Faculteit Wetenschappen
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The biogeochemical cycle of metals in natural freshwater tidal marshes and in flood control areas

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De biogeochemische cyclus van metalen in natuurlijke zoetwaterschorren en gecontroleerde overstromingsgebieden

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General introduction

1.1. Metal contamination

Metals are natural constituents of the earth's crust. In biogeochemical cycles these elements are exchanged between different biotic and abiotic (soil, air, water) compartments. Most metals play an essential role in certain parts of the metabolic systems of living organisms. However, metals are often needed only in very small amounts and elevated concentrations can result in toxicity and adverse effects (Luoma and Rainbow, 2008; Weis et al., 2011). Widespread mining and use greatly modified the global metal distribution. The cumulative industrial release of metals in the environment is enormous and pervasive and largely overwhelms natural geochemical cycles (Nriagu, 1996). This has resulted in significant metal contamination of many ecosystems and resources and an accelerating accumulation of metals in the natural as well as the human food chain (Nriagu and Pacyna, 1988). Although several adverse effects have been known for a long time, exposure to metals continues, and is even increasing in some parts of the world (Järup, 2003; Luoma and Rainbow, 2008).

Large estuaries are often found to be 'hotspots' for pollution. As transitional zones between terrestrial and marine waters they concentrate the runoff from large land surfaces. Additionally, areas surrounding estuaries are often densely populated or accommodate industry and harbor activities. Metal contamination has been reported in estuaries from all over the world (Abrahim and Parker, 2008; Church et al., 2006; Overesch et al., 2007; Pan and Wang, 2012; Pope and Langston, 2011; Reis et al., 2009).

The Schelde estuary (Belgium) is a typical example of an estuarine habitat with elevated metal concentrations in surface water, suspended solids, sediments and embanked areas (Baeyens et al., 2005; Du Laing et al., 2007a; Regnier and Wollast, 1993; Vandecasteele et al., 2002a; Vandecasteele et al., 2003a). The flourishing mining activities and huge nonferrous industries in Northern France (*Metaleurop*), metal processing and other industries around Ghent and Antwerp (including *Metallurgy Hoboken*) and waste water discharged from large agglomerations (Lille, Antwerp, Brussels) resulted in high metal emission into the Schelde river and estuary (Gao et al., 2012). The fast increase in metal concentrations was initiated in the beginning of the 20th century with a pollution maximum between 1960 and 1980 (Zwolsman et al., 1993). After 1980, atmospheric and aquatic emission as well as metal concentrations in the surface water drastically decreased, which is mainly attributed to emission reductions of the industry and the implementation of waste water treatment plants (Gao et al., 2012). However, tons of toxic metals are stored in the estuarine sediments of the Schelde as a legacy of past contamination (Baeyens et al., 2005). Estuaries often act as a sink for suspended solids and associated trace metals (Millward and Glegg, 1997; Monbet, 2006; Rozan and Benoit, 2001; Walling and Owens, 2003). Also in the Schelde estuary, it was calculated that up to 90% of the trace metal influx is stored within the estuary (Baeyens et al., 1997a; Baeyens et al., 2005; De Smedt et al., 1997). Elevated metal concentrations were reported in sediments from subtidal (Bouezmarni and Wollast, 2005) and intertidal areas (Du Laing et al., 2007a). In addition, disposal of contaminated dredged sediments resulted in contamination of alluvial plains (Vandecasteele et al., 2002a; Vandecasteele et al., 2003a). Sediments from the most upstream, freshwater stretch of the estuary are most contaminated since the major metal influx originates from upstream pollution (Northern France) and because contaminated riverine particles are diluted with less contaminated marine water and suspended solids in the brackish and marine part of the estuary (Gao et al., 2012; Regnier and Wollast, 1993). Despite the observed decrease in surface water metal concentrations during the last three decades, a release or increasing availability of these sediment-bound metals during disturbance events or changing environmental conditions can be of concern (Eggleton and Thomas, 2004). Anthropogenic (e.g., dredging, waste water treatment, embankment) or natural (e.g., bioturbation, plant growth, storms) events can alter the mobility, availability or toxicity of metals (De Jonge et al., 2012a; De Jonge et al., 2012b; Hedge et al., 2009; Jacob and Otte, 2003a; Maddock et al., 2007; Peterson et al., 1996; Simpson et al., 1998; Zhuang et al., 1994).

Two ‘events’ which currently take place in the Schelde estuary and which are expected to have an effect on flows, mobility and availability of metals in Schelde sediments are explored in present work: (1) Implementation of flood control areas with controlled reduced tide (CRT) and depolderization projects and (2) improving oxygen conditions in the surface water.

1.2. Habitat restoration in the Schelde estuary

Estuarine and coastal zones are rich, diverse and productive (Barbier et al., 2011; Costanza et al., 1997; Worm et al., 2006). Yet, their deterioration due to human activities is intense and is still increasing (Lotze et al., 2006). This has resulted in the loss of biodiversity and critical ecosystem services such as fisheries, provision of nursery habitats, water quality or coastal protection from flooding and storm events (Barbier et al., 2011; Koch et al., 2009; Worm et al., 2006). In the Schelde estuary, the quality and quantity of valuable estuarine habitat decreased considerably last century (Meire et al., 2005). *Eutrophication,* elevated concentrations of contaminants and anaerobic conditions resulted in a highly polluted system (Meire et al., 2005; Van Damme et al., 2005). Land reclamation, dredging to deepen the navigation channel and building of dikes caused a loss of intertidal habitat and an increase in tidal amplitude (Meire et al., 2005).

With recognition of their essential role for human and marine life, estuaries and coastal zones have become the focus of efforts to develop ecosystem-based management and large-scale restoration strategies. In the Schelde estuary, the *Long Term Vision* was defined as ‘the development of a healthy and multifunctional estuarine water system that can be utilized in a sustainable way for human needs’ (Van den Bergh et al., 2005). To comply with EU-conservation goals and compensate for habitat loss by industrial development, thousands of hectares of tidal marsh need to be restored in the estuary. Yet, space is scarce and restoration is often in conflict with e.g. habitation, agriculture or harbor expansion.

The morphological changes in combination with a global change induced sea level rise and storm event frequency resulted in an increase of the annual maximum high water level with 1 meter last century (Coen, 2008). With an expected sea level rise of 60 cm in 2100, the flood risk was calculated to increase fivefold by the end of this century (Broekx et al., 2011). After the large floods in 1976 in the Schelde estuary, the *Sigmaplan* was initiated and provides now a framework for flood control combined with a higher ecological value. Managed realignment and controlled flooding of formerly embanked areas have already proven to be effective and economically viable strategies to combine flood control and wetland restoration in one and the same area (French, 2006). In the Schelde estuary, dike heightening and the implementation of flood control areas (FCA) should prevent flooding of the densely populated hinterland, while a reduced spring-neap tidal cycle (controlled reduced tide, CRT) introduced in these areas enables estuarine habitat to develop (Cox et al., 2006; Maris et al., 2007).

1.3. Habitat restoration and metal contamination

The implementation of CRT’s in a metal contaminated estuary can have an effect on metal behavior in several ways. Intertidal areas are often found to accumulate trace metals by means of sedimentation (Rozan and Benoit, 1999; Walling and Owens, 2003). Based on the strong association of metals with suspended particulate matter in the Schelde estuary (Bouezmarni and Wollast, 2005; Regnier and Wollast, 1993) and the high sedimentation rates in CRT’s (Vandenbruwaene et al., 2011), also these areas are expected to be sinks for trace metals. Implementation of CRT’s might consequently result in contamination of the site. Yet, historical contamination may have resulted in elevated metal concentrations in the embanked areas suitable for restoration (Vandecasteele et al., 2002a; Vandecasteele et al., 2003a). In addition, re-introduction of the tide might influence the mobility, availability and toxicity of historically present as well as newly introduced trace metals. Inundation is expected to influence soil Eh, pH or salinity which are determinant factors in controlling metal availability (Borch et al., 2010; Du Laing et al., 2007b; Du Laing et al., 2008b; Du Laing et al., 2009b; Gambrell et al., 1991; Gambrell, 1994; Speelmans et al., 2007). Consequently, the implemented CRT may also be a new pollution source when the mobility of historically accumulated metals increases and are released to the surface water.

Plants colonizing the CRT’s can significantly influence the behavior or potential toxicity of metals (Weis and Weis, 2004). For instance, roots of wetland plants which decrease pH or oxidize their rhizosphere can result in local mobilization of trace metals (Almeida et al., 2004; Jacob and Otte, 2004; Vervaeke et al., 2004; Wright and Otte, 1999). Additionally, trace metals, immobilized in the soil, can be accumulated in aboveground plant tissues which can enlarge food chain contamination (Reboreda and Caçador, 2007) or accumulated metals can be excreted by leaves in a more available form (Burke et al., 2000; Weis et al., 2002). Finally, metal containing litter, often with increasing concentrations during decomposition, can be taken up by detritus feeders or can be transported to the estuary (Caçador et al., 2009; Windham et al., 2004).

1.4. The Schelde estuary: a recovering system

Nowadays, the Schelde estuary is recovering from severe contamination. The load of nutrients and most toxic substances decreased, while oxygen conditions improved (Cox et al., 2009; Van Damme et al., 2005). Between 1960 and 2000, the Schelde estuary was characterized by a persistent hypoxia (Cox et al., 2009). Under these reducing conditions, trace metals in sediments as well as in SPM were found to be mainly associated with sulfides (Van Eck and De Rooij, 1993). The formation of insoluble metal sulfides is found to play a major role in reducing availability and mobility of various metals (Lee et al., 2000b; Yu et al., 2001). The amount of acid extracted sulfides (acid volatile sulfides or AVS) and simultaneously extracted metals (SEM) has been proposed as a predictor for the toxicity of Ag, Cd, Cr, Cu, Ni, Pb and Zn (Berry et al., 2004; Di Toro et al., 1990; Yoo et al., 2004). The implementation of waste water treatment plants last decade resulted in a drastic increase in oxygen concentrations in the surface water of the freshwater part of the estuary (Cox et al., 2009). Since AVS is sensitive to oxidation and metal-sulfide precipitation in sediments is a reversible process, the higher oxygen concentrations in the surface water can result in dissociation of metal-sulfide complexes with a subsequent increase in metal mobility and release from the sediment into the surface water (De Jonge et al., 2012a; De Jonge et al., 2012b; Kelderman and Osman, 2007; Petersen et al., 1997; Van Eck and De Rooij, 1993; Zhuang et al., 1994). A recent increase in the dissolved concentrations of Cd, Cu and Zn in the Sea Scheldt, while the emissions decreased, might indicate that remobilization of metals from anoxic sediments occurs (Gao et al., 2012).

1.5. Objectives and outline

Many estuarine and coastal regions are facing similar problems: historical contamination, intensified flood risk and need for habitat restoration. Consequently, flooding of formerly embanked areas as a measure for flood control and habitat restoration is expected to be implemented increasingly (French, 2006). However, processes in these often impaired sites are complex and still poorly understood (Bryan and Langston, 1992; Spencer and Harvey, 2012). This work aims to get more insight in the effect of CRT implementation on the flows, mobility and availability of metals. In addition, we explored if the improving oxygen conditions in the surface water can result in remobilization of sediment bound metals. For this aim, metal behavior was investigated in a pilot CRT (Lippenbroek) and compared with adjacent embanked polders and 'natural' tidal marshes.

After this general introduction on habitat restoration in the metal contaminated Schelde estuary (**Chapter 1**), the next chapter investigates metal flows through the estuary (**Chapter 2**). In that part we focus on the contribution of tidal marshes to the filter function of estuaries with respect to trace metals. In addition, the hypothesis that the implementation of CRT's and de-embankments, planned along the Schelde estuary, will enlarge metal accumulation by overbank sedimentation was tested.

In **Chapter 3**, spatial distribution of historical trace metal contamination in the pilot CRT and adjacent embanked polders and 'natural' tidal marshes, as well as changes in metal concentrations during the first three years after the re-introduction of the tide, was surveyed. Differences in metal availability in these same areas are discussed in **Chapter 4**, based on the sequential extractions, [SEM-AVS] and metal concentrations in different tissues of dominant plant species. In **Chapter 5**, metal accumulation was determined in the dominant plant species which colonized the CRT.

The effect of flooding frequency on metal concentrations in the sediment, pore water and the roots of common reed (*Phragmites australis*) was investigated in a natural tidal marsh in the freshwater stretch of the estuary (**Chapter 6**).

The last two chapters are based on experiments which explore the effect of increasing oxygen concentrations on metal mobility and availability in hypoxic sediments. In a first experiment, we explored if high surface water oxygen concentrations resulted in higher metal availability for the macrophyte *Myriophyllum aquaticum* (**Chapter 7**). In a second experiment, the effect of surface water aeration on SEM-AVS concentrations and metal remobilization from anoxic, contaminated sediments was investigated (**Chapter 8**).

The last chapter summarizes the most important results of the current thesis and explores the implications for CRT implementation, design and management in contaminated estuaries (**Chapter 9**).

CHAPTER 2

Estuaries as filters: the role of tidal marshes in trace metal removal.

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Abstract

Flux calculations demonstrate that many estuaries are natural filters for trace metals. Yet, the underlying processes are poorly investigated. In present study, it was hypothesized that intertidal marshes contribute significantly to the contaminant filter function of estuaries. Trace metal concentrations and sediment characteristics were measured along a transect from the subtidal, over an intertidal flat and marsh to a restored marsh with controlled reduced tide. Metal concentrations in the intertidal and restored marsh were found to be a factor two to five higher than values in the subtidal and intertidal flat sediments. High metal concentrations and high accretion rates indicate a high metal accumulation capacity of the intertidal marshes. Overbank sedimentation in the tidal marshes of the entire estuary was calculated to remove 25% to 50% of the riverine metal influx, even though marshes comprise less than 8% of the total surface of the estuary. In addition, the large scale implementation of planned tidal marsh restoration projects was estimated to almost double the natural trace metal storage capacity of the estuary.

2.1. Introduction

When trace metals are discharged in aquatic systems they can be transported to the ocean, where management and remediation of contaminants become more difficult or impossible. Riverine input is calculated to be the major source of metals in the Greater North Sea (OSPAR, 2009) (Oslo/Paris convention for the Protection of the Marine Environment of the North-East Atlantic). Consequently, reduction of riverine metal fluxes is an important measure to protect marine ecosystems. First, the direct discharge of contaminants into the aquatic system has to be reduced by strict legislation and control. In addition, filtering processes by natural systems during the river-sea continuum can reduce the input of contaminants into the sea. Estuaries, which are transitional zones between terrestrial and marine waters, are found to be successful filters for contaminants (Andrews et al., 2008; Cave et al., 2005; Millward and Glegg, 1997; Monbet, 2006). Mass balances of trace metals in the Schelde estuary (Belgium, SW Netherlands (e.g. (Baeyens et al., 1997a; De Gieter et al., 2005; De Smedt et al., 1997; Ouboter et al., 1997) and other estuaries (e.g. (Audry et al., 2007; Millward and Glegg, 1997; Monbet, 2006; Thévenot et al., 2007) have been studied before. It was calculated that a large part of the trace metals accumulated in the high turbidity zone of the Schelde estuary (Baeyens et al., 1997a). However, estimations on the specific contribution of tidal marshes to this accumulation and the overall filter function of the Schelde or other estuaries with respect to trace metals are scarce.

In present study it is hypothesized that tidal marshes play an important role in the metal filter function of estuaries. When entering the estuary, trace metals are mainly associated with fluvial suspended matter (SPM) (Bouezmarni and Wollast, 2005). SPM and associated metals in estuaries accumulate mainly in areas with low hydrodynamic energy (Regnier and Wollast, 1993; Temmerman et al., 2004b; Van Damme et al., 2009). Tidal flooding of marshes results often in gradual accretion (Temmerman et al., 2004a; Vandenbruwaene et al., 2011), which is expected to be important for the removal of trace metals from the surface waters in estuaries. These marshes are characterized by vegetation, sediments rich in litter and low flow velocities during flooding which promotes sedimentation of the fine grained fraction of suspended solids. This fraction is often rich in organic matter and clay particles and is known to display a high affinity for trace metals (Bouezmarni and Wollast, 2005; Du Laing et al., 2009c). Despite post-depositional diagenetic metal mobility and possible release to the surface water, the organic rich, hypoxic sediments of marshes are generally considered to be sinks for metals (Spencer et al., 2003; Weis and Weis, 2004; Zwolsman et al., 1993). In a first part of present study, sediment characteristics and metal concentrations of surface sediments from a subtidal zone, an intertidal flat, an intertidal marsh and a restored marsh were investigated on one location in the Schelde estuary, in order to evaluate differences in the filtering capacity of these areas. In a second part, the contribution of tidal marshes to the filter function of the estuary was calculated for the entire Schelde estuary.

. The following hypotheses are tested:

- Sediments deposited on tidal marshes are generally higher in clay, organic matter and trace metal content compared to subtidal and intertidal flat sediments.
- Metal accumulation in tidal marshes contributes significantly to the contaminant filter function of estuaries.
- The implementation of tidal marsh restoration projects, planned along the Schelde estuary, will enlarge metal removal by overbank sedimentation.

2.2. Material and methods

2.2.2. Sampling

The study is carried out in the Schelde estuary (Belgium and The Netherlands), a turbid, macrotidal and eutrophic system (Meire et al., 2005) with elevated metal concentrations in surface water, and associated with suspended solids and sediments (Baeyens et al., 1997b; Du Laing et al., 2007a; Teuchies et al., 2012a; Vandecasteele et al., 2003a). For the first part, sediments were sampled along a transect from the subtidal, over a tidal flat and marsh to a restored marsh with controlled reduced tide (CRT) (Beauchard et al., 2011; Cox et al., 2006; Maris et al., 2007) all located in the freshwater tidal zone at 51°05’10’’N; 4°10’20’’E (Fig. 1). Samples were taken in summer (August 2009, not subtidal) and winter (December-January 2009). Subtidal sediment was sampled with a Reineck box corer (0-5 cm). The upper cm of the tidal flat was sampled with a plastic spoon every 5 m from the marsh edge to the water line (60 m in winter, 35 m in summer). In order to quantify sediment deposition rates, sediment samples were collected with sediment traps (PVC plates, ⌀ 20 cm) on the tidal marsh (13 locations evenly distributed over the distance from the marsh edge to the dike) and on the CRT (16 locations randomly spread over the area). Due to the high stream velocity above the tidal flat, no sediment traps could be used in this area. On every location, in the marsh and CRT, one trap with paper filter and one without filter were collected after 28 days exposure time (2 spring-neap tidal cycles). Sediment traps with filter were used to collect and handle deposited sediments more easily: sediments were deposited on pre-dried and weighed filters. These filters with sediments were collected, oven dried (60 °C, until constant weight) and weighed. Sediments for analysis were collected from traps without filter. All sediment samples were transported cooled in polyethylene recipients from the field to the laboratory.

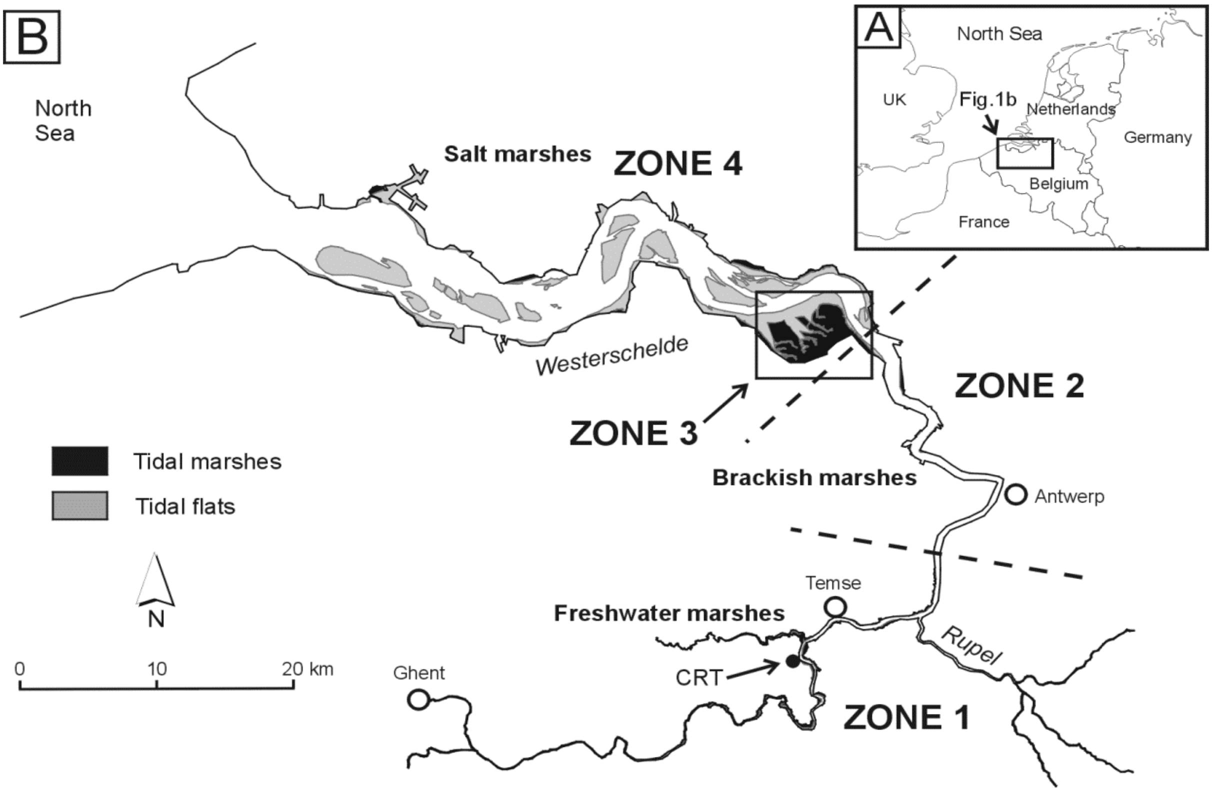


Figure 1. Location of the Schelde estuary (A) and the study area (CRT) within the estuary (B).

2.2.2. Analyses

For grain size analysis a subsample (1 g) of fresh sediment was boiled in 5 mL hydrogen peroxide (H_2O_2, 30%) to remove the organic matter. The clay (<2µm) silt (2-63 µm) and sand (>63µm) fraction of this mixture was determined with a laser diffraction particle size analyzer (Malvern S, Malvern Instruments Ltd, Worcestershire, UK). Organic matter (OM) was estimated through loss on ignition. For this purpose, the weight difference of oven dried (105 °C) and incinerated sediment (after 6h exposure at 550 °C) was determined. Trace metal concentrations were determined in a mixture of an air dry subsample (0.2 g) with nitric acid (5mL HNO_3 69%, Merck EMSURE® for traces analysis) and hydrogen peroxide (5 mL H_2O_2 30%, VWR Int. AnalaR NORMAPUR® for traces analysis) after hot block digestion. Metal concentrations were measured after filtration (cellulose mixed ester, 0.45 µm, Chromafil®) using an ICP-OES (inductively coupled plasma - optic emission spectroscopy, iCAP 6300 Duo SERIES Thermo Fisher Scientific®, Waltham, USA). Analytical accuracy was achieved by the use of blanks and certified reference material for sediment (Institute for Reference Materials and Measurements (IRMM), BCR® № 320, channel sediment) included in each series of sampled for metal analysis. Values from reference samples were lower but within 90% of the certified values for As, Cd and Zn, and within 80% for Cu, Ni, Pb and Mn. Recovery of Cr was low (50%). Variation between recoveries was very low for all elements.

2.2.3. Model calculations

A long-term marsh sedimentation model, MARSED, has been used to estimate the temporal variation in metal accumulation when a young, low elevation marsh evolves to a high equilibrium elevation (Fig. 4). The use and validation of this model is extensively described in (Struyf et al., 2007; Temmerman et al., 2003a; Temmerman et al., 2004b). This model simulates sediment deposition rates based on the input of SPM concentrations and flooding frequency and duration, which depends on the tidal marsh height and river water level. The model is extended to simulate long term variation in metal deposition as a young, low marsh evolves to a high equilibrium marsh. As an example, the average Cd concentration in sediments from freshwater marshes of the Schelde estuary (7.19 µg g^{-1}) calculated in this study (see below) is used as input for the model.

2.2.4. Budget calculations

In a second part of the manuscript the amount of trace metals which accumulated annually in tidal marshes was calculated for the entire Schelde estuary (tidally influenced tributaries not included due to insufficient data) (Table3). The budget calculations are based on surface areas (own data from GIS), sediment accretion rates (own data, data from governmental agencies and from literature) and metal concentrations in tidal marshes and suspended solids (own data including concentrations from the first part of this manuscript and governmental data) (Table SI for overview). The estuary was divided in four zones with similar characteristics in terms of metal concentrations and sedimentation. The first zone consists of freshwater marshes, which reach from km 160 in the estuary (Ghent) to km 85 (Fig. 1). This zone is most polluted since no dilution with marine water or sediments occurs. Zone 2 comprises all brackish marshes from km 85 up to the large brackish marsh of *Saeftinghe* (km 58, Dutch – Belgian border). Downstream of the border, the estuary becomes wider and the influence of tidally introduced marine water and sediments is much larger (Verlaan, 2000). Due to its large surface (2225 ha) the brackish marsh of *Saeftinghe* was considered as a distinct zone (zone 3). Zone 4 covers all brackish and salt marshes from Saeftinghe towards the mouth of the estuary (Westerschelde). Additionally, future metal accumulation was estimated in flood control areas with controlled reduced tide (CRT) and de-embankments projects which will be implemented along the fresh and brackish reach of the estuary.

The surface area of tidal marshes was based upon information of the vegetated tidal zones as available in GIS. The surface area of the future CRT's and de-embankments is a total of operational areas (8 and 50 ha respectively), areas under construction (300 ha CRT) and areas approved by the Flemish government in 2010 (650 and 500 ha respectively) all as part of the Sigma plan.

Data on marsh accretion (m y^{-1}) for zone 1 and 2 are based on the measurements from the *Flemish research institute for nature and forest* (INBO*)*: marsh accretion between October 1997 and January 2000 was measured with respect to a white kaolin clay layer in the marsh soil on 15 freshwater marsh locations and on 16 brackish marsh locations. Marsh accretion in zone 3 and 4 was calculated from changes in volume (i.e. surface area and elevation) of these areas based on digital elevation model (DEM) maps from 1963, 1992, 2002, 2004, 2009 and 2010 for *Saeftinghe* marsh (zone 3) and from 2004, 2009 and 2010 for zone 4. The DEMs for the periods before 2000 were computed from theodolite surveying with a minimum density of 1 point per 0.75 ha, and for the periods after 2000 based on LiDAR surveys with a minimum density of 1 point per 16 m² (e.g., (Temmerman et al., 2004b).

Sedimentation rates are in good agreement with other values reported in the Schelde estuary based on e.g. dating of sediment cores (Temmerman et al., 2004b; Zwolsman et al., 1993). Long term accretion in the CRT's is based on measurements and modeling of Vandenbruwaene et al. (2011). For the de-embanked areas the average of accretion in zone 1 and 2 was used. The bulk density value is based on measurements from Temmerman et al. (2004b). Since no spatial trend in bulk density was observed along the estuary, an average bulk density of 494 +/- 122 kg m³ (n=17) was used for all zones.

Trace metal values are based on concentration measurements from sediment traps and in superficial (0-10 cm) sediments sampled between 2000 and 2010 in 6 marshes (n=120) for zone 1, in 2 marshes (n=16) for zone 2 (personal data and data from INBO) (SI Table 1 for values). Values and trends in metal concentrations found in these zones are in accordance with available results from previous research (Baeyens et al., 1997a; Du Laing et al., 2007a; Van Alsenoy et al., 1993; Zwolsman, 1999). From *Saeftinghe* marsh onwards the estuary becomes wider and marine influence increases drastically (Fig. 1). In this zone 95% of the sediments are found to have a marine origin (Verlaan et al., 1998; Verlaan, 2000). Due to a lack of recent data on metal concentrations in marsh sediments from the Westerschelde (from border to mouth), trace metal values in zone 3 and 4 are based on concentrations measurements in suspended solids sampled from 2005 to 2010 at 2 locations (km 58 and 35; n = 155) for *Saeftinghe* marsh (zone 3) and on 4 locations for zone 4 (km 35, 19, 0 and -10; n = 173) (data from *the Dutch ministry for infrastructure and environment, Rijkswaterstaat*). The values observed are within the range of metal concentrations in surface sediments from Westerschelde marshes (zone 4) reported by (Beeftink et al., 1982), lower (approximately half) of the concentrations reported in marshes by (Zwolsman et al., 1993) and slightly lower than suspended solid concentrations measured in 1995 (Baeyens et al., 1997a). This observation is in accordance with the expected decrease in sediment-bound metal concentrations due to a general improvement in water quality over the last decades (Baeyens et al., 2005; Van Damme et al., 2005).

For each zone total metal accumulation in the marshes A_m (kg y^{-1}) is calculated:

$$A_m = \Delta H * \rho * C_m * S$$

With ΔH = average annual accretion of the marsh (m y^{-1}), ρ = average bulk density (10^3 kg dw m^{-3}), C_m = average metal concentration (g $(10^3\ kg)^{-1}$ dw) and S = total surface of all marshes in the zone (m²). Values for A_m are large and expressed as 10^3 kg y^{-1}.

The total metal accumulation of marshes in the Schelde estuary was compared with riverine metal input (Table 4). Fluxes based on literature were found for the period 1981-1995. To compare with more recent fluxes, basic calculations for the period 2005-2010 were carried out: the average freshwater discharge (km 95, downstream from the Rupel, n=84; 104 ± 52 m³ s^{-1} was multiplied with the average total metal concentrations (km 85 – 110; n=200; data from the *Flemish Environment Agency*, VMM). This is a rough calculation which probably underestimates the influx since metals deposited before this point and influx after this point are not taken into account. Yet, this calculated riverine input (e.g. 2.60 ton Cd y^{-1}) is higher than the added influx (1.53 ton Cd y^{-1}) from the three major tributaries (Bovenschelde, 0.73 ton Cd y^{-1}; Dender 0.58 ton Cd y^{-1}; Rupel 0.22 ton Cd y^{-1}. T he same method is used before for the Schelde (Baeyens et al., 2005).

2.2.3. Statistical analysis

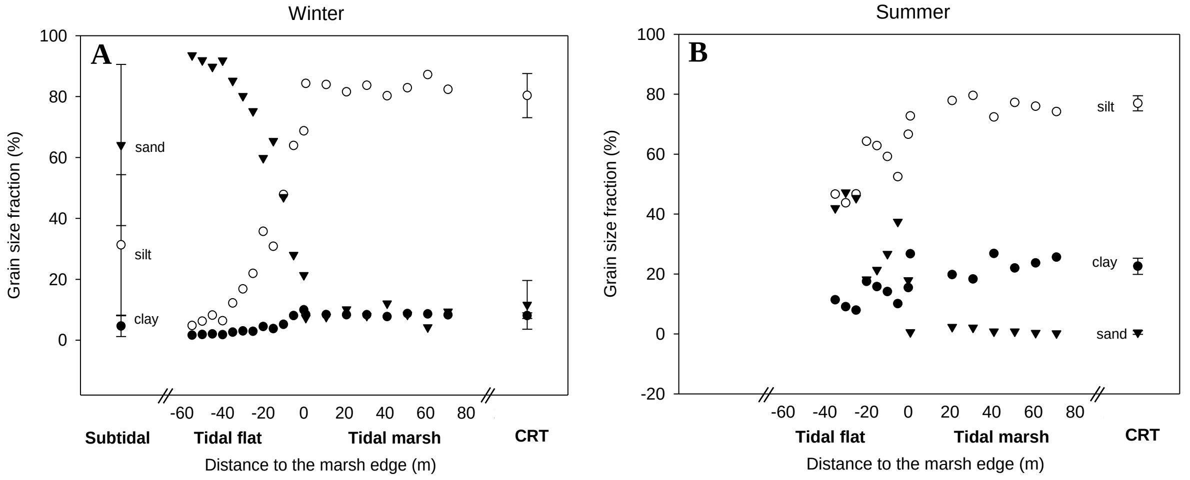
The significance of differences between the sampled areas (subtidal – tidal flat – tidal marsh – CRT) in metal concentration and sediment or metal accumulation within a season was tested with a one way analysis of variance test (ANOVA). Differences between seasons within an area were tested with a paired t-test. Normality of the data was tested with the Shapiro-Wilk test prior to analysis. The Pearson correlation-coefficient was used to determine correlations between different metals and sediment characteristics over all areas and both seasons.

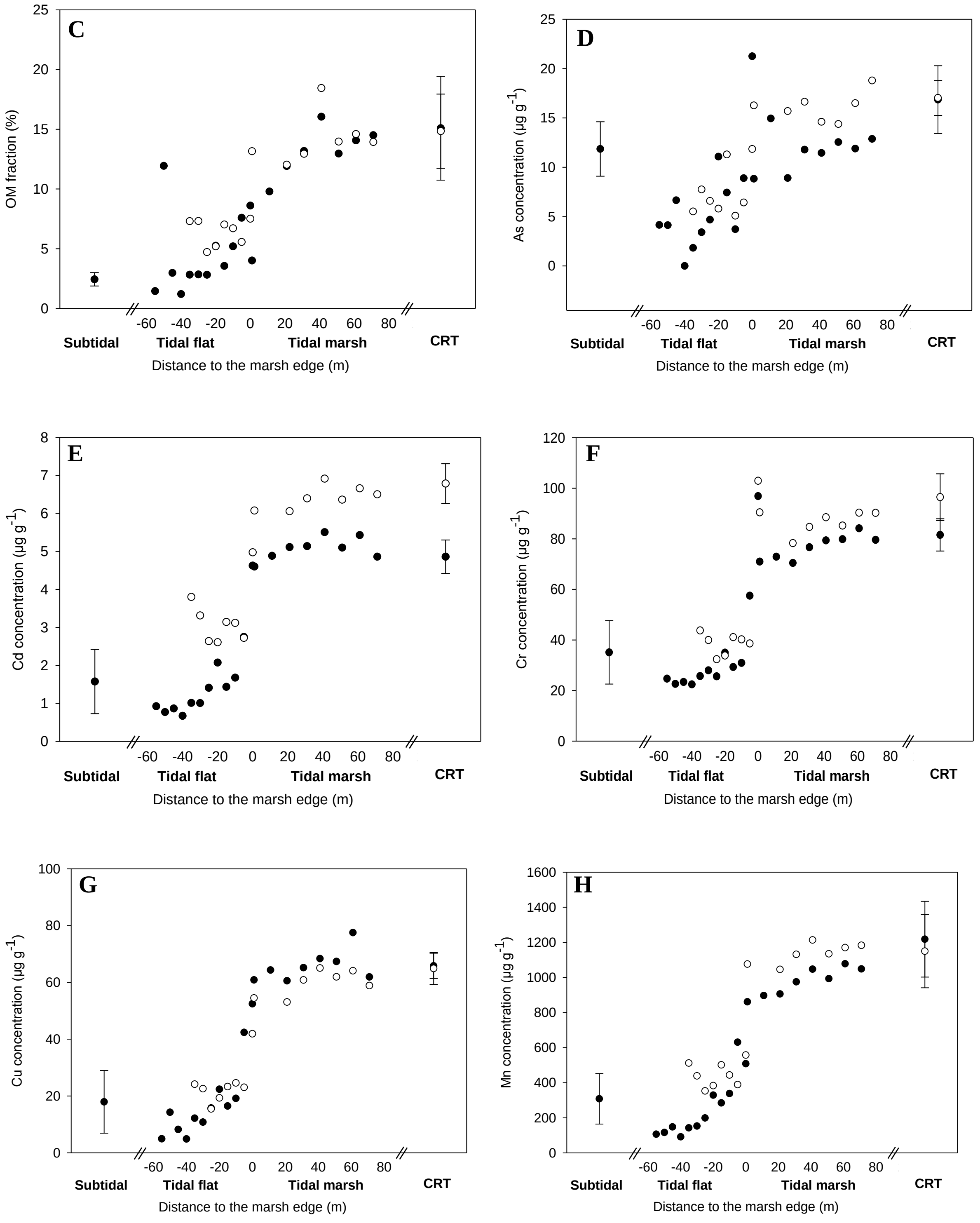
2.3. Results

2.3.1. Spatial distribution and seasonal differences in trace metals in deposited sediments

In general, clay, silt, OM and metal concentrations were not significantly different between the subtidal and tidal flat sediments. In these sediments grain size and metal concentrations were lower, they increased towards the marsh edge and were significantly higher (*p*<0.05, one way ANOVA) in the natural marsh and CRT in both seasons (Fig. 2, Table 1). Trace metal concentrations in the marsh and CRT were a factor 2-5 higher compared to concentrations in tidal flat sediments. The opposite trend, higher values in the tidal flat, was observed for the sand content. Differences between the marsh and CRT were small and only significantly (p<0.05) higher concentrations in the CRT were found for As, Cr and Cu. Spatial distribution was found to be similar for all metals studied (significant correlation, *p*<0.001, Table 2) and all metal concentrations were positively correlated with clay, silt and OM content and negatively with sand (*p*<0.001).

Grain size distribution and metal concentrations were different between sediments deposited in summer and winter (Fig 2, Table 1). In general, sediments sampled during the summer campaign had a smaller grain size and higher metal concentration. These differences were most pronounced in the tidal marsh (all *p*<0.05, paired t-test) where clay, silt and metal concentrations were higher in summer, except for Cu and Zn concentrations which were higher in winter. Differences were large e.g. for clay with 8% dw in summer and 23% dw in winter and for Cd with 4.9 µg g^{-1} in winter and 6.2 µg g^{-1} in summer. Due to a smaller dataset and more spatial variation (gradient from the river to the marsh edge) these differences were less distinct in the tidal flat and only significant for the grain size parameters.




C
OM fraction (%)
0
5
10
15
20
25
-60 -40 -20 0 20 40 60 80
Subtidal
Tidal flat
Tidal marsh
CRT
Distance to the marsh edge (m)
D
As concentration (µg g^{-1})
0
5
10
15
20
25
-60 -40 -20 0 20 40 60 80
Subtidal
Tidal flat
Tidal marsh
CRT
Distance to the marsh edge (m)
E
Cd concentration (µg g^{-1})
0
1
2
3
4
5
6
7
8
-60 -40 -20 0 20 40 60 80
Subtidal
Tidal flat
Tidal marsh
CRT
Distance to the marsh edge (m)
F
Cr concentration (µg g^{-1})
0
20
40
60
80
100
120
-60 -40 -20 0 20 40 60 80
Subtidal
Tidal flat
Tidal marsh
CRT
Distance to the marsh edge (m)
G
Cu concentration (µg g^{-1})
0
20
40
60
80
100
-60 -40 -20 0 20 40 60 80
Subtidal
Tidal flat
Tidal marsh
CRT
Distance to the marsh edge (m)
H
Mn concentration (µg g^{-1})
0
200
400
600
800
1000
1200
1400
1600
-60 -40 -20 0 20 40 60 80
Subtidal
Tidal flat
Tidal marsh
CRT
Distance to the marsh edge (m)

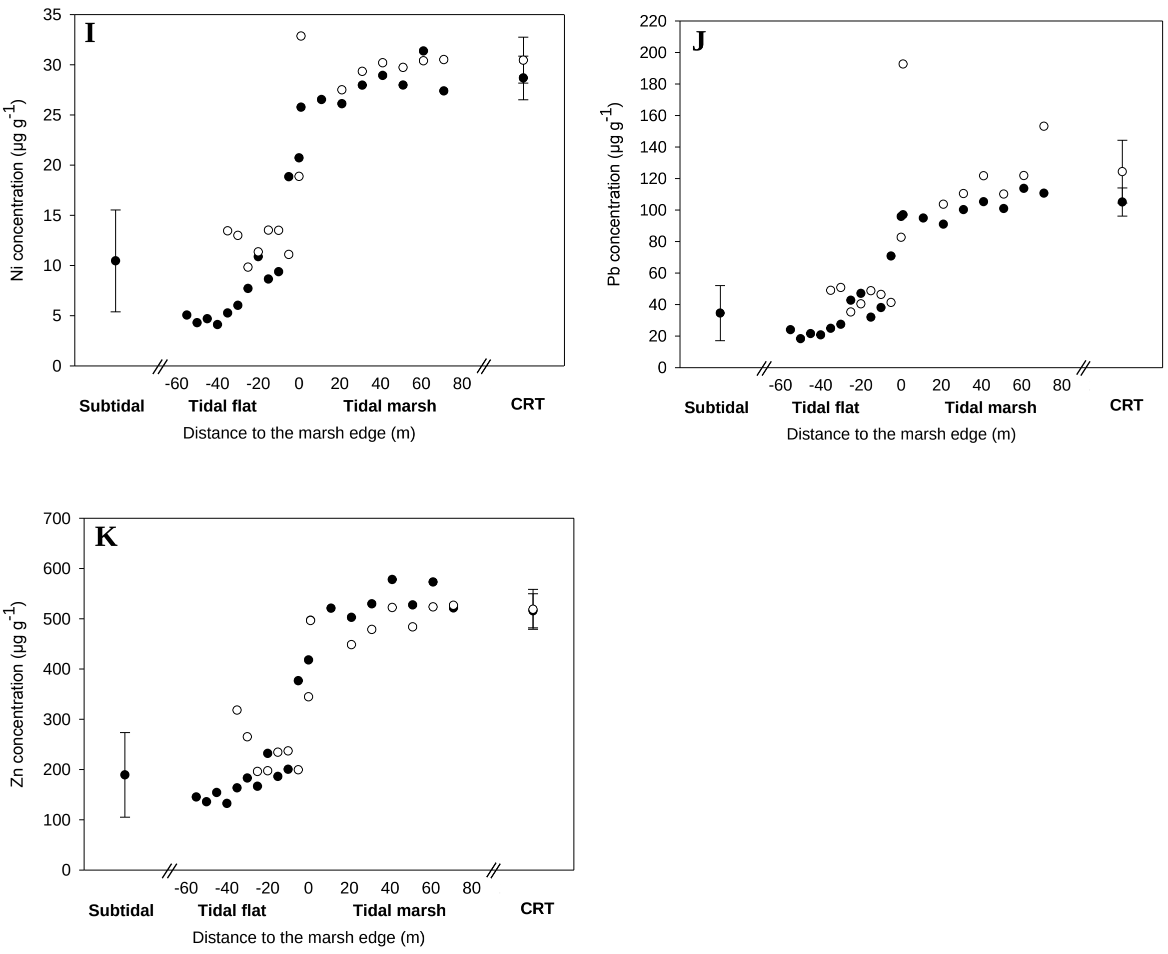


Figure 2. Grain size in winter (A), summer (B), organic matter (C) and metal concentrations (D-K) in estuarine sediments. For OM and metal concentrations: black for winter, white for summer. Average values with standard deviation for subtidal (n=4, only winter) and restored marsh (CRT, n=16) sediments.

Table 1. Average metal concentrations (µg g^{-1}) and sediment characteristics (% dw) in the different areas for winter and summer. Significant differences ($p<0.05$) between seasons within an area are underlined and differences between areas within a season are indicated with letters (a, b, c for winter; x, y, z for summer).

	Subtidal	**Tidal Flat**		**Tidal Marsh**		**CRT**	
	Winter	**Winter**	**Summer**	**Winter**	**Summer**	**Winter**	**Summer**
As	$11.9^{(abc)}$	$7.79^{(a)}$	$7.54^{(x)}$	$11.7^{(b)}$	$15.0^{(y)}$	$15.9^{(c)}$	$17.0^{(y)}$
Cd	$1.58^{(a)}$	$2.00^{(a)}$	$3.29^{(x)}$	$4.90^{(b)}$	$6.22^{(y)}$	$4.76^{(b)}$	$6.77^{(y)}$
Cr	$35.1^{(a)}$	$41.1^{(a)}$	$46.6^{(x)}$	$74.3^{(b)}$	$83.7^{(y)}$	$78.7^{(b)}$	$97.0^{(z)}$
Cu	$17.9^{(a)}$	$23.9^{(a)}$	$24.3^{(x)}$	$63.2^{(b)}$	$57.7^{(y)}$	$63.8^{(b)}$	$64.3^{(z)}$
Mn	$308^{(a)}$	$323^{(a)}$	$447^{(x)}$	$942^{(b)}$	$1.10\ 10^{3(y)}$	$1.18\ 10^{3(b)}$	$1.17\ 10^{3(y)}$
Ni	$10.5^{(a)}$	$10.9^{(a)}$	$13.1^{(x)}$	$26.9^{(b)}$	$29.1^{(y)}$	$27.8^{(b)}$	$31.2^{(y)}$
Pb	$34.6^{(a)}$	$47.4^{(a)}$	$49.4^{(x)}$	$97.6^{(b)}$	$121^{(y)}$	$101^{(b)}$	$135^{(y)}$
Zn	$189^{(a)}$	$241^{(a)}$	$249^{(x)}$	$515^{(b)}$	$477^{(y)}$	$500^{(b)}$	$514^{(y)}$
Clay	$4.62^{(a)}$	$4.99^{(a)}$	$12.7^{(x)}$	$8.32^{(b)}$	$23.3^{(y)}$	$8.06^{(b)}$	$22.6^{(y)}$
Silt	$31.3^{(a)}$	$37.2^{(a)}$	$55.3^{(x)}$	$82.2^{(b)}$	$75.5^{(y)}$	$80.3^{(b)}$	$77.0^{(y)}$
Sand	$64.1^{(a)}$	$57.8^{(a)}$	$32.0^{(x)}$	$9.51^{(b)}$	$1.26^{(y)}$	$11.6^{(b)}$	$0.46^{(y)}$
OM	$2.44^{(a)}$	$4.84^{(a)}$	$6.42^{(x)}$	$12.3^{(b)}$	$14.0^{(y)}$	$14.6^{(b)}$	$15.2^{(y)}$

Table 2. Correlation coefficients (R-values) between the different metals and sediment characteristics. All correlations were significant ($p<0.001$).

	Cd	**Cr**	**Cu**	**Mn**	**Ni**	**Pb**	**Zn**	**clay**	**silt**	**sand**	**OM**
As	0.762	0.814	0.768	0.762	0.79	0.693	0.759	0.518	0.578	-0.694	0.729
Cd		0.949	0.889	0.847	0.927	0.852	0.895	0.794	0.676	-0.906	0.805
Cr			0.921	0.859	0.937	0.868	0.919	0.679	0.71	-0.873	0.807
Cu				0.901	0.956	0.805	0.988	0.487	0.865	-0.893	0.843
Mn					0.959	0.845	0.906	0.575	0.728	-0.835	0.941
Ni						0.909	0.959	0.646	0.782	-0.911	0.904
Pb							0.814	0.724	0.585	-0.803	0.853
Zn								0.496	0.843	-0.881	0.848

2.3.3. Metal accumulation in natural tidal marshes and CRT areas

The average yearly sediment deposition rate, as measured with the sediment traps, was found to be higher ($p<0.05$, one way ANOVA) in the CRT (24 ± 26 kg m^{2} y^{-1}) than in the adjacent marsh (3.6 ± 2.2 kg m^{2} y^{-1}) (Fig. 3). Variation between traps within the CRT was large. This spatial variation in sedimentation rates is mainly related to spatial variations in the marsh surface elevation: the lower the elevation, the higher the frequency, duration and depth of tidal inundations, which results in higher sedimentation rates.

The large sediment accumulation in the CRT coincided with a significant higher metal deposition compared to the marsh ($p<0.05$ for all metals in winter and summer, one way ANOVA). No significant differences (*p*>0.05, paired t-test) between the amount of accumulated sediments or metals were found between winter and summer in both areas.

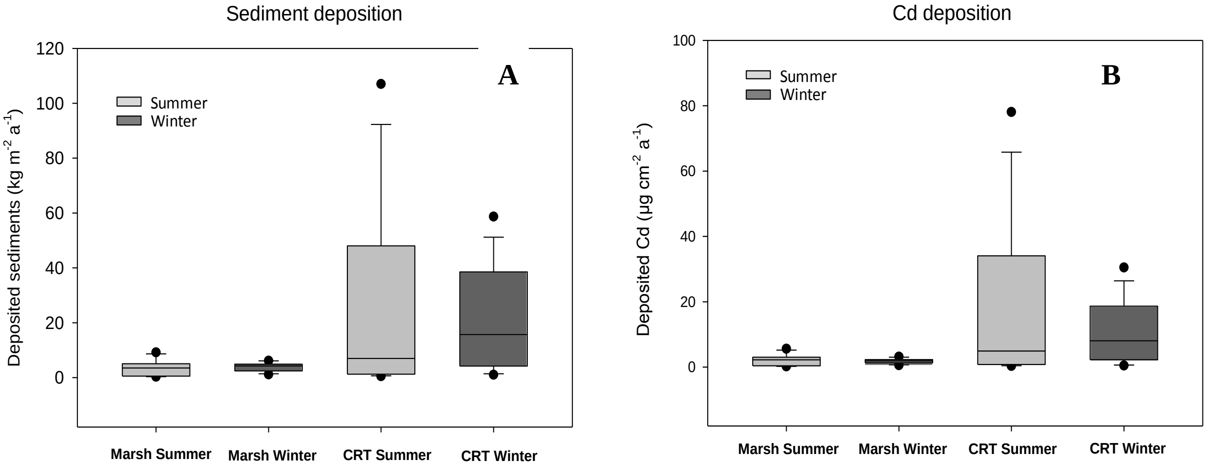


Figure 3. Box plots (median, 25th, 75th percentile and standard deviation) of seasonal difference in deposited sediments (A) and deposited Cd (B) in a tidal marsh and marsh CRT.

The effect of marsh surface elevation on sediment and metal accumulation is further illustrated by modeling of the temporal variation in metal accumulation of a young, low elevated marsh developing towards its high elevated equilibrium state (Fig. 4). Average freshwater tidal marsh Cd concentrations (see 2.2 and SI Table 1) were used as an example. Low marsh elevation resulted in high sedimentation rates and high Cd accumulation (almost 40 µg cm^{-2} y^{-1}). With increasing marsh elevation, tidal inundation frequency, duration and depth diminished, so that sediment and Cd accumulation rapidly decreased and became constant (around 5 µg Cd cm^{-2} y^{-1}) after 20 years, when marsh elevation attained an equilibrium with the mean high water level.

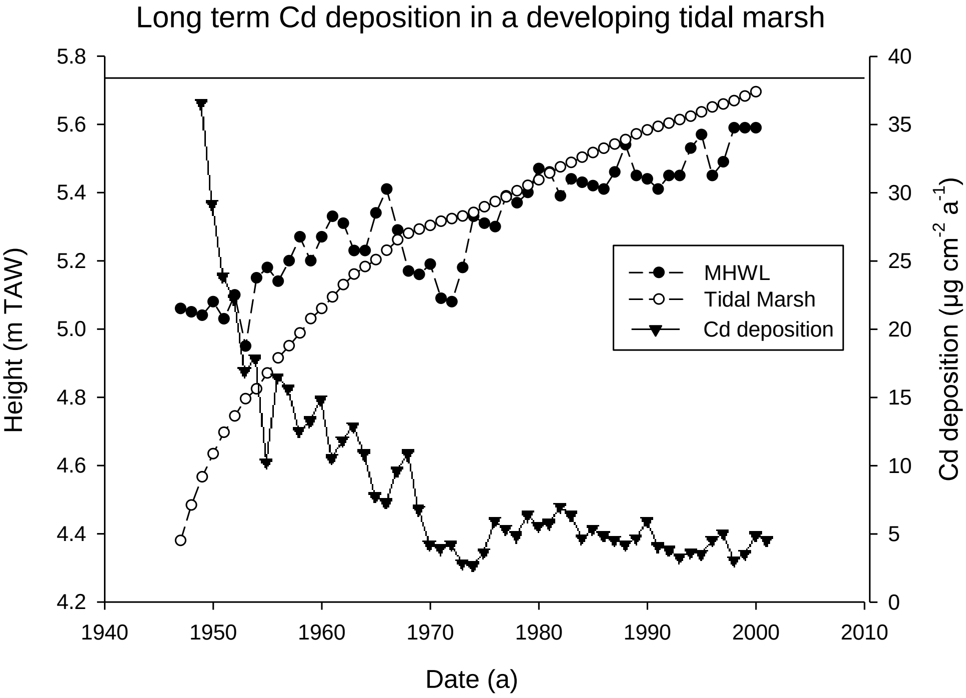


Figure 4. Modelled evolution of marsh elevation and Cd deposition. Elevation of the marsh and mean high water level (MHWL), both in m TAW (Belgian reference height) on the left Y-axis. Cd deposition (µg cm^{-2} y^{-1}) on the right Y-axis.

Average metal concentrations in surface sediments of tidal marshes were estimated for the different estuarine zones (see 2.2.). A general trend existed with the highest metal concentrations in the freshwater marshes and decreasing values towards the mouth of the estuary (Table 3). This trend was absent for As and Mn and less clear for Cr and Ni. Average sedimentation rates were similar in the different zones (6.5 – 9.3 kg m² y^{-1}) but were found to be double (16.3 kg m² y^{-1}) in the CRT areas. This resulted in the highest metal deposition rates per surface unit in the CRT’s. In the different zones, the rate of metal deposition was highest in the freshwater marshes except for As. Due to the high estimated sedimentation rates and the significant surface area (950 ha) planned to be implemented, the total metal retention by future CRT’s is calculated to be comparable to the metal accumulation in all present marshes in the Schelde estuary. For the different zones of the existing marshes, metal accumulation is highest in *Saeftinghe* marsh, which can be attributed to its large surface area (2225 ha).

Metal deposition in the marshes of the Schelde removes a considerable amount of trace metals from the surface water (23% - 53% of the riverine input) (Table 4). With the implementation of marsh restoration projects as foreseen in the next decades, this fraction will roughly double (39% - 90%).

Table 3. Average metal concentrations (µg g^{-1}), calculated metal deposition per surface unit (µg cm^{-2} y^{-1}) and estimated total accumulated metals in the different zones of the Schelde estuary (10^3 kg y^{-1}). The average estimated sedimentation rate (kg m^{-2} y^{-1}) and total surface for the marshes (in 2012) and expected future areas displayed.

		Zone 1	**Zone 2**	**Zone 3**	**Zone 4**		
		Freshwater marshes	Brackish marshes	*Saeftinghe* marsh	Brackish/Salt marshes	Future CRT's	Future marshes
Concentration (µg g^{-1})	As	17.8	23.7	22.5	17.9	20.8	20.8
	Cd	7.19	3.53	2.77	0.779	5.36	5.36
	Cr	110	63.5	99.2	81.7	86.9	86.9
	Cu	94.6	72.4	50.2	25.6	83.5	83.5
	Mn	1077	835	1285	884	956	956
	Ni	33.7	23.7	29.1	24.7	28.7	28.7
	Pb	133	80.2	76.4	48.0	106	106
	Zn	788	350	300	147	569	569
Sedimentation (kg m^{-2} y^{-1})		**9.36**	**8.07**	**6.48**	**7.52**	**16.3**	**8.71**
Metal deposition (µg cm^{-2} y^{-1})	As	16.7	19.1	14.6	13.5	33.8	18.1
	Cd	6.73	2.85	1.79	0.585	8.74	4.67
	Cr	103	51.2	64.2	61.4	142	75.8
	Cu	88.6	58.4	32.5	19.2	136	72.8
	Mn	1008	673	832	664	1558	833
	Ni	31.6	19.1	18.9	18.6	46.8	25.0
	Pb	124	64.7	49.5	36.1	174	92.8
	Zn	737	283	195	111	928	496
Surface (ha)		**262**	**153**	**2225**	**619**	**950**	**550**
Metal deposition (ton y^{-1})	As	0.436	0.293	3.24	0.834	2.64	0.995
	Cd	0.176	0.0436	0.399	0.0362	0.68	0.257
	Cr	2.71	0.783	14.3	3.80	11.1	4.17
	Cu	2.32	0.893	7.24	1.19	10.6	4.00
	Mn	26.4	10.3	185	41.1	122	45.8
	Ni	0.826	0.292	4.19	1.15	3.65	1.38
	Pb	3.25	0.989	11.0	2.23	13.5	5.10
	Zn	19.31	4.32	43.3	6.85	72	27.3

2.4. Discussion

2.4.1. Trace metal accumulation in a freshwater tidal area

Due to large-scale embankments and dredging, the Schelde estuary has often a deep and narrow streambed channel and a large surface of high dynamic areas characterized by high flow velocities and high physical stress (Meire et al., 2005). In these zones sedimentation of larger particles as well as erosion occurs which explains the very high sand (> 63µm) content in the investigated tidal flat and subtidal sediments (Fig. 2A). Frequent sedimentation measurements on this tidal flat demonstrated irregular sedimentation-erosion cycles (Beauchard, unpublished data) in contrast to constant accretion observed in most tidal marshes (Temmerman et al., 2004a). The coarse fraction (> 32 µm) of Schelde sediment was found to contain mainly quarts, calcite and feldspar which are minerals with a low affinity for trace metals (Bouezmarni and Wollast, 2005) explaining the lower metal concentrations in the investigated subtidal and tidal flat sediments (Fig. 2).

Trace metal concentrations in sediments deposited on the tidal marsh and CRT were a factor two to five higher compared to the tidal flat (Fig. 2). Lower flow velocity in these areas, due to lower water depths and hydraulic friction by the marsh vegetation (Temmerman et al., 2012), resulted in deposition of a fine-grained fraction. This fraction consisted mainly of clay, silt and organic matter and is known to have a high affinity for trace metals: clay particles can bind trace metals directly due to charged surfaces and act, as other small particles, as high-surface-area carriers which are coated with interlayered mixtures of organic matter and Fe and Mn oxides which form a solid surface with a strong affinity for metals (Luoma and Rainbow, 2008). The pattern for As is slightly different from the other metals with higher subtidal concentrations, yet also this metalloid is found to be associated mainly with Fe and Mn (hydr)oxides, organic matter and clay particles (Masson et al., 2009).

Sediments deposited in winter had a larger grain size and lower metal concentrations for most elements (Fig. 3 and Table 1). Lower winter metal concentrations in surface sediments of an urban estuary can be explained by resuspension of fine sediments caused by higher turbulence in winter (Buggy and Tobin, 2008). Also in the Schelde estuary a strong seasonal pattern is observed in riverine and overmarsh SPM concentrations and sediment deposition rates on marshes, with higher values in winter, however no differences in grain size were reported in these studies (Fettweis et al., 1998; Struyf et al., 2007; Temmerman et al., 2003b). No difference in sediment quantity between summer and winter was observed in present study, which may be because seasonal differences were only based on one spring-neap tidal cycle for both seasons.

As in other studies, trace metals were found to be mainly associated with the fine grained, organic rich fraction of the suspended solids (Table 2) (Du Laing et al., 2009c). When sediments are transported to the sea, a substantial part of this fine fraction and associated metals can accumulate within the estuary (Verlaan et al., 1998). Based on the constant accretion and the fine and organic characteristics of the deposited sediments in the marsh and CRT of present study, these areas are expected to be important for the contaminant filter function of estuaries.

