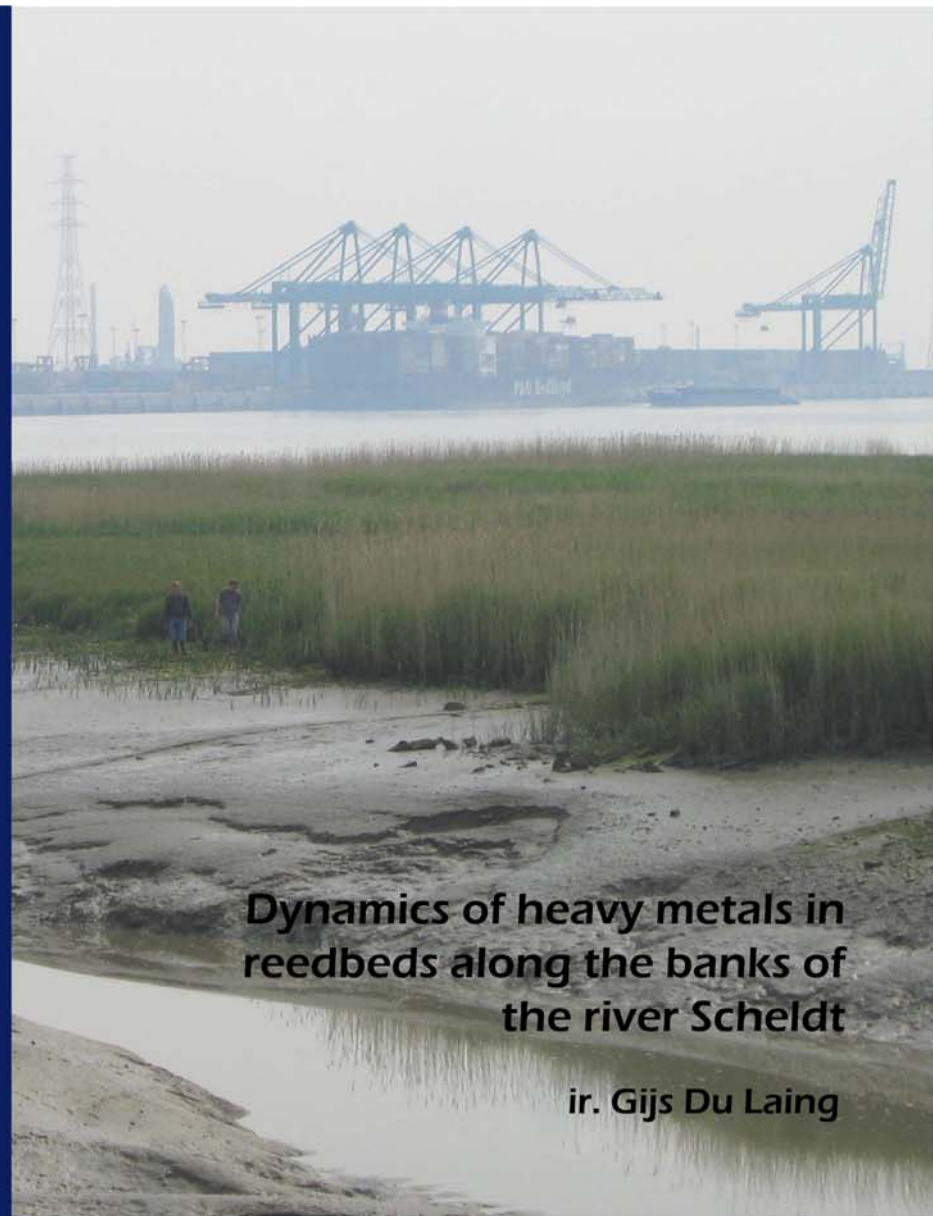DYNAMICS OF HEAVY METALS IN REEDBEDS ALONG THE BANKS OF THE RIVER SCHELDT

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Dynamics of heavy metals in reedbeds along the banks of the river Scheldt

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DYNAMICS OF HEAVY METALS IN REEDBEDS ALONG
THE BANKS OF THE RIVER SCHELDT

Thesis submitted in fulfillment of the requirements
for the degree of Doctor (PhD) in Applied Biological Sciences

WOORD VOORAF

Eerst en vooral had ik graag iedereen bedankt die rechtstreeks, of onrechtstreeks, bijgedragen heeft tot het tot stand komen van dit werk. In eerste instantie zijn dit mijn promotoren, Prof. dr. ir. Filip Tack en Prof. dr. ir. Marc Verloo. Ik zou hen in het bijzonder willen bedanken voor de mogelijkheden die ze mij geboden hebben om dit werk te voltooien en voor het vertrouwen dat ze me gegeven hebben om me op zelfstandige wijze mijn onderzoek te laten uitvoeren. Verder wil ik ook de andere leden van de leescommissie, Prof. dr. ir. Pascal Boeckx, Prof. dr. ir. Eric de Deckere en dr. ir. Bart Vandecasteele, danken voor het kritisch en zorgvuldig nalezen van dit werk. Tevens wil ik ook Prof. dr. N. De Pauw (voorzitter), Prof. dr. ir. André Maes en Prof. dr. ir. Hubert Verplancke, de overige leden van de examencommissie, bedanken voor de beoordeling van dit proefschrift.

Verder wil ik ook de (ex-)collega's Agnes, Alex, Ann, Annelies, Annick, Dieter, Dries, Els, Erik, Katty, Martin, Nicolas, Pieter, Ria, Sri, Sukris, Steven en Virginia van het Labo voor Analytische Chemie en Toegepaste Ecochemie bedanken voor de leuke samenwerking en Ria, Steven en Martin in het bijzonder ook voor hun logistieke en technische ondersteuning bij de analyses en bemonsteringen. Zonder hun steun was het me ongetwijfeld nooit gelukt om mijn onderzoeksactiviteiten met een flinke brok onderwijs en dienstverlening te combineren. Enkele thesisstudenten hebben hiertoe ook een hun steentje bijgedragen: Annelies, Benjamin, Dieter, Jo, Jochen, Marjan, Pieter, Rina en Wouter. Bedankt! In het bijzonder dank ik jullie voor het opvrolijken en helpen relativeren van het wetenschappelijke wereldje, omdat jullie bergen werk verzet hebben met jullie handen en voeten in de verontreinigde smurrie en omdat jullie op voldoende zelfstandige wijze de nodige gegevens konden verzamelen. Pieter en Wouter, de bemonsteringsplaats "Duvel 1,6 euro" staat trouwens nog steeds in mijn GPS (51°15'12" NB, 4°16'64"), maar de databank moet dringend eens geactualiseerd worden.

Ook wil ik Diederik, Bart, Gunther, Roeland en Korneel danken voor de steeds leuke uitstapjes en/of bereidheid tot grensoverschrijdende samenwerking, Margot voor haar hulp bij de fluorescentiemetingen, de laboranten van INBO Geraardsbergen voor hun bijdrage in de textuuranalyses en de beheerders van de schorren om de proefvelden ter beschikking te stellen. De Administratie voor Waterwegen en Zeewezen wil ik ook danken om mij bij aanvang van mijn onderzoek voor een korte periode de toestemming te geven om met mijn wagen af en toe eens de Scheldedijk op te rijden om mijn monsters en materiaal in te laden.

Bij deze wil ik dan ook de vriendelijke zwaantjes danken om mij geen bekeuring te geven toen ze mij gedurende de overige 6 jaar betrapten terwijl ik op de Scheldedijken rondreed zónder vergunning. Hopelijk kan onze samenwerking in de toekomst verder gezet worden.

Verder uiteraard nog het WETPOL-team: Annelies, Anke, Diederik, Dieter en Els, bedankt om mij bijgestaan te hebben bij het realiseren van één van mijn "zotte" ideeën… Ik denk dat wij nu terecht mogen zeggen dat we meer dan een jaar serieus gewerkt hebben aan de PR van ons labo, de faculteit en de universiteit. Hopelijk kunnen we de opgebouwde internationale contacten blijvend onderhouden en vloeien hieruit in de toekomst nog heel wat leuke momenten en samenwerkingsverbanden voort. De foto's van onze recentste congresdeelname in Lissabon bewijzen alvast dat we op de goeie weg zijn, nietwaar Els? En de tweede WETPOL-editie in Estland is alvast ook veelbelovend, allen daarheen!

Verder nog een woordje van dank voor mijn naaste omgeving. Dank aan al mijn vrienden voor hun hulp om van mijn studententijd één groot feest te maken. Ook aan mijn ouders en mijn zus, voor hun steun gedurende mijn hele studieloopbaan en in het bijzonder ook voor hun logistieke steun tijdens de hectische laatste maanden van mijn doctoraat. En Cassandra, omdat je er steeds was de afgelopen jaren en omdat je jouw agenda in mijn superdrukke laatste maanden volledig hebt willen afstemmen op mijn doctoraat. En, last but not least, onze kleine Casper, omdat je de laatste weken van het schrijfwerk opgevrolijkt hebt met je altijd aanwezige lach. Ik hoop dan ook dat ik binnenkort wat tijd kan vrijmaken om het gebrek aan vrije tijd van de laatste maanden ten dele in te halen en des te meer te genieten van je lach…

Gijs, 15 november 2006

Zie ik de lichtjes van de Schelde
Dan gaat m'n hart wat sneller slaan
Ik weet dat jij op mij zult wachten
En dat je aan de kaai zult staan
Zie ik de lichtjes van de Schelde
Is 't of ik in je ogen kijk
Die zo heel veel liefs vertellen
Dan ben ik als een prins zo rijk

Wannes Van De Velde & Hans De Booij, naar: Bobbejaan Schoepen (1952)

Contents

List of abbreviations

AFDM	Ash-free dry mass
ALT	Alternately flooded soil
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
AVS	Acid-volatile sulphide
BC	Background concentration
CRM	Certified reference material
CRS	Chromium reducible sulphur
CEC	Cation exchange capacity
DM	Dry mass
DOC	Dissolved organic carbon
EC	Electrical conductivity
EDTA	Ethylenediaminetetra-acetic acid
F-AAS	Flame atomic absorption spectrometer
GF-AAS	Graphite furnace atomic absorption spectrometer
IC	Inorganic carbon
ICP-OES	Inductively coupled plasma optical emission spectrometer
L:S	Liquid:solid
OM	Organic matter
ORP	Oxidation reduction potential
PE	Polyethylene
PER	Permanently flooded soil
PVC	Polyvinyl chloride
S.D.	standard deviation
SEM	Simultaneously extracted metals
TEAP	Terminal electron-accepting processes
TOC	Total organic carbon
WL	Water level
w/w	Weight/weight

1.
General introduction

1 General introduction

1.1 Floodplains and intertidal zones along the river Scheldt: opportunities and risks

During decades, parts of the river Scheldt have been straightened to improve the possibility to navigate. Dikes have been constructed to protect inhabited areas against flooding and to impolder part of the previously flooded river banks, hereby decreasing the area that is subjected to tidal fluctuations of the water level. This gradually decreased the water holding capacity of the river and its surroundings. In addition, construction of sewers and paved areas increasingly shortcuts rainwater to the rivers and the sea level is rising. All this is causing increased incidences of flooding of inhabited areas. These floodings can occur during heavy rainfall, when sewers and streams cannot absorb the rainwater anymore, or during storm weather at spring-tides, as extremely high water levels in the Scheldt river can be reached when (north)western storm winds impel extra sea water into the estuarine funnel. This resulted in massive floods in the Netherlands (1953), which were acted upon with the ambitious Delta-plan. In Belgium, the Sigma-Plan was an answer to the major floods along the Scheldt river in 1976.

Controlled flooding areas are important components of these river management plans. The construction of controlled flooding areas along the river is aimed to provide additional temporary water storage during events of high rainfall and discharge. This allows to attenuate high peak water levels and thus reduces the chances of flooding. By permanently keeping the groundwater levels high in parts of these flooding areas instead of just flooding them occasionally, the government also hopes to restore valuable wetland habitats. However, suspended particles in the river water, especially the smallest ones, tend to bind heavy metals. Sedimentation of suspended matter in these wetlands could therefore create a significant risk for metal accumulation in these valuable habitats, especially as metals are not biodegradable. Removal and cleaning of all accumulated polluted sediments quickly may prove to be an unrealistic option in the short term because of the large costs involved.

Long lasting sedimentation of suspended particles already resulted in the formation of mudflats along the river banks. Rooting of especially reed plants on these banks and

subsequent accumulation of organic matter in the upper sediment layer caused the creation of marshes. These are considered to act as a geochemical filter removing both inorganic and organic pollutants from the river. Many pollutants are biologically degraded. However, metals cannot be biologically degraded and are expected to accumulate in various ecosystem compartments.

1.2 Aims and scope

Management of river water through the restoration or creation of wetlands must account for potential hazards related to contamination present in the river water and in the wetlands. However, these are currently not taken into account. Predicting potential metal accumulation in controlled flooding areas or newly created wetlands under various management scenarios requires a thorough knowledge of metal behaviour in intertidal marshes. In this study, we aimed to assess the pollution status of recent sediment deposits in the intertidal reedbeds, and to identify the most important factors that affect metal accumulation and mobility in these marshes. This can be used for the development of management oriented models for predicting the metal fate, which should allow to estimate environmental risks related to metal accumulation in sediments and floodplains of the river Scheldt, considering that the removal and/or cleaning of all contaminated sediments is not feasible.

Following objectives can be distinguished:

- to identify the major factors that affect metal accumulation in the upper sediment layer of intertidal areas along the river Scheldt;
- to interpret short-term temporal and spatial variations of metal concentrations in the upper intertidal sediment profile;
- to elucidate the effect of salinity on metal mobility in the upper sediment layer of intertidal zones along the Scheldt;
- to assess the effects of litter decomposition on metal mobility in the upper sediment layer of intertidal zones along the Scheldt;
- to identify the influences determining metal uptake by reed plants growing in intertidal zones along the Scheldt;

- to assess the relations between the water table level and pore water metal concentrations at different depths in intertidal Scheldt sediments.

1.3 Outline of this work

An overview of past and present research on metal accumulation and fate in marshes is presented in **Chapter 2**. The literature review briefly discusses the Scheldt Estuary as study area, e.g. its location, hydrology, metal fate in its surface water and bottom sediments, salinity and sediment dynamics in tidal flats and marshes and the marsh ecology. Processes and properties affecting metal fate in soils and sediments are subsequently discussed, with the focus on factors affecting dissolved metal concentrations in intertidal sediments. Major attention is given to precipitation/dissolution processes upon changing redox conditions, such as the role of Fe/Mn oxides and sulphur cycling, carbonates and pH. The effect of plant growth on metal fate in wetlands is also briefly discussed. Finally, physicochemical methods that are commonly used to assess metal fate in floodplains and marshes are reviewed.

In **Chapter 3,** the factors affecting recent metal accumulation in the upper sediment layer of intertidal areas along the river Scheldt are identified. Therefore, metal contents in the intertidal sediments and a range of physico-chemical sediment properties were analysed at several sampling sites along the river. A regression analyses revealed the factors which significantly affect the metal accumulation.

Many authors reported effects of salinity, organic matter and the hydrological regime on metal mobility, bioavailability and toxicity in soils, sediments and surface waters. In **Chapter 4**, the effect of a changing flooding water salinity on the mobility and availability of metals in the upper layer of intertidal Scheldt sediments was studied in a greenhouse experiment. Floodplain soils and intertidal sediments were subjected to flooding with water of different salinities. Differences between metal concentrations in the pore water of these treatments were identified and elucidated in relation to the different salinities and physicochemical soil and sediment properties. In the first part of **Chapter 5**, results of a greenhouse experiment aiming at the assessment of potential effects of litter decomposition on metal mobility and availability in intertidal sediments will be discussed. This greenhouse experiment was

supplemented by a field experiment to study metal fate upon litter decay and decomposition under more natural conditions. Metal mobility in the surface layer of a calcareous, dredged-sediment derived soil as affected by the hydrological regime is interpreted and discussed in **Chapter 6**.

The intertidal areas of the Scheldt river are predominantly vegetated by reed plants. Metal uptake by these plants is likely one of the major pathways of metal transfer from soils and sediments to the food chain. How metal uptake by these plants could be affected by the factors which also affect the metal mobility is discussed in **Chapter 7**.

In **Chapter 8**, the impact of the water table level on metal concentrations in the pore water of intertidal sediments is assessed. A greenhouse experiment was set up in which metal concentrations in the pore water were measured at different sampling depths below the surface of selected soil and sediment columns as a function of time, upon adjusting the water table to fixed levels below the surface.

Identification of the factors which might affect metal mobility and availability in intertidal reed beds of the Scheldt is supplemented by a field validation in **Chapter 9**. Metal concentrations in the pore water were measured at different sampling depths below the surface of eleven selected intertidal reed beds of the Scheldt estuary. Observed variations of metal concentrations in the pore water were interpreted in relation to the factors which were previously found to affect metal fate, i.e. salinity, organic matter, water table level, reduction status, etc.

Finally, **Chapter 10** summarises the main findings and conclusions. Future research perspectives are presented.

2.

Metal fate in intertidal wetlands of the Scheldt estuary: a literature review

2 Metal fate in intertidal wetlands of the Scheldt estuary: a literature review

2.1 The river Scheldt

The river Scheldt sources in the north of France in the vicinity of St.-Quentin, at a height of 100 meters above sea level. It pours into the North Sea near Vlissingen about 355 kilometers further (Baeyens et al., 1998). The river is subject to tidal fluctuations in the estuarine part, which extends from the mouth to the city of Ghent, about 160 kilometers inland. The Scheldt can be divided into three zones. The part between the source and Ghent is called the Upper Scheldt. This part is not subject to tidal fluctuations. The Sea Scheldt is the part between Ghent and the Dutch-Belgian border while the remaining section is called the Western Scheldt. Valuable nature areas are situated in the estuarine part of the basin and along the tributaries. These involve fresh, brackish and saltwater intertidal marshes. The Scheldt estuary is especially unique, as it accommodates one of the only remaining freshwater tidal zones in Europe. Yet, areas along the river are densely populated and industrialised (Stronkhorst, 1993). Approximately 7 million people live in the river basin, with urban areas near Lille (France), Ghent, Brussels and Antwerp. The largest industrial areas are concentrated near Lille, Antwerp, along the canal from Ghent to Terneuzen, and near Vlissingen. As a result, the river Scheldt and its branches are also used as a major drain for industrial and domestic wastes (Baeyens et al., 1998). A satellite picture of the river mouth and the Western Scheldt is presented in Fig. 2.1.

Fig. 2.1. Satellite picture of part of the Scheldt estuary between Vlissingen and Antwerp (source: www.scheldeschorren.be)

2.2 Hydrology of the Scheldt basin

About 10^9 m³ of seawater flows into the estuary near Vlissingen during each tidal cycle. Near Antwerp and Dendermonde, this amount has decreased to about 70 million and 6 million m³, respectively. The average difference between the water level at high and low tide is 3.85 m near the river mouth. Near Antwerp, this has increased to 5.15 m. It then decreases again to about 1.89 m in Gentbrugge, just before a lock complex near Ghent (Claessens, 1988). The river Scheldt, as well as its branches, are rain-fed. The discharge of these rivers varies considerably with minimal discharges occurring in summer and autumn, and maximum ones in winter and spring (Baeyens et al., 1998). During decades, parts of the river Scheldt have been straightened to improve the possibility to navigate. Dikes have been constructed to protect inhabited areas against flooding and to impolder part of the river banks. All this has decreased the surface area that is subject to tidal fluctuations. As a result, the water holding capacity of the river and its surroundings has decreased and flooding of habited areas increasingly occurs. Moreover, expansion of sewers and paved areas resulted in fast diversion of rainwater to the rivers and the sea level is rising, which further facilitates flooding events. These floodings can occur during heavy rainfall, when sewers and streams cannot absorb the rainwater anymore, or during storm weather at spring-tides, as extremely high water levels in the Scheldt river can be reached when (north)western storm winds impel extra sea water into the estuarine funnel. Some major floodings in the southwestern part of The Netherlands (1953) and in Rupelmonde (1976) incited the Dutch and Flemish government to take measures against the flooding, which were consolidated in the Delta-plan in the Netherlands and the Sigma-Plan in Flanders. Parts of these plans involve the construction of controlled flooding areas along the river to temporarily capture rainwater and river water when necessary. By permanently keeping the groundwater levels high in parts of these areas, instead of just flooding them occasionally, the government also hopes to restore valuable wetland habitats. There is however a risk for sedimentation of suspended matter from the river water in these flooding areas, which could pose problems regarding losses of water holding capacity on a longer term, and for the accumulation of pollutants, such as heavy metals.

2.3 Suspended sediments

The concentration of suspended particles in the river water is subject to large temporal variations. It ranges from a few mg L^{-1} up to 200 mg L^{-1} (Van Damme et al., 2001). These suspended particles, especially the smallest ones, tend to bind heavy metals (Millward & Liu, 2003). Mixing of salty seawater with fresh water causes large amounts of suspended matter with adsorbed pollutants to flocculate near Antwerp (Temmerman, 1988; Heip, 1988). This results in a turbidity maximum in the river water and enhanced sedimentation of suspended matter.

2.4 Metal fate in the Scheldt river water

Metals in the water column can be either dissolved or associated with solid material (Betty et al., 1996). The latter comprises a diverse array of components, ranging from clays to metal oxides to organic detritus. Under anoxic conditions, the formation of sulphides seems to primarily affect the metal fate (Eggleton & Thomas, 2004). Baeyens et al. (1998) identified some peculiar characteristics of the Scheldt estuary, which might affect the fate of metals in the river water:

1. the Scheldt is a tide-governed estuary due to the low river flow leading to large residence times;
2. the upper estuary receives large inputs of biodegradable organic matter that induces anoxic conditions in the water column during summer;
3. considerable and direct supply of toxic pollutants occurs in the upper estuary as a result of the diverse activities by the industrial park around Antwerp;
4. the anoxic zone, the area of pollutant inputs and the zone of maximum turbidity coincide geographically, making it very difficult to distinguish between their individual effects on the metal distribution and behaviour.

Paucot & Wollast (1997) reported very high Cd, Cr, Cu, Pb and Zn contamination levels in the water column of the Scheldt estuary in the early 90s. Observed particulate metal concentrations in the freshwater part were found to significantly exceed the background

concentrations in river waters, world averages and values for various European estuaries which are well known to be polluted. Zwolsman and Van Eck (1993) reported severe trace metal pollution in the Scheldt estuary at the end of the fifties. A major reduction of the pollution by As, Cr, Hg, Pb, and Zn has however occurred in the seventies, and a reduction of Cd and Cu in the eighties, but Ni pollution has increased. Also according to Baeyens (1998), dissolved and particulate metal concentrations assessed in the Scheldt estuary in the period 1978-1995 clearly indicated that the levels of all metals had decreased significantly. A comparison with other estuaries classifies the Scheldt as being moderately polluted for all metals in the dissolved phase, but as being fairly high polluted in the particulate phase, especially for Cd. However, even the dissolved metal concentrations in the Scheldt were found to be between 10 and 50 times higher than total metal concentrations of unpolluted seas and oceans. According to De Smedt et al. (1998), only a small part of these metals ultimately reaches the North sea, due to deposition in the sediment. Some model calculations indicated that several decades will pass until a significant improvement in bottom sediment quality will be achieved without removing and/or cleaning the sediments, even after a 100% reduction of the loading of pollutants (Van Maldegem et al., 1993).

Metal sorption to the solid particles decreases with decreasing pH, decreasing particle concentrations and/or increasing salinity (Turner, 1996; Hatje et al., 2003). Paucot & Wollast (1997) observed desorption of Cd, Cu and Zn from the particulate phase during estuarine mixing in the Scheldt estuary, whereas Mn was removed from the dissolved phase and Ni and Cr behave conservatively. Moreover, Baeyens et al. (1998) reported that dissolved metal concentrations decreased in anoxic conditions because of precipitation as metal sulphides, especially during the summer months in the upper Scheldt estuary, whereas dissolved metal concentrations tended to increase in the lower estuary because of desorption and oxidation processes. Increased oxygen concentrations during the winter months in the upper estuary may lead to sulphide oxidation. This causes the metals to dissolve, but subsequently coprecipitation and adsorption of metals on ironhydroxides again reduces the dissolved concentrations. Stronkhorst (1993) concluded that most trace metals in the water column of the Scheldt estuary showed a decrease of the ecological risk downstream with increasing salinity. However, risk values for copper and cadmium were lower at the Belgian-Dutch border than downstream as a result of the anoxic conditions. Arsenic and lead were found to have negligible risk values. Nickel is regarded a relatively critical metal in the Western Scheldt because a large percentage of the total concentration is dissolved. Zinc also appeared

to be a critical metal in the Western Scheldt with environmental concentrations ranging up to 25 µg L^{-1}. Although zinc concentrations at Vlissingen were reported to affect only 0.1 % of the species, the concentrations at the Belgian-Dutch border would cause an effect to approximately 4 % of the organisms.

2.5 Bottom sediments and dredging operations

The annual deposition of sediments in waterways in Flanders that requires removal for nautical or maintenance purposes is estimated at 1.4 million ton dry weight (De Cooman et al., 2004). Dredged sediments have been deposited on land in the past, as this practice was deemed very useful for adding plant nutrients to agricultural soils. Also for that reason, so-called summer dikes were constructed to allow for spontaneous deposition of nutrient-rich river sediments on floodplains along the river during the winter, which were used for agriculture during summer. However, only 8 % of the bottom river sediments in Flanders is considered not to be contaminated from a purely chemical point of view. Reference metal contents in bottom sediments are 11 mg kg^{-1} DM for As, 0.38 mg kg^{-1} DM for Cd, 17 mg kg^{-1} DM for Cr, 8 mg kg^{-1} DM for Cu, 0.05 mg kg^{-1} DM for Hg, 11 mg kg^{-1} DM for Ni, 14 mg kg^{-1} DM for Pb and 67 mg kg^{-1} DM for Zn, according to the Flemish Environment Agency (De Cooman & Detemmerman, 2003). Remarkably, these references do not seem to be related to the physicochemical sediment properties, such as clay or organic matter contents. Only 1 % is considered as non-polluted when also the biological and ecotoxicological quality of the sediments are also taken into account in the Flemish "Triad" approach (Fig. 2.2). Due to their pollution status, sediments could also be dredged for the purpose of environmental remediation, whereas dredged sediments cannot automatically be regarded as usable soil additives anymore (Van Der Burght, 1994). As a result, most sediments were deposited in confined disposal sites during the last 20 years.
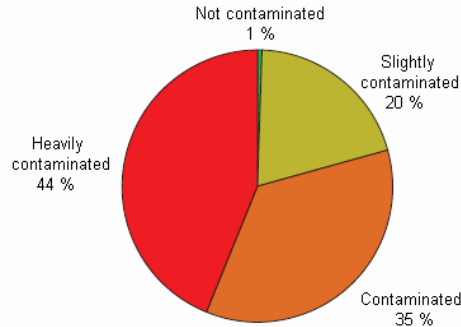
Fig. 2.2. Pollution status of bottom sediments in Flanders (Peeters et al., 2005)

In Flanders, dredged sediments must meet certain quality criteria before they can be disposed on land and re-used as soil. Quality criteria reflect maximum allowable contents of contaminants (Table 2.1).

Table 2.1. Maximum allowable metal contents in dredged sediments (mg kg^{-1}) for deposition on land and re-use as soil substrate (Vlarebo, 1996).

Land use *	As	Cd	Cr	Cu	Hg	Pb	Ni	Zn
I	19	0.8	37	17	0.55	40	9	62
II, III, IV, V	27	1.2	78	109	1.5	120	55	300

* I Nature, II Agriculture, III Residential, IV Recreation, V Industry

These concentrations must be corrected for clay and organic matter contents according to (Vlarebo, 1996):

N (x,y) = N(10,2).(A+B.x+C.y)/(A+B.10+C.2)   (Equation 2.1)

With   N (x,y) = Metal contents from Table 2.1
         x = clay content (%)
         y = organic matter content (%)
         A, B, C = metal specific coefficients (Table 2.2)

Table 2.2. Metal specific coefficients to be used in Equation 2.1

	A	B	C
As	14	0.5	0
Cd	0.4	0.03	0.05
Cr	31	0.6	0
Cu	14	0.3	0
Hg	0.5	0.0046	0
Pb	33	0.3	2.3
Ni	6.5	0.2	0.3
Zn	46	1.1	2.3

Vandecasteele et al. (2002) identified dredged sediment landfills along the upper Scheldt river upstream the city of Ghent. Up to 82% of the sediments were found to be polluted by at least one of the metals Cd, Cr, Pb or Zn, whereas Cu and Ni were of no environmental concern. Concentrations of Cd, Cr and Zn were, in 10 % of the cases, higher than 26, 1900 and 2800 mg kg^{-1}, respectively. Contents of Pb and Cu were significantly lower for sediments disposed after 1965, but no indication for improvement of sediment quality with time was observed for Cd, Cr and Zn. The finest sediment fractions have the highest affinity to bind heavy metals. Deposition of these polluted sediments can be very harmful for the environment. When dredged sediments are subjected to drying and oxidation after disposal, chemical transformation can affect their mobility and availability (Gambrell, 1994). Singh et al. (2000) found that leaching of metals from different types of disposed, polluted sediments per unit of surface area is 2 to more than 20 times higher than that in surface runoff. There is especially a high risk for metal leaching to lower laying soil layers when dredged sediments are alternately subjected to wet and dry conditions (Tack et al., 1998). Metal transformation upon flooding and drying will be further discussed in paragraph 2.8.

2.6 Floodplains, tidal flats and marshes

2.6.1 Sediment dynamics

Land adjacent to the river can experience occasional flooding. In that way, floodplains can be formed by the deposition of sediment carried by river as it floods. Some areas in the estuary

have been frequently flooded for a long time, which resulted in a long lasting sedimentation of suspended particles. These areas are called tidal flats if they are submerged during every high tide and exposed again by the ebb. Tidal flats are called mud flats if they contain significant amounts of mud. Over time these tidal flats get banked up higher until they are only submerged by spring tides and vegetation starts to develop. The so-created marshes are crossed with gullies, allowing the water to penetrate deep into them during every tidal cycle (Fig. 2.3).

Sediment accretion rates for five European coastal wetlands were between 0.27 and 1.90 cm year^{-1} (Callaway et al., 1998). Accretion rates were consistently higher at low-marsh sites than at high-marsh sites. This trend was expected from previous studies that also have evaluated the effect of elevation and distance from creeks on accretion rates in coastal wetlands (e.g. Hatton et al., 1983; Stoddart et al., 1989).

Above the sediment surface of these marshes, the concentration of suspended sediments are high during the floods at the beginning of the tidal cycle, and decrease afterwards. This indicates that suspended sediments permanently settle; minimal re-suspension takes place during ebb (Temmerman, 1988; Van Maldegem et al., 1993). Some authors however reported erosion and loss of marsh area due to increasing water levels (Ward et al., 1998; Kearny & Stevenson, 1991; Spencer, 2002). Braskerud (2001) emphasized the importance of vegetation in capturing suspended matter in constructed wetlands. Sedimentation was found to increase and re-suspension to decrease with increasing vegetation cover. Ultimately, the height of the sediments surface generally reaches an equilibrium. Pethick (1981) registered an asymptotic relationship between the elevation and the age of salt marshes in the UK. Temmerman et al. (2003a) concluded that the sediment accumulation rate decreases if the sediments surface reaches a height of about 10 to 20 cm above the average water level at high tide. If the average water level at high tides increases, however, sediments can also continue to accumulate (Orson et al., 1998).
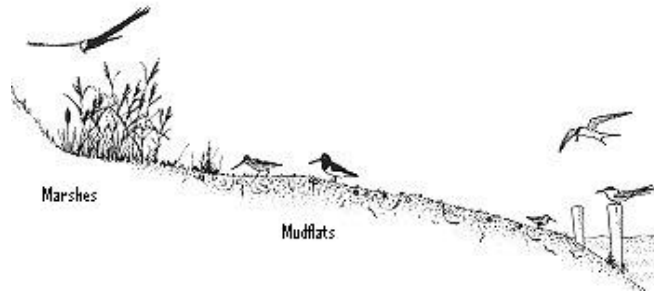
Fig. 2.3. Marshes and mudflats (source: www.scheldeschorren.be)

Temmerman et al. (2003b) noted that spatial and temporal sedimentation patterns do not differ much between freshwater and saltwater marshes of the Scheldt estuary. The much higher vegetation density at freshwater marshes does not seem to be more efficient in tempering the water flow. Temporal sedimentation patterns were found to be mainly affected by tidal action, which does not differ between the saltwater and freshwater marshes in the Scheldt estuary.

2.6.2 Salinity

As referred to before, a longitudinal salinity gradient is observed along the Scheldt river. Salinity decreases from the sea up to the mouth of the tributary Durme, where chloride concentrations stabilize around 100 mg L^{-1} in the surface water. The longitudinal salinity profile is primarily determined by the magnitude of river discharge, whereas the tidal action contributes to a lesser degree. As a result, significant salinity variations in the surface water are observed. For example, the lower riverine part from Ghent to Antwerp is a tidal fresh water river during winter and spring, whereas the water turns brackish between Antwerp and the Rupel mouth during summer and autumn (Baeyens et al., 1998). Bear (1964) noted that actual salinity effects on soils and plants are determined by the salinity of the pore water of intertidal sediments, rather than the salinity of the flooding water. Lau & Chu (1999) observed higher salinities in that pore water compared to the flooding water. The pore water salinity differs between mudflats and marshes. Salt contents are relatively constant in the lower mudflats as they are regularly flooded, whereas the salinity of the upper marsh sediments is subjected to temporal variations. Thus, in terms of the water and salt budget, surface water and groundwater are interrelated. Post (2005) however reported that simulation of the interaction between the two is not a trivial matter. Groundwater responds much slower than

does surface water, and this presents a problem of different time scales when coupling surface water and groundwater models. Relatively little work has been carried out in this field. As a result, salt contents and salinity variations in the pore water of intertidal sediments cannot be easily predicted from the salinities of the surrounding surface waters.

2.6.3 Marsh ecology

When tidal flats get banked up higher, pioneer plants will first start to colonize the marsh. This evolves to a dense vegetation of marsh plants and herbs and finally a floodplain forest can develop. Because the plants contribute to organic detritus accumulation and entrap sediment particles, they play an important role in the steadily rising level of the marshes (DeLaune et al., 1981). Wetland plants do possess various characteristics that enable them to survive periodic conditions of soil saturation and accompanying changes in soil chemistry. These changes include oxygen consumption and depletion which poses additional stress on the roots. Oxygen is needed to maintain aerobic respiration and to oxidize reducing compounds in the rhizosphere (Armstrong et al., 1994). Accumulation of various soil phytotoxins may lead to injury to certain species (Pezeshki, 2001). These toxins are by-products of soil reduction, such as organic components, soluble sulphide species and reduced Fe and Mn. The inhibitory effect of H_2S on cytochrome oxidase is disruptive to aerobic respiration and excess cytosolic Fe and Mn is believed to be harmful to enzymatic structures (Drew, 1990). Negative effects of elevated sulphide concentrations on leaf photosynthetic capacity have also been demonstrated in several wetland plant species, and could be attributed to e.g. alterations in activity of photosynthetic enzymes (Pezeshki, 2001). Moreover, sulphide injuries have been reported in hypoxic roots and rhizomes of common reed (Koch et al., 1990; Furtig et al., 1996). Flooding was also reported to decrease overall biomass production and changing root to shoot ratios (Pezeshki, 2001).

Most plants that naturally occur in wetlands are well-adapted to endure periods of soil oxygen deficiency. They may however differ in their ability to withstand certain levels of intense soil reducing conditions (Pezehski, 2001). Many wetland plants have an extensive oxygen transport system (aerenchyma tissue) in roots, stems, and leaves. This system allows the plant to transport the oxygen to the roots (Armstrong et al., 1994).

According to the marsh ecology, salt, brackish and freshwater marshes can be distinguished. Only salt-tolerant plants can grow on a salt marsh. On the brackish water marshes, also salt-tolerant plants can be observed. However, when they get banked up higher and if they are not grazed, a climax vegetation of reed plants (*Phragmites australis*) will occur. At freshwater marshes, the climax vegetation consists of reed in the lower laying parts of the marshes and a softwood floodplain forest (containing e.g. willows) on the embankments. Thus, the brackish and freshwater marshes along the Scheldt river between Ghent and Antwerp are mainly vegetated by reed plants and willows. Reported maximal aboveground biomass in healthy reed stands varies between 152 (Rolletschek et al., 1999) and 7700 g DM m^{-2} (Rolletschek & Hartzendorf, 2000), but is typically around 1000 g DM m^{-2} (Soetaert et al., 2004). Thus, reed beds are highly productive (Windham & Lathrop, 1999). The maximal aboveground reed stocks are generally assumed to be within 85-100 % of net annual aboveground production (Gessner et al, 1996; Brix et al., 2001). A variable part of this aboveground production ends up as dead litter. That may constitute a considerable portion of the total detrital mass in the habitat and enters the diet of animals (Boschker et al., 1995).

Soetaert et al. (2004) modeled growth and carbon allocation in two reed beds in the Scheldt estuary at Saeftinghe and Burcht from 1996 till 1998. At their peak, the aboveground biomass was 587-1678 g DM m^{-2} at the high-salinity site (13.3 psu), whereas it was 1116-2179 g DM m^{-2} at a low salinity site (1.6 psu). More than 60 % of the biomass was located underground. In 1996, biomasses were 2-3 times lower than in the other 2 years. This was caused by a retarded growth initiation, which was due to lower temperatures early 1996. The architecture of the plants was also affected by salinity, with a higher shoot density (about 50 % more shoots), a better-developed root system (15 % of total belowground biomass compared to 5 %) and more, but smaller leaves at the higher salinity site. Primary production accounted for about 80% of all growth processes and rhizome remobilization for almost 20 %. Within a year, some 44 % (oligohaline) and 36 % (mesohaline) of new assimilates produced by photosynthesis accumulated as dead litter. The other part was assimilated by the plant, either to provide energy for growth (23 %) or for maintenance costs (33 and 41 % at the oligo- and mesohaline sites, respectively). Calculated annual turnover rates of aboveground biomass, rhizomes and roots were 100, 62 and 73 %, respectively.

The bed of the tidal flats and marshes is also teeming with animal life. Small animal species, such as cockles, rag worms and clams can be found by millions in the mud. At high tide they

filter plankton out of the water. At low tide they bury themselves deep down for safety, which does however not guarantee survival. At low tide, the shoals and even more the tidal flats provide a lavish banquet for thousands of birds. And at high tide, when these areas are largely under water, they are a source of food for larger bottom dwellers and fish (Oosterschelde National Park, 2004). As these animals and plants form the base of the food web and live in very close contact with the sediments in marshes and tidal flats, fate of accumulated metals in these sediments should be thoroughly studied to ensure the health of the entire ecosystem.

2.7 Processes and properties affecting metal fate in soils and sediments

Measuring total metal contents in soils and sediments is not sufficient to assess short term environmental risks, as they do not reflect metal mobility, reactivity, or bioavailability (Rieuwerts et al., 1998; Sauvé et al., 2000). This mainly depends upon the metal speciation and fractionation. Gambrell (1994) distinguished a number of general chemical forms of metals in soils and sediments that differ in their mobility and plant availability. These include:

- water-soluble metals, as free ions, inorganic or organic complexes;
- exchangeable metals;
- metals precipitated as inorganic compounds, including insoluble sulphides;
- metals complexed with large molecular-weight humic materials;
- metals adsorbed or occluded to precipitated hydrous oxides;
- metals bound within the crystalline lattice structure of primary minerals.

Recent studies that examined the relationship between bioavailability of heavy metals in soils, toxicological impact and potential transfer of heavy metals through the food chain have focused on the soil solution phase. These soil solutions are in intimate contact with the solid phase and are therefore very much influenced by the mineral equilibria, sorption reactions and complexation processes (Sauvé et al., 2000). It is therefore very important to focus on adsorption/desorption, precipitation/dissolution and complexation/decomplexation equilibria when studying mobility of metals in soils and sediments. As some preconditions, such as pH, redox potential, cation exchange capacity (CEC), organic matter content and microbial activity, can rapidly change due to alternating environmental conditions, true thermodynamical equilibrium is never achieved. Kinetics are therefore also of primary

importance. Some relevant processes, factors and preconditions affecting metal fate in intertidal sediments will be extensively discussed in the following paragraph.

2.8 Factors affecting metal mobility and availability in intertidal sediments

Salomons et al. (1987) divided the sediment-water system in three parts: an oxic layer, an anoxic layer and an oxic-anoxic interface. In the oxic surface water and sediment layers, stable phases of trace metals appear to be especially adsorbed species. Important sorbents are Fe/Mn hydroxides, carbonates, organic matter and clay minerals, whereas especially salinity and soluble organic components in the pore water might affect desorption from these phases. These factors and processes will be discussed in paragraphs 2.8.1 to 2.8.3. The oxic-anoxic interface and the anoxic layer beneath the oxic layer play major roles in the potential flux of trace metals from the sediments to the surface waters. The oxic-anoxic interface is situated in the water column of some stratified lakes and basins, and in the sediments of well-mixed water bodies. In intertidal sediments, it should be situated in the upper sediment layer, just below the oxic layer. At the oxic-anoxic interface and in the anoxic layer, redox-sensitive processes occur, which mainly result in the inclusion of metals in precipitates or the dissolution of metal-containing precipitates. Kinetics of these processes are of great importance if the location of the oxic-anoxic interface is subject to change due to changing water table levels. During the last decades, some studies focused on the kinetics of oxidation processes and metal release upon gradual oxidation of dredged sediments after land disposal or during resuspension of sediments in waterways, whereas studies on the kinetics of reduction processes upon flooding of initially oxidized soils and sediments are rather scarce. Moreover, only few researchers focused on the metal (im)mobilization kinetics in the highly dynamic upper intertidal sediment layer, which might be subject to rapidly fluctuating water table levels. The changing metal mobility as a result of fluctuating redox conditions will therefore be extensively discussed in paragraph 2.8.4.

2.8.1 Adsorption and desorption

Clay particles can be negatively charged by e.g. isomorphous substitution during the formation of the minerals: Si^{4+} is replaced by Al^{3+} or Al^{3+} is replaced by Fe^{2+} or Mg^{2+}. At the surface of these minerals, also hydroxyl groups occur of which the charges depend on the pH. Also the surface charge of organic matter is pH dependent, as a result of the abundance of e.g. acetate groups. The charge per mass unit depends on the type of organic matter. Iron and alumina oxides do not have a net charge near their iso-electric point, but they are positively or negatively charged at respectively lower or higher pH. Electropositively charged elements can be attracted to negatively charged surfaces of organic matter, clay particles and iron and alumina oxides, which determine the cation exchange capacity or CEC (Evans, 1989). A high CEC therefore reduces metal mobility and availability but increases metal cation retention by the sediments. A study of a number of river systems showed that for Cd, Zn and Ni, the Fe/Mn hydroxides were the main carriers under oxic conditions, whereas the organic fraction is most important for Cu (Salomons et al., 1987). Prokop et al. (2003) studied metal mobility and toxicity in land disposed sandy sediments with a pH between 4.4 and 6.1. Speciation calculations predicted that Cd and Zn would remain mainly bound on the clay fraction. However, they did not find a relationship between clay content and metal behaviour (distribution and leachability). On the other hand, a correspondence between TOC and leachability was observed: a higher Cd and Zn leaching rate was noted in sediments with lower organic carbon content, which also had a lower pH. Leaching significantly increased at a pH below 4.5. In the simplest form, the sorption mechanism assumes a single stage in which dissolved metals in the bulk solution attain rapid equilibrium with weak binding sites on the surfaces of particles. However, a second, slower stage can also be hypothesized in which the surface-bound metals may undergo solid-state reactions, by which they migrate on the particle surface to higher-energy binding sites. The time constants of the intra-particle reactions are however relatively long and may cover the range of estuarine flushing times. This suggests that if the second stage is accounted for, it is unlikely that a dissolved constituent will be in true equilibrium with the particulate phase during typical estuarine flushing times (Millward & Liu, 2003).

Fig. 2.4 shows a set of parallel reactions to sites of increasing binding energy on a non-porous surface and a set of consecutive reactions involving the serial migration into the matrix of a porous solid, referred to as "aging" (Ford et al., 1997).
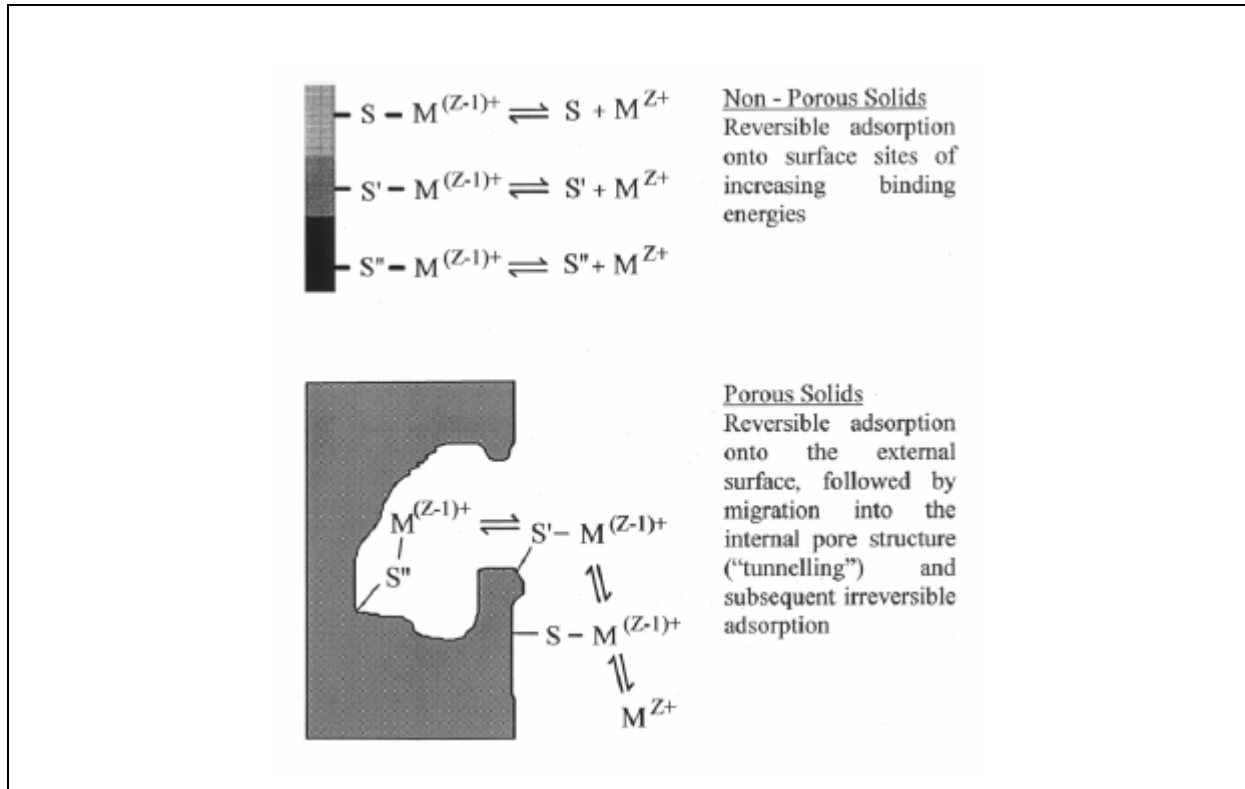
Fig. 2.4. Sorption mechanisms for uptake of dissolved metals by non-porous and porous solids. S, S' and S" are binding sites of increasing energy and M^{z+} is a dissolved metal (Millward & Liu, 2003).

2.8.2 Effect of salinity

Tidal variations in the Scheldt estuary result in varying salinities of the river water and pore water of the sediments. This can affect metal fate in sediments in different ways. Due to increases of chloride concentrations when inland fresh river water mixes with seawater, heavy metals may mobilise from the sediments as soluble chloride-complexes (Hahne & Kroontje, 1973). This is especially the case for Cd as the stability and solubility of cadmium chloride complexes is relatively high and the affinity for sorption to the solid soil phase is low (Doner, 1978; Comans & Van Dijk, 1988). Upon the formation of these complexes, the activity of free Cd^{2+} in the solution will decrease and desorption will increase. Only the Cd which is rather weakly bound to the solid soil phase will be desorbed as a result of chloro-complexation (Paalman et al., 1994). An increase of the salinity is however also associated with an increase

in the concentrations of major elements (Na, K, Ca, Mg) which compete with heavy metals for the sorption sites (Tam & Wong, 1999). According to Khattak et al. (1989), the addition of Ca-salts results in a higher release of exchangeable metals in the soil solution compared to the addition of Na-salts, which are less competitive for sorption. Balls et al. (1994) observed increasing Cd desorption from the particulate phase with increasing salinities in the Forth estuary (Scotland), whereas Gerringa et al. (2001) found that high salinities resulted in a faster release of Cd from CdS during the oxidation of reduced Scheldt sediments. This release was found to be stimulated by both formation of chloride complexes and ion exchange. Gambrell et al. (1991b) also studied salinity effects during the oxidation of reduced metal-polluted brackish marsh sediments. Soluble Cd, Cr and Cu were found to increase with increasing salinity, whereas the salinity did not significantly affect Ni and Pb mobility. According to Millward & Liu (2003), the extent of metal desorption from sediments which were suspended in seawater followed the general order Ni > Cd > Zn > Cu, whereas Lores & Pennock (1998) concluded that salinity affected the binding of metals to humic acids in the Suwannee River. Binding of Cd, Cr, and Zn to humic acids decreased to less than 20 % at salinities increasing up to 15 psu. Cu binding however decreased to 27 % at a salinity of 3 psu, whereas this again increased to 60 % at a salinity of 15 psu. This would be due to complex interactions between salinity, humic acids, pH and Cu, which have also been noted by Gerringa et al. (1998) in the saline part of the Scheldt estuary.

The salinity affects dissolved metal speciation and toxicity in surface waters. Verslycke et al. (2003) reported a decreasing toxicity to the estuarine mysid *Neomysis integer* upon increasing the salinity from 5 to 25 ‰ and attributed this to lower concentrations of the free ions of Hg, Cd, Cu, Zn, Ni and Pb. The salinity effect was strongest for Cd and Pb and was attributed to the complexation with chloride ions. The toxicity of Ni, Cu, and Zn was affected to a smaller extent by salinity. Greger et al. (1995) studied the effect of salinity on the uptake of Cd by the submerged macrophyte *Potamogeton pectinatus* from both water and sediments. They constructed a tentative model for Cd uptake by aquatic plants, which is represented in Fig. 2.5. Cd uptake from the water was found to decrease when increasing the salinity up to 10 ‰. However, in the presence of sediments, Cd uptake increased with increasing salinity. This clearly illustrates that increasing salinity promotes Cd desorption from the sediment, hence increases Cd concentrations in the water column and Cd uptake by organisms. It however also promotes the formation of Cd chloride complexes in the water column, which seem to be less bio-available. A higher Cd bioavailability and toxicity with decreasing salinity was indeed

also observed for other organisms, such as mussels (Fischer, 1986; Stronkhorst, 1993) and invertebrates (McLusky et al., 1986). Zanders & Rojas (1996) concluded that the accumulation of Cd in the tissues of fiddler crabs is also enhanced under conditions of low salinity surface water. They did not only attribute this effect to increased concentrations of Cd^{2+} forms, but also cited the decreased competition of Ca^{2+} for transport sites (e.g. Wright & Frain, 1981), permeability changes of the animal's surfaces in response to environmental salinity levels (e.g. Chan et al., 1992), and the source of the metal (either dietary or in solution, e.g. Pratap & Wendelaar-Bonga, 1993) as factors affecting Cd uptake by organisms under changing salinities.
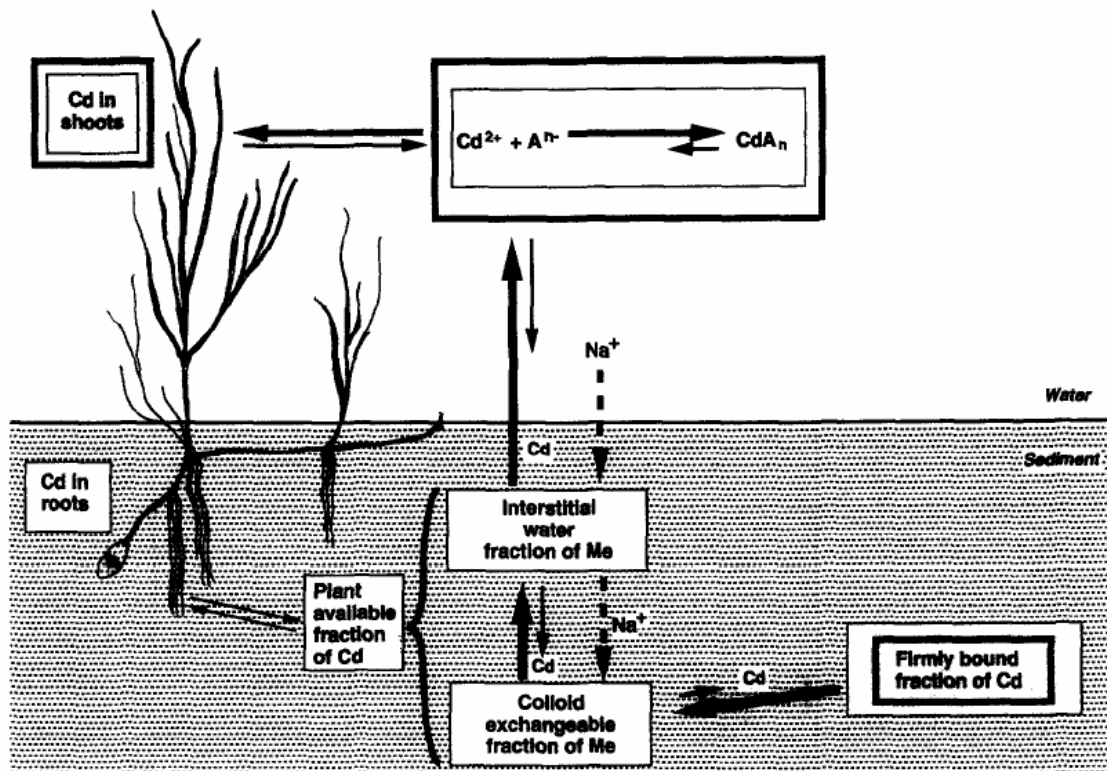
Fig. 2.5. A tentative model of Cd translocation in a macrophyte-water-sediment system at increasing salinity of the water. Change in concentration of Cd denoted with bold squares and changes in equilibrium denoted with bold arrows (Greger et al., 1995).

2.8.3 Adsorption, complexation and chelation to organic matter

Soetaert et al. (2004) found that some 36 to 44 % of new assimilates produced by reed plant photosynthesis ultimately accumulated as dead litter within a year in two reed beds of the Scheldt estuary. Upon decomposition of this litter, metals may be bound to organic matter

fractions due to adsorption, complexation and chelation, i.e. complexation by coordination with multidentate ligands (Alvim Ferraz & Lourenço, 2000). Dissolved organic ligands, such as low to medium molecular weight carboxylic acids, amino acids and fulvic acids, might form soluble metal complexes. As a result, metal desorption from the soil was reported to be enhanced, e.g. for Ni by Tipping et al. (1998) and Wells et al. (1998), for Pb, Cu and Zn by Alvim Ferraz & Lourenço (2000), and for Hg, Cr, Cu and As, but not for Cd and Zn, by Kalbitz & Wennrich (1998). Kalbitz & Wennrich (1998) moreover stated that a different composition of dissolved organic matter and the soil solution can result in a different affinity of metal species with dissolved organic matter. According to Charlatchka & Cambier (1999), especially Pb was found to be complexed by organic acids in a flooded soil. Reversely, high molecular weight organic matter compounds in the solid soil phase can reduce the metal availability (Gambrell, 1994). Moreover, micro-organisms in the rhizosphere of wetland plants can accumulate metals (Scholes et al., 1999). Decho (2000) reported a significant role of extracellular microbial polymeric secretions (microbial biofilms) in intertidal systems, both in the binding and concentration of metal contaminants, as in the trophic transfer of metals to the food web. According to Kunito et al. (2001), these exopolymers were especially produced in large quantities by Cu-resistant bacteria in the rhizosphere of reed plants, which suggest its involvement in the detoxification of Cu. The presence of organic matter can thus both decrease or increase metal mobility, depending on organic matter composition and physico-chemical soil characteristics. As annual biomass production is very high in intertidal reed beds, contents and decomposition rates of organic matter are very high and variable in the upper sediment layer and the presence of organic matter is expected to substantially affect metal fate.

2.8.4 Precipitation/dissolution of metals upon changing redox conditions

2.8.4.1   Redox processes occurring during flooding

Redox reactions can be represented as follows:

$$Ox + n\ e^- + m\ H^+ \leftrightarrow Red + H_2O \qquad \text{(Equation 2.1)}$$
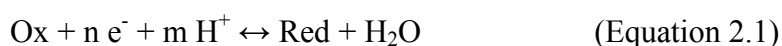
Ox = oxidised form of a compound

Red = reduced form of a compound

e^- = electron

H^+ = proton

The redox potential is a quantitative measure for electron availability in biological and chemical systems. Redox potential measurements may thus be used to indicate the degree of oxidation or reduction of a system.

Water saturation during extended periods of time usually results in changes in chemical properties of sediments and soils, as well as in microbial populations and processes. These changes can greatly affect the chemical mobility and bioavailability of metals. When a soil is flooded, diffusion of oxygen from the air to the soil is strongly impeded. As water fills up the pores and micro-organisms and plants continue to use dissolved oxygen, the oxygen concentration decreases according to the following reaction:

$$O_2 + 4\ H^+ + 4\ e^- \leftrightarrow 2\ H_2O \qquad \text{(Equation 2.2)}$$
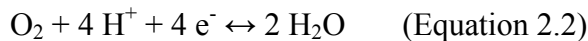
As the soil becomes more reduced, nitrate nitrogen is the next component to be removed, as it is commonly used as an electron acceptor for microbial respiration when oxygen is no longer available. This occurs at a redox potential of about 225 mV at a pH of 7 (Gambrell et al., 1991a). This can be represented as:

$$NO_3^- + 2\ e^- + 2\ H^+ \leftrightarrow NO_2^- + H_2O \qquad \text{(Equation 2.3)}$$
$$2\ NO_2^- + 7\ e^- + 8\ H^+ \leftrightarrow N_2 + 4\ H_2O \qquad \text{(Equation 2.4)}$$
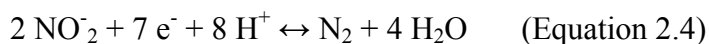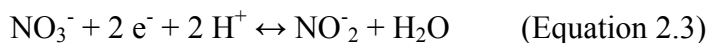
Microbial denitrification rates can be affected by the presence of metals. Sakadevan et al. (1999) found that the addition of relatively small amounts of Cu and Zn (100 mg kg^{-1}) increases denitrification rates, whereas larger amounts of Cd, Cu and Zn (500 mg kg^{-1} Cd and Cu and 1000 mg kg^{-1} Zn) reduced denitrification rates.

When the redox potential is further decreasing, also manganic manganese will be reduced to the manganous form at around 200 mV.

$$\text{E.g.: } MnO_2 + 2\ e^- + 4\ H^+ \leftrightarrow Mn^{2+} + 2\ H_2O \qquad \text{(Equation 2.5)}$$
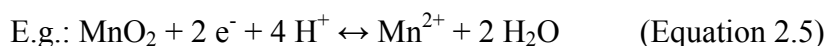
Just above 100 mV, also ferric iron is reduced to the more mobile and soluble ferrous form:

E.g.: $Fe_2O_3 + 2\ e^- + 6\ H^+ \leftrightarrow 2\ Fe^{2+} + 3\ H_2O$ (Equation 2.6)
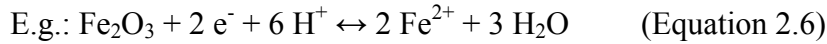
Amorphous oxides are more easily reduced compared to well crystallized forms. As long as these iron oxides are present, the redox potential remains relatively stable. The reductions can occur purely chemically, but this proceeds quite slowly.

There is a considerable gap in reduction of major mineral components until sulphate sulphur is reduced to sulphide forms at about -150 mV (Gambrell et al., 1991a). This reaction is also catalyzed by micro-organisms and occurs at redox potentials below -150 mV:

$SO_4^{2-} + 8e^- + 8H^+ \leftrightarrow S^{2-} + 4H_2O$ (Equation 2.7)
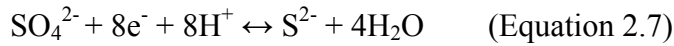
The sulphides can bind to free protons to form H_2S:

$2H^+ + S^{2-} \leftrightarrow H_2S$ (Equation 2.8)

Also other reduced sulphur compounds (e.g. polysulphides, such as pyrite FeS_2, and elemental sulphur) can be produced (Billon et al., 2001).

Finally, after the consumption of sulphates, methane formation is initiated from the reduction of carbon dioxides and certain organic acids at around -200 mV. This only occurs in soils which have been flooded for a long time:

$CO_3^{2-} + 8e^- + 10H^+ \leftrightarrow CH_4 + 3H_2O$ (Equation 2.9)
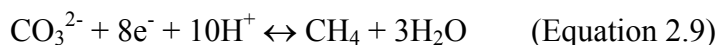
An overview of possible interactions and factors affecting metal mobility and availability in soils of wetlands, dominated by a reed plant vegetation, is given in Fig. 2.6.
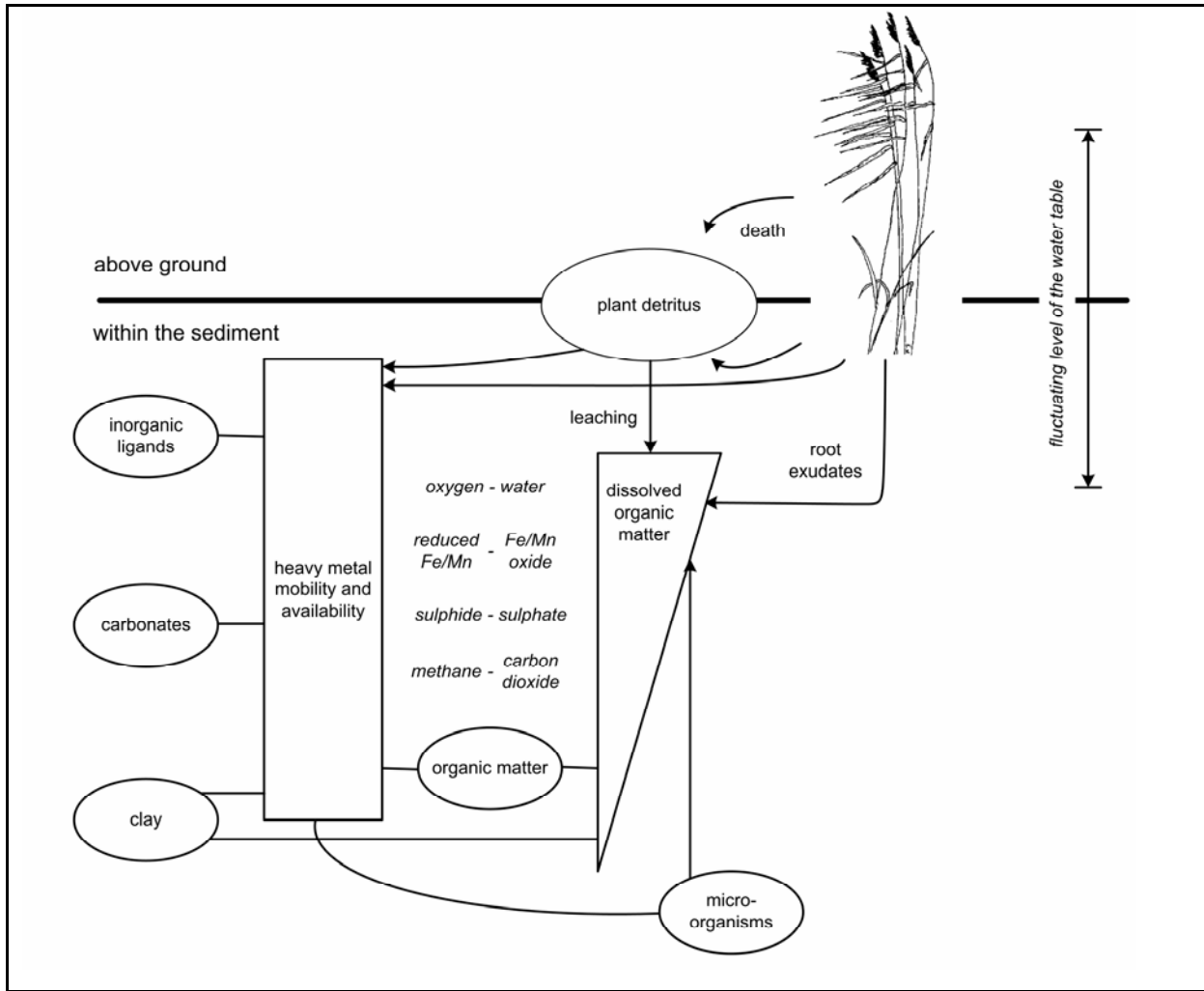
Fig. 2.6. Overview of possible interactions and factors affecting metal mobility and availability in soils of wetlands, dominated by a reed plant vegetation

Some authors report the oxidation and reduction of electron acceptors to occur sequentially when controlling the redox potential at certain levels, e.g. by automatically adding small amounts of O_2. Patrick & Jugsujinda (1992) e.g. reported no overlap in the oxidation or reduction of NO_3^- and Mn^{4+}, and only little overlap in oxidation and reduction of Mn^{4+} and Fe^{3+}. Theoretically, electron acceptors with higher redox potentials will be reduced first (Zehnder & Stumm, 1988). However, as reduction processes are catalyzed by a wide range of micro-organisms, the reduction processes do not always occur sequentially and some overlap can be observed under more natural conditions. Peters & Conrad (1996) showed that anaerobic bacterial activities, i.e. NO_3^- reduction, Mn^{4+} reduction, Fe^{3+} reduction, SO_4^{2-} reduction and methanogenesis all became active simultaneously in all of their tested upland soils as soon as they were incubated under natural, anoxic conditions.

Moreover, accurately determining the redox status is not easy. Tanji et al. (2003) distinguished three methods for assessing the redox status. Firstly, redox potentials can be measured by using a Pt-electrode. In practice, the redox electrodes most commonly used consist of a clean, bright platinum wire or button in contact with the soil solution, and a reference electrode, which completes the electrochemical cell. Redox potential data originating from these measurements are useful for determining the intensity of reduction. One can predict the occurrence of many soil chemical and biological processes affecting transformations and availability of trace metals. However, some theoretical limitations exist along with procedural difficulties when using Pt-electrodes. In natural systems, there are many redox couples present, and not all redox couples are chemically interactive with others. Unless the concentration of a given redox couple is relatively high, inert electrodes (as Pt) used for redox measurements are however not specific for a specific redox couple. The redox electrode therefore responds to the collective electrochemical potentials of all redox couples present and the measured redox potential is generally a mixed potential which reflects a weighted average of the potentials contributed by each of the redox couples present (DeLaune & Reddy, 2005). Results of redox potential measurements should therefore be mainly qualitatively interpreted (Bohn, 1971). Moreover, in oxidized soil or water systems, redox potential is poorly posed due to the low concentration of redox-active components. This contributes to the sometimes observed instability and both short- and long-term drift of the reading. Data on oxygen contents are therefore more useful in oxidized soils, while redox potential measurements are of more value in anaerobic systems (Gambrell et al., 1991a).

A second method to assess the redox status is based on measuring TEAP or terminal electron-accepting processes (Tanji et al., 2003). The microbial electron acceptor consumption, intermediate concentration of H_2 and accumulation of reduced compounds are simultaneously determined and a hierarchical decision tree is used to asses the redox status. This method however is based on complicated sampling and time-consuming analyses procedures. Finally, there is a possibility to calculate the oxidative capacity (OXC) to characterize the redox status of a soil, which is defined as the subtraction of the sum of the oxidized and reduced chemical forms. By using this method it is possible to distinguish between oxic, post-oxic and sulphidic stadiums.

A change in redox conditions may affect soil pH. During reduction, protons are being consumed, whereas during oxidation acidification tends to occur. In turn, these pH changes

might affect metal mobility and availability. A pH drop prevents the transfer of most trace metals to the sediment and/or causes a desorption from the sediments (e.g. Salomons et al., 1987; Gambrell et al., 1991; Calmano et al., 1993). A higher activity of protons reduces the negative surface charge of organic matter, clay particles and iron and alumina oxides and increases the solubility of precipitates, such as sulphides. Fig. 2.7 presents general, combined effects of redox potential and pH on metal mobility. As can be observed in that figure, metals do not react identically on redox and pH changes. As already mentioned above, the valence state of manganese and iron will change during flooding, which can affect their mobility. Marsh sediments also tend to rapidly reduce the very toxic Cr(VI) to less toxic trivalent Cr (Pardue & Patrick, 1995). Moreover, Simpson et al. (2000) observed the reduction of Cu(II) to Cu (I) under slightly alkaline conditions and negative redox potentials, whenever a suitable electron donor species is present. In natural sediments, numerous electron donors (e.g. Fe(II), sulphur compounds), and bacteria acting as catalysts, may be actively involved in the Cu(II) to Cu(I) reduction process, subsequently leading to Cu_2S precipitation. Changes of the valence state as a result of redox changes in natural sediments are however not observed for most of the metals including Cd and Zn, which are more important from an eco-toxicological point of view. However, their mobility can also be significantly affected by redox changes. This can be a result of e.g. redox-induced changes to the metal-binding capacity of humic materials, insoluble metal sulphide formation (or sulphide oxidation) and changes in Fe/Mn-oxyhydroxides, which are known to be effective in immobilizing some metals under oxidizing conditions. Cadmium e.g. is reported to be much more mobile and plant-available in oxidized soils and sediments compared to reduced conditions (Gambrell et al., 1991a&b).
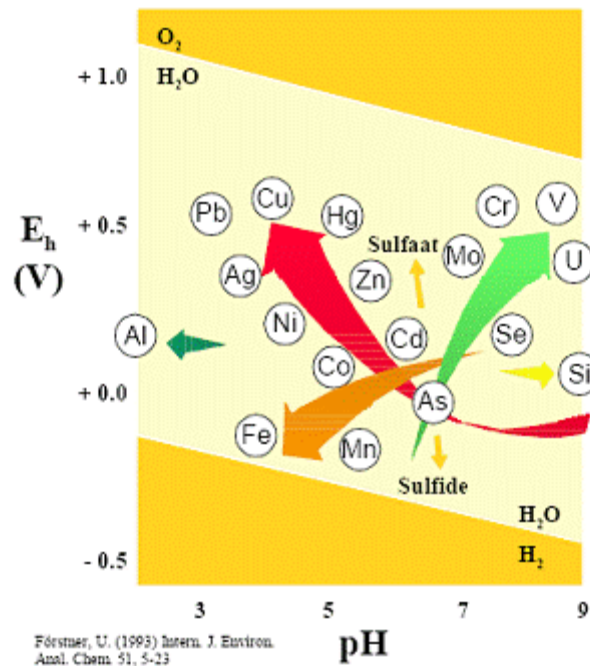
Fig. 2.7. Schematic representation of most important effects of redox potential and pH on metal mobility; arrows point towards increasing mobility (Förstner, 1993)

2.8.4.2   Metal fate as affected by oxidation and reduction of Fe and Mn

In the availability of oxygen, Fe and Mn will precipitate as Fe or Mn oxides. Heavy metals can co-precipitate or adsorb to these oxides, which reduces their availability. Ni was e.g. reported to primarily co-precipitate with Mn oxides by Zwolsman et al. (1993) in salt marsh sediments from the Western Scheldt, whereas Guo et al. (1997) reported an increasing affinity between Fe and Mn oxides and As, Cd, Cr and Zn with increasing sediment redox potential. Reduction and solubilisation of these oxides can in turn have important consequences for the mobility of metals. This has been reported for Pb, Zn and Cd by Davis & Kent (1990) and for Ni by Kashem & Singh (2001). Guo et al. (1997) found decreasing association of Cd, Cr and Zn with Fe and Mn oxides upon decrease of the redox potential. The association of Cr, Cd and Zn with insoluble large molecular humic substances subsequently increased, as well as the association of Cd and Zn with carbonates and sulphides. The stability of Fe^{2+} and iron (hydr)oxides primarily depends on a combination of redox potential and pH of the sediment. The nearly amorphous $Fe(OH)_3$ minerals (ferrihydrite) reduce at a higher redox potential for a given pH than do the crystalline minerals of FeOOH (goethite) or Fe_2O_3 (hematite). Generally, Fe occurs in soluble forms in an acid environment. When a neutral pH is

approached, Fe only can exist in solution at low redox potentials, or as a soluble organic complex in oxic soils. Some bacteria can catalyze these reductions (Lovley & Phillips, 1988b). Some authors, such as Lovley & Phillips (1988a), reported that the reduction of Fe^{3+} does not begin before all Mn^{4+} is depleted. However, Patrick & Jugsujinda (1992) and Peters & Conrad (1996) observed at least some overlap.

2.8.4.3   Metal fate as affected by sulphur cycling

Sulphate reduction is initiated by microbial communities at quite low redox potentials, below -150 mV. It leads to the formation of elemental sulphur and sulphides. The formation of metal sulphide precipitates can occur if the sedimentary medium becomes (super)saturated with respect to the sulphides of reduced metal forms. Iron is usually the most predominant element which generates sulphide precipitates. Indeed, Fe is released from reducible minerals such as oxides/oxihydroxides within the sedimentary layers, in which sulphate reduction occurs, to give ferrous iron. This subsequently reacts with the dissolved H_2S to produce amorphous FeS and/or crystallized FeS (such as mackinawite and greigite), which are considered as precursors for pyrite formation (FeS_2) (Billon et al., 2001).

