards, were recorded.

Physico-chemical analyses

The sediments were analyzed for total Cd, Cu and Zn. Additional measurements included grain size distribution, organic carbon, nitrogen and $CaCO_3$ content. In the <63 µm fraction of the sediment, metals were extracted with 1 M HCl. During aging of the sediment and during the experiment, water samples were taken regularly to follow possible leaching.

Water samples for total metal analysis were taken in acid washed polyethylene bottles and acidified to pH=2. The samples were stored at -20 °C. Before analysis, the water samples were U.V. irradiated during four hours to destroy organic material. Total (dissolved and particulate) Cd, Cu and Zn were measured with Differential Pulse Anodic Stripping Voltammetry with an EG & G hanging mercury drop electrode, connected with a 384B Polarographic Analyzer. Samples for dissolved organic carbon (DOC) analysis were taken by

filtering through pretreated glass fibre filters using a glass syringe with filter set. $HgCl_2$ was added to overcome bacterial activity. Until analysis, the samples were stored at 4 °C. Dissolved organic matter was destroyed by UV irradiation and persulfate and determined as CO_2 colorimetrically (Schreurs, 1978).

Sediments were sampled in polyethylene bags and stored at 4 °C until processing. Within five days after sampling, a part of the sediment was sieved through a 63 µm mesh screen for HCl extraction.

For the extraction procedure, sediment out of 10-15 ml of the sieved slurry was collected under nitrogen pressure through acid-cleaned 0.45 µm cellulose-nitrate filters (Sartorius). 3x5 ml 1 M HCl (Merck, p.a.) were applied sequentially to the filter and allowed to pass through for a total of 30 minutes. The 15 ml HCl were collected in acid-cleaned 20 ml p.e. bottles. The extracts were analysed for metals using an atomic absorption spectrometer (AAS). A flame AAS Perkin Elmer Model 2380 was used for Zn, Mn, Fe and higher levels of Cd and Cu. The low level Cd and Cu concentrations were measured using a Perkin Elmer Model 3030 with graphite furnace and a Zeeman background correction system. Identically filtered slurry samples were freeze-dried to assess the sediment dry weight.

The remaining unsieved sediment was freeze-dried for further analysis. The samples were analyzed for grain size distribution, particulate organic carbon (POC), total nitrogen, $CaCO_3$ and total metals. After separation of clay and sand particles, removal of organic material and carbonates, grain size distribution was assessed with a laser diffraction technique, (Malvern Particle Sizer type 3600 Ec). POC and total N were measured on a Carlo Erba Nitrogen/Carbon analyzer, type NA 1500. $CaCO_3$ was measured volumetrically.

For the total metal analysis, the freeze-dried samples were ground in agate bowls. 500 mg of sediment was destructed with *aqua regia* in closed PTFE beakers in a microwave oven (Nieuwenhuize *et al.*, 1991). The samples were measured with AAS as described above.

Macoma balthica were allowed to clean their guts for 12 hours in filtered seawater. Until dissection, they were kept frozen at -20 °C. After dissecting, freeze-drying and grinding with a mortar, the *Macoma* tissue samples (around 400 mg each) were digested in a mixture of 8 ml HNO_3 and 2 ml HCl in the microwave oven (Nieuwenhuize and Poley-Vos, 1989). The samples were measured with AAS as described above. BCR mussel tissue (CRM no 278) was used as reference material. For the spiked aged sediments, the relation between metal accumulation and sediment quality (sediment type as well as pollution level) was tested in a 2-way Analysis of Variance (Sokal and Rolf, 1970).

RESULTS

Experiment 1

As adsorption in the sandy low organic sediment was expected to be less efficient than in the silty high organic sediment, the Cu concentration in the stock solution used for spiking the sandy sediment was twice the concentration, used for spiking the silty sediment. This resulted in an actual Cu increase of 0.74 to 2.6 mg/kg DW in the sandy sediment and of 7.9 to 12.0 mg/kg DW in the muddy sediment. Sediment concentrations did not change significantly during the ten days' experiment. *Macoma* accumulated Cu during the first two days in all treatments, including the controls (Fig. 5.2). In the spiked sandy sediment, tissue copper concentration increased significantly. In the muddy sediments no further accumulation was noticed, although total copper content in the muddy sediment was much higher than in the sandy sediment. During the ten day exposure time, no mortality was observed.

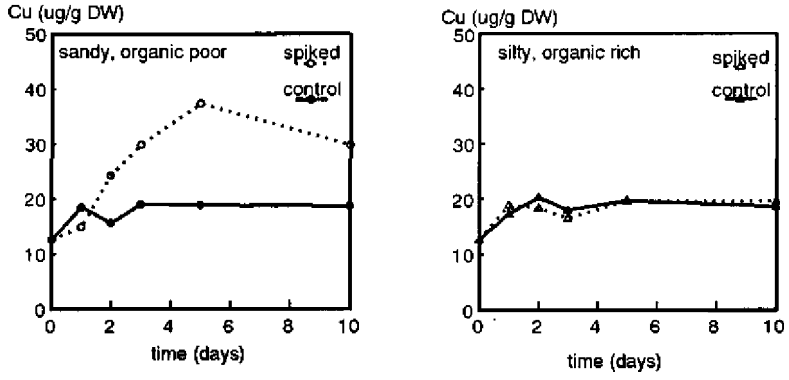

Figure 5.2 Cu accumulation in *M. balthica* after exposure in freshly spiked sediments in experiment 1. The points are the average of two replicate samples.

Experiment 2

Sediment and water

The dissolved organic carbon (DOC) concentration in the overlying water during the accumulation experiment varied from 1.83 to 2.75 mg/l in June and 1.28 to 1.95 mg/l in August. There was no significant difference between the different water baths. During the experiment, total metal concentrations in the water did differ between the water baths: concentrations in the water bath with control sediments varied for Cu between 3.3 and 10 nM.

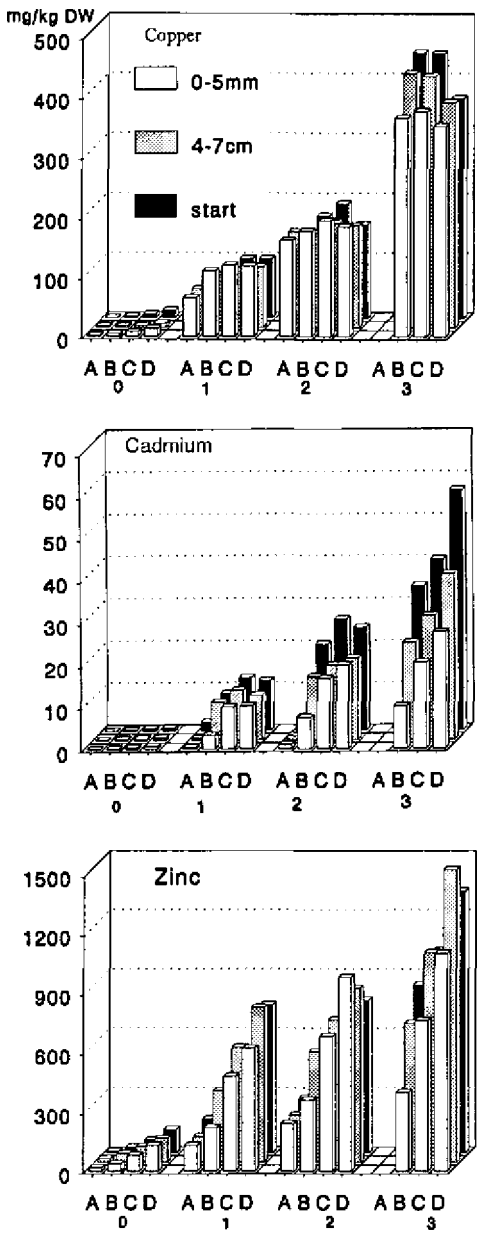

Figure 5.3 Metals in the sediment immediately after spiking (black bars), in the top 5 mm (light grey bars) and 4-7 cm layer (dark grey bars) after 5 months aging in experiment 2.

With level 1 polluted sediments between 8.9 and 19.4 nM. With level 2 pollution between 12.5 and 15.7 nM and with the highest pollution level (3) between 18.8 and 25.3 nM. Total Cu concentration in the supply water varied between 6.5 and 8.8 nM. Cd was not detectable (less than 0.05 nM) in the supply water and the control box. In the polluted-sediment water baths, Cd concentrations varied between 0.17 and 0.41 nM. Zn concentrations in the supply water varied between 45 and 76 nM. In the control bath between 21 and 86 nM; in the pollution level 1 bath between 70 and 103 nM; in the level 2 bath between 128 and 190 nM and in the level 3 bath between 94 and 228 nM.

In Figure 5.3, the metal concentrations immediately after spiking (*start*) are presented together with the top 5 mm and the 4-7cm layer after 5 months' aging. The other sediment characteristics are given in table 3. The initial concentrations (immediately after spiking) in the 100% silty organic rich sediment (D) were most consistent with the theoretical concentrations of metals in the spiked sediments (Table 5.2). In the sandier sediments, adsorption was less efficient due to the few binding places. For Cu however, adsorption turned out to be very efficient in the sandier sediments as well. For comparison with the initial situation, Cd was leached from the sediment during the aging period. The Cd content in the deeper layer was decreased by an average of 28 % during the aging period. In the top layer the concentration had decreased to less than half the initial concentration. Cu

concentrations in the 4-7 cm layer were decreased slightly in all sediments after the aging period. In the top 5 mm layer of pollution levels 1 and 2 however, Cu concentrations were higher than in the 4-7cm layer. For Zn, a relative increase in the deeper layer of the more silty sediments was observed, whereas the top layer contained relatively less Zn compared with the initial situation.

Accumulation and effects in Macoma balthica

-Burrowing behaviour in aged sediments

The burrowing behaviour in the polluted sediments depended very much on the type of sediment used. In the 100 % sandy organic low sediment (A), a distinct difference in burrowing behaviour was observed between contamination level 2 and the lower levels (Figure 5.4a). It has to be noted that sediment A_3 was not used in the experiments because of precipitation of metals salts (see materials and methods). Both the factor sediment and the factor pollution level were significant ($P<0.005$) in accounting for the difference in burrowing behaviour. In the sandy spiked series and the most polluted low silt series (A_1, A_2 and B_3), some animals were still not burrowed after 100 minutes. In all more or less silty sediment mixtures (B,C and D), the effect of metal contamination could not be distinghuished (Figures 5.4b-d).

The burrowing behaviour in the control of the sandier substrates A and B was slightly different from the muddier substrates C and D (Figure 5.4e). However, these differences were not significant.

-Mortality, growth and condition

No mortality occurred in the control (0) series. From the burrowed clams in the most polluted sandy series (A_1, A_2 and B_3), the majority of the animals appeared on the sediment surface within five days, indicating that they were in a severely stressed situation (Figures 5.5a-c). Within the first ten days of the experiment, the moribund animals started to die in A_2 and B_3. In A_1 mortality started after 11 days. On day 17, these sediment series were taken out of the experiment because the majority of the animals had died. It was concluded that Cu was causing mortality in these experiments (see further). In the remaining series, no mortality occurred during the experiment, except B_2 and B_3 where 2-6% had died by the end of the 57-day exposure. During the exposure, no increase in length was observed in any of the experimental groups. The dry weight of the clams had not decreased significantly compared to the starting situation (Table 5.4). However, the *Macoma* in A_1 and A_2 that were almost

dead had a much lower dry weight (19.2 and 17.5 mg) compared with the controls (27.6 ± 2.4 mg). Although mortality occurred in B_3, dry weight was still 22.7 ± 11.1 mg.

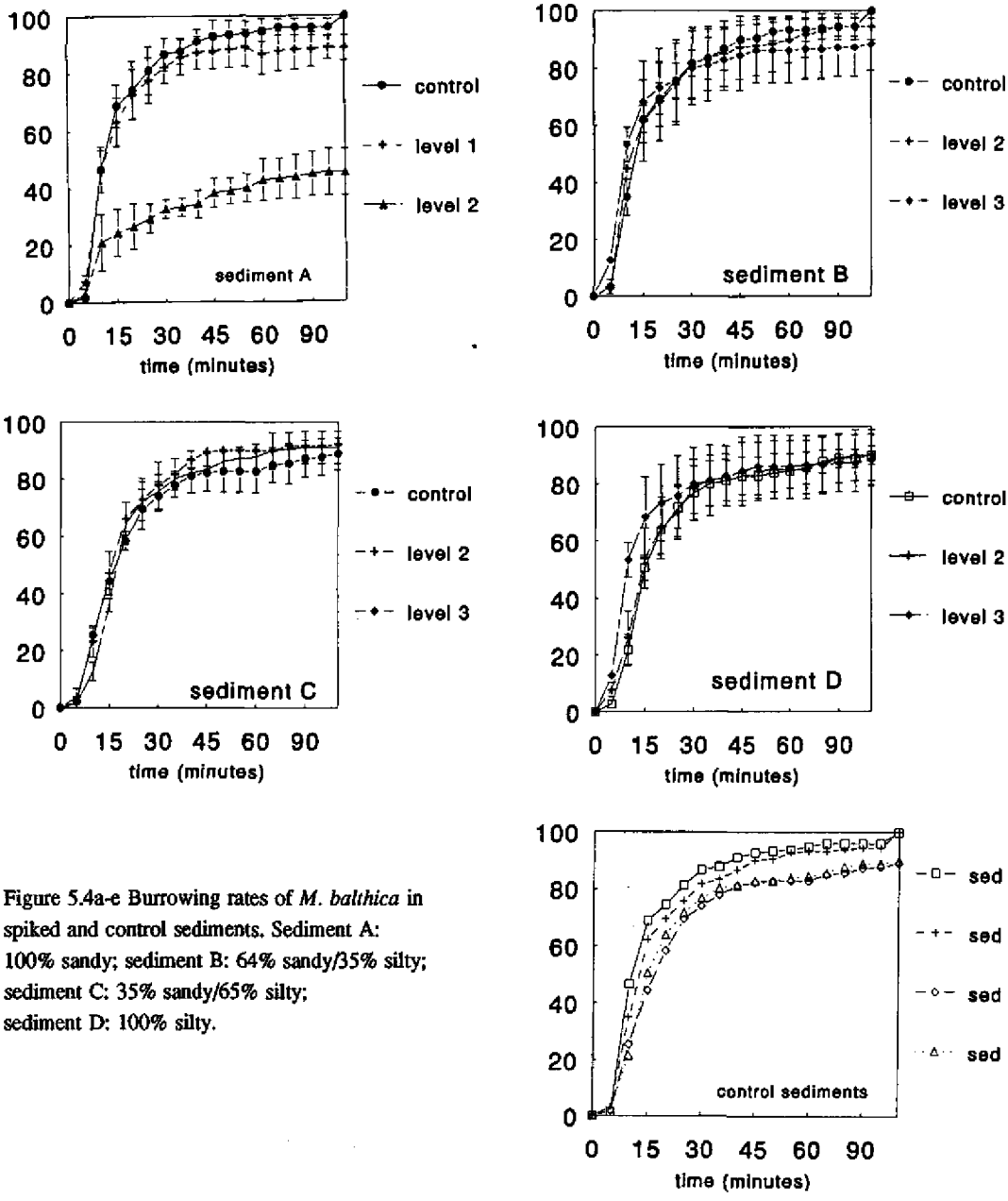

Figure 5.4a-e Burrowing rates of *M. balthica* in spiked and control sediments. Sediment A: 100% sandy; sediment B: 64% sandy/35% silty; sediment C: 35% sandy/65% silty; sediment D: 100% silty.

-Metal accumulation in relation to sediment quality

With the spiked unaged sediments of experiment 1, a significant increase in tissue-Cu levels was found in the sandy low organic sediment. In the silty high organic sediment, no effects could be observed, although total sediment-Cu concentrations were higher than in the sandy sediment (see figure 2).

In the control aged sediments (experiment 2) accumulation was not significantly different (Figures 5.6a-c). For the spiked sediments, accumulation was lower, the higher the concentration of clay particles and organic matter content. Within one pollution level, the difference between the different sediment types was most obvious with Cu: the type of sediment accounted to a large extent for the differences in Cu accumulation. In the sandy substrates A_1, A_2 and B_3 Cu levels in *M. balthica* turned out to be extremely high, in spite of the shorter accumulation time.

With the aged sediments, the relation between metal accumulation and sediment quality was tested in a 2-way ANOVA. The sandiest sediment (A) could not be used in the analysis because in the A_3 case no data were available. The A_1, A_2 and B_3 sediment accumulation time was shorter, so they could not be compared in the statistical analysis. For Cu, more than for Cd or Zn, sediment properties influenced metal uptake. Differences in accumulation were very significant ($P<0.001$) for sediment type as well as for pollution level. For Cd, only the pollution level was significant in accounting for differences in accumulation ($P<0.001$). For Zn, both the

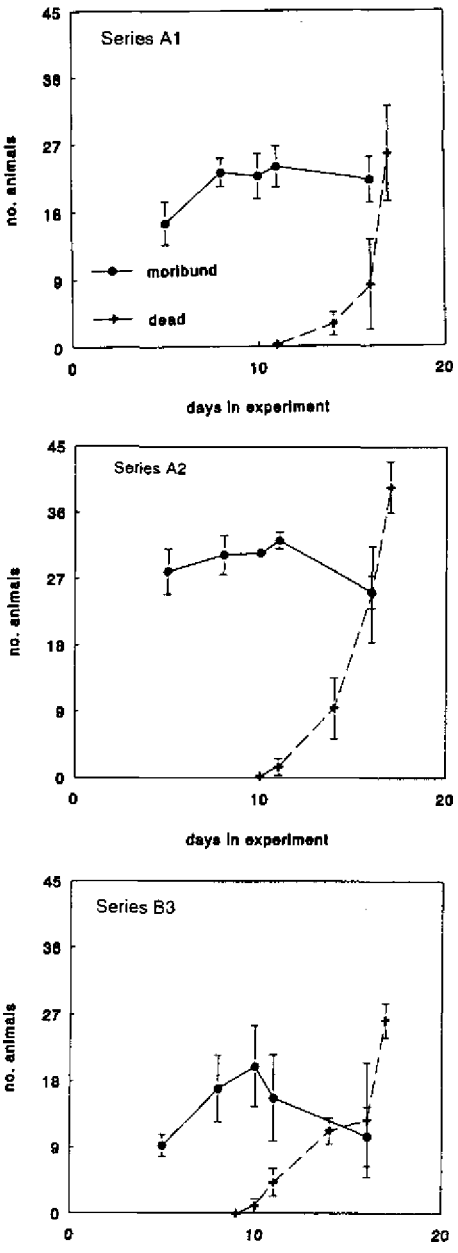

Figure 5.5 Mortality of *M. balthica* in the sandy sediments of experiment 2.

sediment type and the pollution level were significant (P<0.001): the muddier the sediment, the less accumulation was observed.

No relation was found between total metal contents in the sediment on the one hand and tissue levels on the other hand. A better relation was found if a correction was made for the fraction <63 µm or the HCl-extracted fraction (see Figure 5.7). For Cd, the best relation between tissue concentrations and sediment was found after correction for the <63 µm fraction. The linear relation can be described as:

$$[Cd]_{Macoma} = 0.09\ [Cd]_{<63\mu m} + 0.63 \quad R=0.92 \quad P<0.001 \qquad (1)$$

A linear relation was also found for Zn after the Zn content was corrected for the fraction <63 µm:

$$[Zn]_{Macoma} = 0.12\ [Zn]_{<63\mu m} + 240 \quad R=0.95 \quad P<0.001 \qquad (2)$$

The tissue-copper concentrations were best correlated with the 1 M HCl exchangeable sediment fraction <63 µm. However, the 100% sandy sediments were not included!

$$[Cu]_{Macoma} = 0.28\ [Cu]_{HCl\ exch.} + 16 \quad R=0.97 \quad P<0.001 \qquad (3)$$

DISCUSSION

Metal accumulation

As the design of experiment 1 and 2 was rather different, the results cannot be compared directly. Unfortunately, it was practically not possible to test the sediments in experiment 2 immediately after spiking. Therefore, the results from a preliminary experiment with comparable animals and sediments were presented to point at the possible effects of a different experimental protocol. It was striking, that effects were observed at much lower metal concentrations in the freshly spiked sediments. In experiment 1, the overlying water was not refreshed. It is very likely that accumulation from the overlying water took place, as most probably much Cu had been leaching to the overlying water. However, also the difference in sediment composition of the sandy, organic low sediment in experiment 1 and 2 could have played a role.

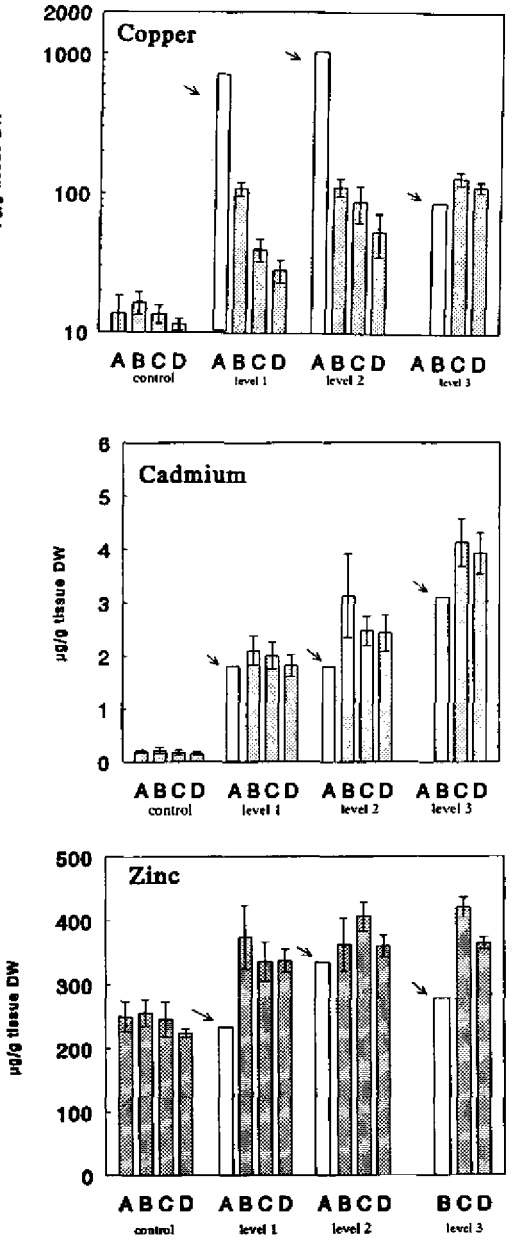

Figure 5.6 Cd, Cu and Zn accumulation in *M. balthica* after exposure to aged sediments. The arrows point at cases with 17 days exposure.