2.4.2. Trace metal accumulation in marshes of the entire estuary

Annual metal deposition per marsh surface area in the Schelde estuary varied considerably between the investigated zones (Table 3.). Metal accumulation is highest in the CRT's, mainly due to their high sedimentation rates. The CRT is characterized by a stagnant phase during flood and a large spatial variation in elevation (Beauchard et al., 2011) which resulted in deposition of 24 ± 26 kg dw sediment m^{-2} a^{-1} (Fig. 4). Elevation differences are expected to decrease in time and the CRT will progressively evolve towards a flattened platform with sedimentation rates of approximately 16 kg dw sediment m^{-2} y^{-1} (Vandenbruwaene et al., 2011), which is still double of the estimated sedimentation rates in other marshes in the estuary. However, differences in locations in the estuary, initial topography or inlet/outlet configurations can result in other sediment accretion rates in future CRT areas.

Variation in sediment deposition in tidal marshes is strongly related to tidal inundation characteristics which, on its part are determined mainly by marsh elevation (Temmerman et al., 2003b). Our modeled results demonstrated that fast accretion rates occur in young, low elevated marshes (Fig. 5). The rapid rise of the marsh elevation results in lower inundation frequency, duration and depth with lower accretion rates as a consequence. On the long term (after about 2 to 3 decades) accretion rates in the marshes of the Schelde are found to be in equilibrium with the mean high water level rise in the estuary (Temmerman et al., 2004b). The decrease in sedimentation rates during marsh development coincided with an eightfold decrease in Cd deposition after twenty years. In the existing natural marshes metal accumulation per surface area is largest in the freshwater region, while differences between brackish marshes, *Saeftinghe* marsh or salt marshes are smaller (Table 3). The calculated Cd deposition per surface area in the freshwater marshes (zone 1; 6.3 µg cm^{-2} y^{-1}) was more than 10 times higher compared to deposition in salt marshes (zone 4; 0.59 µg cm^{-2} y^{-1}). These differences can be attributed to variation in the load, characteristics and metal concentrations of SPM and to differences in sedimentation rates which exist along the estuarine gradient. Riverine SPM and associated metals undergo changes due to mixing with marine water and sediments (Regnier and Wollast, 1993). A decrease in metal concentrations in SPM observed towards the mouth is mainly due to dilution of contaminated riverine particles with less contaminated marine particles (Regnier and Wollast, 1993).

This is also observed in the estimated concentrations in the marshes of the different zones of the Schelde estuary for most elements (Table 3). However, this pattern obtained by conservative mixing can be changed by modifications in the distribution coefficient K_D (ratio of particulate versus dissolved trace metals) due to environmental variables in the surface water such as salinity, pH or redox potential (Chaudry and Zwolsman, 2008). An increase in dissolved Cd, Cu and Zn is often observed with the influence of marine water: The hypoxic conditions in the upper estuary promote the precipitation of dissolved Cd, Cu and Zn with sulfides while the dissolution of these complexes occurs under the oxic conditions of marine water (Paucot and Wollast, 1997). Additionally, the formation of metal-chloride complexes increases the mobility of mainly Cd and Zn (Gerringa et al., 2001; Paucot and Wollast, 1997). Dissolved Cu is mainly associated with organic ligands which are progressively mineralized during transport to the sea, resulting in higher dissolved concentrations (Regnier and Wollast, 1993). Dissolved Mn concentrations display an opposite trend, with lower concentrations in the lower estuary due to precipitation as oxy-hydroxides under the oxic conditions of marine water (Paucot and Wollast, 1997). Also As and to a lesser degree Cr and Ni concentrations are found to behave similar to Mn which can be attributed to association of these elements with Fe and Mn oxy-hydroxides under oxic conditions (Baeyens et al., 2007; De Gieter et al., 2005). Baeyens et al. (1997b) found that Cr, Ni and Pb exhibited a conservative mixing behavior in the Schelde estuary. These trends from literature are in good agreement with the difference in trace metal concentrations estimated in the marshes of the different zones of the Schelde estuary (Table 3). Due to the stagnant phase during high tide in CRT’s, deposition of a finer fraction with higher metal concentrations can be expected. However, almost no differences in grain size, OM or metal concentrations between the CRT and the adjacent marsh were observed (Table 1).

Table 4. Estimated input and output of trace metals in the Schelde estuary (tons per year), based on literature and own calculations ([a](Baeyens et al., 1997a); [b](De Smedt et al., 1997); [c](Baeyens et al., 2005); [d]This study). Total metal deposition (tons per year) and metal removal in marshes of the entire estuary compared the estimated riverine input (%), for the period 2005-2010 and for a future scenario with an additional surface area of restored marshes implemented.

in 10^3 kg per year	**Period**	**As**	**Cd**	**Cr**	**Cu**	**Mn**	**Ni**	**Pb**	**Zn**
Freshwater input	1981-1983[a]	-	12.36	-	123.6	-	-	125	386
	1990[b]	-	-	99.3	62.5	-	12.9	120	349
	1995-1998[c]	-	3.86	-	51.2	-	33.0	94.0	283
	2005-2010[d]	22.5	2.60	41.1	40.0	723	23.8	50.9	253
Marine output	1981-1983[a]		0.770		11.1			14.4	42.4
	1995-1998[c]		0.271		6.66		8.2	6.99	25.3
Annual metal deposition marshes	2005-2010[d]	5.06	0.655	21.6	11.6	271	6.46	17.5	73.8
	future[d]	8.70	1.59	36.8	26.3	438	11.5	36.1	173
% removal by marshes	2005-2010[d]	23	25	53	29	38	27	34	29
	future[d]	39	61	90	66	61	48	71	68

2.4.3. Trace metal fluxes in the estuary

The calculated metal accumulation per surface area unit are found to be higher in the Schelde marshes compared to other marshes as summarized by (Rozan and Benoit, 1999). This can be attributed to the relatively high metal concentrations in SPM and high sedimentation rates in the Schelde estuary. However, the total metal accumulation in the marshes of an estuary depends largely on the total surface of the marshes. Less than 8% of the total surface area of the Schelde estuary consists of tidal marshes, 3300 ha, of which the brackish marsh *Saeftinghe* comprises 60% (Table 3). In order to protect the hinterland against flooding and to restore estuarine nature a large surface (950 ha) of flood control areas with controlled reduced tide (CRT) and marsh restoration projects (550 ha) are under construction or planned, mainly in the fresh- and brackish water part of the Schelde estuary (Sigmaplan 2012: personal communication). For the entire estuary an annual estimated amount of 0.7 ton Cd, 18 ton Pb and 76 ton Zn are deposited in the marshes (Table 4). In a future scenario, after implementation of an additional surface of 1500 ha of intertidal areas, metal accumulation in the Schelde estuary is calculated to be 1.6 (e.g. Mn) up to 2.3 times higher (e.g. Cd, 1.6 ton). Riverine Cd input was calculated to be 2.6 ton per year. The assessed estuarine metal removal by deposition in tidal marshes ranged from 23% (As) up to 53% (Cr) and increases up to 39% (As) - 90% (Cr) with the implementation of 1500 ha of marsh restoration projects. However, it must be noted that extrapolation of the results from the small CRT to the planned large scale restoration projects may not be completely correct since implementation of these areas may change the sediment and metal balance of the estuary.

Mass balances of trace metal transport through the Schelde estuary have been calculated before and are closely linked to sediment fluxes. SPM fluxes originating from the river basin are mainly deposited in the upper estuary and SPM concentrations downstream of *Saeftinghe* marsh consist nearly for 90% of material originating from the sea (Verlaan et al., 1998). A large fraction of fluvial SPM is found to be deposited in the high turbidity zone of the estuary (between Antwerp and the border, zone 2) (Verlaan, 2000). Bottom sediments are found to be rich in silt and clay in this zone, while outside this stretch, bottom sediments consist mainly of sand, as observed in the investigated subtidal and tidal flat sediments of present study (Wartel, 1977). The yearly riverine input of sediments is variable and estimated to be on average 214 ± 99 10^6 kg for the period 1992 – 2007 (Claus et al., 2009), 430 10^6 kg by (Baeyens et al., 1997b) and 800 10^6 kg by (Sas and De Jonghe, 1993). Our calculations on sediment accumulation were 230 10^6 kg per year in present marshes and 470 10^6 kg per year after the implementation of marsh restoration projects. Since these values comprise accumulation of marine and fluvial sediments, comparison is difficult. Yet, we can conclude that sedimentation in marshes may have an important effect on sediment balances in the estuary.

In a comparable way as for SPM, the estuary acts as a sink for trace metals and only a small fraction of the riverine input reaches the sea (Baeyens et al., 2005; Ouboter et al., 1997). The results of different studies estimating metal fluxes through the Schelde estuary are very similar (Baeyens et al., 1997a; Baeyens et al., 2005; De Smedt et al., 1997; Van der Kooij, 1982; Van Eck et al., 1991). The total riverine Cd input is estimated at 12 tons per year in 1981-1983 and 3.9 ton per year in 1995-1998 while the marine output remained low, around 0.8 and 0.3 ton per year respectively (Table 4). Metal input estimations decrease with time, determined by the general decline in metal pollution from 1960 to recent years (Baeyens et al., 2005; Zwolsman et al., 1993). Linked to the SPM fluxes, Baeyens et al. (2005) calculated that about 60% of the particulate metal phase is deposited in the maximum turbidity zone, while the marine output flux ranges from 7% (Cd) to 24% (Ni) of the input flux. These studies indicate that up to 90% of the trace metals are removed from the surface water in the Schelde estuary. Based on the calculations of present study, accumulation by tidal marshes contributes for about 25% - 50% to this filtering function of this estuary while they comprise less than 8% of the total surface of the estuary. Sedimentation in subtidal zones and dredging with land disposal are other main sinks for contaminants associated with SPM (Ouboter et al., 1997). In order to ensure access of larger container ships to the Antwerp harbor, deepening of the navigation channel and maintenance dredging is large and dredged volumes increased from ± 7 10^6 m^3 fresh material y^{-1} between 1950 and 1970 up to an average of ± 15 10^6 m^3 fresh material y^{-1} recent years (Flemish government). However, most of the dredged material is dumped again in the estuary, and only a small part (e.g. in 2007, ± 3%, i.e. 300 10^3 m^3 fresh material) is disposed on land (Flemish Government, 2007).

2.4.4. Uncertainties of budget calculations

The flux calculations executed in this study are based on best available data. Certain processes and assumptions which may have had an effect on the results are considered. Trace metal concentrations in SPM or superficial sediment layers are used to calculate metal accumulation in marshes. However, a difference in SPM and deposited sediments may exist and processes as atmospheric deposition and postdepositional diagenetic mobility can change metal concentrations in superficial marsh sediments (Zwolsman et al., 1993).

The uncertainties of metal budget estimates are usually hard to assess (Monbet, 2006). The variables used to calculate fluxes display a certain variability which resulted in a large uncertainty on the final flux values. Trace metal concentrations in SPM or marsh sediments had an average relative error (based on the standard deviation) of 30%. Relative errors of accretion rates and bulk density were 40% and 25% respectively. Additionally, freshwater discharge and water metal concentrations, used to calculate riverine metal input, both had a relative error of 50%. Calculation of metal removal by marshes and propagation of the uncertainties resulted in a large relative error of 180%. This emphasizes that given fluxes are not exact numbers. Yet, values used for the calculations and estimated fluxes were plausible and in accordance with the literature.

2.4.5. Estuaries as filters for trace metals.

The Schelde estuary is found to be an effective filter for trace metals, reducing the metal input into the marine environment. Total metal fluxes in the North Sea were calculated within the framework of the OSPAR Commission, a treatise on ‘protecting and conserving the North-East Atlantic and it resources’ (OSPAR, 2009). A total riverine Cd input of 50 tons in 1990 and 20 tons in 2006 was estimated for the Greater North Sea (OSPAR region II). Additionally, 23 and 13 tons were deposited atmospherically in this region in 1990 and 2006 respectively. With an estimated Cd flux of 0.3 ton to the North Sea in 1995 (Table 4), the Schelde estuary contributes only for a very small fraction to metal fluxes in this region. The catchment areas from the Elbe and Maas/Rhine, which are much larger, have an annual influx of respectively 3 and 4 tons Cd in to the North Sea (2000-2006).

Different studies demonstrate the filtering capacity of estuaries with respect to trace metals. Large metal retention was observed in the Humber estuary, UK (10-97%) (Cave et al., 2005; Millward and Glegg, 1997) and in the Morlaix River estuary, France (86% for Pb) (Monbet, 2006). In the Gironde estuary (France), metal removal was small, and the estuary acted as a source for dissolved metals as Cd and Cu (Audry et al., 2007). Also in the Seine river basin (France), retention was small (0.2 ton Cd) compared to the inflow (3.4 tons Cd) (Thévenot et al., 2007). In last study, a small fraction (0.8%)

of the retention was attributed to metal accumulation in floodplain soils of this river system (Thévenot et al., 2007). Larger metal removal (19% Cu – 45% Zn) by overbank sedimentation in the well developed floodplains of the river Swale (UK), contaminated by historical mining activities are reported (Walling and Owens, 2003). A salt marsh in the Quinnipiac estuary (USA) retained 6% (Cd) up to 17% (Pb) of the riverine metal input (Rozan and Benoit, 1999) and (Rozan and Benoit, 2001) estimated a metal removal of 20%-30% of the riverine input by salt marshes in the same region. Despite the low surface area of tidal marshes in the Schelde estuary, comparable values for metal retention by marshes (23% – 53%) were estimated (Table 3). High turbidity and increasing MHWL in the Schelde estuary might have caused high annual sediment accretion rates and hence metal accumulation per surface unit in Schelde marshes (Table 2) compared to values reported (Rozan and Benoit, 1999). In present study, the implementation of 1500 ha of intertidal areas was calculated to roughly double metal accumulation in the estuary, with an extra storage of about 100 ton Zn y^{-1} or 20 ton Pb y^{-1}. Andrews et al. (2008) calculated an extra storage of 6 ton Zn y^{-1} and 3 ton Pb y^{-1} as a result of a managed realignment scenario in which 26000 ha of intertidal areas will be restored in the Humber estuary (UK). These values are much lower, which can be attributed to the lower metal concentrations and lower accretion rates (1 mm y^{-1}) used (Andrews et al., 2008). Also Andrews et al. (2006) calculated an increase up to 100% of the current annual storage of trace metals in the Humber estuary (UK) in a large realignment scenario. Comparable to present study, it was concluded that the storage of contaminants in the realignment sites enlarges the natural trace metal storage capacity of the estuary, which can improve the long-term water quality and avoid cleaning costs (Andrews et al., 2006; Cave et al., 2005).

The filter function of estuaries reduces contamination of the marine ecosystem, but results on the other hand in accumulation of pollutants and severe contamination of the estuarine habitat. Estuaries from all over the world are found to be contaminated (Birch, 2011; Overesch et al., 2007; Pan and Wang, 2012; Pope and Langston, 2011) which can result in toxicity and adverse effects on the biota living in the estuary (Weis et al., 2011).

2.5. Conclusions

The results of this study indicate that overbank sedimentation in tidal marshes removes a substantial fraction (about 30%) of the riverine metal influx in the Schelde estuary, even though marshes comprise less than 8% of the total surface of the estuary. Metal accumulation is largest in freshwater marshes since dilution with less contaminated marine water resulted in lower metal concentrations in deposited sediments of brackish and salt marshes. Consequently, freshwater marshes have a higher filtering potential and accordingly are often most contaminated. The implementation of 1500 ha flood control areas and tidal marsh restoration projects planned along the Schelde estuary was calculated to almost double the metal accumulation capacity by overbank sedimentation, mainly caused by their high accretion rates. However, high uncertainties indicate that calculated values should be considered as order of magnitude estimates.

Fluxes of trace metals were found to be closely related to transport of suspended solids. The constant accretion in Schelde marshes, caused by the MHWL rise, determines to a large extent the metal accumulation capacity of the marshes. Sedimentation in subtidal areas of the maximum turbidity zone, with land disposal after dredging, is expected to be another important sink for sediments and associated contaminants in the Schelde estuary.

Supporting Information. **Table 1**.

Metal concentrations in sediments (from cores or sediment traps) used for the calculation of metal deposition in the Schelde estuary. INBO = *Flemish (Belgium) governmental research institute for nature and forest*. RWS = *the Dutch ministry for infrastructure and environment, Rijkswaterstaat*.

ZONE	location	km	sampling	date	source	As	Cd	Cr	Cu	Mn	Ni	Pb	Zn
1	**Appels**	**128**	**core**	**2008**	**INBO**	13.74	2.05	49.42	26.34	n.a.	14.84	50.83	250.16
	Zele	**123**	**core**	**2008**	**INBO**	17.60	7.65	221.92	116.59	n.a.	39.28	142.25	846.10
						17.07	7.93	184.55	106.58	n.a.	36.21	133.08	799.68
						17.84	7.67	219.81	119.17	n.a.	39.88	139.71	863.80
						32.34	6.56	240.99	71.09	n.a.	26.39	134.57	784.56
	Gr Schor Hamme	**111**	**core**	**2008**	**INBO**	19.88	6.22	124.61	87.10	n.a.	35.91	114.64	637.92
						21.29	7.28	153.33	96.93	n.a.	34.14	124.34	711.19
						20.09	7.83	153.26	102.14	n.a.	39.46	124.79	753.47
						21.74	6.43	130.18	88.88	n.a.	35.43	113.62	637.73
						28.14	13.70	218.37	142.12	n.a.	52.12	166.36	997.39
						19.15	7.19	117.80	82.86	n.a.	31.14	103.39	685.19
	De plaat	**103**	**sed trap**	**2010**	**own data**	16.27	6.08	90.44	54.49	1075.8	32.85	192.66	496.42
						15.69	6.06	78.28	53.09	1045.8	27.50	103.65	448.33
						16.64	6.40	84.70	60.83	1131.5	29.34	110.44	478.71
						14.61	6.92	88.52	65.04	1213.3	30.20	121.74	522.36
						14.38	6.36	85.24	61.95	1134.4	29.73	110.09	483.70
						16.49	6.66	90.32	64.09	1169.6	30.39	121.88	523.56
						18.79	6.50	90.26	58.85	1182.8	30.51	153.16	526.68
						11.49	5.23	66.52	47.60	876.9	23.16	84.35	393.67
						13.64	5.64	74.69	53.38	994.3	26.06	98.18	425.24
						12.94	6.24	83.11	54.96	1066.9	29.62	109.57	449.83
						14.43	6.36	89.11	60.83	1156.8	30.34	121.99	493.22
						8.83	4.60	70.98	60.87	860.9	25.77	97.00	496.93
						14.95	4.88	72.90	64.33	896.8	26.53	94.90	520.87
						8.91	5.11	70.44	60.55	905.8	26.11	91.03	502.71
						11.78	5.14	76.68	65.19	974.7	27.97	100.25	529.60
						11.45	5.51	79.37	68.38	1047.0	28.93	105.31	578.04
						12.55	5.10	79.84	67.37	992.9	27.97	100.95	527.35
						11.89	5.43	84.14	77.52	1077.6	31.36	113.75	573.01
						12.87	4.86	79.57	61.91	1048.6	27.39	110.65	521.59
						10.10	3.42	54.43	41.51	674.9	19.22	68.38	376.50
						14.55	4.19	67.65	55.92	838.5	24.72	86.22	469.53
						11.60	4.52	70.74	61.53	892.9	25.47	91.88	498.79
						12.67	5.25	77.70	66.87	994.1	28.67	104.60	551.65
						9.72	5.66	81.66	69.95	1035.0	29.62	104.31	551.31
	Kijkverdriet	**94**	**core**	**2005**	**own data**	19.02	5.49	100.98	77.94	n.a.	35.29	135.29	471.57
						28.43	7.84	104.90	92.35	n.a.	40.69	150.00	526.47
						25.00	7.02	110.58	84.04	n.a.	38.56	149.04	520.19
						20.39	6.80	107.77	75.63	1631.1	36.99	148.54	498.06
						29.41	7.65	103.92	83.43	n.a.	37.16	147.06	521.57
						29.95	7.25	120.77	57.87	n.a.	43.29	163.29	547.83
						29.27	6.24	106.34	82.05	n.a.	40.20	142.44	514.15
						32.51	7.98	126.11	82.76	n.a.	42.36	168.47	590.15
						36.45	8.28	120.20	77.04	1497.5	41.77	152.71	582.27
						27.86	8.06	124.38	77.71	1810.9	40.60	157.21	543.28
						20.69	7.00	118.23	88.08	1211.8	37.73	152.71	628.57
						19.32	7.63	93.04	79.03	1516.9	33.24	132.37	578.74
						22.33	8.06	128.16	92.91	1368.9	39.90	162.14	644.66
						31.53	12.22	141.87	128.08	1300.5	43.84	187.19	724.14
						15.92	7.66	107.46	85.27	969.2	33.93	144.28	602.99
						23.53	7.75	104.90	78.04	1333.3	33.63	143.14	602.94
						31.00	8.20	127.00	98.10	1310.0	40.80	164.00	638.00
						23.90	8.98	122.93	88.39	1161.0	39.22	157.07	666.34
						24.04	8.37	118.27	81.92	1105.8	36.15	149.04	591.35

ZONE	location	km	sampling	date	source	As	Cd	Cr	Cu	Mn	Ni	Pb	Zn
1	**Notelaer**	**93**	**sed trap**	**2001**	**own data**	13.90	7.97	134.32	77.63	1065.3	36.50	137.10	754.05
						n.a.	6.34	93.16	116.28	1620.9	36.50	208.60	990.84
						14.07	6.43	108.54	142.71	1175.9	32.56	140.70	668.34
						n.a.	5.34	85.97	116.23	1591.6	28.90	152.88	1560.21
						16.89	7.05	112.27	94.09	1549.9	35.07	150.02	768.01
						12.77	8.45	107.07	137.52	1375.2	35.36	143.42	911.59
						15.72	8.45	111.00	96.95	1208.3	34.97	146.37	992.14
						n.a.	6.31	109.92	98.73	1262.1	33.49	141.48	1078.88
						17.43	8.81	113.32	106.54	1046.0	37.00	135.59	804.84
						13.09	7.55	113.80	82.98	1107.8	38.57	133.94	705.94
						11.03	8.72	110.28	87.42	1022.6	37.89	123.31	706.77
						n.a.	7.76	112.78	93.53	800.8	37.35	137.61	920.85
						14.20	7.95	138.19	78.37	838.6	38.05	136.30	780.88
						13.85	8.42	151.31	84.60	1012.3	39.74	145.98	815.13
						n.a.	8.06	140.65	78.57	834.5	37.32	138.77	808.25
						n.a.	7.43	137.33	100.41	1230.9	37.13	143.44	826.04
						n.a.	6.89	105.96	89.18	1421.6	30.64	137.75	1589.40
						7.91	6.13	102.87	88.72	719.1	37.09	120.67	668.64
						n.a.	3.67	32.59	287.17	2240.3	16.70	164.97	246.44
						4.64	7.79	117.76	158.55	1066.3	34.59	161.34	1010.66
						19.11	7.75	112.53	143.31	1072.2	34.61	140.13	757.96
						13.71	9.40	110.62	180.13	1018.1	34.17	145.86	919.24
						15.22	8.66	107.52	167.46	924.8	32.45	140.82	1065.65
						n.a.	6.99	111.97	124.29	1222.4	33.80	147.92	1212.12
						15.79	8.58	110.51	97.29	985.7	35.92	130.24	756.78
						11.68	8.37	106.08	102.19	874.9	35.62	136.25	695.86
						n.a.	7.90	106.33	91.75	945.8	36.86	129.62	2096.20
						n.a.	8.99	102.46	98.60	890.3	36.35	118.90	2107.30
						n.a.	7.53	112.37	90.31	1072.2	36.70	141.24	1443.30
						n.a.	7.79	129.58	86.76	817.8	37.56	138.97	1314.55
						n.a.	8.09	143.55	82.89	971.4	37.99	138.15	1219.64
						n.a.	8.17	137.83	79.56	846.0	36.98	136.88	1254.75
						n.a.	4.42	65.09	71.09	783.6	23.06	107.42	2407.58
						n.a.	6.57	69.60	121.60	793.2	22.72	119.50	1667.76
						13.22	6.04	103.87	154.86	972.6	31.26	135.03	618.51
						16.51	7.53	108.36	122.81	1403.5	33.13	153.77	987.62
						19.93	7.47	110.61	98.85	1016.4	34.18	140.51	633.78
						18.00	8.60	103.00	173.00	943.0	32.80	147.00	691.00
						19.53	8.37	105.12	123.72	920.9	32.65	145.12	776.74
						14.53	6.59	114.29	122.03	1259.1	35.35	151.09	910.41
						18.30	7.96	104.30	91.49	951.5	34.49	129.00	660.57
						12.41	5.79	98.66	85.01	781.8	33.92	120.99	695.97
						4.22	6.97	105.54	113.98	839.1	34.62	127.70	1108.18
						n.a.	7.61	128.78	97.56	688.8	36.00	134.63	792.20
						5.45	6.87	127.59	70.99	868.0	32.93	133.04	672.85
						13.90	7.32	123.26	71.55	760.9	34.20	124.19	742.35
						n.a.	7.06	130.39	118.63	1058.8	35.29	139.22	837.25
						n.a.	6.79	100.51	80.69	1098.2	29.69	123.78	1563.52
						15.14	13.72	101.92	195.76	1876.9	32.19	178.61	981.84
						21.42	8.36	112.19	122.39	1152.5	34.17	145.84	784.29
						22.41	9.80	104.58	156.86	1083.1	33.80	155.00	714.29
						15.04	9.49	104.32	99.62	1043.2	30.92	137.22	921.05
						n.a.	9.43	112.91	99.17	981.8	35.84	137.46	981.84
						14.00	8.40	104.53	92.95	861.4	34.81	127.86	751.28
						n.a.	7.62	106.72	78.61	1010.0	35.64	128.63	769.89
						12.68	8.10	97.17	77.07	882.9	32.88	114.15	739.51
						n.a.	8.19	101.68	89.73	802.6	35.04	117.47	1145.11
						17.10	8.19	126.01	86.50	714.7	37.89	137.71	777.68
						n.a.	7.98	137.66	82.29	884.8	36.31	137.66	760.10
						n.a.	8.23	134.04	81.28	787.0	36.00	135.95	780.28
						n.a.	5.37	102.33	82.46	766.0	30.40	97.37	789.87
						n.a.	5.88	87.26	100.75	910.7	26.63	114.12	1971.18
						21.62	9.59	116.54	103.38	968.0	38.63	138.16	773.50
			core	**2008**	**INBO**	35.11	4.86	107.85	146.93	957.1	35.34	151.79	429.08
						36.72	7.12	112.32	153.48	1586.0	44.19	154.34	730.58
						19.78	5.46	164.15	121.36	n.a.	25.61	103.98	637.69

ZONE	location	km	sampling	date	source	As	Cd	Cr	Cu	Mn	Ni	Pb	Zn
2	**Schor oude doel**	**62**	**core**	**2008**	**INBO**	13.19	1.91	45.85	23.95	n.a.	12.23	41.62	235.65
						51.58	3.83	89.53	151.69	957.02	36.15	135.39	439.67
						22.90	1.81	42.57	87.59	872.19	22.76	66.43	262.53
						14.95	2.05	29.03	52.10	620.84	16.15	48.14	224.34
						27.60	3.89	69.89	115.13	1278.77	34.57	104.11	437.98
						27.82	2.90	57.75	105.43	1134.41	29.25	87.40	369.16
						27.63	3.35	62.94	104.66	1002.17	30.91	93.42	402.61
						22.93	2.58	47.03	92.10	1051.71	25.73	72.29	320.26
	Kruibeke	**80**	**core**	**2005**	**own data**	26.21	5.36	80.55	60.38	750.63	24.86	83.43	381.93
						20.35	3.51	68.54	50.99	692.30	20.95	77.07	355.64
						20.33	4.52	62.84	45.75	604.60	18.44	70.38	338.01
						18.16	3.19	62.67	44.75	655.88	18.50	70.26	336.54
						19.48	5.00	74.42	56.99	695.17	22.59	81.50	369.77
						20.47	3.51	70.98	53.25	669.25	21.68	80.01	359.19
						23.96	4.97	72.38	53.61	705.48	20.82	80.60	361.01
						22.09	4.15	78.34	59.46	827.27	23.01	90.45	412.84
3/4	**Border**	**58**	**SPM**	**2005-2008**	**RWS**	29	3.50	110	72	1500	33	100	400
						31	3.90	110	70	1400	31	100	430
						32	3.70	100	64	1300	29	95	400
						35	3.70	100	65	1200	30	95	420
						33	4.20	94	72	1200	31	100	460
						33	3.80	110	65	1400	33	96	410
						32	3.80	110	63	1500	33	99	390
						32	3.70	120	65	1500	37	110	400
						30	3.20	110	61	1700	31	100	370
						29	3.10	100	56	1800	31	93	370
						30	2.80	120	59	1800	38	99	360
						31	2.60	100	54	1600	30	90	340
						31	3.30	100	57	1400	29	90	350
						28	3.70	100	58	1500	30	86	360
						28	3.70	100	58	1500	30	89	340
						29	2.80	90	51	1600	27	82	340
						30	3.00	94	55	1400	28	83	340
						29	3.00	91	54	1400	26	81	340
						30	4.10	99	60	1400	28	88	370
						28	3.60	87	51	1400	25	75	320
						30	4.40	90	55	1400	25	80	320
						30	3.30	92	52	1400	26	77	310
						32	3.70	88	51	1400	26	82	330
						33	4.20	99	63	1400	29	90	360
						31	3.90	95	54	1300	27	79	340
						30	4.80	100	65	1300	30	95	410
						21	4.40	100	63	1600	31	86	400
						24	5.10	100	65	1400	29	88	430
						22	5.00	110	69	1500	34	94	460
						22	4.50	100	68	1600	31	85	440
						23	4.70	110	69	1400	33	90	440
						20	4.40	93	61	1400	28	82	420
						18	4.70	110	70	1200	35	94	450
						18	4.30	100	65	1400	30	91	410
						22	4.50	100	64	1300	29	89	400
						17	4.00	97	57	1400	28	87	370
						20	4.30	100	62	1400	30	91	360
						21	4.10	100	62	1500	31	91	360
						22	4.00	99	62	1600	29	90	360
						23	4.20	110	68	1700	32	94	370
						26	3.40	93	54	1600	25	81	320
						27	4.40	100	63	1700	29	90	370

ZONE	location	km	sampling	date	source	As	Cd	Cr	Cu	Mn	Ni	Pb	Zn
3/4	**border**	**58**	**SPM**	**2005-2008**	**RWS**	25	4.10	100	62	1600	31	92	360
						23	3.30	96	52	1500	28	84	340
						20	3.70	100	65	1500	32	92	360
						19	4.00	100	63	1600	30	93	390
						17	3.30	97	59	1400	29	81	370
						21	4.40	110	68	1500	30	92	400
						21	3.90	100	61	1400	28	86	380
						21	4.80	110	71	1500	30	91	440
						23	4.20	99	63	1500	28	85	420
						24	4.30	95	59	1400	28	82	400
						21	4.90	100	67	1400	30	90	460
						23	4.40	110	69	1400	30	89	440
						24	6.50	100	76	1500	30	97	480
						20	6.10	100	78	1500	31	99	510
						11	5.50	100	74	1400	29	95	480
						16	5.10	100	68	1500	29	89	440
						20	4.90	100	70	1400	29	93	440
						20	4.60	96	65	1300	29	91	420
						21	4.70	96	66	1400	28	90	420
						20	4.30	98	63	1400	28	93	410
						18	4.30	100	65	1100	29	97	420
						20	4.10	99	57	1600	30	90	430
						20	3.40	93	50	1500	28	81	390
						22	3.30	92	54	1500	29	86	390
						22	3.70	91	54	1500	28	84	390
						21	3.70	95	59	1500	27	87	390
						23	3.90	94	61	1300	28	89	410
						18	3.60	96	61	1500	28	88	410
						17	3.70	92	58	1400	26	81	400
						19	3.80	100	62	1400	29	87	410
						19	4.20	95	63	1400	27	85	430
						20	4.80	110	72	1400	32	96	480
						22	4.90	110	80	1400	31	91	480
						21	5.10	100	84	1400	32	95	550
						22	4.50	99	78	1300	30	90	470
						24	3.90	91	63	1500	28	82	430
						n.a.	4.50	89	53	1400	29	83	440
						n.a.	4.40	94	52	1209	31	82	420
						n.a.	4.70	100	55	1418	30	83	430
						n.a.	4.50	110	55	1270	32	85	450
						n.a.	4.60	100	72	1260	31	94	390
						n.a.	4.10	110	65	1460	32	90	360
						n.a.	3.80	100	61	1340	29	85	330
						n.a.	3.40	91	50	1260	29	79	330
						n.a.	3.30	78	48	1195	25	69	320
						n.a.	4.30	100	68	1380	30	89	360
						n.a.	4.30	100	62	1140	33	100	390
						n.a.	4.20	99	58	1200	32	89	360
						n.a.	4.50	100	70	1200	32	94	390
						n.a.	4.10	99	62	1250	31	94	370
						n.a.	3.90	94	59	1320	29	88	350
						n.a.	4.20	100	65	1380	32	95	380
						n.a.	3.60	81	48	1505	26	75	300
						n.a.	3.30	94	54	1350	30	84	320
						n.a.	3.60	91	53	1340	29	81	330
						n.a.	4.00	100	64	1100	32	93	360
						n.a.	4.60	130	82	1090	39	110	420
						n.a.	4.00	97	57	1230	30	83	350
						n.a.	4.70	110	67	1380	32	94	400
						n.a.	4.80	110	71	1370	33	100	430
						n.a.	3.80	100	64	1230	32	89	360

ZONE	location	km	sampling	date	source	As	Cd	Cr	Cu	Mn	Ni	Pb	Zn
3/4	**Hansweert**	**35**	**SPM**	**2005-2008**	**RWS**	21	1.40	100	33	1209	28	67	220
						24	1.70	110	66	1418	32	81	280
						22	1.50	110	31	1270	30	72	210
						22	1.20	100	30	1260	29	70	200
						23	1.50	110	35	1460	30	74	240
						20	1.50	95	32	1340	27	63	220
						18	1.40	91	52	1260	26	54	190
						18	1.40	92	26	1195	27	55	185
						22	1.60	100	31	1380	28	67	220
						17	0.94	87	24	1140	24	53	160
						20	1.00	92	27	1200	26	56	190
						21	1.10	96	28	1200	27	61	190
						22	1.20	100	30	1250	29	65	200
						23	1.40	110	34	1320	31	69	210
						26	1.90	110	37	1380	33	71	240
						27	2.00	120	39	1505	33	78	260
						25	1.90	110	41	1350	31	74	260
						23	1.70	98	56	1340	29	67	240
						20	1.30	91	28	1100	26	56	200
						19	1.50	93	26	1090	26	53	190
						17	1.30	87	24	1230	26	50	190
						21	1.70	110	170	1380	35	67	235
						21	1.10	110	27	1370	31	61	190
						21	1.20	100	27	1230	30	61	180
						23	1.80	100	34	1270	30	68	220
						24	1.60	110	40	1300	31	71	230
						21	1.30	98	29	1080	29	61	200
						23	1.70	97	34	1150	29	68	225
						24	2.30	110	38	1290	32	77	250
						20	1.30	92	28	1040	28	64	190
						11	3.20	37	11	270	12	15	190
						16	1.10	71	41	1050	22	46	180
						20	1.40	98	33	1187	27	61	197
						20	1.20	100	35	1290	30	63	190
						21	1.60	100	32	1380	29	67	210
						20	1.30	100	30	1360	29	65	190
						18	1.20	95	27	1270	27	66	170
						20	1.50	98	32	1220	27	68	200
						20	1.40	100	36	1220	28	67	190
						22	1.50	110	38	657	31	75	230
						22	1.50	110	33	805	30	71	230
						21	1.10	100	30	842	29	68	200
						23	1.40	110	32	1160	31	76	230
						18	1.10	76	29	930	23	53	190
						17	1.30	91	27	960	27	57	190
						19	1.40	100	28	810	30	59	190
						19	1.20	90	27	940	26	58	190
						20	1.20	100	150	810	28	62	200
						22	1.30	110	34	1045	29	64	200
						21	1.00	95	40	1000	26	56	170
						22	1.20	98	40	770	28	64	200
						24	1.40	110	37	1220	32	74	220

ZONE	location	km	sampling	date	source	As	Cd	Cr	Cu	Mn	Ni	Pb	Zn
4	**Terneuzen**	**19**	**SPM**	**2005-2008**	**RWS**	14	0.57	69	16	970	19	39	110
						17	0.65	78	19	1040	22	48	130
						17	0.77	83	21	920	23	50	140
						21	0.95	100	27	790	30	64	180
						18	0.72	83	20	770	23	50	150
						15	0.92	96	20	860	23	45	160
						14	0.78	74	19	750	21	39	140
						16	0.94	82	20	980	24	43	150
						15	0.77	72	18	740	20	37	140
						17	0.85	88	24	1230	25	49	155
						18	0.89	90	30	1000	26	50	160
						15	0.66	81	19	960	21	43	130
						21	0.95	100	27	860	31	68	190
						19	0.78	87	22	780	25	54	150
						21	1.00	93	25	710	27	54	170
						19	0.97	87	24	720	24	51	160
						18	0.86	77	22	760	22	44	145
						17	0.73	72	19	330	21	43	140
						17	0.83	78	38	900	22	46	160
						19	0.93	81	22	1050	23	45	160
						16	0.97	80	21	980	24	43	160
						14	0.56	70	17	1010	21	37	120
						19	0.75	99	24	920	30	55	170
						18	0.73	100	24	1090	29	53	160
						19	0.77	89	24	790	27	52	150
						17	0.74	83	21	1110	24	48	140
						16	0.66	76	19	679	23	44	130
						16	0.69	71	18	559	21	43	130
						16	0.78	72	19	523	21	43	130
						17	0.73	74	19	710	22	47	130
						11	1.20	44	14	700	12	20	120
						14	0.83	67	19	780	21	40	140
						17	0.86	83	23	780	24	51	160
						18	0.84	85	26	820	26	51	150
						17	1.10	83	22	700	25	47	150
						16	0.76	81	22	730	23	46	140
						16	0.82	85	23	830	25	53	140
						14	0.71	74	18	670	20	42	120
						18	1.10	95	30	760	26	62	160
						17	0.57	83	24	720	23	48	130
						15	0.70	76	18	610	20	42	120
						21	1.10	100	31	740	29	67	190
						20	1.00	110	25	750	29	64	180
						16	0.64	68	16	750	21	38	140
						15	0.75	77	24	770	23	47	140
						15	0.66	83	19	670	22	40	120
						13	0.58	68	16	800	18	34	110
						15	0.60	71	17	770	21	39	120
						15	0.65	75	18	810	21	42	120
						19	0.72	91	23	770	26	52	150
						15	0.54	75	18	750	20	40	110
						19	0.75	90	30	770	27	55	150

ZONE	location	km	sampling	date	source	As	Cd	Cr	Cu	Mn	Ni	Pb	Zn
4	**Vlissingen**	**0**	**SPM**	**2005-2008**	**RWS**	17	0.51	76	17	800	21	45	120
						14	0.50	63	15	660	17	36	100
						13	0.39	59	13	680	16	32	90
						19	0.42	80	16	690	24	47	130
						19	0.51	70	15	530	21	41	120
						14	0.76	73	18	730	22	39	140
						13	0.59	83	81	880	26	39	140
						15	0.73	75	17	630	22	39	130
						14	0.67	67	16	730	19	35	120
						14	0.60	70	17	810	20	39	120
						16	0.64	80	22	800	24	44	140
						16	0.44	68	15	740	20	40	110
						19	0.38	86	17	920	26	50	130
						19	0.37	77	16	657	23	47	120
						16	0.52	67	14	480	19	36	110
						19	0.55	84	18	680	24	46	130
						20	0.43	85	19	743	26	51	140
						22	0.52	79	28	565	24	48	150
						19	0.48	94	68	560	30	47	140
						23	0.65	84	17	730	26	38	130
						14	0.63	71	61	620	23	39	150
						15	0.55	75	85	670	23	40	130
						18	0.47	79	16	690	24	43	120
						17	0.35	78	170	615	23	45	120
						17	0.47	88	17	650	25	45	130
						19	0.41	83	18	n.a.	25	49	130
						18	0.49	83	19	n.a.	25	48	140
						17	0.48	71	17	n.a.	22	43	120
						18	0.45	75	17	n.a.	23	46	120
						17	0.63	74	18	n.a.	23	46	120
						14	0.59	60	14	n.a.	17	30	120
						14	0.52	63	39	n.a.	20	37	120
						16	0.47	73	29	n.a.	25	44	150
						18	0.36	60	47	n.a.	22	34	120
						17	0.57	66	16	n.a.	20	38	110
						15	0.58	75	20	n.a.	23	42	120
						14	0.60	81	19	n.a.	22	43	120
						16	0.54	78	17	n.a.	22	44	110
						18	0.49	84	18	n.a.	24	48	130
						17	0.74	91	26	n.a.	27	54	140
						18	0.59	87	35	n.a.	26	51	130
						19	0.36	85	18	n.a.	25	50	130
						19	0.46	83	24	n.a.	25	50	130
						17	0.48	48	15	n.a.	16	30	110
						14	0.51	71	100	n.a.	22	40	120
						16	0.58	82	19	n.a.	25	46	125
						17	0.48	81	39	n.a.	23	45	120
						17	0.51	98	230	n.a.	29	46	120
						16	0.45	77	43	n.a.	23	44	120
						15	0.38	73	17	n.a.	19	37	100
						16	0.42	67	32	n.a.	21	40	110
						18	0.55	82	62	n.a.	25	48	130
	Wielingen	**-10**	**SPM**	**2005-2008**	**RWS**	18	0.44	76	16	n.a.	22	46	120
						28	0.51	61	23	n.a.	19	30	140
						15	0.39	78	17	n.a.	25	36	130
						17	0.31	83	22	n.a.	25	46	120
						17	0.33	67	14	n.a.	21	38	100
						17	0.38	66	16	n.a.	24	38	130
						16	0.42	175	140	n.a.	65	41	130
						17	0.36	69	19	n.a.	21	38	110
						19	0.32	87	17	n.a.	27	50	130
						15	0.40	65	15	n.a.	20	37	110
						18	0.31	65	25	n.a.	21	33	93
						16	0.37	72	14	n.a.	21	40	98
						17	0.35	72	15	n.a.	21	43	110
						15	0.39	54	17	n.a.	18	29	99
						17	0.36	73	14	n.a.	22	40	100
						15	0.37	65	13	n.a.	19	33	93

CHAPTER 3

Evolution of sediment metal concentrations in a tidal marsh restoration project.

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Abstract

The combination of flood prevention and tidal marsh restoration will be implemented on a large scale in the Schelde estuary (Belgium). Densely populated and industrialized, this estuary was found to be severely contaminated with trace metals. In this study we evaluated the effect of tidal restoration on sediment trace metal concentrations. To asses historical contamination of embanked-, a restored- and natural tidal areas, deep sediment cores were sampled while the evolution of metal concentrations was determined by means of superficial samples taken during 10 sampling campaigns spread over the first 3 years of the restoration project. Metal concentrations in the natural tidal marsh reflected the estuaries' contamination history. Fertilization by irrigation caused high metal concentrations in superficial soil layers of some embanked areas. However, reintroduction of the tide resulted in deposition of a new sediment layer with lower metal concentrations, comparable to the natural tidal marsh. Despite diagenetic mobility of manganese no diagenetic movements of the trace metals were observed during these first three years. Removal of metals from the estuary and burial of contaminated sediments in the restored site emphasizes the potential of these restoration projects to decrease metal contamination risks. However, more research under field conditions on the effects of changes in land use and inundation related changes in metal bioavailability is needed to draw clear conclusions on the environmental consequences.

3.1. Introduction

Global change induced sea level rise and increased storm event frequency, combined with embankments and channel deepening have intensified flood risk in many estuaries worldwide. As these coastal areas are often densely populated and economically important, flood protection is of major importance. Managed realignment and controlled flooding of formerly embanked areas have proven to be effective and economically viable strategies for flood control (French, 2006; Hey and Philippi, 1995; Zedler and Kercher, 2005). Moreover, the possibility to combine tidal marsh restoration in the same area makes this technique even more valuable. Since tidal areas are one of the most valuable ecosystems in the world which provide many ecosystem services (Costanza et al., 1997), storm flood mitigation by expanding the surface of tidal areas will additionally improve other economic, social and ecological functions of estuaries.

To comply with EU-conservation goals and compensate for habitat loss by industrial development, thousands of hectares of tidal marsh need to be restored in the Schelde estuary (Belgium and the Netherlands). Many of these areas will be restored by means of the controlled reduced tide technique (CRT, (Beauchard et al., 2011; Cox et al., 2006; Maris et al., 2007)). In this approach, embanked areas provide storage capacity during storm tides, while a spring-neap tidal cycle introduced in these areas enables estuarine habitat to develop (Jacobs et al., 2009; Maris et al., 2007).

However, severe pollution reported in many estuaries should to be taken into account when tidal marshes are being restored. By concentrating the runoff from their entire catchment basin, estuaries are hotspots for persistent contaminants. The Schelde estuary is a typical example, with high trace metal concentrations in surface water, suspended matter and sediments (Baeyens et al., 2005; Teuchies et al., 2008; Vandecasteele et al., 2002a). Also embanked polders are expected to contain elevated metal concentrations. These areas in the fresh water tidal range of the estuary were periodically flooded during winter to provide nutrient-rich suspended solids to the agricultural fields. Since metals are known to show a high affinity for suspended particulate matter (Regnier and Wollast, 1993) this irrigation technique often resulted in deposition of metal contaminated sediments.

When reconnecting these embanked polder sites to tidal inundation in restoration projects this might have important consequences for metal concentrations and availability in the embanked area for several reasons. First, tidal areas are known to accumulate trace metals by means of sedimentation (Rozan and Benoit, 1999). Since metal concentrations in surface water decreased considerably during the last decades, it is hypothesized that concentrations in newly deposited sediments will be lower than the concentrations from historical depositions in the embanked area. Secondly, inundation of former agricultural land will result in hydrology related physical-chemical changes which can alter metal mobility (Du Laing et al., 2009c). Increasing mobility might consequence in higher metal bioavailability to flora and fauna. Additionally mobility determines the ability of diagenetic movements of metals in historically and newly deposited sediments. Manganese- and iron oxides as well as sulfides are the most important binding phases which can reduce metal mobility in sediments (Du Laing et al., 2009c; Gambrell, 1994). Inundation is expected to reduce the concentration of oxides while sulfides might become more important in reducing metal mobility (Teuchies et al., 2011). However, the oxidation-reduction conditions are depending on many factors e.g. flooding frequency and duration, plant growth, water oxygen concentrations or organic matter content. And thirdly, changes in land use, from agriculture to nature and recreation, and introduction of new species, for example wetland plants or edible fish, might change the potential risk of present contaminants.

Projects where flood mitigation and tidal marsh restoration are combined have been implemented increasingly. Metal contamination, often present in estuaries where this technique is applied, cannot be ignored. The objectives of this study were:

- To explore differences in trace metal contamination between natural tidal areas and polder areas suitable for marsh restoration.
- To examine changes in sediment trace metal concentrations during the first three years of a CRT pilot project in the Schelde estuary (Belgium).
- To discuss the effect of flooding on metal mobility in the polder soil and deposited fresh sediments.

3.2. Materials and methods

3.2.1. Study area

The Schelde estuary (Belgium) is a turbid, macrotidal and eutrophic system (Meire et al., 2005) which gets a considerably large input of trace metals (Baeyens et al., 2005). The study area (51°05’10’’N; 4°10’20’’E) is situated in the freshwater zone of the estuary (Fig. 1). Lippenbroek (LB) is an experimental restored area designed for multidisciplinary research on a new tidal marsh restoration technique (Cox et al., 2006; Jacobs et al., 2008; Jacobs et al., 2009; Maris et al., 2007). This technique combines flood control and marsh restoration: water storage in embanked areas enlarges storage capacity during storm tides whereas a reduced tide is introduced by a simple system of culverts enabling estuarine habitat to develop in the flood control area (Maris et al., 2007).

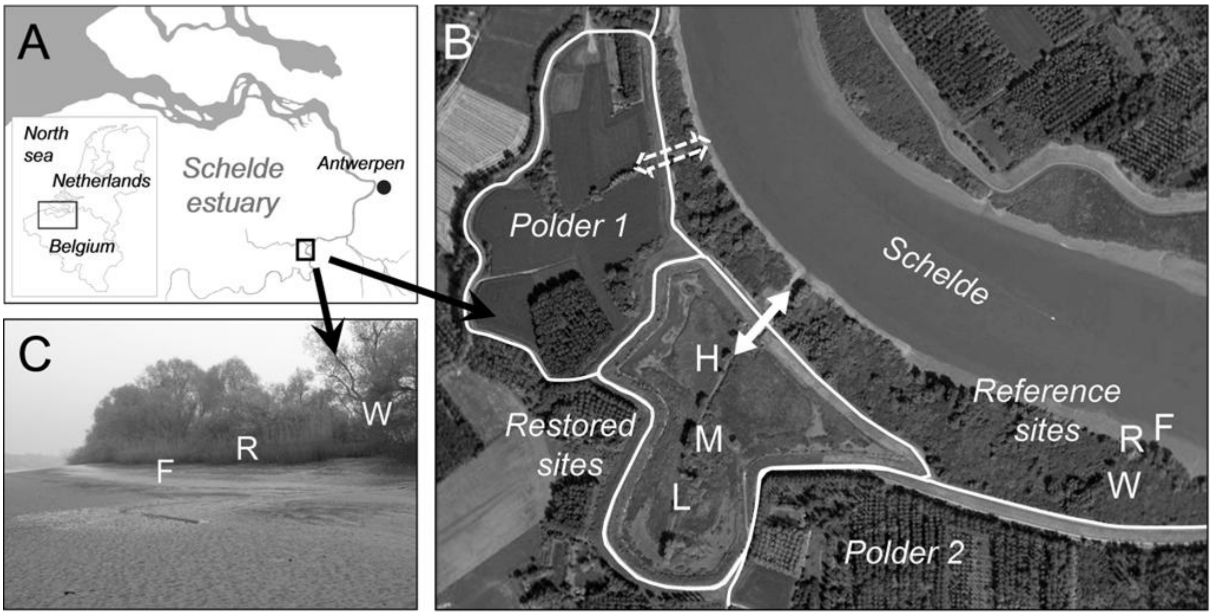

Figure 1. A) Location map of the study area. B) Aerial photograph of the restored-, adjacent reference- and polder areas. Reference site W = high willow tidal marsh; R = low reed tidal marsh; site F = tidal flat. Sites in the restored marsh have high (H), mid (M) or low (L) elevation.

Prior to restoration, this embanked area consisted of cropland with a central ditch for drainage of the polder. A valve allowed drainage during summer and sporadic irrigation with Schelde water during winter. Agricultural activities were abandoned in 2003. In 2004 – 2005 dikes and culverts were constructed and the polder received its definitive tidal regime on March 1st 2006. The tidal amplitude in the estuary (5.2 m at this location) is reduced in the area to 0.9 m and high water levels are lowered in order to optimally restore a tidal gradient in the embanked area (for extensive hydrological description, see Beauchard et al., 2011).

In the restored area, three sampling sites covering a flooding gradient were considered: a low-elevation site (L), a mid-elevation site (M) and a high-elevation site (H). Three reference sites were monitored in the adjacent estuary; these include a tidal flat without vegetation (F), a low marsh site overgrown with *Phragmites australis* (common reed, R) and a site in the high marsh overgrown with *Salix sp.* (willow, W). Figure 2 displays the morphological and hydrological characteristics of the sites. In the restored sites, sediment deposition was rapid and elevation-dependant, with a higher rate at site L, leading to a progressive flattening of the polder surface. The agricultural soil at site L was covered by a large layer of estuarine sediments in the first three years (for more details, see Vandenbruwaene et al., 2011).

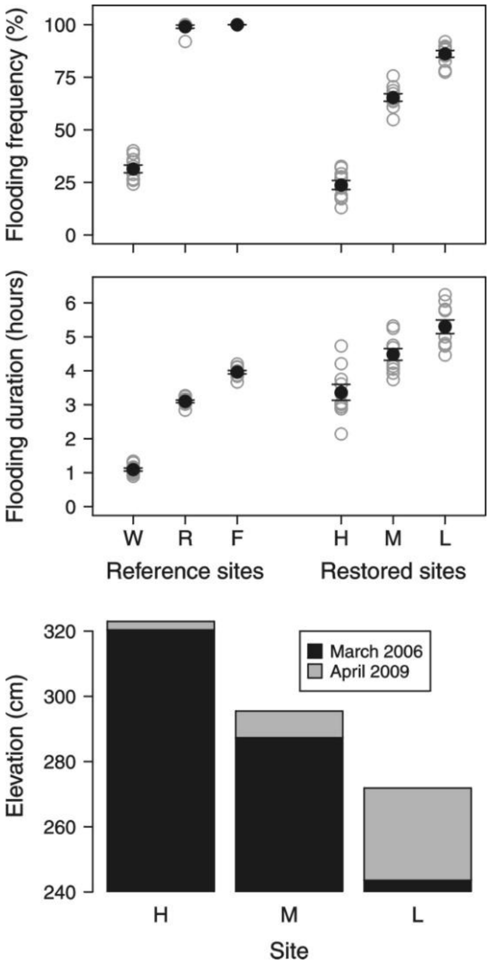

Figure 2. Flooding duration and frequency in restored and reference sites and elevation change at reference sites; flooding frequency was defined by the number of inundating tides as a percentage of the total number of tides. Black points represent mean values (± SE) of 13 seasonal mean values from spring 2006 until spring 2009 (grey points). Elevation is measured compared to the Belgian see level reference.

Additionally, two sites were sampled in adjacent polders (= embanked areas) with different inundation and metal contamination history. One sampling site was situated in a polder to the NW of the restored area (P1). A second sampling site was situated in a polder to the SE of the restored area (P2). Both polders were still cultivated (maize). On a historical map of 1790 polder 2 (P2) was embanked without a direct connection between the polder and the river. As in the restored area, a valve between the embanked P1 and the estuary existed and was used to fertilize by means of irrigation. In P2, this practice had ceased before start of the ninetieth century. Between 2006 and 2009 no inundation in both studied polders (P1, P2) has been recorded.

3.2.2. Sampling

In June 2007, 1 m deep sediment cores were taken with a shell bit (5 cm Ø) (not in site R). At each site, two replicates, each based on four mixed cores, were taken randomly in a 2 meter radius. The cores were sliced (0-5; 5-10; 10-15; 15-20; 20-30; 30-40; 40-50; 50-60; 60-80; 80-100 cm) and sections were mixed per depth.

In the restored and reference areas, monitoring was carried out during 9 sampling campaigns from April 2006 to April 2008 (once every three months), plus one additional campaign in April 2009. Three soil strata were considered: 0-5 cm, 5-10 cm and 10-15 cm. A shell bit (1 cm Ø) was used for soil extraction and strata were separated, each based on 10 to 15 mixed cores. Sediment resistance was measured in situ with a hand penetrometer (Eijkelkamp, 06.01.SA).

3.2.3. Laboratory analyses

Sediment samples were separately stored in polyethylene recipients. In the lab, they were immediately weighed, air dried and ground. A subsample was weighed, oven dried (105 °C) to a constant weight and then reweighed to estimate sediment water content and to correct measured metal concentrations when expressed per g dry mass (DM).

Granulometry of the samples was determined with a laser diffraction particle size analyzer (Malvern S, Malvern Instruments Ltd, Worcestershire, UK). Content of clay (< 4 µm), very fine silt (4-8 µm), fine silt (8-16 µm), medium silt (16-31 µm), coarse silt (31-63 µm) and sand (> 63 µm) and mean grain size were determined. Organic matter (OM) was estimated through loss on ignition. For this purpose, weight difference of oven dried (105 °C) versus incinerated sediment (6h at 550 °C) was determined.

Air-dried soil was used for determination of total metal concentration (As, Cd, Cu, Cr, Ni, Pb, Zn and Mn) after “hot block digestion” for which 5 mL nitric acid (HNO_3 69% Normapur Trace Analysis Cd Hg Pb) and 5 mL of hydrogen peroxide (H_2O_2 27% for electrical purpose) are added to 0.2 g of sample. This mixture was heated (30min at 100 °C), cooled overnight and heated again (60 min at 150 °C). Metal concentrations in the resulting mixture were measured after filtration using inductively coupled plasma (ICP) type radial plasma iris/charge injection device (CID) (Thermo). Analytical accuracy was achieved by the use of blanks and certified reference material for sediment (Institute for Reference Materials and Measurements (IRMM), BCR® № 320, channel sediment) included in all series of metal analysis in order to verify the analytical procedure. All values from the reference samples were lower but within 90% of the certified values for As, Cd and Zn, and within 80% for Cu, Ni, Pb and Mn. Recovery of Cr was low (50%). Variation between recoveries was very low for all elements.

3.2.4. Data analysis

3.2.4.1. Time effect on trace metal concentrations

The significance of differences in metal concentrations between spring 2006 and spring 2009 was assessed by Wilcoxon's signed-rank tests. The results obtained for As, Cd, Cr, Ni, Pb and Zn were very similar but differed from patterns observed for Mn. To minimise the amount of graphs, Zn and Mn concentrations were used to illustrate the observed trends among the monitoring sites while the graphs of the other elements are displayed in supplementary material. .

3.2.4.2. Relationship between sediment characteristics and metal concentrations

Given the general affinity between trace metals and the physicochemical properties of the sediment, the relationship between sediment characteristics and trace metals was explored. For this, a co-inertia analysis (Dray et al., 2003) was applied on the dataset. The data were sorted in two tables sharing the same lines (10 seasons × 6 sites × 3 strata), which were then standardized. In the co-inertia principle, the two tables define two multidimensional point clouds which are combined in order to construct a system of axes which maximize the common information between them. Statistically, the similarity between the two patterns was assessed by the Rv coefficient which is equivalent to a simple Pearson's correlation coefficient between two variables (Escoufier, 1973). Its significance was tested by a Monte-Carlo procedure, based on 9999 random permutations of the table lines (Heo and Gabriel, 1998). Computations and graphical representations were performed using R software (R Development Core Team). The co-inertia analysis was realized under the *ade4* package.

