Afforestation of dredged material: fate of metals under different tree species

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AFFORESTATION OF DREDGED MATERIAL: FATE OF METALS UNDER DIFFERENT TREE SPECIES

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Dit doctoraat kwam tot stand als ’bijproduct’ van twee projecten, één in opdracht van AWZ en één in opdracht van het Havenbedrijf van Antwerpen. Deze twee projecten waren beiden zeer toepassingsgericht en behandelden thema’s zoals: welke boomsoorten kunnen groeien op baggerslib, hoe moet de aanplanting en het beheer van baggerdijken gebeuren, is er een ecotoxicologisch risico, etc. Toch was er binnen deze projecten ruimte voor een wetenschappelijke onderbouw en zo is er uiteindelijk dit doctoraat gekomen. Maar een doctoraat maak je niet alleen, daarom hartelijk dank aan iedereen die me op eender welke wijze hielp en steunde bij de voorbereiding van dit werk! Een aantal mensen wil ik graag in het bijzonder vermelden.

Mijn promotoren Kris Verheyen en Filip Tack zijn nog niet zo lang betrokken bij mijn onderzoek. Toch hebben ze hun stempel gedrukt op dit werk en ik wil ze allebei bedanken voor hun steun, hun input en de mogelijkheden die ze me geboden hebben. Ze hebben beiden een gezonde ambitie en een gezonde kijk op wetenschap. Kris moet ik nog eens speciaal bedanken want hij heeft de doorslag gegeven in de beslissing om dit doctoraat toch te maken. Professor Lust wil ik bedanken omdat hij er mee voor gezorgd heeft dat de projecten er gekomen zijn.

De ‘baggercel’ van het eerste uur verdient een speciale vermelding: Sebastiaan, Pieter en Anja. De kruisbestuiving van ideeën en de samenwerking maakten het werk bijzonder aangenaam en maakte dat we samen echt vooruitgang konden maken. Maar ook al mijn andere collega’s van het laboratorium voor bosbouw wil ik hartelijk bedanken (er zijn er in de voorbije jaren een hele reeks gepasseerd). Er was en is op het labo een sfeer van samenwerking waarbinnen onderlinge kritiek een soort drijvende kracht is om onszelf steeds in vraag te stellen en te verbeteren. Dit is niet altijd gemakkelijk maar wel efficiënt. Sebastiaan, Lieven en An waren, in mijn beginjaren, de drijvende krachten van het labo. Zij ijverden constant voor samenwerking, kwaliteit en publicaties. Zonder hen zou dit
doctoraat er nooit gekomen zijn. Ook hartelijk dank aan Lotte voor haar bijdrage aan het ‘Broekpolder’-hoofdstuk.

De laboranten, Luc en Greet, wil ik bedanken, niet alleen voor de analyses, maar ook voor hun algemene inzet voor het labo en het kwaliteitssysteem. Mede dank zij jullie is er een goed functionerend kwaliteitssysteem.

En dan Karien natuurlijk! Zonder haar werkte ik waarschijnlijk zelfs niet op het labo, maar ook nog veel meer. Robbe en Hasse hebben ook hun aandeel gehad, zij het dat ze eerder hebben tegengewerkt dan geholpen. Vaak kwam ik door hun toedoen ’s morgens moeër op het werk dan dat ik er de vorige avond vertrokken was. En als ik ’s avonds of op mijn ‘viervijfde’ nog wou werken werd dat dikwijls zeer professioneel geboycot. Ze hebben me alleszins genoeg afleiding bezorgd.

En wat er verder nog nodig is om een doctoraat tot stand te brengen en wat vaak vergeten wordt: een grote dosis geluk. Geluk dat je de mogelijkheden gekregen hebt, en dan bedoel ik de financiële en professionele mogelijkheden, maar ook de verstandelijke en fysieke mogelijkheden. Het is belangrijk af en toe eens stil te staan bij het feit dat veel mensen dit geluk niet hebben en om allerlei redenen hun ‘project’ in hun leven niet kunnen waarmaken.

Nog een kleine bedenking: de mensheid zal waarschijnlijk nooit alles kunnen weten. Om alles te begrijpen zijn onze hersenen vermoedelijk te klein. Maar als de wetenschap aan het zelfde tempo verder gaat zullen we op een dag wel alles weten wat we kunnen weten. Op die dag zullen Science en Nature failliet gaan.
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<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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</thead>
<tbody>
<tr>
<td>AAS</td>
<td>Atomic Absorption Spectrophotometry</td>
</tr>
<tr>
<td>BCF</td>
<td>Bio Concentration Factor</td>
</tr>
<tr>
<td>BV</td>
<td>Bank Vole</td>
</tr>
<tr>
<td>C</td>
<td>Carbon</td>
</tr>
<tr>
<td>$C_{plant}$</td>
<td>Metal concentration in the harvested component of the plant biomass</td>
</tr>
<tr>
<td>$C_{soil}$</td>
<td>metal concentration in the soil volume</td>
</tr>
<tr>
<td>$C_{target}$</td>
<td>target concentration</td>
</tr>
<tr>
<td>CS</td>
<td>Common Shrew</td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of variation</td>
</tr>
<tr>
<td>DAW</td>
<td>Dry Ash Weight</td>
</tr>
<tr>
<td>DM</td>
<td>Dry Matter</td>
</tr>
<tr>
<td>DW</td>
<td>Dry Weight</td>
</tr>
<tr>
<td>EC</td>
<td>Electrical Conductivity</td>
</tr>
<tr>
<td>FAAS</td>
<td>Flame Atomic Absorption Spectrophotometry</td>
</tr>
<tr>
<td>FW</td>
<td>Fresh Weight</td>
</tr>
<tr>
<td>H</td>
<td>Soil Horizon</td>
</tr>
<tr>
<td>ICP/OES</td>
<td>Inductively Coupled Plasma/Optical Emission Spectroscopy</td>
</tr>
<tr>
<td>ICP-AES</td>
<td>Inductively Coupled Plasma–Atomic Emission Spectrometry</td>
</tr>
<tr>
<td>L</td>
<td>Liver</td>
</tr>
<tr>
<td>LOI</td>
<td>Loss On Ignition</td>
</tr>
<tr>
<td>MER</td>
<td>Metal Extraction Ratio</td>
</tr>
<tr>
<td>$M_{plant}$</td>
<td>Mass of harvestable aboveground biomass</td>
</tr>
<tr>
<td>$M_{rooted\ zone}$</td>
<td>Mass of the soil volume rooted by the tree</td>
</tr>
<tr>
<td>n</td>
<td>Number of samples</td>
</tr>
<tr>
<td>OC</td>
<td>Organic Carbon</td>
</tr>
<tr>
<td>OM</td>
<td>Organic Matter</td>
</tr>
<tr>
<td>PC</td>
<td>Principal component</td>
</tr>
<tr>
<td>PCA</td>
<td>Principal Component analysis</td>
</tr>
<tr>
<td>$R^2$</td>
<td>Coefficient of determination</td>
</tr>
<tr>
<td>$R^2_{adj}$</td>
<td>Adjusted coefficient of determination</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>--------------------------------------</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>VLAREA</td>
<td>Flemish decree on Waste Management</td>
</tr>
<tr>
<td>VLAREBO</td>
<td>Flemish Decree on Soil Sanitation</td>
</tr>
<tr>
<td>WB</td>
<td>Whole Body</td>
</tr>
<tr>
<td>WM</td>
<td>Wood Mouse</td>
</tr>
<tr>
<td>Y</td>
<td>Year</td>
</tr>
</tbody>
</table>
Due to pollution of the Belgian inland waterways, sediments are often polluted with contaminants such as heavy metals (Geuzens et al. 1998). The periodic dredging of these waterways is a continuous source of large volumes of contaminated dredged materials which have to be treated or discarded (Förstner and Calmano 1998). In 1999, the total amount of sediment to be dredged in Flemish waterways was estimated at 27.6 $10^6$ m³ with a cost of dredging of € 305 $10^6$ (Cauwenberghs 2000). As more technical remediation techniques such as sediment washing, vitrification, and thermal treatment are often not yet economically and technologically feasible (Rulkens et al. 1998, Mulligan et al. 2001), the current option for treatment of contaminated sediment is disposal in confined landfills. This results in the establishment of fertile but contaminated sites with little beneficial uses.

In the past extended areas along our waterways were used for the disposal of contaminated dredged sediments. For example, more than 425 ha of contaminated sediments are located along the shores and in alluvial plains of the main Flemish waterways Scheldt and Leie (Vandecasteele et al. 2004a). Currently, many of upland disposed dredged sediments are used for agricultural purposes and natural habitat creation (Vandecasteele et al. 2002)

Afforestation of these sites is a common technique. Planting trees initiates soil development and nutrient cycling and improves the visual appearance of the site (Glimmerveen 1996). Dickinson (2000) argues that there is sufficient evidence to consider the use of trees in the reclamation of polluted soils as part of a realistic, integrated, low-cost, ecologically sound and sustainable reclamation strategy for
contaminated land. Afforestation is an opportunity to bring a large number of brownfield sites into productive use, which otherwise would be prohibitively expensive to remediate. The canopy layer, forest floor and root system are thought to form a ‘green cap’ which provides physical stabilization of the soil (Glimmerveen 1996, Pulford and Watson 2003). Trees might immobilize metals due to this ‘green cap’, the formation of stable organic matter and the decrease of soil water fluxes. This approach of immobilization of pollutants using plants is termed phytostabilization.

An other possibility that is often referred to is to use trees for phytoextraction. This technology is aimed at removing heavy metals from the site by repeated coppicing of the trees. Several authors postulate that trees have the ability to remove significant amounts of heavy metals from the soil (Landberg and Greger 1996, Ernst 1996, Dickinson 2000). Willow and poplar are considered best suited for this task because of their strong nature to coppice, their high capacity for Zn and Cd uptake, and their high biomass production (Schnoor et al. 1996, Greger and Landberg 1999, Robinson et al. 2000, Roselli et al. 2003).

Depending on the tree species traits, tree species might be more suited for phytostabilization or phytoextraction. Whenever phytostabilization or phytoextraction is chosen as remediation technique, the risk of pollutant dispersal into the environment should be minimized. Trees might indeed have metal immobilizing capacities but on the other hand, trees might mobilize metals due to pH changes, formation of soluble organic matter and eventual uptake of metals. These effects are species-specific because tree species have different effects on soil parameters as pH and organic matter content and exhibit different element uptake patterns (Finzi et al. 1998, Augusto et al. 2002, Reich et al. 2005). The global effect of trees on soil metals and the inter-specific differences between species is still not yet fully investigated and understood. The present research studies the effects of different tree species on the fate of metals in the forest-soil system of afforested dredged sediments. The objectives are:
(1) To evaluate and compare different tree species within the scope of reclamation and phytoremediation of metal polluted dredged sediment derived soils.

(2) To quantify the reallocation of soil metals in the ecosystem on the short and longer term under different tree species.

To reach these aims, 1 to 33-year-old plantations on dredged sediments were studied (Fig. 0.1). The first two chapters deal with the uptake and tolerance of different tree species, aged one to three years old, growing on dredged sediment. The following three chapters study the reallocation of metals in the ecosystem and the tree species effect on this reallocation after 10 and 33 years of tree growth. The last chapter discusses the interpretation of plant analysis data.

The first part of the dissertation is dedicated to the differences between species concerning the uptake of and tolerance to metals in the sediment. In Chapter 1, growth and metal uptake in the leaves is assessed for different tree species (black alder, white poplar, sycamore maple, common ash and black locust) on a (young) mound constructed from dredged sediments. Foliar samples of the tree species were collected and analyzed and the possibilities for phytostabilization and phytoextraction and the possible risks of metal cycling are discussed. A pot experiment was conducted to assess growth, survival and uptake of metals in dredged sediment with increased concentrations of Cd and Zn (Chapter 2).

The second part of the dissertation is concerned with the reallocation of metals in the dredged sediment ecosystem on the middle-long term (10 to 30 years). Metal concentrations in soil, willow leaves and small mammals were assessed on three disposal sites for dredged material with different pollution degree (Chapter 3). Comparative information is reported on Cd and Zn concentrations in small mammals, vegetation i.e. willow leaves, and NH₄OAc-EDTA extractable concentrations in soil.
The risk on food-chain transfer was assessed using a bio-magnification model. Emphasis was laid on Cd and Zn because these elements have been shown to be the most transferable heavy metals from soil to plants and because of the elevated uptake of Cd and Zn by the willows.

Metal compartmentalization in the soil-tree system was investigated on a hydraulically raised site for dredged sediment that was planted 10-years before with poplars. The effect of high Cd and Zn uptake on the Cd and Zn concentrations in the soil profile are presented and discussed in Chapter 4. The preconditions for metal accumulation in the soil profile are discussed.

Chapter 5 presents long term data on the effects of different tree species on the compartmentalization of metals and on some soil characteristics that determine metal mobility. Differences between tree species with reference to the distribution of metals in the soil profile are relevant for assessment of the risk and for further research on phytostabilization. The redistribution of metals in the soil profile under four different tree species was investigated on a site for dredged material that was planted 33-years before sampling.

Based on the acquired experience, Chapter 6 deals with the correct use and correct interpretation of leaf analyses as part of phytoextraction or biomonitoring studies.

In the ‘Conclusions’ chapter, results and insights from the previous chapters are combined to discuss phytoextraction, phytostabilization, methodology and risks for metal mobilization.
Fig. 0.1. Schematic overview of the thesis
Chapter 1

In situ measurements of metal uptake by five tree species growing on a young site for dredged sediment: phytoremediation possibilities*

Abstract

Five tree species (Acer pseudoplatanus L., Alnus glutinosa L. Gaertn., Fraxinus excelsior L., Populus alba L. and Robinia pseudoacacia L.) were planted on a mound constructed of dredged sediment. The sediment originated from a brackish river mouth and was slightly polluted with heavy metals. This study evaluated the use of trees for site reclamation by means of phytoextraction or phytostabilization of metals. Although the brackish nature of the sediment caused slight salt damage, overall survival of the planted trees was satisfactory. Robinia and white poplar had the highest growth rates. Ash, maple and alder had the highest survival rates (>90%) but showed stunted growth. Ash, alder, maple and Robinia contained normal concentrations of Cd, Cu, Pb and Zn in their foliage. As a consequence these species reduce the risk of metal dispersal and are therefore suitable species for phytostabilization under the given conditions. White poplar accumulated high concentrations of Cd (8.0 mg kg⁻¹) and Zn (465 mg kg⁻¹) in its leaves and might therefore cause a risk of Cd and Zn input into the ecosystem because

of autumn litter fall. This species is thus unsuitable for phytostabilization. Despite elevated metal concentrations in the leaves, phytoextraction of heavy metals from the soil by harvesting stem and/or leaf biomass of white poplar will not be a realistic option. It will require an excessive amount of time to be effective as at most 0.12% of the total Cd stock in the upper 1 m soil can be removed.

**Introduction**

Rivers and harbor docks are dredged on a regular basis to provide shipping traffic efficiency. In Flanders, the dredged material is for the most part polluted with both heavy metals and organic pollutants. Remediation techniques are often economically unacceptable because of the large volume of contaminated sediment (Förstner and Calmano 1998). Traditionally, dredged sediment is hydraulically raised in confined disposal facilities. To decrease the space required for storage, water can be removed from dredged material using a gravity-driven approach and the resulting material can be disposed into mounds. This technique results in a 50 to 114% increase in the amount of material that can be stored per unit area (Luyssaert et al. 2001).

Afforesting of polluted sites is part of a realistic, low-cost, ecologically-sound and sustainable reclamation strategy for bringing polluted sites into productive use (Dickinson 2000). Planting trees on these sites initiates soil development and nutrient cycling and improves the visual appearance of the site (Glimmerveen 1996). Trees are expected to be suitable for achieving extensive and long-term phytoextraction or phytostabilization. Many authors postulate that trees have the ability to remove significant amounts of heavy metals from the soil (Landberg and Greger 1996, Ernst 1996, Dickinson 2000). This technology, termed phytoextraction, removes heavy metals from the site by repeated coppicing of the trees. Willow and poplar are
Chapter 1

considered best suited for this task because of their strong nature to coppice, their high capacity for Zn and Cd uptake, and their high biomass production (Schnoor et al. 1996, Greger and Landberg 1999, Robinson et al. 2000, Roselli et al. 2003).

Other authors state that the feasibility of phytoextraction by planting trees is doubtful (Felix 1997, Salt et al. 1998, Robinson et al. 2003) since this technique may require an excessive amount of time and since no tree species are known to accumulate all of the most environmentally toxic metals such as Pb, Cd or As. At the same time, phytoextraction has environmental consequences, since heavy metals are brought into circulation via litter fall (Glimmerveen 1996, Robinson et al. 2000), causing dispersal in the ecosystem.

Phytostabilization is an alternative remediation technique, not aimed at extraction of the metals, but rather aimed at fixation of heavy metals in the soil. Tree growth stabilizes contamination either by immobilization or by preventing migration (Vangronsveld et al. 1995). Direct stabilization of the soil is achieved by root growth and the establishment of a litter layer and a vegetation cover (Ross 1994). The increased organic matter content increases soil aggregation and water holding ability which, together with increased interception and evapotranspiration, reduces leaching losses to the ground water (Schnoor 2000, Pulford and Watson 2003).

Whenever phytostabilization or phytoextraction is chosen as remediation technique, the risk of pollutant dispersal into the environment should be minimized. According to Ross (1994), the input of metals into the food web is potentially more harmful than metal leaching into the groundwater. Recycling of heavy metals through litter fall and litter decomposition can thus be an important pathway for input into the food web (Ross 1994).
The aim of this study was to determine which tree species are able to grow on mounds constructed from dredged sediment and to evaluate the possibilities and limitations for phytoextraction and phytostabilization of these mounds.

**Materials and methods**

**Site description**

In Antwerp, Belgium, dewatered dredged sediment from the river Scheldt was used to construct a mound of 9 m height in 1995. In March 1997, a rectangular area of 20 by 90 m on the flattened top of the mound was planted with 5 tree species: two pioneer species, black alder (*Alnus glutinosa* L. GAERTN.) and white poplar (*Populus alba* L.); two climax species, sycamore maple (*Acer pseudoplatanus* L.) and common ash (*Fraxinus excelsior* L.); and one fast growing exotic species, black locust (*Robinia pseudoacacia* L.). The experimental area was divided in three blocks of 30 by 20 m and each block was divided in 6 connected plots of 10 by 10 m. Two-year old seedlings were planted in March 1997, 2 years after construction of the mound. For each tree species, seedlings were planted at a triangular spacing of 1.5 m in three randomly chosen plots, one in each block. In each block, the remaining plot was left unplanted resulting in three unplanted plots on the site.