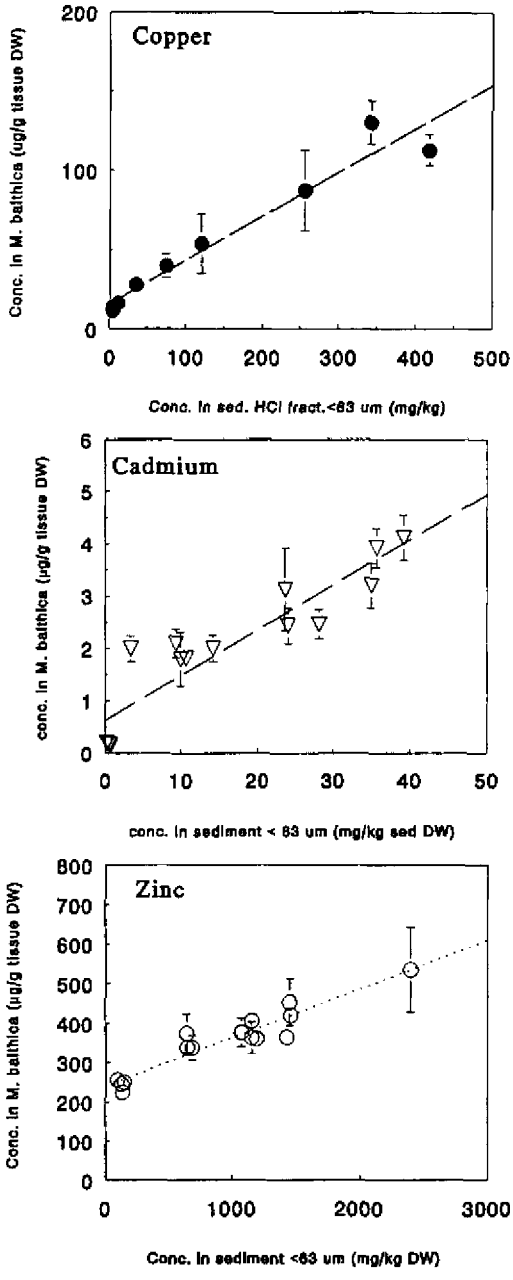

Figure 5.7 Relation between metal concentrations in the sediment and accumulation in *Macoma balthica*. The equations are given in the text.

These observations agree with Bryan (1985), who observed a decreased accumulation of Zn and Cu by *Scrobicularia plana*, tested in sediments with flowing seawater instead of stagnant aerated water.

Both in experiment 1 and 2, metal availability from the silty organic rich spiked sediments was low. These results are in accordance with Luoma and Jenne (1977) who found a slow rate of uptake of sediment-bound metals by *M. balthica*, compared with the rates of solute metal uptake.

In the present study, no lethal body burden was reached within the 57 days of exposure, except copper in the most sandy sediments. With heavily polluted natural sediments mentioned by McGreer (1982), there was no evidence of toxicity for *Macoma balthica* due to metal bioaccumulation. *Macoma* in natural sediment from Dalhousie Harbour with metal loads of Cu 55.5, Zn 4033, Pb 243.9 and Cd 6.7 mg/kg air dry (% silt = 67) had mean tissue concentrations of 50.2, 1962, 94.6 and 5.8 µg/g DW respectively, indicating that *M. balthica* can survive in polluted sediments (Ray *et al.*, 1981).

From both experiments 1 and 2, it was obvious that trace metals are more available from the sandier sediment (see Fig. 5.2 and 5.6b). In contrast to the silty organic rich sediments, mortality was observed in the sandy organic low sediments within ten days in experiment 2.

Compared with Cd and Zn, the uptake of Cu was even more efficient in the sandier sediments. The available Cd and Zn are apparently the adsorbed fraction, because of the relation with the < 63 µm fraction. A reason for the relatively high Cu uptake from the sandy sediments could be the fact that Cu merely adsorbs onto organic matter. Organic matter is often considered the most important component binding Cu in soils and sediments (Flemming and Trevors, 1989). As adsorption was very effective, especially in the sandy sediments, *Macoma balthica* could have ingested high amounts of Cu while picking organic material from the sediment. This effect was also observed with an other deposit feeder: *Macoma inquinata*: in a static system with sediment and added copper, more than 50% of the added Cu became bound to the organic fraction of the sediment. The deposit feeding clam approximately doubled in Cu body burden, whereas copper remained unavailable for a suspension feeding clam, *Protothaca staminea* (Crecelius *et al.*, 1982). Also accumulation in the suspension feeding cockle *Cerastoderma edule* was not influenced by Cu-spiked sandy sediment (Chapter Six). Experiments using ^{64}Cu indicated that Cu accumulation from food particles can be very efficient (Chapter Three).

TREATMENT	LENGTH (mm)	SD	DRY WEIGHT (mg)	CADMIUM (ug/g DW)	SD	COPPER (ug/g DW)	SD	ZINC (ug/g DW)	SD	LEAD (ug/g DW)	SD
start	13.06	(0.17)	27	0.31		16.6		242		1.2	
A-0	13.18	(0.48)	27.6	0.20	(0.03)	13.7	(4.9)	250	(23.4)	0.77	(0.15)
B-0	13.52	(0.14)	27.6	0.22	(0.06)	16.5	(3.0)	256	(21.0)	1.11	(0.19)
C-0	13.24	(0.23)	25.3	0.18	(0.05)	13.7	(2.1)	246	(27.7)	1.23	(0.11)
D-0	13.27	(0.08)	26.5	0.16	(0.03)	11.6	(1.1)	224	(7.0)	1.35	(0.10)
A-1	13.28	(0.59)	19.2	2.00	(0.24)	716.3	(95.8)	375	(35.4)	1.2	(0.16)
B-1	13.01	(0.16)	24.5	2.10	(0.27)	108.5	(12.2)	374	(49.6)	1.04	(0.20)
C-1	13.35	(0.16)	25.9	2.00	(0.25)	40.1	(7.3)	336	(30.6)	0.91	(0.07)
D-1	13.37	(0.10)	25.6	1.82	(0.21)	28.3	(5.2)	336	(18.2)	1.21	(0.21)
A-2	13.43	(0.62)	17.5	1.80	(0.51)	1060	(287)	535	(106.7)	1.53	(0.49)
B-2	13.33	(0.43)	27.6	3.13	(0.78)	112.2	(15.9)	362	(41.3)	1.26	(0.30)
C-2	13.25	(0.20)	25.6	2.47	(0.28)	87.5	(25.4)	405	(22.6)	1.18	(0.15)
D-2	13.05	(0.13)	27.3	2.43	(0.34)	54.0	(18.6)	360	(17.1)	1.47	(0.30)
-	-	-	-	-	-	-	-	-	-	-	-
B-3	13.08	(0.73)	22.7	3.20	(0.43)	87.7	(19.2)	452	(59.1)	1.1	(0.14)
C-3	13.19	(0.26)	24.8	4.12	(0.44)	130.3	(13)	420	(15.6)	1.07	(0.15)
D-3	13.26	(0.28)	26.0	3.92	(0.38)	113	(10)	364	(9.3)	1.25	(0.3)

Table 5.3 Length, weight and metal content of M. balthica after exposure to aged sediments during 57 days.
Length was the average of 20 individuals, weight was the average of 45 individuals except for the case where mortality had occured.
The standard deviation is given in parentheses. A1, A2 and B3 were taken out of the experiment after 17 days.

The differences in accumulation rate between the metals can also be explained by differences in metal regulation by *M. balthica.* For bivalves, metallothioneins (MT) are believed to play a key role in metal regulation (Roesijadi, 1992). The ability to retain metals through binding to proteins makes bivalves efficient accumulators of metals. However, with *Macoma balthica* observations have failed to demonstrate the occurrence of MT in *M. balthica* after Cd or Cu exposure (Langston and Mingjiang Zhou, 1987). Most of the cytosolic Cd or Cu was bound to High Molecular Weight proteins. Only with Pacific populations of *M. balthica* uptake of Cu, Ag and Zn in metallothionein-like proteins has been reported (Johansson *et al.*, 1986). Differences in metal regulation between East Atlantic and Pacific species could possibly be explained by genetic differences, as enzym patterns (determined by gel-electroforesis) showed distinct differences between East Atlantic and West Atlantic populations of *Macoma balthica* (Meehan, 1985). Considering the geographical isolation, a genetic difference between Pacific and East Atlantic populations is imaginable. As a compensation for the absence of a recognized detoxifying protein, slow uptake of Cd was suggested as an adaptation to survive in contaminated areas (Langston and Mingjiang Zhou, 1987).

Because the clams were exposed to a mixture of metals, it is not easy to assess which metal was responsible for the mortality. Considering metal body burdens, then Cu body burden has exceeded the lethal level by far in the sandy sediments (Table 5.4). From previous (unpublished) experiments, it is known that mortality will follow with a body burden exceeding 120 $\mu g\ g^{-1}$ DW. Above this level, the regulation capacity (whatever mechanism that may be) was obviously exceeded, with toxic effects as a result. This is in contrast with San Francisco Bay populations, where much higher Cu tissue levels in healthy *Macoma balthica* are known (up to 800 μg^{-1} DW). For *Macoma balthica* however, a large variation in intra- and inter-population metal sensitivity is known (Luoma *et al.*, 1983).

For Cd and Zn, body burdens of up to 4 $\mu g\ g^{-1}$ DW (Cd in C_3 and D_3) and 500 $\mu g\ g^{-1}$ DW (Zn in C_3) respectively, were not lethal (Table 5.4 and Figure 5.6). This indicates that the Zn and Cd body burdens in A_1, A_2 and B_3 could not have been responsible for the early mortality (Figures 5.6a-c). Based on the metal tissue levels, it is concluded that the clams died because of high copper loads.

Tissue Cu concentrations were extremely high in moribund and dead specimens in the sandy sediments. The very high tissue concentrations can be explained by selective feeding on organic particles (see above). Obviously, the moribund clams were less efficient in cleaning their guts than healthy clams. It is most probable that the very high Cu body burdens were affected by Cu loads from organic particles in the intestines.

Burrowing behaviour

As far as burrowing behaviour is concerned, the results of the present study are remarkable, compared with previous reports. Although the sediments were spiked with relatively high loads of heavy metals, burrowing response was much lower than reported elsewhere. Burrowing behaviour of Littleneck clams (*Protothaca staminea*) in sediments with Cu additions of only 2-20 µg/g sediment DW caused an increase in the effective burrowing time (ET_{50}) (Phelps *et al.*, 1983, 1985). Yet, burrowing times with aged spiked sediment or a natural sediment containing twice as much naturally occurring Cu were not significantly different.

The described sediments were low in organic carbon content (comparable with our sediment A). The difference with the results presented in our study may be due to a different sensitivity of *Protothaca staminea*, compared with *Macoma balthica*.A more obvious explanation is the difference in spiking procedure: in the present experiment, considerable aging time was taken into consideration, whereas the effects mentioned in the *Protothaca staminea* tests were only observed in freshly spiked sediments. Effects in these sediments were obviously caused by metals from the dissolved phase instead of metals from the sediment. Results obtained with this type of experiments have little relevance for natural situations when metals are in equilibrium with the sediment. It may be concluded that this spiking approach is questionable.

Reports on burrowing tests with natural sediments from different locations also give different results: in tests, carried out by McGreer (1979), with *Macoma balthica* using contaminated sediments from a mudflat gradient (Fraser River estuary, British Columbia), effects were clearly demonstrated: inhibition of burrowing behaviour occurred in all sediments compared to the control. This sublethal effect was attributed to Cd and Hg, for which linear regressions of the individual metals versus the burrowing response time were shown to be significant.

In another reported burrowing test, reburrowing after 48 hours of exposure to contaminated sediments from San Francisco Bay did not give significant differences, although the heavy metal pollution level was comparable with the Fraser River mudflat (Chapman *et al.*, 1987). The reason for differences in the reported burrowing tests in natural sediments could possibly be explained by differences in sediment handling: in the latter example, much attention was paid to the preservation of the sediment, while in the tests where effects were reported, no mention was made of careful handling and storage. An effect that could have occured during sediment handling and transport is oxidation. Cu and Cd is reported to leach from the sediment amongst others as a result of aerobic breakdown of organic matter after introduction of oxygen in anaerobic sediment (Gerringa, 1990).

In a recent review, it was recognized that with in situ toxicity tests large differences in test responses occur. They were attributed to alteration of toxicant partitioning due to different sampling methods, manipulations in the laboratory and temporal effects (Burton Jr, 1991). The results of the burrowing test presented here also point to the importance of adhering to careful sediment preparation.

Water and sediment

For the spiked aged sediments (experiment 2), a difference between Cu on the one hand and Cd and Zn on the other hand could be observed. Cu adsorption on the sediment was almost as effective in the sandy sediments as it was in the muddy sediments. Obviously sufficient binding sites were available (Figure 5.3). Adsorption of Zn and Cd however depended very much on the sediment type: the more small particles and the more organic matter in the sediment, the more metal was adsorbed on the sediment. The observed decrease of Cd in the sediment could be the result of leaching (Gerringa, 1990). Leaching was most effective from the top layer, as the Cd content in the 0-5mm layer was lower than in the 4-7cm layer, even in spite of a relative increase in the concentration of particles <63 µm, compared with the 4-7 cm layer. As Cu leaching is mostly dependent on the Cu complexing capacity of the overlying water and degradation of organic matter (Gerringa, 1990), little Cu leaching was expected. This is in agreement with the results.

The total metal concentrations in the overlying water during the experiment turned out to be slightly elevated in the polluted sediment cases, compared with the control sediments and the inflowing water. However, the elevated concentrations were still that low, that regarding metal accumulation, no serious influence was to be expected from the overlying water (Johansson *et al.*, 1986; Langston and Mingjiang Zhou, 1987).

The effects of aging

After the aging period in experiment 2, the deeper sediment was coloured blackish-grey, indicating a reduced environment. In this reduced environment, Cu and Cd availability will be decreased through binding with sulphides.

It is known that Acid Volatile Sulfide (AVS) concentrations potentially affect metal availability (Di Toro *et al.*, 1992). AVS are a reactive solid-phase sulfide pool that apparently binds some metals, thus reducing toxicity. Whenever anoxic sediments are exposed to air during collection and processing, AVS are volatized. Cd toxicity in sediments has been shown to be inversely related to AVS complexation. As Cu and Zn also form metal sulfide precipitates that are more insoluble than iron sulfide, it is likely that the results found for Cd

apply to these metals (Burton Jr, 1991). In our study, AVS were probably volatized during mixing, but restored during the aging period.

The results about Cu in reduced sediments are not in agreement with reported field observations: very high concentrations of Cu in *M. balthica* have been found in relatively uncontaminated sites that were anoxic (Luoma and Bryan, 1982). One explanation for this remarkable finding was that tissue Cu might be precipitated and accumulated as sulfide during periods of anoxia (Bryan and Langston, 1992). The results from our study indicate that Cu sulphides are not likely to be accumulated from the sediment, as accumulation should have been highest in the most organic rich and anoxic sediment. However, if the overlying water were anoxic as well, the situation would be different. It is known that with reduced oxygen tension in the water, copper uptake is enhanced, possibly due changes in Cu speciation as a result of pH change (Neuhoff, 1983; Neuhoff and Theede, 1984). In areas with unexpected high Cu concentrations in tissues of deposit feeders, it is very well likely that oxygen tension in the water influences Cu uptake more than redox variation in the sediment.

Uptake from the water or from the sediment?

It has proven very difficult to assess the importance of sediments as a source of metals to benthic organisms: as sediments include both interstitial water and particulate phases, the problem of determining biological availability is complex. Because of this complex situation, experimental setups are not easy to design; data on sediment toxicity are consequently limited.

To discriminate between water and the sediment is one problem. Discrimination between the interstitial and the overlying water is another question in sediment toxicity studies. It is reported that trace metals present in interstitial water rather than bound to particles appeared to be responsible for sediment toxicity in amphipods (Swartz *et al.*, 1985). However, with the deposit feeding tellinid bivalve *Macoma nasuta* interstitial water constituted very little (4 %) of the total amount ventilated by the animal (Winsor *et al.*, 1990). In static toxicity tests with an equilibrated sediment-water system, accumulation from the water phase will govern the accumulation from the sediment. This was the situation in experiment 1. With amphipods, it was argued that solid-phase LC_{50} values for sediment Cu correspond to LC_{50} values for dissolved Cu in the overlying water at equilibrium (Cairns *et al.*, 1984).

However, in a natural setting compared with a laboratory situation, there is no stagnant water column on the sediment. Therefore, in addition to accumulation from the water phase, direct accumulation from the sediment should be considered as an additional pathway for metal uptake for deposit feeders like *Macoma balthica*, that ingest sediment and organic particles with their long siphons. This can be concluded from the Cu accumulation results:

although concentrations in the overlying water were low, accumulation from the sandy sediments was considerable.

CONCLUSION

The present study gives an example of the effects of aging on burrowing behaviour and survival in metal spiked sediments. The control sediments used in this study were representative for Dutch estuarine sediments. As only few metal toxicity effects were found with much higher pollution levels, one might conclude that *Macoma balthica* in Dutch estuaries is not yet exposed to threatening toxic concentrations. However, the situation described in this experiment is only representative for undisturbed reduced sediments, with little exchange between the sediment and the overlying water.

The situation would be completely different if the sediment (e.g., harbour sludge) were disturbed on a large scale, for example through dredging activities. In that case, metals would go partly into solution and organisms could accumulate metals from the overlying water. High metal concentrations, especially copper, do occur in Westerschelde sediments, however mostly in harbours and areas where estuarine organisms are not present because of the low salinity. Disturbance of these regions might form a serious risk for less polluted areas downstream.

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Chapter Six

The influence of sediment, food and organic ligands on the uptake of dissolved copper by *Macoma balthica* in long-term experiments.

ABSTRACT

The sediment dwelling bivalve *Macoma balthica* was exposed to dissolved copper in a flow-through system in long-term experiments. Compared with another sediment dwelling bivalve, the suspension feeder *Cerastoderma edule* (cockle), *Macoma* accumulated copper from the sediment, while the cockles did not. When dwelling in silty, organic rich sediment, *Macoma* accumulated less from the water, whereas accumulation in the cockle was not influenced by the sediment type.

In natural Oosterschelde water, total ligand concentrations were around 100 nequivalent copper, with a free Cu^{2+} concentration of $6.3x10^{-14}$ M (pCu 13.20). Addition of 400 nM Cu (25 µg/l) resulted in a free Cu^{2+} concentration $2.19x10^{-8}$ M (pCu 7.66). Addition of low concentrations of EDTA caused a large reduction of Cu uptake, which was confirmed with the calculated cupric ion activity. The calculations point at the importance of considering the chemical speciation of dissolved copper in toxicity studies.

When algae were added to the exposure water, *Macoma* accumulated significantly more copper, although the filtration rates in the algae-dosage were decreased. This points at an important contribution of food-associated copper to the overall accumulation by *Macoma balthica*.

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INTRODUCTION

Sediment-dwelling bivalves are often suggested as useful monitor organisms for polluted sediments. As a consequence, emphasis in laboratory research with these animals has been laid on sediment toxicity. In the field situation however, metal availability from the sediment might be very low, in spite of the often very strong pollution, as was shown in Chapter Five. In such situations, metal uptake from the overlying water will most probably govern uptake from the sediment.

The sensitivity of bivalve molluscs for copper is well known, regarding the large volume of literature available (Hodson *et al.*, 1979). The majority of toxicity studies are based on short term exposures and have an LC_{50} as endpoint. Fewer studies deal with uptake kinetics at a sublethal level. These uptake studies suffer from difficulties with the interpretation of dose-effect relationships, considering the complicated speciation measurements. Inorganic, but most of all organic complexation of copper causes a decrease of the concentration of biologically available metal. At very low, sublethal copper dosages, a large fraction of the added metal might become unavailable for uptake because complexing ligands are still unsaturated at these low concentrations. At high (acutely toxic) concentrations, these ligands are saturated and thus interfere less in dose-effect relationships. The problems with complexation are also the reason why static bioassays with environmentally relevant (= not acutely toxic) copper concentrations can hardly be carried out properly. Between two refreshments the bioavailable concentration will not only decrease because of adsorption onto walls, but also through complexation with ligands, present in natural water or secreted by the organisms (see Chapter Four).

Next to dissolved copper, food-associated copper possibly contributes to the overall uptake of copper. In Chapter Three, it was demonstrated in short term experiments with ^{64}Cu labelled algae that food-associated copper could contribute for a significant part to the overall metal body burden of *M. balthica*. However, the presence of food can influence metal body burdens in several ways. Besides direct metal absorption from digested food, the accumulation and elimination of Cu will depend via several mechanisms on food *concentrations*. Absence, or a too low concentration of food will cause strongly reduced ventilation rates (Riisgård and Randlov, 1981; Hummel, 1985) Consequently, less copper will diffuse through the gills. The presence of food will influence the physiological and metabolic condition (Bayne *et al.*, 1988), so accumulation as well as elimination rates of metals can be increased (Borchardt, 1983). To evaluate tissue metal concentrations from animals in the field, accumulation of copper from the sediment, the overlying water and from food, has been studied in combined, long-term experiments.

In the first experiment, Cu accumulation in two different sediment-dwelling bivalves species is compared: the deposit feeder *Macoma balthica* (balthic tellin) and the suspension feeder *Cerastoderma edule* (cockle). Although both species often co-occur in estuaries, the mode of feeding (deposit- or suspension) might influence the relative contribution of various metal uptake routes.

Also the origin and history of the experimental bivalves might influence metal uptake rates and toxicity, as was demonstrated for example with *Macoma balthica* from San Fransisco Bay (Luoma *et al.*, 1983). Therefore in the second experiment, accumulation in *Macoma balthica* from a sandy location in the relatively clean Oosterschelde Sea-arm was compared with accumulation in *Macoma balthica* origining from a silty, organic rich location in the more polluted Westerschelde estuary.

In the third experiment, Cu accumulation and elimination was studied in starved and fed individuals to evaluate the influence of food. Additionally, in the second and third experiment the influence of EDTA on the accumulation and elimination of Cu was studied.

MATERIALS AND METHODS

Experimental setup

The experiments were carried out at the field station of the Tidal Waters Division (Ministry of Transport and Public Works) which is situated near the storm surge barrier at the mouth of the Oosterschelde estuary, the Netherlands (Figure 1.1). At the station, seawater (salinity 32 ‰) is pumped at a rate of 20 m^3 h^{-1} from an inlet 200 meters from the shore, close to the storm surge barrier. For the experiments, the water was filtered through a 2 m^3 sand bed.

For experiment 1, circular Plexiglass exposure tanks (20 l) were used. In the exposure tanks, a Plexiglass chamber was placed 5 cm from the bottom and filled with sediment up to the rim (10 cm height). Sediments were always frozen before use to kill indigenous animals. The chamber was divided into 4 compartments, which enabled sampling without sediment disturbance in the remaining compartments. Particles were prevented to settle down on the bottom of the exposure tank by keeping the water in circulation with a magnetic stirrer.