Sulphate reduction was reported to occur below a redox potential of -150 mV according to Gambrell et al. (1991a) or -220 mV according to Ross (1989). Experimental studies in subtidal marine sediments (Westrich & Berner, 1984) have shown that in the presence of high levels (> 5 mM) of sulphate, typical in the marine environment, organic carbon availability most often limits further sulphate reduction. As a result, marine sediments are often depleted in degradable organic matter. There is still sulphate in their pore waters, whereas all reducible iron is converted to iron sulphide. The methanogenic state is reached only at very great depths (Salomons et al., 1987). Sulphates may be limiting at higher depths in some highly organic marine and brackish sediments, where sulphate is exhausted by sulphate-reducing bacteria, or in freshwater environments, where sulphate concentrations are lower. Moreover, the formation of pyrite is probably not favoured in freshwater systems (van den Bergh & Loch, 2000). A model of Lovley & Klug (1986) predicts that sulphate reduction in freshwater lake sediments becomes limited by insufficient sulphate at sulphate concentrations in the pore water of below 30 μmol L^{-1}. At low sulphate concentrations, sulphate reducers are sulphate-limited and methanogenic bacteria out-compete sulphate reducers for common substrates (White et al., 1989). According to Salomons et al. (1987), the lower sulphate concentrations in

freshwater environments result in only a partial transformation of the iron hydroxides to iron sulphides, whereas the methanogenic state is reached relatively fast and the available reducible iron is precipitated as siderite ($FeCO_3$). Sulphate reduction is moreover also known to be inhibited by the presence of Fe reducing micro-organisms (Lovley & Phillips, 1987).

The degradation of organic matter and the transformation of iron hydroxides to iron sulphides and siderite ($FeCO_3$) in the anoxic layer causes a reduction in the total adsorption capacity. Alternatively, the production of hydrogen sulphide and the high alkalinity makes the formation of stable metal sulphides, or carbonates in the absence of sulphate, possible. Numerous papers have indeed been written about the possible formation of trace metal sulphides and reaction of trace metals with iron sulphide minerals. The authors have suggested that during the sulphidisation process some trace metals, that are liberated from sedimentary components such as organic matter and metal oxides, can adsorb or coprecipitate with FeS minerals or can precipitate directly as discrete/separate solid phases (Billon et al., 2001). Cu, Zn and Cd are especially expected to occur as CuS, ZnS and CdS in marine and estuarine anoxic sediments, as sulphate is highly available in these environments. This was concluded from e.g. adsorption experiments with and without sulphide present. Zn, Cu, Ni and above all Cd were moreover found to be only weakly pyritized, whereas Mn is well pyritized (Huerta-Diaz et al., 1998; Billon et al., 2001). Guo et al. (1997) moreover observed increasing association of Cd and Zn with sulphides, next to carbonates and insoluble large molecular humic substances with decreasing redox potentials, whereas Cr association was restricted to insoluble large molecular humic substances. Zwolsman et al. (1997) found lower dissolved metal concentrations in a part of the Scheldt estuary during spring and summer, which were attributed to sulphide formation as a result of the creation of anoxic conditions. Salomons et al. (1987) calculated the influence of the sulphide concentration on the speciation and solubility of Zn, Cu and Cd in a solution with 5000 mg Cl L^{-1} and an alkalinity of 50 mmol L^{-1}. The effect of the presence of chlorides on the solubility of Cd, Cu and Zn and the effect of the presence of hydroxides and (bi)carbonates on the solubility of Zn were only observed at low sulphide concentrations ($< 10^{-3}$ µg L^{-1} for Cd, $< 10^{-2}$ µg L^{-1} for Cu and $< 10^{-1}$ µg L^{-1} for Zn). At higher sulphide concentrations, the formation of soluble bi- and polysulphide complexes again increased the metal solubility. This was observed from about 10^5 µg L^{-1} for Cd, 10^3 µg L^{-1} for Cu and 10 µg L^{-1} for Zn. As a result, sulphide concentrations do not affect Cd solubility in a relatively broad concentration range ($10^{-3} - 10^5$ µg L^{-1}).

Dissolved metals in oxic environments might be complexed by organic matter, as mentioned in paragraph 2.8.3. However, in pore waters of anoxic environments, (bi)sulphide ions might compete with the dissolved organic matter. Salomons et al. (1987) performed a number of calculations to determine whether organic complexation is an important factor for metal solubility in sulphidic pore waters of estuarine sediments. They found that organic matter does not complex Cu, Zn and Cd to an appreciable extent in the presence of (bi)sulphides. No differences in pore water concentrations of sorption experiments were found for Cr and As, whether sulphide was present or not. In both cases, dissolved concentrations could be described by adsorption processes only. From this, Salomons et al. (1987) concluded that Cr and As do not form solid sulphide compounds, which was confirmed by Guo et al. (1997).

Charlatchka & Cambier (1999) studied immobilization of Cd, Pb and Zn in a well-buffered flooded soil by controlling the redox potential in soil-water suspensions during 14 days and by analyzing the leachates of an undisturbed block of topsoil, which was flooded during one year. They concluded that flooding at first increases Cd, Pb and Zn mobility by decreasing the pH and reducing the Fe and Mn oxides, whereas extensive flooding periods again lead to fixing of these trace metals, rather by re-adsorption or precipitation than by formation of insoluble sulphides. They derived the latter conclusion from the fact that sulphides were not measured in concentrations above 0.05 mg L^{-1} in the leachates. Very low sulphide concentrations in the leachates however do not prove the absence of sulphide precipitates in the solid soil fraction as sulphides of Cd, Pb and Zn are very insoluble. White et al. (1989) indeed observed sulphate reduction in the upper sediment layer of a lake, but they did not measure dissolved sulphide concentrations above 1 μmol L^{-1}, which should be attributed to the low solubility of metal sulphides.

Initially reduced sediments can be temporarily or permanently re-oxidized by processes such as (Gambrell, 1994; Eggleton & Thomas, 2004):

- bioturbation, or the disruption of the integrity of the flooded soil surface through benthic organisms that displace and suspend materials in the surface layer;
- re-suspension due to more turbulent flow conditions (underwater currents, seasonal flooding or storms);
- dredging and land-disposal;
- or permanent drainage of wetlands, lowering of the water table level.

Mainly due to the oxidation of sulphides, solubility and leaching of most heavy metals increase when very reduced sediments are oxidized (DeLaune & Smith, 1985; Singh et al., 1998). At a high pH, oxidation of metal sulphides is expected to be purely chemical, whereas it can be microbiologically mediated at much lower pH values (Salomons et al., 1987). Metals co-precipitated with or adsorbed to FeS and MnS are rapidly oxidized, due to their relative solubility in oxic conditions (Allen et al., 1993; Simpson et al., 1998; Caetano et al., 2002). More stable sulphide-bound metals (such as CuS and pyrite) are however unlikely to be oxidized in the short term due to their slower oxidation kinetics (Caetano et al., 2002). Following sulphide oxidation, the released Fe and Mn can be rapidly re-precipitated and deposited as insoluble oxides/hydroxides, to which newly released metals can become adsorbed at varying rates and extents (DiToro et al., 1990; Caetano et al., 2002; Eggleton & Thomas, 2004).

Tack et al. (1998) reported strong metal mobility increases for Cd, Cu, Pb and Zn in dredged sediments, while leachability of Fe decreases and Mn, Ni and Co are mainly unaffected. Gambrell et al. (1991b) found increasing Cd, Cu, Ni and Zn solubilities upon oxidation of a brackish contaminated sediment at acid and near-neutral pH levels, whereas this was not observed for Cr. Only little Pb mobilization was observed at intermediate redox potentials. Calmano et al. (1993) and Zoumis et al. (2001) reported the binding forms of Cd, Cu, Pb and Zn to change from stronger bound oxidisable fractions to weaker bound carbonate and exchangeable fractions during sediment oxidation, which significantly increased dissolved Cd and Zn concentrations. Stephens et al. (2001) could correlate the leaching patterns of heavy metals in sediments during drying to sulphide:sulphate ratios, which confirmed the role of sulphide oxidation. Van den Berg et al. (1998a) also attributed increased metal concentrations in the pore water of a freshwater estuarine soil during summer months to sulphide oxidation as a result of the decreasing groundwater levels. Gerringa et al. (2001) reported an increased dissolution of Cd compared to Zn upon oxidative dissolution of Cd and Zn sulphides at high salinities in the Scheldt estuary. This was attributed to a combination of the lower solubility of CdS compared to ZnS and the formation of stabile Cd chloride complexes with increasing salinities, as mentioned in paragraph 2.8.2. The increased metal mobility upon oxidation of initially reduced sediments also seems to be reflected in an increased plant availability. Thus, Vandecasteele et al. (2005b) found lower Cd concentrations in the leaves and bark of the wetland plant species *Salix cinerea* with increasing duration of submersion periods in the field. An upland hydrological regime also resulted in elevated Cd and Zn concentrations in the

leaves under greenhouse conditions, compared to a wetland hydrological regime. Moreover, initially submerged soils emerging only in the second half of the growing season resulted in elevated Cd and Zn foliar concentrations at that time, whereas foliar Zn concentrations were high at a sandy-textured oxic plot with low soil metal concentrations (Vandecasteele et al., 2005a). Such effect of reducing conditions on metal availability was also reported by others for other plant species (Gambrell, 1994).

From the above, it is clear that it is important to know whether metal concentrations in the pore water are determined by precipitation-dissolution reactions or by adsorption processes. With precipitation-dissolution processes the concentrations will not depend on the total metal content in the sediment and an increased input in the system will not affect the concentrations in the pore waters. When adsorption is the main process which binds the metals to the sediments, the increased input can cause an increase in metal concentrations in the pore waters, whereas a decrease in adsorption capacity will also cause an increase in metal levels in the pore waters (Salomons et al., 1987).

2.8.4.4 Induced metal fluxes between the oxic and anoxic layer and the oxic-anoxic interface

Calculations using concentration gradients of heavy metals indicate that diffusive fluxes between the sediment and the surface water contribute to concentrations in the surface water (van den Berg et al., 1998b). The differences in composition between the oxic and anoxic sediment layers causes the transport of several components. When the oxic-anoxic interface is situated in the sediment, the composition of the oxic sediment layer will also be changed due to the flux from the anoxic layer. Important in this respect is the enrichment of iron, manganese and phosphorus in the oxic surface layer. This effect and the differences in kinetic parameters between oxidation and reduction reactions determines the transport of components from the anoxic layer to the surface waters (Salomons et al., 1987). As a result of the generally low concentrations of metals in the pore waters of the anoxic layer, fluxes to the oxic layers will be low. However, physical processes such as consolidation and bioturbation can enhance the upward flux. Simpson et al. (2002) studied the effect of tides, bioturbating organisms, and periods of anoxia on metal fluxes from contaminated harbor sediments in a shallow tidal estuarine bay, together with capping technology options for the containment of metal contaminants. More frequent short periods of air exposure followed by renewal of overlying water resulted in a large increase in the zinc flux from the sediments. Moreover,

fluxes were found to be greater in the presence of sediment-dwelling organisms. If the organisms were removed, and re-colonizing organisms later added, their bioturbation activities initially lowered zinc fluxes, but fluxes gradually reached steady state at the higher levels seen previously. Capping materials were found to physically isolate contaminated sediments, provide a binding substrate for metals released from the sediment, and create an anoxic environment below the cap, which stimulates the formation of insoluble metal sulphides. A 5 mm layer of clean sediment was the most effective capping material in reducing zinc fluxes. A 10 mm layer of a zeolite/sand mixture also greatly reduced these fluxes, but significant breakthrough of zinc occurred after 2 weeks, whereas a 20 mm layer of sand was not effective. The presence of organisms disturbed capping materials and increased zinc fluxes again. Finally, Simpson et al. (2002) concluded that capping materials should have a depth of more than 30 cm to minimize organisms burrowing to contaminated sediments beneath.

2.8.5 Carbonates and pH in calcareous sediments

A drop in pH prevents the transfer of most trace metals to the sediment and/or causes a desorption from the sediments (e.g. Salomons et al., 1987; Gambrell et al., 1991; Calmano et al., 1993). At low pH, the negative surface charge of organic matter, clay particles and iron and alumina oxides is reduced, and various compounds such as carbonates and sulphides become more soluble. The presence of carbonates in calcareous sediments constitutes an effective buffer against a decrease in pH. Moreover, carbonates may also directly precipitate metals (e.g. Gambrell, 1994; Guo et al., 1997; Charlatchka & Cambier, 2000). These carbonates can be geochemically or biogenically formed and deposited as part of the sediments in tidal flats, floodplains or marshes. However, decalcification can also occur and complete decalcification may result in acidification of the pore water. When the pore-water reaches a certain pH, heavy metals may rapidly become mobilized. Because decalcification and subsequent mobilization of contaminants are fairly slow in calcareous soils, decalcification of hydric soils is regarded as a chemical time bomb (van den Berg & Loch, 2000).

Two main causes for decalcification of the top layer of hydric soils are oxidation of iron sulphides under aerobic conditions and increased CO_2 pressure in the soil during

waterlogging. Carbon dioxide is produced in the soil when organic matter is decomposed aerobically, by methanogenesis and by respiration from roots. Saturated conditions inhibit the escape of CO_2 to the atmosphere. Consequently, decalcification in aerated soils (or soil layers) is much slower than that in saturated soils (or soil layers). Increased CO_2 pressure in the soil results in calcium carbonate dissolution according to:

$$CaCO_3 + CO_2 + H_2O \rightarrow Ca^{2+} + 2\ HCO_3^- \quad \text{(Equation 2.10)}.$$
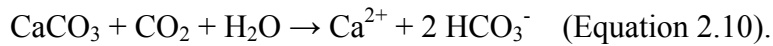
Vegetation may stimulate decalcification of the soil, especially in the rhizosphere. Both living roots and micro-organisms are sources of CO_2, and dead roots are a source of easily decomposable organic matter. Oxidation of sulphides may be an important decalcification process in soils subject to periodic oxidation and reduction. The oxidation of FeS during aeration of the soil can be schematized in two reaction steps:

$$FeS + 2\ O_2 \rightarrow Fe^{2+} + SO_4^{2-} \qquad\qquad \text{(Equation 2.11)}$$
$$4\ Fe^{2+} + O_2 + 10\ H_2O \rightarrow 4\ Fe(OH)_3 + 8\ H^+ \qquad \text{(Equation 2.12)}.$$
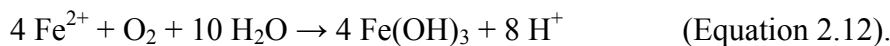
For each molecule of FeS oxidized, two protons are produced, whereas subsequent precipitation of Fe(III) hydroxides leads to an even higher production of protons. In calcareous soils the decrease in pH is buffered by dissolution of calcium carbonate:

$$CaCO_3 + H^+ \leftrightarrow Ca^{2+} + HCO_3^- \qquad\qquad \text{(Equation 2.13)}.$$

Evidently, the intensity of decalcification due to sulphide oxidation varies with the amount of sulphide formed in the sediment, and thus on the duration of waterlogging. Depending on the site-specific hydrological conditions, approximately 0.1-0.3 % of calcium carbonate may be dissolved per year by a combination of the two processes mentioned above (van den Berg & Loch, 2000).

2.8.6 Plant growth

Wetland plants can oxidize the sediments in the root zone through movement of oxygen downwards through aerenchyma tissue (Moorhead & Reddy, 1988). This oxidation can

remobilize the metal contaminants, thus increasing the otherwise low availability of metals in wetland sediments (Weis & Weis, 2004). Some species of mangroves were found to oxidize the rhizosphere, reducing sulphide contents and enhancing metal concentrations in the exchangeable form (De Lacerda et al., 1993). Wright & Otte (1999) found that *Typha latifolia* oxidised the rhizosphere, but this did not increase pore water metal concentrations. It caused a pH decreased within 1 cm of the roots and it increased the concentration of soluble zinc near and beneath the roots, while another plant, *Glyceria fluitans*, had only little effect on sediment chemistry. A striking feature of roots of some wetland plants is the presence of metal-rich rhizoconcretions or plaque on the roots (Vale et al., 1990). These structures are composed mostly of iron and manganese hydroxides and other co-precipitated metals, and are often referred to as "iron plaque". The main element, iron, could originate from the microbial reduction of Fe oxides in the zone around the roots, the oxidation of Fe sulphides or from $FeCO_3$, which is unstable under oxic conditions (St-Cyr & Crowder, 1988). Together with Fe, also other metals are mobilized from the reduced anoxic sediments and concentrated in the oxidized microenvironment around the roots (Doyle & Otte, 1997). Their concentrations can reach 5-10 times the concentrations seen in the surrounding sediments (Sundby et al., 1998). There have been conflicting reports as to whether the presence of the plaque reduces or increases the uptake of metals by the plants (Weis & Weis, 2004). The presence of these concretions appeared to reduce the amount of zinc taken up by *Aster tripolium* (Otte et al., 1989) and the amount of manganese taken up by *Phragmites australis* (Batty et al., 2000). The plaque might have acted as a physical barrier, although the barrier was not effective at low pH conditions. At higher pH conditions the presence of plaque enhanced Cu uptake into roots. In *Typha latifolia* (cattail), the presence of iron plaque did not reduce the uptake of toxic metals (Ye et al., 1998a). Iron plaque moreover increased zinc uptake by rice (*Oryza sativa*) and movement into shoots (Zhang et al., 1998).

Metal remobilisation may also result from the excretion of plant exudates (Xu & Jaffe, 2006). Another factor that can affect the accumulation of metals in wetland plants is the presence of microbial symbionts, such as rhizosphere bacteria. Mycorrhizae, symbiotic fungi associated with roots, can provide an interface between the roots and the soil, increasing the absorptive surface area of root hair. They might be effective at assimilating metals that are present at toxic concentrations in the soil, thus restricting metal uptake by the plants. However, they were also reported to increase metal uptake by wetland plants (Weis & Weis, 2004). Moreover, Decho (2000) reported a significant role of extracellular microbial polymeric

secretions (microbial biofilms) in intertidal systems, both in the binding and concentration of metal contaminants, as in the trophic transfer of metals to the food web. According to Kunito et al. (2001), these extracellular microbial polymeric secretions were especially produced in large quantities by Cu-resistant bacteria in the rhizosphere of reed plants, which suggest its involvement in the reduction of the bioavailability of Cu.

2.9 Physicochemical assessment of metal fate in sediments and soils of floodplains and tidal marshes

2.9.1 Extraction of labile metal fractions

Environmental legislation is still based on the measurement of total metal amounts rather than on actual exposure concentrations in many countries. Considering the extreme variation in soil physicochemical properties, total concentrations however do not represent biological availabilities and toxicities of metals in different soils (Tack & Verloo, 1995; Boyd & Williams, 2003). To assess more readily available metal fractions under field conditions, procedures to extract more labile metal fractions using "soft" extractants (e.g. 0.01 M $CaCl_2$) have therefore become an important aspect of many environmental monitoring programs. A wide range of procedures have been developed, all of which have their own advantages and disadvantages (Rauret, 1998; Meers, 2005). Their use primarily depends upon the questions which need to be answered. When using most of these extraction procedures, redox conditions of the samples can not be preserved due to the specific sample pre-treatment which is needed: sediment sampling, drying, grinding and/or homogenization. Moreover, small-scale spatial and temporal variations cannot be adequately studied under natural conditions because the methods are destructive. As a result, several important features characterising metal accumulation and affecting metal fate in floodplain soils can be lost when subjecting floodplain soils or sediments to most of these procedures.

There is a general agreement that uptake by organisms is related to particular metal fractions in the pore water rather than the total concentration in the soils or sediments (Sauvé et al., 1996). Most recent studies examining relationships between availability of heavy metals in soils, toxicological impacts on soil fauna and flora, and the potential transfer of heavy metals

through the food chain have therefore focused on extracting the soil solution (Tye et al., 2003). Chapman *et al.* (2002) concluded that pore water testing and analyses can be effective tools provided their limitations are well understood by researchers and managers. Carr and Nipper also concluded from a SETAC (Society of Environmental Toxicology and Chemistry) Technical Workshop on sediment pore water toxicity testing: "Determination of chemical concentrations in pore waters is recommended, in addition to the regular contaminant measurements conducted in the whole sediment as a means of providing information on routes and levels of exposure, aiding in the interpretation of test results, and identifying sources of toxicity. It is however nearly impossible to avoid artifacts and chemical changes when removing pore water from sediment. Sampling, extraction, and storage techniques are critically important for achieving the most field-representative samples of pore water." (Carr & Nipper, 2001 in: Förstner, 2004).

Bufflap & Allen (1995a) distinguished four means of extracting sediment pore water. Two of the methods, squeezing and centrifugation are *ex situ* and require the removal of soil or sediment from the natural system. The other two, vacuum filtration and dialysis, have the advantage that they can be performed *in situ*, thus avoiding redox changes during handling. Knight et al. (1998) also listed some additional disadvantages of centrifugation: it is time consuming and the collected sample often requires further filtration or centrifugation. In addition to each method having its own advantages and disadvantages, several general sources of error can alter pore water chemical concentrations: oxidation, sediment sampling, metal contamination, temperature artefacts and filtration. If samples are not filtered during or after extraction, the residual particles can cause errors in two ways: interferences during the analytical procedure and sorption of metals to the particles altering the metal concentrations in the pore water (Bufflap & Allen, 1995b). Also, sample acidification for temporary storage would result in solubilization of metals which were previously adsorbed to colloidal particles. However, if filters are used, these filters can also be a source of error as they can adsorb or release metals. For example, ceramic suction cups for vacuum filtration are known to suffer from adsorption of charged groups, particularly trace metals, phosphorus and ammonium groups (Wenzel & Wieshammer, 1995; Grossman & Udluft, 1991). Such effects may be particularly important when filtering weak extractants with low overall ionic strength, compared to stronger salt solutions for which the filters are usually designed.

If soils and sediments are too dry and pore water cannot be collected, water can be used as an extractant to estimate the mobile and potentially bioavailable metal fraction. In that case, it is again very difficult to study small-scale spatial and temporal variability as the method is destructive and there is a significant risk for changing the redox conditions as sediments have to be removed from their natural environment. Moreover, different liquid to solid ratios (L:S) and equilibration times can be applied, which can make comparison of data between different studies very difficult (Tack et al., 2002).

Rhizon soil moisture samplers (type MOM, Rhizosphere Research Products, Wageningen, The Netherlands) have therefore recently been designed. These samplers aim at smoothly extracting soil solutions during field experiments under the natural moisture conditions, meanwhile filtrating the sample without losses due to adsorption to the filter material. They may allow simultaneous and sequential sampling of soil pore water at different depths of the soil profile in situ in a non-destructive way (Luo et al., 2003). They consist of a filter tube with a diameter of 2.5 mm and a length of 10 cm, which is connected to a PVC/PE tube. The filter tube consists of a polyethersulfone filter membrane with a pore diameter of <0.2 μm. To extract pore water, a vacuum can be built up inside the sampler by connecting a 10 mL syringe or a vacuum tube to the PVC/PE tube. Because of their small size, repeated sampling at high spatial resolutions in soil profiles should be possible. Knight et al. (1998) also stated that these samplers would have no cation exchange capacity. Dessication can however lead to reduced sampling volumes (Schröder, 2005).

Schröder (2005) tried to find out if pore water sampling by using Rhizon soil moisture samplers is a good starting point for assessing risks related to metal pollution in floodplain soils. He compared results of Rhizon extraction with $CaCl_2$ extraction and concluded that metal concentrations in pore water of the oxic, upper sediment layer and $CaCl_2$ extractions showed a surprisingly good agreement. Systematic differences between both could be explained by differences in pH and DOC concentrations, especially due to the larger pore size of the filters used in $CaCl_2$ extraction procedure (0.45 μm compared to <0.2 μm). Both methods are found to have their advantages and a choice for one of the methods depends on the question that should be assessed.

2.9.2 Sequential extraction procedures

Next to pore water extraction among other single extraction procedures, the optimised three step sequential extraction procedure developed by the Measurement and Testing Programme of the European community (Rauret et al., 1999; Sahuquillo et al., 1999) is worth mentioning as an extraction procedure which can be used when assessing metal fate in floodplains on a longer term. It is an operationally defined fractionation method that aims to extract the acid extractable metals in a first step, which includes metals that are associated with carbonates. In a second step, metals of the reducible fraction are released, which are mainly associated with Fe and Mn oxides. In the last step, the oxidisable fraction is extracted, which primarily includes metals that are associated with organic matter or precipitated as sulphides. In that way, each step simulates a possible change in environmental conditions. By conducting this procedure, one should be able to assess which part of the total metal pool will be released on a longer term upon the creation of e.g. oxidising or reducing conditions in soils. This procedure however also has some major reported disadvantages: it is very time-consuming, the used extraction reagents are not completely selective, the experimental conditions cannot be extrapolated to natural conditions and metal redistribution can occur during the sample preparation and analysis (e.g. Nirel & Morel, 1990; Hirner, 1992; Whalley & Grant, 1994; Tack & Verloo, 1996; Luo & Christie, 1998; Davidson et al., 1999; Gomez-Ariza et al., 1999; Sahuquillo et al., 1999). The fact that the procedure is operationally defined should thus be taken into account very carefully when interpreting the results of such sequential extraction procedures. Conclusions derived from the application of the procedure should probably be restricted to the longer term assessment of changes in metal fractionation and mobility, instead of the prediction of short term changes upon changing environmental conditions.

2.9.3 Acid-volatile sulphides (AVS) and simultaneously extracted metals (SEM)

According to e.g. Kornicker & Morse (1991), Di Toro et al. (1992), Morse & Arakaki (1993), Huerta-Diaz et al. (1998) and Billon et al. (2001), iron monosulphide (FeS) is often considered the most reactive of the sulphide phases in sediments although pyretic sulphide phases (FeS_2) can also be abundant and reactive toward trace metals. The formation of pyrite is however not favoured in freshwater systems (van den Bergh & Loch, 2000). Trace metals entering anoxic sediment environments can thus be expected to react to form sulphide phases

according to: $M^{2+} + FeS_{(s)} \leftrightarrow MS_{(s)} + Fe^{2+}$, where M = e.g. Cd, Cu, Ni, Pb, Zn, Co, Hg (Simpson et al., 2000). Because the solubility of these metal sulphides is very low, sediments with an excess of reactive sulphide to trace metals will exhibit very low dissolved metal concentrations in pore waters. The reactive sulphide fraction may be estimated by measuring the acid-volatile sulphide (AVS) content of the sediment (e.g. 30-min extraction with 1 M HCl), whereas the simultaneously extracted metal (SEM = $\sum$ Cd, Cu, Ni, Pb, Zn) content approximates the reactive trace metal fraction (Di Toro et al., 1992; Allen et al., 1993). The less reactive, intermediary oxidized sulphur compounds, such as pyrite (FeS_2) and elemental S, are however also partially recovered during AVS determination. These could be determined by preliminary reduction using Cr(II) and are referred to as Chromium Reducible Sulphur or CRS (Tack et al., 1997a; Billon et al., 2001).

Once the reactive sulphide phase, which is determined as AVS (predominantly FeS), has been exhausted in the absence of other binding phases, the metals are expected to appear in the pore waters in order of decreasing metal sulphide solubility, that is Ni, Zn, Pb, Cd and then Cu (Di Toro et al., 1992). Berry et al. (1996) provided evidence for this relationship for each of the metal ions Cd, Cu, Ni, Pb and Zn (Simpson et al., 2000). Simultaneously extracted metals might however also include metals which are associated with other binding phases, e.g. some metals associated with clay, carbonate or organic matter fractions. If simultaneously extracted metal amounts are therefore found to be smaller than acid-volatile sulphide amounts, one can state that most heavy metals are expected to be immobile and not available. Vice versa, if simultaneously extracted metal amounts exceed the acid-volatile sulphide amounts, mobile and easily available heavy metals might, but not necessarily, be abundant. Some authors, such as Di Toro et al. (1992) and van den Hoop et al. (1997), state that no lethal toxic effects on organisms should be expected if the SEM/AVS ratio is smaller than one. Di Toro et al. (1990) initially showed that lethal toxic effects of cadmium spiked marine sediment on amphipods can successfully be predicted by considering the Cd/AVS ratio, whereas estimation of the toxicity based on the total metal content of the sediment failed. Lethal toxic effects were found to be absent for Cd/AVS ratios smaller than one, but appeared to be present at Cd/AVS ratios exceeding one. Comparable results were afterwards also observed for freshwater sediments (e.g. Ankley et al., 1991), sediments contaminated with Cu, Pb, Ni and Zn (e.g. Di Toro et al., 1992; Ankley et al., 1993; Casas & Crecelius, 1994), and also for other organisms (Carlson et al., 1991) (van den Hoop et al., 1997).

AVS concentrations in Dutch freshwater and marine sediments were found to range from non-detectable (< 0.1 µmol g^{-1}) to approximately 50 µmol g^{-1} dry sediment. AVS is mainly present in excess of SEM, which means that the heavy metals could all be bound by sulphides (van den Hoop et al., 1997).

Sequential extractions have some major disadvantages, some of which were mentioned above. Peltier et al. (2005) therefore advised to use sequential extraction procedures only on anoxic sediments in conjunction with (1) parallel extractions (e.g. AVS/SEM determinations) or (2) more accurate, direct speciation methods (e.g. X-ray absorption spectroscopy) to assess the 'true' sulphide speciation of metals. Vanthuyne & Maes (2006) therefore recently started to develop a new, very promising method to determine the likelihood of trace metal sulphide associations in anoxic sediments. The method combines (1) one day oxidation kinetics modeling in the presence of excess EDTA (pH 8) and (2) correction for the 1 day anoxic EDTA-soluble fraction. In contrast to the AVS/SEM method, it can estimate more accurately the sulphide-associated fraction of trace metals (e.g. Cu) and it also includes a correction for the carbonate and/or hydroxide-associated metals. Despite the fact that X-ray absorption spectroscopy analysis is more powerful for heavily polluted sediments, major benefits of the developed method are its easy-availability, cheapness and rapidity. In addition, it is also a suitable tool to verify whether the same sulphide speciation of trace metals is obtained in anoxic sediments by commonly used sequential extraction procedures, which generally lack selectivity. The oxidation kinetics modeling approach is also interesting in the sense that it allows to distribute the trace metals over 4 fractions, namely C0, C1, C2 and a non-oxidisable fraction, which correspond respectively to (1) the fraction immediately dissolved in the background EDTA solution under anoxic conditions (C0), (2) the fraction associated with 'quickly-oxidisable' FeS phases (C1), (3) the fraction associated with discrete, more 'slowly-oxidisable' trace metal sulphides (C2) and (4) the fraction associated with sedimentary phases on which oxidation has no effect. Furthermore, the obtained oxidation kinetics modeling data contain important information on the trace metal reactivity under changing environmental conditions (e.g. oxidation rate of metal sulphide phases) (Vanthuyne & Maes, 2006).

2.9.4 Modeling

Geochemical modeling is being considered as the most convenient way to describe the complex interactions and equilibriums, which were described in paragraph 2.8. However, most geochemical models which predict metal fate quite accurately are only applicable to study metal partitioning in surface waters and under steady state conditions in non-saturated, oxic soil layers. For example, Van Gils et al. (1993), Betty et al. (1996), Turner (1996), De Smedt et al. (1998) and Ouboter et al. (1998) modeled metal partitioning and transport in estuarine surface waters.

Schröder (2005) was however - as far as we know - the first researcher who aimed to develop geochemical models which can predict solid-solution partitioning of heavy metals and As in embanked floodplain soils, which are subject to rapidly alternating environmental conditions. He stated that careful modeling of speciation and adsorption processes can be a useful tool for the investigation and understanding of metal availability in river floodplain soils. However, the prediction capabilities of his geochemical models still seemed to be limited regardless extensive modeling efforts and data collection to validate the models. With a fully parameterized model, he was able to predict Zn pore water concentrations of the oxic, upper layer of an artificially contaminated sandy floodplain soil within a 0.5 log-unit for 90% of all samples. Prediction capabilities were however quite poor in samples having a pH below 7. Only Cd and Zn contents in the soil solution were found to be sufficiently described by geochemical models up to a depth of about 40-50 cm below the sediments surface: observed values deviated less than a factor 10 in the largest part of this part of the soil profile. Observed Cu, As, Fe and Mn contents however seemed to be at least one log unit higher or lower than the predicted contents in a significant part of the soil profile. For Cu, acceptable results were limited to the oxic upper 20 cm sediment layer. For Pb, a yet unknown mineral, labeled as PbX, should first be added to the geochemical model to allow for acceptable prediction levels. It seemed impossible to accurately model metal fate under more reduced conditions, whereas models aiming to predict metal fate in the permanently oxic upper soil layer were found to be somewhat more successful. The results pointed out that metals strongly bound to organic matter could be more accurately predicted than metals mainly present as a mineral. Moreover, yet unavailable, detailed information regarding the sorption capacity and presence of colloidal Fe and Mn in pore water was reported to be essential to improve the prediction of solid-solution partitioning of a number of metals. The role of the non-equilibrium processes is also

not negligible in floodplain soil systems. A lack of data on kinetics of reactions affecting metal fate in floodplain soils, e.g. sulphide formation and Fe/Mn oxide reduction, therefore currently also seems to limit actual prediction capabilities of the geochemical models, especially in the area just above and below the groundwater table. Geochemical models therefore now only have a heuristic value in our opinion. They can be used for sensitivity analysis, exploring gaps in understanding, and they can serve as a guide for designing further experimental studies or sampling campaigns, but not to accurately predict metal fate in the upper layer of intertidal sediments, which are being alternately flooded. Aiming to contribute to the development of management-oriented models with higher prediction capabilities, we will further focus on the kinetics of reactions affecting metal fate in the upper layer of floodplain sediments in some of the following chapters.

3.

Factors affecting metal contents of intertidal Scheldt sediments

3 Factors affecting metal contents of intertidal Scheldt sediments

3.1 Introduction

Due to the industrial and urban development, pressure on the Scheldt estuary is high. As a result, the surface area of the intertidal marshes has decreased and a significant amount of the existing intertidal flats have been contaminated with heavy metals. The temporarily flooded sediments and their associated plant litter are expected to be major sinks for them (Du Laing et al., 2006). Metal accumulation in the sediments is determined by input coming from discharge of industrial and urban sewage or by atmospheric deposition in the wetland ecosystems, but also by the extent by which the substrate is capable to bind and release metals. This is governed by soil and sediment characteristics such as pH, cation exchange capacity, organic matter contents, redox conditions and chloride contents. These properties determine the type and stability of metal sorption or precipitation, which are also related to the metal mobility, bioavailability and potential toxicity (Schierup and Larsen, 1981a and Du Laing et al., 2002). Revealing the most important factors determining the metal contamination levels of intertidal flats can therefore contribute to a more sustainable development of wetland ecosystems and can help to evaluate whether and under which conditions wetland creation is a safe option. This chapter aimed to do so for the tidally influenced reed beds in the Scheldt estuary. Total metal contents of the upper 20 cm sediment layer were determined in 26 intertidal reed beds along the river Scheldt and the sediments were characterized for total metal contents and a range of physico-chemical properties (clay, loam and sand content, organic matter content, pH, carbonate content, chloride content, conductivity, acid volatile sulphide content). Factors affecting the metal contents in this upper sediment layer were identified. Only surface sediment samples were sampled as we aimed to focus on risks for metal accumulation in newly created wetlands, studying only the most recent depositions. Moreover, these sediments mainly reflect the recent anthropogenic influences, particularly industrial activity, and the interaction between the soil and biosphere is most obvious in this upper sediment layer (Swennen et al., 1998). We also aimed to assess the short-term temporal

and spatial variations of metal contents in the upper 100 cm intertidal sediment profile and to identify the factors affecting metal accumulation in these deeper sediment layers.

3.2 Factors affecting metal contents in the upper sediment layer

3.2.1 Materials and methods

The upper 20 cm sediment layer was sampled at 26 intertidal reed beds along the river Scheldt. The sediments were characterized for total metal contents and a range of physico-chemical properties (clay, loam, sand, organic matter, carbonate, chloride and acid volatile sulphide content, pH and conductivity). Factors affecting the metal contents were identified.

3.2.1.1   Study sites

The study was carried out in the part of the Scheldt estuary downstream the city of Ghent which is subjected to tidal influences (Fig. 3.1). All study sites are tidal marshes vegetated by a monospecific stand of common reed, *Phragmites australis* (Cav.) Trin. ex Steud.
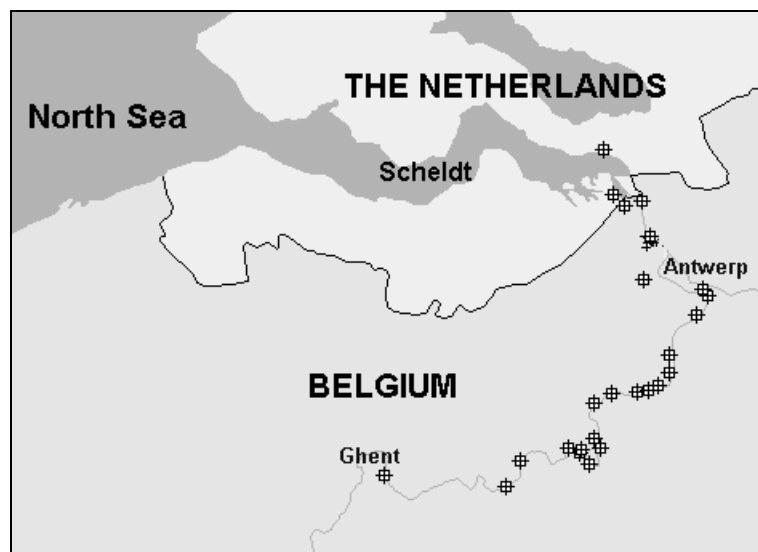
Fig. 3.1. Situation of the sampling sites along the tidal river Scheldt

3.2.1.2   Sampling procedure

In August 2002, on each location three samples of approximately 1 kg were taken from the upper 20 cm of the intertidal sediments. The samples were taken at low tide randomly a few meters from each other. They were transported to the lab in plastic bags, air-dried during three weeks and then ground in a hammer-cross beater mill (Gladiator BO 3567).

3.2.1.3   Analyses

All used glassware and containers were washed in a laboratory dishwasher, subsequently soaked overnight in 5 % ultrapure 65 % HNO_3 and rinsed with deionised water. The pH was measured in a 1/5 sediment/distilled water suspension after equilibration during 18 h (Van Ranst et al., 1999). The conductivity was measured in the filtrate of a 1/5 sediment/distilled water suspension, shaken for ½ h (Van Ranst et al., 1999). The organic matter content was determined by measuring the weight loss after incineration of oven-dried samples (2 h at 450°C). The carbonate content was determined by back-titration with 0.5 M NaOH of an excess 0.25 M H_2SO_4 added to 1 g of sediment (Nelson, 1982). Texture analyses were conducted on samples obtained by mixing equal amounts (30 g) of the three samples at each location. They were performed at the Research Institute for Nature and Forest (INBO) using laser diffraction (Coulter LS200, Miami, FL) after removing organic matter and carbonates by adding hydrogen peroxide and an acetate buffer solution respectively. To determine the chloride content 10 g of sediment was suspended in 50 ml of 0.15 M HNO_3 and shaken for 30 minutes. The filtrate was titrated with 0.05 M $AgNO_3$ using potentiometric end-point detection (Van Ranst et al., 1999). Acid volatile sulphide (AVS) determination involved acidification of fresh sediment samples and trapping the evolved H_2S in a Zn-acetate solution. Trapped sulphide was determined by iodometric titration (Tack et al., 1997a). The cation exchange capacity was measured by percolation of 150 ml 1 M NH_4OAc through a percolation tube filled with a mixture of 5 g sediment and 35 g quartz sand, followed by washing through the excess with 300 mL denatured ethanol. The exchangeable ammonium ions were then eluted with 500 mL 1 M KCl and analysed in the percolate by means of a steam distillation (Tecator Kjeltec System 1002 Distilling Unit). The exchangeable cations were also analysed in this percolate using flame atomic absorption spectrometry (Varian AA-1475, Varian, Palo Alto, CA) (Van Ranst et al., 1999). Pseudo-total metal contents were determined by aqua regia extraction (Ure, 1990). The metal contents in the extracts were

analysed using ICP-OES (Varian Vista MPX, Varian, Palo Alto, CA). CRM 277 (estuarine sediment) was also analysed using the same method. Results varied from 92.5 to 101.7 % of the certified values. Statistical analyses involved the calculation of Pearson correlation coefficients between the average analyses results obtained for each parameter and sampling site, using SPSS 12.0 (2003).

3.2.2 Results and discussion

3.2.2.1   Sediment properties

Physico-chemical sediment properties are summarized in Table 3.1. Chloride concentrations and conductivity both generally decreased with increasing distance from the river mouth (Fig. 3.2). Moreover, these parameters were strongly positively correlated ($r = 0.983$, $p < 0.001$). The influence of the salty seawater can be clearly distinguished at sampling locations up to about 75 km from the river mouth. Up to 75 km from the river mouth, the river water still has a significant chlorinity (above 0.5 g L^{-1}) at both low and high river discharge rates. Further from the mouth, the surface water is not affected anymore by salty seawater during high river discharges (Baeyens et al., 1998).

Table 3.1. Summary statistics of metal concentrations and sediment properties in the upper sediment layer of 26 intertidal marshes along the river Scheldt (n = 78)

	Mean	Median	S.D.	Min	10th perc.	90th perc.	Max
Cd (mg kg^{-1} DM)	7.63	7.49	4.46	0.46	1.68	12.95	21.85
Cr (mg kg^{-1} DM)	134	147	56	26	45	195	244
Cu (mg kg^{-1} DM)	97	96	72	4	19	166	513
Ni (mg kg^{-1} DM)	36.5	38.4	15.0	7.6	13.1	58.0	70.7
Pb (mg kg^{-1} DM)	143	146	76	13	37	218	459
Zn (mg kg^{-1} DM)	595	650	298	37	132	868	1501
% Clay	31.8	35.1	12.9	4.7	13.1	46.5	47.8
% Silt	42.8	51.0	16.9	0.3	17.9	59.1	65.5
% Sand	25.4	9.4	29.0	0.3	1.1	70.5	95.0
% OM	11.6	12.2	5.8	1.1	2.9	19.9	23.3
% CaCO$_3$	7.6	7.1	2.3	2.5	4.9	10.5	13.4
Cl (mg kg^{-1} DM)	393	21	800	<2	<2	1754	3993
pH	7.61	7.52	0.33	7.16	7.28	8.08	8.68
EC (μS cm^{-1})	730	530	718	130	250	1790	3720

The pH of the sediments varied between 7.2 and 8.7. The high carbonate contents indicate a significant buffering capacity of these sediments against acidification. In the pH range of the sampled sediments, metal solubility is expected to be minimal. In addition to the pH effect, metals may precipitate as carbonates which additionally explains a decreased metal availability (e.g. Guo et al., 1997). Carbonates can be geochemically or biogenically formed and deposited as part of the sediments in tidal flats, floodplains or marshes. Yet, the buffering capacity provided by carbonates in certain conditions may prove to be limited in the longer term. Van den Bergh and Loch (2000) reported intense decalcification at a tidally influenced freshwater wetland area in The Netherlands. This was attributed to increased CO_2 pressure during waterlogging and sulphide oxidation under aerobic conditions. They calculated that approximately 0.1-0.3 % calcium carbonate may be dissolved per year, depending on site-specific hydrological conditions. They estimated that in a worst case scenario at the sites with the lowest carbonate contents, only 8 years would be needed to deplete all carbonates under changing hydrological conditions. The subsequent pH decrease could cause metal release.
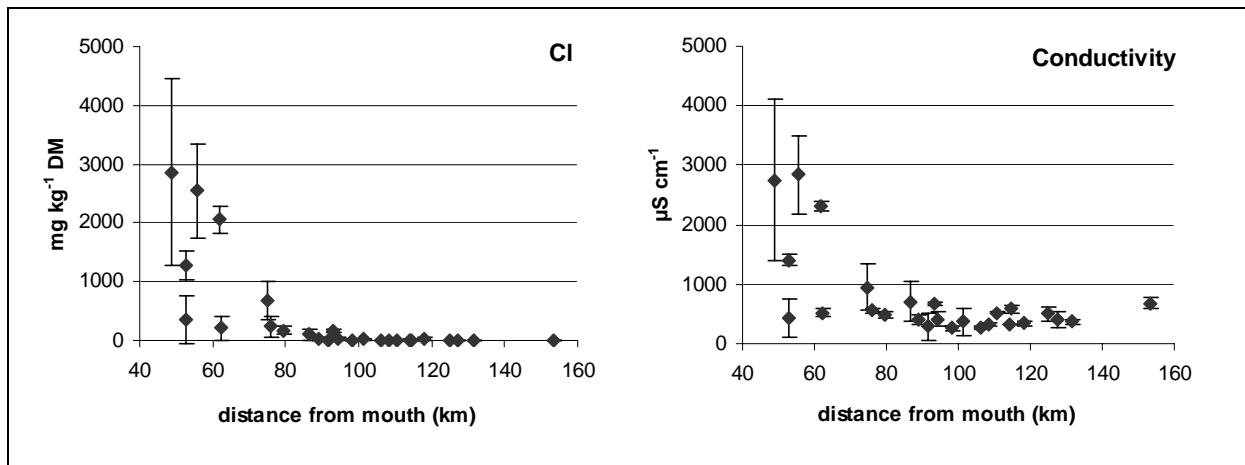
Fig. 3.2. Chloride concentrations and conductivity in the upper sediment layer of 26 intertidal marshes along the river Scheldt as a function of the distance from the river mouth, error bars represent standard deviation at each sampling site (n= 3)

Generally, clay contents increased and sand contents decreased with increasing distance from river mouth (Fig. 3.3). Vandecasteele et al. (2003) also observed such gradient in the texture of alluvial sediment-derived soils along the Sea Scheldt. However, the spatial variability of suspended sediment settling within each site somewhat masks this trend. Vandecasteele et al. (2003) and Meers et al. (2005) reported a texture gradient when dredged sediments are hydraulically pumped into a disposal site after being brought into suspension. On intertidal marshes, similar texture gradients can be expected. Sandy particles will settle near their point

of introduction, while clayey sediment particles are transported further away from the inlet, where the water flow rate decreases. However, there were also some sites at which high sand contents should be attributed to relatively recent infrastructure works, e.g. the very high sand content at 76 km from the river mouth is due to the presence of an artificially created beach next to the sampling site.
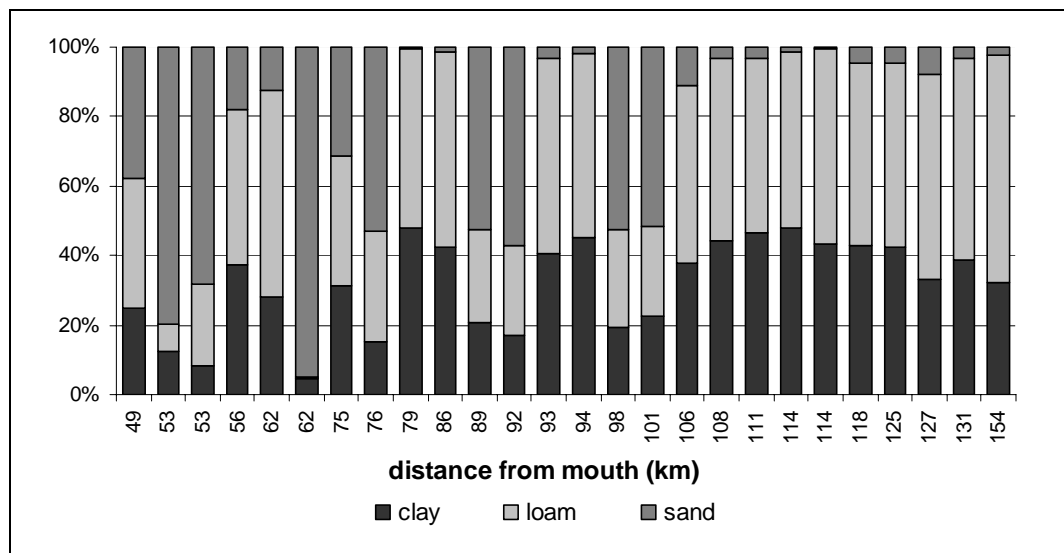
Fig. 3.3. Texture of the upper sediment layer of 26 intertidal marshes along the river Scheldt as a function of the distance from the river mouth

The organic matter concentrations were variable, ranging from 1.6 up to 21.0 % and were significantly correlated with clay concentrations ($r = 0.920$, $p < 0.001$). This could be due to their similar settling characteristics, slower organic matter decomposition by limited oxygen diffusion in clayey sediments and/or adhesion of organic molecules to clayey sediments. Total metal concentrations are depicted in Fig. 3.4 as a function of the distance to the river mouth. Also indicated are site specific background values and remediation levels for upland soils according to the Flemish Environmental legislation (Vlarebo, 1996). The background values and remediation threshold levels are different at each site because they are function of the organic matter and clay contents. The Cd concentrations often exceeded the remediation values, whereas the Ni concentrations leaned towards the background values. The sediments were moderately contaminated by Cr, Cu and Zn. Pb concentrations were quite high at some locations. The contamination did not reveal any clear trend as a function of the distance to the river mouth. The metal concentrations could also be compared with the Flemish reference metal concentrations in river bottom sediments, according to the Flemish Environment Agency (De Cooman & Detemmerman, 2003). These are 0.38 mg kg^{-1} DM for Cd, 17 mg kg^{-1}

DM for Cr, 8 mg kg^{-1} DM for Cu, 11 mg kg^{-1} DM for Ni, 14 mg kg^{-1} DM for Pb and 67 mg kg^{-1} DM for Zn. Remarkably, these reference values are however not related to the physicochemical sediment properties, such as clay or organic matter contents.
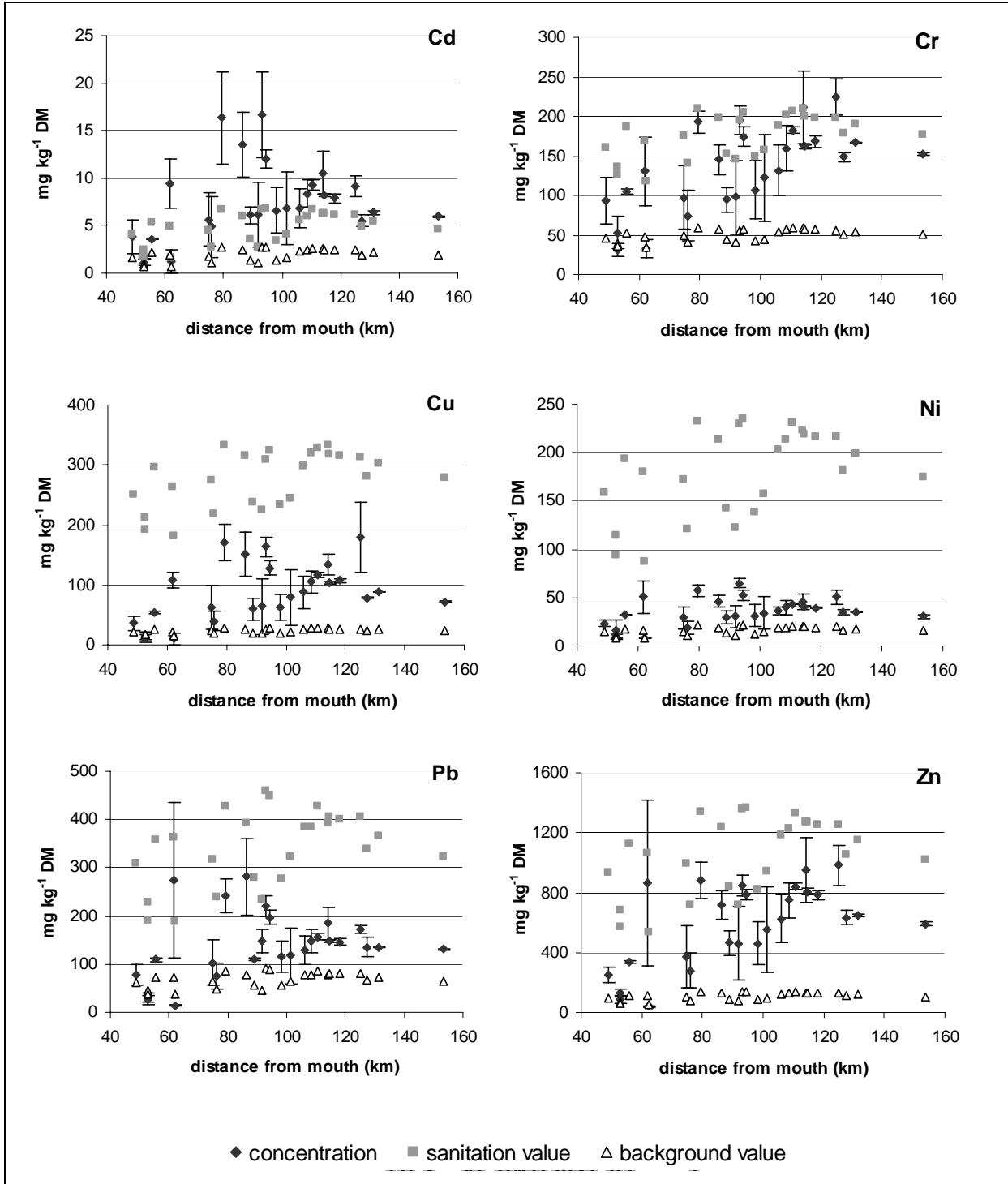
Fig. 3.4. Total metal concentrations (diamond) and site specific sanitation threshold levels (squares) and background values (triangles) as a function of the distance to the river mouth; error bars represent standard deviation at each sampling site (n= 3).

3.2.2.2   Origin and fate of metals in the river Scheldt

Most of the metal contamination in the upper sediment layer of inter-tidal flats is expected to originate from the deposition of polluted sediment particles from the adjacent surface waters. Paucot and Wollast (1997) reported very high Cd, Cr, Cu, Pb and Zn contamination levels in the water column of the Scheldt estuary in the early 90s. Particulate metal concentrations in the freshwater part of the estuary significantly exceeded the background concentrations in river waters, world averages and values for various European estuaries which are well known to be polluted. According to Baeyens (1998), dissolved and particulate metal concentrations assessed in the Scheldt estuary in the period 1978-1995 have yield a clear indication that the levels of all metals decreased significantly. A comparison with other estuaries classifies the Scheldt as being moderately polluted for all metals in the dissolved phase, but as being fairly high polluted in the particulate phase, especially for Cd. The differences between metal concentrations in dissolved and particulate phase in the Scheldt estuary are discussed by Baeyens et al. (1998). They reported anoxic conditions to decrease dissolved metal concentrations as a result of metal sulphide precipitation, especially during the summer months in the upper Scheldt estuary, whereas desorption and oxidation processes may increase the dissolved metal concentrations in the lower estuary. Increased oxygen concentrations during the winter months in the upper estuary may lead to sulphide oxidation. This will lead to the solubilization of metals, which subsequently are subject to coprecipitation and adsorption on iron hydroxides or other components. De Smedt et al. (1998) stated that only a small part of the metals ultimately reaches the North sea, due to the deposition of the particles. As a result, Van Eck (1999) reported significant metal pollution levels in bottom sediments of the Western Scheldt, the Dutch part of the Scheldt estuary. Mean Cd levels were found to exceed natural background levels by a factor of about 40. For Cu, Pb and Zn, this enrichment ratio was 5, whereas average Cr and Ni contents did not exceed background levels. Some model calculations indicated that several decades will pass until a significant improvement in bottom sediment quality will be achieved without removing and/or cleaning the sediments, even after a 100 % reduction of the loading of pollutants (Van Maldegem et al., 1993).

Van Eck (1999) attributed the high Cd levels in the sediments to a wide range of sources. Metallurgic and ore processing industries (phosphate and fertilizer production) were characterized as point sources, whereas use of batteries, paints and plastics constitute

diffusive sources. He stated that 95 % of the total Cd amounts in the Western Scheldt would originate from the Sea Scheldt section of the river. The largest input would come from industries that are situated near Rupelmonde and the port of Antwerp. They would contribute for more than 75 % to the total Cd emission in the Sea Scheldt. Affluent rivers might also contribute to the metal input. Near Rupelmonde, the river Rupel e.g. empties into the river Scheldt. The water of the river Rupel can contain pollutants originating from e.g. the cities of Brussels and Mechelen as it is connected to the Brussels-Scheldt canal and the river Zenne via the river Dijle. The Flemish government (MIRA, 2005) reported that wastewater of households now significantly contributes to the surface water pollution for Cd (6.3 %), Cr (21.2 %), Cu (17.2 %), Ni (19.3 %), Pb (15.4 %) and Zn (11.8 %). Up to 37.7 % of the Cd loads in surface waters of the Flemish region can be directly attributed to industrial activities (mainly metallurgy, chemistry, paper and cardboard industries). Industrial activities are less important sources for Cr, Cu, Ni. The use of antifouling paints at shipyards is however reported to contribute to an important extent to the Cu loads. Non-specific sources, such as corrosion of building materials, use of wood preservatives, atmospheric deposition, soil erosion and leaching were also significant sources for most of the metals. For Cu, Pb, Ni and Zn, significant parts of the surface water loads could however not be attributed to certain activities. Some sources have not yet been identified properly, such as corrosion of metals from hulls of old ships or from the anodes (mainly Zn) used to protect hulls from corrosion, metal leaching from antifouling paints, metal fluxes from ground water to surface water and the use of metal-containing pesticides by households. Emmerson et al. (1997) attributed recently increasing Cu and Zn concentrations in waters of the Blackwater Estuary (UK) to the increasing use of Cu as a replacement for Sn in antifouling preparations for boats and the use of Zn sacrificial anodes on pleasure craft.

3.2.2.3   Comparison of metal contents in superficial intertidal sediments

Some metal concentrations in superficial sediments of intertidal areas all over the world have been compiled in Table 3.2. Most authors found a relationship between metal contents and organic matter or clay contents. Lowest organic matter and metal contents were found in mangrove sites. Metal concentrations in the upper intertidal sediment layer of the Scheldt estuary agreed well with those of the other European sampling locations, i.e. the Biesbosch in the Netherlands (Van den Berg et al., 1998) and the Tees estuary in the UK (Jones and Turki, 1997). Some authors also studied metal fate and the history of metal accumulation in

sediments of the Scheldt estuary. Vandecasteele et al. (2002) and Vandecasteele et al. (2003) e.g. focussed on old dredged sediment landfills and alluvial plains along the upper Scheldt river upstream the city of Ghent and the Sea Scheldt between Ghent and Berlare. Up to 82% of the samples originating from the upper Scheldt were found to be polluted by at least one of the metals Cd, Cr, Pb or Zn, whereas Cu and Ni were reported to be of no environmental concern. Concentrations of Cd, Cr and Zn were, in 10 % of the cases, higher than 26, 1900 and 2800 mg kg^{-1} DM, respectively. Concentrations of Pb and Cu were significantly lower for sediments disposed after 1965, but no indication for improvement of sediment quality with time was observed for Cd, Cr and Zn. For the Sea Scheldt, up to 96% of the sediment-derived soils (ca. 120 ha sampled) was found to be polluted by at least one of the metals Cd, Cr, Zn or Pb, whereas Cu and Ni were again reported to be of less environmental concern. Concentrations of Cd, Cr and Zn were, in 10 % of the cases, higher than 14, 1400 and 2200 mg kg^{-1} DM, respectively. Vandecasteele et al. (2003) concluded that relative to the Upper Scheldt, a larger proportion of the sediment-derived soils along the Sea Scheldt are polluted (96 versus 82%), but the overall pollution level is lower. We observed even lower concentrations in the superficial intertidal sediment layer in the Sea Scheldt and part of the Western Scheldt. The 90th percentiles in our study were found to be 13, 195 and 868 mg kg^{-1} for Cd, Cr and Zn, respectively. The daily tidal cycles may enhance metal release from the sediments and deposition of cleaner sediments originating from the North Sea in the tidal part of the Scheldt estuary. Verlaan (2000) indeed already found that the marine fraction accounted for less than 10 % of the total bottom sediment amounts in the upper estuary, whereas this sharply increased from 10 to 75 % between Lillo (Antwerp) and Saeftinghe (Belgian-Dutch border). Moreover, the Upper Scheldt river is not subject to tidal influence, and significant fluxes of industrial wastes, originating mainly from northern France, may facilitate accumulation of metals in the sediments. Callaway et al. (1998) studied heavy metal chronologies between 1900 and 1990 at the St. Annaland marsh in the Eastern Scheldt, near the mouth of the river Scheldt. They sampled the upper 50 cm sediment layer at two sampling points (low and high marsh) and used ^{137}Cs dating to relate 1 cm sediment slices to sediment deposition during certain time periods, taking into account that the sediment accretion rate was 0.68 cm year^{-1} at the low marsh and 0.34 cm year^{-1} at the high marsh. They found that Pb, Cu and Cr deposition was highest in the mid-1960s, with Cu showing a sharp increase between 1985 and 1990. The peak concentrations were approximately five times greater than the concentration from the bottom of the core, indicating a very significant increase in pollutant levels in this area. There were also increases in Cd and Ni concentrations; however

these increases were not as large, and there was no sharp decrease in these metals since the mid-1960s. Zwolsman et al. (1993) found very similar trends in metals from two salt marshes that they sampled in the Western Scheldt. They documented peak concentrations of Cd, Cr, Cu, Pb, and Zn in approximately 1961-1965, although peak concentrations were lower than in the cores which were collected by Callaway et al. (1998) at the St. Annaland marsh. In addition, more recent peaks in Cu concentration were also found in their cores. It was not clear whether the decrease in pollutants at the St. Annaland marsh should be attributed to hydrological changes due to flood protection projects in the south-western area of the Netherlands or to a regional drop in pollutant levels. Zwolsman et al. (1993) attributed concentration decreases in the Western Scheldt to changes in pollutant inputs, not to hydrological changes, because the Western Scheldt has not been hydrologically altered as the Eastern Scheldt.

Table 3.2. Some metal and organic matter/carbon concentrations in superficial sediments of intertidal areas which were reported in literature

Reference	Location	Metal concentration (mg kg^{-1} DM)						Organic matter
		Cd	Cr	Cu	Ni	Pb	Zn	
Spencer (2002)	5 UK estuaries (Humber, Solway, Medway, Forth, Thames)		30-212	7-86	21-55	25-127	59-319	
Shriadah (1999)	Mangroves (United Arab Emirates)	3.1-6.9	8-19	5-29	15-109	13-50	5-22	ND – 2.13 % OC
Ong Che (1999)	Mudflat (Mai Po, Hong Kong)	1.1-1.4	20-75	51-87	44-87	69-220	130-308	11.81 ± 0.66 % OM
Jones and Turki (1997)	Tees Estuary (UK)	2.6-9.8	36-577	25-262	21-49	37-680	65-777	3.96 - 12.41 % OC
Van den Berg et al. (1998)	Biesbosch (Netherlands)							% OC :
	Location 1	9.7		214		529	1140	14.4
	Location 2	1.8		55		215	420	3.2
	Location 3	2.2		59		246	470	3.1
Ranjbar (1998)	Anzali wetland (Iran)	1.2		87.5	52.6	51.8	278.5	
Tam and Wong (1996)	Mangroves (Hong Kong, China)	0.73		16.3	1.7		40	1.8 ± 0.1 % OM
		3.91		44.3	10.9		143	9.2 ± 0.5 % OM

3.2.2.4   Factors affecting metal contents in intertidal Scheldt sediments

To assess the human impact on metal pollution of the intertidal Scheldt sediments, enrichment ratios were plotted as a function of the distance to the river mouth (Fig. 3.5). They were defined as the ratio of total metal concentrations in the sediments to their predicted background values, calculated according to the Flemish environmental legislation (Vlarebo, 1996) based on site specific organic matter and clay contents. Most metal concentrations are elevated at locations between 92 and 95 km from the river mouth. These sites are situated near an affluent which may supply a significant amount of pollutants, originating from e.g. the city of Brussels, and just downstream a former shipyard (at 98 km from the river mouth) where metal-based paints have been used for several decades during the construction of boats. This shipyard was however closed in 1994. Spencer (2002) also reported elevated metal concentrations near shipyards of the Medway Estuary (Kent, UK). In the freshwater zone of the estuary, two other sites are also remarkably contaminated. One is situated next to an industrial area in Hoboken (86 km from the river mouth) at which metallurgic activities have been performed for decades. The other one is located at 79 km from the river mouth, situated also downstream this industrial area, and on the crossing of a major Belgian motorway and the ring road of the city of Antwerp. Lead in particular may accumulate in waters surrounded by a network of roads and bridges (Gallagher et al., 1996; Evans et al., 2003), whereas mining, mineral processing and manufacturing are well known anthropogenic trace metal pollutant sources in industrialised areas. Jones et al. (2003) reported that a zinc refinery was the major source of trace-element contamination in the Derwent estuary in Tasmania. Pollutants were added to the estuary during bulk handling at the refinery wharf, by proximal atmospheric fallout from bulk handling, from stockpiles or stack emissions, and/or from surface water and groundwater discharge. The contaminants were distributed downstream from the refinery by combined fluvial and tidal activity, while the latter also caused an upstream movement.

In the brackish part of the estuary, one site can also be distinguished which is seriously contaminated, especially by Cd (9.4 mg kg^{-1} DM), Pb (274 mg kg^{-1} DM) and Zn (864 mg kg^{-1} DM). It is situated at 62 km from the river mouth in the middle of the industrial areas of Antwerp harbour, with metallurgic activities nearby. Cd and Cr concentrations are also often very close to the remediation thresholds at several other sites although these elevated levels can not be related to specific point-sources.
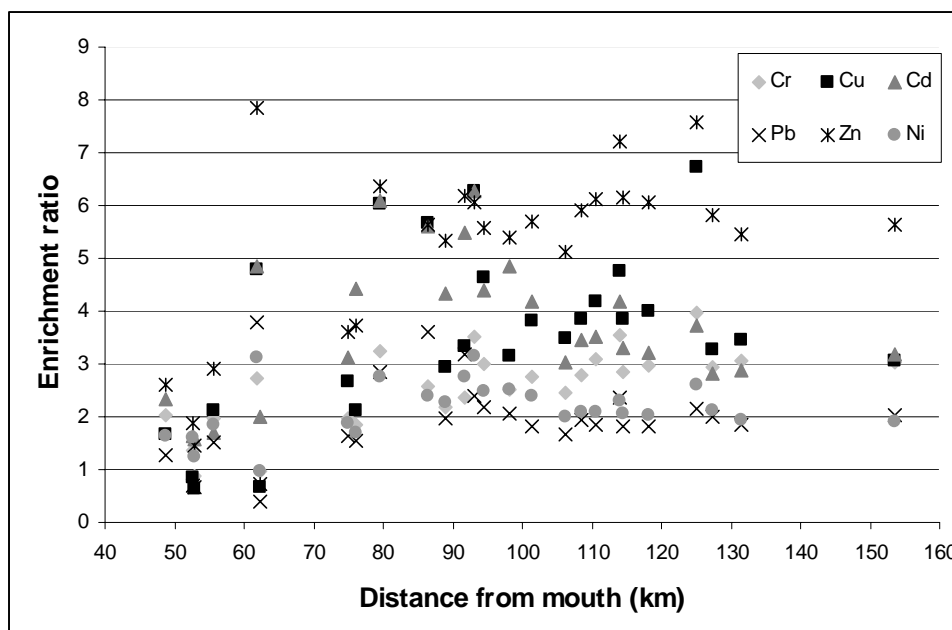
Fig. 3.5. Enrichment ratios for metals in intertidal sediments of the Scheldt plotted as a function of the distance to the river mouth. They are defined as the ratio of the observed total metal concentrations to the predicted background values according to the Flemish environmental legislation (Vlarebo, 1996)

Pearson correlations between metal concentrations and sediment properties are presented in Table 3.3. Chloride concentrations or conductivities were not correlated with metal concentrations. Significant, positive correlations were however observed between metal concentrations and clay, loam or organic matter concentrations. Generally, correlation coefficients decreased in the following order: organic matter – metal content > clay - metal content > loam – metal content. Van Eck (1999) already reported a strong association of metals with clayey particles and organic matter in the Scheldt estuary. Other authors, such as Suzuki et al. (1989), Gallagher et al. (1996), Callaway et al. (1998) and Shriadah (1999), also found significant relationships between a number of heavy metals and organic carbon. Moreover, metals and organic carbon analyses for different grain-size fractions showed higher levels in the finest fraction (<63 μm) (Shriadah, 1999). This indicates that complexation with organic matter may play an important role in the distribution patterns of metals in wetlands. From the high correlation between Cd, Cr, Cu, Pb and Zn concentrations, Callaway et al. (1998) moreover drew the rather extreme conclusion that these metals have similar sources and behave similarly in the sediment column.

One might expect that metals leach from the sediments with decreasing pH (e.g. Gambrell et al., 1991), which should result in positive correlations between pH and total metal concentrations. However, these correlations were found to be negative in our study. This

might be attributed to the pH fluctuating in a rather narrow range (7.2 to 8.7), as the sediments are well-buffered. Moreover, a decreased pH of intertidal Scheldt sediments seems to reflect increased acidification with increased organic matter concentrations, which results in a significant, negative correlation between organic matter concentrations and pH (R = -0.712, p < 0.001). This acidification might result from increased CO_2 and organic acid production during the decomposition of organic matter. The negative correlations between metal concentrations and pH might result from the combination of the significant negative correlation between the organic matter concentration and pH on the one hand and the significant positive correlations between organic matter and metal concentrations on the other hand. Organic complexation thus affects metal accumulation to a greater extent than the slight pH variations in the well-buffered intertidal sediments of the Scheldt estuary.

Table 3.3. Correlations between some sediment characteristics (pH, carbonate concentrations, conductivity, chloride concentrations, organic matter, clay, loam and sand concentrations) and metal concentrations (n = 26) in the superficial intertidal sediment layer of the Scheldt estuary

		pH	Carbonate	Conductivity	Cl	OM	Clay	Loam	Sand
Cd	Correlation	-.540(**)	.212	-.230	-.305	.823(**)	.739(**)	.627(**)	-.699(**)
	Sig.	.004	.299	.258	.130	.000	.000	.001	.000
Cr	Correlation	-.813(**)	.063	-.252	-.339	.883(**)	.910(**)	.811(**)	-.883(**)
	Sig.	.000	.759	.213	.091	.000	.000	.000	.000
Cu	Correlation	-.662(**)	.166	-.245	-.332	.871(**)	.841(**)	.719(**)	-.800(**)
	Sig.	.000	.417	.227	.098	.000	.000	.000	.000
Ni	Correlation	-.658(**)	.224	-.103	-.188	.920(**)	.828(**)	.746(**)	-.807(**)
	Sig.	.000	.272	.617	.359	.000	.000	.000	.000
Pb	Correlation	-.522(**)	.334	-.031	-.124	.765(**)	.713(**)	.731(**)	-.749(**)
	Sig.	.006	.095	.880	.547	.000	.000	.000	.000
Zn	Correlation	-.772(**)	.153	-.260	-.347	.872(**)	.866(**)	.806(**)	-.862(**)
	Sig.	.000	.455	.199	.083	.000	.000	.000	.000

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

When metal concentrations are plotted against clay or organic matter concentrations, some locations can be distinguished at which metal concentrations are significantly higher then expected from the correlation between clay or organic matter and metal concentrations (e.g. significantly above the regression line in Fig. 3.6). These locations coincide with those situated near the point-sources that were described above.
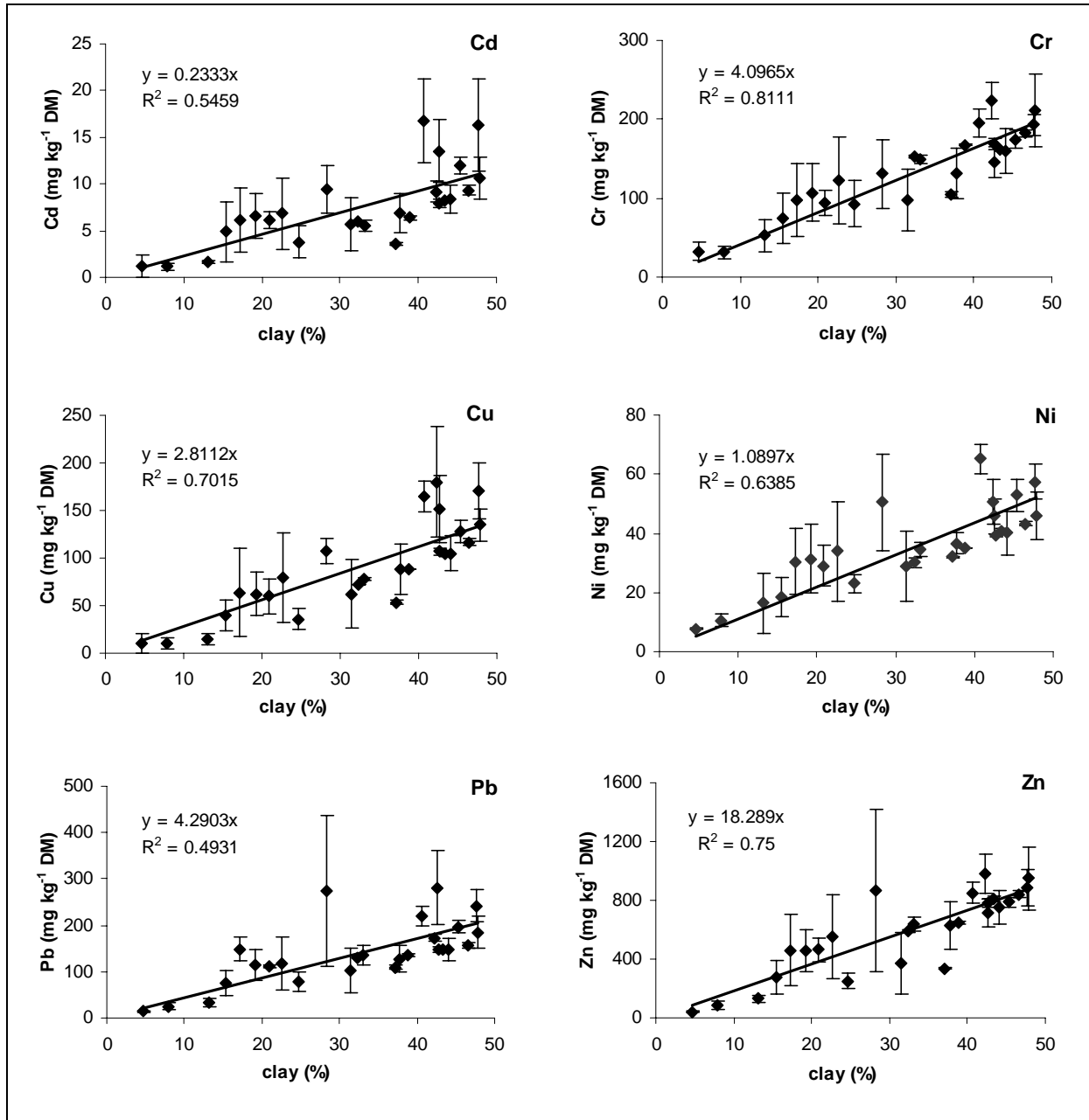
Fig. 3.6. Correlations between metal concentrations and clay concentrations (intercept with Y-axis set to zero) in the superficial intertidal sediment layer of the Scheldt estuary

As already mentioned, Cd and Cr concentrations are often very close to the remediation thresholds at a lot of sites where the elevated levels can not be related to specific point-sources. At these sites, Cd and Cr concentrations are not significantly higher than values predicted from the clay concentrations. Some locations can also be distinguished where metal concentrations are significantly lower than predicted from the regression between metal and clay or organic matter concentrations (e.g. significantly below the regression line in Fig. 3.6). Several of these sites are situated in the higher salinity part of the estuary. They are subjected

to flooding by less polluted seawater. Due to increases of chloride concentrations when inland fresh river water mixes with seawater, heavy metals may mobilise from the sediments as soluble chloride-complexes (Hahne and Kroontje, 1973). An increase of the salinity is also associated with an increase in the concentrations of major elements (Na, K, Ca, Mg), which compete with heavy metals for the sorption sites (Tam and Wong, 1999). This was supported by a stepwise linear regression analysis that included pH, clay, carbonate, organic matter, chloride and AVS concentrations as predictor variables (Table 3.4). For most metals, the procedure retained organic matter as most significant predictor variable. Thus, organic matter contents seemed to primarily affect Cd, Cu, Ni, Pb and Zn contents, whereas Cr contents were mainly determined by clay contents. Chloride concentrations were also included in the regression equation for Cu and Zn, as they still significantly ($p < 0.05$) contribute to explaining additional variability after inclusion of organic matter, although the effect of the organic matter is much more significant. The latter is confirmed by the calculation of the standardised regression coefficients (Beta-values) for both the model predicting Cu concentrations (0.843 for the organic matter and -0.226 for the chloride concentration) and for the model predicting Zn concentrations (0.842 for the organic matter and -0.241 for the chloride concentration). The regression coefficients for chlorides were negative, which indicates a decreasing trend in metal contents with increasing chloride contents. This was also the case for Cd and Cr, but chloride concentrations were not included in the regression equation, as their effect was just not significant at the 0.05 level. The effect of chloride contents was far from significant for Pb and Ni, which indicates that Pb and Ni concentrations are not affected by variations in chloride concentrations.