**Sediment analysis**

Samples of the dredged sediment were taken in May 1997 at two soil depths (0-15 cm and 30-45 cm) at 36 points regularly spread over the planted area to permit physical and chemical characterization. The samples were dried at 45°C until constant weight. The pH-KCl of a 1/5 sediment/KCl solution was determined with an ion-specific electrode (Loeppert and Suarez 1996). The percentage of organic carbon in the
sediment was determined with the method of Walkley and Black (Kalra and Maynard 1991), total N with the modified method of Kjeldahl (Bremner 1996), CaCO$_3$ with the simple titrimetric procedure (Loeppert and Suarez 1996). Electrical conductivity (EC) was measured potentiometric on a 1/5 sediment/water solution (Kalra and Maynard 1991) and total P colorimetric after coloring the extract using Scheel reagent (Van Ranst et al. 1999). The elements K, Cd, Cu, Fe, Mn, Pb, and Zn were extracted from the sediment sample by a digestion in aqua regia (ISO 11466). Subsequently the element concentrations of the solute were determined using flame atomic absorption spectrophotometry. Beside chemical characteristics, the percentage of sand was determined for six samples of the upper soil layer (0-15 cm) by wet sieving after destruction of CaCO$_3$, organic matter, FeO$_2$ and MnO$_2$ and after chemical dispersal to separate the individual sediment particles.

**Tree sampling**

Tree height, height growth and survival rates were determined in November 1998 after the second growing season. The experiment was untimely stopped in the winter of 1999, owing to the removal of the experimental area as a consequence of harbor expansion. Therefore the planned follow-up of the experiment is not available. Tree foliage on all planted plots was sampled in August 1998. Two bulk samples of five trees each were taken at each plot. Consequently the foliar composition of each tree species was represented by 6 bulk samples. Leaf samples were taken from all leaves of the upper third of the crown. The leaf samples were dried at 45 °C until constant weight and ashed for 4 h at 450°C. During ashing the temperature was gradually raised to avoid Cd volatilization. Afterward, the ash was digested in concentrated HNO$_3$. The solute was then analyzed for Cd, Cu, Pb and Zn concentration by flame atomic absorption spectrophotometry.
Results

Sediment characteristics

The concentrations of most elements were similar at 0-15 cm as at 30-45 cm (p<0.05; Table 1.1 and 1.2). The similarity of the soil characteristics reflects the high uniformity of the dredged sediment and indicates that distinct soil horizons are not yet present. The high electrical conductivity and high Na concentrations of the dredged material are explained by the estuarine origin of the sediment. In addition, Na and EC were the only variables which were significantly lower (p<0.05) in the top layer (0-15) than in the deeper layer (30-45 cm) of the sediment. The gradient is likely caused by leaching of the salts out of the top layer.

Table 1.1. Mean and standard deviation of the soil characteristics (n=72 except for sand where n= 7)

<table>
<thead>
<tr>
<th>Depth (cm)</th>
<th>0-15</th>
<th>30-45</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sand (%)</td>
<td>30 ± 5</td>
<td>-</td>
</tr>
<tr>
<td>pH(KCl)</td>
<td>7.1 ± 0.1</td>
<td>7.1 ± 0.1</td>
</tr>
<tr>
<td>N (mg kg⁻¹ DW)</td>
<td>2,019 ± 267</td>
<td>1,885 ± 286</td>
</tr>
<tr>
<td>P (mg kg⁻¹ DW)</td>
<td>2,441 ± 412</td>
<td>2,209 ± 385</td>
</tr>
<tr>
<td>K (mg kg⁻¹ DW)</td>
<td>9,155 ± 532</td>
<td>9,159 ± 840</td>
</tr>
<tr>
<td>Org. C. (%)</td>
<td>6.2 ± 0.8</td>
<td>6.2 ± 0.8</td>
</tr>
<tr>
<td>CaCO₃ (%)</td>
<td>9.0 ± 2.5</td>
<td>8.9 ± 2.9</td>
</tr>
<tr>
<td>Na (mg kg⁻¹ DW)</td>
<td>457 ± 102</td>
<td>823 ± 205</td>
</tr>
<tr>
<td>EC₁:₅ (mS m⁻¹)</td>
<td>87.9 ± 33.8</td>
<td>219.3 ± 57.1</td>
</tr>
<tr>
<td>Fe (mg kg⁻¹ DW)</td>
<td>54,202 ± 6,254</td>
<td>53,866 ± 6,061</td>
</tr>
<tr>
<td>Mn (mg kg⁻¹ DW)</td>
<td>683 ± 77</td>
<td>650 ± 69</td>
</tr>
</tbody>
</table>
The dredged sediment also had a high pH and a high nutrient stock. According to reference data for heavy metals in uncontaminated surface soils calculated on the world scale (Kabata-Pendias and Pendias 1992; Table 1.2), Cu concentrations were in the normal range, Pb and Zn concentrations were relatively high, and Cd concentrations were more than threefold higher than the upper range. The Cd, Cu, Pb and Zn concentrations were below Flemish legislation limits for re-use of waste material (Table 1.2).

<table>
<thead>
<tr>
<th>Depth (cm)</th>
<th>0-15</th>
<th>30-45</th>
<th>Reference data</th>
<th>Legislation limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cd</td>
<td>5.7 ± 0.8</td>
<td>5.9 ± 0.7</td>
<td>0.08-1.61</td>
<td>10</td>
</tr>
<tr>
<td>Cu</td>
<td>54.2 ± 6.3</td>
<td>53.9 ± 6.1</td>
<td>4-100</td>
<td>375</td>
</tr>
<tr>
<td>Pb</td>
<td>75.2 ± 11.2</td>
<td>74.3 ± 8.1</td>
<td>1.5-70</td>
<td>1250</td>
</tr>
<tr>
<td>Zn</td>
<td>358 ± 40</td>
<td>359 ± 46</td>
<td>9-362</td>
<td>1250</td>
</tr>
</tbody>
</table>

**Growth and survival rate**

Two groups could be depicted when survival rates and height growth were considered. More than 90% of the alder, ash and maple seedlings were still living two years after planting (Table 1.3), but these species showed stunted growth. The second group,
existing of Robinia and white poplar, showed higher mortality but also higher growth. Mortality of these two species was lower than 30%. The average height growth of Robinia and white poplar measured more than 50 cm (Table 1.3). On almost 31% of the maple and almost 80% of the ash trees necrosis of the leaf margins was observed, a typical symptom of salt stress (Munns 2002). The foliage of the other species did not show visual symptoms of salt stress.

Table 1.3. Survival rate and growth (cm) of the planted trees after the second growing season

<table>
<thead>
<tr>
<th></th>
<th>% survival</th>
<th>Growth (cm)</th>
<th>SD</th>
<th>10% percentile</th>
<th>90% percentile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alder</td>
<td>92</td>
<td>5</td>
<td>31</td>
<td>-23</td>
<td>27</td>
</tr>
<tr>
<td>Ash</td>
<td>98</td>
<td>2</td>
<td>8</td>
<td>-3</td>
<td>8</td>
</tr>
<tr>
<td>Maple</td>
<td>99</td>
<td>5</td>
<td>11</td>
<td>-2</td>
<td>15</td>
</tr>
<tr>
<td>Robinia</td>
<td>83</td>
<td>53</td>
<td>39</td>
<td>9</td>
<td>97</td>
</tr>
<tr>
<td>White poplar</td>
<td>71</td>
<td>54</td>
<td>39</td>
<td>13</td>
<td>86</td>
</tr>
</tbody>
</table>

**Foliar element concentrations**

Foliar heavy metal concentrations were measured at the end of the second growing season (Table 1.4). The observed concentrations were within the normal range which was compiled from different literature references (Table 1.5), except for white poplar. White poplar foliar concentrations of Cd and Zn exceeded the normal range (Table 1.4 and 1.5). The foliar Cd and Zn concentrations for white poplar were higher than the Cd and Zn concentrations in the sediment.
Table 1.4. Foliar metal concentrations the second year after planting (mg kg\(^{-1}\) DW, n=6)

<table>
<thead>
<tr>
<th>Species</th>
<th>Cd</th>
<th>Cu</th>
<th>Pb</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alder</td>
<td>&lt;0.23*</td>
<td>5.8 ± 0.9</td>
<td>5.0 ± 0.5</td>
<td>65 ± 12</td>
</tr>
<tr>
<td>Ash</td>
<td>0.3 ± 0.3</td>
<td>12.4 ± 1.8</td>
<td>5.0 ± 1.2</td>
<td>26 ± 8</td>
</tr>
<tr>
<td>Maple</td>
<td>0.5 ± 0.3</td>
<td>5.9 ± 1.3</td>
<td>4.5 ± 1.6</td>
<td>74 ± 48</td>
</tr>
<tr>
<td>Robinia</td>
<td>&lt;0.23*</td>
<td>8.3 ± 1.2</td>
<td>2.3 ± 0.3</td>
<td>45 ± 5</td>
</tr>
<tr>
<td>White poplar</td>
<td>8.0 ± 2.0</td>
<td>3.8 ± 0.4</td>
<td>3.3 ± 0.6</td>
<td>465 ± 125</td>
</tr>
</tbody>
</table>

*: Determination limit

Table 1.5. Reference data for foliar analyses (mg kg\(^{-1}\))

<table>
<thead>
<tr>
<th>Species</th>
<th>Cd</th>
<th>Cu</th>
<th>Pb</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal ranges in plants (1)</td>
<td>0.05-0.2</td>
<td>5-30</td>
<td>5-10</td>
<td>27-150</td>
</tr>
<tr>
<td>Normal ranges in plants (2)</td>
<td>0.1-2.4</td>
<td>5-20</td>
<td>0.2-20</td>
<td>1-400</td>
</tr>
<tr>
<td>Normal range in plant material (3)</td>
<td>0.2-0.8</td>
<td>4-15</td>
<td>0.1-10</td>
<td>8-400</td>
</tr>
<tr>
<td>Robinia pseudoacacia (6)</td>
<td>0.05</td>
<td>7.0</td>
<td>.8</td>
<td>30</td>
</tr>
<tr>
<td>Populus nigra (5)</td>
<td>0.25 ±0.07</td>
<td>5.1 ± 0.6</td>
<td>2.3 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>Populus spp. (4)</td>
<td>14.2</td>
<td></td>
<td></td>
<td>67.1</td>
</tr>
</tbody>
</table>

(1) Kabata-Pendias and Pendias (1992)
(2) Alloway (1995)
(3) Ross (1994)
(4) Reuter and Robinson (1997)
(5) Djingova et al. (1996)
(6) Bargagli (1998)
Discussion

Tree growth and survival

Despite high sodium concentrations in the sediment, mortality of the planted seedlings was limited to at most 30%. Elevated salt concentrations in soils are reported to cause reduced growth rate and reduced photosynthetic leaf area of the plant due to necrosis of the leaf margins (Kozlowski 1997, Munns 2002). No specific literature was found for the planted species, but for Prunus salicina, Catlin et al. (1993) reported a salt tolerance threshold for fruit yield of 26 mS m⁻¹, markedly different from the reported EC-values (154 ± 81 mS m⁻¹).

Because salts wash out of the soil over time, seedling growth will improve during subsequent years. On a mound constructed in the beginning of 1992 of the same dredged material as used in this experiment and planted with the same species immediately after construction, growth clearly improved after some years (Figure 1.1; Mertens & Lust, 1999). For sycamore maple and common ash, the growth was still limited after the third growing season, but increased from the fourth growing season. Growth ameliorated probably because plants overcame the ‘plant shock’ and because salts washed out of the soil. Soil washing might cause salts to leach to the groundwater. As a consequence the location of the mound should be chosen carefully or appropriate measures should be taken to prevent leaching to the groundwater.

Phytostabilization

The risk of environmental harm will be reduced by choosing tree species that do not accumulate heavy metals, because access to the contaminating metals will be reduced (Glimmerveen 1996, Pulford and Watson 2003). Recycling of mainly leaf bound heavy metals through leaf fall should be avoided to minimize the risk of spreading heavy metals into the environment. This was shown by Mertens et al. (2001) and Beyer et al.
(1990), both on heavily polluted disposal sites for dredged sediment. On sites covered with reed (*Phragmites australis* [Cav.] Trin. Ex Steud), that did not accumulate metals in its aboveground biomass, Beyer et al. (1990) found no elevated metal concentrations in living biota. Elevated concentrations of Cd were found in small mammals living on disposal sites covered with willow (Mertens et al. 2001). Cadmium was thought to be introduced into the food web by willow litter fall.

![Figure 1.1. Height of trees growing on a dredged material mound constructed and planted in 1992 (Mertens & Lust, 1999)](image-url)
Alder, ash, maple and Robinia showed low foliar heavy metal concentrations and might therefore be suited for phytostabilization purposes on the experimental site. On the 33-year-old site, studied in Chapter 5, the concentrations in the litter of ash and maple were similar to the concentrations that were measured in these young trees. Also Roselli et al. (2003) found alder and maple to display low metal concentrations in their above-ground tissues on a site with heavy metal contaminated composted sewage sludge. Although the mobility of heavy metals can be increased by the formation of chloride complexes when high amounts of chloride ions are present (Bauske and Goetz 1993), no elevated concentrations in the leaves of these tree species were found.

An aspect that is often overlooked in the discussions on phytoremediation of contaminated sites with trees is the knowledge that forestry tends to mobilize pollutants through progressive soil acidification (Mayer 1998). When the acid neutralizing capacity is used, pH will decrease, leading to an elevated mobility of the metals (Tack et al. 1996). Due to the high CaCO₃ concentration in the soil, the mound seems not threatened by acidification in the short term. Luyssaert et al. (2001) calculated on a mound constructed with the same dredged material that it would take between 465 and 840 years before acid neutralizing capacity of the upper 0.5 m is used. Cappuyns et al. (2004) combined data of acid deposition in Flanders with acid neutralizing capacity values obtained from pH_stat leaching tests to predict soil acidification in the upper 20 cm of several disposal sites for dredged material in Flanders. It would take between 250 and 600 years to have substantial pH decrease (from about 7.5 to 6) in the sediment.

The solubility of Zn in dredged sediment started increasing from pH lower than 6, for Cd it started beneath pH 4 and for Cu and Pb it started beneath pH 2 (Tack et al. 1996). Alder and Robinia are nitrogen fixing species, which will cause an accelerated decline in soil pH (Johnson and Lindberg 1992). The acid production by N₂-fixing legumes was reported to range from 0.2 to 2.7 mol H⁺kg⁻¹ biomass produced (Tang et al. 1999). Assuming an acid production of 2.7 mol H⁺kg⁻¹ and a biomass production of 5 tons ha⁻¹
Chapter 1

1, it could mean that the acid neutralizing capacity is used in 570 years instead of 1150 years. It should hereby be stated that N\textsubscript{2}-fixing will probably be low because N\textsubscript{2}-fixing decreases for high nitrogen content of the soil (Tang et al. 1999). As a consequence, acidification is expected to be lower than estimated. On the short term acidification of the soil is not an immediate threat on brackish sediment mounds. The consequences of acidification are further investigated and discussed in Chapter 5.

Phytoextraction

The possibilities and limitations for heavy metal phytoextraction were estimated on the basis of leaf concentrations. Exact calculations require metal concentrations in stem-wood, bark and branches as well as the biomass of those components. For the extraction of metals from the soil by harvesting plant material, trees are needed that tolerate the pollution, coppice readily and take up high amounts of metals in the plant material. White poplar meets these three conditions, and high concentrations of Cd and Zn were found in the leaves of white poplar trees in this study.

It turned out however that white poplar is not suited to clean the soil of Cd and Zn pollution by harvesting tree biomass within a realistic length of time. When only leaves are harvested, an estimated leaf production of 2.5 ton DM ha\textsuperscript{-1} y\textsuperscript{-1} would yearly export 20 g Cd ha\textsuperscript{-1} and 1163 g Zn ha\textsuperscript{-1}. This is only about 0.04% of the total stock of both Zn and Cd in the upper 1 m of the soil. Cd concentrations in the wood of willows and poplars are reported to be 1 to 5 times lower than leaf concentrations, but woody biomass production is approximately double the production of litter (Riddell-Black 1994, Schnoor et al. 1996, Greger and Landberg 1999, Eriksson and Ledin 1999, Robinson et al. 2000, Klang-Westin and Eriksson 2003). This means that if woody biomass is harvested together with the leaves, at most 0.12% of the total Cd stock in the upper 1 m soil can be removed per year.
Moreover, the use of white poplar, and other poplar or willow species, for phytoremediation has the disadvantage that the soil will only be cleaned from Cd and Zn, not from other heavy metals. If trees are used, coppicing systems have to be developed that prevent recycling of metals in the system via annual leaf fall.

**Conclusion**

White poplar accumulated large amounts of Cd and Zn and might cause a risk because of recycling of Zn and Cd through litter fall. Ash, alder, maple and Robinia are suited for reclamation of dredged sediment mounds. The visual appearance is enhanced and the trees stabilize the waste. The risk of recycling of Cd, Cu, Pb and Zn in the ecosystem is minimal as these species do not accumulate elevated concentrations of these metals. Alder and Robinia will more rapidly decrease the pH because of nitrogen fixation, but because of the high CaCO₃ content of the soil it will still take 570 years before acid neutralizing capacity of the upper 0.5 m is used. Alder, ash and maple displayed stunted growth because of high soil salt concentrations. Because salt leaches from the soil and because of the high survival rates, better growth is expected the forthcoming years.

An estimate of the possibilities for phytoextraction suggested that none of the five investigated species was suited for phytoextraction of all soil metals. Even when using white poplar for the extraction of Cd and Zn, an excessive amount of time will be required to purify the sediment. The risk for recycling of the metals is an important issue that should be considered.
Chapter 2

Greenhouse experiment with different tree species: growth and metal uptake with increasing Cd and Zn levels

Abstract

Metal uptake (Cd, Cu, Ni, Pb, Zn), growth and survival of 5 tree species were evaluated in two greenhouse pot experiments, one with increasing concentrations of Cd (5.8-36.1 mg kg$^{-1}$) and one with increasing concentrations of Zn (355-1871 mg kg$^{-1}$). The survival rate of alder and ash was least affected by elevated Cd and Zn concentrations. Increased Cd concentration in the soil only resulted in an increase of the Cd concentration in the leaves of white poplar. Elevated Zn concentrations in the soil increased the Zn concentrations in the leaves of both white poplar and alder. The Zn concentrations in alder leaves were about half the concentrations in white poplar leaves. An increase of the Zn concentrations in the soil seemed to slightly increase the Cd concentrations in the leaves of white poplar. Other interactions between the metals were not found.
**Introduction**

Survival rate and metal concentrations in the leaves of most tree species, growing on the dredged sediment mound described in Chapter 1, did not seem to be affected by the metals in the sediment. Only Cd and Zn concentrations in the leaves of white poplar were high. The dredged sediment was polluted with metals, but not to a high extent, and metal uptake by the trees might have been hampered by the high pH, Ca concentration and clay content.