3.3. Results

3.3.1. Deep cores: historical contamination

Large differences in metal concentrations were observed between sites and depths (Fig. 3). Zn concentrations were higher in P2 than in P1 where values did not exceed 60 µg g^{-1} DM. In P2, concentrations decreased with depth from more than 1500 µg g^{-1} DM near the surface to circa 400 µg g^{-1} DM in the deepest layer. In site W, the concentration increased with depth from 400 µg g^{-1} DM near the surface to more than 1000 µg g^{-1} DM in the deepest sediment layer. Lower concentrations were measured in site F, which never exceeded more than 600 µg g^{-1}. In the restored area, sites L and M profiles were found to be similar. An abrupt increase occurred from top sediment (500 µg g^{-1} DM) to 30 cm deep (circa 1200 µg g^{-1} DM), followed by a slight decrease toward the deepest layer (circa 900 µg g^{-1} DM). In site H, the concentration was globally lower, and decreased in a progressive way, from higher values in top sediment (800 µg g^{-1} DM) to 100 µg g^{-1} DM in the deepest layer.

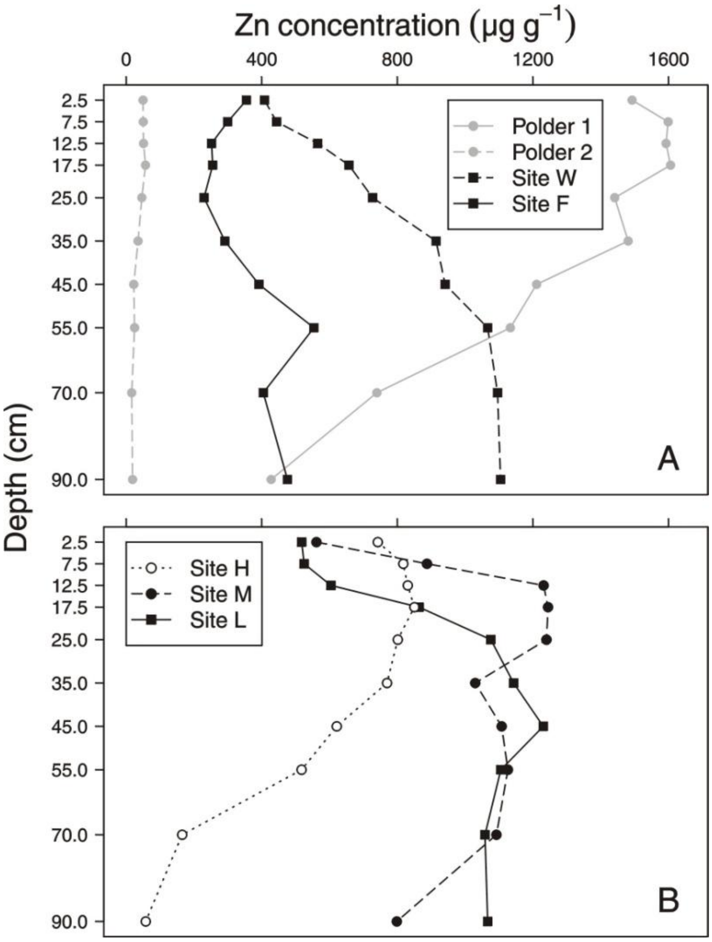

Figure 3. Average soil Zn concentration in polder and reference sites (A) and in the restored sites (B) in June 2007. . Polder 1, NW and Polder 2, SE of the restored area. Reference site W = willow tidal marsh; site F = tidal flat. Sites in the restored marsh have high (H), mid (M) or low (L) elevation.

3.3.2. Long term data from superficial cores: effect of tidal restoration.

3.3.2.1. Trace metal concentrations

In general, metal concentrations in the reference area were lower than those in the restored area (Table 1). Only Cd concentrations exceeded the Flemish soil sanitation threshold for nature areas in site R and W. No significant changes in metal concentrations between 2006 and 2009 were observed in the reference sites.

In the restored area, general trends in soil metal concentrations were comparable for As, Cd, Cr, Cu, Ni, Pb and Zn. High values were observed in the restored area in 2006. Cd and Cr concentrations exceeded their sanitation thresholds in all sites. At that time, sites L and M were the most contaminated with exceeded sanitation thresholds for Pb, Zn (M, L) and As (only site M). In 2009, all metal concentrations, except for Cd, were below the sanitation threshold in site L. Sanitation thresholds were still exceeded in deeper layers in site H (Cd, Cr) and site M (As, Cd, Cr, Pb, Zn). Trends in this group of metals can be distinguished from trends observed for Mn concentrations, which increased significantly in the restored sites between 2006 and 2009.

Temporal trends of all metal concentrations are appended to supplementary material (Fig. S1 and Fig. S2). Zn and Mn concentrations illustrate the main trends (Fig. 4). In spring 2006, Zn concentrations in the restored area were higher than in the reference sites (Fig. 4). An overall decrease was observed in the restored sites during the 3 years of tidal influence. The decrease was prompt in all soil strata of site L, resulting in a decrease from more than 1200 µg g^{-1} DM to lower values in al strata (circa 500 µg g^{-1} DM) in 2009. The decrease was comparable in the upper layer in site M but less pronounced in the deeper layers which resulted in stratification. In 2009, Zn concentrations in the upper layers were similar in all restored sites and comparable with values in the reference site W (between 500 and 600 µg g^{-1} DM). Overall Zn concentrations in the reference sites did not exhibit significant changes during the study period. Nevertheless, increasing values were found in the top soil between 2006 and 2009. Globally, Zn concentrations in site W (circa 500 µg g^{-1} DM) were higher than in site R (circa 400 µg g^{-1} DM) and site F (circa 250 µg g^{-1} DM).

Contrary to Zn, average soil Mn concentrations in the restored sites increased significantly between 2006 and 2009. This trend was observed for all strata of site L and M. Stratification resulted in higher values in the upper layer in all restored sites. Comparable to Zn, increasing Mn concentrations in the reference sites were found in the upper 5 cm between 2006 and 2009. Again, Mn concentrations were higher in site W (circa 800µg g^{-1} DM) compared to site R (circa 600 µg g^{-1} DM) and site F (circa 400 µg g^{-1} DM).

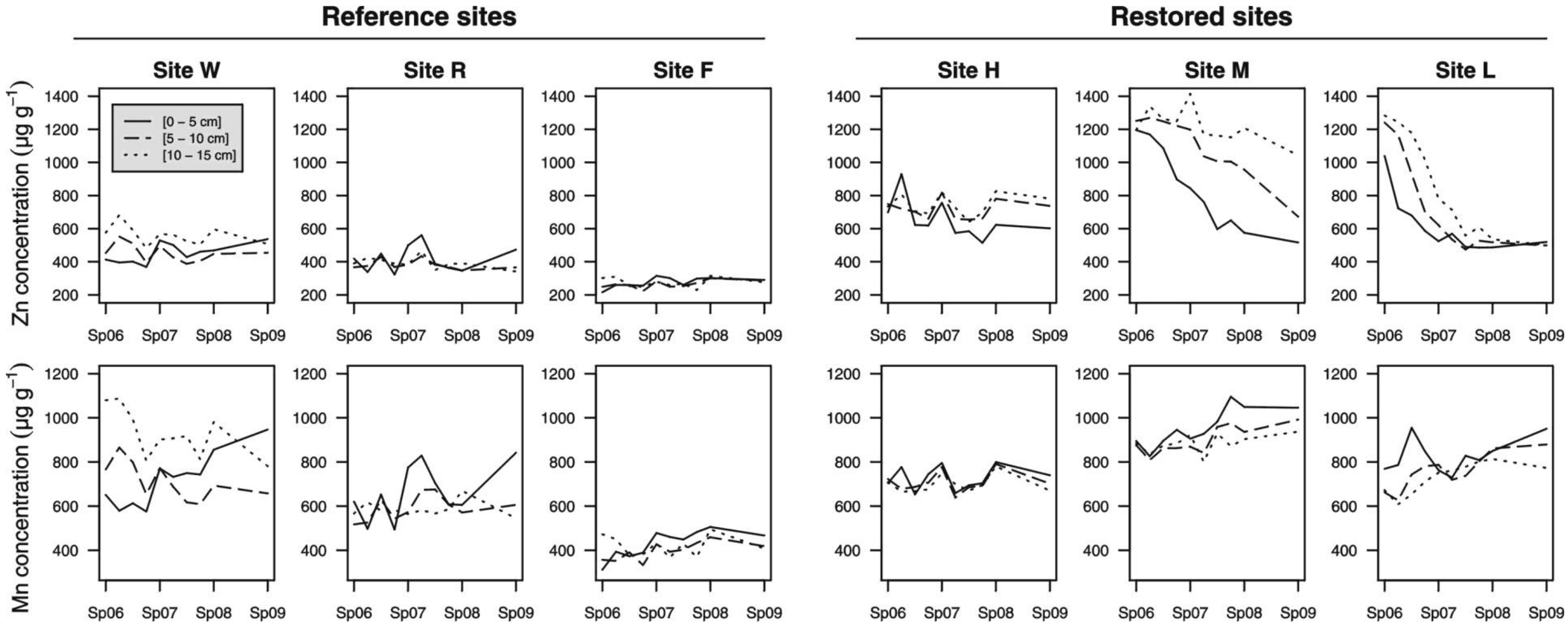

Figure 4. Temporal evolution of Zn (above) and Mn (below) concentrations on different depths ((0-5; 5-10; 10-15) cm) in the reference sites (left) and in the restored sites (right). The graphs show the 10 sampling seasons from spring 2006 to spring 2009. Graphs for all elements are provided in supplementary material.

Table 1: Total metal soil concentrations (µg g^{-1} dry mass) in the restored and reference area in April 2006 and April 2009. Asterisks indicate a significant difference between 2006 and 2009 according to Wilcoxon's signed-rank test. Significance level: *, 0.050; **, 0.010. Flemish soil sanitation thresholds for nature areas are added. Values exceeding the sanitation thresholds in bold.

		Reference sites										Restored sites											Soil sanitation threshold
		Site W			Site R			Site F			Mean value	Site H			Site M			Site L			Mean value		
		[0-5cm]	[5-10cm]	[10-15cm]	[0-5cm]	[5-10cm]	[10-15cm]	[0-5cm]	[5-10cm]	[10-15cm]		[0-5cm]	[5-10cm]	[10-15cm]	[0-5cm]	[5-10cm]	[10-15cm]	[0-5cm]	[5-10cm]	[10-15cm]			
As	2006	14.2	9.38	21.0	12.3	11.7	12.5	2.59	12.7	14.6	12.3	28.2	50.7	36.8	**65.5**	**90.3**	**79.8**	30.7	49.6	**66.5**	55.4		60.0
	2009	16.6	9.38	11.7	5.97	5.63	15.8	12.4	11.4	12.1	11.2	26.1	46.8	53.0	12.6	15.7	67.2	11.7	11.7	22.1	29.7	*	
Cd	2006	**4.82**	**5.56**	**7.35**	**4.92**	**4.24**	**4.75**	2.13	2.65	3.52	**4.44**	**7.98**	**8.58**	**8.50**	**15.1**	**16.2**	**15.3**	**14.3**	**17.6**	**18.0**	**13.5**		4.00
	2009	**7.02**	**5.56**	**6.35**	**5.48**	**4.32**	**4.17**	3.24	3.36	3.02	**4.72**	**7.00**	**8.50**	**9.00**	**6.54**	**8.77**	**13.5**	**6.38**	**6.43**	**6.54**	**8.07**	*	
Cr	2006	69.7	86.8	117	67.3	60.5	68.8	35.1	41.6	50.5	66.4	**144**	**160**	**157**	**311**	**333**	**317**	**363**	**510**	**533**	**314**		130
	2009	94.5	79.3	97.7	73.5	59.9	55.8	45.9	45.8	45.7	66.5	115	**151**	**169**	88.5	**139**	**254**	88.8	89.8	92.4	**132**	*	
Cu	2006	51.3	59.6	83.3	50.2	43.8	45.5	18.1	20.2	33.2	45.0	74.6	76.7	79.8	132	141	137	117	135	137	114		230
	2009	63.5	54.4	64.4	48.6	39.5	34.9	25.6	24.7	25.0	42.3	60.4	72.1	77.9	58.6	80.2	118	59.2	59.7	61.3	71.9	**	
Ni	2006	20.2	24.4	31.7	19.2	17.6	18.9	8.79	10.5	14.1	18.4	21.3	22.7	23.2	32.9	33.1	32.7	34.2	36.1	35.0	30.1		93.0
	2009	30.8	24.6	29.2	24.9	20.1	18.1	14.9	14.8	13.8	21.2	23.7	25.1	25.0	29.1	31.3	34.8	29.1	27.6	27.1	28.1		
Pb	2006	89.8	110.3	144.2	83.3	69.0	90.3	38.6	41.2	63.5	81.1	169	193	182	**307**	**326**	**307**	**202**	**244**	**261**	**243**		200
	2009	112.1	93.5	116.3	86.6	72.3	62.1	62.9	50.4	50.0	78.5	143	185	200	104	148	**250**	99.5	105	104	149	*	
Zn	2006	413	454	576	418	367	390	216	249	302	376	699	748	733	**1.19x10^{3}**	**1.25x10^{3}**	**1.20x10^{3}**	**1.04x10^{3}**	**1.24x10^{3}**	**1.28x10^{3}**	**1.04x10^{3}**		860
	2009	537	454	507	474	366	341	290	288	277	393	602	738	782	516	672	**1.04x10^{3}**	520	498	499	652	*	
Mn	2006	651	766	1079	620	517	567	312	357	472	593	705	723	708	895	875	883	769	662	672	766		–
	2009	947	657	781	842	606	544	467	418	408	630	739	702	670	1.05x10^{3}	991	937	951	879	773	854	*	

3.3.2.2. Relationship between soil characteristics and metal concentrations

The co-inertia analysis shows a strong co-structure between the sediment characteristics and trace metal concentrations (Fig. 5). The two first axes encompassed a high part of the variances of the two point clouds (98.7 % and 99.3 % for respectively sediment characteristics and trace metals), and the correlation between the two configurations was found to be highly significant ($Rv = 0.67$; $p < 0.0001$).

The first axis accounted for a system effect (Fig. 5C and 5D), opposing the restored area against the reference sites, and organizing the reference sites from high (W, close to the restored sites) to low elevation (F). From the reference sites to the restored sites, this axis accounted for a clear granulometric gradient. The sediment in the restored sites was characterized by a finer granulometry and a higher amount of organic matter compared to sediments in sites R and F, with a higher mean grain size and higher percentage of sand (see supplementary material Table S1 for detailed values).

The second axis highlighted two aspects. First, it organised the CRT sites along a flooding gradient, from site H to site L resulting according to sediment resistance (higher in site H) and water content (higher in site L). Secondly, a time effect was added to this flooding gradient. The axis shows a change in the restored sites from the first to the last spring season (Fig. 5F and 5H), particularly at low elevation (site L) which resulted in a clear soil homogenization in spring 2009 (no distinction between the strata). The flooding process resulted in sediments with a higher water and silt content. Reference sites did not exhibit such a temporal change (Fig. 5E and 5G).

Trace metals were found to covary positively on the first axis, pointing at higher concentrations in the restored sites as previously observed (Fig. 5B). Higher metal concentrations and a higher content of clay, fine silt and organic matter occur at the same sites. On the second axis, two groups opposed: As, Pb, Cr, Zn, Cd; and Cu, Ni, Mn. The concentrations in the first group discriminated sites H and M from site L and characterized the temporal change from the first to the last spring season. In the second group, the increase in Mn concentrations was consistent. For Cu, high temporal variations made interpretation difficult. The decrease in Ni concentration in the restored sites was not significant.

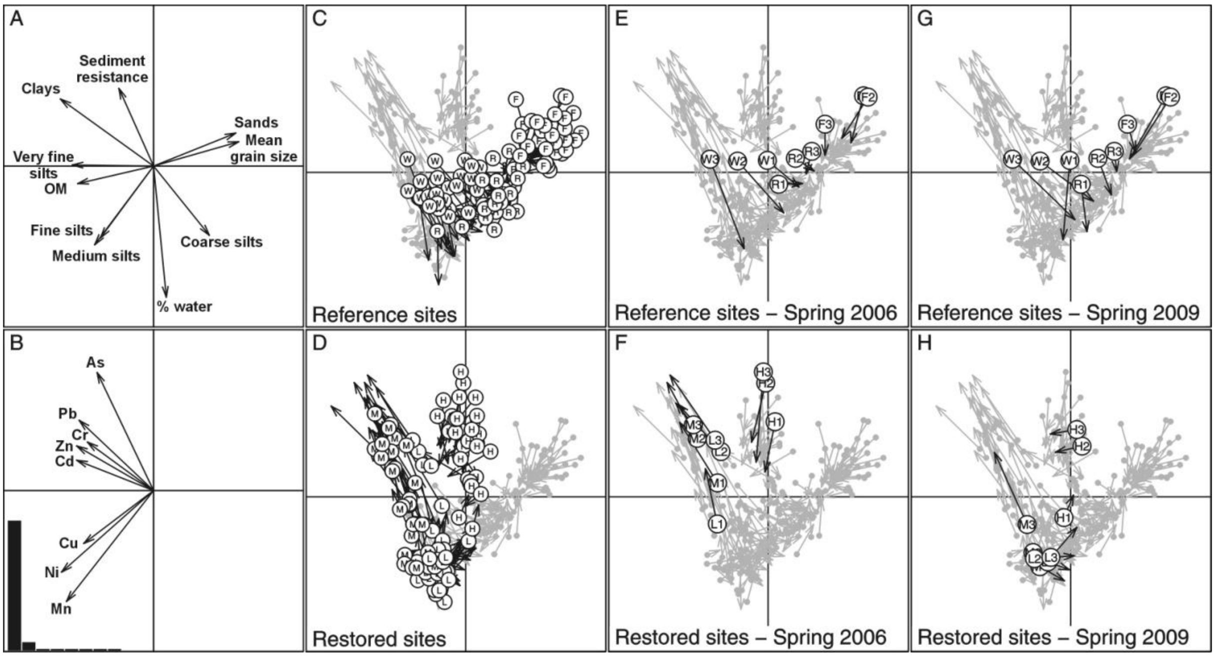

Figure 5. Co-inertia analysis. Bottom-left corner, eigenvalues diagram; axis 1 (horizontal), 94.7%; axis 2 (vertical), 5.1%. A and B) soil characteristics and trace metal covariance. C to H) Co-structure between the patterns of soil characteristics (circles) and trace metals (arrow tip); each arrow represents a sample (season-site-stratum); arrow length indicates the lack of fitting. Pearson's correlation coefficients between the coordinates (grey points versus their respective arrow tip) on the first axis: $r = 0.88$, $p < 0.0001$. On the second axis: $r = 0.71$, $p < 0.0001$. E to H) numbers in labels indicate the soil strata: 1, 0-5 cm; 2, 5-10 cm; 3, 10-15 c

3.4. Discussion

3.4.1. Spatial distribution of trace metals is linked to historical inundation practice

High trace metal concentrations in the sediment of tidal and embanked areas were found (Table 1). Cd concentrations exceeded the Flemish soil sanitation thresholds for nature areas, up to a factor 8 in the superficial polder soil. Also Cr, Pb and Zn sanitation thresholds were exceeded. As, Cd, Cr, Cu and Pb concentrations exceeded the serious effect levels (SEL) in the superficial polder soil (de Deckere et al., 2011). A long history of trace metal pollution in the Schelde estuary has been reported before (e.g. Baeyens et al., 2005). Anthropogenic activities resulted in increasing surface water metal concentrations from the beginning of the 20th century, maximum values around the seventies and decreasing concentrations again last decades (Baeyens et al., 1997b; Zwolsman et al., 1993). Deposition of contaminated suspended solids on tidal areas, land disposal of dredged sediments or winter inundation for agricultural purposes resulted in metal contamination of a large number of areas adjacent to the Schelde estuary (Du Laing et al., 2009d; Vandecasteele et al., 2002a). Trace metal concentrations in these areas are determined by (1) the estuaries' surface water contamination (2) the inundation history of the area (3) diagenetic processes and (4) atmospheric deposition (Zwolsman et al., 1993).

Tidal marshes near the Schelde are known to accrete according to the mean high water level rise in the estuary (Temmerman et al., 2003a). The continuous deposition of metal contaminated suspended sediments results in vertical profiles of trace metal concentrations in tidal areas which can reflect long term contamination history in estuaries (Canavan et al., 2007; Church et al., 2006; Lesven et al., 2010). Also the decrease in Zn concentrations towards the surface, found in the tidal marsh, might be a reflection of decreasing water Zn concentrations during the last decades (Fig. 3). With accretion of 1-2 cm y^{-1}, reported in the freshwater part of the estuary (Temmerman et al., 2004a) the maximum Zn concentration observed between 60 and 90 cm depth then corresponds with the maximum pollution of the estuary between 1960 and 1970 as observed by Andrews et al. (2008) and Zwolsman et al. (1993). Floodplain sediments proved to be efficient in scavenging trace metals with low post-depositional mobility (Du Laing et al., 2008a; Frohne et al., 2011; Lesven et al., 2008; Rennert and Rinklebe, 2010). However trace metal mobility caused by diagenetic processes might significantly alter the vertical profile expected from anthropogenic influence (Zwolsman et al., 1993). Diagenetic modifications are controlled by the availability and behavior of metal scavenging fractions which is in turn determined by conditions such as the redox potential (Eh) and pH.

Higher flow velocities above tidal flats result in sediments with larger grain size and less organic matter compared to tidal marshes. Since trace metals show a high affinity for small sized clay and organic particles (Chapagain et al., 2009; Du Laing et al., 2007a; Rinklebe et al., 2007), metal concentrations in the tidal flat were found lower compared to the tidal marsh.

Low Zn concentrations were also found in the polder area (P2) which was not longer subjected to flooding in the 60ties (Fig. 3). In this area, tidal inundation with deposition of sediments stopped before a significant contamination with trace metals of the Schelde occurred. However, Cu and Pb concentrations in the superficial layers of the polder were found to be three times higher compared to the deeper layers. Atmospheric deposition or fertilization could explain these higher values. Contrary to the tidal marsh, the highest Zn concentrations in the contaminated polder area (P1) were measured in the superficial soil layers. Fertilization by means of winter irrigation with estuarine surface water was applied in the 20th century. A sluice allowed metal contaminated surface water to flood the polder. Trace metals in the surface water, mainly adsorbed to suspended particulate matter (Millward and Liu, 2003; Regnier and Wollast, 1993), were deposited on the agricultural soil. Deposited metals may be trapped in Mn and Fe oxides near the surface, preventing downward migration. The ongoing agricultural activities in embanked areas with high metal concentrations in superficial soil layers might be of concern (Overesch et al., 2007; Vandecasteele et al., 2006).

3.4.2. Temporal changes of trace metals

Restoration of tidal inundation resulted in a decrease of metal concentrations in the superficial sediment layers (Fig. 4). Metal concentrations in the top soil, exceeding the sanitation threshold before inundation, decreased towards concentrations below the threshold (except for Cd) after three years of tidal inundation. A metal concentration decrease after re-inundation, due to metal release to the surface water by solubilization of Mn and Fe oxides under reducing conditions, has been reported by Emmerson et al. (2000). However, no decrease in Zn concentrations in the 'original agricultural soil' of the restored site was observed in our study. The rate of Zn decrease in the top soil is higher at lower sites which experience a higher sedimentation rate. For instance, the decrease was faster and stronger at low elevation where 30 cm of new sediments were deposited compared to a slow decrease in the upper 5 cm of the highest site, which after three years was still a mixture of agricultural soil and new sediments. Consequently, the direct burial of the contaminated soil with less contaminated sediments seems to be the main process explaining the metal concentration decrease.

Controlled Reduced Tide (CRT) is a technique which effectively restores a complete tidal gradient in formerly embanked and lowered areas, but some differences in tidal characteristics between CRT sites and estuarine tidal areas persist (Beauchard et al., 2011). This might engender differences in soil characteristics and metal concentrations of deposited sediments (Du Laing et al., 2009c). For instance, the lower current velocities and prolonged stagnant phase at high tide allow fine grained suspended sediments and organic matter to settle down (Fig. 5) (Vandenbruwaene et al., 2011). Since metals display a high affinity to fine-grain sediments as well as to organic matter, higher metal concentrations in the restored area can be expected (Rinklebe et al., 2007). However, Zn concentrations in newly deposited sediments of the restored area and the reference tidal marsh W were found to be comparable. Additionally, the differences in tidal characteristics which exist within the restored area did not result in differences in Zn concentrations of newly deposited sediments.

Contrary to Zn, an immediate increase in sediment Mn concentrations was observed after the tide was restored (Fig. 4). Additionally, Mn concentrations in newly deposited sediments differed between depths. A concentration profile with higher Mn concentrations in the upper 5 cm of the newly deposited sediments was observed. This indicates that Mn concentrations are determined by diagenetical processes. Dissolution of Mn oxides in deeper sediment layers and precipitation with oxides near the sediment water interface (SWI) can result in enrichment profiles which do not reflect the historical input of anthropogenic sources (Douglas and Adeney, 2000).

Mn concentrations also increased in the upper sediment layer of the reference tidal marsh. Also here diagenetic mobility is expected to be the predominant process. Increasing oxygen concentrations in the estuary's surface water observed last years (Cox et al., 2009) might have resulted in upward migration of Mn ions and precipitation of Mn oxides near the SWI (Teuchies et al., 2011). Mn oxides are known to be an important binding phase for trace metals (Adams et al., 2009; Li et al., 2009). Diagenetic mobility of Mn can result in similar movement of other trace elements and precipitation in the same diagenetic enrichment zones (Emmerson et al., 2000; Zwolsman et al., 1993). Increasing Zn concentrations in the upper sediment layer of the reference tidal marsh, following the increase of Mn, might indicate that Zn mobility is determined by the diagenetic movement of Mn in the reference marsh. Increased metal mobility caused by surface water oxygenation can be an indication for increasing metal availability and transfer to the estuary (Teuchies et al., 2011; Zoumis et al., 2001).

Based on our results, the reintroduction of tidal inundation in an embanked area is expected to result in deposition of a uniform sediment layer with metal concentrations comparable to concentrations found in superficial layers of an adjacent tidal marsh. At present conditions, no influence of the diagenetically governed concentration changes of Mn on the mobility of other trace metals was apparent in the soil of the experimental CRT sites. Also, Emmerson et al. (2000) and Macleod et al. (1999) reported that penetration of surface water and the changes which occur in the soil after re-inundation are slow processes. Additionally, other binding phases in the soil might prevent migration of metal ions with Mn. Organic matter, Fe-oxides, clay particles and sulfides are expected to be important binding phases (Gambrell, 1994).

Depending on the contamination level of the site as well as the estuary, tidal restoration will result in an in- or decrease in trace metal concentrations in the superficial soil layers of the inundation area. Deposition of sediments is the main process of metal removal in estuaries (Andrews et al., 2008; Rozan and Benoit, 1999). As reported by Cave et al. (2005), managed realignment has a potential to increase the area available for sediment deposition. Consequently, the creation of tidal areas by means of CRT’s has a potential to improve long term water and sediment quality (Cave et al., 2005) and can avoid cleaning costs of a contaminated area (Andrews et al., 2006).

3.4.3. Metal availability

The evolution of total metal concentrations during a tidal restoration project has been investigated. However, the availability of these metals will determine the final ecological impact. Since tidal inundation can alter availability of present metals, potential effects will be discussed based on literature and available data. Most studies on the impact of managed realignment on trace metal availability are situated in salt water areas (Blackwell et al., 2004; Emmerson et al., 1997; Emmerson et al., 2000; Emmerson et al., 2001; Macleod et al., 1999). Intrusion of brackish or saline surface water was found to influence metal availability (Du Laing et al., 2008b; Emmerson et al., 2001; Speelmans et al., 2007). Also flooding in itself, independently of salinity, can change metal availability (Du Laing et al., 2009b; Speelmans et al., 2007). Inundation is expected to influence soil Eh, which is a determinant factor in controlling metal availability (Gambrell et al., 1991). A decrease in Eh due to flooding might result in reduction of sulfates to sulfides and the formation of insoluble metal sulfides which can reduce availability of certain trace metals in the soil (Frohne et al., 2011; Gambrell et al., 1991; Teuchies et al., 2008). The prolonged inundation time which characterizes the CRT regime in the restored area, can be favorable for metal sulfide precipitation. However, not all toxic metals behave similarly. For example a high mobility of As, Sb (antimony) and Ni was found at low Eh (Antić-Mladenović et al., 2011; Frohne et al., 2011). Additionally, periodical flooding, controlled by the tidal amplitude, was found to be insufficient to create the reducing conditions in which sulfate reduction occurs (Du Laing et al., 2007b). Additionally, surface water aeration expected in CRT areas (Maris et al., 2007) might decrease the potential of metal sulfide precipitation. Under prevailing conditions, Mn and Fe oxides might be more important in controlling metal availability in the soil and newly deposited sediments of the restored area. Moreover, flooding might result in dissolution of Mn and Fe oxides in deeper sediment layers, with increasing metal availability as a consequence. Metal availability in tidal areas might be subjected to large variations. During stagnant pore water phases at low or neap tide, metal release to the pore water can increase the availability while a water flow through the soil during inundation can flush the released metals (Rennert and Rinklebe, 2010). Increasing Mn concentrations found in the superficial soil layers, subsequent to flooding, might be an indication for increased mobility. However, no diagenetic mobility of other trace metals was observed. As summarized by French (2006), changes occurring in the soil are slow and situated mainly near the surface and in newly deposited sediments. However, an immediate change in groundwater levels after tidal flooding was observed (Fig. S3).

The restoration of the tide resulted in a quick colonization with typical freshwater tidal marsh vegetation (Jacobs et al., 2009). Plant growth can, depending on the species, significantly increase or decrease trace metal availability in the soil (Reboreda and Caçador, 2007; Vinagre et al., 2008). Additionally, metal uptake by plants can enhance transfer to the food chain (Caçador et al., 2009). Compared to metal stocks in the soil, plant uptake is often small (Overesch et al., 2007; Teuchies et al., 2008). Still, the high variability in plant metal transfer, depending on species and conditions, can locally result in high concentrations (Overesch et al., 2007). *Salix sp*. (willow) and *Phragmites australis* (common reed) are the predominant species in the freshwater part of the Schelde estuary. Willow species are known to accumulate trace metals resulting in high foliar concentrations (Vandecasteele et al., 2002b). In contrary, stabilization of metals in the rhizosphere and a lower allocation to leaf tissues in reed plants has been reported (Du Laing et al., 2009b; Weis and Weis, 2004). On the other hand, decomposing litter of most plant species, including reed, becomes highly enriched in metals. This can result in higher availability and high metal exposure to benthic invertebrates (Du Laing et al., 2008a; Weis and Weis, 2004) and fish species (Chandra Sekhar et al., 2004).

3.5. Conclusions

Irrigation with contaminated water from the Schelde during the last decades resulted in high trace metal concentrations in the agricultural soils of embanked areas adjacent to the estuary. Reintroduction of tidal flooding in these areas was followed by the deposition of a less contaminated uniform sediment layer. Despite diagenetic mobility of manganese no diagenetic movements of the trace metals were observed during the first three years. Metal concentrations in these newly deposited sediments were comparable to concentrations in sediments measured in the surface layer of an adjacent tidal marsh. Additionally to the significant decrease in top soil metal concentrations in the restored area, the sediment accumulation can contribute to metal removal from the estuary. Implementation of almost 1000 ha of CRT areas, planned along the Schelde estuary, might significantly improve water and sediment quality in the estuary. Based on the results it is still unclear what the consequences are for metal availability and long term mobility in the restored area.

CHAPTER 4

Land use changes and metal mobility: multi-approach study on tidal marsh restoration in a contaminated estuary.

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Abstract

Inundation of formerly embanked areas in order to combine flood control and tidal marsh restoration will be applied increasingly. However, areas suitable for the implementation are often found to be contaminated. Re-inundation of metal contaminated soils can have consequences on total metal concentrations as well as metal mobility. In this study, metal mobility in a tidal marsh restoration project was evaluated based on the modified BCR sequential extraction method, concentrations of Acid Volatile Sulfides (AVS) and Simultaneously Extracted Metals (SEM) and metal concentrations in plants. The results obtained from the sequential extraction suggest an increase in metal mobility following inundation due to reduction of Fe and Mn oxides and the subsequent release of associated metals. However, the contradictory results between sequential extraction and [SEM-AVS] may indicate that redistribution of the metals to the mobile fraction can be caused by sample processing. High AVS concentrations in newly deposited sediments in the restored marsh may indicate that the formation of insoluble metal sulfide complexes will reduce metal mobility on the longer term. Processes following inundation of metal contaminated land are complex and more in situ research is needed to get a better insight in the risks involved.

4.1. Introduction

Estuarine and coastal intertidal zones are rich, diverse and productive systems which deliver a wide range of ecosystem services (Barbier et al., 2011; Costanza et al., 1997). Flood hazard regulation is one of the main benefits supplied to society. Human impacts, mainly overexploitation and habitat destruction, resulted in degradation of these valuable ecosystems worldwide (Lotze et al., 2006). Managed realignment or controlled flooding of formerly embanked areas have proven to be effective and economically viable strategies to combine flood control and wetland restoration in one and the same area (Beauchard et al., 2011; French, 2006; Jacobs et al., 2009; Zedler and Kercher, 2005). Yet, estuaries act as a sink for many toxic substances and historical contamination, land disposal of contaminated dredged sediments or irrigation of embanked areas with contaminated river water resulted in elevated concentrations of metals in many areas suitable for estuarine marsh restoration (Emmerson et al., 2000; Teuchies et al., 2012a; Vandecasteele et al., 2003a).

Inundation of contaminated land in order to restore wetlands will be applied increasingly; yet, processes in these often impaired sites are still poorly understood (Spencer and Harvey, 2012). Reintroduction of the tide in a metal contaminated polder is expected to have consequences on total metal concentrations as well as on metal availability. Sedimentation, erosion and remobilization caused by tidal flooding can result in changes in total metal concentrations (Teuchies et al., 2012a). Additionally, it is hypothesized that flooding related changes in environmental conditions will influence the availability of both essential and toxic elements by altering the forms in which they occur (Speelmans et al., 2010). Factors controlling metal mobility as pH, redox potential and the availability of complexing ligands (both organic and inorganic) and particle surfaces for adsorption are expected to be influenced by tidal flooding (Gambrell, 1994). Furthermore, also the land use change itself might change the transfer of metals into the food chain. Ceasing agriculture activities on the contaminated field will directly reduce human exposure while establishment of a freshwater tidal marsh with other plant and animal species as well as reconnection of the area to the estuary might enlarge the potential risk to the larger ecosystem.

Processes which occur after re-inundation of contaminated land are complex and their effect on metal availability hard to predict. Current knowledge is mainly based on laboratory studies, whereas the use of in situ measurements is important to get insight in the complex multi-factorial field situation (French, 2006). It is generally accepted that the distribution, mobility, biological availability and toxicity of metals depend not simply on the concentrations, but on their chemical and physical forms (Ankley et al., 1996; Rao et al., 2008). The determination of trace metals associated with different geochemical fractions (e.g. carbonates, oxides, sulfides and organic matter bound or residual fractions) gives information which can be valuable for a thorough environmental impact assessment (Passos et al., 2010). In fractionation studies, metals associated with 'operationally defined fractions' are based on the application of a series of chemical extractants with increasing reactivity (Bervoets et al., 1997; Kersten and Forstner, 1986; Rauret et al., 1989; Tessier et al., 1979). The results of fractionation studies are useful in making inferences on metal behavior and its responses to environmental changes (e.g. pH or Eh) in order give insight in long term adverse effects (Forstner, 1993; Hass and Fine, 2010). However, these methods have several drawbacks such as the limited selectivity of extractants, the possible redistribution of metals during sample preparation or extraction or the inability to distinguish between sulfides or organic matter associated metal fractions (Hass and Fine, 2010).

Formation of insoluble metal sulfides in hypoxic sediments plays a major role in reducing sediment metal availability and transfer to the surface water, hence the difference of acid extracted sulfides (acid volatile sulfides or AVS) and simultaneously extracted metals (SEM) is often used as a proxy for prediction of trace metal toxicity in the sedimentary environment (Di Toro et al., 1992; Lee et al., 2000c).

In present study, the evaluation of changes in the potential hazard of metals following inundation are based on either the determination of (1) total metal concentrations, (2) metal fractionation and (3) [SEM-AVS] as well as based on (4) metal concentrations in different tissues of dominant plant species. Sediment cores from the restored marsh after one year of tidal influence, as well as from an embanked area and a natural tidal marsh were validated according to these four different approaches.

4.2. Materials and methods

4.2.1. Study area

The Schelde estuary (Belgium and The Netherlands) is a turbid, macrotidal and eutrophic system (Meire et al., 2005) with elevated trace metal concentrations in surface water, suspended solids and sediments (Baeyens et al., 2005; Du Laing et al., 2007a; Vandecasteele et al., 2003a). The study area (51°05'10''N; 4°10'20''E), located in the freshwater tidal zone (Fig. 1), is an experimental restored tidal marsh (8.2 ha) (Beauchard et al., 2011; Cox et al., 2006; Jacobs et al., 2009; Maris et al., 2007; Vandenbruwaene et al., 2011). This formerly agricultural site has been yearly flooded as a fertilization technique during the 1960's and 70's by which heavily polluted sediments were brought in (Teuchies et al., 2012a).

In the restored area, three sampling sites covering a flooding gradient were considered: a low-elevation site (L), a mid-elevation site (M) and a high-elevation site (H). Two natural reference sites were sampled; a tidal flat without vegetation (F) and a site in the high marsh overgrown with *Salix sp.*

(willow, W) (Fig. 1). Additionally, an adjacent polder (=embanked area) was sampled in order to compare the restored marsh with the situation prior to inundation. The polder was still cultivated (maize). As in the restored area, an irrigation valve between the embanked polder P and the estuary resulted in elevated metal concentrations (Teuchies et al., 2012a).

4.2.2. Sampling

In June 2007, one year after restoration of the marsh, 1 m deep sediment cores were taken with a shell bit (5 cm Ø). At each site, 8 cores were taken randomly in a 2 meter radius. The cores were sliced at 10, 20, 30, 40, 60 and 80 cm. Sediments from four cores were mixed per depth, resulting in two replicates. Sediments were stored cooled without air in polyethylene recipients. In the lab, subsamples for nutrient analysis were immediately weighed and processed, for SEM and AVS frozen at -20 °C and for sequential extraction, metal concentrations and certain sediment characteristics air dried prior to analysis.

At each site (except F) the two or three most dominant plant species were sampled; (site P): *Populus* sp., *Sambucus nigra*, *Zea mays*; (site H): *Epilobium hirsutum*, *Urtica dioica*; (site M): *Lythrum salicaria*, *Polygonum lapathifolium*; (site L): *Phragmites australis*, *L. salicaria*, *Salix* sp.; (site W): *Impatiens glandulifera*, *Salix* sp., *U. dioica.* Three individuals, including roots, of each species were sampled randomly at every site as close as possible to the sediment core location, thoroughly rinsed with river water in the field as well as with demineralized water in the laboratory, dried for 48 h at 70 °C, weighed and ground.

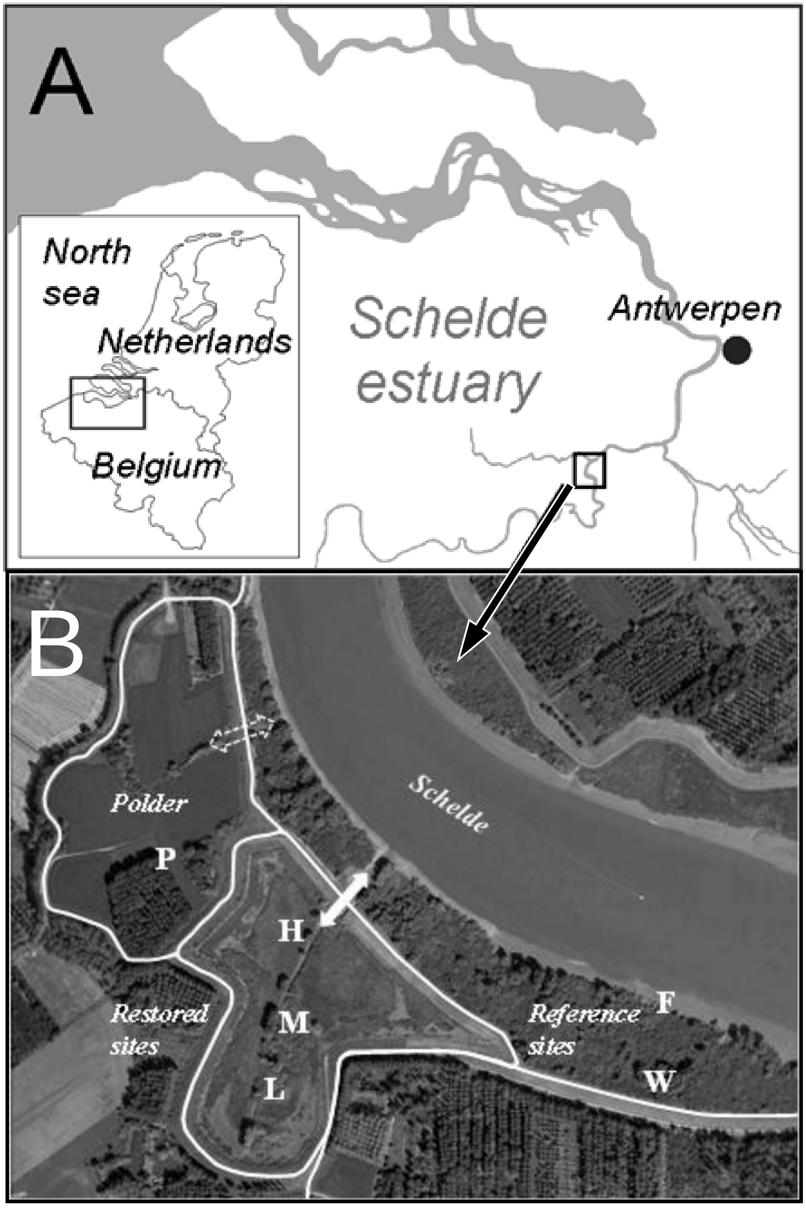


Figure 1. A) Location map of the study area. B) Aerial photograph of the restored (Low, Medium, High site), reference (Willow marsh, Flat) and polder area (see text for details).

4.2.3. Laboratory analyses

4.2.3.1. Sediment characteristics

Nutrient concentrations were measured colorimetrically using a SAN++ Flow Analyser (Skalar, FAS, SA 20/40, Skalar Analytical B.V., Breda, the Netherlands) after extractions of wet sediment with potassium chloride (KCl, 1 M; 1:2.5 w/v) for ammonium (NH_4^+), nitrite and nitrate (NO_2^- + NO_3^-) and with ammonium acetate EDTA (ethylenediaminetetraacetic acid) (1 M; 1:5, w/v) for phosphate (PO_4^{3-}). Soil pH-H_2O were measured in the supernatant solution from a soil extraction with distilled water.

A subsample of the air dried sediment was weighed, oven dried (105 °C) to a constant weight and then reweighed to estimate the moisture content (MC). Oven dried samples were incinerated for 6 h at 550 °C and weighed to estimate the organic matter (OM) concentration (loss on ignition). Clay content (< 2 µm) was determined with a laser diffraction particle size analyzer (Malvern S, Malvern Instruments Ltd, Worcestershire, UK).

The redox potential (mV) was measured in the field (in 2010, 3 years after the soil samples were taken) with a stainless steel measuring probe against a Ag/AgCl reference electrode connected to a multimeter in each site at 2, 5, 10, 15 and 20 cm depth after stabilization (10 min).

4.2.3.2. Sediment sequential extraction and metal concentrations

Air dried sediments (both replicas mixed, n=1) were subjected to the modified BCR sequential extraction procedure (Rauret et al., 1999) involving three operationally defined fractions: (1) exchangeable and acid-soluble (Ex-M), (2) reducible (Red-M) and (3) oxidizable (Ox-M), and a fourth ‘‘residual’’ fraction (Res-M) step (for detailed information on the extraction procedure see Table S1). All extractions were performed by shaking in a mechanical, end-over-end shaker. After extraction, the supernatant was separated by centrifuging (20 min, 3000 rpm). Between extraction steps, the residue was washed with ultrapure water, shaken for 15 minutes and centrifuged (20 min, 3000 rpm) and the supernatant was carefully discarded. Blanks were used during the sequential extraction procedure and the concentrations were processed accordingly. A subsample of the air dried soil (0.2 g) as well as the air dried residue after the sequential extraction were used for determination of total metal concentration through hot block digestion method for which 5 mL nitric acid (HNO_3, 69% Normapur, VWR) and 5 mL of hydrogen peroxide (H_2O_2, 27% for electrical purpose, VWR) were added to the sample. The mixture was heated (30 min at 100 °C), cooled overnight and heated again (60 min at 150 °C). Total amount of the metal removed at each step of the procedure (Sum of extracted metals) was compared with the results from the pseudo-total digestion (Total metal) for the recovery during sequential extraction procedure (Figure S1):

$$\%\,Recovery = \frac{\Sigma(metal\ extracted\ \ through\ SEP)}{Total\ metal\ (digested)} X\ 100\%$$

4.2.3.3. SEM-AVS

Acid volatile sulfides (AVS) and simultaneously extracted metals (SEM) were determined following the purge-and-trap method (Allen et al., 1993); fresh sediment was stirred for 2 hours with hydrochloric acid (HCl, 37% Normapur, VWR) under constant N_2 flow. The S^{-} ions trapped in a NaOH solution were measured photospectrometically (UV-160, Shimadzu, Kyoto, Japan). In the sediment-acid mixture, metal concentrations were measured and SEM was calculated as the molar sum of Cd, Cr, Cu, Zn, Pb and Ni. The difference, on a molar basis, of SEM-AVS is displayed as [SEM-AVS].

4.2.3.4. Plants

Plant metal concentrations were measured after microwave digestion of an over dried (60°C) subsample (around 0.5 g), in a mixture of HNO_3 (65%, Suprapur®, Merck) and H_2O_2 (30%, Suprapur®, Merck) (20:1, v/v).

Plant and sediment (total and SEM) metal concentrations were measured using inductively coupled plasma (ICP) optical emission spectroscopy (OES), (Thermo Jarrell Ash IRIS, Thermo Fisher Scientific, Waltham, USA). Analytical accuracy was achieved by the use of blanks and certified reference material for sediment (Institute for Reference Materials and Measurements (IRMM), BCR® № 320, channel sediment) and plants (IRMM, BCR® no 060, aquatic plant (*Lagiosiphon major*)) included in all series of metal analysis in order to verify the analytical procedure. For sediment, all values from the reference samples were lower but within 90% of the certified values for As, Cd and Zn, and within 80% for Cu, Ni, Pb and Mn. Recovery of Cr was low (50%). For plants, average recovery ranged from 81% to 89 % for Cd, Cu, Mn, Pb and Zn. Variation between recoveries was very low for all elements.

4.2.4. Statistical analysis

Normality of the data was tested with the Shapiro-Wilk test prior to analysis. The significance of differences in sediment characteristics and plant metal concentrations between sites was tested with an analysis of variance test (ANOVA). The Pearson correlation-coefficient was used to determine the relation between AVS or [SEM-AVS] concentrations and the Ex-M and Ox-M fractions.

4.3. Results

4.3.1. Site characteristics

No flooding occurred in the polder area. Flooding frequency (inundating tides as % of all tides) was 30% in the tidal marsh (W), 100% in the tidal flat (F) and about 20%, 70% and 80% in the sites of the restored marsh (H, M and L respectively). Flooding duration was longer in the restored marsh (3.5, 4.5 and 5.5 h for H, M and L) than in the natural sites (1 and 4 h for F and W) (for details see (Beauchard et al., 2011)).

The pH values in all sites were within the range of 7.1 – 7.7 with significantly ($p<0.05$) higher values in the polder (P) and highest site of the restored marsh (H) (Table 1). Redox potential values (measured 4 years after restoration) decreased with increasing inundation frequency, with a value in site H which was significantly ($p<0.05$) higher than the other sites. Sites with lower redox values (L and F) had significantly ($p<0.05$) higher PO_4^{3-} and NH_4^+ concentrations while the driest site (P) had significantly ($p<0.05$) higher NO_3^- and NO_2^- concentrations. OM and clay content were significantly ($p<0.05$) lower in the tidal flat (F) compared to most other sites. In general, metal concentrations are highest in the polder area, intermediate in the restored marsh and lowest in the natural intertidal areas, with the lowest values in the tidal flat.

4.3.2. Metal fractionation

The exchangeable fraction (Ex-M) was highest for Cd, Mn and Zn and low for As, Cr, Cu and Pb (Fig. 2.). The residual fraction (Res-M) was highest for As, Ni and Pb but relatively low for the other elements. For most metals, the oxide bound (Red-M) metals were the major fraction and this was particularly high for Mn and Zn. The oxidizable fraction (Ox-M) was mainly high in the restored sites and low in the polder site, in particular for As, Cr and Cu. Ex-M exhibited most variation, with often increasing values with depth to a maximum value after which values decreased again. High Ex-M was found in the tidal marsh (for Cd, Ni, Mn, Zn) and in the low elevation site of the restored marsh (for Cd and Mn). Lowest Ex-M values were observed in the polder site, except for As.

Table 1. Average values for soil parameters and metal concentrations in the upper 30 cm layer for the different sites (n=10, standard deviation in brackets). Redox (upper 20 cm, n=5). Values with the same letter are not significantly different (p<0.05), letter a indicates always the smallest number.

variable	Unit	**P**	**H**	**M**	**L**	**W**	**F**
OM	%	11.9 (0.5)c	7.28 (1.05)ab	10.3 (1.3)bc	9.86 (0.73)bc	10.6 (3.0)c	4.28 (1.43)a
MC		30.6 (2.3)a	26.9 (1.3)a	42.9 (15.2)bc	52.3 (12.6)c	47.8 (5.6)bc	38.3 (7.4)ab
Clay		19.8 (6.0)c	12.7 (1.7)bc	12.8 (1.7)bc	11.4 (0.8)ab	10.9 (1.2)ab	6.87 (1.75)a
pH$_{(H_2O)}$	/	7.36 (0.11)b	7.76 (0.15)c	7.18 (0.35)a	7.19 (0.07)a	7.27 (0.11)ab	7.09 (0.09)a
PO_4	µg g^{-1} fw	188 (50)ab	102 (36)a	256 (201)ab	664 (122)c	315 (286)b	935 (98)d
NH_4		2.32 (4.11)a	0.76 (0.69)a	1.16 (0.98)a	38.2 (11.5)c	4.03 (1.88)a	22.8 (11.0)b
$NO_2 + NO_3$		15.5 (10.0)b	2.49 (1.66)a	1.12 (0.73)a	0.39 (0.27)a	1.09 (0.68)a	0.54 (0.32)a
Redox	mV	/	-33.8 (77.7)a	-177 (54)b	-191 (76)b	-148 (21)b	-222 (167)b
As	µg g^{-1} dw	46.8 (17.5)b	41.0 (7.96)ab	57.1 (17.5)b	22.7 (11.9)a	14.9 (6.8)a	10.7 (0.4)a
Cd		26.1 (2.2)d	10.4 (0.7)bc	14.9 (3.8)c	10.5 (4.0)bc	8.35 (3.46)ab	3.58 (1.30)a
Cr		710 (104)d	189 (29)bc	271 (101)c	227 (160)bc	131 (47)ab	48.2 (10.4)a
Cu		173 (31)d	93.5 (6.0)b	135 (31)c	90.7 (24.0)b	89.2 (33.6)b	29.8 (12.3)a
Mn		706 (210)b	773 (39)bc	889 (66)d	760 (124)bc	854 (185)cd	409 (149)a
Ni		40.0 (4.1)d	27.0 (0.9)b	34.5 (3.6)cd	30.2 (3.3)bc	33.8 (10.7)c	14.4 (3.8)a
Pb		323 (46)d	215 (11)c	291 (72)d	162 (49)b	148 (39)b	64.2 (21.5)a
Zn		1640 (140)e	839 (40)c	1160 (280)d	758 (252)c	592 (170)b	308 (88)a

Table 2. Average metal concentrations in plant tissues for three different species occurring in two different sites. Significant differences in bold, with p-value levels: *<0.05.

	site	**As**	**Cd**	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Pb**	**Zn**
Salix sp.	**L**	2.00	5.12	**5.66***	19.39	896	112	3.32	3.01	229
	W	0.73	4.82	**3.74***	16.01	480	55.2	1.86	2.31	259
L. salicaria	**L**	6.25	0.812	5.96	9.04	7.16 10^3	342	**2.93***	**8.66***	79.9
	M	3.25	1.49	4.84	11.1	2.45 10^3	210	**1.69***	**4.49***	80.6
U. dioica	**W**	1.00	0.35	4.51	13.6	919	48.8	1.80	2.78	42.0
	H	0.86	0.61	4.94	9.52	475	35.6	1.30	2.37	37.5

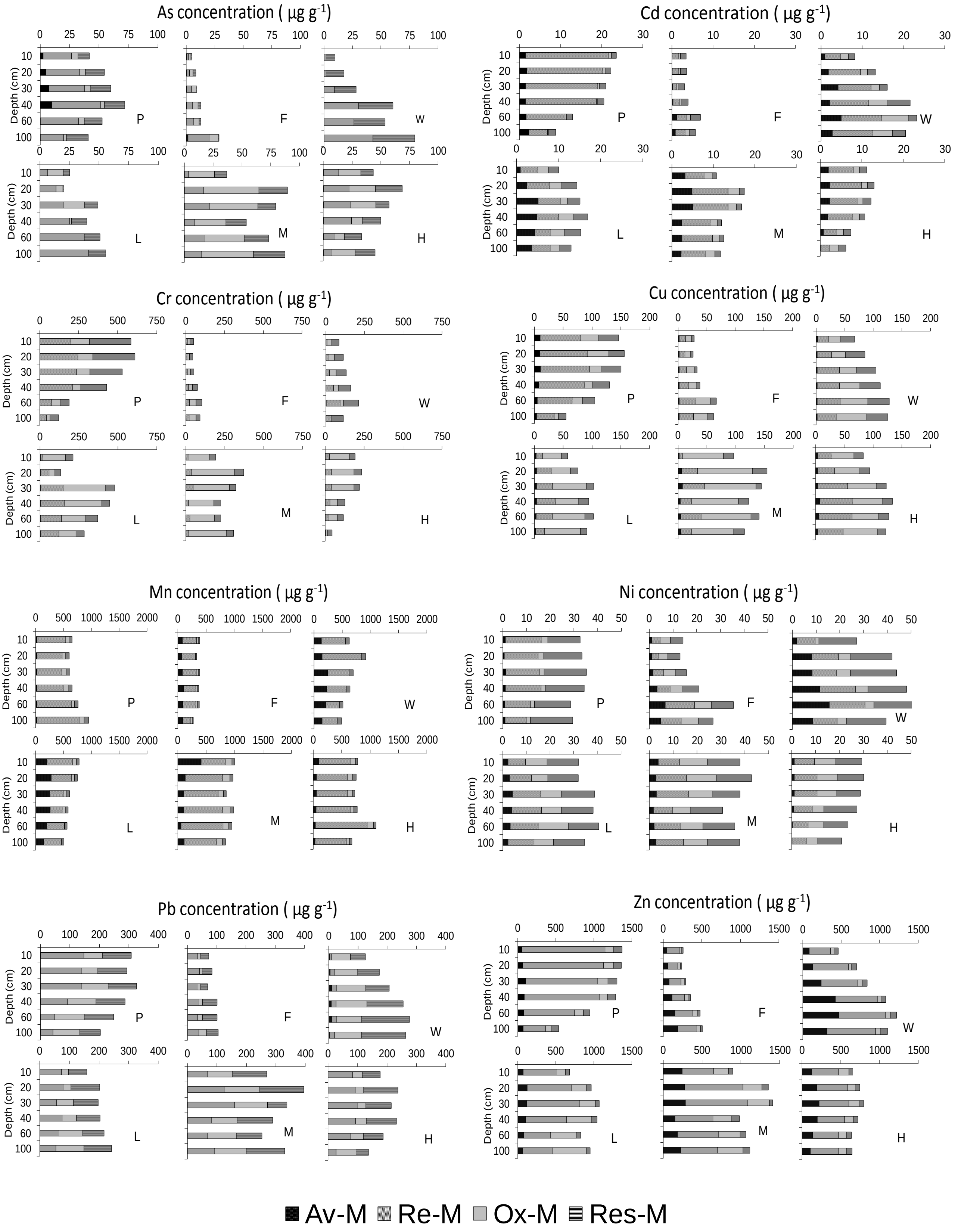


Figure 2. Metal concentrations (µg g^{-1}) in different fractions. Results from different depths (cm) and sites (polder, P; tidal flat, F; tidal marsh, W; restored marsh with low, L; moderate, M; and high, H elevation).

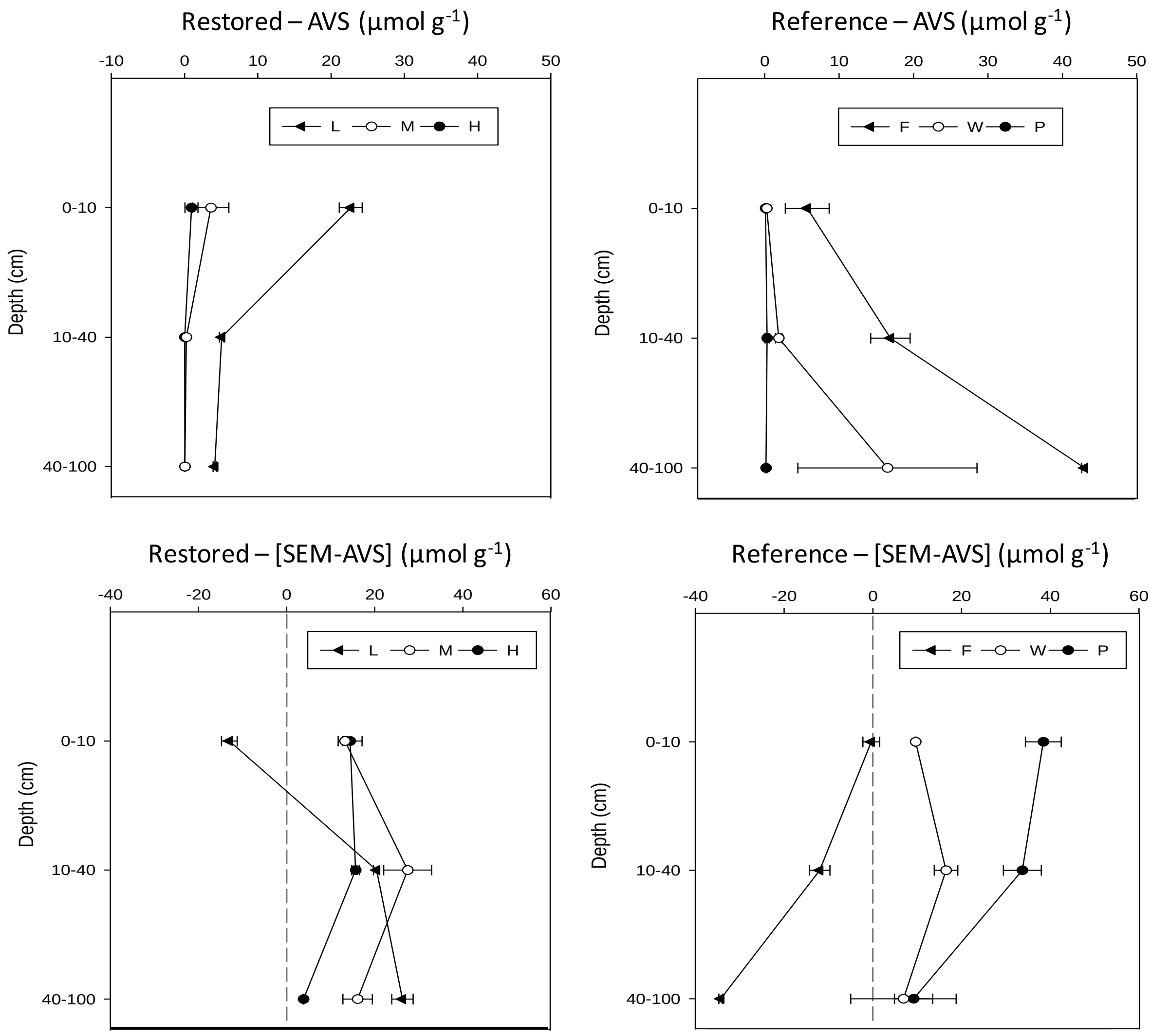


Figure 3. Average (n=2, both values displayed as error bar) AVS and [SEM-AVS] concentrations in the restored area (A and C) and the reference and polder areas (B and D).