For experiment 2 and 3, polyethylene transport containers functioned as water baths (42b x 60l x 30h cm). For details on this exposure system, see Chapter Five. Filtered seawater, $CuCl_2$ solution, and (in experiment 3) algal culture were pumped through the system continuously. The refreshment rate of the water amounted to 3 times per hour. With all experiments, control exposures without Cu addition were run simultaneously with the Cu additions. For each experiment, animals were collected from intertidal mudflat locations in

either the Westerschelde or the Oosterschelde. They were allowed to get accustomed to the laboratory situation for two weeks. Only 3- and 4 year old individuals (av. length 13 mm ±1,5 mm) were used in the experiments.

Experiment 1. In each compartment, 20 *Macoma balthica* and 10 *Cerastoderma edule*, all origining from the mudflat Dortsman (Oosterschelde, see Figure 1.1) were exposed to dissolved Cu (320 nM, or 20 µg/l for the first 20 days and 160 nM ± 60 nM for the following 88 days), while inhabiting sandy, organic low, sediment (Jacobahaven-beach sand, Figure 1.1), or silty, organic rich sediment (Oesterput, Oosterschelde). Additionally, the sandy sediment was spiked with extra copper (2 µg/g sediment Dry Weight). This spiked sediment is called sand ++. For details on the spiking procedure see Chapter Five. The sediment composition and Cu concentration is given in Table 6.1.

	[Cu]sed		POC		CaCO3	
	ug/g DW	sd	%	sd	%	sd
silty control	9.3	0.7	2.23	0.09	20.03	0.22
silty copper	12.1	2.3	2.31	0.18	20.9	0.24
sandy control	0.7	0.3	0.16	0.06	1.7	0.27
sandy copper	1.2	0.3	0.15	0.03	1.8	0.19
sandy++	1.7	0.4	0.16	0.06	1.7	0.27

Table 6.1 Particulate Organic Carbon, $CaCO_3$ and Cu concentration in the sediment in experiment 1.

The animals that were exposed in the spiked sediment did not receive additional copper through the water. The animals were fed 3 times a week with *Phaeodactylum tricornutum* from a batch culture. The exposure ran from April until July during 108 days. The time series were carried out in quadruplicate, except the sandy spiked sediments series which were carried out in duplicate.

Experiment 2: Exposure chambers were filled with *Macoma balthica* origining from the Oosterschelde (Dortsman) or from the Westerschelde (Paulinapolder). The animals were burrowed in beach sand (50 individuals per chamber). In each water bath, 8 Oosterschelde and 8 Westerschelde chambers were placed at random. The chambers were moved twice a week to compensate for possible differences in location in the water bath. The first bath received filtered sea water. The second received filtered seawater with 1000 nM EDTA as complexing agent. The third received 250 nM Cu with 250 nM EDTA. For this experiment, the animals were not acclimatized to the experimental conditions, because a redistribution of metals in the tissue could occur before the exposure started. The exposure started in September and lasted for 70 days. It was followed by an elimination period of 10 days.

Experiment 3: *Macoma balthica* were exposed to copper with and without continuous supply of food. The clams were expected to change their water ventilation activity (or filtration rate) in response to the different feeding conditions. It is suggested that starved bivalves have reduced filtration rates and consequently accumulate less metals from the water. Differences in filtration activity might seriously influence the rate of uptake of contaminants. Therefore, filtration rate was controlled during the experiment. The filtration rate is defined as the amount of water that is filtered by the gills per unit of time. This depends on the pumping rate (also called clearance rate) and the particle retention efficiency:

Filtration rate (ml/ind/h) = Clearance rate (ml/ind/h) * Retention (% food)

If the particles would not be retained with optimal efficiency, this is not a correct measure for pumping (or water ventilation) activity. However, *Phaeodactylum tricornutum* is well known as a food source for bivalves, and concentrations were so no overloading of the gills took place (see results). Consequently, retention in these experiments was considered to be 100%. This filtration rate was measured by assessing the concentration of particles > 2.3 µm before and after switching off the food and water supply during a certain period. Control measurements (to assess losses through sinking) were carried out in containers without animals. Filtration rate was calculated as described in Chapter Four.

Every water bath was filled with 16 sediment chambers, each containing 25 *M. balthica* individuals, origining from the mudflat Paulinapolder in the Westerschelde Estuary. Additional continuous mixing of the water was provided by a stirring rod with a poly-ethylene propeller. Container 1 received a nominal Cu concentration of 400 nM (25 µg/l) via the water during 18 days. The exposure was followed by an elimination period of 35 days. Container 2 was identical to 1 but received algae from a continous system. These algae (*Phaeodactylum tricornutum*) were grown in an 18 l continuous culture under a 12/12 light/dark regime in filtered seawater, supplied with synthetic medium according to Kester (Kester *et al.*, 1967). Steady state conditions in the culture were reached at an algal concentration of $1{,}6*10^6$ cells/ml. Algae that were added to contaminated water, received 40 nM Cu extra in their culture medium. During the elimination period, algae were grown without extra Cu. The algae were added to the experiment at a rate of 125 ml per hour. Algal concentration in the exposure container was $21 * 10^3$ cells/ml.

Container 3 received a nominal concentration of 400 nM Cu together with 250 nM EDTA during 53 days. Container 4 was identical to 3 but started with elimination after 18 days. A final container without Cu dosage was used as a control. Except container 2, the clams did not receive any algal food during this experiment. The experiment was carried out during May and June.

Physico-chemical analyses

Water samples for metal analysis were taken in acid washed polyethylene bottles and acidified to pH=2 after filtration. The samples were stored at -20 °C. Before analysis, the water samples were U.V. irradiated during four hours to destroy organic material. Total (dissolved and particulate) Cd, Cu and Zn were analyzed as described in Chapter Four. Ligand concentration (L_{asv} and L_{csv}) and conditional stability constant K' were assessed as described in Chapter Four, with the difference that here catechol was used instead of salicylaldoxime (SA) as added ligand with cathodic stripping voltammetry. With catechol, ligand groups with a moderate Cu binding strength (log K' around 8-10) are determined. The measured total dissolved copper concentration were used in combination with the measured ligand concentrations and binding strength to calculate the concentrations of the different copper species in the experiments. For this calculation, an updated version of the chemical speciation program MINEQL (Westall *et al.*, 1976) was used (MINEQL$^+$, version 2.1) with pH fixed at 7.8.

Sediment was sampled in p.e. bags and stored at -20 °C until processing. For sediment and tissue, Cu was measured using a Perkin Elmer Model 3030 with graphite furnace and a Zeeman background correction system. The samples were analyzed for grain size distribution, particulate organic carbon (POC), total nitrogen, $CaCO_3$ and total metals as described in Chapter Five. *Macoma balthica* were allowed to clean their guts for 12 hours in filtered seawater. Until dissection, they were kept frozen at -20 °C. After dissecting, they were treated and analyzed as in Chapter Five.

RESULTS

Experiment 1

During the exposure period, the dryweight of all *C. edule* had increased, although there was a significant difference between the animals that had received copper (Figure 6.1). From these, the animals that were burrowed in sand had a lower dryweight than those from the silty sediment. Although the continuous refreshment of the water caused a quick dilution of the algal suspension, food supply was obviously sufficient for the cockles. The dryweight of *Macoma balthica* had decreased slightly during the experiment. No differences between the various exposures were found.

Although the copper concentration in the water had not been constant during the exposure period, distinct differences in Cu accumulation patterns were found. Initial tissue Cu concentrations in *C. edule* were much lower than those in *M. balthica* (Figure 6.2). With the cockles buried in sand, high mortality and a sharp increase in tissue copper concentrations was observed within three weeks, probably due to the initially elevated copper dosage. The cockles that were left over, could recover from the high initial dose. Initially, metal levels also increased in the cockles that were buried in silty sediment. Also these levels gradually returned to the normal low value. Cu content in control cockles had decreased slightly during the experiment. Accumulation in cockles that were buried in sandy, Cu spiked sediment was not different from the control cockles in both sediments.

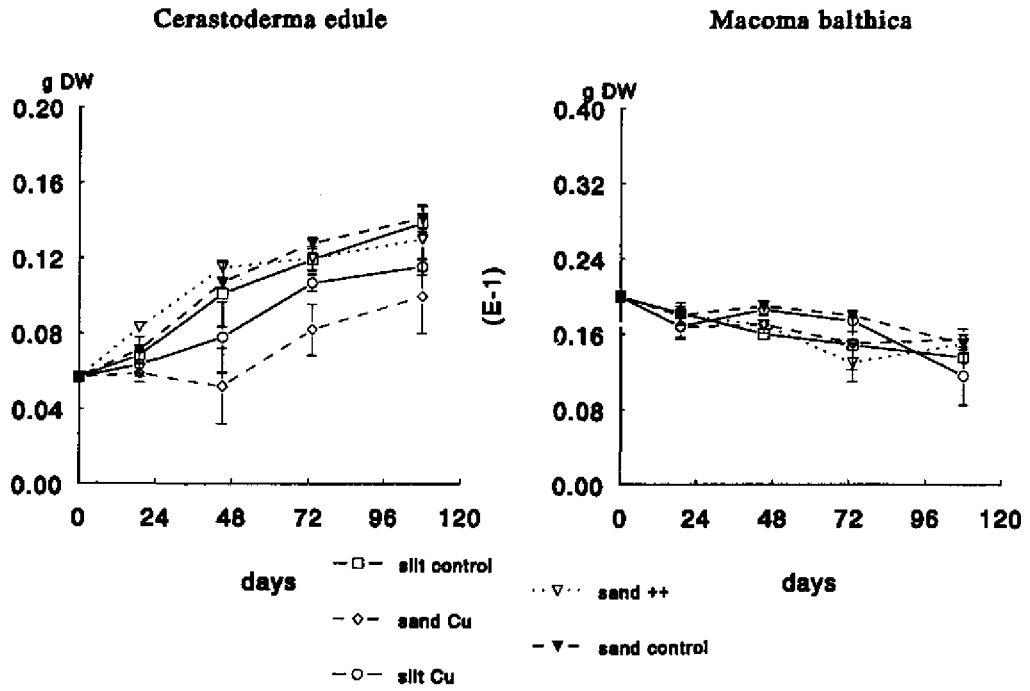

Figure 6.1 Course of tissue dry weight of *Cerastoderma edule* and *Macoma balthica* during experiment 1. The animals were exposed to Cu in the overlying water (**sand Cu** and **silt Cu**), to Cu-spiked sediment (**sand ++**), or to control water and sediment (**sand control** and **silt control**).

Also *M. balthica* accumulated Cu while being burrowed in the sandy substrate. Accumulated concentrations were very high, compared with *C. edule*. However, no mortality occurred. Contrary to the cockles, *M. balthica* accumulated a significant amount of Cu from the spiked sandy sediment. On the other hand, accumulation of Cu while being buried in silty sediment was not different from the controls, in spite of the Cu dosage in the overlying water.

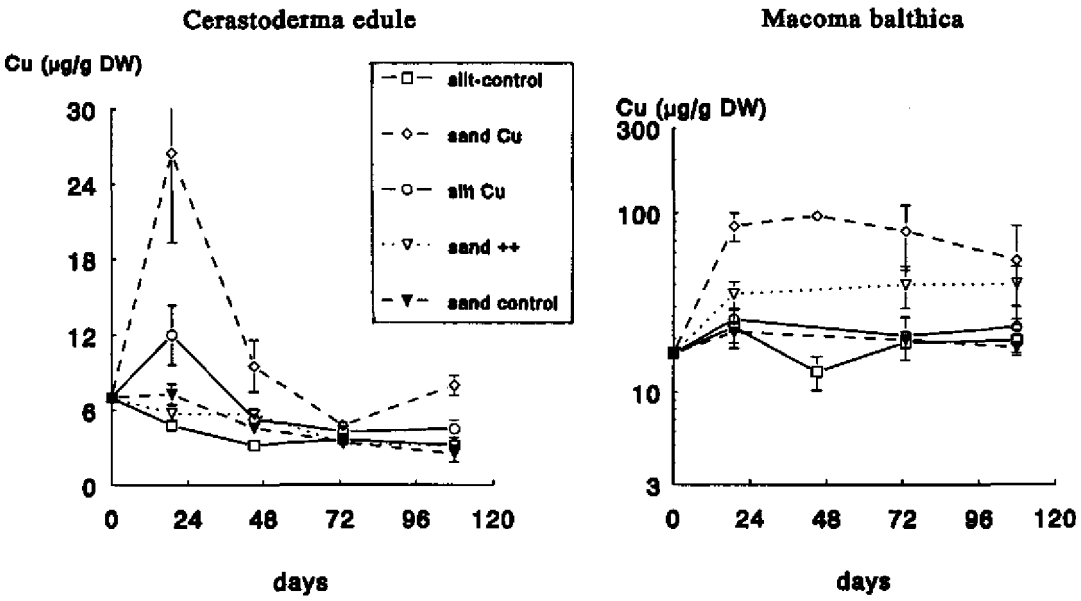

Figure 6.2 Tissue copper concentrations in *Cerastoderma edule* and *Macoma balthica* in experiment 1. The animals were exposed to Cu in the overlying water (*sand Cu* and *silt Cu*), to Cu-spiked sediment (*sand* ++), or to control water and sediment (*sand control* and *silt control*).

Experiment 2

The initial tissue Cu content of Westerschelde individuals was higher than the Cu content of Oosterschelde individuals. After an initial slight decrease in metal content, tissue levels remained relatively constant in all clams exposed to seawater with EDTA (Figure 6.3). This constant level was lowest in OS animals. WS animals also had a very constant level when exposed in control seawater (without any addition of EDTA or Cu). OS animals however, showed a slightly increased concentration during a prolonged time when exposed to control water. Both in OS and WS animals Cu tissue levels increased quickly in the Cu-exposure bath. Within a few weeks, these levels stabilized, and even decreased in Westerschelde animals. In Oosterschelde animals, a decrease in Cu tissue levels during the elimination period was observed. This was not the case in Westerschelde animals.

Experiment 3

In experiment 3, the dryweight of the individuals in both the fed and unfed containers had not decreased during the 53 days experiment. During the exposure period, some mortality occurred in the Cu-exposed containers (Table 6.2). With EDTA addition and in the control situation, no mortality occured. During the elimination period, mortality decreased to zero. During the exposure period, accumulation of Cu was linear in all dosage combinations.

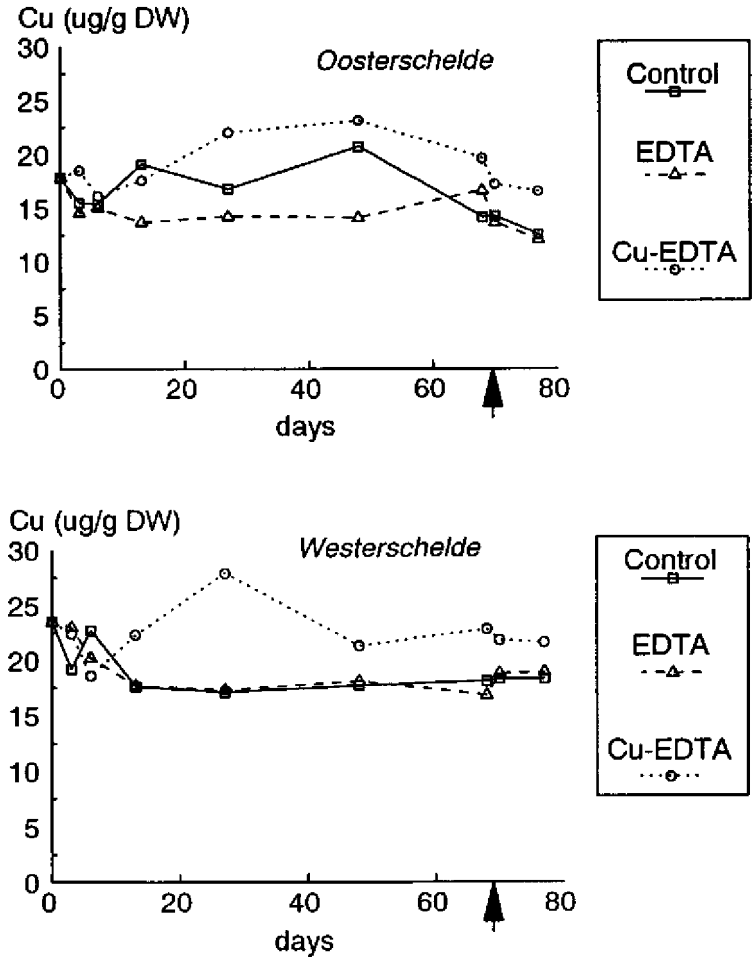

Figure 6.3 Tissue copper concentrations of animals origining from the Oosterschelde or from the Westerschelde. The arrows mark the start of the elimination period.

The copper accumulation rate in the fed container was significantly higher, compared with the unfed container (Figure 6.4). The loss rate of copper amounted to 1 to 3.6% Cu per day. The measured total dissolved copper and ligand concentrations were used for the calculation of the concentration of the different copper species (summarized in Table 6.3). In experiment 2, Cu^{2+} concentrations were very low, with pCu (-log free ion activity) around 13 in the control and EDTA containers. All the copper that was present, was complexed with the strong ligand (C_{csv}). Addition of extra EDTA did not have any influence on the Cu^{2+} concentration. With 250 nM Cu added, both the strong and the weaker natural ligands (L_{csv} and L_{asv}) were saturated, as was EDTA. Due to saturation of the organic ligands, the major fraction of copper was present as inorganic ligand (more than 22%) or as free ion (10.9 %).

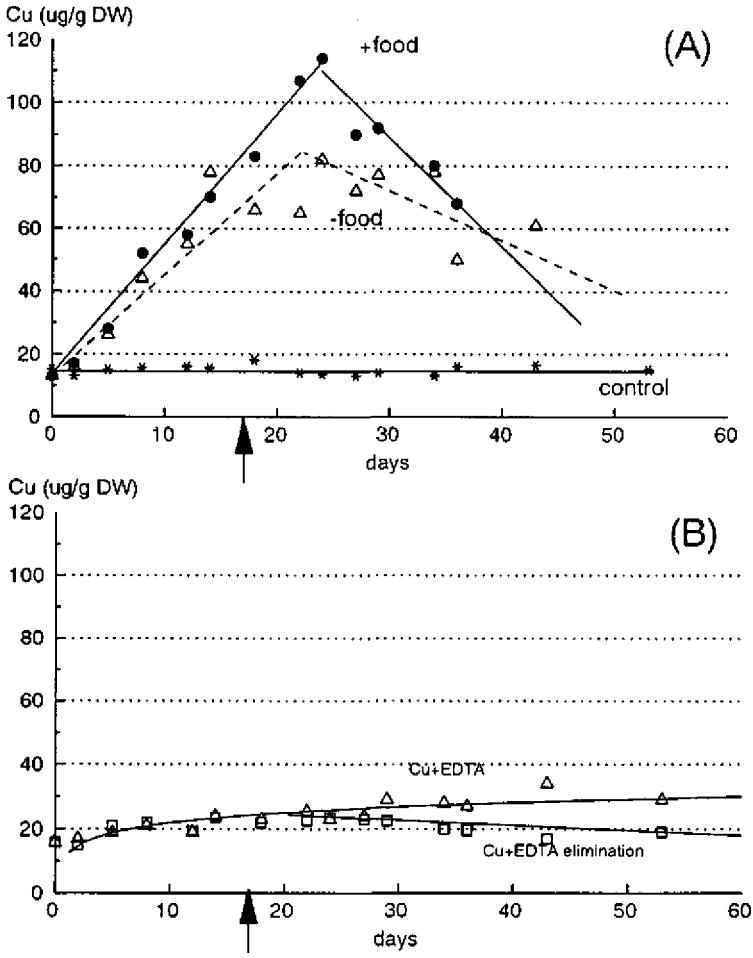

Figure 6.4 Copper accumulation and elimination during exposure to 400 nM Cu with or without Cu-enriched algae (A) or to 400 nM Cu with 250 nM EDTA (B). The arrows mark the start of the elimination period.

	accumulation								elimination		
Day in experiment:	4	5	7	8	9	10	14	17	25	32	40
Control	0	0	0	0	0	0	0	0	0	0	0
Cu+EDTA	0	0	0	0	0	0	0	0	0	0	0
Cu+Food	0	0	4	6	8	8	13	18	27	31	32
Cu	0	0	8	12	13	15	22	28	40	44	45

Table 6.2 Cumulative percentual mortality of *Macoma balthica* exposed to 400 nM Cu alone, 400 nM Cu with 250 nM EDTA, or 400 nM Cu with copper-enriched algae.

	INPUT			OUTPUT (in M)									
	nM	neq	neq	-log		-log		-log		-log		-log	
	[Cu-t]	[L asv]	[L csv]	[Cu2+]	%total	[CuL asv]	%total	[CuL csv]	%total	[anorg.]	%total	[CuEDTA]	%total
Control	7	80	13	13.29	<1	11.13	<1	8.16	99.9	12.83	<1	-	-
EDTA	7	80	13	13.20	<1	11.30	<1	8.16	99.9	12.75	<1	12.04	<1
EDTA-C	250	80	13	8.52	10.9	7.12	27.3	7.75	6.4	7.2	22.5	7.24	19.5
A (EDTA	400	100	30	7.85	3.6	7.03	23.8	7.52	7.6	7.44	10.3	6.67	53.5
B (EDTA	400	280	35	8.48	<1	6.67	54.7	7.46	8.9	8.02	1	6.89	33
C	400	100	30	6.70	50.8	7.02	24.3	7.52	7.6	7.20	16.1	-	-
D	400	280	35	7.66	5.2	6.57	63.3	7.46	8.3	7.20	16.1	-	-

exp.2

Control = Oosterschelde water. Input values: Cu=7 nM; Zn=40 nM; Ni=14.7 nM; Mn=10.9 nM; Fe=5.47 mM.
log K' asv = 9; log K' csv = 13; [L asv] and [L csv] are the average of 3 measurements during the experiment. salinity = 32 g/l; pH
EDTA = Control + 1000 nM EDTA
EDTA-Cu = Control + 250 nM EDTA + 250 nM Cu

exp.3

A = Oosterschelde water in experiment 3. Composition as in experiment 2, but [Fe] = 100 nM and [EDTA] = 262 nM.
[L asv] and [L csv] are the average of field measurements in the Oosterschelde in May and June
B = as A, but [L asv] and [L csv] are the maxima of field measurements in the Oosterschelde in May and June
C = as A, but without EDTA
D = as B, but without EDTA

Table 6.3 Measured and calculated concentrations of different copper species and natural organic ligands in experiment 2 and 3. Only the major inorganic Cu species ($CuCl^+$, $CuCO_{3aq.}$, $CuOH^+$ and $Cu(OH)_{2aq}$) are added up in the table.