In practice, also more polluted dredged sediment sites are afforested, as afforestation is a common reclamation technique. It is therefore important to know which tree species are tolerant to high metal concentrations and which species accumulate metals in the leaves. The relation between different metal concentrations in the soil and in the leaves should be assessed for each tree species to evaluate the risk of food chain contamination. Vandecasteele et al. (2005b) evaluated the growth and metal uptake of two willow clones in a greenhouse pot experiment with dredged sediment. Increasing metal concentrations did not cause growth inhibition, but willow foliar Cd concentrations were strongly correlated with soil Cd concentrations. Madejon et al. (2005) found a significant correlation between Cd and Zn concentrations in the soil and in poplar leaves. For other tree species, few data are available.

A pot trial was set up to study the effect of increasing Cd and Zn concentrations in the soil on 5 tree species. Cd and Zn were chosen because these metals were taken up in high amounts by willows and poplars (see Chapter 1). The five tree species tested in this greenhouse pot experiment are the species that were planted on the site studied in Chapter 1: black alder (*Alnus glutinosa* L. GAERTN.), white poplar (*Populus alba* L.),
sycamore maple (*Acer pseudoplatanus* L.), common ash (*Fraxinus excelsior* L.) and black locust (*Robinia pseudoacacia* L.)

Although pot trials are not suited to make extrapolations to the site level (Giller et al. 1998; see also Discussion), this pot experiment is useful to allow for a better understanding of the relative differences in metal uptake and tolerance between the tree species.

**Materials and methods**

Undrained containers (diameter 19 cm, height 21 cm, content 4 l) were filled with dredged sediment that was collected from the dredged sediment mound described in Chapter 1. The chemical properties of the used dredged sediment, same as the mound, are summarized in Table 1.1 and Table 1.2. The treatments were blank dredged sediment and four different concentrations of Cd added to the sediment (Table 2.1). The same setup was repeated for four concentration levels of Zn. For every treatment and every tree species, four pots were prepared and planted in April 1997. Three of these pots were planted with two seedlings and one pot was planted with one seedling, resulting in 7 trees for each treatment and species. The planted seedlings of ash, alder, maple, Robinia and white poplar were 1 year old. Four concentration steps were made for both Cd and Zn by adding 1 l of a solution of Zn or Cd sulfate. Table 2.1 shows the concentrations in this solution and the resulting concentration in the soil of the pots. The pots were placed in a greenhouse and watered according to their needs. For each tree, the length of the main shoot was determined after the first and the second growing season.
Leaves were sampled for analysis of metals in the second growing season (1999). It was not possible to take leaf samples for metal analysis from each tree because of limited growth of the trees. Even pooling of the leaves of the two trees of one pot gave often not enough material for analysis. When possible, one sample was taken in the second half of August and as much samples as possible were obtained collecting litter fall in October. In both cases, the leaves of both trees of one pot were pooled to obtain one sample. Not enough leaf material was available for analysis of the leaves of maple, for ash only 1 sample was taken in August.
Leaf samples were dried, digested and analyzed for Cd, Cu, Pb and Zn with the same methods as described in Chapter 1. No differences could be found between results of litter fall and leaves (paired comparison) and these results were presented and discussed together. No formal statistical analysis was performed because of the limited data set and because all data could be visually presented.

**Results and discussion**

**Survival and growth**

The data on the survival of the different tree species in the treatments with Cd and Zn suggest that alder and ash are more tolerant to elevated levels of Cd and Zn in the soil than the other tree species (Fig. 2.1 and 2.2). Almost no mortality was recorded for these species and increased Cd and Zn concentrations did not affect the survival rate of these species, except for the highest level of Zn which caused total mortality for all species. The survival rate of white poplar, Robinia and maple seemed severely affected by the addition of Cd, although there was at least one tree for every species that survived at the highest Cd level (Fig. 2.1). Increased Zn concentrations seemed to have caused higher mortality of maple but there was no clear correlation with the survival rate of white poplar and Robinia (Fig. 2.2). The survival rate for the latter two species was lowest in the lowest treatment and at the level of the blank treatment in the higher concentration steps. Maple seemed clearly most affected by metals from all species.
Fig. 2.1. Number of surviving trees in the treatment with increasing Cd concentrations

Fig. 2.2. Number of surviving trees in the treatment with increasing Zn concentrations
Addition of Zn or Cd to the soil had little effect on tree growth (Fig. 2.3 and 2.4). The response of plants to heavy metals seems hard to predict (Tyler et al. 1989). In laboratory experiments exposing organisms to small amounts of toxic elements, scientists often recorded for example an increase of growth, especially in the beginning of the experiment. The results for tree growth of this experiment should be treated with care. The pots were rather small and were not fertilized. Therefore the nutrient pool was limited which might be the reason why no growth differences between the species were found.

Fig. 2.3. Height of the trees in the treatment with increased Cd concentration (Cd treatment: 0 = 5.8 mg kg$^{-1}$; 1 = 5.9 mg kg$^{-1}$; 2 = 6.1 mg kg$^{-1}$; 3 = 8.9 mg kg$^{-1}$; 4 = 36.1 mg kg$^{-1}$)
Metal uptake with increasing Cd and Zn levels

Leaf analysis

Increased Cd concentrations in the sediment seemed only to increase the concentrations in white poplar leaves (Fig. 2.5). For the other tree species, the Cd concentration in the leaves remained rather constant or lower than the detection limit (0.23 mg kg⁻¹). No interactions with other metals appeared as the other metal concentrations remained more or less constant over the soil Cd range. Increased Zn concentrations in the soil caused the Zn concentrations in the leaves of poplars to increase, but also Zn concentrations in leaves of alder increased (Fig. 2.6).

For Robinia and ash, the Zn concentrations did not increase with increasing soil Zn concentrations. The data for Cd concentrations in the leaves suggest that increased Zn concentration in the soil might slightly increase the Cd concentration in the leaves of white poplar. This increase is very limited, from about 1 mg kg⁻¹ to about 2 mg kg⁻¹.
The data for Cu suggest that an increase in Zn concentration in the soil caused a decrease of Cu concentrations in the leaves of alder. For the other species and the other elements, the soil Zn concentration did not seem to influence the leaf metal concentrations.

The Cd and Zn concentrations in the highest treatments are high compared to normal values. Nevertheless, most species do not exhibit elevated leaf concentrations in these treatments. Planting these species on extremely polluted sites will not enhance the risk for input of metals in the food web via uptake by the trees. White poplar however does show elevated concentrations in the leaves when soil concentrations increase. Therefore this specie should not be planted on highly polluted sites.

Metal uptake data obtained from pot experiments may not be directly extrapolated to possible uptake in field conditions. Plants growing in greenhouses on soils that are enriched with metals in general take up higher amounts of metals than the same plants growing on the same soil in the field (Impens et al., 1985). Moreover, when soils are artificially contaminated using metal salts, the metal speciation will largely differ to that from the field where the contamination may have been introduced in a different form and was allowed to react with the soil contaminants over an extended period of time. Other important factors are the limited dimensions of the pots, influencing rooting of the medium and limiting nutrient availability. Also growing conditions in the greenhouse are significantly different from site conditions.
Fig. 2.5. Leaf concentrations of Cd, Cu, Pb, Ni, Zn (mg kg\(^{-1}\)) for the different treatment levels of Cd. For the leaf Cd concentration, a number of data is missing because data were lower than detection limit (0.23 mg kg\(^{-1}\))
Fig. 2.6. Leaf concentrations of Cd, Cu, Pb, Ni, Zn (mg kg⁻¹) for the different treatment levels of Zn. For the leaf Cd concentration, a number of data is missing because data were lower than detection limit (0.23 mg kg⁻¹)
For most species where no enhanced uptake was observed, concentrations in the pot trial were not different from the concentrations measured in the field, except for Cd and Zn concentrations in white poplar being lower than in the field. Root uptake might have been hampered due to the small dimensions of the pots and due to limited availability of nutrients as no fertilization was added. Although pot trials are not suited to predict the behavior of the trees in the field, the experiment clearly shows differences in the uptake pattern of species. Robinia and ash do not take up more metals when soil metal concentration is increased, while white poplar shows a clear response to increased soil Cd and Zn concentrations. Increased soil Zn concentrations affect Zn concentrations in the leaves of alder, but increased Cd concentration seems to have little effect.

**Conclusions**

It is often stated that trees are rather tolerant to soil metal pollution, but this pot trial indicated that there are some differences between the tree species. Alder and ash seemed to be more tolerant to elevated levels of Cd and Zn in the soil than poplar and maple. Almost no mortality was recorded for alder and ash and increased Cd and Zn concentrations in the soil did not affect the survival rate of these species, except for the highest level of Zn which caused total mortality for all species. The survival rate of white poplar, Robinia and maple seemed severely affected by the addition of Cd. Tree height seemed to be hardly affected for any species by increased soil metal concentrations.

An increased Cd concentration in the soil did only increase the Cd concentration in the leaves of white poplar. The other species did not show any change of the leaf concentrations due to the Cd treatments. Elevated Zn concentration in the soil increased the Zn concentrations in the leaves of white poplar and alder. The concentrations in
Chapter 2

alder were about half the concentrations in white poplar. Elevated Zn concentrations seemed to slightly affect the Cd concentrations in the leaves of white poplar. Concentrations of Cu, Ni and Pb did not seem affected by the treatments with increased Cd and Zn in the soil. No conclusions can be drawn for maple, as not enough leaf tissue was available for analysis.
Chapter 3
Cd and Zn concentrations in small mammals and willow leaves on disposal facilities for dredged material*

Abstract

Disposal sites for dredged material are often polluted with heavy metals. The uptake of Cd and Zn by small mammals and willow trees was assessed on three sites with a different pollution degree. Detailed soil sampling showed a huge variation in soil characteristics within the sites, typical for disposal sites for dredged material. This variation made risk assessment and interpretation of soil chemical analyses complicated. Small mammals were thought to give a more integral description of the contaminant level because they cover a large area. Cd concentrations in small mammals were elevated compared with background levels whereas Zn concentrations were rather low. There were no significant differences between sites in Cd or Zn levels in animals. Leaf analysis showed a clear response to the pollution degree of the sites. The calculations using the BIOMAG model indicated that the Cd of the soil caused low risk for predators. However, the elevated Cd and Zn concentrations in the willow leaves on the polluted sites and the high Cd concentrations in the small mammals on all sites indicated that ecosystem development should be carefully considered.

**Chapter 3**

**Introduction**

Dredged material of Western European rivers is often polluted with heavy metals. This contaminated dredging sludge is not suitable for agricultural purposes, and is therefore dumped in disposal facilities. Spontaneous ecosystem development as well as forestation of these sites makes it necessary that bioavailability and accumulation of heavy metals in these ecosystems are estimated and that the risk for dispersion and ecological damage is assessed.

The potential toxicity of pollutants is strongly determined by the speciation of the elements. In this respect, total soil analysis, using a strong acid for the digestion, is inadequate to assess the availability and the toxicity of contaminants for humans and wildlife (Talmage and Walton 1991, Whitworth et al. 1999). Bioavailability of heavy metals in metal-contaminated soils is strongly determined by physical, chemical and biological aspects (Ross 1994, Ernst 1996).

Confined disposal facilities for dredged material have a strong texture gradient due to different sedimentation velocities: during hydraulic raising, sand will settle near the nozzle while clay will settle further away. This particle size gradient causes a variety in soil physical, chemical and biological parameters and will result in strongly varying availabilities of pollutants, making risk assessment, only based on soil parameters, even more complicated.

As biological organisms respond to the bioavailable amount of pollution and integrate contaminant exposure spatially, temporally, and across media, environmental risk should be assessed based on analysis of biota (Beyer et al. 1990, Talmage and Walton 1991, Abdul Rida and Bouché 1997). Plant analysis gives information on the bioavailability of elements in the rooting zone whereas small mammals respond to a larger area. Small mammals are often used as bio-indicators of pollution, residues
being determined in either whole body or in specific organs (Talmage and Walton 1991, Shore 1995).

The objectives of this chapter are to assess the uptake of cadmium and zinc by small mammals and willow trees on disposal sites for dredged material and to discuss the risk for these organisms. Comparative information is reported on Cd and Zn concentrations in small mammals, vegetation i.e. willow leaves, and NH₄OAc-EDTA extractable concentrations in soil. The risk on food-chain transfer was assessed using a biomagnification model. Stress was laid on Cd and Zn because these elements have been shown to be the most transferable heavy metals from soil to plants (Labrecque et al. 1995) and because these elements are taken up by the willows in excessive amounts.

**Materials and methods**

Three disposal sites with a different pollution degree were investigated. The sites were all hydraulically raised and were mainly covered with willow trees (*Salix fragilis* L.). The disposed material was dredged from freshwater rivers or canals. Stand history and characteristics of the disposal sites are shown in Table 3.1. Herbaceous vegetation did not exceed 10 % of soil surface, humus form taxa was mull. In the centre of each site, an experimental plot was defined of 20 by 20 m, covered with *S. fragilis* L.. In each of these plots, 12 soil samples from the upper layer (0-15 cm) were taken randomly and analyzed for pH(KCl), organic carbon and exchangeable Cd and Zn. Organic carbon was analyzed using the Walkley and Black method; exchangeable Cd and Zn were extracted using NH₄OAc-EDTA and determined with flame atomic absorption spectroscopy (FAAS).
Table 3.1. Stand history and characteristics of the disposal sites for dredged material

<table>
<thead>
<tr>
<th>Site</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface area (m²)</td>
<td>110,000</td>
<td>50,000</td>
<td>60,000</td>
</tr>
<tr>
<td>Origin of sediment</td>
<td>Canal broadening</td>
<td>Channel maintenance</td>
<td>Channel maintenance</td>
</tr>
<tr>
<td>Stand vegetation</td>
<td>Spontaneous willow</td>
<td>Planted willow</td>
<td>Planted willow and poplar</td>
</tr>
</tbody>
</table>

The soil at site 2 was sampled in detail to assess the variability of its chemical variables. 134 samples were collected from a grid at 0-15 cm depth and analyzed for pH(H₂O), pH(KCl), organic carbon and total Cd and Zn concentrations. Cd and Zn were extracted using HNO₃. To determine pH and organic carbon, the same methods were used as described above.

In the first week of September 1997, 12 trees were sampled in each of the experimental plots. Leaf samples were taken from the upper third of the crown, ashed, dissolved in HNO₃ and analyzed for Zn and Cd concentrations by FAAS.

During 12 days at the end of October 1997, 6 days at the beginning of January 1998 and 6 days at the beginning of March 1998 small mammals were collected using conventional snap traps baited with pieces of carrot and peanut butter. Reported average habitat areas of the trapped species amounted 2,250 m² for wood mice (*Apodemus sylvaticus* L.), 500 to 2,000 m² for bank voles (*Clethrionomys glareolus* Schreber) and 400 to 650 m² (max. 1,150 m²) for common shrew (*Sorex araneus* L.) (Lange et al. 1994). Because these habitat areas are smaller than the surface areas of the disposal sites (Table 3.1) it was assumed that the trapped small mammals fed mainly on the disposal sites.

The liver of the small mammals was removed as described by Talmage and Walton (1991) and Shore and Douben (1994). Both the livers and the rest of the small
mammals were destructed separately using HNO\textsubscript{3} as described by Niazi et al. (1993), the extract was analyzed for Cd and Zn by FAAS. Fresh weight (FW) concentrations were used because we considered this as having most toxicological and ecological relevance. To convert fresh weight concentrations to dry weight, Talmage and Walton (1991) used multiplication factors 3.4, 3.67 and 3.5 for the liver of respectively wood mouse, bank vole and common shrew, and 3.2, 3.16 and 3.12 for whole body concentrations of wood mouse, bank vole and common shrew.

The Cd concentrations of kidneys of tawny owl (\textit{Strix aluco} L.) and weasel (\textit{Mustela nivalis} L.) feeding on small mammals trapped on site 3 were calculated using the BIOMAG model as described by Gorree et al. (1995). The toxicological part of this model uses the concentrations in the food as an input and calculates the internal concentrations of the pollutant in organs of the target species known to accumulate this pollutant, e.g. the kidney or the liver. In the case of cadmium, the concentration in the kidney is calculated using a one-compartment model:

\[ Q(t) = dfi \cdot food \cdot \frac{c_{up}}{c_{out}} \cdot (1 - e^{-c_{out}t}) \cdot cf_{kidney} \]

Where \( Q \) is the concentration in the target organ; \( t \) the exposure time; \( dfi \) the daily food intake per gram DW; \( food \) the concentration in food of target animal; \( c_{up} \) an uptake constant; \( c_{out} \) an excretion constant; \( cf_{kidney} \) the concentration factor kidney/body.

Tawny owl and weasel were chosen because their diet contains predominantly small mammals. As a worst-case scenario, the highest concentrations in small mammals were used as model input: namely the Cd concentrations in January on site 3 and data of site 2 for common shrew. Input data that were necessary for modeling but that were not measured in this study, were gathered from Lowe (1980) and Gorree et al. (1995) and
are given in Table 3.2. As Tawny owl is a generalist predator, we assumed that its diet reflects the average abundancy of each specie as determined with the life traps.

Table 3.2. Input parameters for calculation of the internal concentrations of Cd in the kidneys of weasel and tawny owl

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Weasel</th>
<th>Tawny owl</th>
</tr>
</thead>
<tbody>
<tr>
<td>t</td>
<td>d</td>
<td>700</td>
<td>1780</td>
</tr>
<tr>
<td>dfi</td>
<td>g g⁻¹ DW d⁻¹</td>
<td>0.095</td>
<td>0.10</td>
</tr>
<tr>
<td>cup</td>
<td>-</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>cout</td>
<td>-</td>
<td>0.0035</td>
<td>0.0035</td>
</tr>
<tr>
<td>cfkidney</td>
<td>-</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>food</td>
<td>µg g⁻¹ DW</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% common shrew in diet</td>
<td></td>
<td>5.8</td>
<td>13.4</td>
</tr>
<tr>
<td>% bank vole in diet</td>
<td></td>
<td>85</td>
<td>13.4</td>
</tr>
<tr>
<td>% wood mouse in diet</td>
<td></td>
<td>9.7</td>
<td>73.2</td>
</tr>
</tbody>
</table>

Data were tested for normality and one-way analysis of variance was performed using non-parametric Tamhane post-hoc tests for multiple comparison. Outliers were not removed.