Table 3.4. Results of a stepwise linear regression for metal (Cd, Cr, Cu, Ni, Pb and Zn) concentrations in superficial intertidal Scheldt sediments as a function of pH, clay, carbonate, organic matter, chloride and AVS concentrations. R represents the correlation coefficient and Sig. F the significance of the overall model, whereas the p-values between brackets in the last column represent the significance of the individual regression coefficients (= Sig. t).

	R	Sig. F	Regression equation
Cd	0.823	<0.001	Cd (mg kg^{-1} DM) = 0.186 (p=0.870) + 0.627 (p<0.001) × OM (%)
Cr	0.910	<0.001	Cr (mg kg^{-1} DM) = 17.440 (p=0.146) + 3.625 (p<0.001) × Clay (%)
Cu	0.900	<0.001	Cu (mg kg^{-1} DM) = 4.819 (p=0.666) + 7.660 (p<0.001) × OM (%) – 13.255 (p=0.021) × Cl (g kg^{-1} DM)
Ni	0.920	<0.001	Ni (mg kg^{-1} DM) = 7.815 (p=0.006) + 2.414 (p<0.001) × OM (%)
Pb	0.765	<0.001	Pb (mg kg^{-1} DM) = 26.186 (p=0.233) + 9.797 (p<0.001) × OM (%)
Zn	0.904	<0.001	Zn (mg kg^{-1} DM) = 109.656 (p=0.089) + 43.730 (p<0.001) × OM (%) – 80.688 (p=0.013) × Cl (g kg^{-1} DM)

Concentrations predicted by using these equations are plotted against observed concentrations in Fig. 3.7. Some sites can still be distinguished at which observed metal concentrations are higher than predicted. They again coincide with locations near the point-sources which were described above. However, two more sites were now identified as possible point-sources. One is situated just a few hundred metres upstream a wastewater treatment plant of which the effluent flows into the river Scheldt. The other one is situated near an old shipyard at which steel cables are still being produced.
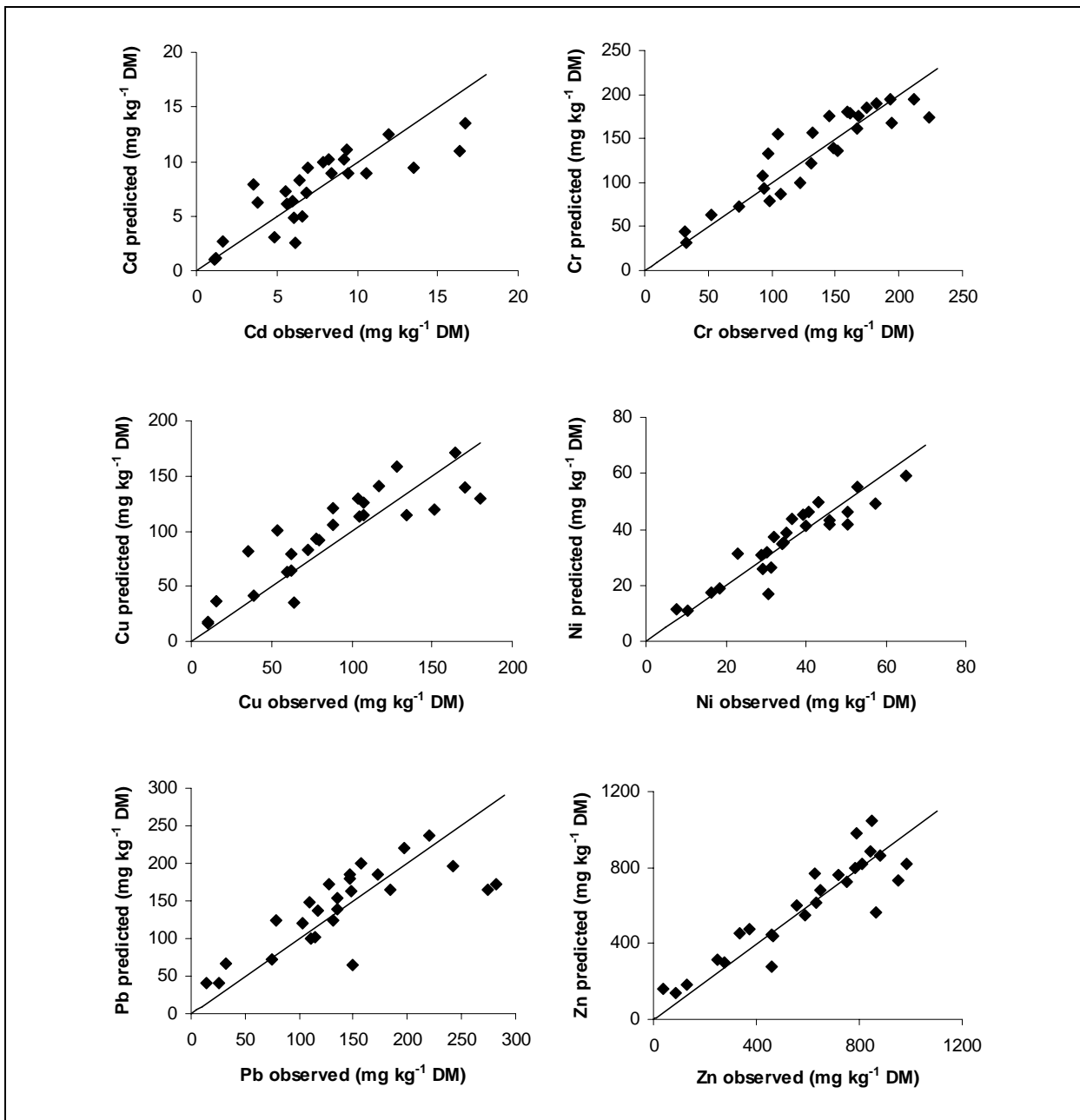
Fig. 3.7. Relationships between metal concentrations predicted by using the equations from Table 3.4 and observed metal concentrations in superficial intertidal Scheldt sediments

3.2.3 Conclusion

The superficial intertidal sediment layer of the river Scheldt is significantly contaminated with trace metals. The Cd concentrations often exceed the Vlarebo remediation values for nature areas, whereas the sediments are moderately contaminated by Cr, Cu and Zn. Lead concentrations occasionally are high. Nickel concentrations however lean towards background values. Significant positive correlations were observed between metals, organic matter and clay concentrations in the superficial layer of intertidal Scheldt sediments. So metal contents can be predicted from clay or organic matter contents. Metal concentrations were somewhat higher then predicted at sites within a range of a few km from specific point-sources of metals (shipyards, industrial areas with metallurgic activities, major motorways), whereas they were lower than predicted at sites which are regularly subjected to flooding by high salinity water.

3.3 Temporal and spatial variations in the upper sediment layer

3.3.1 Materials and methods

At the end of May 2003, sediments were sampled at 3 sampling locations: Doel, Kijkverdriet and Konkelschoor, at 55.7, 94.4 and 131.4 km from the river mouth, respectively. Sampling was repeated in the beginning of August, October, December 2003 and February 2004. By means of an auger, three sediment cores (Ø 3 cm) were sampled at each sampling location within a distance of approximately 2 meters from each other. Sediment was sampled from four depths, 0-20 cm, 20-40 cm, 40-60 cm and 60-100 cm below the sediments surface. To conduct a more detailed sampling of the upper sediment layer, a cylindrical PVC tube (height = 150 mm, diameter = 200 mm) was hammered in the ground, dug up, placed on a PVC plate and transported to the lab. The upper layer of these sediment cores was sliced into 3 sections: 0-2 cm, 2-5 cm and 5-10 cm. All sediment samples were dried in the laboratory at 50 °C to constant weight, ground using a hammer-cross beater mill (Gladiator BO 3567) and stored for further analyses. For the identification of sampling depths, the surface of the sediments was used as a reference. As the sampling sites could bank up during the sampling period, sediment accretion rates were also measured. Therefore, litter was removed at three plots (30x30 cm) on each sampling location in October. White-coloured kaolin clay was used to cover the sediments surface. At each sampling time, sediment deposition above the white layer was measured. The sediment accretion rate was found to be less than 0.2 cm between October 2003 and April 2004. Sediment deposition thus probably only slightly affects metal contents in the upper sediment layer on short term at intertidal marshes, which are only flooded at spring tides. Callaway et al. (1998) also found rather low sediment accretion rates at marshes in the Eastern Scheldt. They measured 0.68 cm year^{-1} at the low marsh and 0.34 cm year^{-1} at the high marsh.

Metal contents were determined in all samples of August. At the other sampling times, proportionally mixed samples of each combination of sampling location and depth were analysed for metals. Organic matter, carbonate, chloride contents and pH were determined in the samples of May, August and October, whereas acid-volatile sulphide (AVS) contents were determined in mixed samples of the 0-20, 20-40, 40-60 and 60-100 cm depths of October,

December and February. All used glassware and containers were washed in a laboratory dishwasher, subsequently soaked overnight in 5 % ultrapure 65 % HNO_3 and rinsed with deionised water. The pH was measured in a 1/5 sediment/distilled water suspension after equilibration during 18 h (Van Ranst et al., 1999). The conductivity was measured in the filtrate of a 1/5 sediment/distilled water suspension, shaken for ½ h (Van Ranst et al., 1999). The organic matter content was determined by measuring the weight loss after incineration of oven-dried samples (2 h at 450°C). The carbonate content was determined by back-titration with 0.5 M NaOH of an excess 0.25 M H_2SO_4 added to 1 g of sediment (Nelson, 1982). Texture analyses were conducted on samples obtained by mixing equal amounts (30 g) of the three samples at each location. They were performed at the Research Institute for Nature and Forest (INBO) using laser diffraction (Coulter LS200, Miami, FL) after removing organic matter and carbonates by adding hydrogen peroxide and an acetate buffer solution respectively. To determine the chloride content 10 g of sediment was suspended in 50 ml of 0.15 M HNO_3 and shaken for 30 minutes. The filtrate was titrated with 0.05 M $AgNO_3$ using potentiometric end-point detection (Van Ranst et al., 1999). Acid volatile sulphide (AVS) was determined on fresh sediment samples by conversion of sulphide to H_2S and absorption in a Zn-acetate solution, followed by a back titration (Tack et al., 1997a). Pseudo-total metal contents were determined by aqua regia extraction (Ure, 1990). The metal contents in the extracts were analysed using ICP-OES (Varian Vista MPX, Varian, Palo Alto, CA). To detect significant effects of sampling time, sampling location, sediment profile depth and possible interactions between these factors on the variables which were mentioned above, variance analyses were conducted using SPSS 12.0 (2003).

3.3.2 Results and discussion

Sampling location, sampling depth and sampling time all had a significant effect on chloride concentrations ($p < 0.001$ for all factors). Moreover, there was significant interactions between all factors. Chloride concentrations decreased with increasing distance to the river mouth from an average of 2940 mg kg^{-1} DM at Doel to 81 mg kg^{-1} DM at Konkelschoor, consistent with the decreasing influence of salty seawater. The chloride concentrations also decrease significantly with increasing sampling depth. This decrease was particularly pronounced at Doel, where a clayey sediment layer covers a sandy subsurface layer low in organic matter. Salts are expected to leach more easily from the sandy subsurface sediments.

Additionally, the salty tidal flooding water primarily comes into contact with the upper sediment layer. As this upper sediment layer is rather clayey and impermeable, seepage towards lower sediment layers is hampered.

Temporal variations of chloride concentrations in the sediments are significant, but rather small. Chloride concentrations in the river water are significantly higher during low discharge rates, when the salinity front is moving upstream (Baeyens et al., 1998). As a consequence, the Sea Scheldt between Ghent and Antwerp is a tidal fresh water river during winter and spring, whereas it turns brackish between Antwerp and the Rupel river mouth (100 km upstream) during summer and autumn (Baeyens et al., 1998). The salinity of the intertidal sediments was significantly lower in May compared to August and October at all sampling sites, reflecting these changes in the salinity of the river water. The effect decreased with increasing distance from the river mouth because salinity of the river water is not the only factor determining the salinity of intertidal sediments. River water mixes in variable proportions with groundwater and rainwater, whereas in the upper sediment layer, salts can be concentrated following evapotranspiration. Bear (1964) noted that salinity effects on soils and plants are determined rather by the salinity of the pore water of intertidal sediments than by the salinity of the flooding water. Lau & Chu (1999) observed higher salinities in that pore water compared to the flooding water. Numerical simulation of the interaction between the groundwater and the surface water is not a trivial matter as groundwater responds much slower than surface water (Post, 2005). This presents a problem of different time scales when coupling surface water and groundwater models. Thus, hydrological models are not yet able to adequately predict water fluxes and salinities in the transition zone between rivers and upland soils, which makes it even more difficult to explain seasonal and temporal salinity variations in intertidal sediments.

Sampling location, time and depth, as well as interactions between them, all are significant in explaining variability in the organic matter concentration ($p < 0.001$ for all factors). The significance of location effects increased with increasing sampling depths. The organic matter supply to the upper sediment layer, e.g. due to litter accumulation and belowground biomass production, and the organic matter decomposition rate in this layer thus seem to depend only little on the sampling location, whereas different leaching and decomposition rates of organic fractions at the different sites probably determine organic matter concentrations at higher depths. Indeed, the organic matter concentrations were highest at Kijkverdriet, where the

organic matter decomposition rate is expected to be rather low as a result of the anoxic conditions. In contrast, the organic matter concentrations were lowest in the deeper sediment layers of Doel. The sediments are more sandy and permeable at this site, which results in a lower retention of organic fractions.

The significance of sampling time effects in contrast to the location effect decreased with increasing sampling depth. The temporal variability of organic matter concentrations in the upper sediment layer is quite difficult to assess. At Konkelschoor, average concentrations in that layer were highest in May, whereas at Kijkverdriet and Doel they were highest in August. They are probably determined by a variety of opposing factors, such as organic matter accumulation in the upper sediment layer just after the growing season of reed plants as a result of reed plant decay and subsequent litter decomposition (Du Laing et al., 2006) and variable belowground biomass production rates of reed plants (Soetaert et al., 2004).

Sampling time and depth ($p < 0.001$) were significantly in explaining the variability in carbonate concentrations. Average carbonate concentrations significantly decreased with depth. Additionally, effects of sampling depth significantly depended on the sampling location. In Doel, carbonate concentrations decreased from 11.4 % in the upper 0-2 cm sediment layer to 4.7 % in the 60-100 cm sediment layer, in Kijkverdriet they decreased from 7.3 to 5.4 % and in Konkelschoor from 7.0 to 6.1 %. Measured changes in carbonate concentrations might however be attributed to the operational analysis procedure. Some sediments, such as those of Kijkverdriet and Konkelschoor contain a significant amount of sulphides, whereas those of Doel do not (Fig. 3.9). During drying of the sulphide-rich samples preceding the analyses, the sulphides might be oxidised, simultaneously releasing protons and lowering the carbonate contents and pH, as mentioned in chapter 2. For each molecule of FeS oxidised, two protons are produced (van den Berg & Loch, 2000). Differences between the highest and lowest average AVS concentrations along the sediment profile are below 48 mmol S^{2-} kg^{-1} DM. Upon oxidation of such amount, 96 mmol H^+ kg^{-1} DM is released, which consumes less than 1 % of $CaCO_3$ in the sediment. So this could only partly account for the observed decreases of carbonate concentrations with sampling depths, which totalled 0.9 to 6.7 %.

The decreasing carbonate concentrations at higher depths may also result from an increasing CO_2 pressure. CO_2 is produced during organic matter decomposition, whereas diffusion to the

atmosphere is expected to be hampered upon flooding, especially in clayey sediments. Decalcification might occur according to the following reaction (Van Den Bergh & Loch, 2000):

$$CaCO_3 + CO_2 + H_2O \rightarrow Ca^{2+} + 2\ HCO_3^- \ .$$
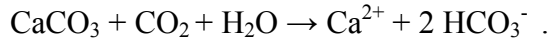
The significantly higher carbonate concentration in the upper sediment layer of especially Doel might be related to the sampling site being located nearer to the sea. As a result, carbonates could settle during tidal flooding. Moreover, the sediments in Doel are rather sandy and contain little organic matter. This should lead to a lower CO_2 and organic acid production and a more efficient exchange of CO_2 with the atmosphere. This in turn would limit decalcification (van den Bergh and Loch, 2000). Temporal settling fluctuations could explain differences in the carbonate concentrations of the upper 0-2 cm sediment layer at Doel. The carbonate concentration was highest in August at 13 %, and lowest in May (9 %). Breakdown of settled carbonates as a result of CO_2 and organic acid production associated with the litter decay and decomposition at the end of the growing season could constitute an additional factor explaining this decrease.

Although the pH varied in narrow ranges (7.16 - 8.68), there were significant differences in pH between sampling locations and sampling depths ($p < 0.001$), but not between different sampling times ($p = 0.065$). Interaction between sampling location and depth were significant ($p < 0.001$), whereas interactions of sampling depth and location with time were not ($p = 0.205$ and 0.075, respectively). pH changes as a function of depth thus depend on the location. The average pH was higher at Doel (7.85) compared to Kijkverdriet and Konkelschoor (7.35). Moreover, the pH clearly increased with increasing depth at Doel, whereas no clear profile was observed at Kijkverdriet and Konkelschoor.

Measured pH changes might also partly be attributed to the operational analysis procedure, as the pH was measured on dry samples and protons are released upon oxidisation of sulphides. The impact on pH is however expected to be very low, as the sediments are still buffered well by the presence of carbonates and bicarbonates. The calculated maximum pH decrease due to sulphide oxidation was less than 0.1. Bicarbonate are thus probably also formed by natural processes. A lower permeability of sediments and/or a higher organic matter decomposition rate can indeed lead to a higher CO_2 pressure, resulting in bicarbonate formation and a small

pH decrease in the presence of carbonates. Moreover, bicarbonates are expected to leach more easily from sandy sediments during tidal flooding, which may prevent the pH from significantly decreasing upon organic matter decomposition. The permeability of the sediments is indeed lower at Kijkverdriet and Konkelschoor compared to Doel, whereas the organic matter concentration is also higher. Moreover, organic matter and clay concentrations decrease with increasing depth at Doel.

Short-distance variations in metal concentrations between sampling points situated approximately 2 metres from each other were rather low for Cd and Cu (Fig. 3.8). Short-term temporal variations could not be identified due to practical restrictions as sampling could only be performed at low tide. However, they are also expected to be rather low. Sediment accretion rates were found to be very low. Metals are expected to migrate rather slowly through the sediment profile, especially in sediments containing a significant amount of clay and organic matter, such as those of Kijkverdriet and Konkelschoor. Moreover, superficial flooding during only a limited period of time is not expected to significantly affect ground water levels and flows, as the superficial sediment layer is rather clayey and impermeable.
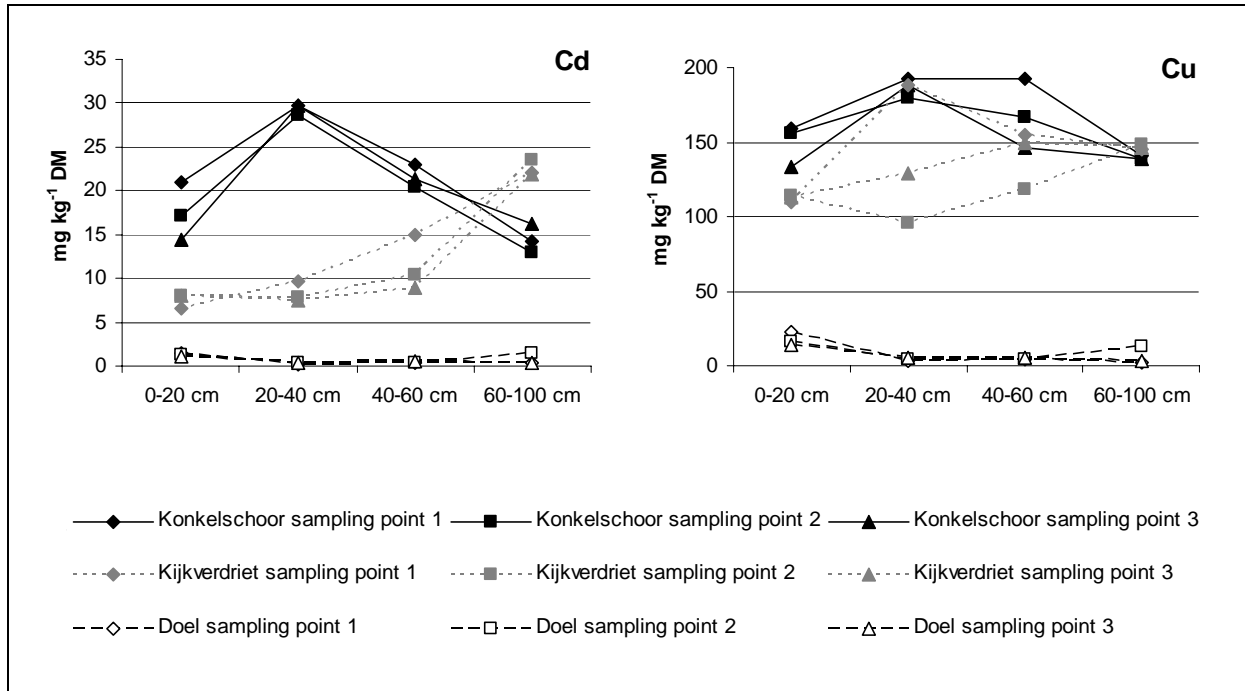

Fig. 3.8. Short-distance variations of metal concentrations within the sediment profiles of Konkelschoor, Kijkverdriet and Doel in August (sampling points 1, 2 and 3 situated approximately 2 m from each other)

Sampling location, time, depth and most of their interactions all were significant in explaining variability in metal concentrations. Sampling time was not a significant factor for Fe and Cu concentrations (p = 0.932 and 0.307, respectively). However, significant interaction effects of time with depth and location were observed also for these elements, which indicates that the occurrence of time effects depended on the sampling depth and location. Metal concentrations were significantly lower in Doel compared to Konkelschoor and Kijkverdriet. This might be attributed to the generally lower organic matter and clay concentrations and the higher chloride concentrations, as previously mentioned when discussing metal concentrations in the upper 0-20 cm sediment layer (*see* 3.2).

Iron and Mn concentrations generally decreased with increasing depth at all sampling locations. The more elevated Fe and Mn concentrations in the upper layer is likely related to more oxic conditions that favour precipitation of Fe and Mn as oxides (Callaway et al., 1998). However, Fe and Mn concentrations also showed a peak at higher depths in Kijkverdriet and Konkelschoor, causing a significant depth-location interaction effects for Fe and Mn concentrations. These peaks might be attributed to the presence of sulphides at the higher depths in Kijkverdriet and Konkelschoor. These induce the formation of insoluble Fe and Mn sulphide precipitates. Sampling depth and location-depth interaction effects were also significant for Cd, Cr, Cu, Pb and Zn concentrations. Cadmium and Zn concentrations reached a maximum at 20-40 cm below the sediments surface at Kijkverdriet, whereas they decreased with increasing depth at Doel and kept increasing with increasing depth at Konkelschoor. At Kijkverdriet, the most significant increase was situated between 20 and 40 cm below the sediments surface, whereas the steepest increase was found in the 60-100 cm layer for Konkelschoor. Some authors (e.g. Giordano et al., 1992 & Zwolsman et al., 1993, in: Monterroso et al., 2003) stated that the vertical distribution of metal concentrations in contaminated sediments can generally be attributed to anthropogenic inputs. Geochronologies or "contamination histories" of wetlands could thus be reconstructed through the collection and analysis of profiles of sediment from selected areas (Fox et al., 1999). As a result, the extent and the history of pollution in estuarine and coastal areas has been estimated from profiles of trace contaminants in sediment cores by several authors (e.g. Santschi et al., 2001 & Bay et al., 2003). They often rely on ^{137}Cs dating (a fallout product of nuclear testing) and normalization of metal concentrations to Al or Li concentrations (e.g. DeLaune et al., 1978, Pardue et al., 1992, Callaway et al., 1998, Spencer, 2002, Wang et al., 2004). However, according to Spencer et al. (2003), salt marshes in macro-tidal estuaries retain a time-

integrated or "smoothed" signal rather than a record of discrete pollutant inputs, due to extensive sediment reworking. They found that Fe and Mn have been significantly remobilised and that these diagenetic processes have slightly modified the vertical distributions of Cu, Pb and Zn in a dated salt-marsh core from the macro-tidal Medway estuary (southeast England). They thus concluded that an accurate chronology of metal input to salt marsh sediments can be difficult to assess because of the potential early-diagenetic mobility of both the radionuclides which are often used for dating and the contaminants of interest. Moreover, some specific characteristics of the intertidal marshes, such as the fluctuating water table levels, the occurrence of emergent vegetation and bioturbation, might facilitate metal redistribution within the upper sediment profile layer. As a result, one should be very careful when attributing metal distribution within the upper sediment profile to the pollution history in intertidal zones.

We would attribute the decreases of metal concentrations with increasing depth at Doel mainly to the significant decrease of organic matter and clay with depth. This in turn might be related to e.g. reed plant rooting and litter accumulation in the upper sediment layers. Thus, De Lacerda et al. (1997) found increased Cd and Zn concentrations in the upper sediment layer of a site vegetated by *Spartina alterniflora*, compared to adjacent mud flats without plant cover. The metal concentrations at 5-15 cm depth were 4 times higher at the vegetated site. Similar results were found at sites vegetated with *Spartina maritima* by Caçador et al. (1996). They concluded that roots influence metal concentrations in marsh sediments because they produce complex organic compounds and oxidise the rhizosphere. The formation of iron plaque in the oxidised rhizosphere also might contribute to increased metal concentrations (Sundby et al., 1998). Also decomposing reed litter was found to cause metal enrichment in the upper layer (Larsen & Schierup, 1981). This will be further discussed in Chapter 5.

Moreover, the higher sulphide contents in Kijkverdriet and at higher sampling depths in Konkelschoor (Fig. 3.10) suggest precipitation of metals as sulphides (Billon et al., 2001), which might explain the increasing metal concentrations with increasing depths. In Kijkverdriet, a significant amount of sulphides is already found in the upper 0-20 cm sediment layer, whereas at Konkelschoor, significant amounts are found from about 60 cm below the surface. Metals that leached from the upper sediment layer are thus expected to be especially concentrated in the higher sediment layers at Kijkverdriet and in the lower layers at Konkelschoor. White et al. (1989) also observed peaks of sulphur contents in freshwater lake

sediments and attributed these to sulphate reduction and Fe sulphide precipitation. However, these peaks were situated at much lower depths, between 5 and 10 cm below the surface of the sediments. The average water table level is however situated at higher depths in intertidal sediments. Moreover, intertidal sediments are subject to alternating hydrological conditions, which does not facilitate sulphide formation in the upper sediment layers. As a result, sulphate reduction rates are indeed expected to increase more gradually with depth in the intertidal sediments, compared to the bottom sediments of rivers or lakes. Moreover, peak sulphur concentrations are also expected to occur at higher depths.

Cadmium and Zn usually show similar sorption and mobilisation characteristics in upland soils. When all sediment samples of Doel (0-100 cm), which are the least reduced and contain low sulphide amounts, are included in a linear regression analysis, the correlation between Cd and Zn concentrations is indeed found to be very significant (Fig. 3.9). The slope of the regression line indicates that both elements are accumulated in the same ratio as in the upper 20 cm sediment layer of most intertidal marshes of the Scheldt estuary (*see* paragraph 3.2). Moreover, the intercept of the regression equation does not seem to be significant, which could be expected when assuming that these metals are both mainly associated with clay and organic matter. When the sediment samples of other sampling points Kijkverdriet and Konkelschoor, which have more elevated AVS contents, are subjected to separate linear regression analyses, the slope of the regression line was found to decrease and the intercept became more significant, compared to Doel (Fig. 3.9). This effect is most pronounced at Kijkverdriet, the sampling site with the highest AVS content. Cadmium is found to be preferentially accumulated compared to Zn. This might be attributed to the additional presence of a pool in which Cd is preferentially accumulated. Sulphides might constitute this pool, as the solubility of CdS is much lower compared to ZnS. Moreover, only a small amount of sulphides is needed to precipitate a significant portion of all Cd present. Accumulation of Cd in this "sulphide pool" might be promoted by the higher chloride contents at Kijkverdriet. These promote leaching of especially Cd from the upper sediment layers to the lower layers as soluble Cd chloride complexes and thus favour Cd migration and precipitation at greater depth. Zn chloride complexes are indeed less stable than Cd chloride complexes. This coincided with the calculations and observations of Gerringa et al. (2001). They concluded that the formation of dissolved Cd chloride complexes when oxidation occurs at high salinities increases the mobility of especially Cd, causing a decrease in the Zn over Cd ratio of the total dissolved concentrations.

Bi-plots for other combinations of elements showed similar relationships. Preferential accumulation in the "sulphide pool" at the higher sediment depths however seems to be restricted to Cd and Zn, which can probably be explained by their overall higher mobility and rate of leaching from low AVS sampling points. Moreover, plots for other elements were often more scattered as sorption and mobilisation characteristics in the absence of sulphides does not show as much agreement as between Cd and Zn.
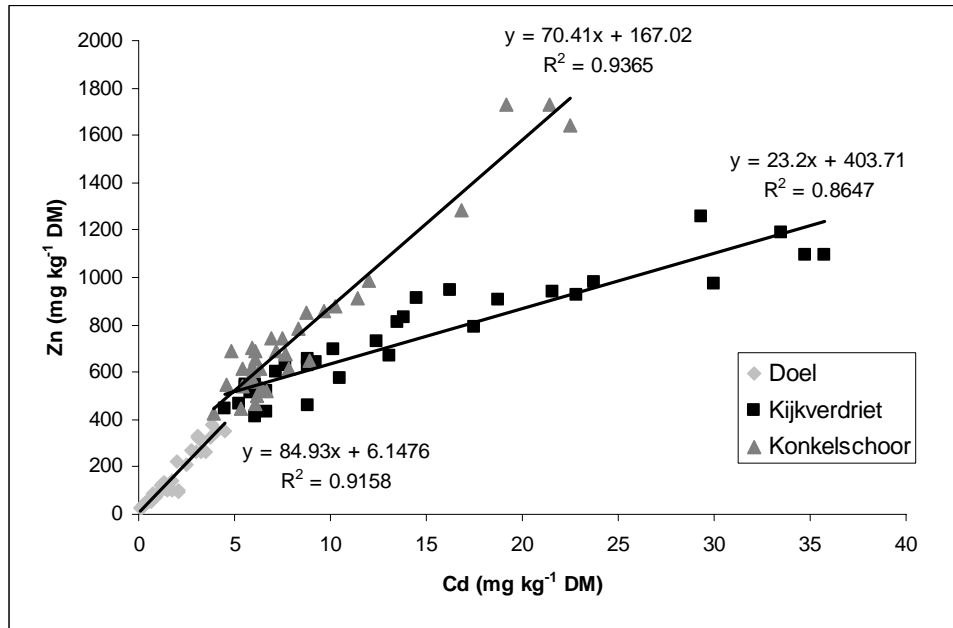
Fig. 3.9. Correlation between Cd and Zn contents in intertidal sediment samples (0-100 cm below the surface) of Doel, Kijkverdriet and Konkelschoor

Temporal variations in sulphide contents can be expected and might influence metal accumulation and mobility. In the 40-100 cm layer of Doel, AVS concentrations decreased from low, but detectable concentrations in October and December (3 – 5.5 mmol kg^{-1} DM) to very low concentrations (<0.8 mmol kg^{-1} DM) in February. This coincided with metal concentrations that also decreased from October to February, as illustrated for Cu in Fig. 3.10. Temporal changes of low sulphide contents may thus indeed be related to variations in metal contents. Sulphide oxidation and subsequent metal release might be caused by oxygen diffusion into the sediment during a period of lower levels of the water table. Moreover, reed plant decay and litter decomposition in the upper sediment layers during fall, which also seem to result in increased Cu concentrations in the 0-5 cm layer (Fig. 3.10), might result in an increased oxygen consumption and thus lead to more anoxic conditions at higher depths in

October-December compared to February. Ongoing leaching of Cu from the litter to the lower layers or increased sulphide precipitation due to increased sulphide formation results in the maximal Cu concentration being reached in February for the 5-10 cm layer and in May at the higher depths. At the highest depths, similar profiles are observed for Cd, Pb and Zn. However, the increased Cu concentrations in the upper sediment layer in October are not accompanied by increased Cd and Zn concentrations. This might be due to a weaker association of Cd and Zn with the litter as the stability of Cd/Zn-organic matter complexes is lower (Kalbitz & Wennrich, 1998).

At both Kijkverdriet and Konkelschoor, Cu concentrations did not follow fluctuating sulphide concentrations as strongly. At these higher sulphide levels, sulphide production may not anymore constitute a limiting factor for Cu sulphide formation. Vice versa, oxidation of part of the sulphides will not primarily affect Cu sulphide contents. Indeed, total molar contents of metals with the lowest metal sulphide solubility products (Cu, Cd, Pb, Ni, Zn) were lower than the total molar sulphide contents. Thus, Cu sulphide formation should be mostly determined by Cu mobility instead. In October, Cu contents were lowest at the highest depths but highest in the upper layer. The strong complexation of Cu by organic matter may result in a redistribution of Cu within the profile towards lower depths when reed plant decay and litter accumulation are intensive. The increasing Cu concentrations from October to February at higher sampling depths might additionally be linked to gradual leaching of organic Cu-complexes from the litter, which accumulates in the upper sediment layer during fall and winter. The decreasing Cu concentrations between May and October at these higher sampling depths might in turn be related to an increasing organic matter decomposition during the hotter summer months, in absence of organic matter leaching from higher sediment layers.
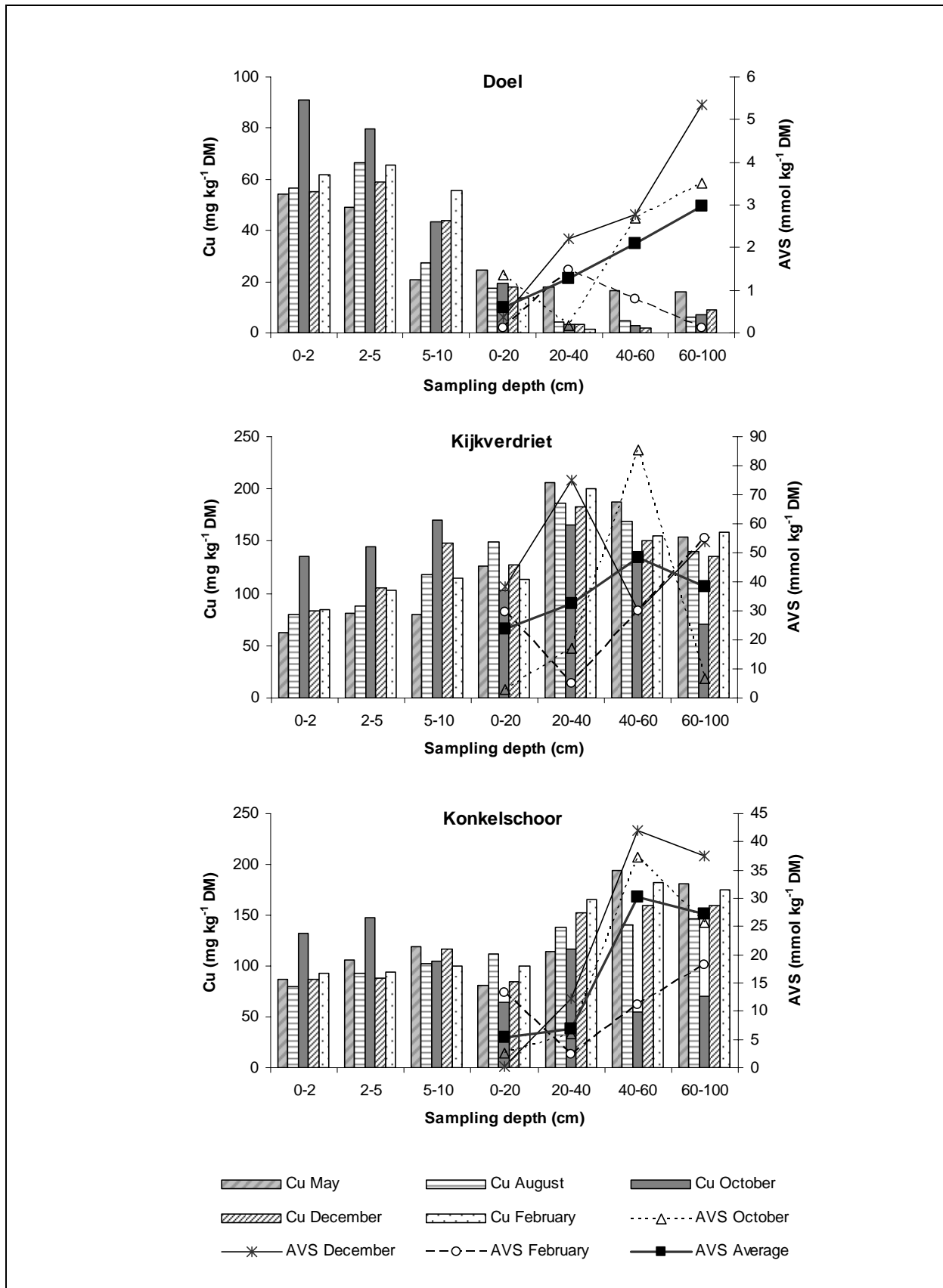
Fig. 3.10. Temporal fluctuations of Cu and AVS concentrations in the 0-100 cm intertidal sediment layer of Doel, Kijkverdriet and Konkelschoor as a function of sampling depth (AVS not measured on samples 0-2, 2-5 and 5-10 cm)

Fe, Mn and probably Ni (Zwolsman et al., 1993) could also be released rapidly upon reduction of Fe/Mn oxides during the period of fast litter decomposition. Moreover, cations could be released from carbonates as a result of increasing CO_2 pressure, as previously mentioned. Several other elements could also directly leach from the litter during decomposition. All these cations can be transported relatively fast within the sediment profile and can compete with Cu for sorption onto the solid sediment fractions. This can result in lower Cu contents at higher depths. Upon further depletion of easily available organic matter, decreasing Fe/Mn oxide reduction and carbonate breakdown rates, redistribution and re-adsorption of Cu onto the sediments can occur, leading again to higher Cu concentrations at higher depths from December onwards. Cd, Cr, Pb and Zn are expected to act similarly as Cu, because their profiles were very similar.

3.3.3 Conclusion

Most variations of sediment properties in intertidal zones could be attributed to the sampling location. The sampling location primarily determines texture and chloride, carbonate and organic matter contents, which in turn affect metal accumulation. Metal accumulation, which is primarily due to association of metals with organic matter and clay in the surface sediment layer, seems to be supplemented by an accumulation as sulphide precipitates at higher sediment depths. The depth at which sulphide precipitation significantly contributes to metal accumulation depended on the sampling location, and varied from less than 5 cm in clayey, organic sediments to more than 1 m in sandy sediments. Temporal variations of Cu, Cd, Pb and Zn concentrations can only be linked to newly formed sulphides or sulphide oxidation in the sites with the lowest sulphide contents. At sampling sites containing high sulphide amounts, variations should be primarily attributed to metal exchange and the presence of mobile metal complexes. Litter decomposition at the end of the growing season could hereby play a significant role.

3.4 Conclusion

The superficial intertidal sediment layer of the river Scheldt is significantly contaminated with trace metals. The Cd concentrations often exceed the Vlarebo remediation values for nature areas, whereas the sediments are moderately contaminated by Cr, Cu and Zn. Lead concentrations occasionally are high. Nickel concentrations however lean towards background values. Most variations of sediment properties in intertidal zones could be attributed to the sampling location. The sampling location primarily determines texture and chloride, carbonate and organic matter contents, which in turn affect metal accumulation. Significant positive correlations were observed between metals, organic matter and clay concentrations in the superficial layer of intertidal Scheldt sediments. So metal concentrations can be predicted from clay or organic matter concentrations in this sediment layer. Metal concentrations were somewhat higher then predicted at sites within a range of a few km from specific point-sources of metals (shipyards, industrial areas with metallurgic activities, major motorways), whereas they were lower than predicted at sites which are regularly subjected to flooding by high salinity water. Metal accumulation, which is primarily due to association of metals with organic matter and clay in the surface sediment layer, seems to be supplemented by an accumulation as sulphide precipitates at higher sediment depths. The depth at which sulphide precipitation significantly contributes to metal accumulation depended on the sampling location, and varied from less than 5 cm in clayey, organic sediments to more than 1 m in sandy sediments. Temporal variations of Cu, Cd, Pb and Zn concentrations can only be linked to newly formed sulphides or sulphide oxidation in the sites with the lowest sulphide contents. At sampling sites containing high sulphide amounts, variations should be primarily attributed to metal exchange and the presence of mobile metal complexes. Litter decomposition at the end of the growing season could hereby play a significant role.

4.

Effect of salinity on metal mobility

4 Effect of salinity on metal mobility

4.1 Introduction

A longitudinal salinity gradient can be observed along the Scheldt river. Salinity decreases from the sea up to the mouth of the tributary Durme, where chloride concentrations stabilize around 100 mg L^{-1} in the surface water. The longitudinal salinity profile is primarily determined by the magnitude of river discharge, whereas the tidal action contributes to a lesser degree. As a result, significant salinity variations in the surface water are observed. E.g., the lower riverine part from Ghent to Antwerp is a tidal fresh water river during winter and spring, whereas the water becomes brackish between Antwerp and the Rupel mouth during summer and autumn (Baeyens et al., 1998). The presence of salts can affect soils and sediments in different ways, from affecting the sorption of exchangeable cations to changing physical soil characteristics.

NaCl is a strong electrolyte. The chlorine anion is a stronger metal binding ligand than NO_3^- and ClO_3^-. Some authors (Khattak et al., 1989 & Smolders & McLaughlin, 1996) reported an increased mobilisation of Mn and Cd upon the addition of Cl^- compared to NO_3^- and ClO_3^-. Results of both laboratory (Paalman et al., 1994) and field experiments (Hatje et al., 2003) illustrated the increasing Cd mobilisation with increasing salinities. By chlorocomplexation, the activity of free Cd^{2+} in the soil solution is expected to decrease and desorption is expected to increase (Paalman et al., 1994 & Hatje et al., 2003). The speciation shifts towards $CdCl^+$, $CdCl_2$, $CdCl_3^-$ and $CdCl_4^{2-}$ forms (Helmke, 1999). The effects of salinity are not confined to the complexation capacity of the anions. When Ca^{2+} and Mg^{2+} are abundant in the soil solution, metals will also be mobilised from particles as a result of competition between these divalent cations and the metal ions (Paalman et al., 1994). These driving forces are important for Zn and Cd in particular (Millward & Liu, 2003). Paalman et al. (1994) and Khattak et al. (1989) showed that the cation of added salts significantly influenced Cd and Mn mobilisation. The Ca^{2+}-ion competed with Cd^{2+} for sorption to suspended matter, whereas this was not observed for Na^+ (Paalman et al., 1994). Similar results were obtained by Khattak et al. (1989) for Mn. Moreover, Khattak et al. (1989) and Naidu et al. (1994) found an effect of

salinity on soil pH, which might in turn affect metal mobility. A decreasing pH was observed when the salinity of the soil solution increased.

Lores & Pennock (1998) concluded that salinity can affect the binding of metals with humic acids. The percentage of Cd, Cr and Zn which was bound to humic acids drastically decreased during initial salinity increases, whereas it was not affected anymore by continued salinity increases at high salt contents. This was however not the case for Cu. The percentage of Cu bound to humic acids decreased when the salinity increased up to 3 g Cl$^-$ L^{-1}. This percentage again increased when the salinity rose above 3 g Cl$^-$ L^{-1}, which suggested complex interactions between humic acids, salinity, pH and Cu. Increasing salinities can possibly lead to changes in the conformation of humic acids, which can release new binding sites for Cu. Such complex interactions between salinity, organic matter, pH and Cu have also been noted by Gerringa et al. (1998) in the saline part of the Scheldt estuary.

Van Eck (1999) moreover reported decreasing concentrations of Cd in suspended matter with increasing salinities in the Western Scheldt and part of the Sea Scheldt. He attributed this effect not only to direct metal release from suspended matter due to increasing salinities, but also to a higher sulphide content, which facilitates metal precipitation, in the freshwater part of the estuary. Seasonal changes in the association of Cd with suspended matter were also related to the changing oxygen conditions of the surface water. However, a significantly decreasing metal sorption to suspended matter with increasing salinities is also observed in the absence of sulphides, in the oxygen-rich environment of the Western Scheldt. Gerringa et al. (2001) found that high salinities resulted in a faster release of Cd from CdS during the oxidation of reduced Scheldt sediments. This release was found to be stimulated by both formation of chloride complexes and ion exchange. In this chapter, we aim to identify effects of salinity on metal mobility in the intertidal Scheldt sediments. We focussed on the kinetics of metal mobility changes upon the creation of flooded conditions.

4.2 Materials and methods

4.2.1 Experimental setup

A part of the upper 0-30 cm soil or sediment layer was excavated at 4 sites: Kramp, Kijkverdriet, Galgenschoor and Lippensbroek. The sites were selected based on their significant pollution levels, and varying characteristics and physico-chemical properties (Chapter 3). Kramp, Kijkverdriet and Galgenschoor are intertidal marshes, which are regularly flooded by Scheldt river water. Galgenschoor is situated in the brackish part of the Scheldt estuary, whereas Kramp is situated in the freshwater part. Kijkverdriet is situated at the boundary between the freshwater and the brackish zone (Fig. 9.1, Galgenschoor referred to as "Lillo"). Lippensbroek is situated between Kramp and Kijkverdriet. It was a polder at the time of sampling, but it is now being used as an experimental, pilot-scale controlled flooding area. Scheldt water now regularly inundates the polder by means of a lock system. To improve the readability, the soil of Lippensbroek will however also be referred to as a "sediment" when it is pooled together with the sediments of other sampling sites in the next paragraphs. The salinity of the flooding water at the sampling sites varies from about 0.7 g NaCl L^{-1} near Kramp to 5.5 g NaCl L^{-1} near Galgenschoor.

A part of the upper 30 cm sediment layer was excavated at each site. Plant roots and litter were removed. To homogenize the sediment, it was sieved over a sieve of 8 mm mesh width and intensively mixed. The fraction larger than 8 mm was discarded. The homogeneous sediment was distributed over 3 recipients of 32 x 24 x 20 cm. Every recipient was provided with a sediment layer of 11 cm thick. The recipients were stored in a greenhouse at a temperature controlled between 15 and 25°C and a light regime of 16 hours light per day. The recipients were flooded with water of different salinities. Water was added until the water level was situated at 5 cm above the sediment surface. Solutions of the desired salinity were prepared by dissolving NaCl in deionised water in amounts of 0.5 (low salinity), 2.5 (middle salinity) and 5 g NaCl L^{-1} (high salinity), respectively. Different amounts of flooding water were added to the sediments to reach saturation as the sediments were characterised by different initial water contents and densities. As a result, the salinities of the sediments which were finally reached, differed. Therefore, Na and Cl contents in the pore water were monitored extensively as they are expected to affect metal fate rather than the salinity of the

flooding water. Evaporation and removal by sampling were compensated for by adding deionised water weekly to ensured that water levels were maintained at 5 cm above the sediment surface without affecting total salt amounts in the recipients. This amounted on average to about 0.5 L of distilled water per recipient each week. Yet, sampling did remove salts from the setup. This effect was neglected as approximately 10 % of the added water and salt amounts were removed after the 8 months that the experiment lasted. It was not feasible to duplicate the experimental setup as we aimed to conduct the experiment with different salinities and types of sediments, meanwhile maintaining a relatively high temporal sampling resolution. Therefore, the effect of sediment heterogeneity was diluted by duplicating the sampling procedure (see sampling section).

To study the effect of salinity on metal bioavailability, duckweed (*Lemna minor*) was transplanted to the recipients around day 150. After a growth period of 4 weeks, the plants were harvested and analysed for metal contents.

4.2.2 Sampling

Each recipient was fitted with four Rhizon soil moisture samplers (type MOM, Rhizosphere Research Products, Wageningen, The Netherlands) to sample pore water and surface water, as described in Chapter 2. Two samplers were installed horizontally at 5 cm below the sediment surface to sample pore water. The two other samplers were installed to sample surface water at 2.5 cm above the sediment surface, coinciding with 2.5 cm below the water surface (Fig. 4.1). To install them, holes were made in the recipient walls, through which the samplers were injected into the sediment. Silicone was used to ensure that all connections were waterproof.
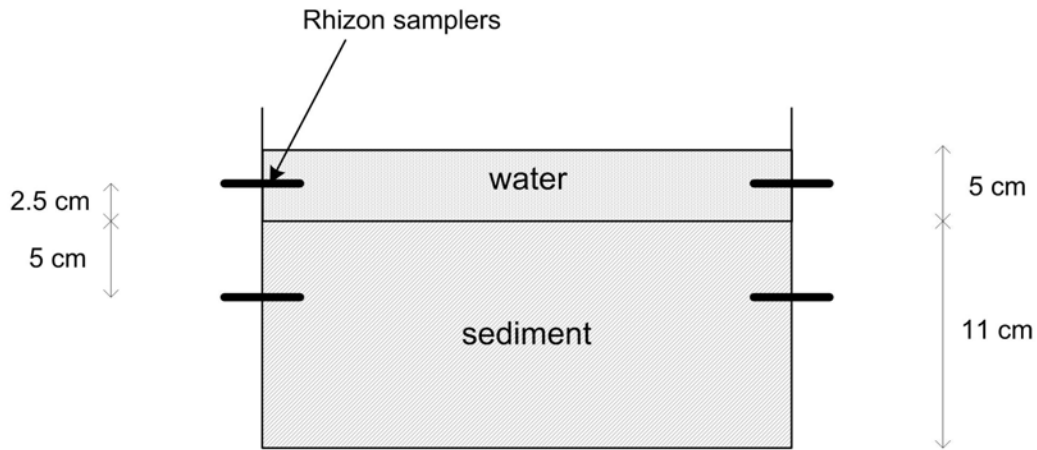
Fig. 4.1. Experimental setup

During filling of the recipients, some samples were taken of the sediment of each recipient to analyse the physico-chemical sediment properties. Around days 150 and 250, several core samples were taken from the sediment of each recipient to check for possible changes in these physico-chemical sediment properties during the experiment. To homogenise the sediments preceding the analyses, they were dried overnight at 70°C and ground in a hammer-cross beater mill. This was however not done for the determination of acid volatile sulphide (AVS) contents, as drying can cause oxidation of the sulphides.

4.2.3 Analyses

All glassware and containers used were washed in a laboratory dishwasher, subsequently soaked overnight in 5 % ultrapure 65 % HNO_3 and rinsed with deionised water. Pseudo-total metal contents in the sediments were determined by an aqua regia destruction (Ure, 1990) and analysed with ICP-OES (Varian Vista MPX, Varian, Palo Alto, CA). Acid volatile sulphide (AVS) was determined on fresh sediment samples by transformation of sulphide into H_2S and absorption in a Zn-acetate solution, followed by a back titration. This method is described by Tack et al. (1997a). The pH was measured in a 1/5 sediment/distilled water suspension after equilibration during 18 h (Van Ranst et al., 1999). The conductivity was measured in the filtrate of a 1/5 sediment/distilled water suspension, shaken for ½ h (Van Ranst et al., 1999). The organic matter content was determined by measuring the weight loss after incineration of

oven-dried samples (2 h at 450°C). The carbonate content was determined by back-titration (with 0.5 M NaOH) of an excess 0.25 M H_2SO_4 added to 1 g of sediment (Nelson, 1982). Texture was determined at the Research Institute for Nature and Forest (INBO) using laser diffraction (Coulter LS200, Miami, FL) after removing organic matter and carbonates by adding hydrogen peroxide and an acetate buffer solution respectively. To determine the chloride content 10 g of sediment was suspended in 50 ml of 0.15 M HNO_3 and shaken for 30 minutes. The filtrate was titrated with 0.05 M $AgNO_3$ using potentiometric end-point detection (Van Ranst et al., 1999). The cation exchange capacity was measured by percolation of 150 ml 1 M NH_4OAc through a percolation tube filled with a mixture of 5 g sediment and 35 g quartz sand, followed by washing through the excess with 300 mL denatured ethanol. The exchangeable ammonium ions were then eluted with 500 mL 1 M KCl and analysed in the percolate by means of a steam distillation (Tecator Kjeltec System 1002 Distilling Unit). The exchangeable cations were also analysed in this percolate using ICP-OES (Varian Vista MPX, Varian, Palo Alto, CA) (Van Ranst et al., 1999).

During the first 140 days of the experiment, pore water of the sediments was extracted by both Rhizon samplers approximately twice a week. Some samples were taken occasionally between day 140 and day 250. The samples were acidified with concentrated HNO_3 to keep the metals in solution and to solubilise metals which precipitated during sampling. Samples of both samplers were poured together in a recipient and stored in a refrigerator before analysis. All samples were analysed for Cd, Cu, Ni, Fe, Mn and Zn using GF-AAS (Varian SpectraAA-800/GTA-100, Varian, Palo Alto, CA) and ICP-OES (Varian Vista MPX, Varian, Palo Alto, CA). To check whether the operational analyses procedures could be affected by the salinities under investigation, standards were prepared in solutions of 0.5, 2.5 and 5 g NaCl L^{-1} and analysed after calibration with non-saline standards. Analyses results for all metals were found to decrease with increasing salinities in a range from 1 up to 10 %, which should be taken into consideration when interpreting the results. At least every two weeks during the first 140 days, a sample was also analysed for Ca, Mg, Na and K contents. The TOC-, NO_3^-, SO_4^{2-} and Cl^- contents were regularly determined on non-acidified samples directly after sampling. Surface water was extracted 9 times during the entire experiment, using the two Rhizon samplers installed above the sediment surface. They were also analysed for Cd, Cu, Ni, Fe, Mn, Zn, Ca, Mg, Na, K, TOC, NO_3^-, SO_4^{2-} and Cl^-. TOC contents in the surface and pore water samples were measured with a TOC-analyser (TOC-5000, Shimadzu, Tokyo Japan). Cl^- contents were determined by titration with 0.05 M $AgNO_3$ using potentiometric

end-point detection. NO_3^- and SO_4^{2-} contents were measured with a Metrohm configuration consisting of a 761 Compact Ion Chromatograph equipped with a 788 IC Filtration Sample Processor and an anion exchange column (IC-AN-Column Metrosep A supp 4, Metrohm Ion Analysis, Switzerland).

The redox potential of the sediments was measured with a HI 3090 B/5 electrode that was inserted permanently into the sediment. One electrode was placed in each recipient at 5 cm below the sediment surface. For each measurement, the electrode was temporarily connected to a HI 9025 meter of HANNA instruments. During the first four months, the redox potential was measured almost daily, afterwards twice a week. The measured value was corrected to the Standard Hydrogen Electrode as a reference by adding the difference between the redox potential measured in a ZoBells solution (0.033 M $K_3Fe(CN)_6$ and 0.033 M $K_4Fe(CN)_6$ in 0.1 M KCl) and the theoretical value of + 428 mV.

The duckweed plants were analysed in their entirety for metal contents without separating roots from leaves. They were thoroughly rinsed with distilled water and dried overnight at 50°C. About 0.5 g of plant material was weighed into a beaker and 5 ml of ultra pure HNO_3 were added. The beaker was heated on a hot plate at 130 $^\circ$C for one hour. After heating, a total of 4 ml H_2O_2 was added to each sample and then heated for 10 minutes. The suspensions were filtered in 50 ml volumetric flasks using filter papers (S&S blue ribbon, Schleicher & Schuell). The filtrates were diluted to the mark using 1 % HNO_3. Cd, Cu, Ni, Zn, Fe and Mn contents were determined using GF-AAS (Varian SpectraAA-800/GTA-100, Varian, Palo Alto, CA) and ICP-OES (Varian Vista MPX, Varian, Palo Alto, CA).

4.3 Results and discussion

Some initial metal contents and physico-chemical properties of the sediments are presented in Table 4.1. Clay and organic matter contents were highest in Kijkverdriet, which also resulted in the highest CEC. $CaCO_3$ concentrations ranged from 7.9 to 9.2 % and accounted for a pH in the neutral to slightly alkaline range (7.33 to 8.02). Chloride contents and conductivity rapidly decreased when moving upstream from Galgenschoor over Kijkverdriet to Kramp, coinciding with the decreasing influence of salty seawater. As the sediments were excavated

during summer, those of Kijkverdriet were also subject to flooding by brackish river water and contain a relatively high chloride content. Lippensbroek was not flooded by river water, which resulted in chloride concentrations below the detection limit of 0.02 g kg^{-1}. The Kijkverdriet sediment was very clayey and the organic matter content was very high. This resulted in a more reduced state, a much higher initial AVS content and a much lower redox potential. All sediments were polluted by metals. Cd concentrations exceeded the background levels for soils according to the Flemish legislation (Vlarebo, 1996) by a factor ranging from 2 to 5. Cu concentrations exceeded them by a factor in the range of 4 to 5 and Ni by a factor from 2 to 3. Zn concentrations were 4 to 8 times higher than the background values. Cu, Ni and Zn concentrations did however not exceed the soil sanitation threshold values according to the Flemish legislation (Vlarebo, 1996). Cd concentrations significantly exceeded the sanitation thresholds imposed on nature areas in Kijkverdriet, Kramp and Lippensbroek, whereas these thresholds were just not reached in Galgenschoor.

Table 4.1. Metal concentrations and some physico-chemical properties of the sediments (mean ± standard deviation)

	Galgenschoor	Kijkverdriet	Kramp	Lippensbroek
Cd (mg kg^{-1} DM)	4.3 ± 0.1	11.5 ± 0.2	11.2 ± 0.1	8.5 ± 0.1
Cu (mg kg^{-1} DM)	82 ± 1	132 ± 3	125 ± 1	95 ± 0
Ni (mg kg^{-1} DM)	32.2 ± 0.3	53.8 ± 0.6	40.2 ± 0.1	32.6 ± 0.3
Zn (mg kg^{-1} DM)	413 ± 4	696 ± 10	947 ± 7	696 ± 1
Clay (%)	32.4 ± 1.2	49.4 ± 0.9	41.5 ± 0.8	39.9 ± 0.3
Loam (%)	48.1 ± 1.1	43.6 ± 0.5	40.1 ± 0.9	45.4 ± 0.6
Sand (%)	19.5 ± 0.2	7.1 ± 1.1	18.4 ± 1.3	14.7 ± 0.8
CaCO$_3$ (%)	7.9 ± 0.4	8.9 ± 0.1	9.2 ± 0.3	7.9 ± 0.1
OM (%)	15.4 ± 0.1	28.8 ± 0.3	15.1 ± 0.6	12.2 ± 0.4
pH	7.93 ± 0.05	7.33 ± 0.00	8.02 ± 0.01	7.63 ± 0.06
EC (µS cm^{-1})	3970 ± 61	1058 ± 15	216 ± 15	85 ± 1
Cl$^-$ (g kg^{-1} DM)	71.29 ± 0.18	10.19 ± 0.47	0.62 ± 0.03	<0.02 ± 0.00
CEC (cmol(+) kg^{-1})	22.9 ± 0.3	37.7 ± 1.0	28.2 ± 0.0	27.0 ± 0.4
AVS (mg kg^{-1} DM)	45 ± 17	995 ± 216	42 ± 37	51 ± 63
Redox (mV)	+465	-168	+466	+470

Between 0.2 and 1.8 % of total Ni was found to be in the exchangeable fraction. This ranged between 0.6 and 2.2 % for Cu. Cd was predominant in the exchangeable fraction, with 12, 16 and 22 % of total Cd for Lippensbroek, Kramp and Galgenschoor, respectively. This was however not the case in Kijkverdriet, where the exchangeable fraction accounted for only 1.9

% of the total Cd amount. This might be partly due to the formation of insoluble and stable Cd sulphide precipitates as a result of the highly reduced state (e.g. Salomons et al., 1987; Simpson et al., 2000; Guo et al., 2001). The low Cd amounts in the exchangeable fraction of the Kijkverdriet sediment might also have been promoted by the very significant abundance of Mn in this fraction. While only 0.2 to 1.0 % of the total Mn was found to be exchangeable in the sediments of Lippensbroek, Kramp and Galgenschoor, this percentage amounted to 9.1 in Kijkverdriet. As Cd tends to form stable and insoluble sulphides, soluble Mn created upon reduction of the Kijkverdriet sediment probably replaces Cd at negatively charged sediment surfaces. Zn is also quite well represented in the exchangeable fractions of the sediments of Galgenschoor and Kramp (4.3 and 3.3 %), whereas it is less abundant in exchangeable fractions of the sediments of Kijkverdriet and Lippensbroek (0.9 and 1.0 %).

Most physico-chemical sediment properties did not change significantly during the first 150 days of the experiment. However, some remarkable shifts in the Lippensbroek soil were observed. $CaCO_3$ concentrations decreased from 7.9 ± 0.1 to 5.1 ± 0.4 %, whereas organic matter concentrations also decreased from 12.2 ± 0.4 to 9.5 ± 0.2 %. A relatively fast decomposition of organic matter probably results in acidification and breakdown of the carbonates, which in turn might affect metal mobility (van den Bergh and Loch, 2000). These effects were also observed to a smaller extent in the sediments of Galgenschoor and Kramp, whereas they were not significant in the Kijkverdriet sediment.

For the middle and high salinity treatments, average chloride and Na concentrations in the pore water were lower in the treatments of Kramp and Kijkverdriet, compared to Lippensbroek (Table 4.2). Differences were thus observed between the salinity of the pore water and the salinity of the flooding water, which has also been observed in field condition by Lau & Chu (1999). The lower initial water content in Lippensbroek indeed implied that a greater amount of saline water had to be added to reach saturated conditions. Chloride and Na concentrations were highest in the pore water of the Galgenschoor sediment, where they were also less affected by the salinity of the flooding water. This could be attributed to the higher initial salinity as this sediment originated from the brackish part of the estuary. TOC concentrations were quite variable (Table 4.2), but no clear trends or significant differences between treatments could be observed.

Table 4.2. Cl⁻, Na and TOC concentrations in the pore water of 4 different sediments flooded with water of 3 different salinities (mean of samples taken at different sampling times ± standard deviation)

Sediment	Treatment	Cl^- (g L^{-1})	Na (g L^{-1})	TOC (mg L^{-1})
Galgenschoor				
	Low salinity	4.16 ± 0.67	2.06 ± 0.33	5.9 ± 2.4
	Middle salinity	4.60 ± 0.80	2.32 ± 0.4	5.7 ± 1.3
	High salinity	5.68 ± 0.80	2.68 ± 0.39	6.6 ± 1.4
Kijkverdriet				
	Low salinity	0.44 ± 0.05	0.20 ± 0.02	11.6 ± 6.9
	Middle salinity	0.81 ± 0.07	0.35 ± 0.05	13.2 ± 7.1
	High salinity	1.40 ± 0.17	0.65 ± 0.09	14.9 ± 5.1
Kramp				
	Low salinity	0.21 ± 0.01	0.12 ± 0.02	4.3 ± 1.5
	Middle salinity	0.84 ± 0.07	0.35 ± 0.09	3.5 ± 2.2
	High salinity	1.45 ± 0.19	0.61 ± 0.15	5.4 ± 1.8
Lippensbroek				
	Low salinity	0.26 ± 0.02	0.08 ± 0.01	3.4 ± 2.8
	Middle salinity	1.28 ± 0.12	0.46 ± 0.05	6.7 ± 2.4
	High salinity	3.01 ± 0.30	1.03 ± 0.09	9.2 ± 2.6

Fe and Mn concentrations in the pore water are plotted as a function of time in Fig. 4.2. In Kijkverdriet, Fe and Mn concentrations were already high from the start of the experiment, and subsequently decreased. The highly reduced state of the Kijkverdriet sediment at the time of sampling probably implies the dominance of Fe/Mn-oxides reducing microbial colonies (Lovley & Phillips, 1988b), which promoted rapid reduction of Fe/Mn-oxides and formation of soluble Fe^{2+} and Mn^{2+}.

The decrease in Fe and Mn concentrations in the pore water may be due to continuing formation of insoluble Fe- and Mn-sulphides during the reduction of sulphates. Sulphides were abundant in the Kijkverdriet sediment from the beginning of the experiment (Table 4.1). The hypothesis of continuing sulphide formation was also confirmed by the redox potentials. In the Kijkverdriet sediment, the low redox potential at the beginning of the experiment (-168 mV) points to favourable conditions for sulphate reduction. The further decrease during the first 10 days of the experiment towards a quite stable value in the range of -260 to -280 mV indicated that Fe and Mn oxide reduction probably ended. Mn concentrations however suddenly increased around day 250. Due to the very high evaporation rates as a result of increased temperatures at that moment, the water table level lowered significantly in a very

short time. Moreover, a lot of fresh water had to be added to compensate this evaporation. As a result, increased oxygen concentrations probably resulted in a rapid oxidation of Mn-sulphides.

In Galgenschoor, Kramp and Lippensbroek, Mn concentrations first increased, followed by a decrease. The increase could be attributed to the activity of Fe/Mn oxides reducing microbial communities and resulting formation of soluble Mn^{2+} and Fe^{2+}. The solubilisation of Mn directly started after the beginning of the experiment. A peak was reached between day 50 and day 150, after which the Mn concentrations started to decrease. Significant Fe mobilisation only started after a lag period of about 50 days, which coincided with the period of increasing Mn concentrations. Some authors, such as Lovley & Phillips (1988a), indeed already reported that the reduction of Fe^{3+} does not begin before all Mn^{4+} is depleted. In the Lippensbroek soil however, Fe mobilisation started from the beginning of the experiment. This upland soil probably contains more easily reducible types of Fe-oxides and/or less easily reducible types of Mn-oxides. The Fe concentrations start to decrease after day 175 in the pore water of Kramp and Lippensbroek, whereas they keep increasing in the pore water of Galgenschoor. The decreases in the second part of the experiment (after day 100) could be attributed to the formation of insoluble Fe- and Mn-sulphides upon reduction of sulphates, which probably takes the upper hand. This hypothesis is strengthened by the observation of decreasing sulphate contents (Fig. 4.3) accompanied by a decrease in redox potentials. No significant sulphate reduction was expected to occur in the beginning of the experiment, based on the initially high redox potentials (+465, +466 and +470 mV for Galgenschoor, Kramp and Lippensbroek, respectively). During the experiment, redox potentials decreased to reach a final values in the range of -250 to -280 mV. The rate of the decrease depended upon the treatment. In all Kramp sediments, the decrease was rather gradual and the -150 mV value was not reached before day 60. This redox value is expected to coincide with dominating sulphate reduction, according to Gambrell et al. (1991). The redox potential already reached -150 mV between day 10 and day 20 in the Lippensbroek soil, with the decrease rate slowing down afterwards. A similar evolution was observed in the Galgenschoor sediment, but the period of fast redox potential decrease lasted until day 20 to 40.
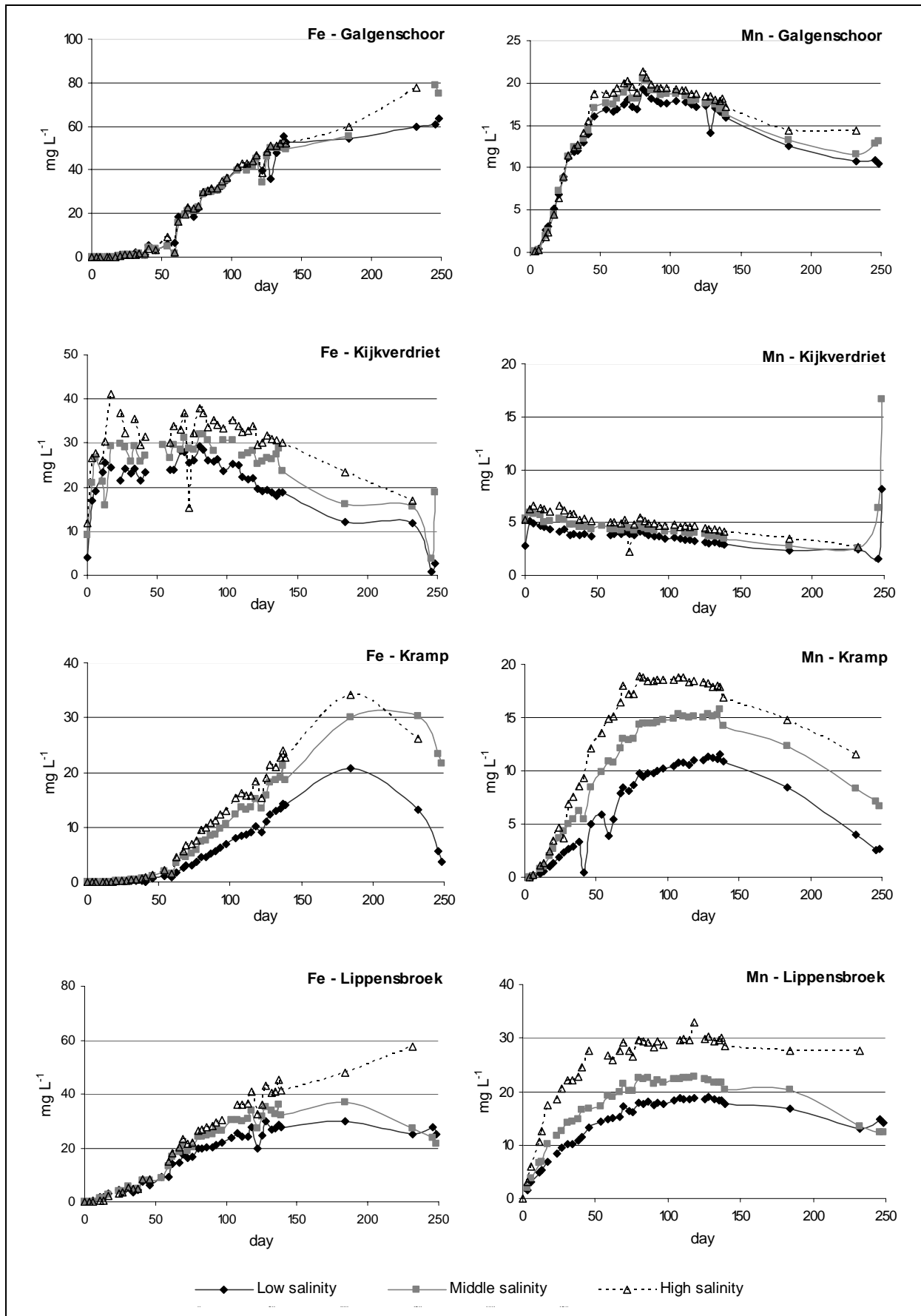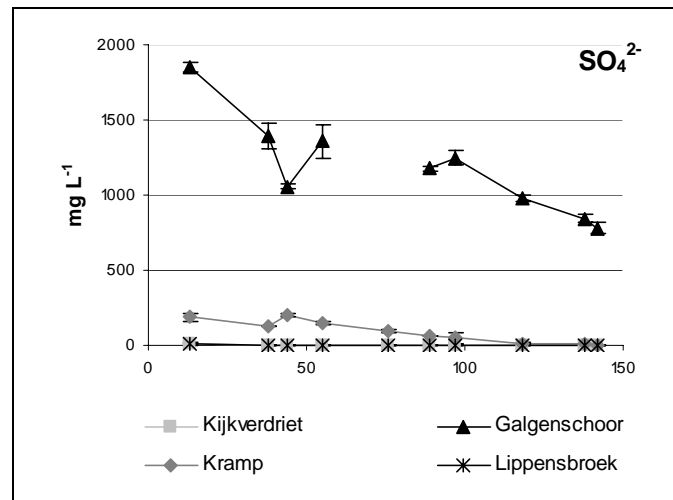
Fig. 4.2. Evolution of Fe and Mn concentrations in the pore water of 4 different sediments flooded with water of 3 different salinities, as a function of sampling time

The faster decrease of redox potentials in Galgenschoor compared to Kramp is reflected in the peak of Mn contents being reached earlier. Mn concentrations of Lippensbroek however did not decrease as fast as in Kramp or Galgenschoor regardless the fast decreases of the redox potential to below -150 mV. This is probably due to the lower sulphate availability (Fig. 4.3), which might be limiting for sulphide formation. Some authors, such as Salomons et al. (1987) indeed already reported limited sulphate reduction in freshwater environments, due to the lower sulphate availability. The hypothesis of sulphide formation is confirmed by an increase of up to 200 mg AVS kg^{-1} DM in the sediment of Galgenschoor after 250 days, coinciding with the significantly decreasing sulphate concentrations in solution (Fig. 4.3). It is however not confirmed for the sediments of Kramp and Lippensbroek, as no significant sulphide formation was detected. For a 10 mg L^{-1} decrease of Fe or Mn concentrations in the pore water, a sulphide production of about 6 mg S^{2-} L^{-1} pore water is needed. The method which was used to detect AVS was not sensitive enough to correctly detect such low concentrations, as the detection limit was about 10 mg S^{2-} kg^{-1} sediment. Not only increasing sulphide formation, but also decreasing availability of easily reducible Mn-oxides is expected to facilitate the decrease of Mn concentrations in the pore water. According to Salomons et al. (1987), the lower sulphate concentrations in freshwater environments may result in only a partial transformation of the iron hydroxides to iron sulphides, whereas the methanogenic state is reached relatively fast and part of the available reducible iron is converted to siderite ($FeCO_3$).

