Metal accumulation in intertidal litter through decomposing leaf blades, sheaths and stems of *Phragmites australis*

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Abstract

Metal contents of decomposing leaf blades, leaf sheaths and stems of common reed (*Phragmites australis*) were monitored by a litter bag method on the sediment of an intertidal brackish marsh in the Scheldt estuary (The Netherlands). On monthly intervals, two litter bags were retrieved from the marsh during 9 months for both leaf blades and sheaths and during 16 months for stems. All samples were dried, weighed and analysed for ash and Cd, Cu, Cr, Ni, Pb and Zn contents. Most concentrations increased considerably during the decomposition. Generally, also a very important net metal inflow into the litter bags could be observed. The inflow was highest for leaf blades. High correlations between ash contents and metal concentrations for leaf blades suggest that the increase of leaf blade metal contents can be due to physicochemical sorption of dissolved metals and an important infiltration of mud particles, which were not removed by rinsing the leaf blades with distilled water preceding the analyses. For stems, smaller amounts of inflowing ash and even outflowing ash amounts were found, which suggests that inflow of inorganic particles is not the major factor determining metal accumulation by stems on medium term. Ergosterol concentrations in stem tissue however proved to be correlated with metal contents, which suggests a significant role of fungal litter colonizers in metal accumulation. For leaf sheaths, the effects of physicochemical sorption, infiltration of mud particles and incorporation by microbial litter colonizers do not seem to be as pronounced as for stems and leaf blades.

Keywords: Sediments; Fungi; Organic matter; Common reed; Wetlands; Scheldt estuary

1. Introduction

anthropogenically introduced into the environment (Hart, 1982). A major fraction of the elements entering the sediment system will rapidly be fixed onto the solid phase, where a number of physical and chemical properties will determine the strength of metal retention. A small proportion of the metals dissolves and becomes available for plant uptake. Plant uptake directly reduces the input of metals into adjacent waters (Chen et al., 2000; Vandecasteele et al., 2005). Plants can provide a sink if during decomposition metals are bound to the litter by passive sorption on organic surfaces or by physiological mechanisms of microbial colonizers of the litter (Gadd, 1992, 1993; Ledin, 2000). However, the litter can also act as a metal source when microbial activity mobilizes metals (e.g. Gadd, 1993) or when it becomes available to deposit feeders. Several studies suggest that metals in litter are available to deposit feeders and, thus, can enter estuarine food webs (Weis and Weis, 2004). Consumption of detritus which contains metals can cause metal accumulation and deleterious effects in higher trophic levels (Dorgelo et al., 1995; Du Laing et al., 2002; Weis et al., 2002). The role of plant sequestration of metals into long-term sinks depends on the rate of uptake into the plant, rates of translocation and retention within individual tissue types, and the rate and mode of tissue decomposition (Catallo, 1993; Kadlec and Knight, 1996).

Metal uptake and distribution has been examined in *Phragmites australis* (Cav.) Trin. ex Steud. (common reed) (Larsen and Schierup, 1981; Schierup and Larsen, 1981; Gries and Garbe, 1989; Peverly et al., 1995; Keller et al., 1998; Windham et al., 2003; Soudek et al., 2004). All studies report the highest amounts of metals in the roots, while leaf tissue has the second highest concentrations followed by stems and rhizomes. A small fraction of metals is released in the environment through leaf tissue during the growing season (Burke et al., 2000). Once the reed tissue enters the litter layer and decomposition proceeds, increasing metal concentrations can be observed (Larsen and Schierup, 1981; Kufel, 1991; Windham et al., 2004). Besides the uptake of metals by organisms involved in decay, the fate of metals after organic matter decomposition is unsure. Several authors claim that upon mineralization, the metals previously bound to the organic matter will be remobilised into the environment (Alloway, 1995). Others claim that metals will be transferred from the more available fractions to e.g. highly insoluble organic complexes with strongly humified litter and thus sequestered in long-term sinks (Paré et al., 1999).