4.3.3. Sulfide bound metals [SEM-AVS]

Acid volatile sulfides (AVS) were not observed in the polder soil (Fig. 3). AVS concentrations were significantly higher in the tidal flat (F) ($p<0.001$) and concentrations increased with depth in both natural tidal areas (W, F). High AVS and low SEM concentrations resulted in a negative [SEM-AVS] values (concentration difference on a molar base) from the surface up to 1 m depth in the tidal flat (F). [SEM-AVS] values were positive over the entire depth in the tidal marsh (W). AVS concentrations increased with inundation frequency in the restored marsh with values in the lowest site (L) significantly higher than both other sites ($p=0.03$ for both sites). Except for higher AVS concentrations in the upper layer of the low elevation site (L), all other values in the restored marsh were below 10 µmol g^{-1}. In the restored marsh, negative [SEM-AVS] values were only measured in the upper layer of the low elevation site (L).

4.3.4. Plant metal concentrations

Differences in metal concentrations between tissues are rather small and mainly in the order roots > leaves > stems (Fig. 4). Except for As concentrations in the roots of the plants in site L, all metal concentrations in plant tissues were lower than average surface sediment metal concentrations. Average metal concentrations in the dominant plant species were not different between the sites for Cd, Cu, Ni and Zn, while As, Fe and Pb concentrations were significantly ($p<0.05$) higher in the low elevation site (L) and Mn higher in the low and mid elevation sites (L, M). Cr concentrations were significantly ($p<0.05$) higher in plant tissues from the polder (P) compared to sites H and W. Large differences within and among plant species resulted in high standard deviations.

Salix sp. metal concentrations were higher in site L compared to site W (significant for Cr) (Table 2). Differences in *L. salicaria* metal concentrations between site L and M were not consistent among different metals. For most metals concentrations were higher in site L (significant for Ni, Pb). No significant differences in *U. dioica* metal concentrations between site W and H were found.

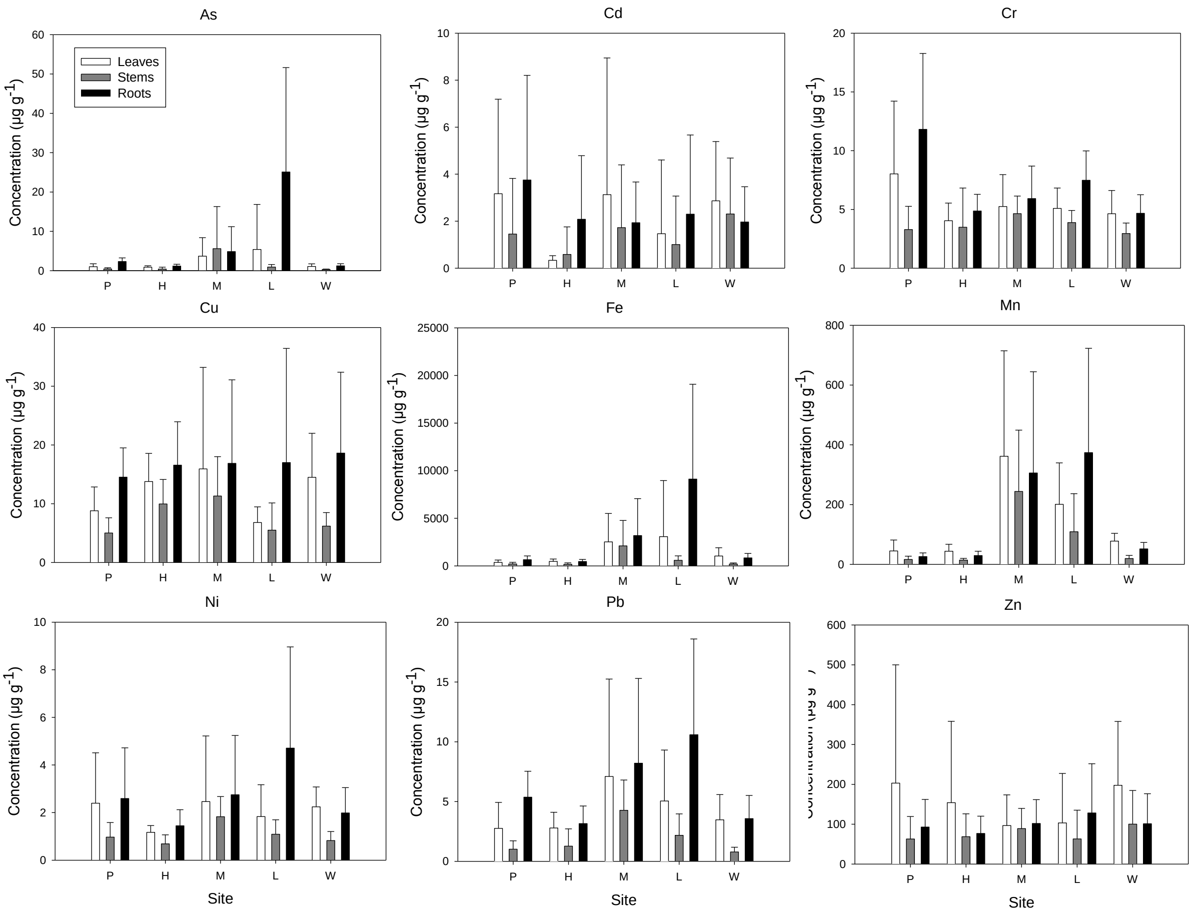


Figure 4. Average (n=6-9) metal concentrations with standard deviation in leaves, stems and roots of the most dominant plant species in the different sites (polder, P; tidal flat, F; tidal marsh, W; restored marsh with low, L; moderate, M; and high, H elevation).

4.4. Discussion

4.4.1. Metal contamination of the area

The sediments of the investigated areas are contaminated with trace metals (Table 1). The *Flemish soil sanitation thresholds for nature areas* were exceeded for Cd, Pb and Zn (Flemish Government, 2008) and As, Cd, Cr, Cu and Pb concentrations exceed their *severe effect levels*, based on the concentration at which only 5% or less of a certain set of invertebrate species should be able to survive (de Deckere et al., 2011). The low relative content of metals in the residual phase (Res-M) is indicative for a high level of anthropogenic pollution (Passos et al., 2010). However, also Pb, with higher concentrations in the residual fraction largely exceeds natural background levels (Luoma and Rainbow, 2008). Tidal flooding and irrigation of embanked agricultural soils with contaminated river water and disposal of dredged sediments resulted in contamination of many areas adjacent to the Schelde estuary (Du Laing et al., 2007a; Vandecasteele et al., 2003a).

4.4.2. Effect of tidal marsh restoration on metal mobility

The elevated metal concentrations measured in the embanked polder soil were found to be mainly located in the upper 40 cm of the soil, which are biologically most relevant (Fig 5). Yet, based on the results of the sequential extractions, a low metal availability is suggested (Fig 2.). The major part of the metals in the soil of this area was associated with Red-M suggesting association with Fe and Mn (hydr)oxides under the aerobic conditions. This resulted in very low metal concentrations in the most mobile, exchangeable fraction (Ex-M). The study of Teuchies et al. (2012) (Teuchies et al., 2012a) in the same study area reported that tidal marsh restoration in these polder areas resulted in burial of the contaminated former polder soil, due to the deposition of a uniform, less contaminated layer. Yet, inundation caused substantial changes in the soil, which also affected metal mobility (Fig. 5). A gradient in redox conditions with low values in the low elevation site L (= most inundated), was indicated by the high ratio of NH_4^+ to NO_2^- and NO_3^- and the high extractable PO_4^{3-} concentrations, which are released from their Fe complexes when $Fe^{(III)}$ is reduced to $Fe^{(II)}$ (Table 1) (Mitsch and Gosselink, 2000). Based on the results of the sequential extraction, these conditions caused the dissolution of the Fe/Mn-hydroxides and associated metals which caused a shift to higher metal mobility (Ex-M), which was comparable to the mobility in sediments from the natural tidal marsh (Fig. 2). In general a shift from Red-M in the polder area to Ox-M in the restored marsh is observed. A higher mobility was also indicated by increasing Mn concentrations in the upper sediment layers of the restored marsh and can be attributed to flooding related reducing conditions resulting in an upward migration of the reduced $Mn^{(II)}$ ions and precipitation again as Mn-oxides near the oxic surface layers (Teuchies et al., 2012a).

An increase in metal mobility in the sediments following inundation of an embanked area after managed retreat was found before (Emmerson et al., 2000; Kolditz et al., 2009). Physicochemical processes which occur following re-introduction of the tide are complex and different conditions prevailing in other sites or estuaries can result in a different metal behavior (Bryan and Langston, 1992; French, 2006). In Essex, a managed retreat site in the UK, changes in metal mobility were found to be slow and mainly situated near the surface, in newly deposited sediments (Emmerson et al., 2000; Macleod et al., 1999). Also on the longer term, the former land surface was a barrier which limits physicochemical changes in deeper layers (Spencer et al., 2008). Differences in metal fractions between the polder and restored marsh sediments in the present study were observed up to a depth of 1 m after one year. Also based on the comparability in metal concentrations in the different fractions between the restored marsh and the natural tidal areas, we can conclude that changes in metal mobility are fast and over a large depth. An immediate and large drop in pH (from 7 to 3) following breaching, but only lasting for several weeks, observed by (Blackwell et al., 2004) could result in a temporarily increase in metal mobility. Also, intrusion of brackish or salt water can cause a higher metal mobility and extractability (Du Laing et al., 2008b; Macleod et al., 1999; Speelmans et al., 2007; Wong et al., 2010). Yet, this did not result in significant leaching of metals to the environment (Emmerson et al., 2001). Not all metals behave similarly. Comparable to present study, mobility in estuarine or marine sediments is often higher for Cd, Zn and Mn and low for Pb (Delgado et al., 2011; Morillo et al., 2004; Pertsemli and Voutsa, 2007). Low Pb Ex-M in the restored marsh may be caused by its strong co-precipitation with Fe and Mn oxides while a low Cu Ex-M and high Ox-M can be attributed to its high affinity for organic material (Diaz-de Alba et al., 2011; Passos et al., 2010). Low Cr Ex-M in the restored marsh can be attributed to the reduction of $Cr^{(VI)}$ to the less mobile $Cr^{(III)}$ under the reducing conditions caused by flooding (Du Laing et al., 2009c).

As described above, a short term increase in metal mobility is often observed. Yet, a lower metal mobility in wetland compared to dryland conditions is generally described (Gambrell, 1994; Jacob and Otte, 2003a; Odum, 2000; Vandecasteele et al., 2005b). In waterlogged or frequently flooded soils, sulfate may be reduced to sulfide (Mitsch and Gosselink, 2000). The formation of insoluble metal sulfides is found to play a major role in reducing sediment metal availability and transfer to the surface water under anoxic conditions (De Jonge et al., 2012a; Di Toro et al., 1992; Lee et al., 2000c; Teuchies et al., 2011; van der Geest and Paumen, 2008). The reducing conditions in the tidal flat, caused by diurnal flooding, resulted in acid volatile sulfide (AVS) concentrations which were found to be high enough to complex the pool of Cd, Cr, Cu, Ni, Pb and Zn (simultaneously extracted metals, SEM) which might decrease their mobility (Fig. 3). The same conditions ([SEM-AVS] < 0) were observed in the upper sediment layer of the most inundated site (L) of the restored marsh (Fig. 5). Since accretion in this site was more than 10 cm in the first year, the 0-10 cm layer consisted entirely of newly deposited sediments. The prolonged inundation period which characterizes the tidal regime of the restored marsh (controlled reduced tide, CRT) may be favorable for metal sulfide precipitation. The AVS concentrations were higher than values reported in 84 other streams spread over Western Europe (Burton et al., 2007), comparable with marine mudflat sediments (Di Toro et al., 1990) and in the lower range of concentrations found in other Belgian watercourses (De Jonge et al., 2009). We may conclude that in newly deposited sediments and under the conditions created in restored marsh, the presence of sulfides may reduce the mobility of ‘SEM metals’, even more than in the natural marsh. As observed by (Charlatchka and Cambier, 2000) flooding is expected to result in an initial increase in metal mobility caused by the dissolution of Mn and Fe oxides after which mobility will decrease again due to (co)precipitation with (Fe)sulfides when the Eh reduces further (Du Laing et al., 2009c; Speelmans et al., 2007).

When AVS exceeds SEM values ([SEM-AVS] < 0), metal concentration are expected to be high in Ox-M and low in Ex-M. A general trend of higher Ox-M in the restored marsh compared to the polder may emphasize that the reducing effect of flooding resulted in the reduction of sulfates to sulfides and (co)precipitation of metals. However, in both natural tidal areas (F, W), Ex-M increased with increasing AVS concentrations, indicating a higher mobility when [SEM-AVS] decreased. Also Ox-M was not remarkable higher when AVS concentrations were high. In general, AVS or [SEM-AVS] was not found to correlate significantly with Ex-M or Ox-M as expected (Table S2). This may be attributed to the redistribution of metals when soil samples were dried during processing in the laboratory (Forstner, 1993; Rao et al., 2008). Sulfides may be oxidized, by which associated metals are released and extracted with the first, most mobile metal fraction (Ex-M). This emphasizes that results from sequential extraction procedures must be treated with caution and preferably combined with other measures of metal mobility, availability or toxicity.

4.4.3. Consequences for phyto-availability

Higher As, Fe, Mn and Pb concentrations were measured in plant tissues of most inundated sites of the restored marsh (M, L) (Fig. 4). Based on the sequential extraction data a higher availability due to dissolution of Fe and Mn oxides and associated metals due to flooding was indeed expected in the restored marsh. Also the specific redox chemistry of As implies a higher mobility when, under reducing conditions, $As^{(V)}$ is reduced and the more mobile and toxic $As^{(III)}$ will be the predominant form (Du Laing et al., 2009a; Frohne et al., 2011; Sharma and Sohn, 2009). Metal concentrations (especially Fe and As) were very high in the roots of typical wetland plants (especially *P. australis* and *L. salicaria*). This can be attributed to the precipitation of Fe and Mn-oxides and associated metals on the plant roots (known as *root plaque*) under the oxidizing conditions maintained in the rhizosphere and can be a reflection of higher metal availability in the vicinity of the roots (Armstrong and Armstrong, 1988; StCyr and Campbell, 1996; Teuchies et al., 2008; Teuchies et al., 2012b).

Based on these processes and the sequential extraction data, also the mobility of e.g. Cd and Zn is expected to increase while no differences in plant tissue concentrations of these elements was observed for the different sites. Adsorption on other fractions, e.g. the newly formed sulfides might have reduced their plant uptake (Gambrell, 1994; Morse and Luther Iii, 1999). Cd and Zn concentrations were found to be higher in *Salix* sp. and *Populus* sp. grown under oxidizing compared to reduced conditions (Vandecasteele et al., 2005b; Vandecasteele et al., 2007). High Cr in plant tissues of the polder area may be caused by their high concentrations in the polder soil while also the presence of the oxidized and more toxic and mobile $Cr^{(VI)}$ may have contributed to an elevated uptake (Du Laing et al., 2009c). Yet, since different plant species were sampled in the different sites, it is difficult to distinguish between species specific uptake mechanisms and soil availability. *Sambucus nigra* and *Populus* sp., sampled as two of the most dominant plant species in the polder area were characterized by high Cr and Cd concentrations respectively (Table S3). Despite higher metal concentrations in the polder area, low bioaccumulation by maize (Table S5) resulted in low metal concentrations in the aboveground plant tissues. Tidal marsh restoration coincides with the establishment of a species rich wetland vegetation which can alter metal transfer from soil to plant tissues and transfer to the estuarine food chain.

4.4.4. Conclusions: implications for marsh restoration

The consequences of tidal inundation on metal availability are complex and not straightforward (Fig. 5) (Bryan and Langston, 1992). Based on the results of the sequential extraction we can conclude that tidal flooding of a polder, in order to restore the tidal marsh, resulted in dissolution of Fe/Mn-hydroxides and associated metals, which caused a general shift to higher metal mobility. But redistribution of metals from sulfide precipitates towards the mobile fraction (Ex-M) during sample processing may have resulted in an overestimation of the mobile metal fraction. Metal sulfide precipitates, mainly measured in the freshly deposited sediments, are expected to reduce metal mobility on the long term. However, the formation of metal sulfide complexes is expected to reduce the toxicity of a subset of metals (Cd, Cr, Cu, Ni, Pb, Zn) (Berry et al., 2004; Lee et al., 2000c) while a higher mobility of e.g. the metalloid As can be expected under reducing conditions (Sharma and Sohn, 2009). In addition, metal availability measured in bulk sediments, based on sequential extractions or the SEM-AVS model, is not necessarily related to metal uptake and toxicity for biota. For example ingestion of contaminated sediments by invertebrates, the specific conditions created at the root-sediment interface of macrophytes or species specific uptake by plants can have a large influence on metal accumulation (Almeida et al., 2004; De Jonge et al., 2010; De Jonge et al., 2012b; McLaughlin et al., 2000; Mourier et al., 2011; Teuchies et al., 2012b). Also the improving oxygen conditions in the Schelde estuary (Cox et al., 2009) may reduce metal sulfide precipitates in the sediment on the longer term (De Jonge et al., 2012a; De Jonge et al., 2012b; Teuchies et al., 2011).

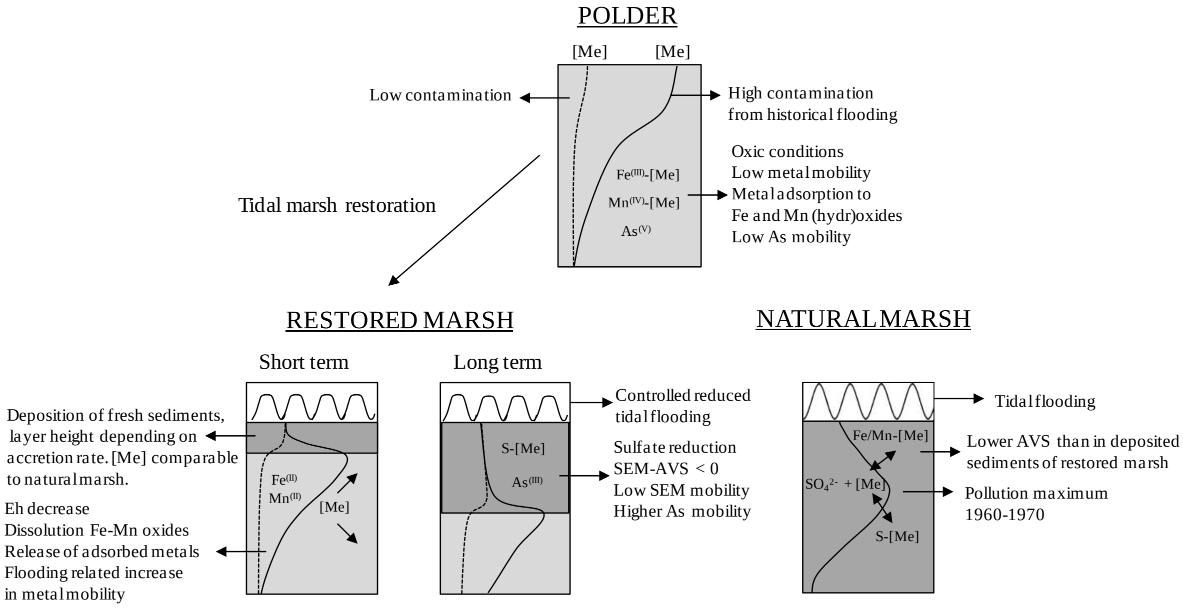


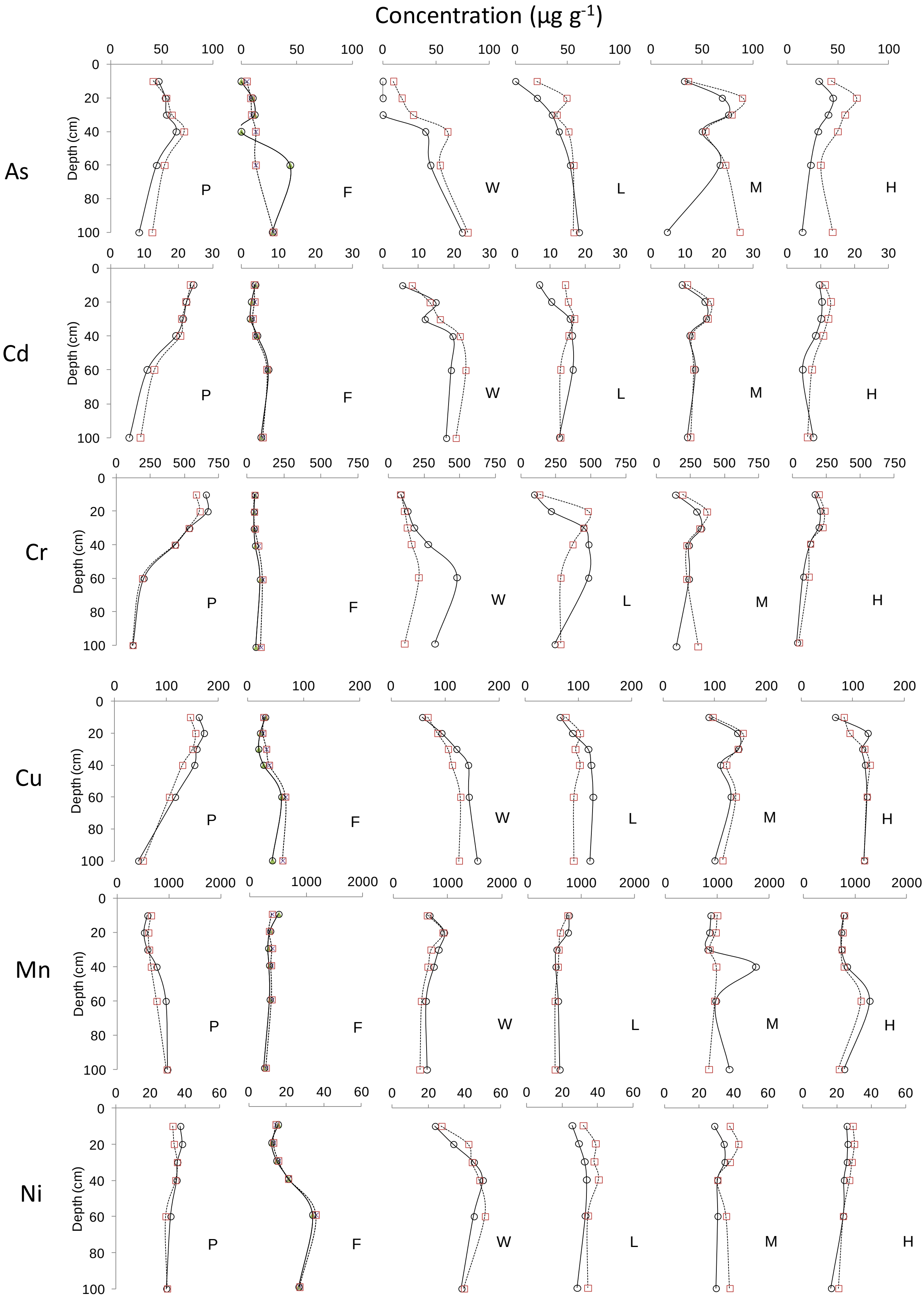
Figure 5. Conceptual graph of the main metal related processes which occured in the soil and sediment of a polder area, restored marsh and natural tidal marsh in present study. Lines in the figures indicate metal concentrations ([Me]) in the soil from the surface up to a depth of 1 meter.

Different conditions prevailing in other sites or estuaries can result in a different metal behavior (French, 2006). Changes in metal availability and release are expected to occur fast after re-inundation and temporal changes in metal mobility can be expected. Therefore it is suggested that in situ measurements of metal availability occur with a short time interval especially in the first months of tidal flooding. Since the results of the BCR sequential extraction method and [SEM-AVS] were rather contradictory, the additional use of direct measurements of availability (e.g. with diffusion gradient in thin film (DGT) membranes or pore water metal concentrations) are recommended

Supporting information

Table S1. Extraction steps executed in this study following the modified BCR sequential extraction method (Rauret et al., 1999).

Fraction	**Phase Targeted**	**Extraction reagent/ Conditions / extraction time**
Step1 (Ex-M) Exchangeable / acid-soluble	adsorbed and exchanged	1. 40 ml of 0.11 mol l^{-1} Acetic acid added to 1 gm of sediment; 2. Extraction carried out by shaking for 16 hours 3. Aliquot separated by centrifugation 4. Residue washed with 20 ml distilled water (shaking for 15 minutes followed by centrifugation) . Supernatant was discarded.
Step 2 (Red-M) Reducible	Bound to iron and manganese oxides	1. 40 ml of 0.5 mol l^{-1} Hydroxylamine hydrochloride(pH adjusted to 1.5 with HNO_3) added to the residue of step 1; 2. Extracted by shaking for 16 hours. 3. Aliquot separated by centrifugation 4. Residue washed with 20 ml distilled water (shaking for 15 minutes followed by centrifugation) . Supernatant was discarded.
Step 3 (Ox-M) Oxidizable	Bound to sulphide and organic matter	1. 10 ml 8.8 mol l^{-1} H_2O_2 (pH 2-3) added to residue from step 2. 2. Digested at room temp for 1 hr with occasional shaking followed by digestion at 85± 2ºC for 1 hr. till the volume reduces to 3 ml 3. Additional 10 ml of H_2O_2 added and, digested for 1 hr . The volume is reduced to 1 ml 4. 50 ml of 1.0 M Ammonium acetate added after cooling, extracted by shaking for 16 hours 5. Aliquot separated by centrifugation 6. Residue washed with 20 ml distilled water (shaking for 15 minutes followed by centrifugation). Supernatant was discarded.


Concentration (μg g-1)
As
Cd
Cr
Cu
Mn
Ni
Depth (cm)
0
20
40
60
80
100
0 50 100
0 10 20 30
0 250 500 750
0 100 200
0 1000 2000
0 20 40 60
P
F
W
L
M
H

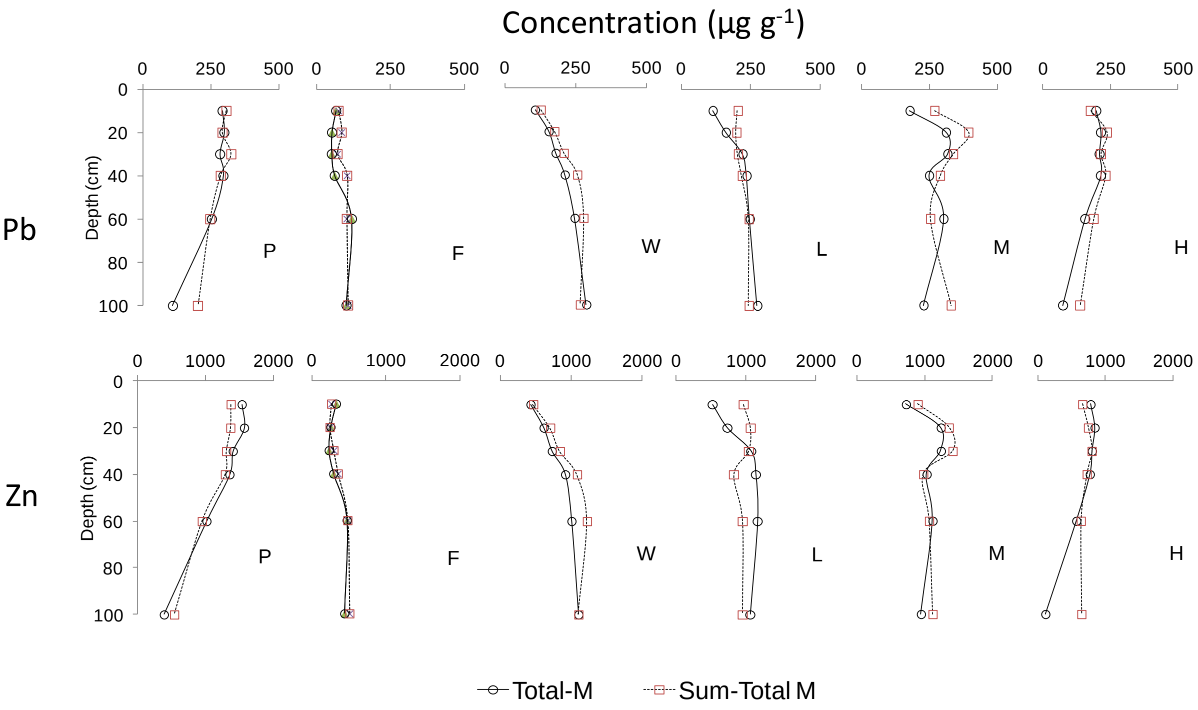


Figure S1. Total amount of the metal removed at each step of the procedure (Sum of extracted metals) compared with the results from the pseudo-total digestion (Total metal) for the recovery during sequential extraction procedure.

Table S2. Pearson correlations between AVS or [SEM-AVS] and the exchangeable (Ex-M) or oxidizable (Ox-M) fractions of the different metals. Significant correlations (*p*<0.05) bold.

		As	**Cd**	**Cr**	**Cu**	**Mn**	**Ni**	**Pb**	**Zn**
AVS	**Ex-M**	-0.088	-0.218	0.061	-0.444	0.102	0.388	0.120	-0.002
	Ox-M	-0.224	0.087	-0.251	-0.259	-0.324	0.105	**-0.475**	**0.481**
[SEM-AVS]	**Ex-M**	0.346	**0.503**	0.151	0.652	-0.039	-0.174	-0.045	0.097
	Ox-M	0.159	0.063	0.434	0.369	0.242	-0.045	**0.603**	-0.296

Table S3. Average (n=3-6) metal concentrations in different plant tissues.

Species	tissue	As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn
E. hirsutum	L	0.937	0.402	4.05	16.7	463	44.9	1.13	3.22	70.9
	S	0.277	0.239	2.28	10.4	126	15.4	0.733	1.01	82.2
	R	1.36	3.20	5.44	18.8	462	27.6	1.33	3.68	107
I. glandulifera	L	1.04	2.10	3.87	13.2	689	57.7	1.70	2.58	126
	S	0.305	1.48	2.79	3.23	157	10.2	0.680	0.554	56.3
	R	1.26	1.95	5.26	9.39	1.21 10^3	62.7	1.51	5.24	74.1
L. salicaria	L	2.50	0.448	5.48	8.79	3.36 10^3	206	2.10	6.05	65.7
	S	1.93	1.46	4.65	10.2	1.92 10^3	230	1.80	4.21	74.5
	R	8.81	1.63	5.84	11.6	7.75 10^3	359	2.75	8.55	99.5
P. australis	L	1.06	0.128	3.33	4.18	412	151	0.705	1.60	25.5
	S	0.503	0.123	3.40	2.20	250	44.7	0.600	0.928	15.9
	R	41.9	0.405	7.65	7.12	8.29 10^3	298	3.50	11.2	58.8
P. lapathifolium	L	0.712	0.276	3.37	7.98	581	408	1.12	4.68	72.6
	S	1.47	0.509	3.41	5.89	718	166	1.05	1.95	67.5
	R	4.71	2.55	8.77	30.8	3.66 10^3	550	5.76	17.2	144
Populus sp.	L	0.738	6.19	5.35	9.36	296	64.4	4.09	1.60	432
	S	0.276	3.58	2.44	5.94	126	14.6	1.45	0.605	108
	R	2.57	7.83	15.2	18.2	449	21.0	4.20	5.28	154
Salix sp.	L	0.643	6.10	3.79	7.59	453	86.4	1.65	2.38	370
	S	0.643	4.33	3.39	8.90	378	59.9	1.07	1.78	187
	R	2.34	4.55	6.25	34.2	1.08 10^3	89.1	4.50	3.58	203
S. nigra	L	1.55	1.52	10.2	10.2	503	29.7	1.63	4.52	66.5
	S	0.858	0.307	5.41	7.34	441	27.6	1.07	2.01	55.4
	R	2.50	2.13	15.2	13.8	498	18.0	1.65	4.31	82.9
U. dioica	L	1.31	0.337	5.56	16.0	1.33 10^3	71.3	2.27	3.98	44.3
	S	0.493	0.576	4.39	7.34	261	17.6	0.840	1.27	35.7
	R	0.933	0.402	3.77	11.6	543	37.1	1.54	2.31	37.1
Z. mays	L	0.104	0.151	2.93	2.36	80.8	34.9	0.292	0.622	15.4
	S	0.169	0.127	1.83	1.58	85.2	9.98	0.273	0.531	18.8
	R	1.94	0.524	3.82	11.5	1.18 10^3	42.9	1.84	7.15	24.0

Table S4. Standard deviation of the average metal concentrations in different plant tissues (see Table S3).

Species	tissue	As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn
E. hirsutum	L	0.379	0.216	1.12	1.4	128	5.9	0.05	1.06	8.9
	S	0.074	0.078	0.40	5.3	35	2.6	0.386	0.34	72.2
	R	0.43	3.44	1.25	6.3	172	10.2	0.28	1.53	31
I. glandulifera	L	0.16	0.01	0.67	6.0	189	13.3	0.25	0.88	30
	S	0.071	0.15	0.45	1.03	7	0.5	0.155	0.106	18.3
	R	0.62	0.41	2.07	1.67	$0.64\ 10^3$	21.0	0.49	2.57	8.4
L. salicaria	L	1.92	0.184	1.73	2.03	$5.78\ 10^3$	141	1.25	4.04	18.7
	S	1.26	2.50	1.22	7.1	$2.44\ 10^3$	210	0.82	2.26	52.9
	R	10.81	1.50	2.77	7.3	$10.59\ 10^3$	385	2.30	8.66	59.6
P. australis	L	0.92	0.081	1.12	0.75	271	129	0.314	1.17	8.5
	S	0.146	0.093	0.70	0.10	146	14.0	0.135	0.574	6.2
	R	31.9	0.085	1.73	1.36	$2.92\ 10^3$	143	1.82	3.46	6.8
P. lapathifolium	L	0.160	0.037	0.31	1.56	165	36	0.30	3.31	10.3
	S	2.03	0.157	1.91	0.93	910	103	0.59	1.83	44.1
	R	3.79	1.82	2.41	18.4	$2.99\ 10^3$	438	2.23	1.46	81
Populus sp.	L	0.392	5.20	3.78	1.97	158	52.8	2.44	1.28	383
	S	0.064	3.03	0.30	1.44	41	8.2	0.65	0.126	73
	R	0.45	5.15	4.0	4.1	135	4.8	3.01	1.98	68
Salix sp.	L	0.305	2.73	1.04	2.78	181	26.6	0.27	1.15	90
	S	0.680	2.41	1.26	3.31	356	80.8	0.41	1.97	51
	R	2.10	3.57	2.19	22.1	$1.08\ 10^3$	102.0	5.41	1.63	135
S. nigra	L	0.18	0.39	0.3	1.7	41	5.6	0.28	0.34	19.5
	S	0.154	0.035	2.61	1.68	167	14.6	0.05	0.34	20.3
	R	1.65	2.21	7.0	6.1	297	6.8	0.71	2.83	44.7
U. dioica	L	0.71	0.196	2.37	8.3	$0.99\ 10^3$	36.8	1.22	2.54	19.3
	S	0.504	1.280	3.36	2.08	188	10.5	0.540	1.59	30.5
	R	0.525	0.266	1.42	4.3	334	19.5	0.80	1.31	17.6
Z. mays	L	0.034	0.020	0.22	0.38	43.418	31.6	0.036	0.301	6.3
	S	0.045	0.009	0.38	0.24	34.279	4.10	0.085	0.376	6.3
	R	0.86	0.074	0.44	3.5	$0.31\ 10^3$	8.2	0.58	1.26	3.8

Table S5. Average transfer coefficients (soil – aboveground plant tissue) for the 3 different areas. For the Polder only values from *Zea mays* used.

	As	Cd	Cr	Cu	Mn	Ni	Pb	Zn
Polder (maize)	0.003	0.005	0.003	0.011	0.028	0.007	0.002	0.011
Restored marsh	0.070	0.115	0.019	0.099	0.201	0.049	0.017	0.104
Tidal marsh	0.046	0.310	0.029	0.116	0.057	0.045	0.014	0.251

CHAPTER 5

Role of plants in metal cycling in a tidal wetland: implications for phytoremidiation.

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Abstract

Accumulation of 9 metals in 29 plant species was quantified in a restored tidal wetland on a contaminated site. Transfer coefficients between sediment and aboveground plant tissues were lower than in many other systems; from 0.013 (Pb) to 0.189 (Mn). A minor fraction of the sediment metal pool cycled through the aboveground vegetation (≤0.02%). However, during the four years of this study, species composition changed, and plant biomass as well as the metal pool in the vegetation increased (≤0.12%). Succession to either a willow dominated brushwood or a monospecific reed stand can further enlarge this pool (≤2.5%). Since the amount of trace metals in the wetland soil or in suspended solids deposited during tidal flooding are some orders of magnitude larger than the vegetation pool, phytoextraction is not applicable. To reduce toxic metal distribution to the wider environment, species with low accumulation, e.g. *Scirpus maritimus* or *Typha latifolia*, are preferred.

5.1. Introduction

In estuaries or coastal zones, managed realignment and controlled flooding of formerly embanked areas have proven to be effective and economically viable strategies to combine flood control and wetland restoration in one and the same area (Beauchard et al., 2011; French, 2006; Jacobs et al., 2009; Zedler and Kercher, 2005). However, historical contamination, land disposal of contaminated dredged sediments or irrigation of embanked areas with contaminated river water resulted in high concentrations of toxic metals in many areas suitable for the implementation of these projects (Emmerson et al., 2000; Teuchies et al., 2012a; Vandecasteele et al., 2003a). Inundation of contaminated land in order to restore wetlands will be applied increasingly; yet, processes in these often impaired sites are still poorly understood (Spencer and Harvey, 2012).

Wetlands filter out toxic metals from the surface water, often immobilize them in their sediments and on the long term return them to the geological part of the earth cycle (Odum et al., 2000; Prasad et al., 2006; (Gambrell, 1994). Plants colonizing the restored wetlands can significantly influence the behavior or potential toxicity of historically present or newly introduced metals (Weis and Weis, 2004). Plants contribute to the filter function of tidal wetlands in several ways: (1) plants will reduce stream velocity and thereby increase the sedimentation of particles and associated pollutants (Bal et al., 2011)(Prasad et al., 2006). (2) The high metal adsorption capacity of litter concentrates trace metals from the surface water onto the marsh surface litter layer (Orson et al., 1992; Zawislanski et al., 2001). (3) The release of exudates and oxygen from roots, and the input of organic matter as litter, can modify the sediment biogeochemistry in such a way that the retention capacity of the marsh is increased (Almeida et al., 2011; Caçador et al., 1996; Choi et al., 2006).

However, plants can also enlarge the toxicity of trace metals in wetland soils (Weis and Weis, 2004). For instance, roots of wetland plants which decrease pH or oxidize their rhizosphere can result in local mobilization of trace metals (Almeida et al., 2004; Jacob and Otte, 2004; Vervaeke et al., 2004; Wright and Otte, 1999). Trace metals, immobilized in the soil, can be accumulated in aboveground plant tissues which can enlarge food chain contamination (Reboreda and Caçador, 2007) or accumulated metals can be excreted by leaves in a more available form (Burke et al., 2000; Weis et al., 2002). Finally, containing litter metals, often with increasing concentrations during decomposition, can be taken up by detritus feeders or can be transported to the estuary (Caçador et al., 2009; Windham et al., 2004). Whether plants will mobilize or immobilize metals in wetland sediments depends on a large combination of factors and is difficult to predict. However, the advantages of vegetation appear to outweigh the possible effects of small scale remobilization in the rhizosphere (Jacob and Otte, 2003b). The use of wetlands to treat metal contaminated surface water or remediate sites with elevated soil metal concentrations is widely investigated and implemented (Bert et al., 2009;

Rai, 2008; Salt et al., 1998; Vymazal, 2011). Phytoextraction by accumulation of metals in harvestable plant parts becomes only effective with the presence of plants that either hyperaccumulate metals or have a large annual yield (Keller in Prasad). In most natural wetlands, allocation of metals from sediments to aboveground tissues is low and harvesting the entire vegetation is often not desirable. Phytostabilization-immobilizing metals in belowground plant tissues or sediments- mostly is the preferable approach for remediation of contaminated wetlands with the use of plants (Duarte et al., 2010; Weis and Weis, 2004).

Translocation of trace metals from sediment to aboveground plant tissues differs greatly between species (Deng et al., 2004; Fitzgerald et al., 2003). Knowledge about the differences in trace metal accumulation between plant species is essential to evaluate the effect of plant growth on toxic metal cycling within marshes and to develop appropriate management options (Tack and Vandecasteele, 2008; Weis and Weis, 2004). Additionally, this knowledge is valuable in the risk assessment of metal toxicity in tidal marsh restoration projects in contaminated sites. Therefore, the aim of this study was:

- To investigate differences in metal accumulation among common wetland plant species.
- Compare the total aboveground trace metal pool in plants of a tidal marsh with the pool in sediments and incoming suspended solids.
- Evaluate the possibilities for phytoextraction and phytostabilization in a contaminated tidal marsh.

This paper presents concentrations of 8 metals and As in the aboveground tissues of 29 common wetland plant species and calculates the total aboveground trace metal pool for the entire marsh.

5.2. Material and methods

5.2.1. Study area

The Schelde estuary (Belgium and The Netherlands) is a turbid, macrotidal and eutrophic system (Meire et al., 2005) with high trace metal concentrations in surface water, suspended solids and sediments (Baeyens et al., 2005; Du Laing et al., 2007a; Teuchies et al., 2012a; Vandecasteele et al., 2002a). The study area (51°05’10’’N; 4°10’20’’E) is located in the freshwater tidal zone. The investigated site is an experimental restored freshwater tidal marsh (8.2 ha) (Beauchard et al., 2011; Cox et al., 2006; Jacobs et al., 2009; Maris et al., 2007). The formerly agricultural site has been yearly flooded as a fertilization technique during the 1960’s and 70’s by which heavily polluted sediments were brought in (Teuchies et al., 2012a).

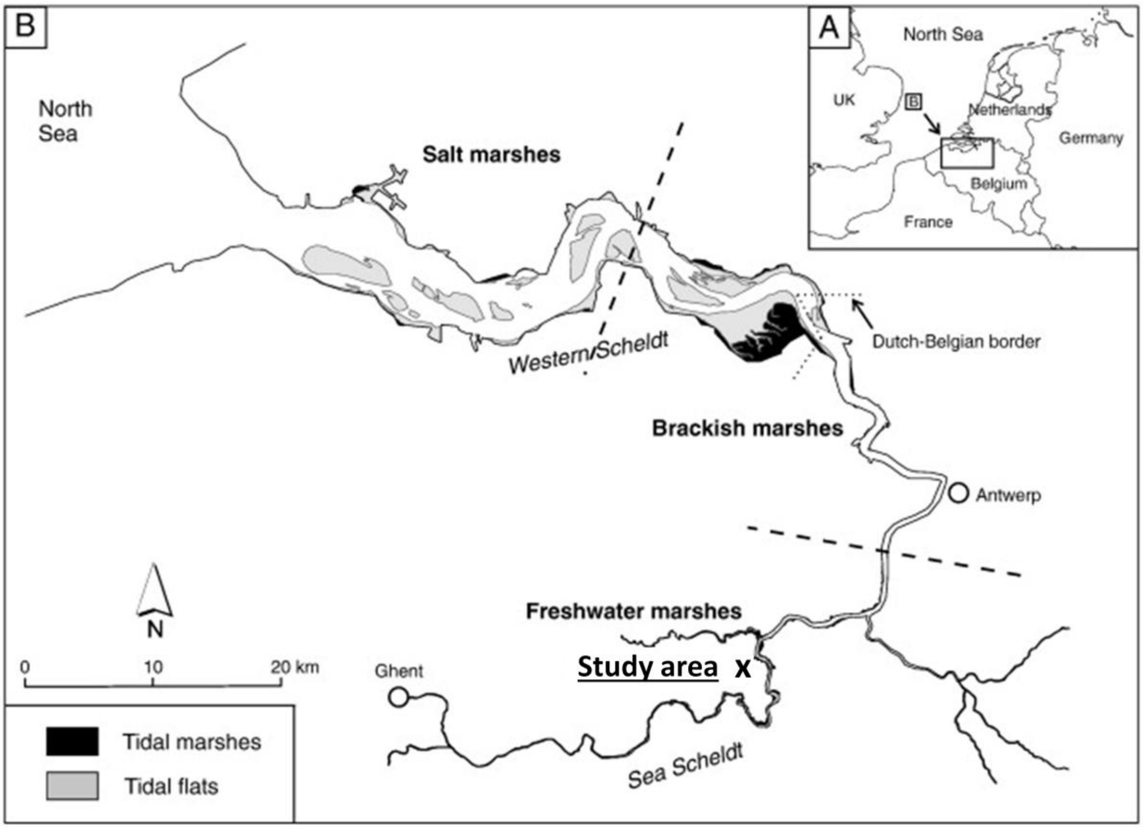

Figure 1. Location of the Schelde estuary (A) and the study area (x) within the estuary (B).

5.2.2. Sampling

5.2.2.1. Plant metal concentrations

Metal (Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn) and semimetal (As) concentrations were determined in the 29 most dominant plant species of the tidal marsh. Plants were sampled during four campaigns, in April and October 2007 and 2008. At each campaign, five individual plants (aboveground tissues, stems and leaves) per species were collected randomly throughout the marsh. For *Salix* sp. higher than 1 m only one branch instead of the entire plant was harvested. Plant samples were thoroughly rinsed with river water in the field as well as with demineralized water in the laboratory, dried for 48 h at 70 °C, weighed and ground.

5.2.2.2. Plant biomass estimation

The biomass of the 28 (excluding algal mats, *Vaucheria* sp.) most dominant plant species was estimated for the entire area during simultaneous campaigns (April and October 2007-2008) as well as during two additional campaigns (September 2009 and 2011). For this purpose the marsh was divided into homogenous polygons (± 80). In each polygon, dominant species' cover (species with at least 20% cover) was estimated. In order to link these estimates to biomass, total leaf area per species (i.e. the total sum of leaf area per surface area, can be more than 100%) was estimated instead of projected leaf area (maximum 100%). The cover-biomass relationship for each species (f_{Sp}) was determined by means of a linear model based on cover and plant dry weight obtained from 0.25 m² plots (Flombaum and Sala, 2007) (Jacobs et al., submitted). In these 0.25 m² plots, plant cover was estimated and subsequently harvested dried and weight for each species separately. Data per species were pooled for all sampling campaigns (at least 20 cover-biomass estimates per species). The collected data on cover, biomass and metal concentrations for the 28 dominant species allowed to estimate the metal pool per species MeP_{Sp} (1) and the total metal pool of the area MeP_{Total}(2):

$$MeP_{Sp} = \sum_{i=1}^{n} CA\, f_{Sp}\, [Me]_{Sp} \qquad (1)$$

$$MeP_{Total} = \sum_{i=1}^{n} MeP_{Sp} \qquad (2)$$

Where C is the estimated cover (%) of a species in a certain polygon, A the surface area (m²) of the polygon, f_{Sp} the species specific factor (g (% cover m²)$^{-1}$) to convert estimated cover (%) into biomass (g) calculated from the cover and biomass data from the 0.25 m² plots, n the number of polygons where the species was found and $[Me]_{Sp}$ the metal concentration (µg g^{-1}dw) of the concerning species. The total metal pool was calculated as the sum of MeP_{Sp} for all 28 dominant species (n). The total metal pool for 2009 and 2011 (no metal concentrations available) was calculated with average metal concentrations measured in plants from 2007-2008.

Average metal concentrations (n=180) in the sediment are based on seasonal measurements in different layers (0-5, 5-10 and 10-15 cm depth) from 2006 to 2009 at 3 different locations (Teuchies et al., 2012a). Displayed transfer coefficients (TC, ratio metal concentration plant/sediment) are based on average sediment concentrations and average plant concentrations measured in 2007 and 2008. The total metal content of the upper 30 cm of the marsh soil, which is assumed as important for phyto-availability and phyto-extraction as most plants roots are situated in this layer, is calculated with an average bulk density of 800 kg m^{-3} measured in the marsh.

The biomass and trace metal pools were also estimated for several possible future scenarios: a monospecific stand of *P. australis* or *T. latifolia* (biomass based on average values measured in monospecific plots of these plant species) or a *Salix* sp. forest (biomass based on literature data).

5.2.3. Laboratory analyses

Plant metal concentrations were measured after hot block digestion: 5 mL nitric acid (HNO_3 69%, Merck EMSURE® for traces analysis) was added to 0.5 g dw (dry weight) plant material. The mixture was heated (30 min at 100 °C) and cooled overnight, 5 mL of hydrogen peroxide (H_2O_2 30%, VWR Int. AnalaR NORMAPUR® for traces analysis) was added, heated again (60 min at 150 °C) and 5 mL of hydrochloric acid (HCl, 32% Merck EMSURE® for traces analysis) was added after which the mixture was heated a last time (15 min at 100 °C). Metal (Cd, Cr, Cu, Fe, Mn, Pb, Ni and Zn) and semimetal (As) concentrations in the resulting mixture were measured after filtration (cellulose mixed ester, 0.45 µm, Chromafil®) using an ICP-AES (inductively coupled plasma - atomic emission spectroscopy, iCAP 6300 Duo SERIES Thermo Fisher Scientific®, Waltham, USA). Blanks and certified reference material for plants (Institute for Reference Materials and Measurements, IRMM, BCR® no 060, aquatic plant (*Lagiosiphon major*)) were included in all series of metal analysis to verify analytical accuracy. All measured metal concentrations were lower than the certified values and average recoveries varied between 81% for Pb to 89% for Cd.

5.2.4. Statistical analyses

Normality of the data was tested with the Shapiro-Wilk test prior to analysis. The significance of differences between seasons or years was tested with a three way analysis of variance test (ANOVA) with variables species, year and season. Not all plant species were sampled each campaign since the dominance of plants changed during succession in the marsh. Consequently, differences between years or seasons were tested only with concentrations in plant species that were sampled in both years and both seasons respectively. The Pearson correlation-coefficient was used to determine correlations between different metal concentrations over all species and campaigns.

5.3. Results

5.3.1. Metal concentrations in plants.

Plant Cr concentrations were significantly higher in spring (p=0.0373) while Mn and Zn concentrations were significantly higher in autumn (p=0.0365 and p=0.0108 respectively) (Table 1). Other differences in the average plant metal concentrations between spring and autumn were small and not consistent. Plant metal concentrations were higher in 2008 compared to 2007 (except for Mn).

Table 1. Average (n=67-98) metal concentrations in plants in the different seasons and years. Significant differences between seasons or years bold, with significant levels $p < 0.05$: *; $p < 0.01$: **.

	As	**Cd**	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Pb**	**Zn**
spring	0.626	0.656	**4.55***	8.37	626	**119***	0.781	1.94	**49.5***
autumn	0.626	0.438	**3.27***	8.98	590	**149***	0.734	1.90	**56.6***
2007	0.593	**0.517***	3.54	9.00	516	149	**0.615***	1.68	**49.9***
2008	0.644	**0.766***	3.86	8.56	635	127	**0.851***	1.98	**59.0***

Highest metal concentrations were measured in the macroalgal species *Vaucheria* sp. (Table 2). The concentrations were 3 (Cd) to 14 times (As) higher than average concentrations of all other plant species. The ‘possible toxicity range’ (Prasad et al. 2006) is exceeded for As, Cr, Cu, Mn, Pb and Zn. Despite the high concentrations, values do not exceed average sediment concentrations and transfer coefficients (TC) sediment-algae ranged from 0.23 (Cr) up to 0.84 (Mn). Algae concentrations are not further considered in the result section.

The variation in average metal concentrations was highest for Cd where the highest concentration (*Salix* sp.) was more than 33 times higher than the lowest concentration (*Phragmites australis*) (Table 2). Variation was much lower for Zn, Ni and As (factor 6). The variation *within* plant species was also rather high (Table S1), especially in *P. australis* and *Salix* sp..

In general, metal accumulation was lowest in monocot, highly productive wetland species (*Typha latifolia* < *Scirpus maritimus* < *Glyceria maxima* < *Juncus maritimus* < *P. australis*) (Table 2). Metal concentrations in *Salix* sp. were lower than concentrations in most other species in the marsh except for Cd and Zn which were three times higher than the average concentration in all other plant species. Highest concentrations were found in some terrestrial plants (e.g. *Cirsium arvense* or *Ranunculus repens*) and small macrophytes (e.g. *Veronica* sp., *Alisma* sp. or *Juncus articulatus*). Highest values were measured in *Veronica anagallis-aquatica* for As, Cr and Pb, in *J. articulatus* for Ni and in *Alisma* sp. for Cu.

Average TC’s were highest for Cd (0.095), Cu (0.093), Zn (0.095) and Mn (0.189) and low for Pb (0.013). All average metal concentrations in the dominant plant species were within the ‘normal range’ and the lower value of the ‘possible toxicity range’ was only exceeded for Mn (two species) and for Cr (eight species) (Table 2) (Prasad). The maximum concentrations measured in the plant species exceeded this lower value of the ‘possible toxicity range’ for Cu (7 species), Mn (10 species), Zn (11 species) and almost in every species (24) for Cr (Table S3).

Table 2. Average trace metal concentrations in sediment (n=180) and plants (n=5-20). Plant concentrations exceeding the possible harmful concentration underlined. Plant species ordered from low to high metal accumulation.

	As **$\mu g\ g^{-1}$**	**Cd** **$\mu g\ g^{-1}$**	**Cr** **$\mu g\ g^{-1}$**	**Cu** **$\mu g\ g^{-1}$**	**Fe** **$\mu g\ g^{-1}$**	**Mn** **$\mu g\ g^{-1}$**	**Ni** **$\mu g\ g^{-1}$**	**Pb** **$\mu g\ g^{-1}$**	**Zn** **$\mu g\ g^{-1}$**
Sediment	29.6	6.77	130	91.6	n.a.	702	25.4	133	543
Possibly harmful conc	5-20	5-30	5-30	20-100	n.a.	300-500	10-100	30-300	100-400
Typha latifolia	0.275	0.0831	1.34	4.43	212	**483**	0.309	0.555	26.5
Scirpus maritimus	0.376	0.233	2.06	4.22	248	288	0.361	0.834	24.9
Glyceria maxima	0.604	0.071	2.98	4.37	378	123	0.351	1.02	36.6
Juncus maritimus	0.234	0.970	0.75	8.02	128	48.6	0.359	0.462	41.9
Phragmites australis	0.339	0.060	2.98	4.64	194	110	1.043	0.592	37.9
Epilobium hirsutum	0.437	0.139	1.65	7.52	314	60.5	0.449	1.13	40.3
Rumex conglomeratus	0.563	0.358	2.47	5.49	492	64.7	0.596	1.53	29.8
Lythrum salicaria	0.432	0.173	2.20	6.81	328	115	0.489	1.02	59.6
Juncus effusus	0.425	1.79	1.93	7.43	288	140	0.347	0.931	52.9
Polygonum lapathifolium	0.611	0.184	2.94	4.77	449	230	0.388	1.37	40.8
Phalaris arundinacea	0.676	0.109	**5.28**	6.23	499	81.8	0.495	1.31	38.1
Salix sp.	0.419	2.03	1.40	4.42	359	53.1	0.402	1.05	132
Urtica dioica	0.688	0.136	4.63	8.24	666	44.6	0.742	2.38	31.2
Persicaria hydropiper	0.743	0.255	2.33	5.78	686	**302**	0.602	2.08	45.8
Lycopus europaeus	0.592	0.138	2.42	11.8	443	67.4	0.725	1.53	74.3
Plantago major	0.405	0.173	**8.03**	9.90	672	127	0.846	1.90	43.2
Bidens cernua	0.656	1.86	1.36	13.4	140	171	0.628	0.659	95.0
Rumex obtusifolius	0.777	0.508	2.70	7.52	803	73.4	0.796	2.37	39.9
Poa sp.	0.626	0.175	4.61	8.13	846	87.5	1.05	2.48	37.7
Symphytum officinale	0.695	0.227	2.82	9.74	590	50.2	0.777	2.16	51.0
Bidens frondosa	0.454	1.88	3.42	11.1	159	76.3	0.962	0.76	70.1
Pulicaria dysenterica	0.743	1.40	**5.43**	7.64	714	86.2	0.831	2.41	39.9
Alisma sp.	0.589	0.647	**5.18**	15.3	630	206	0.798	2.02	62.5
Ranunculus repens	0.721	0.846	**7.76**	14.1	644	111	0.866	2.06	45.1
Veronica beccabunga	0.895	0.463	**7.33**	8.29	$1.05\ 10^3$	103	1.11	3.18	54.4
Juncus articulatus	0.734	1.54	4.66	14.8	760	218	1.39	2.42	69.6
Veronica anagallis-aquatica	1.45	0.576	**8.44**	12.0	$1.72\ 10^3$	134	1.31	5.38	58.9
Cirsium arvense	0.904	0.896	**7.17**	13.5	$1.06\ 10^3$	64.8	1.24	3.34	63.6
Vaucheria sp.	**17.5**	2.08	**29.2**	**38.2**	$13.8\ 10^3$	**588**	8.84	**36.2**	297
Average	1.19	0.690	4.67	9.58	$1.01\ 10^3$	149	1.00	2.94	60.0
Average without algae	0.609	0.640	3.80	8.56	552	133	0.72	1.75	51.6
Transfer coefficient	0.0403	0.102	0.0361	0.105	n.a.	0.212	0.0395	0.0221	0.111
Transfer coefficient (without algae)	0.0206	0.095	0.0293	0.0934	n.a.	0.189	0.0285	0.0132	0.0950

5.3.2. Biomass and metal pools in the marsh.

The estimated total plant biomass doubled, from 3.18 ± 0.87 tons ha^{-1} in autumn 2007 to 9.44 ± 2.15 tons ha^{-1} in autumn 2011 (Fig. 2). The estimated metal pool increased accordingly, with the assumption that metal concentrations in 2007-2008 are a reliable estimation for metal concentrations in 2009 and 2011. During the investigated period the marsh was in an early successional stage. The pioneer species *Persicaria hydropiper*, *Epilobium hirsutum*, *Lythrum salicaria* and *Urtica_dioica*, contributed substantially to the total plant biomass as well as the total aboveground metal stock (Table 3). A decrease in the contribution of these species to the total plant biomass is occurring as the marsh evolves to a climax succession stage. Macrophyte species which become more dominant have lower metal concentrations: the estimated biomass of *P. australis* increased five-fold between autumn 2007 and 2011 (0.49 ± 0.11 to 2.72 ± 0.63 tons ha^{-1}) and threefold for *T. latifolia* (0.60 ± 0.24 to 1.75 ± 0.62 tons ha^{-1}). *G._maxima* and *S._maritimus* were absent before 2008 after which their biomass increased considerably. The total estimated biomass of *Salix* sp. was found to decrease during the investigated period. However, difficulties and large errors in biomass estimations of individual *Salix* trees compared to monospecific stands of annual plant species rather than an actual decrease in biomass of *Salix* sp. is assumed. An increase in the number and biomass of *Salix* trees was clearly observed between 2007 and 2011. Since the amount of Cd accumulated in *Salix* sp. biomass constitutes almost 60% of the total Cd content in the marsh plants, the decrease in total Cd content can be attributed to a decrease in the estimated biomass of *Salix*_sp.