**Results**

The detailed sampling at site 2, given in Table 3.3, revealed that all soil parameters except pH varied strongly over the site. This confirms the expectation that the different sedimentation velocities created a heterogeneous soil. Under such heterogeneous soil conditions, the use of a biomonitor that covers a large area might be crucial to get an
integral idea of the pollution and the risk. Besides, organic carbon was significantly correlated with Cd ($R^2_{adj}=0.68; p<0.001$) and Zn concentration ($R^2_{adj}=0.88; p<0.001$).

Table 3.3. Soil characteristics based on the detailed sampling of site 2 (0-15 cm depth, n=134)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Mean</th>
<th>SD</th>
<th>CV (%)</th>
<th>Min.</th>
<th>Max.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Cd (mg kg$^{-1}$)</td>
<td>5.26</td>
<td>5.05</td>
<td>96</td>
<td>0.05</td>
<td>22.90</td>
</tr>
<tr>
<td>Total Zn (mg kg$^{-1}$)</td>
<td>528</td>
<td>365</td>
<td>69</td>
<td>1</td>
<td>1760</td>
</tr>
<tr>
<td>% C</td>
<td>2.8</td>
<td>1.9</td>
<td>67</td>
<td>0.1</td>
<td>5.6</td>
</tr>
<tr>
<td>% clay*</td>
<td>54.8</td>
<td>27.5</td>
<td>50</td>
<td>12.0</td>
<td>95.0</td>
</tr>
<tr>
<td>pH-H$_2$O</td>
<td>7.0</td>
<td>-.2/+.5</td>
<td>7</td>
<td>6.5</td>
<td>8.4</td>
</tr>
<tr>
<td>pH-KCl</td>
<td>6.9</td>
<td>-.2/+.5</td>
<td>7</td>
<td>6.5</td>
<td>7.5</td>
</tr>
</tbody>
</table>

All three experimental plots had a significantly different pH whereas the organic carbon content of the most polluted site was significantly higher compared to the least polluted sites (Table 3.4 and Fig. 3.1). Fig. 3.1 shows the Cd and Zn concentrations in the willow leaves, in the NH$_4$OAc-EDTA-soluble soil fraction and in small mammal tissue. The numbers of small mammals trapped are summarized in Table 3.5. To meet instrument requirements the limited amount of liver tissue that was dissolved in strong acid had to be diluted. This caused solution concentrations of Cd in the liver of wood mice and bank voles to be under quantification limit. The solution quantification limit corresponds with an average concentration in liver of 5.3 mg Cd kg$^{-1}$ FW. Therefore, whole body Cd concentrations represented in Fig. 3.1 do not include liver and are slightly underestimated.
Table 3.4. pH-KCl and percentage organic carbon (% C) on the experimental plots of
the three sites (0-15 cm depth, N=12) and an indication of homogeneous subsets for
p=0.05

<table>
<thead>
<tr>
<th>Site</th>
<th>Mean</th>
<th>SD</th>
<th>Homogeneous subsets</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH-KCl</td>
<td>1</td>
<td>7.4</td>
<td>-0.1 / +0.2</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>7.2</td>
<td>-0.1 / +0.2</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>6.7</td>
<td>-0.1 / +0.1</td>
</tr>
<tr>
<td>% C</td>
<td>1</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.6</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>2.8</td>
<td>0.8</td>
</tr>
</tbody>
</table>

The sites showed the same rank order of Cd or Zn concentration for all types of
analyses, i.e. soil, leaf, small mammal and liver. For each type of analysis, the
concentrations found on the three sites were mutually compared in Table 3.6. This
comparison showed that the mean difference between the sites is largest and most
significant, using leaf analysis. For the analyses of the small mammal species the
differences between the sites were mostly not significant.

No influence of capture period or sex was found on the Zn and Cd concentrations in
small mammal tissue. The weight of the small mammals had little influence on the Cd
concentration in the small mammals: as the weight of the small mammals increased,
the whole body Cd concentration slightly decreased. Nevertheless, the correlations
were very weak (R²<0.35) and not significant.

Cd concentrations in predators, calculated using the BIOMAG model, amounted to
4.55 mg kg⁻¹ DW for the kidneys of tawny owl and 6.37 mg kg⁻¹ DW for the kidneys of
weasel.
Fig. 3.1. Average Cd and Zn concentrations in soil extract using NH4OAc-EDTA at 0-15 cm depth, leaves of S. fragilis, liver and whole body concentrations of mice on the experimental plots of the sites. WM: wood mouse; BV: bank vole; CS: common shrew
### Table 3.5. Number of mice trapped on the experimental plots of the three sites

<table>
<thead>
<tr>
<th>Site</th>
<th>Wood mouse (<em>Apodemus sylvaticus</em>)</th>
<th>Bank vole (<em>Clethrionomys glareolus</em>)</th>
<th>Common shrew (<em>Sorex araneus</em>)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>28</td>
<td>-</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>23</td>
<td>6</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>71</td>
<td>13</td>
<td>13</td>
</tr>
</tbody>
</table>
Table 3.6. Comparison of the concentrations found on the three sites using the Tamhane post hoc test

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Site (I)</th>
<th>Site (J)</th>
<th>Cd Mean Diff. of I-J (mg kg⁻¹)</th>
<th>SD</th>
<th>p</th>
<th>Zn Mean Diff. of I-J (mg kg⁻¹)</th>
<th>SD</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil</td>
<td>1</td>
<td>2</td>
<td>-3.4</td>
<td>.25</td>
<td>.209</td>
<td>-33.3</td>
<td>11.0</td>
<td>.005</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>3</td>
<td>-1.90</td>
<td>.25</td>
<td>.000</td>
<td>-156.7</td>
<td>11.0</td>
<td>.000</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3</td>
<td>-1.56</td>
<td>.24</td>
<td>.000</td>
<td>-123.4</td>
<td>10.7</td>
<td>.000</td>
</tr>
<tr>
<td>Leaves</td>
<td>1</td>
<td>2</td>
<td>-2.57</td>
<td>.58</td>
<td>.000</td>
<td>-254.7</td>
<td>57.3</td>
<td>.000</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>3</td>
<td>-4.69</td>
<td>.58</td>
<td>.000</td>
<td>-544.4</td>
<td>57.3</td>
<td>.000</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3</td>
<td>-2.12</td>
<td>.58</td>
<td>.017</td>
<td>-289.7</td>
<td>57.3</td>
<td>.002</td>
</tr>
<tr>
<td>La wood mouse</td>
<td>1</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-2.2</td>
<td>1.2</td>
<td>.246</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-3.6</td>
<td>1.3</td>
<td>.017</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-1.4</td>
<td>1.2</td>
<td>.556</td>
</tr>
<tr>
<td>L bank vole b</td>
<td>1</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-4.3</td>
<td>1.7</td>
<td>.029</td>
</tr>
<tr>
<td>L common shrew b</td>
<td>1</td>
<td>2</td>
<td>-21.5</td>
<td>15.8</td>
<td>.200</td>
<td>-2.3</td>
<td>1.9</td>
<td>.278</td>
</tr>
<tr>
<td>WB wood mouse</td>
<td>1</td>
<td>2</td>
<td>-0.00</td>
<td>.03</td>
<td>.999</td>
<td>-1.5</td>
<td>1.5</td>
<td>.750</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>3</td>
<td>-0.03</td>
<td>.03</td>
<td>.755</td>
<td>-2.5</td>
<td>1.6</td>
<td>.299</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3</td>
<td>-0.04</td>
<td>.03</td>
<td>.517</td>
<td>-1.0</td>
<td>1.5</td>
<td>.843</td>
</tr>
<tr>
<td>WB bank vole b</td>
<td>1</td>
<td>3</td>
<td>0.00</td>
<td>.01</td>
<td>.992</td>
<td>-2.7</td>
<td>2.4</td>
<td>.279</td>
</tr>
<tr>
<td>WB common shrew b</td>
<td>1</td>
<td>2</td>
<td>-1.18</td>
<td>.61</td>
<td>.083</td>
<td>-4</td>
<td>2.6</td>
<td>.889</td>
</tr>
</tbody>
</table>

*a* Liver  
*b* student t-test because only two groups were available  
*c* Whole body
**Discussion**

*Salix* is a genus known for its high uptake of Cd and Zn (Landberg and Greger 1994). Typical background concentrations in terrestrial plants are 1-400 mg Zn kg\(^{-1}\) DW (Ross 1994, Nissen and Lepp 1997) and 0.2-0.8 mg Cd kg\(^{-1}\) DW (Ross 1994). Nissen and Lepp (1997) found values from 75 to 306 mg Zn kg\(^{-1}\) DW in the leaves of 8 *Salix* species on not-contaminated reference soils. For *S. fragilis* in particular, they found 82 mg Zn kg\(^{-1}\) DW. The leaves of the willows of site 1 contained 102 mg kg\(^{-1}\) DW Zn and 0.86 mg kg\(^{-1}\) DW Cd. This means that, from this respect, the experimental plot of site 1 can be considered as almost normal, while site 2 and 3 showed increased concentrations of Zn and Cd (Fig. 3.1). The disposed material of site 1 was indeed normal soil, from broadening the canal (Table 3.1), although it had been in contact with polluted water of the canal.

According to Talmage and Walton (1991) the three small mammal species i.e. wood mouse, bank vole and common shrew are suitable to be used as biomonitors. Compared to data compiled by Talmage and Walton (1991) on non-polluted sites (Table 3.7), Zn concentrations in liver and whole body tissue were rather low for all species. Even on the most polluted site (site 3) Zn concentrations were lower than background. The reason for this might be that the sites had a high pH in the range of 7 to 8, typical for dredged material in the studied region. According to Ullrich et al. (1999), there seems to be a threshold pH value for Zn of about 6 below which a significant increase in the exchangeable fraction was observed. Moreover, Zn is an essential element and can, to a certain extent, be regulated in living organisms by homeostatic mechanisms (Alloway and Ayres 1997).
Cd and Zn uptake in small mammals and willow leaves

Table 3.7. Cd and Zn concentrations in mice trapped on reference sites by Talmage and Walton (1991), data are converted to fresh weight (mg kg⁻¹ FW)

<table>
<thead>
<tr>
<th></th>
<th>Cd</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Whole body</td>
</tr>
<tr>
<td>Wood mouse</td>
<td>0.15-0.26</td>
<td>0.09</td>
</tr>
<tr>
<td>Bank vole</td>
<td>-</td>
<td>0.06</td>
</tr>
<tr>
<td>Common shrew</td>
<td>0.83-7.26</td>
<td>0.38-1.28</td>
</tr>
</tbody>
</table>

Cd concentrations in small mammals were high compared to literature data (Table 3.7). This indicates that Cd was available for the small mammals on all sites, resulting in an elevated tissue concentration. Cd is relatively labile, even in slightly alkaline soils with a pH range of 7-8 (Ullrich et al. 1999). Especially the Cd concentrations in common shrew were very high, a phenomenon that is also reported by other studies. The different food habits of this species are said to be the major cause (Hunter et al. 1987, Talmage and Walton 1991, Ross 1994). The diet of common shrew consists mainly of invertebrates, insects and molluscs while wood mice and bank voles are mainly herbivores (Lange et al. 1994).

Although the small mammals took up Cd, the BIOMAG model did not predict accumulation in the food chain. Gorree et al. (1995) stated that the no-effect concentration of Cd in kidneys of predators is about 150 mg kg⁻¹ DW, a lot higher than the calculated values of 21.9 mg kg⁻¹ DW in tawny owl and 6.37 mg kg⁻¹ DW in weasel.

The analyses of small mammals, soil and leaves all indicated that site 1 had lowest pollution degree for Cd and Zn while site 3 had highest. This indicated a correlation
between soil concentration and concentrations in plants and animals. Shore (1995) reviewed studies on Cd, Pb and F residues in small mammals and found a correlation between total soil Cd concentration and Cd residues in liver of common shrew (n = 10; R² = 0.671; p<0.005). When applied to the data on the total concentration of site 2, the regression of Shore predicted a Cd concentration in the liver of common shrew of 66.3 mg kg⁻¹ FW. This is surprisingly close to the measured concentration of 68.3 mg kg⁻¹ FW. The regression will probably be less accurate for site 2 and 3. Because no total concentrations were measured on this site, this cannot be verified exactly.

Willow leaf analysis showed the biggest and most significant differences in Cd and Zn concentration (Table 3.6) compared to analyses of soil and small mammals. The NH₄OAc-EDTA extractable Cd and Zn concentrations in the soil fraction were measured because this was thought to be a good measure for the bioavailable amount in the soil. This method was slightly less sensitive while small mammal analysis could hardly detect any differences between the sites. However, analyses of small mammals give a better overall view because of the larger area that small mammals cover.

**Conclusion**

Three sites were selected with different soil concentrations of Cd and Zn. Cd levels, but not Zn, were elevated in small mammals compared with background levels, but there were no significant differences between sites in Cd or Zn levels in animals. In contrast, there was inter-site variation in contaminant levels in soil and in willow leaves. Small mammals were not found to accumulate Zn, probably because of the effect of homeostasis. Cd, on the other hand, was clearly accumulated, even on the site where soil and leaf analysis did not indicate a pollution problem.
The calculations using the BIOMAG model indicated that the Cd in the soil of the investigated disposal facilities probably caused low risk for predators. However, the elevated Cd and Zn concentrations in the willow leaves on site 2 and 3 and the high Cd concentrations in the small mammals on all the sites indicated that ecosystem development should be considered carefully.

Acknowledgements

The authors would like to thank the Flemish government for giving permission to trap shrews (Soricidae spp.).
Chapter 4
Redistribution of Cd and Zn in the upper soil layer after 10 years of poplar growth

Abstract

Biota can play a significant role in metal cycling. We determined the metal content in soil and biomass compartments in a 10-year-old poplar plantation on a disposal site for dredged material. Because of the strong texture gradient, two plots with different soil texture were sampled. In both plots, the poplars took up large amounts of Cd and Zn (8.1 mg Cd kg\(^{-1}\) DM and 475 mg Zn kg\(^{-1}\) DM in the fresh leaves). When the estimated concentrations of the decomposed litter were higher than the soil concentrations, the concentrations in the upper soil layer increased due to redistribution of the metals via litter fall. This occurred for Cd and Zn on the least polluted part of the site and the increase even caused the Cd concentrations for this horizon to exceed the legislative limits. Risk assessment should not only account for the concentrations in the soil, but also for the amounts of metals in the biota and the redistribution caused by the biota.
Introduction

Dredged material from Western European rivers is often contaminated with metals and other pollutants. Because this contamination limits its possible uses, the dredged sediment is often dumped on land in confined disposal facilities. Afforestation is a common restoration technique for such sites (Luyssaert et al. 2001, Vandecasteele et al. 2002). Most poplars (Populus spp.) and willows (Salix spp.) are well adapted to the sediment-derived soils and are often used for afforestation. Both genera are well-known accumulators of Cd and Zn in aboveground biomass compartments (Pulford and Watson 2003, Mertens et al. 2004, Vandecasteele et al. 2004b) and are said to be suited for soil sanitation by phytoextraction of soil Cd (Meers et al. 2005, Dickinson and Pulford 2005). Nevertheless, the high uptake of Cd and Zn might pose a risk for the dispersal of these metals to the food web (Mertens et al. 2004).

Accumulation of metals in leaves causes recycling in the food web through litter fall and litter decomposition (Kuzovkina and Quigley 2005). Besides the uptake of metals, tree growth has the potential to change the solubility and availability of metals in the soil through root respiration, root exudation, the formation and decomposition of organic matter, the stimulation of soil life and acidification of the soil (Hinsinger 2000, Vervaeke et al. 2004). These changes in solubility and availability can result in a redistribution of the metals in the soil-tree system.

Many studies on metal fluxes and dynamics report laboratory experiments (e.g. Tack et al. 1996, Marseille et al. 2000, Han et al. 2003, Ekvall and Greger 2003). These controlled experimental results need to be tested and validated in the field over extended periods of time, because the behavior of chemical contaminants in soils involves numerous processes which operate simultaneously and vary continuously over time (Hesterberg 1998). The aim of our study is to describe the distribution of trace
metals, especially Cd and Zn, between different compartments of the soil-tree system of a 10-year-old poplar plantation on dredged sediment. Especially the soil distribution of metals after 10-years of poplar growth is focused on.

**Materials and methods**

**Description of the site**

Two sample plots were established in March 2003 on a 280 m long and 20 m wide disposal site for hydraulically raised dredged river sediments. The site is located in Menen (Belgium) and was raised with sediments from the river Leie up to 2 m thick. It was planted with 2-year-old rooted poplar cuttings (*Populus interamericana* ‘Boulare’ and ‘Beaupré’) in the spring of 1993, 1 year after disposal. The trees were planted following a 2 x 2 m square design.

Hydraulic raising of a sediment disposal site causes a texture gradient to be established across the site. Coarse particles settle near the pipe exhaust, while silt and clay settles further away from the inlet. Along this gradient, two sample plots were established with different soil texture, one at the beginning of the site, near the inlet (further called “inlet plot”) and one at the end of the site, near the outlet (further called “outlet plot”). The plots were rectangular (25 m x 20 m) and the distance between the two plots was 215 m.

**Soil characterization**

Soil samples were collected on both sampling plots in March 2003, 10 years after planting. At that time, no litter layer was distinguished and soil samples were taken
from 3 soil horizons. The upper sampling horizon (H₁) was about 3 cm thick and had a marked darker color than the soil underneath. This layer consisted of mineral soil intensely mixed with organic material. The second horizon sampled (H₂) was located at about 3-20 cm depth. This layer was well-structured and aggregated and had a lighter color than H₁. The third horizon (H₃) was situated beneath H₂ and was less structured and less rooted and was sampled at 40 to 50 cm depth. Four replicates were taken in each sampling plot. Each plot was divided in 4 quadrants. In the centre of each quadrant, soil samples were collected with an auger in triplicate within a 2 m radius around each centre point and then pooled for each horizon.

Soil samples were dried at 40°C in a forced air oven to a constant weight. Aggregates were broken and the soil was passed through a 2 mm sieve. Total nitrogen content was analyzed using the Kjeldahl method (Bremner 1996). The pH(KCl) was measured in a 1/5 soil/KCl solution (1 mol l⁻¹) using an ion-specific electrode (Loeppert and Suarez 1996). Soil texture was measured using the conventional pipette method (Labex 8903-11-2-1). Loss on ignition (LOI) was determined after ashing during 4 h at 450°C and weighing the residue.