This study aims to assess the role of decomposing organic matter in wetland metal cycles and the role of different factors affecting metal fate in the litter layer. Heavy metal contents of decomposing leaf blades, stems and sheaths were monitored by a standard litter bag method in an intertidal zone of the Scheldt estuary. Unlike previous research studying metal dynamics during reed decomposition (Larsen and Schierup, 1981; Kufel, 1991; Windham et al., 2004), we additionally investigated the possible interaction between the prominent fungal colonization (Van Ryckegem et al., 2005a,b) and the observed metal dynamics. Common reed was selected as the test plant species as this is a widespread, dominant species in many aquatic ecosystems. It forms dense stands that are among the most productive ecosystems in temperate areas. Moreover, reed plants are only lightly grazed in the living state and the greatest part of the primary production ultimately enters detrital systems (Polunin, 1982), where fungi prove to be the dominant microbial reed decomposers (>90% of microbial production) both in the litter layer (Findlay et al., 2002) and in the water (Komínková et al., 2000). We aimed to design an experiment in which natural, environmental conditions in a brackish marsh are represented as much as possible during the decomposition.

2. Materials and methods

2.1. Study site

The study was carried out in a brackish tidal marsh of the Scheldt estuary called 'Schor van Doel'. It faces the 'Hertoginpolder' close to 'Saeftinghe Marsh', just across the Belgium border in The Netherlands (51°21^0 N, 4°14^0 E). It is vegetated by a monospecific stand of common reed, *P. australis*. Leaf blade, leaf sheath and stem biomass production was found by Soetaert et al. (2004) to be 320 ± 131, 125 ± 16 and 445 ± 16 g m^{-2}, respectively (mean ± standard deviation). The estimated sedimentation rate is about 3 cm year^{-1}. Metal contents in the upper 5 cm sediment layer were found to be 2.52–4.77 mg kg^{-1} for Cd, 89–213 mg kg^{-1} for Cr, 54.1–90.9 mg kg^{-1} for Cu, 10.3–98.9 mg kg^{-1} for Ni, 82–152 mg kg^{-1} for Pb and 210–377 mg kg^{-1} for Zn (expressed on dry mass basis). Organic matter and carbonate contents varied from 14.7% to 22.1% and from 6% to 13%, respectively (w/w). Conductivities between 1.77 and 9.52 mS cm^{-1}, pH between 7.1 and 7.8 and chloride contents between 1.9 and 11.5 g kg^{-1} dry mass were observed. All metal, carbonate, organic matter and chloride contents were highest in the upper 5 cm sediment layer. Lower concentrations were observed at higher depths.

2.2. Experimental setup

Samples originated from freshly harvested plants (with vital microbial colonization). The experimental period started when most senescent leaves and culms of the reed plants were expected to fall down onto the marsh sediments. For culms this is a rather arbitrary

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

2.3. Analyses

Ash contents were determined by measuring the weight loss after incineration of the oven dried samples (4 h at 450°C). Mass loss data were fitted to the exponential model, $m_t = m_0 e^{-kt}$, where m_t is the litter dry mass remaining after time t, m_0 is the original mass and k the breakdown coefficient. Analysis of covariance (ANCOVA; general linear model procedure, SAS Statistical Package version 8.2, 1999) on log-transformed data was used to compare the breakdown rates between litter types. For the analyses of metal contents, samples were ground in a hammer-cross beater mill and homogenised. Five grams of each sample was weighed to the nearest 0.1 mg on an analytical balance (Sartorius BP221S, Sartorius, Göttingen, Germany), placed into a 100 ml Pyrex beaker and treated with 10 ml ultra-pure 65% HNO_3. The beaker was covered with a watch-glass and the suspension was heated up to 130 °C for 1 h. Four milliliters of 20% H_2O_2 was added in aliquots of 1 ml. After cooling, the suspension was filtered (S&S, blue ribbon, Schleicher & Schuell, Dassel, Germany) in a 50-ml volumetric flask and diluted to the mark. In all extracts Cd, Cu, Cr, Ni, Pb and Zn contents were measured using F-AAS (flame atomic absorption spectrometry, Varian SpectrAA-1475, Palo Alto, CA, USA) or GF-AAS (graphite furnace atomic absorption spectrometry, Varian SpectrAA-800/GTA-100, Palo Alto, CA, USA). The analyses were performed according to Du Laing et al. (2003). The concentrations of Fe, Mn, Ca, Mg, K and Na were also measured as they can interact with adsorbed fractions of heavy metals. F-AAS was used to measure Fe, Mn, Ca and Mg contents and flame emission photometry (Eppendorf Elex 6361, Hamburg, Germany) to measure K and Na contents. Statistical analyses consisted of the calculation of correlations according to the Pearson correlation coefficients method, using SPSS 12.0 (2003). Modified box-plots were constructed using SPSS 12.0 (2003) to represent distribution of data graphically.