Fe and Mn mobilisation are both significantly facilitated by increasing salinity. The effects of salinity on Fe and Mn mobility were significant for the sediments of Kijkverdriet and Kramp (Table 4.3). For the Lippensbroek soil, the effect of salinity on the Mn mobility was significant at the 0.05 level, but the effect on the Fe mobility not. Furthermore, salt addition does not significantly affect Fe and Mn mobility in the Galgenschoor sediment (p = 0.995 and p = 0.461, respectively), which was already very saline before adding the saline flooding water (Table 4.2). According to Khattak et al. (1989), salt addition does not affect the reduction of Mn oxides, but it induces a pH decrease. In combination with reducing conditions, this low pH creates a favourable environment for solubilisation of Mn, whereas Mn^{2+} - Ca^{2+}/Na^+ exchange reactions prevent the sorption of Mn to soil particles. Free Mn will immediately form soluble $MnCl_2$ in the presence of chlorides (Khattak et al., 1988, Hatje et al., 2003).

Table 4.3. Significance of the effect of salinity on metal concentrations in the pore water (p-values of oneway Anova)

	Galgenschoor	Kijkverdriet	Kramp	Lippensbroek
Fe	0.995	0.000	0.039	0.085
Mn	0.461	<0.001	<0.001	<0.001
Cd	0.393	0.254	0.030	<0.001
Cu	0.600	0.029	0.357	0.878
Ni	0.889	0.159	0.092	0.060
Zn	0.525	0.863	0.207	<0.001

Fig. 4.3. Evolution of SO_4^{2-} concentrations in the pore water of the sediments of Kijkverdriet, Galgenschoor, Kramp and Lippensbroek (mean of all salinity treatments ± standard deviation)

Cd concentrations in the pore water of the different sediments are plotted as a function of sampling time in Fig. 4.4. In the reduced, sulphide-rich sediment of Kijkverdriet, Cd concentrations in the pore water continuously remained below the Flemish background level of 1 µg L^{-1}. This can be attributed to the fact that Cd-sulphides are highly insoluble (e.g. Salomons et al., 1987; Zumdahl, 1992; Simpson et al., 2000; Guo et al., 2001). In the initially oxidised sediments, increasing Cd concentrations are observed during the first 20 days. This is followed by a rapid decrease until day 50, after which the Cd concentrations remained very low.

The Cd concentrations in the pore waters of Galgenschoor and Kramp rapidly decreased to below the background value of 1 µg L^{-1}. For the Lippensbroek soil however, it took about 150 days to reach the background value. The peak concentrations, which were reached before day 20, often significantly exceeded the Flemish remediation standard for ground water (5 µg L^{-1}).

The decreasing concentrations in the second part of the experiment can be attributed to Cd-sulphide formation. This hypothesis is strengthened by the fact that background values were not easily reached in the Lippensbroek soil after day 50. In that soil, sulphide formation was probably hampered due to the low sulphate availability (Fig. 4.3).
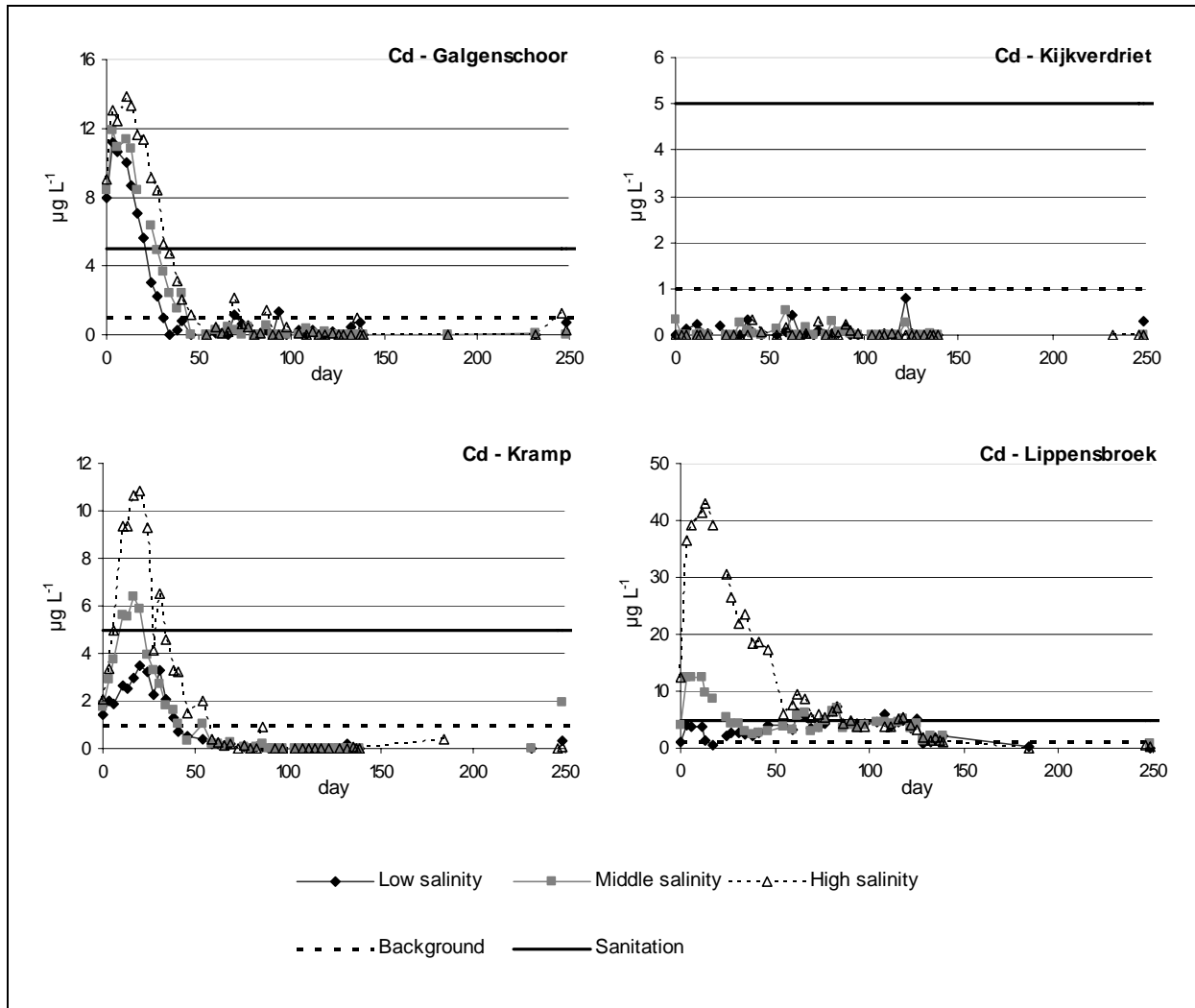
Fig. 4.4. Cd concentrations in pore water (µg L^{-1}) of 4 different sediments flooded with water of 3 different salinities, as a function of sampling time; background levels and sanitation thresholds for groundwater according to Vlarebo (1996)

The Cd concentrations were primarily affected by the salinity of the flooding water in Kramp and Lippensbroek, the sediments from the freshwater part of the estuary that initially were oxidised (Table 4.3). Increasing salinity clearly resulted in an increasing Cd mobility. This has also been reported by other authors (Paalman et al., 1994 & Hatje et al., 2003). It coincided with the observation of Gerringa et al. (2001). They found that high salinities resulted in a faster release of Cd from CdS during the oxidation of reduced Scheldt sediments.

This release was found to be stimulated by both formation of chloride complexes and ion exchange. The effect is most significant in the Lippensbroek soil ($p < 0.001$), for which the salinity treatment caused a very strong increase in salinity of the pore water compared to the sediments natural conditions. A Cd concentration of up to 45 µg L^{-1}, which coincides with 9 times the sanitation treshold, was observed after flooding with high salinity water. Even when the Lippensbroek soil was subjected to a salinity of only 0.5 mg NaCl L^{-1}, the remediation threshold was regularly exceeded. This suggests that subjecting polluted freshwater sediments or soils to a saline environment may, at least temporarily, create a significant environmental hazard. Increasing the salinity of the flooding water of the already saline Galgenschoor sediment did however not influence Cd mobility significantly ($p = 0.393$), but it also did not affect the salinity of the pore water, as has previously been discussed. In contrast with the mobility of Fe and Mn, Cd mobility in the Kijkverdriet sediment was not significantly affected by the salinity ($p = 0.254$), probably because of the much lower solubility of Cd sulphides compared to sulphides of Fe and Mn (Zumdahl, 1998).

To check whether sulphide abundance and formation interfere with the effect of salinity on Cd mobility, the highest observed Cd concentration in the pore water of each treatment during the first 50 days was plotted as a function of the average Cl^{-} concentration in the pore water (Fig. 4.5). The decreasing slope of the linear regression curve indeed suggests that the effect of pore water salinity on Cd mobility decreases when sulphides are abundant or being formed. Kijkverdriet sediment initially contained a significant amount of sulphides, which explains the low and constant Cd concentrations in the sediment solution that are largely unaffected by salinity. Contrarily, the largest effect of salinity on Cd mobility was observed for Lippensbroek, where sulphate availability was limiting for sulphide formation. Sulphides were initially not detected in Kramp, but were slowly being formed during sulphate reduction (Fig. 4.3). This increasing effect of sulphides might explain the somewhat smaller salinity effect in this sediment. In the Galgenschoor sediment, sulphides were rapidly being formed (Fig. 4.3), which resulted in an even lower effect of salinity, and thus a smaller slope compared to the one of Kramp. The suppression of salinity effects on metal mobility in the abundance of sulphides was already observed by Salomons et al. (1987). They calculated the influence of the sulphide concentration on the speciation and solubility of Zn, Cu and Cd in a solution with a chlorinity of 5000 mg L^{-1} and an alkalinity of 50 meq L^{-1}. The effect of the presence of chlorides on the solubility of Cd, Cu and Zn was indeed only observed at low sulphide concentrations.
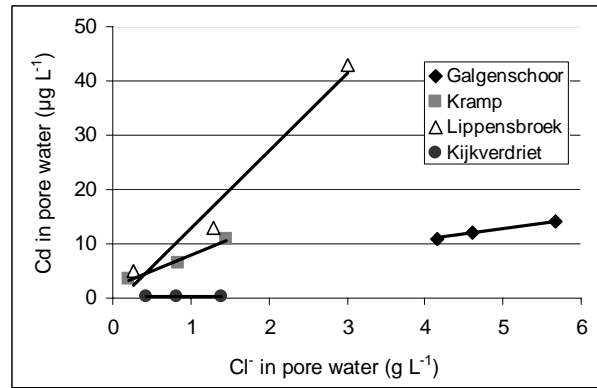
Fig. 4.5. Highest observed Cd concentration in the pore water of each treatment during the first 50 days plotted as a function of the average Cl⁻ concentration in the pore water

The Cu concentrations in the pore water rarely exceeded background levels (Fig. 4.6). They were lowest in the initially reduced sediment of Kijkverdriet and exhibited a decreasing trend with time in the sediments which were initially more oxidised. Contrary to Cd, the decreasing trend starts from the beginning of the experiment. This might be due to the lower solubility product of Cu sulphides (Zumdahl, 1998) but also to the strong association of Cu with organic matter (e.g. Kalbitz & Wennrich, 1998; Alvim Ferraz & Lourenço, 2000), of which the decomposition rate significantly decreases under more reducing conditions (e.g. Reddy & Patrick, 1975). Moreover, coinciding with the evolution of redox potentials, the decrease of Cu contents was slowest in Kramp, which suggests that the decreasing trend is indeed initiated by sulphide formation. Moreover, Cu concentrations reach very low values in the Lippensbroek soil after day 50, which was not observed for Cd. This might again be attributed to the lower solubility product of Cu-sulphides compared to Cd-sulphides, which especially becomes important in conditions of limiting sulphate availability (Fig. 4.3). Indeed, contrary to Cd, salinity does not significantly affect Cu mobility (p = 0.600, 0.357 and 0.878 for Galgenschoor, Kramp and Lippensbroek, respectively). This could be due to the lower stability of Cu-chloride complexes combined with the lower solubility product of Cu-sulphides, which would both explain a lower Cu solubility. Little attention should be given to the significant salinity effect in the Kijkverdriet sediment, as most of the data for that sediment are very close to the detection limit of 2 $\mu g\ L^{-1}$.

Fig. 4.6. Cu concentrations in pore water (µg L^{-1}) of 4 different sediments flooded with water of 3 different salinities, as a function of sampling time; background levels and sanitation thresholds for groundwater according to Vlarebo (1996)

Ni concentrations increased from the beginning of the experiment in pore water of the initially oxidised sediments (Fig. 4.7). The evolutions of Ni concentrations are very similar to those of Fe and Mn, which suggests that Ni is mainly associated with Fe/Mn-oxides. The direct mobilisation after the beginning of the experiment, which was also observed for Mn but not for Fe, and the fact that Ni mobilisation does not accelerate from day 50 in contrast with the Fe mobilisation both suggest that Ni is primarily associated with Mn oxides. Ni was indeed already reported to primarily co-precipitate with Mn oxides in salt marsh sediments from the Western Scheldt (Zwolsman et al. (1993) in: Callaway et al., 1998). This is also reflected in the lower Pearson correlation coefficient of 0.361 between Ni and Fe compared to 0.781 between Ni and Mn. Whereas the Mn mobilisation was significantly affected by the salinity, Ni mobility was not (p = 0.889, 0.159, 0.092 and 0.060 for Galgenschoor, Kijkverdriet,

Kramp and Lippensbroek, respectively). This could be due to a combination of the higher stability of Mn-chlorides compared to Ni-chloride complexes and the lower solubility of Ni-sulphides compared to Mn-sulphides. The decreasing Ni concentrations in the second part of the experiment may indeed be due to the continuing formation of insoluble Ni-sulphides during the reduction of sulphates (e.g. Salomons et al., 1987; Simpson et al., 2000). Probably for the same reason, the Ni concentrations in pore water of the initially reduced Kijkverdriet sediment were continuously low. They however suddenly increase around day 250. As already mentioned when discussing the evolution of Mn concentrations, oxygen supply due to increased evaporation rates at the end of the experiment probably have lead to a rapid oxidation of sulphides and solubilisation of associated metals. Higher salinities clearly seem to promote this solubilisation.
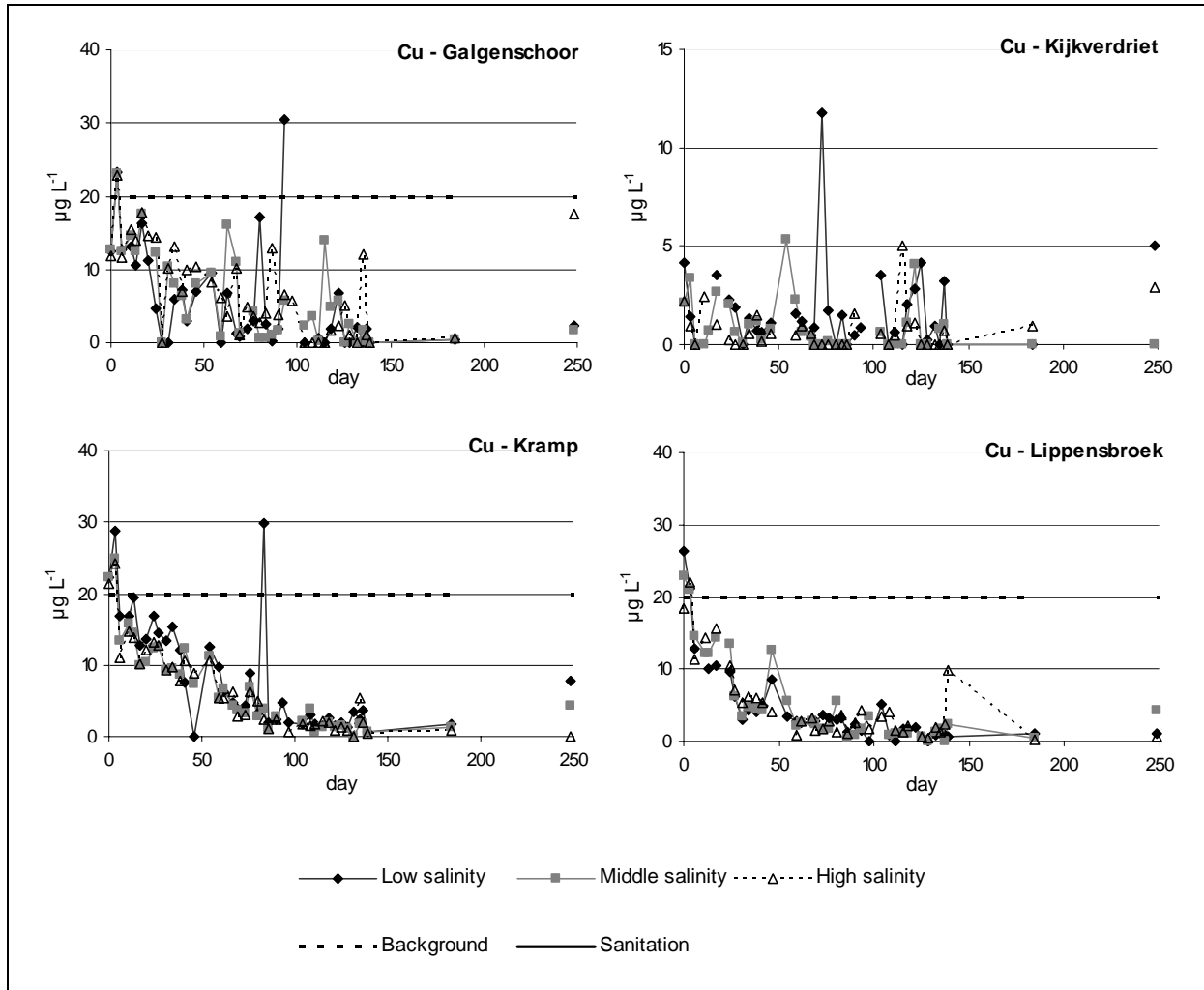

Fig. 4.7. Ni concentrations in pore water (µg L^{-1}) of 4 different sediments flooded with water of 3 different salinities, as a function of sampling time; background levels and sanitation thresholds for groundwater according to Vlarebo (1996)

Zn concentrations generally exceeded background values in the first part of the experiment, but they remained far below the threshold values for sanitation. They decreased after a relatively small initial increase. Trends seem to be similar to those of Cd, but fluctuations were much larger. According to Millward & Liu (2003), Cd and Zn mobilisation are subject to the same driving forces: chlorocomplexation and competition with Ca, Mg and Na. Their similar mobilisation behaviour is confirmed by the significant Pearson correlation between both metal concentrations in the pore water (R = 0.490). Zn concentrations however more slowly decreased in the second part of the experiment, compared to Cd concentrations. This was also observed by Simpson et al. (2000) and can be attributed to the significantly higher solubility of Zn sulphides than Cd sulphides. As for Cd, Fe and Mn, mobilisation was promoted by increasing salinities in sediments originating from the freshwater part of the estuary. For the Lippensbroek soil, this salinity effect was significant (p < 0.001). Increasing salinities also promoted Zn mobilisation in the sediment of Kramp during the first 75 days. In the second part of the experiment, however, the highest Zn concentrations were found in the pore water of the low salinity treatment, which caused the overall effect to be evaluated as non significant in statistical analysis (p = 0.207).
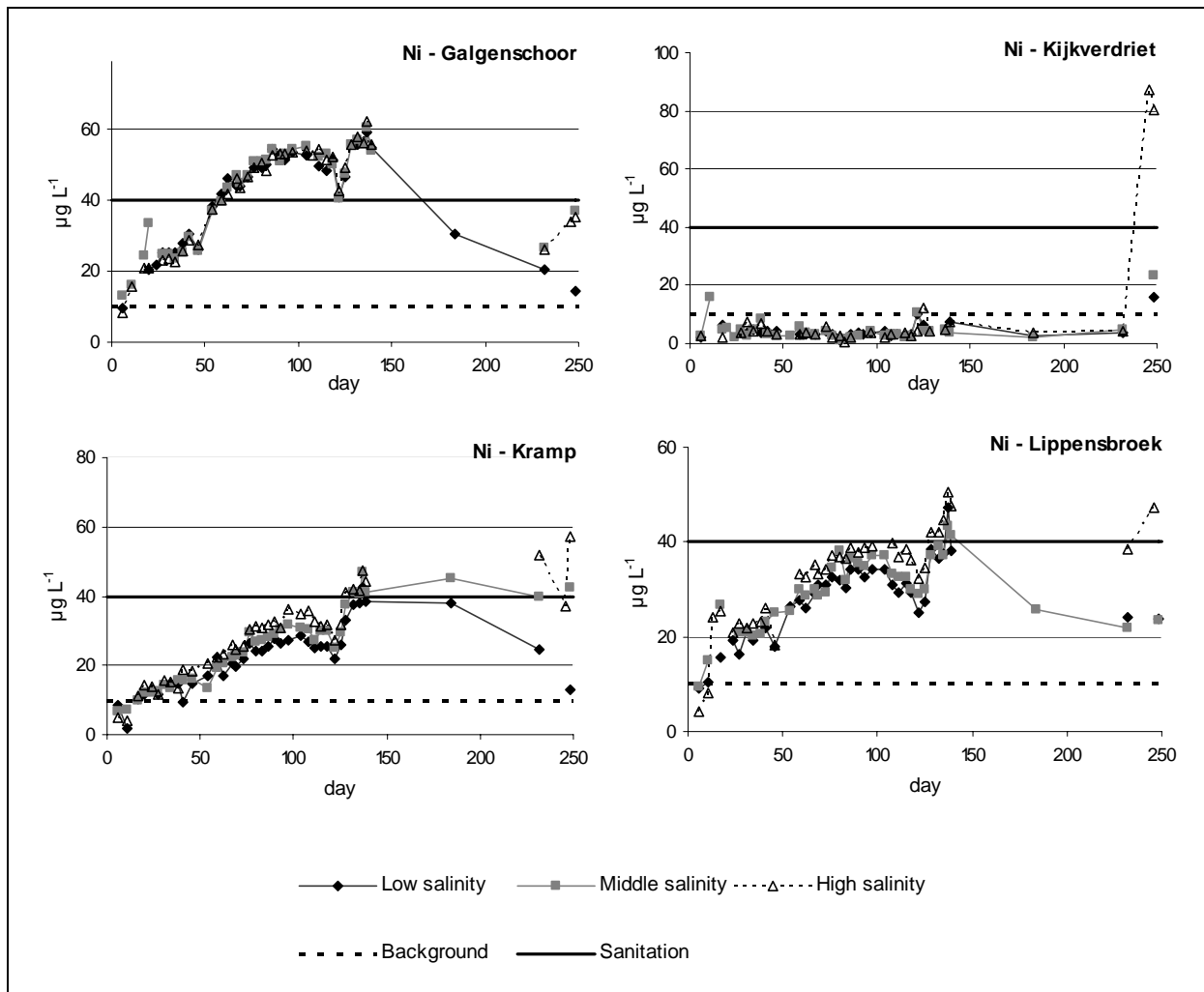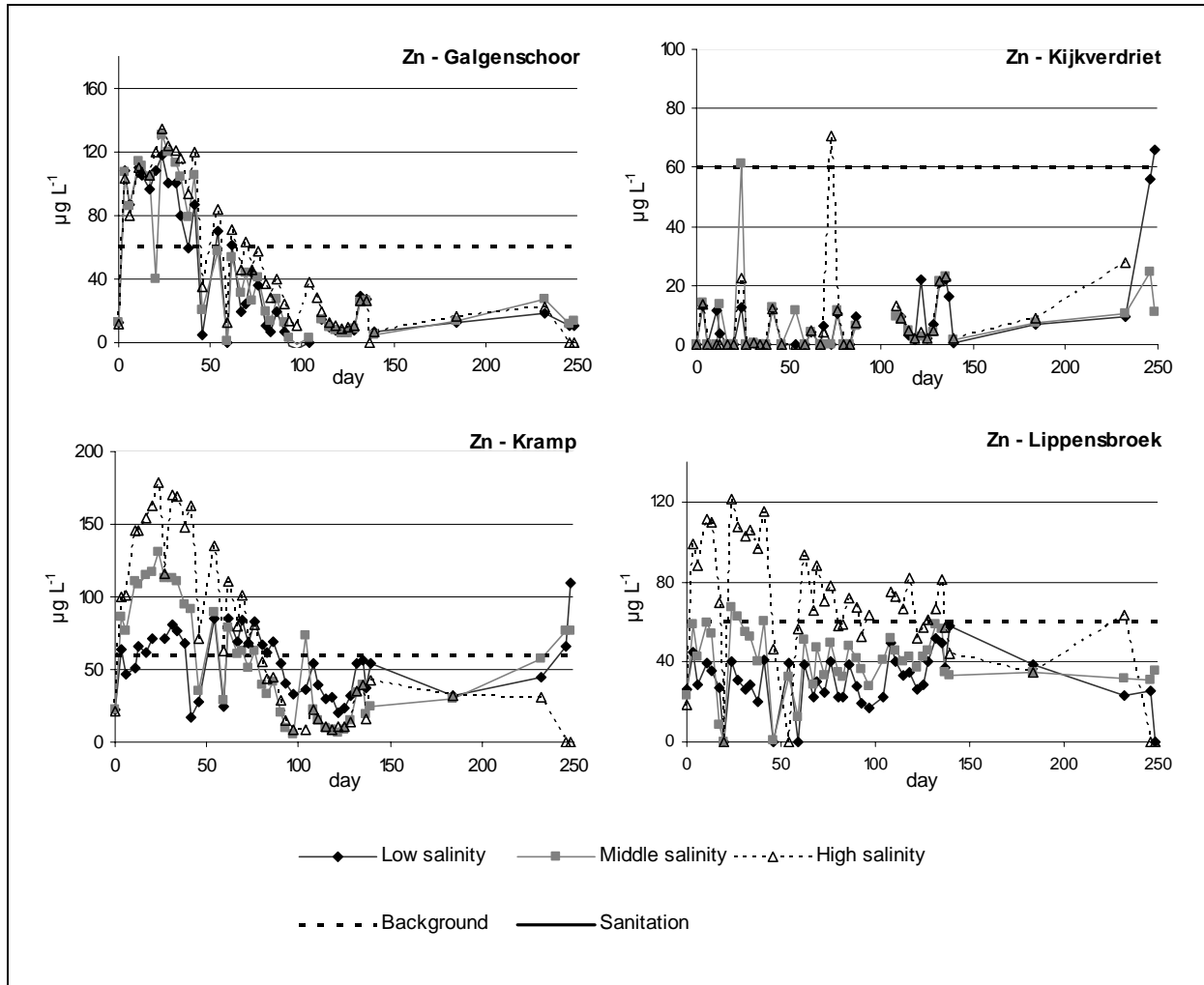
Fig. 4.8. Zn concentrations in pore water (µg L^{-1}) of 4 different sediments flooded with water of 3 different salinities, as a function of sampling time; background levels and sanitation thresholds for groundwater according to Vlarebo (1996)

Cd, Cu and Ni concentrations in the surface water samples and the Flemish surface water quality standards are presented in Table 4.4. Cd concentrations in the surface water were highly variable and generally lower than in the pore water of the sediments. If contacted with the reduced, sulphide-rich Kijkverdriet sediment, the Cd concentrations meet the quality standard of 1 µg L^{-1} defined by the Flemish environmental legislation. However, when sediments from the freshwater part of the estuary were contacted with water of salinities above 2.5 g NaCl L^{-1}, the Cd concentrations in the surface water often significantly exceeded the quality standard, and even an outlier of 42 µg L^{-1} was measured once. Coinciding with Cd concentrations in the pore water, the concentrations in the surface water are significantly affected by the salinity of the flooding water in the initially oxidised, low-salinity sediments (p < 0.001 for Lippensbroek and Kramp). They were not significantly affected in the already

saline sediment of Galgenschoor (p = 0.279) or the sulphide-rich sediment of Kijkverdriet (p = 0.459). The temporal variations of Cd concentrations in the surface water and the effect of salinity are clearly demonstrated in Fig. 4.9. The significant temporal variations can be attributed to the surface water being exposed more to fluctuations in temperature, evapotranspiration, biomass production, etc. compared to the pore water. Zn concentrations rarely exceeded the detection limit and are therefore not presented in Table 4.4. Cu and Ni concentrations never exceeded their quality standards of 50 μg L^{-1}. They were subjected to large variations, but they are not significantly affected by salinity. Cu concentrations were significantly lower and Ni concentrations significantly higher in surface water that was in contact with the reduced Kijkverdriet sediment, compared to the other, more oxidised sediments. Fe and Mn concentrations were also very high in the surface water above this sediment. They reached peak concentrations up to 78 mg L^{-1} for Fe and 29 mg L^{-1} for Mn at day 41. This might be attributed to the reduction of Fe/Mn-oxides. As a result, associated Ni might have been released, as was also already observed in the pore water. Fe and Mn concentrations however significantly decreased after day 41, which might be due to the formation of sulphides, which probably also account for the low Cd and Cu concentrations.

Table 4.4. Cd, Cu and Ni concentrations in the surface water samples of 4 different sediments flooded with water of 3 different salinities (mean of samples taken at different sampling times ± standard deviation) and the Flemish surface water quality standards

Sediment	Treatment	Cd (μg L^{-1})	Cu (μg L^{-1})	Ni (μg L^{-1})
Galgenschoor				
	Low salinity	0.85 ± 0.53	13.5 ± 4.9	5.7 ± 3.7
	Middle salinity	1.15 ± 0.80	12.6 ± 4.6	5.5 ± 3.6
	High salinity	6.20 ± 13.41	13.4 ± 3.8	12.4 ± 22.7
Kijkverdriet				
	Low salinity	0.06 ± 0.13	2.6 ± 2.8	9.4 ± 2.7
	Middle salinity	0.15 ± 0.24	3.8 ± 2.5	10.7 ± 3.3
	High salinity	0.25 ± 0.49	4.8 ± 4.9	10.3 ± 3.1
Kramp				
	Low salinity	0.69 ± 0.41	15.8 ± 6.1	7.6 ± 13.5
	Middle salinity	1.24 ± 0.61	15.4 ± 3.6	5.5 ± 4.0
	High salinity	2.50 ± 1.29	15.4 ± 4.0	3.9 ± 3.8
Lippensbroek				
	Low salinity	0.57 ± 0.22	16.5 ± 4.3	3.9 ± 2.1
	Middle salinity	1.56 ± 0.54	15.9 ± 4.0	2.4 ± 1.7
	High salinity	4.56 ± 1.95	16.4 ± 5.1	4.2 ± 3.8
Flemish surface water quality standard		1	50	50

Not only the Cd concentration in surface and pore water, but also the uptake of metals by duckweed was affected by the salinity. The Cd concentrations in duckweed increased by a factor 4 when the salinity of the flooding water increased from 0.5 to 5 g NaCl L^{-1}, reaching up to a maximum of 126 mg kg^{-1} DM in the Lippensbroek treatment. A highly significant Pearson correlation (R = 0.934) was found between Cd concentrations in the duckweed and Cd concentrations in the surface waters at day 137, just before transplanting the duckweed to the recipients. Greger et al. (1995) studied the effect of salinity on the uptake of Cd by the submerged macrophyte *Potamogeton pectinatus* from both water and sediments. Cd uptake from the water was found to decrease when increasing the salinity up to 10 ‰. However, in the presence of sediments, Cd uptake increased with increasing salinity. This clearly illustrates the fact that increasing salinity promotes Cd desorption from the sediment, which results in increased Cd concentrations in the water column and thus an enhanced Cd uptake by organisms. It however also promotes the formation of Cd chloride complexes in the water column, which seem to be less bio-available, especially at higher salinities. As a result, a decreasing bioavailability and toxicity of Cd with increasing salinity of the surface water was found for a series of aquatic organisms (e.g.Fischer, 1986; McLusky et al., 1986; Stronkhorst, 1993; Zanders & Rojas, 1996; Verslycke et al., 2003).

The duckweed plants were however not able to survive in the surface water above the Kijkverdriet sediment. This might be attributed to the very reduced state of this sediment, which probably accounted for the accumulation of various phytotoxins, that are by-products of sediment reduction, such as organic components, soluble sulphide species and reduced Fe and Mn (Pezeshki, 2001). The Cu and Zn concentrations in the duckweed were slightly affected by the salinity in the treatments of Lippensbroek and Galgenschoor. As the total Cu concentrations in the surface water and the pore water were however not influenced by the salinity, changes in speciation of Cu might have caused an increased bio-availability without affecting the mobility. The Ni uptake by duckweed was not affected by the salinity.
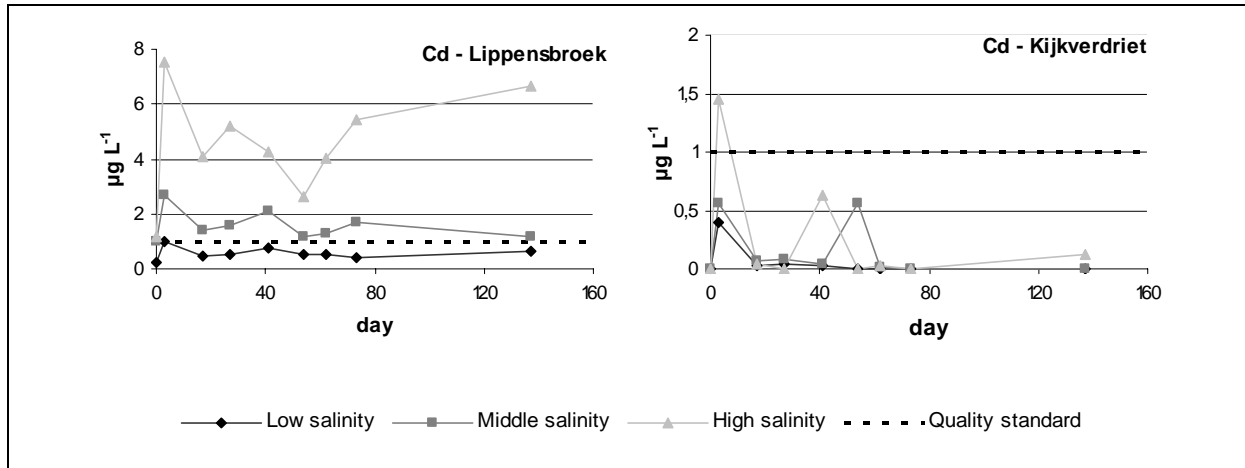
Fig. 4.9. Temporal variation of Cd concentrations in the surface water samples of the sediments of Lippensbroek and Kijkverdriet, flooded with water of 3 different salinities; quality standard represents the Flemish surface water quality standard

4.4 Conclusion

Salinity primarily enhances Cd mobility. Cd concentrations in pore water of soils and sediments and surrounding surface waters significantly exceeded sanitation tresholds and quality standards during flooding of initially oxidized sediments. The effect was observed already at lower salinities of 0.5 g NaCl L^{-1}. This implies that risks related to Cd leaching to ground water and Cd uptake by organisms would be created when constructing flooding areas in the metal-polluted zones between Temse and the Scheldt river mouth. These risks can be reduced by inducing sulphide precipitation because Cd is then immobilised as sulphide and not anymore reacting to changes in salinity. This could be achieved by permanently flooding the polluted sediments, because sulphates are sufficiently available in the river water of the brackish part of the estuary. Microbial sulphate reduction is not expected to be limited by organic matter availability as biomass turnover is very high in intertidal reedbeds. Stimulation of sulphide formation should however be done with great caution as accidental oxidation of the sulphide-rich sediments could also create a significant risk for metal mobilisation, which is facilitated by increasing salinity. In that way, a "chemical time bomb" can indeed be created. Especially in the freshwater sediments and soils, low sulphate availabilities might be limiting for sulphide formation.

5.

Effect of decomposing litter on metal mobility

5 Effect of decomposing litter on metal mobility

5.1 Introduction

When mudflats get banked up or when controlled flooding areas are being constructed, emergent vegetation, such as reed plants and willows, can colonise the marshes. Uptake of metals by these plants directly reduces the metal input into the adjacent waters. Moreover, as these plants are characterised by a very high biomass production and turnover, the upper soil layer will be enriched by organic matter originating from rooting and the decay and decomposition of plant parts. As such, these plants can especially provide a sink if during decomposition metals are bound to the litter by passive sorption on the organic surfaces or by physiological mechanisms of microbial colonizers of the litter. However, the litter can also act as a metal source when microbial activity mobilises metals or when it becomes available to deposit feeders. Several studies suggest that metals in litter are available to deposit feeders and thus can enter estuarine food webs (Weis & Weis, 2004). Consumption of metal-laden detritus can cause metal accumulation and deleterious effects in higher trophic levels (Dorgelo et al., 1995; Du Laing et al., 2002; Weis et al., 2002). The role of plant sequestration of metals into long-term sinks depends on the rate of uptake by the plant, rates of translocation and retention within individual tissue types, and the rate and mode of tissue decomposition (Catallo, 1993; Kadlec & Knight, 1996).

This chapter aims to assess the role of decomposing organic matter in wetland metal cycles. In paragraph 5.2, results of a greenhouse experiment aiming at the assessment of potential effects of litter decomposition on metal mobility and availability will be discussed. This greenhouse experiment was supplemented by a field experiment to study metal fate upon litter decay and decomposition under more natural conditions. The results of this experiment are discussed in paragraph 5.3.

5.2 Greenhouse experiment

5.2.1 Materials and methods

5.2.1.1   Experimental setup

Soil was excavated in August 2003 at Lippensbroek, which is a polder of about 13 hectares, situated near the river Scheldt in Hamme. The soil is heavily polluted by metals. Scheldt water regularly inundates the polder by means of a lock system since 2005. As such, it is now being used as an experimental, pilot-scale controlled flooding area.

A part of the upper 50 cm soil layer was excavated. Plant roots and litter were removed. To homogenize the soil, it was sieved over a sieve of 8 mm mesh width and the fraction larger than 8 mm was removed. This soil was intensively mixed with different types and concentrations of organic matter originating from reed stems, reed leaves and willow leaves. Reed plants and willows are the most abundant plant species on freshwater and brackish-water marshes along the river Scheldt. They are characterised by a high biomass production rate. The plant material was collected in Kijkverdriet along the river Scheldt and in Drongen along the river Leie, at the end of the growing season. Sheaths of the reed plants were removed from the leaves. The plant material was dried in an oven at 50 °C and ground in a hammer-cross beater mill (SM 2000 Retsch GmbH & Co., Haan, Germany) to pieces smaller than 2 mm.

The first treatment consisted of the blank soil (1), to which no organic matter was added. In the second (2) and third (3) treatment, 3.5 % and 7 % of reed leaves were added to the soil on a dry weight base, respectively. In the fourth (4) treatment, 7 % of willows leaves were added to the soil on a dry weight base. This enabled us to compare the effects of reed and willow leaves. In the fifth (5) and sixth (6) treatment, 10 % and 20 % of reed stems were added to the soil on a dry weight base, enabling a comparison between the effect of reed leaves and stems. The fact that concentrations chosen for reed stems are about 3 times higher than those for reed leaves is based on the 3 times higher biomass production of reed stems compared to leaves at the Scheldt marshes (Soetaert et al., 2004).

Each mixture was distributed over 2 recipients of 32 x 24 x 20 cm. The recipients had drainage outlets with a 3 mm diameter at the bottom. Geotextile was put on the bottom of the recipients to prevent soil export through the drainage outlets. Every recipient was provided with a soil layer of 10 cm thickness. The soils in the recipients could be alternately flooded and drained by putting the recipients in and taking them out of a larger recipient (48 x 32 x 22 cm) filled with deionised water. Each mixture was subjected to 2 flooding regimes: one was permanently flooded (PER) and one was alternately 2 weeks flooded and 1 week emerged (ALT). The permanently and alternately flooded regimes represent the situation at younger and older marshes, respectively.

During the flooded periods, the water table was kept at 5 cm above the soil surface. Evaporation was compensated for by regularly adding deionised water. During the emerged periods of the alternately flooded regimes, the recipients filled with soil were lifted from the larger recipients filled with water and allowed to drain. The percolates were collected, analysed and replaced by freshly prepared deionised water before starting each new flooded period. All recipients were put in a greenhouse at a temperature controlled between 15 and 25°C. An overview of the different treatments is given in Table 5.1, whereas a scheme of the experimental setup is plotted in Fig. 5.1. It was not feasible to duplicate the experimental setup as we aimed to conduct the experiment with different types and concentrations of organic matter, meanwhile maintaining a relatively high temporal sampling resolution. Therefore, the effect of soil heterogeneity was diluted by duplicating the sampling procedure (see sampling section).

Table 5.1. Overview of the different treatments

	Permanently flooded	Alternately 2 weeks flooded and 1 week emerged
blank soil	1 PER	1 ALT
3.5 % reed leaves added	2 PER	2 ALT
7 % reed leaves added	3 PER	3 ALT
7 % willow leaves added	4 PER	4 ALT
10 % reed stems added	5 PER	5 ALT
20 % reed stems added	6 PER	6 ALT
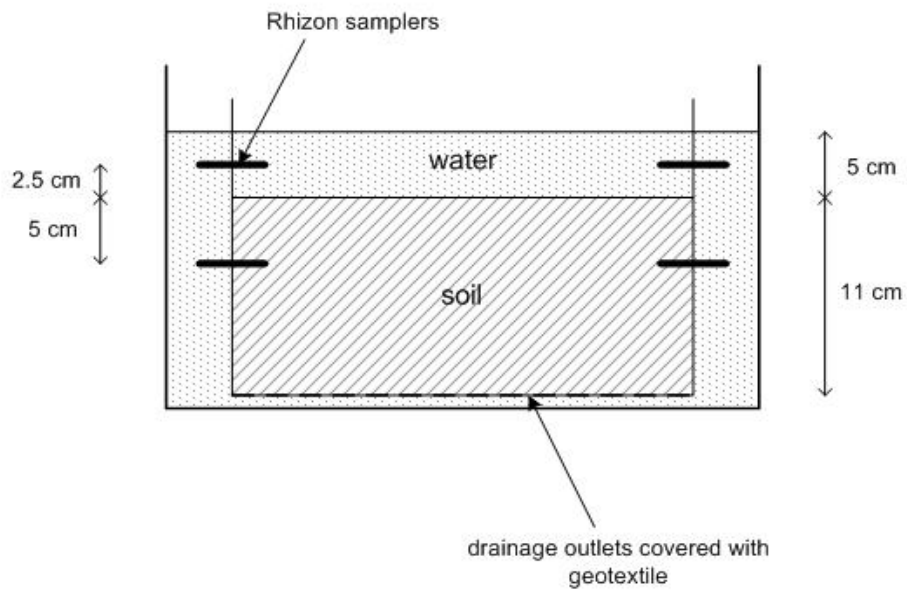
Fig. 5.1. Scheme of the experimental setup

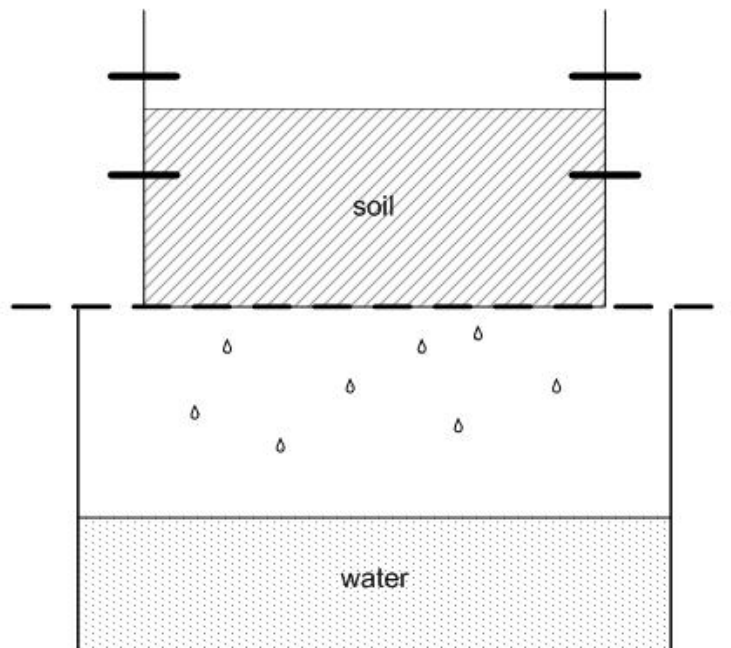To study the effect of organic matter addition on plant bioavailability, reed plants, duckweed and willow cuttings (*Salix cinerea*) were transplanted to the recipients around day 150. Each recipient was divided into 3 compartments by inserting plastic partition walls in the soils. One compartment was not planted to allow for continued pore water sampling without interference with growing plant roots. All aboveground biomass of the reed plants was abscised. Two of the reed plant root plugs and seven willows cuttings were planted in the soils of the other two compartments of each treatment, whereas the floating duckweed plants were transplanted to the surface water of the permanently flooded soils. Around day 250, sublethal plant stress which might be induced due to the movement of metal ions into photosynthetic tissues (Windham et al., 2003) was checked by measuring the chlorofyl a fluorescence of reed and willow leaves by means of Pulse Amplitude Modulation (PAM) Fluorometry (Macinnis-Ng & Ralph, 2002). The willows, reed plants and duckweed were harvested afterwards, to investigate whether their metal uptake and biomass production rate were affected by the different treatments.

5.2.1.2   Sampling

Each recipient was fitted with four Rhizon soil moisture samplers (type MOM, Rhizosphere Research Products, Wageningen, The Netherlands) to sample pore water and surface water. These samplers aim at smoothly extracting soil solutions during field experiments under the natural moisture conditions, meanwhile filtering the sample. They consist of a filter tube with a diameter of 2.5 mm and a length of 10 cm, which is connected to a PVC/PE tube. The filter tube consists of a polyethersulfone filter membrane with a pore diameter of <0.2 μm. To extract pore water, a vacuum can be built up inside the sampler by connecting a 10 mL syringe or a vacuum tube to the PVC/PE tube (Fig. 5.2). Two samplers were installed horizontally at 5 cm below the soil surface to sample soil pore water. The two other samplers were installed to sample surface water at 2.5 cm above the soil surface, coinciding with 2.5 cm below the water surface (Fig. 5.1). To install them, holes were made in the recipient walls, through which the samplers were injected into the soil. Silicone was used to ensure that all connections were waterproof.
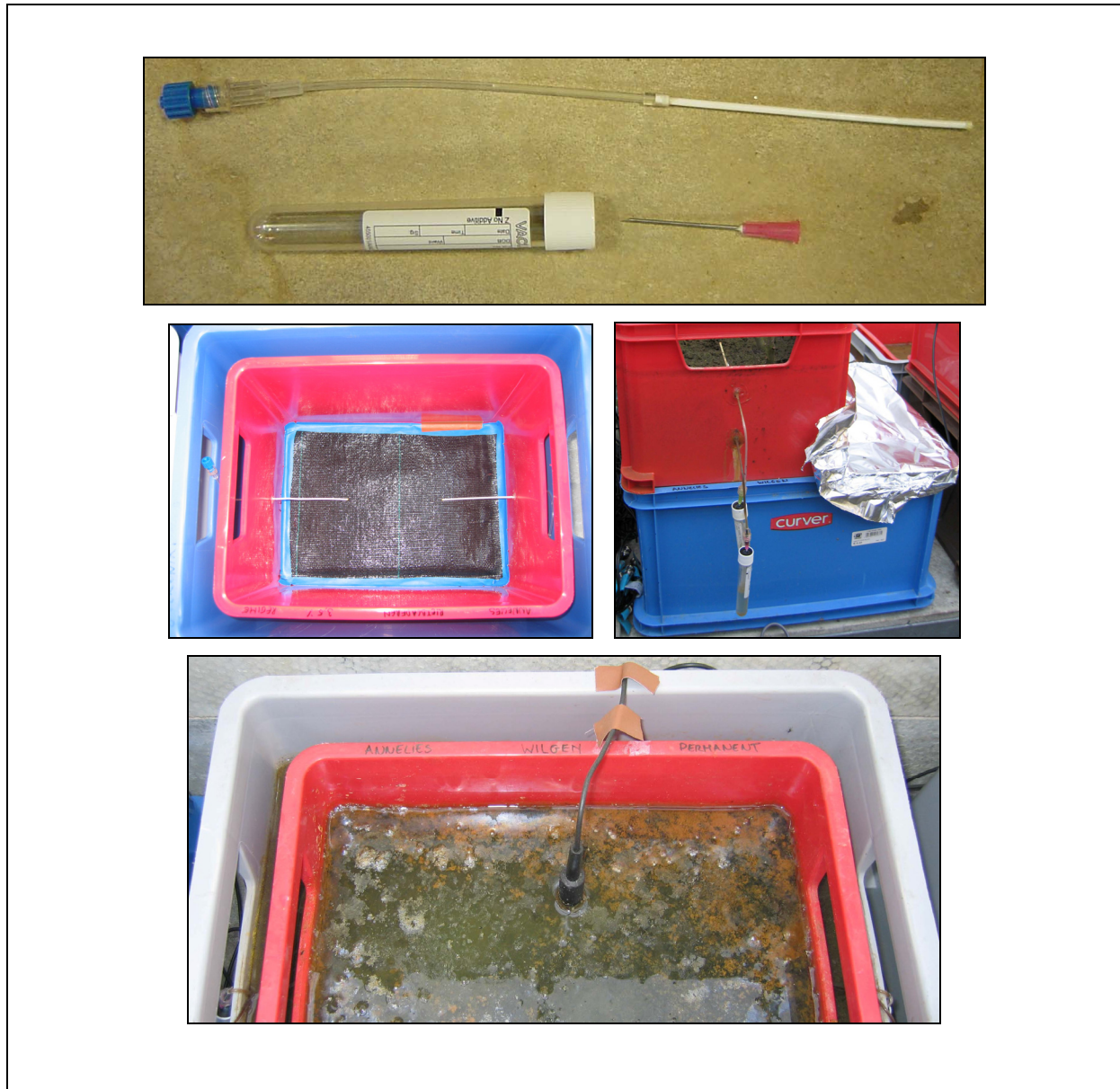
Fig. 5.2. Pictures of the experimental setup, above: Rhizon soil moisture sampling set, middle left: recipient before filling with soil, middle right: pore water sampling during emerged period, below: redox electrode during flooded period

During the filling of the recipients, soil samples were taken to analyse the physico-chemical soil properties. Around days 150 and 250, several core samples were taken from the soil of each recipient to check for possible changes in these physico-chemical soil properties during the experiment. To homogenise the soils preceding the analyses, they were dried overnight at 70°C and ground in a hammer-cross beater mill. This was however not done for the determination of acid volatile sulphide contents, as drying can induce oxidation of the sulphides (Salomons et al., 1987). At the end of the experiment, willow leaves, reed plants

and duckweed were harvested. Duckweed plants were thoroughly washed with deionised water and entirely analysed for metal contents without separating roots from leaves. Leaves and stems of the reed plants were abscised from the belowground plant parts, whereas leaves and stems of the willows were abscised from the cuttings. They were thoroughly washed with deionised water. Reed and willow stems were separated from the leaves. All individually collected leaves and stems were pooled into willow leaf, willow stem, reed leaf and reed stem material of each treatment. This was subsequently dried overnight at 70°C, weighed, ground in a hammer-cross beater mill and thoroughly homogenised preceding the analyses.

5.2.1.3 Analyses

All glassware and containers used were washed in a laboratory dishwasher, subsequently soaked overnight in 5 % ultrapure 65 % HNO_3 and rinsed with deionised water. Pseudo-total metal contents in the soils were determined by an aqua regia destruction (Ure, 1990) and analyses with ICP-OES (Varian Vista MPX, Varian, Palo Alto, CA). Acid volatile sulphide (AVS) was determined by transformation of sulphide to H_2S and absorption in a Zn-acetate solution, followed by a back titration (Tack et al., 1997a). The pH was measured in a 1/5 sediment/distilled water suspension after equilibration during 18 h (Van Ranst et al., 1999). The conductivity was measured in the filtrate of a 1/5 sediment/distilled water suspension, shaken for ½ h (Van Ranst et al., 1999). The organic matter content was determined by measuring the weight loss after incineration of oven-dried samples (2 h at 450°C). The carbonate content was determined by back-titration (with 0.5 M NaOH) of an excess 0.25 M H_2SO_4 added to 1 g of sediment (Nelson, 1982). Texture was determined at the Research Institute for Nature and Forest (INBO) using laser diffraction (Coulter LS200, Miami, FL) after removing organic matter and carbonates by adding hydrogen peroxide and an acetate buffer solution respectively. To determine the chloride content, 10 g of sediment was suspended in 50 ml of 0.15 M HNO_3 and shaken for 30 minutes. The filtrate was titrated with 0.05 M $AgNO_3$ using potentiometric end-point detection (Van Ranst et al., 1999). The cation exchange capacity was measured by percolation of 150 ml 1 M NH_4OAc through a percolation tube filled with a mixture of 5 g sediment and 35 g quartz sand, followed by washing through the excess with 300 mL denatured ethanol. The exchangeable ammonium ions were then eluted with 500 mL 1 M KCl and analysed in the percolate by means of a steam distillation (Tecator Kjeltec System 1002 Distilling Unit). The exchangeable cations

were also analysed in this percolate using ICP-OES (Varian Vista MPX, Varian, Palo Alto, CA) (Van Ranst et al., 1999).

During the first 140 days of the experiment, soil pore water was extracted by both Rhizon samplers approximately twice a week in flooded periods and three times a week in emerged periods. Between day 140 and day 250, some samples were taken occasionally. The samples were acidified with concentrated HNO_3 to keep the metals in solution and to solubilise metals which precipitated during sampling. Samples of both samplers were poured together in a recipient and stored in a refrigerator preceding the analyses. All samples were analysed for Cd, Cu, Ni, Fe, Mn and Zn contents using GF-AAS (Varian SpectraAA-800/GTA-100, Varian, Palo Alto, CA) and ICP-OES (Varian Vista MPX, Varian, Palo Alto, CA). To check for soil homogeneity, metal contents in the solutions of both samplers were once determined separately in the beginning of the experiment (Table 5.2). Deviations of results originating from both samplers were generally below 20 %, although occasionally deviations up to 50 % were observed. As analyses results coincided quite well for most samplers, a sufficiently high soil homogeneity and similar experimental conditions around both samplers were assumed.

Table 5.2. Comparison of metal concentrations in pore water samples of both Rhizon samplers

Treatment	Cd (μg L^{-1})		Cu (mg L^{-1})		Fe (mg L^{-1})	
	rhizon 1	rhizon 2	rhizon 1	rhizon 2	rhizon 1	rhizon 2
1PER	0.9	0.8	0.05	0.04	< 0.1	< 0.1
2PER	24.1	25.5	0.36	0.41	0.21	0.17
3PER	38.3	65.8	0.45	0.57	0.59	0.71
4PER	50.9	49.7	0.25	0.29	0.60	0.72
5PER	13.5	15.5	0.18	0.25	0.40	0.51
6PER	31.5	38.8	0.34	0.41	1.47	1.56
1REG	0.7	1	0.05	0.05	< 0.1	< 0.1
2REG	19.9	24.4	0.33	0.35	0.21	0.24
3REG	44.2	46.1	0.47	0.55	0.68	0.48
4REG	64.5	67.8	0.31	0.30	0.68	0.87
5REG	23.5	15.3	0.31	0.30	0.54	0.50
6REG	35.3	36.5	0.37	0.38	1.39	1.51
	Mn (mg L^{-1})		Ni (mg L^{-1})		Zn (mg L^{-1})	
	rhizon 1	rhizon 2	rhizon 1	rhizon 2	rhizon 1	rhizon 2
1PER	< 0.005	< 0.005	< 0.002	< 0.002	< 0.04	< 0.04
2PER	4.03	3.95	0.17	0.15	0.36	0.35
3PER	9.44	13.20	0.36	0.50	1.01	1.31
4PER	18.80	19.75	0.31	0.37	0.93	1.04
5PER	5.70	6.69	0.10	0.11	0.25	0.24
6PER	15.28	16.43	0.19	0.21	0.79	0.94
1REG	< 0.005	< 0.005	< 0.002	< 0.002	< 0.04	< 0.04
2REG	3.74	4.47	0.13	0.15	0.31	0.36
3REG	10.69	9.11	0.39	0.35	1.09	0.96
4REG	20.30	25.30	0.30	0.41	1.09	1.24
5REG	8.92	6.51	0.15	0.11	0.40	0.29
6REG	15.38	15.80	0.20	0.21	0.86	0.90

At least every two weeks during the first 140 days, a sample was also analysed for Ca, Mg, Na and K contents. The TOC, NO_3^-, SO_4^{2-} and Cl^- contents were regularly determined on non-acidified samples directly after sampling. Surface water was extracted 9 times during the entire experiment, using the two Rhizon samplers installed above the soil surface, and also analysed for Cd, Cu, Ni, Fe, Mn, Zn, Ca, Mg, Na, K, TOC, NO_3^-, SO_4^{2-} and Cl^- contents. TOC contents in the surface and pore water samples were measured with a TOC-analyser (TOC-5000, Shimadzu, Tokyo Japan). Cl^-, NO_3^- and SO_4^{2-} contents were measured with a Metrohm configuration consisting of a 761 Compact Ion Chromatograph equipped with a 788 IC Filtration Sample Processor and an anion exchange column (IC-AN-Column Metrosep A supp 4, Metrohm Ion Analysis, Switzerland).

The redox potential of the soil was measured with a HI 3090 B/5 electrode that was inserted permanently into the soil, to avoid disturbing the soil redox state before each measurement. One electrode was placed in each recipient at 5 cm below the soil surface (Fig. 5.2). For each measurement, the electrode was temporarily connected to a HI 9025 meter of HANNA instruments. During the first four months, the redox potential was measured almost daily, afterwards twice a week. The measured value was corrected to the Standard Hydrogen Electrode as a reference by adding the difference between the redox potential measured in a ZoBells solution (0.033 M $K_3Fe(CN)_6$ and 0.033 M $K_4Fe(CN)_6$ in 0.1 M KCl) and the theoretical value of $+ 428$ mV.

To measure the chlorofyl a fluorescence of reed and willow leaves, leaf clips were attached to the 4th, 5th and 6th leaf from the top of the same branch of a plant. In that way, a leaf zone was adapted to darkness for 20 minutes. Subsequently, an optical cable was attached to the leaf clip and the leaf clip was opened, allowing light pulses (pulse frequency 600 Hz) originating from the PAM-2000 fluorescence meter to reach the leaf. Fluorescence emitted by the leaf was measured and $\Delta F/F_m$' was defined as the effective quantum yield of photochemical energy conversion in PS2 (Genty et al., 1989).

Plant samples were analysed for metal contents by weighing 0.5 g in beakers. Five ml of ultra pure HNO_3 were added, after which the suspension was heated on a hot plate at 130 ^{0}C for one hour. After heating, a total of 4 ml hydrogen peroxide was added to each sample and then heated for 10 minutes. The suspensions were filtered in 50 ml volumetric flasks using filter papers (S&S blue ribbon, Schleicher & Schuell). The filtrates were diluted to the mark using

1% HNO_3. Cd, Cu, Ni, Zn, Fe and Mn concentrations were analysed on duckweed samples, whereas only Cd and Cu concentrations were analysed on reed plant and willow samples, using F-AAS (Varian Spectra AA-1475, Varian, Palo Alto, CA) and GF-AAS (Varian SpectraAA-800/GTA-100, Varian, Palo Alto, CA). As it is very difficult to completely digest reed plant material preceding the analyses, destruction methods were compared and quality control results were described by Du Laing et al. (2003). To be able to assess variations which should be attributed to the analytical procedure, the analysis procedure was also replicated 4 times on 3 selected samples for both Cd and Cu, which indicated that the relative variation coefficient never exceeded 10 %.

5.2.2 Results and discussion

The used soil contained 7.8 ± 0.4 % $CaCO_3$, 10.4 ± 0.1 % OM, 39.9 ± 0.3 % clay, 45.4 ± 0.6 % loam, and 14.7 ± 0.8 % sand. The pH was 7.60 ± 0.04 % and the EC 77.5 ± 4.7 μS cm^{-1}. It contained significant amounts of metals: Cd 8.8 ± 0.1 mg kg^{-1} DM, Cu 94 ± 1 mg kg^{-1} DM, Ni 38.5 ± 0.1 mg kg^{-1} DM, Zn 781 ± 2 mg kg^{-1} DM, Fe 37772 ± 237 mg kg^{-1} DM and Mn 1199 ± 2 mg kg^{-1}. Eleven % of total Cd, 1.4 % of total Cu, 0.8 % of total Zn, <0.4 % of total Ni, 0.2 % of total Mn and 0.01 % of total Fe were found in the exchangeable cation fraction. Some characteristics of the organic matter which was added to the soils are summarised in Table 5.3. The metal concentrations seem to be within a normal range for reed plants and willows (Chapter 7 and Vandecasteele et al., 2006). The willow leaves obviously had accumulated Cd and Zn, whereas reed leaves were higher in Fe and Mn than willow. Contents of Cd, Ni and Zn were lower in the reed leaves compared to the stems. Reed plants however seem to concentrate some essential elements such as Fe, Mn and Cu in their leaves. The favourable carbon:nitrogen ratio of willow leaves should permit a fast decomposition. In contrary, an external nitrogen source will be needed for a fast and complete decomposition of reed stems and leaves. Polunin (1982) indeed found that the addition of nitrate (and phosphate) to reed leaf litter led to increased decay rates, and increased aerobic respiration of natural reed litter in the laboratory. Moreover, Gessner (2000) observed lower decomposition rates and higher nitrogen and phosphorus accumulation in decomposing reed stems, compared to leaves.

Table 5.3. Characteristics of the organic matter which was added to the soils (mean ± standard deviation)

	Reed leaves	Willow leaves	Reed stems
OM (%)	89.5 ± 0.1	89.1 ± 0.1	97.0 ± 0.1
C (%)	51.9 ± 0.1	51.7 ± 0.1	56.3 ± 0.1
C:N (w/w)	20.9 ± 0.3	12.3 ± 0.0	167 ± 15
Cd (mg kg^{-1} DM)	0.01 ± 0.01	2.93 ± 0.08	0.02 ± 0.00
Cu (mg kg^{-1} DM)	6.0 ± 0.4	9.0 ± 1.0	3.0 ± 0.4
Fe (mg kg^{-1} DM)	142 ± 7	96 ± 3	64 ± 5
Mn (mg kg^{-1} DM)	167 ± 7	33 ± 0	14 ± 1
Ni (mg kg^{-1} DM)	1.0 ± 0.1	2.1 ± 0.4	3.7 ± 0.2
Zn (mg kg^{-1} DM)	20 ± 11	221 ± 1	23 ± 2

In all soils, the redox potential dropped from more than 200 mV to around -200 mV during the first 20 days of the experiment (data not shown). The latter value may already facilitate reduction of sulphates to sulphides (Gambrell et al., 1991a). It decreased faster in the soils to which organic matter was added compared to the blank soil. For the 2 PER and 3 PER treatments, an extra redox potential drop from -220 mV to -275 mV was observed around day 60, which could coincide with values facilitating CO_2 reduction (Gambrell et al., 1991a). A similar redox potential drop was observed around day 80 in the 4 PER and 5 PER treatments. The last soil for which the redox potential drop is observed is the soil to which 20 % reed stems were added (6 PER). The redox potential decreased rather gradually between day 80 and 120 in this soil.

Total organic carbon (TOC) concentrations in the pore water were measured until day 136 (Fig. 5.3). They stayed relatively stable or slightly increased during the first part of the experiment in the permanently flooded soils, after which they significantly decreased. Between day 2 and day 136, the total organic carbon (TOC) concentrations decreased for the different treatments as following: 1 PER: from 44 to 18 mg L^{-1}; 2 PER: from 1138 to 130 mg L^{-1}; 3 PER: from 1960 to 187 mg L^{-1}; 4 PER: from 2996 to 329 mg L^{-1}; 5 PER: from 976 to 239 mg L^{-1}; and 6 PER: from 1997 to 394 mg L$^-$. The initial TOC concentrations seem to increase with increasing amounts of added reed stems or leaves. When TOC concentrations were normalised to added organic matter amounts, they also seemed to decrease in the following order: willow leaves > reed leaves > reed stems. This might be attributed to a decreasing decomposability due to an increasing C:N proportion (Table 5.3), which indicates an increasing need for an external nitrogen source for a fast and complete organic matter decomposition. It might also be attributed to the higher amounts of recalcitrant organic

fractions in reed stems compared to leaves, which hamper a fast decomposition (Hietz, 1992; Gessner, 2000). The supply and transfer rate of electron acceptors might also become bottlenecks for complete decomposition upon addition of high amounts of easily decomposable organic matter, which is expected to result in TOC accumulation in the pore water. Moreover, the concentration abatements seem to start more rapidly with decreasing contents of reed stems, or when leaves were added compared to stems. This might be attributed to the faster depletion of easily decomposable material. In the alternately flooded treatments, similar TOC concentrations in the pore water were observed as in the permanently flooded soils at the beginning of the experiment. However, a decreasing trend could already be observed from the third week onwards due to the TOC export upon percolation and removal of the percolate. Moreover, organic matter decomposition is expected to proceed faster when increasing the number of alternate aerobic and anaerobic periods (Reddy & Patrick, 1975). As a result, TOC concentrations in the pore water were significantly lower in the alternately flooded soils at the end of the sampling period. Total organic matter contents in the solid soil fractions were also measured around day 150. About 12.5 % of the total organic matter amount seem to have disappeared in both the permanently and the alternately flooded blank soil. Between 44 and 54 % of the added organic matter amounts was broken down in all treatments to which leaves were added. No significant differences were observed between reed and willow leaves, which suggests that a possible faster decomposition of willow leaves during the first weeks is overtaken during the later period. For reed leaves, the removed organic matter amounts were slightly higher in the alternately flooded treatments (2 ALT and 3 ALT) compared to the permanently flooded treatments (2 PER and 3 PER), whereas the opposite was observed upon willow leaf addition.

Reed stems decompose slower than leaves, a feature noticed before (e.g. Hietz, 1992; Gessner, 2000). Moreover, much more variation is observed for the treatments to which reed stems were added: 40, 21, 7 and 15 % of the added amounts had disappeared in 5 PER, 6 PER, 5 ALT and 6 ALT, respectively. Except for 5 ALT, total exported organic matter amounts were however similar to those of the leaf treatments, as added organic matter amounts were also higher. Remarkably, lower amounts seem to have disappeared in the alternately flooded treatments. Measurements of percolated TOC amounts indicate that they do not seem to account for more than 12 % of the total organic matter amounts which have disappeared during the first 150 days. Ongoing decomposition during the percolation, which

could last for over a day, might however have resulted in an underestimation of percolated amounts.
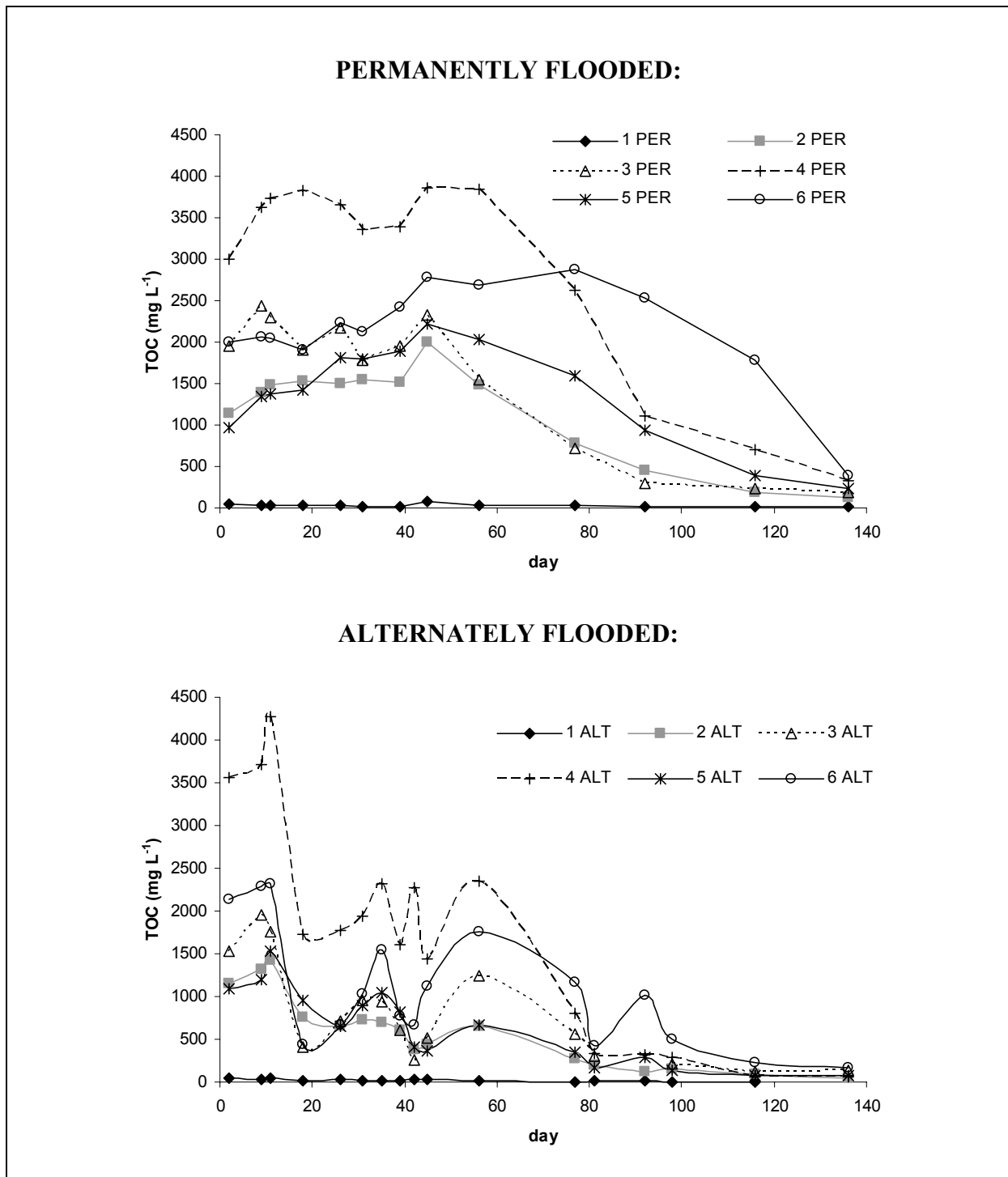

Fig. 5.3. TOC concentrations in the pore water of an alternately (ALT) and permanently (PER) flooded soil to which different concentrations and types of organic material (1-6, see Table 5.1) were added, as a function of time upon flooding

Measured TOC amounts in the percolates totalled 61, 358, 512, 2096, 574 and 2408 mg kg^{-1} soil DM for the six ALT treatments, respectively. They were especially high in the willow leaf treatment (4 ALT) and the 20 % reed stem treatment (6 ALT) during the first 80 days. These were also the treatments in which the pore water TOC concentrations were the highest. The higher release rate of soluble organic matter fractions probably caused the transfer of electron acceptors to become a limiting factor for complete decomposition, which resulted in an increased export through the percolates.