Pseudo- total Cd, Cr, Cu, Pb, and Zn concentrations were determined by digesting the sediment in an aqua regia solution (3 g sample in 21 ml HCl, 12 mol l⁻¹, and 7 ml HNO₃, 15.8 mol l⁻¹) under reflux for 2 hours (ISO 11466), and then analyzing the solution by flame atomic absorption spectrophotometry (Varian SpectrAA 220).

**Biomass**

The aboveground biomass (DM stem wood, twigs and leaves) was estimated for both plots based on stem diameters at 1.3 m height using the biomass equations of Johansson et al. (1999) constructed for stands of *Populus tremula* L. in Sweden.
In each sampling plot, four healthy trees with average diameter were selected for sampling, one in each quadrant. Wood samples were obtained by augering small cores with a Pressler borer at 1.3 m height and bark was sampled with a fine knife (March 2003). Leaves were collected from the upper third of the crown using telescopic pruning scissors in August 2003. The leaf samples were not rinsed before drying because the total leaf concentration was assumed to be relevant for the eventual changes in soil concentration. The wood, bark and leaf samples were dried at 70°C in a forced air oven to constant weight, subsequently milled and sieved and 0.5 g sample was digested in 2 ml concentrated HClO₄ (11.6 mol l⁻¹) and 12 ml concentrated HNO₃ (14 mol l⁻¹) during 2.5 hrs on a heating block. Wood and bark solutions were analyzed for Cd, Cr, Cu, Pb and Zn by flame atomic absorption spectrophotometry (Varian SpectrAA 220), leaf solutions were analyzed with inductively coupled plasma atomic emission spectroscopy (ICP/OES - Perkin-Elmer OPTIMA 3300 DV).

**Data analysis**

Means of soil layers were compared using the non-parametric Mann-Whitney U test. Following Moran (2003), the p-values were not corrected for multiple comparisons.

**Results**

The plots were selected on both ends of the dredged sediment disposal site (10 m away from the edge) to get an idea of metal compartmentalization over the entire site (Table 4.1). The soil of the inlet plot contained more than 85 % sand while silt and clay dominated the texture of the outlet plot. The top layer of the soil was most affected by biological activity. This was reflected in slight but significantly lower pH-values in the
Cd and Zn redistribution in the soil under poplar after 10-years

upper horizon (H1) than in the deeper horizons, this difference being more pronounced in the inlet plot. The soil LOI and N concentration were for both plots markedly higher in the upper horizon compared to deeper layers.

Total biomass of the inlet plot was estimated to be 81 ton wood ha\(^{-1}\) (stems and twigs) and 2.2 ton DM leaves ha\(^{-1}\). Higher production was observed on the more clayey outlet plot, estimated at 179 ton wood ha\(^{-1}\) and 3.3 ton DM leaves ha\(^{-1}\).

Table 4.1. Soil texture (%), pH-KCl and nitrogen concentration (mg kg\(^{-1}\) DM) in the three soil horizons (n=4), *: the value is significantly different from the value of H3 at p<0.05 (H1=0-3 cm depth; H2=3-10 cm depth; H3=40-50 cm)

<table>
<thead>
<tr>
<th>Horizon</th>
<th>&lt; 2 µm</th>
<th>2-50 µm</th>
<th>&gt; 50 µm</th>
<th>pH-KCl</th>
<th>LOI</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inlet</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H1</td>
<td>6 ± 2</td>
<td>8 ± 3</td>
<td>86 ± 4</td>
<td>7.1 ± 0.1 *</td>
<td>6.2 ± 2.4 *</td>
<td>2185 ± 735 *</td>
</tr>
<tr>
<td>H2</td>
<td>4 ± 1</td>
<td>5 ± 1</td>
<td>91 ± 2</td>
<td>7.4 ± 0.1 *</td>
<td>1.6 ± 0.3 *</td>
<td>528 ± 127 *</td>
</tr>
<tr>
<td>H3</td>
<td>4 ± 1</td>
<td>7 ± 1</td>
<td>89 ± 2</td>
<td>7.6 ± 0.1</td>
<td>1.1 ± 0.1</td>
<td>338 ± 42</td>
</tr>
<tr>
<td><strong>Outlet</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H1</td>
<td>39 ± 7</td>
<td>53 ± 5</td>
<td>8 ± 4</td>
<td>6.9 ± 0.1 *</td>
<td>19.2 ± 3.1 *</td>
<td>7347 ± 1108 *</td>
</tr>
<tr>
<td>H2</td>
<td>39 ± 22</td>
<td>51 ± 18</td>
<td>10 ± 6</td>
<td>7.1 ± 0.1</td>
<td>10.7 ± 1.3</td>
<td>3613 ± 423</td>
</tr>
<tr>
<td>H3</td>
<td>28 ± 11</td>
<td>63 ± 9</td>
<td>10 ± 5</td>
<td>7.1 ± 0.0</td>
<td>10.8 ± 1.0</td>
<td>3461 ± 363</td>
</tr>
</tbody>
</table>

The Cr, Cu and Pb concentrations in the biomass compartments (Table 4.2) were in the range of reference values compiled from different literature references (see Table 1.5). The Cd and Zn concentrations on the other hand were high compared to these reference values (Cd up to 30 times higher), but comparable to results of former studies on poplars on dredged sediment (Mertens et al. 2004, Vandecasteele et al. 2003). When white poplar was compared to other tree species on a site for dredged material, it took
up elevated amounts of Cd and Zn while the other tree species did not show abnormal concentrations in leaves or wood (Mertens et al. 2004).

<table>
<thead>
<tr>
<th></th>
<th>Cd</th>
<th>Cr</th>
<th>Cu</th>
<th>Pb</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inlet</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>bark</td>
<td>7.4 ± 1.3</td>
<td>(&lt; 5.3)</td>
<td>(&lt; 6.6)</td>
<td>11.7 ± 2.2</td>
<td>294 ± 30</td>
</tr>
<tr>
<td>wood</td>
<td>3.4 ± 0.7</td>
<td>9.6 ± 1.2</td>
<td>(&lt; 6.7)</td>
<td>(&lt; 9.3)</td>
<td>68 ± 18</td>
</tr>
<tr>
<td>leaf</td>
<td>6.9 ± 2.7</td>
<td>1.7 ± 0.3</td>
<td>10.9 ± 0.8</td>
<td>6.5 ± 1.7</td>
<td>475 ± 129</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Cd</th>
<th>Cr</th>
<th>Cu</th>
<th>Pb</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Outlet</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>bark</td>
<td>8.1 ± 1.8</td>
<td>(&lt; 5.1)</td>
<td>(&lt; 6.5)</td>
<td>(&lt; 9.1)</td>
<td>227 ± 47</td>
</tr>
<tr>
<td>wood</td>
<td>3.8 ± 0.5</td>
<td>12.1 ± 10.1</td>
<td>(&lt; 6.7)</td>
<td>(&lt; 9.3)</td>
<td>56 ± 10</td>
</tr>
<tr>
<td>leaf</td>
<td>8.1 ± 0.9</td>
<td>1.6 ± 0.6</td>
<td>10.9 ± 1.2</td>
<td>4.8 ± 0.5</td>
<td>402 ± 95</td>
</tr>
</tbody>
</table>

On the inlet plot, total soil concentrations of Cd and Zn in H1 were significantly higher than in the deeper horizons (Table 4.3). For the other elements on the inlet plot and for all elements on the outlet plot, no significant differences were found between the soil horizons.

Estimates of the metal amounts in soil, wood and leaves are presented in Table 4.4. The amount of metals in the soil was calculated using a density of 1300 kg m⁻³ and a depth of the rooted zone of 0.5 m. Amounts in the biomass compartments were calculated by multiplying the amount of wood or leaves with the corresponding biomass concentrations.
Cd and Zn redistribution in the soil under poplar after 10-years

Table 4.3. Concentrations of metals in the different soil layers (mg kg\(^{-1}\) DM) and loss on ignition (LOI; %) (*: the value is significantly different from the value of H\(_3\) at p<0.05; H\(_1\)=0-3 cm depth; H\(_2\)=3-10 cm depth; H\(_3\)=40-50 cm)

<table>
<thead>
<tr>
<th></th>
<th>Cd</th>
<th>Cr</th>
<th>Cu</th>
<th>Pb</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inlet</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H(_1)</td>
<td>2.7 ± 0.9(^a) *</td>
<td>44 ± 6</td>
<td>47 ± 5</td>
<td>91 ± 13</td>
<td>397 ± 66 *</td>
</tr>
<tr>
<td>H(_2)</td>
<td>1.1 ± 0.1  *</td>
<td>39 ± 5</td>
<td>44 ± 5</td>
<td>74 ± 6</td>
<td>261 ± 9</td>
</tr>
<tr>
<td>H(_3)</td>
<td>0.9 ± 0.1</td>
<td>41 ± 7</td>
<td>54 ± 11</td>
<td>82 ± 11</td>
<td>246 ± 37</td>
</tr>
<tr>
<td><strong>Outlet</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H(_1)</td>
<td>15.5 ± 2.8(^a)</td>
<td>215 ± 30(^a)</td>
<td>175 ± 33</td>
<td>300 ± 38</td>
<td>1404 ± 219(^a)</td>
</tr>
<tr>
<td>H(_2)</td>
<td>15.1 ± 2.1(^a)</td>
<td>236 ± 25(^a)</td>
<td>182 ± 21</td>
<td>340 ± 37(^a)</td>
<td>1510 ± 185(^a)</td>
</tr>
<tr>
<td>H(_3)</td>
<td>15.7 ± 2.1(^a)</td>
<td>237 ± 23(^a)</td>
<td>174 ± 17</td>
<td>341 ± 35(^a)</td>
<td>1532 ± 175(^a)</td>
</tr>
</tbody>
</table>

\(^a\): the concentration is higher than the limit for area for nature conservation purposes (VLAREBO 1998)

Table 4.4. Amounts of metals (g ha\(^{-1}\)) in the standing wood biomass, leaf biomass and the upper 0.5 m soil

<table>
<thead>
<tr>
<th></th>
<th>Cd</th>
<th>Cr</th>
<th>Cu</th>
<th>Pb</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>inlet</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>soil</td>
<td>6500</td>
<td>260000</td>
<td>318500</td>
<td>507000</td>
<td>1647750</td>
</tr>
<tr>
<td>wood</td>
<td>282.2</td>
<td>796.8</td>
<td>556.1</td>
<td>771.9</td>
<td>5644</td>
</tr>
<tr>
<td>leaf</td>
<td>15.2</td>
<td>3.7</td>
<td>24.0</td>
<td>14.3</td>
<td>1045</td>
</tr>
<tr>
<td><strong>outlet</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>soil</td>
<td>100100</td>
<td>1537250</td>
<td>1157000</td>
<td>2213250</td>
<td>9886500</td>
</tr>
<tr>
<td>wood</td>
<td>600.4</td>
<td>1896</td>
<td>1058.6</td>
<td>1469.4</td>
<td>8848</td>
</tr>
<tr>
<td>leaf</td>
<td>30.0</td>
<td>5.9</td>
<td>40.3</td>
<td>17.8</td>
<td>1487</td>
</tr>
</tbody>
</table>
**Discussion**

Soil characteristics were expected to be more or less homogeneous over the soil profile depth because intense mixing occurred within the sampled profile (only 50 cm) at the time of hydraulically raising (see also Vandecasteele et al. 2005a). This is reflected in the similarity in soil texture with depth (Table 4.1) and in the concentrations of Cr, Cu and Pb (Table 4.3). Yet, Cd and Zn concentrations in the upper soil layer (H1) on the inlet plot were significantly higher than in the deeper horizons. The latter observation may suggest that within the past 10 years, a significant redistribution of Cd and Zn in the soil profile of the inlet plot has occurred, leading to an increase of the concentration of Cd and Zn in the upper soil layer (H1) and/or a decrease of the concentrations in the deeper layers. The question is why a redistribution was found for Cd and Zn, and solely on the inlet plot?

A decrease of Cd and Zn concentrations in the deeper soil layers might be caused either by leaching or uptake by plant roots. Significant leaching of metals from these dredged sediment derived soils is not expected. These sediments are highly buffered against acidification due to the presence of high amounts of CaCO3 (Singh et al. 2000, Luyssaert et al. 2001, Cappuyns et al. 2004). Leaching tests performed on similar dredged sediment derived soils led to the conclusion that metal migration would be very small and of no environmental concern (Tack et al. 1998, Tack et al. 1999). Additionally, leaching would cause a depletion of metal concentrations in the top layer, as pH in this layer was lowest. Uptake is an other process that might have decreased the soil concentration, but as the amounts of metals that are taken up in the biomass compartments are small compared to the amount of metals in the soil, uptake will have had limited effect. Because of the rooting depth of poplar, uptake is expected to occur throughout the sampled profile (0-50 cm). Uptake and leaching thus eventually would lead to a slight decrease over the sampled profile. An accumulation of metals in the top
layer, however, must be attributed to significant amounts of these metals in litter. High amounts of metals are taken up from the soil, are transported to the canopy and are recycled to the forest floor by litter fall. The litter decomposes and will be mixed with the top soil by the biota and by soil perturbation.

But is it possible that this process results in redistribution of metals in the soil profile, only on the inlet plot and not on the outlet plot? In fact there are two main conditions that have to be fulfilled before the concentration of a given soil layer can increase. The first condition is obviously that the total amount of input metals in this top layer is greater than the output by leaching and uptake. This was not measured but the fact that redistribution occurred on the inlet plot suggests that on this plot this input flux was indeed higher than the output flux. The second condition is that the concentration of the input material must be higher than the concentration in the soil. The litter decomposes and will be mixed with the top soil. The concentration in this top layer can only increase if the concentration in the decomposed litter is higher than the concentration in the soil. To calculate the degree of decomposition of the litter, we used the single exponential decay model (Olson 1963; Wieder and Lang, 1982):

\[ W_t = W_0 e^{-kt} \]  

In which \( W_t \) and \( W_0 \) are litter masses at time \( t \) and time \( 0 \); \( k \) is the decomposition constant (yr\(^{-1}\)). Taylor (1989) found \( k \)-values of 0.191 for *Populus tremuloides*. Based on the model and supposing a constant litter production over the period of 10-years, it was calculated that about 58% of the litter was decomposed at the time of sampling. Berg et al. (2003) found that in boreal area the decomposition of poplar leaves (*Populus tremula*) proceeds to a certain ‘limit value’ (=fraction of litter that does not decompose) of 55% of the dry weight after three years (Berg et al. 2003). Assuming 42% of the litter remaining, metal concentrations will at least double during decomposition from fresh leaves to decomposed litter. Thus, Cd and Zn concentrations of the decomposed litter material would become in the order of 15 mg Cd kg\(^{-1}\) and 800
mg Zn kg\(^{-1}\) dry weight. These concentrations are significantly higher than the soil concentration levels of the inlet plot. On the outlet plot, the Cd concentrations in the litter are in the same order of magnitude as the soil, and for Zn they are below soil concentration levels.

The soil concentration in H\(_1\) was estimated with a simple model calculation to verify our hypothesis that leaf concentrations might determine metal concentrations in the top soil. The topsoil H\(_1\) is assumed to consist of original sediment mixed with decomposed litter. The concentration of this soil layer can be calculated from the amount of metals in this original sediment and in the decomposed litter that are present in the topsoil as shown by formula 1, assuming that there was no output from H\(_1\) through leaching or uptake and no input through aerial deposition. The amount of metals in the decomposed litter is equal to the amount of metals in the litter that has been produced.

\[
C_{H1} = \frac{(C_H \times W_s + C_{leaf} \times W_{leaf} \times T)}{W_{H1}}
\]  

(4.2)

Where \(C_{H1}\) is the concentration in H\(_1\); \(C_H\) is the concentration of the original soil, at the start of the experiment; \(W_s\) is the weight of the mineral soil in H\(_1\); \(C_{leaf}\) is the concentration in the leaves; \(W_{leaf}\) is the weight of leaves that is produced annually per unit area; \(T\) is the time trees have been growing in years. The weight of the mineral soil in H\(_1\) is the weight of H\(_1\) minus the weight of the decomposed litter in this soil layer:

\[
W_s = W_{H1} - W_{DL}
\]  

(4.3)

Where \(W_{H1}\) is the weight of the upper soil layer per unit area; \(W_{DL}\) is the weight DM of the decomposed litter in H\(_1\) per unit area.

\(C_{H1}\) cannot be calculated exactly as we do not know to what extent the leaves have been decomposed, we do not know the proportion of litter and proportion of soil in H\(_1\) and we also do not know the exact depth to which the litter is mixed in the soil. Several
assumptions had to be made: annual litter fall and leaf concentrations were assumed to be constant over the past 10 years, and all the decomposed litter was distributed in the upper 3 cm of the soil profile. We assumed $C_H$ to be best estimated by the concentration in $H_3$. The soil density was assumed to be 990 kg m$^{-3}$ on the inlet plot and 630 kg m$^{-3}$ on the outlet plot as calculated with the pedotransfer function given by Harrison and Bocock (1981) for topsoils. This function calculates the density based on LOI (data in Table 4.1). $W_{DL}$ was assumed to be 42% of the amount of leaves (modeled by formula 4.1) and $T$ was 10 years. Leaf biomass estimates were low compared to other data on poplar plantations on fertile soils. We also calculated $C_{HI}$ using leaf biomass production of 4.8 ton DM ha$^{-1}$ y$^{-1}$ as was measured by Deckmyn et al. (2004) in a plantation of 3 years old. The results of the calculations are presented in Table 4.5.

Calculated concentrations in $H_1$ were generally slightly underestimated as compared to the measured concentrations (Table 4.5). Nevertheless, the calculation also results in elevated concentrations of Cd and Zn in $H_1$ of the inlet plot and little differences for the other elements and on the outlet plot. In spite of the limitations and simplifications, the results of the calculations suggest that high leaf concentrations can result in increased surface soil concentrations, depending on the soil conditions.