3. Results and discussion

3.1. Weight loss and organic matter decomposition

The residual ash-free dry weights are depicted in Fig. 1.

The time for 50% weight loss was approximately 7 months for sheaths, 7 months for leaf blades and 15 months for stems. This coincides with exponential breakdown rates k of 0.0039, 0.0035 and 0.0026 for respectively leaf sheaths, leaf blades and stems. The breakdown coefficient given for stems presents the decay rate after the initial lag period of 6 months during which there was no decomposition. ANCOVA suggested no difference in breakdown rate between leaf sheaths and leaf blades ($P > 0.05$), while stems showed a significantly slower breakdown ($P < 0.01$; even if the lag period was kept out of the analysis) compared to both leaf blades and sheaths, a feature noticed before (e.g. Hietz, 1992; Gessner, 2000).

The ash content of the leaf blades increased considerably during the first 6 months of decomposition, from 7% to around 50% (Fig. 1). The high ash content of leaf blade samples compared to other plant parts is probably caused by the large surface area and specific decay characteristics. Leaf blades show a decay pattern which is visually characterized by a collapse and removal by shredder invertebrates of leaf mesophyll between the longitudinal vascular bundles. Between those fine ridges of vascular bundles, fine clayey sediments infiltrate and accumulate, making leaves efficient traps for inorganic substances or mud particles difficult to remove. Ash content of stems and, more pronounced, of leaf sheaths gradually increases during the decomposition process with little variation during the experimental period $4.7 \pm 1.2\%$ and $12.6 \pm 1.1\%$ for stems and sheaths,

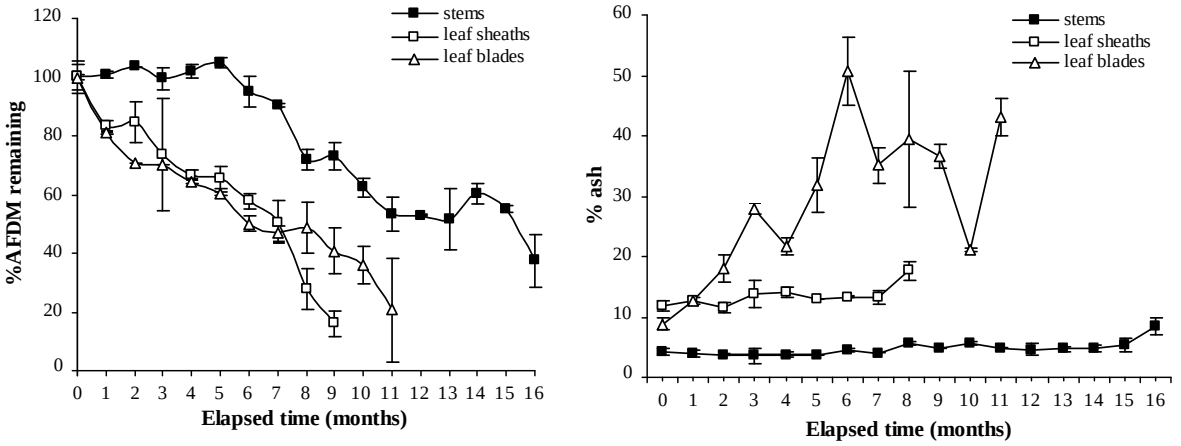

Fig. 1. Ash-free dry mass (AFDM) remaining and ash content (%, mean ± standard deviation) in the litter bags during decomposition of stems, leaf sheaths and leaf blades of *Phragmites australis* in a brackish tidal marsh. Experiment started in December 2001 for leaf sheaths and stems and in October 2001 for leaf blades (Van Ryckegem et al., 2005a,b).