The estimated amount of trace metals that cycled yearly within the aboveground vegetation ranged from 2.44 g Cd ha^{-1} up to 1.92 kg Fe ha^{-1} which is a very small fraction of the metals in the upper 30 cm of the sediment (range from 0.002% for Pb to 0.053% for Mn) (Table 3). In a future scenario with *P. australis* dominance this fraction could increase to 0.006% (Pb) and up to 0.202% (Mn). In case of a *Salix*_sp. forest (with an estimated biomass of 200 tons ha^{-1} for a 20 year old stand (Van Nevel et al., 2011; Vervaeke et al., 2001)) the trace metal stock is roughly 40 times higher than in the scenario of a monospecific reed stand (Table 3). Especially for Cd, the stock (400 g Cd ha^{-1} in a 20 year old stand) and yearly cycled amount (20 g Cd ha^{-1}) is much larger in the *Salix*_sp forest (Fig 2.).

Table 3. Average weight and average absolute metal concentrations in plants. The eight plant species with the largest contribution displayed (% of contribution compared to the entire marsh given). Values for future scenarios with monospecific stands and the proportion of metals in plants compared to sediments are displayed.

	weight ton ha^{-1}	**As g ha^{-1}**	**Cd g ha^{-1}**	**Cr g ha^{-1}**	**Cu g ha^{-1}**	**Fe g ha^{-1}**	**Mn g ha^{-1}**	**Ni g ha^{-1}**	**Pb g ha^{-1}**	**Zn g ha^{-1}**
Glyceria maxima	0.308	0.186	0.022	0.918	1.35	117	38.0	0.108	0.315	11.3
Urtica dioica	0.267	0.184	0.036	1.24	2.20	178	11.9	0.198	0.635	8.34
Typha latifolia	0.669	0.184	0.056	0.896	2.96	142	323	0.206	0.371	17.7
Lythrum salicaria	0.481	0.208	0.083	1.06	3.27	158	55.1	0.235	0.488	28.6
Salix sp.	0.715	0.300	1.45	1.00	3.16	257	38.0	0.288	0.750	94.7
Epilobium hirsutum	0.776	0.339	0.108	1.28	5.83	243	47.0	0.348	0.878	31.3
Phragmites australis	1.08	0.366	0.0649	3.21	5.00	209	118	1.12	0.638	40.8
Persicaria hydropiper	0.675	0.502	0.172	1.57	3.90	463	204	0.406	1.41	30.9
Total	5.36	2.48	2.44	12.4	31.2	1.92 10^{3}	887	3.15	5.98	285
% of total	93	91	82	90	89	92	94	92	92	93
Future *P. autstralis*	30.9	10.5	1.86	92.2	144	6.00 10^{3}	3.40 10^{3}	32.3	18.3	1.17 10^{3}
Future *T. latifolia*	45.6	12.5	3.79	61.0	202	9.66 10^{3}	2.20 10^{3}	14.1	25.3	1.21 10^{3}
Future *Salix sp.*	200	71.6	562	291	396	168 10^{3}	6.47 10^{3}	77.9	340	18.0 10^{3}
Sediment (0-30 cm) kg ha^{-1}		71.1	16.2	311	220	na	1.68 10^{3}	60.9	319	1.30 10^{3}
% of sediment (present)		0.003	0.015	0.004	0.014	na	0.053	0.005	0.002	0.022
% of sediment (*P. australis*)		0.015	0.011	0.030	0.065	na	0.202	0.053	0.006	0.090
% of sediment (*Salix*)		0.101	3.458	0.094	0.180	na	0.384	0.128	0.107	1.378

5.4. Discussion

5.4.1. Metal accumulation in marsh plants

The sediments of the investigated tidal marsh are highly contaminated with trace metals (Table 2). Average Cd concentrations exceed the ‘Flemish soil sanitation threshold for nature areas’ and average Cr and Cu concentrations exceed their ‘serious effect levels’ (de Deckere et al., 2011). Anthropogenic contamination is large, mainly for Cd, Pb and Zn, with concentrations exceeding the ‘average shale’ 31 (Cd), 7 (Pb) and 6 (Zn) times (Luoma and Rainbow, 2008). The measured concentrations of all metals exceeded their preindustrial concentrations from floodplain sediments in Belgium between 1.6 times (Cr) and 18 times (Cd) (Maes, 2007). Highest metal concentrations were measured in the former agricultural soil, while marsh restoration resulted in deposition of a uniform, less contaminated layer (Teuchies et al., 2012a).

Plant metal concentrations were higher in 2008 compared to 2007. This may be attributed to the earlier start of the autumn in 2007: plants had a lower leaf/stem biomass ratio when sampled, and metal concentrations are often higher in leaves compared to stems (Odum, 2000; Vymazal et al., 2007).

Also, an increase in metal availability caused by flooding related processes in the contaminated agricultural soil (Gambrell, 1994; Teuchies et al., 2012a) might have resulted in larger plant uptake in 2008 (Vandecasteele et al., 2002b). However, differences in season and year are based on a small amount of replicas per species. Since plants are not harvested on similar locations each year, differences in concentrations may be caused by the spatial difference in metal concentration in the sediment.

Highest metal concentrations were measured in the macroalgal species *Vaucheria* sp. (Table 1). The high concentrations may be attributed to a high metal biosorption capacity of algae mats (Odum, 2000), which is linked to the exchange of mainly Ca-ions with trace metals at certain functional groups on the algal cell wall (Michalak and Chojnacka, 2010). The biomass of a dense mat of *Vaucheria* sp. was measured (49 ± 28 g m^{-2}) and is much lower than the estimated biomass of a dens stand of *P. australis* (3000 ± 700 g m^{-2}) or *T. latifolia* (4500 ± 1800 g m^{-2}). Additionally, the surface area of mudflats covered with *Vaucheria* sp. in the marsh was generally low. The contribution of these algae to the total metal accumulation in the marsh is difficult to assess, mainly due to high temporal variation in algal biomass, but is expected to be low. This species is therefore not further considered in the discussion.

Despite the elevated metal concentrations in the sediment, low metal accumulation by the investigated plant species was indicated, since all concentrations were within the ‘normal range’, none of the plant species reached the ‘hyperaccumulation thresholds’ and the TC’s in the marsh were relatively low compared to general values reported by Prasad et al. (2006). This might indicate a lower metal phyto-availability in the marsh sediments compared to dryer upland soils. The specific conditions created in wetlands, high clay and organic matter, buffered pH and low redox potential values, are indeed found to reduce metal availability (Gambrell, 1994). The higher TC or phytoavailability for Cd, Cu and Zn, as observed in present study, is found in many systems, including tidal marshes and is caused by their lower sorption capacity to soil components, (Almeida et al., 2004; Deng et al., 2004; Gambrell, 1994; Overesch et al., 2007). The high TC of Mn, which is an essential element of many enzyme systems, hence needed in a larger amount, can also be caused by preferential uptake (Kabata-Pendias and Pendias, 2001).

Wetland plants are often found to have a high metal tolerance and high concentrations are found in tissues of several species, including *Typha* sp. and *Phragmites* sp., growing in wetlands receiving acid mine drainage (Deng et al., 2004) (Odum et al., 2000). In present study, metal accumulation was lowest in monocot, highly productive wetland species. Much higher *T. latifolia* metal concentrations and transfer coefficients (TC’s) were found in an artificial lagoon and a natural stream with lower or similar sediment metal concentrations (Carranza-Álvarez et al., 2008; Sasmaz et al., 2008). Metal concentrations in *T. latifolia* and *Scirpus* sp. from present study were much lower than most values in these species from different studies summarized by Carranza-Álvarez et al. (2008). Also TC’s for *G. maxima* growing in a pond receiving motorway runoff were found to be higher than in present study (Sriyaraj and Shutes, 2001). Metal concentrations in sediment and *P. australis* samples were comparable with data from other tidal marshes along the Schelde estuary (Du Laing et al., 2009d). Comparable to present study, high TC’s for Cd and Zn are often found in *Salix* sp. (Pulford and Watson, 2003a). Higher metal concentrations, including Cd and Zn, were reported in *Salix* sp. growing on contaminated dredged sediment derived soils near the Schelde (Vandecasteele et al., 2002b; Vandecasteele et al., 2005a). Metal concentrations in sediment and *U. dioica* were within the range of values reported by Notten et al. (2006). Zn concentrations in plants from present study were generally much lower than values reported in most of the 27 plant species growing on a metal contaminated site (Moreira et al., 2011).

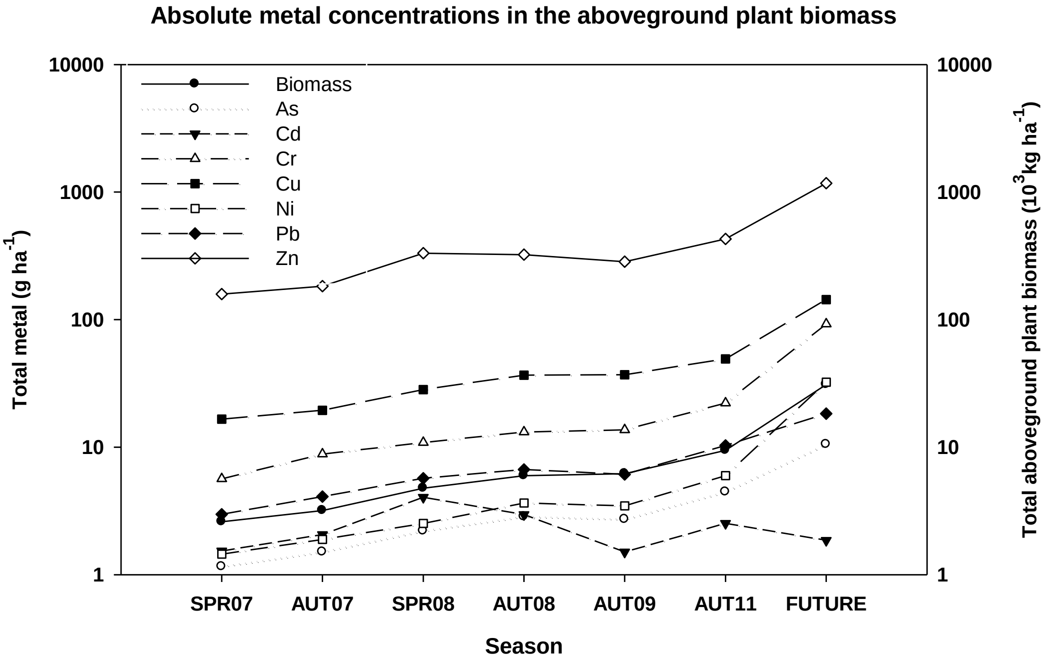

Figure 2. Estimates of the total metal content and total biomass of the aboveground plants for the different sampling seasons. Future is a scenario with a monospecific reed stand covering the entire tidal marsh.

5.4.2. Phytoremidiation in a tidal marsh?

5.4.2.1. Phytoextraction

Phytoextraction, a technique which removes toxic elements by concentrating them in harvestable plant parts, becomes effective with the use of plants that either hyperaccumulate toxic metals or have a large annual yield (Prasad et al., 2006). None of the 28 plant species from the marsh of present study reached the '*hyperaccumulation thresholds'* (Prasad et al., 2006; (Lasat, 2002) and despite a high annual biomass of many plant species dominant in the marsh, only a small fraction of the trace metals present in the sediment were accumulated in the total plant biomass during the early successional stage of the tidal marsh in the present study (Table 3). The observed increase in biomass between 2007 and 2011 caused an increase in the metal standing stock of the aboveground vegetation. This with the assumption that trace metal concentrations in plants were not considerably different between 2007-2008 and 2011, since the total metal pool in 2011 was calculated with the available metal concentrations in the plants of 2007-2008. Since most plant species are annuals and *Salix* sp. concentrates most metals in leafs, the accumulation of metals during successive years may not be of large importance. Changes in metal mobility in the sediment may influence metal accumulation in plants, but even if changes in metal concentrations occurred, the total plant metal pool will remain small.

The calculated aboveground metal standing stock in the early successional stage was roughly a factor 10 smaller than calculated stocks in a constructed wetland planted with *Phalaris arundinacea* (Vymazal et al., 2010). The aboveground biomass and metal content of natural saltwater marsh vegetation were comparable to the stocks of the monospecific stands of *P. australis* and *T. latifolia* calculated in present study (Caçador et al., 2009). *Salix*-dominated tidal forest is the climax succession stage in natural tidal marshes of the freshwater part of the Schelde estuary, and might also become predominant in the restored marsh (Jacobs et al., 2009). Succession to *Salix* sp. forest will result after 20 years in a stock of trace metals stored in the woody structures of the trees which is roughly 50 times higher than in the early successional stage. Especially the estimated total amount of Cd and Zn are high but still comparable to calculated stocks in a 10 year old aspen stand on contaminated soil (Van Nevel et al., 2011). Management of the restored marsh can influence the competitive outcome of *P. australis* versus *Salix* sp.. Winter mowing and willow-chopping or sluice configuration adjustments which increases flooding duration and frequency will promote reed growth (Jacobs et al., 2009). A marsh which is covered by a monospecific stand of *P. australis* in a managed scenario will result in a lower metal stock in the aboveground vegetation.

Phytoextraction by *Salix* sp. is often suggested but mostly found to be too slow to be realistic (Pulford and Watson, 2003a; Van Nevel et al., 2007). Only for removal of Cd and Zn, which are accumulated in higher amounts, this technique may be applicable in certain situations (Meers et al., 2007). (Mertens et al., 2006) found a maximum *Salix* sp. extraction ratio for Cd; they calculated an annual uptake of 1.6% of the amount of Cd in the upper 0.5 m of the soil in the aboveground biomass, which means that the biomass should be harvested annually for 50 years to meet the criteria for reuse as soil in Flanders. In present study not even 3% of the sediment Cd pool is accumulated after 20 years. Besides, the average estimated constant deposition of metal contaminated suspended solids during tidal flooding in marshes from the Schelde (± 5 kg dw sediments m^{-2} a^{-1}) (Temmerman et al., 2004a) results in an input of trace metals which exceeds the total metal content in the present vegetation on average 300 times (Fig. 3 for Cd). Accordingly, the effect of metal accumulation in the aboveground vegetation on the global metal cycle was negligible in the marsh, for all scenarios.

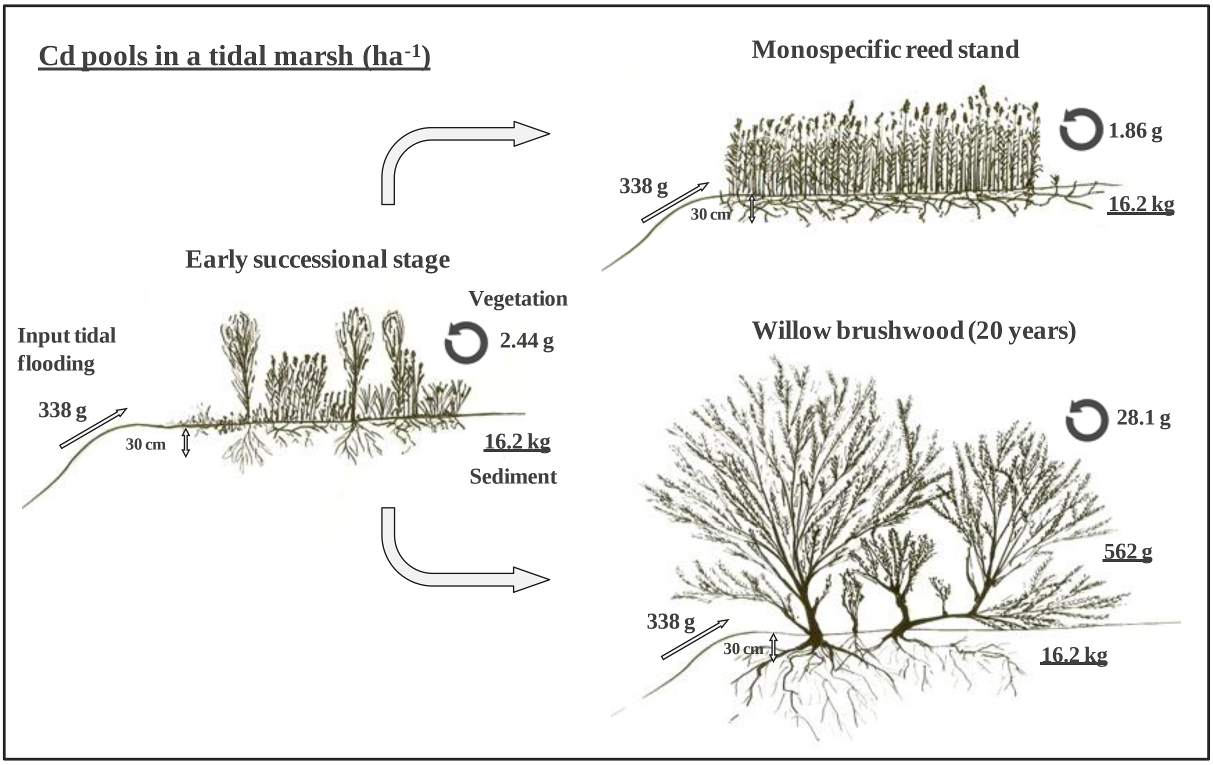

Figure 3. Conceptual scheme of annual flow and pools of Cd in a tidal marsh in an early successional stage and two possible climax situations (reed and willow). Estimated pools (g or kg Cd ha^{-1}) underlined (sediment top 30 cm and wood in the willow stand). Estimated annual flows for inflow with tidal flooding and for the aboveground vegetation displayed (g Cd ha^{-1}).

5.4.2.2. Phytostabilization

Movement of trace metals from contaminated sediments to above ground plant tissues can significantly increase their availability to other organisms (Prasad et al., 2006). Direct metal excretion from plant leaves and consumption of metal contaminated living plant tissues as well as litter can contribute to the risk of food chain contamination (Bert et al., 2009; Burke et al., 2000; Weis et al., 2002). High accumulation of Cd and Zn in *Salix* sp. leaves might cause transfer to the food chain via direct consumption of leaves or via metal contaminated litter (Vandecasteele et al., 2002b). Leaf beetles showed elevated body Cd concentrations when foraging on poplar trees growing on contaminated sediment derived soils in the same estuary (Schelde) (Vandecasteele et al., 2003b). Also other studies demonstrate that mainly Cd may result in elevated body concentrations, e.g. in gastropods (*Cepaea nemoralis*) (Jordaens et al., 2006) or small mammals (Mertens et al., 2001). In the study of Mertens et al. (2001), Cd soil concentrations comparable to this study (5.26 µg g^{-1}) resulted in elevated liver concentrations of shrews (>60 µg g^{-1}), yet model calculations predicted no food chain accumulation and a low risk for predators. Additionally, metal contaminated litter can result in top soil contamination and metal transport to the estuary (Caçador et al., 2009; Mertens et al., 2007; Van Nevel et al., 2011). An estimated yearly litter fall of 10 tons ha^{-1} (Mertens et al., 2006; Van Nevel et al., 2011) results in a yearly input of almost 30 g litter associated Cd ha^{-1} and 900 g litter associated Zn ha^{-1}. Also the large annual biomass production of a monospecific reed stand can result in a large input of e.g. litter associated Cr and Ni.

Consequently, trace metal accumulation in aboveground plant tissues may increase the possible toxicity of present trace metals. On the other hand metal sequestration in belowground sediments and roots and low transfer to aboveground plant tissues, i.e. phytostabilization, may be a preferable situation (Duarte et al., 2010; Salt et al., 1998; Weis and Weis, 2004). The lower TC’s and the extensive root system of the tall emergent macrophytes which are predominant in the marsh (*P. australis, T. latifolia, G. maxima, S. maritimus*) may contribute to this process. However, the wetland plant and sediment specific biogeochemistry can also result in belowground metal mobilization, mainly in the vicinity of roots (Almeida et al., 2008; Kissoon et al., 2010; Otte et al., 2004). This can be attributed mainly to Eh changes in the rhizosphere induced by oxygen release from their roots (Jacob and Otte, 2003b; Weis and Weis, 2004). Wetland plants rooting in anoxic sediments often maintain aerobic root respiration via internal oxygen transport through aerenchyma (Vartapetian and Jackson, 1997). An excess of oxygen in the roots can leak out (known as radial oxygen loss, ROL) resulting in an oxidized rhizosphere (Armstrong and Armstrong, 1988; Wright and Otte, 1999). Metals are mobilized from the reduced sediments and can precipitate with Fe and Mn (oxy)hydroxides on the root surface (‘root plaque’) which results in high rhizoconcentrations on a small scale or even in elevated concentrations in bulk sediments from a vegetated marsh compared to non vegetated sediments (Almeida et al., 2004; Doyle and Otte, 1997; Teuchies et al., 2008; Vervaeke et al., 2004). Thus the anoxic, reduced and organic rich sediments of a wetland often act as a sink, while plant activities as belowground mobilization and accumulation in aerial tissues can turn the marsh into a source (Vervaeke et al., 2004; Weis and Weis, 2004). However, mobilization is often found to be local and the advantages of plant growth which can enlarge the capacity of a tidal marsh to sequester trace metals belowground are found to be more important on a large scale and longer term (Jacob and Otte, 2003b).

5.5. Conclusions

Changes in land use can considerably modify the potential risk of metal contaminated sites. The conversion of agricultural field to restored wetlands might reduce human food chain contamination. Transfer coefficients in the marsh were found to be low compared to many other studies and only a very small fraction (≤0.05%) of the sediment metal pool cycled annually through the aboveground vegetation. Moreover, the estimated amount of trace metals deposited on a tidal marsh was about 300 times larger than the vegetation pool. Accordingly, phytoextraction is not applicable. Plant growth might result in higher toxicity of present metals by local changes in availability or food chain contamination via consumption of living plant tissues as well as litter with elevated metal concentrations. Therefore plants with minimal uptake of metals into the aerial tissues, which were found to be monocot, highly productive wetland species (e.g. *Typha latifolia, Scirpus maritimus* or *Phragmites australis*) and which may reduce distribution of toxic metals to the wider environment or transfer into the food chain, are preferable.

Supplementary data

Table S1. Standard deviation of the average values for trace metal concentrations in plants presented in Table 2.

species	As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Typha latifolia	0.228	0.053	1.62	1.51	205	195	0.202	0.525	6.63
Scirpus maritimus	0.162	0.198	1.46	3.63	160	181	0.162	0.474	12.3
Glyceria maxima	0.438	0.043	1.61	2.76	440	31.9	0.268	0.971	13.0
Juncus maritimus	0.114	0.371	0.239	2.97	55.1	20.9	0.130	0.150	10.8
Phragmites australis	0.287	0.083	3.93	3.72	264	105	1.83	0.756	16.7
Epilobium hirsutum	0.208	0.058	0.917	3.42	258	48.6	0.264	0.616	13.4
Rumex conglomeratus	0.249	0.402	2.18	3.78	249	26.9	0.308	0.730	15.7
Lythrum salicaria	0.220	0.206	1.91	2.70	197	58.0	0.214	0.510	25.7
Juncus effusus	0.185	1.50	1.77	3.20	174	76.5	0.257	0.545	20.8
Polygonum lapathifolium	0.386	0.218	2.88	2.04	224	63.3	0.286	0.761	18.6
Phalaris arundinacea	0.764	0.094	4.18	2.40	558	54.1	0.586	1.36	20.3
Salix sp.	0.358	2.81	1.46	1.98	843	32.4	0.390	1.70	89.8
Urtica dioica	0.448	0.092	3.52	4.27	653	31.8	0.576	1.97	15.1
Persicaria hydropiper	0.539	0.181	1.93	2.20	751	143	0.582	2.33	12.5
Lycopus europaeus	0.443	0.081	1.96	5.70	449	53.0	0.430	1.42	46.1
Plantago major	0.458	0.184	6.64	5.71	340	79.1	0.442	1.29	28.6
Bidens cernua	0.258	0.726	0.734	1.80	43.1	92.4	0.157	0.147	33.4
Rumex obtusifolius	0.850	0.436	2.89	3.27	1151	55.4	0.743	2.99	15.1
Poa sp.	0.475	0.173	2.71	6.04	1442	82.1	1.61	3.57	30.3
Symphytum officinale	0.464	0.154	2.02	4.05	517	28.4	0.482	1.53	19.9
Bidens frondosa	0.166	1.37	0.759	5.50	60.7	27.9	0.238	0.344	36.3
Pulicaria dysenterica	0.743	0.975	4.99	1.74	858	45.9	0.776	2.73	11.8
Alisma sp.	0.304	0.597	10.433	6.20	551	87.6	0.647	1.74	24.1
Ranunculus repens	0.450	0.520	5.38	5.40	424	66.9	0.689	1.27	12.7
Veronica beccabunga	0.687	0.277	4.85	2.41	779	70.5	0.676	2.26	17.3
Juncus articulatus	0.455	1.41	2.00	4.57	337	108	0.860	0.982	24.1
Veronica anagallis-aquatica	1.00	0.431	5.53	7.47	1407	69.1	1.11	4.41	22.8
Cirsium arvense	0.521	0.772	6.88	4.42	672	30.5	0.725	1.97	22.3
Vaucheria sp.	2.86	0.515	9.70	7.65	2517	209	1.63	8.46	76.6

Table S2. Minimum of measured values for trace metal concentrations in plants presented in Table 2.

species	As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Typha latifolia	0.030	0.006	0.210	2.11	64.8	179	0.161	0.121	11.6
Scirpus maritimus	0.224	0.040	0.224	1.72	74.3	86.8	0.197	0.303	14.9
Glyceria maxima	0.203	0.024	1.33	1.93	78.6	73.0	0.192	0.447	24.7
Juncus maritimus	0.130	0.572	0.460	4.71	82.3	27.4	0.176	0.333	30.0
Phragmites australis	0.073	0.006	0.942	0.184	37.4	7.65	0.095	0.137	14.3
Epilobium hirsutum	0.241	0.039	0.350	3.23	107	17.9	0.270	0.524	20.2
Rumex conglomeratus	0.199	0.041	0.820	1.83	123	30.3	0.201	0.622	12.8
Lythrum salicaria	0.119	0.042	0.613	2.20	46.2	16.2	0.237	0.378	25.5
Juncus effusus	0.148	0.235	0.459	1.04	114	36.1	0.216	0.071	28.4
Polygonum lapathifolium	0.224	0.009	0.935	1.91	147	151	0.284	0.566	26.8
Phalaris arundinacea	0.237	0.018	1.33	2.55	86.7	49.8	0.152	0.143	21.1
Salix sp.	0.099	0.037	0.231	1.53	68.0	14.8	0.136	0.279	24.9
Urtica dioica	0.203	0.037	0.660	4.14	69.9	8.14	0.050	0.416	16.2
Persicaria hydropiper	0.328	0.071	0.615	2.22	108	116	0.097	0.357	20.1
Lycopus europaeus	0.037	0.062	0.686	0.046	60.1	17.2	0.505	0.215	34.0
Plantago major	0.066	0.063	0.829	3.77	113	39.6	0.234	0.192	16.7
Bidens cernua	0.389	1.18	0.564	11.5	74.0	50.3	0.389	0.404	51.0
Rumex obtusifolius	0.108	0.114	0.389	2.66	82.9	22.9	0.167	0.374	18.2
Poa sp.	0.025	0.064	1.71	0.02	100	31.0	0.176	0.524	16.5
Symphytum officinale	0.269	0.084	0.511	3.84	129	25.8	0.259	0.618	28.4
Bidens frondosa	0.192	0.701	2.74	4.59	82.3	32.8	0.756	0.408	39.1
Pulicaria dysenterica	0.150	0.284	1.48	4.57	244	35.5	0.156	0.740	23.7
Alisma sp.	0.220	0.161	1.13	4.67	53.7	47.8	0.103	0.171	32.2
Ranunculus repens	0.228	0.314	1.77	5.67	109	21.9	0.120	0.282	25.5
Veronica beccabunga	0.294	0.017	0.990	3.77	365	28.6	0.268	0.981	29.0
Juncus articulatus	0.076	0.196	1.60	5.85	247	89.4	0.508	0.804	36.5
Veronica anagallis-aquatica	0.476	0.225	1.24	3.58	278	41.2	0.349	0.919	27.7
Cirsium arvense	0.325	0.258	1.12	7.73	134	26.1	0.887	0.395	33.0
Vaucheria sp.	15.1	1.60	19.0	28.1	11173	350	7.40	26.1	206

Table S3. Maximum of measured values for trace metal concentrations in plants presented in Table 2. Plant concentrations exceeding the possible harmful concentration underlined

species	As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Typha latifolia	1.07	0.210	7.24	7.45	907	899	0.628	2.65	40.5
Scirpus maritimus	0.726	0.646	4.55	9.65	564	667	0.545	1.63	56.0
Glyceria maxima	1.45	0.163	7.20	9.44	1500	182	1.02	3.74	60.6
Juncus maritimus	0.423	1.57	1.13	11.8	237	88	0.561	0.655	55.5
Phragmites australis	1.27	0.375	19.0	11.0	1219	476	8.47	3.59	65.2
Epilobium hirsutum	0.980	0.280	3.52	18.2	1090	225	0.919	2.73	72.5
Rumex conglomeratus	1.11	1.39	9.82	13.8	963	132	1.18	2.85	68.8
Lythrum salicaria	1.02	1.00	9.16	11.6	745	235	0.840	2.31	118
Juncus effusus	0.892	4.94	8.45	11.7	602	340	0.945	1.99	110
Polygonum lapathifolium	1.24	0.565	7.91	7.41	741	304	0.757	2.48	72.6
Phalaris arundinacea	2.04	0.255	10.6	8.41	1463	178	1.49	3.64	66.7
Salix sp.	1.78	10.91	6.98	9.12	3815	122	1.89	7.91	394
Urtica dioica	1.97	0.387	16.2	20.5	2863	117	1.99	8.44	64.4
Persicaria hydropiper	2.22	0.727	7.35	8.88	2896	534	2.16	8.92	61.7
Lycopus europaeus	1.53	0.311	6.51	19.0	1578	205	1.72	5.04	193
Plantago major	1.23	0.643	25.4	18.6	1173	276	1.64	3.93	95.9
Bidens cernua	1.09	2.94	2.22	16.0	195	297	0.780	0.771	129
Rumex obtusifolius	3.09	1.60	10.5	14.1	4123	222	2.75	10.9	68.8
Poa sp.	1.39	0.522	7.94	18.2	4821	302	5.50	12.0	101
Symphytum officinale	1.84	0.538	7.18	19.2	1959	130	1.72	6.28	89.2
Bidens frondosa	0.613	3.64	4.48	18.0	222	105	1.28	1.24	129
Pulicaria dysenterica	2.61	3.36	16.2	10.3	2968	186	2.84	9.53	56.4
Alisma sp.	1.03	2.53	43.6	27.2	2003	315	2.19	6.46	126
Ranunculus repens	1.99	2.39	17.8	24.7	1899	244	2.66	5.72	74.0
Veronica beccabunga	2.38	1.03	19.9	13.6	3045	283	2.70	9.11	100
Juncus articulatus	1.76	5.16	8.34	22.8	1432	514	3.28	3.90	124
Veronica anagallis-aquatica	4.18	1.79	20.2	39.2	5331	283	3.72	15.1	96.3
Cirsium arvense	1.91	3.38	29.8	21.2	2140	114	2.60	6.27	100
Vaucheria sp.	21.31	2.72	41.5	46.5	17157	876	11.4	46.0	371

Table S4. Average (n=min 5) metal concentrations (µg g^{-1}) per species per campaign.

species	year	season	As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Alisma sp.	2007	autumn	0.56	0.31	2.49	13.36	373.41	245.68	0.44	1.21	45.95
	2008	spring	0.51	0.95	8.62	16.11	767.04	180.22	0.80	2.38	64.68
	2008	autumn	0.70	0.63	3.74	16.30	721.72	195.88	1.16	2.39	76.40
Bidens cernua	2008	autumn	0.66	1.86	1.36	13.40	140.07	170.71	0.63	0.66	95.01
Bidens frondosa	2008	autumn	0.45	1.88	3.42	11.08	159.36	76.32	0.96	0.76	70.11
Cirsium arvense	2007	spring	0.92	1.26	6.72	10.58	1096.83	55.99	1.25	3.78	60.22
	2007	autumn	0.58	0.77	7.59	17.01	614.49	52.98	0.91	1.97	63.78
	2008	autumn	1.21	0.66	7.20	12.82	1458.33	85.35	1.57	4.26	66.90
Epilobium hirsutum	2007	spring	0.44	0.13	2.23	6.96	292.37	48.11	0.46	1.14	32.69
	2007	autumn	0.29	0.08	0.60	7.18	148.07	46.10	0.18	0.68	38.61
	2008	spring	0.53	0.14	1.91	5.96	458.57	77.35	0.46	1.40	34.74
	2008	autumn	0.43	0.18	1.45	9.83	289.99	64.81	0.59	1.12	54.48
Glyceria maxima	2008	spring	0.73	0.07	3.47	3.05	598.65	128.55	0.39	1.39	28.66
	2008	autumn	0.48	0.07	2.49	5.68	157.57	117.79	0.31	0.65	44.52
Juncus articulatus	2007	spring	0.38	1.09	4.75	13.71	528.37	237.67	0.93	1.76	57.59
	2007	autumn	1.76	0.41	8.13	13.33	1431.64	305.27	1.88	3.90	71.68
	2008	spring	0.69	2.24	3.72	14.94	797.10	184.50	0.94	2.42	70.44
	2008	autumn	1.04	1.74	4.61	16.78	873.60	202.37	2.59	3.01	87.63
Juncus effusus	2007	spring	0.33	1.71	3.35	8.40	265.55	121.42	0.37	0.88	46.84
	2007	autumn	0.44	0.78	1.51	6.58	206.14	194.92	0.22	0.64	44.19
	2008	spring	0.47	2.40	1.38	7.35	346.92	118.76	0.40	1.12	61.18
Juncus maritimus	2007	autumn	0.20	0.84	0.82	11.75	114.47	88.18	0.18	0.65	40.30
	2008	autumn	0.24	1.00	0.74	7.27	130.66	40.66	0.40	0.42	42.19
Lycopus europaeus	2007	spring	0.98	0.19	3.89	9.47	860.84	82.74	0.98	2.80	56.48
	2007	autumn	0.44	0.10	2.10	10.42	270.63	86.59	0.59	0.95	56.63
	2008	autumn	0.36	0.12	1.26	15.62	196.68	32.73	0.60	0.84	109.90
Lythrum salicaria	2007	spring	0.44	0.11	3.13	5.65	511.48	132.42	0.63	1.49	47.04
	2007	autumn	0.61	0.15	1.39	4.89	314.02	140.73	0.34	1.03	56.11
	2008	spring	0.35	0.31	2.55	7.64	292.55	93.07	0.43	0.88	70.94
	2008	autumn	0.34	0.09	1.66	8.90	201.54	96.61	0.58	0.69	62.00
Persicaria hydropiper	2007	autumn	0.73	0.19	2.47	6.24	608.42	291.46	0.48	1.93	44.47
	2008	spring	1.37	0.26	4.30	7.97	1641.36	214.39	1.13	4.97	53.72
	2008	autumn	0.51	0.32	1.41	4.44	382.01	347.85	0.51	1.07	43.91
Phalaris arundinacea	2008	spring	0.68	0.11	5.28	6.23	498.57	81.78	0.50	1.31	38.13
Phragmites australis	2007	spring	0.20	0.03	2.08	6.16	169.74	143.19	0.40	0.41	43.95
	2007	autumn	0.42	0.02	2.01	3.18	192.06	119.54	0.40	0.68	22.50
	2008	spring	0.39	0.14	1.84	8.98	303.73	87.29	0.56	0.93	54.31
	2008	autumn	0.35	0.05	5.99	0.25	110.63	89.46	2.81	0.35	30.76
Plantago major	2007	spring	0.23	0.10	6.58	7.18	501.56	110.31	0.60	1.70	25.98
	2007	autumn	0.58	0.24	9.49	12.61	841.81	144.36	1.09	2.10	60.35
Poa sp.	2007	spring	0.52	0.11	5.46	6.34	424.39	54.71	0.70	1.36	27.10

	2007	autumn	0.73	0.24	3.75	9.92	1268.00	120.37	1.39	3.61	48.22
Pulicaria dysenterica	2007	autumn	0.51	0.96	2.57	7.29	396.89	88.78	0.49	1.35	31.93
	2008	autumn	0.93	1.76	7.72	7.92	968.32	84.09	1.10	3.26	46.28
Ranunculus repens	2007	spring	0.95	1.23	10.79	20.84	807.89	110.71	1.19	2.55	51.22
	2007	autumn	0.56	0.45	2.68	11.80	459.49	150.33	0.31	1.70	29.34
	2008	spring	0.70	0.77	8.68	11.12	629.59	118.76	0.54	1.88	40.73
	2008	autumn	0.58	0.70	5.85	11.38	568.72	88.49	1.09	1.90	49.50
Rumex conglomeratus	2007	autumn	0.57	0.25	1.81	4.38	383.51	53.97	0.46	1.43	29.35
	2008	spring	0.52	0.33	3.11	7.40	498.39	88.16	0.48	1.44	28.59
	2008	autumn	0.60	0.50	2.36	4.31	592.86	47.28	0.86	1.74	31.75
Rumex obtusifolius	2007	autumn	1.04	0.15	3.36	7.70	919.35	85.41	0.69	2.76	40.61
	2008	spring	0.84	0.51	2.71	8.43	1006.03	86.15	0.82	2.64	43.68
	2008	autumn	0.57	0.68	2.35	6.30	492.04	51.57	0.82	1.83	34.72
Salix sp.	2007	spring	0.34	0.86	1.19	3.03	136.35	57.18	0.24	0.62	55.99
	2007	autumn	0.30	1.55	1.34	3.17	124.36	45.24	0.21	0.45	123.48
	2008	spring	0.36	2.89	1.03	6.48	262.27	49.20	0.48	1.04	166.80
	2008	autumn	0.74	3.01	2.18	5.17	1050.27	62.80	0.76	2.34	195.77
Scirpus maritimus	2008	spring	0.39	0.34	2.19	7.05	349.28	377.96	0.33	1.04	32.37
	2008	autumn	0.36	0.12	1.93	1.39	146.82	197.46	0.39	0.63	17.36
Symphytum officinale	2007	spring	0.70	0.24	3.68	10.30	616.76	43.23	0.99	2.05	52.08
	2007	autumn	0.93	0.30	3.23	11.68	838.35	70.59	0.76	2.84	63.44
	2008	autumn	0.45	0.15	1.56	7.24	313.58	36.72	0.58	1.59	37.56
Typha latifolia	2007	spring	0.09	0.07	3.10	4.23	149.10	409.96	0.38	0.39	25.18
	2007	autumn	0.30	0.06	0.51	5.13	143.62	444.43	0.11	0.48	25.13
	2008	spring	0.40	0.10	1.09	4.71	276.15	466.67	0.33	0.82	26.46
	2008	autumn	0.30	0.11	0.82	3.51	292.73	619.87	0.45	0.54	29.47
Urtica dioica	2007	spring	0.71	0.15	4.62	10.33	704.08	49.86	1.03	2.66	35.41
	2007	autumn	0.58	0.07	6.19	8.24	592.96	51.45	0.71	2.43	30.22
	2008	spring	0.50	0.13	3.13	7.23	451.34	31.69	0.64	1.61	28.99
	2008	autumn	0.97	0.19	4.57	7.16	916.02	45.45	0.58	2.81	30.31
Vaucheria sp.	2007	autumn	17.54	2.08	29.24	38.16	13848.61	588.14	8.84	36.22	296.89
Veronica anagallis-aquatica	2007	spring	0.60	0.62	4.76	8.44	469.81	62.00	0.52	1.58	34.88
	2007	autumn	1.27	0.36	6.38	10.32	1540.24	150.55	1.23	5.13	56.33
	2008	spring	1.96	0.71	12.68	10.54	2494.00	161.17	1.78	7.34	62.72
	2008	autumn	1.76	0.61	8.33	19.86	2038.60	145.20	1.50	6.55	80.27
Veronica beccabunga	2007	spring	2.10	0.56	7.85	10.71	1997.37	140.21	1.74	5.40	59.14
	2007	autumn	0.62	0.27	5.77	8.71	856.70	119.38	1.05	3.02	44.65
	2008	spring	0.67	0.53	7.95	7.35	857.62	83.15	0.96	2.59	57.91

CHAPTER 6

Influence of tidal regime on the distribution of trace metals in a contaminated tidal freshwater marsh soil colonised with common reed (*Phragmites australis*).

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Abstract

A historical input of trace metals into tidal marshes fringing the river Scheldt may be a cause for concern. Nevertheless, the specific physicochemical form, rather than the total concentration, determines the ecotoxicological risk of metals in the soil. In this study the effect of tidal regime on the distribution of trace metals in different compartments of the soil was investigated. As, Cd, Cu and Zn concentrations in sediment, pore water and in roots were determined along a depth profile. Total sediment metal concentrations were similar at different sites, reflecting pollution history. Pore water metal concentrations were generally higher under less flooded conditions (mean is (2.32 ± 0.08) µg Cd L^{-1} and (1.53 ± 0.03) µg Cd L^{-1}). Metal concentrations associated with roots (mean is 202.47 ± 2.83 mg Cd kg^{-1} and 69.39 ± 0.99 mg Cd kg^{-1}) were up to 10 times higher than sediment (mean is 20.48 ± 0.19 mg Cd kg^{-1} and 20.42 ± 0.21 mg Cd kg^{-1}) metal concentrations and higher under dryer conditions. Despite high metal concentrations associated with roots, the major part of the metals in the marsh soil is still associated with the sediment as the overall biomass of roots is small compared to the sediment.

6.1. Introduction

Estuaries and their fringing tidal marshes support many important ecosystem functions (Meire et al., 2005). As a transfer zone between the riverine and marine habitat this system plays an important role in biogeochemical element cycles and is characterised by a high biological productivity and biodiversity (Costanza et al., 1997; Daily et al., 1997). Unfortunately, often the design of river ecosystems to satisfy human needs has deteriorated their quantitative and qualitative natural ecosystem values. In addition, the continuous rise of industrialization during the last 200 years in Europe has resulted in an enormous increase of production, use and release of trace metals to the environment (Nriagu, 1996). Once released into the aquatic environment, a part of those metals are transferred to sediments by the processes of adsorption onto suspended matter and sedimentation (Zwolsman et al., 1993). Sedimentation of polluted particles results in high metal contents in the soil of tidal marshes, which are generally considered as a sink (Hart, 1982). Despite a gradual amelioration of the river Scheldt's water quality (Soetaert et al., 2006; Van Damme et al., 2005) the high historical input of metals into the marsh soil may still pose a risk to ecosystem functioning (Vandecasteele et al., 2002b; Vandecasteele et al., 2004).

The specific physicochemical form, rather than total content, determines bioavailability and ecotoxicological risk of metals in soil (Larsen and Schierup, 1981; Tack and Verloo, 1995). Metals in pore water are generally considered as the most bioavailable fraction in the soil (Ankley et al., 1994; Gambrell, 1994; Wenholz and Crunkilton, 1995). The fraction of metals in pore water depends on environmental conditions, such as pH, redox potential, and organic content (Du Laing et al., 2002; Gambrell, 1994). The hydrological regime might affect these soil conditions (van den Berg et al., 2000) and consequently metal bioavailability. For the management of metal-polluted wetlands, it is important to get more insight in the effect of flooding on the partitioning and bioavalability of trace metals in the soil (Du Laing et al., 2007b). Common reed (*Phragmites australis*) is a widely distributed species in tidal marshes (Van der Werff, 1991). Due to the presence of aerenchyma, transport of oxygen from shoot to roots occurs. Oxygen diffuses from root to rhizosphere where it can re-oxidize reduced elements (Armstrong et al., 2000). When ferrous iron encounters O_2 in the rhizosphere, it is subjected to oxidation and precipitation as ferric oxyhydroxide on the root surface (Armstrong, 1982). Root plaques may also contain a variety of other metals and metalloids (Batty et al., 2000).

Besides their natural values, the use of wetlands as filters has gained great interest in the past decades (Reboreda and Caçador, 2007). Tidal wetlands can also act as a buffer during high discharge or storm events minimizing the flooding danger of inhabited areas (Day et al., 2003). Due to a better understanding of the importance of tidal marshes in the estuarine ecosystem (Gribsholt et al., 2005;

Struyf et al., 2006) and the urgent need for flood control, the implementation of controlled inundation areas with controlled reduced tide has recently gained more recognition (Cox et al., 2006; Meire et al., 2005). In these areas, attaining safety objectives can be combined with the extension of the tidal marshes (Meire et al., 2005). The restoration of such new tidal systems requires an understanding of the potential risks of metals in tidal systems. Until now most studies focused on above ground biomass metal content. Nevertheless, the investigation of the below ground metal distribution along a depth profile is important to get more insight in the potential ecological risk of metal contamination in freshwater tidal wetlands.

The aim of this study is to assess the effect of hydrological regime on the partitioning of metals over the different compartments sediment, pore water and roots in a metal-polluted freshwater tidal marsh soil under field conditions. Therefore water, soil and root samples of reed were collected in a freshwater tidal wetland along the river Scheldt during one growing season. Total metal concentrations were determined in these samples.

6.2. Materials and methods

6.2.1. Study area and sampling

The partitioning of metals over different compartments in the soil was investigated in a highly metal-contaminated freshwater tidal marsh along the Sea Scheldt (for a description of the estuary see Meire et al., 2005). The marsh “Kijkverdriet” is situated near Steendorp (51°07’ N, 4° 15’ E). The tidal range is approximately 6 meters. Practical salinity unit (psu) varies between 0.3 en 0.8 during winter and between 0.4 and 2.3 during summer. Two sites approximately 500 m distant from each other with different inundation characteristics were compared. The vegetation at both sites consists of dense reed. With a mean (August, September, October, November) above ground biomass of 2155 (± 155) kg m^{-2} in site 1 and 1556 (± 179) kg m^{-2} in site 2.

At each site levels of groundwater table and flooding water were measured every 5 minutes by piezometer divers during a period of 43 days (Piezometer datalogger type Diver, Eijkelkamp Agrisearch Equipment. Data analysis with EnviroMon software, version 1.5.07, Van Essen Instruments). At both sites core samples and pore water samples were taken in August, September, October, November 2005 and in February 2006. All samples were taken in a 5 m radius. Pore water samples were taken using rhizons (a vacuum tube connected with a 10 cm long porous rod inserted into the soil). Triplicate pore water samples were taken every 10 cm until a depth of 50 cm at site 1 and, due to the hard clayish substrate, until 40 cm at site 2. At each site and each sampling moment 2 times 2 adjacent core samples (60 cm deep, Ø 8.5 cm) were taken randomly bordering the piezometers and rhizons. They were divided into 6 equal parts, corresponding to 10 cm sections. The two adjacent cores were mixed and the depth sections were analyzed separately.

Roots, collected in the same cores, were separated from the sediment by thorough rinsing with artificial medium hard fresh water (770 µM $NaHCO_3$, 77 µM KCl, 0.5 mM $MgSO_4$, 2.0 mM $CaCl_2$) (OECD, 1993) through a 500 µm sieve. In a white basin filled with water, roots were separated from organic matter with a pair of tweezers. The root plaque could not be removed by this method. Therefore, measured metal contents associated with the roots contain both, metals taken up and metals associated with the root plaque. Besides, due to the root plaque, it was not possible to separate living and death roots. Root metal concentrations were determined for each depth section separately.

Rhizome metal concentrations were found to be 10 to 20 times lower compared to root metal concentrations. (Windham et al., 2003) assumed that rhizome metal concentrations are actively kept low. Additionally, in contrast to roots, rhizomes do not contribute to plant uptake and do not lose oxygen to their immediate environment (Peverly et al., 1995). As their effect on metal partitioning in the marsh soil will accordingly be minimal, we will not discuss rhizome metal concentrations in this article.

6.2.2. Analysis

All pore water samples were analysed for Fe, Mn, As, Cd, Cu, and Zn concentration using ICP type radial plasma iris/CID (THERMO, Breda, The Netherlands). Pore water samples taken in February were additionally analysed for pH and nutrients (NO_2, NO_3, NH_4, PO_4 and SO_4) using a segmented flow analyzer (SKALAR, Breda, The Netherlands). Due to small sample volumes at site 2 the nutrient pore water concentration could only be determined up to a depth of 30 cm.

All sediment samples were analyzed for metal concentration. Sediment samples taken in October and November were additionally analyzed for different soil properties. Sediment samples were oven dried (40° C), grounded and sieved (500µm). For digestion 5 mL nitric acid (HNO3 69% Normapur Trace Analysis Cd Hg Pb) and 5 mL hydrogen peroxide (H_2O_2 27% for electrical purpose) are added to 0.2 g soil. This is heated to 100 °C for 30 min and to 150 °C for 60 min. Metal concentration (Mn, As, Cd, Cu, and Zn) was determined using ICP type radial plasma iris/CID (THERMO, Breda, The Netherlands). The cation exchange capacity (CEC) was determined at pH 7 with Ammonium Acetate using the Chapman method. Therefore 25 g fresh soil was washed with NH_4OAc. After removing excess of saturation solution with 95% ethanol the adsorbed NH_4 was extracted by leaching the soil with 1 M KCl. The concentration of NH_4-N in the KCl extract was determined by colorimetry

(Chapman, 1965). The moisture content was determined by measuring the weight loss from a sediment sample after drying for 14 hours at 105 °C. Grain size distribution was determined using laser diffractometry (Malvern S, Malvern Instruments Limited, Enigma Business Park, Worcestershire, United Kingdom). The organic matter content was determined by measuring weight loss after incineration of oven dried samples for 1 h at 550° C. The $CaCO_3$ content was measured by back-titration with 1 N NaOH of an excess of 1 N HCl added to 1 g oven dried (40° C) sediment (Ryan et al., 2001). Using a redox electrode system as described by the international standard (ISO11271:2002(E)) the redox potential was measured every 10 cm from a depth of 5 cm until a depth of 45 cm.

Root samples were oven dried (70° C). The dried tissues were weighed and ground into powder. Dried samples were digested using a mixture of ultra pure HNO_3 and H_2O_2 (1:5) following a microwave digestion procedure (Blust et al., 1988). Metal concentrations (As, Cd, Cu and Zn) of root samples were determined using Inductively Coupled Plasma – Atomic Emission Spectrophotometer (ICP-AES, Varian Liberty series II).

Root and sediment metal concentrations were calculated on a dry weight basis and expressed as mg g^{-1}. All samples were analysed in batches with blanks. Analytical accuracy was determined using certified reference material of the Community Bureau of Reference, i.e. standard for trace elements in river sediment (CRM 320) and olive leaves (CRM 62). Recoveries were within 10 % of the certified values.

6.2.3. Calculation and statistical analysis

The total metal content per square meter in the 60 cm top layer of the tidal marsh was calculated. Using the moisture content and the accepted theoretical weight of water (1.00 g cm^{-3}) organic matter (1.47 g cm^{-3}) and the inorganic fraction (2.65 g cm^{-3}) the volume of the pore water and the weight of the sediment per square meter were calculated. Using the concentration of metals in pore water and sediment, the total content was determined.

The statistical analysis was carried out using the SAS statistical package (The SAS ® System, SAS institute Inc., Cary, NC, USA). Metal concentrations (sediment, pore water, roots) in both sites were compared with a three-way ANOVA with site, depth and month as categorical variables. The Tuckey a posteriori test was used to evaluate the differences between the two sites for each depth interval. Because soil properties were determined only for 2 months, values in both sites were compared with a two-way ANOVA with site end depth as categorical variables.

6.3. Results

6.3.1. Site characteristics

For a 43 day period the height of the water according to the surface is presented in Fig. 1. Inundation frequency and water level during flood events were higher at site 1. In the period of measuring, site 1 was inundated 36 times compared to 17 times at site 2. During this period site 1 was inundated 38 h 40 min and site 2 was inundated 22 h and 50 min. Flood events lasted 20 min longer at site 1 and the water level was approximately 15 cm higher compared to site 2.

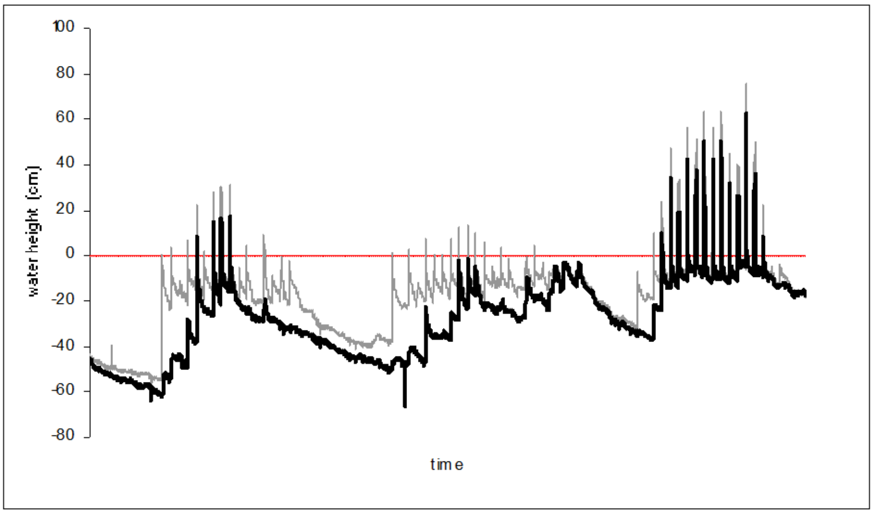

Figure 1. The height of the flooding water according to the surface during a 43 day period in both sites. With = site 1 and ▬ = site 2. ▬

Moisture content at site 1 was, with values up to 250% almost twice as high as at site 2 (Table 1). Organic matter content (± 25% at site 1 and ± 20% at site 2) and sand content (8-12 % at site 1 and 5-10% at site 2) were significantly higher at site 1 (Table 1). The lower inundation frequency at site 2 coincided with a significantly higher content of silt (68-71% at site 1 and 73-76% at site 2) and clay (14-17% at site 1 and 16-19% at site 2) and a significantly higher CEC (10-15 cmol (+) kg^{-1} (centimol positive ions per kg fresh soil) at site 1 and 15-20 cmol (+) kg^{-1} at site 2) compared to site 1. Due to the significant site*depth interaction for $CaCO_3$ and pH the difference between the two sites was investigated for each depth with an a posteriori test. Site 2 pH was significantly higher in the 30-40 depth interval ($p = 0.048$) and site 2 $CaCO_3$ content was again significantly higher in the 30-40 depth interval ($p = 0.0282$).

Table 1. Statistical analysis results for the soil properties, p values given. Two-way ANOVA with site and depth as categorical variables. Non significant interactions are not shown (> 0.05).

variable	MC	OM	$CaCO_3$	Clay	silt	sand	CEC	pH
site	<0.0001	<0.0001	<0.0001	0.0439	<0.0001	<0.0001	<0.0001	0.0266
depth	0.0234	0.1304	0.0001	0.0011	0.4507	0.0489	0.4578	0.0514
site*depth	-	-	0.0206	-	-	-	-	0.0125

Root biomass reached maximum values in the 10 cm top soil of the marsh (Fig. 3). In this layer the biomass is nearly 200 g m^{-2} at site 1 and 120 g m^{-2} at site 2. With increasing depth the root biomass decreased until approximately 70 g m^{-2} in the 50 to 60 cm soil layer. Differences in root biomass between the two sites were not significant in any different depth layer.

Both direct measurements of the redox potential and pore water nutrient concentrations gave a picture of the redox potential in the soil. The redox potential values at site 1 were lower and decreased fast from 250 mV near the surface to -100 mV at 15 cm depth to almost -200 mV on a depth of 45 cm (Fig. 4). The redox potential at site 2, also 250 mV near the surface, decreased to approximately 150 mV on a depth of 45 cm.

Higher values of NH_4 in the deeper sediment layers at site 1 and higher NO_3 and SO_4 values in the upper sediment layers of site 2 were the observed. The Fe and Mn pore water concentration increased with depth and decreased again at site 1 from a depth of 40 cm (Fig. 4). Differences between two sites Fe and Mn pore water concentrations were not significant (all $p > 0.05$).

6.3.2. Trace metal concentrations

Statistical analysis (Table 2) showed significant interactions between site, depth and month for different metals in the different soil compartments. With an a posteriori test significance between sites was examined for each metal on each depth (results not shown, explained in the text) to accurately compare metal partitioning between the sites.

In this study, the explanation of the variation between different months is not a priority. It is therefore decided to present (Fig. 5) and discuss differences in metal concentrations in pore water, sediment and roots between sites based on mean values (calculated over different months) and the standard deviation. Besides, as cores were taken in different places at each month, it is not clear if variation between months is a temporal or a spatial factor.