In experiment 3, two different concentrations of L_{asv} and L_{csv} have been used for the calculation of the pCu. This difference in natural ligand concentration caused an almost 10 times difference in the concentration of bioavailable copper (Cu^{2+}): pCu going from 6.70 with the average to 7.66 with the high ligand concentration. Also the presence of EDTA (situation A and B) causes the concentration of bioavailable copper to be reduced largely. This was also demonstrated in the uptake by *Macoma balthica* (Figure 6.4): in the situation with EDTA, uptake rates were far less than without EDTA.

Although the water in the -non food- situation was filtered, still a measurable concentration of particles larger than 2.3 µm was present (Table 6.4). The particle concentration was reduced by the sand bed filter to 1/4 of the concentration in raw sea water. It was expected that the particle concentration was at such a level that clearance rate and feeding activity of the animals was reduced. Surprisingly, the filtration rate of these animals was much higher than the clearance rate in fed animals (Table 6.4). Optimum curves were assessed to find the relation between food concentrations and filtration rates. These curves could be described with the function

$$Filtr = A * e^{-\frac{1}{F} * \frac{(F-O)^2}{R^2}}$$

Where
- **Filtr**= filtration rate in ml/h/ind
- **A**= constant
- **F**= Food concentration (cells/ml)
- **O**= Optimum food concentration (cells/ml)
- **R**= Range (cells/ml)

The parameters values for the function were assessed by non-linear parameter estimation in SYSTAT. The optimum curve for clams that were suffering low food conditions appeared to be shifted towards a lower optimum concentration. Clams from a nursery stock at the field station receiving unfiltered seawater had their optimum shifted to a higher optimum. From the parameter values, only the optimum food concentration (O) was significantly different between the treatments. In Chapter Seven, this function is applied in a model description of feeding and growth of *Macoma balthica*.

Accumulation of dissolved copper in long-term experiments

Sea water:	Filtered	Filtered + algae	Unfiltered
Particles > 2.3 um/ml	5000	22000	21000
Filtration rate (ml/ind/h)	15.2	5.1	

Table 6.4 Particle concentrations and filtration rates after 12 days exposure in filtered seawater or seawater with algae.

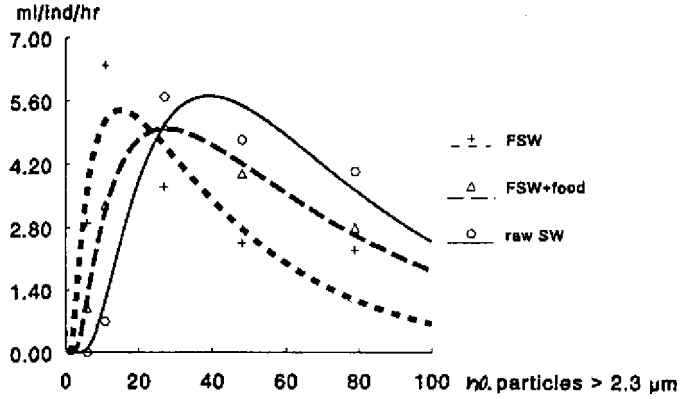

Figure 6.5 Optimum curves of clearance rates at varying particle densities

DISCUSSION

In experiment 1, dryweight of the suspension feeding *Cerastoderma edule* had increased, whereas this was not the case with *M. balthica*. Interspecific competition between *Macoma balthica* and *Cerastoderma edule* is not obvious (Kamermans *et-al.*, 1992). In other experimental situations, varying from no food addition to continuous supply, *M. balthica* dryweight always remained rather constant, even after months. With regard to this constant dryweight and considering the low mortality rates in control situations, *M. balthica* can be regarded as an ideal experimental organism. Tissue metal contents in *C. edule* were low (close to the detection limit), compared to *M. balthica*. Comparable Cu accumulation in tissues of *C. edule* and *M. balthica* were observed by Jenner and Bowmer (1990). The mortality in *C. edule* indicated a very high sensitivity of this species for copper.

Although both bivalve species are sediment-dwelling, a remarkable difference in uptake was observed: apparently, accumulation in cockles is less depending on the substrate they are inhabiting: while being burrowed in silty sediment, *Cerastoderma* accumulated Cu from the water, whereas *Macoma balthica* was able to keep its tissue concentration at control levels (Figure 6.2). On the other hand, *M. balthica* accumulated Cu from sandy spiked sediment,

while *C. edule* did not at all. Because the Cu dosage had not been constant during the exposure, no conclusions can be drawn from the accumulation- and elimination patterns. Yet, the difference between *Macoma balthica* and *Cerastoderma edule* was obvious.

In spite of the low concentrations of bioavailable copper (pCu around 13), no Cu elimination occured in experiment 2. Copper accumulation rate was not proportional to the increase in the available fraction: at calculated free Cu^{2+} concentrations of more than 1000 times the control situation (see Table 6.3), tissue levels were elevated, but remained constant. So at these concentrations, still some regulation seems functional. Although for *Macoma balthica*, the presence of metallothioneins as regulating mechanism has not been demonstrated (Langston, 1987), it is obvious that *Macoma* is well capable to control its internal Cu levels to a certain extent. The elevated initial tissue levels and the decrease of Cu after a few weeks in WS animals points at a higher basic concentration of metal regulating proteins in WS animals. This is very well possible, because the Westerschelde estuary has a considerable pollution history, which could have caused selection of the more metal resistant individuals. Only the last decade, heavy metal input in the estuary has decreased largely (Van Eck *et al.*, 1991).

The difference between the accumulation pattern in animals exposed to EDTA can possibly be explained by a higher clearance rate in Oosterschelde individuals. Considering the high concentration of organic material in the water and the sediment, it can be supposed that food availability at Paulinapolder (Westerschelde) is high, compared with the Dortsman (Oosterschelde). If the animals are adapted to their original food situation, then filtration rates in Oosterschelde animals will be higher than in Westerschelde animals. Preliminary observations on filtration rates confirmed this conjecture.

The free copper ion concentration in control Oosterschelde water (pCu 13) was comparable with control situations reported elsewhere (Dodoo *et al.*, 1992). Although in experiment 2 and 3 theoretically sufficient EDTA was present to complex all copper, only a fraction was complexed. This can be explained by the competition with other ions in the seawater (mainly manganese and iron). In experiment 2 and 3, it is demonstrated that in Oosterschelde water with average ligand concentrations, about 100 nM Cu (6.3 µg/l) will be complexed with organic ligands. If dissolved Cu concentrations exceed this concentration, the amount of biologically available copper increases sharply.

This complexation of Cu at low experimental dosages can be the cause of misinterpretation of results: presumed regulation of Cu in molluscs at low ambient levels actually is the reduction of available copper due to complexation with unsaturated ligands. For example, at Cu concentrations above 15 µg/l, behavioural response in *Dreissena*

polymorpha was attributed to accumulation, whereas below this level, the absence of a response was ascribed to regulation (Kraak *et al.*, 1992) At these low Cu concentrations however, ligands in lake water are obviously not yet saturated (Verweij *et al.*, 1989). In literature, bioavailability has been shown to be related to the cupric ion activity and not the concentration of chelated copper (Zamuda and Sunda, 1982). Also physiological effects were related to the free Cu ion concentration, and not the total dissolved copper concentration (Sanders *et al.*, 1983; Meador, 1991).

Several authors contribute a positive correlation relation between food concentration and metal uptake rates to feeding activity (Borchardt, 1983; Riisgård *et al.*, 1987). It is reported that bivalves even close their shells in response to low algae concentrations, thus reducing the water transport through the mantle cavity (Riisgård and Randlov, 1981). In the present experiment however, it was demonstrated that this relation is not as direct as it seemed. It was shown that *Macoma balthica* can shift its filtration optimum, thereby seeing to a garantueed food supply under varying conditions. The optimum shift has been demonstrated before (Hummel, 1985). This shift can explain the increased clearance rate in the not feeded situation. Because the filtration rate was not elevated in fed clams, the extra Cu accumulation should be contributed to food-associated Cu. The increased elimination rate with the fed animals is probably the result of a general increase in metabolic activity.

If Cu uptake through the dissolved phase would be the major process, then an increased uptake was expected in the -not feeded- situation, regarding the higher water filtration rates. In spite of the lower clearance rate with fed *M. balthica*, Cu accumulation was higher in these individuals. This would imply that Cu associated with food is available for uptake, and contributes in this case for a significant part to the overall Cu uptake by *Macoma balthica*. This finding is in agreement with observations on uptake of Cu from ^{64}Cu spiked algae (see Chapter Three).

The nutritional value of the particles in the two food situations was different as well. In filtered seawater, a considerable fraction will consist of material with low nutritional value, whereas the algae can be digested very efficiently. This difference will also contribute to differences in Cu uptake in the gut.

CONCLUSION

Cu uptake from the water by *Macoma balthica* is influenced by many factors. A comparison between the filter feeder *Cerastoderma edule* and *Macoma balthica*, revealed that differences in uptake were related to their feeding behaviour. *Macoma* accumulated copper

from sandy spiked sediment, while the cockles did not. When dwelling in silty, organic rich sediment, *Macoma* accumulated less from the water, whereas accumulation in the cockle was not influenced by the sediment type.

Because Cu in natural waters is largely complexed with organic ligands, the assessment of Cu toxicity and accumulation rates of low Cu concentrations is complicated. Variation in the natural ligand concentration at low experimental dosages has a large influence on the free cupric ion acitivity. The distribution of Cu over the various organic and inorganic ligands demonstrated once more that total dissolved concentrations do not give any indication of the toxic copper concentration.

The variation in filtration rates hampers the assessment of the Cu uptake through food. Yet, it is concluded from these long term experiments that food might contribute for a large part to the overall Cu uptake by *Macoma balthica*. However, more research is necessary, in particular on the relation between clearance rates and metal uptake rates, to be able to give a more detailed picture of metal uptake through water and through food.

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Chapter Seven

Concentrations of selected heavy metals in benthic diatoms and sediment in the Westerschelde Estuary

ABSTRACT

A method for the measurement of trace metal contents in benthic diatoms is described. The method was applied for the assessment of cadmium, lead, copper and zinc in benthic diatoms in the polluted Scheldt Estuary. In highly polluted areas, the bioconcentration factor (metals in diatom/metals in sediment) in diatoms was < 1. In relatively clean areas, the bioconcentration factor was mostly > 1. Considering the feeding habits of deposit feeding bivalves, a considerable amount of metals can possibly be accumulated through feeding on benthic diatoms.

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Manuscript in preparation

INTRODUCTION

In recent years a multitude of data has been compiled on contaminant levels in marine and estuarine environments. As for the biota, a lot of information is available on accumulation and effects of heavy metals in birds, fish and benthic macrofauna (e.g., (Salomons and Förstner, 1984; Rand and Petrocelli, 1985; Bryan and Langston, 1992). Accumulation of heavy metals in aquatic flora has mostly been studied in benthic macroalgae, in particular in relation to the use as a biological monitor (Gutknecht, 1963; Bryan *et al.*, 1980; Phillips, 1990).

With respect to microalgae, the response of planktonic species on heavy metals has been studied extensively in cultured populations (Thomas *et al.*, 1977; Canterford, 1979; Romeo and Gnassia-Barelli, 1985). Also heavy metal contents in plankton in the field situation has been studied (Knauer and Martin, 1973; Revis *et al.*, 1989). As far as we know, no data are available on the concentrations of contaminants in the lowest benthic trophic level, the benthic microflora.

Benthic microalgae are an important food supply for numerous deposit feeding intertidal species. It is quite well possible that this microflora might play an important role in the accumulation of contaminants through coastal food chains. Then in the framework of bioaccumulation studies, knowledge on the contaminant concentrations of this benthic microflora is essential.

The reason for the lack of data on contaminants in benthic microalgae might be connected with sampling methods. With the usually applied methods like decantation it is difficult to extract the sediment completely from the microalgae. The aim of this research is to assess the concentration of the heavy metals Cd, Cu, Pb and Zn in benthic diatoms and sediments along an estuarine gradient. For this, an algal collection technique based on phototaxis, is used. It enables collection of benthic microalgae without sediment particles.

MATERIALS AND METHODS

The area

The collection of samples took place along a gradient in the Scheldt Estuary (also called Westerschelde) and at three locations in the Oosterschelde (Figure 1.1). The Scheldt Estuary is heavily polluted, mainly caused by untreated domestic and industrial waste water from cities such

as Brussels, Antwerp and Ghent (for a review, see van Eck et al.; (1991)). It is a very dynamic area with tidal differences of 4 to 5 metres and a salinity gradient from the North Sea to the river Scheldt.

The Oosterschelde is considered as a relatively clean sea arm. It is hardly mixed with fresh water and salinity levels range from 28.6-32.3‰ (Heip, 1989). The sea arm stays in direct contact with the North Sea through a storm surge barrier.

Sampling and analytical procedures

In the sediment, both 'free-living' (epipelic) and 'immobile' (epipsamnic) benthic diatoms can be distinghuised (Round, 1971). Generally, epipelic diatoms are rather large (> 20 µm), whereas epipsamnic diatoms are mostly smaller species (5-20 µm). The latter are firmly attached to sand grains and consequently hardly able to move or migrate through the sediment. They can mostly be found in sandy, exposed locations. The epipelic species dominate in sheltered habitats where they will not easily be suspended (Colijn and Dijkema, 1981; Daemen and De Leeuw-Vereecken, 1985; Sabbe and Vijverman, 1991).

For several decennia it is known, that free living diatoms can be isolated from a damp mud surface by laying a piece of cloth on the surface and allowing time for the algae to move up into it by positive phototaxis (Eaton and Moss, 1966). The method used in this study is based on this concept and comprises a combination of lens cleaning tissues and planktonic gauze as described by Vos (1989).

The diatoms were collected between May and August 1991. On intertidal mudflats, epipelic diatoms tend to move to the surface when the water withdraws. Locations with high concentrations of these diatoms have a typically brown colouring, in particular on sunny days. At these brownish coloured sites, two layers of lens tissue (Whatman, 46x27 cm) were spread out on 10-30 spots per sampling area. On top of the tissues one layer of planktonic gauze (20x30 cm engineered sieve cloth Monodur-Polyamid, mesh opening 80 µm) was placed. After 30-45 minutes the gauzes were collected and directly taken to the laboratory, where the diatoms were washed out with filtered water from the sampling site. The diatom solution was sieved over a piece of gauze (120 µm) to remove any fibres from the lens tissues. Further, they were concentrated by centrifugation. The diatoms were collected by filtering the concentrated suspension over pre-weighed, acid-washed cellulose-acetate filters (0.45 µm, Sartorius). The content of one algal concentrate was distributed over several filters. The filters were stored at -20 °C. After freeze-drying and dry-weight assessment, the filters were destroyed in a low

temperature asher and redissolved in 65% HNO_3 (Merck Suprapur). Metals were measured with graphite furnace atomic absorption spectroscopy (GFAAS) furnished with a Zeeman background correction using graphite tubes with L'vov platforms. In a pilot study on the applicability of various tissue methods (Vos, 1989), at least 100 sheets of plankton gauze were needed to obtain sufficient dry material for one analysis. By collecting the diatoms on cellulose-acetate filters, we could reduce the necessary number of sheets drastically to less than 10 per analysis.

At each location, sediment samples were taken from the upper 10 cm layer with a corer (diameter 20 mm). With a bone spatula, the top layer of the area near the lens tissues was scraped to assess metals in the upper 2 mm of the sediment. Metals, Particulate Organic Carbon and Nitrogen, $CaCO_3$ and grain size distribution in the sediments were analyzed as described in Chapter Five.

RESULTS AND DISCUSSION

The sediments in the Westerschelde had a high percentage of small particles, whereas in the Oosterschelde, sediments were typically more sandy (see Table 7.1). Although the sediments were not normalized for a certain standard fraction, a distinct metal pollution gradient was recognized, going from the mouth of the estuary to the more riverine part of the Scheldt Estuary (location 4 to 11, Figure 1.1). As expected, metal concentrations in sediments from the Oosterschelde locations were lower than in Westerschelde samples (location 1 to 3). The more sandy sediment texture also contributes to the lower metal concentrations.

The observed heavy metal concentrations in sediments agree with previous reports (Wollast *et al.*, 1985; Baeyens *et al.*, 1988; Regnier *et al.*, 1989), be it that at location 9 markedly high lead levels were found in the top as well as in the 0-10 cm layer. The high lead concentrations have to be attributed to the industrial activity along the estuary.

Cadmium concentrations in the diatoms varied between less than 0.1 and 2 µg/g DW (Figure 7.1). Although cadmium concentrations in the sediment could amount up to 7.9 µg/g DW (location 9), levels in the diatoms were very low and the bioconcentration factor was < 1. At the less polluted sites however, the bioconcentration factor (Cd diatom/Cd sediment) was > 1.
For lead also low concentration factors were found: although concentrations in the sediment were rather high, levels in the diatoms were not more 24.4 µg/g DW. Yet, an almost linear relation was found between lead in the sediment and lead in the diatoms, not regarding location 9.

Location	No.	Layer	(%) POC	(%) Ntot	(%) CaCO3	(ug/g) Cd	BCF	(ug/g) Pb	BCF	(ug/g) Cu	BCF	(ug/g) Zn	BCF	(%) <63um	(%) <16.3um
Jacobahaven	1	0-10 cm	0.19		21.1	0.06	21.7	3.2	1.50	3.1	3.3	10	4.4		
	1	0-2 mm	0.2		14.4	0.08	16.3	2.8	1.71	1.7	6.1	10	4.4		
Kattendijke	2	0-10 cm	0.26		6.1	0.33	3	4.6	1.04	1.3	13.8	27	1.4		
	2	0-2 mm	0.57	0.03	6	0.33	3	4.4	1.09	1.2	14.9	27	1.4		
Stroodorp	3	0-10 cm	1.02	0.06	9.4	0.27	1.5	9.6	0.09	2.3	1.5	34	1.1		
	3	0-2 mm	0.96	0.07	7.3	0.28	1.4	5.8	0.16	2.4	1.4	29	1.3		
Rammekes	4	0-10 cm	1.16	0.06	7.5	0.09	6.67	16.2	0.51	6.7	1.28	41	1.28		
	4	0-2 mm	0.21		14.3	0.49	1.23	35.2	0.23	17	0.50	111	0.47		
Ellewoutsdijk	5	0-10 cm	1.17	0.11	16.4	0.82	0.17	35.7	0.15	20.4	0.30	123	0.62	61	28
	5	0-2 mm	1.26	0.15	215	0.61	0.23	30.2	0.18	17.2	0.35	109	0.70	88	52
Kruiningen-haven	6	0-10 cm	0.95	0.07	15.9	0.65	1.85	20.6	0.63	13.6	1.02	78	1.30	82	53
	6	0-2 mm	1.08	0.18	22	0.8	1.50	31.6	0.41	20	0.69	123	0.83	86	57
Baalhoek	7	0-10 cm	0.49	0.06	12.5	0.8	1.60	20.4	0.62	12.2	1.23	94	0.86	55.6	19
	7	0-2 mm	1.05	0.1	16.8	1.2	1.07	39.1	0.33	19.5	0.77	142	0.57	62	21
Appelzak	8	0-10 cm	1.83	0.1	5.1	2	0.23	40.6	0.27	20.9	0.42	126	0.81		
	8	0-2 mm	1.17	0.02	15.7	5.5	0.08	116	0.10	81.6	0.11	430	0.24		
Doel	9	0-10 cm	2.37	0.15	9.9	6.9	0.015	775	0.01	195.2	0.08	56	0.55		
	9	0-2 mm	2.29	0.06	16.2	7.9	0.013	352.5	0.02	96.3	0.16	47	0.65		
Lillo	10	0-10 cm	1.54	0.22	15	7.8	0.09	88.5	0.17	88.5	0.14	350	0.52	86.6	45.3
	10	0-2 mm	1.81	0.18	15.7	5.7	0.13	56.5	0.27	47	0.26	234	0.78	78.9	36.7
Burcht	11	0-10 cm	1.44	0.12	15.6	3.5	0.40	55.6	0.68	51	0.52	206	0.45	64.4	25.6
	11	0-2 mm	0.57	0.05	13.4	4.6	0.30	90	0.42	57.3	0.46	270	0.34		

Table 7.1 Characteristics and concentrations of Cd, Pb, Cu and Zn in the sediment. For each location, the bioconcentration factor (BCF; metals in diatoms/metals in sediment) is given. The locations are marked in Figure 1.1.

At location 9 (not shown in Figure 7.1), Pb concentration in the diatoms was 7.25 (± 3.59) µg/g DW, whereas sediment levels were more than 350 µg/g DW! For zinc, concentrations in diatoms were varying from 20 up to more than 200 µg/g DW. Here also, a linear relation could be found between metals in diatoms and metals in the sediment. Like with cadmium, the variation in copper concentrations in the diatoms was not as large as the variation in the sediment: concentrations varied between 3 and 29 µg/g DW. Only in the Oosterschelde locations, the bioconcentration factor was > 1. These concentrations are in the range that has been found for the pelagic species *Dytilum brightwellii* (Rijstenbil and Poortvliet, 1992). The relation with sediment concentrations was less obvious than for lead or zinc.

Except for copper, which was lower in our samples, the observations made by Vos in the Westerschelde estuary (1989) were within the range of these measurements. Reports on metal contents in pelagic phytoplankton samples were also in the range of the benthic diatom measurements presented here (Knauer and Martin, 1973). It was suggested that exceptional high peaks in metal contents could be caused by dinoflagellate blooms, as dinoflagellates tended to take up higher amounts of Cu per unit of algal biomass than did other algae. Dinoflagellates blooms (*Amphidinium* spp.) are known to occur in the Westerschelde (K. Sabbe, pers. comm.), so these could cause a deviation from expected Cu values.

Generally, metal contents in diatoms from the most upstream location (11) were highest, while metal concentrations in the sediment were not the highest. This can be due to a shift in diatom species caused by a changed physical environment (grain size, salinity). In the Westerschelde, a clear relation between grain size and the distribution of benthic diatom assemblages has been found (Sabbe and Vijverman, 1991). The species most abundant in the Westerschelde are *Navicula* spp., *Delphineis* spp., *Cymatosira* spp., *Cyclotella* spp., *Thalassiosira* spp. (often settled down) and *Gyrosigma* spp. At the more brackish areas (8), typically centricate diatom species are abundant (*Cyclotella* spp., *Thalassiosira*). Another explanation for the elevated values at location 11 can be a relatively high availability of metals from the overlying or interstitial water.

The succes of collecting diatoms in the field is partly depending on weather conditions: because of the phototaxis reaction, the diatom yield will be best at sunny days. On rainy days, collection is not recommended because of the possible contamination by sediment grains, caused by splashing raindrops.

A disadvantage of the method is that only epipelic diatom species are collected, so the data give a representation of only a part of the benthic microalgal community. Epipelic diatom species form a substantial part of the diet of the deposit feeder *Macoma balthica* (Hummel, 1985; Kamermans, 1993).