respectively. This is probably due to the softening of the tissue becoming more susceptible to mud infiltration and invertebrate shredder activity causing micro relief at the tissue surface.

3.2. Metal contents

Initial metal contents in leaf blades, leaf sheaths and stems are presented in Table 1. Except for Cr and Zn, initial metal concentrations are lowest in stem tissue.

The changes of metal contents in leaf blades, sheaths and stems are depicted in Fig. 2. Most concentrations expressed on dry weight basis increased considerably during decomposition.

Increases in metal concentrations in plant litter were also observed by other authors. Larsen and Schierup (1981) found increasing Zn, Pb and Cd concentrations in *Phragmites* leaf blades during decomposition in the littoral zone of a sewage-polluted and a non-polluted lake. However, Cu contents were relatively constant. Windham et al. (2004) found increasing Cr, Cu, Hg, Pb and Zn concentrations in decomposing leaves and stems of reed plants in contaminated marshes.

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

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

Increasing metal concentrations do not prove that the litter bags act as a sink for metals. Therefore, the overall metal pools in the decomposing organic matter were estimated by multiplying the measured litter weights by their metal concentrations. The calculated amounts after 7 months, associated with leaf blades, stems and sheaths, are presented in Table 2 as a proportion to the initial amounts. Generally, a very important net metal inflow could be observed during these first 7 months. The inflow was highest for leaf blades. After 7 months, metal amounts associated with leaf blades were between 4.8 and 13.6 times higher than the initial amounts. Leaf sheath tissue accumulated up to between 1.5 and 10.0 times the initial amounts, whereas this proportion was found to be between 0.9 and 7.6 for stems. These data indicate that the litter bags were indeed important sinks for heavy metals.

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

Kufel (1991) however observed decreasing Pb and Mo amounts per litter bag during decomposition of reed plant material. The litter bags used by Kufel however were submerged in littoral water with low metal pollution without contact with the sediment surface, which may account for a less significant inflow of metals. Windham et al. (2004) found highly variable patterns when calculating metals pools within the litter bags. They however did not observe net metal accumulation