It cannot be excluded that aerial deposition also contributed to elevated Cd and Zn concentrations in the leaves. Although it can be assumed that the major fraction originated from uptake from the soil. High uptake of Cd and Zn from the soil by poplars has been reported before and was related to the soil concentration (see e.g. Vandecasteele et al. 2003, Madejon et al. 2004). When white poplar was compared to other tree species on a site for dredged material, it took up elevated amounts of Cd and Zn while the other tree species did not show abnormal concentrations in leaves or wood (Mertens et al. 2004). Total atmospheric deposition of Cd and Zn in forests in 1994 in the Netherlands, was estimated to be averagely 1.2 g Cd ha$^{-1}$ y$^{-1}$ and 41 g Zn ha$^{-1}$ y$^{-1}$ (Bronswijk et al. 2003). This is low compared to the amounts of metals in the leaves (Table 4.4).
Chapter 4

Table 4.5. Calculated concentrations in H₁ (mg kg⁻¹)

<table>
<thead>
<tr>
<th>Leaf biomass (ton DM ha⁻¹)</th>
<th>Cd</th>
<th>Cr</th>
<th>Cu</th>
<th>Pb</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inlet</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial conc.</td>
<td>0.9</td>
<td>41</td>
<td>54</td>
<td>82</td>
<td>246</td>
</tr>
<tr>
<td>Calculated</td>
<td>2.2a</td>
<td>1.4</td>
<td>40</td>
<td>53</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>2.2a</td>
<td>2.0</td>
<td>38</td>
<td>52</td>
<td>77</td>
</tr>
<tr>
<td>Measured after 10 years</td>
<td>2.7 ± 0.9</td>
<td>44 ± 6</td>
<td>47 ± 5</td>
<td>91 ± 13</td>
<td>397 ± 66</td>
</tr>
<tr>
<td><strong>Outlet</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial conc.</td>
<td>15.7</td>
<td>237</td>
<td>174</td>
<td>341</td>
<td>1532</td>
</tr>
<tr>
<td>Calculated</td>
<td>3.3a</td>
<td>16.0</td>
<td>220</td>
<td>163</td>
<td>316</td>
</tr>
<tr>
<td></td>
<td>4.8b</td>
<td>16.1</td>
<td>212</td>
<td>158</td>
<td>306</td>
</tr>
<tr>
<td>Measured after 10 years</td>
<td>15.7 ± 2.1</td>
<td>237 ± 23</td>
<td>174 ± 17</td>
<td>341 ± 35a</td>
<td>1532 ± 175</td>
</tr>
</tbody>
</table>

a: As calculated using the biomass equation of Johansson et al. (1999)
b: As reported by Deckmyn et al. (2004) for 3-year-old poplar stands

If atmospheric deposition is also added to the upper soil layer H₁, equation (4.2) can be extended to:

\[ C_{H1} = (C_{H1}^* (W_{H1} - W_{DL}) + C_{leaf}^* W_{leaf}^* T + AD^* T) / W_{H1} \]  (4.4)

where AD is annual atmospheric deposition per unit area. When the atmospheric deposition data of Bronswijk et al. (2003) were entered in this equation, the metal concentrations in H₁ increased with no more than 0.04 mg kg⁻¹ Cd and 1.4 mg kg⁻¹ Zn on the inlet plot, and 0.06 mg kg⁻¹ Cd and 2.2 mg kg⁻¹ for Zn.
An increase of the Cd concentration in the upper soil layer was thought to be possible on a great part of the site. The Cd concentrations in the decomposed litter (=concentration in the leaves / limit value) were about equal to the soil concentration in the outlet plot. As the soil concentrations of the site decreased from the outlet plot towards the inlet plot, the concentration in the decomposed litter might be higher than the soil concentration on most part of the plots. An increase of the Cd concentration in the upper soil layer might therefore be possible on a great part of the site. This will of course depend on the (unknown) output fluxes due to root uptake and leaching.

**Implications for risks and management**

The accumulation of Cd and Zn in the top layer as a result of cycling through plant uptake and leaf fall may increase ecotoxicological risks towards organisms living in these soils. Elevated concentrations of Cd were found in small mammals living on dredged sediment sites covered with willow (Mertens et al. 2001). Willow spp give evidence of comparable uptake characteristics as poplars and cadmium was thought to be introduced into the food web by willow litter fall. It seems therefore that this increase of metal concentrations in the upper soil layer should be avoided. Poplar spp thus appear not suited for introduction on contaminated dredged sediment-derived soils. Maple (Acer pseudoplatanus), alder (Alnus glutinosa), ash (Fraxinus excelsior) and black locust (Robinia pseudoacacia) did not take up elevated amounts of metals on dredged sediment (Mertens et al. 2004) and seem better suited to be planted on these sites.

Although the observed increase of Cd and Zn in H1 was limited to a restricted soil layer (only 3 cm thick) and limited to a part of the site, the results of this investigation may have consequences for the practical management of polluted sites. It seems for example that the accumulation of Cd and Zn can lead to an exceedance of the legislative limits.
Observed concentrations in H1 on the inlet plot exceeded limits for soils in use for agriculture or nature conservation areas according to the Flemish Decree on Soil Sanitation (VLAREBO 1996). The concentrations in the deeper horizons did not exceed these limits, indicating that original concentrations in the soil profile might have been lower than legislative limits (limit for Cd: 1.6 mg kg⁻¹). Exceedance of the limits means that according to Flemish legislation, the polluted soil layer has to be remediated. The relevancy for this site is limited as a large part of the site will have exceeded the limits from the beginning, but it shows that, depending on site characteristics and legislative limits in force, poplar growth might cause a site to exceed legislative limits.

The accumulation in the surface soil might also arise when willows or poplars are used for phytoextraction of metals without annual harvesting of the leaves. Dickinson and Pulford (2005) state that clean-up of Cd-contaminated land can be achieved through cultivation and harvest of selected clones of short-rotation coppice willow within a realistic crop lifecycle. This clean-up is only possible at sites with low-level contamination of Cd (Klang-Westin & Eriksson 2003, Dickinson and Pulford 2005). For these slightly polluted sites, the leaf concentration will be most probably higher than the soil concentration. Therefore, although the Cd concentration over the entire soil profile will decrease, the concentration of Cd in the surface soil layer might increase, depending on the output fluxes (leaching and uptake). It is therefore very important that leaf litter is annually harvested, as was also stated by Dickinson and Pulford (2005). On the other hand, accumulation in a small soil layer might constitute a strategy for subsequent clean-up with traditional methods as only the upper thin soil layer will have to be removed.

Legislative limits and risk assessment are often based on total concentrations in the soil. However, this investigation shows that it should also account for the concentrations in the biota and the redistribution caused by the biota. The analysis of soil and biomass samples is an important tool for risk assessment, but these samples are
only random samples at one moment in time. The metal distribution in the soil horizon might contain more integrated information over a longer time period and is a result of all the processes that took place in the past. This investigation shows that risk assessment should account for the role of plants in metal cycling and the wider ecotoxicological implications of this redistribution process should be investigated.

**Conclusion**

Depending on the site characteristics, accumulation of Cd and Zn in the leaves of poplar may cause accumulation of Cd and Zn in the upper soil horizon, resulting in concentrations exceeding legislative limits. Increase of the soil concentrations are possible when the concentrations in decomposed litter are higher than the soil concentrations and when the input flux of metals is higher than the output flux by leaching and uptake. This study revealed again that poplars might not be suited for planting on sites for dredged material. Other species that do not accumulate metals in their aboveground biomass such as maple, ash, Robinia and alder will have to be chosen. When poplars (or willows) are used for phytoextraction, leaf harvesting might be indispensable.

**Acknowledgements**

The authors are grateful to Elisabet Vila de Abadal-Castilla, Luc Willems and Greet De bruyn for their technical support.
Chapter 5

Tree species effect on the redistribution of soil metals after 33-years of tree growth

Abstract

Phytostabilization of metals in polluted soils using trees is often promoted although it is not clear what influence different tree species have on the mobilization of soil metals. Four tree species (*Quercus robur, Fraxinus excelsior, Acer pseudoplatanus, Populus ‘Robusta’*) were planted in a monoculture plot experiment on a site for dredged sediment and soil and biomass compartments were sampled 33-years after planting. Poplar took up high amounts of Cd and Zn and this was associated with increased Cd and Zn concentrations in the upper soil layer. The other species contained normal concentrations of Cd, Cu, Cr, Pb and Zn in their tissues. Oak acidified the soil more than the other species and acidification caused a decrease of the concentration of metals in the upper soil layer due to leaching. The pH under poplar was lower than expected and associated with high carbon concentrations in the top soil. This might possibly be assigned to retardation of the litter decomposition due to the elevated concentrations of Cd and Zn in the litter and upper soil layer.
Tree species effect after 33-years

Introduction

Metal polluted sites for dredged sediments are mostly set aside. These sites are often afforested or develop spontaneously into woodland. Growth of trees might induce several changes in the soil characteristics, and forests showed to play an important role in metal cycling and partitioning (Watmough et al. 2005, Andersen et al. 2004).

Trees are often postulated to be suited for phytostabilization, as they might stabilize metals in the soil. The vegetation cover and root growth will protect the soil surface from dispersal by wind and water erosion (Eviner & Chapin 2003). Elevated evapotranspiration in forests reduces the flow of water through the soil and therefore might reduce the amount of metals that leach from the soil to the ground and surface waters (Glimmerveen 1996, Pulford and Watson 2003). Garten (1999) modeled the effect of a forest cover on the loss of $^{90}$Sr by leaching from contaminated soil, mainly in shallow subsurface flow, and showed that such losses were reduced by approximately 16% under trees relative to grass. This was attributed to the greater rate of evapotranspiration by the trees. Plants can also enhance retention of elements by binding them with phenolics (Hattenschwiler & Vitousek 2000), or root mucilage (Glinski & Lipiec 1990).

On the other hand, tree growth might enhance metal mobility. Species that decrease pH increase metal solubility (Finzi et al. 1998) and can enhance leaching of metals. Plant species influence metal availability by promoting low-molecular-weight organic acids through root exudation or litter decomposition. These acids act as ligands and, depending on the type exuded, have different effects on metal solubility and speciation (Jones & Darrah 1994). Organic acids rich in humic acid form water-soluble complexes with metals, increasing solubility (Marschner 1995). Preferential uptake of metals from the soil in the aboveground biomass actively mobilizes the metals and transports them to the crown canopy and the litter layer, two important parts of the food web.
Plant species differ in their effect on the biogeochemical cycles of metals through e.g. organic acid input, cation uptake, and effects on soil pH and redox (Finzi et al. 1998, Eviner & Chapin 2003). The aim of this study was to investigate if important differences between tree species could be found in metal compartmentalization on a disposal facility for dredged sediment after 33 years of tree growth. The metal concentrations in the different compartments are the net result of many biogeochemical interactions that took place over the preceding 33 years. The interest of this study is that different tree species can be compared on identical, new soil. At the time of planting, the site was very homogeneous and no soil profile had yet developed. This is in contrast to most polluted sites where a developed soil profile is gradually polluted from the surface soil down. Four broadleaved species had been planted: poplar (Populus ‘Robusta’), oak (Quercus robur), ash (Fraxinus excelsior) and maple (Acer pseudoplatanus). Poplar is a species that takes up high amounts of Cd and Zn in its biomass (Vandecasteele et al. 2003, Mertens et al. 2004, Laureijsen et al. 2004) and this can be compared to the other species showing normal concentrations (Mertens et al. 2004). Oak tends to acidify the soil more than for example ash and maple because of high lignin content in the litter and low amount of Ca in the litter (Augusto et al. 2002, Reich et al. 2005). Ash and maple have good litter quality and will promote fast decomposition, lower production of acids and formation of stable humus (Reich et al. 2005).

**Materials and methods**

**Site description**

This study area was a disposal site for hydraulically raised dredged harbor sediments near the harbor of Rotterdam (The Netherlands). It covers an area of 2.5 ha and was raised in the period 1960-1967. It was raised in 4 layers resulting in a package of 7 m
thick dredged sediment. The last layer was raised in 1966-1967 and was 2 m thick. In 1970 the site was planted and, due to dewatering, the sediment layer depth had decreased to 5.3 m (Oosterbaan and van den Berg 2002). In 1981, 8 samples spread over the site were taken and analyzed for texture and metal concentration (Table 5.1). The soil contained a rather high amount of clay (about 30 %). The metal concentrations in the two sampling depths were not different.

Table 5.1. Results of soil sampling and analysis of 1981 (n=8; Peeters 1994)

<table>
<thead>
<tr>
<th>Depth</th>
<th>&lt;2 µm</th>
<th>2-63 µm</th>
<th>&gt;63 µm</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-2 cm</td>
<td>30.9 ± 1.5</td>
<td>37.5 ± 2.6</td>
<td>31.6 ± 2.4</td>
</tr>
<tr>
<td>25-30 cm</td>
<td>31.4 ± 1.5</td>
<td>39.8 ± 1.3</td>
<td>28.9 ± 2.1</td>
</tr>
</tbody>
</table>

Four blocks (20 m by 200 m) were planted in 1970-1972, each one with one of the following tree species: poplar (Populus ‘Robusta’), oak (Quercus robur), ash (Fraxinus excelsior) or maple (Acer pseudoplatanus). The trees were planted at distances of 1.8 m in the row and 2.75 m between the rows (densities of about 1970 trees ha⁻¹). In between shrubs and auxiliary trees were planted. In one half of each block main tree species and shrubs were thinned few times, the other half remained unthinned. In 2003, these shrubs and auxiliary trees had densities ranging from 480 to 1500 individuals per ha. The sampling places for the further investigation were selected so that no shrubs or auxiliary trees grew over it, aimed at observing primarily the effect of the main tree species.
Both the biomass and soil were sampled and analyzed in August 2003. At that time, the trees were in their 33rd growing season. Based on the results of this sampling, an extended soil sampling was done in July 2005.

**Biomass**

All biomass measurements were done in 2003. Stem biomass was calculated by multiplying the stem volume and the wood densities for the given species (densities given by Rijsdijk and Laming (1994). The stem volume was determined based on the measurements of the stem diameter at 1.3 m height (transect, n=36 per species) and the tree height measurements (n=12 per species, equally distributed over the diameter classes) using the wood yield tables of Faber and Tiemens (1975) for poplar and Dagnelie et al. (1999) for the other tree species. The biomass of the branches was assumed to be one third of the stem wood biomass (Oosterbaan and van den Berg 2002).

From each tree species, 4 healthy trees with average diameter, equally spread over the length of the blocks were selected for sampling. Near every sample tree, autumn litter fall was collected with circular traps of 0.24 m². The traps were emptied every month from September to November 2003 and weight was determined after drying at 70°C in a forced air oven to constant weight. Wood was sampled by augering small cores with a Prezzler auger at 1.3 m height and bark was removed with a fine knife in March 2004. Samples were dried at 70°C in a forced air oven to constant weight. Subsequently the samples were milled and sieved before analysis. The metals were extracted by heating 0.5 g sample in 10 ml 65% HNO₃ p.a. and 2 ml 70% HClO₄ p.a. during 2 hours of heating. Extracts were diluted and analyzed for Ca, Cd, Cr, Cu, Pb and Zn on ICP/OES (Perkin-Elmer OPTIMA 3300 DV). Samples were not rinsed before drying.
Soil

In 2003, soil samples were collected at the same places where biomass samples were taken. Thus 4 replicates in each block were taken. The litter layer (less than 1 cm thick) was removed. The upper horizon (H1) was sampled, this horizon was about 5 cm thick and had a marked darker color than the soil underneath. The second horizon sampled (H2) started directly under H1 and was about 4 to 15 cm thick. This layer was well-structured and aggregated and had a lighter color than H1. The third horizon (H3) was less structured and less rooted and started directly under H2. This horizon was sampled at 40 to 50 cm depth.

Soil samples taken in 2003 were dried at 40°C in a forced air oven to a constant weight. Aggregates were broken and the soil was passed through a 2 mm sieve. Soil pH was determined in a 1/5 sediment/water solution and in a 1/5 sediment/KCl solution (1mol l⁻¹) with an ion-specific electrode. Pseudo-total Ca, Cd, Cr, Cu, Pb, and Zn contents were determined by digesting the sediment in an aqua regia solution (3 g sample in 21 ml HCl, 12 mol l⁻¹, and 7 ml HNO₃, 15.8 mol l⁻¹) under reflux for 2 hours and diluting the filtrate in 100 ml water (ISO 11466), and then analyzing the solution by ICP/OES.

Based on this first sampling campaign a second sampling campaign was executed in July 2005. For a more robust statistical analysis of the differences between the species, 8 replicates were taken in each block, evenly spread over the block (against 4 in the first campaign). For poplar only 4 samples could be taken because a large part of the site had been felled. Pseudo-total Ca, Cd, Cr, Cu, and Zn were determined in the same way as in the first campaign. Pb was not measured this time because no tree species effect on Pb in the soil had been found in the first campaign. Only two soil depths (H1 and H3) were sampled because in 2003, little or no difference was found between H2 and H3. An estimation of a mobile pool of Cd, Cr, Cu and Zn was made by extracting the sediment with NH₄OAc-EDTA (1.8615 g EDTA and 38.5 g ammonium acetate diluted to 0.5 l and set to pH 7 with 1M NH₄, 5 g sample in 50 g extractant) and by
analyzing with ICP/OES. Soil pH was determined in a 1/5 sediment/water solution and in a 1/5 sediment/KCl solution with an ion-specific electrode. Organic carbon was determined using Walkley and Black (Kalra and Maynard 1991).

**Data analysis**

The differences between the tree species were tested using non-parametric analysis of variance (Kruskal-Wallis), and the non-parametric Mann Whitney U test was used for pairwise comparison. Soil data of the two sampling campaigns (2003 and 2005) were not pooled to avoid pseudo-replication. The soil data of 2003 were used as a preliminary study. In the results and discussion data, only the soil data of 2005 are presented and discussed. A principal component analysis (PCA) of the soil results was used for the discussion of the global effect of the tree species on the soil characteristics. Without being restricted by assumptions of normally distributed data or independence of variables, principal component analysis can be used for describing and interpreting complex biological and chemical data (Gauch 1982). By least-square regressions through the multidimensional space created by the original variables, new, more complex variables, called principal components (PC), are formed. The first PC created describes the largest variation in the data set. The second PC describes the maximum of the residual variation and so on. Since the second PC is orthogonal to the first, they are independent.
Results

pH and organic material

Soil pH in the upper soil layer H₁ was significantly different under different tree species (Table 5.2). The pH in this soil layer was lowest under oak (pH-KCl 5.2) and highest under ash (6.9). The pH in H₁ generally was 1 to 2 units lower in comparison to H₃. No tree species effect could be found on the pH in the deeper soil layers, except for a small difference in pH-H₂O between oak and maple in H₃.

Also for organic carbon content, a clear species effect was recognized: oak and poplar had high carbon contents in the upper soil layer (oak 23.5% and poplar 23.2%) and ash had significantly lower carbon content (17.1%). Again no tree species effect could be found in the deeper soil layer. For all data on pH, %C and Ca concentration, there was a significant difference between H₁ and H₃.