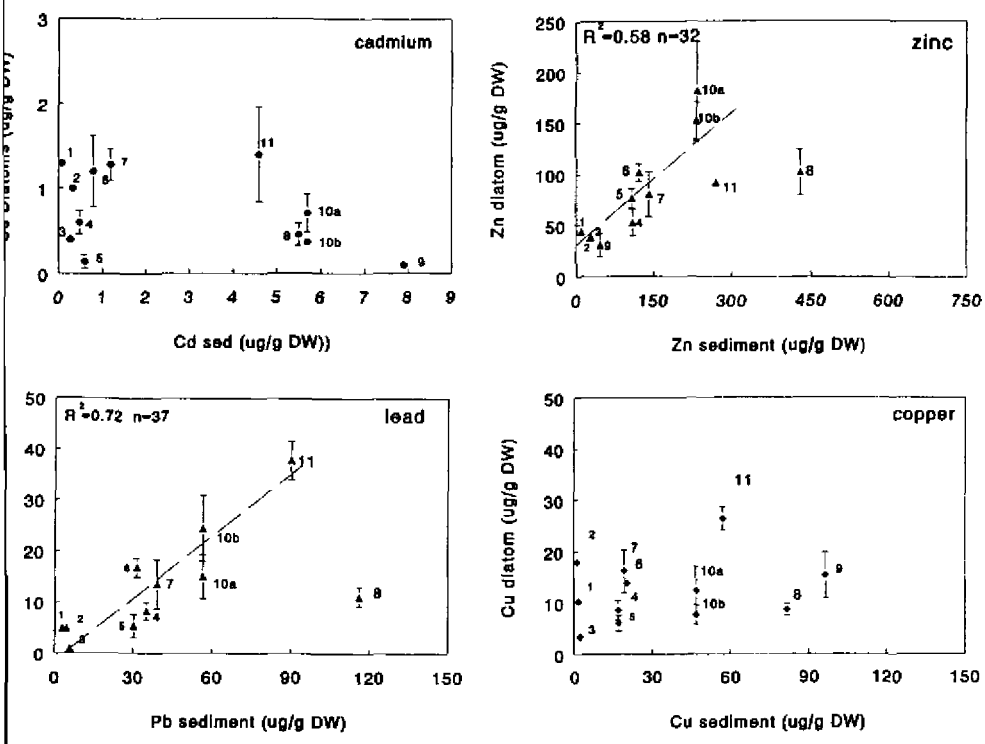

Figure 7.1 Relation between metals in the sediment and metals in benthic diatoms.

With the described lens-tissue technique it was possible to collect benthic diatoms without contamination through sediment particles. Considering the agreement with the analyses made by Vos, it was concluded that our modifications on the analytical procedure did not have any influence on the results.

The described analytical method using cellulose-acetate filters is only applicable for metal analysis. If organic micropollutants were to be studied, more phytoplankton material is needed to obtain sufficient dry weight.

The actual role of food associated heavy metals for the overall accumulation by bivalves is discussed. For mussels, food is considered to contribute for a minor fraction (less than 5%) to the metal levels in the bivalve (Borchardt, 1983; Riisgård *et al.*, 1987; Amiard-Triquet *et al.*, 1988; Ettajani *et al.*, 1991). For deposit feeding bivalves like *Macoma balthica* food might contribute more to the overall metal uptake, because filtration rates are much lower: deposit feeders select from a concentrated source, whereas suspension feeders have to concentrate a very dilute source (Gilbert, 1977; see also Chapter three). If a *M. balthica* individual (30 mg) would consume 3 mg benthic diatoms (10% of body weight) per day with an average Cu content of 15 µg/g DW, then metal intake rate would be 0.045 µg/day (= 1.5 ppm increase per day; around 8% of the tissue metal content in clams from unpolluted regions), taking the view that the diatom-associated Cu would be 100% biologically available. In practice, metal availability will not be 100%, and the clam will also loose Cu through elimination. A substantial metal contribution via food is nevertheless well imaginable.

More research is needed on the influence of algal growth rates, season, and species composition on metal content of an algal sample. Whether concentrations in benthic diatoms are governed by metal concentrations in the overlying water or in the pore water, is also a question that remains to be solved. Also the actual contribution of benthic diatoms to contaminant accumulation in deposit feeders remains uncertain as the diet of deposit-feeders consists of both benthic and (settled down) pelagic food material.

CONCLUSION

By using the lens-tissue technique, enabling diatoms to move upwards in plankton gauze sheets, we were able to collect epipelic diatoms without sediment particles. The collection method is very suitable for pollution studies, especially for the assessment of heavy metal contents. In general, metal concentrations were varying in a smaller range than total metal concentrations in the sediment. Bioconcentration factors were often > 1 in low polluted areas, while in highly polluted areas, bioconcentration factors were << 1. In spite of the lower bioconcentration factors, the diatoms might very well contribute to the overall metal accumulation in benthic deposit feeders, because (unlike sediment particles) they have a high food value.

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Chapter eight

A simulation model for growth and heavy metal uptake by the deposit feeder *Macoma balthica.*

ABSTRACT

In order to describe metal accumulation in *Macoma balthica*, a dynamic simulation model was developed. The model comprises a 'growth' submodel, describing the variation in dryweight of *M. balthica* during the year, and a 'metal' submodel, describing the accumulation and elimination of cadmium and copper from the water (dissolved), food (particulate) and the sediment. The 'growth' submodel was calibrated with data from a field survey. During 2 years, animals and sediment were collected from 5 locations on an intertidal mudflat, varying in sediment composition. In an initial model description, growth of *M. balthica* was related to the Chl-*a* fraction of particulate organic carbon in water as well as in the sediment. This resulted in a growth lag in springtime, and high growth rates in the summer period. In the field situation, increase in dryweigth only took place from February until around June. This growth could only be modelled if *Macoma balthica* was allowed to feed and grow on detritus, even if this food would be of a relatively low quality.

Tissue metal concentration depended for the larger part on dissolved metal concentrations and on growth dilution, in particular for cadmium. Sediment composition influenced metal uptake in the sense that in sandier environments, animals spend a relatively large time suspension feeding. In that case, the contribution of dissolved metals was relatively large, compared with the more muddy substrates. Both in the model and in the field situation, sediment metal concentrations contributed only partly (copper) or hardly (cadmium) to the overall metal uptake, except for accidental high metal concentrations on the sandy locations.

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INTRODUCTION

In aquatic environments, contaminant concentrations in bivalve tissues can be used as a monitor for environmental pollution. For the marine water phase, *Mytilus edulis* is a commonly used species. For sediment monitoring, bivalve deposit feeders are interesting because they live in direct contact with the sediment. However, the results that have been published on the relation between the degree of pollution of the sediment and accumulation in bivalves like *Scrobicularia plana* or *Macoma balthica* so far are not very promising: the bivalves did not accumulate in relation to their environment, and only poor relations were established (Luoma and Bryan, 1982; Bryan and Langston, 1992). This has amongst others geochemical causes: metal bioavailability from the sediment is substantially influenced by sediment composition, redox situation and binding strength of contaminants with different sediment elements (Jenne and Luoma, 1977; Campbell *et al.*, 1988; Luoma, 1989). As *Macoma balthica* is exposed directly to the sediment, metal accumulation is supposed to be related to the (biologically available) metal concentration in the sediment. However, *M. balthica* is also in close contact with the overlying water through its siphons. For this reason, it can accumulate metals through food that origins either from the overlying water (when suspension feeding) or from the sediment top layer (when deposit feeding). While sucking up particles, *Macoma balthica* filters considerable amounts of (overlying) water. These routes of uptake are often neglected when studying bioavailability of metals from the sediment.

Other biological factors that influence metal uptake should not be underrated. For example, deviations from the expected concentrations have been shown to be related to differences in tolerance of populations (Luoma *et al.*, 1983). Apart from these interspecies differences in sensitivity (tolerance), animals from different locations experience different food conditions, which influence the animals' condition and metabolic activity, and consequently metal accumulation and elimination rates. For example, with *Mytilus edulis* it has been demonstrated that cadmium uptake rates are influenced by food availability (Borchardt, 1983). Moreover, the mode of feeding could also influence metal accumulation: bivalve deposit feeders are able to switch between deposit and suspension feeding, which might influence the amount of metals accumulated from either the sediment or the overlying water.

To discriminate between and to estimate the role of some major biological mechanisms that will influence metal uptake by *M. balthica*, a dynamic simulation model for trace metal uptake has been developed. The metabolic activity of an organism is used as the basis for metal uptake. Consequently, the model for *Macoma balthica* is composed of a submodel for growth and a submodel for metal uptake. Some basic elements of the process formulation of

this physiologically structured model are derived from descriptions of mussel feeding and growth in the Oosterschelde estuary (Klepper, 1989; Herman, 1993) and a model that described cadmium and copper accumulation in *Mytilus edulis* (Van Haren *et al.*, 1990). Because limited information was available on growth determining factors, the growth submodel was calibrated with dryweight data from an intensive monitoring program at an intertidal mudflat in the Westerschelde estuary. This mudflat shows a large gradient in sediment composition over only a small distance. Salinity and degree of pollution are average, so it can function as a representative mudflat for a large part of the estuary. To discriminate between seasonal effects, sampling was carried out intensively during two successive years.

For the input of the metal submodel, parameters were assessed experimentally (Chapters five and six). The metal submodel was validated with data on tissue metal contents in the field monitoring program.

MATERIALS AND METHODS

Field monitoring program

The animals were collected from the intertidal mudflat Baalhoek (Figure 1.1, Page 14). From the dike to the channel, the mudflat measures approximately 300 m. The highest location, bordering the dike, has a high fraction of small particles and organic material. Going towards the channel, the sediment becomes increasingly sandy. The samples were taken on 5 sites along the sediment gradient, in a straight line from the dike towards the channel. The distance between location 1 and 2 and between 2 and 3 amounted 20 m. The oter intervals were 40 m. The tidal range on this mudflat is relatively small, the highest sampling location being 10 cm below mean tidal level (MTL) and the lowest 115 cm below MTL.

Sample collection and handling

Monthly from March until August and bimonthly from August until March, samples were collected. Sampling always took place in the first week of each month. *M. balthica* was collected by sieving the upper 15 cm of the mudflat with a nylon net. The animals were always collected just after the water had withdrawn from the area. In the laboratory, the animals were allowed to empty their guts for 24 hours in filtered (Whatman GF/C) Westerschelde water, collected at the same location. Until dissection, they were kept frozen at -20 °C. Shell length (L) was measured with a calliper along the longest axis to the nearest 0.01 mm.

The collected animals were separated in 3 age groups. The age was assessed on the basis of the winter-rings on the shell. The first group contained 2 year old animals. The second group contained 3 year old animals and the third group contained 4 and 5 year old animals. Younger or older animals were not used. Dry weight and tissue metal content were assessed for every age group. After dissection, the tissues were freeze-dried.

Simultaneously with the collection of the animals, samples were taken from the sediment. The 0-10 cm layer was collected with a corer (diameter 20 mm). Samples from the top 3-mm were taken by scraping gently over the sediment surface with a bone spatula. Until processing, the sediment samples were stored at -20 °C.

Water samples were taken in the beginning of each month during high tide. For metal analysis, an acid cleaned poly-ethylene bottle was filled at a distance of appr. 3 mtr from the shore with help of a glass fibre pole. A second water sample was taken for the measurement of Chlorophyll-*a*, particulate (POC) and dissolved (DOC) organic carbon. Chlorinity and temperature were measured on the sampling location. Analyses on tissue, sediment and water were carried out as described in Chapter five.

MODEL DESCRIPTION: CARBON UPTAKE AND GROWTH (SUBMODEL *GROWTH*)

Feeding strategy of Macoma balthica

Feeding activity is associated with food quality, food availability and water temperature. *Macoma balthica* is classified as a deposit feeder, as it feeds by 'vacuum cleaning' the sediment surface with its long movable siphons (Brafield and Newell, 1961). Although *M. balthica* is a deposit feeder, food can be derived from the overlying water as well: while eating from the sediment, water is taken in simultaneously. Stomach contents of *Macoma* from the Dutch Waddenzee indicated that a substantial part of the ingested diatom species had a pelagic origin (Hummel, 1985c; Kamermans, 1993). Some authors even relate growth in *Macoma balthica* directly to Chl-*a* concentration in the overlying water (Hummel, 1985c; Zwarts, 1991). However, the actual contribution of pelagic or benthic food sources will most probably depend on the relative abundance of either source. In early publications, it has been suggested that *M. balthica* is a deposit feeder at low water and a suspension feeder when covered by tide (Brafield and Newell, 1961). Also a temperature dependence has been suggested, implying a reduced time of deposit feeding at temperatures above 10 °C, and consequently reduced uptake of food (De Wilde, 1975). In aquaria as well as from field

experimental data, it has been demonstrated that *Macoma* from muddy sand sediment was deposit feeding, whereas clams from sandy sediment were filter feeding (Olafsson, 1986).

Recently, it has been postulated that hydrodynamic conditions are a major determinant of feeding behavior, where with increasing water velocity, the deposit-feeding radius of *Macoma* decreased. At a high flow regime, it might be more profitable to switch from deposit feeding to suspension feeding (Levinton, 1991).

These different hypotheses seem incompatible, but there is considerable overlap: the observations in the Waddensea were mostly carried out in relatively sandy sediment habitats (Kamermans, 1993). High flow regimes co-occur generally with sandy sediment, whereas low flow regimes are more common with muddy sand sediments. The common feature in all these hypotheses is that at a certain time, *Macoma* will feed on the energetically most efficient food source.

In the model, *Macoma balthica* will consume material from the sediment as well as from the overlying water, because even if *M. balthica* is deposit feeding 100% of its feeding time, still food from the overlying water might be ingested. Yet, benthic material, i.e. detritus, bacteria and benthic diatoms, is considered to form the major food source (Lopez and Levinton, 1987). The contribution of these sources is depending on the nutritional value. Compared with other deposit feeding species (e.g. polychaetes), the ingestion rate of inorganic sediment in bivalves is remarkably low. It has been suggested that this is due to the capability of a high degree of selection (Cammen, 1980). Indeed, *Macoma balthica* has been demonstrated to preferentially select particles with organic coatings (Taghon, 1982). Therefore in the model, ingested material is expressed as Particulate Organic Carbon (POC). The concentration Particulate Organic Carbon in either the water (POCWAT) or the sediment (POCSED) will determine the uptake rate. Because POCWAT is normally expressed in mg/l, whereas POCSED is given in g/kg Dry Weight (or in weight percentage of the sediment), a conversion factor is used.

$$POC = SEDFAC * POCSED + POCWAT \qquad (1)$$

where	**POCSED**	Particulate organic carbon in the sediment	(mg/g DW)
	SEDFAC	Conversion from g/kg to mg/l	(g/l)
	POCWAT	Particulate organic carbon in the water	(mg/l)
	POC	Total particulate organic carbon for Macoma	(mg/l)

In certain conditions, it will be energetically profitable to switch to pure suspension feeding. This switch is depending on the ratio between available food from the sediment and

the overlying water. If this ratio is below a certain level in the model, POC is POCWAT and POCSED is 0. As a consequence, *Macoma balthica* will spend proportionally more time suspension feeding in sandy habitats.

$$if(\frac{POCSED}{POCWAT}<LIM)thenPOC=POCWAT \quad (2)$$

where **LIM** threshold level for switch to suspension feeding (g/l)

Clearance rate

In the model, uptake of particles from the overlying water (filtration rate) is a function of the concentration of POC (particulate organic carbon) and the clearance rate (volume of water pumped per unit of time). This clearance rate is a temperature regulated function, depending on the size of the animal with an allometric relation (Bayne, 1976).

$$TEMPC=Q10CR^{(\frac{TEMP-10}{10})} \quad (3)$$

where **TEMPC** temperature effect on clearance rate (-)
CR clearance rate (l/d) (l/d)

The clearance rate is depressed at high food concentrations (Widdows *et al.*, 1979; Riisgård and Randlov, 1981; Hummel, 1985b; Bayne *et al.*, 1988). The description for the optimum filtration curve of *Macoma balthica* is given in Chapter six.

$$RED=e^{-\frac{1}{POC}*\frac{(POC-CP)^2}{WID^2}} \quad (4)$$

where **RED** Reduction of filtration rate, depending on food concentrations (-)
CP POC concentration for which clearance rate is maximum (mg/l)
WID POC concentration determining the rate of reduction (mg/l)

$$CR=A_c*(W^{B_c})*TEMPC*RED \quad (5)$$

where $\mathbf{A_c}$ constant (l/d/mg DW)
$\mathbf{B_c}$ constant (-)
W dryweight individual organism (mg DW)

During emersion, it will be more difficult for *M. balthica* to continue feeding. Observations on Chlorophyll-*a* intake showed that the major part was taken in during submersion (Hummel, 1985c; Kamermans, 1993).

$$FILTR = FL * POC * CR \quad (6)$$

where	**FILT**	filtered particles	(mg/d)
	FL	time period per tide flooded (0 < FL < 1)	(-)

The filtered particles are selected in several steps. The first step of particle selection are the gills: 'quality' particles are concentrated and passed to the labial palps. Large particles are removed by ciliary sorting mechanisms and ejected as pseudofaeces (PSF) via the inhalant opening. The proportion of material that is rejected is expressed with a treshold level for ingestion.

$$PSFR = \max\left(0, \left(1 - \frac{TR_{ps}}{POC}\right)\right) \quad (7)$$

$$PSF = PSFR * FILTR \quad (8)$$

where	**PSFR**	proportion that is rejected	(-)
	PSF	pseudofaeces	(mg/d)
	TR_{ps}	treshold level for ingestion	(mg/l)

The ingested material will not be assimilated totally, because only a fraction actually consists of edible material. This edible fraction (EDFR) will be larger during the summer period (because of the higher turnover rates) than in the winter. The input of EDFR is given as a forcing function. The assimilated material is expressed as follows:

$$ASS = EDFR * (FILTR - PSF) \quad (9)$$

where	**ASS**	assimilated material	(mg/d)
	EDFR	edible fraction	(-)

The unused material is released through the exhalant siphon as faeces:

$$FAEC = (1 - EDFR) * (FILTR - PSF) \quad (10)$$

where	**FAEC**	faeces	(mg/d)

Respiration

The assimilated material is used for metabolic processes, reproduction and growth. The metabolic processes are summarized in a temperature depending first order function for basal respiration rate. As these are depending on enzyme activity, the temperature dependence is expressed as a Q10 function. The energy costs for the activity of the animal (assimilation-dependent rate), are not incorporated in the model formulation, as this has shown to be not more than 3% for mussels (Widdows and Hawkins, 1989).

$$TEMPR = Q10R^{\left(\frac{TEMP-10}{10}\right)} \quad (11)$$

$$RES = A_r * W^{B_r} * TEMPR \quad (12)$$

where **TEMPR** temperature effect on respiration (-)
Q10R Q10 factor for respiration (-)
RES respiration (mg/d)
A_r constant (1/mg DW/d)
B_r constant (-)

From February on until around May, energy is put in gonadal material, which is lost through spawning. The fraction that is used for spawning (SPFR) is a weight dependent function, where SPAWN as a forcing function indicates during which period energy is put in spawning (data derived from Hummel (1985a)). The total energy put in spawning is depending on the actual weight of the animal.

$$SPFR = A_s * W^{B_s} * SPAWN \quad (13)$$

$$SP = SPFR * W \quad (14)$$

where **SPFR** fraction used for spawning (-)
A_s constant (1/mg DW/d)
B_s constant (-)
SPAWN time period in which spawning takes place (-)
SP energy put in spawning (mg/d)

The scope for growth is the resultant of assimilated minus respirated material:

$$SFG=ASS-RES \tag{15}$$

where **SFG** scope for growth (mg/d)

For the actual growth, weight lost through spawning is substracted from the scope for growth.

$$\frac{dW}{dt}=SFG-SP \tag{16}$$

As in the field situation growth has shown to be unaffected by a salinity range (Thompson and Nichols, 1988), any influence of salinity on growth was not included in this submodel.

METAL UPTAKE AND ELIMINATION (SUBMODEL METAL)

For metal uptake, three different routes are considered: uptake of dissolved metals through the gills (DISFLUX), uptake of metals associated with food (FFLUX) and metal uptake from the sediment (SEDFLUX).

Dissolved metal uptake

Uptake through the gills is surface dependent. Heavy metal bioavailability will be determined by the chemical form of the metal, i.e. metal speciation. For metal input from the water, total dissolved concentrations are used instead of a more accurately defined biologically available fraction. It is recognized that, in particular for copper, total dissolved metal is not representing the available fraction. However, no directly measurable bioavailable fraction can be defined so far.

Also salinity and oxygen tension of the overlying water will influence metal uptake through changes in copper speciation as well as through influence on metabolic activity (Akberali, 1978; Neuhoff, 1983; McLusky *et al.*, 1986). Within the scope of this model, it is not possible to differentiate between these factors as their role is rather complex (see Chapter four and (Neuhoff, 1983; Wright and Zamuda, 1987). The metal uptake rate is a dimensionless constant and is a summary of the above mentioned influences. It will vary for each situation.

The only source of dissolved metals in the model is the overlying water. Although for the assessment of sediment toxicity, pore water is considered to play a key role (Giesy *et al.*,

1990; Burton Jr, 1991), it seems to be of minor importance with respect to bivalve deposit feeders: experiments with the clam *Macoma nasuta* indicated that pore water contributes for not more than 4% to the overall ventilation of water (Winsor *et al.*, 1990).
For the estimation of parameters for dissolved (GILLEXCH) and particulate (GUTEXCH) copper uptake rates, data from long term laboratory experiments with different food conditions were used (Chapter six). The parameters were estimated with simplified version of the metal submodel, where dryweight, sediment input and filtration rate were fixed (see further). Parameter values for cadmium uptake were derived from Sarala Devi (1990) and McLeese (1984).

Influence of food on metal uptake

According to Borchardt (1983), the contribution of food to the overall metal accumulation is supposed to be mainly through the stimulation of ventilation activity: a positive correlation exists between food concentration and the uptake of trace metals. For the model, the relation between clearance rate and metal uptake rate has a Michaelis-Menten limitation, suggesting that at very low clearance rates (when no food is available) metal uptake is reduced and that at high clearance rates, metal uptake is independent from the clearance rate, because exchange processes at the gill membrane surface are slower than water refreshment through the gills.

$$DISFLUX = GILLEXCH * FL * \left(\frac{CR}{B_m + CR}\right) * M_{DISS} * W^{-\frac{1}{3}} \qquad (17)$$

where	**DISFLUX**	metal accumulated from the water	(µg/g DW/d)
	GILLEXCH	uptake factor water	(-)
	$\mathbf{B_m}$	constant	(-)
	$\mathbf{M_{diss}}$	concentration total dissolved metal	(µg/l)

Although for filter feeding bivalves, metal concentrations in food seem to play minor role in metal uptake rates, this theory may not hold for deposit feeding bivalves (see Chapter four).

Uptake from food takes place in the gut, where only nutritive particles will be ingested. Material of lower nutritive value will be expelled as faeces or pseudofaeces before any digestion has taken place. Therefore, the bioavailability of metals, associated with (pseudo)faeces, is considered to be neglectible.