Sulphate concentrations in the pore water ranged from 17 to 81 mg L^{-1} in the beginning of the experiment for the blank soil and the soils to which reed plant material was added (data not shown). They increased with increasing organic matter concentration, which suggests that sulphate was leaching from the added organic matter. A much higher concentration of 1194 mg L^{-1} was found in the soil to which willow leaves were added. This concentration even increased up to 5498 mg L^{-1} at day 26. These differences in sulphate leaching rates between willows and reed plants can not yet be attributed to differing sulphur concentrations in the plants as these were not measured. Moreover, insufficient data are currently available in the literature to accurately assess the factors affecting sulphur concentrations in willows and reed plants. During the rest of the experiment, sulphate concentrations first decreased to about 1 mg L^{-1} in all treatments. Subsequently, they slightly increased again from around day 75 onwards in all permanently flooded soils to which organic matter was added and in the alternately flooded soils to which leaves were added. These changes were however not observed in the blank. Sulphate concentrations permanently remained relatively high in the alternately flooded blank, whereas they decreased and remained low in the permanently flooded blank. Sulphate reduction was confirmed by the detection of acid-volatile sulphides (AVS) in the soil. Just before planting the reed and willow plants around day 150, AVS contents in the six PER treatments totalled 47, 81, 101, 104, 80 and 21 mg kg^{-1}, respectively. Acid-volatile sulphides were also detected in the alternately flooded soils. However, produced amounts were lower, except for the soil to which 20 % reed stems were added (6 PER). For the six PER treatments, they totalled 9, 29, 16, 51, 43 and 90 mg kg^{-1}, respectively. Acid-volatile sulphides were not detectable anymore (<2 mg kg^{-1}) in the permanently flooded blank soil (1 PER) around day 250, so after planting the reed and willow plants. Average AVS contents were then 33 mg kg^{-1} in 2 PER and 3 PER, 128 mg kg^{-1} in 4 PER, 19 mg kg^{-1} in 5 PER and 28 mg kg^{-1} in 6 PER. AVS contents around day 250 totalled 15, 14 and 20 mg kg^{-1} in 2 ALT, 3 ALT and 4 ALT, the alternately flooded soils to which leaves were added, whereas

some acid-volatile sulphides (5 mg kg^{-1}) were still detected in the alternately flooded blank (1 ALT). No AVS was however detected anymore (<2 mg kg^{-1}) in 5 ALT and 6 ALT, the alternately flooded soils to which reed stems were added. Addition of willow leaves thus significantly enhanced AVS formation compared to the addition of reed leaves, whereas addition of reed stems generally resulted in a lower AVS production compared to leaves. These differences should probably be attributed to a combination of differing sulphate release rates and decomposability of the added organic matter, as mentioned above. The fact that smaller amounts of sulphides were formed in 6 PER (20 % reed stems) compared to 5 PER (10 % reed stems) might be due to the Fe oxide reducing microbial communities still taking the upper hand in 6 PER around day 140. Sulphate reduction is indeed known to be inhibited by the presence of Fe reducing micro-organisms (Lovley & Phillips, 1987). Moreover, a combination of continuously high Ni and Zn concentrations in the pore water as a result of the ongoing Fe and Mn oxide reduction, which will be discussed later, could induce toxic effects that could hamper the development of microbial sulphate reducing communities. Poulson et al. (1997) indeed found that a total activity of $Ni^{2+}_{(aq)} + Zn^{2+}_{(aq)}$ greater than approximately $2.5 \cdot 10^{-5}$ M is toxic to *Desulfovibrio desulfuricans* at a pH of 7.2. This threshold activity coincides with the order of magnitude of molar Ni + Zn concentrations in the pore water of the 6 PER treatment in the beginning of the experiment. Sulphide contents decreased in the second part of the experiment in all permanently flooded soils, except the one to which willow leaves were added. A decreasing availability of sulphates and easily decomposable organic matter probably resulted in a decreasing sulphate reduction rate. Growing reed plants moreover transport oxygen to their root zone (Weis & Weis, 2004), which probably also promoted the oxidation of sulphides.

Fe and Mn concentrations in the pore water are plotted as a function of time in Fig. 5.4. In all permanently flooded soils to which organic matter was added (2-6 PER), Mn concentrations immediately increased to reach a peak before day 50. For Fe, concentrations increased more gradually and peak concentrations were reached somewhat later. Fe and Mn concentrations in pore water of the blank (1 PER) were much lower and increased more steadily until day 100. The observed peak Fe and Mn concentrations in the pore water were too high (from around 70 to 750 mg L^{-1}) to be primarily attributed to leaching from the decomposing organic matter. The supplementation of organic carbon thus seems to promote Fe/Mn-oxide reduction by micro-organisms. Mn-oxide reduction indeed already starts at higher redox potentials and is followed by Fe-oxide reduction (Lovley & Phillips, 1988a).
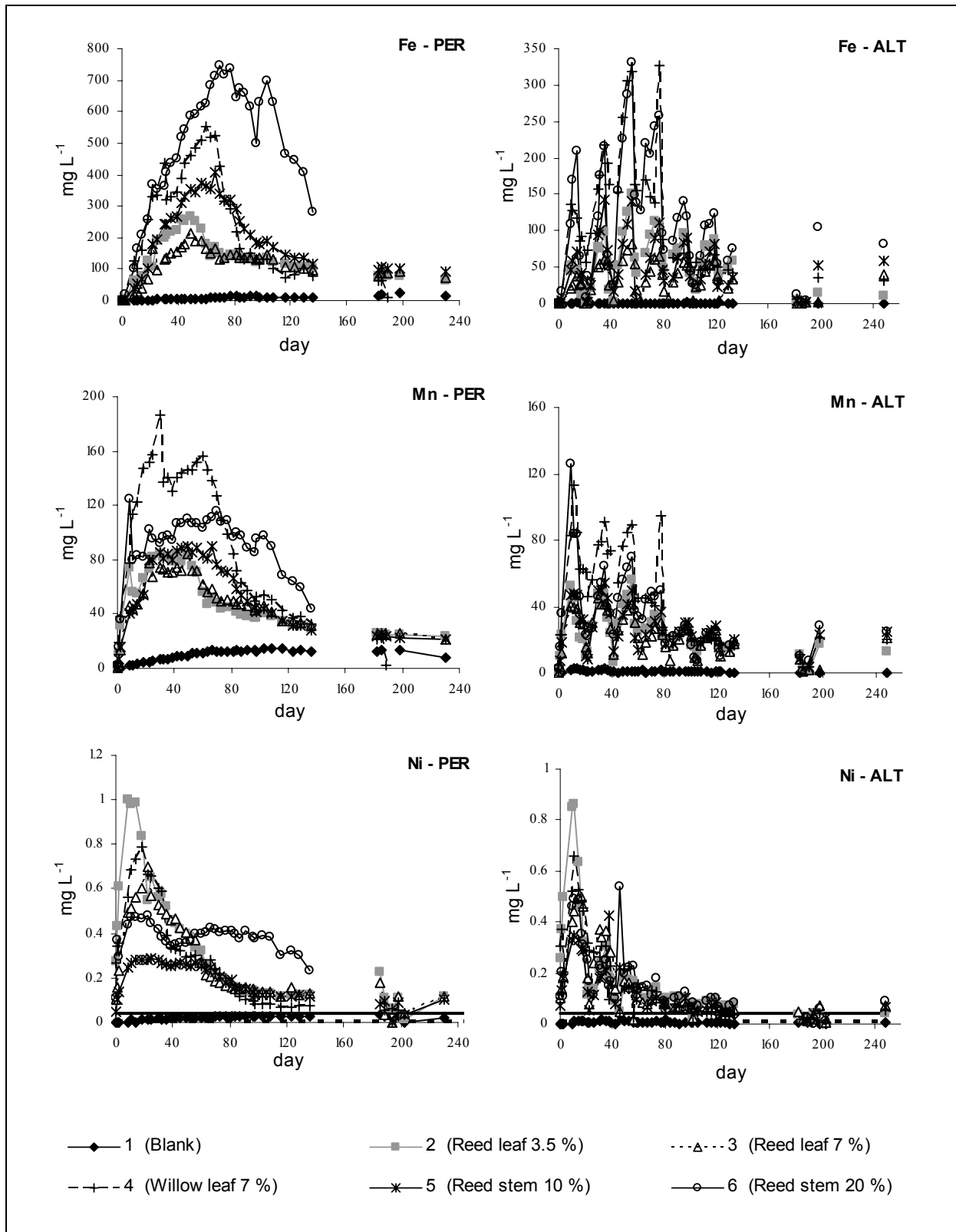
Fig. 5.4. Evolution of Fe, Mn and Ni concentrations in the pore water of an alternately (ALT) and permanently (PER) flooded soil to which different concentrations and types of organic material (1-6) were added, as a function of time upon flooding

Peak Fe and Mn concentrations were normalised to the added percentage of organic matter for each treatment. These normalised concentrations were lowest for the reed stems and were double as high for willow leaves compared to reed leaves. The potential to mobilise Fe and Mn thus seems to decrease in the following order: willow leaves > reed leaves > reed stems. This coincides with a significantly increasing C:N proportion (Table 5.3), which indicates an increasing need for an external nitrogen source for a fast and complete organic matter decomposition. Stems also contain higher amounts of recalcitrant organic fractions, which hampers a fast decomposition (Hietz, 1992; Gessner, 2000).

The decreasing Fe and Mn concentrations in the second part of the experiment can be attributed to the combination of decreasing Fe and Mn oxide reduction rates and an increasing formation of sulphide precipitates due to an increasing dominance of sulphate reducing micro-organisms. The significant redox potential drops towards values which facilitate sulphate reduction and are accompanied by drops of Fe and Mn concentrations in the pore water. The highest peak for Mn was observed in the 4 PER treatment. However, it did not coincide with the plot of Fe concentrations, for which the highest peak was reached in the 6 PER treatment. This can be attributed to the significant amounts of sulphates that leach from willow leaves during the first days of the experiment. As a result, sulphide formation may start more rapidly in 4 PER. As Fe sulphides are much less soluble compared to Mn sulphides, this may lead to a more rapid decrease of Fe concentrations in the pore water compared to Mn concentrations when willow leaves are added. Moreover, the different organic matter types may differ in their capacity to re-adsorb Fe and Mn upon release. Drops in Fe and Mn concentrations which were mentioned above were much higher compared to the amount of sulphides which were formed (on a molar basis). This confirms that Fe and Mn might indeed have been subject to other processes, such as a re-adsorption by the soil or biotic compartments or migration to more oxic parts of the experimental setup and subsequent precipitation as oxides. Another possibility is that sulphides were formed which are not determined in the operational AVS analysis procedure, such as pyrite (Tack et al., 1997a; Billon et al., 2001). Moreover, sulphide losses due to partial oxidation of the soil between sampling and analysis might have resulted in a discrepancy between the sulphide amounts which were present and those which were measured. In the alternately flooded soils, Fe and Mn concentrations were also lowest in the blank and highest in the soils to which willow leaves (4 ALT) and 20 % reed stems (6 ALT) were added. The general trend coincides with the one of the permanently flooded soils. Peak concentrations are highest around day 60, after which they decrease. However, concentrations

fluctuate significantly during each tidal cycle. For all treatments, the highest concentrations are observed at the end of each flooded period, whereas they rapidly drop upon drainage and start to increase again from the moment the soils are flooded again. They are partly exported from the soil through the percolates and partly expected to oxidise and precipitate as oxides during drainage, whereas the Fe/Mn oxide reducing micro-organisms mobilise them again during flooding. As a result of these alternating conditions, peak concentrations are not as high as in the permanently flooded treatments. Whereas the highest observed Fe and Mn concentrations were 744 mg L^{-1} and 186 mg L^{-1} in the permanently flooded soils, they only reached 319 mg L^{-1} and 126 mg L^{-1} in the alternately flooded soils, respectively. Fe and Mn concentrations also seemed to slightly increase again at the end of the experiment, especially in 5 ALT and 6 ALT. This might be attributed to the depletion of sulphides which was mentioned above. The abundance of sulphides might result in Fe and Mn being more susceptible to reductive dissolution during the flooded periods. Differences between Mn concentrations in the permanently and alternately flooded soils were relatively smaller than differences between Fe concentrations. This might be attributed to the fact that Mn oxide reduction takes place earlier upon flooding, as already mentioned above.

Ni concentrations increased substantially during the first weeks, after which they also decreased (Fig. 5.4). In all treatments to which organic matter was added, Ni concentrations exceeded the Flemish sanitation threshold for ground water, especially in the beginning of the experiment, although the sanitation threshold for concentrations in the solid soil fraction was not exceeded. During the first 3 weeks, observed peak concentrations reached up to 20 times the sanitation threshold. After the first 180 days, they still regularly exceeded the sanitation threshold. The evolution of Ni concentrations was very similar to the one of Mn. Ni was also rapidly released upon flooding, suggesting the association of Ni with Mn oxides and release upon reduction of these oxides. Ni was indeed already reported to primarily co-precipitate with Mn oxides in salt marsh sediments from the Western Scheldt (Zwolsman et al., 1993). However, Ni concentrations decreased more rapidly afterwards, especially in the soils to which leaves were added. In these soils, formation of significant amounts of sulphides was indeed observed, and Ni sulphides have a much lower solubility than Mn sulphides. This is additionally supported because only a small decrease of Ni concentrations has been observed during the first 140 days in 6 PER, the treatment where only little sulphides were formed.

In both the permanently and alternately flooded treatments to which organic matter was added, Cd concentrations in the pore water reached a peak at the second day of the experiment, whereas no peak was observed in the blank treatment. Peak concentrations reached up to 70 $\mu g\ L^{-1}$, coinciding with 14 times the Flemish sanitation threshold for ground water, and increased with increasing organic matter concentrations. They were highest in the 3, 4 and 6 treatments (7 % reed and willow leaves and 20 % reed stems), which clearly coincides with peaks of Mn and Fe concentrations. Upon reduction of Fe and Mn oxides, Fe and Mn might replace exchangeable Cd fractions at the negatively charged soil surface and Cd associated with these oxides might also be released (Davis & Kent, 1990; Guo et al., 1997). The concentrations subsequently decreased very fast to around background levels in the permanently flooded soils (PER). This might be attributed to Cd sulphide formation, as Cd sulphides are very insoluble and only small amounts of sulphides are needed to completely precipitate all Cd from the pore water (e.g. Salomons et al., 1987; Simpson et al., 2000). The Cd concentrations remained very low in the alternately flooded soils to which leaves were added (2 ALT, 3 ALT and 4 ALT), but still reached quite high concentrations in the alternately flooded blank (1 ALT) and the soils mixed for 20 % with reed stems (6 ALT). Moreover, Cd concentrations still exceeded the sanitation thresholds during emerged periods at the end of the experiment in treatment 5 ALT, the alternately flooded treatment of soil mixed for 10 % with reed stem material. In 1 ALT and 6 ALT, the highest concentrations were found in the emerged periods, whereas in 5 ALT, the highest concentrations were found during flooding. 1 ALT, 5 ALT and 6 ALT are all treatments in which sulphide depletion occurred in the second part of the experiment. Under the condition of complete sulphide depletion in 5 ALT, Cd concentrations might be susceptible again to release upon reductive dissolution of Fe and Mn oxides during flooding. This would explain that pore water concentrations are highest during flooded periods. As sulphide depletion was still incomplete in 1 ALT and 6 ALT, Cd concentrations alternately increased upon sulphide oxidation in emerged periods and decreased again as a result of resumed sulphate reduction during flooded periods. The Cd concentrations in the percolates seem to decrease significantly with time. The total amount of Cd collected in the percolates until day 126 was 4.1 $\mu g\ kg^{-1}$ DM for 1 ALT, 11.5 $\mu g\ kg^{-1}$ DM for 2 ALT, 31.5 $\mu g\ kg^{-1}$ DM for 3 ALT, 6.5 $\mu g\ kg^{-1}$ DM for 4 ALT, 19.1 $\mu g\ kg^{-1}$ DM for 5 ALT and 31.2 $\mu g\ kg^{-1}$ DM for 6 ALT. This coincided with 0.05 to 0.36 % of total soil contents. Addition of reed leaves (2 ALT and 3 ALT) obviously resulted in higher Cd export compared to the addition of reed stems (5 ALT and 6 ALT) in the first weeks of the experiment. Their higher decomposition rate probably facilitates a faster Cd release.
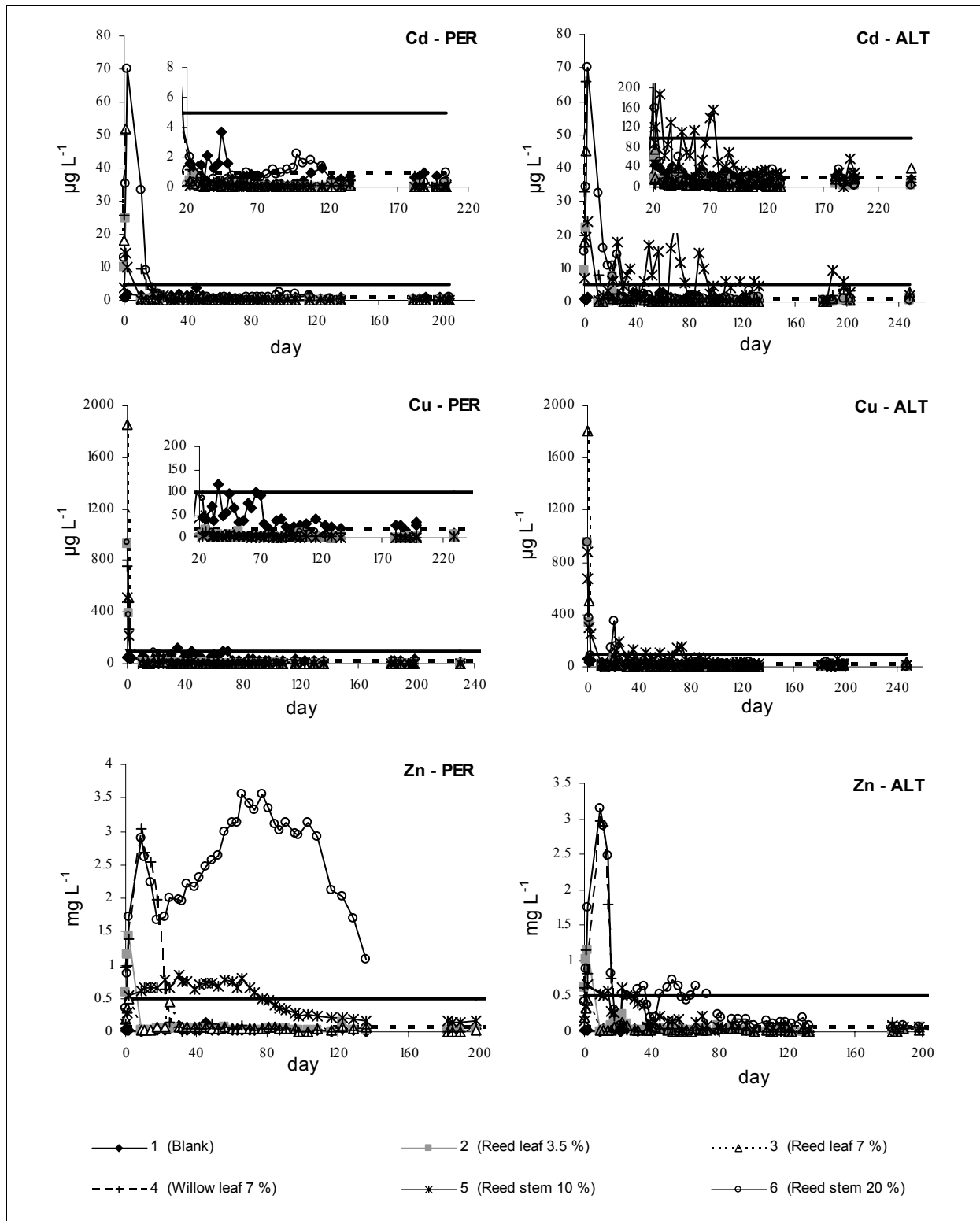
Fig. 5.5. Cd, Cu and Zn concentrations in the pore water of an alternately (ALT) and permanently (PER) flooded soil to which different concentrations and types of organic material (1-6) were added, as a function of time upon flooding

However, this Cd export decreased to below blank levels around day 100 for 2 ALT and 3 ALT. This was not the case for 5 ALT and 6 ALT, which might be attributed to the higher re-adsorption rates by the soil or biotic compartments, higher sulphide production rates and/or decreasing Fe/Mn oxide reduction rates upon depletion of Fe/Mn oxides and easily decomposable organic matter. Cd contents were higher in percolates of 3 ALT compared to 4 ALT during the entire experiment. Cd concentrations in the willow leaves themselves were however much higher than in the reed leaves, which indicates that the Cd release upon organic matter decomposition is probably opposed by other processes when willow leaves were added, such as the much higher sulphide production rate. This is facilitated by the more favourable C:N proportion of willow leaves and the much faster sulphate release, which was previously already mentioned.

The changes in Cu concentrations with time were very similar to those of Cd, which suggests that Cu and Cd availability are affected by the same factors. Cu concentrations were also very high (up to 1852 μg L^{-1}) during the first days for both permanently and alternately flooded treatments, whereas they also regularly exceeded the Flemish sanitation threshold for ground water in the treatment 5 ALT. Contrary to Cd, Cu release during the first days was much lower for the willow leaf treatments compared to the reed leaf treatments. Moreover, Cu concentrations in the pore water decreased more rapidly than the Cd concentrations. As already mentioned, metal release is expected to be counteracted by sulphide precipitation and re-adsorption during these first days and sulphide formation is highest in the willow leaf treatment. As the solubility of Cu sulphides is lower than the solubility of Cd sulphides (Zumdahl, 1992), Cu contents are indeed expected to be affected more rapidly in the beginning of sulphide formation.

Zinc contents also increased during the first days and decreased afterwards, although less rapidly compared to Cd and Cu. On the one hand, solubility of Zn sulphides is higher than solubility of Cd and Cu sulphides (Zumdahl, 1992). On the other hand, released Zn amounts are much higher than Cd or Cu, which implies that much more sulphides are needed to completely precipitate released Zn. Zn concentrations increased to above 3.5 mg L^{-1}, which represents 7 times the Flemish sanitation threshold. As for Cd, Zn release might be a result of Fe and Mn release upon Fe and Mn oxide reduction (Davis & Kent, 1990; Guo et al., 1997). The higher Zn concentrations in the pore water of the willow leaf and reed stem treatments (4, 5 and 6) indeed coincided with higher Fe and Mn concentrations. They however rapidly

decreased in 4 PER (willow leaves), whereas it took a longer time to reduce them to below sanitation thresholds in the reed stem treatments (5 PER, 6 PER). A small drop could be observed between day 10 and day 20 in the 6 PER treatment, coinciding with the drop in the 4 PER treatment. However, the Zn concentration increased again in 6 PER after that initial drop. Sufficient amounts of sulphides to precipitate all released Zn were probably produced in 4 PER from day 10 onwards, due to the higher initial release of sulphates, as mentioned above. As only small amounts of sulphates were released in 6 PER, sulphide production probably failed to rapidly reduce the high Zn concentrations. This could in turn have induced a toxic effect on the development of microbial sulphate reducing communities, possibly in combination with the high Ni, Fe, and Mn concentrations, which could have led to the increasing Zn concentrations in 6 PER after the initial small drop. Such toxic effects at concentrations in the same order of magnitude as the Zn concentrations we have observed were reported by Poulson et al. (1997). As a result, measured sulphide contents were also significantly lower in 6 PER around day 150, as mentioned above. In the alternately flooded soils (ALT), the Zn concentrations rapidly decreased during the first 3 hydrological cycles (i.e. 9 weeks). This might probably also be attributed to their export through the percolates, which indeed totalled 12 mg for 4 ALT, 17 mg for 5 ALT and 30 mg for 6 ALT for the first 3 hydrological cycles and afterwards decreased as a function of time. Metal concentrations in the pore water during the second part of the experiment also seem to depend on the concentration of added reed stem material. Zn concentrations were significantly higher when 20 % of reed stems (6) were added compared to when only 10 % were added (5). In contrast, Cd and Cu concentrations were highest when only 10 % reed stems were added to the alternately flooded soils. This might again be attributed to counteracting metal release, re-adsorption and sulphide precipitation, which all seem to be promoted by organic matter addition. In the alternately flooded soils, sulphides are expected to oxidise during emerged periods. Especially at lower sulphide contents (5 ALT), Cd and Cu concentrations in the pore water are affected as sulphide availability becomes a bottleneck for Cd and Cu sulphide formation upon oxidation of sulphides. Zn is however released in much higher molar amounts and the solubility of Zn sulphides is higher. As a result, increasing Zn release with increasing organic matter contents cannot be sufficiently counteracted by increased sulphide precipitation or re-adsorption.

Cadmium concentrations in duckweed which was transplanted to the permanently flooded treatments around day 150 decreased from 21 mg kg^{-1} DM in the blank to 4.6 ± 2.7 mg kg^{-1}

DM when organic matter was added to the soil, whereas Cu concentrations also decreased significantly, from 80 to 14 ± 2.4 mg kg^{-1} DM. Zn concentrations decreased less substantially, from 212 to 137 ± 34 mg kg^{-1} DM, whereas Fe, Mn and Ni concentrations increased from 6481, 5529 and 10.0 mg kg^{-1} DM to 15581 ± 4136, 6209 ± 1664 and 14.7 ± 3.4 mg kg^{-1} DM, respectively. This coincided with the Cd and Cu concentrations in the surface waters just before inoculating them with duckweed (day 136). These were significantly higher in the blank (1.818 µg L^{-1} and 30.2 µg L^{-1}, respectively), compared to the treatments to which organic matter was added (<0.1 µg L^{-1} and <2 µg L^{-1}, respectively). Moreover, Fe, Mn and Ni concentrations in the surface water were lower in the blank (<0.1 mg L^{-1}, 0.42 mg L^{-1} and 0.01 mg L^{-1}, respectively) compared to the treatments to which organic matter was added (107 ± 72 mg L^{-1}, 18.0 ± 9.8 mg L^{-1}, and 0.11 ± 0.06 mg L^{-1}, respectively), which also coincided with the variations of duckweed concentrations. Zn concentrations in the surface water fluctuated around the detection limit (0.04 mg L^{-1}). Metal concentrations in the duckweed thus seem to be primarily determined by their concentrations in the surface water. Fe and Mn concentrations in the surface water in turn were affected by increased Fe/Mn oxide reduction upon addition of organic matter. Increased tissue Fe and Mn concentrations after creation of reducing conditions in wetland soils have indeed also been observed for other wetland plants (Pezeshki, 2001). Sulphide formation and organic complexation upon addition of organic matter probably caused the decrease of Cd and Cu concentrations in the plants, as in the pore water. Ni concentrations in the surface water probably also were influenced to an extent by Fe/Mn oxide reduction, but the effect was smaller compared to Fe and Mn. This is probably due to the lower solubility of Ni sulphides and their smaller molar contents, which should result in a relatively higher precipitation of Ni sulphides upon addition of organic matter. Vice versa, produced sulphide amounts are too low to precipitate all Zn as Zn sulphides, which results in a relatively lower abatement of Zn concentrations in duckweed upon addition of organic matter, compared to Cd and Cu. Duckweed biomass production decreased from 0.29 g DM to 0.17 ± 0.06 g DM per recipient upon addition of organic matter, which should probably be attributed to the decreased nutrient availability as a result of e.g. nitrate reduction and the precipitation of micronutrients (e.g. Cu and Zn) as sulphides. A very reduced state can moreover account for the accumulation of various soil phytotoxins that are by-products of soil reduction, such as organic components, soluble sulphide species and reduced Fe and Mn (Pezeshki, 2001).

Biomass production and metal concentrations in transplanted reed plants and willows which have grown on the soils for about 100 days from day 150 onwards are represented in Table 5.4. Biomass production varied significantly, by a factor 10, 16, 12 and 33 for reed stems, reed leaves, willow leaves and willow stems, respectively. In the permanently flooded soils, biomass production was highest in the blank treatment (1 PER). Upon addition of small organic matter amounts (2 PER and 5 PER), they dropped, which might again be attributed to a decreased nutrient availability as a result of e.g. nitrate reduction and the precipitation of micronutrients (e.g. Cu and Zn) as sulphides. Upon addition of higher amounts of organic matter (3 PER, 4 PER and 6 PER), biomass production increased again. Increasing availability of organic complexing agents might now determine metal mobilisation and availability. In the alternately flooded treatments, biomass production was significantly higher on the soils to which leaves were added (2 ALT, 3 ALT and 4 ALT), coinciding with the soils which still contained some sulphides at the end of the experiment. Metals released upon gradual oxidation of sulphides during emerged periods might form a continuous source of micronutrients for the plants. Cu concentrations decreased with increasing amounts of added organic matter. Complexation of Cu by soil organic matter probably reduces the bioavailability of Cu. This effect seems to be more pronounced for reed stems compared to reed leaves. Moreover, baseline Cu concentrations were significantly higher in reed leaves compared to stems. Whereas Cu concentrations varied by a factor 11 in reed stems, they only varied by a factor 5 in reed leaves. The latter might be due to active Cu transport from the stems to the leaves as Cu plays a significant role in photosynthesis. Leaf biomass production indeed also increased with increasing Cu concentrations in these leaves. The reed stem biomass production increased with increasing Cd concentrations. This might be deduced to an increasing reed stem production with increasing Zn concentrations. Zn concentrations in the biomass were not measured, but are indeed accumulated in the stems as Zn has an essential function in the biosynthesis of the plant growth hormone indolyl-3-acetic acid, which is primarily active in the stems (Schierup & Larsen, 1981a). Moreover, factors affecting Zn uptake are expected to simultaneously affect Cd uptake, due to their very similar fate in the environment. In contrast to Cu, Cd concentrations in stems indeed only varied by a factor 11, whereas they varied by a factor 17 in the leaves. This suggests active incorporation in the stems. Whereas Cd and Cu concentrations in willow leaves only varied by a factor 3 and by a factor 5 in willow stems, willow leaf and stem biomass production varied by a factor 12 and 33, respectively. Thus, concentrations of Cd and Cu in willows varied much less compared to both metal concentrations in reed plants and willow biomass production. The experimental

growth period was possibly not sufficiently long to allow for metal translocation from willow cuttings to stems and leaves to be significantly overruled by metal uptake from the environment. Moreover, metals might have been actively accumulated by the willows in the treatments with the lowest environmental metal availability, which also might have contributed to the smaller differences between the metal concentrations of the willows in the different treatments.

Movement of metal ions into photosynthetic tissues can induces stress (Windham et al., 2003). Photosynthesis, however, seems to be far less affected than biomass production under our experimental conditions. Only small variations of $\Delta F/F'_m$ have been observed regardless the larger variations of metal concentrations in leaves. Mendelssohn et al. (2001) also found chlorophyll fluorescence to be relatively insensitive to increasing levels of cadmium in two other wetland plants, *Typha domingensis* and *Spartina alterniflora*. Tolerance to metals can be achieved by restricting metal transport across the root endodermis, and removing any mobile ions in the xylem by means of storage in cell walls and vacuoles, or binding by metallothioneins or phytochelatins (Windham et al., 2003). Lower Cu concentrations in the reed leaves however seem to result in a slightly lower fluorescence in our study, which might reflect the fact that Cu is a micronutrient which is involved in the photosynthesis process. The lowest value (0.742) was found in reed leaves of the 5 ALT treatment, which however did not coincide with the lowest Cu concentrations in the leaves. In this treatment, a peak of Cd concentrations in the pore water of the soil, which reached up to 9.4 μg L^{-1} and lasted for over 10 days, was however observed during the growth period. Reed plants might be sensitive for such peaks, contrary to willows which are expected to be more resistant to higher metal concentrations. Moreover, willows are expected to actively accumulate and translocate micronutrients in conditions of low availability, as already mentioned above.

Table 5.4. Biomass production (g DM per recipient), Chlorophyl a fluorescence (ΔF/Fm') and metal concentrations (mg kg^{-1} DM) in reed plants and willows (S = stem, L = leaf) planted on an alternately (ALT) and permanently (PER) flooded soil to which different concentrations and types of organic material (1-6, see Table 5.1) were added

	Biomass (g)				Chl a fluorescence (ΔF/Fm')		Cu concentration (mg kg^{-1} DM)				Cd concentration (mg kg^{-1} DM)			
	Reed		Willow		Reed	Willow	Reed		Willow		Reed		Willow	
	S	L	S	L	L	L	S	L	S	L	S	L	S	L
1 PER	4.3	9.4	8.7	9.7	0.834	0.815	12.4	8.8	3.8	5.4	0.054	0.037	3.6	4.1
2 PER	0.9	2.1	1.1	1.8	0.813	0.797	2.5	4.5	4.0	4.8	0.051	0.054	0.9	2.6
3 PER	1.9	6.6	1.7	3.4	0.807	0.811	3.9	4.5	3.7	6.6	0.032	0.023	1.2	2.7
4 PER	2.1	5.9	4.0	6.5	0.829	0.824	1.9	3.7	5.6	5.6	0.034	0.015	1.2	3.5
5 PER	0.9	1.3	0.4	1.1	0.807	0.796	2.3	3.4	10.5	10.0	0.053	0.037	2.6	6.0
6 PER	3.5	4.5	1.5	3.6	0.790	0.809	1.6	3.1	6.5	6.5	0.066	0.024	1.6	3.4
1 ALT	1.6	3.4	13.1	11.9	0.826	0.779	17.2	14.4	5.8	6.9	0.087	0.047	3.7	3.7
2 ALT	7.9	21.4	11.1	9.6	0.830	0.811	13.0	10.0	2.9	5.7	0.158	0.106	2.0	2.4
3 ALT	8.9	20.5	12.5	13.3	0.828	0.824	9.7	7.8	2.6	6.0	0.106	0.054	0.7	2.1
4 ALT	6.5	12.1	10.2	11.4	0.825	0.808	7.2	7.6	3.2	16.2	0.075	0.036	0.9	2.3
5 ALT	1.9	2.3	2.7	4.4	0.742	0.831	3.1	4.6	5.0	5.3	0.014	0.030	2.2	3.3
6 ALT	1.5	2	1	2	0.815	0.830	2.7	5.2	7.6	6.2	0.025	0.262	2.0	3.3

5.2.3 Conclusion

During the first weeks after organic matter supply, Cd, Cu, Ni and Zn concentrations in pore water significantly exceeded sanitation thresholds for ground water. The Ni concentrations however also significantly exceeded its sanitation threshold during a longer period of time upon addition of any type and amount of organic matter, which was also the case for Zn upon addition of reed stem material. Willow leaf material is rapidly decomposed, which results in high Fe and Mn oxide reduction rates, subsequently resulting in high metal concentrations in the pore water. Upon addition of willow leaves, also huge amounts of sulphates were released, which induced the formation of sulphides and precipitation of metal sulphides. As sufficient sulphide amounts are produced, all metal concentrations in the pore water can be reduced to acceptable concentrations within the first weeks. Metal release also increased with increasing added amounts of reed stems. However, lower sulphates amounts were released. As a result, insufficient amounts of sulphides can be produced to precipitate all metals and reduce Ni, Zn, Fe and Mn concentrations to within acceptable limits in a reasonable amount of time. This in turn seems to reduce sulphate reduction rates. As such, a vicious circle seems to be produced which is characterised by only slowly decreasing Zn, Ni, Fe and Mn concentrations, when high amounts of low-sulphate releasing reed stem material are added to a permanently flooded

soil. When the soils were alternately flooded, the metals were exported through the percolates. Total metal export by the percolates significantly increased when organic matter was added. The export was concentrated in the first weeks after organic matter addition. As a result, concentrations of most metals in the pore water were more rapidly reduced to within acceptable limits compared to permanently flooded soils. About five months after flooding the initially oxidised soil, both metal concentrations and growth of duckweed, reed plants and willows still seem to be affected by organic matter addition, whereas photosynthesis is less affected. Metal concentrations of duckweed were affected by metal concentrations in the surface water which is in close contact with the sediments. These in turn seem to be affected by Fe/Mn oxide reduction and sulphide precipitation. Metal concentrations of reed leaves and stems seem to be regulated by the plants, whereas metal translocation from the cuttings seems to affect the concentrations in willow leaves. Plant biomass production is expected to be primarily affected by changing nutrient availability upon organic matter addition, whereas peak concentrations of metals which are already toxic at low concentrations, such as Cd, also might induce sublethal plant stress.

5.3 Field monitoring

Adapted from: Du Laing, G., Van Ryckegem, G., Tack, F.M.G. & Verloo, M.G. (2006). Metal accumulation in intertidal litter through decomposing leaf blades, sheaths and stems of *Phragmites australis*. Chemosphere 63, 1815-1823.

Heavy metal contents of decomposing leaf blades, stems and sheaths of reed plants (*Phragmites australis*) were monitored by a standard litter bag method in an intertidal zone of the Scheldt estuary. Unlike previous research studying metal dynamics during reed decomposition (Larsen and Schierup, 1981; Kufel, 1991; Windham et al., 2004), we additionally investigated the possible interaction between the prominent fungal colonization (Van Ryckegem et al., submitted) and the observed metal dynamics. Fungi prove to be the dominant microbial reed decomposers (> 90% of microbial production) both in the litter layer (Findlay et al., 2002) and in the water (Komínková et al., 2000).

5.3.1 Materials and methods

5.3.1.1   Study site

The study was carried out in a brackish tidal marsh of the Scheldt estuary called 'Schor van Doel'. It faces the 'Hertoginpolder' close to 'Saeftinghe Marsh', just across the Belgium border in The Netherlands (51° 21′ N, 4° 14′ E). It is vegetated by a monospecific stand of common reed, *Phragmites australis.* Leaf blade, leaf sheath and stem biomass production was found by Soetaert et al. (2004) to be 320 ± 131, 125 ± 16 and 445 ± 16 g m^{-2}, respectively (mean ± standard deviation). The estimated sedimentation rate is about 3 cm year^{-1}. Metal contents in the upper 5 cm sediment layer were found to be 2.52 – 4.77 mg kg^{-1} for Cd, 89 - 213 mg kg^{-1} for Cr, 54.1 - 90.9 mg kg^{-1} for Cu, 10.3 - 98.9 mg kg^{-1} for Ni, 82 - 152 mg kg^{-1} for Pb and 210 - 377 mg kg^{-1} for Zn (expressed on dry mass basis). Organic matter and carbonate contents varied from 14.7 to 22.1% and from 6 to 13%, respectively (w/w). Conductivities between 1.77 and 9.52 mS cm^{-1}, pH between 7.1 and 7.8 and chloride contents between 1.9 and 11.5 g kg^{-1} dry mass were observed. All metal, carbonate, organic matter and

chloride contents were highest in the upper 5 cm sediment layer. Lower concentrations were observed at higher depths.

5.3.1.2   Experimental setup

Samples originated from freshly harvested plants (with vital microbial colonization). The experimental period started when most senescent leaves and culms of the reed plants were expected to fall down onto the marsh sediments. For culms this is a rather arbitrary choice because they show a variable period of standing decay. Therefore brown lower leaves were collected from the marsh prior to shedding in October 2001; culms were collected in December 2001, once fully senescent, by cutting out sections comprising two nodes and one internode 1 m above the sediment, with the leaf sheath surrounding the stem. This timing and collecting method was chosen to reflect a natural decomposition on the marsh sediment (Gessner, 2000). Plastic litter bags with a large enough mesh to allow fluent transport of water, sediments and organisms (35 × 20 cm; mesh 4 mm) were filled with 5.0 g fresh weight of leaves and 50.0 g fresh weight of cut culm sections and anchored by hooked bars on the intertidal sediment the day after collecting. On monthly intervals 2 litter bags of each type (leaves and culms) were retrieved from the marsh. Samples were immediately transported in a cool box to the laboratory, where they were gently but thoroughly rinsed with distilled water to remove adhering clay and macro-invertebrates. Stems and leaf sheaths were separated and processed separately during the entire study. All samples were dried at 40°C during 72 h, weighed and analysed for ash and heavy metal contents. Sampling was ended after 11 months for the leaves and 16 months for the culms, as due to the advanced state of decomposition not enough sample was left to allow representative samplings. Moreover, analysis of the leaves for metal contents was already ended after 9 months, as not enough material was left for both metal and ash analyses. Before replacing the samples on the marsh in the beginning of the experiment, ash and heavy metal contents were determined on five samples of leaves, stems and sheaths to allow for a precise estimation of the variability of initial contents.

5.3.1.3   Analyses

Ash contents were determined by measuring the weight loss after incineration of the oven dried samples (4 h at 450°C). Mass loss data were fitted to the exponential model, $m_t = m_0 e^{-kt}$, where m_t is the litter dry mass remaining after time t, m_0 is the original mass and k the

breakdown coefficient. Analysis of covariance (ANCOVA; general linear model procedure, SAS Statistical Package version 8.2, 1999) on log-transformed data was used to compare the breakdown rates between litter types. For the analyses of metal contents, samples were ground in a hammer-cross beater mill and homogenised. Five gram of each sample was weighed to the nearest 0.1 mg on an analytical balance (Sartorius BP221S, Sartorius, Göttingen, Germany), placed into a 100 mL Pyrex beaker and treated with 10 mL ultra-pure 65% HNO_3. The beaker was covered with a watch-glass and the suspension was heated up to 130°C for 1 h. Four ml 20% H_2O_2 was added in aliquots of 1 mL. After cooling, the suspension was filtered (S&S, blue ribbon, Schleicher & Schuell, Dassel, Germany) in a 50-mL volumetric flask and diluted to the mark. In all extracts Cd, Cu, Cr, Ni, Pb and Zn contents were measured using F-AAS (flame atomic absorption spectrometry, Varian SpectrAA-1475, Palo Alto, CA, USA) or GF-AAS (graphite furnace atomic absorption spectrometry, Varian SpectrAA-800 / GTA-100, Palo Alto, CA, USA). The analyses were performed according to Du Laing et al. (2003). The concentrations of Fe, Mn, Ca, Mg, K and Na were also measured as they can interact with adsorbed fractions of heavy metals. F-AAS was used to measure Fe, Mn, Ca and Mg contents and flame emission photometry (Eppendorf Elex 6361, Hamburg, Germany) to measure K and Na contents. Statistical analyses consisted of the calculation of correlations according to the Pearson correlation coefficients method, using SPSS 12.0 (2003). Modified box-plots were constructed using SPSS 12.0 (2003) to represent distribution of data graphically.

5.3.2 Results and discussion

5.3.2.1   Weight loss and organic matter decomposition

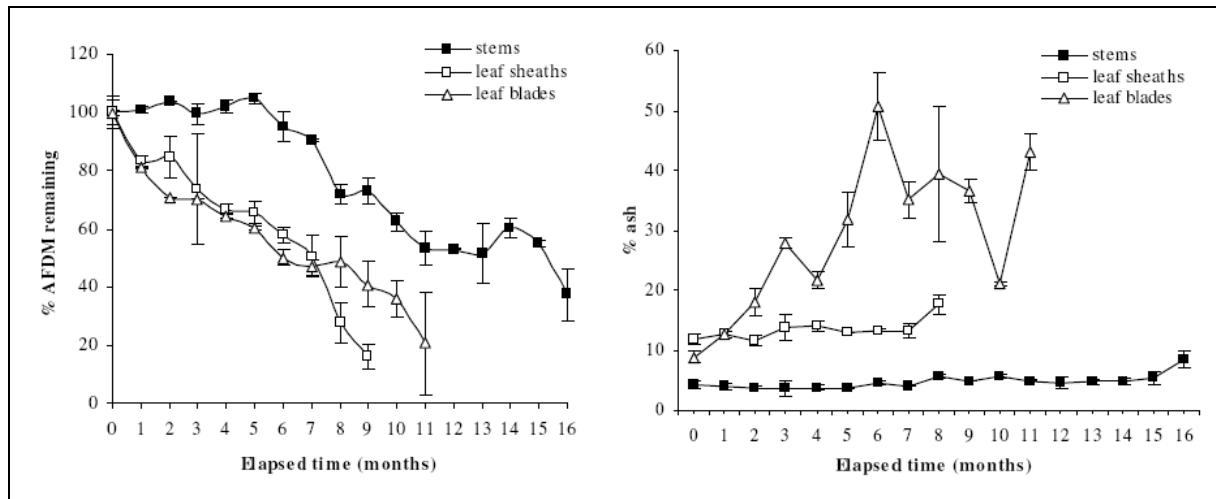The residual ash-free dry weights are depicted in Fig. 5.6.

Fig. 5.6. Ash-free dry mass (AFDM) remaining and ash content (%, mean ± standard deviation) in the litter bags during decomposition of stems, leaf sheaths and leaf blades of *Phragmites australis* in a brackish tidal marsh. Experiment started in December 2001 for leaf sheaths and stems and in October 2001 for leaf blades.

The time for 50% weight loss was approximately 7 months for sheaths, 7 months for leaf blades and 15 months for stems. This coincides with exponential breakdown rates k of 0.0039, 0.0035 and 0.0026 for respectively leaf sheaths, leaf blades and stems. The breakdown coefficient given for stems presents the decay rate after the initial lag period of six months during which there was no decomposition. ANCOVA suggested no difference in breakdown rate between leaf sheaths and leaf blades ($P > 0.05$), while stems showed a significantly slower breakdown ($P < 0.01$; even if the lag period was kept out of the analysis) compared to both leaf blades and sheaths, a feature noticed before (e.g. Hietz, 1992; Gessner, 2000).

The ash content of the leaf blades increased considerably during the first six months of decomposition, from 7 to around 50% (Fig. 5.6). The high ash content of leaf blade samples compared to other plant parts is probably caused by the large surface area and specific decay characteristics. Leaf blades show a decay pattern which is visually characterized by a collapse and removal by shredder invertebrates of leaf mesophyll between the longitudinal vascular bundles. Between those fine ridges of vascular bundles, fine clayey sediments infiltrate and accumulate, making leaves efficient traps for inorganic substances or mud particles difficult to remove. Ash content of stems and, more pronounced, of leaf sheaths gradually increases during the decomposition process with little variation during the experimental period 4.7 ± 1.2% and 12.6 ± 1.1% for stems and sheaths, respectively. This is probably due to the softening of the tissue becoming more susceptible to mud infiltration and invertebrate shredder activity causing micro relief at the tissue surface.

5.3.2.2   Metal contents

Initial metal contents in leaf blades, leaf sheaths and stems are presented in Table 5.5. Except for Cr and Zn, initial metal concentrations are lowest in stem tissue.

Table 5.5. Initial metal contents of leaf blades, stems and sheaths (mg kg^{-1} DM, mean ± standard deviation, n = 5)

	Leaf blades	Stems	Sheaths
Cd	0.159 ± 0.016	0.033 ± 0.008	0.146 ± 0.023
Cr	1.48 ± 0.53	1.42 ± 0.29	1.39 ± 0.37
Cu	6.26 ± 0.25	2.95 ± 0.55	8.89 ± 1.54
Ni	1.32 ± 0.35	0.45 ± 0.14	0.82 ± 0.23
Pb	4.62 ± 0.87	0.34 ± 0.08	3.12 ± 0.51
Zn	40.9 ± 6.1	38.4 ± 14.1	35.3 ± 1.7

The changes of metal contents in leaf blades, sheaths and stems are depicted in Fig. 5.7. Most concentrations expressed on dry weight basis increased considerably during decomposition. Increases in metal concentrations in plant litter were also observed by other authors. Larsen and Schierup (1981) found increasing Zn, Pb and Cd concentrations in *Phragmites* leaf blades during decomposition in the littoral zone of a sewage-polluted and a non-polluted lake. However, Cu contents were relatively constant. Windham et al. (2004) found increasing Cr, Cu, Hg, Pb and Zn concentrations in decomposing leaves and stems of reed plants in contaminated marshes.
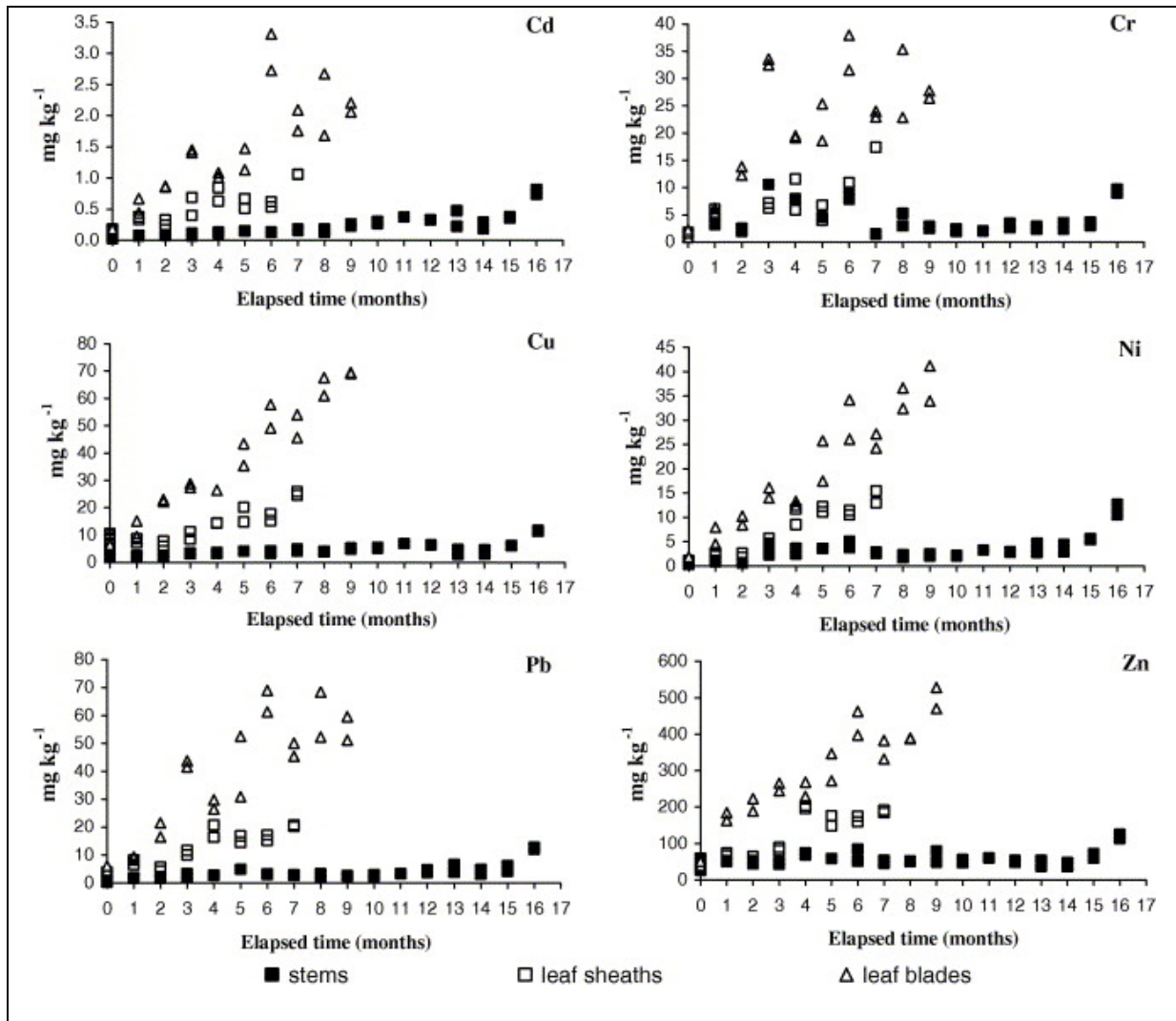
Fig. 5.7. Changes of metal contents (mg kg^{-1} DM) during decomposition of stems, leaf sheaths and leaf blades of *Phragmites australis* in a brackish tidal marsh. Experiment started in December 2001 for leaf sheaths and stems and in October 2001 for leaf blades.

Increasing metal concentrations do not prove that the litter bags act as a sink for metals. Therefore, the overall metal pools in the decomposing organic matter were estimated by multiplying the measured litter weights by their metal concentrations. The calculated amounts after 7 months, associated with leaf blades, stems and sheaths, are presented in Table 5.6 as a proportion to the initial amounts. Generally, a very important net metal inflow could be observed during these first 7 months. The inflow was highest for leaf blades. After 7 months, metal amounts associated with leaf blades were between 4.8 and 13.6 times higher than the initial amounts. Leaf sheath tissue accumulated up to between 1.5 and 10.0 times the initial amounts, whereas this proportion was found to be between 0.9 and 7.6 for stems. These data indicate that the litter bags were indeed important sinks for heavy metals.

Table 5.6. Proportions of calculated Cd, Cr, Cu, Ni, Pb and Zn amounts, associated with leaf blades, stems and sheaths in a litter bag to the mean initial amounts associated with leaf blades, stems and sheaths in a litter bag. Duplicate analysis result from two different litter bags which were both retrieved after 7 months from the marsh

	Leaf blades	Stems	Sheaths
Cd	7.3 - 8.6	4.2 – 4.8	3.8 – 3.9
Cr	10.2 -10.7	0.9	6.6
Cu	4.8 - 5.7	1.3 – 1.5	1.5 – 1.5
Ni	12.1 - 13.6	5.2 – 5.6	8.4 – 10.0
Pb	6.5 - 7.1	7.1 – 7.6	3.4 - 3.5
Zn	5.4 – 6.2	1.1 – 1.3	2.8 – 2.9

Increases of the metal contents could be attributed to different factors, such as contamination by sediment particles, passive sorption onto recalcitrant organic fractions and active accumulation by microbial colonizers (Breteler et al., 1981; Gadd, 1993; Zawislanski et al., 2001; Kovacova and Sturdik, 2002; Weis and Weis, 2004).

Kufel (1991) however observed decreasing Pb and Mo amounts per litter bag during decomposition of reed plant material. The litter bags used by Kufel however were submerged in littoral water with low metal pollution without contact with the sediment surface, which may account for a less significant inflow of metals. Windham et al. (2004) found highly variable patterns when calculating metals pools within the litter bags. They however did not observe net metal accumulation in the litter bags during their experiments. It should be noted that metal accumulation observed in litter bag experiments significantly depends upon the environmental conditions (e.g. salinity, pollution degree), and experimental conditions (e.g. submerged or littoral, mesh size of the litter bags, treatment of the plant litter), which makes comparison between different studies difficult.

The top layer of the marsh sediments can be periodically resuspended by tidal wave action, which increases the risk of infiltration of mud particles into the litter bags, especially if the bags are incubated in close contact with the sediment layer. The concentrations of metals in the top layer of the marsh sediment are much higher than those recorded in the initial plant litter. Contamination with sediment particles can therefore easily constitute a major source of error in determining low litter metal concentrations. To test whether metal contents of the litter could be influenced by adhering mud particles, correlations between ash contents and

metal concentrations were calculated (Table 5.7). If trapping of sediment particles which contain much metals is important, significant correlations between increasing metal contents and increasing ash contents can be expected, as sediment particles consist mainly of ash. For leaf blades with highly increasing ash contents (Fig. 5.6), the correlations were significant at the 0.01 level for all elements, whereas for the sheaths and stems the correlations were not significant for most of the elements. The increase of leaf blade metal contents may thus be due to an important infiltration of mud particles, which were not removed by rinsing the leaf blades with destilled water preceding the analyses.

Table 5.7. Correlations between ash and heavy metal contents in litter bags for leaf blades, stems and sheaths

	Leaf blades	Stems	Sheaths
Cd	0.986 **	0.638 *	0.525 [ns]
Cr	0.899 **	-0.449 [ns]	0.540 [ns]
Cu	0.886 **	0.587 *	0.430 [ns]
Ni	0.906 **	-0.058 [ns]	0.613 [ns]
Pb	0.979 **	0.032 [ns]	0.693 [ns]
Zn	0.906 **	0.106 [ns]	0.674 [ns]

** Correlation is significant at the 0.01 level (2-tailed); * Correlation is significant at the 0.05 level (2-tailed); [ns] Correlation is not significant

The proportion of metals in the ash inflow was also estimated. Ash inflow was calculated at each sampling time as the difference between ash amounts and initial ash amounts in the litter bags, associated with leaf blades, stems and sheaths, respectively. Similarly, metal inflow at each sampling time was calculated as the difference between metal amounts and initial metal amounts in the litter bags, associated with leaf blades, stems and sheaths, respectively. The percentage of metals in the ash inflow was then calculated at each sampling time for leaf blades, stems and sheaths, respectively: $100 \times$ (metal inflow/ash inflow). The values were also calculated for Fe, Mn, Ca, Mg, K and Na as these elements can interact with adsorbed fractions of heavy metals. For the leaf blades, a substantial ash inflow was observed. Percentages of most metals in the ash inflow were very high in the first month and then decreased (Fig. 5.8). The inflow of ash with relatively low and stable metal concentrations between the second and the ninth month can be due to trapping of certain sediment fractions with relatively low metal contents. In that case, the inflow of ash with higher metal concentrations during the first month could be attributed to combined trapping of the same

kind of sediment fractions and a faster physicochemical sorption of dissolved metals onto the remaining organic matter.
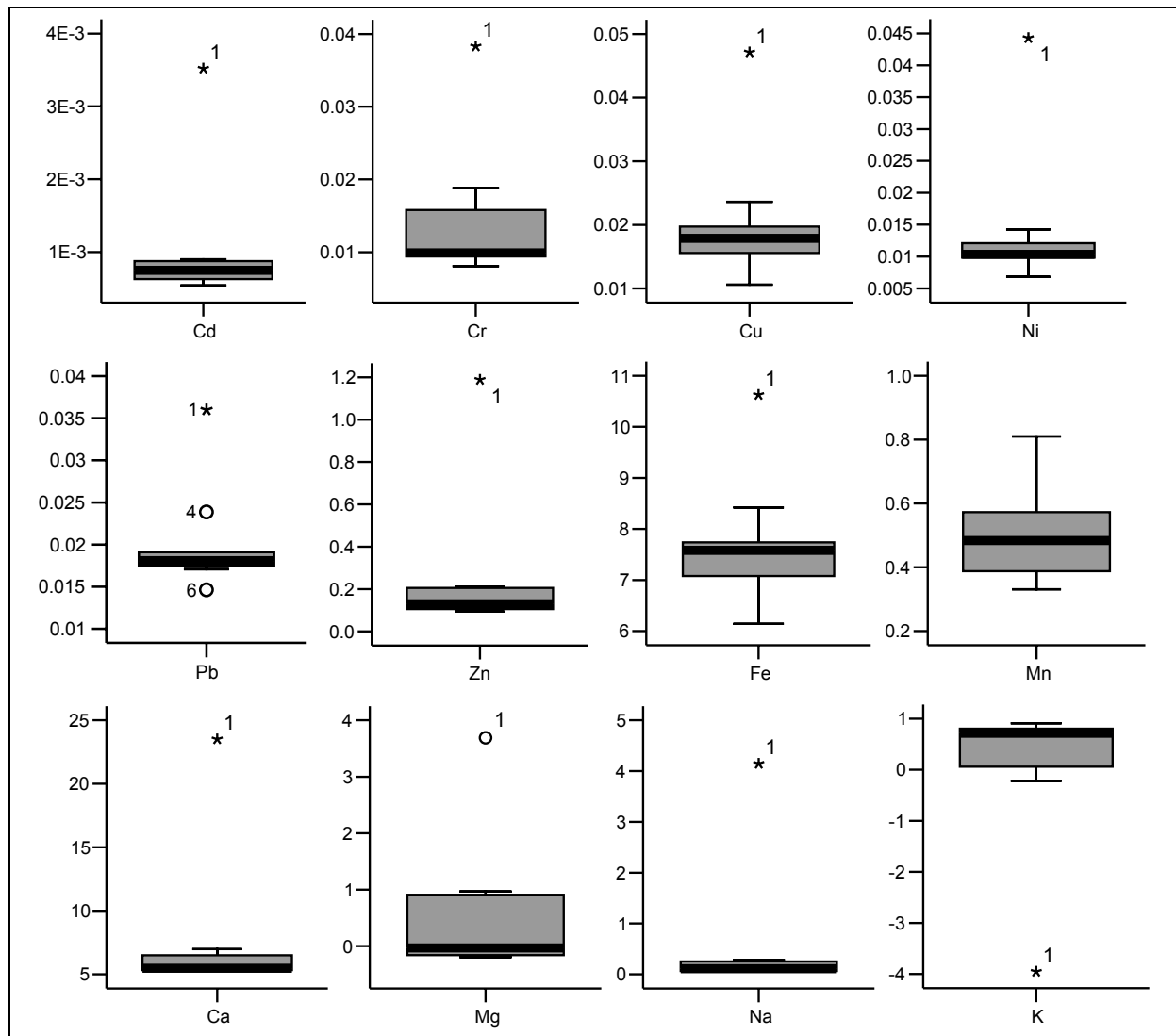

Fig. 5.8. Modified box-plot diagrams showing for leaf blades the distribution of metal concentrations in the ash inflow (%) with the initial values as a basis. The centre line shows the median of the values. Outliers are indicated with a circle and extremes with an asterix. They are labeled by sampling time (number of months from the beginning of the experiment).

For leaf sheaths and stems, smaller amounts of inflowing ash, and for stems even continuously outflowing ash amounts, were found during most of the experiment. Metal contents in the inflowing and outflowing ash are fluctuating, which again suggests that inflow of inorganic particles is not the major factor determining metal accumulation by leaf sheaths and stems on medium term. Other mechanisms such as active accumulation by microbial organisms could then be more important. Windham et al. (2004) continued a quite similar

litter bag experiment up to 24 months. They found that adsorption and accumulation of fine sediment cannot be the major cause of increasing metal concentrations on longer term, especially as accumulation was found to be higher at sites which were less contaminated, from which they concluded that microbial action is likely one of the major mechanisms responsible for the enrichment.

To test whether metal accumulation could be due to incorporation by microbial litter colonizers, we calculated correlations between metals and fungal biomass (ergosterol concentrations) within the same plant litter. Fungal dynamics in the decomposing litter are discussed by Van Ryckegem et al. (submitted), demonstrating that fungi are dominant decomposers during aerobic decay of *Phragmites australis*. They contribute up to 10% of ash free litter mass. However, patterns are different between plant parts. Leaf blades had highest fungal colonization in the canopy in a hanging position prior to shedding. After entering the litter layer fungal biomass decreased spectacularly but recovered partially. Leaf sheaths showed a similar pattern but the drop in fungal biomass after falling was less pronounced. Final concentrations were the highest recorded during decay. Stems showed no detectable fungal colonization in a standing position and fungal biomass gradually increased during the decomposition process in the litter layer. Correlations between fungal biomass (ergosterol concentrations) and metals are depicted in Table 5.8. No significant correlations were found for leaf blades and low correlations were demonstrated for the leaf sheaths. However, fungal biomass proved to be highly correlated with metal contents in stem tissue except for Cr and Zn, both elements of which the contents stayed relatively stable during the experimental period (Table 5.6). This correlation suggests an involvement of fungal activity in metal accumulation in stem tissue by a) direct incorporation in fungal mass (Ledin, 2000); b) enhanced binding of metals to the decomposing litter due to complexation between extracellular fungal products and metals (Gadd, 1993); c) by induced changes in litter quality by mineralization, e.g. increasing availability of phenolic units as lignin depolymerises, offering many potential metal-binding sites (e.g. Senesi et al., 1987, Ledin, 2000). To clarify which mechanisms could drive metal accumulation under influence of microbial decomposers, more specific research is needed.

Table 5.8. Correlations between fungal biomass (ergosterol) and heavy metal contents in litter bags for leaf blades, stems and sheaths

	Leaf blades	Stems	Sheaths
Cd	-0.393^{ns}	0.741^{**}	0.660^{*}
Cr	-0.106^{ns}	0.003^{ns}	0.640^{*}
Cu	-0.341^{ns}	0.624^{**}	0.274^{ns}
Ni	-0.280^{ns}	0.491^{**}	0.249^{ns}
Pb	-0.246^{ns}	0.577^{**}	0.348^{ns}
Zn	-0.314^{ns}	0.295^{ns}	0.211^{ns}

$**$ Correlation is significant at the 0.01 level (2-tailed); $*$ Correlation is significant at the 0.05 level (2-tailed); ^{ns} Correlation is not significant

5.3.3 Conclusion

Most metal contents in reed litter increased considerably during decomposition. As reed biomass turnover is very high, metal accumulation by litter could be an important parameter to monitor concerning the metals transfer at the base of the food chain in intertidal reed beds. Fungal activity may be important in immobilizing metals in decomposing stem tissue. This could also be the case for leaf blades, but for this tissue type the effect of fungal activity on metal concentrations is found to be overridden by the passive metal sorption and trapping of sediment particles and associated metals. Both factors seem to be of intermediate importance for leaf sheaths.

5.4 Conclusion

As a result of the decomposition of reed plants and willow leaves at the end of the growing season, significant amounts of organic matter are supplied to the upper sediment layer. During the first weeks after organic matter supply to some intertidal sediments under greenhouse conditions, Cd, Cu, Ni and Zn concentrations in pore water of the sediments were found to significantly exceed sanitation thresholds for ground water. Upon addition of organic matter, also sulphates were however released, which induced the formation of sulphides and precipitation of metal sulphides. As sufficient sulphide amounts were produced when willow leaves were added, all metal concentrations in the pore water could be rapidly reduced to acceptable concentrations. Lower sulphates amounts were released when reed stems were added. As a result, insufficient amounts of sulphides were produced to precipitate all metals and reduce Ni, Zn, Fe and Mn concentrations to within acceptable limits in a reasonable amount of time. When the soils were alternately flooded, the metals were exported through the percolates. Total metal export by the percolates significantly increased when organic matter was added. The export was concentrated in the first weeks after organic matter addition.

Most metal contents in reed litter itself increased considerably during decomposition under field conditions. Fungal activity may be important in immobilizing metals in decomposing stem tissue. This could also be the case for leaf blades, but for this tissue type, the effect of fungal activity on metal concentrations is found to be overridden by the passive metal sorption and trapping of sediment particles and associated metals. Both factors seem to be of intermediate importance for leaf sheaths.

6.

Influence of hydrological regime on metal mobility

6 Influence of hydrological regime on metal mobility

6.1 Introduction

Hydrological regime and average water storage period might be key parameters in management scenarios for contaminated wetlands. The hydrological regime affects the pH, redox potential and indirectly also the organic matter and $CaCO_3$ content of the soil (van den Berg and Loch, 2000). It can be controlled fairly easily. An adequate regime is not only important for the development of a valuable wetland ecosystem, but it is also crucial to responsibly manage metal pollution in wetland soils. The aim of this chapter is to assess and quantify the effect of different hydrological regimes on the pore water metal concentration in a calcareous metal-polluted sediment-derived soil. The results are relevant to assist in the selection of an adequate hydrological regime when managing, protecting or restoring wetlands in overbank sedimentation zones with important metal contamination and similar physicochemical soil characteristics.

6.2 Materials and methods

6.2.1 Experimental setup

A greenhouse experiment was set up. Soil was collected at a polluted overbank sedimentation zone called 'Merelbeekse Scheldemeersen', situated on the right bank of the Upper Scheldt. The site is a pasture on an alluvial soil formerly superficially excavated for clay reclamation.

Because of this, the soil became more susceptible to flooding and subsequent overbank sedimentation. The top 20 cm of the soil of 20 locations was sampled and was analysed for total metal content. The location with the highest metal content was selected to sample soil for the greenhouse experiment. After removal of the vegetation, the top 20 cm of the soil was excavated with a shovel over an area of 1 m². The soil was passed over a sieve with 4 mm mesh width. The fraction larger than 4 mm was removed. Five recipients of 32 x 24 x 20 cm were filled with soil at a height of 10 cm. The recipients were equipped with drainage outlets with a 3 mm diameter at the bottom. Geotextile was put on the bottom of the recipients to minimise loss of soil through the drainage outlets. The recipients were put in a greenhouse at a controlled temperature between 15 and 25°C and with a light regime of 16 hours light per day.

The soils in the recipients were subjected to different flooding regimes by putting them in and taking them out of a larger box (48 x 32 x 22 cm) filled with deionised water. This water was acidified to pH 4 to simulate the most significant effects of acidic rainfall. The water that causes flooding in nature is not always originating from rainfall but also from seepage and river water that contains several ions and nutrients. It was however acidified to mimic a "worst case scenario", as most metals are expected to become more mobile in acid conditions.

The recipients were subjected to multiple hydrological regimes (R): (R 1) permanently flooded, (R 2) alternately two weeks flooded and one week emerged, (R 3) alternately two days flooded and eight days emerged, (R 4) alternately two days flooded and two days emerged, (R 5) continuously on field capacity. All treatments were intensively monitored during 96 days. These regimes were chosen to simulate different parts of a wetland or different possible flooding strategies. The area has different elevations and the hydrological regime of the overbank sedimentation zone under study can be regulated with locks. Thus, different parts of the terrain are expected to be exposed to different flooding regimes. These can include permanent flooding, short emerged periods, short flooded periods, a tidal regime or no flooding at all.

During the flooded periods, the water table was kept at 5 cm above the soil surface by adding some deionised water (pH 4) if necessary. Near field capacity was reached by watering the soil regularly until a point was reached where water tended to percolate. For each new flooded

period of the alternating regimes, fresh acidified, deionised water was used. After each flooded period, the water was allowed to percolate for two days. The amount of water that had percolated was measured and after mixing, a sample was taken. The recipient was prepared for another flooded period by emptying and cleaning it with distilled water.

Each container was fitted with two 10 cm Rhizon MOM soil moisture samplers (Eijkelkamp, Giesbeek, NL) to extract the soil solution in vacuum tubes over a porous filter compartment with a pore size of 0.1 μm. The Rhizon samplers were installed horizontally 5 cm below the soil surface. The pore size of 0.1 μm is operationally chosen to extract metals which can be considered as available for uptake by plant roots. Pore water samples were taken every two days. During the filling of the recipients, a total of 100 g soil was sampled in several small amounts to check the homogeneity of the soil.

6.2.2 Analyses

Dry matter content of the soil was determined by measuring the weight loss before and after drying the soil at 105°C for 48 hours. Texture of the soil samples was determined using laser diffraction (Coulter LS200, Miami, FL). Total organic carbon (TOC) in the soil was measured with a TOC analyzer equipped with a solid sample module (Shimadzu 5050A Solid Sample Module Analyser, Shimadzu, Kyoto, Japan). $CaCO_3$ content was determined by back-titration with 0.5 M NaOH of an excess of H_2SO_4 added to 1 g air-dried sediment (Van Ranst et al., 1999). Pseudo-total metal concentrations in the soil were determined after *aqua regia* destruction (Ure, 1990) and analysis of the elements Cd, Cu, Cr, Fe, Mn, Ni, Pb and Zn using Inductive Coupled Plasma Optical Emission Spectrometry (ICP-OES) (Varian Vista MPX, Varian, Palo Alto, CA). Acid volatile sulphur (AVS) was determined by volatilisation of sulfides after addition of 12 M HCl to the sediment. The evolved H_2S was trapped in a Zn-acetate solution, and determined by iodometric titration (Tack et al., 1997a). The redox potential was measured with a HI 3131B electrode that was inserted in the soil, equilibrated for 10 minutes and connected to a HI 9025 meter of HANNA instruments. The measured reading was converted to a redox potential with respect to the Standard Hydrogen Electrode by adding the difference of the measurement of the redox potential in a ZoBells solution (0.0033 M $K_3Fe(CN)_6$ and 0.0033 M $K_4Fe(CN)_6$ in 0.1 M KCl) with the theoretical value of + 428 mV.

Pore water samples were acidified to pH 2 with concentrated HNO_3 and combined into a polystyrene recipient. Metal analysis was performed using ICP-OES for Fe, Mn and Zn, and using Graphite Furnace Atomic Absorption Spectrometry (GF-AAS) (SpectrAA-800/GTA-100, Varian, Palo Alto, CA) for Cd, Cu, Cr, Ni and Pb. The TOC content in the percolate was determined with a Total Organic Carbon Analyser (TOC-5000, Shimadzu Corporation, Tokyo, Japan). After acidification with HNO_3, the samples of the percolate were analyzed for the metals as before.

6.2.3 Soil and pore water quality

Heavy metal concentrations in soil and pore water were evaluated with reference to the Flemish Decree on Soil Sanitation (Vlarebo, 1996). Background total concentration levels (BC) in Flemish soils are defined as these concentrations commonly found in soils that are considered clean, and are a function of soil organic matter and clay content (Tack et al., 1997b). Sanitation thresholds for different land use classes in the Flemish regulations indicate threshold levels for both the solid part of the soil and groundwater above which action must be taken to remediate the soil. Sanitation thresholds for the solid part of the soil are differentiated according to land use: nature, forestry and agriculture (class I+II), habitation (class III), sports and recreation (class IV) and industry (class V).

6.2.4 Statistics

Pearson correlation coefficients were calculated between the metal concentrations in the pore water of the different treatments. To compare the differences between the pore water concentrations of the different regimes, the first 96 days were divided in periods of about 20 days, coinciding with the duration of the largest hydrological cycle in R 2. Three different periods were considered: Period 1: day 34 - 52, period 2: day 54 - 74 and period 3: day 76 - 96. The first 32 days were not included in the statistical analysis to exclude transitional effects during the initial period of the experiment. Mean concentrations were subsequently compared between treatments for each period and between periods for each treatment. An ANOVA was conducted to assess the significance of the effects "period" and "regime" as factors. The

SIDAK multiple comparison method was used to identify significant differences between means.

6.3 Results

6.3.1 Soil characteristics

The soil used in the experiment can be identified as a silty clay loam soil according to the USDA soil texture triangle (clay: 35%, loam: 58% and sand: 7%). The pH of the soil was 7.51 ± 0.03. The soil contained a fair amount of $CaCO_3$ and organic matter and was contaminated with Cd, Cr, Pb and Zn (Table 6.1). The low rsd's indicate that the soil used in the experiment was sufficiently mixed before filling the recipients for the different treatments. Cd concentrations exceeded the Flemish soil sanitation thresholds (Table 6.2) for inhabited areas (III). Zn concentrations were still below the sanitation threshold for recreational purposes (land use class IV) but Cr concentrations exceeded that value. Concentrations of Pb, Cu and Ni did not exceed sanitation thresholds but nevertheless were markedly higher than baseline concentrations in Flanders.