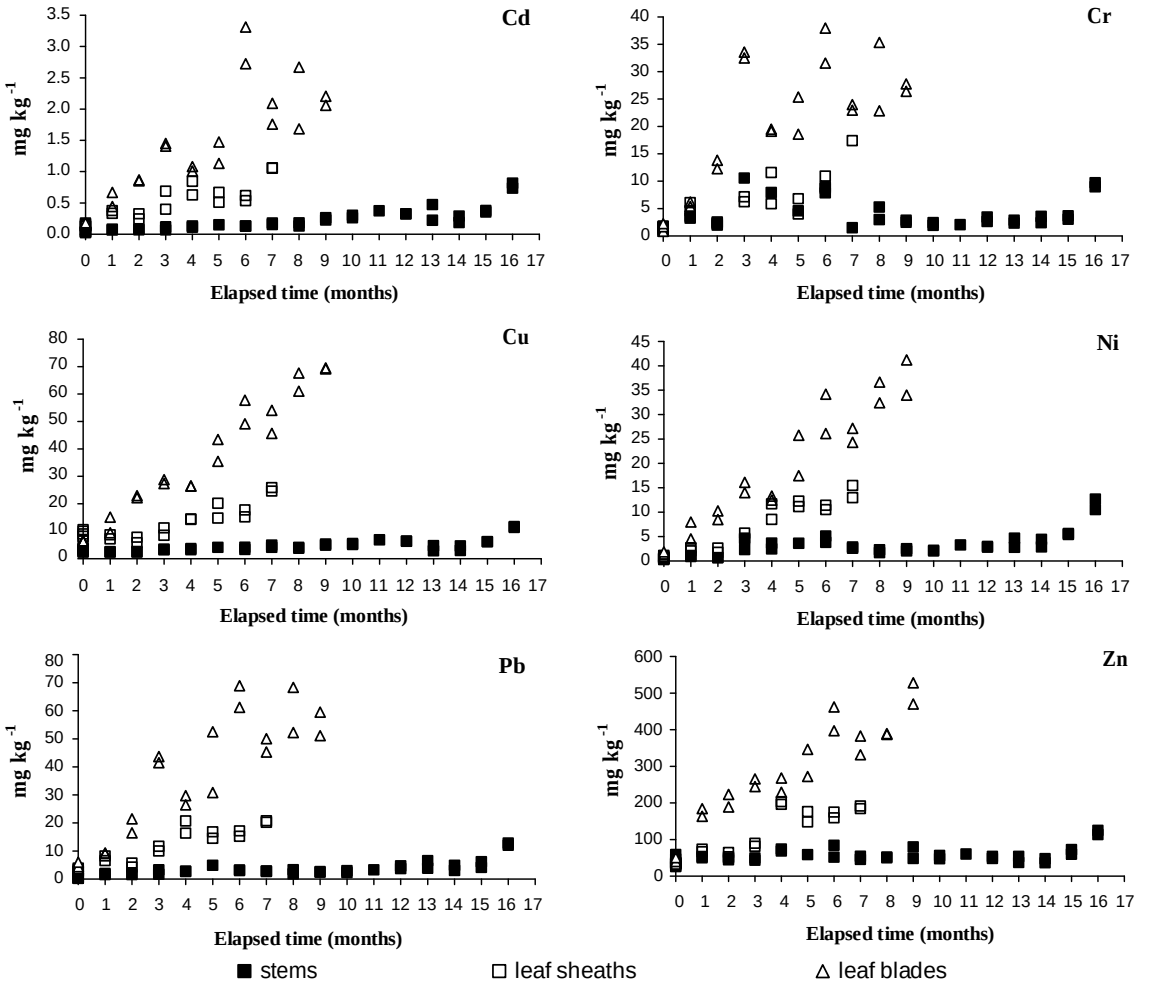

Fig. 2. Changes of metal contents (mg kg^{-1} dry mass) during decomposition of stems, leaf sheaths and leaf blades of *Phragmites australis* in a brackish tidal marsh. Experiment started in December 2001 for leaf sheaths and stems and in October 2001 for leaf blades.

Table 2
Proportions of calculated Cd, Cr, Cu, Ni, Pb and Zn amounts, associated with leaf blades, stems and sheaths in a litter bag to the mean initial amounts associated with leaf blades, stems and sheaths in a litter bag

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

Duplicate analysis result from two different litter bags which were both retrieved after 7 months from the marsh.

in the litter bags during their experiments. It should be noted that metal accumulation observed in litter bag experiments significantly depends upon the environmental conditions (e.g. salinity, pollution degree), and experimental conditions (e.g. submerged or littoral, mesh size of the litter bags, treatment of the plant litter), which makes comparison between different studies difficult.

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

Table 3
Correlations between ash and heavy metal contents in litter bags for leaf blades, stems and sheaths

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

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

centrations were calculated (Table 3). If trapping of sediment particles which contain much metals is important, significant correlations between increasing metal contents and increasing ash contents can be expected, as sediment particles consist mainly of ash. For leaf blades with highly increasing ash contents (Fig. 1), the correlations were significant at the 0.01 level for all elements, whereas for the sheaths and stems the correlations were not significant for most of the elements. The increase of leaf blade metal contents may thus be due to an important infiltration of mud particles, which were not removed by rinsing the leaf blades with distilled water preceding the analyses.