Significant differences in sediment metal concentrations between the two sites occurred only for Cu in sediment layers 40-50 cm ($p = 0.002$) and 50-60 cm ($p = 0.004$) and for Zn in the upper 0-10 cm ($p = 0.003$) and 10-20 cm ($p = 0.013$), with higher concentrations at site 2 (Fig. 5). Minimum sediment As, Cd, Cu and Zn concentrations in site 1 (27.8 ± 1.7 g kg^{-1}; 7.3 ± 0.3 g kg^{-1}; 79.1 ± 2.8 g kg^{-1}; 531.6 ± 11.4 g kg^{-1}) and site 2 (23.6 ± 1.7 g kg^{-1}; 8.4 ± 0.5 g kg^{-1}; 91.1 ± 5.1 g kg^{-1}; 630.9 ± 14.9 g kg^{-1}) were found in the upper 10 cm. The concentration increased with increasing depth. Maximum sediment Cd and Cu concentrations were found at a depth of approximately 40 cm in site 1 (34.1 ± 2.2 mg kg^{-1}; 193.1 ± 9.8 mg kg^{-1}) and site 2 (33.5 ± 0.6 mg kg^{-1}; 198.3 ± 5.3 mg kg^{-1}) whereupon a decrease in concentration took place. Maximum sediment As and Zn concentrations were found at a depth of 60 cm in site 1 (125.4 ± 7.5 mg kg^{-1}; 1103.9 ± 160.6 mg kg^{-1}) and in site 2 (130.5 ± 11.8 mg kg^{-1}; 1143.6 ± 45.2 mg kg^{-1}) (Fig. 5).

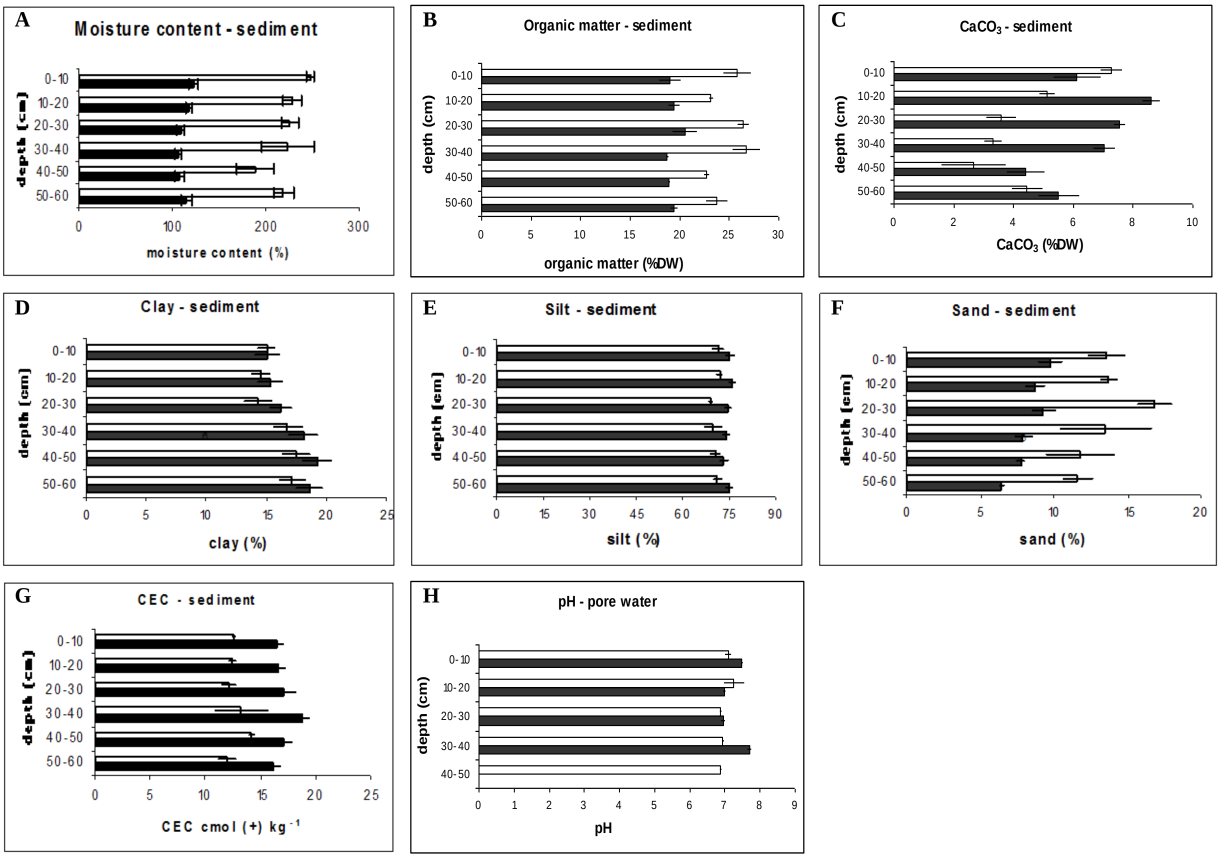

Figure 2. Properties of the soil (mean values ± standard error; moisture content, $CaCO_3$, clay, silt, sand content and CEC $n = 24$; organic matter $n = 12$; pH $n = 10$). □ = site 1, higher inundation frequency, ■ = site 2, lower inundation frequency.

Table 2. Statistical analysis results for metal concentration, p values given. Three-way ANOVA with site, depth and month as categorical variables. Non significant interactions are not shown ($p > 0.05$).

	SEDIMENT		POREWATER		ROOTS	
metal	**variable**	**p-value**	**variable**	**p-value**	**variable**	**p-value**
As	site	0.6911	site	<0.0001	site	0.1226
	depth	<0.0001	depth	<0.0001	depth	<0.0001
	month	0.0244	month	<0.0001	month	0.3627
	site*depth	0.0066	site*depth	<0.0001	site*depth	<0.0001
	month*depth	0.0161	month*depth	<0.0001		
			site*month	<0.0001		
			site*month*depth	<0.0001		
Cd	site	0.9126	site	0.0092	site	<0.0001
	depth	<0.0001	depth	0.0002	depth	<0.0001
	month	0.0902	month	0.0009	month	0.0101
	site*month	0.0112			site*depth	<0.0001
					site*month	0.0338
Cu	site	0.0007	site	<0.0001	site	<0.0001
	depth	<0.0001	depth	0.0606	depth	<0.0001
	month	0.686	month	<0.0001	month	0.0545
	site*month	0.0370	site*depth	<0.0001	site*depth	<0.0001
			month*depth	0.0149		
			site*month	0.1049		
			site*month*depth	0.0078		
Zn	site	<0.0001	site	<0.0001	site	0.0056
	depth	<0.0001	depth	0.0195	depth	<0.0001
	month	0.6473	month	<0.0001	month	0.1062
	site*month	0.0425			site*depth	<0.0001
	month*depth	0.0259			site*month	0.0352
Fe			site	0.8085	site	0.0330
			depth	<0.0001	depth	<0.0001
			month	0.1782	month	0.8245
					site*depth	<0.0001
Mn	site	<0.0001	site	0.0064	site	0.0015
	depth	0.0045	depth	<0.0001	depth	<0.0001
	month	0.4169	month	0.0910	month	0.9009
	site*depth	0.0060			site*depth	<0.0001

Although profiles of metal concentrations in the sediment were similar at both sites, metal concentrations in pore water differed considerably between sites and metals. The concentration of As at site 1 was at every depth lower than the detection limit (0.03 mg L^{-1}). Except for a high mean concentration of approximately 0.5 mg L^{-1} measured in the 30-40 cm interval, also at site 2 As concentrations were near to the 0.03 mg L^{-1} detection limit. The Cd concentration in the pore water increased from a minimum in the upper soil layer near to the 0.002 mg L^{-1} detection limit at both sites to a maximum on a depth of 30 to 40 cm at site 1 (0.0031 ± 0.0015 mg L^{-1}) and site 2 (0.0057 ± 0.0005 mg L^{-1}) In this zone the concentration was significantly higher at site 2 ($p = 0.006$). For Cu, concentrations in pore water reached only in the upper layer of the soil values higher than the detection limit (0.002 mg L^{-1}), with a higher value at site 2 (0.0052 ± 0.0010 mg L^{-1}) compared to site 1 (0.0014 ± 0.0004 mg L^{-1}). Zn pore water concentrations varied between 0.05 mg L^{-1} and 0.07 mg L^{-1} in site 1. Similar to the other metals Zn concentrations in the pore water were higher at site 2 where a maximum was found in the 10-20 cm layer (0.15 ± 0.03 mg L^{-1}). These differences were significant in the zones from 10 to 20 cm ($p < 0.001$) and from 20 to 30 cm ($p = 0.016$).

Metal concentrations associated with the roots of the reed plants were high compared to sediment metal concentrations. Cd concentrations associated with the roots in the 10-20 cm layer of site 2 were found to be 372 ± 53 mg kg^{-1} which is more then10 times the sediment Cd concentration in the same layer. Fig. 5 shows a general trend of increasing root metal concentration until a depth of approximately 30 cm at site 1 and approximately 40 cm at site 2, accordingly reaching higher values in the dryer site 2 in deeper sediment layers. In the 40-60 cm layer, the As concentration in the roots was significantly higher at site 2 ($p < 0.001$) with maximum values up to 400 mg kg^{-1}. The Cd and Cu concentration in the roots at site 2 were significantly higher in the zone from 20 to 60 cm depth (all $p < 0.001$) with maximum values of 372 ± 53 mg kg^{-1} and 871 ± 81 mg kg^{-1} compared to site 1 maxima of 105 ± 26 mg kg^{-1} and 241 ± 56 mg kg^{-1} . The maximum root Zn concentration was in site 2 4989 ± 555 mg kg^{-1} then in site 1 (4061 ± 456 mg kg^{-1}). The differences in Zn root metal concentration between the two sites were significant in the 30-40 cm ($p = 0.022$), 40-50 cm ($p < 0.001$) and 50-60 cm ($p = 0.002$) layer with, analogous to the other metals, higher values at site 2.

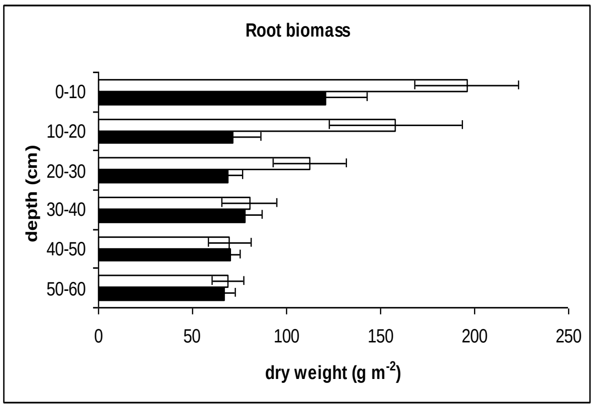

Figure 3. Root dry weight (mean over time ± standard error, n = 10) in the 60 cm top layer of the marsh soil (g m^{-2}). □ = site 1, higher inundation frequency, ■ = site 2, lower inundation frequency.

Even though metal concentrations associated with the roots were locally very high, the total metal content in the sediment was approximately 200 times higher than metals associated with the roots (Fig. 6). For the 60 cm deep layer we sampled, this resulted for both sites in sediment contents in the order of 21 x 10^3 mg m^{-2} for As, 6 x 10^3 mg m^{-2} for Cd, 45 x 10^3 mg m^{-2} for Cu and 250 x 10^3 mg m^{-2} for Zn. The total Cd and Cu root content differed significantly between the two sites ($p = 0.0002$ and $p < 0.0001$), with higher values in site 2 (90.1 ± 5.7 mg Cd m^{-2}; 232.7 ± 11.2 mg Cu m^{-2}) compared to site 1 (45.7 ± 7.5 mg Cd m^{-2}; 124.5 ± 18.2 mg Cu m^{-2}). Total Zn root content was significantly ($p = 0.03$) higher in site 1 (1817 ± 209.3 mg Zn m^{-2}) compared to site 2 (1576.5 ± 171.3 mg Zn m^{-2}). Difference between site 1 (62.5 ± 7.6 mg m^{-2}) and site 2 (78.0 ± 6.4 mg m^{-2}) root As content were not significant ($p = 0.014$). Despite high differences between As and Zn pore water content between site 1 (7.4 ± 0.0 mg As m^{-2}; 28.1 ± 3.0 mg Zn m^{-2}) and site 2 (50.9 ± 20.0 mg As m^{-2}; 61.4 ± 15.4 mg Zn m^{-2}) they were not found significant ($p = 0.06$; $p = 0.07$). Cd and Cu pore water content were small in site 1 (0.7 ± 0.2 mg Cd m^{-2}; 0.5 ± 0.1 mg Cu m^{-2}) and site 2 (1.1 ± 0.4 mg Cd m^{-2}; 0.8 ± 0.2 mg Cu m^{-2}).

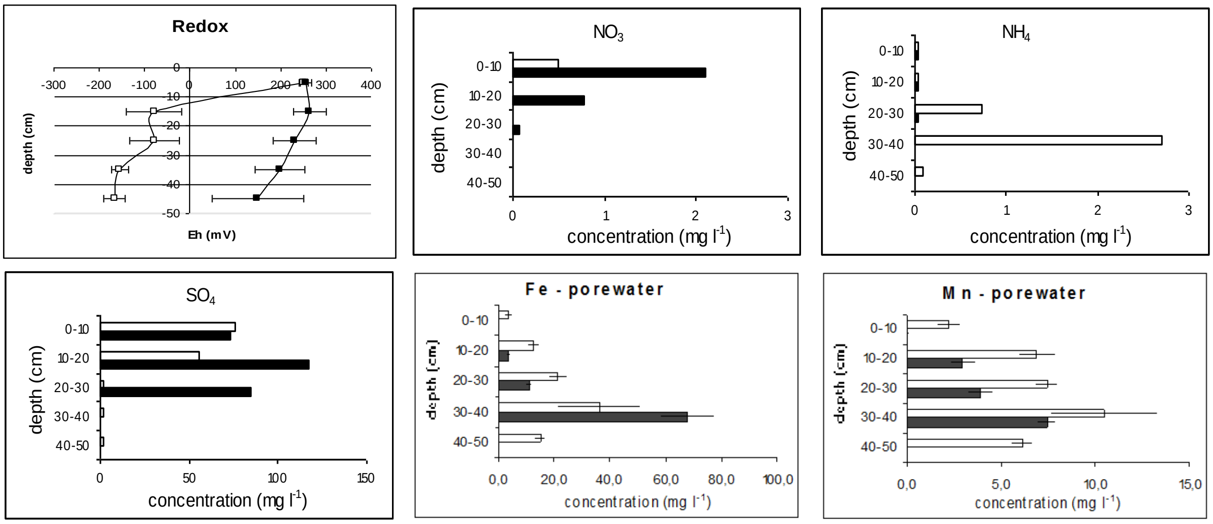

Figure 4. Graphs indicative for the redox potential. Direct measurement of the redox potential with □ = site 1 and ■ = site 2 given for different depths (n = 3). Nutrient (n = 1), Fe (n = 10) and Mn (n = 10) concentrations in pore water for depth sections of 10 cm. □ = site 1, ■ = site 2.

6.4. Discussion

6.4.1. Trace metals in the Scheldt estuary.

The Scheldt estuary is characterised by a long history of contamination (Baeyens et al., 2005; Oenema et al., 1988; Zwolsman et al., 1993). An important part of the metal load in the river system is adsorbed onto the fine-grained material in suspension and settles in the intertidal areas (Regnier and Wollast, 1993). Consequently; metal concentrations in sediments of the tidal marsh were found to be high. Maximum Cd concentrations (34 mg kg^{-1}) were found to be higher than maximum values measured in 11 different European estuaries (maximum12 mg kg^{-1}) sited by Zwolsman et al. (1996). Maximum Cu (198 mg kg-1) and Zn (1143 mg kg -1) concentrations were found to be higher than the maxima found in 50% of the other European estuaries (Zwolsman et al., 1996). Diagenetic modifications and bioturbation can change the partitioning of metals between compartments in tidal marsh soils (Cornwell, 1986; Gendron et al., 1986; Zwolsman et al., 1993). Nevertheless, the distribution of metals measured can be a reflection of pollution history (Vandecasteele et al., 2003a; Zwolsman et al., 1993). The increasing metal concentration with depth in the marsh soil seems to follow the trend of the decreasing metal pollution during the last 40 years. The shift in sediment metal concentrations beyond the depth of 30-40 cm demonstrates the pollution maximum mid sixties as shown by Zwolsman et al. (1993). Due to its old age, the tidal marsh achieves an equilibrium in sedimentation patterns, with equal sedimentation and erosion quantities everywhere in the marsh as a consequence (Temmerman et al., 2004a).This can explain the similar metal concentration profiles in the sediments at both sites.

6.4.2. Pore water metal concentration.

Even though total metal concentrations in the sediment are high and similar in both sites it is assumed that it is actually the pore water concentration that establishes the bioavailability of these contaminants in the soil (Hall and Pulliam, 1995; Tack et al., 1998; Wood and Shelley, 1999).The difference in tidal regime between the two sites results in dissimilarities for certain soil characteristics and processes and consequently, in differences in metal pore water concentrations. Hereby pH and redox potential are considered as the major characteristics in determining the physicochemical occurrence of metals in the soil (Gambrell, 1994; Vandecasteele et al., 2002a). The pH might have a major effect on metal pore water concentrations but it is considered important particularly when it drops below 6.5 (Gambrell et al., 1991; Jackson et al., 1993). However, since soil pH was neutral and did not significantly differ between the two sites, it could not be responsible for possible differences in pore water concentration of the measured metals.

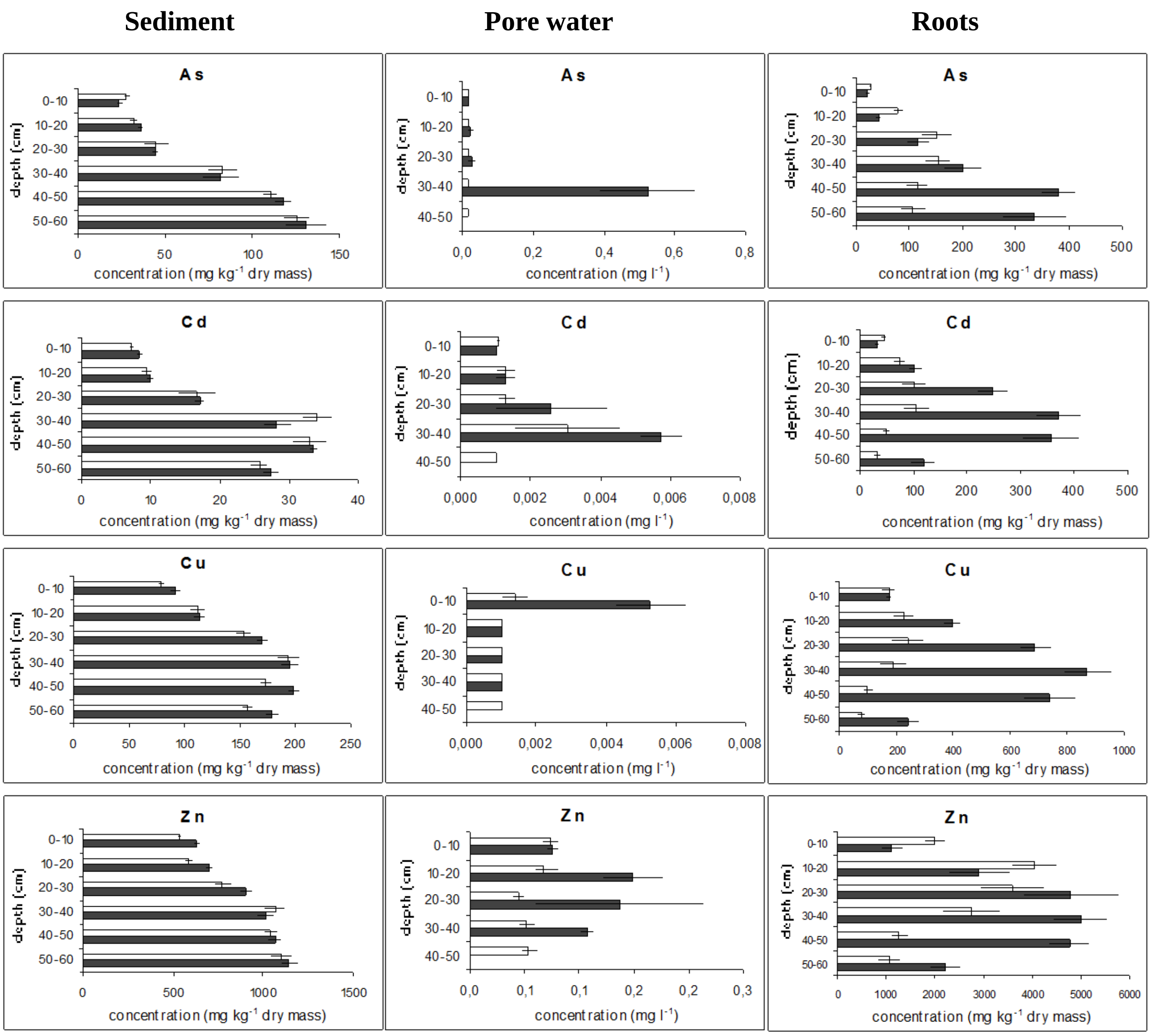

Figure 5. Total As, Cd, Cu and Zn contents (mean over time ± standard error, root and sediment n = 10, pore water n = minimum 5, maximum 15) in sediment, pore water and roots for depth sections of 10 cm. □ = site 1, higher inundation frequency, ■ = site 2, lower inundation frequency. Pore water detection limits: As = 0.03 mg l^{-1}, Cd = 0.002 mg l^{-1}, Cu = 0.002 mg l^{-1}, Zn = = 0.001 mg l^{-1}. Half of the detection limit is used as an approaching value for concentrations lower than the detection limit.

Generally trace metals co-precipitate with Fe-sulphides under reducing conditions (Van den Berg et al., 1998b). Accordingly in most studies it is found that the mobility of metals is lower under flooded, and consequently reducing, conditions (Gambrell, 1994; Tack and Verloo, 1995; Vandecasteele et al., 2005b). The lower metal pore water concentrations at site 1, mainly at major depths, reflect this.

Due to a large adsorption capacity of clay minerals and organic matter, i.e. high cation exchange capacity (CEC), metal mobility decreases with higher clay and organic matter content in the soil, although this adsorption is reversible (Fergusson, 1990). The CEC is significantly higher in site 2 (higher pore water concentration) (Table 1) and is therefore probably not a major factor in affecting the metal pore water concentration in this study.

With the exception of Cu, trace metal concentrations in the pore water are low near the surface. In this oxic sediment layer, metals are assumed to adsorb to Fe and Mn oxyhydroxides. At greater depth metals are released into pore water due to the reductive dissolution of Fe and Mn oxyhydroxides (Finney and Huh, 1989; Jackson, 1998; Park and Jaffé, 1996; Zwolsman et al., 1993). These patterns are visible in the Cd and site 2 Zn pore water profiles. The low pore water As concentration in the upper layers could be attributed to the strong affinity of this trace element for oxyhydroxides (Masscheleyn et al., 1991).

At greater depth and more reducing conditions, formation of sulphides can occur (Van den Berg et al., 1998b). At site 1, where floods take place more frequently, the sediment has lower redox potentials and sulphides are probably present at a lower depth compared to site 2. Metals, mainly iron, can precipitate with these sulphides and the concentrations of all trace metals can decrease due to coprecipitation (Ankley et al., 1991; Giblin et al., 1986; Masscheleyn et al., 1991; Moore et al., 1988; Van den Berg et al., 1998b). This process may be responsible for of the decrease in As and Cd concentration in the deeper pore water at site 1 and the decrease in the Zn concentration from a depth of 20 cm at site 2. High AVS (Acid-Volatile Sulphide) values measured by (Du Laing, 2006) in the study area indicate the possible importance of metal sulphide precipitation at this marsh.

Low Cu concentrations in the pore water can be attributed to the high organic matter content in the marsh soil, because Cu has a great affinity for organic matter (Jackson, 1998; Vandecasteele et al., 2002a). Higher Cu concentrations in the pore water of the upper 10 cm at site 2 can be due to a higher decomposition rate of organic matter in this oxic layer, resulting in the release of the bound metals (Zhang et al., 1995).

6.4.3. Trace metals associated with Phragmites australis roots.

The significant interaction in metal concentration values between site and depth indicates the importance of sampling at different depth intervals instead of taking mixed soil samples. Metal concentrations associated with the roots are higher than sediment metal concentrations, for Cd even up to 10 times. Still, this does not mean that measured metals are taken up by the plants roots. Diffusion of oxygen out of the roots can cause the oxidation of the rhizosphere (Armstrong et al., 2000; Jaynes and Carpenter, 1986; Stcyr and Crowder, 1989). This oxidizing environment can then result in precipitation of Fe and Mn oxides on the root surface. A metal concentration gradient can be built up with a movement of dissolved metal ions towards the roots. Coprecipitation and adsorption of other metals can then result in a root plaque with a high concentration of all trace metals (Doyle and Otte, 1997; StCyr and Campbell, 1996).

The depth profiles of root metal concentration are comparable for all metals (Fig. 5). The availability of dissolved metal ions, mainly Fe^{2+} and Mn^{2+} might be the determining factor. Hereby, the decreasing redox potential in the soil plays a crucial role. As previously mentioned, the dissolution of Fe and Mn oxyhydroxides can result in an increase of Fe^{2+} and Mn^{2+} ions with increasing depth (Finney and Huh, 1989; Jackson, 1998; Zwolsman et al., 1993). More reducing conditions at a greater depth can result over again in a decrease of the availability of dissolved ions due to precipitation with sulfides (Van den Berg et al., 1998b). Because the redox potential at the more frequently inundated site 1 is lower (-166 ± 23 mV in the 40-50 cm layer) compared to site 2 (149 ± 100 mV in the 40-50 cm layer), formation of metal sulfides is probably more important at this site. This might result in a lower concentration of dissolved metal ions in site 1 pore water and may be the cause of the lower metal concentration associated with the roots of site 1 from a depth of nearly 30 cm. The differences between metal concentrations associated with the roots of *Phragmites australis* at the two sites indicate the dissimilarity in metal behavior caused by a difference in submersion. The metal concentration associated with the roots may be a reflection of the bioavailability of metals in the soil.

It is not clear which fraction of this high metal load is taken up by roots, can be transported to aboveground biomass and enter the food chain by herbivory. St-Cyr & Campbell (1996) found that the major part of the metal root concentration is situated on the roots (plaque) and not in the roots (in most cases more than 80% for Fe, Mn and Zn). Due to precipitation with Mn and Fe oxyhydroxides on the roots, metals become less bioavailable (Weis and Weis, 2004), consequently high metal root plaque concentrations may result in phytostabilization. On the other hand, when roots die, oxygen loss will stop. The reducing environment will dissolve the oxy-hydroxides and dead roots can now act as a metal source.

6.4.4. Distribution of metals in the soil.

Despite metal concentrations associated with the roots being higher then sediment metal concentrations, the main pool of metals in the tidal marsh was found to be the sediment (Fig. 6), due to the higher mass of the sediment compared to the roots. Consistent with the metal concentrations (Fig. 5) the total metal content in pore water and roots are higher in the dryer site 2. Nevertheless, due to a higher pore water volume (Fig. 2) and a higher root biomass (Fig. 3) in site 1 the differences in total metal content in pore water and roots are not as pronounced as shown in the metal concentration graphs (Fig. 5). Root biomass did not differ significantly between sites but the different values still affects the calculation.

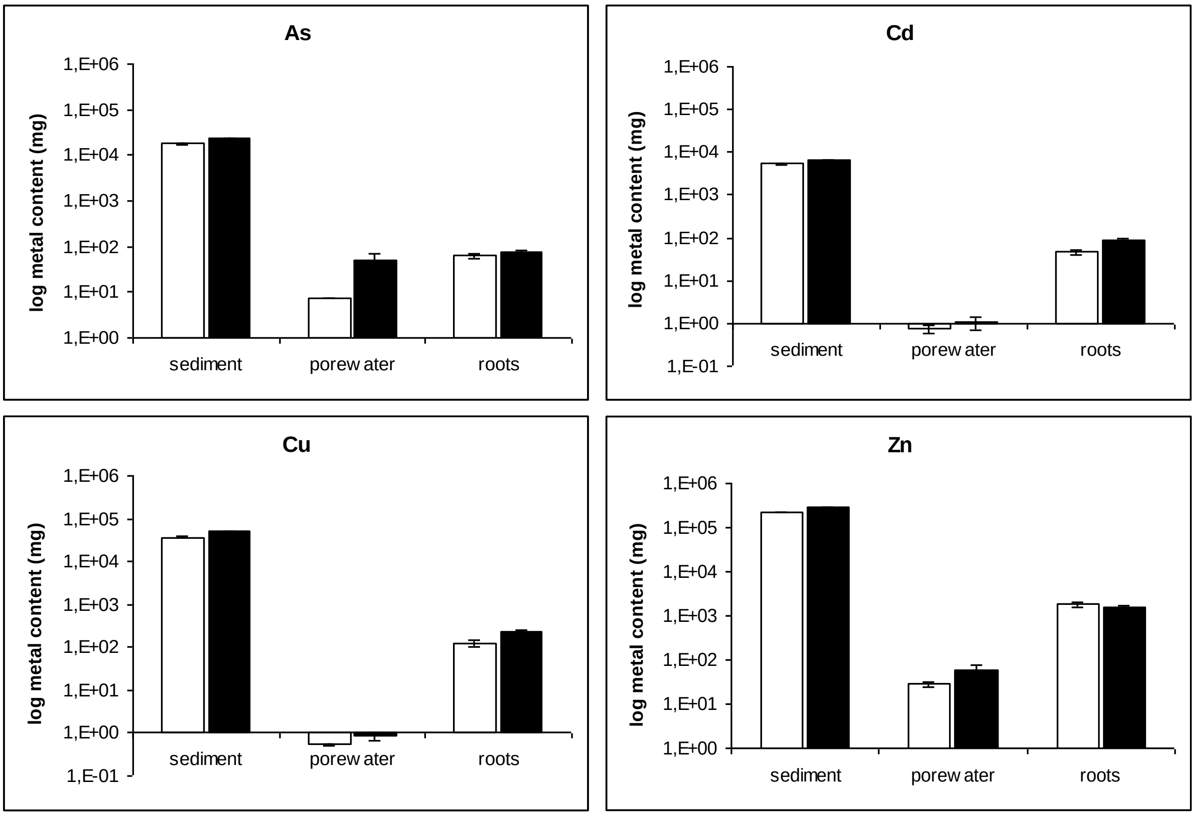

Figure 6. The calculated total content of metals and their partitioning between sediment pore water and roots in the 60 cm top layer of the marsh soil (per m^2). □ = site 1, higher inundation frequency, ■ = site 2, lower inundation frequency. All error bars are shown but very small on this logarithmic scale

6.5. Conclusions

High trace metal concentrations were measured in the soil. The down core distribution of the metals seems to reflect pollution history with a maximum pollution from the mid sixties situated at a depth of 30-40 cm. A restricted quantity of these persistent contaminants reaches the pore water and can be readily available for plant uptake. Differences in redox potential, due to a difference in tidal regime at the two sites, leads to variances in metal pore water concentrations with generally lower values in the site with a higher inundation frequency. A loss of oxygen from the roots can create an oxidizing rhizosphere leading to precipitation of metal oxyhydroxides on their surface with a very high metal concentration accordingly. The hydrological regime has an influence here as well, with a lower metal concentration in the more inundated site. Despite the high metal concentrations associated with the roots, due to their low total biomass the major part of the metals is still associated with the sediment.

CHAPTER 7

Can Acid Volatile Sulfides (AVS) influence metal concentrations in the macrophyte *Myriophyllum aquaticum*?

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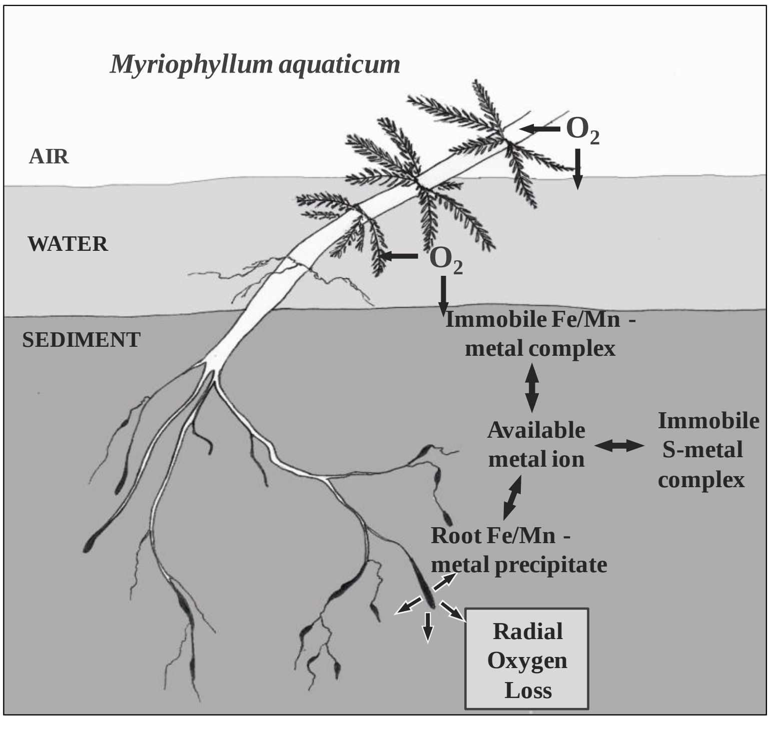
Abstract

The difference between the molar concentrations of simultaneously extracted metals (SEM) and acid volatile sulfides (AVS) is widely used to predict metal availability towards invertebrates in hypoxic sediments. However, this model is poorly investigated for macrophytes. The present study evaluates metal accumulation in roots and stems of the macrophyte *Myriophyllum aquaticum* during a 54 day lab experiment. The macrophytes, rooting in metal contaminated, hypoxic and sulfide rich field sediments were exposed to surface water with 40% or 90% oxygen. High oxygen concentrations in the 90% treatment resulted in dissolution of the metal-sulfide complexes and a gradual increase in labile metal concentrations during the experiment. However, the general trend of increasing availability in the sediment with time was not translated in rising *M. aquaticum* metal concentrations. Processes at the root-sediment interface, e.g. radial oxygen loss (ROL) or the release of organic compounds by plant roots and their effect on metal availability in the rhizosphere may be of larger importance for metal accumulation than the bulk metal mobility predicted by the SEM-AVS model.



7.1. Introduction

Metal toxicity is a potential risk for many aquatic ecosystems worldwide (Luoma and Rainbow, 2008). When released into surface water, the major part of these elements will accumulate in bottom sediments (Eggleton and Thomas, 2004). In organic rich, anoxic sediments the formation of insoluble metal sulfides is found to play a major role in reducing sediment metal availability and transfer to the surface water (Lee et al., 2000b; Yu et al., 2001). The amount of acid extracted sulfides (acid volatile sulfides or AVS) and simultaneously extracted metals (SEM) is proposed as a predictor for toxicity of certain trace metals in sediments (cadmium (Cd), copper (Cu), lead (Pb), nickel (Ni), zinc (Zn), chromium (Cr) and silver (Ag)) (Berry et al., 2004; Di Toro et al., 1990; Yoo et al., 2004). In this model, the sediment is predicted to be non toxic when, on a molar basis, AVS concentrations exceed SEM concentrations ([SEM-AVS] < 0) and is mainly used to evaluate metal toxicity towards aquatic macro-invertebrates (Allen et al., 1993; Berry et al., 1996; Di Toro et al., 1992; Lee and Lee, 2005) but, has only poorly been evaluated with respect to metal bioavailability and toxicity to macrophytes.

Recent studies indicate that certain aquatic invertebrates can accumulate sediment-bound metals when an excess of AVS is observed up to levels which largely exceed tissue concentrations from organisms of uncontaminated sediments (De Jonge et al., 2009; Lee et al., 2000a). This has been shown for benthic invertebrates which can be exposed to high metal concentrations due to the increased availability of ingested sediment-bound metals caused by the specific conditions within the animals' guts (lower pH and presence of digestive enzymes and surfactants) (De Jonge et al., 2011). Also for aquatic plants it can be expected that metal exposure is not necessarily related to metal availability in bulk sediments (Jacob and Otte, 2003a). Wetland plants and macrophytes rooting in anoxic sediments often maintain aerobic root respiration via internal oxygen transport through aerenchyma (Vartapetian and Jackson, 1997). An excess of oxygen in the roots can leak out (known as radial oxygen loss, ROL) resulting in an oxidized rhizosphere (Armstrong and Armstrong, 1988). This can result in oxidation and dissolution of sulfides and consequently in the mobilization of metals near the roots while low metal mobility can still predominate in the reducing bulk sediment (Almeida et al., 2008).

Metal-sulfide precipitation in sediments is a reversible process. Natural or anthropogenic governed sediment disturbance events as well as changes in the sediment redox potential of undisturbed sediments can result in dissociation of metal-sulfide complexes (Eggleton and Thomas, 2004). A subsequent increase in metal mobility and release into the surface water can be expected (De Jonge et al., 2012a; Teuchies et al., 2011). In this study an experiment was performed where natural anoxic, AVS rich sediments were exposed to both aerated and nitrogen-purged surface water under natural flow conditions. Macrophytes from a genus known to have the ability to create an oxidized rhizosphere (*Myriophyllum aquaticum*) (Flessa, 1994; Laskov et al., 2006) were included. Following objectives were investigated:

- Is metal uptake by the macrophytes influenced by the sulfide concentrations in the bulk sediment?
- Are changes in metal mobility, caused by surface water aeration, reflected in macrophyte metal concentrations?
- Will radial oxygen loss by the macrophytes rooting in the sediment result in higher metal mobility in the bulk sediment?

7.2. Materials and methods

7.2.1. Sediment sampling site

The experiment was performed with natural sediments, sampled in November 2009 in a small brook (Moerbeek) in Belgium (50°96’24.42” N, 3°67’46.32” E). The brook is characterized by low stream velocity, low oxygen (7.0 ± 1.5% saturation) and high OM concentrations (17 ± 1.5% dw). High sediment AVS and metal concentrations in the sediment of this stream have been reported before (De Jonge et al., 2010). Sediment (0-10 cm) was sampled using a stainless steel Petit Ponar grab sampler (Wildco cat. No. 1728; 235 cm^2) and immediately transported in closed polypropylene containers (10 L). In the laboratory, the sediment was homogenized and large organic debris was removed.

7.2.2. Myriophyllum pre-culture

Prior to the start of the experiment parrot feather water milfoil (*Myriophyllum aquaticum* (Vell.) Verdc.) plants were grown in artificial sediment (74% dw quartz sand, 5% dw peat powder, 20% dw kaolin clay , 1% dw $CaCO_3$) at 20 °C and constant light (25 W m^{-2}). Plants were multiplied, starting from 10 individuals from the German Institute of Hydrology, by cutting plants in individual head whorls which are planted in the water saturated artificial sediment. Plants of 21 days old were transplanted in the sediment of the experimental containers five days prior to the first sampling day in order to let the sediment and plants stabilize. During this period the oxygen concentrations of both treatments were kept at 40% saturation.

7.2.3. Experimental setup and sampling

Two experimental containers (PVC, 60 x 80 x 40 cm) were filled with 72 L sediment. Prior to the first sampling sediments stabilized for one week under N_2 atmosphere and for 10 days with 24 L medium-hard reconstituted freshwater with 40% O_2 saturation (OECD, 2004; 294 mg L^{-1} $CaCl_2.2H_2O$; 123.25 mg L^{-1} $MgSO_4.7H_2O$; 64.75 mg L^{-1} $NaHCO_3$; 5.75 mg L^{-1} KCl). In each container 24 replicate PVC cores (⌀ 8 cm, depth15 cm) were inserted in the sediment, four for each sampling day. Ten water milfoil plants were planted in half of the replicate cores in both containers. When the experiment started the oxygen in the surface water of one container was kept at 40% while in the second treatment O_2 saturation raised to 90%. Oxygen levels were kept constant (40.7 ± 6.9% and 90.0 ± 2.9%) using an O_2-stat system (R362, Consort, Turnhout, Belgium). In order to approach the natural situation a surface water flow of 10 L min^{-1} was created (Fig. S1). The experiment was performed in a climate chamber at 20 °C (Type WT15′/+5DU-WB, Weiss Technik, Reiskirchen-Lindenstruth, Germany) and a light (12h) – dark (12h) cycle (25 W m^{-2}).

Sediment and plants were sampled after 0, 2, 5, 12, 32 and 54 days. On each sampling day four replicate sediment cores (⌀ 8cm, depth15 cm), two with and two without plants, were randomly removed from the containers. The ten water milfoil plants in two of these cores were removed carefully, rinsed with artificial freshwater, divided in above and belowground parts, weighed and oven dried at 60°C in open polypropylene vials. The roots and stems of three plants from every core were used for metal analysis. Sediment cores were sliced at 1, 4, 8 and 15 cm depth and frozen at -20 °C in N_2-purged polypropylene vials until analysis. Sediment metal mobility was measured in the same cores on the same depth sections using Diffusive Gradients in Thin films (DGT) (Zhang et al., 1995). Loaded DGT sediment probes (0.80 mm open pore diffusive gel; 0.40 mm Chelex gel; 1.8 cm x 15 cm window) were purchased from DGT Research Ltd (Lancaster, UK). In order to prevent oxygenation, the probes were deoxygenated by immersing them in a N_2-bubbled suspension of 5 g/L Chelex-100 (Sigma) in 0.01 M NaCl solution for 12 h. Immediately after degassing the probes were inserted in the four replicate cores 24 h prior to their sampling.

7.2.4. Laboratory analyses

The pH was measured in the supernatant of a sediment KCl extraction (1 M; 1:5, w/v). Organic matter (OM) was estimated through loss on ignition. For this purpose, weight difference of oven dried (105 °C) versus incinerated sediment (6h at 550 °C) was determined. The clay content (< 4 µm) was determined with a laser diffraction particle size analyzer (Malvern S, Malvern Instruments Ltd, Worcestershire, UK). Nutrient concentrations were measured colorimetrically using a SAN++ Flow

Analyser (Skalar, FAS, SA 20/40, Skalar Analytical B.V., Breda, the Netherlands) after extractions of wet sediment with H_2O (1:2.5, w/v) for sulfate (SO_4^{2-}), with KCl (1 M; 1:2.5 w/v) for ammonium (NH_4^+), nitrite and nitrate ($NO_2^- + NO_3^-$) and with ammonium acetate EDTA (ethylenediaminetetraacetic acid) (1 M; 1:5, w/v) for phosphate (PO_4^{3-}) concentration measurements.

AVS were extracted for 1 h from 10 g wet sediment using 10 mL of 1 N hydrochloric acid (HCl, Suprapur®, Merck; 37%) under constant N_2 atmosphere (Leonard et al., 1996). The volatile hydrogen sulfide (H_2S) was collected in a sulfide antioxidant buffer (SAOB) in which the amount of free sulfur was measured with an ORION 96-16 ion-selective sulfur electrode (Ionplus, Beverly, MA, USA). In the same extraction metal concentrations were measured and SEM_{Tot} was calculated as the molar sum of Cd, Cu, Zn, Pb, Ni, Cr and Ag/2. A sediment subsample was weighed, oven dried (105 °C) to a constant weight and then reweighed to estimate sediment water content and to correct measured AVS, nutrient and metal concentrations when expressed per g dry weight (dw). The difference of SEM and AVS, on a molar basis is displayed as [SEM-AVS].

Total sediment metal concentrations were determined in a dry sediment – nitric acid (HNO_3, Suprapur®, Merck; 65%) and HCl (1:3, v/v) mixture after microwave (ETHOS 900 Microwave Labstation, Milestone, Italy) digestion in Teflon bombs.[27] After digestion, samples were filtered using a 0.20 µm cellulose acetate filter (Schleicher & Schuell MicroScience GmbH, Dassel, Germany) and diluted with ultrapure water (Milli-Q, Millipore) up to 50 mL.

Plant metal concentrations were measured after microwave digestion of entire below and above ground parts of individual plants in a mixture of HNO_3 and hydrogen peroxide (H_2O_2, Suprapur®, Merck; 30%) (20:1, v/v) (Blust et al., 1988).

After removal, the DGT probes were thoroughly rinsed with Milli-Q and stored into clean plastic bags at 4 °C. The resin gels of the sediment DGT probes were sliced at 1, 4, 8 and 15 cm using a Teflon coated blade and immersed in 1 N HNO_3 (65%, Suprapur®, Merck) and kept at room temperature until analysis. For Cr, only Cr(III) was expected to bind to the chelex resin (Ernstberger et al., 2002).

All trace metals (Al, Ag, As, Cd, Co, Cr, Cu, Pb, Ni, Zn) were analyzed using a high resolution inductively coupled plasma mass spectrometer (HR-ICP-MS; Thermo Scientific, Finnigan element 2, Bremen, Germany). Fe and Mn were measured using an inductively coupled plasma optic emission spectrometer (ICP-OES; Thermo scientific, ICAP 6300 Duo, Waltham, MA). Blanks and certified reference material for sediment (Institute for Reference Materials and Measurements (IRMM), BCR® no. 320, river sediment) and plants (IRMM, BCR® no 060, aquatic plant (*Lagiosiphon major*)) were included in all series of metal analysis to verify analytical accuracy. Recoveries were within 10% of the certified values.

7.2.5. Statistical analyses and calculations

The statistical analysis was carried out using the SAS statistical package (The SAS® System, SAS institute Inc., Cary, NC, USA). Normality of the data was tested with the Shapiro-Wilk test prior to analysis. The significance of differences between treatments and time points was tested with an analysis of variance test (ANOVA). Two or three way ANOVA with variables treatment, time and depth were used where appropriate. The Tuckey a posteriori test was used to evaluate the differences mutually. The Pearson correlation-coefficient was used to determine the relation between labile metal concentrations in the 0-4 cm sediment layer (plant relevant layer) and plant metal concentrations. The relative growth rate (RGR) based on biomass was calculated after Knauer et al. (2008):

$\mathrm{RGR} = [\ln (B_t) - \ln (B_0)]/t$

Where B_t is the biomass at the end of the experiment (time t), B_0 is the biomass at the start of experiment (time 0) and t is the duration of the experiment (days).

7.3. Results

7.3.1. Sediment geochemistry

Average concentrations of general sediment characteristics, nutrient and total metal concentrations in the sediment are displayed in supporting information (Table S1). No significant difference between concentrations at the start and at the end of the experiment existed for most elements. Exceptions are a significant decrease in SO_4^{2-} concentrations (p<0.0001 both treatments) and a significant increase in NH_4^+ concentrations (p=0.002 for the 90% and p=0.004 for the 40% treatment).

The molar differences of SEM and AVS [SEM-AVS] were comparable (± -250 µmol g^{-1} dw) in all layers from both treatments at the start of the experiment (Fig. S2). During the experiment stratification took place with higher [SEM-AVS] values near the surface. The major changes were observed in the 0-1 cm sediment layer from the 90% O_2 treatment. In this layer a significant (p<0.0001) decrease in AVS concentrations from 215 to 50 µmol g^{-1} dw was measured (Fig. 1). [SEM-AVS] increased significantly (p<0.0001) in the 90% O_2 treatment and were significantly higher than concentrations in the same surface layer of the 40% O_2 treatment on day 54 (p=0.002). No differences in AVS or [SEM-AVS] were observed between cores with and without plants (Fig. 1).

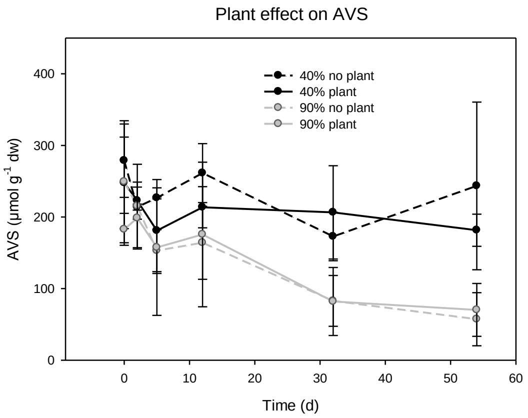


Figure 1. Sediment AVS (µmol g^{-1} dw) concentrations in function of time (d) in the upper sediment layer (0-1 cm) in the 40% treatment (black) and the 90% treatment (gray) for cores with plants (full line) and without plants (dashed line). Average values (n=4) with standard deviation.

7.3.2. Metal mobility

Sediment metal mobility is quantified as labile pore water concentrations measured with DGT membranes (Fig. 2). As for the [SEM-AVS] patterns, labile Ni and Zn concentrations were comparable in all layers of both treatments at the start of the experiment followed by stratification with higher concentrations near the surface. Stratification and the increase in labile concentrations are small in the 40% treatment. In the 90% treatment, a clear stratification in metal mobility of Zn and Ni was observed at the end of the experiment with mobility in the upper sediment layer which was significantly higher compared to the other sediment layers. Contrary to Zn and Ni, differences in labile Fe concentrations between depth layers decreased during the experiment and stratification was reversed, with the lowest concentrations near the surface. No significant differences in labile Mn concentrations between different depth layers, different dates or both treatments were observed.

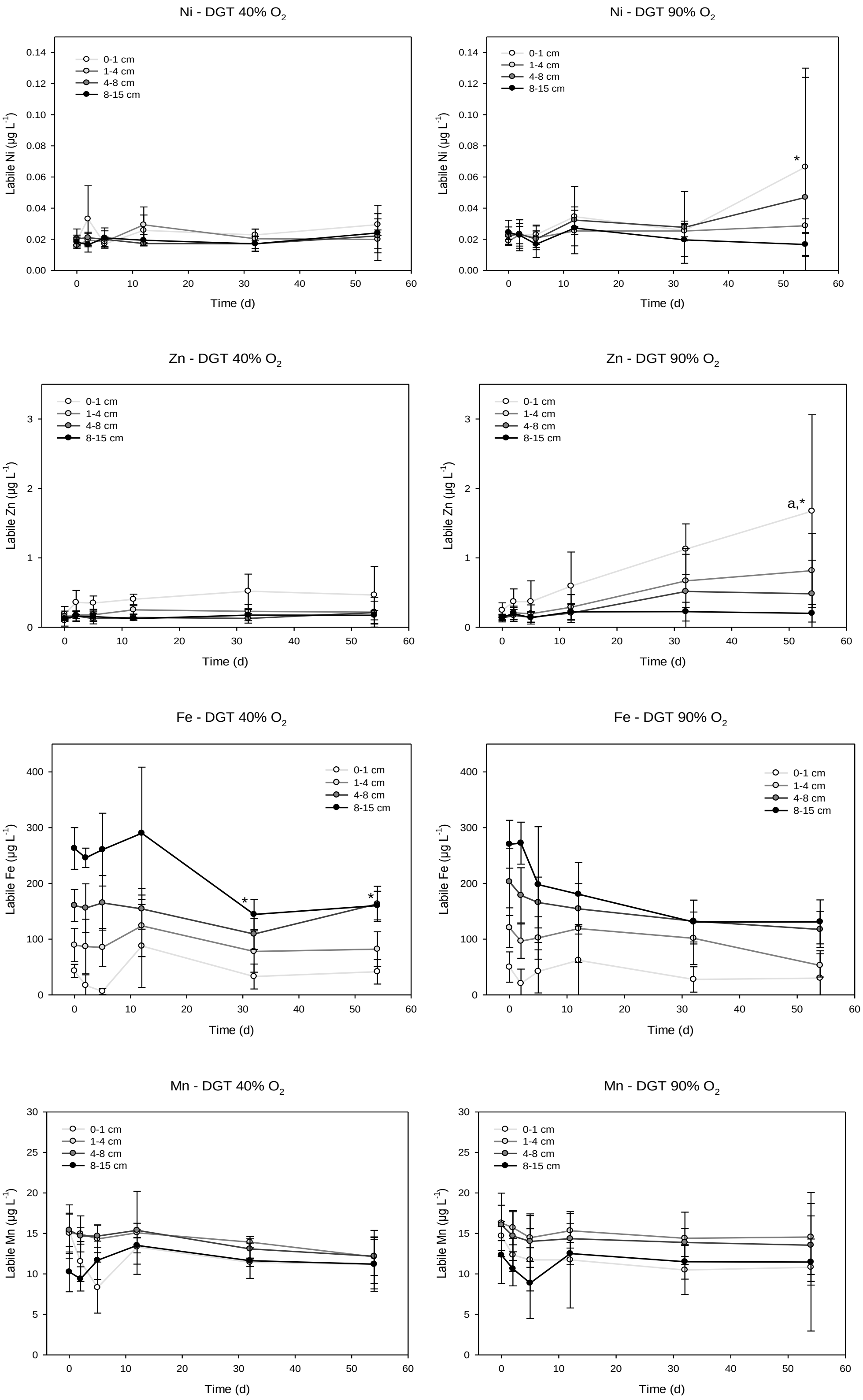


Figure 2. Labile metal concentrations (ng L^{-1}) in function of time (d) in the sediment of the 40% treatment (left column) and the 90% treatment (middle column). Average values (n=4) with standard deviation. * = significant ($p<0.005$) difference from day 0 in the same treatment and sediment layer, a = significant difference ($p<0.005$) between treatments for similar layers and time point.

7.3.3. Myriophyllum aquaticum

In both treatments, Ni, Zn and Fe concentrations in the roots of *Myriophyllum aquaticum* were significantly higher than the concentrations found in the stems (Ni, $p<0.0001$ for both treatments; Zn, $p<0.0001$ (90%) and $p=0.0042$ (40%); Fe $p<0.0001$ for both treatments) (Fig. 3 for Ni, Zn, Fe and Mn, other metals Fig. S4). The opposite, higher concentrations in the stems were found for Mn ($p < 0.0001$ for both treatments). At the start of the experiment, metal concentrations in the plant parts are similar for both treatments. During the experiment, differences between treatments arise. Significantly higher root concentrations in the 90% O_2 treatment for Ni ($p=0.0033$) and Zn ($p=0.0065$) and significantly higher stem Mn concentrations in the 40% O_2 treatment ($p<0.0001$) were observed. The trend in root concentrations in the 90% treatment is similar for Ni, Zn and Fe with high concentrations after the 5 day stabilization period, increasing concentrations the first 5 days followed by a decrease until the end of the experiment (54 days). Comparably, a decrease from the start towards the end of the experiment was observed for stem concentrations of the 90% treatment for Ni (2.5 to 1.2 µg g^{-1} dw), Zn (197.4 to 106.8 µg g^{-1} dw) and Mn (1.6 to 0.9 mg g^{-1} dw). The absolute plant root and stem metal content increased for most metals during the experiments (Fig. S3 for Ni, Zn, Fe and Mn, other metals Fig. S4). Metal values were significantly higher at the end of the experiment compared to the start for Zn (90% stem, $p<0.001$; root, $p=0.007$; 40% stem, $p=0.03$), Ni (90% stem, $p=0.002$), Fe (90% root, $p<0.001$) and Mn (40% stem, $p=0.003$). No differences in absolute plant metal concentrations between treatments were observed. Shoot and root weight increased with about 400% during the experiment without significant treatment differences (Fig. S5).

Most plant Zn and Ni concentrations were lower than their average concentration in the sediment (Fig. 3). In contrast, all plant Mn concentrations are higher than the sediment concentrations. Fe stem concentrations were lower than sediment concentrations while Fe root concentrations were on average 3.75 times higher. Yet, the total Fe content in 10 plants of every experimental core contained only 1.33% of the sediment metal content. For Zn, this is only 0.22%.

A significant negative correlation was found between labile Zn concentrations in the 0-4 cm sediment layer and Zn concentrations in the roots (40%, $r=0.963$, $p= 0.002$ and 90% $r=0.859$, $p=0.03$) and in the stems (90%, $r=0.919$, $p=0.001$) (Fig. 4, Table S2). No significant correlations between labile Zn concentrations or SEM-AVS values in the 0-4 cm sediment layer and absolute Zn values in the plants were found.

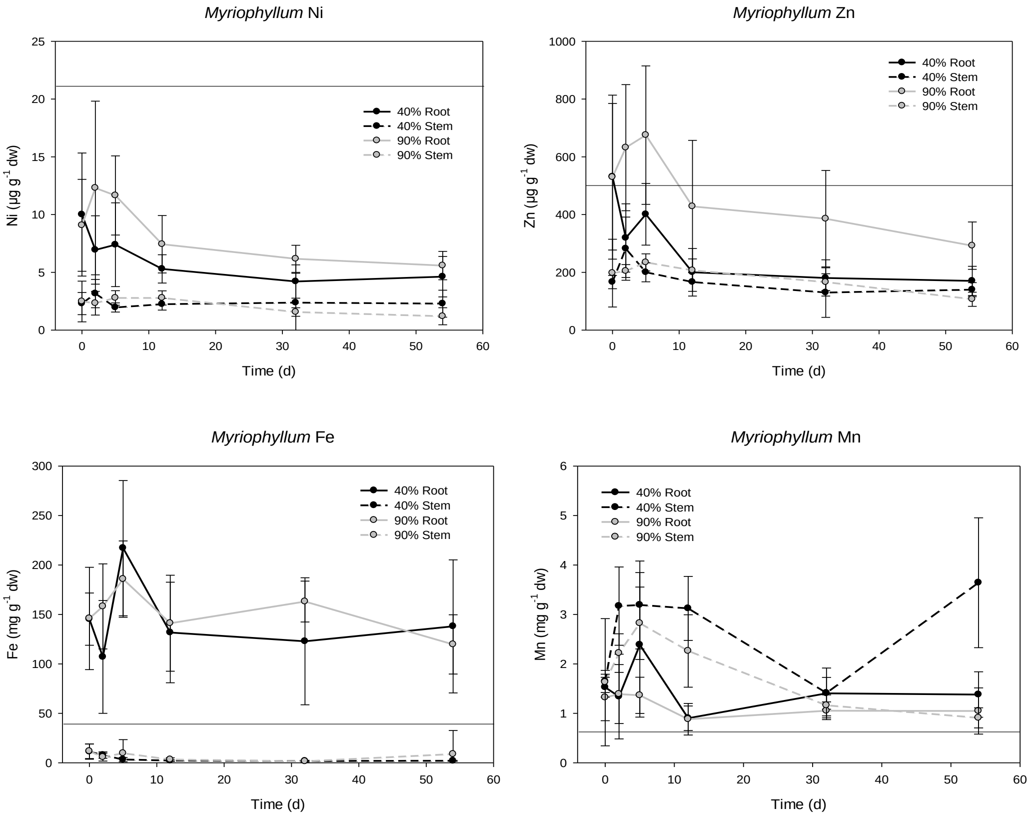


Figure 3. Plant metal concentrations (µg or mg g^{-1} dw) in function of time (d) in roots (full line) and stems (dashed line) in the 40% treatment (black) and the 90% treatment (gray). Average values (n=6) with standard deviation. The solid line indicates average sediment metal concentrations.