Table 6.1. $CaCO_3$, total organic carbon, moisture content and metal concentrations of the soil in the beginning of the test with average values and standard deviations (S.D.) (n = 5)

Treatment	$CaCO_3$ (%)	TOC (%)	Moisture content (%)	Concentration (mg.kg^{-1}dry soil)							
				Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn
average	4.1	10.5	31.5	30.2	740	109	36215	497	36.7	276	1743
sd	0.1	0.3	0.2	0.4	13	2	930	18	0.5	3	21
rsd (%)	1.7	2.6	0.6	1.3	1.7	2.0	2.6	3.6	1.4	1.4	1.2

Table 6.2. Overview of the average soil metal concentration of the five treatments at the beginning of the experiment, the background concentration (BC) and the soil sanitation values in Flanders for the five different land use classes (I to V)

	Concentration (mg kg^{-1} dry soil)					
	Cd	Cr	Cu	Pb	Ni	Zn
BC	2	52	25	66	16	108
I + II	4.9	183	288	328	181	1047
III	14.6	422	576	1147	852	1745
IV	36.6	703	721	2457	997	1745
V	73.1	1124	1153	4095	1269	5235
Soil	30.2	740	109	276	37	1743

6.3.2 Redox potential

The change in redox potential with time was very similar for R 1, the continuously flooded treatment, and R 4, the treatment with a fast alternation between flooding and emerged state (Fig. 6.1). In both treatments, the redox potential dropped to approximately 50 mV and then remained fairly constant. However, for R 1 this value was reached more rapidly than in R 4. For R 2, subjected to longer periods of flooded and emerged state, the redox potential also dropped but it kept fluctuating around 150 mV after thirty days. In treatment R 3, a periodic pattern that matches the hydrological regime was established from day fifty on. For R 5, the redox potential fluctuated between 500 and 600 mV throughout the experimental period.
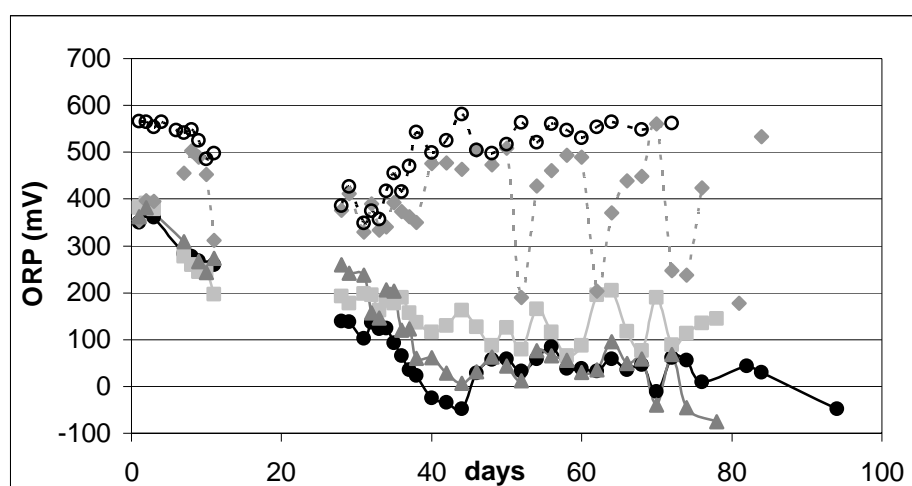

Fig. 6.1. Soil redox potential (ORP, mV) of the five treatments (R 1: ●, R 2: ▲, R 3: ◆, R 4: ■ and R5:○)

6.3.3 Pore water concentrations

Metal concentrations in the pore water varied in different ways between the regimes and between the different elements (Fig. 6.2 to Fig. 6.5). Pore water metal concentrations in uncontaminated soils reported in other studies are listed in Table 6.3, along with the background concentration and soil sanitation value for groundwater according to Flemish regulations (Vlarebo, 1996). The average pore water concentrations over three defined periods (day 34-52, 54-74 and 76-96) are summarized in Table 6.4. Different letters denote statistically different subsets between the regimes for each period (small letters, $p < 0.05$) and between periods for each regime (capital letters, $p < 0.05$). Differences between flooding regimes were highly significant. There was however no significant effect of the period nor was there a significant interaction between period and hydrological regime, for both Cr and Cu. Therefore, no distinction was made in Table 6.4 between the three periods. For Cd, Fe, Mn, Ni and Zn, there was a significant interaction between the period and the regime.

Table 6.3. Reference values for metal pore water concentrations ($\mu g \ L^{-1}$)

Soil type	Cd	Cr	Cu	Ni	Pb	Zn	Source
loam and clay, pH > 7	0.4-3.6	0.4-54	16-58	10-72	0.4-2.5	15-57	Meers, 2005
sand and loam, pH < 7	0.7-8.5	0.4-54	27-176	6-18	0.8-7.4	115-1035	Meers, 2005
Reference soils	0.3-4.7		54-242		1-147	71-1376	Hobbelen et al., 2004
sand and loam, pH > 7	0.4-1.7		15-30			55-85	Hobbelen et al., 2006
clay and loam, pH > 7	0.5-3.4		17-122			48-212	Hobbelen et al., 2006
background value	1	20	10	10	5	60	Vlarebo, 1996
sanitation threshold	5	50	100	40	20	500	Vlarebo, 1996

Table 6.4. Average pore water concentrations of Fe, Mn, Zn, Cr, Cu, Cd and Ni. Different letters denote statistically different subsets (p < 0.05) between regimes for each period (small letter) and between periods for each regime (capitals) (period 1: day 34 - 52, period 2: day 54 – 74, period 3: day 76 – 96; regimes: see legend of Figures 2-5).

	Period	Regime				
		1	2	3	4	5
Fe	1	8.8 [cA]	0.4 [aA]	0.1 [aB]	2.6 [bA]	0.1 [aA]
	2	14.0 [cB]	0.2 [aA]	0.0 [aA]	4.2 [bB]	0.1 [aA]
	3	21.1 [cC]	0.2 [aA]	0.0 [aAB]	7.5 [bC]	0.1 [aA]
Mn	1	3.3 [dA]	0.7 [bA]	0.0 [aA]	1.5 [cA]	0.0 [aA]
	2	3.8 [dB]	0.6 [bA]	0.0 [aA]	1.7 [cA]	0.0 [aA]
	3	4.7 [dC]	0.5 [bA]	0.0 [aA]	2.2 [cB]	0.0 [aA]
Zn	1	0.12 [bC]	0.12 [bA]	0.06 [aA]	0.11 [bC]	0.16 [cA]
	2	0.10 [bcB]	0.11 [cA]	0.05 [aA]	0.09 [bB]	0.15 [dA]
	3	0.08 [bA]	0.12 [cA]	0.06 [aA]	0.07 [aA]	0.17 [dA]
Cr	1-2-3	27 [c]	15 [a]	16 [a]	14 [a]	23 [b]
Cu	1-2-3	4 [a]	47 [b]	54 [b]	13 [a]	69 [c]
Cd	1	0.8 [aB]	6.2 [cA]	3.0 [bA]	5.5 [cB]	8.3 [dA]
	2	0.3 [aA]	5.7 [dA]	2.4 [bA]	3.7 [cA]	8.1 [eA]
	3	0.1 [aA]	7.4 [cA]	2.3 [abA]	3.3 [bA]	9.8 [dB]
Ni	1	28 [dA]	13 [bA]	10 [aA]	16 [cA]	11 [abA]
	2	29 [dA]	13 [bA]	9 [aA]	15 [cA]	11 [abA]
	3	33 [dB]	13 [bA]	8 [aA]	19 [cA]	10 [aA]

The Pb concentrations in all regimes were below the detection limit (< 2 μg L^{-1}) during the entire experimental period (data not shown). Pore water concentrations of Cd in some of the regimes (Fig. 6.2, part A) frequently exceeded soil sanitation values for groundwater (5 μg L^{-1}). Cu concentration patterns (Fig. 6.2, part B) were similar to those of Cd. However, Cd, concentrations initially increased during ten days, a behaviour that was not observed for Cu. The concentrations for the regime that was continuously submerged (R 1) and the regime that alternated each two days (R 4) subsequently decreased. The fluctuations in the concentrations of the alternating regimes (R 2 and 3) corresponded with the changes in the moisture regime. For R 3, the concentrations dropped to background concentration levels when the soil was submerged. A maximum was reached when the soil was emerged. Except for Cd in the beginning, maxima did not exceed the sanitation thresholds. The fluctuations for R 2 were obviously larger and the maxima higher than for R 3. Moreover, the average Cd concentrations were significantly higher for R 2 compared to R 3, whereas this was not the case for Cu (Table 6.4). For R 2, the Cd concentrations exceeded the sanitation threshold two to three times, whereas they just reached this standard for Cu. In the regime at field capacity (R 5), the Cd concentrations were significantly higher compared to the permanently flooded

or alternating regimes (Table 6.4). Moreover, they were above the sanitation threshold during the whole experimental period. Similarly, the average Cu concentration was clearly highest in R 5. Permanently emerged conditions thus caused significantly higher concentrations of Cd and Cu in the pore water.
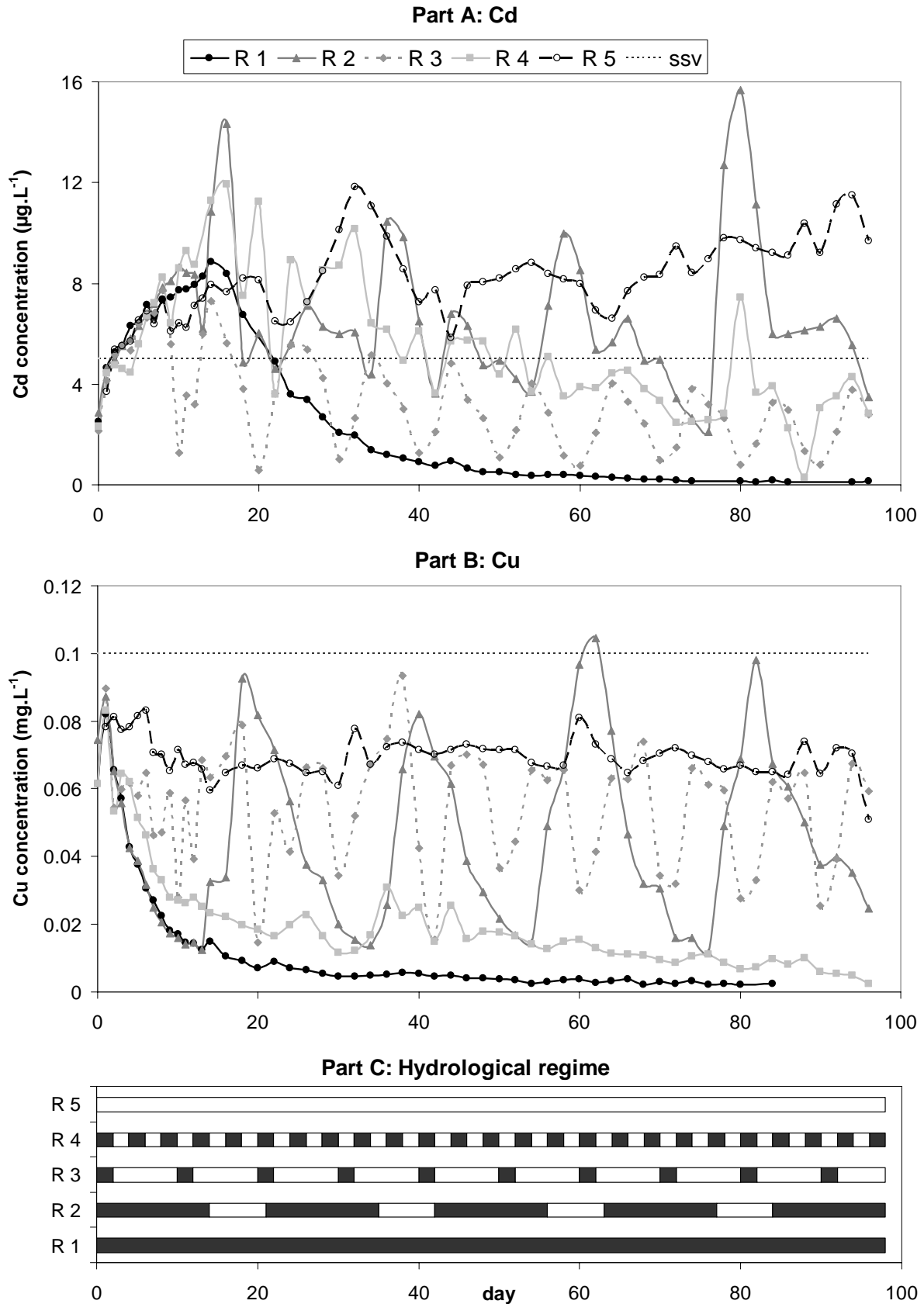
Part A: Cd

Part B: Cu

Part C: Hydrological regime

Fig. 6.2. Part A + B: Cd and Cu concentrations in the pore water (R 1: ●, R 2: ▲, R 3: ◆, R 4: ▪ and R 5: ○, soil sanitation value for groundwater: ⋯). Part C: Hydrological regime is denoted with white for dry periods and black for submerged periods

The changes in the pore water concentration of Mn (Fig. 6.3, part A) were for every regime very similar to the changes in the Fe concentration (Fig. 6.3, part B). Large differences between the regimes were observed (Table 6.4). In the treatments where due to longer periods of submersion and/or shorter dry periods, more reducing conditions were established, Fe and Mn concentration in the pore water markedly increased, in particular for R 1 and R 4 (Fig. 6.1). In treatment R 2, with a prolonged, two week flooded period, Fe and Mn concentrations also increased while the soil is submerged. The fluctuations were larger for Mn compared to Fe. However, average concentrations were significantly lower compared to R 1 and R 4. The remaining two regimes (R 3 and R 5) characterised by more dominant oxidising conditions, exhibited significantly lower concentrations of Fe and Mn in the pore water.
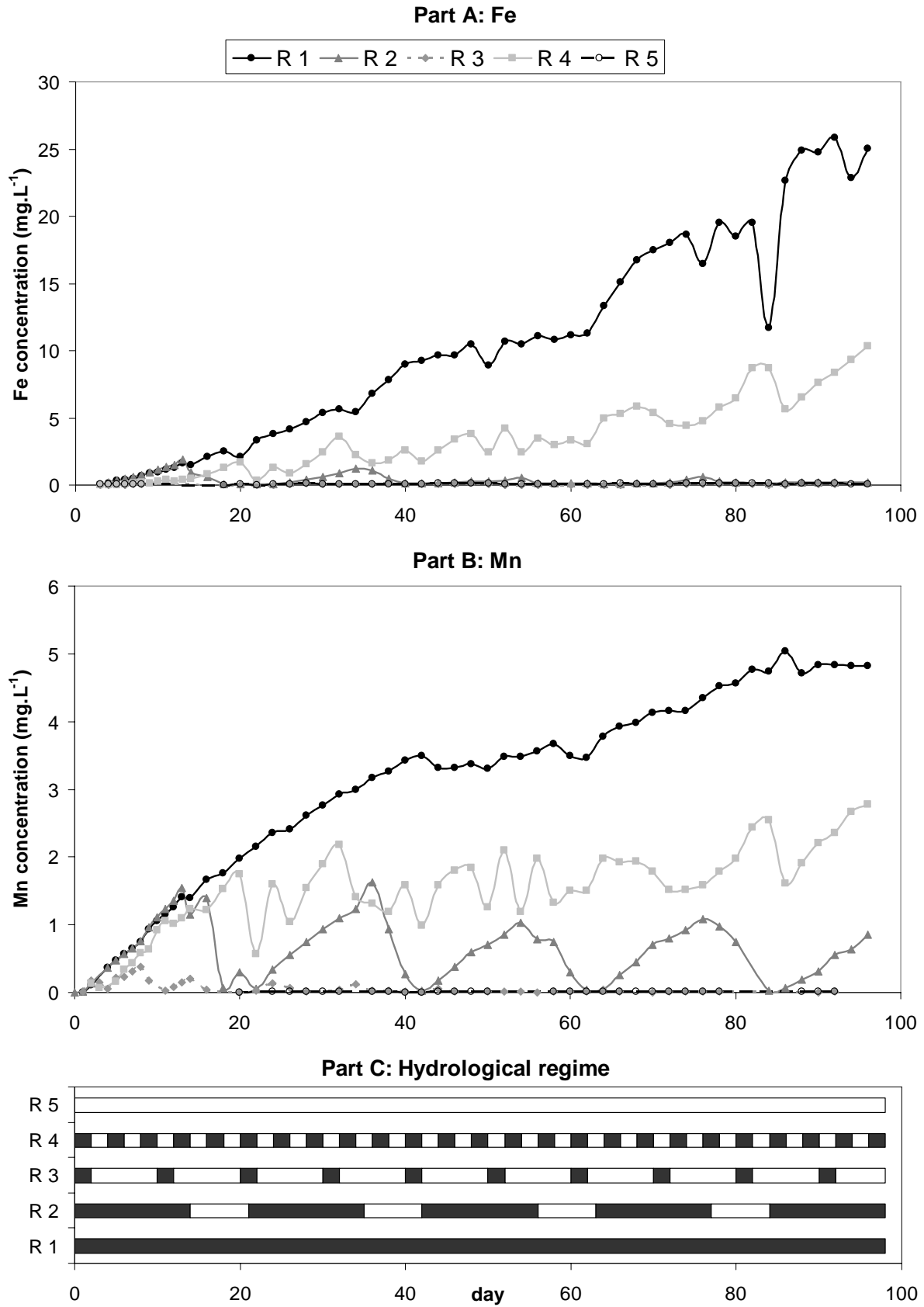
Fig. 6.3. Part A + B: Fe and Mn concentrations in the pore water (R 1: ●, R 2: ▲, R 3: ◆, R 4: ▪ and R 5: ○). Part C: Hydrological regime is denoted with white for dry periods and black for submerged periods

The Ni concentrations of every regime remained below the sanitation threshold (Fig. 6.4, part A). In the permanently flooded regime (R 1), Ni concentrations increased steadily and stabilised around 30 µg L^{-1} after two months. The concentrations in the pore water of R 3 were significantly lower and fluctuated with the same periodicity as the moisture regime. The minima coincided with the periods when the soil was submerged. The maxima were measured when the soil was emerged and were similar to the levels of R 5 (10 µg L^{-1}). Also for R 2, the periodic fluctuations coincided with the periodic changes in the moisture regime. The maximum coincided with the end of the wet period.

The differences between the regimes were rather small for Cr concentrations (Fig. 6.4, part B and Table 6.4). In the permanently flooded soil (R 1), a maximum was reached after about 55 days, after which the Cr concentration continued to fluctuate between 20 and 30 µg L^{-1}. The concentration in the pore water of R 5 was the highest in the beginning, but then dropped and showed a similar pattern as the concentration in the pore water of R 1 after thirty days. Average concentrations in the alternating regimes were significantly lower compared to R 1 and R 5. The pattern was periodic with maxima at the end of the dry periods and minima at the beginning of the wet periods. Average Cr concentrations did not differ significantly between R 2, R 3 and R 4 (Table 6.4), although the maxima which were reached seem to be highest in R 2. In this treatment, Cr concentrations continued to fluctuate between 10 and 30 µg L^{-1}, although one higher peak concentration was also reached around day 60.

Part A: Ni

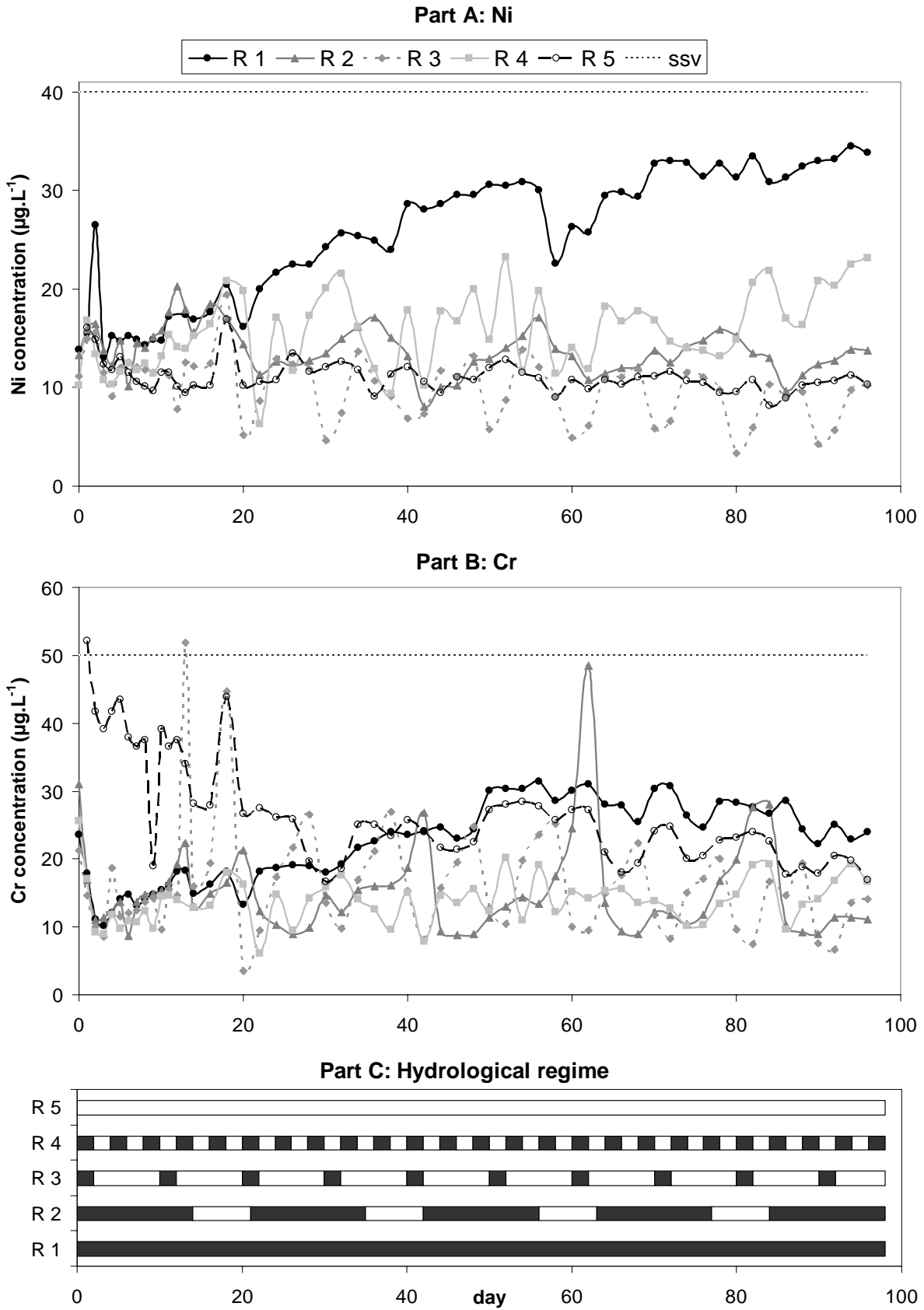
Part B: Cr

Part C: Hydrological regime

Fig. 6.4. Part A + B: Ni and Cr concentrations in the pore water (R 1: ●, R 2: ▲, R 3: ◆, R 4: ▨ and R 5: ○, soil sanitation value for groundwater: ⋯). Part C: Hydrological regime is denoted with white for dry periods and black for submerged periods

The changes in Zn concentrations in the R 1 and R 4 treatments were very similar (Fig. 6.5, part A). After a relatively short period of concentration increases in the beginning, the Zn concentrations began a slow descent to stabilise around concentrations somewhat lower than 0.1 mg L^{-1}. The average Zn concentration of 0.06 mg L^{-1} in R 3 was significantly lower compared to the other treatments (Table 6.4). The periodic fluctuations around that average can be related to the moisture regime. Peaks occurred on the fourth or fifth day of the drying period and the lowest concentrations were measured when the soil was submerged. Also for R 2, we saw a cyclic pattern. Here the maxima occurred on the first day of the dry cycle and the minima on the last day of the wet cycle. The concentration in the pore water of R 5 was significantly higher and relatively constant in the second half of the experiment: between 0.15 and 0.2 mg L^{-1}. All Zn concentrations remained below the sanitation threshold, however.

Ca concentrations in the pore water of the permanently flooded regime (R 1) continuously increased, whereas they were stable, but relatively high, in the soil which was kept at field capacity (R 5) (Fig. 6.5, part B). In R4, concentrations fluctuated randomly. In R 2 and R 3, Ca increased during submerged periods, but after an initial lag period rapidly decreased during the emerged periods.

Part A: Zn

Part B: Ca

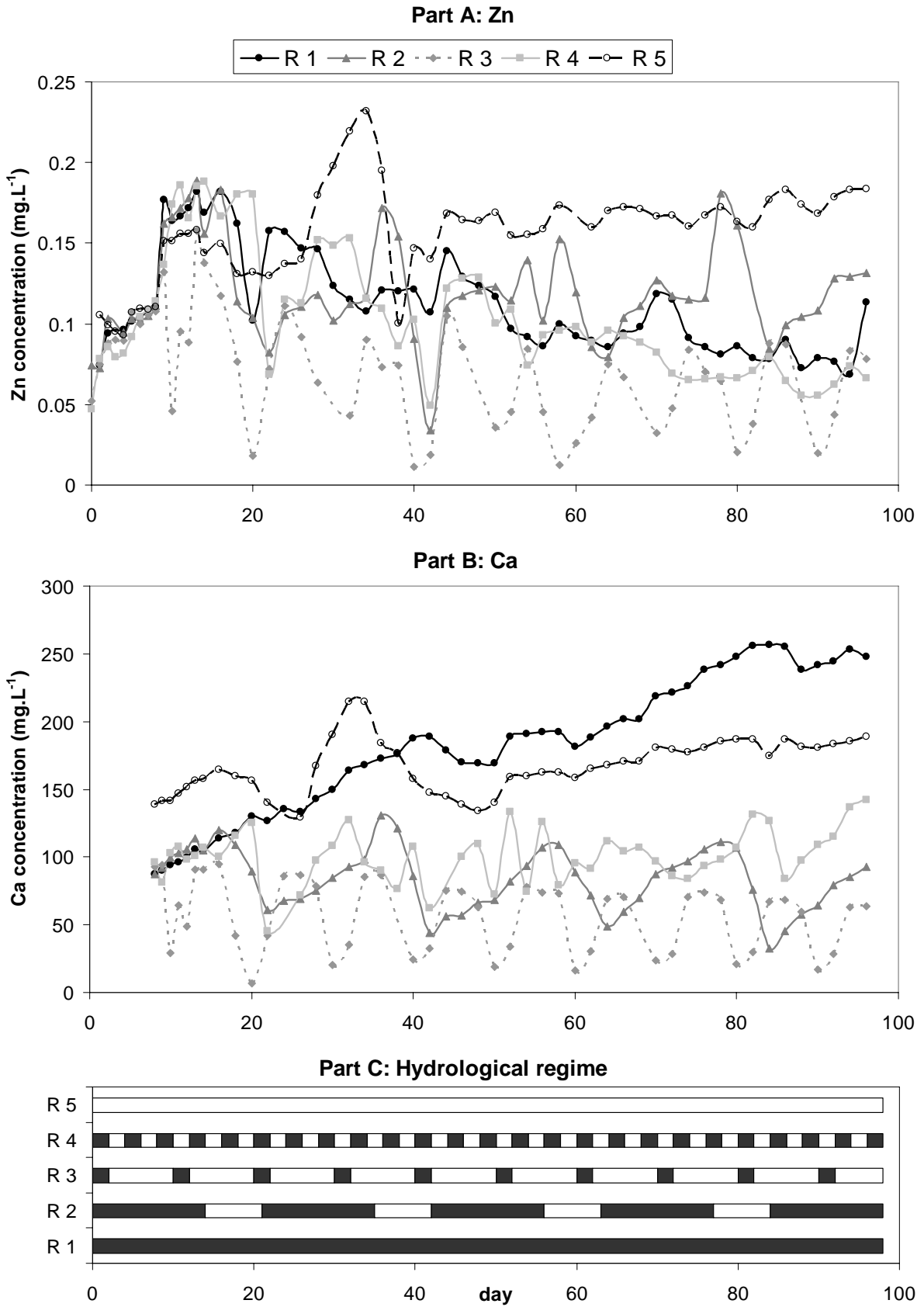
Part C: Hydrological regime

Fig. 6.5. Part A + B: Zn and Ca concentrations in the pore water (R 1: ●, R 2: ▲, R 3: ◆, R 4: ▨ and R 5: ○). Part C: Hydrological regime is denoted with white for dry periods and black for submerged periods

6.4 Discussion

No significant changes in $CaCO_3$ and TOC were observed after three months. This does not exclude that these soil components have been involved in various processes. However, significant changes might become detectable only after more extended periods. It is important to account for long term effects, e.g. decalcification, of a hydrological regime applied to a metal contaminated site. For example, $CaCO_3$ contributes a significant buffering capacity to the soil. When acidification exceeds the buffering capacity of the soil, a decrease in the soil pH can lead to an increased mobility of metals (Gambrell, 1994). According to van den Berg and Loch (2000), the oxidation of previously formed iron sulphides and increased carbon dioxide pressure in soils with alternating hydrological regimes can have a significant effect on the decalcification of a hydric soil.

Although a significant decrease of $CaCO_3$ contents was not detectable, significant Ca levels in solutions suggest it dissolves to some extent. CO_2 liberated during microbial decomposition of organic matter might contribute to $CaCO_3$ dissolution according to

$$CaCO_3 + CO_2 + H_2O \rightarrow Ca(HCO_3)_2 \qquad \text{(Equation 1)}$$

Differences in soil solution Ca between the moisture regimes (Fig. 6.5) might be related to differences in removal of CO_2 from the system. In soils, the CO_2 pressure can easily become tenfold that to the atmospheric CO_2 pressure because of the slow diffusion of gasses through pore water (Lindsay, 1979). When a dry period is applied, pore water will percolate and formed bicarbonates are exported from the soil. Subsequently, pores are filled with air containing low atmospheric levels of CO_2. Thus, CO_2 pressure is temporarily decreased. Accordingly, carbonate dissolution is slowed down which is reflected in lower Ca levels during the dry periods in treatments R 2 to R 4. The consistently higher levels of Ca in the R 1 and R 5 treatments can thus reflect higher CO_2 levels in these soils, caused by the absence of tidal dynamics. Additionally, more constant conditions in these treatments might cause the microbial activity to be more stable. This would explain a more steady and intensive production of acidity than in the soils with alternating regime.

In flooded soils, oxygen is consumed and increasingly, alternate electron acceptors such as nitrate and iron/manganese oxides, are used by microbial communities to acquire energy for their growth. This causes the redox potential to decrease during flooded periods (Fig. 6.1). Sulphate may become an electron acceptor and be converted to sulphide at Eh values below 200 mV (Ross, 1989). Our redox values did not drop below − 150 mV. Moreover, sulphide contents measured after 100 days remained below 10 mg S^{2-} kg^{-1} dry soil. According to Lovley & Phillips (1987), sulphate reduction can be inhibited by Fe reducing micro-organisms. The increasing Fe and Mn pore water concentrations indeed indicate that the reduction of the oxides was still ongoing. Finally, the sulphate concentration in the soil might have been too low to facilitate the formation of significant sulphide amounts. Sulphate concentrations were not measured. The low initial electrical conductivity of the soil (EC: 142 μS cm^{-1} compared to the average of 900 μS cm^{-1} for recent dredged sediment derived soils in the Upper Scheldt) however points towards sulphate leaching from the soil prior to its use in the experiment.

The increase in Fe and Mn concentration in the pore water during a submerged period is attributed to the microbial reduction of Fe and Mn oxides (Gambrell, 1994). The active reduction of Fe and Mn oxides was particularly pronounced in R 1, 2 and 4 (Fig. 6.3). Mn oxides are reduced at a higher redox potential than Fe oxides (Brümmer, 1974). This is reflected in Mn showing a faster response than Fe. In R 2, that involves two-week periods of flooding, manganese clearly reacted to the periodic reducing conditions. It showed a cyclic increase and decrease in concentrations. Iron concentrations, however, did not markedly react to the changes between dry and submerged states. In the treatment with flooding events of only 2 days (R 3), the Mn response was also very weak. The emerged periods in that regime appeared sufficiently long to return the system to a fully oxidised state. During dry periods, the redox potential increased above 500 mV (Fig. 6.1). Moreover, such a long emerged periods are expected to reduce the amounts and activities of Fe and Mn oxide reducing microbial communities on the medium or long term.

In treatment R 4, with dry and submerged cycles of only 2 days, conditions were sufficiently reducing to allow for a significant dissolution of both Mn and Fe. A net increase in Fe and Mn concentrations in the pore water resulted, which approached the behaviour of Fe and Mn concentrations in the pore water of the permanently flooded soil (R 1). The durations of the

emerged and the flooded periods thus primarily determine the response of Fe and Mn concentrations in the pore water. These observations are reflected in a very significant, positive correlation between Fe and Mn concentrations in both R 1 and R 4 (Table 6.5).

The pore water concentration can be considered as a measure of relative metal mobility. For some elements, a transitional phase in the pore water concentration was observed during the first weeks, irrespective the hydrological regime. Afterwards, all metals except Cr showed different constant or periodic concentration patterns that depended on the hydrological regime. Different factors might control the release of metals to a varying extent. As such, reduction of Fe and Mn oxides may cause co-precipitated trace elements to be released. This might be the case for Ni. It exhibits a pattern similar to that of Fe and Mn. Moreover, positive correlations between Fe, Mn and Ni for R 1, R 2 and R 4 (Table 6.5) were high and significant. The linear correlation between Ni and Mn is apparent from Fig. 6.6 (Part A). Yet, differences between the soil that was kept permanently on field capacity (R 5) and the one that was kept flooded (R 1) were lower for Ni (factor 2 – 3, Table 6.4) compared to Fe or Mn (factor > 80, Table 6.4). Moreover, several observations during emerged conditions (such as R 5) deviate from the linear regression line at the intercept with the Y-axis in Fig. 6.6 (Part A). It is hypothesised that Ni release under more oxic conditions is associated with $CaCO_3$ dissolution. Ca was also significantly released under the emerged conditions of R 5 (Fig. 6.5, part B). Moreover, the response of Ni to the 2 days of flooding in R 3 is more pronounced than that of Fe and Mn. The daily release of Ni was higher in the submerged periods of R 3 (about 2 μg L^{-1} day^{-1}) compared to R 2 (about 1 μg L^{-1} day^{-1}). This did not coincide with the release of Fe and Mn, but with the release of Ca (Fig. 6.5, part B). The correlation between Ni and Ca is rather weak (Fig. 6.6, part B). However, a very good fit (Fig. 6.6, part C) is obtained by including both Ca and Mn in a multiple regression equation (Equation 2):

$$Ni\ (\mu g\ L^{-1}) = 7.934 + 4.280 \times Mn\ (mg\ L^{-1}) + 0.023 \times Ca\ (mg\ L^{-1}) \quad (R = 0.961, p < 0.001)$$

The equation indicated that only a small part of the variation should be attributed to the variation of Ca concentrations, which confirms the dominant role of Fe/Mn oxide reduction processes in Ni mobilisation.
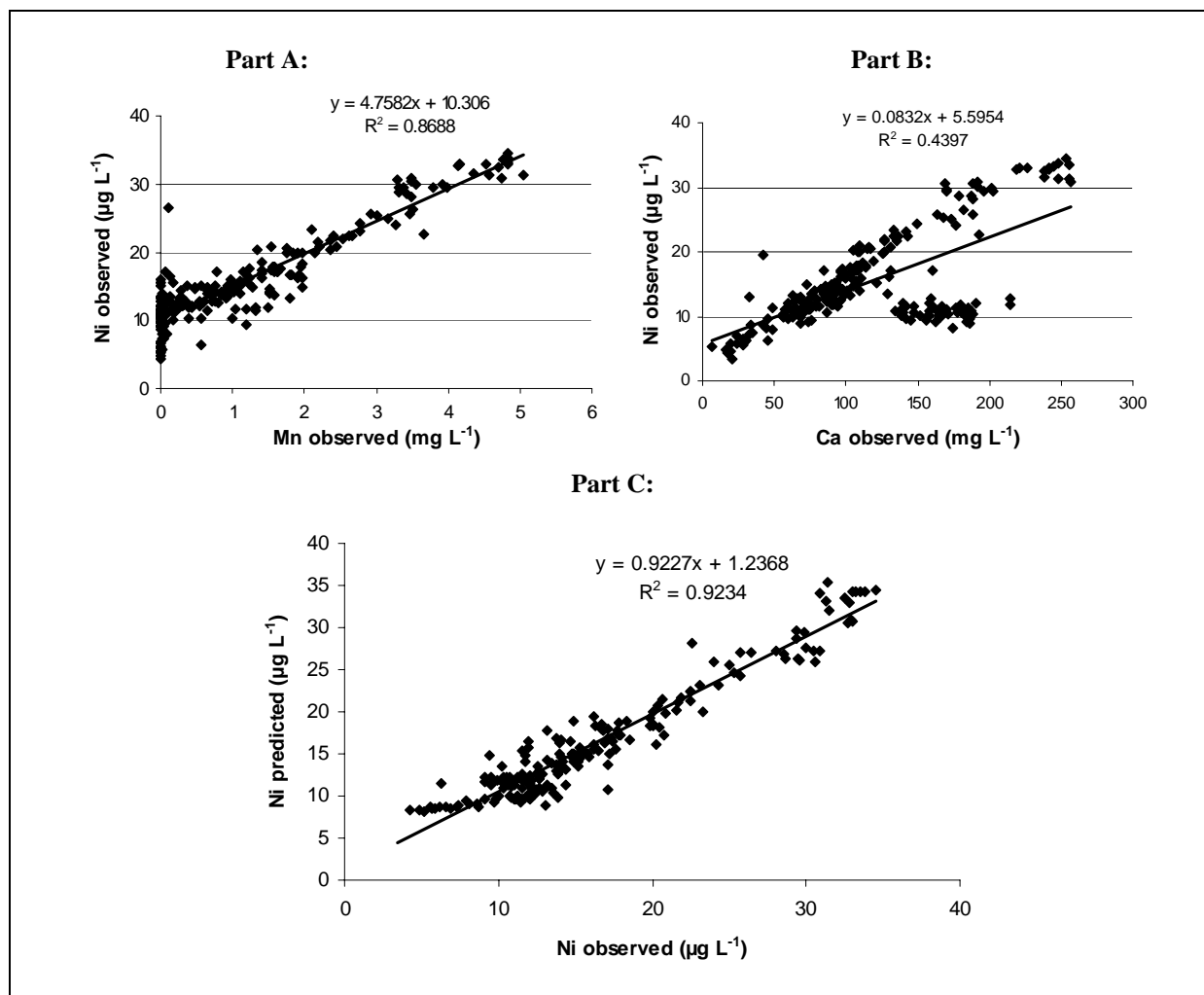
Fig. 6.6. Correlation between (Part A) Ni and Mn concentrations in the pore water, (Part B) Ni and Ca concentrations in the pore water, (Part C) Observed and predicted Ni concentrations in the pore water when using equation 2

Only for R 1 and R 4, and not for R 2, similar correlations with Fe and Mn concentrations were found for Cr in the pore water (Table 6.5). The overall correlation between Cr and Fe or Mn concentrations was lower compared to Ni, whereas the correlation of Cr with Ca concentrations was stronger ($R = 0.579$). The correlation coefficient of the linear model that predicts Cr concentrations in the pore water from Ca and Mn concentrations was substantially lower than for Ni ($R = 0.649$ versus 0.961). Other factors thus also appear important in determining Cr dissolution. One of these factors might be the more complex redox chemistry of Cr. According to Masscheleyn et al. (1992), Cr(VI) is known to be rapidly reduced to Cr (III) in the presence of organic matter and/or Fe(II). They however also reported Cr(III) to be oxidised to Cr(VI) in natural waters and soils, which might be catalysed by the presence of Fe and/or Mn oxides. Trivalent Cr might precipitate as $Cr(OH)_3$. The trivalent Cr in solution can

however also form soluble complexes with organic components, which keeps Cr in solution. In turn, these soluble Cr(III)-DOC complexes could also sorb to sediments, which might remove Cr(III) from solution. These counteracting reactions could explain the mobilisation under both oxidised and reduced conditions (Guo et al., 1997). They could thus also account for the relatively small differences between the different moisture regimes and the observation that Cr concentrations cannot be easily predicted from Fe, Mn and Ca concentrations.

Table 6.5. Pearson correlation coefficients (r) for the correlations (p < 0.001) between the metal concentrations in the pore water of R 1 and R 4, between the Fe, Mn and Ni concentrations in the pore water of R 1, 2 and 4, between the Cr, Fe, Mn and Ni concentrations in the pore water of R 1 and 4, correlations between the Cd and Zn concentrations in the pore water of every regime and for the correlations of Cd and Zn with the Fe, Mn, Ni and Cr concentrations in the pore water of R 1 and with the Fe concentrations in the pore water of R 4 (n = at least 40)

	r		r		r
Cd.1 - Cd.4	0.56	Mn.1 - Mn.4	0.75	Ni.1 - Ni.4	0.49
Cu.1 - Cu.4	0.95	Fe.1 - Fe.4	0.92	Zn.1 - Zn.4	0.76
Fe.1 - Mn.1	0.87	Fe.2 - Mn.2	0.79	Fe.4 - Mn.4	0.84
Fe.1 - Ni.1	0.86	Fe.2 - Ni.2	0.69	Fe.4 - Ni.4	0.64
Mn.1 - Ni.1	0.87	Mn.2 - Ni.2	0.69	Mn.4 - Ni.4	0.80
Cr.1 - Fe.1	0.68	Cr.1 - Mn.1	0.81	Cr.1 - Ni.1	0.80
Cr.4 - Fe.4	0.46	Cr.4 - Mn.4	0.69	Cr.4 - Ni.4	0.67
Cd.1 - Zn.1	0.64	Cd.1 - Fe.1	-0.73	Zn.1 - Fe.1	-0.68
Cd.2 - Zn.2	0.63	Cd.1 - Mn.1	-0.87	Zn.1 - Mn.1	-0.51
Cd.3 - Zn.3	0.89	Cd.1 - Ni.1	-0.83	Zn.1 - Ni.1	-0.52
Cd.4 - Zn.4	0.88	Cd.1 - Cr.1	-0.87	Zn.1 - Cr.1	-0.46
Cd.5 - Zn.5	0.74	Cd.4 - Fe.4	-0.57	Zn.4 - Fe.4	-0.58

In the permanently flooded soil where continuous reducing conditions were established, the concentrations of Cd and Cu steadily decreased to achieve very low values. In the other regimes, concentrations decreased rapidly during flooded periods then raised again when the soil was emerged.

Although total AVS remained very low throughout the experiment, this particular behaviour of the elements to reducing conditions should probably be attributed to their interaction with sulphides. In the permanently flooded soil, metals would become immobilised as sulphides. Oxidation of these sulphides during emerged periods which last for more than 2 days would result in the oxidation of sulphides and release of Cu and Cd to the pore water. Indeed, the 2-day emerged period in R 2 seems could be too short to allow a sufficient amount of oxygen to

penetrate into the soil and initiate sulphide oxidation. As a result, Cd and Cu concentrations in the pore water were also steadily decreasing in that treatment.

Some factors point against the hypothesis of sulphide formation. First, formation of significant sulphide amounts was not detected. Moreover, a theoretical redox potential value of less than -220 mV is needed for sulphide formation to proceed (Ross, 1989). However, other authors reported that sulphate reduction can be initiated at higher redox potentials, such as below -100 mV, especially in the presence of Fe and Mn oxide reduction which can result in the occurrence of mixed potentials (Kashem & Singh, 2001). Thus, it is not excluded that sulphides controlled the metal behaviour, even when present in minute amounts. The detection limit of sulphides was 10 mg S^{2-} kg^{-1} DM. When the concentration drop in R 1 or R 4 is converted to mg/kg sediment it can be estimated that 1 and 20 μg S^{2-} kg^{-1} DM are sufficient to precipitate the metals. Other sulphides, such as FeS and MnS, are orders of magnitude more soluble than CdS, CuS and ZnS under reducing conditions and therefore are not expected to be formed significantly. The solubility product of FeS is in the order of 10^{-18}, whereas it is 10^{-27} for CdS, 10^{-36} for CuS and 10^{-25} for ZnS. Similarly, NiS formation is expected to occur under reducing conditions, yet a net Ni mobilisation was observed under reducing conditions. Ni precipitation by a limited amount of sulphide is probably overruled by Ni release upon reduction of Fe and Mn oxides in reducing conditions.

The conclusions of this study are restricted to the upper soil layer (0-10 cm). Similar factors probably affect metal fate at lower soil layers. However, the time needed to establish reducing or oxidising conditions during different events of flooding and drying will differ. Similar soil characteristics as these of the studied soil have been encountered on several dredged sediment affected areas next to the upper Scheldt river (Vandecasteele et al., 2004). Extrapolation of the findings to salt march ecosystems is less certain, however because in these environments, the influence of considerable amounts of cations and metal complexing anions becomes very important (Du Laing et al., 2002). Moreover, the presence of larger amounts of sulphates in brackish and sea.

6.5 Conclusions

The hydrological regime to which a metal-polluted overbank sedimentation zone is subjected has a pronounced influence on the metal concentration in the pore water of the upper soil layer. Flooding conditions lead to an increase of the Fe, Mn, Ni and Cr mobility and a decrease in the Cd, Cu and Zn mobility in the upper soil layer. Keeping the soil at field capacity resulted in a low pore water concentration of Fe, Mn and Ni while the Cd, Cu, Cr and Zn pore water concentrations increased. Alternating hydrological conditions resulted in fluctuating metal concentrations in the pore water. If the flooding lasts for a maximum of two days and is followed by a longer emerged period or when the soil is kept continuously on field capacity, release of most metals appears primarily related to Ca release. The metals could be mobilised as a result of Ca release, or they might be subject to the same factors which affect Ca release, such as the dissolution of carbonates. In conditions that allow sufficiently strong reducing conditions to be established, there is evidence that the mobility of Cd, Cu, and to a lesser extent Zn, is primarily affected by formation and re-oxidation of sulphides, whereas Ni would mainly be mobilised as a result of Fe/Mn oxide reduction. Interpretation and prediction of Cr fate is less clear from the current data as its behaviour is complicated by its occurrence in a wide range of oxidation states and species. To prevent the Cd concentrations in the pore water to exceed the sanitation thresholds and reduce the risks related to Cd mobilisation, a permanently flooded situation seems most suitable. However, this might result in elevated Ni and Cr concentrations in the pore water.

7.

Metal uptake by reed plants
(*Phragmites australis*)

7   Metal uptake by reed plants (*Phragmites australis*)

7.1   Introduction

While many engineering studies of treatment wetlands adopt a black box approach analyzing levels in the influent and effluent, more must be known about the patterns and processes of metal uptake, distribution and removal by different species of wetland plants. The extent of uptake and how metals are distributed within wetland plants can have important effects on the residence time of metals in plants and in wetlands, and the potential release of metals. This information is needed to better understand these systems and to assure that the wetlands do not themselves eventually become sources of metal contamination to surrounding areas (Weis and Weis, 2004).

Metal uptake and distribution has been examined in common reed plants (*Phragmites australis* (Cav.) Trin. ex Steud.) by Larsen and Schierup (1981), Schierup and Larsen (1981a&b), Gries and Garbe (1989), Peverly et al. (1995), Keller et al. (1998) and Windham et al. (2003). Most studies report the highest amounts of most metals in the roots, while leaf tissue has the second highest concentrations followed by stems and rhizomes. Schierup and Larsen (1981a&b) and Peverly et al. (1995) however found high concentrations of Zn aboveground, while other metals were retained in the roots and rhizomes. Restriction of upward metal movement into shoots can be a tolerance mechanism of wetland plants, next to restricting uptake, sequestering metals in tissues or cellular compartments (e.g. central vacuoles) that are insensitive to them and translocation of excessive metals into old leaves shortly before their shedding (McCabe et al., 2001, Windham et al., 2003 and Weis & Weis, 2004). The protective mechanisms against metal toxicity differ between plant species (Fediuc & Erdei, 2002). A small fraction of metals is also found to be released in the environment through leaf tissue during the growing season (Burke et al., 2000). Weis and Weis (2004) reviewed some factors affecting metal uptake by wetland plants. Ye et al. (1997) compared different population of *Phragmites australis*, one from a contaminated mine site and three from clean sites, and found similar Zn, Pb and Cd uptake. The metal-contaminated population thus did not seem to modify its uptake or distribution of metals as a response to the

contaminated environment. Schierup and Larsen (1981a&b) investigated the uptake of heavy metals by reed plants in a polluted and a non-polluted Danish lake. Metal contents in reed plants of the non-polluted lake were higher than those in the polluted lake. They concluded that uptake by the plants is determined by factors, such as pH and redox potential rather than by total pollutant levels in sediments and surface waters. Ye et al. (1998b) observed smaller biomass production and greater metal accumulation (especially Zn) in *Phragmites australis* seedlings grown for 90 days on a metal-contaminated sediment under flooded conditions compared to dry conditions. The redox potential facilitated Fe and Mn oxide reduction, but not sulphide formation, which might suggest that these metals were released upon the reduction of Fe/Mn oxides. According to Lehtonen (1989), *Phragmites australis* showed the weakest response to acid lake water in its metal accumulation, compared to the species *Nuphar lutea* and mosses growing at the bottom of the lakes. This was attributed to the fact that not all sediment layers from which reed plants obtain their nutrients are affected by acidification. *Phragmites* indeed obtains its nutrients from sediments alone, whereas *Nuphar lutea* obtains nutrients from both water and sediments and the mosses from the water alone. Windham et al. (2001) observed increased Pb uptake and translocation to aboveground parts of reed plants when adding Pb to sediments. Keller et al. (1998) however found no correlations between concentrations of extractable metals from marsh sediments and concentrations in roots of reed plants, and suggested that factors other than labile concentrations in sediments control metal uptake and storage in roots.

We aimed to check whether metal concentrations in reed plants along the river Scheldt coincide with those previously reported at other sampling sites and whether used analyses procedures could interfere with the experimental results. Moreover, we tried to find significant relationships between sediment properties and metal concentrations in reed stems, aiming at the identification of the environmental factors which could significantly affect metal uptake by these plants. Reed stems were selected to study the factors affecting metal uptake, as metal concentrations analysed in roots and rhizomes may reflect some proportion of metals that are merely adsorbed onto the root surface rather than within the root tissue (Hall & Pulliam, 1995 and Weis & Weis, 2004). Leaves might be more susceptible to atmospheric deposition compared to stems, due to their larger external surface area. Moreover, Windham et al. (2003) (in: Weis and Weis, 2004) reported that only 4 to 20 % of the aboveground metals in reed plants were found in leaf tissue, as stem biomass production is much higher.

7.2 Materials and methods

The study was carried out in the part of the Scheldt estuary that is subjected to tidal influences, downstream the city of Ghent (Fig. 3.1). All 26 study sites are tidal marshes vegetated by a monospecific stand of common reed, *Phragmites australis* (Cav.) Trin. ex Steud. Most of them are inundated at spring-tide only. Three reed plants were randomly harvested at each sampling site in August 2002. The reed plant samples in the current study were fractionated into rhizomes, roots, leaves and stems and plumes. Leaf sheaths were removed and all leaves of each plant were pooled. The samples were washed thoroughly with deionised water, dried at 70 ºC for 48 h, ground in a hammer-cross beater mill and homogenized. Plant samples were analysed for metal contents by weighing 1 g in beakers. Five ml of ultra pure HNO_3 were added, after which the suspension was heated on a hot plate at 130 ^0C for one hour. After heating, a total of 4 ml hydrogen peroxide was added to each sample and then heated for 10 minutes. The suspensions were filtered in 50 ml volumetric flasks using filter papers (S&S blue ribbon, Schleicher & Schuell). The filtrates were diluted to the mark using 1% HNO_3. Cd, Cr, Cu, Ni, Pb and Zn concentrations were analysed using ICP-OES (Varian Vista MPX, Varian, Palo Alto, CA) and GF-AAS (Varian SpectraAA-800/GTA-100, Varian, Palo Alto, CA). As it is very difficult to completely digest reed plant material preceding the analyses, destruction methods were compared and quality control results were described by Du Laing et al. (2003). The upper sediment layer was also sampled and analysed, as was already described in Chapter 3.

7.3 Results and discussion

Metal concentrations in the leaves and stems of *Phragmites australis* at the intertidal marshes along the river Scheldt are presented in Fig. 7.1. Some metal concentrations in *Phragmites australis* from other sites are presented in Table 7.1 and Table 7.2, for leaves and stems respectively. Differences between different studies should not only be attributed to different pollution levels and physico-chemical sediment, water or sediments characteristics at the sampling sites, but also to differing sampling times. Most other authors (e.g. Schierup & Larsen, 1981a) also sampled reed plants for metal analysis during summer months, whereas some authors studied metal fluctuations in reed plants over the complete growing season (e.g. Larsen and Schierup, 1981, Scholes et al., 1999, Weis et al., 2003). Weis and Weis (2004) concluded that it is very difficult to generalize about seasonal changes in metal levels, since they appear to vary greatly with the metal. However, individual leaves were found to acquire greater concentrations of metals over their life span. Moreover, Weis et al. (2003) moreover noted that a great variation among leaves within the same plant at any given time of sampling can be observed, which suggests that it is important to analyse a large number of leaves and not assume that a few are representative of the plant as a whole (Weis and Weis, 2004). Moreover, we found that the different procedures used for the destruction of reed plant material preceding the analyses significantly affected the observed metal concentrations (Du Laing et al., 2003). This could be due to the fact that reed plant matrices are difficult to destruct as they contain e.g. high amounts of lignin and cellulose (Lenssen et al., 1999). The effects depended upon the metal and sample type, which implies that drawing accurate conclusions based on a comparison of metal concentrations between different studies should be done with great caution. Based on the similar order of magnitude of the concentrations, we can however conclude that the environmental risks related to reed plant material decomposition and consumption are similar to those at most of the other sites, for which results have been reported in the literature.
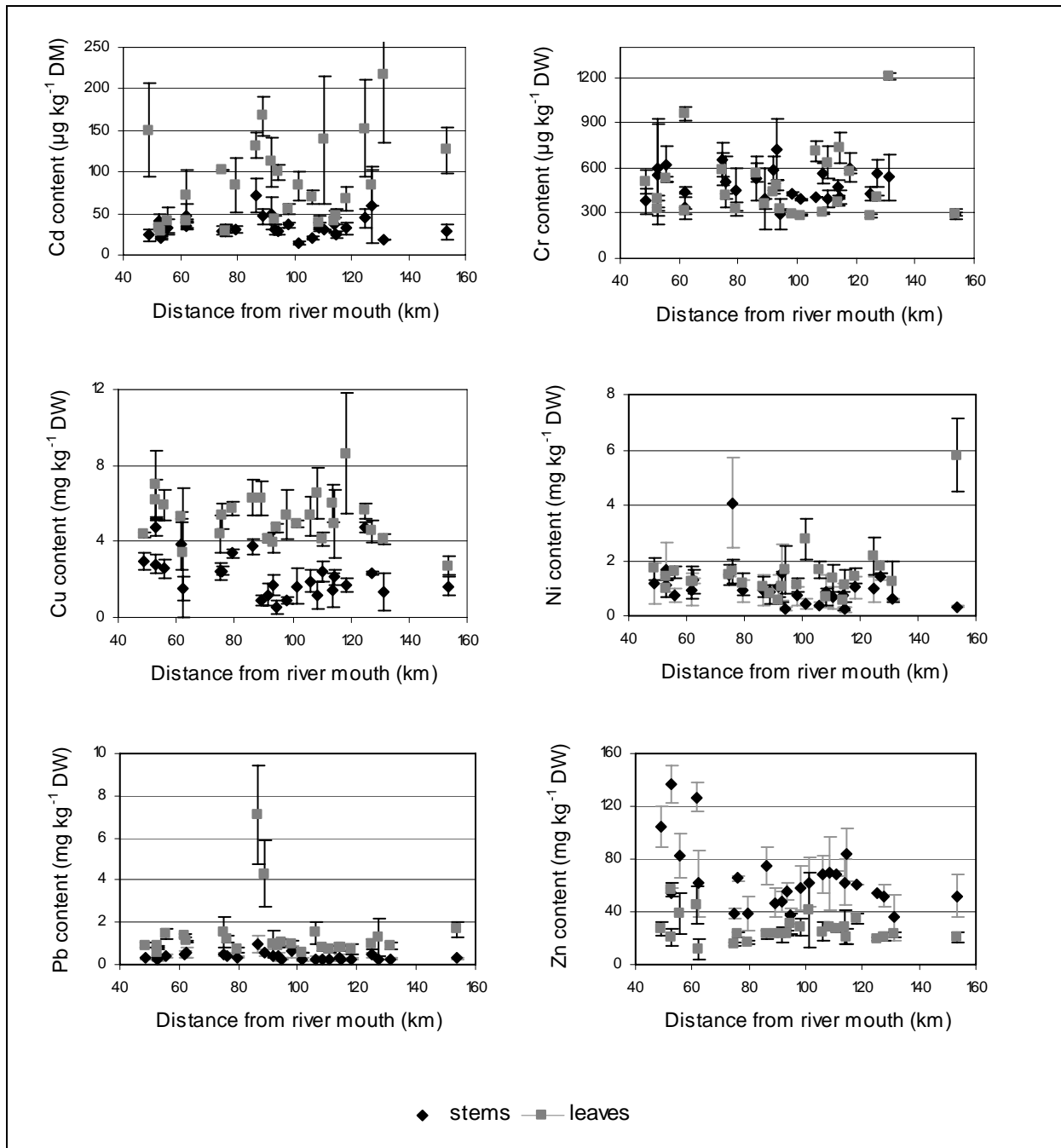
Fig. 7.1. Metal concentrations in leaves and stems of *Phragmites australis* at intertidal marshes along the river Scheldt

Table 7.1. Reported metal concentrations (mg kg^{-1} DM, range, average or average ± standard deviation) in leaves of *Phragmites australis*

Reference	Location	Cd	Cr	Cu	Ni	Pb	Zn	Fe	Mn
Abdel-Shafy et al. (1994)	Wastewater treatment plant	0.9-1.0	2.3-3.8	6.3-7.7	2.7-4.1	8.0-12.5	386-477	185-222	
	Control	0.5	0.9	7.0	2.2	10.0	339	329	
Baudo et al. (1985)	Lake Mezzola, Italy		2.6-220	3.2-7.8			11-40	140-1600	120-740
Keller et al. (1998)	Danube Delta, Romania		0.1±0.0		0.3±0.1	0.2±0.0			
Larsen (1983)	Mid-Jutland, Denmark					<1.0	33.7		
Lehtonen (1989)	Espoo, Finland								
	Lake 1 (Hauklampi)	<0.1		2.0±0.7			25 ± 8	83 ± 34	51 ± 11
	Lake 2 (Kurkijärvi)	<0.1		2.0±0.9			14 ± 2	64 ± 13	38 ± 13

Table 7.2. Reported metal concentrations (mg kg^{-1} DM, range, average or average ± standard deviation) in stems of *Phragmites australis*

Reference	Location	Cd	Cr	Cu	Ni	Pb	Zn	Fe	Mn
Abdel-Shafy et al. (1994)	Wastewater treatment plant	0.4-0.5	1.6-3.1	3.4-5.1	1.2-2.0	1.5-2.5	184-329	80-155	
	Control	0.4	0.8	3.9	2.0	6.1	257	185	
Baudo et al. (1985)	Lake Mezzola, Italy		0.7-110	1.1-7			7-34	49-330	39-270
Larsen (1983)	Mid-Jutland, Denmark	<0.03		2.4		<1.0	48		
Lehtonen (1989)	Espoo, Finland								
	Lake 1 (Hauklampi)	<0.1		1.5±0.5			130±30	19±6	18±10
	Lake 2 (Kurkijärvi)	<0.1		1.4±0.7			55±25	19±4	13±5

Most metal concentrations were significantly higher in the leaves compared to the stems. Zn concentrations were however highest in the stems, which has also been observed by Larsen (1983) and Lehtonen (1989). Zn would accumulate there as it has an essential function in the biosynthesis of the plant growth hormone indolyl-3-acetic acid, which is primarily active in the stems (Schierup and Larsen, 1981a). A baseline level of 40 mg Zn kg^{-1} DM is observed,

which seems to indicate a minimum level needed for the plant. However, Zn concentrations up to 140 mg kg^{-1} DM are found at some sites. Sediments of all these sites exhibited high chloride contents, which suggests that salinity might affect the Zn uptake. Uptake of the other elements does not seem to be affected by the salinity. This might be attributed to a combination of the higher solubility of Zn sulphides, the higher amount of sulphides needed to precipitate all Zn and the relatively high stability of Zn chloride complexes. Whereas the mobility of Cd is also expected to be increased by salinity, this increase may be compensated by a reduction of the mobility due to sulphide formation in the deeper sediment layers, as discussed in Chapter 6. Moreover, as Cd is not an essential element, plants may be able to selectively eliminate it from their tissues. Remarkably high Pb concentrations in the leaves were observed at two sites. The nearby roadways and metallurgic activities probably account for elevated atmospheric Pb deposition on the leaves. Moreover, a controlled flooding area is being constructed nearby. During summer, the digging and transport of dry, contaminated soil during the construction works might have increased atmospheric deposition of soil particles on the leaves. Metal concentrations in the plumes ranged from 19 to 117 µg kg^{-1} DM for Cd, from 98 to 408 µg kg^{-1} DM for Cr, from 3.1 to 7.0 mg kg^{-1} DM for Cu, from 0.5 to 2.3 mg kg^{-1} DM for Ni, from 0.4 to 4.5 mg kg^{-1} DM for Pb and from 36 to 132 mg kg^{-1} DM for Zn. Relative variations were highest for Pb, suggesting the importance of atmospheric deposition.

There were no clear relations between total metal concentrations in the sediments and the reed stems. Cd concentrations in the stems varied by a factor 5, between 14 and 72 µg kg^{-1}, whereas Cd concentrations in the sediments varied by a factor 15, between 1.1 and 16.7 mg kg^{-1}. Zn concentrations in the stems varied by a factor 4, between 37 and 137 mg kg^{-1}, whereas Zn concentrations in the sediment varied by a factor 24, between 40 and 950 mg kg^{-1}. Cu concentrations in the stems varied by a factor 10, between 0.5 and 4.8 mg kg^{-1}, whereas they varied by a factor 17 in the sediment, between 10 and 171 mg kg^{-1}. Ni concentrations in the stems vary by a factor 21, between 0.2 and 4.1 mg kg^{-1}, whereas Ni concentrations in the sediment only vary by a factor 3, between 88 and 235 mg kg^{-1}. Cr concentrations in the stems vary by a factor 2, between 0.29 and 0.72 mg kg^{-1}, whereas Cr concentrations in the sediment varied by a factor 7, between 31 and 224 mg kg^{-1}. Finally, Pb concentrations in the stems vary by a factor 5, between 0.21 and 0.99 mg kg^{-1}, whereas Pb concentrations in the sediment vary by only a factor 2, between 189 and 461 mg kg^{-1}.

Aiming to identify the environmental factors that could significantly affect metal uptake by these plants, we calculated transfer coefficients K_d for each sampling site as the ratio of a metal concentrations in the leaves or stems of the reed plants (mg kg^{-1} DM) to the total metal concentrations in the sediments (mg kg^{-1} DM). These were plotted as a function of the distance to the river mouth (Fig. 7.2).
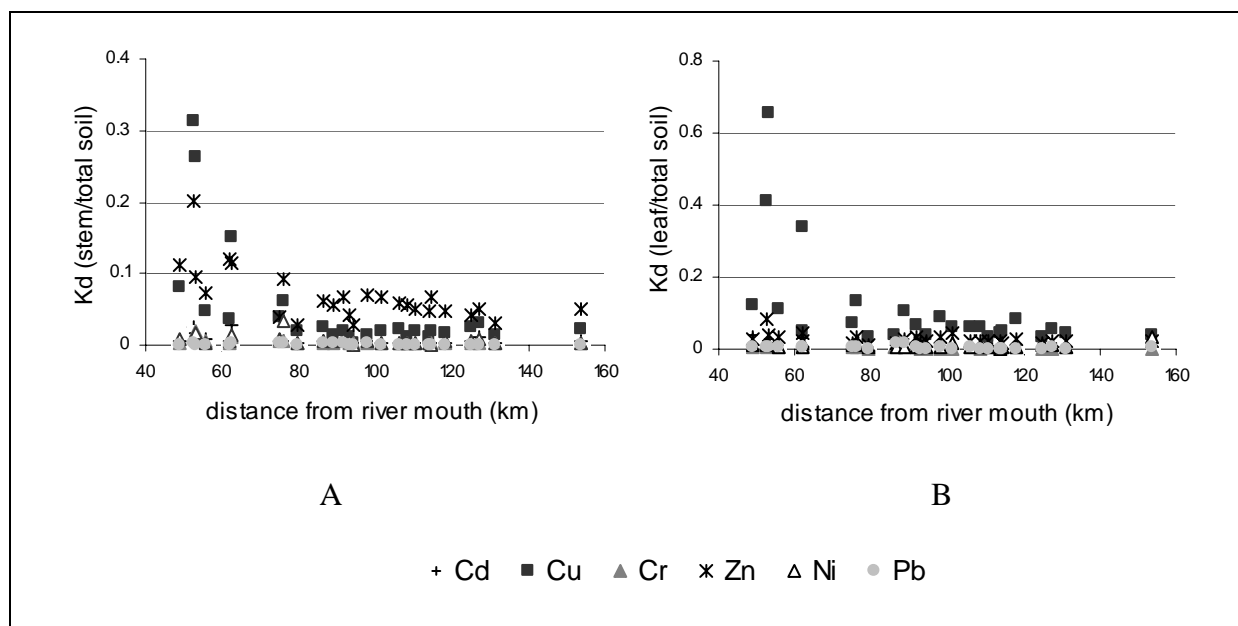

Fig. 7.2. Transfer coefficients, calculated as the ratio of a metal concentration in the stems (A) or leaves (B) of the reed plants (mg kg^{-1} DM) to the total metal concentrations in the sediments (mg kg^{-1} DM), as a function of the distance from the river mouth

Transfer coefficients of Cu and Zn are generally higher than those of the other elements. Moreover, Cu and Zn transfer from sediments to stems is significantly higher at some sites near the river mouth. Sediments of these sampling sites have low clay and organic matter contents, which implies also low total metal concentrations. As Cu and Zn are essential elements, they seem to be more actively translocated to the aboveground plant parts at these sites. Moreover, Zn uptake might be affected by the salinity, as already mentioned above. Cu also seems to be actively transported to the leaves, whereas this is not the case for Zn. This might be attributed to the functions of these metals in the plant: Cu is needed for the functioning of plastocyanin in the leaves, an enzyme which is used for electron transfer in photosynthesis, and in the enzyme cytochrome oxidase which is used during respiration, whereas Zn is mainly used for the production of growth hormones in the stems.

These conclusions should be interpreted with great caution as we could not discriminate between internal uptake and external sorption to the plant although plant parts were thoroughly washed preceding the analyses. As the proportion of external surface area to the total weight is higher for leaves compared to stems, leaves are probably more significantly affected by external sorption due to atmospheric deposition. However, external sorption could also affect the metal contents in the stems, as they are in closer contact with the polluted sediments.

Transfer coefficients for the leaves were higher than those for the stems. Moreover, Windham et al. (2003) reported that only 4 to 20 % of the aboveground metals in reed plants were found in leaf tissue, as stem biomass production is much higher. We therefore further focussed on the stems when aiming to assess environmental factors affecting the metal uptake by reed plants. For Cd and Zn, K_d coefficients for transfer from exchangeable metal fractions in the sediments (Chapter 3) to the reed stems were plotted as a function of clay contents of the sediments in Fig. 7.3. Metal transfer to aboveground plant parts was inversely related to the clay content. A lower sorption capacity due to a lower clay content might decrease the capacity of the sediments to immobilize Cd and Zn and thus increase the plant availability. Moreover, as Zn is an essential element, the plants might actively promote Zn release from the sediment and accumulate Zn when the Zn concentration in the sediment is rather low, which is the case if clay and organic matter contents are low, as mentioned in Chapter 3. This may then also induce an increased Cd uptake as the fate of Cd and Zn in the sediments and their uptake mechanisms are quite similar. Finally, the less clayey sediments are expected to be susceptible to more rapidly fluctuating water table levels, which leads to occasional oxidation of sulphides and an increased metal availability. Vandecasteele et al. (2005b) also found lower Cd concentrations in the leaves and bark of the wetland plant species *Salix cinerea* with increasing duration of submersion periods in the field. Under greenhouse conditions, an upland hydrological regime resulted in elevated Cd and Zn concentrations in the leaves compared to a wetland hydrological regime. Moreover, initially submerged sediments emerging only in the second half of the growing season resulted in elevated Cd and Zn foliar concentrations at that time, whereas foliar Zn concentrations were high at a sandy-textured oxic plot with low sediment metal concentrations (Vandecasteele et al., 2005a). This kind of effect of reducing conditions on metal availability was also reported by others for other plant species, such as Gambrell (1994). It was already mentioned in Chapter 6 and will be further discussed in Chapters 8 and 9. For Zn, the inverse relationship between these

transfer coefficients and the clay contents is less clear, as salinity also seems to affect the uptake. Higher than expected Zn transfer coefficients were observed for the 4 sites with a sediment chloride content above 1000 mg kg^{-1}, regardless their clay content. When sites with sediment chloride contents above 1000 mg kg^{-1} were omitted from the dataset, the inverse relationship becomes more clear (Fig. 7.3, black boxes). Chloride contents below 700 mg kg^{-1} (or conductivities below 1000 µS cm^{-1}) did not seem to affect Zn uptake. No clear relationships were found between Cd and Zn transfer coefficients and chloride contents or conductivities for the complete dataset or only the freshwater part of the estuary (conductivity < 1000 µS cm^{-1}).
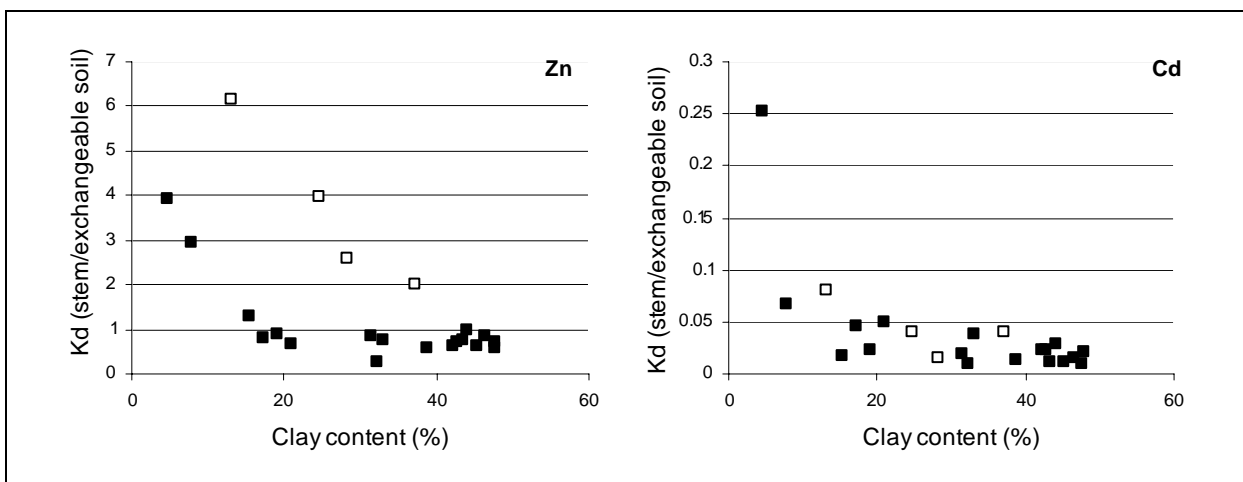

Fig. 7.3. Transfer coefficients for Cd and Zn, calculated as the ratio of a metal content in the stems of the reed plants (mg kg^{-1} DM) to the exchangeable metal contents in the sediments (mg kg^{-1} DM), as a function of the clay content of the sediments (black boxes = sediments contain less than 1000 mg Cl^{-} kg^{-1} DM, white boxes = sediments contain more than 1000 mg Cl^{-} kg^{-1} DM).

The K_d coefficients for transfer of Cd and Zn from exchangeable metal fractions in the sediments to the reed stems can be significantly predicted by the following linear regression models:

$$\log K_{d,Cd} = -0.147 - 1.102 \times \log Clay \qquad \text{(Eq. 1, } R = 0.793, \text{ p} < 0.001)$$
$$\log K_{d,Zn} = 1.187 - 0.804 \times \log Clay \qquad \text{(Eq. 2, } R = 0.632, \text{ p} = 0.002)$$

where $K_{d,X}$ represents the transfer coefficient of metal X (dimensionless) calculated as the ratio between concentrations in the stem and extractable concentrations in the sediment. *Clay* represents the clay content in %.

The intercept was not significant for Cd, whereas it was significant for Zn (p < 0.001). This reflects the essential role of Zn in the growth of reed plants, which might lead to active Zn uptake and accumulation in the stems at lower exchangeable concentrations in the sediment. The prediction of Zn was significantly improved by adding chloride concentration to the model:

$$\log K_{d,Zn} = 1.000 + 0.239 \times Cl^- - 0.756 \times \log Clay \quad \text{(Eq. 3, } R = 0.881, \text{ p} < 0.001)$$

where $K_{d,X}$ represents the transfer coefficient of metal X (dimensionless) calculated as the ratio between concentrations in the stem and extractable concentrations in the sediment. *Clay* represents the clay content in % and Cl^- the chloride content in g kg^{-1} DM.

The intercept, clay and chloride contents all significantly affected log $K_{d,Zn}$ (p < 0.001). Standardised regression coefficients (Beta) were 0.615 for Cl^- and -0.594 for log *Clay.* Salinity thus again seems to promote Zn uptake, whereas increasing clay contents reduced it. Replacing the chloride content by conductivity slightly reduces the prediction capability of the model (R = 0.857). Prediction capabilities for Cd uptake could not be significantly improved by adding chloride contents to the model, which suggests that Cd uptake by reed plants is not affected by salinity. Moreover, correlation coefficients could not be significantly improved by replacing the clay contents by organic matter contents or adding organic matter contents to the model.

