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

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HIGHLIGHTS
► Metal mobility was investigated in a tidal marsh restoration project.
► Sequential extractions, SEM–AVS and metal concentrations in plants were studied.
► Results suggest a short term increase in metal mobility due to inundation.
► Metal–sulfide precipitation is expected to reduce mobility on the longer term.

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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 the reduction of Fe and Mn oxides and the subsequent release of associated metals. However, the differences in 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 different conditions prevailing in other sites or estuaries can result in different behavior of the trace metals. More in situ research is needed to get a better insight in the risks involved.

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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, many areas suitable for estuarine marsh restoration are contaminated with trace metals (Emmerson et al., 2000; Teuchies et al., 2012a). Estuaries often act as a sink for trace metals, which accumulate in their sediments. Embanked areas might be contaminated by deposition of contaminated suspended solids before embankment, land disposal of contaminated dredged sediments, or irrigation of with contaminated river water (Vandecasteele et al., 2003).

Inundation of contaminated land in order to restore wetlands will be applied increasingly; yet, processes in these sites, which are often largely changed by their previous land use, 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. Tidal flooding can result in deposition of suspended solids, erosion of upper soil layers or diagenetic mobility of metals in the soil, which might change the total metal concentrations in the upper soil layers of the restored...
sites (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 chemical forms in which they occur (Speelman et al., 2010). Factors controlling metal mobility such 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, the land use change itself might impact on the transfer of metals into the food chain. Cessing agriculture activities on the contaminated soil of the pre-breached sites will directly reduce human exposure, while establishment of a freshwater tidal marsh with wetland 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 effects on metal availability are 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., 2000).

The objective of present study is to evaluate changes in metal mobility and availability when tidal marsh habitat is being restored in a contaminated embanked site. The results of this study are useful to develop appropriate management options or plant future tidal marsh restoration projects. In the present study, the potential metal bioavailability is based on 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.

2. Materials and methods

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, 2007b; Vandecasteele et al., 2003). 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 1960s and 70s 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). 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)).

2.2. Sampling

In June 2007, one year after restoration of the marsh, 1 m deep soil 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. Soil from four cores were mixed per depth.

Fig. 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).
resulting in two replicates. The soil was 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 soil 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.; and (site W): Impatiens glandulifera, Salix sp., U. dioica. Three individual plants or three large branches of different individuals for the trees, as well as their roots, which were carefully excavated, of each species were sampled randomly at every site as close as possible to the soil core location, thoroughly rinsed with river water in the field as well as with demineralized water in the laboratory. All leaves, roots and rhizomes were removed manually or with stainless-steel scissors from the stem, dried for 48 h at 70 °C, weighed and ground. Roots and rhizomes were processed together.

2.3. Laboratory analyses

2.3.1. Soil characteristics

Nutrient concentrations were measured colorimetrically using a SAN+™ Flow Analyzer (Skalar, FAS, SA 20/40, Skalar Analytical B.V., Breda, The Netherlands) after extractions of wet soil with potassium chloride (KCl, 1 M; 1:2.5, w/v) for ammonium (NH₄⁺), nitrite and nitrate (NO₂⁻ + NO₃⁻) and with ammonium acetate EDTA (ethylenediaminetetraacetic acid) (1 M; 1:5, w/v) for phosphate (PO₄³⁻). Soil pHₕ₂ₒ was measured in the supernatant solution from a soil extraction with distilled water (1:5, w/v) after 24 h.

A subsample of the air dried soil was weighed, oven dried (105 °C) to a constant weight and then reweighed to estimate the moisture content (MC). Oven dried samples were then 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 Pt 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).

2.3.2. Soil sequential extraction and metal concentrations

Air dried soil (both replications, n=1) was 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 min 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 was used for determination of total metal concentration through hot block digestion method for which 5 mL nitric acid (HNO₃, 69% Normapur, VWR) and 5 mL of hydrogen peroxide (H₂O₂, 27% for electrical purpose, VWR) were added to the sample (Curran and Avrett, 2004). 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 (Fig. S1):

\[
\% \text{Recovery} = \frac{\sum (\text{metal extracted through SEP})}{\text{Total metal (digested)}} \times 100\%.
\]

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 soil was stirred for 2 h with hydrochloric acid (HCl, 37% Normapur, VWR) under constant N₂ flow. The S⁻ ions trapped in a NaOH solution were measured photospectrometrically (UV-160, Shimadzu, Kyoto, Japan). In the soil-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].