In some model descriptions the particulate fraction of metals in water is regarded as the food-associated metal source. However, this fraction consists for only a minor (and not well

defined) part of nutritive material. Moreover, deposit feeders will depend for a considerable part on bottom-related food material. To define a more correct measure for food-related sources, the metal contents of benthic diatoms, which form part of the diet of *M. balthica*, were used as input for metal flux through food in the model (Chapter seven). Although only very limited information is available on metals associated with diatoms, the metal content of benthic and pelagic food sources is considered to be comparable (Chapter seven).

$$FFLUX = GUTEXCH * ASS * M_{FOOD} / W \qquad (18)$$

where	**FFLUX**	metal accumulated from food	(µg/g DW/d)
	GUTEXCH	uptake factor food	(-)
	$\mathbf{M_{food}}$	metal concentration in food	(µg/g)

Metal availability from the sediment

Because uptake mechanisms from sediment-associated metals (SEDFLUX) are not sufficiently understood (e.g. (Luoma and Bryan, 1982; Campbell *et al.*, 1988; Luoma, 1989)), this uptake process is regarded as a 'black box'. Uptake factors (FAC_m) are derived empirically. For the biologically available metal content in the sediment, the fraction < 63µm was considered to be a more realistic estimate than the total concentration (Chapter five). For parameter estimation of uptake of copper and cadmium from the sediment, data were derived from long term experiments in with spiked aged sediments (Chapter five). As with the parameters for disflux, the parameters were estimated with simplified version of the metal submodel, where now dryweight, water input, food input and filtration rate were fixed (see further). Although a detailed description of biogeochemical processes is preferred, no further refinement in more or less available fractions was made, because of lacking input data.

$$SEDFLUX = FAC_M * M_{SED} * W^{-\frac{1}{3}} \qquad (19)$$

where	**SEDFLUX**	metal accumulated from sediment (µg/g DW/d)
	$\mathbf{FAC_m}$	uptake factor from sediment
	$\mathbf{M_{sed}}$	metal concentration in sediment (µg/g)

Metal accumulation and elimination

Like in other bivalve molluscs, a metal detoxifying mechanism will be functional in *Macoma balthica*. A generally accepted concept for the detoxification pathway is complexing

of the metal ion with a metallothionein-like protein, followed by elimination in the hepatopancreas through lysosomes (Simkiss *et al.*, 1982; Viarengo *et al.*, 1984; Roesijadi, 1992). Although investigations have failed to demonstrate the functioning of metallothionein-like proteins (Langston and Mingjiang Zhou, 1987), some mechanism may be assumed, regarding the relatively high tissue metal concentrations in *Macoma balthica*, compared with *Cerastoderma edule* (Chapter Six).

It is generally known that metal detoxification mechanisms are induced at exposure to elevated metal concentrations. Therefore in the model, elimination is linearly related to the accumulated tissue metal concentration. The metal binding proteins have major function in the homeostasis of essential metals. Therefore, baseline concentration for copper is given (M_{CRIT}).

$$ELIFLUX = ELIRATE * (M_{TISS} - M_{CRIT}) \qquad (20)$$

where	**ELIFLUX**	elimination from organism	(µg/g DW/d)
	ELIRATE	elimination factor, depending on metal	(1/d)
	$\mathbf{M_{tiss}}$	metal concentration in organism	(µg/g)
	$\mathbf{M_{crit}}$	baseline concentration of essential metals	(µg/g)

Changes in metal concentration in *Macoma balthica* are the result of uptake and elimination fluxes:

$$\frac{dM_{tiss}}{dt} = DISFLUX + FFLUX + SEDFLUX - ELIFLUX \qquad (21)$$

The model was implemented in SENECA 2.0 (De Hoop *et al.*, 1993). Data on POC in water and sediment, water temperatures, and dryweigth of the animals from the monitoring program were used as input for *calibration* (parameter estimation in SENECA) with the submodel growth. Metals in the overlying water and in the sediment, and the results from the growth submodel were used to run simulations in the submodel metal. Input data for dissolved cadmium were derived from the WAKWAL monitoring program (Tidal Water Division), with a correction factor, derived from a measuring program on the Westerschelde (Zwolsman, 1994). Dissolved copper was monitored monthly by the Centre for Estuarine and Coastal Ecology. The model simulations were compared with the data on tissue metal concentrations of the 2-year old age group of the collected animals (the *validation* of the model).

date	01/16	03/05	04/02	05/21	06/06	07/04	09/04	11/07	01/09	03/07	04/11	05/07	06/05	07/11	08/07	10/31
Cadmium (ug/g DW)																
location 1	1.45	2.18	2.86	1.14	0.74	0.51	0.78	0.9	1.1	2.1	0.58	0.8	1.8	1.1	1	1.98
2						0.51	0.38	0.43	0.71	1.9	0.89	0.87	0.68	1	0.57	0.79
3	0.64	0.21	1.07	0.28	0.4	0.26	0.24	0.26	0.4	0.34	0.66	0.65	0.32	0.44	0.46	0.45
4	0.23	0.22	0.32	0.11	0.21	0.12	0.08	0.06		0.1	0.12	0.07	0.07	0.06	0.13	0.15
5	0.09	0.11	0.10	0.06	0.11	0.14	0.05	0.06		2.3	0.29	0.04	0.06	0.06	0.2	0.09
Copper (ug/g DW)																
location 1	8.16	28.03	24.63	14.78	8.24	5.7	8.6	9.1	22.6	17.4	7	18.9	13.9	23.1	13	32
2						5.9	5.2	5.9	9.1	19.7	8.5	18.2	12.1	20.7	7.8	7.9
3	3.96	5.25	12.99	3.88	4.39	3.2	4	4.1	6.3	10	5.2	15.6	12.1	11	5.8	6
4	1.50	2.34	3.62	1.83	2.19	1.2	1.7	1.1		6.1	1	6.3	4	2.7	1.1	2.1
5	0.42	1.29	1.44	1.03	1.39	2.7	1.1	0.34		3.3	1.6	5.4	2	6.9	0.76	1.2
POC (%)																
location 1	1.27	1.21	2.56	1.64	1.28	0.46	0.75	0.91	1.02	0.8	0.37	0.44	0.96	1.24	0.95	0.99
2						0.67	0.55	0.67	0.85	1	0.8	0.54	0.61	1.17	0.6	0.61
3	1.38	0.49	1.14	0.63	0.62	0.42	0.41	0.42	0.25	0.5	0.74	0.37	0.4	0.61	0.29	0.56
4	0.51	0.37	0.56	0.36	0.47	0.18	0.16	0.18	0.15	0.16	0.37	0.12	0.17	0.25	0.17	0.14
5	0.17	0.11	0.35	0.18	0.43	0.2	0.14	0.2	0.1	0.09	0.1	0.08	0.14	0.16	0.11	0.12
% < 63 um																
location 1	65.1	80.1	72.0	49.1	52.2	48.5	51.8	48.7	62.6	39.7	41.4	59.9	75.7	83.8	78	
2						39.5	38.5	31.8	34.2	33.9	45.2	62.1	56.6	67	52.9	
3	39.4	35.9	44.2	28.2	34.3	26.5	29.4	21.5	22.1	20.9	42.9	35	33.3	40.6	43.6	
4	28.5	26.3	27.1	17.7	16.9	16	12.5	12.3	13.7	14.8	12.6	10.2	13.5	13.1	18.4	
5	17.3	15.4	13.9	10.3	14.7	16.2	8.5	10.1	10.2	9.6	11.9	10.3	10.1	10.5	11.6	

Table 8.1 Sediment characteristics, Cd and Cu concentrations during the simulation period. These data were used as input for the model.

SUBMODEL GROWTH

PARAMETER	act. value	units
Q10CL	2	-
Q10R	1.08	-
LIM	0.6	-
AC	0.094	l/d/mg DW
BC	0.45	-
SEDFAC	0.58	g/l
WID	9.07	mg/l
CP'	7.4...1.6	-
TRESHPS	6.07	mg/l
AR	0.017	mg DW/mg
BR	0.61	-
AS	0.0003	1/mg DW/d
BS	0.5	-
FLOOD'	5.5...7.0	-

SUBMODEL METAL

PARAMETER	act. value		units
GUTEXCH Cu	1	5	-
FACCU	0.02	0.02	-
GILLEXCH Cu	0.8	2.1	-
ELIRATE Cu	0.05	0.05	1/d
CUCRIT	12	12	ug/g DW
AM	1	1	-
BM	0.00032	0.00032	l/d
GUTEXCh Cd	0.12	0.12	-
FACCD	0.0098	0.0098	-
GILLEXCH Cd	1.6	1.6	-
ELIRATE Cd	0.03	0.03	1/d

Table 8.2 Parameter values for submodel 'Growth' and submodel 'Metal'. For 'Metal' the initial and the adapted values are given. CP' and FLOOD' are site dependent.

RESULTS

Calibration - growth

The density of *Macoma balthica* in the field was very much related to the sediment composition. The highest density was found in the muddiest sediment. The 2- and 3- years animals were most abundant on every site.

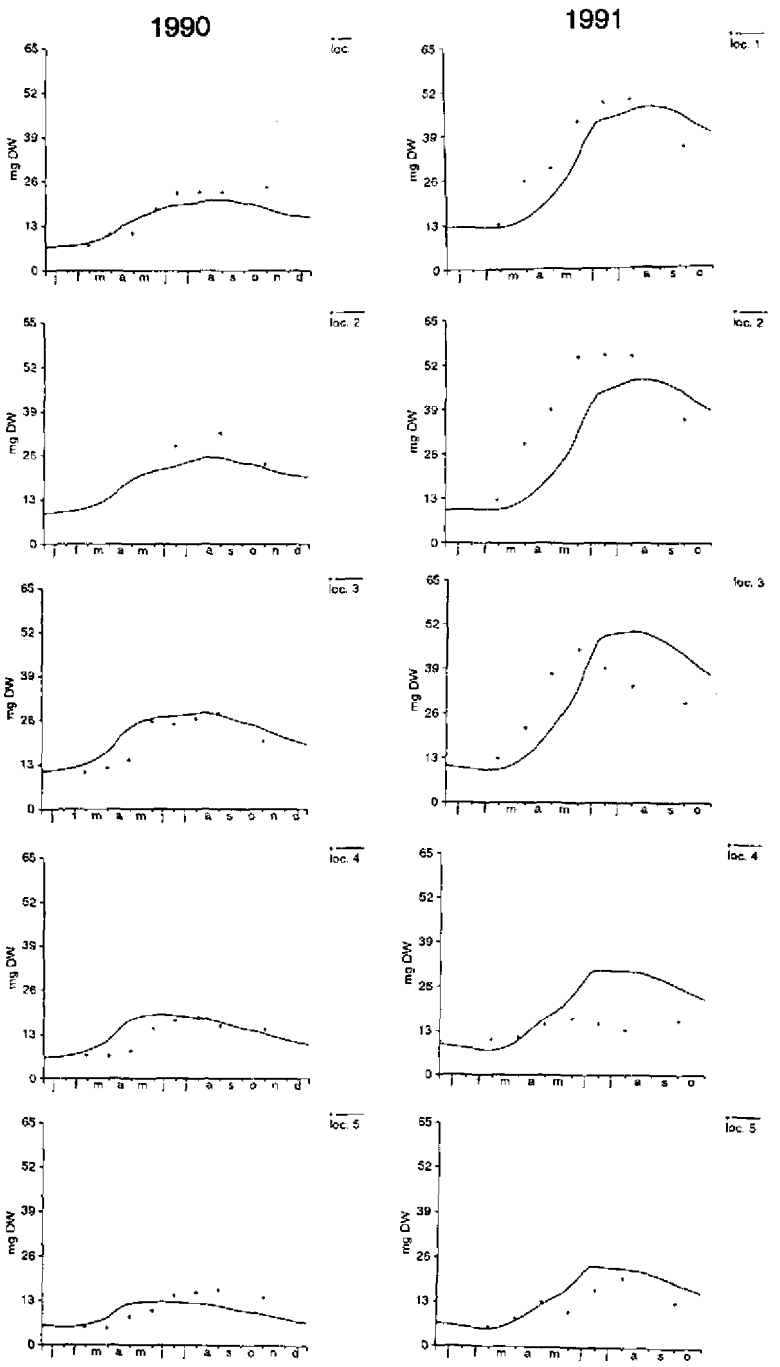

Figure 8.1 Simulation of tissue dry weight of 2-year old *M. balthica* at locations 1 to 5 in 1990 and 1991.

Shell length and tissue weigth of each age group was also very much related to the sediment composition. In spite of the shorter submersion time, the largest animals were found on site 1 and 2, with a gradual decrease of size towards the sandier locations.

In 1990, the dry weight of the animals showed a slight decrease in early spring but increased sharply in late spring (Figure 8.1). From the summer on, the dry weigth decreased slowly to a minimum in the winter. In 1991 a similar growth curve was observed, but maximum dry weights were much higher. This increase was obvious in particular on the muddy locations. With the model description, growth could be described reasonably, although in the natural situation weight decrease is larger than would be expected from the model simulation. About 30% of the filtered material was assimilated. At the more food abundant muddy locations relatively more material was expelled as pseudofaeces. The Scope for Growth of *Macoma balthica* was only positive from February until around July (Figure 8.2).

Parameter estimation for metal uptake

Simplified versions of the submodel 'metal' were used to estimate parameter values for uptake and elimination. For this, data from Chapter Five (sediment) and Chapter Six (water and food) were used. For cadmium and copper uptake from the sediment, a passable fit was obtained for different degrees of sediment pollution (Figure 8.3). Also for copper from the water and food, parameter values were obtained that gave a good fit in clean as well as in polluted situations (figure 8.4). The filtration rate was kept constant for both food situations.

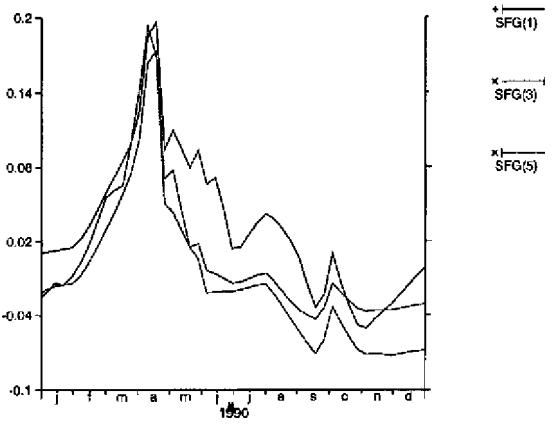

Figure 8.2 Scope for Growth of *M. balthica* in 1990 at 3 locations, going from silty (1) to sandy (5).

Validation - metal accumulation

Cadmium. For cadmium a very clear seasonal tendency was found in the animals (Figure 8.5). A peak around March was followed by a steep decline in cadmium concentration in the tissue until a minimum around June. From then, the concentration slowly increased until the maximum in the early spring in the following year.

The total accumulation in 1991 was distinctly lower than in 1990. The maximal accumulation (appr. 2.5 µg/g) was found in animals from site 5 in the spring of 1990. The lowest cadmium content (less than 0.5 µg/g) was found in individuals from site 1 during the late spring of 1991. On the sandy locations, variations in the cadmium concentrations in the sediment were reflected in the clams (see March 1991). In spite of the lowest total cadmium concentration in the sediment, the highest tissue cadmium levels were nearly always found in the sandiest sediment. According to the model simulation, cadmium in the water was largely determining the overall accumulation (Figure 8.5).

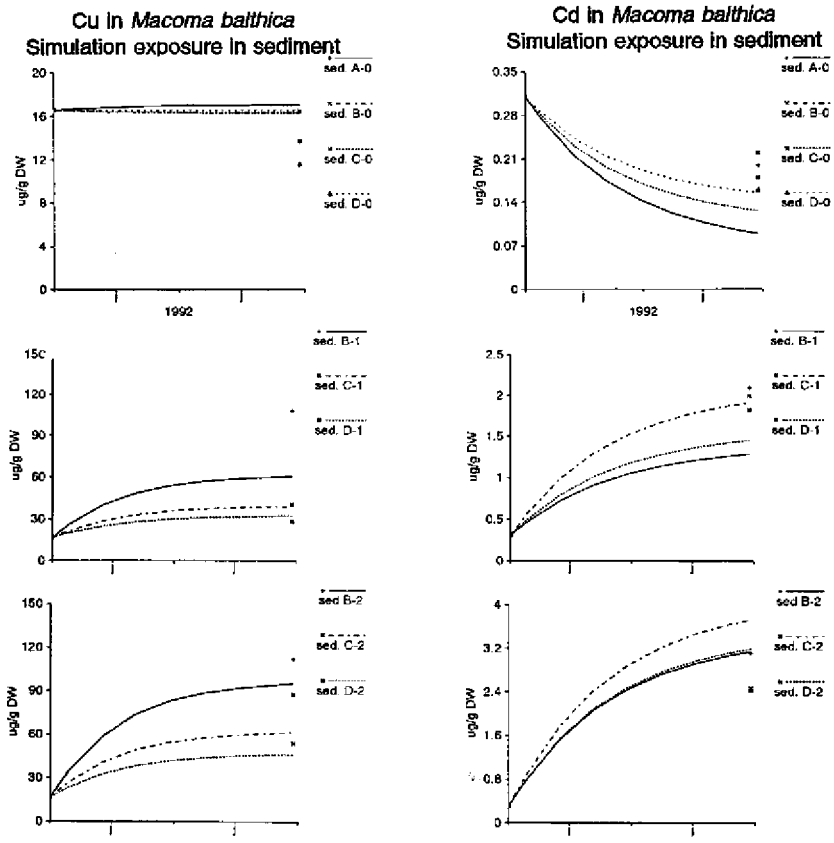

Figure 8.3 Parameter estimation for Cd and Cu uptake from the sediment. The starting and final tissue concentrations after the 57 day exposure are derived from Chapter Five.

Copper. For copper, hardly any seasonal influence could be observed in the field data. In 1990, a slight increase could be observed in the early spring, followed by a very gradual decrease until the late autumn (Figure 8.8). In 1991 however, no seasonal pattern could be seen. In 1990, the highest copper content was always found in the muddiest sediments (site 1), whereas the concentration in animals from site 5 always was much lower. In 1991 however, copper in site 1 animals was lower than in 1990, whereas much higher concentrations were found in site 5 animals. Remarkable were the peak concentrations that occured in samples from site 5 in April and May.

With the model simulation, the experimental parameters for dissolved metal uptake gave too high Cu tissue concentrations for the field situation (Figure 8.9). Therefore, the values for dissolved uptake and food uptake were adapted, to obtain a more realistic representation of the field situation. According to the model simulation, all compartments contributed to a certain extent to the overall uptake. However, on the sandy locations, water was relatively more important (Figure 8.7).

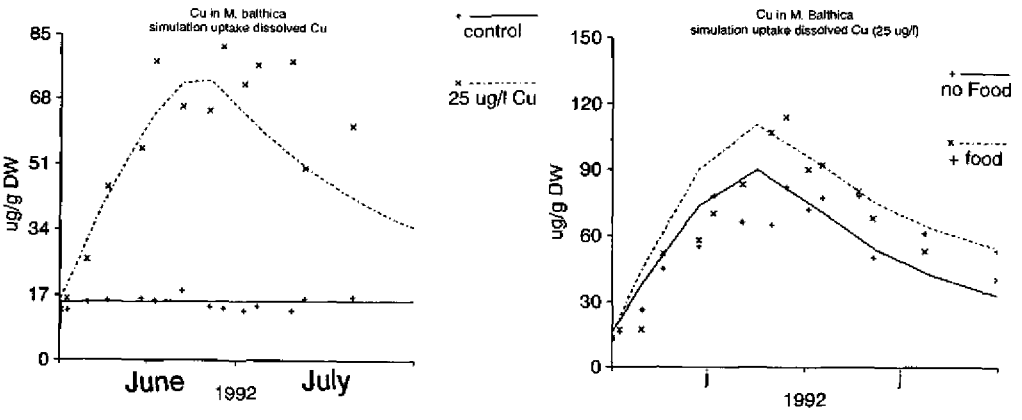

Figure 8.4 Parameter estimation for dissolved and particulate Cu uptake. The tissue concententrations are derived from Chapter Six.

DISCUSSION

Submodel growth

From the available literature, it was difficult to obtain a clear image of the food uptake process by *Macoma balthica*. In particular, information on the edible fraction of ingested

material and the production of (pseudo)faeces is rather varying. *M. balthica* is reported to process daily 11-25% of its body weight organic matter (Bubnova, 1972). The amount of filtered organic matter in the model simulation was in the same range. The proportion of organic material expelled as faeces or pseudofaeces was varying during the year, but on the whole, it was in the range described by Hummel (1985a). Deviations from the starting point of the increase in dryweight can be explained by the energy that is put into shell growth at the start of the growing period. Shell growth is not incorporated in the model.

Temperature or food limitation?

The observed growth pattern in *M. balthica* was very similar to growth observations in the Wadden Sea (Beukema and De Bruin, 1977; Beukema *et al.*, 1985; Zwarts, 1991). The rapid increase in dryweigth during April, May and June and the subsequent decrease in dryweight was explained by a combination of food deprivation and peak energy demands due to the high water temperature (Beukema *et al.*, 1985; Hummel, 1985a, b; Zwarts, 1991). Indeed, the increased food supply with the spring bloom and the subsequent low Chl-a levels during the summer period in the Wadden Sea correlate very well with the increase and decrease in dryweight in *M. balthica*.

Following the theory as described above, chl-*a* related matter was because of its intrinsic food value (Lopez and Levinton, 1987) supposed to determine yearly growth in the Westerschelde. However, the explanation for the growth pattern of *M. balthica* did not hold in the Westerschelde Estuary. Because primary production in this estuary is light-limited, a dual algal blooming period (i.e. a large spring and an smaller autumn bloom) is only observed in the mouth of the estuary. At the monitoring location, chl-*a* concentrations in the water hardly increase before May and only reach a peak during June and July, followed by a small peak in September. Yet, dry weight increase of *M. balthica* is very fast from April on, and stops in July. Preliminary model simulations based on the chl-*a* assumption resulted in a lag in growth during spring, and a dryweight peak in August. The increased respiration during the summer period was not sufficient to reach a negative scope for growth. For the Westerschelde estuary, it was obvious that the theory, based on research in the Wadden Sea, did not hold.

The only way obtain growth during springtime, was to allow *Macoma* to feed and grow on detritus, in the model expressed as Particulate Organic Carbon (POC). POC shows a distinct peak during the early spring in the Westerschelde, in particular in the more brackish part of the estuary (Soetaert, pers. comm.). This resulted in an acceptable representation of growth, in spite of the relatively low nutritional value of POC.