A similar inverse relationship was observed for Cr, Cu and Ni between transfer from the total metal pool in the sediments to the stems and sediment organic matter content (Fig. 7.4). As Cr, Cu and Ni have a high affinity for organic matter, a lower sorption capacity due to a lower organic matter content might decrease the capacity of the sediments to immobilize them and thus increase the plant availability. Moreover, the plants might actively promote Cu, Cr and Ni release from the sediment by the excretion of root exudates (Xu & Jaffe, 2006) and accumulate Cu, Cr and Ni when their concentration in the sediment is rather low. This is the case if clay and organic matter contents are low, as mentioned in Chapter 3. Finally, higher organic matter contents may lead to higher consumption rates of electron acceptors, resulting in a more reduced state of the sediments and higher sulphide amounts at lower sediment

depths. This in turn might result in the precipitation of metals which makes them less available, as was already mentioned in Chapter 6 and will be further discussed in Chapters 8 and 9.
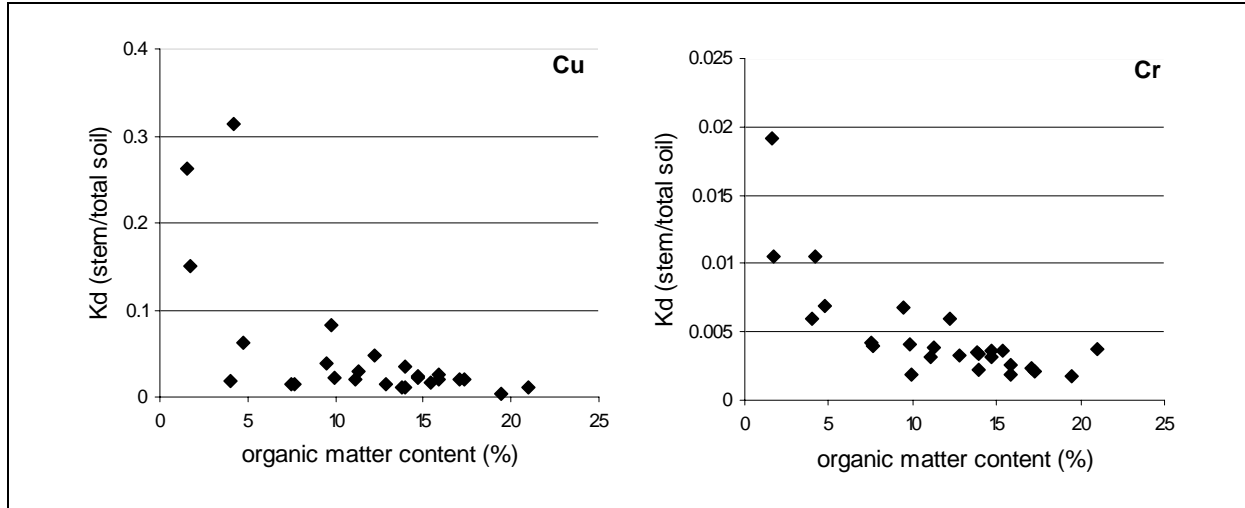

Fig. 7.4. Transfer coefficients for Cu and Cr, calculated as the ratio of a metal concentration in the stems of the reed plants (mg kg^{-1} DM) to the total metal concentrations in the sediments (mg kg^{-1} DM), as a function of the organic matter content of the sediments.

The coefficients for transfer of Cu, Cr and Ni from the total metal pools in the sediments to the reed stems can be significantly predicted by the following linear regression models:

$$\log K_{d,Cu} = -0.480 - 1.085 \times \log OM \qquad \text{(Eq. 4, } R = 0.745, \text{ p} < 0.001\text{)}$$

$$\log K_{d,Cr} = -1.680 - 0.730 \times \log OM \qquad \text{(Eq. 5, } R = 0.856, \text{ p} < 0.001\text{)}$$

$$\log K_{d,Ni} = -1.494 - 0.823 \times \log OM \qquad \text{(Eq. 6, } R = 0.711, \text{ p} < 0.001\text{)}$$

where $K_{d,X}$ represents the transfer coefficient of metal X (dimensionless) calculated as the ratio between concentrations in the stem and total concentrations in the sediment. *OM* represents the organic matter content in %.

Regression coefficients are 0.745 for Cu (p < 0.001), 0.856 for Cr (p < 0.001) and 0.711 for Ni (p < 0.001). The intercepts all significantly contributed to the model (p < 0.001 for Cr and Ni and p = 0.028 for Cu). Based on its large slope compared to the intercept, Cu transfer seems to be most importantly affected by organic matter contents. Prediction capabilities could not be significantly improved by replacing organic matter by clay contents or adding

clay contents to the model, which confirms the role of organic matter in Cu, Cr and Ni uptake by the reed plants. Prediction capabilities for Cu and Cr could be significantly improved by adding chloride contents to the models (R = 0.856 and 0.884 respectively):

$$\log K_{d,Cu} = -0.603 + 0.222 \times Cl^- - 1.054 \times \log OM \quad \text{(Eq. 7, } R = 0.856, \text{ p} < 0.001)$$

$$\log K_{d,Cr} = -1.718 + 0.069 \times Cl^- - 0.721 \times \log OM \quad \text{(Eq. 8, } R = 0.884, \text{ p} < 0.001)$$

where $K_{d,X}$ represents the transfer coefficient of metal X (dimensionless) calculated as the ratio between concentrations in the stem and total concentrations in the sediment. *OM* represents the organic matter content in % and Cl^- the chloride content in g kg^{-1} DM.

The intercept (p = 0.004 for Cu and p < 0.001 for Cr), log OM (p < 0.001 for Cu and Cr) and chloride contents (p = 0.001 for Cu and p = 0.017 for Cr) all significantly affected log $K_{d,Cu}$ and log $K_{d,Cr}$. Standardised regression coefficients (Beta) in the regression model of log $K_{d,Cu}$ were -0.724 for log *OM* and 0.421 for Cl^-. These were found to be -0.844 and 0.224, respectively, in the regression model of log $K_{d,Cr}$. This might suggest a role of the salinity in the Cu and Cr uptake by reed plants. Adding chloride contents to the model does however not significantly improve the prediction capability for Ni. The good fit between observed and predicted K_d values using equations 3, 6, 7 and 8 is presented in Fig. 7.5.

Finally, the transfer coefficient of Pb between the total metal pool in the sediments and the reed stems can be significantly (p = 0.001) predicted by the following linear regression model:

$$\log K_{d,Pb} = -2.183 - 0.547 \times \log Clay \quad \text{(Eq. 9, } R = 0.630, \text{ p} = 0.001)$$

where $K_{d,X}$ represents the transfer coefficient of metal X (dimensionless) calculated as the ratio between concentrations in the stem and total concentrations in the sediment. *Clay* represents the clay content in %.

The correlation coefficient (0.630) is much lower compared to those of the models which were constructed for Cd, Cu, Cr, Ni and Zn. Prediction capabilities could not be improved by adding extra parameters to the model.
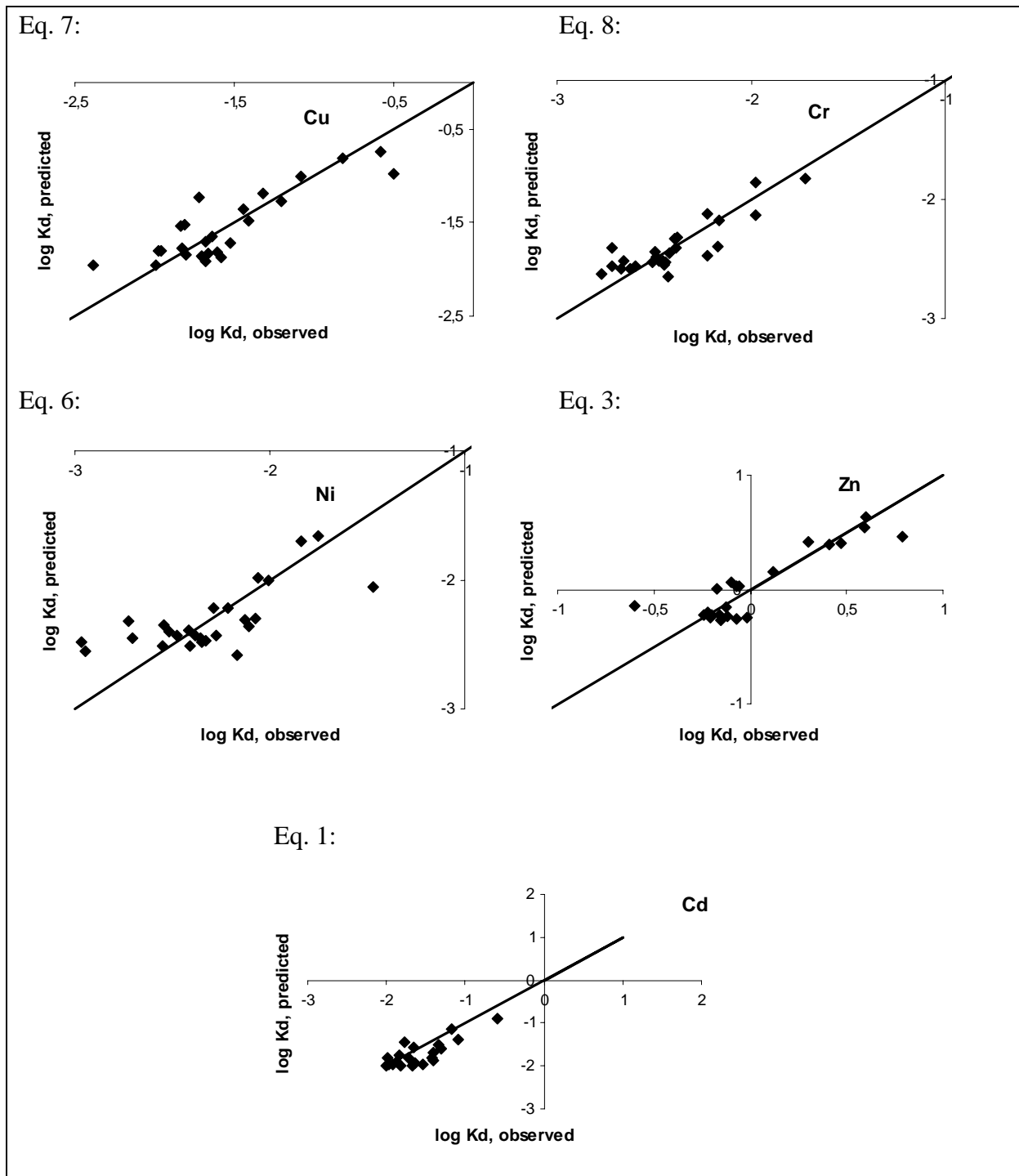
Fig. 7.5. Relationship between observed and predicted Kd values using equations 3, 6, 7 and 8

7.4 Conclusion

Concentrations of metals in reed plants are low. They tend to be higher in the leaves than in the stems, with the exception of Zn. Only Zn is primarily accumulated in the stems, which might be attributed to its role in the biosynthesis of a plant growth hormone. Uptake of metals by reed plants and their transfer to the stems seems to be affected by clay and organic matter contents of the marsh sediments. The higher the clay and organic matter contents, the lower the uptake and transfer to the aboveground plant parts. This might be attributed to a higher sorption capacity, which reduces the metal availability. Additionally, high clay and organic matter contents promote the creation of more reduced conditions and subsequent sulphide precipitation, which reduces the metal availability at lower sampling depths. Moreover, active metal release from the sediment and increased metal uptake might be promoted by the plants at lower total metal concentrations in the sediment, which are related to lower clay and organic matter contents. Next to the effect of clay and organic matter, salinity enhances the uptake of some metals, such as Cr, Ni and Zn.

8.

Effect of water table level on metal mobility

8 Effect of water table level on metal mobility

8.1. Introduction

In Chapter 2, we discussed possible effects of oxidation and the creation of reducing conditions on metal mobility in intertidal sediments, whereas in Chapters 2 to 7 we focussed on assessing the factors affecting metal mobility and availability in the superficial sediment layer. In this chapter, the relationship between the water table level and the metal mobility and availability in the upper 1 m layer of intertidal Scheldt sediments will be assessed. A greenhouse experiment was set up in which pore water metal concentrations were measured at different sampling depths (10, 30, 60 and 90 cm) below the surface of a soil and 4 intertidal sediments as a function of time, upon adjusting the water level to 0, 40 and 80 cm below the surface of the soil and sediments. The highest water table levels can generally be observed at the younger marshes, whereas a lower mean water table level characterises the older marshes, which are already banked up higher. Moreover, the effect of the water table level on metal mobility and availability is important during the construction of controlled inundation areas in metal-polluted regions, as the water table level of these floodplains can be manipulated by e.g. constructing dikes and using locks.

8.2. Materials and methods

8.2.1 Experimental setup

A part of the upper 40 cm intertidal sediment layer was excavated at Lillo, Bornem, Kijkverdriet and Konkelschoor, whereas this was also done for the upper 40 cm soil layer at Lippensbroek. Konkelschoor, Kijkverdriet, Bornem and Lillo are intertidal marshes, which are vegetated by reed plants and regularly flooded by Scheldt river water. Lillo is situated in the brackish part of the Scheldt estuary, whereas Konkelschoor is situated in the freshwater part. Kijkverdriet and Bornem are situated at the boundary between the freshwater and the brackish zone. Lippensbroek is situated near Bornem. It was a polder at the time of sampling,

but it is now being used as an experimental, pilot-scale controlled flooding area. As a result, Scheldt water now regularly inundates this polder by means of a lock system. To improve the readability, the soil of Lippensbroek will however also be referred to as a "sediment" when it is pooled together with the sediments of other sampling sites. The sediments were transported to the lab in plastic recipients, where they were thoroughly homogenized. Plant roots and rhizomes were removed by hand picking.

Fifteen PVC tubes with a diameter of 16 cm and a length of 1 m were closed at the bottom. Rhizon soil moisture samplers (type MOM, Rhizosphere Research Products, Wageningen, The Netherlands) equipped with extension tubes were installed vertically inside the tubes at fixed distances from the bottoms of the tubes. It was not feasible to duplicate the experimental setup as we aimed to conduct the experiment with different water levels and types of sediments, meanwhile maintaining a relatively high temporal sampling resolution. Therefore, the effect of sediment heterogeneity was diluted by duplicating the sampling procedure, i.e. two samplers were installed at each sampling depth in each tube, at least 15 cm away from each other. Three tubes were filled up with each type of sediment up to 95 cm of the bottom, meanwhile taping the Rhizon samplers to the walls of the tubes so that the 10-cm long filter tubes of the samplers were situated at fixed distance intervals of 5-15, 25-35, 55-65 and 85-95 cm below the sediment surface. These sampling intervals will be referred to as 10, 30, 60 and 90 cm below the sediment surface. Before filling the tubes with sediments, perforated PVC tubes with a diameter of 32 mm were installed in the middle of the tubes. These wells allowed to measure and control the level of the water table. They were covered with a stopper after the installation to avoid penetration of significant oxygen amounts into the sediments. The recipients were stored in a greenhouse at a temperature controlled between 15 and 25°C and a light regime of 16 hours light per day.

Each sediment was subjected to three different levels of the ground water table. To achieve this, deionised water was added just above the sediment surface (i.e. completely waterlogged) (referred to as WL-0), and 40 (WL-40) and 80 cm (WL-80) below the surface. The level of the water table was assessed measuring the water height in the wells twice a week. It was adjusted if necessary by drainage or addition of distilled water.

8.2.2 Sampling

The first samplings of pore water took place two weeks after the beginning of the experiment to allow the sediments to settle around the samplers. Pore water was sampled after 2 weeks, 2 months, 4 months, 6 months and 10 months by connecting vacuum tubes to the sampler's extension tubes. Samples collected by the two samplers which were installed at each depth of each treatment were poured together in a recipient. During the filling of the recipients, three samples of each sediment were also taken to determine the physico-chemical properties. They were dried overnight at 70°C and ground in a hammer-cross beater mill (Gladiator BO 3567). They were however not dried and grinded for the determination of acid volatile sulphide contents, as drying can induce the oxidation of the sulphides.

8.2.3 Analyses

All glassware and containers were washed in a laboratory dishwasher, subsequently soaked overnight in 5 % ultrapure 65 % HNO_3 and rinsed with deionised water. Pseudo-total metal contents in the sediments were determined by an aqua regia destruction (Ure, 1990) and analyses with inductively coupled plasma – optical emission spectrometry (ICP-OES, Varian Vista MPX, Varian, Palo Alto, CA). Acid volatile sulphide contents (AVS) were determined on fresh sediment samples by conversion of sulphide to H_2S and absorption in a Zn-acetate solution, followed by a back titration, as described by Tack et al. (1997a). The pH was measured in a 1/5 sediment/distilled water suspension after equilibration during 18 h (Van Ranst et al., 1999). The conductivity was measured in the filtrate of a 1/5 sediment/distilled water suspension, shaken for ½ h (Van Ranst et al., 1999). The organic matter content was determined by measuring the weight loss after incineration of oven-dried samples (2 h at 450°C). The carbonate content was determined by back-titration (with 0.5 M NaOH) of an excess 0.25 M H_2SO_4 added to 1 g of sediment (Nelson, 1982). Texture was determined at the Research Institute for Nature and Forest (INBO) using laser diffraction (Coulter LS200, Miami, FL) after removing organic matter and carbonates by adding hydrogen peroxide and an acetate buffer solution respectively. To determine the chloride content, 10 g of sediment was suspended in 50 ml of 0.15 M HNO_3 and shaken for 30 minutes. The filtrate was titrated with 0.05 M $AgNO_3$ using potentiometric end-point detection (Van Ranst et al., 1999). The cation exchange capacity was measured by percolation of 150 ml 1 M NH_4OAc through a

percolation tube filled with a mixture of 5 g sediment and 35 g quartz sand, followed by washing through the excess with 300 mL denatured ethanol. The exchangeable ammonium ions were then eluted with 500 mL 1 M KCl and analysed in the percolate by means of a steam distillation (Tecator Kjeltec System 1002 Distilling Unit). The exchangeable cations were also analysed in this percolate using ICP-OES (Varian Vista MPX, Varian, Palo Alto, CA) (Van Ranst et al., 1999).

The total organic carbon (TOC), inorganic carbon (IC), NO_3^-, SO_4^{2-} and Cl^- concentrations in the pore water were determined on non-acidified samples directly after sampling. TOC and IC concentrations were measured with a TOC-analyser (TOC-5000, Shimadzu, Tokyo Japan). Cl^-, NO_3^- and SO_4^{2-} concentrations were measured with a Metrohm configuration consisting of a 761 Compact Ion Chromatograph equipped with a 788 IC Filtration Sample Processor and an anion exchange column (IC-AN-Column Metrosep A supp 4, Metrohm Ion Analysis, Switzerland). Subsequently, the pore water samples were acidified with a few drops of concentrated HNO_3 to keep the metals in solution and to solubilise metals which precipitated during sampling. They were analysed for Cd, Cr, Cu, Fe, Mn, Ni, Zn, Ca, Mg, Na and K contents using graphite furnace – atomic adsorption spectrometry (GF-AAS, Varian SpectraAA-800/GTA-100, Varian, Palo Alto, CA) and ICP-OES (Varian Vista MPX, Varian, Palo Alto, CA).

8.3. Results and discussion

8.3.1. Sediment properties

Initial metal contents and physico-chemical properties of the sediments are presented in Table 8.1. Organic matter concentrations were highest in Kijkverdriet (24.2 %) and lowest in Lippensbroek (8.5 %). The sediments of Kijkverdriet, Bornem and Konkelschoor consisted for more than 50 % of clay, and only the sediments of Lippensbroek and Lillo contained a significant amount of sand. $CaCO_3$ concentrations ranged from 4.1 to 9.6 %. The pH ranged from 7.19 to 7.41. Chloride contents and conductivities rapidly decrease when moving upstream from Lillo to Konkelschoor, coinciding with the decreasing influence of salty

seawater. Lippensbroek was not flooded by river water, which explains the low chloride contents. The Kijkverdriet sediment contained some AVS (9.5 ± 2.7 mg kg^{-1} DM), whereas AVS amounts in the other sediments were below the detection limit of 2 mg kg^{-1} DM.

Pseudo-total concentrations of Cr, Cu, Ni, Pb and Zn did not exceed the soil sanitation threshold values according to the Flemish legislation (Vlarebo, 1996), but they were higher than the background values. Cadmium concentrations significantly exceeded the sanitation thresholds imposed on nature areas in Kijkverdriet, Bornem and Lippensbroek, whereas these levels were just not reached in Lillo and Konkelschoor. The high Cd concentrations might be attributed to the wide variety of Cd sources in the Scheldt estuary, as described in Chapter 3. Exchangeable amounts expressed as a percentage of total contents varied between 10.9 and 23.6 % for Cd, between 1.2 and 1.9 % for Cu, between 1.1 and 1.5 % for Ni, and between 2.5 and 5.0 % for Zn. These percentages were lowest in Kijkverdriet and highest in Lillo.

Table 8.1. Metal concentrations and some physico-chemical properties of the sediments (mean ± standard deviation, n = 3, except for clay, loam and sand contents)

	Lillo	Kijkverdriet	Bornem	Konkelschoor	Lippensbroek
Cd (mg kg^{-1} DM)	4.0 ± 0.1	8.5 ± 0.4	6.9 ± 0.2	5.4 ± 0.1	7.7 ± 0.2
Cr (mg kg^{-1} DM)	71 ± 1	138 ± 7	124 ± 2	158 ± 1	169 ± 3
Cu (mg kg^{-1} DM)	90 ± 5	105 ± 4	106 ± 2	96 ± 1	92 ± 1
Ni (mg kg^{-1} DM)	28.5 ± 0.5	51.3 ± 2.9	35.3 ± 0.3	32.2 ± 0.4	32.1 ± 0.9
Pb (mg kg^{-1} DM)	97 ± 6	150 ± 8	136 ± 3	123 ± 1	222 ± 56
Zn (mg kg^{-1} DM)	388 ± 22	534 ± 21	595 ± 12	573 ± 4	638 ± 15
Clay (%)	33.6	50.6	54.1	51.0	39.9
Loam (%)	46.3	48.2	45.6	48.5	46.1
Sand (%)	20.1	1.2	0.3	0.5	14.0
CaCO$_3$ (%)	9.6 ± 0.4	5.3 ± 0.8	7.0 ± 1.6	5.3 ± 0.3	4.1 ± 1.1
OM (%)	14.1 ± 0.5	24.2 ± 0.9	14.5 ± 0.2	14.2 ± 0.4	8.5 ± 0.1
pH	7.35 ± 0.03	7.25 ± 0.04	7.36 ± 0.04	7.19 ± 0.02	7.41 ± 0.05
EC (μS cm^{-1})	1765 ± 134	304 ± 28	264 ± 10	293 ± 10	81 ± 16
AVS (mg kg^{-1} DM)	<2	9.5 ± 2.7	<2	<2	<2
Cl$^-$ (g kg^{-1} DM)	3.46 ± 1.45	0.53 ± 0.18	0.32 ± 0.07	0.17 ± 0.11	0.10 ± 0.02
CEC (cmol(+) kg^{-1})	24.4 ± 1.0	41.8 ± 2.8	22.1 ± 0.6	32.3 ± 1.0	24.5 ± 0.8

8.3.2. Pore water

Total organic carbon (TOC) concentrations in the pore water increased during the first 6 months in all treatments and at all sampling depths, after which they again decreased towards the 10th month (Fig. 8.1). The only exception was the completely waterlogged Lippensbroek soil, where concentrations were still increasing in the 10th month. TOC concentrations reached their peak at sampling points below the water table. Complete organic matter decomposition is indeed hampered under reducing conditions. In Lillo, peak concentrations around 350 mg L^{-1} were observed, whereas they did not exceed 200 mg L^{-1} below the water table in Kijkverdriet, Bornem, Konkelschoor and Lippensbroek. The inorganic carbon (IC) concentrations however kept increasing up to the 10th month (Fig. 8.1), probably due to the dissolution of CO_2 produced during organic matter decomposition (Hellings et al., 2000; van den Berg & Loch, 2000). They also substantially increased below the water table, towards peaks between 400 and 600 mg L^{-1} in Kijkverdriet, Bornem and Konkelschoor. Peak concentrations in Lillo did however not exceed 400 mg L^{-1}.

Organic matter decay and subsequent net release of soluble organic matter fractions can thus be observed during the first 6 months. Release of soluble organic substances seems to overrule their decomposition to CO_2. As decomposition is not complete, not only increasing CO_2 concentrations, but also increasing TOC concentrations are observed in the pore water. Upon time, the energetic efficiency of microbial decomposers and their organic matter decomposition rate are expected to decrease, but also organic matter decay rates are decreasing and subsequent release of soluble organic molecules is slowing down. As a result, TOC concentrations in the pore water decrease again, but CO_2 keeps accumulating until an equilibrium is observed. This equilibrium is determined by CO_2 accumulation, CO_2 diffusion, which is hampered under waterlogged conditions, and CO_2 exchange with the atmosphere at the sediment surface. The higher peak TOC concentrations in Lillo might be attributed to slower organic matter decomposition due to the higher salinity. Additionally, higher salt contents may increase the stability of soluble organic matter fractions. The lower initial TOC concentration and the lower IC concentrations in the Lippensbroek soil might be due to its lower initial moisture content, lower organic matter content and more permeable texture, as it was initially an upland soil. As a result, oxygen might penetrate deeper into the soil for a longer time, which might slow down the use of alternative electron acceptors upon organic matter decomposition.
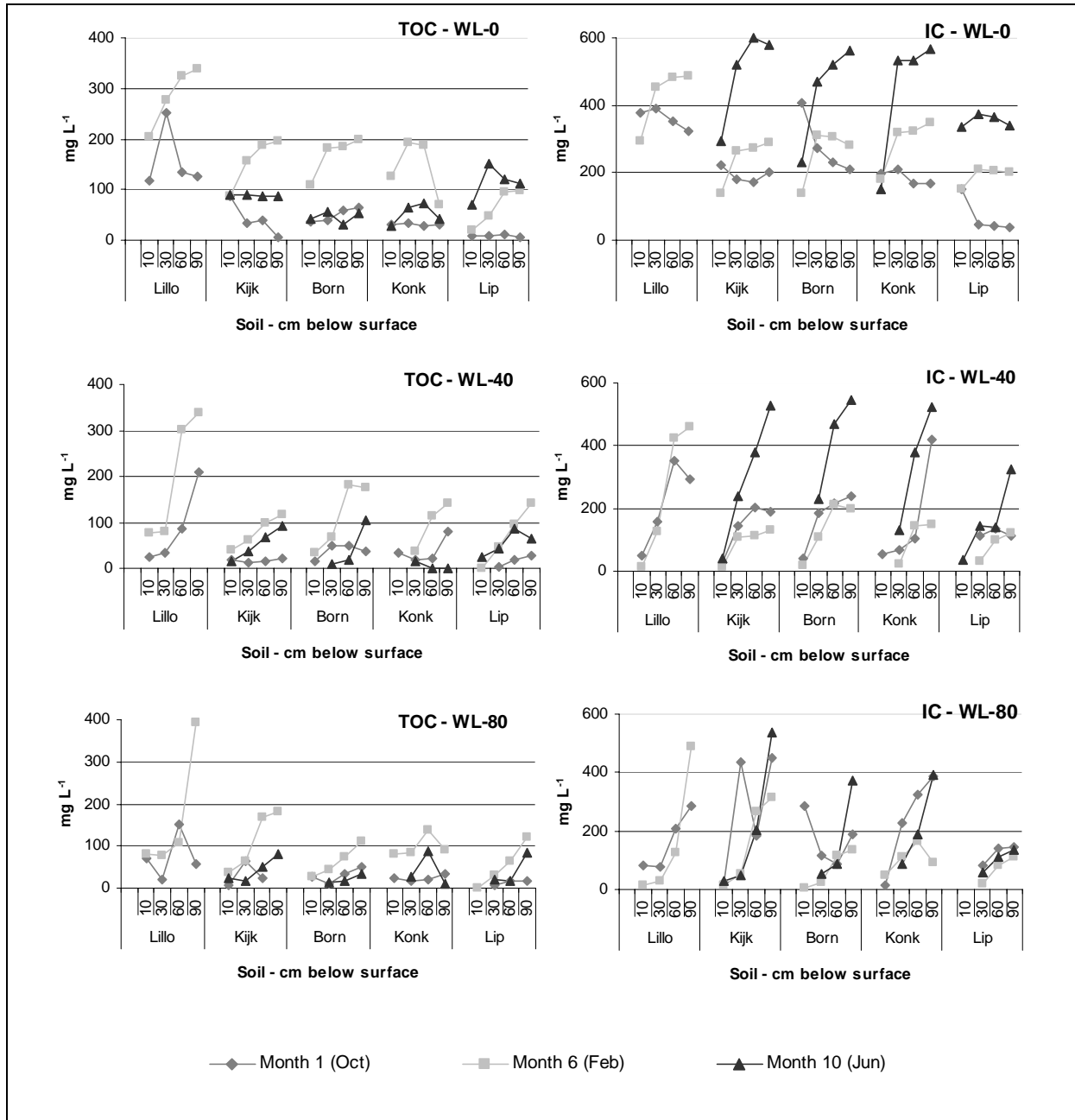
Fig. 8.1. Total organic carbon (TOC) and inorganic carbon (IC) concentrations at different sampling depths (10, 30, 60 and 90 cm) below the surface of 5 sediments (Lillo: Lillo, Kijk: Kijkverdriet, Born: Bornem, Konk: Konkelschoor, Lip: Lippensbroek) as a function of time, upon adjusting the water level to 0 (WL-0), 40 (WL-40) and 80 (WL-80) cm below the sediment surface (missing values: samples of month 10 in Lillo)

Significant amounts of sulphates were found after the first month (Fig. 8.2). Sulphate concentrations fluctuated around 100 mg L^{-1} in Kijkverdriet, Bornem and Lippensbroek, but were somewhat higher in Lillo and Konkelschoor. The lower the water table level, the higher the observed sulphate concentrations were after the first month. A depth profile, showing decreasing sulphate concentrations with increasing depths, was already established in WL-40 and WL-80 after the first month. Below and just above the water table, the sulphate

concentrations also significantly decreased with time. In that way, the depth profile developed more clearly upon time, showing significantly increasing sulphate concentrations with increasing sampling height above the water table, starting from more than 30 cm above the water table. In WL-0, the sulphate concentrations were already very low at 10 cm below the water table and generally seemed to decrease with time at all of the sampling depths. Observed sulphate concentrations are expected to result from sulphate reduction and subsequent sulphide precipitation in anoxic environments, sulphate transport from oxic towards anoxic zones (by e.g. diffusion), sulphate release from decomposing organic matter and sulphate uptake by organisms. Sulphide oxidation is not expected to significantly contribute to increasing sulphate concentrations in the beginning of the experiment, as measured sulphide amounts were very low. In the WL-0 treatment of the sediments of Kijkverdriet, Bornem, Lippensbroek and Konkelschoor, all sulphates seem to have disappeared at all sampling depths after 6 months. This was also the case for the 30, 60 and 90 cm sampling depths of the WL-40 treatments of Kijkverdriet, Lippensbroek and Bornem, whereas it took 10 months in Konkelschoor at the depth of 30 cm. At 10 cm depth, sulphate concentrations were not reduced to below detection limits within the first 10 months. In WL-80, relatively fast reductions were observed at the 60 and 90 cm sampling depths. In Kijkverdriet, Bornem and Konkelschoor, sulphate concentrations at 30 and 60 cm depth increased to above initial levels in the 10th month, whereas the amount of representative pore water samples at 10 cm depth was too low as the sediment was too dry.
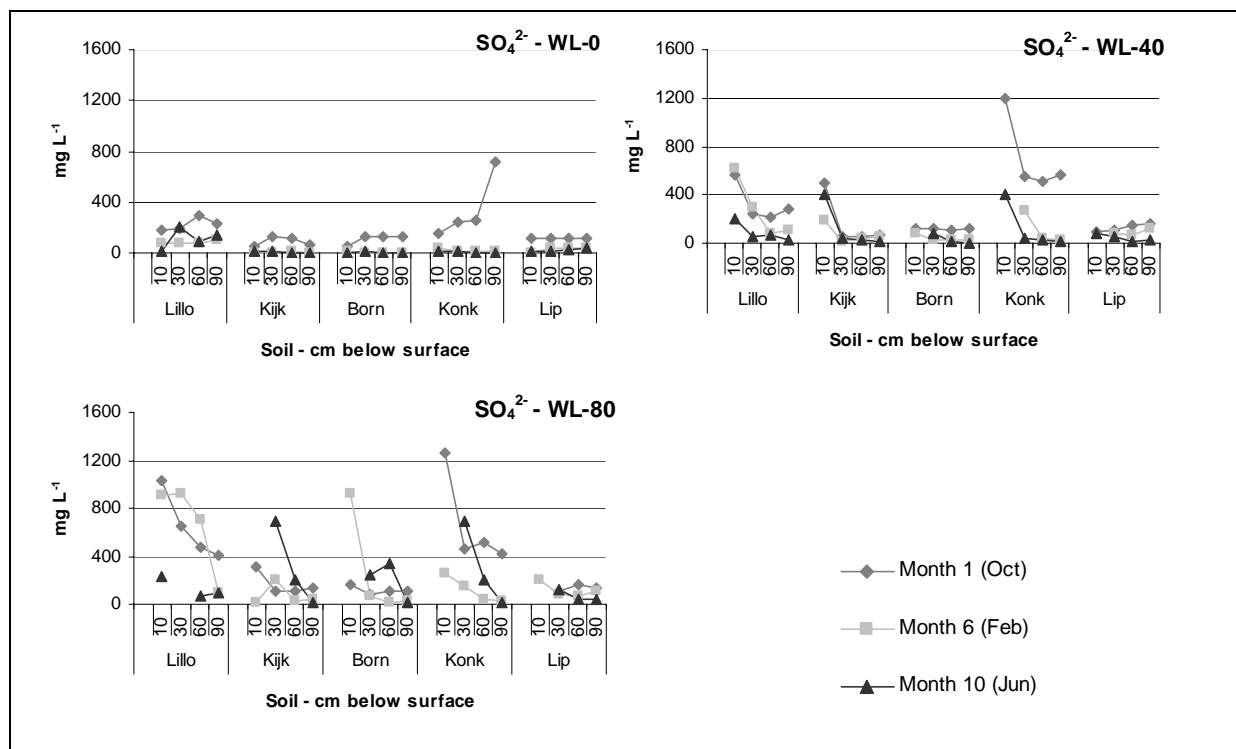
Fig. 8.2. Sulphate concentrations at different sampling depths (10, 30, 60 and 90 cm) below the surface of 5 sediments (Lillo: Lillo, Kijk: Kijkverdriet, Born: Bornem, Konk: Konkelschoor, Lip: Lippensbroek) as a function of time, upon adjusting the water level to 0 (WL-0), 40 (WL-40) and 80 (WL-80) cm below the sediment surface

Mg, Na and K concentrations were significantly higher in the pore water of the Lillo sediment, compared to the other sediments, as that sediment originated from the brackish part of the estuary. Ca (Fig. 8.3) and Mg concentrations increased with time at all sampling points which were situated both more than 10 cm below the sediment surface and below the water table. Carbon dioxide which is produced upon organic matter decomposition is being accumulated below the water table and the sediment surface, as diffusion towards the atmosphere is hampered. Solubilisation of CO_2 subsequently leads to increased calcium or magnesium bicarbonate concentrations, as e.g. carbonates are being decomposed according to the following reaction: $CaCO_3 + CO_2 + H_2O \rightarrow Ca(HCO_3)_2$ (van den Berg & Loch, 2000). The Ca in this reaction might also originate from the pool of cations which were sorbed to the negatively charged sediment complex. Increases of Ca and Mg concentrations in the pore water are indeed accompanied by increased inorganic carbon concentrations (Fig. 8.1).
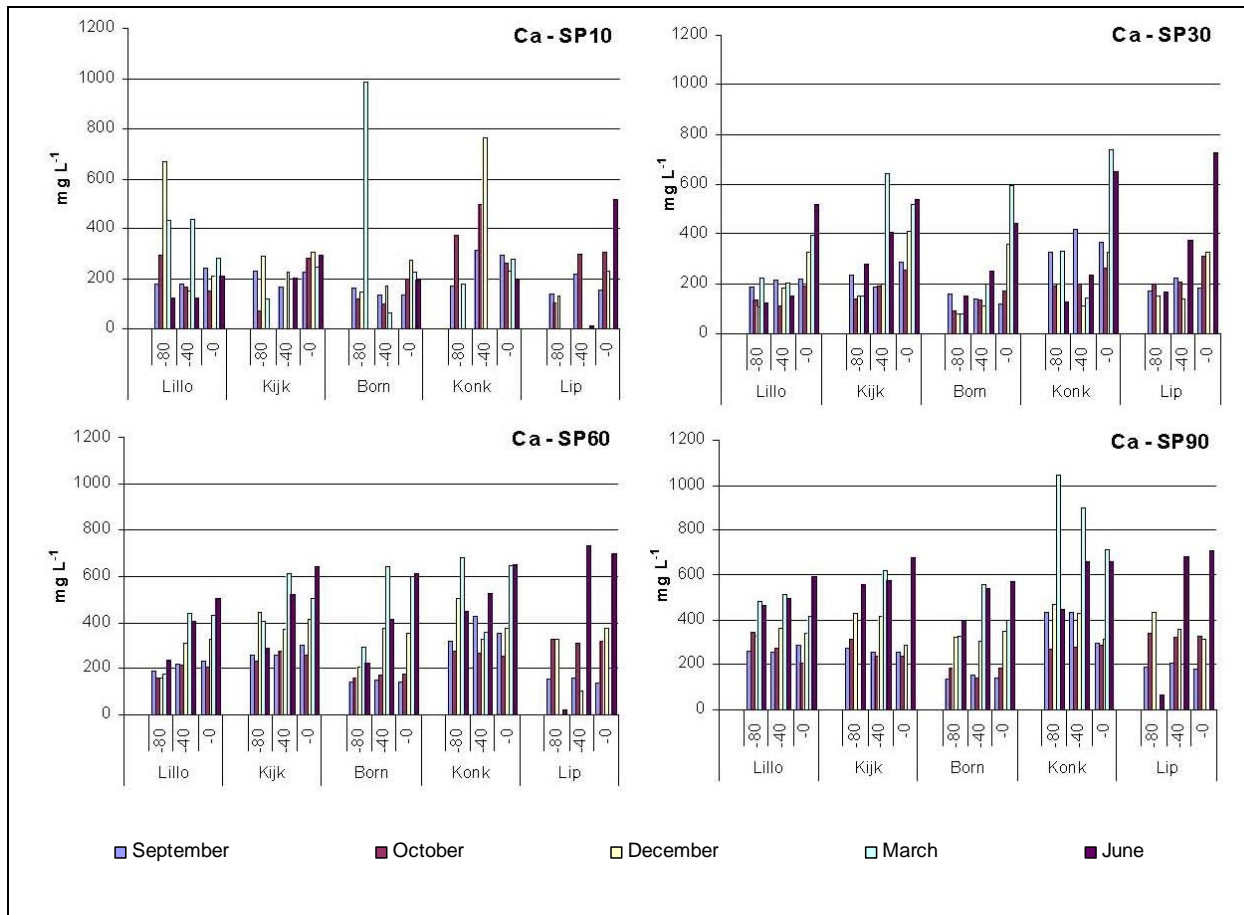
Fig. 8.3 Pore water Ca concentrations as a function of time at 4 sampling points (SP-10, SP-30, SP-60 and SP-90: sampling points at 10, 30, 60 and 90 cm below the sediment surface) in 5 sediments (Lillo: Lillo, Kijk: Kijkverdriet, Born: Bornem, Konk: Konkelschoor, Lip: Lippensbroek) upon adjusting the water table to 3 levels (-80, -40 and -0: 80, 40 and 0 cm below the sediment surface)

Fe and Mn concentrations were obviously higher at sampling points below the water table (Fig. 8.4). At 10 and 30 cm below the sediment surface, significant Fe and Mn amounts were only found in the pore water when the sediment column was completely waterlogged up to its surface. In Lillo, Konkelschoor and Kijkverdriet, Mn concentrations below the water table were already significant in the first month, which indicates an initially already quite reduced state and the abundance of Mn oxide reducing microbial communities. In Kijkverdriet, Konkelschoor, Lillo and Bornem, steady state concentrations seem to be reached within the first 6 months, or the concentrations even again started to decrease after an initial peak. In Lippensbroek, Fe and Mn concentrations were however very low in the beginning of the experiment and still seem to continue to increase after 10 months. Moreover, Mn concentrations reached the highest concentrations in Lippensbroek, whereas peak Fe concentrations were the lower compared to the other sediments. As Lippensbroek was the only upland soil, it had a lower initial moisture content, a lower organic matter content and a

more permeable texture, which also resulted in lower initial TOC concentration and lower IC concentrations (Fig. 8.1). As a result, oxygen might penetrate deeper into the soil for a longer period of time, which might slow down the use of alternative electron acceptors for organic matter decomposition. Moreover, as this concerned an upland soil, organic matter probably has reached a more advanced decomposition state and Fe, Mn and sulphate reducing microbial communities probably were not yet abundant in the beginning of the experiment. In that way, it takes more time to reach maximum Mn oxide reducing capacities. Moreover, microbial communities will develop in a relatively strict sequence under these conditions, which is determined by their energetic efficiencies. The lower initial sulphate concentrations in the Lippensbroek soil could also have hampered the development of sulphate reducing communities and the formation of sulphides, which is a major counteracting factor for Fe and Mn release. Sulphate concentrations below the water table indeed less rapidly decreased in Lippensbroek, compared to the other sediments. At 60 cm below the surface, the Fe and Mn concentrations were elevated when the water table was kept at 40 cm below the sediment surface or when the sediment column was completely waterlogged up to the surface. When it is lowered to 80 cm below the surface, Mn concentrations however also still reached quite high concentrations in all sediments, whereas this was also the case for Fe concentrations in Kijkverdriet and Konkelschoor. At sufficiently high sampling depths, Fe and Mn oxide reduction thus also occurs above the water table, which indicates that an unsaturated sediment column of 60 cm might also be able to create reducing conditions. At 90 cm below the surface, Fe and Mn concentrations increased with time and subsequently decreased or reached steady state conditions. Neither the profile nor peak concentrations seemed to depend on the water table level at this sampling depth.
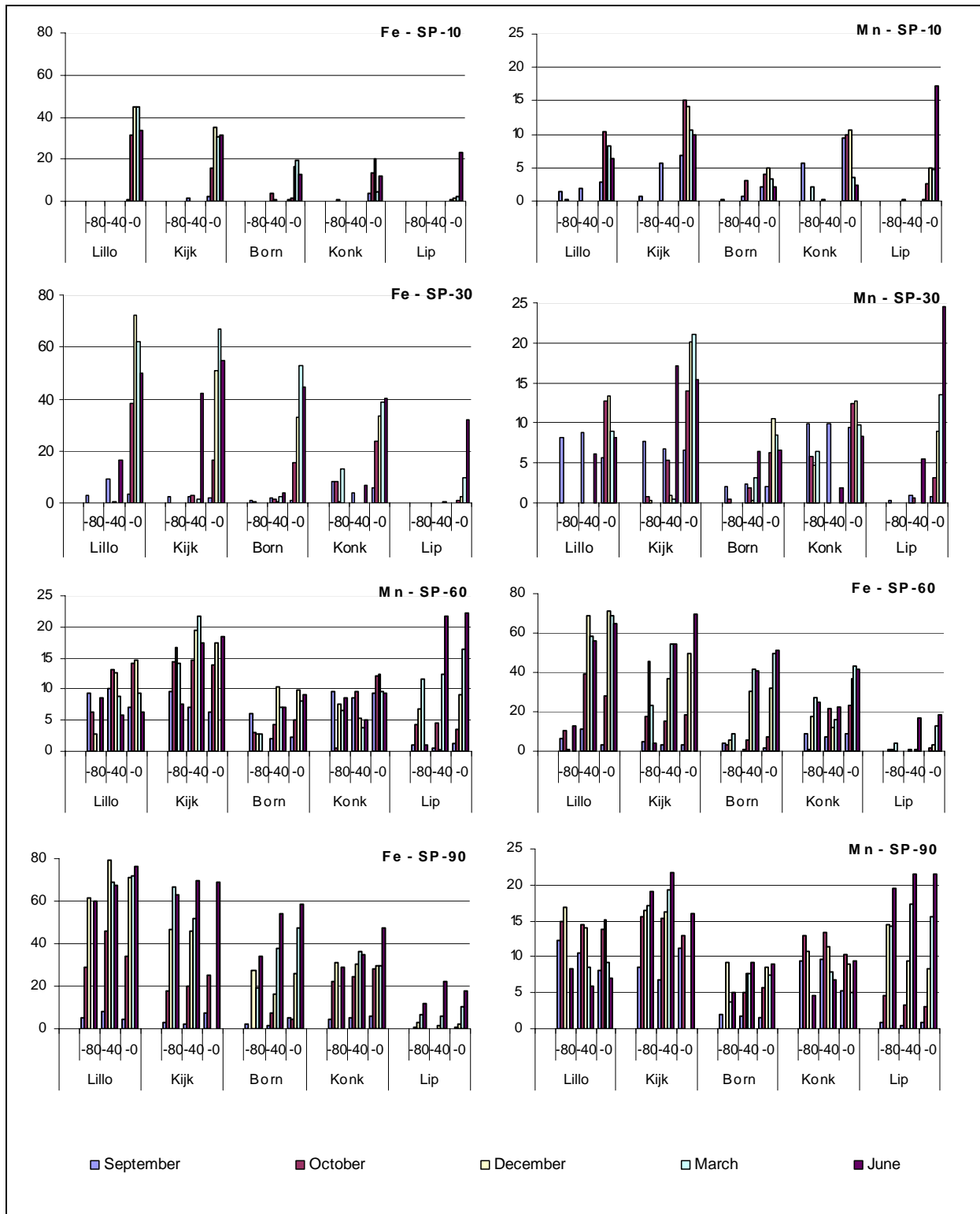
Fig. 8.4. Pore water Fe and Mn concentrations (mg L^{-1}) as a function of time at 4 sampling points (SP-10, SP-30, SP-60 and SP-90: sampling points at 10, 30, 60 and 90 cm below the sediment surface) in 5 sediments (Lillo: Lillo, Kijk: Kijkverdriet, Born: Bornem, Konk: Konkelschoor, Lip: Lippensbroek) upon adjusting the water table to 3 levels (-80, -40 and -0: 80, 40 and 0 cm below the sediment surface)

Cd concentrations at 10 cm below the sediment surface (Fig. 8.5) often exceeded sanitation thresholds in the sediment originating from Lillo when the groundwater table was situated at 40 or 80 cm below the sediment surface. This sediment had the lowest total Cd concentration, but it was also the most saline one. Increasing salinity thus seems to result in an increasing Cd mobility, which has also been reported by other authors (e.g. Hatje et al., 2003) and has been discussed in Chapter 4. According to most authors (e.g. Paalman et al., 1994 & Millward & Liu, 2003), Cd mobilisation is subject to chlorocomplexation and competition with Ca, Mg and Na for sorption to solid soil fractions. In the current experiment, the Cd concentrations rapidly increased during the first 6 months. Neither chloride contents nor Ca, Mg and Na concentrations did increase in a similar way during the first 6 months. This suggests that small, non-detectable (< 2 mg AVS kg^{-1} DM) amounts of sulphides might have been present at the beginning of the experiment, which hampered Cd mobilisation. Upon oxidation of these sulphides, Cd mobilisation is effectuated and enhanced by chlorocomplexation and competition by cations for sorption to the solid sediment fractions. This coincided with the observations of Gerringa et al. (2001). They found that high salinities resulted in a faster release of Cd from CdS during the oxidation of reduced Scheldt sediments, which was found to be stimulated both by formation of chloride complexes and ion exchange. The oxidation rate of the upper 10 cm sediment layer seems to increase when lowering the water table. Whereas Cd concentrations increased to about 7 µg L^{-1} after 10 months when the water table was lowered to only -40 cm, they increased up to 13 µg L^{-1} when the water table was lowered to -80 cm. In the other sediments, Cd concentrations in the pore water rarely exceeded background levels during the entire experimental period. However, some mobilisation was also observed in Konkelschoor after 6 months. When the water table was kept at 80 cm below the surface, pore water sampling at 10 cm depth became impossible at the higher summer temperatures in June (upon 10 months of oxidation), as the sediment was becoming too dry.

The Cr concentrations in the pore water at 10 cm below the sediment surface (Fig. 8.5) only once exceeded the background level. These were higher in Lillo, which suggests that Cr mobilisation is also promoted by an increasing salinity. Moreover, Cr concentrations increased as a function of time when the sediment was kept completely waterlogged. The behaviour of Cr and the quantity that is found in the pore water is expected to result from a complex series of counteracting mechanisms. According to Masscheleyn et al. (1992), Cr(VI) is known to be rapidly reduced to Cr (III) in the presence of organic matter and/or Fe(II). They however also reported Cr(III) to be oxidised to Cr(VI) in natural waters and soils, which

might be catalysed by the presence of Fe and/or Mn oxides. Trivalent Cr might precipitate as $Cr(OH)_3$. However, the trivalent Cr in solution can also form soluble complexes with organic components, which keeps Cr in solution. In turn, these soluble Cr(III)-DOC complexes could also sorb to sediments, which might remove Cr(III) from solution. These counteracting reactions could explain the mobilisation under both oxidised and reduced conditions (Guo et al., 1997). The Cu concentrations at 10 cm below the sediment surface (Fig. 8.5) were also highest in Lillo, although they did not exceed sanitation thresholds. The differences with Cu concentrations at other sampling sites were not as significant as for Cd. Moreover, they only slightly increased in the -80 cm and -40 cm water table treatments of Lillo during the first 6 months, which suggests that Cu is less affected by salinity than Cd, as already seen in Chapter 4. However, sulphide oxidation also seems to be a primary factor affecting Cu mobilisation, as significant increases of Cu concentrations in the pore water were observed in the WL-80 treatment of Konkelschoor during the first 6 months of oxidation. The Zn concentrations at 10 cm below the sediment surface (Fig. 8.5) rarely exceeded background values. As for Cd and Cu, the highest values were found in the saline sediment originating from Lillo, which suggests that there is also an effect of salinity on the Zn mobility.
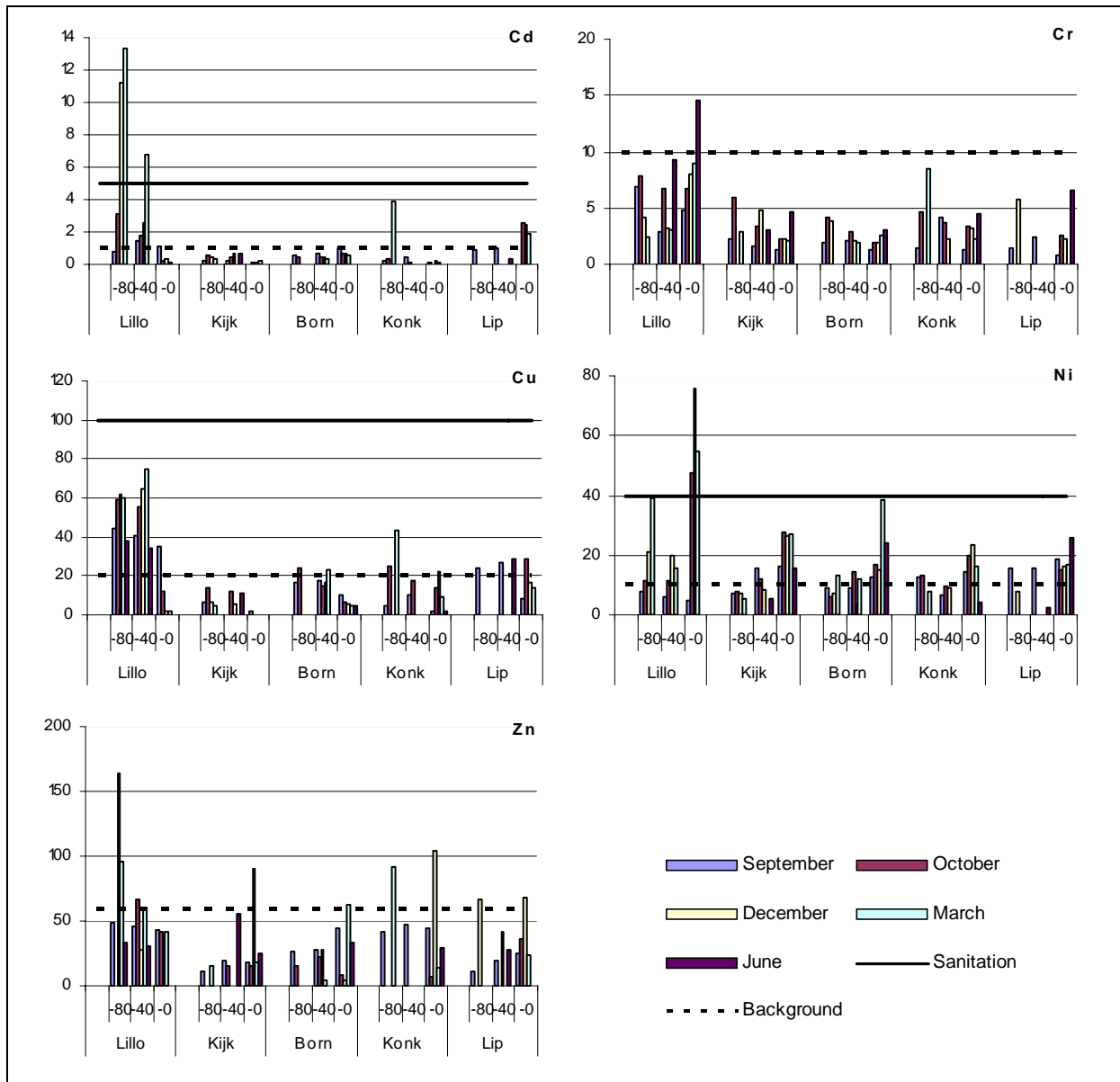
Fig. 8.5. Pore water Cd, Cr, Cu, Ni and Zn concentrations (µg L^{-1}) as a function of time in 5 sediments (Lillo: Lillo, Kijk: Kijkverdriet, Born: Bornem, Konk: Konkelschoor, Lip: Lippensbroek) at 10 cm depth upon adjusting the water table to 3 levels (-80, -40 and -0: 80, 40 and 0 cm below the sediment surface)

The Ni concentrations at 10 cm below the sediment surface (Fig. 8.5) were highest when the sediment was waterlogged. Co-precipitated Ni might indeed be released upon Fe and Mn oxide reduction (Kashem & Singh, 2001). As was seen for Cd, Ni concentrations also increased with time and fluctuated more significantly in Lillo, which suggests that Ni mobility is also slightly affected by salinity. In the relatively reduced sediments of Kijkverdriet and Konkelschoor, Ni concentrations again decreased after an initial increase, as was also observed for Mn and Zn, which might indicate precipitation as sulphides.

At 30 cm below the surface of the Lillo sediment, Cd concentrations decreased to background levels when the water table fluctuated around a depth of 40 cm. However, when the water table was lowered to 80 cm below the sediment surface, Cd concentrations still increased and exceeded the sanitation thresholds after 6 months. At the end of the experiment, the Cd concentrations also substantially increased at depth of 30 cm in the sediments of Lippensbroek, Konkelschoor and Bornem when the water table was kept at 40 cm below the sediment surface. This effect was most pronounced for Lippensbroek. Thus, Cd seemed to accumulate just above the water table. Ca, Mn, Ni and Fe concentrations were at that time also rapidly increasing in these sediments due to Fe and Mn oxide reduction and CO_2 accumulation below the water table, as previously mentioned. Released Ca, Mn, Ni and Fe might thus compete with Cd for sorption to the solid sediment fractions, as mentioned in previous chapters. As a result, Cd could be released from the solid sediment fraction to the pore water of the sediment. Just below the water table, this Cd release might be counteracted by sulphide precipitation if a small amount of sulphides is present, whereas Cd which migrates to just above the water table remains in solution. Moreover, as the -30 cm sampling point was just 10 cm away from the water table in WL-40, a temporal lowering of the water table in the hotter month of June could also have resulted in the oxidation of (part of) the sulphides, which have been formed. Cr concentrations increased again as a function of time when the sediments were kept completely waterlogged up to their surface. The highest concentrations were however found when the water table was kept at 40 or 80 cm below the soil surface in the Lippensbroek soil. The mobilisation of Cr under both oxidising and reducing conditions is expected to result from a quite complex series of counteracting mechanisms, as mentioned above. Cu concentrations were again highest above the water table at all sampling sites. Highest values were again observed in Lillo, and lowest values were found in the very reduced sulphide-rich and organic sediment of Kijkverdriet, again confirming the roles of salinity and sulphide precipitation. Ni concentrations again exceeded sanitation thresholds in the completely waterlogged Lillo sediment, but they now also increased towards sanitation thresholds in the other sediments. As for Cd, Zn concentrations substantially increased in the sediments of Lippensbroek, Konkelschoor and Bornem at the end of the experiment when the water table was kept at 40 cm below the sediment surface, which might be attributed to sulphide oxidation upon fluctuating water table levels and competition with Ca, Mn, Ni and Fe for sorption to the solid sediment fraction as a result of Fe and Mn oxide reduction and solubilisation of CO_2.
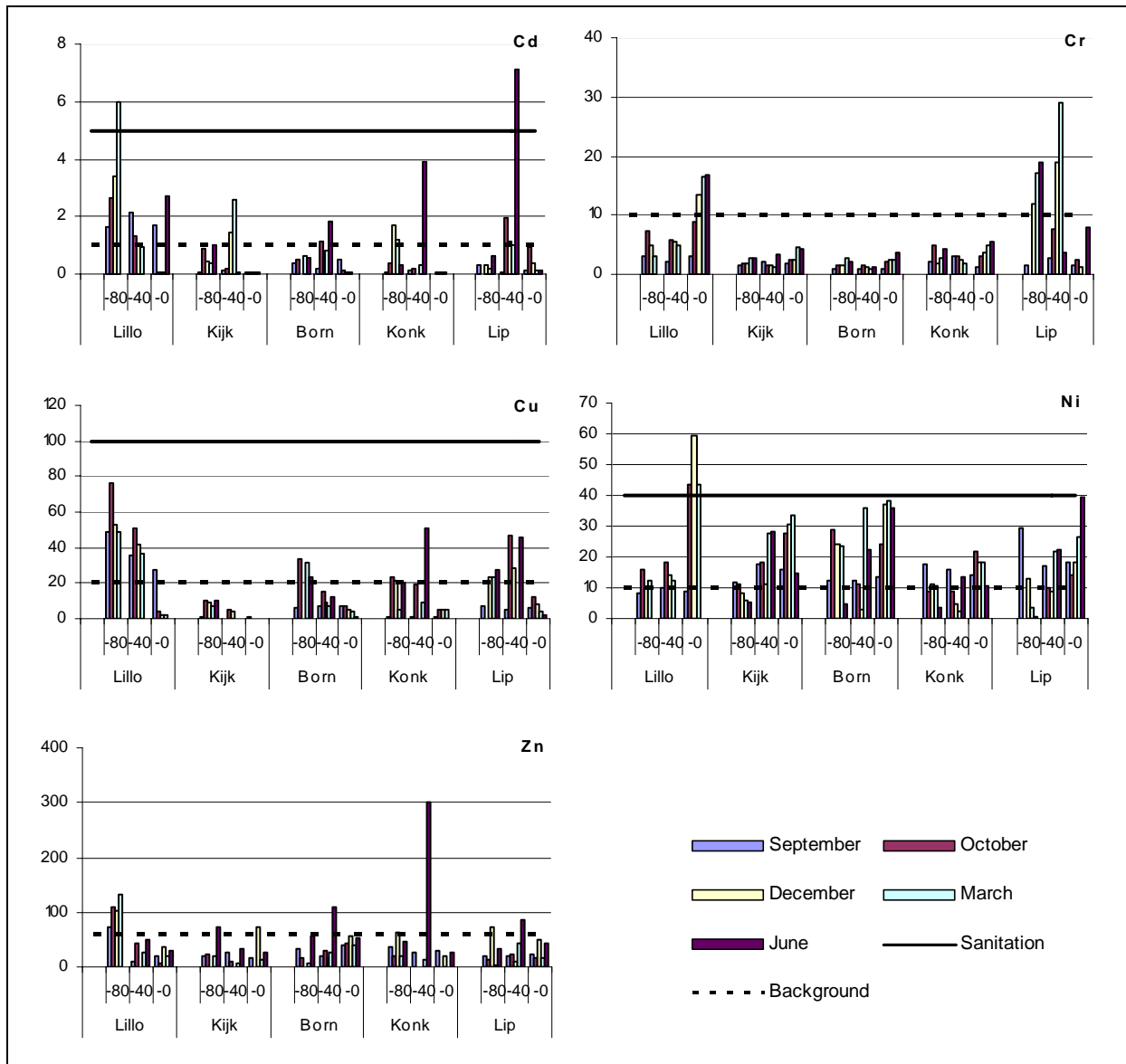
Fig. 8.6. Pore water Cd, Cr, Cu, Ni and Zn concentrations (μg L^{-1}) as a function of time in 5 sediments (Lillo: Lillo, Kijk: Kijkverdriet, Born: Bornem, Konk: Konkelschoor, Lip: Lippensbroek) at 30 cm depth upon adjusting the water table to 3 levels (-80, -40 and -0: 80, 40 and 0 cm below the sediment surface)

The Cd concentrations decreased to below sanitation thresholds at 60 cm below the surface of the Lillo sediment (Fig. 8.7). In the sediments of Kijkverdriet, Bornem and Konkelschoor, they did not exceed background values. They were however somewhat higher when the water table was kept at a level of 80 cm below the surface of the Bornem sediment. As this is a very sandy sediment, oxygen might penetrate somewhat deeper into this sediment. Cd concentrations were also high at 60 cm depth when the water table of the Lippensbroek soil was regulated at 40 or 80 cm below the surface, and even exceeded the sanitation threshold once. These high concentrations around the water table level should again be attributed to a slightly fluctuating water table, which may induce sulphide oxidation, or to the high Ca and

Mn concentrations just below the water table, which might be due to the increased CO_2 pressure and the reduction of Mn oxides as a result of organic matter decomposition. This might result in competition with Cd for sorption to the sediment matrix and subsequent Cd release, which is not counteracted by sulphide precipitation as a sufficiently reduced state has not yet been reached. The latter is due to the fact that oxygen penetrates deeper and for a longer time into the Lippensbroek soil, as already mentioned above. Cr concentrations in the pore water again increased below the water table as a function of time, when the water level was kept at 40 cm below the sediment surface or when the sediment was completely waterlogged. Salinity again seems to affect Cr mobility, as Cr concentrations in the pore water were significantly higher in Lillo. Similar patterns as for Cd were again seen for Cu and Zn, which confirms that Cu and Zn mobility are probably affected by similar factors. Ni concentrations at 60 cm depth regularly peaked towards sanitation threshold values when the sediment is completely waterlogged or when the water table is kept at a depth of 40 cm. As for Fe and Mn, they however also reached quite high values at a depth of 60 cm when the water table was kept at 80 cm below the sediment surface. A sediment column of 60 cm might also be able to create reducing conditions, which favour Ni mobilisation. The Ni concentrations were also again highest in Lillo, which confirms the role of the salinity.
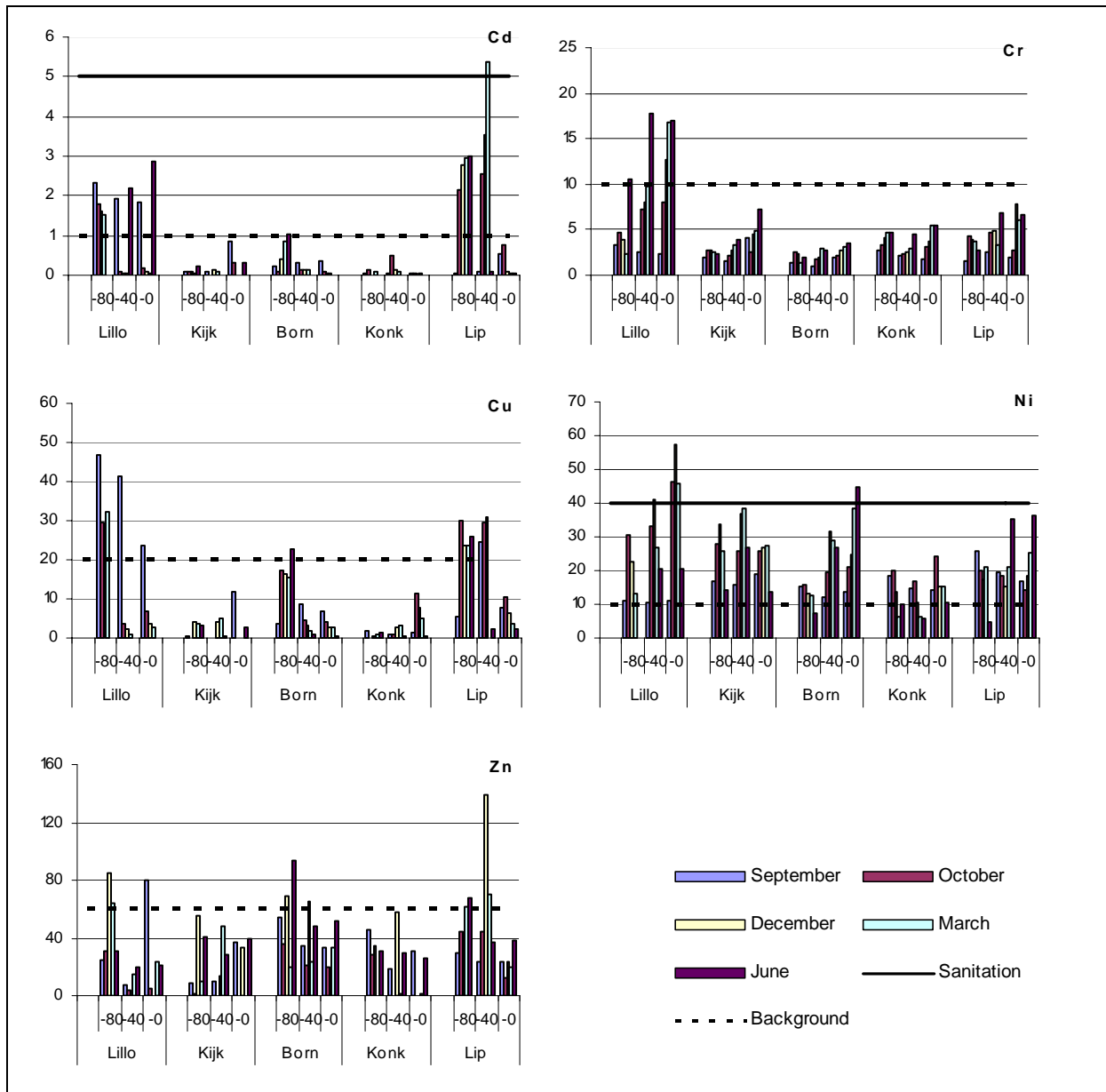
Fig. 8.7. Pore water Cd, Cr, Cu, Ni and Zn concentrations ($\mu g\ L^{-1}$) as a function of time in 5 sediments (Lillo: Lillo, Kijk: Kijkverdriet, Born: Bornem, Konk: Konkelschoor, Lip: Lippensbroek) at 60 cm depth upon adjusting the water table to 3 levels (-80, -40 and -0: 80, 40 and 0 cm below the sediment surface)

Finally, the lowest Cd, Cu, and Zn concentrations were found at the deepest sampling point, situated at a depth of 90 cm below the sediment surface (Fig. 8.8). They only occasionally exceeded background values. Sulphide precipitation probably takes the upper hand at this sampling depth, which is confirmed by indeed substantially decreasing sulphate concentrations towards the end of the experiment (Fig. 8.2). Cr concentrations however significantly increased in all treatments as a function of time and they were again highest in Lillo, which confirms the role of reducing conditions (Masscheleyn et al., 1992; Guo et al., 1997) and salinity (Gambrell et al., 1991b; Lores & Pennock, 1998) in increasing the Cr

mobility. Ni concentrations were very similar to the ones at a sampling depth of 60 cm, and thus they again regularly exceeded sanitation thresholds.
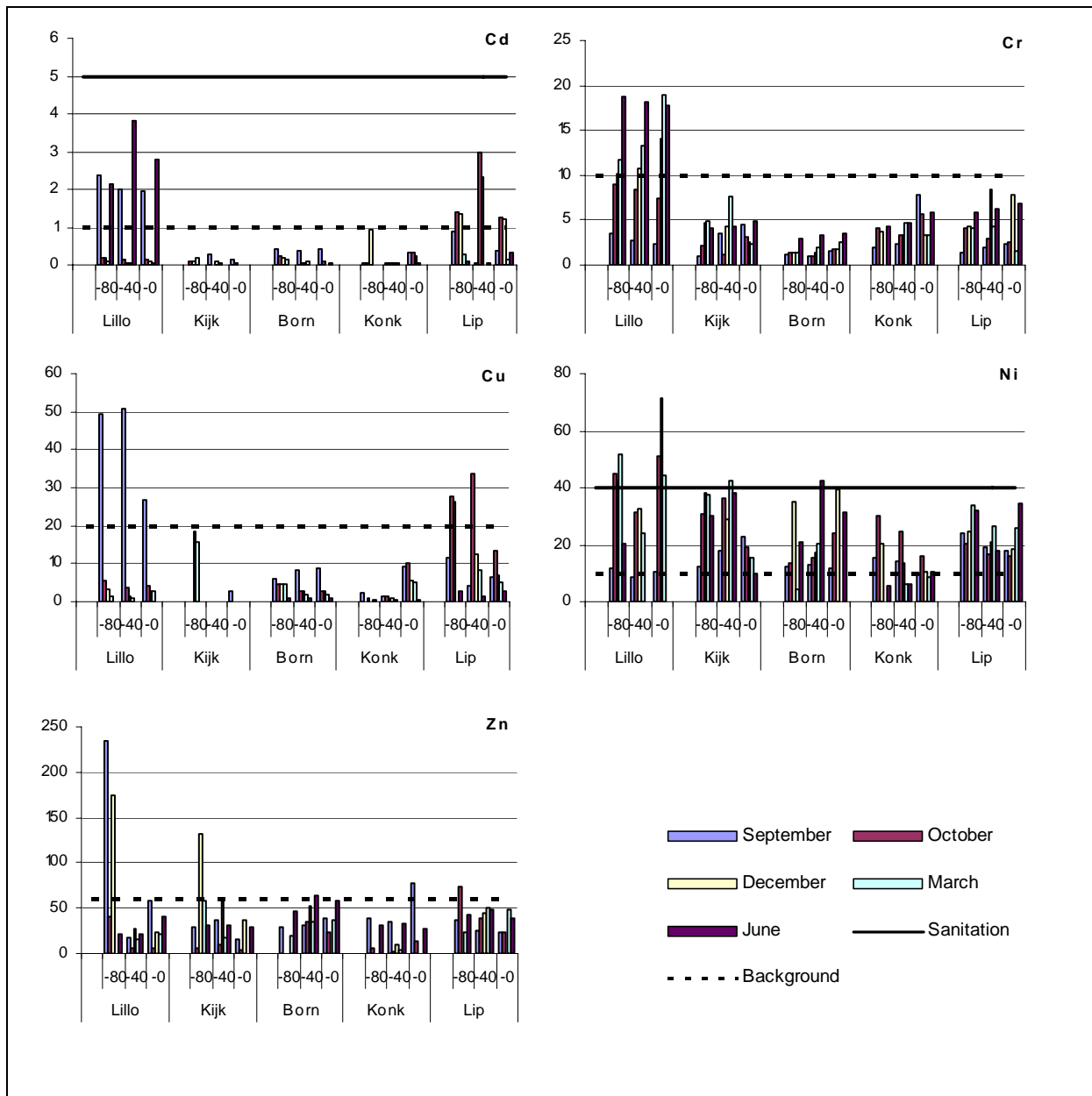

Fig. 8.8. Pore water Cd, Cr, Cu, Ni and Zn concentrations (μg L^{-1}) as a function of time in 5 sediments (Lillo: Lillo, Kijk: Kijkverdriet, Born: Bornem, Konk: Konkelschoor, Lip: Lippensbroek) at 90 cm depth upon adjusting the water table to 3 levels (-80, -40 and -0: 80, 40 and 0 cm below the sediment surface)

8.4. *Conclusion*

Nickel and Cr release are facilitated by reductive conditions, and were slightly promoted by increasing salinities. These reducing conditions can occur below the water table, or above the water table, but at a sufficiently high sampling depth (more than 30 cm below the sediment surface). Cr is however also mobilised under more oxic conditions, as it can also form complexes with organic substances. Cu, Zn and especially Cd are all primarily released above the water table under high salinity conditions. Cd, Cu and Zn are also released below or just above the water table when organic matter is being decomposed, resulting in Ca, Mn, Ni and/or Fe release upon CO_2 accumulation and Fe and Mn oxide reduction, without being accompanied by sulphide production. Under greenhouse conditions, it takes about 2 months before the Flemish soil sanitation threshold levels for groundwater are reached for Ni or Cd concentrations in the pore water, upon the creation of waterlogged or emerged conditions, respectively. However, the total Ni concentrations in the sediments did not exceed any soil sanitation threshold for the solid soil fraction. Slight water level fluctuations on short term might thus result in metals becoming locally available just above or below the water table. These fluctuations are not expected to create a significant overall metal mobilisation, but they might affect plant uptake.

9.

Field monitoring of metal concentrations in the pore water

9 Field monitoring of metal concentrations in the pore water

9.1 Introduction

In the previous chapters, factors affecting the metal mobility in intertidal sediments of the river Scheldt were identified by conducting greenhouse experimental trials. In the current chapter, the findings are validated under field conditions. Groundwater monitoring pipes and pore water samplers were installed at 11 different sampling sites along the river Scheldt. Water table levels were measured and physico-chemical sediment and pore water characteristics were determined at different sampling depths (10, 30, 60 and 90 cm) below the sediment surface. The relationships between the redox status of the sediments, their other physico-chemical characteristics and the sampling depth were assessed, after which the concentrations of Cd, Cr, Cu, Ni and Zn in the pore water were discussed as a function of these parameters.