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 HNO₃ (65%, Suprapur®, Merck) and H₂O₂ (30%, Suprapur®, Merck) (20:1, v/v).

Plant and soil (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® no 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 soil, 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.

2.3.5. Statistical analysis

Normality of the data was tested with the Shapiro–Wilk test prior to analysis. The significance of differences in soil 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.

3. Results

3.1. Site characteristics

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) lower PO₄³⁻ and NH₄⁺ concentrations while the driest site (P) had significantly (p<0.05) higher NO₃⁻ and NO₂⁻ concentrations. OM and clay contents 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.
3.2. Metal fractionation

The exchangeable fraction (Ex-M) was highest for Cd, Mn and Zn (up to 30, 45 and 40% of the summed fractions respectively) and low for As, Cr, Cu and Pb (<15, 1.8 and 5% respectively) (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 (on average 66 and 56% respectively). The oxidizable fraction (Ox-M) was mainly high in the restored sites (60% on average for Cu) and low in the polder site (18% on average for Cu), 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.

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 \( \mu \text{mol g}^{-1} \). In the restored marsh, negative [SEM–AVS] values were only measured in the upper layer of the low elevation site (L).

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 sites L and M were not consistent among different metals. For most metal concentrations were higher in site L (significant for Ni, Pb). No significant differences in U. dioica metal concentrations between sites W and H were found.

4. Discussion

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; Teuchies et al., 2012a; Vandecasteele et al., 2003).

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 sequential extractions, the availability of most metals is lower in the polder than in the other investigated sites (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 lower metal concentrations in the most mobile, exchangeable fraction (Ex-M). Yet, due to the high total metal concentrations in the polder soil, this available fraction is, for e.g. Cd, higher than in sediments from several other field sites (Diaz-de Alba et al., 2011; Emmerson et al., 2000; Passos et al., 2010). The study of 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). The results of the sequential extraction might indicate that these conditions have caused the dissolution of the Fe/Mn-hydroxides and associated metals which caused a shift to higher metal mobility (Ex-M), which was

![Fig. 2. Metal concentrations (mg kg$^{-1}$) 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).](image-url)
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 might also be 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 soil following inundation of an embanked area after managed retreat has been observed before (Emmerson et al., 2000; Kolditz et al., 2009). Physicochemical processes which occur following re-introduction of the tide are complex and it is obvious that different conditions or soil characteristics prevailing in other sites or estuaries can result in a different metal behavior (Bryan and Langston, 1992; Du Laing et al., 2009b; 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). In the present study, we might 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. Flooding may as well result in dissolution of calcium carbonates which might reduce the buffer capacity and pH and hence increase metal mobility on the longer term (Du Laing et al., 2007b). The experimental site of present study is located in the freshwater stretch of the estuary, whereas many managed realignment sites are situated in brackish or marine zones. Intrusion of brackish or salt water can cause a higher metal mobility and extractability (Du Laing et al., 2008a; 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 Voutsas, 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., 2009b).

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, 2003; Odum, 2000; van der Geest and León Paumen, 2008; Vandecasteele et al., 2005). 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; Teuchies et al., 2011; van der Geest and León 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).

![Fig. 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).](image-url)

**Table 2**

Average metal concentrations (mg kg⁻¹) in plant tissues for three different species occurring in two different sites, standard deviation in brackets (n=9). Significant differences given in bold (p<0.05).