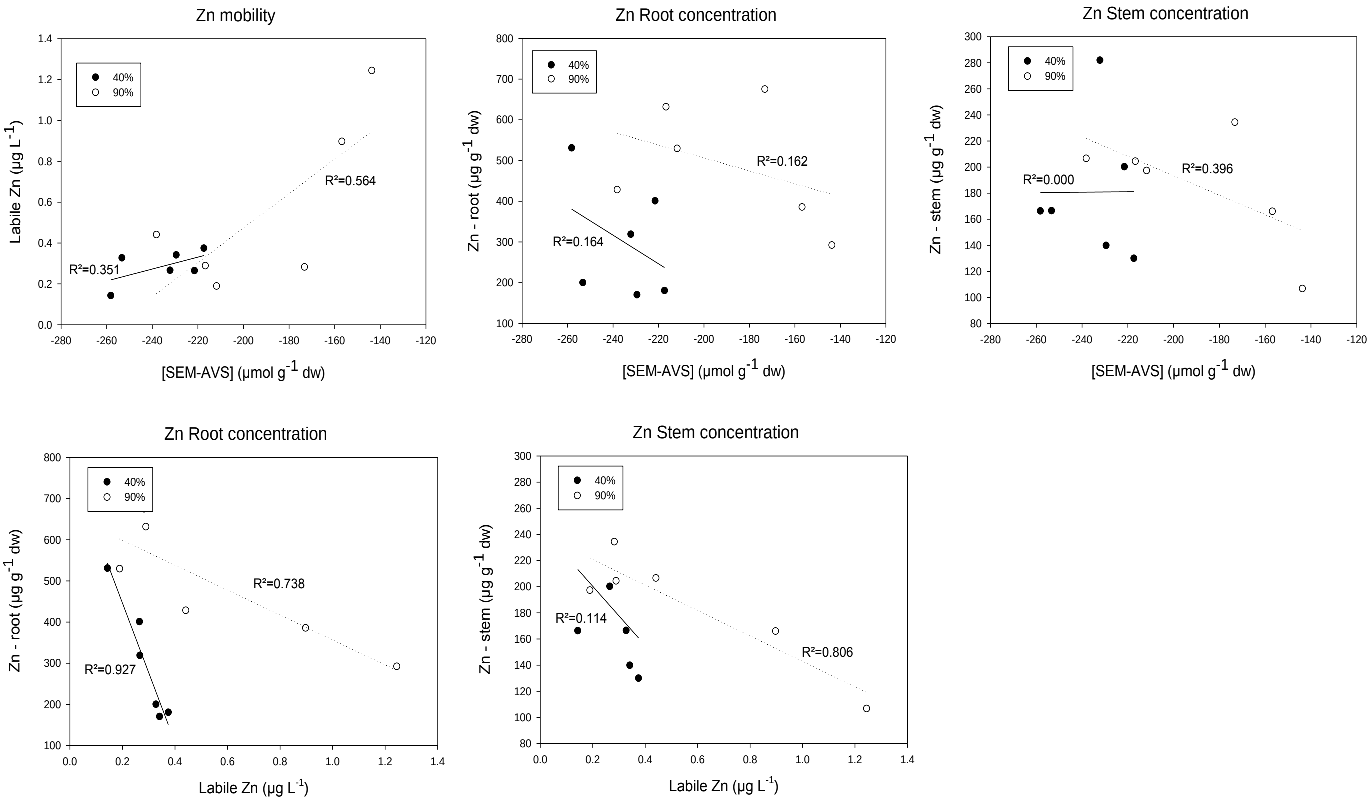


Figure 4. Relation between [SEM-AVS] (µmol g^{-1} dw) and DGT – measured labile Zn concentrations (µg L^{-1}) and Zn concentrations in roots and stems of *Myriophyllum* (µg g^{-1} dw), R² added, see Table S2 for significance of relationships.

7.4. Discussion

7.4.1. Effect of surface water aeration on SEM-AVS and metal mobility

Surface water aeration in the 90% oxygen treatment resulted in a considerable decrease in AVS concentrations. Yet, the oxidation of sulfides was limited to superficial sediments with an average decline of 60% after 54 days in the upper cm of the sediment. In contrast with this experiment, which imitated the natural conditions of sediments with a surface water flow, the decrease in AVS concentrations occurred much faster in aeration-resuspension experiments. An AVS decrease up to 90% after 6 hours and 99.5% after 4 days of resuspension and aeration are reported (Maddock et al., 2007; Simpson et al., 1998). However, our results emphasize that also under undisturbed conditions, high surface water oxygen concentrations can result in a substantial AVS breakdown on a relatively short period. The abiotic changes in the sediment of this experiment are more thoroughly discussed by De Jonge et al. (2012) (De Jonge et al., 2012a).

The precipitation of sulfides with metals is found to reduce mobility and toxicity of Ag, Cd, Cr, Cu, Ni, Pb and Zn (Allen et al., 1993; Di Toro et al., 1992; Yoo et al., 2004). Consequently, the oxidation of sulfides under oxic conditions can reduce the metal precipitation capacity of sediments (Teuchies et al., 2011). Also in this experiment the oxidation of sulfides in the superficial sediment layer of the 90% oxygen treatment corresponded with an increase in labile Ni and Zn concentrations in the sediment pore water. However, according to the SEM-AVS model, metal mobility is expected to increase particularly when the concentration of SEM exceeds the AVS concentration [SEM-AVS] > 0 (Di Toro et al., 1990). Despite the aeration, an excess of AVS compared to SEM was measured throughout the experiment. The anoxic conditions and high organic load of the stream where the sediments were sampled resulted in very high AVS values compared to other streams spread over Western Europe, (Burton et al., 2007) and comparable to the highest values found in Flemish lowland rivers by De Jonge et al. (2010). Since the effect of oxygen in the surface water on sediment sulfide oxidation occurs particularly on the sediment water interface, an excess of SEM compared to AVS, which was not measured in the upper cm, is expected in this small superficial interface layer. Fe and Mn constitute an important part of sulfide metal precipitations in the sediment. Yet, no increase in labile Fe and Mn concentrations following sulfide oxidation was observed. Moreover, lowest labile Fe and Mn concentrations were found in the more oxic sediment surface layers. This may emphasize the importance of Fe and Mn oxide precipitation near the sediment water interface which can reduce their mobility (Zhuang et al., 1994).

7.4.2. Effect of Myriophyllum on AVS concentrations

An effect of plant growth on AVS concentrations can be expected. Macrophytes which increase the sediment redox potential and thereby change redox related biogeochemical processes are reported before (Jaynes and Carpenter, 1986; Wigand et al., 1997). *Myriophyllum* sp. were found to create an oxidized zone in their rhizosphere by means of oxygen release from their roots, known as Radial Oxygen Loss (ROL) (Flessa, 1994). This may result in AVS oxidation around the roots. Yet, no significant differences in AVS concentrations between cores with and without *M. aquaticum* plants were observed in this study. The ability to transport oxygen from surface water or air to the roots, release it into the rhizosphere and hence alter the redox potential differs between macrophyte species (Aldridge and Ganf, 2003). Oxygen diffusion from *Myriophyllum* sp. roots was found to be limited to certain root zones with a maximum oxidized area of 0.25 mm up to 1mm around the roots (Flessa, 1994; Laskov et al., 2006). The root density in this experiment may be not large enough to cause an effect on the overall sediment AVS concentrations in our experimental cores. Additionally, high oxygen demand of the experimental sediment, which was rich in organic matter, had high sulfide concentrations and a low redox potential, may have decreased the oxidizing range of the roots (Carpenter et al., 1983).

7.4.3. Myriophyllum sp. metal concentrations

A broad range of metal concentrations in *Myriophyllum* plants can be found in literature (Table 1.). Rapid biosorption from the water phase onto the plant surface often results in high *Myriophyllum* concentrations in phytoextraction experiments (Ridvan Sivaci et al., 2004; Yan et al., 2010). Metal concentrations in *Myriophyllum* plants growing in natural sediments display large variations. *M. aquaticum* was found to have the highest Zn concentrations compared to 15 other macrophyte species from urban creeks (Cardwell et al., 2002). In that study, bioconcentration was high, with root Zn concentrations up to 33 times higher than sediment concentrations. Their stem Zn concentrations were 1458 µg g^{-1} while *M. spicatum*, sampled in Slovenian lakes with comparable Zn sediment concentrations were only 15 µg g^{-1} (Grudnik and Germ, 2010). Feiler et al. (2006) determined an EC50 value of 697 µg g^{-1} dry sediment for Zn, based on growth of *M. aquaticum* whorls in a sediment contact test. Despite the elevated sediment metal concentrations, no visible harmful effects on *M. aquaticum* plants were noticed in the present study. However, the relative growth rate based on biomass (0.026 d^{-1}, calculated after Knauer et al. (2008) was lower than growth rates from *M. aquaticum* (average 0.111 d^{-1}) and *M. spicatum* (average 0.052 d^{-1}) in different experiments. Since growth depends on a large amount of growing conditions and no control treatment without trace metals was included in this study this comparison should be treated with caution.

Table 1. Comparison of average *Myriophyllum* Zn concentrations from different sites and experiments. Values of sediment and plant in µg g^{-1} DM, water values (µg L^{-1}) are concentrations at the start of the experiments.

Study type	Plant species	Exposure	Sed.	Water	Stem	Root	Entire plant	Reference
Exp. with field sediments	*M. aquaticum*	54 days	800	6	183	395	n.a.	This study
Field samples	*M. aquaticum*	n.a.	128	n.a.	1458	4296	n.a.	(Cardwell et al., 2002)
Field samples	*M. spicatum*	n.a.	139	2	15	n.a.	n.a.	(Grudnik and Germ, 2010)
Field samples	*M. spicatum*	n.a.	1405	156	n.a.	n.a.	414	(Samecka-Cymerman and Kempers, 2004)
Biosorption experiment	*M. spicatum*	160 min	n.a.	10000	n.a.	n.a.	4500	(Keskinkan et al., 2003)
Biosorption experiment	*M. spicatum*	120 min	n.a.	9900	n.a.	n.a.	4000	(Lesage et al., 2007)
Biosorption experiment	*M. spicatum*	12 weeks	n.a.	1000	n.a.	n.a.	2883	(Lesage et al., 2008)
Biosorption experiment	*M. aquaticum*	21 days	n.a.	28060	n.a.	n.a.	549	(Kamal et al., 2004)

7.4.4. [AVS-SEM] or DGT measurements to predict plant metal availability?

The large differences in *Myriophyllum* sp. metal concentrations when sediment contamination is comparable may emphasize the importance of sediment geochemistry controlling metal mobility and uptake by macrophytes. As discussed before, precipitation with sulfides is proposed to be the predominant determinant of toxicity in anoxic sediments (Allen et al., 1993). In this study, [SEM-AVS] approaching zero resulted in an increase in mobile Zn and Ni pore water concentrations under high surface water oxygen concentrations. Yet, this gradual increase in metal availability, predicted by lower [SEM-AVS] and higher DGT measured mobility was not reflected in higher *M. aquaticum* metal. Moreover, plant metal concentrations decreased towards the end of the experiment.

This may indicate that metal mobility measured in the sediment is not the dominant factor affecting metal uptake by plants. First, plants may actively regulate metal uptake at their root membranes or sequester accumulated metals to prevent harm or translocation to sensitive tissues (Prasad, 2005). Secondly, the release of organic and inorganic compounds from roots to the adjacent sediments can significantly change metal availability in the rhizosphere. The release of oxygen (ROL) from macrophyte roots can locally oxidize sulfides resulting in a higher metal mobility in the rhizosphere which does not inevitability reflect the overall availability measured in the bulk sediments (Fig. 5) (Almeida et al., 2004). This metal mobilization often results in (co)precipitation as metal-(hydr)oxides on the root surface, known as ‘root plaque’ (Jacob and Otte, 2003a). Since Fe deposition is found to be the dominant geochemical process at the root surface (Li et al., 2011) the very high Fe root concentrations may indicate the presence of a root plaque on the *M. aquaticum* plants in the experiment. Additionally, thickenings perceptible near the apical root zones of the *M. aquaticum* plants from this study were assumed root plaques (Fig. 5).

Despite the overall negative correlations found between metal availability in the sediment and metal concentrations in the macrophytes, higher Ni and Zn root concentrations observed in the 90% oxygen treatment may be a reflection of the higher metal mobility as measured by DGT's in this treatment (StCyr and Campbell, 1996; Teuchies et al., 2008). Surface water aeration can result in metal sulfide oxidation near the sediment water interface which can cause additional Ni^{2+} and Zn^{2+} ions available to migrate and coprecipitate with Fe and Mn oxides in the root plaques (StCyr and Campbell, 1996). Comparable to roots, metal adsorption on the aboveground plant parts may be determined by their availability. The lower stem Mn concentrations in the 90% O_2 treatment may be a reflection of a reduced amount of available Mn^{2+} ions in the surface water caused by precipitation of Mn oxides on the water sediment interface in the aerated treatment.

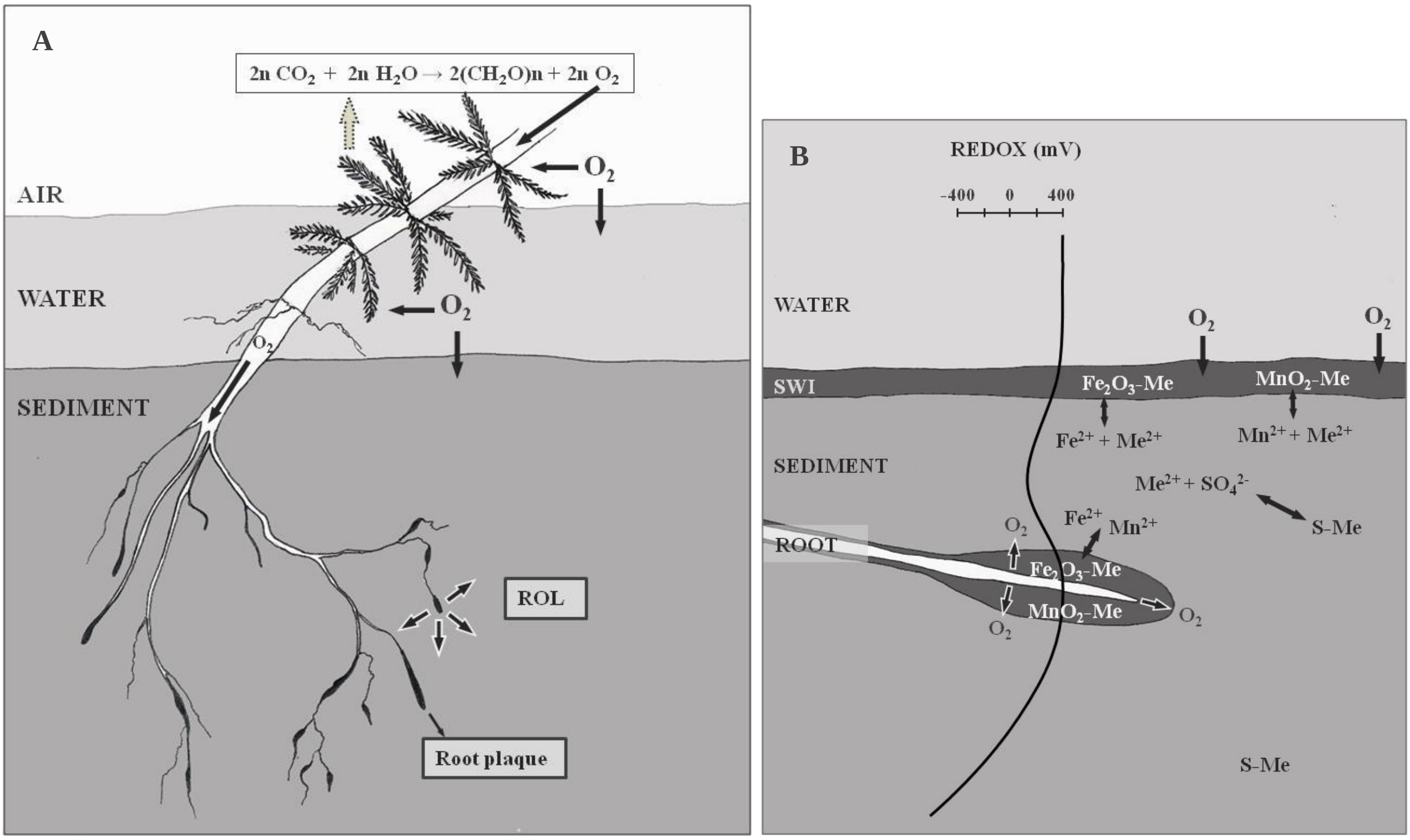


Figure 5. Conceptual drawing of oxygen transport through the air-water-sediment-plant system (A) and the effect of oxygen and redox gradients near the sediment-water-interface (SWI) and near the apical root zone on trace metal mobility (B).

The results of the present study suggest that precipitation of metals in the root plaque and on the plant surface can be a reflection of metal availability. Yet, this does not imply higher metal uptake by plants. The ‘root plaque’ can act as a physical barrier for mobile metals in the sediment resulting in a lower susceptibility to metal toxicity (Li et al., 2011; Liu et al., 2009). Additionally, plants can react on external factors, such as high metal concentrations, by decreasing their root permeability through changing their anatomical structure or morphology (Cheng et al., 2010). Metal sulfide precipitation is considered to seriously reduce toxicity of anoxic, metal rich sediments. However, this assumption should be treated with caution. The precipitation process was shown to be reversible. Additionally, small scale differences in metal availability caused by macrophytes may result in a high exposure to free metal ions even when an excess of AVS compared SEM is measured in the bulk sediments. Based on the results of this study, measuring [SEM-AVS] or DGT mobile metal concentrations was found to be unsatisfactory to assess metal availability to plants in anoxic sediments. The complexity of processes at the root-sediment interface and their effect on metal availability in the rhizosphere makes it difficult to predict metal toxicity towards plants based on the SEM-AVS model. These findings are important regarding risk assessment of contaminated anoxic sediments in which trace metals are generally considered to be less harmful. However, other outcomes may arise under different conditions: e.g. an increase in metal uptake by plants may occur when SEM largely exceed AVS values or plants may have a larger effect on sediment AVS when metal concentrations are lower. We believe that future work should focus on detailed processes in the rhizosphere related to [SEM-AVS] and metal mobility.

Supporting information

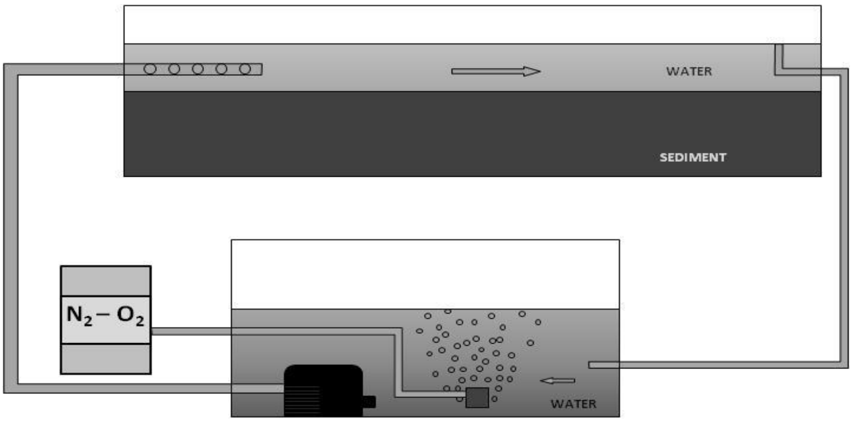


Figure S1. Experimental set-up. The upper basin contains field sediment, plants and artificial fresh surface water. An overflow was discharged in a lower basin where oxygen concentrations were controlled. A pump (10 L min^{-1}) creates a flow through both basins.

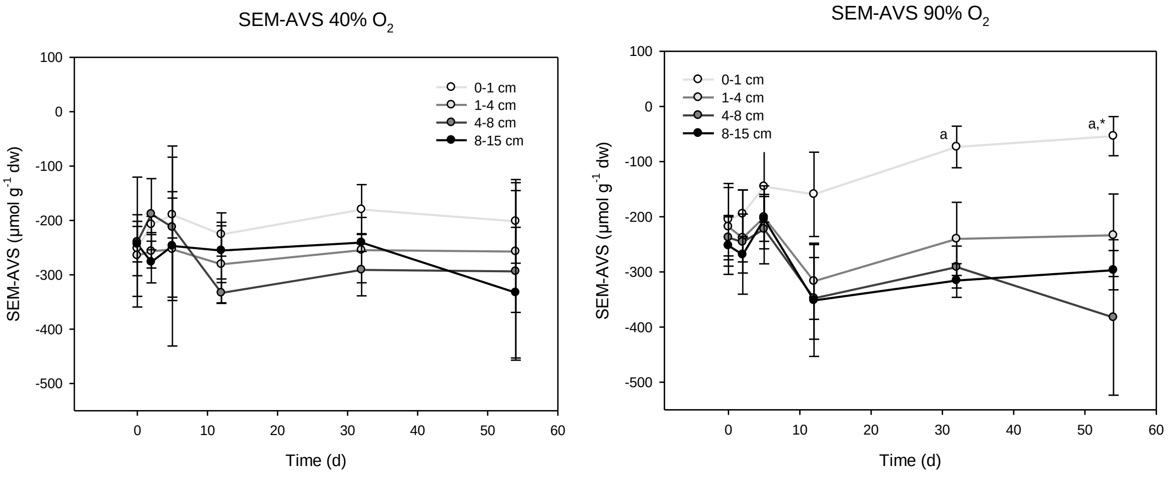
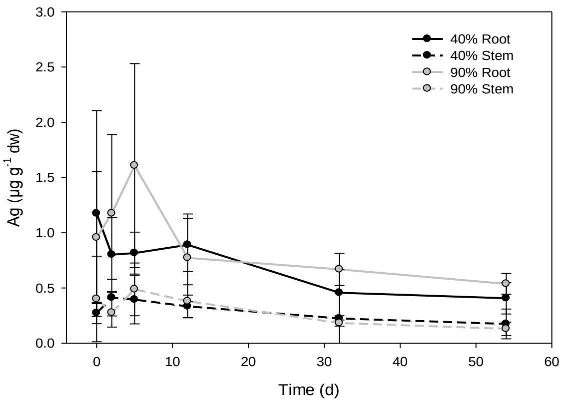
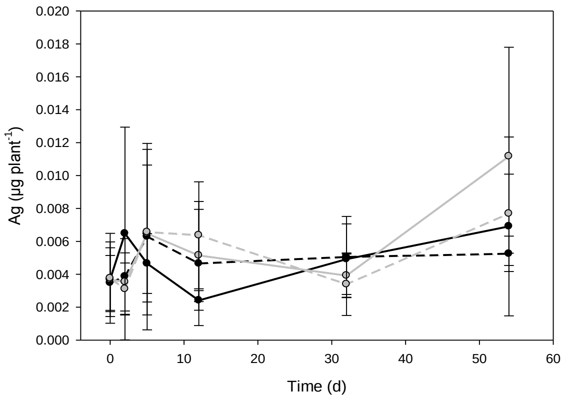


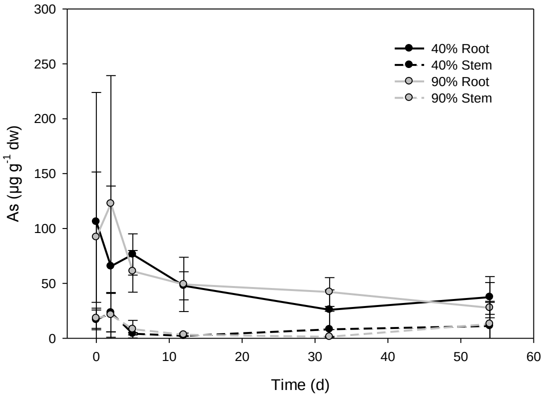
Figure S2. Sediment SEM-AVS (µmol g^{-1} dw) concentrations for both treatments in different depth layers in function of time (d). Average values (n=4) with standard deviation, a = significant ($p<0.005$) difference from day 0 in the same treatment and sediment layer, * = significant difference ($p<0.005$) between treatments for similar layers and time point.



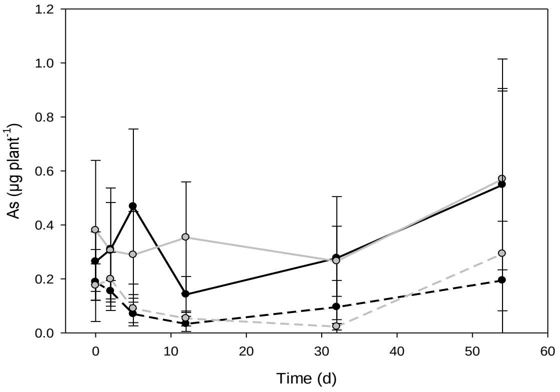
Myriophyllum Ag



Myriophyllum Ag - absolute



Myriophyllum As



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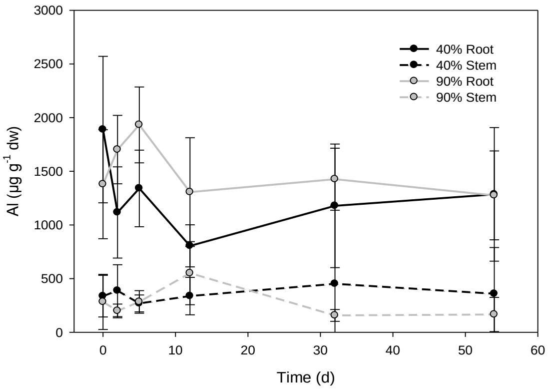
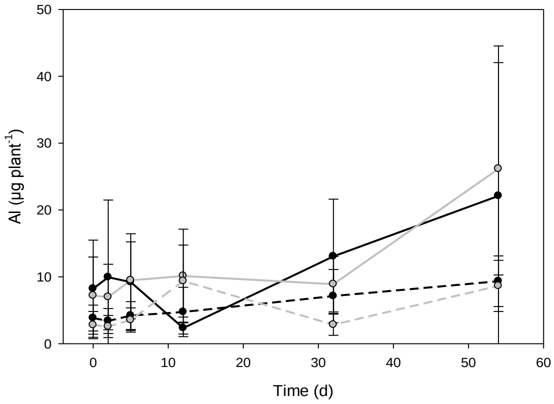
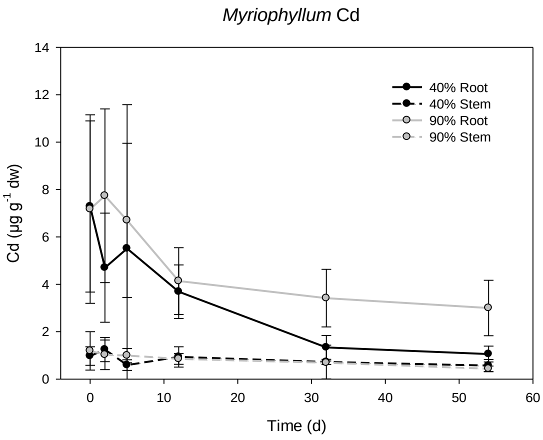
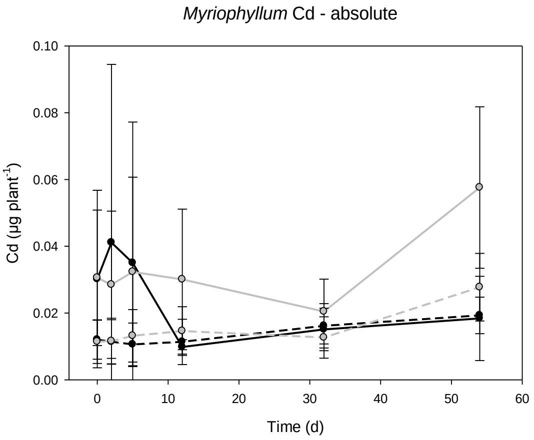


Myriophyllum Al



Myriophyllum Al - absolute


Myriophyllum Cd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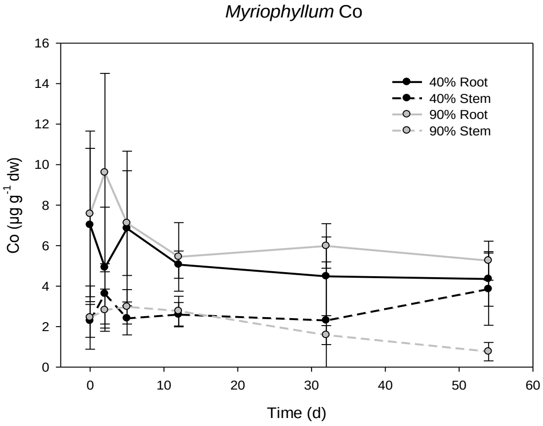
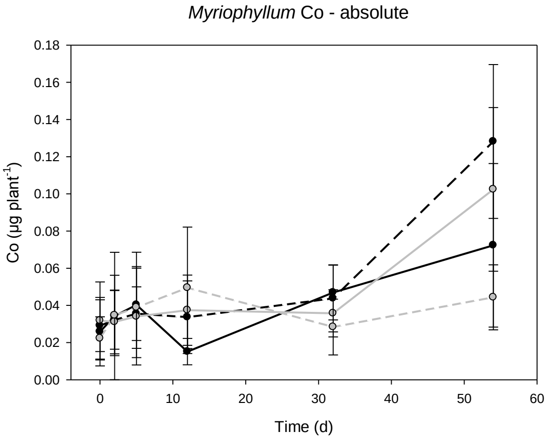
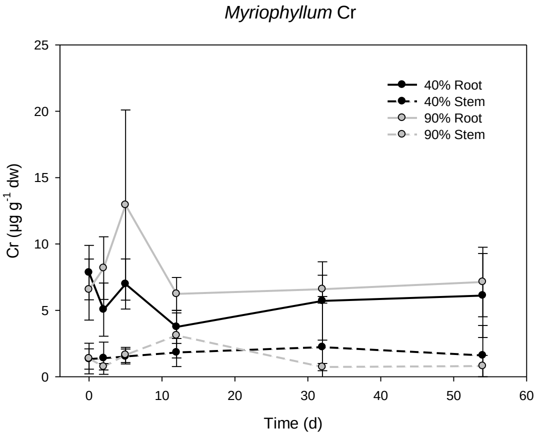
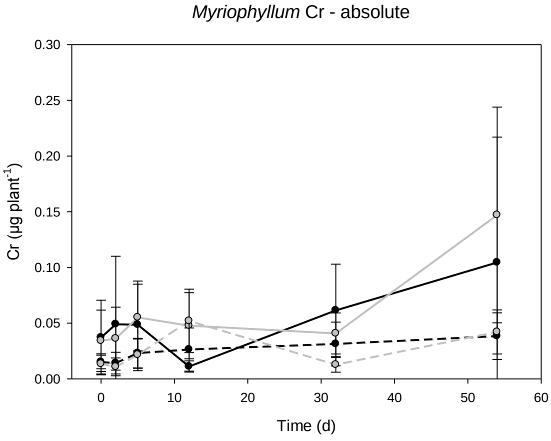

Myriophyllum Co


Myriophyllum Co - absolute


Myriophyllum Cr


Myriophyllum Cr - absolute

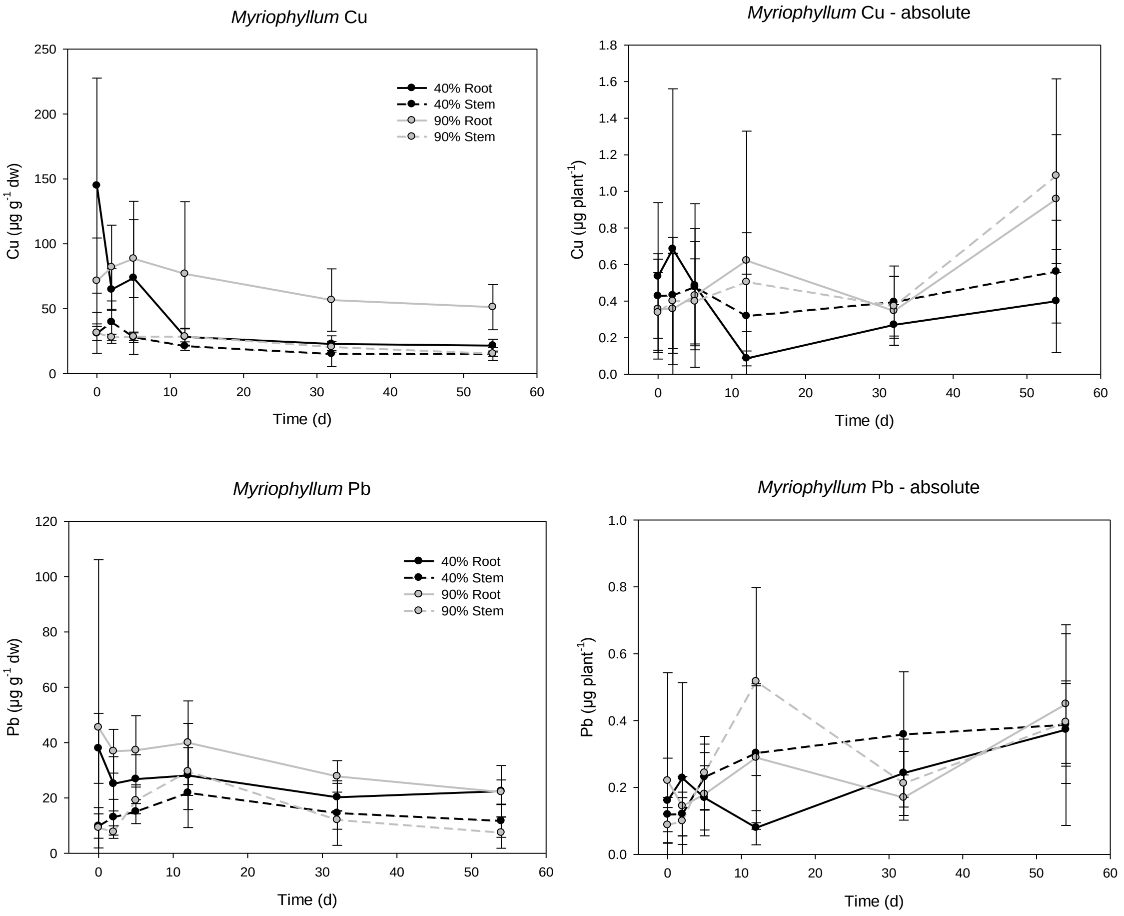


Figure S3. Plant metal concentrations (µg or mg g^{-1} dw) and absolute metal content (µg $plant^{-1}$) in function of time (d) in roots (full line) and stems (dashed line) in the 40% treatment (black) and the 90% treatment (gray). Average values (n=6) with standard deviation.

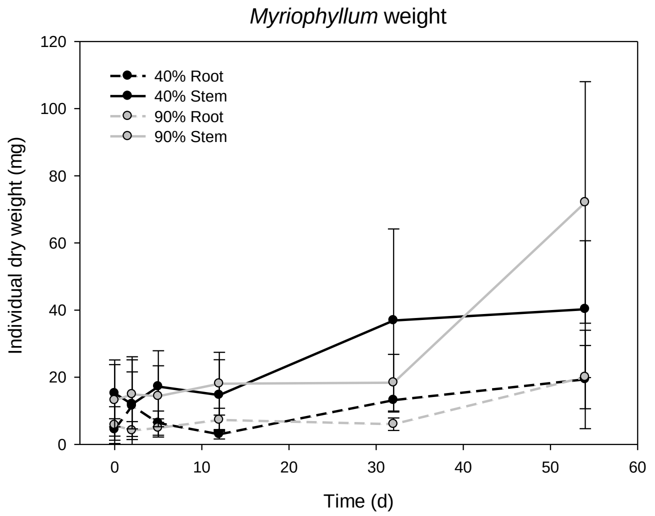


Figure S4. Dry weight of roots (dashed) and stems (full line) of individual plants in function of time (d) in the 40% (black) and the 90% (gray) treatment. Mean values (n=6) with standard deviation.

Table S1. General characteristics and total metal concentrations in the sediment. Average over depth and treatment (n = 72) with standard deviation.

sediment characteristics			
pH	6.57 ± 0.12	Al (mg g^{-1})	11.08 ± 1.26
OM (%)	17.07 ± 1.46	Ag (µg g^{-1})	4.73 ± 1.16
clay content (%)	3.74 ± 0.70	As (µg g^{-1})	22.49 ± 3.74
moisture content (%)	74.00 ± 0.85	Cd (µg g^{-1})	2.24 ± 0.27
SO_4^{2-} (mg g^{-1})	0.48 ± 0.36	Co (µg g^{-1})	7.83 ± 0.76
PO_4^{3-} (mg g^{-1})	3.04 ± 0.44	Cr (µg g^{-1})	54.78 ± 6.21
NO_3^- + NO_2^- (mg g^{-1})	< 0.10	Cu (µg g^{-1})	99.62 ± 17.07
NH_4^+ (mg g^{-1})	0.15 ± 0.02	Ni (µg g^{-1})	21.28 ± 2.75
Fe (mg g^{-1})	39.39 ± 3.98	Pb (µg g^{-1})	103.14 ± 18.55
Mn (mg g^{-1})	0.60 ± 0.08	Zn (µg g^{-1})	800.66 ± 113.60

Table S2. Pearson correlations between metal availability measures, plant metal concentrations (upper part) and absolute plant metal content (lower part). The r-values and significance levels are presented, significant correlations bold, n = 6 (time points); *p* < 0.05: *; *p* < 0.01: **.

	40% treatment		**90% treatment**	
	Root Zn conc	Stem Zn conc	Root Zn conc	Stem Zn conc
Labile Zn 0-4 cm	**-0.963****	-0.338	**-0.859***	**-0.919****
SEM-AVS 0-4 cm	-0.406	0.005	-0.402	-0.629
	Root Zn abs	Stem Zn abs	Root Zn abs	Stem Zn abs
Labile Zn 0-4 cm	-0.276	0.474	0.674	0.806
SEM-AVS 0-4 cm	0.507	0.593	0.474	0.522

CHAPTER 8

The effect of waste water treatment on river metal concentrations: removal or enrichment?

Johannes Teuchies, Lieven Bervoets, Tom Cox, Patrick Meire, Eric De Deckere.

Journal of Soils and Sediments 11 (2011) 364 – 372.

Abstract

Purpose Discharge of untreated domestic and industrial waste in many European rivers resulted in low oxygen concentrations and contamination with trace metals, often concentrated in sediments. Under these anoxic conditions the formation of insoluble metal sulfides is known to reduce metal availability. Nowadays implementation of waste water treatment plants result in increasing surface water oxygen concentrations. Under these conditions sediments can be turned from a trace metal sink into a trace metal source.

Materials and methods In an ex situ experiment with metal contaminated sediment, we investigated the effect of surface water aeration on sediment metal sulfide (acid volatile sulfides, AVS) concentrations and sediment metal release to the surface water. These results were compared with long term field data, where surface water oxygen and metal concentrations, before and after the implementation of a waste water treatment plant, were compared.

Results and discussion Aeration of surface water in the experimental setup resulted in a decrease of sediment AVS concentrations due to sulfide oxidation. Metals, known to precipitate with these sulfides, became more mobile and increasing dissolved metal (arsenic (As), cadmium (Cd), copper (Cu)) concentrations in the surface water were observed. Contrary to As, Cd or Cu, manganese (Mn) surface water concentrations decreased in the aerated treatment. Mn ions will precipitate and accumulate in the sediment as Mn oxides under the oxic conditions. Field data, however demonstrated a decrease of all total metal surface water concentrations with increasing oxygen concentrations following the implementation of the waste water treatment plant.

Conclusions The gradual decrease in surface water metal concentrations in the river before the treatment started and the removal of metals in the waste water treatment process could not be countered by an increase in metal flux from the sediment as observed in the experiment.

8.1. Introduction

Metal contamination is one of the most complex issues of today’s contamination problems. Widespread uses of metals, the legacies of past contamination and new technologies continue to pose important ecological risks for aquatic environments across the earth (Luoma and Rainbow, 2008). In the course of the last decades a gradual decrease of the surface water metal concentrations has been observed in Europe. Nevertheless, metal contaminated river sediments can still constitute an ecological threat. Since trace metals show a high affinity for organic and inorganic particles, these contaminants are often historically concentrated in the sediments (Du Laing et al., 2009c; Hart, 1982; Regnier and Wollast, 1993). The formation of insoluble metal sulfides is found to play a major role in reducing sediment metal availability and transfer to the surface water under anoxic conditions (Di Toro et al., 1990; Di Toro et al., 1992). The ratio of acid extracted sulfides (AVS, Acid Volatile Sulfides) and Simultaneously Extracted metals (SEM) is proposed as a predictor for availability of some divalent metals (cadmium (Cd), cupper (Cu), lead (Pb), nickel (Ni) and zinc (Zn)) (Allen et al., 1993; Di Toro et al., 1992).

Anthropogenic activities (dredging, waste water treatment) or natural induced changes (seasonality, bioturbation, storms) might result in an increase of oxygen levels in both surface water and sediment (Peterson et al., 1996; Zhuang et al., 1994). Under these conditions metal sulfides may be oxidized and sediments can be turned from a trace metal sink into a trace metal source (Maddock et al., 2007; Petersen et al., 1997). Resuspension of anoxic sediments in oxic surface water, caused by dredging and dumping or natural events can enhance this process and can result in a larger dissolved metal flux to the surface water and increased metal availability (Eggleton and Thomas, 2004; Hedge et al., 2009).

The aim of this study was to investigate the effect of aeration and resuspension of sediments on the mobility of metals to the water column. Therefore a controlled experiment was conducted and results were compared with long term data from the river Zenne as test case. The river Zenne flows through Brussels, Belgium, and the basin is situated in one of the most densely populated areas of Europe. Untreated domestic and industrial waste water has been discharged in the river for decades, resulting in severe contamination of water and sediment with micro pollutants such as trace metals and in a decreased oxygen level in the surface waters. In the long term experiment with ex situ river Zenne sediment cores, we investigated the effect of surface water aeration on sediment SEM/AVS concentrations and sediment metal release to the surface water. These results were compared with long term field data, where river Zenne surface water oxygen, metal and suspended solid concentrations before and after the implementation of the waste water treatment plant in March 2007 were compared.

8.2. Material and methods

8.2.1. Sampling site

Sediment and water have been sampled May 2006, downstream of Brussels near to Weerde (50°58´07.96” N, 4°28´47.27” E). First the water was sampled and stored in big barrels for three days at 4°C to enable settling of suspended material. One week later the sediment was sampled at the same location with an Eckman grab sampler. The sediment (9% < 2 µm, 2 µm < 44 % < 63µm) at the site contained a high concentration of metals (As: 18 µg g^{-1}dry mass, Cd: 4.5 µg g^{-1}dry mass, Cr: 65 µg g^{-1}dry mass, Cu: 194 µg g^{-1}dry mass, Hg: 3.0 µg g^{-1}dry mass, Ni: 43 µg g^{-1}dry mass, Pb: 398 µg g^{-1}dry mass, Zn: 970 µg g^{-1}dry mass).

8.2.2. Experimental setup

In order to approach the natural situation, this experiment was executed in sediment cores with a layer of surface water, in a climate chamber with constant temperature (20°C). First the water was added to 15 individual PVC cores (diameter 24 cm, height 40 cm). Immediately after sampling, sediments were homogenized, distributed over the cores with water and given 12 hours to settle before the start of the experiment. This resulted in a sediment layer of 20 cm and a surficial water layer of 15 cm. Surface water levels were replenished regularly with demineralized water in order to maintain the initial level.

Rhizons (pore water sampler, Eijkelkamp) were inserted horizontally through holes in each core in order to sample pore water (2.5 cm sediment depth). An air pump was placed in the surface water of each core. Differences in air pump flow rate (none, low and high) resulted in different oxygen concentrations and suspended solid load: 3 treatments with 5 replicates. The 3 different treatments investigated consisted of 1) ANOXIC: a non aerated treatment with low oxygen and suspended solid concentrations, 2) OXIC-CLEAR: a low aerated treatment with high oxygen and low suspended solid concentrations and 3) OXIC-TURBID: a high aerated treatment with high oxygen and resuspension resulting in high suspended solid concentrations (Fig. 1).

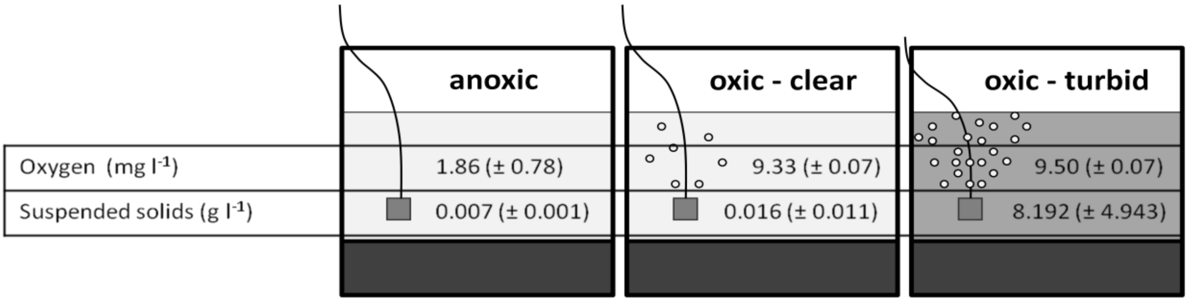


Figure 1. Mean oxygen and suspended solid concentrations with the standard error (n=5) for the 3 different treatments in the core experiment

8.2.3. Sampling

Water and sediment samples were taken from the batch tests after 1.5 year of treatment. Previous to sampling surface water was gently mixed and basic water characteristics were measured (oxygen, suspended solid concentration, pH and conductivity). Surface water was sampled with 10 mL syringes. The upper 5 cm of the sediment was sampled with a small (1 cm diameter) sediment corer. In each core 5 sediment samples were taken randomly and mixed. In order to extract pore water from the sediment a vacuum pump was connected to the rhizon samplers.

8.2.4. Analyses

Oxygen concentrations, pH and conductivity were measured using a WTW Oxi 315i and WTW pH/Cond 340i set. Surface water suspended solid (ss) concentrations were measured by filtering from 0.05 l up to 1 l, depending on the ss concentration, over a preweighed filter (Wathman, glass fibre, particle retention 1.2 µm). Filters and blanks were dried at 105° and weighed again. Total metal concentrations (arsenic (As), Cd, chromium (Cr), Cu, manganese (Mn) and Pb) were measured in both pore and surface water of the batch tests. Since rhizon water samplers with standard 0.1 µm pores are used to sample pore water, this water phase can be assumed dissolved. Water samples for total metal concentrations (10 mL) were acidified with 100µL nitric acid (HNO_3 69% Normapur Trace Analysis Cd Hg Pb) and then filtered (cellulose ester, 0.45 µm pore diameter). Water samples for dissolved metal concentrations measured in the surface water of the batch tests were first filtered and then acidified. Water metal concentrations were determined using an Inductively Coupled Plasma Mass Spectrometer (ICP-MS, Varian UlatraMass 700), with Germanium (Ge) as internal standard.

In the batch tests sediment AVS, SEM (Cd, Cu, Ni, Pb and Zn) and total metal concentrations (As, Cd, Cr, Cu and Pb) were measured. Immediately after sampling, a part of the wet sediment is weighed and oven dried (40°C). The rest of the sediment is weighed and immediately used for AVS and SEM determination following the purge-and-trap method of Allen et al. (1993); wet sediment was stirred for 2 hours with hydrochloric acid (HCl, 37%, Normapur) to dissociate metals and sulfides. The H_2S gas formed by this reaction was lead through a NaOH solution by the constant N_2 flow. The S^- ions were measured photospectrometically in the solution in order to determine the AVS concentration. In order to determine SEM concentrations, metal concentrations in the sediment-acid mixture were measured after filtration using ICP type radial plasma iris/CID (THERMO).

Dried sediments are grinded and used for determination of total metal concentration after 'hot block digestion'. For digestion 5 mL nitric acid (HNO3 69% Normapur Trace Analysis Cd Hg Pb) and 5 mL of hydrogen peroxide (H_2O_2 27% for electrical purpose) are added to 0.2 g of soil. This mixture is heated up to 100 °C for 30 min, cooled overnight and again heated at 150 °C during 60 min. Sediment metal concentrations were determined using ICP type radial plasma iris/CID (THERMO). Blanks and certified reference material for sediment (IRMM, BCR® № 320, river sediment) and water (IRMM, BCR® № 610, groundwater) were included in all series of metal analysis in order to verify the analyze procedure.

8.2.5. Field data

The findings from the experiment were compared with field data from the river Zenne itself. The 1164 km² river basin covers one of the most densely populated areas of Europe. With his 103 km length the Zenne passes through Brussels, Belgium, before discharging itself into the Rupel and subsequently into the Schelde estuary. For decades, waste water was discharged untreated into the river with high pollution and low oxygen concentrations as a consequence. In March 2007 a waste water treatment plant with a capacity 1,100,000 inhabitant's equivalent became operative, resulting in an instant increase of the water quality. Surface water data from before and after the treatment started were investigated. All the long term surface water data from the Zenne were collected from the database of the Flemish Environment Agency (www.vmm.be/geoview). Measuring and sampling frequency varied depending on the parameter and sampling location.

8.2.6. Statistical analysis

The statistical analysis was carried out using the SAS statistical package (The SAS® System, SAS institute Inc., Cary, NC, USA). The significance of differences between treatments was tested with an ANOVA. The Tuckey a posteriori test was used to evaluate the differences mutually.

8.3. Results

8.3.1. Experimental results

The ANOXIC treatment had low oxygen (1.86 mg l^{-1}) and low suspended solid (0.004 g l^{-1}) concentrations. The OXIC-CLEAR treatment had high oxygen (9.33 mg l^{-1}) and low suspended solid concentrations (0.014 g l^{-1}). The OXIC TURBID treatment had high oxygen (9.50 mg l^{-1}) and high suspended solid (8.165 g l^{-1}) concentrations (see Fig. 1). Total metal concentrations in the experimental sediment for all treatments were 8.05 ± 2.15 µg g^{-1} dry mass (As), 6.09 ± 0.31 µg g^{-1} dry mass (Cd), 213.18 ± 10.20 µg g^{-1} dry mass (Cu), 529.14 ± 25.64 µg g^{-1} dry mass (Pb) and 240.30 ± 15.51 µg g^{-1} dry mass (Mn). No significant differences between sediment total recoverable metals in the upper 5 cm were found among treatments. The 1.5 year period of surface water aeration resulted in significant lower sediment AVS concentrations in the OXIC-CLEAR (1.74 mmol kg^{-1}, $p<0.001$) and the OXIC-TURBID (0.59 mmol kg^{-1}, $p<0.001$) treatments compared to the ANOXIC treatment (35.51 mmol kg^{-1}) (Fig. 2). ANOXIC treatment SEM concentrations were significantly lower compared to the OXIC-CLEAR ($p=0.0148$) and the OXIC-TURBID ($p=0.0049$) treatments (see Fig. 2). The SEM/AVS ratio which is lower than 1 (0.75 $\pm$ 0.17) in the ANOXIC treatment indicated the presence of an excess of sulfides which can precipitate with trace metals in the sediment. Higher SEM/AVS ratios for the OXIC-CLEAR (29.50 $\pm$ 12.20) and OXIC-TURBID (73.26 ± 17.23) treatment can indicate a higher metal availability in the sediment.

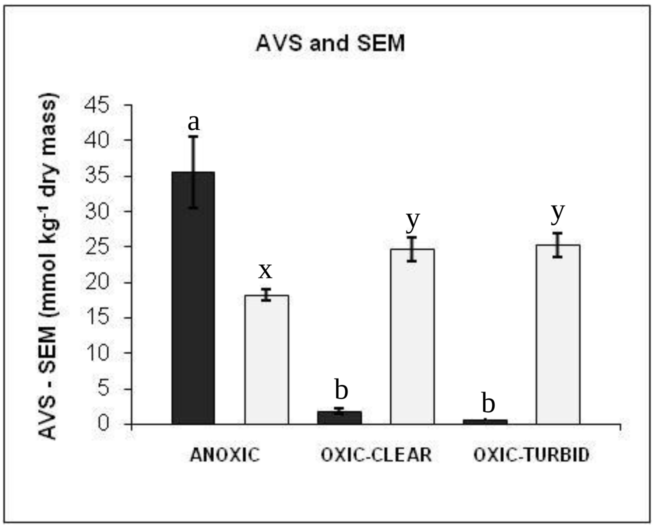


Figure 2. AVS (dark) and SEM (light) concentrations in the experiment top 5 cm sediments after 1.5 year of treatment. Mean values (n=5) with standard error. Both variables expressed on the same axes with same units

A significant increase in dissolved metal concentrations in the surface water of the experimental cores with increasing aeration level was found for As ($p=0.0010$), Cd ($p=0.0003$) and Cu ($p=0.0017$) (Fig. 3). The opposite trend, decreasing dissolved metal concentrations with increasing aeration level, was found for Mn ($p=0.0241$) (see Fig. 3).

Surface water total metal concentrations are from 54 (Mn) up to 566 (Cd) times higher in the OXIC-TURBID treatment compared to the ANOXIC treatment (Table 1). Despite these high values, differences in concentrations between treatments were not significant ($p>0.05$ for all metals). The variation in total metal concentrations within the treatments, especially in the OXIC-TURBID, was very high. For example total Pb concentrations in the OXIC-TURBID treatment surface water varied from 131 µg l^{-1} to 12930 µg l^{-1} between cores. Related to these results, also suspended solid concentrations showed a very high variation within treatments. Suspended solid concentrations in the surface water of the OXIC-TURBID treatment varied from 0.03 mg l^{-1} to 22.23 mg l^{-1}. Consequently, a positive, highly significant correlation between surface water total metal and suspended solid concentration in the experiment was found ($p<0.0001$ for all metals).

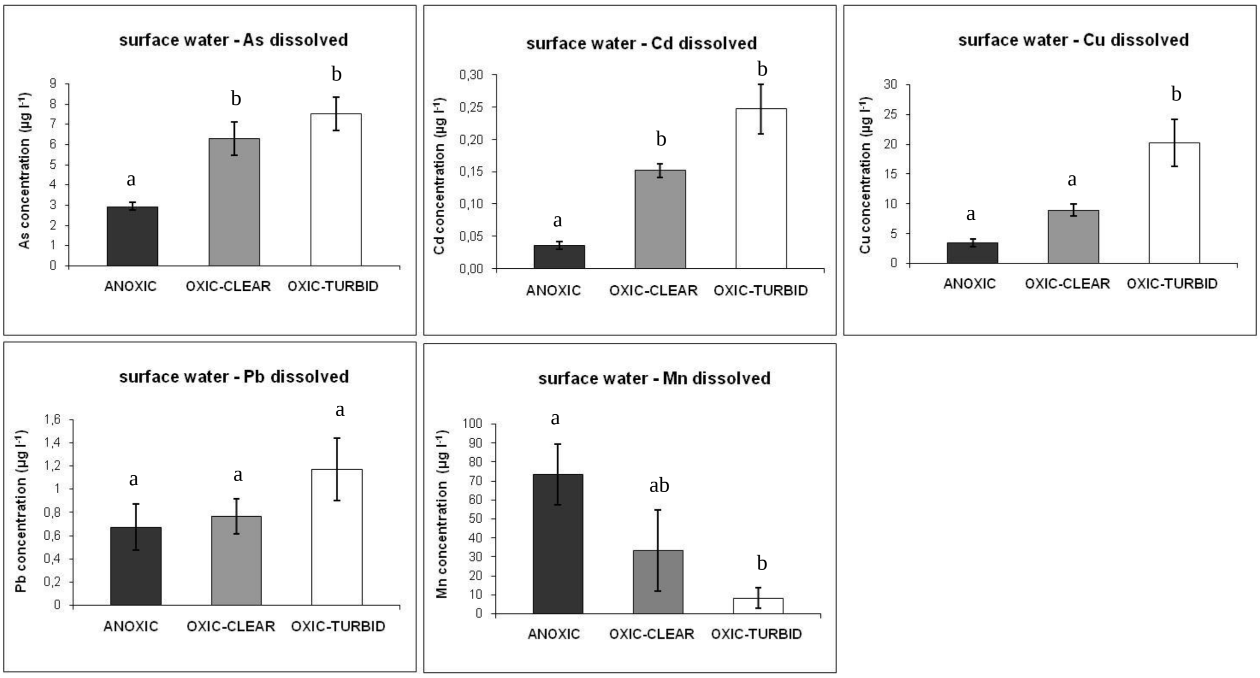


Figure 3. Surface water dissolved metal concentrations in the experiment after 1.5 year of treatment. Mean values (n=5) with standard error

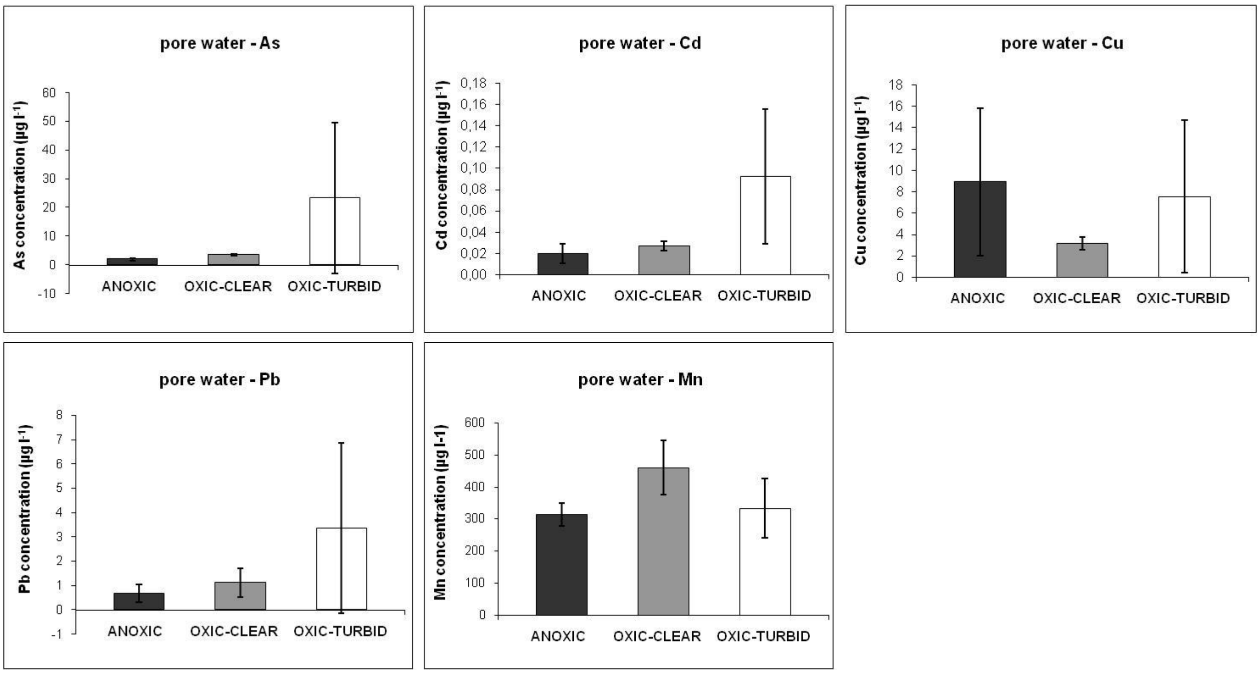


Figure 4. Pore water metal concentrations on 2.5 cm depth in the experiment sediments after 1.5 year of treatment. Mean values (n=5) with standard error.