The proportion of metals in the ash inflow was also estimated. Ash inflow was calculated at each sampling time as the difference between ash amounts and initial ash amounts in the litter bags, associated with leaf blades, stems and sheaths, respectively. Similarly, metal inflow at each sampling time was calculated as the difference between metal amounts and initial metal amounts in the litter bags, associated with leaf blades, stems and sheaths, respectively. The percentage of metals in the ash inflow was then calculated at each sampling time for leaf blades, stems and sheaths, respectively: 100 - (metal inflow/ash inflow). The values were also calculated for Fe, Mn, Ca, Mg, K and Na as these elements can interact with adsorbed fractions of heavy metals. For the leaf blades, a substantial ash inflow was observed. Percentages of most metals in the ash inflow were very high in the first month and then decreased (Fig. 3). The inflow of ash with relatively low and stable metal concentrations between the second and the ninth month can be due to trapping of certain sediment fractions with relatively low metal contents. In that case, the inflow of ash with higher metal concentrations during the first month could be attributed to combined trapping of the same kind of sediment fractions and a faster physicochemical sorption of dissolved metals onto the remaining organic matter.

For leaf sheaths and stems, smaller amounts of inflowing ash, and for stems even continuously outflowing ash amounts, were found during most of the experiment. Metal contents in the inflowing and outflowing ash are fluctuating, which again suggests that inflow of inorganic particles is not the major factor determining metal accumulation by leaf sheaths and stems on medium term. Other mechanisms such as active accumulation by microbial organisms could then be more important. Windham et al. (2004) continued a quite similar litter bag experiment up to 24 months. They found that adsorption and accumulation of fine sediment cannot be the major cause of increasing metal concentrations on longer term, especially as accumulation was found to be higher at sites which were less contaminated, from which they concluded that microbial action is likely one of the major mechanisms responsible for the enrichment.

To test whether metal accumulation could be due to incorporation by microbial litter colonizers, we calculated correlations between metals and fungal biomass (ergosterol concentrations) within the same plant litter. Fungal dynamics in the decomposing litter are discussed by Van Ryckegem et al. (2005a,b), demonstrating that fungi are dominant decomposers during aerobic decay of *P. australis*. They contribute up to 10% of ash free litter mass. However, patterns are different between plant parts. Leaf blades had highest fungal colonization in the canopy in a hanging position prior to shedding. After entering the litter layer fungal biomass decreased spectacularly but recovered partially. Leaf sheaths showed a similar pattern but the drop in fungal biomass after falling was less pronounced. Final concentrations were the highest recorded during decay. Stems showed no detectable fungal colonization in a standing position and fungal biomass gradually increased during the decomposition process in the litter layer. Correlations between fungal biomass (ergosterol concentrations) and metals are depicted in Table 4. No significant correlations were found for leaf blades and low correlations were demonstrated for the leaf sheaths. However, fungal biomass proved to be highly correlated with metal contents in stem tissue except for Cr and Zn, both elements of which the contents stayed relatively stable during the experimental period (Table 2). This correlation suggests an involvement of fungal activity in metal accumulation in stem tissue by (a) direct incorporation in fungal mass (Ledin, 2000); (b) enhanced binding of metals to the decomposing litter due to complexation between extracellular fungal products and metals (Gadd, 1993) and (c) by induced changes in litter quality by mineralization, e.g. increasing availability of phenolic units as lignin depolymerises, offering many potential metal-binding sites (e.g. Senesi et al., 1987; Ledin, 2000). To clarify which mechanisms could drive metal accumulation under influence of microbial decomposers, more specific research is needed.