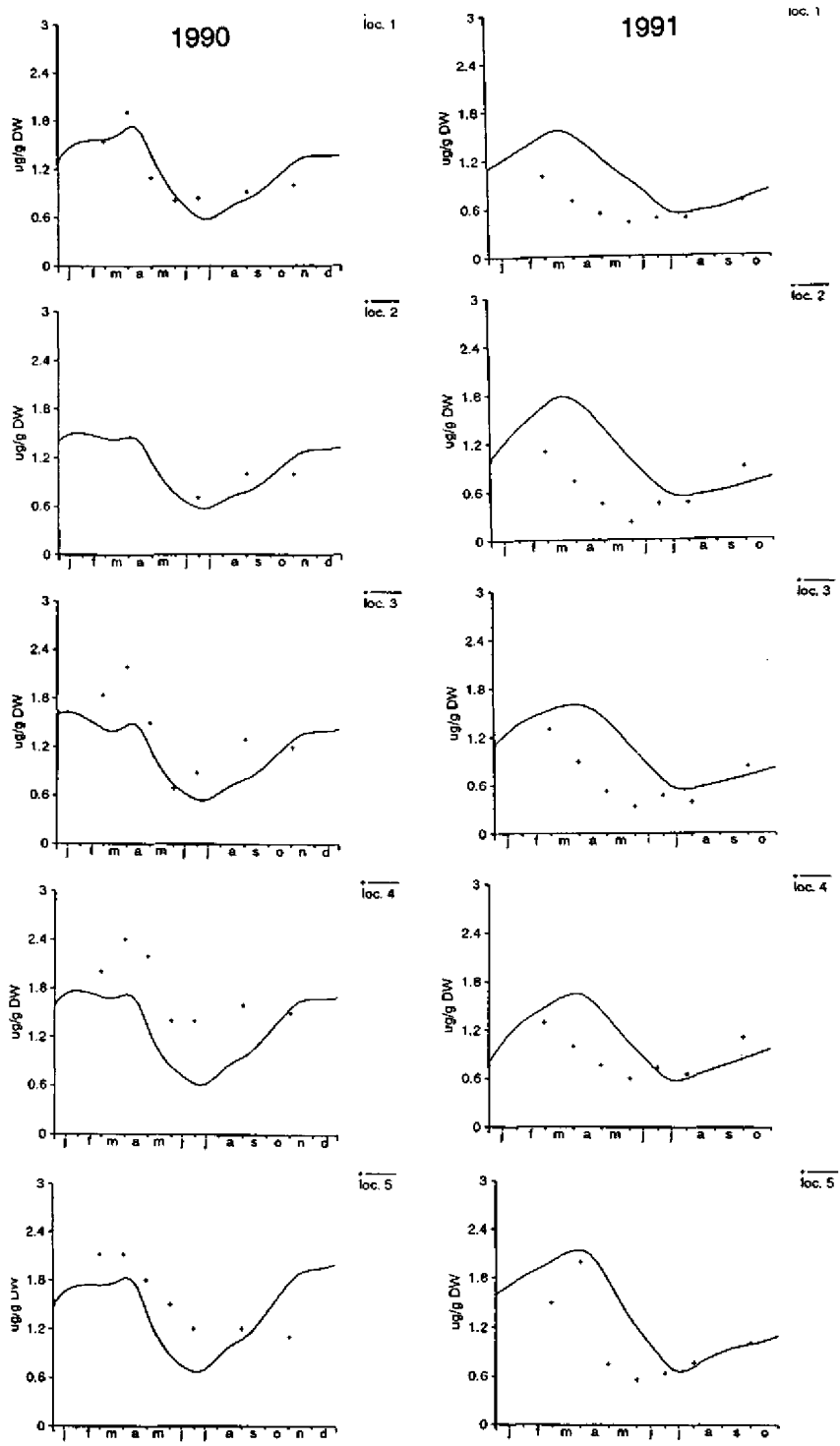

Figure 8.5 Simulation of tissue cadmium levels in 2-year old *M. balthica* from locations 1 to 5 in 1990 and 1991.

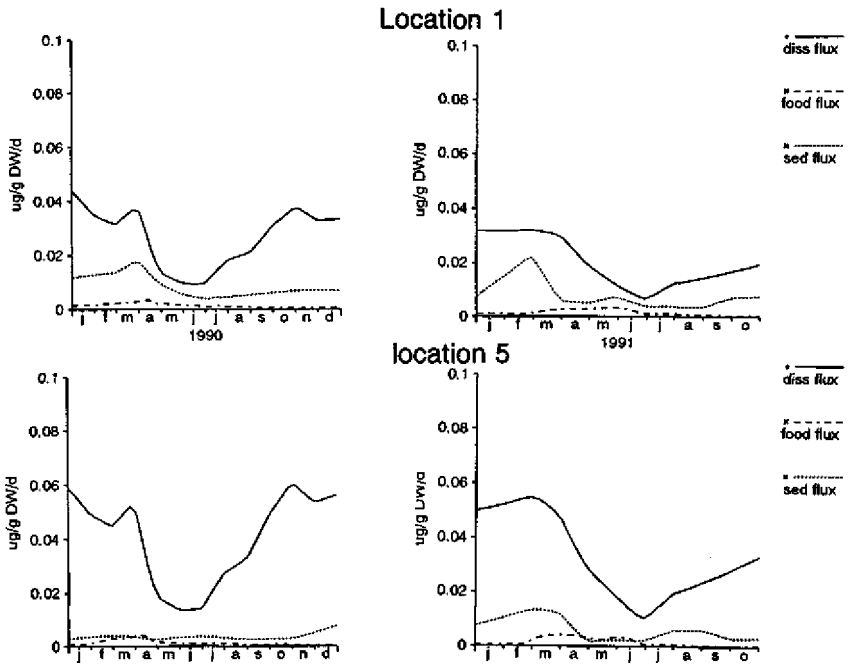

Figure 8.6 Fluxes of cadmium at location 1 and 5.

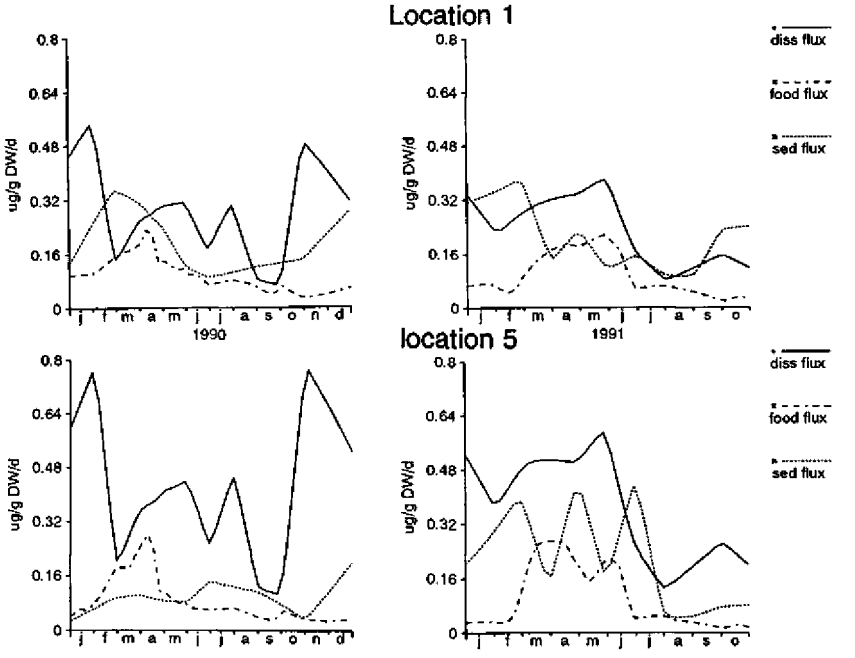

Figure 8.7 Fluxes of copper at location 1 and 5.

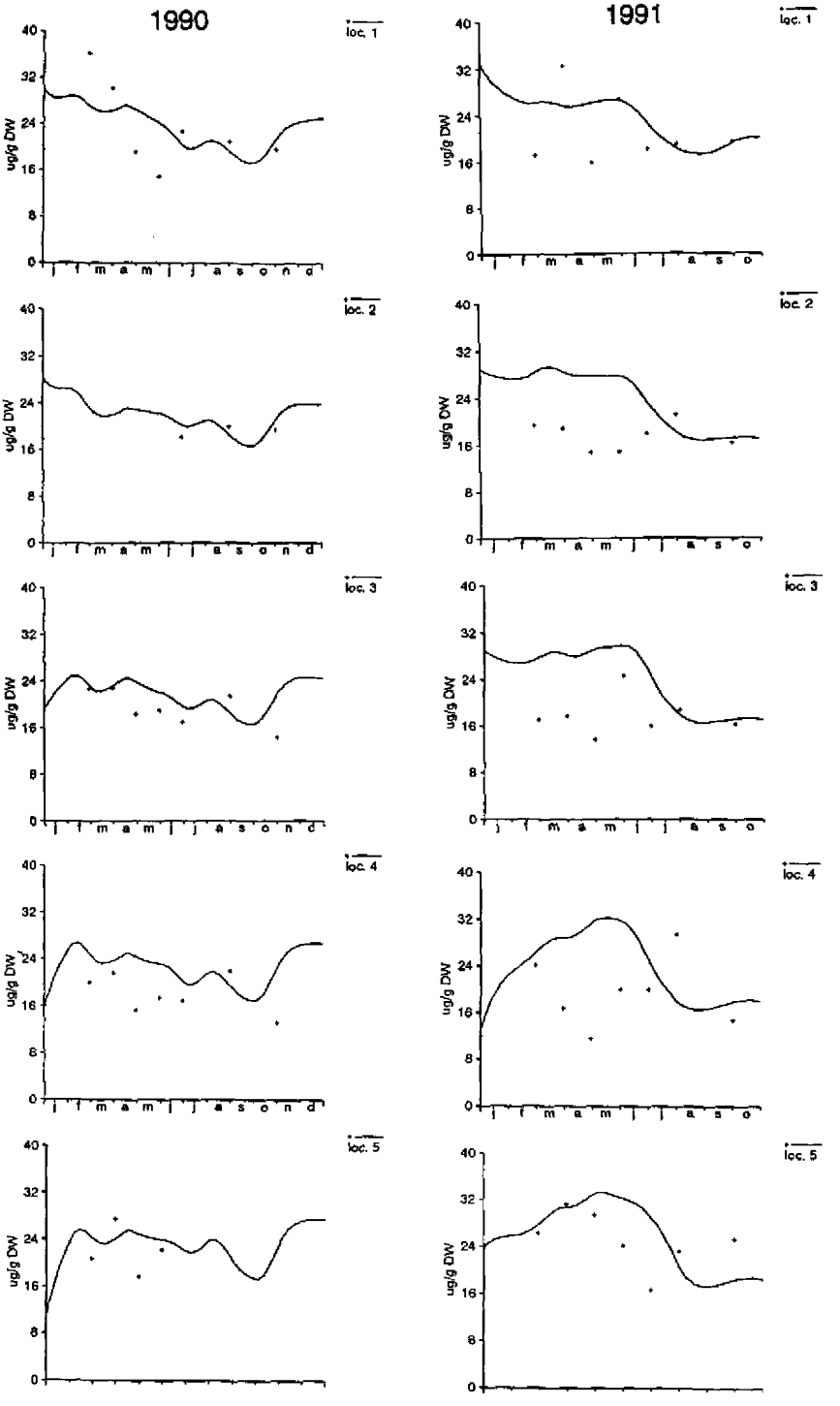

Figure 8.8 Simulation of tissue copper levels in 2-year old *M. balthica* from locations 1 to 5 in 1990 and 1991.

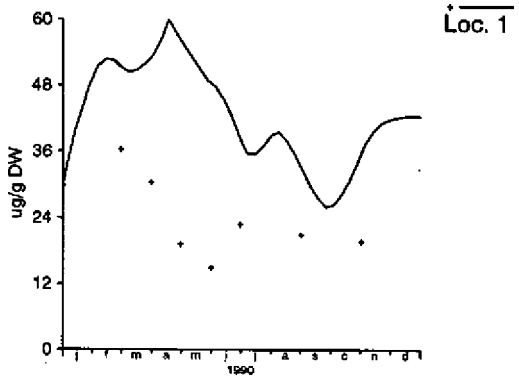

Figure 8.9 Simulation of tissue Cu concentrations in the field, using the parameters from the laboratory experiments.

An explanation for growth in springtime that was not worked out in the model simulation, is the nutritive value of microfytobenthos. It is known that for these algae, the peak in primary production in the Westerschelde is earlier than in the water column. Incorporating this information in the model would imply that *Macoma balthica* spends more time deposit feeding instead of suspension feeding. Unfortunately, sufficiently detailed information on Chl-*a* concentrations of the microfytobenthos was not available.

Submodel metal

Water. The parameters for dissolved copper uptake as assessed in the laboratory were not representative for the field situation. Complexation of copper explains this phenomena: in exposure experiments, very low copper concentrations will not result in accumulation, due to complexation with unsaturated ligands (see also Chapter Four and Six). Only at higher copper levels, a linear dose-response (=accumulation) curve will be found, because then the ligands are saturated (the start of the linearity of the response curve will depend on the ligand concentration in the water, see figure 8.10). In the Westerschelde, dissolved copper levels are generally in the lower part of the dose-response curve. So, due to the non-linearity, dose-response relationships as assessed in the laboratory can not be simply extrapolated to a field situation. Because in the laboratory experiments, the biologically available fraction was calculated, we could have used these more realistic data for the parameter estimation. However, in that case the bioavailable fraction in the Westerschelde should be used as input for the model. At this moment, only total dissolved Cu concentrations are known. Organic

ligand characteristiscs in Westerschelde water during the year are being investigated at present (Gerringa, in preparation).

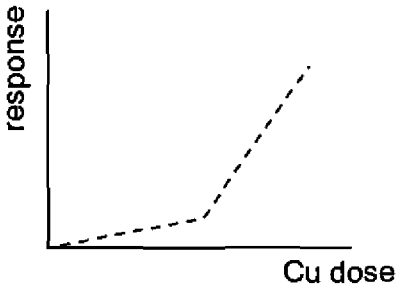

Figure 8.10 Schematic presentation of a dose-response curve for copper.

In the model simulation, a considerable part of the accumulated copper originated from ingestion via food. This is in agreement with the results from Chapters three and six, where a possible contribution of food was suggested. If this model is run with a filter feeding bivalve, then the contribution from water will be much larger, due to the relatively high ventilation rates. The experimental results on copper uptake by filter feeders agree with this (Ettajani, 1991). In Figure 8.11, the influence of clearance rate on the contribution of food to the overall metal uptake is illustrated. The relative contribution of food decreases with increasing filtration rates.

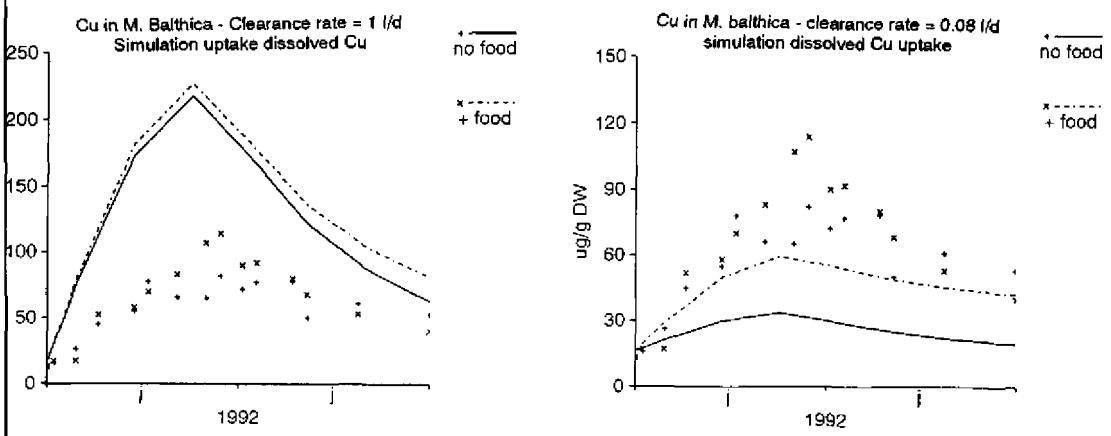

Figure 8.11 The relative contribution of food to the overall Cu uptake at high and low clearance rates.

Sediment. The results from the field observations and model simulation show that in general, metal availability from the sediment is rather low, compared with availability from the water and from food. Only in the sandy locations, variation in the sediment (cadmium in particular) were reflected in the organisms. This was also simulated by the model, in spite of

the simplified representation of the available metal fraction. The observations in Chapter five, pointing at a low metal availability from the sediment, can be held in the simulation of metal accumulation in the field, at least for the locations investigated in the present study.

It is recognized that more understanding of the biogeochemical processes governing metal accumulation is necessary. Recently, some progress in this field has been described (Tessier *et al.*, 1993). However, in areas with a relatively low metal bioavailability from the sediment, metal uptake from the overlying water and food will be the major pathway.

From the model simulation it is obvious that food availability from either the sediment or the water will determine the behaviour of *Macoma balthica*. At low total food concentrations, ventilation rates will be slightly increased, resulting in a larger contribution of dissolved copper to the overall uptake. If benthic food sources have distinctly higher metal concentrations than pelagic sources, then accumulation will be higher when deposit feeding. However, the overall contribution of the sediment to accumulation is low, in particular for cadmium. If sediment particles with associated metals are ingested, they will mostly be expelled as pseudofaeces, before they are subjected to any digestion (in which case a labile metal fraction could become available for uptake).

To conclude, by building a simulation model, the biological factors that influence metal concentrations in deposit feeding bivalves could be quantified. For the situation in the Westerschelde estuary, The Netherlands, it was obvious that dissolved cadmium concentrations, which show a seasonal trend, largely determined tissue concentrations. For copper, hardly any seasonal trends could be observed. Next to dissolved copper, sediment and food associated copper contributed significantly to the body burden.

With the construction of the simulation model, also gaps in the knowledge on metal uptake and elimination kinetics became evident. In particular, the extrapolation of copper uptake rates from the laboratory to the field situation. Without a well defined measure for the influence of dissolved organic ligands on copper complexation, assessment of uptake from the water is hardly possible. Also the role of feeding activity (and consequently filtration rate) on the uptake of metals from other sources than food, still is poorly understood. Accumulation rates in laboratory situations might strongly depend on such variables. As long as these relationships are not understood, it will remain very difficult to extrapolate laboratory results to the field situation. Provided that reliable parameter values for the contribution of food and the bioavailable dissolved fraction to uptake are available, this model description can be a useful tool for the interpretation of tissue metal contents of deposit feeding bivalves that are applied in environmental monitoring programs.

Acknowledgements - D. de Jong (Tidal Water Division, Middelburg) kindly provided benthic chlorophyll-*a* data for the estimation of the edible fraction. J. Kromkamp (NIOO) allowed us to use his dataset for the assessment of the pelagic edible fraction in the Westerschelde. J. Sinke (NIOO) was responsible for the routine measurements in the water column. Data on trace metal concentrations in the Westerschelde Estuary were provided by the Tidal Water Division of the Ministry of Transport and Public Works, Middelburg. The support of the Laboratory for Sediment and Particle analysis (J. Nieuwenhuize, C. Poley-Vos, J. van Liere and Y. Maas) is greatly acknowledged.

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Summary and conclusion

In coastal and estuarine regions of Western Europe, concentrations of contaminants in the sediment are often relatively high compared to the waterphase. Consequently, these contaminants might form a serious threat to animals living in and associated with the sediment.

As a biological monitor for sediment pollution, the benthic deposit feeding bivalve *Macoma balthica* could possibly be used as an alternative for the commonly used mussel *Mytilus edulis*. However, a problem with sediment monitoring is that many sediment associated species (including *Macoma balthica*) do not accumulate metals relative to the total concentrations measured in the sediment. *Macoma balthica* lives buried in the sediment. It feeds by picking up particles from the sediment surface with long movable siphons. Consequently, heavy metals can be accumulated from the sediment, but also from overlying and pore water and through food. There is still very little known about the relative importance of these uptake pathways of metals for *Macoma balthica*. The aim of this research was to assess the contribution of the major uptake routes of heavy metals to the body burdens in *Macoma balthica*.

The importance of the different uptake pathways was assessed with a multi-level approach. Short-term laboratory experiments using the radiotracer ^{64}Cu were carried out at the Interfaculty Reactor Institute in Delft. Long term accumulation studies were carried out with a flow-through system at the field station Jacobahaven of the Tidal Water Division. In addition, an intensive monitoring program in the field was carried out for assessment of the actual situation. With the uptake experiments, emphasis has been laid on the study of copper. In all experiments, environmentally realistic concentrations of metals were used.

Food

To study the role of food in Cu accumulation by bivalves, algae spiked with the radiotracer ^{64}Cu can be used. With spiked algae however, redistribution of Cu between the dissolved and the particulate phase hampers the assessment of the contribution of food. In Chapter Two, a method is described to overcome this problem of redistribution. By adding excess EDTA to the seawater, the biological availability of dissolved Cu was minimized. The effectiveness of complexation by EDTA was controlled through adsorption on *Macoma balthica* shells and uptake in *Macoma balthica* tissue.

In Chapter Three, this method was used to assess the availability of the radioisotope ^{64}Cu from phytoplankton and water. As far as we know, this isotope has never been used before in marine food chain studies. As a model food source the marine diatom *Phaeodactylum tricornutum* was allowed to accumulate ^{64}Cu for one day and fed to the clams. Excess EDTA was added to prevent uptake of dissolved ^{64}Cu that could be leaking from the labelled

diatoms. In control experiments, unlabelled diatoms were fed to *M. balthica* in the presence of dissolved ^{64}Cu (with and without EDTA) in order to assure a similar filtration activity. In repeated experiments with varying particulate/dissolved copper ratios, uptake through food always turned out to be at least as efficient as uptake from the water. It was concluded that Cu, associated with food, is well available for uptake by *Macoma balthica.*

Also in a flow-through system, experiments have been carried out with copper-spiked food (Chapter Six). When copper-spiked algae were added to the exposure water, *Macoma* accumulated significantly more copper. In literature, increased metal uptake with the presence of food is explained by an increased filtration activity. For this reason, filtration rates were measured during the experiment. Filtration rates in the algae-dosage were decreased, compared with the non-feeded situation. These results led again to the conclusion that food-associated copper contributed significantly to the overall accumulation.

Because it was demonstrated that metals in food could contribute to the overall accumulation by *Macoma balthica*, metal concentrations in benthic diatoms were assessed on several locations along the Westerschelde Estuary (Chapter Seven). By using the lens tissue technique, enabling diatoms to move upwards in plankton gauze sheets, we were able to collect epipelic diatoms without sediment particles. In highly polluted areas, the bioconcentration factor (metals in diatom/metals in sediment) in diatoms was less than 1, whereas in relatively clean areas, the bioconcentration factor was mostly higher than 1. Total metal concentrations were high enough (for copper about 12 microgram per gram dryweight) to contribute significantly to the tissue levels of *Macoma*, also if the food-associated metals are only partly bioavailable.