9.2 Materials and methods

9.2.1 Sampling

The study was carried out in the part of the Scheldt estuary between Berlare and Doel, which is subjected to tidal influences. Eleven study sites were selected (Fig. 9.1). They all consist of tidal marshes, vegetated by especially common reed, *Phragmites australis* (Cav.) Trin. ex Steud. (Fig. 9.2). Most of them are only inundated at high or spring-tides.
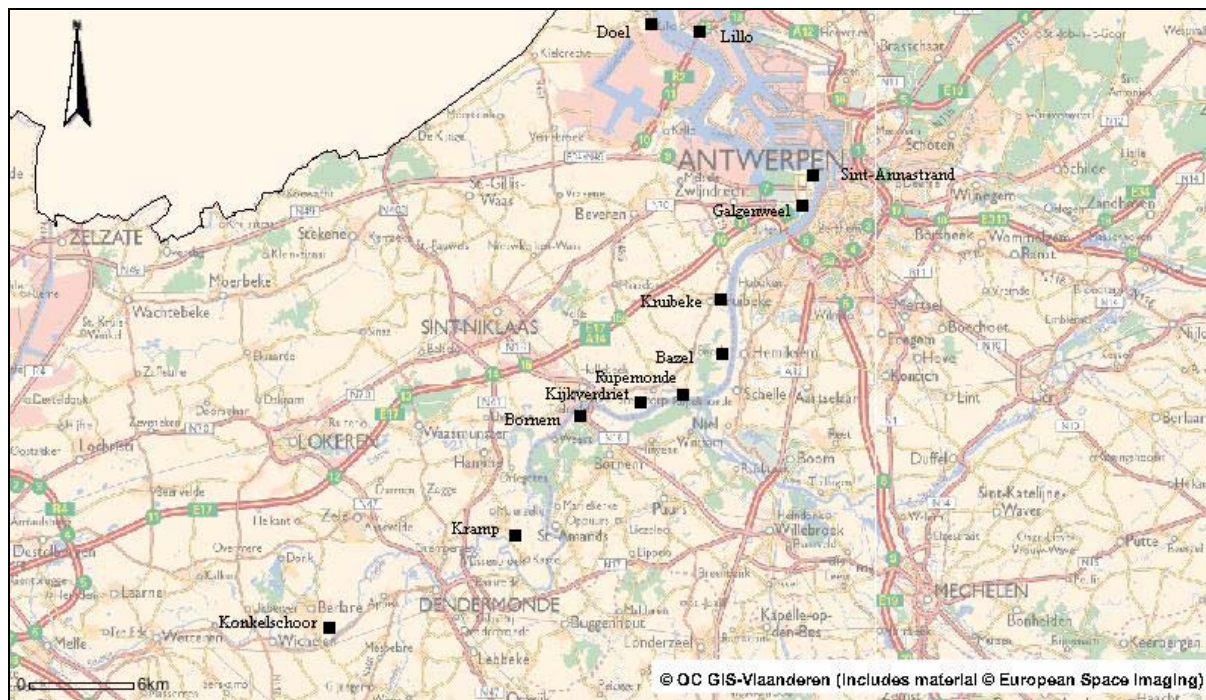
Fig. 9.1. Location of the sampling sites

The marshes "Konkelschoor" in Berlare, "Kramp" in Kastel, "Bornem" in Bornem and "Kijkverdriet" in Steendorp are situated in the freshwater part of the estuary, at 131.39, 114.45, 98.11 and 94.39 km from the river mouth, respectively. A series of marshes is situated near Rupelmonde, Bazel and Kruibeke, which will be referred to as "Rupelmonde", "Bazel" and "Kruibeke". These are situated at 91.71, 88.94 and 86.35 km from the river mouth, respectively. They have recently been primarily disturbed by the construction of the largest controlled flooding area in Flanders, which should protect the inhabited areas near the river Scheldt from flooding. The "Galgenweel" marsh in Burcht is located at 79.45 km from the mouth. When moving downstream, it is the first marsh which is significantly affected by brackish water. The marsh near "Sint-Annastrand" (75.99 km from the mouth) is rather small and very sandy, as it situated near an artificially created beach in the city of Antwerp. The "Lillo" marsh (also called "Galgenschoor") is located in the middle of the harbor of Antwerp, at 61.82 km from the river mouth. The "Doel" marsh is the sampling site which is situated closest to the river mouth (55.66 km) and near the Dutch-Belgian border. Together with the "Saeftinghe" marsh, just across the border in The Netherlands, it forms the largest brackish-water marsh in Europe. Within the "Doel" marsh, 3 sampling sites were selected at a distance of approximately 10 m from each other, aiming at the assessment of within-marsh variability. These will be referred to as Doel A, Doel B and Doel C. Assessing within-marsh variability at all sampling locations was not feasible as we primarily aimed to assess metal concentrations

levels in the pore water of a representative series of intertidal sediments along the Scheldt estuary, meanwhile maintaining an acceptable temporal sampling resolution.

Fig. 9.2. Some pictures of sampling sites: Bornem (above left), Kijkverdriet (above right), Galgenweel (below left), and Sint-Annastrand (below right)

To sample pore water, Rhizon soil moisture samplers (type MOM, Rhizosphere Research Products, Wageningen, The Netherlands) were equipped with extension tubes and taped vertically on the outside of PVC tubes at fixed distances from the lower part of the tubes. On each tube, three samplers were installed at each distance. Using an auger which had the same diameter as the tubes, a 1-m deep hole was made at a distance of about 10 m from the dike at each sampling site in August 2005. The tubes were installed in the holes, so that the 10-cm long filter tubes of the Rhizon samplers are situated at distance intervals of 5-15, 25-35, 55-65 and 85-95 cm below the sediment surface. These distances will be referred to as 10, 30, 60 and 90 cm below the sediment surface. The tube itself was hermetically sealed using a stopper after the installation to avoid oxygen penetration into the sediment through the tube. This

experimental setup allowed a relatively cheap in situ sampling and filtration of pore water at several sediment depths during low tides, in analogy with the sampling procedure which was used during the greenhouse experimental trials (e.g. Chapter 8).

The first samplings took place one month after installation of the tubes to allow the sediments to settle around the samplers. To extract pore water, a vacuum was applied to the samplers by connecting a 10 mL vacuum tube to the extension tubes. The pore water was collected in these vacuum tubes. The tubes were brought to the laboratory, where the contents of the three tubes of each depth were combined. These samples were stored at 4°C preceding the analyses. The pore water sampling was repeated approximately every two months. A separate groundwater monitoring pipe was installed about 1 m from the pore water samplers up to a depth of 1.5 m below the sediment surface to be able to measure the water table level. The way both tubes were installed at the Kramp marsh is shown in Fig. 9.3.

Fig. 9.3. Experimental setup at Kramp (left: perforated groundwater monitoring pipe to measure water table level, right: tube with fixed Rhizon soil moisture samplers to sample pore water at multiple sampling depths)

At the beginning of the samplings, three sediment cores were sampled with an auger within a distance of 2 meters from the pore water sampler. The sediments were sampled at low tide. The sampling points were situated randomly a few meters from each other, taking into account the site heterogeneity at the sampling time. Four depths were sampled: 0-20 cm, 20-40 cm, 40-70 cm and 70-100 cm below the sediment surface. The samples were transported to

the lab in plastic bags. Part of the samples was air-dried during three weeks and subsequently ground in a hammer-cross beater mill (Gladiator BO 3567).

9.2.2 Analyses

All glassware and containers used were washed in a laboratory dishwasher, subsequently soaked overnight in 5 % ultrapure 65 % HNO_3 and rinsed with deionised water. Pseudo-total metal contents in the sediments were determined by an aqua regia destruction (Ure, 1990) and analyses with ICP-OES (Varian Vista MPX, Varian, Palo Alto, CA). Acid volatile sulphide (AVS) contents were determined by transformation of sulphides to H_2S and absorption in a Zn-acetate solution, followed by a back titration, as described by Tack et al. (1997a). The pH was measured in a 1/5 sediment/distilled water suspension after equilibration during 18 h (Van Ranst et al., 1999). The conductivity was measured in the filtrate of a 1/5 sediment/distilled water suspension, shaken for ½ h (Van Ranst et al., 1999). The organic matter content was determined by measuring the weight loss after incineration of oven-dried samples (2 h at 450°C). The carbonate content was determined by back-titration (with 0.5 M NaOH) of an excess 0.25 M H_2SO_4 added to 1 g of sediment (Nelson, 1982). Texture was determined at the Research Institute for Nature and Forest (INBO) using laser diffraction (Coulter LS200, Miami, FL) after removing organic matter and carbonates by adding hydrogen peroxide and an acetate buffer solution respectively. To determine the chloride content 10 g of sediment was suspended in 50 ml of 0.15 M HNO_3 and shaken for 30 minutes. The filtrate was titrated with 0.05 M $AgNO_3$ using potentiometric end-point detection (Van Ranst et al., 1999). The cation exchange capacity (CEC) was measured by percolation of 150 ml 1 M NH_4OAc through a percolation tube filled with a mixture of 5 g sediment and 35 g quartz sand, followed by washing through the excess with 300 mL denatured ethanol. The exchangeable ammonium ions were then eluted with 500 mL 1 M KCl and analysed in the percolate by means of a steam distillation (Tecator Kjeltec System 1002 Distilling Unit). The exchangeable cations were also analysed in this percolate using ICP-OES (Varian Vista MPX, Varian, Palo Alto, CA) (Van Ranst et al., 1999). Most parameters were determined on all samples. CEC and AVS contents were however measured on mixed samples, composed of equal sample amounts of the three sampling points at each sampling location and depth. Most analyses were conducted on samples which were dried and grinded. However, fresh samples

were used for the determination of acid volatile sulphide contents, as drying can induce the oxidation of the sulphides.

The total organic carbon (TOC), inorganic carbon (IC), NO_3^-, SO_4^{2-} and Cl^- concentrations in the pore water were determined on non-acidified samples directly after sampling. TOC and IC concentrations were measured with a TOC-analyser (TOC-5000, Shimadzu, Tokyo Japan). Cl^-, NO_3^- and SO_4^{2-} concentrations were measured with a Metrohm configuration consisting of a 761 Compact Ion Chromatograph equipped with a 788 IC Filtration Sample Processor and an anion exchange column (IC-AN-Column Metrosep A supp 4, Metrohm Ion Analysis, Switzerland). Subsequently, the pore water samples were acidified with a few drops of concentrated HNO_3 to keep the metals in solution and to solubilise metals which precipitated during sampling. They were analysed for Cd, Cr, Cu, Fe, Mn, Ni, Zn, Ca, Mg, Na and K contents using graphite furnace – atomic adsorption spectrometry (GF-AAS, Varian SpectraAA-800/GTA-100, Varian, Palo Alto, CA) and ICP-OES (Varian Vista MPX, Varian, Palo Alto, CA). Every time the pore water was sampled, the level of the water table was also measured twice with an interval of one day, by putting a scaled lath in the groundwater monitoring pipe.

To detect significant effects of sampling depth and location on the metal concentrations in the solid sediment fractions, variance analyses were conducted using SPSS 12.0 (2003).

9.3 Results and discussion

Sediment characteristics for all sampling points are summarised in Table 9.1. The pH was near to neutral and relatively stable. It varied between 7.36 and 8.16 and was slightly higher in the upper layer, probably because of acid production upon organic matter decomposition. The organic matter contents indeed fluctuated within a wider range at a depth of 10 cm, compared to the deeper sediment layers. Moreover, a net reduction of electron acceptors (such as sulphates) might be expected at higher sampling depths. Protons are trapped upon reduction of these electron acceptors, which increases the pH. The relatively low pH fluctuations might be attributed to the high carbonate buffering capacity. $CaCO_3$ concentrations indeed varied

between 1.8 and 17.7 %. Chloride contents and conductivities significantly decreased with increasing distance to the river mouth. Average, median and maximum AVS concentrations clearly increased with depth. At Rupelmonde AVS concentrations were very low, even at the 90 cm depth. The sediments at Rupelmonde were most sandy of the studied sites and contained the lowest organic matter amounts. These conditions do less favour strongly reducing conditions.

Table 9.1. Some characteristics of the sampled sediments at the different sampling depths (10-30-60-90 cm below the sediment surface): pH, EC, CaCO₃, chloride, OM and clay content (average: average, stdev: standard deviation, med: median, min: minimum, max: maximum)

depth	pH	EC	Chlorides	CaCO$_3$	OM	Clay	AVS
		(μS cm^{-1})	(g Cl^{-} kg^{-1} DM)	(%)	(%)	(%)	(mg kg^{-1} DM)
10 cm							
average	7.58	1733	1.63	9.03	14	40.3	32
stdev	0.16	1410	1.63	4.35	5.53	10.9	41
med	7.56	1108	0.97	7.87	14.9	40.9	13
min	7.36	292	0.02	2.5	4.67	22.8	2
max	7.89	4810	4.75	17.7	25.4	53.9	127
30 cm							
average	7.74	1623	1.61	7.05	11.5	35	46
stdev	0.25	1338	1.85	3.42	6.23	15.3	43
med	7.77	1263	0.64	6.66	11.1	38.8	33
min	7.36	209	0.001	1.82	2.96	13.6	<2
max	8.16	5077	6.19	12.4	22.5	58.2	132
60 cm							
average	7.76	1698	1.54	7.58	11.5	38.6	103
stdev	0.23	1180	1.68	2.7	5.71	14.4	110
med	7.76	1346	0.59	8.15	11.4	42.9	65
min	7.43	269	0.02	2.15	2.4	11.5	<2
max	8.08	4140	4.94	12	20.8	58.9	402
90 cm							
average	7.78	1790	1.54	8.99	10.4	38.7	144
stdev	0.22	1260	1.71	3.65	4.15	13.3	142
med	7.87	1133	0.28	9.67	10.8	44.8	99
min	7.43	299	0.001	2.73	2.21	12.3	3
max	8.09	3903	5.01	15	15.2	54.5	533

Cadmium, Cr, Cu, Ni and Zn total concentrations in the sediment varied significantly with sampling depth and location ($p < 0.001$). However, they did not differ significantly between the three Doel sampling sites, situated at a short distance (10 m) from each other. They were lowest in Doel, Rupelmonde and Bazel, where the sediments contained little clay and organic matter. They were often highest in Kijkverdriet and Bornem, and also very high in Galgenweel, Konkelschoor and Kramp, coinciding with the sites at which the highest average

clay and organic matter contents were observed. At the freshwater sampling sites of Kijkverdriet and Bornem, the sediments were also rich in sulphides, which also promotes metal accumulation. Moreover, Galgenweel is the first sampling location which is situated in the brackish water part of the estuary, when moving downstream. Polluted particles are expected to settle there as a result of flocculation upon increasing salinity (Temmerman, 1988; Heip, 1988). Total concentrations of Cd, Cr, Cu, Ni and Zn, and also spatial variations, significantly increased with increasing sampling depth. Whereas Cd, Cr, Cu, Ni, and Zn contents varied by factors of 7, 4, 6, 4 and 4 between the lowest and the highest concentrations at a depth of 10 cm, these factors became 50, 24, 33, 6, and 13, respectively, at a depth of 90 cm. Cadmium and Cu concentrations thus seemed mostly affected by sampling depth. These elements generally occur in relatively low concentrations, but their sulphides have a very low solubility product, which suggests the role of sulphide precipitation. Moreover, whereas metal concentrations increased with increasing sampling depth, clay and organic matter contents generally decreased with increasing sampling depth. In chapter 3, significant positive correlations were found between metal concentrations in the 0-20 cm sediment layer and organic matter and clay contents for 26 sampling sites, which indicated that metal accumulation in the upper sediment layer is primarily determined by organic matter and clay contents. Such significant positive correlations were now again observed for the sampling points at 10 cm below the sediment surface (Fig. 9.4). Other factors however seem to affect metal accumulation at higher depths, as correlations are not linear anymore when moving to the deeper sediment layers. The higher the organic matter or clay content, the more observed metal concentrations deviated from the concentrations which were predicted based on the relationships between metals, organic matter and clay contents at the 10 cm sampling point. For Cd, Cu, Ni and Zn, the highest deviations were found at sampling points at which the sediments also contained significant AVS amounts, whereas the lowest deviations were found at sampling points at which the sediments contained very little or no sulphides. This again suggests the role of sulphide precipitation in Cd, Cu, Ni and Zn accumulation at higher sampling depths. However, it cannot be excluded that a historically higher pollution grade affected the metal contents locally by covering of strongly polluted sediments with more recent, less polluted sediments. For Cr, the amount of deviations was much lower. Moreover, the highest deviations were not found at the sulphide-rich sites, nor at the highest sampling depth, but rather at 60 cm below the sediment surface. Cr enrichment at that sediment layer is probably a result of historical deposition of polluted sediments that accumulated at higher sampling depths, and/or the fact that Cr exists in a wide range of oxidation states and species,

which all have a different precipitation behaviour and affinity for sorption to the solid fraction (Banks et al., 2006; Guo et al., 1997; Masscheleyn et al., 1992). This was already mentioned in the previous chapters.
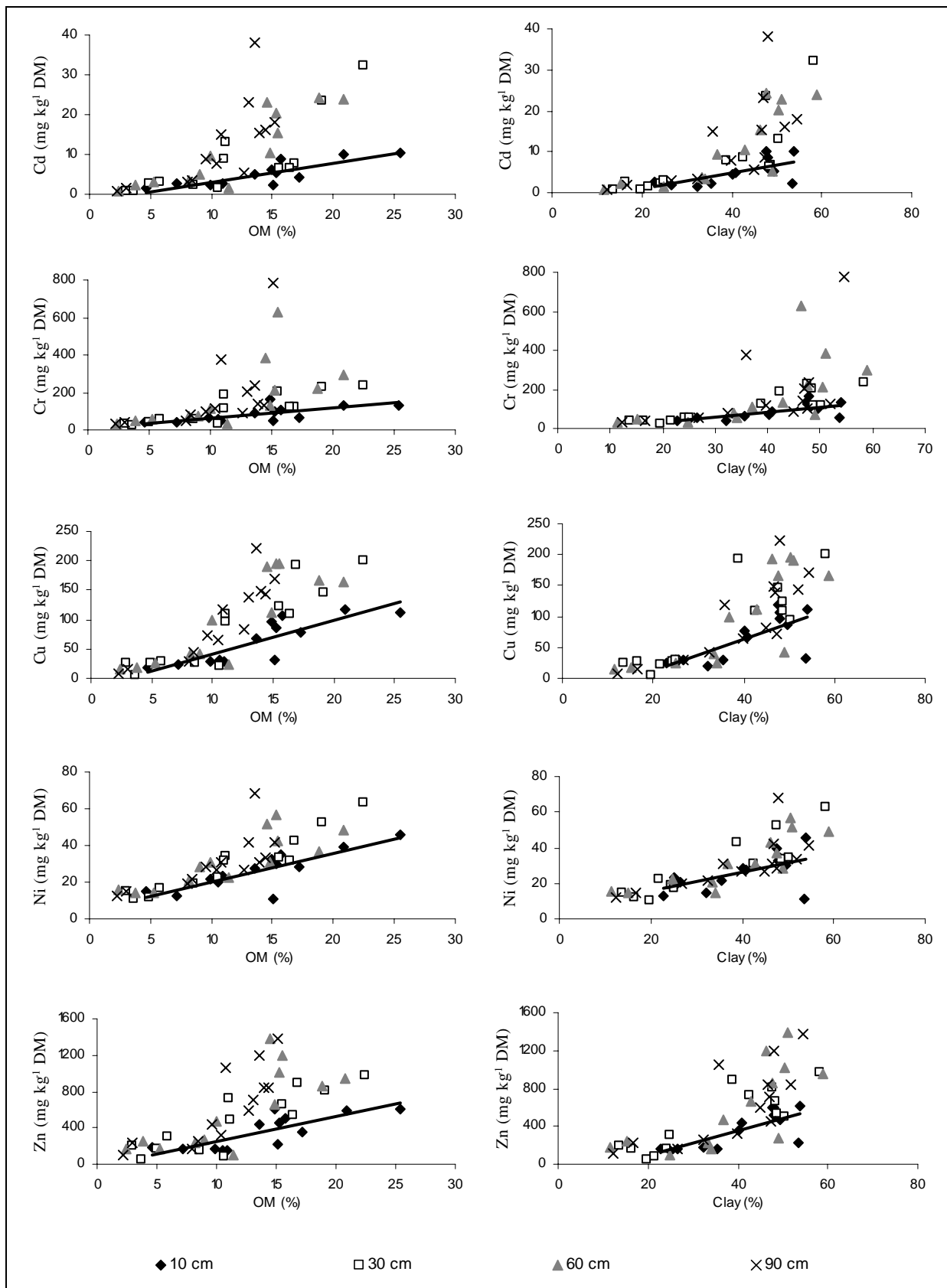
Fig. 9.4. Relationship between metal contents and organic matter and clay contents as a function of sampling depth. The line represents the linear regression between metal contents and organic matter or clay contents at the 10 cm sampling point.

At most of the sampling sites, the level of the water table fluctuated only slightly during the 6 measurements between October 2004 and March 2005 and in September 2005. The water level stayed above 30 cm below the sediment surface at Doel, Lillo, Sint-Annastrand, Galgenweel, Kruibeke, Bazel, Kijkverdriet, Bornem and Konkelschoor. It however dropped to 83 cm and 47 cm below the surface in Rupelmonde and Kramp, respectively. Moreover, the water table was found at higher depths in June at most of the sampling locations. The lowest level, 98 cm below the sediment surface, was measured at Rupelmonde, the only sampling site of which the sediments contained little or no sulphides. In contrast, the water table level remained high in June in Bornem and Kijkverdriet (less than 10 cm below the sediment surface), both sampling sites more elevated sulphides contents. At Kruibeke, Bazel and Konkelschoor, the water table was at only 12 to 35 cm below the surface in June, while it was at between 50 to 90 cm at the other sampling sites. The lowest water table levels in June were mainly observed in the more sandy sediments.

Only in Kijkverdriet and Kruibeke, significant Fe and Mn concentrations were found in the pore water at 10 cm below the sediment surface at all sampling times. Mn concentrations did not exceed 5 mg L^{-1}, whereas Fe concentrations did not exceed 25 mg L^{-1}. Significant Mn concentrations in the pore water of this sediment layer were also occasionally observed in Bornem and Bazel. As a result, the sediments of Kijkverdriet and Kruibeke can be considered as the most reduced ones, followed by Bornem and Bazel. These sites indeed also contained significant amounts of sulphides in their upper sediment layer (22-127 mg kg^{-1} DM). These were also all sampling locations at which the water table level always remained above 30 cm below the sediment surface during the entire experimental period. At 30 cm below the surface, Mn concentrations occasionally increased to above 3 mg L^{-1} at most sampling sites, which points at the beginning of Mn oxide reduction. They however remained low at Kramp, Galgenweel and Rupelmonde, where the sediments contained very low sulphide amounts (less than 5 mg kg^{-1} DM). Only at Kruibeke and Kijkverdriet, Mn concentrations did not vary much with sampling depth. They were continuously high at the depths of 60 and 90 cm at most sampling sites, whereas lower concentrations were reached occasionally at Kramp, Galgenweel and Rupelmonde. Fe concentrations at these depths were highest in January and lowest in June. Lowest concentrations were again found at Kramp, Galgenweel and Rupelmonde. The sediments at Kramp, Galgenweel and Rupelmonde thus appeared to be the most oxidised ones, whereas those of Kruibeke and Kijkverdriet were the most reduced, followed by Bornem and Bazel.

In October 2004 and September 2005, a significant linear correlation could be observed between sulphate and chloride concentrations at the sampling depths of 10 and 30 cm (Fig. 9.5). The highest chloride and sulphate concentrations were found at the more brackish sampling sites. In the upper sediment layer, sulphate and chloride concentrations thus reflect the decreasing salinity of the flooding water with increasing distance to the river mouth. At a depth of 60 cm, sulphate concentrations are often lower than the values which are predicted based on the chloride:sulphate ratio of the above sediment layer. These values are situated below the regression line in Fig. 9.5 and could be a result of increased sulphide formation due to increased sulphate reduction. Observations near or above the line are however also occasionally present and probably indicate no sulphide reduction or perhaps a net sulphide oxidation. These higher sulphate concentrations were indeed observed in the more oxidised sediments (e.g. Galgenweel and Rupelmonde) and also in other sandy sediments, subjected to more significantly fluctuating levels of the water table and containing less organic matter (e.g. Doel, Sint-Annastrand). As a result, oxygen can probably also penetrate deeper into these sediments. At the sampling depth of 90 cm, sulphate concentrations were generally much lower than predicted from chloride concentrations, which indeed points towards sulphide formation as a result of sulphate reduction, although higher sulphate concentrations were also observed occasionally.
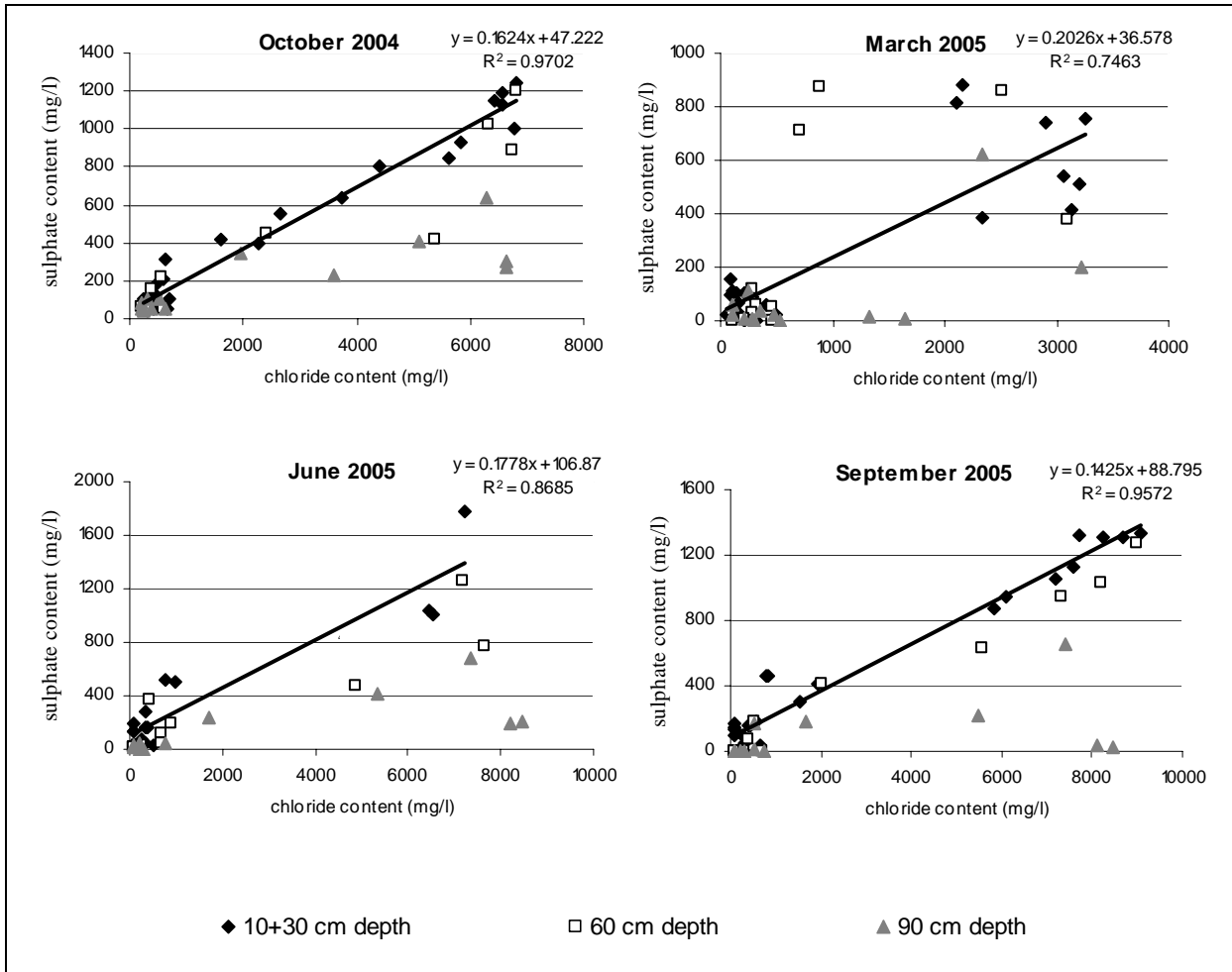
Fig. 9.5. Relationship between sulphate and chloride concentrations in the pore water at sampling depths of 10, 30, 60 and 90 cm and linear regression relationship between chloride and sulphate concentrations for the samples of 10 and 30 cm depth in October 2004, and March, June and September 2005

TOC and IC concentrations in the pore water increased with increasing sampling depth. The TOC concentrations were obviously higher in March 2005 at all sampling depths (Fig. 9.6), compared to October 2004 (Fig. 9.6), June 2005 and September 2005. Organic molecules originating from the decay of organic matter which is deposited on the sediments during fall and winter might leach towards the lower sediment layers between October and March. This leaching may be facilitated by lower temperatures in March, which slow down their decomposition. Higher temperatures and occasional lowering of the water table, might then again lead to ongoing decomposition and lower TOC concentrations in June.
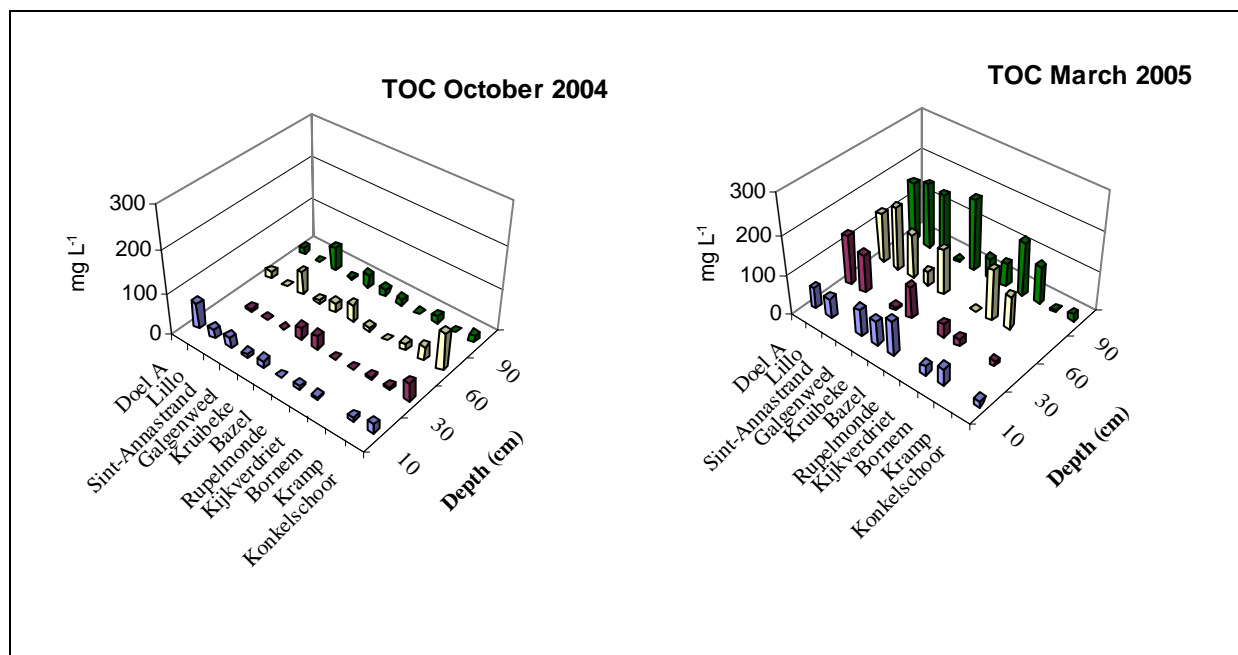
Fig. 9.6. TOC concentrations in the pore water (mg L^{-1}) as a function of sampling depth and location in October 2004 and March 2005

At all sampling depths, average Cd, Cr, Cu, Ni and Zn concentrations in the pore water did not differ significantly between the three Doel sampling sites (Doel A, Doel B and Doel C), situated at a short distance (10 m) from each other. Therefore, only the results of Doel A are discussed in the following paragraphs.

Cd concentrations only occasionally exceeded background values in October 2004 (Fig. 9.7). The number of exceedances was significantly higher in June 2005. The exceedances were obviously highest above the water table at the brackish sampling sites (Doel, Lillo, Sint-Annastrand and Galgenweel), although total Cd concentrations in the sediment were relatively low at these sampling points. This again confirms the role of salinity in Cd mobilisation in the absence of sulphides, as discussed in Chapter 4. Moreover, the peak concentrations coincided with those found in the greenhouse experiments of Chapter 8. Elevated concentrations were also found at a depth of 30 cm in Kramp and Rupelmonde, both freshwater sites at which the average water table level was situated at 40 cm below the sediment surface and where the water table level never went higher than 24 cm below the surface. Values for the sampling point at 10 cm below the sediment surface were missing at the brackish sampling sites due to the fact that the sediments were too dry to allow pore water sampling. However, lower concentrations were found at that sampling point in Kramp and Rupelmonde. The highest

concentrations were thus found just above the average water table level, coinciding with the results of greenhouse experiments as discussed in Chapter 8. Just below the sediment surface and above the average water table level, precipitation of Fe and Mn oxides might induce co-precipitation of Cd during the lower water table levels in June.
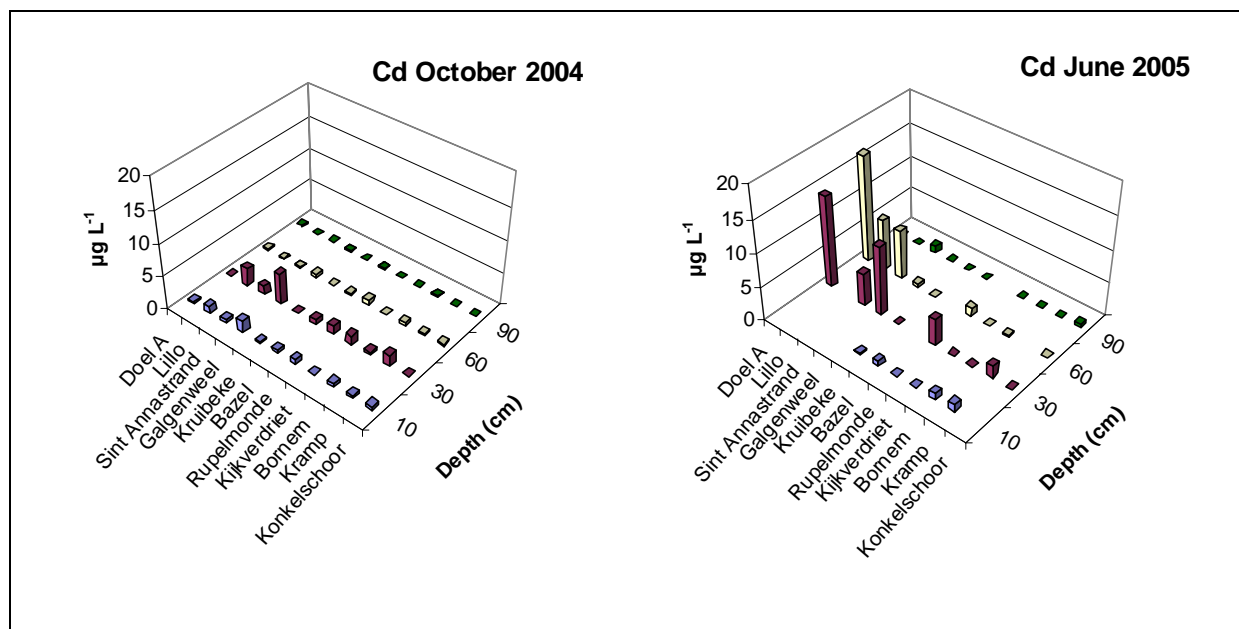

Fig. 9.7. Cd concentrations in the pore water (µg L^{-1}) as a function of sampling depth and location in October 2004 and June 2005

The Cr concentrations in the pore water were significantly higher at the highest sampling depths (60 and 90 cm below the sediment surface), compared to the depths of 10 and 30 cm below the sediment surface (Fig. 9.8). This should be attributed to the fact that Cr exists in a wide range of oxidation states and species, which all have a different precipitation behaviour and affinity for sorption to the soil matrix (Banks et al., 2006; Guo et al., 1997; Masscheleyn et al., 1992). According to Masscheleyn et al. (1992), Cr(VI) is known to be rapidly reduced to Cr (III) in the presence of organic matter and/or Fe(II). They however also reported Cr(III) to be oxidised to Cr(VI) in natural waters and soils, which might be catalysed by the presence of Fe and/or Mn oxides. Trivalent Cr might precipitate as Cr(OH)$_3$. The trivalent Cr in solution can however also form soluble complexes with organic components, which keeps Cr in solution. The role of these organic components in the mobilisation of Cr is confirmed by measuring the lowest concentrations in the deeper sediment layers of Rupelmonde and Galgenweel. These were indeed both sites at which the sediments contained very low TOC concentrations in the pore water also at the higher sampling depths. Moreover, Cr

concentrations in the pore water were significantly higher in March 2005, compared to the other sampling times. This can also be linked to the increased TOC concentrations (Fig. 9.6). The latter was attributed to the leaching of organic molecules originating from the decay of organic matter which is deposited on the sediments during fall and winter. Increased Cr mobility upon reduction at increased sampling depths below the water table was also observed in the greenhouse experimental trials of Chapter 8. However, average Cr concentrations in the pore water were higher under field conditions. This might be attributed to a continuous Cr supply by the tidal flooding water, the continuous supply of dissolved organic carbon or the release of complexing agents by wetland plants (Xu & Jaffe, 2006). The latter might facilitate the uptake of Cr by the plants, meanwhile increasing Cr mobility in soil (Banks et al., 2006).
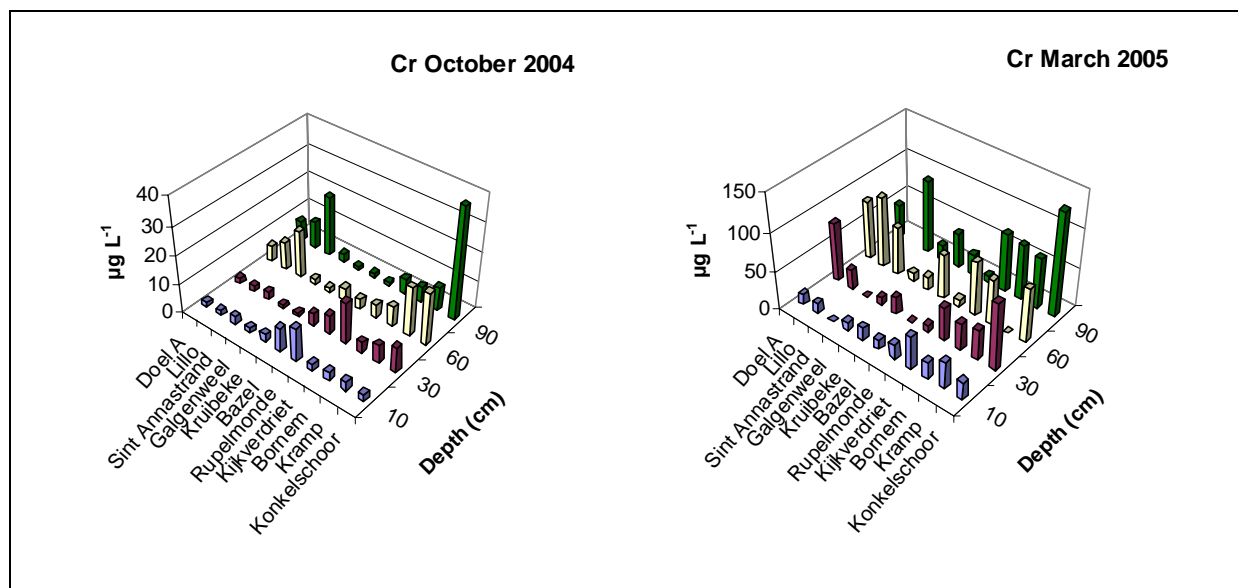

Fig. 9.8. Cr concentrations in the pore water (μg L^{-1}) as a function of sampling depth and location in October 2004 and March 2005

The Cu concentrations only exceeded sanitation thresholds at 60 and 90 cm below the sediment surface of the brackish sampling sites in March. This coincided with significantly higher TOC concentrations (Fig. 9.6), which was attributed to the leaching of organic molecules originating from the decay of organic matter, deposited on the sediments during fall and winter. Cu indeed is known for its affinity to form complexes with organic molecules (e.g. Alvim Ferraz & Lourenço, 2000; Kalbitz & Wennrich, 1998). Average Cu concentrations in the pore water however significantly (p = 0.000) decreased with increasing sampling depth, from 7.1 μg L^{-1} at 10 cm to 2.3 μg L^{-1} at 90 cm below the sediment surface. As a result, there is an inverse relation between Cu concentrations in the pore water and total

Cu concentrations in the sediment. The more oxidised state of the upper sediment layer hampers precipitation of Cu as Cu sulphide, leading to higher concentrations in the pore water. This is especially important for Cu, as Cu sulphide has a much lower solubility product compared to the other metal sulphides. At 10 cm below the sediment surface, the highest Cu concentration was observed at Kramp in October 2004. The clayey sediments were then subject to a water table level of about 40 cm below the sediment surface. This might have promoted sulphide oxidation at 10 cm below the sediment surface, which was not accompanied by rapid leaching towards higher or lower sediment layers as the sediments are rather clayey. Peak Cu concentrations coincided with those found in the greenhouse experiment of Chapter 8.

At 10 cm below the sediment surface, average Ni concentrations in the pore water did not differ significantly between the sampling locations. At 90 cm below the surface, they were significantly lower at the sites which contained significant sulphide amounts (Lillo, Kijkverdriet, Kruibeke, Bornem). The Ni concentrations did not decrease as much as a function of sampling depth as Cu. Ni is also expected to be released upon reduction of Fe and Mn oxides, as mentioned in the previous chapters, whereas the solubility product of Cu sulphide is also lower. The Ni concentrations in the pore water exceeded the sanitation threshold of 50 µg L^{-1} in June, both in Galgenweel at 60 and 90 cm and in Kramp at 30 cm below the sediment surface. The sediments at both sampling sites contained much clay, but were subjected to lowering of the water table in June. As a result, Ni is expected to be released upon oxidation of sulphides in June. The oxidation rate was probably higher in the more sandy sediments, as oxygen could penetrate more easily. Thus, at the more sandy sampling sites, Ni might have migrated already towards higher or lower sediment layers and subsequently might have been (co-)precipitated as oxides or sulphides between the samplings of March and June. In the same period, high concentrations in the pore water were still observed in the more clayey sediments. In March, average Ni concentrations in the pore water were significantly higher at all sampling depths, compared to the other sampling times, and sanitation thresholds were also regularly exceeded. As for Cu, this should be linked to the significantly higher TOC concentrations in March (Fig. 9.6). As for Cd and Cu, peak Ni concentrations also coincided with those found in the greenhouse experiment of Chapter 8.

The Zn concentrations in the pore water only got close to sanitation thresholds at the brackish sampling sites in September 2005 (Fig. 9.9) at a depth of 30 cm below the sediment surface,

whereas total Zn concentrations in the sediment were significantly lower at these sampling points. This again confirms the role of salinity in Zn mobilisation. Peak Zn concentrations at these sites were however somewhat higher compared to those found in the greenhouse experiment (Chapter 8). Whereas concentrations in the greenhouse did not exceed 300 µg L^{-1}, they went up to 500 µg L^{-1} in the field. As Zn is a relatively more soluble element in the environment, it is probably conveyed by the flooding water in relatively high amounts during each tidal cycle. Moreover, it has an essential function in the biosynthesis of a plant growth hormone, which is primarily active in the reed stems (Schierup & Larsen, 1981a). As a results, reed plants are expected to excrete substances which can solubilise these micronutrients (Xu & Jaffe, 2006), whereas Zn is also released by the plants during organic matter decay. As observed for Cd, mean Zn concentrations were also highest at a depth of 30 cm and lowest at 90 cm depth. At Kijkverdriet, Konkelschoor, Bazel and Kruibeke, the average Zn concentrations in the pore water were however highest at 10 cm below the sediment surface. These coincided with sites at which average water table level was situated at less than 12 cm below the sediment surface and at which the water table level never went lower than 20 cm below the sediment surface. As for Cd, the highest concentrations were thus found just above the average level of the water table. This also coincided with the results of greenhouse experiments (Chapter 8). Above that water table, precipitation of Fe and Mn oxides might induce co-precipitation of Zn, whereas sulphide precipitation increases with increasing depths below the water table.
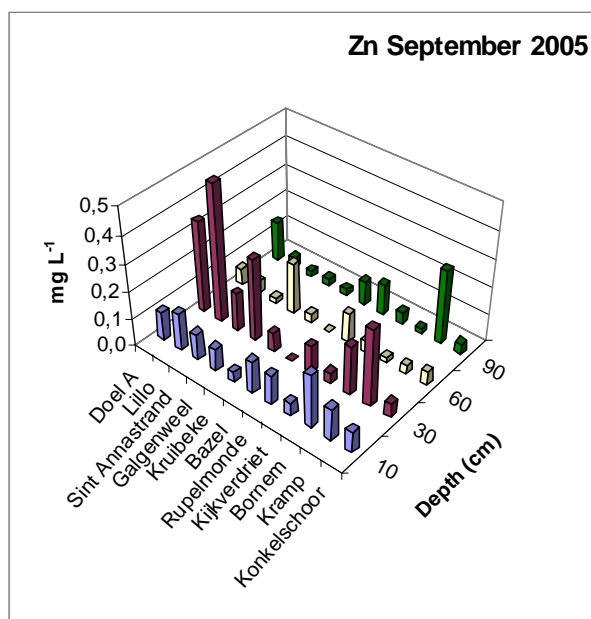

Fig. 9.9. Zn concentrations (mg L^{-1}) in the pore water as a function of sampling depth and location in September 2005

9.4 Conclusion

Metal mobility and availability in the field seemed to be affected by the factors which were identified in previous chapters to affect metal fate significantly. However, the effects were often less clear, as the resolution of measurements in the field was much lower compared to the greenhouse experiments due to sampling restrictions (e.g. samples could only be taken at low tides). As a result, some variations in metal mobility might not have been observed in the field. Moreover, several counteracting factors determine the ultimate metal fate in the field. The sediments were subjected to more moderate conditions in the field compared to the greenhouse. The temperatures in the field are e.g. not as high as under greenhouse conditions. These are expected to primarily affect the microbial reduction rates. In contrary, the sediments in the field are subject to more dynamic variations compared to the greenhouse, such as daily flooding. At most of the sampling sites, the level of the water table fluctuated only slightly during fall, winter and spring. However, the water table level significantly decreased during summer at most of the sites, especially those with more sandy sediments. As a result, the highest sulphide concentrations are found at the sites where the water table level never decreased significantly. These sulphides primarily determine Cd, Cu, Ni and Zn concentrations in the pore water. Organic complexation results in the mobilisation of Cu, Ni and Cr. Soluble organic molecules probably originated from the incomplete decomposition of organic matter at lower temperatures during the winter months. The concentrations of Cd, Ni and Zn in the pore water were moreover affected by Fe/Mn oxide reduction, whereas Cd and Zn concentrations are also affected by the salinity.

10.

General discussion, conclusion and future research perspectives

10 General discussion, conclusion and future research perspectives

10.1 *Factors affecting metal contents of the intertidal Scheldt sediments*

Intertidal Scheldt sediments are quite significantly contaminated with trace metals. The Cd concentrations often exceed the Vlarebo remediation values for nature areas, whereas the sediments are moderately contaminated by Cr, Cu and Zn. Pb concentrations occasionally are high. Ni concentrations however lean towards background values.

Significant correlations were observed between metals, organic matter and clay contents of the superficial layer in intertidal Scheldt sediments. So metal concentrations can be predicted from clay or organic matter contents in this sediment layer. Metal concentrations were somewhat higher then predicted at sites within a range of a few km from specific point-sources of metals (shipyards, industrial areas, major motorways). They were lower than predicted at sites which are regularly subjected to flooding by high salinity water.

Most variations of sediment properties in intertidal zones could be attributed to the sampling location. The sampling location primarily determines texture and chloride, carbonate and organic matter contents, which in turn affect metal accumulation. Metal accumulation, which is primarily due to association of metals with organic matter and clay in the surface sediment layer, seems to be supplemented by an accumulation as sulphides at higher sediment depths. The depth at which sulphide precipitation significantly contributes to metal accumulation depended on the sampling location, and varied from a few centimetres in clayey, organic sediments to more than 1 m in sandy sediments. Temporal variations of Cu, Cd, Pb and Zn concentrations can only be linked to newly formed sulphides or sulphide oxidation at the sites with the lowest sulphide contents. At sampling sites containing high sulphide amounts, variations should be primarily attributed to metal exchange and the presence of mobile metal complexes. Litter decomposition at the end of the growing season could hereby play a significant role.

10.2 Effect of salinity on metal mobility and availability

Increasing salinity is known to reduce bioavailability and toxicity towards aquatic organisms of metals that are present in the water phase. Salinity however will enhance Cd mobilisation from the intertidal Scheldt sediments towards the water phase. Cd concentrations in pore water of sediments and surrounding surface waters significantly exceeded sanitation thresholds and quality standards during flooding of initially oxidized sediments. The effects were observed already at lower salinities of 0.5 g NaCl L^{-1}. This implies that risks related to Cd leaching to ground water and Cd uptake by organisms would be created when constructing flooding areas in the metal-polluted zones between Temse and the Scheldt river mouth. These risks can be reduced by inducing sulphide precipitation because Cd is then immobilised as sulphide and not anymore reacting to changes in salinity. This could be achieved by permanently flooding the polluted sediments, because sulphates are sufficiently available in the river water of the brackish part of the estuary. Microbial sulphate reduction is not expected to be limited by organic matter availability as biomass turnover is very high in intertidal reedbeds. Stimulation of sulphide formation should however be done with great caution as accidental oxidation of the sulphide-rich sediments could also create a significant risk for metal mobilisation, which is facilitated by increasing salinity. Thus, a "chemical time bomb" might indeed be created. Especially in the freshwater sediments and soils, low sulphate availabilities might be limiting for sulphide formation.

10.3 Effect of litter decomposition on metal mobility and availability

As a result of the decay of plants with high biomass production rates (especially reed and willow), a significant amount of litter is supplied to the upper intertidal sediment layer at the end of the growing season. During the first weeks after litter supply, Cd, Cu, Ni and Zn concentrations in pore water significantly exceeded sanitation thresholds for ground water. The Ni concentrations however also significantly exceeded its sanitation threshold during a longer period of time upon addition of any type and amount of organic matter, whereas this was also the case for Zn upon addition of reed stem material. Willow leaf material is rapidly decomposed, which results in high Fe and Mn oxide reduction rates, subsequently resulting in high metal concentrations in the pore water. Upon addition of willow leaves, also large

amounts of sulphates seem to be released, which induced the formation of sulphides and precipitation of metal sulphides. As sufficient sulphide amounts are produced, all metal concentrations in the pore water can be reduced to acceptable concentrations within the first weeks. Metal release also increased with increasing added amounts of reed stems. However, lower sulphates amounts were released. As a result, insufficient amounts of sulphides can be produced to precipitate all metals and reduce Ni, Zn, Fe and Mn concentrations to within acceptable limits in a reasonable amount of time. This in turn seems to reduce sulphate reduction rates. As such, a vicious circle seems to be produced which is characterised by only slowly decreasing Zn, Ni, Fe and Mn concentrations, when high amounts of low-sulphate releasing reed stem material are added to a permanently flooded soil. When the soils are alternately flooded, metals are exported through the percolates. Total metal export by the percolates significantly increases when organic matter is added. The export is concentrated in the first weeks after organic matter addition. As a result, concentrations of most metals in the pore water are more rapidly reduced to within acceptable limits compared to permanently flooded soils. About five months after flooding the initially oxidised soil, both metal concentrations and growth of duckweed, reed plants and willows still seem to be affected by organic matter addition, whereas photosynthesis is less affected. Metal concentrations of duckweed are affected by metal concentrations in the surface water which is in close contact with the sediments. These in turn seem to be affected by Fe/Mn oxide reduction and sulphide precipitation. Metal concentrations of reed leaves and stems seem to be regulated by the plants, whereas metal translocation from the cuttings seems to affect the concentrations in willow leaves. Plant biomass production is expected to be primarily affected by changing nutrient availability upon organic matter addition, whereas peak concentrations of metals which are already toxic at low concentrations, such as Cd, also might induce sublethal plant stress.

Taking into account all effects mentioned above, it could be concluded that the dominating plant species in intertidal wetlands or floodplains can affect the metal fate upon decomposition at the end of the growing season. Willow leaves seem to immobilise metals more significantly upon decomposition compared to reed leaves and stems. To confirm this, the factors affecting sulphur uptake and sulphate release by these plant species should however still be identified. As decomposing reed plant material seems to mobilise metals, harvesting reed plants at the end of the growing season in metal-polluted zones within newly

created wetland areas indirectly might lead to a reduction of the distribution of pollutants within the wetland.

During decomposition under field conditions, most metal contents in reed litter increased considerably. As reed biomass turnover is also very high, metal accumulation by litter could be a very important parameter to monitor and take into account when discussing metal transfer at the base of the food chain in intertidal reed beds. There are indications that fungal activity is an important factor determining metal accumulation in decomposing stem tissue. This could also be the case for leaf blades, but for this tissue type the effect of fungal activity on metal concentrations is found to be overruled by passive metal sorption and trapping of sediment particles and associated metals. Both factors are of intermediate importance for leaf sheaths.

10.4 Effect of the hydrological regime on metal mobility

The hydrological regime to which a metal-polluted overbank sedimentation zone is subjected has a pronounced influence on the metal concentration in the pore water of the upper soil layer. Flooding conditions lead to an increase of the Fe, Mn, Ni and Cr mobility and a decrease in the Cd, Cu and Zn mobility in the upper layer of a calcareous soil. Keeping the soil at field capacity resulted in a low pore water concentration of Fe, Mn and Ni while the Cd, Cu, Cr and Zn pore water concentrations increased. Alternating hydrological conditions resulted in fluctuating metal concentrations in the pore water. When the flooding lasts for a maximum of two days and is followed by a longer emerged period or when the soil is kept continuously on field capacity, release of most metals appears primarily related to Ca release. The metals could be mobilised as a result of Ca release, or they might be subject to the same factors which affect Ca release, such as the dissolution of carbonates. In conditions that allow sufficiently strong reducing conditions to be established, there is evidence that the mobility of Cd, Cu, and to a lesser extent Zn, is primarily affected by formation and re-oxidation of sulphides, whereas Ni would mainly be mobilised as a result of Fe/Mn oxide reduction.

10.5 Factors affecting metal uptake by reed plants

Concentrations of metals in reed plants are low. They tend to be higher in the leaves than in the stems, with the exception of Zn. Only Zn is primarily accumulated in the stems, which might be attributed to its role in the biosynthesis of a plant growth hormone. Uptake of metals by reed plants and their transfer to the stems seems to be affected by clay and organic matter contents in the sediment. The higher the clay and organic matter contents, the lower the uptake and transfer to the aboveground plant parts. This might be attributed to a higher sorption capacity, which reduces the metal availability. Additionally, high clay and organic matter contents promote the creation of more reduced conditions and subsequent sulphide precipitation, which reduces the metal availability at lower sampling depths. Moreover, active metal release from the sediment and increased metal uptake might be promoted by the plants at lower total metal concentrations in the sediment, which are related to lower clay and organic matter contents. Next to the effect of clay and organic matter, salinity enhances the uptake of some metals, such as Cr, Ni and Zn.

10.6 Effect of water table level on metal availability

Nickel and Cr release are facilitated by reductive conditions, and were slightly promoted by increasing salinities. These reducing conditions can occur below the water table, or above the water table, but at a sufficiently high sampling depth (more than 30 cm below the sediment surface). Cr is however also mobilised under more oxic conditions, as it can also form complexes with organic substances. Cu, Zn and especially Cd are all primarily released above the water table under high salinity conditions. Cd, Cu and Zn are also released below or just above the water table when organic matter is being decomposed, resulting in Ca, Mn, Ni and/or Fe release upon CO_2 accumulation and Fe and Mn oxide reduction, without being accompanied by sulphide production. Under greenhouse conditions, it takes about 2 months before the Flemish soil sanitation threshold levels for groundwater are reached for Ni or Cd concentrations in the pore water, upon the creation of waterlogged or emerged conditions, respectively. However, the total Ni concentrations in the sediments did not exceed any soil sanitation threshold for the solid soil fraction. Slight water level fluctuations on short term might thus result in metals becoming locally available just above or below the water table.

These fluctuations are not expected to create a significant overall metal mobilisation, but they might affect plant uptake.

10.7 Field validation

Overall, the same factors could be shown to affect metal fate in field conditions. However, the effects were often less clear, as the resolution of measurements in the field was much lower compared to the greenhouse experiments due to sampling restrictions (e.g. samples could only be taken at low tides). As a result, some variations in metal mobility might not have been observed in the field. Moreover, several counteracting factors determine the ultimate metal fate in the field. The sediments and soils were subjected to more moderate conditions in the field compared to the greenhouse. The temperatures in the field are e.g. not as high as under greenhouse conditions. These are expected to primarily affect the microbial reduction rates. In contrary, the sediments in the field are subject to more dynamic variations compared to the greenhouse, such as daily flooding. At most of the sampling sites, the level of the water table fluctuated only slightly during fall, winter and spring. However, the water table level significantly decreased during summer at most of the sites, especially those with more sandy sediments. As a result, the highest sulphide concentrations are found at the sites where the water table level never decreased significantly. These sulphides primarily determine Cd, Cu, Ni and Zn concentrations in the pore water. Organic complexation results in the mobilisation of Cu, Ni and Cr. Soluble organic molecules probably originated from the incomplete decomposition of organic matter at lower temperatures during the winter months. The concentrations of Cd, Ni and Zn in the pore water were moreover affected by Fe/Mn oxide reduction, whereas Cd and Zn concentrations are also affected by the salinity.

10.8 Future research perspectives

The following sections present some perspectives for future research based on the current experiences and findings.

1. Kinetics of metal mobility changes should be further studied (e.g. effects of temperature) and incorporated in existing geochemical models, which are currently

based on steady-state conditions. As a final result, models should be constructed which are able to predict the real-time speciation and mobility of metals in soils and sediments under rapidly alternating hydrological conditions (e.g. at the oxic-anoxic interface). Also other elements should be studied thoroughly, as they could expose a different behaviour (e.g. As).

2. During the greenhouse experimental trials and the field monitoring, cations and chlorides were found to significantly affect the mobility and availability of metals (especially Cd). This might result in risks related to metal mobilisation during the construction of floodplains in brackish parts of the estuary. However, the brackish estuarine waters also contain sulphates, which might in turn reduce the metal mobility as a result of their reduction to sulphides in the wetland soils. The contribution of both counteracting factors to the ultimate metal mobility should be identified more extensively under different environmental circumstances in different parts of the estuary.

3. Assessment of the relationship between soil physical properties and chemical properties under conditions of alternating hydrological conditions. Emersion after submersion leads to aeration, which subsequently induces the oxidation of sulphides. The way the emersion affects the aeration depends on e.g. the physicochemical soil properties and the duration of emersion and submersion periods, which should be quantified.

4. The depth at which sulphate reduction occurs in the upper sediment layer depends on the flooding regime and water table fluctuations. A more profound monitoring of water table levels seems necessary. Models should be constructed which reveal the relationship between the flooding regime and water table level in different types of wetland soils. This should enable us to translate metal pollution control to concrete management procedures which can easily be applied in the field.

5. Metals are expected to be released to an important extent upon complete decalcification, whereas Van den Bergh and Loch (2000) already reported intense decalcification at tidally influenced freshwater wetland areas in The Netherlands. This decalcification seems to be promoted by alternating hydrological conditions, but could

also be affected by future sea level rises. Decalcification under changing hydrological conditions should therefore be further studied. Models could be constructed which are able to predict carbonate formation and deposition on the one hand and decalcification rates on the other hand in wetland sediments which are subject to changing hydrological conditions.

6. Some techniques could be applied to reduce the risks associated with the high metal contents of intertidal and floodplain soils and sediments, such as:

   a. Adding carbonates where nearly complete decalcification is observed;

   b. Capping sediments to hamper sulphide oxidation. This could be done e.g. by covering them with non-polluted sediment or soil layers;

   c. Supplying sulphates to the upper sediment layers during the first weeks of litter decay. This might lead to a more rapid reduction of elevated metal concentrations in the pore water, as a result of faster sulphide formation. Factors affecting sulphur concentrations in different plants and sulphate release from decomposing plant material and the role of atmospheric sulphate deposition could be assessed, as well as the feasibility of supplying sulphates by supplying organic matter containing large amounts of sulphur;

   d. Keeping the water level and sulphate contents in the flooding water permanently high in metal-polluted zones within newly created wetland areas in the brackish or saline zone of the estuary;

   e. Harvesting reed plants at the end of the growing season in metal-polluted zones within newly created wetland areas, as it might lead to a reduction of the pollutant distribution within the wetland.

The effect of such interventions on metal mobility, bioavailability and toxicity should however first be extensively studied in the laboratory and the field.

7. Due to ongoing purification of wastewaters, the oxygen conditions of surface waters are gradually improving. This might however result in a gradual or sudden metal release from the sediments, as a result of the oxidation of sulphides. The effects of wastewater purification and improving oxygen conditions in the surface water on metal fate in intertidal and bottom sediments should be further studied. To predict metal fate under such conditions, one should e.g. be able to discriminate between oxygen consumption due to the decomposition of naturally occurring organic matter

on the one hand and organic matter which originates from sewage discharges on the other hand. Moreover, oxygen diffusion and metal fluxes between the sediments and the surrounding surface waters and between the anoxic and oxic sediment layers should be modelled for different types of sediments which are contacted with surface waters of variable composition under different hydrological regimes.

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SUMMARY

In this study, we aimed to assess the pollution status of recent sediment deposits in the intertidal reedbeds along the banks of the river Scheldt, to be able to predict potential metal accumulation in controlled flooding areas and new wetlands. Moreover, we aimed to identify the most important factors that affect metal accumulation and mobility in these marshes. This can be used for the development of management oriented models for predicting the metal fate, which should allow to estimate environmental risks related to metal accumulation in sediments and floodplains of the river Scheldt, considering that the removal and/or cleaning of all contaminated sediments is not feasible.

The superficial intertidal sediment layer of the river Scheldt is significantly contaminated with trace metals. The Cd concentrations often exceed the Vlarebo remediation values for nature areas, whereas the sediments are moderately contaminated by Cr, Cu and Zn. Lead concentrations occasionally are high. Nickel concentrations however lean towards background values. Significant positive correlations were observed between metals, organic matter and clay concentrations in the superficial layer of intertidal Scheldt sediments. So metal concentrations can be predicted from clay or organic matter concentrations in this sediment layer. Metal concentrations were somewhat higher then predicted at sites within a range of a few km from specific point-sources of metals (shipyards, industrial areas with metallurgic activities, major motorways), whereas they were lower than predicted at sites which are regularly subjected to flooding by high salinity water. In the deeper sediment layers, sulphides seem to play an important role in the metal accumulation, next to organic matter and clay.

An increased salinity increased the transfer of metals, especially Cd, from the intertidal sediments towards the water phase. The Cd concentrations in the pore water of soils, sediments and surrounding surface waters exceeded the sanitation thresholds and quality standards upon flooding of initially oxidised soils with brackish water. In the presence of significant sulphide amounts, Cd however stays immobile and is not affected anymore by salinity changes.

As a result of the decomposition of reed plants and willow leaves at the end of the growing season, significant amounts of organic matter are supplied to the upper sediment layer. During the first weeks after organic matter supply to some intertidal sediments under greenhouse conditions, Cd, Cu, Ni and Zn concentrations in pore water of the sediments were found to significantly exceed sanitation thresholds for ground water. Upon addition of organic matter, also sulphates were however released, which induced the formation of sulphides and precipitation of metal sulphides. As sufficient sulphide amounts were produced when willow leaves were added, all metal concentrations in the pore water could be rapidly reduced to acceptable concentrations. Lower sulphates amounts were released when reed stems were added. As a result, insufficient amounts of sulphides were produced to precipitate all metals and reduce Ni, Zn, Fe and Mn concentrations to within acceptable limits in a reasonable amount of time. When the soils were alternately flooded, the metals were exported through the percolates. Total metal export by the percolates significantly increased when organic matter was added. The export was concentrated in the first weeks after organic matter addition.

Most metal concentrations in reed litter itself increased considerably during decomposition under field conditions. Fungal activity may be important in immobilizing metals in decomposing stem tissue. This could also be the case for leaf blades, but for this tissue type, the effect of fungal activity on metal concentrations is found to be overridden by the passive metal sorption and trapping of sediment particles and associated metals. Both factors seem to be of intermediate importance for leaf sheaths. Also under field conditions, organic complexation was found to contribute to the mobilisation of Cu, Ni and Cr in the sediment. Soluble organic molecules probably originated from the incomplete decomposition of organic matter at lower temperatures during the winter months.

The hydrological regime to which a metal-polluted overbank sedimentation zone is subjected was found to have a pronounced influence on the metal concentration in the pore water of the upper soil layer. Flooding conditions lead to an increase of the Fe, Mn, Ni and Cr mobility and a decrease in the Cd, Cu and Zn mobility in the upper soil layer of a calcareous soil or sediment. Alternating hydrological conditions resulted in fluctuating metal concentrations in the pore water. Reduction and oxidation of Fe and Mn, breakdown of carbonates and the formation and re-oxidation of sulphides were the main controlling processes. Slight fluctuations of the groundwater table level can result in a locally elevated metal availability

just above or below the groundwater table. This however is not expected to result in an overall metal mobilisation. On most of the intertidal marshes, the level of the groundwater table fluctuated only slightly during fall, winter and spring. It however sharply dropped to higher sediment depths during summer, especially on marshes with sandy sediments. This could result in the oxidation of metal sulphides and subsequent release of metals.

Concentrations of metals in reed plants are low. They tend to be higher in the leaves than in the stems, with the exception of Zn. Uptake of metals by reed plants and their transfer to the stems seems to be affected by clay and organic matter contents of the marsh sediments. The higher the clay and organic matter contents, the lower the uptake and transfer to the aboveground plant parts. Next to the effect of clay and organic matter, salinity enhances the uptake of some metals, such as Cr, Ni and Zn.

SAMENVATTING

In deze studie was het de bedoeling om de verontreinigingsgraad van recente sedimentafzettingen in de rietvelden langs de oevers van de Schelde in te schatten, dit om de voorspelling van potentiële metaalaccumulatie in gecontroleerde overstromingsgebieden en nieuwe moerasgebieden mogelijk te maken. Het was tevens de bedoeling de belangrijkste factoren die metaalaccumulatie en –mobiliteit in deze intergetijdenzones beïnvloeden te identificeren en bij te dragen tot de ontwikkeling van beleidsgerichte modellen die als doel hebben het metaalgedrag te voorspellen. Dit zou ons moeten toelaten de milieurisico's gerelateerd met metaalaccumulatie in oeverzones van de Schelde te reduceren, zonder alle verontreinigde sedimenten te moeten verwijderen en saneren.

De bovenste laag van de intertidale Scheldesedimenten bleek vrij sterk met metalen verontreinigd te zijn. De Cd-gehaltes overschrijden dikwijls de saneringswaarden die opgelegd zijn aan natuurgebieden, terwijl de sedimenten matig verontreinigd zijn met Cr, Cu en Zn. De Pb-gehaltes zijn tevens vrij hoog op enkele locaties. De Ni-gehaltes benaderen echter de achtergrondwaarden. Significante correlaties werden waargenomen tussen metaalgehaltes en gehaltes aan organisch materiaal en klei in de bovenste sedimentlaag. Als dusdanig kunnen metaalgehaltes voorspeld worden gebruik makend van de gehaltes aan klei en organisch materiaal. De metaalgehaltes waren iets hoger dan voorspeld op locaties binnen enkele kilometers van specifieke puntbronnen (scheepswerven, industriegebieden, belangrijke autowegen). Ze waren lager dan voorspeld in sedimenten die regelmatig overstroomd worden door zoutrijk water. In diepere sedimentlagen speelden, naast de gehaltes aan organisch materiaal en klei, sulfiden een belangrijke rol in de metaalaccumulatie.

Een toenemende saliniteit verhoogt de transfer van metalen, in het bijzonder van Cd, van de intertidale sedimenten naar de waterige fase. De Cd-gehaltes in het poriënwater van bodems, sedimenten en omliggende oppervlaktewaters overtroffen de saneringsgrenzen en kwaliteitsstandaarden bij overstroming van initieel geoxideerde gronden met brak water. In aanwezigheid van voldoende sulfiden blijft Cd echter immobiel en reageert het niet meer op een wijziging van de saliniteit.

Ten gevolge van het afsterven van planten met een erg hoge biomassaproductie (in het bijzonder rietplanten en wilgenbladeren) worden significante hoeveelheden strooisel toegevoegd aan de bovenste sedimentlagen op het einde van het groeiseizoen. Gedurende de eerstvolgende weken na toediening van organisch materiaal afkomstig van rietbladeren, wilgenbladeren en rietstengels aan enkele sedimenten onder serrecondities namen de gehaltes aan Cd, Cu, Ni en Zn in het poriënwater van de bovenste sedimentlaag sterk toe. Uit dit organisch materiaal werden echter tevens sulfaten vrijgesteld, die op termijn deze vrijstelling teniet kunnen doen door sulfiden te vormen, die met de metalen neerslaan. Gezien bij de toediening van wilgenbladeren voldoende sulfidenhoeveelheden geproduceerd werden, werden alle metaalconcentraties in het poriënwater snel gereduceerd tot aanvaardbare concentraties. Kleinere sulfaathoeveelheden werden vrijgesteld bij toediening van organisch materiaal afkomstig van rietstengels. De hoeveelheid gevormde sulfiden leek onvoldoende om alle metalen neer te slaan en Ni, Zn, Fe en Mn concentraties te reduceren tot binnen aanvaardbare grenzen in een redelijke tijdspanne. Wanneer de bodem alternerend overstroomd werd, werden de metalen geëxporteerd via het percolaat. De totale export nam significant toe wanneer organisch materiaal toegevoegd werd. Ze was geconcentreerd tijdens de eerste weken na toediening van organisch materiaal.

De concentraties van de meeste metalen in rietstrooisel namen sterk toe bij afbraak onder veldomstandigheden. Er waren aanwijzingen dat fungale activiteit in belangrijke mate de metaalaccumulatie in ontbindende rietstengels beïnvloedde. Passieve sorptie van metalen en sedimentpartikels leek echter belangrijker voor metaalaccumulatie in de rietbladeren die aan decompositie onderhevig waren. Tevens werd onder veldomstandigheden vastgesteld dat organische complexatie resulteert in de mobilisatie van Cu, Ni en Cr in het sediment. Oplosbare organische moleculen waren vermoedelijk afkomstig van de onvolledige afbraak van organisch materiaal bij lagere temperaturen tijdens de wintermaanden.

Het hydrologische regime waaraan een metaalverontreinigde grond onderworpen wordt, beïnvloedt de metaalconcentraties in het poriënwater van de bovenste bodemlaag in belangrijke mate. Overstroming leidde tot een verhoogde mobiliteit van Fe, Mn, Ni en Cr en een verlaagde mobiliteit van Cd, Cu en Zn in een kalkrijke bodem. Afwisselende hydrologische condities leidden tot fluctuerende metaalconcentraties in het poriënwater. Reductie en oxidatie van Fe en Mn, afbraak van carbonaten en de vorming en heroxidatie van sulfiden waren hierbij de belangrijkste sturende processen. Lichte fluctuaties van het niveau

van de watertafel kunnen resulteren in een lokale verhoging van de metaalbeschikbaarheid net boven of onder de watertafel. Deze worden echter niet verwacht aanleiding te geven tot een algehele metaalmobilisatie. Op de meeste schorren leek de stand van de grondwatertafel tevens slechts licht te variëren tijdens de herfst, winter en lente. Deze daalde echter significant tijdens de zomer, in het bijzonder op de schorren met zandige sedimenten, wat aanleiding kan geven tot de oxidatie van metaalsulfiden en de vrijstelling van metalen.

De metaalconcentraties in de rietplanten waren laag. Ze bleken in de bladeren echter hoger te zijn dan in de stengels, met uitzondering van Zn. Opname van metalen door rietplanten en hun transfer naar de stengels bleek beïnvloed te worden door de gehaltes aan klei en organisch materiaal in het sediment. Hoe hoger deze gehaltes, hoe lager de opname en transfer naar bovengrondse plantendelen. Naast klei en organisch materiaal beïnvloedde het zoutgehalte tevens de metaalopname voor een aantal elementen, zoals Cr, Ni en Zn.

Curriculum Vitae

Gijs Du Laing (1977) graduated in 2000 as Bio-engineer with a specialization in Chemistry at Ghent University. In the final year of his studies, he was awarded by the price of the Covenant of Graduated Bio-engineers Ghent. Subsequently, he started his PhD work on "Dynamics of heavy metals in reed beds along the banks of the river Scheldt" in 2000, first as a PhD grant holder of the BOF research fund of Ghent University, afterwards as a member of the assisting academic personnel of Ghent University. The research was conducted at the Laboratory of Analytical Chemistry and Applied Ecochemistry (Department of Applied Analytical and Physical Chemistry, Faculty of Bioscience Engineering, Ghent University). In 2006, he also acquired an Environmental Coordinator (Type A) Certificate at Ghent University.

During the six year period of his PhD research, he actively guided about 15 students to a successful conclusion of their master thesis. He also participated in 11 international conferences and co-founded the International Symposium on Wetland Pollutant Dynamics (WETPOL). The latter is an international symposium dedicated to the issues of human caused pollution in wetlands, and to the importance of wetlands in controlling that pollution. The first edition took place in September 2005 and attracted 165 participants from over 40 different countries. He already acted as a (co-)author of 11 publications in peer reviewed international journals (A1) and as a guest editor of a special issue of Science of the Total Environment.

As a member of the assisting academic personnel, he assisted in the training sessions of several courses, such as Analytical Inorganic Chemistry, Environmental Chemistry, Soil Chemistry, Soil Pollution and Soil Protection. Moreover, he spent a significant amount of time by providing internal and external departmental services. As a member of the Environment, Safety and Hygiene Committee, he also coordinated the waste management at the Faculty of Bioscience Engineering (Ghent University).

Gijs Du Laing is the author or co-author of the following publications.

Publications in international peer reviewed journals (A1)

Vandecasteele, B., **Du Laing, G.** & Tack, F.M.G. (2007). Effect of submergence-emergence sequence and organic matter or aluminosilicate amendment on metal uptake by woody wetland plant species from contaminated sediments. Environmental Pollution, 145, 329-338.

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