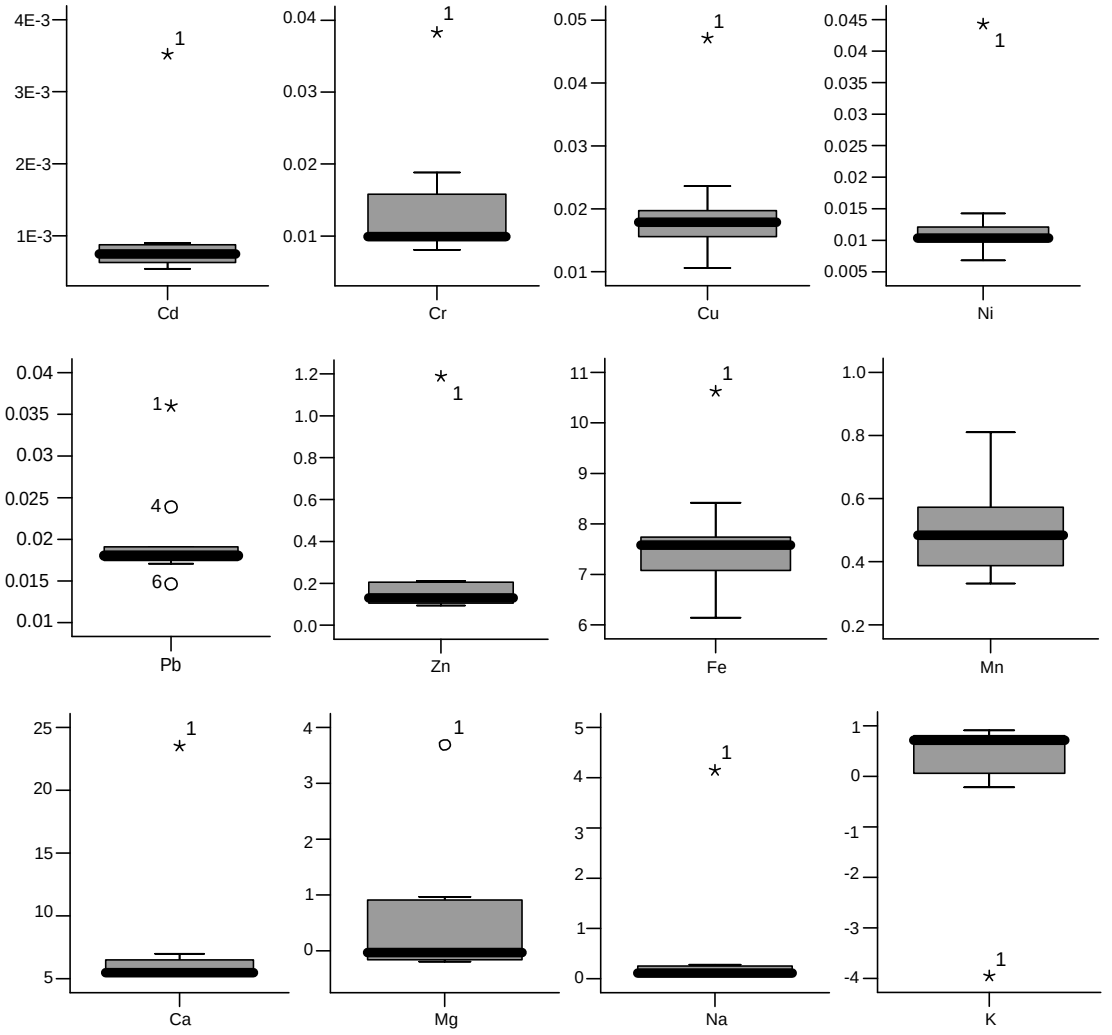

Fig. 3. Modified box-plot diagrams showing for leaf blades the distribution of metal concentrations in the ash inflow (%) with the initial values as a basis. The centre line shows the median of the values. Outliers are indicated with a circle and extremes with an asterisk. They are labelled by sampling time (number of months from the beginning of the experiment). Outliers are defined as cases with values between 1.5 and 3 box lengths and extremes as cases with values more than 3 box lengths from the upper or lower edge of the box. The box length is the inter-quartile range.

Table 4
Correlations between fungal biomass (ergosterol) and heavy metal contents in litter bags for leaf blades, stems and sheaths

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

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

4. Conclusion

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

factors seem to be of intermediate importance for leaf sheaths.

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