A significant ($p<0.0001$) increase in pH with increasing aeration level was observed. pH values raised from 6.70 ± 0.03 in the ANOXIC treatment to 7.05 ± 0.01 in the OXIC-CLEAR treatment and again to 7.39 ± 0.10 in the OXIC-TURBID treatment. Significant ($p=0.0025$) differences in surface water conductivity between the ANOXIC treatment (451 ± 141 µs cm^{-1}), the OXIC-CLEAR (1996 ± 164 µs cm^{-1}) and the OXIC-TURBID treatment (1451 ± 325 µs cm^{-1}) were observed.

No significant differences between pore water concentrations in the three different treatments were found ($p>0.05$ for all metals). A high variability between samples from one treatment can be observed (Fig. 4).

Table 1. Surface water total metal concentrations (µg l^{-1}) after 1.5 year of treatment. Mean values with standard error.

	ANOXIC	**OXIC-CLEAR**	**OXIC-TURBID**
As	**4.17 (+/- 0.52)**	**6.86 (+/- 0.84)**	**294.04 (+/- 140.41)**
Cd	**0.12 (+/- 0.04)**	**0.21 (+/- 0.03)**	**66. 02 (+/- 34.50)**
Cu	**8.12 (+/- 2.51)**	**11.80 (+/- 2.13)**	**3200.70 (+/- 1610.48)**
Pb	**11.04 (+/- 4.62)**	**8.77 (+/- 3.75)**	**5777.43 (+/- 2771.17)**
Mn	**71.90 (+/- 14.27)**	**37.27 (+/- 21.75)**	**3860.70 (+/- 2010.76)**

8.3.2. Field results

Waste water treatment resulted in an instant increase in Zenne surface water oxygen concentrations from an average of 1.96 ± 0.16 mg l^{-1} in the 18 years before purification to 6.30 ± 0.20 mg l^{-1} in the 3 years after purification (Fig. 5). Especially the periods with low oxygen concentrations during summer, observed before purification started, disappeared. The suspended solid concentration decreased with purification (Fig. 6).

The total Cu and Pb concentrations in the Zenne surface water were generally decreasing after 1990 (Fig. 7). No clear long term trend was observed for total As, Cd and Mn concentrations in the Zenne surface water (see Fig. 7). From waste water purification in 2007 onward, a noticeable decrease in most examined total metal (Cd, Cu, Pb, Mn) concentrations was observed (see Fig. 7). Similarly as in the experiment, significant positive correlations between the total metal concentrations and the suspended solids in the Zenne surface water were found (Cd ($p=0.0098$), Cu ($p=0.0026$), Pb ($p=0.0116$), Mn ($p=00256$)). Unfortunately, the data on dissolved metal concentrations in the Zenne surface water were limited. The dissolved concentrations were measured for Mn and Cu but not enough samples were taken in order to draw clear conclusions on differences before and after waste water treatment (Fig. 8)

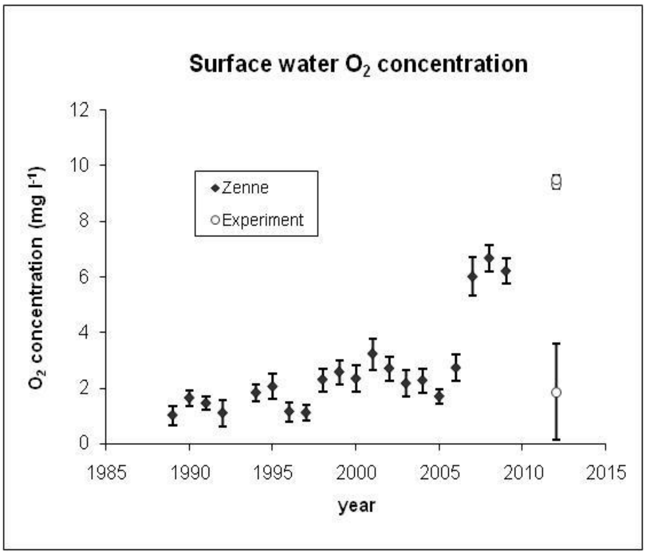


Figure 5. Zenne (●) and experiment (○) surface water oxygen concentrations. Zenne concentrations measured weekly at 1 point in the river. Year mean values with standard errors.

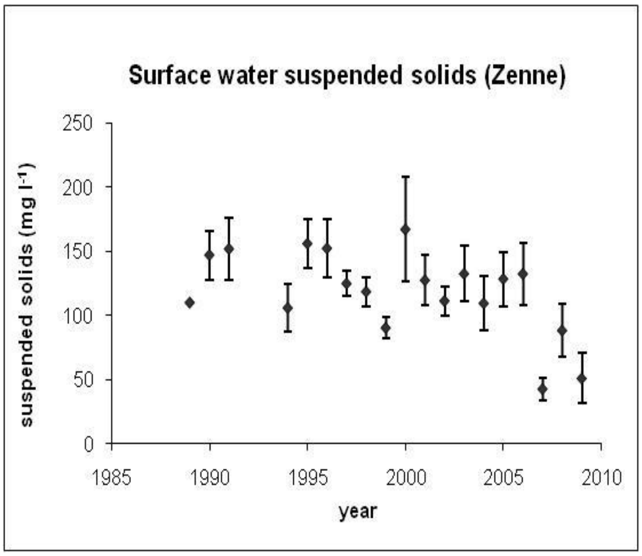


Figure 6. Zenne surface water suspended solid concentrations measured semi-monthly at 1 point in the river. Year mean (n = 24) values with standard errors

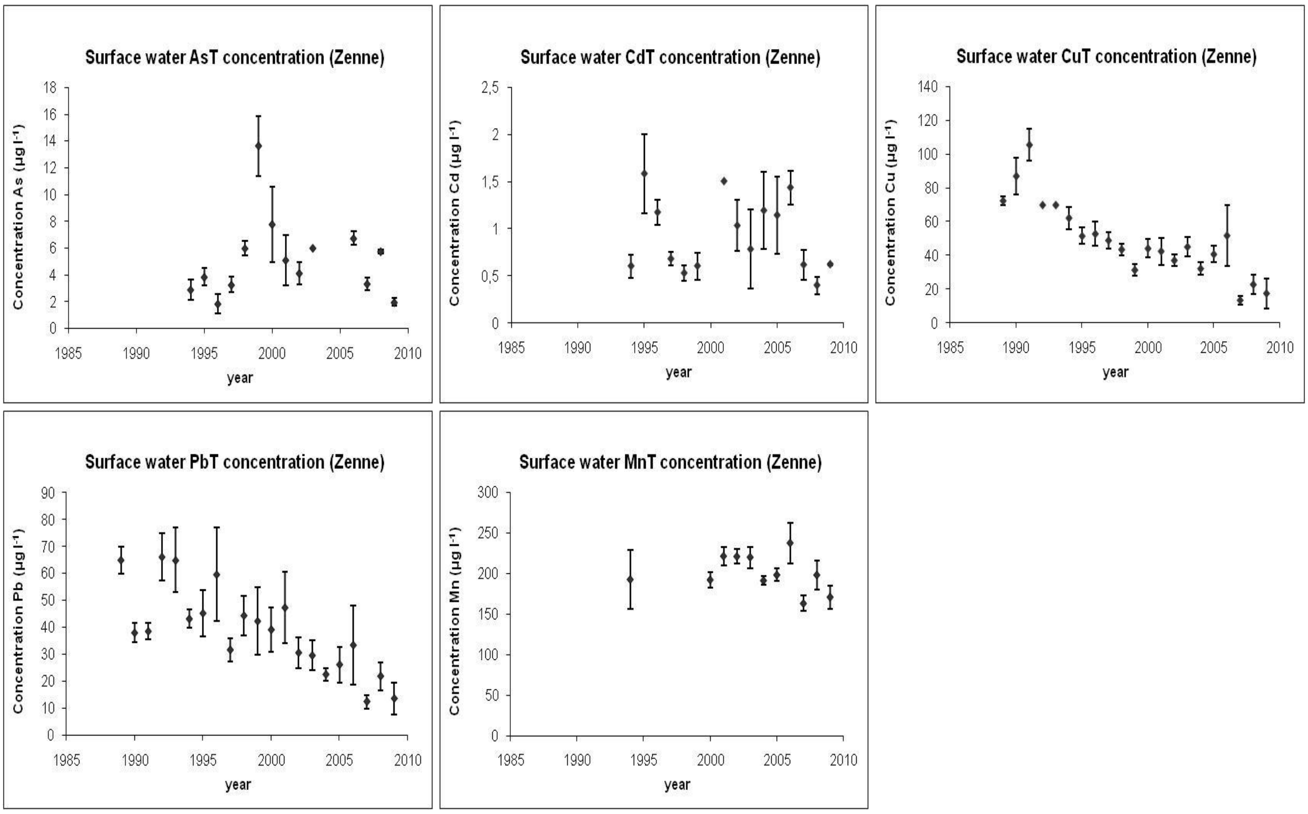


Figure 7. Zenne surface water total metal concentrations (1 sampling point). Measuring frequency differs between metals and years. Year mean (n between 1 and 24) values with standard error

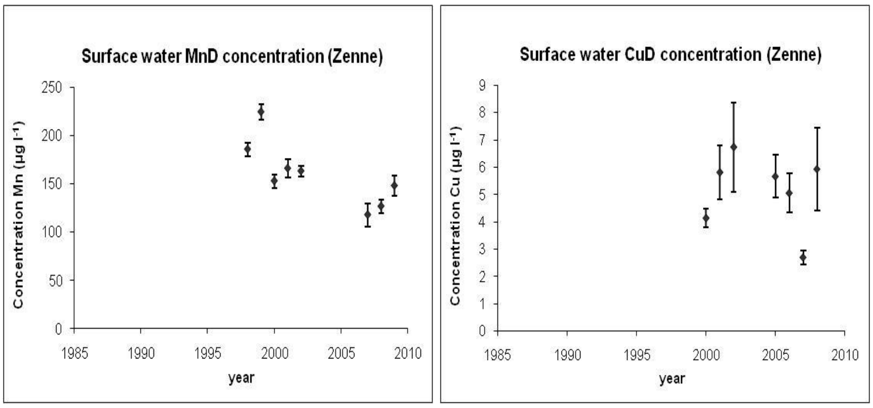


Figure 8. Zenne surface water dissolved metal concentrations (1 sampling point). Measuring frequency differs between metals and years. Year mean (n between 2 and 24) values with standard error.

8.4. Discussion

Drainage of untreated waste water in the river Zenne caused severe contamination. Sediment metal concentrations were high and exceeded Flemish quality standards (for reuse as soil) (QS) 11 times (Zn), 2 times (Cu), up to 40 (Cr) and even more than 50 times (Pb) (Flemish Government, 2008). Despite these high values, all surface water total metal concentrations besides Zn dropped below Flemish QS in recent years (Flemish government Vlarem, 2000).

Additionally, contaminated effluents caused decades of low oxygen concentrations in the surface water of the Zenne. Surface water low oxygen concentrations in the ANOXIC treatment in the experiment resulted in an excess of sulfides compared to SEM in the sediment. Since sulfides precipitate with some trace metals, a lower availability can be expected under the ANOXIC conditions (Allen et al., 1993; Di Toro et al., 1990; Di Toro et al., 1992). Additionally, lower SEM values were observed in the ANOXIC treatment. During 1.5 year of anaerobic conditions pyrite may be formed. This strong sulfite-metal combination, which will not be released entirely during the AVS-SEM extraction (Morse, 1994) can explain the lower SEM values found in the ANOXIC treatment sediments. As observed in the Zenne, surface water oxygen concentrations increased promptly after the waste water treatment plant of Brussels was started up. The experimental results showed that these higher oxygen concentrations can result in a drastic decrease in sediment AVS concentrations. In both OXIC treatments surface water oxygen penetrated the sediment and sulfide precipitates in the top soil layer were oxidized. Higher trace metal availability in sediment and surface water can be a consequence (Hartley and Dickinson, 2010; van den Berg et al., 1998a).

Furthermore, metal flux to the overlying water was influenced by surface water aeration in the experiment. An increase of dissolved metals in the surface water (As, Cd and Cu) was observed in the cores which had been subjected to aeration and resuspension. Cd and Cu are known to (co)precipitate with sulfides (Di Toro et al., 1992; Morse and Rickard, 2004). Sulfides will be oxidized to sulfate under the aerobic conditions and (co)precipitated Cd and Cu will be released and can diffuse to pore- and surface water.

Despite a difference in chemical behavior compared to Cd, Cu and Pb the metalloid As showed the same trend and was enriched in the surface water under the oxic conditions in the experiment as well. Depending on pH and redox potential (Eh), As^{V} or As^{III} will be the predominant form (Sharma and Sohn, 2009). In the anoxic cores the more toxic form As^{III} will dominate (Sharma and Sohn, 2009). Comparable with Cd and Cu, As^{III} can be retained associated with sulfides, predominantly as arsenopyrite, in sulfur rich sediments, under reduced conditions (Bose and Sharma, 2002; Keimowitz et al., 2005). Oxidation of these As-sulfide precipitates can result in a release of As to pore water and surface water (Keimowitz et al., 2005), explaining the higher dissolved As concentrations in the oxic cores.

As observed in the experiment for Cu metal remobilization can be more pronounced when suspended solid concentrations are high. Metal sulfide precipitates can be transported as suspended solids in the water column. The contact surface between oxic surface water and metal sulfides is larger in suspended solids compared to bottom sediments. High suspended solid concentrations are found in turbid rivers or during short term resuspension events. Dredging activities, storm events or bioturbation can cause resuspension of anoxic metal contaminated sediments into oxic surface waters. This can result in a very fast increase in metal availability due to metal sulfide oxidation (Cappuyns et al., 2006; Hedge et al., 2009; Saulnier and Mucci, 2000).

Contrary to the other metals, decreasing surface water dissolved Mn concentrations, subsequent to aeration and resuspension were observed in the experiment. Soluble Mn^{II} ions will precipitate and accumulate in the sediment as Mn oxides under the oxic conditions in the aerated cores. (Li et al., 2009) found Mn oxides to be the most important component controlling the adsorption of metals in superficial sediments. As concluded by (Zhuang et al., 1994) the transfer of sediment bound metals to the surface water as a consequence of sulfide oxidation could be much larger if no other binding phases such as Mn and Fe oxides are available. Since dissolved Cd, Cu, Pb and As concentrations increased in both OXIC treatments, a higher contribution of sulfide compounds in controlling metal mobility compared to the binding phases under oxic conditions can be assumed under these experimental conditions. In addition the higher surface water conductivity in both OXIC treatments indicates higher surface water ion concentrations.

In addition to sediments, metals in the surface water can be precipitated with sulfides as well, behaving as suspended solids. Oxic conditions can dissolve these complexes and the dissolved metal fraction in the surface water can increase (Simpson et al., 1998). This can be confirmed by the observed increase of the dissolved/total metal concentration ratio in the OXIC CLEAR treatment compared to the ANOXIC treatment. Higher dissolved metal concentrations can indicate higher metal availability (Luoma and Rainbow, 2008).

Together with the redox potential, the pH is one of the key factors controlling metal mobility (Gambrell, 1994). (Di Nanno et al., 2007; Satawathananont et al., 1991) found a pH decrease caused by oxidation of metal sulfides. Since metals are more mobile under low pH conditions, this process can add to the mobilization effect of metal sulfide oxidation (Tack et al., 1996). This is not in accordance with the higher pH values observed in the OXIC treatments of the experiment. Despite these higher pH values metal mobility can be assumed to be higher in both OXIC treatments since dissolved As, Cd and Cu concentrations were higher.

Higher metal mobility in the sediment causing transfer to the surface water was also expected to have an effect on the pore water concentrations. Due to a high variability between replicas no significant trends were observed. Except for Mn, where pore water concentrations exceeded considerably surface water concentrations, pore- and surface water dissolved metal concentrations were more or less in the same order of magnitude. With differences in pore- and surface water metal concentrations, fluxes of dissolved metals might be expected. Flux calculations, based on the experimental data and translated in the field situation can give more insight in the importance of metal remobilization in natural systems. However, sediment – water fluxes are determined by adsorption, precipitation and coprecipitation with organic matter, Mn- and Fe oxyhydroxides and metal sulfides. These processes show distinct variability with depth in the sediment, mainly driven by the redox potential (Boudreau, 1999). As such, pore water concentrations from one specific depth in a sediment core (pore water was sampled at 2.5 cm depth) will not suffice to calculate fluxes through the sediment water interface. Flux calculations would require a full diagenetic model (Boudreau, 1996; Boudreau, 1999) which would take re-reduction or re-oxidation, preventing the flux, into account.

No differences in surface water total metal concentrations between the ANOXIC and OXIC-CLEAR treatment were found. Total As, Cd and Cu concentrations in these treatments were slightly higher than the dissolved fractions. The total Pb concentration, an element known to show even more than other metals high affinity for suspended matter (Luoma and Rainbow, 2008), is at least by a factor 10 higher compared to the dissolved phase. All Mn found in the surface water of these treatments was dissolved. On the other hand, the OXIC-TURBID treatment resulted in a considerable increase in total metal concentrations. However, the increase was not significant. This can be attributed to the high variability observed in the surface water total metal concentrations. Most trace metals occurring in surface water are known to show a high affinity for particulate matter (Regnier and Wollast, 1993). A positive, highly significant correlation between surface water total metal and suspended solid concentration in the experiment was found. The total metal concentration in the surface water depends on the amount of suspended sediments in the sample. Consequently, the high variability in suspended solid concentrations between treatments and replicas explains the total metal concentration variability.

Metal concentrations in suspended solids of the experiment were calculated and found to be in the same order of magnitude to sediment metal concentrations which indicates that the total metal concentration was determined by the amount of surface sediment in suspension. Suspended solid metal concentrations in the river Zenne were calculated and found to be roughly a factor 5 higher than if this were only sediments in suspension. Metals have a high affinity for fine grained suspended solids and organic matter. These fractions are preferably transported in the water column while heavier particles will settle down.

Higher trace metal mobility subsequent to surface water aeration was observed in the experiment. Decades of anoxia and metal polluted sediments makes the Zenne a feasible candidate for metal remobilization. Nevertheless, waste water treatment and hence surface water oxygen increase goes together with a decrease in Zenne surface water total metal concentrations. The removal of the metal rich suspended solids in the river Zenne by the waste water treatment process probably caused the removal of trace metals. Dissolved Cu concentrations in the river Zenne are lower than the concentration in the OXIC CLEAR treatment. Additionally, the dissolved Cu concentration and the dissolved/total Cu ratio in the Zenne did not change clearly after the waste water treatment plant became operative. This can indicate that metal availability in the river will not alter drastically with waste water treatment. However, based on the scarce data on dissolved metal concentrations in the Zenne, we could not draw clear conclusions on a probable effect of aeration causing a higher dissolved metal fraction and hence a higher availability in the sediments or the water column.

8.5. Conclusions

Aeration of surface water resulted in metal sulfide oxidation in superficial sediments and suspended solids in the experiment. This caused higher dissolved surface water trace metal concentrations. Waste water treatment in the river Zenne resulted in an increase in surface water oxygen concentrations. However, a decrease of total metal surface water concentrations was observed subsequent to waste water treatment. From the results of this research we can conclude that the gradual decrease in metal surface water concentration in the Zenne before the treatment started and the removal of suspended solid bound metals during the waste water treatment process could not be countered by an increase in metal flux from the sediment as observed in the experiment. Additionally, the limited results on dissolved metal concentrations indicated no noticeable change in metal availability following waste water treatment in the Zenne. Sediment metal release due to sulfide oxidation can be too slow to be observed in the field. A low residence time and high dilution can mask observations of metal remobilization in the field.

CHAPTER 9

Synthesis: Trace metal dynamics in the Schelde estuary: a system recovering from severe contamination

Large estuaries are often found to be ‘hotspots’ for pollution. As transitional zones between terrestrial and marine waters they concentrate the runoff from large land surfaces. Additionally, areas surrounding estuaries are often densely populated or provide industry and harbor activities. The Schelde estuary is a typical example where eutrophication, elevated concentrations of contaminants, anaerobic conditions and land reclamation resulted in a highly degraded system (Meire et al., 2005; Van Damme et al., 2005). Elevated metal concentrations were reported in surface water, suspended solids and sediments of the estuary (Baeyens et al., 1997b; Bouezmarni and Wollast, 2005; Regnier and Wollast, 1993; Vandecasteele et al., 2003a; Zwolsman et al., 1993). But, the Schelde estuary is recovering. The load of nutrients and most of toxic substances decreased, while oxygen conditions improved (Cox et al., 2009; Van Damme et al., 2005). However, despite the lower metal concentrations in surface water of the Schelde estuary last decades, tons of toxic metals are stored in the estuarine sediments as a legacy of past contamination (Baeyens et al., 2005). Up to 90% of the trace metal influx is stored within the estuary (Baeyens et al., 1997a; Baeyens et al., 2005; De Smedt et al., 1997). Release or increasing availability during disturbance events or changing environmental conditions are of concern (Eggleton and Thomas, 2004; Kelderman and Osman, 2007). Present work focused on two ‘events’ in the Schelde estuary which are expected to have an effect on flows, mobility and availability of metals in Schelde sediments:

1. Large scale implementation of flood control areas with controlled reduced tide (CRT) (950 ha) and depolderization projects (550 ha) are planned along the Schelde estuary. Is re-introduction of the tide in these areas, which are found to be often metal contaminated, of concern, since changes in total metal concentrations as well as metal mobility and availability can be expected?

2. Under the anoxic conditions, which prevailed between the years 1960 and 2000 in the freshwater stretch of the Schelde estuary (Cox et al., 2009), certain trace metals formed insoluble complexes with sulfides (Di Toro et al., 1990; Morse and Luther Iii, 1999; Van Eck and De Rooij, 1993). We explored if the improving oxygen conditions in the surface water can result in an increase in metal mobility and availability in the estuarine sediments.

9.1. Trace metal concentrations in CRT's.

Estuarine and coastal intertidal zones are rich, diverse and productive systems which deliver a wide range of ecosystem services (Barbier et al., 2011; Costanza et al., 1997). Human impacts, mainly overexploitation and habitat destruction, resulted in degradation of these valuable ecosystems worldwide (Lotze et al., 2006). In many estuaries the need for restoration is acknowledged but space is scarce and restoration is often in conflict with e.g. habitation, agriculture or harbor expansion. The need for flood hazard regulation is often increasing; land reclamation, channel deepening and high dikes, combined with global change induced sea-level rise results in high flood risk of populated regions surrounding estuaries. Managed realignment or controlled flooding of formerly embanked areas have proven to be effective and economically viable strategies to combine flood control and wetland restoration in one and the same area (Beauchard et al., 2011; French, 2006; Jacobs et al., 2009; Zedler and Kercher, 2005). After the large floods in 1976 in the Schelde estuary, the 'Sigmaplan' was initiated and provides now a framework for flood control combined with a higher ecological value. The implementation of CRT's is an important part of this project. In this approach, embanked areas provide storage capacity during storm tides to prevent flooding of the hinterland, while a reduced spring-neap tidal cycle introduced in these areas enables estuarine habitat to develop (Cox et al., 2006; Maris et al., 2007). Yet, land disposal of contaminated dredged sediments or irrigation of embanked areas with contaminated river water resulted in elevated metal concentrations in many polder areas suitable for the realization of CRT's (**Chapter 3**) (Vandecasteele et al., 2003a). Also in the investigated pilot CRT (Lippenbroek) the polder soil was found to contain elevated metal concentrations with Cd concentrations exceeding the *Flemish soil sanitation threshold* up to a factor 5. It was found that the reintroduction of the tide in this pilot CRT resulted in burial of the contaminated polder soil due to the deposition of a uniform, less contaminated sediment layer. Based on differences in tidal characteristics between CRT's and natural tidal areas, e.g. a prolonged stagnant phase at high tide and lower current velocities (Beauchard et al., 2011), differences in soil characteristics and metal concentrations can be expected. However, metal concentrations in deposited sediments were comparable to an adjacent 'natural' tidal marsh (**Chapter 3, 2**). In addition to the significant decrease in surface soil metal concentrations in the CRT, this accumulation of sediment-associated metals was found to contribute to metal removal from the estuary. The implementation of 1500 ha flood control areas and tidal marsh restoration projects planned along the Schelde estuary was calculated to almost double present metal accumulation by overbank sedimentation in tidal marshes, mainly caused by their high accretion rates (**Chapter 2**) (Vandenbruwaene et al., 2011). About 10 kg Cd, 150 kg Pb and 750 kg Zn is deposited yearly in the 8 ha large CRT pilot project.

9.1.1. Trace metal availability in CRT's

Flooding of the contaminated polder soils can significantly change the mobility and availability of both, essential and non-essential metals (Gambrell et al., 1991). Factors controlling metal mobility such as the redox potential, pH and the availability of complexing ligands (both organic and inorganic) and particle surfaces for adsorption are expected to be influenced by tidal flooding (Gambrell, 1994). Moreover, the specific hydrological and geomorphological conditions created in CRT's can result in processes which are different from natural tidal marshes (Beauchard et al., 2011; Spencer and Harvey, 2012).

In a non-flooded polder, adjacent to the pilot CRT, elevated trace metal concentrations were mainly located in the upper 40 cm (Figure 1). Based on the results of a sequential extraction procedure, a low metal availability is suggested (**Chapter 4**). The major part of the metals in the soil of this area was associated with Fe and Mn (hydr)oxides under the aerobic conditions, which resulted in very low metal concentrations in the most mobile, exchangeable fraction (Ex-M). In the CRT, one year of tidal flooding resulted in a shift to higher metal mobility (Ex-M), which was comparable to the mobility in sediments from the natural tidal marsh. Flooding related reducing conditions may have caused the dissolution of the Fe/Mn-hydroxides and associated metals. But redistribution of metals from sulfide precipitates toward the mobile fraction (Ex-M) during sample processing may have resulted in an overestimation of the mobile metal fraction. Yet, a higher mobility was also indicated by increasing Mn concentrations in the upper sediment layers of the restored marsh and can be attributed to flooding related reducing conditions resulting in an upward migration of the reduced $Mn^{(II)}$ ions and precipitation again as Mn-oxides near the oxic surface layers. No diagenetic mobility of other metals was observed during the first three years of tidal flooding (**Chapter 3**). Comparable to the total metal concentrations, also metal mobility in sediments, based on the sequential extractions, was found to be comparable among the CRT and natural marshes.

An increase in metal mobility in the sediments following inundation of an embanked area after managed retreat was found before (Emmerson et al., 2000; Kolditz et al., 2009). As observed by (Charlatchka and Cambier, 2000) also in the CRT, inundation is expected to result in an initial increase in metal mobility caused by the dissolution of Mn and Fe oxides after which mobility will decrease again due to (co)precipitation with (Fe)sulfides when the Eh reduces further (Du Laing et al., 2009c). Also in a natural freshwater marsh, frequent inundation was found to result in lower metal availability compared to a higher site in the same marsh (**Chapter 6**). The formation of insoluble metal sulfides is found to play a major role in reducing sediment metal availability and transfer to the surface water under anoxic conditions (Di Toro et al., 1992; Lee et al., 2000c) (**Chapter 7**). The reducing conditions in the newly deposited sediments of the CRT resulted in acid volatile sulfide

(AVS) concentrations which were found to be high enough to complex the pool of Cd, Cr, Cu, Ni, Pb and Zn (simultaneously extracted metals, SEM) (**Chapter 4**). The prolonged inundation period and the low drainage capacity of the compacted polder soil, which characterizes the CRT, created conditions with high sulfide concentrations which may, on the longer term, reduce the availability of 'SEM metals', more than in the natural marsh.

The physical-chemical processes which occur following re-introduction of the tide are complex (Bryan and Langston, 1992). Different conditions prevailing in other sites or estuaries can result in a different metal behavior (French, 2006). In Essex, a managed retreat site in the UK, changes in metal mobility were found to be slow and mainly situated near the surface and in newly deposited sediments (Emmerson et al., 2000; Macleod et al., 1999), while differences in metal fractions between the polder and CRT sediments in the present study were observed up to a depth of 1 m after one year. An immediate and large drop in pH (from 7 to 3), but only lasting for several weeks, following breaching (Blackwell et al., 2004) could result in a temporarily increase in metal mobility. Also restoration in salt water regions can result in different processes which may affect metal mobility (Du Laing et al., 2009c; Gambrell et al., 1991). In the Schelde estuary, marsh restoration in higher salinity zones is planned and differences along the estuarine gradient can be expected. First, processes in the surface water (e.g., mixing, flocculation, desorption) can change metal concentrations associated with SPM. In the Schelde estuary a decrease in surface water metal concentrations with increasing salinity is observed and mainly due to dilution of contaminated riverine particles with less contaminated marine particles (Regnier and Wollast, 1993), which results in lower metal concentrations in deposited sediments (**Chapter 2**). Secondly, the intrusion of salt water in metal contaminated soils will affect metal mobility. Competition for binding sites between the salt water cations (Na^+, K^+, Ca^{2+}, Mg^{2+}) as well as complexation with anions (Cl^-, SO_4^{2-}) can result in a higher metal mobility, mainly Cd, and release to the surface water (Du Laing et al., 2008b; Speelmans et al., 2007). Managed retread and flooding with salt water in the Essex site resulted in adsorption of Pb, Cr and Cu, while Cd was released to the surface water (Macleod et al., 1999). A general increase in the mobile metal fraction following inundation with salt water was observed in lab experiments with sediments of the Essex site, but this did not result in significant leaching of metals to the environment (Emmerson et al., 2001).

9.1.2. Trace metal bioaccumulation in CRT's

When tidal marshes are being restored, plant and animal species will usually colonize the site. Transfer of trace metals from the contaminated polder soils to aboveground plant tissues can result in food chain contamination. Additionally, plants change the mobility of metals in wetland sediments (Weis and Weis, 2004). Whether plants mobilize or immobilize metals in the sediment depends on a large combination of factors and is difficult to predict. However, the advantages of vegetation often appear to outweigh the possible effects of small scale remobilization in the rhizosphere (Jacob and Otte, 2003).

The investigated CRT was rapidly colonized with typical wetland plant species (Jacobs et al., 2009). The higher metal concentrations (As, Fe, Mn and Pb) in plant tissues from the restored marsh compared to the polder area may indicate a higher phyto-availability following inundation (**Chapter 4**). Yet, since different plant species in the different areas, it is difficult to distinguish between species specific uptake mechanisms and soil availability. In general, transfer coefficients (TC) between sediments and the aboveground tissues of the most abundant plant species were found to be lower than general values reported by Prasad et al. (2006) (**Chapter 5**). Mainly tall emergent macrophytes which colonized the CRT (reed (*Phragmites australis*), cattail (*Typha latifolia*), reed mannagrass (*Glyceria maxima*) and cosmopolitan bulrush (*Scirpus maritimus*)) were found to transfer low amounts of trace metals to aboveground tissues. This resulted in a very small fraction (≤0.05%) of the top 30 cm sediment metal pool that cycled annually through the aboveground vegetation. These wetland plants often sequester trace metals within their extensive root system. Wetland plants rooting in anoxic sediments often maintain aerobic root respiration via internal oxygen transport through aerenchyma (Vartapetian and Jackson, 1997). An excess of oxygen in the roots can leak out (known as radial oxygen loss, ROL) resulting in an oxidized rhizosphere (Armstrong and Armstrong, 1988; Wright and Otte, 1999). Metals are mobilized from the reduced sediments and often precipitate with Fe and Mn (oxy)hydroxides on the root surface ('root plaque') which results in high metal concentrations associated with the plant roots (**Chapter 6**).

As in many other studies higher TC's are found for Willow species (*Salix* sp.), mainly for Cd and Zn (Pulford and Watson, 2003b; Vandecasteele et al., 2002b; Vandecasteele et al., 2010) (**Chapter 5**). *Salix*-dominated tidal forest is the climax succession stage in natural tidal marshes of the freshwater part of the Schelde estuary, and might also become predominant in the CRT (Jacobs et al., 2009). Succession to *Salix* sp. forest will result in a stock of trace metals stored in the woody structures of the trees which is roughly 50 times higher than in the present early successional stage. This may result in higher bioaccumulation of present metals via consumption of living plant tissues as well as litter with elevated metal concentrations. Leaf beetles showed elevated body Cd concentrations when foraging on poplar trees growing on contaminated sediment derived soils in the same estuary (Schelde) (Vandecasteele et al., 2003b). Also other studies demonstrate that mainly Cd may result in elevated body concentrations, e.g. in gastropods (*Cepaea nemoralis*) (Jordaens et al., 2006) or small mammals (Mertens et al., 2001). In the study of Mertens et al. (2001), Cd soil concentrations comparable to this study (5.26 µg g^{-1}) resulted in elevated liver concentrations of shrews (>60 µg g^{-1}), yet model calculations predicted no food chain accumulation and a low risk for predators. Also metal accumulation by benthic invertebrates, which live in close relationship with the sediments, can be of concern (Van Vliet et al., 2006; Vijver et al., 2007).

Re-submergence of contaminated dredged sediment-derived soils was found to reduce Cd uptake in willow trees, mainly due to the formation of insoluble metal sulfides (Vandecasteele et al., 2010). Also in our study high AVS values in newly deposited sediments in the CRT may reduce metal bioavailability on the longer term (**Chapter 4**). However, lower availability in the sediment did not result in lower concentrations in earthworms in the same study of (Vandecasteele et al., 2010). Species specific exposure routes, mainly ingestion of contaminated sediments, were found be a more important factor in determining metal accumulation in benthic invertebrate species than metal availability in the bulk sediments (Bervoets et al., 1997; De Jonge et al., 2011). Also processes at the root-sediment interface (e.g. ROL) and their effect on metal mobility (e.g. AVS oxidation) can cause high metal availability for plants when [SEM-AVS] > 0 is measured in the bulk sediment (**Chapter 6, 7**). However, Speelmans et al. (2007) found lower metal accumulation in the oligochaete worm *Tubifex tubifex* under permanently flooded conditions while oxidation was associated with a rapid increase of metal accumulation. In the same study, metal accumulation was higher when inundation with brackish water occurred, compared to freshwater conditions (Speelmans et al., 2007). As for metal concentrations and availability, differences in metal uptake can vary along the estuarine gradient. In higher salinity zones, processes in the soil or the occurrence of other plant (in the Schelde mainly *Phragmites australis*, *Scirpus maritimus* and *Spartina* sp. (cordgrass)) or animal species can result in different uptake patterns. Metal concentrations in *Spartina* sp., a helophyte which is dominant in higher salinity zones, were found to be slightly higher than concentrations in *P. australis* from the same locations (Burke et al., 2000; Weis et al., 2003; Weis et al., 2002).

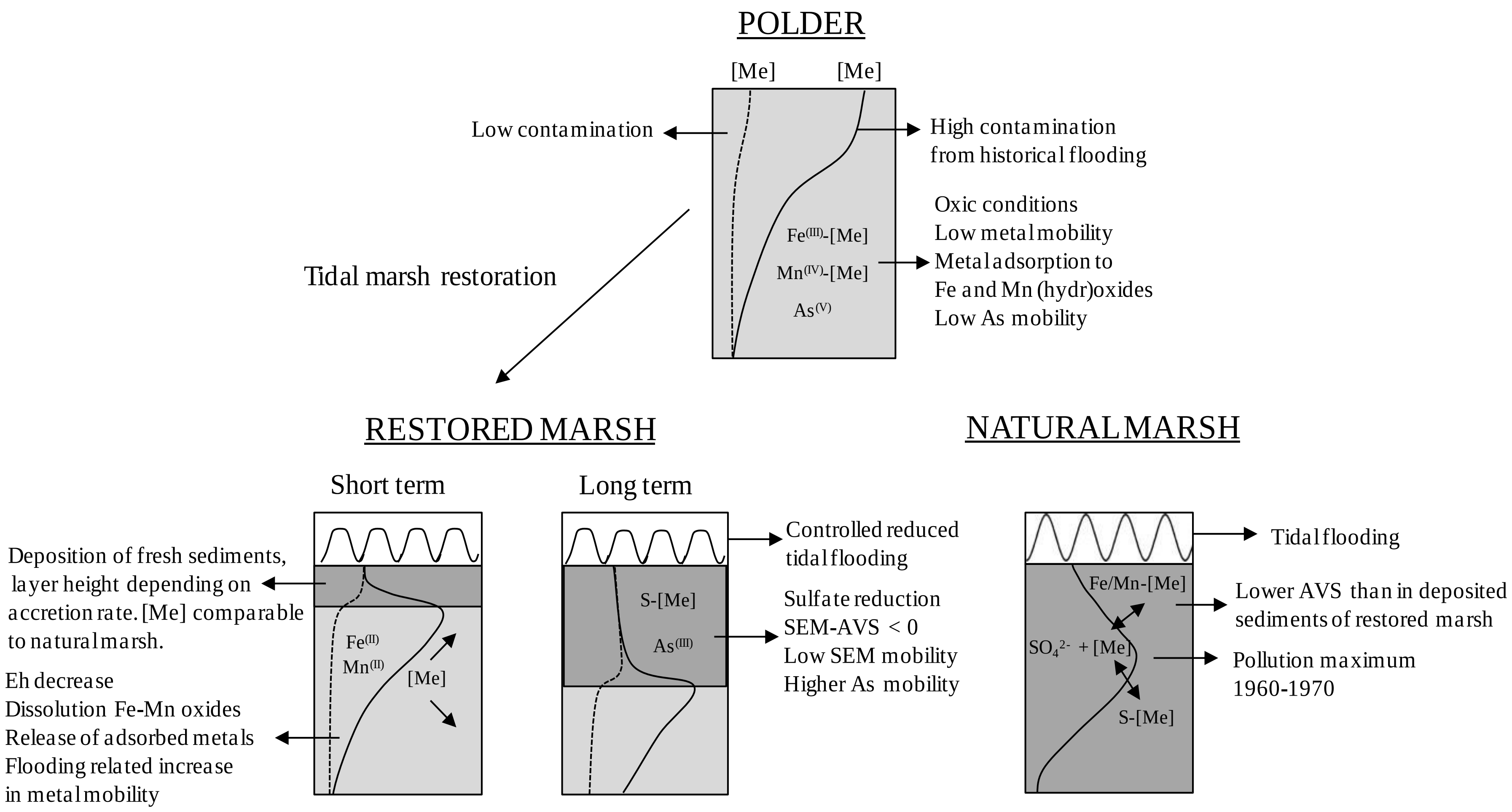


Figure 1. Conceptual graph of the main metal related processes which occurred in the soil and sediment of a polder area, restored marsh and natural tidal marsh in present study. Lines in the figures indicate metal concentrations ([Me]) in the soil from the surface up to a depth of 1 meter.

9.1.3. Implications for CRT management and design.

Trace metal contamination in embanked areas was found to be determined to a large extend by the inundation history of the area (**Chapter 3**). Marshes which were embanked before the Schelde became severely contaminated and where no winter irrigation with contaminated river water occurred did not show elevated metal concentrations in the soil. However, winter irrigation or disposal of dredged sediments resulted in elevated metal concentrations in other embanked areas. Conditions which were found to minimize metal concentrations, mobility, availability and phyto-accumulation were mainly found in the low elevation sites; more frequent inundation resulted in faster accretion and burial of the contaminated polder soil, reducing conditions with high AVS concentrations and the growth of tall macrophytes with low metal accumulation. Despite the temporal increase in metal mobility which can be expected, high flooding frequency in the entire CRT might reduce metal mobility and availability on the longer term (Charlatchka and Cambier, 2000; Gambrell et al., 1991; Gambrell, 1994; Speelmans et al., 2007; Vandecasteele et al., 2010) (**Chapter 3, 4, 5, 6**). A high flooding frequency in the first period after re-inundation after which inlet sluice dynamics are adjusted to obtain a lower flooding frequency in the longer term may be a appropriate management option.

However, the physical-chemical processes which occur following re-introduction of the tide are complex (Bryan and Langston, 1992). Bioaccumulation and toxicity towards biota stays hard to predict (De Jonge et al., 2012b) (**Chapter 7**). Conclusions are not straightforward and caution is recommended. Creating reducing conditions might reduce the mobility of the a subset of metals (Cd, Cr, Cu, Ni, Pb, Zn) (Berry et al., 2004; Lee et al., 2000c) while a higher mobility of e.g. the metalloid As can be expected (Sharma and Sohn, 2009). Removing the metals by digging out the contaminated layer may be worth to consider. Additionally the removal of remnant terrestrial vegetation prior to re-inundation will favor the establishment of typical wetland vegetation (Jacobs et al., 2009). If legislation allows for reuse of the soil, incorporation into the dikes which needs to be constructed around the CRT can be an easy and cheap option to store the contaminated soil. Yet, precautionary measures which might prevent leakage, e.g., capping the soil with clean sediments or asphalt as used in overflow dikes of CRT’s, should be taken (Simpson et al., 2002).

9.2. The effect of oxygen on metal mobility

A large part of the historical trace metal influx is stored within the estuary (Baeyens et al., 1997a; Baeyens et al., 2005; De Smedt et al., 1997). It was found that overbank sedimentation in tidal marshes contributes considerably (about 30%) to this process even though marshes comprise less than

10% of the total surface of the estuary (**Chapter 2**). This estuarine filter function reduces marine contamination, but on the other hand also results in elevated metal concentrations in estuarine sediments. Additionally, the planned large scale implementation of CRT's (950 ha) and depolderization projects (550 ha) will result in a large extra contact surface between contaminated soils and surface water. Between the years 1960 and 2000, the Schelde estuary was characterized by a persistent hypoxia (Cox et al., 2009). Under these reduced conditions trace metals in sediments as well as in SPM were found to be mainly associated with sulfides (Van Eck and De Rooij, 1993). Already in 1993, Van Eck and De Rooij simulated that a future reduction in organic load would result in higher surface water oxygen concentrations and a subsequent increase in dissolved metal concentrations due to sulfide oxidation. Experimental data confirm that this '*chemical time bomb*' may occur, but evidence on alarming effects at larger scale (e.g. estuary) are lacking. Experiments in this manuscript (**Chapter 7, 8**) indicate that high surface water oxygen concentrations result in dissolution of metal sulfide complexes, with a gradual increase in labile metal concentrations within the sediment, a release of metals to the surface water and a higher metal toxicity towards the aquatic organism *Daphnia magna* (De Jonge et al., 2012a; De Jonge et al., 2012b). However, the abrupt increase in oxygen in the surface water of the polluted river Zenne, following the realization of a large waste water treatment plant in Brussels, did not result in total higher surface water metal concentrations in situ (**Chapter 8**).

In the freshwater stretch of the Schelde estuary, annual averaged oxygen concentrations increased from < 2 mg L^{-1} before the year 2000 to almost 8 mg L^{-1} after 2000 (Fig. 2). As expected, based on the results of the experiments, an increase in dissolved Zn concentrations could be observed in this zone (Fig. 3). This may be the result of the combined effect of a decrease in the Zn distribution coefficient K_D (ratio of particulate versus dissolved Zn) and a higher release of sediment bound metals. Also (Gao et al., 2012) reported K_D values for Cd, Cu, Pb and Zn in the Schelde estuary which were 2 to 5 times lower in 2010 compared to the nineties. Additionally, the total metal concentrations in the surface water (Fig. 4) and surface sediments of a tidal marsh (Fig. 5) are found to increase. This may confirm that higher surface water oxygen concentrations result in the dissolution of metal sulfides in the marsh sediments with a higher mobility, upward migration, and precipitation with Fe and Mn hydr(oxides) near the water-sediment interface or release to the surface water as a consequence. This also emphasizes the importance of oxygen in the water on marsh sediments, even though the fact that these sediments are exposed to the air for about 85% of the time (Beauchard et al., 2011). In CRT's surface water oxygen concentrations are expected to be higher than in the water of the adjacent estuary (Maris et al., 2007). Yet, higher AVS and lower redox potential values in the sediments deposited in the CRT compared to the 'natural marsh' indicate that the reducing conditions of the specific hydrological regime might counteract the oxidizing effect of the oxidized surface water (**Chapter 4**).

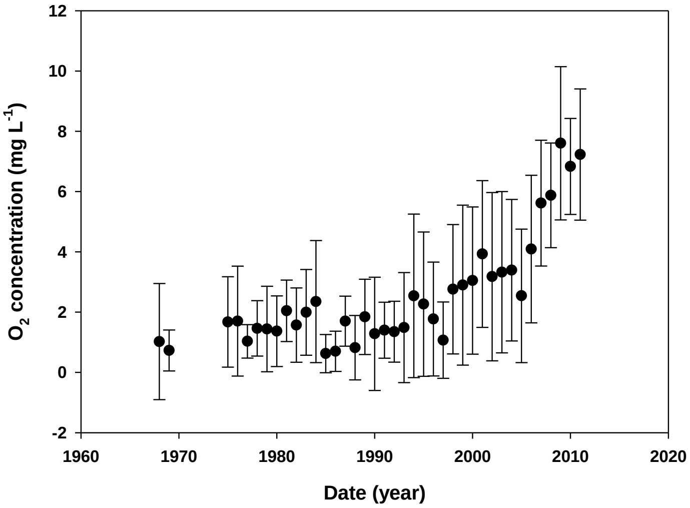


Figure 2. Yearly averaged oxygen concentrations with standard deviation for the freshwater stretch of the Schelde estuary (average from km 160 (Ghent) to km 85 from the mouth).

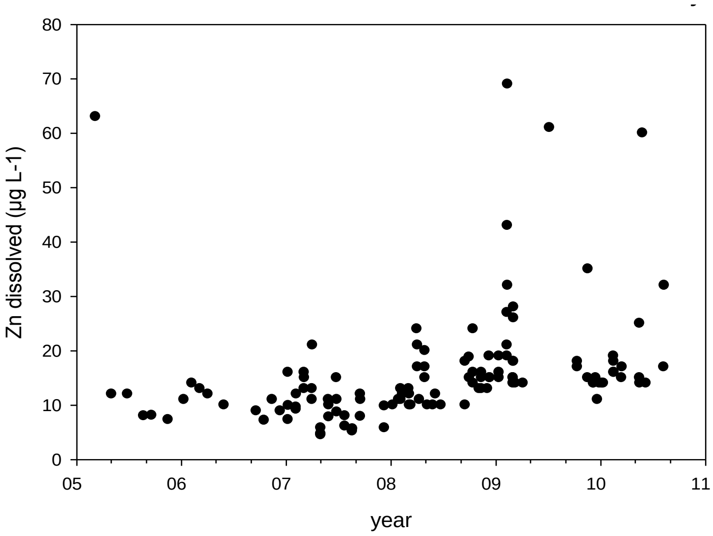


Figure 3. Dissolved Zn concentrations for the freshwater stretch of the Schelde estuary, different stations between km 160 (Ghent) to km 85 from the mouth.

The increasing total Zn concentrations in the Schelde surface water during the last 10 years was only observed around Temse (km 95). As it was observed in the river Zenne, also in the Schelde a large scale release of metals with higher values in the surface water is not expected. Dissolved Zn concentrations are increasing but are still below the quality standard. Other binding fractions as organic matter, carbonates or Fe and Mn hydroxides near the sediment water interface may prevent this flux (Zhuang et al., 1994). However, changes in K_D or metal availability in superficial sediment layers as an effect of the improving oxygen conditions may have implications on the toxicity of the contaminated sediments in the Schelde estuary.

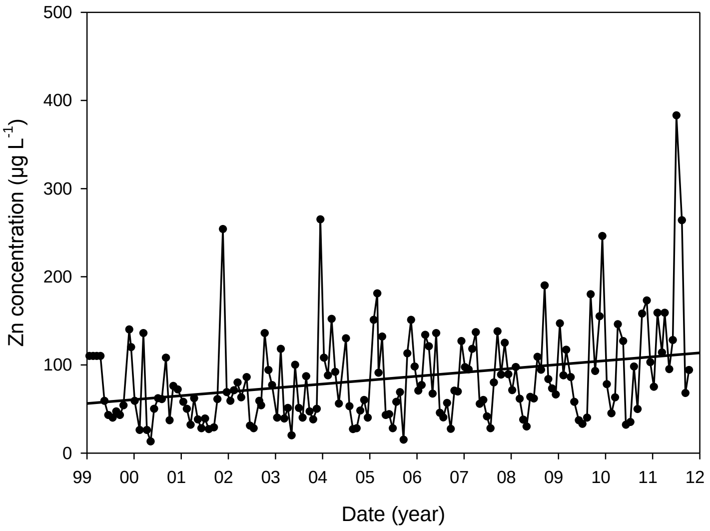


Figure 4. Total Zn concentrations in the surface water at Temse (freshwater, 95 km from the mouth).

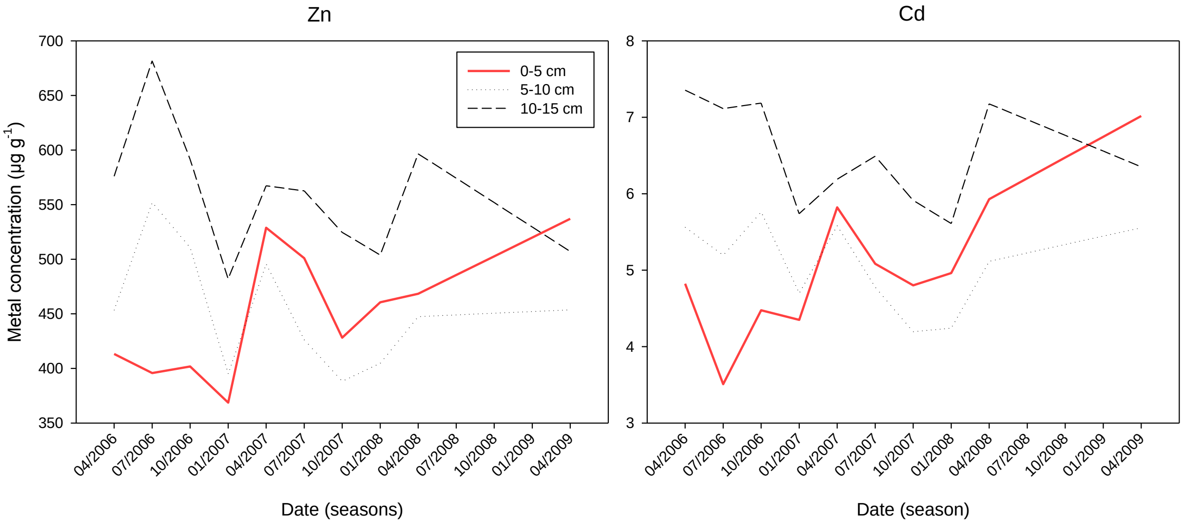


Figure 5. Zn and Cd concentrations in sediments of the upper 15 cm of a freshwater tidal marsh (marsh near CRT Lippenbroek, km 103).

CHAPTER 10

Nederlandse samenvatting

Vele estuaria worden gekenmerkt door een sterke verontreiniging. Als overgangsgebied tussen land en zee worden contaminanten van het gehele stroomgebied geconcentreerd in een relatief kleine zone. Daarbij zijn de omliggende gebieden vaak dicht bevolkt, meestal in combinatie met industriële- en haven-activiteiten. Het Schelde-estuarium vormt een typisch voorbeeld van een systeem waar eutrofiëring, hoge concentraties aan polluenten, quasi zuurstofloze omstandigheden en inpolderingen resulteerden in een sterk gedegradeerd habitat. Metaalverwerkende industrie in Noord Frankrijk, rond Gent en Antwerpen en rechtstreekse lozing van afvalwater van grote steden (Lille, Antwerpen, Brussel) hebben ervoor gezorgd dat het oppervlaktewater en de sedimenten van de Schelde sterk werden verontreinigd met metalen. Het estuarium is zich echter aan het herstellen. De ladingen aan nutriënten en polluenten zoals metalen, PCB’s of PAK’s nemen geleidelijk aan af en zuurstof concentraties nemen toe. Door de sterke affiniteit van metalen voor sedimentpartikels, accumuleerde een groot deel van de enorme historische metaalvervuiling desondanks in de estuarine sedimenten van bodem, slikken en schorren. Door natuurlijke (stormen, plantengroei, bioturbatie) of antropogene (baggeren, ontpolderen) verstoring van gecontamineerde sedimenten kan de mobiliteit, biobeschikbaarheid of toxiciteit van de metalen veranderen. Dit werk heeft als doel om na te gaan of (1) de geplande ontpolderingen in het Schelde-estuarium of (2) de verhoogde zuurstofconcentraties in het oppervlaktewater, een verandering in de risico’s van metalen aanwezig in de sedimenten teweegbrengen.

10.1. Ontpoldering in een metaalgecontamineerd estuarium

Estuaria zijn economisch, maar ook ecologisch erg waardevolle gebieden. Wereldwijd zijn grote oppervlakte estuarien habitat verloren gegaan en staan de resterende gebieden vaak onder sterke druk. Hierbij zijn een belangrijk deel van de biodiversiteit en vele bijhorende ecosysteemfuncties verloren gegaan. Het verlies aan oppervlakte, verdieping van de vaargeul en optrekken van dijken zorgde ook voor een toename van de hoogwaterstanden. In combinatie met een (toekomstige) stijging van de zeespiegel is het overstromingsgevaar in het Schelde-estuarium sterk toegenomen. De nood aan herstel wordt erkend, maar ruimte is vaak schaars. Net als in vele andere estuaria wordt natuurherstel en bescherming tegen overstroming aan elkaar gekoppeld. In het Schelde-estuarium voorziet het Sigmaplan in de aanleg van gecontroleerde overstromingsgebieden; ingepolderde gebieden dienen voor waterberging bij stormtij waarbij herstel van estuarine natuur in deze gebieden kan plaatsvinden door middel van een aangepast sluizensysteem dat zorgt voor een periodieke overstroming, vergelijkbaar met natuurlijke slikken en schorren (gecontroleerd overstromingsgebied met gecontroleerd gereduceerd getij, GGG). Onderzoek voor dit werk werd uitgevoerd in het GGG pilootproject (Lippenbroek).

Het GGG en aangrenzende poldergebieden bleken echter sterk vervuild met metalen (vooral cadmium). Door bevloeiing van akkers met vervuild Scheldewater en het bergen van vervuilde baggerspecie de voorbije decennia werd de bovenste laag van veel polderbodems gecontamineerd. Metaalconcentraties in het oppervlaktewater van de Schelde namen sterk toe tussen 1920 en 1970, waarna de gehalten weer daalden. Door het continue afzetten van sedimenten op de natuurlijke schorren (ongeveer 1-2 cm per jaar) bevindt de laag met hoge metaalconcentraties zich meestal op grotere diepte (50 cm) in deze gebieden. Ook in het GGG Lippenbroek zorgde sedimentatie bij overstroming in het gebied er voor dat de gecontamineerde polderbodem werd bedekt met sedimenten met lagere gehalten aan metalen, vergelijkbaar met deze van de oppervlaktelagen in de natuurlijke schorren. Afzetten van sedimenten en geassocieerde metalen in GGG’s zorgt daarbij voor een verhoogde filterende functie van het estuarium. Verschillende factoren wijzen er echter op dat overstroming van de polderbodem een verhoging van de metaalmobiliteit, en dus ook beschikbaarheid teweeg brengt. Onder oxische omstandigheden in de polderbodem zijn veel metalen gebonden aan mangaan- en ijzer-oxiden. Overstroming in het GGG resulteerde in reductie van de oxiden en vrijkomen van ijzer, mangaan en geassocieerde metalen. Ondanks een stijging in metaalbeschikbaarheid is er echter weinig accumulatie in de bovengrondse delen van de planten die het GGG koloniseerden. Grote helofyten (riet, lisdodde, groot liesgras, zeebies) die een groot aandeel uitmaakten van de totale plantenbiomassa namen zeer weinig metalen op uit de bodem. De hoogste concentratie aan cadmium en zink werd gemeten in wilgen. In de nieuw afgezette sedimenten worden zuurstofloze omstandigheden gecreëerd waarbij sulfaten worden gereduceerd tot sulfiden. Sulfiden vormen moeilijk oplosbare complexen met metalen zodat wordt verwacht dat de mobiliteit op langere termijn terug lager zal zijn. Ook in een nabijgelegen zoetwaterschor zorgde een hogere overstromingsfrequentie voor een lagere biobeschikbaarheid van metalen in de bodem. Op basis van dit onderzoek lijkt de implementatie van een GGG in een metaalvervuilde polder geen onrustwekkende gevolgen te hebben voor metaaltoxiciteit. Een hoge overstromingsfrequentie is preferentieel vermits dit resulteert in hoge sedimentatiesnelheden, omstandigheden waardbij metalen complexeren met sulfiden en de groei van planten die weinig metalen opnemen. De processen zijn echter complex en voorzichtigheid is geboden. Omstandigheden kunnen verschillen langsheen de estuarine gradiënt of in andere estuaria wat kan resulteren in een verschil in metaal mobilitiet of biobeschikbaarheid.

10.2. Invloed van zuurstof op metaalmobiliteit

Van 1960 tot 2000 werd de zoetwatergetijdenzone van de Schelde gekarakteriseerd door anoxische condities. Onder deze omstandigheden kwamen metalen in sedimenten en gesuspendeerde partikels vooral voor in complexen met sulfiden, waardoor hun mobiliteit en biobeschikbaarheid laag is. Door een afname van de organische belasting, vooral door de inwerkingstelling van waterzuiveringsinstallaties, nam de zuurstofconcentratie in het oppervlaktewater de laatste 10 jaar strek toe. In experimenten kwamen we tot de vaststelling dat een hogere zuurstofconcentratie in het oppervlaktewater kan leiden tot een hogere metaalmobiliteit in vervuilde Zenne sedimenten, met migratie naar het oppervlaktewater als gevolg. In de Zenne zelf echter, waar de waterzuivering van Brussel vanaf 2006 zorgde voor een sterke toename van zuurstof in het water, werd dit fenomeen niet waargenomen. Vrijkomen van metalen kan een langzaam proces zijn en wordt eventueel gemaskeerd door het waterzuiveringsproces zelf, waarbij ook metalen uit het water worden verwijderd. In het Schelde-estuarium zijn er wel enkele aanwijzingen die wijzen op een toename in metaalmobiliteit in historisch vervuilde sedimenten: concentraties aan metalen in oppervlaktelagen van schorren en opgeloste metaalgehalte in het oppervlaktewater nemen toe de laatste jaren, terwijl de dalende trend in emissies wordt voortgezet. Concentraties zijn voorlopig nog niet alarmerend, maar verder onderzoek naar mogelijke veranderingen in toxiciteit of mobiliteit zijn aangewezen.

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