Table 5.2. Results for pH(KCl), pH(H₂O), %C and Ca concentration measured in 2005 (n=8), for both sampled soil horizons (H₁= 0-5 cm; H₃ = 40-50 cm). Values followed by the same letter did not differ (p<0.05)

<table>
<thead>
<tr>
<th></th>
<th>pH(KCl)</th>
<th>pH(H₂O)</th>
<th>%C</th>
<th>Ca (mg 100g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H₁</td>
<td>H₃</td>
<td>H₁</td>
<td>H₃</td>
</tr>
<tr>
<td>oak</td>
<td>5.2 ± 0.5c</td>
<td>7.6 ± 0.1a</td>
<td>5.6 ± 0.4c</td>
<td>7.8 ± 0.2a</td>
</tr>
<tr>
<td>ash</td>
<td>6.9 ± 0.2a</td>
<td>7.6 ± 0.2a</td>
<td>7.1 ± 0.2a</td>
<td>8.0 ± 0.1a</td>
</tr>
<tr>
<td>maple</td>
<td>6.6 ± 0.2b</td>
<td>7.7 ± 0.1a</td>
<td>6.8 ± 0.2b</td>
<td>8.0 ± 0.1a</td>
</tr>
<tr>
<td>poplar</td>
<td>6.3 ± 0.5b</td>
<td>7.7 ± 0.1a</td>
<td>6.5 ± 0.5b</td>
<td>7.9 ± 0.1a</td>
</tr>
</tbody>
</table>
Chapter 5

It is remarkable that poplar showed a rather low pH and high C content in the upper layer because poplar is known for having good litter quality that easily degrades and having high Ca content in the litter. The latter was confirmed by the Ca concentrations: the Ca concentration under poplar was not different from ash and maple, only oak showed significantly lower Ca concentrations.

There was a significant correlation between pH, C and Ca concentration in the upper soil layer (Table 5.3). Only the correlation between Ca and C was not significant.

<table>
<thead>
<tr>
<th></th>
<th>pHKCL</th>
<th>pH-H₂O</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH-H₂O</td>
<td>0.99</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>-0.59</td>
<td>* -0.60</td>
<td>*</td>
</tr>
<tr>
<td>Ca</td>
<td>0.80</td>
<td>* 0.78</td>
<td>-0.31</td>
</tr>
</tbody>
</table>

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

Biomass

Data on the amount of biomass produced are necessary for the calculation of the metal stock in the biomass compartments. Ash showed the highest wood production per ha, poplar the lowest (Table 5.4). Standing stock per ha of poplar was low because of more intensive thinning regime typical for poplar management. Mortality of this species was rather high due to attacks by leaf fungi like Melampsora species. Litter production per
ha was estimated from the amount of litter that was caught in the litter traps and litter production seemed highest for maple, followed by ash.

Table 5.4. Biomass of standing wood and litter for each tree species in 2003

<table>
<thead>
<tr>
<th></th>
<th>Wood biomass (t DM ha⁻¹)</th>
<th>Litter biomass (t DM ha⁻¹)</th>
<th>Number of trees ha⁻¹</th>
<th>Volume wood (m³ ha⁻¹)</th>
<th>Wood biomass (kg DM tree⁻¹)</th>
<th>Litter biomass (kg DM tree⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>oak</td>
<td>108 ± 5</td>
<td>2.8 ± 1.3</td>
<td>674</td>
<td>209.3</td>
<td>160 ± 7</td>
<td>4.2 ± 1.9</td>
</tr>
<tr>
<td>ash</td>
<td>189 ± 14</td>
<td>3.1 ± 0.9</td>
<td>530</td>
<td>407.7</td>
<td>360 ± 26</td>
<td>5.8 ± 1.7</td>
</tr>
<tr>
<td>maple</td>
<td>85 ± 5</td>
<td>3.8 ± 0.4</td>
<td>454</td>
<td>189.7</td>
<td>190 ± 11</td>
<td>8.4 ± 0.9</td>
</tr>
<tr>
<td>poplar</td>
<td>67 ± 6</td>
<td>2.2 ± 0.5</td>
<td>178</td>
<td>188.6</td>
<td>380 ± 34</td>
<td>12.4 ± 2.8</td>
</tr>
</tbody>
</table>

Metal and Ca concentrations and contents in the biomass compartments

For all elements (metals and Ca) measured in the biomass compartments, differences could be found between the species (Table 5.5). These differences were greatest for Cd and Zn, the concentrations in poplar being about 5 to 80 times higher than in the other species. The differences between oak, ash and maple were small. The high uptake by poplars was also found in other research. Cd and Zn concentrations in poplars were lowest in the wood and highest in the litter, something that was also found in other research (Greger and Landberg 1999, Klang-Westin and Eriksson 2003).

For Cr and Cu, the differences between the species were rather small and no clear pattern was found. The Pb concentration did not show much variance between the species, except for the bark of poplar that contained much higher concentrations. Also the bark concentration of oak was rather high.
The observed metal concentrations (Table 5.5) were within the normal range which was compiled from different literature sources (Table 1.5), except for poplar. Poplar litter concentrations of Cd and Zn exceeded the normal range and it was the only species in which bio-accumulation occurred. The litter Cd and Zn concentrations for poplar were higher than the Cd and Zn concentrations in the sediment.

Table 5.5. Metal and Ca concentrations in the biomass compartments (mg kg\(^{-1}\)) (n=4). Values followed by the same letter did not differ (\(p<0.05\))

<table>
<thead>
<tr>
<th></th>
<th>Ca</th>
<th>Cd</th>
<th>Cr</th>
<th>Cu</th>
<th>Pb</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wood</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>oak</td>
<td>61 ± 31</td>
<td>(b&lt;0.5)</td>
<td>(b&lt;1.0)</td>
<td>(1.2 \pm 0.2) (1)</td>
<td>(&lt;1.0)</td>
<td>(&lt;5.0)</td>
</tr>
<tr>
<td>ash</td>
<td>86 ± 33</td>
<td>(b&lt;0.5)</td>
<td>(1.1 \pm 0.1) (1)</td>
<td>(1.5 \pm 0.6) (2)</td>
<td>(&lt;1.0)</td>
<td>(13) (3)</td>
</tr>
<tr>
<td>maple</td>
<td>174 ± 51</td>
<td>(a&lt;0.5)</td>
<td>(1.5 \pm 0.6)</td>
<td>(1.2 \pm 0.3) (2)</td>
<td>(&lt;1.0)</td>
<td>(6.1 \pm 0.9) (1)</td>
</tr>
<tr>
<td>poplar</td>
<td>209 ± 44</td>
<td>(a2.5 \pm 0.7)</td>
<td>(1.5) (3)</td>
<td>(ab 1.5 \pm 0.4)</td>
<td>(&lt;1.0)</td>
<td>(71.6 \pm 9.9)</td>
</tr>
</tbody>
</table>

|     |        |          |          |          |          |          |
| Bark|        |          |          |          |          |          |
| oak | 1363 ± 121| \(b<0.5\)| \(2.2 \pm 1.7\) (2)| \(6.6 \pm 1.6\)| \(10.7 \pm 5.2\)| \(ab 16.4 \pm 7.4\) |
| ash | 1477 ± 319| \(b<0.5\)| \(<1.0\)| \(3.3 \pm 0.9\)| \(2.5 \pm 0.5\)| \(11.1 \pm 4.7\) |
| maple| 2561 ± 175| \(a<0.5\)| \(1.13\) (3)| \(6.4 \pm 1.0\)| \(5.7 \pm 1.5\)| \(42.4 \pm 8.3\) |
| poplar| 1332 ± 150| \(b5.6 \pm 0.4\)| \(<1.0\)| \(7.1 \pm 0.9\)| \(23.5 \pm 14.5\)| \(250.9 \pm 29.9\) |

|     |        |          |          |          |          |          |
| Litter fall|        |          |          |          |          |          |
| oak | 1516 ± 115| \(c<0.5\)| \(2.7 \pm 0.7\)| \(6.2 \pm 0.3\)| \(4.4 \pm 0.1\)| \(42.9 \pm 4.0\) |
| ash | 2568 ± 191| \(bc<0.5\)| \(<1.0\) b| \(5.1 \pm 0.7\)| \(4.4 \pm 0.1\)| \(25.6 \pm 1.8\) |
| maple| 3099 ± 109| \(a<0.5\)| \(1.8 \pm 0.2\)| \(3.5 \pm 0.5\)| \(5.4 \pm 0.3\)| \(37.8\) (3) |
| poplar| 2730 ± 75| \(b10.0 \pm 1.6\)| \(1.7\) (3)| \(bc 8.0 \pm 1.1\)| \(4.5 \pm 1.2\)| \(1306.5 \pm 105.5\) |

< : if all values were lower than determination limit, the determination limit was given, preceded by "<" ( ) : the number between brackets is the number of values lower than the determination limit. The determination limits were: 0.5 for Cd; 1.0 for Cr; 1.0 for Cu; 1.0 for Pb and 5.0 for Zn
The amount of elements in the wood and litter were calculated by multiplying the concentrations with the estimated biomass (Table 5.6). These data are used to estimate the flux of elements caused by immobilization in the wood and by litter fall. The amount of metals in the bark was not accounted for because there were no data on the amount of bark. The initial amount of metals in the upper 0.5 m of the soil profile was calculated based on the concentrations measured in 1981 (Table 5.1; Ca concentration was based on the average concentration in H₃) and an estimated soil density of 1300 kg m⁻³.

Table 5.6. Amounts of metals (g ha⁻¹) in woody biomass and litter biomass of the 4 tree species and amount of metals in the upper 0.5 m of the soil (based on data of Table 5.4 and Table 5.5)

<table>
<thead>
<tr>
<th></th>
<th>Ca</th>
<th>Cd</th>
<th>Cr</th>
<th>Cu</th>
<th>Pb</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Wood</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>oak</td>
<td>6534 ± 3340</td>
<td>&lt;54.1</td>
<td>&lt;108</td>
<td>130 ± 22 *</td>
<td>&lt;108</td>
<td>&lt;541</td>
</tr>
<tr>
<td>ash</td>
<td>16160 ± 6152</td>
<td>&lt;94.4</td>
<td>208 ± 24 *</td>
<td>245 ± 78 *</td>
<td>&lt;189</td>
<td>1322 ± 761 *</td>
</tr>
<tr>
<td>maple</td>
<td>14748 ± 4324</td>
<td>&lt;42.6</td>
<td>128 ± 52</td>
<td>94 ± 18 *</td>
<td>&lt;85</td>
<td>494 ± 83 *b</td>
</tr>
<tr>
<td>poplar</td>
<td>13970 ± 2962</td>
<td>168 ± 50</td>
<td>74 ± 15 *</td>
<td>101 ± 29</td>
<td>&lt;67</td>
<td>4819 ± 809</td>
</tr>
<tr>
<td><strong>Leaf fall</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>oak</td>
<td>4244 ± 322</td>
<td>&lt;1.4 **</td>
<td>7.6 ± 1.9</td>
<td>17.4 ± 1.0</td>
<td>12.3 ± 0.2</td>
<td>120 ± 11</td>
</tr>
<tr>
<td>ash</td>
<td>7961 ± 591</td>
<td>&lt;1.6</td>
<td>&lt;3.1</td>
<td>15.8 ± 2.1</td>
<td>13.6 ± 0.4</td>
<td>79 ± 5</td>
</tr>
<tr>
<td>maple</td>
<td>11776 ± 415</td>
<td>&lt;1.9</td>
<td>6.8 ± 0.9</td>
<td>13.3 ± 1.7</td>
<td>20.5 ± 1.0</td>
<td>60 ± 72 *</td>
</tr>
<tr>
<td>poplar</td>
<td>6005 ± 166</td>
<td>22.0 ± 3.4</td>
<td>2.6 ± 0.8 *</td>
<td>17.6 ± 2.5</td>
<td>9.9 ± 2.6</td>
<td>2874 ± 232.1</td>
</tr>
<tr>
<td><strong>Soil</strong></td>
<td>42 . 10³</td>
<td>65650</td>
<td>1326000</td>
<td>1196000</td>
<td>1885000</td>
<td>7579000</td>
</tr>
</tbody>
</table>
The amount of Ca in the litter differs significantly between each tree species (Table 5.6): maple contained the highest amount (almost 12000 g ha\(^{-1}\)) and oak the lowest (about 4000 g ha\(^{-1}\)). The amount of Cd and Zn in the wood and litter of the poplars was much higher than in the other species. High amounts of Cr and Cu were found in the wood of ash, but differences between species in the litter concentrations were rather small. For Cr, Cu and Pb, the differences in the amounts found in the litter were small. For Zn, the lowest amounts in the litter were found in maple and ash.

**Metals in the soil**

*Total* concentrations

The most distinct species effect on the total soil metal concentrations in the soil was the high amount of Cd and Zn in the upper soil layer under poplar (2 to 3 times higher than under the other species; Table 5.7). There was a clear pattern in oak showing the lowest concentrations for all elements. This difference was however not significant, probably due to shortage of replicates. If the data of 2003 and 2005 were pooled, the concentrations under oak were significantly lower than under ash and maple (p<0.05), as were the concentrations of Cr and Cu under poplar (p<0.05).

The concentrations in the deeper soil layer (H\(_3\)) were not different between the species, except for Cr. This difference for Cr was significant between ash and poplar but was rather small and not linked to the concentrations in the upper soil layer or above ground biomass. It might have been caused by differences in the root characteristics.

The concentrations of Cd and Zn in H\(_1\) under poplar were higher than H\(_3\) but for all other cases, the concentration in H\(_1\) was significantly lower than that in H\(_3\) (p<0.05). This difference was highest under oak for all elements.
Table 5.7. Metal concentrations (mg kg\(^{-1}\)) in the soil profiles H\(_1\) (= 0-5 cm) and H\(_3\) (40-50 cm) in 2005 (n= 8). Values followed by the same letter did not differ (\(p<0.05\), per horizon)

<table>
<thead>
<tr>
<th></th>
<th>Cd</th>
<th>Cr</th>
<th>Cu</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H(_1)</td>
<td>H(_3)</td>
<td>H(_1)</td>
<td>H(_3)</td>
</tr>
<tr>
<td>oak</td>
<td>4.5 ± 1.8(^a)</td>
<td>7.7 ± 0.8(^a)</td>
<td>92 ± 46(^a)</td>
<td>195 ± 16(^ab)</td>
</tr>
<tr>
<td>ash</td>
<td>5.9 ± 2.1(^a)</td>
<td>7.6 ± 1.3(^a)</td>
<td>124 ± 36(^a)</td>
<td>181 ± 21(^a)</td>
</tr>
<tr>
<td>maple</td>
<td>5.6 ± 0.5(^a)</td>
<td>7.3 ± 0.6(^a)</td>
<td>129 ± 15(^a)</td>
<td>194 ± 15(^ab)</td>
</tr>
<tr>
<td>poplar</td>
<td>16.7 ± 2.5(^b)</td>
<td>8.1 ± 1.1(^a)</td>
<td>103 ± 31(^a)</td>
<td>204 ± 23(^b)</td>
</tr>
</tbody>
</table>

**Extractable concentrations**

The NH\(_4\)OAc-EDTA extractable concentrations of Cd and Zn in H\(_1\) were significantly highest under poplar (Table 5.8). Very few and only small differences were found further on. Extractable Cu concentrations under maple were higher than under ash and poplar and its Zn concentrations were higher than under ash.

Table 5.8. NH\(_4\)OAc-EDTA extractable concentration (mg kg\(^{-1}\) DW), for both sampled soil horizons (H\(_1\)= 0-5 cm; H\(_3\) = 40-50 cm) in 2005 (n=8). Values followed by the same letter did not differ (\(p<0.05\))

<table>
<thead>
<tr>
<th></th>
<th>Cd</th>
<th>Cr</th>
<th>Cu</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H(_1)</td>
<td>H(_3)</td>
<td>H(_1)</td>
<td>H(_3)</td>
</tr>
<tr>
<td>oak</td>
<td>0.36 ± 0.14(^a)</td>
<td>0.48 ± 0.05</td>
<td>0.10 ± 0.02</td>
<td>0.05 ± 0.00</td>
</tr>
<tr>
<td>ash</td>
<td>0.38 ± 0.20(^a)</td>
<td>0.41 ± 0.13</td>
<td>0.15 ± 0.18</td>
<td>0.19 ± 0.30</td>
</tr>
<tr>
<td>maple</td>
<td>0.46 ± 0.07(^a)</td>
<td>0.45 ± 0.08</td>
<td>0.08 ± 0.01</td>
<td>0.04 ± 0.01</td>
</tr>
<tr>
<td>poplar</td>
<td>1.37 ± 0.27(^b)</td>
<td>0.49 ± 0.05</td>
<td>0.05 ± 0.01</td>
<td>0.04 ± 0.01</td>
</tr>
</tbody>
</table>
Principal component analysis

The principal components analysis (PCA, varimax rotated solution), applied to the soil data set of H1 of 2005 (Table 5.2, Table 5.7 and Table 5.8), reduced the data set to two relevant factors that accounted for 60 % of the variability in the data examined. The first PC explained 33% of the variance in the soil data set and was mainly influenced by Cd and Zn concentrations (Fig. 2.5). The second PC accounted for 27 % of the variance of the data set and was strongly correlated with pH and organic matter related variables: pHKCl (0.94), pH-H2O (0.94), C (-0.78) and Ca (0.65). The factor scores show clear groups of tree species (Fig. 5.1).