Sediment

In semi-field experiments, the accumulation and behavioral effect of heavy metals from different sediment types on the benthic bivalve *Macoma balthica* were assessed (Chapter Five). Sediments with different grain size composition and organic carbon content were spiked with cadmium, copper and zinc and aged for five months, in order to reach equilibrium conditions that would be comparable to the field situation. The maximum metal concentrations in the spiked sediments were comparable with the worst case harbour sludge from Dutch estuarine regions. During the exposure, clean filtered seawater was running continuously over the sediment. The observed effects on burrowing behaviour, mortality and bioaccumulation were to a large extent related to sediment characteristics. The strongest effects and the highest bioaccumulation were observed in sediments with the lowest silt and clay fractions. In sediments with more than 50 % < 20 µm no effects on burrowing behaviour were observed, not even in the highest dosage. In this most polluted sediment tissue body burdens of metals did not reach lethal concentrations. The very low bioavailability of the metals can be explained by the reduced state of the sediment, causing metals to bind with sulphides.

In the experiments, spiked aged sediments were much less toxic than freshly spiked sediments. From the results it was concluded that it is very important to pay close attention to experimental setup, so that the achieved data can be extrapolated to the natural situation in the field.

Water

If sediments actually contribute only for a minor part to the metal levels in *M. balthica*, then the overlying water could be relatively important. In Chapter Four, the radiotracer ^{64}Cu was used to assess the influence of natural organic ligands on the bioavailability of dissolved copper. Biological availability of the ^{64}Cu-complexes was measured by accumulation in the bivalve *Macoma balthica*. The experiments were carried out with water from the relatively clean Oosterschelde sea arm and the relatively polluted Westerschelde estuary. Adsorption onto shells as well as uptake in tissues was assessed at salinities of 10 ‰ and 30 ‰. At a salinity of 10 ‰, uptake of ^{64}Cu was increased, compared with 30 ‰. This increase was only slightly in Westerschelde water, but considerably in Oosterschelde water. Simultaneously with the exposure experiments, ligand characteristics of the natural waters were assessed by anodic and cathodic stripping voltammetry. High ligand concentrations, as occurring in the Westerschelde around February, reduced ^{64}Cu (320 Nm) uptake by more than 50% compared with the Oosterschelde, in spite of the lower salinity. This implied that at high ambient ligand concentrations the influence of salinity on ^{64}Cu uptake was less pronounced.

Also in flow-through systems, copper accumulation was measured using low dissolved copper concentrations. At a dissolved copper concentration of 25 microgram per litre (about 10 times the concentration in the Westerschelde Estuary), the accumulated copper caused mortality within a few weeks. However, it was not possible to extrapolate these, or any other toxicity results to the field situation, because toxicity in the field depends largely on the copper complexing capacity of the water. With the copper concentrations, used in laboratory experiments, organic ligands are mostly saturated, whereas in natural situations this is generally not the case. Only if the copper complexing capacity i.e. the interaction of metals with organic matter in natural waters is well defined, statements can be made on copper toxicity in the field.

Model

In order to describe metal accumulation in *Macoma balthica*, a dynamic simulation model was developed (Chapter eight). The model is composed out of a 'growth' submodel, describing the variation in dry weight of *M. balthica* during the year, and a 'metal' submodel, describing the accumulation and elimination of cadmium and copper from the water

(dissolved), food (particulate) and sediment. The 'growth' submodel was calibrated with data from a field survey. With this survey, animals and sediment were collected during 2 years from 5 locations on an intertidal mudflat, varying in sediment composition. Although *Macoma* was expected to thrive mainly on diatoms, a good fit for the growth submodel could only be achieved if *Macoma balthica* in the model was allowed to feed and grow on detritus, also if the nutritional value was low.

Tissue metal concentrations depended partly on growth dilution, but more on the dissolved metal concentrations. Sediment composition influenced metal uptake in the sense that in sandier environments, animals spend more time suspension feeding which will increase the relative contribution of the overlying water. Both in the model and in the field situation, sediment metal concentrations contributed only partly (copper) or hardly (cadmium) to the overall metal uptake, except for accidental high metal concentrations on the sandy locations.

How harmful are polluted sediments in an estuary like the Westerschelde?

It was demonstrated that high concentrations of copper, cadmium and zinc in silty, organic rich sediments with only an oxidized top layer will cause little risk in undisturbed situations. In the Westerschelde estuary, pore water profiles show maxima of Cd, Cu and Zn near the sediment-water interface (Zwolsman, 1993). *Macoma balthica* hardly ventilates any pore water, so this also does not form any direct risk. However, the sediment and suspended matter act as a source of dissolved trace metals to the water column (Zwolsman, 1993), and in this way, the sediments might form a risk for *Macoma* and other benthic macrofauna. In spite of the low concentration of biologically available copper, uptake from the overlying water was very efficient. Considering the sensitivity of molluscs for copper, any increase in the bioavailable fraction could have serious consequences for mollusc populations in the Westerschelde. This increase is not to be expected from industrial or agricultural discharge. However, water sanitation measures in Brussels and Antwerp in the near future are expected to decrease the zone of anoxia in the eastern part of the estuary. Oxidation of metal sulphides, followed by complexation of the released metals with e.g. chloride, is an imaginable process to occur. Next to this, the concentration and nature of copper complexing ligands might change due to increased bacterial activity in the oxygenated zone. Both processes can cause a change in the concentration of dissolved bioavailable copper downstream, with possible ecotoxicological consequences.

A lot of research on metal accumulation and toxicity has been carried out with commercial species like *Mytilus edulis* and *Crassostrea gigas*. From the present research, it has become clear that although deposit feeding bivalves do resemble other bivalves in many aspects, accumulation (and consequently toxicity) of pollutants is rather different, largely due to the difference in feeding behaviour. *Macoma balthica* has shown to react adequately on

variations in metal levels in the environment and is very well able to accumulate and eliminate considerable amounts of trace metals, without showing obvious signs of stress. With respect to biological monitoring, *Macoma* could very well serve as an alternative for the commonly used biomonitor *M. edulis* in sandy habitats. *Mytilus edulis* is less suitable for sediment monitoring because the animals are not in direct contact with the sediment. The large salinity tolerance and wide distribution of *Macoma balthica* can be regarded as an extra advantage. However, when assessing the harmfulness of sediments using *Macoma* as a biological monitor, contaminant uptake from the overlying water or from food are factors have to be taken into account. If data on metal concentrations in the various compartments are available, the relative contribution of these can be estimated through a model simulation as presented in Chapter eight. In the near future, the biological availability of trace metals from the overlying water is expected to change as a result of oxidation processes in the eastern part of the estuary. *Macoma* would be a very suitable organism to measure the sensitivity of bivalve populations to these changing environmental conditions.

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Samenvatting en conclusie

De bodems in estuaria in West Europa zijn vaak relatief zwaar vervuild in vergelijking met het bovenstaande water. Deze vervuiling, met name van zware metalen, kan een serieuze bedreiging vormen voor het biologisch leven in deze bodems. Het nonnetje, *Macoma balthica*, wordt daarom wel eens voorgesteld als een biologische monitor van verontreinigde bodems in intergetijde-gebieden. Om te kunnen oordelen over de gehaltes aan zware metalen die in nonnetjes gemeten worden, moet bekend zijn via welke wegen het nonnetje die metalen kan opnemen.

Het nonnetje leeft enkele centimeters diep in het sediment. Met een slurfje (de siphon) worden partikeltjes (organisch materiaal en zandkorreltjes) van het bodemoppervlak opgezogen, het zogenaamde *deposit feeding*. Zware metalen zouden dus direct via het sediment opgenomen kunnen worden. Daarbij kunnen ook uit het bovenstaande of uit het poriewater opgeloste metalen worden opgenomen, en kan een aanzienlijk deel via vertering uit het voedsel komen. Er is echter nauwelijks iets bekend over de relatieve bijdrage van deze verschillende opnamewegen (sediment, voedsel en water) aan de accumulatie van zware metalen in nonnetjes. Met het onderzoek dat in dit proefschrift is beschreven, is geprobeerd om meer inzicht te krijgen in het aandeel van de diverse opnameroutes van zware metalen voor het nonnetje.

Voor het onderzoek zijn accumulatie-experimenten uitgevoerd onder gecontroleerde omstandigheden bij het Interfacultair Reactor Instituut te Delft. Hierbij werd de radioactieve isotoop ^{64}Cu gebruikt. Daarnaast zijn langdurige experimenten in een semi-veld situatie uitgevoerd (op het veldstation Jacobahaven van DGW Middelburg) en zijn veldstudies verricht in de (relatief schone) Oosterschelde en de (relatief vervuilde) Westerschelde. Bij de experimenten lag de nadruk op het metaal koper. Steeds zijn er daarbij milieurelevante concentraties gebruikt.

Voedsel

De koper-isotoop ^{64}Cu werd gebruikt om de opneembaarheid van koper uit voedsel (algen) vast te stellen. Daarvoor werden de algen 'opgeladen' door ze enige tijd in zeewater met ^{64}Cu te laten groeien en vervolgens het loshangende ^{64}Cu er af te wassen. Helaas bleek dat de algen, ondanks de wasbeurt, tijdens het voedsel-experiment toch snel ^{64}Cu verloren. Om te voorkomen dat nonnetjes het opgeloste ^{64}Cu zouden opnemen, is een complexerende stof toegevoegd. Door complexatie wordt de opneembaarheid van het koper-koper-ion drastisch verminderd. Een effectieve complexerende stof is EDTA. Echter, in zeewater verloopt de complexatie erg langzaam door de hoge concentraties van competerende ionen als calcium en magnesium. In Hoofdstuk 2 is beschreven hoe door toevoeging van een overmaat EDTA toch heel snel het meeste koper is te complexeren en zo de opname van

opgelost ^{64}Cu tot een minimum beperkt kan worden. Die methode is gebruikt om opname-experimenten met opgeladen algen uit te voeren, beschreven in Hoofdstuk 3. Het bleek dat ^{64}Cu via algen heel gemakkelijk opgenomen kan worden door nonnetjes. Om te controleren of het toch geen opgelost ^{64}Cu was dat werd opgenomen, werden de schelpen apart van het vlees gemeten. Opgelost ^{64}Cu hecht namelijk erg makkelijk aan de schelpen. Het bleek dat in het voedsel-experiment nauwelijks meetbare hoeveelheden ^{64}Cu op de schelp zaten, terwijl wel veel in het vlees gemeten werd.

Ook op het veldstation zijn experimenten gedaan met koper in het voedsel (Hoofdstuk 6). Als nonnetjes tegelijk met koper in het water continu algen kregen toegediend, werd aanzienlijk meer koper opgenomen zonder de aanwezigheid van voedsel. In de literatuur wordt deze verhoogde opname verklaard door de verhoogde filtratiesnelheid bij aanwezigheid van voedsel. Daarom werd de filtratiesnelheid in dit experiment gecontroleerd. Het bleek dat niet gevoerde nonnetjes juist een hogere filtratiesnelheid hadden, in vergelijking met de wel gevoerde nonnetjes. Uit dit experiment werd daarom geconcludeerd dat koper in voedsel toch aanzienlijk kan bijdragen aan de totale accumulatie van koper in nonnetjes. Deze bevindingen kwamen overeen met de resultaten uit Hoofdstuk 3.

Omdat voedsel mogelijk een belangrijke bron van zware metalen voor nonnetjes is, zijn op verschillende plaatsen in de Westerschelde metaalconcentraties in bodemdiatomeëen bepaald (Hoofdstuk 7). Deze bodemdiatomeëen vormen een belangrijk deel van het voedsel van nonnetjes. Voor de metaalanalyse zijn de diatomeëen verzameld met de lens-tissue methode, waarbij ze via een laagje lens-tissue papier in planktongaas kruipen. Met deze methode wordt verontreiniging via sedimentdeeltjes tot een minimum beperkt. Het bleek dat de metaalconcentraties in de algen redelijk constant waren, ondanks soms zwaar vervuilde bodems. De concentraties waren dermate hoog (voor koper ongeveer 12 microgram per gram drooggewicht) dat ze aanzienlijk kunnen bijdragen aan de weefselconcentraties van nonnetjes, ook als maar een gedeelte werkelijk opneembaar zou zijn.

Sediment

Nonnetjes leven ingegraven in het sediment. Dat zou kunnen betekenen dat ze zich niet in verontreinigde bodems kunnen handhaven. Toch kan dat, zoals blijkt uit Hoofdstuk 5. In een semi-veld experiment konden nonnetjes zich ingraven in sediment dat van te voren was opgeladen met zeer hoge concentraties koper, cadmium en zink. Hoe slibrijker het sediment, des te hoger waren de opgeladen concentraties, omdat slibrijk sediment meer bindingsplaatsen heeft voor metalen. Ondanks die hoge concentraties groeven de nonnetjes zich snel in. Over het sediment stroomde continu schoon Oosterscheldewater. Na 2 maanden bleken ze maar heel weinig cadmium, koper en zink opgenomen te hebben. Een belangrijke reden hiervoor was de veroudering van het sediment: tussen het opladen van het sediment en het ingraven van de nonnetjes was het sediment 5 maanden met rust gelaten. Hierdoor was het sediment in evenwicht met het water en waren de metalen in het slibrijke sediment bij de zuurstofloze

situatie geïmmobiliseerd door de vorming van metaalsulfides. In veldsituaties kan zo'n situatie betekenen dat de biologische beschikbaarheid van zware metalen in het sediment heel laag is. Als er echter zuurstof bij het sediment komt (bijvoorbeeld door baggeren), oxyderen de sulfides en wordt organisch materiaal afgebroken. Hierdoor kunnen de gebonden metalen weer vrijkomen en een bedreiging vormen voor bodemdieren in de Westerschelde. Bij het experiment waren metalen in zandige sedimenten, ook bij lage concentraties, wél erg beschikbaar waren voor de nonnetjes! Uit deze experimenten bleek dat de resultaten in grote mate afhankelijk zijn van de experimentele situatie.

Water

Aangezien metalen in het bovenstaand water een belangrijke bijdrage kunnen leveren aan de opname, is in verschillende experimenten de beschikbaarheid van koper uit het water onderzocht. ^{64}Cu is gebruikt om de biologische beschikbaarheid van opgelost koper in Westerschelde- en Oosterschelde water te vergelijken. Die beschikbaarheid kan namelijk nogal verschillen. De oorzaak hiervan is de vorm waarin koper voorkomt. Er wordt aangenomen dat de ionvorm (Cu^{2+}) de best opneembare kopervorm is. De opneembaarheid van opgelost koper wordt verminderd door het complexeren met allerlei verbindingen zoals bijvoorbeeld EDTA (zie hierboven). EDTA is een kunstmatige complexerende stof, maar er bestaan ook talloze natuurlijke complexerende stoffen, bijvoorbeeld humuszuren. Vooral allerlei opgeloste organische verbindingen (ook liganden genoemd), kunnen zorgen voor een forse verlaging van de biologische beschikbaarheid. Door de grote toevoer van rivierwater is de concentratie complexerende liganden in de Westerschelde relatief hoog. Het is echter moeilijk om het complexerend vermogen van het water in een experiment te bepalen, omdat het in de eerste plaats nauwelijks bekend is hoe die liganden er precies uitzien, maar ook omdat door afbraak en nieuwvorming de concentraties kunnen variëren in de tijd. Met kortdurende experimenten kan die variatie beperkt worden. Voor deze experimenten is ^{64}Cu gebruikt, omdat hiermee binnen enkele uren en bij zeer lage concentraties, opname gemeten kan worden (zie Hoofdstuk 4). Het bleek dat eenzelfde hoeveelheid opgelost ^{64}Cu veel sneller werd opgenomen in Oosterscheldewater (geen rivier-invloed, dus weinig koper complexerende liganden) dan in Westerscheldewater. Als de concentratie complexerende liganden in de Oosterschelde en in de Westerschelde vergelijkbaar was, dan zou juist meer koper uit de Westerschelde worden opgenomen, omdat door het lagere zoutgehalte de opneembaarheid weer beter wordt. Het zout-effect speelde geen rol bij hoge ligand concentraties.

Op het veldstation zijn vervolgens langdurige experimenten met lage koperconcentraties uitgevoerd (zie Hoofdstuk 6). Koperconcentraties lager dan 15 microgram per liter gaven al een verhoging van het kopergehalte in nonnetjes te zien. Bij een concentratie van 25 microgram per liter namen de nonnetjes zo snel het metaal op, dat ze binnen enkele weken

een dodelijke concentratie hadden geaccumuleerd. Hoewel deze concentraties maar een factor 10 hoger zijn dan de gemiddelde gehaltes in de Westerschelde, kunnen de laboratoriumresultaten niet direct naar de veldsituatie worden geëxtrapoleerd. In het veld zullen de organische liganden veelal onverzadigd zijn, en is de biologische beschikbaarheid van het opgelost koper dus heel laag. Met de iets hogere concentraties in laboratoriumexperimenten zijn de organische liganden verzadigd, en neemt de concentratie biologisch beschikbaar koper veel sneller toe. Om uitspraken te kunnen doen over kopertoxiciteit is informatie over de koper complexerende capaciteit van het water dus essentieel.

Model

De resultaten van de hiervoor beschreven experimenten werden gebruikt in een modelbeschrijving van metaalopname door nonnetjes (Hoofdstuk 8). Met uitzondering van de gegevens over opgelost koper (om de hierboven beschreven reden), was dit goed mogelijk. De modeluitkomsten werden getoetst aan gegevens van een intensieve meetserie van sediment en nonnetjes uit de Westerschelde.

Als basis van het model werd de jaarlijkse groei van nonnetjes beschreven, omdat groeiverdunning en eetgewoonten invloed zullen hebben op het metaalgehalte in het dier. Het bleek moeilijk om de groei van nonnetjes te relateren aan chlorophyl-*a* gehaltes in het water, zoals dat voor de Waddenzee gedaan is. Alleen als nonnetjes in het model detritus mochten eten (ook als het van een relatief lage kwaliteit is) was de groei vergelijkbaar met in de Westerschelde waargenomen groei.

Aan de hand van het model kon ook vastgesteld worden welke opnameroute in welke situatie het meest bijdroeg aan de accumulatie van cadmium of koper. Nonnetjes in zandig sediment besteden relatief meer tijd aan suspensie-eten, hetgeen de opname-route van metalen zal beïnvloeden. Het bleek bijvoorbeeld dat koper- en cadmiumgehaltes in het sediment niet méér bijdroegen aan de gehaltes in het dier dan de gehaltes in het water of in voedsel, tenzij de nonnetjes in zandig sediment zaten. Voor cadmium was duidelijk dat het bovenstaand water veruit de belangrijkste bron van opname was.

Hoe schadelijk zijn vervuilde bodems in een estuarium als de Westerschelde?

In Hoofdstuk 5 is aangetoond dat zeer hoge concentraties cadmium, zink, of koper in slibrijke bodems met alleen een geoxideerd toplaagje, een laag risico vormen zolang de bodems niet verstoord worden. Het poriewaterprofiel in bodems in de Westerschelde toont Cd, Cu en Zn maxima bij het grensvlak tussen sediment en water (Zwolsman, 1993).Het nonnetje filtreert echter nauwelijks enig poriewater dus hiervan zal weinig risico te verwachten zijn. Het is echter aangetoond dat de bodem en het zwevend materiaal metalen naleveren aan het water (Zwolsman, 1993). Via de opgeloste fase kunnen nonnetjes dan, ook in benedenstroomse gebieden, makkelijk metalen accumuleren.

Als gevolg van het verbeteren van de afvalwaterzuivering in België zullen de omstandigheden in de Westerschelde mogelijk veranderen. Door de zuurstoftoename in het oostelijk deel van het estuarium kunnen metaalsulfides oxyderen en de metalen vervolgens in oplossing gaan. Met toenemende bacteriële activiteit kan de concentratie en de aard van organische liganden veranderen. Door deze processen kan de concentratie biologisch beschikbaar koper veranderen, met mogelijke gevolgen voor kopergevoelige soorten.

Er is veel toxicologisch onderzoek verricht aan commerciële schelpdiersoorten als de mossel *Mytilus edulis* en de oester *Crassostrea gigas*. Uit het hier beschreven onderzoek is duidelijk geworden dat bij *deposit feeders* (schelpdieren die van de bodem eten, zoals nonnetjes) het aandeel van de verschillende opnameroutes van zware metalen verschilt van die bij *suspension feeders* (schelpdieren die uit het water filteren, zoals mossels). Bij *filter feeders* is de waterroute verreweg het belangrijkst, terwijl bij *deposit feeders* het voedsel en het sediment ook voor een belangrijk deel bijdragen aan de metaalopname. Dit kan verklaard worden door het grote verschil in filtratiesnelheid.

Omdat nonnetjes in direct contact staan met het sediment en omdat ze beschikbaarheid van metalen uit de bodem beter weergeven dan filter feeders (zoals kokkels) zijn ze in gebieden met vervuilde slibrijke bodems een betere biologische indicator dan mosselen, die veel in routine-meetnetten gebruikt worden. Omdat ze gevoelig zijn voor veranderingen in het milieu, en vooral omdat ze veel toleranter zijn voor lage zoutgehaltes dan mosselen, kunnen nonnetjes een minstens zo goede indicatorfunctie vervullen als de laatstgenoemden. Een opname-simulatiemodel, zoals beschreven in Hoofdstuk 8, kan dan een goede indruk geven van het relatieve aandeel van het sediment, het voedsel en water in de metaalaccumulatie door het nonnetje. Het nonnetje zou een zeer geschikte soort zijn om de gevoeligheid van het ecosysteem in de Westerschelde voor de veranderende milieuomstandigheden te meten.

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Curriculum vitae

Christien Absil werd geboren op 23 September 1963 te Heythuysen (Limburg). In 1981 haalde zij haar diploma Gymnasium ß aan de Scholengemeenschap St. Ursula te Horn. In hetzelfde jaar startte zij haar studie Biologie aan de Universiteit Utrecht. In de doctoraalfase werd het hoofdvak Toxicologie uitgevoerd bij MT-TNO in den Helder. Tevens deed zijn de nevenrichting Visserijkunde aan de vakgroep Visteelt en Visserij van de Landbouwuniversiteit Wageningen. Voor dit vak werd in het kader van een Interdisciplinair Studie Project (georganiseerd door de vakgroep Civiele Techniek, Technische Universiteit Delft), een studieperiode van vijf maanden in Maleisië doorgebracht. Verder haalde zij haar 1e graads bevoegdheid in de Didactiek van de Biologie en volgde zij de nevenrichting Botanische Oecologie. In September 1988 werd de studie afgerond. Van Oktober 1988 tot Oktober 1992 heeft zij aan het Centrum voor Estuariene en Mariene Oecologie van het Nederlands Instituut voor Oecologisch Onderzoek aan dit promotieonderzoek gewerkt. De resultaten staan beschreven in dit proefschrift.