<table>
<thead>
<tr>
<th>Site</th>
<th>As (mg kg⁻¹)</th>
<th>Cd (mg kg⁻¹)</th>
<th>Cr (mg kg⁻¹)</th>
<th>Cu (mg kg⁻¹)</th>
<th>Fe (mg kg⁻¹)</th>
<th>Mn (mg kg⁻¹)</th>
<th>Ni (mg kg⁻¹)</th>
<th>Pb (mg kg⁻¹)</th>
<th>Zn (mg kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salix</em> sp.</td>
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</tr>
<tr>
<td>L</td>
<td>2.00 (2.09)</td>
<td>5.12 (4.44)</td>
<td><strong>5.66 (2.29)</strong></td>
<td>19.4 (23.3)</td>
<td>896 (1062)</td>
<td>112 (109)</td>
<td>3.32 (5.35)</td>
<td>3.01 (2.03)</td>
<td>229 (152)</td>
</tr>
<tr>
<td>M</td>
<td>0.73 (0.62)</td>
<td>4.82 (1.42)</td>
<td><strong>3.74 (1.42)</strong></td>
<td>16.0 (14.3)</td>
<td>480 (338)</td>
<td>55.2 (30.4)</td>
<td>1.86 (1.09)</td>
<td>2.31 (1.55)</td>
<td>260 (107)</td>
</tr>
<tr>
<td>W</td>
<td>6.25 (9.11)</td>
<td>0.812 (0.891)</td>
<td><strong>5.96 (2.42)</strong></td>
<td>9.04 (5.78)</td>
<td>9.16 × 10³</td>
<td>342 (324)</td>
<td><strong>2.93 (2.03)</strong></td>
<td><strong>8.66 (7.68)</strong></td>
<td>79.9 (57.0)</td>
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<tr>
<td><em>L. salicaria</em></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>3.25 (5.55)</td>
<td>1.49 (2.39)</td>
<td>4.84 (1.58)</td>
<td>11.1 (7.6)</td>
<td>2.45 × 10³</td>
<td><strong>3.13 × 10³</strong></td>
<td>210 (264)</td>
<td><strong>1.69 (1.06)</strong></td>
<td><strong>4.49 (3.35)</strong></td>
</tr>
<tr>
<td>M</td>
<td>1.00 (0.73)</td>
<td>0.35 (0.53)</td>
<td>4.51 (2.10)</td>
<td>13.6 (7.6)</td>
<td>919 (905)</td>
<td>48.8 (35.8)</td>
<td>1.80 (1.16)</td>
<td>2.78 (2.39)</td>
<td>42.0 (18.6)</td>
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<tr>
<td>W</td>
<td>8.61 (0.56)</td>
<td>0.61 (1.17)</td>
<td>4.94 (3.18)</td>
<td>9.52 (3.10)</td>
<td>475 (321)</td>
<td>35.6 (28.2)</td>
<td>1.30 (0.81)</td>
<td>2.37 (1.82)</td>
<td>37.5 (28.0)</td>
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</tbody>
</table>
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 might 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., 2009b; Speelmans et al., 2007). It should be noted that results of present study were based on samples taken at one moment, whereas temporal changes in metal mobility can be expected. Tidal flooding, spring-neap tidal cycles, seasonality, or long term changes may result in variation in sulfide concentrations, organic matter, CaCO₃, pH or redox potential and hence metal concentrations in the pore water (Du Laing et al., 2007b, 2008b).

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 tidal flooding related increase in metal mobility which associated metals are released and extracted with the first, most mobile metal fraction (Ex-M). This may emphasize that pretreatment of samples prior to sequential extractions can largely modify their distribution and that the obtained results should be treated with caution.

### 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 might indeed be expected in the restored marsh. Also the specific redox chemistry of As implies a higher mobility when, under reducing conditions, As(III) is reduced and the more mobile and toxic As(V) 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, 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., 2005, 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., 2009b). 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 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 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., 2000) 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, 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, 2012b; Teuchies et al., 2011).

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) is recommended.

### Conflict of interest

There is no conflict of interest.

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### Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2013.01.053.

### References


Jacobs S, Beauchard O, Struyf E, Cox T, Maris T, Meire P. Restoration of tidal freshwater vegetation using controlled reduced tide (CRT) along the Schelde Estuary (Belgium). Estuar Coast Shelf Sci 2009;85:368–76.


Spencer KL, Harvey CL. Understanding system disturbance and ecosystem services in restored saltmarshes: integrating physical and biogeochemical processes. Estuar Coast Shelf Sci 2012;106:23–32.


StClY Campbell PGC. Metals (Fe, Mn, Zn) in the root plaque of submerged aquatic plants collected in situ: relations with metal concentrations in the adjacent sediments and in the root tissue. Biogeochemistry 1999;33:45–76.