Discussion

The data of 1981 (Table 5.1) demonstrate that the soil on the site was initially very homogeneous. This might also be concluded from the properties of the deeper soil layer (H3). The concentrations in this soil layer were little different from the data of 1981 and, because there is little difference between the tree species blocks, it can be assumed that tree growth did not have much influence on this soil layer. Although the site was rather homogeneous at the time of planting, the variance in H1 had increased greatly at the time of sampling (Table 5.9). The relative variance for Cd and Zn was up to 10 times higher in H1 in 2005 than in 1981. For Cr and Cu, the variance was about 5 times higher. This increase in variance might have different reasons but an important part of the increase is related to tree growth. For many characteristics that were measured, there was a significant difference between the tree species in H1. It is well known that trees have influence on the soil characteristics as explained in the introduction.
Fig. 5.1. Results of the PCA of the soil data of H1 (0-5 cm) (a) scatter plot of the first two PC-scores of the data; (b) scatter plot of the scores of the variables
Table 5.9. Coefficient of variation (%) for the entire site in 1981 and in $H_3$ (40-50 cm) and $H_1$ (0-5 cm) in 2005

<table>
<thead>
<tr>
<th></th>
<th>pHKCL</th>
<th>Cd</th>
<th>Cr</th>
<th>Cu</th>
<th>Zn</th>
<th>Ca</th>
</tr>
</thead>
<tbody>
<tr>
<td>1981</td>
<td></td>
<td>6.8</td>
<td>6.3</td>
<td>5.4</td>
<td>5.7</td>
<td></td>
</tr>
<tr>
<td>$H_3$</td>
<td>1.6</td>
<td>12.4</td>
<td>10</td>
<td>9.8</td>
<td>9.3</td>
<td>15.4</td>
</tr>
<tr>
<td>$H_1$</td>
<td>12.3</td>
<td>63</td>
<td>31.7</td>
<td>23.5</td>
<td>48.6</td>
<td>28.4</td>
</tr>
</tbody>
</table>

The first two PCs of the PCA accounted for 60% of the variance in $H_1$. On the first axis (PC1), mainly determined by Cd and Zn data, two groups can be distinguished: the poplars, showing high values on this axis, and the other tree species, showing low values (Fig. 5.1). This is most likely linked to the uptake of Cd and Zn in the litter, poplar showing high amounts of Cd and Zn against normal concentrations and normal amounts for the other species. *Populus* spp, and *Salix* spp as well, are generally known to take up Cd and Zn from polluted soil and to accumulate these metals in their biomass, including shoots, leaves and wood, distinctly more than other tree species (Sawidis et al. 2001, Vandecasteele et al. 2003, Brekken and Steines 2004, Laureijsen et al. 2004). The causal relationship between the high uptake of Cd and Zn and an increase of the concentration in $H_1$ is further discussed in the section “Fluxes”.

On the second axis (PC2), which is the axis determined by pH and organic carbon data, oak is the species with the lowest values while ash has the highest values. It is generally known that plant species can differ in their effect on soil pH, base cation saturation and organic matter content, and differences between tree species for these characteristics have been reported in the past (Finzi et al. 1998, Augusto et al. 2002). Base cation leaching and soil acidification are enhanced by species with high litter concentrations of organic acids (particularly tannins) and low litter concentrations of base cations (Ovington 1953, Finzi et al. 1998). According to Raulund-Rasmussen & Vejre (1995), acidification might largely be caused by input of organic acids that
Tree species effect after 33-years

originate from the incomplete decomposition of organic matter. Lower pH under spruce and beech was explained by slower litter decomposition in these species. This leads to the production of organic acids and also delays the return of base cations to the soil sorption complex (Hagen-Thorn et al. 2004).

The surface soil under oak showed lowest pH and Ca concentration and highest carbon content. The scores of oak for PC2 were significantly different of these of ash and maple (p<0.05). The reasons for the low pH were probably the low concentration of Ca in the litter and a retarded decomposition of the litter as could be seen from the high organic carbon content in the soil. Litter of oak growing on loamy sand contained higher concentrations of lignin than maples on the same site (Reich et al. 2005). On former agricultural land (sand to loam) that was afforested, differences in soil pH between oak (Q. robur) and ash (F. excelsior) were not measured after 30-40 years of tree growth (Hagen-Thorn et al. 2004), but the upper mineral soil layer under oak had, in general, lower base saturation than soils under ash (Hagen-Thorn et al. 2004). Muys et al. (1992) found the thickness and quality of holorganic layer and soil pH to be different under different tree species. These effects could be explained by the quality and quantity of the annual litter. Under oak litter accumulated and moder humus formation started, while under ash a well-functioning forest mull was found. Finzi et al. (1998) found lower soil pH under red oak than under white ash.

The PCA shows that ash had a positive effect on the characteristics related to pH and organic matter (PC2), as can also be seen in the soil data tables: ash had highest pH and Ca concentrations and lowest C concentrations. Ash litter has been shown to be easily decomposable and rich in nutrients (Hagen-Thorn et al. 2004), which promoted a high base cation return to the soil. A high content of base cations in the foliage and high leaf susceptibility to leaching led to increased base cation input via throughfall as well (Eviner & Chapin 2003).
The relatively low pH and high carbon content under poplar suggest that litter decomposition under this species might have been retarded. The pH under poplar was 0.6 units lower than under ash. This is remarkable as on a former agricultural loamy soil that was afforested 25-years before measurements were done, the pH in the upper 5 cm of the soil was significantly highest under poplar compared to other species (Table 5.10). Leaf litter from poplars and willows generally has high nutritional quality, facilitating rapid litter decomposition and nutrient release to soils (Cooke and Weih 2005). In another study (Muizenbos, Belgium; Verstraeten 2004) soil pH under ash and maple was compared in two neighboring plots of at least 80-years old on sandy loam. The soil pH-CaCl$_2$ of the upper (5-10 cm) and deeper (10-20 cm) soil layers were comparable on both plots, being slightly higher under poplar in the upper layer.

Table 5.10. pH of the upper 5 cm of the soil on a former agricultural land after 25-years of forest growth (Dossche, 1998; values followed by the same letter did not differ, p<0.05)

<table>
<thead>
<tr>
<th>Species</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poplar</td>
<td>4.97</td>
</tr>
<tr>
<td>Poplar</td>
<td>4.66</td>
</tr>
<tr>
<td>Ash</td>
<td>4.12</td>
</tr>
<tr>
<td>Lime</td>
<td>3.99</td>
</tr>
<tr>
<td>Oak</td>
<td>3.49</td>
</tr>
<tr>
<td>Maple</td>
<td>3.36</td>
</tr>
</tbody>
</table>

Litter Ca concentration in poplar was indeed high, almost double that of the oak litter and higher than the ash litter. The amount of Ca recycled to the soil via litter fall was lower than the amount of maple and ash, but higher than oak. This suggests that the low pH was not mainly caused by depletion of base cations, but rather by the formation of acids. The (organic) carbon content in the upper soil layer was highest compared to the other species, although the amount of litter produced was lowest of all. Both unexpected low pH and high carbon content indicate that the decomposition of the litter might have been retarded.
This retardation might be caused by the high concentrations of Cd and Zn in the litter. Numerous studies also reported retarded decomposition of litter when Cd and Zn were present in elevated amounts (see for example Coughtrey et al. 1979, Derome and Lindroos 1998, Berg et al. 2003). Laskowski et al. (1994) reported a significant decrease in forest litter respiration rate after 4 weeks at levels of 1000 mg kg\(^{-1}\) Zn and 50 mg kg\(^{-1}\) Cd. It was not clear if at lower metal levels the respiration rate would decline. Niklinska et al. (1998) reported significant inhibition of respiration rate at levels from 10 mg kg\(^{-1}\) Cd and levels of about 500-1000 mg kg\(^{-1}\) Zn. It is therefore possible that, at the levels that were measured at this site (10 mg kg\(^{-1}\) Cd and 1300 mg kg\(^{-1}\) Zn in the litter), the decomposition of the poplar litter had decreased. The retarded decomposition might have resulted in a decreased pH and relatively high carbon contents in the soil, almost as high as the content under oak.

The combined effect of soil acidification and high uptake of Cd and Zn makes poplar not desirable to be planted on these sites. The high amount of Cd and Zn in the crown, litter and surface soil layer might cause transport of these metals into the food web. The low pH will probably increase the mobility of these metals in the surface soil as the mobility of both Cd and Zn in soils is predominantly determined by soil pH (Bergkvist et al. 1989). Higher mobility might increase the risk for leaching and uptake.

**Fluxes**

The input and output fluxes determine the metal concentrations and metal amounts in the different soil layers (Fig. 5.2). Output of metals from the soil layers is mainly controlled by leaching and uptake by plant roots. Input in the upper soil layer is determined by input via litter fall and atmospheric deposition. Input via atmospheric deposition was not accounted for in this study. As all species are broadleaved species, because it was assumed that differences in deposition rate between the species are
limited, especially when compared to the differences caused by root uptake and soil acidification.

![Diagram of metal fluxes related to the uptake of metals in the litter](image)

**Fig. 5.2.** Metal fluxes related to the uptake of metals in the litter

The total metal concentrations in H₁ were all lower than H₃, except for Cd and Zn under poplar. Under poplar, high concentrations in H₁ might have been caused by the high input of Cd and Zn by the litter. Whether uptake of metals in the litter causes an increase of the soil concentration depends on two conditions: 1) the concentration in the decomposed litter is higher than the soil concentration and 2) the input flux is greater than the output flux. Decomposition of poplar leaves mostly stops at a ‘limit value’ (=fraction of litter that does not decompose) of 55% of the dry weight (Berg et al. 2003). As the amount of metals remains equal, metal concentrations might double during decomposition from fresh leaves to decomposed litter. The Cd and Zn
concentrations of the decomposed litter will be significantly higher than the soil concentration and an increase of the concentration is possible. This is however only possible if the output fluxes of metals does not exceed the input flux. Output is mainly the result of uptake by the roots and leaching.

The decrease of the metal concentrations in H1 measured under oak, ash and maple might be caused by leaching, uptake by plant roots and addition of less polluted material, e.g. litter with lower metal concentrations. Plant uptake and leaching of heavy metals depend on the mobility and availability of the metals in the soil, which in turn are affected by pH and contents of solid and dissolved organic C (see for example Seuntjens et al. 2002, Dijkstra et al. 2004, Strobel et al. 2005). Low pH favors solubility, and hence mobility. In addition, complexation by dissolved organic C may enhance the solubility of heavy metals. Because of lowest pH under oak and poplar, more metals might have leached from H1 under these species. The metal concentrations in H1 under these species were lowest, except for Cd and Zn under poplar because of high litter concentration. We couldn’t find an effect of pH on the NH4OAc-EDTA extractable concentrations. This extraction is buffered at pH 7 and this might have masked pH effects due to isothermal adsorption/desorption equilibrium.

The soil layer in which acidification occurs was limited (about 5 cm), and because of the high acid neutralizing capacity of the deeper soil layers (Singh et al. 2000, Luyssaert et al. 2001, Cappuyns et al. 2004), the risk for leaching to the groundwater will still be limited. For the estimation of leaching fluxes, information is needed on the soluble metal concentration in the soil and the soil water flux (Moolenaar et al. 1998). The soil water flux also depends on the tree species (Van der Salm et al. 2006).

More detailed information on the fluxes is needed to understand the processes going on and to be able to make predictions for the future. Leaching needs more attention, especially because of risk for groundwater pollution. On the other hand, Ross (1994) stated that the input of metals into the food web is potentially more harmful than metal
leaching into the groundwater. It was recently shown that metals might have a stronger effect on the microbial diversity in the soil than it was generally believed (Gans et al. 2005). The upper soil layer H₁ was only about 5 cm thick but it is a very important layer because it has the highest biological activity and the litter is an important basis of the food web. Therefore special protection of this soil layer might be important and a decrease of the metal concentration might have more ecological advantages than it might pose risks.

**Conclusions**

After 30 years of tree growth, we found a redistribution of metals in the soil profile that was dependant on the tree species. Two processes seem to determine the soil concentration in the upper soil layer: uptake of metals in the leaves and species specific soil acidification. Poplar took up high amounts of Cd and Zn and this resulted in increased concentrations of these metals in the upper soil layer. Oak acidified the soil more than ash and maple and this resulted in higher leaching from the upper soil layer and associated lower metal concentrations in this soil layer. The high amounts of Cd and Zn in the litter and upper soil layer seemed to retard litter decomposition which might have caused unexpected low pH values and high carbon contents under poplar.
Chapter 6
Interpretation of metal concentrations in plant tissue for biomonitoring and phytoextraction studies

Abstract

Some plant species accumulate trace metals from the soil in their aboveground biomass. Therefore, some scientists have concluded that these species are suitable for biomonitoring trace metal concentrations in the soil or for removing excessive trace metals from the soil by means of phytoextraction. A significant correlation between the chemical composition of foliage and soil is not a sufficient condition for using the chemical composition of foliage as a biomonitor for the quality of the soil. This composition can, however, provide additional information to the traditional soil samples. The phytoextraction potential of a plant species cannot solely be evaluated on the basis of the trace metal concentrations in the plant and soil tissue. Data on the depth of the rooting zone, the density of the soil and the harvestable biomass should also be taken into account. Although plant tissue analysis is a useful tool in a wide range of studies and applications, trace metal concentrations in plant tissue cannot be viewed in isolation. Instead it should be analyzed and interpreted in relation to other information such as soil concentrations, rooted zone, biomass production, etc.

Introduction

Chapter 1 partly considered phytoextraction and part of Chapter 3 considered biomonitor. Some discussion is however necessary on these two topics. The uptake of soil metals by plants growing on polluted sites has been the subject of many research studies. Some plant species were reported to accumulate metals from the soil in their aboveground biomass. This observation has led some scientists to conclude that plants can be used to monitor soil pollution or to remediate polluted soils. However, we believe that these conclusions were often based on a superficial evaluation and interpretation of trace metal concentrations in plant tissues.

Bioindicators and biomonitors

An organism that provides qualitative information on the quality of its environment is called a bioindicator and one that provides quantitative information a biomonitor (Bargagli 1998). Bioindicators and biomonitors can be passive or active. The terms bioindicator and biomonitor have been and still are mistakenly used as synonyms. However, the terminological confusion is not the main problem. More important are the logical flaws that have led to an excessive use of the terms bioindicator and biomonitor for what is often no more than a correlation between trace metal concentrations in tree foliage and trace metal concentrations in the soil. Many articles published in a variety of journals assume that as a result of this correlation, plant analysis can be used to monitor soil pollution.

We have selected the article of Madejon et al. (2004) to illustrate some of the logical flaws which occur in scientific literature. Madejon et al. (2004) were interested in the environmental quality of the soil. Therefore soil samples were taken and the Cd and Zn
Metal concentrations for biomonitoring and phytoextraction

centrations determined. On the same site, foliar samples from white poplars (*Populus alba* L.) were also taken and analyzed for Cd and Zn. The foliar Cd and Zn concentrations were reported to be positively correlated to the soil concentrations. The positive correlation persuaded Madejon et al. (2004) to suggest that the leaves of white poplar could be used for biomonitoring Cd and Zn in the soil. Given the definition of a bioindicator and a biomonitor this is a correct statement. However, we fail to see the advantage of using foliar samples to determine soil concentrations whenever it is possible to analyze the soil. Soil analyses are certainly the most straightforward method for assessing the soil quality, whereas using foliar analysis to monitor soil pollution has some practical constraints.

1. Trace metal uptake is dependent on the species or even clonal species (Landberg and Greger 1994). Therefore, monitoring is in practice restricted to areas where the specific species or clone is growing. Consequently the plant or clonal species has to be determined before analysis. In the case of clonal species, determination may be difficult and laborious.

2. At present, no plant species are known that respond to a wide range of elements. In the case of white poplars, biomonitoring is likely to reveal Cd, Zn and As pollution in the soil. However, the same biomonitoring strategy would fail to reveal soil pollution by, for example, Pb and Cu (Mertens et al. 2004). A soil sample which has been analyzed for a wide range of elements would reveal the total concentration for those elements.

3. The trace metal concentration in tree foliage varies with the crown class, stand management, crown dimensions, infections, season, etc. (Luyssaert et al. 2002). Consequently, the variability in trace metal concentrations in tree foliage should be known and understood when designing a suitable leaf sampling procedure. For example, Cd, Zn, Cu and Pb concentrations in tree leaves are reported to vary during the growing season (Luyssaert et al. 2002). If the assessment of the
soil quality is now based on the chemical composition of the foliage, the soil quality in terms of Cd, Zn, Cu and Pb concentrations will also vary during the growing season. Therefore, foliage sampling should be done in a predefined manner, which restricts the possibilities for determining the pollution on a site.

4. Due to the height of trees, collecting foliar samples is often impractical.

Even if the aforementioned difficulties could be overcome, would tree foliage analysis have advantages over soil analysis?

1. It has been argued that bioindicators and biomonitors contain integrated information for a longer time period and over a larger space, whereas a soil sample provides information about the specific sampling time and location. Does this argument hold in the case of Madejon et al. (2004)? Tree roots cover a large soil volume. However, tree roots were reported to avoid zones contaminated with heavy metals. Avoidance was thought to be an efficient survival strategy of the plant (Dickinson et al. 1991, Breckle and Kahle 1992). This means that the tree roots might avoid the hot spots, despite these spots having a considerable influence on the assessment of the soil’s environmental quality (Hsiao et al. 2000). Tree roots stay in the soil year round and are therefore expected to contain time-integrated information. However, Madejon et al. (2004) analyzed the leaves not the roots. Element concentrations in the roots are not simply reflected in the leaves. Foliar concentrations of trace metals are driven by accumulation, antagonism, accretion, resorption and excretion. Consequently, it is unlikely that the tree foliage contains the time-space-integrated information sought.

2. Environmental risk and damage occurs when the metals are available to the living organisms. Analysis of the total soil concentration provides no information about the availability. What does the availability for white poplar
tell us about the availability for other living organisms at the site? Availability to poplars is not the same as availability to other species. Oak or ash, for example, did not accumulate Cd, Cu, Cr, Pb or Zn on sites where white poplars accumulated Cd and Zn (Mertens et al. 2004). This means that the availability is highly dependent on the species. Yet total soil analysis or sequential extractions are not a valid alternative for determining the availability of soil trace metals to living organisms. The availability of trace metals in an ecosystem can only be evaluated by simultaneously analyzing soil and plant samples (Bargagli 1998).

So far, foliar trace metal concentrations have not been successfully predicted from the trace metal concentrations in the soil. The estimation of available concentrations of elements in the soil from the elemental composition of plants, or vice versa, can only be used for overall estimates on a broad scale (Bargagli 1998).

Do the above objections mean that there is no need for foliar analyses? No! Firstly the objections raised for biomonitoring soil quality do not hold for biomonitoring other abiotic components of the ecosystem. Tree foliage has been reported to be a sensitive bioindicator and biomonitor for assessing ozone and sulphur oxide concentrations in remote areas (Bennett 1996) and for assessing large-scale patterns of atmospheric trace metal concentrations (Landolt et al. 1989). In addition, foliar analysis is used as a tool for monitoring the effects of air pollution on the health of forest ecosystems (UN/ECE-EC 1998). Secondly, both leaf and soil analyses are needed when studying the effects of soil trace metals on the chemical composition of foliage or assessing the environmental quality of ecosystems, and these two approaches should be treated as complementary information (Kabata-Pendias and Pendias 1992, Ross 1994, Pilgrim and Hughes 1994). Thirdly, foliar analysis should be considered when assessing the environmental quality of a food web in an ecosystem. After all, foliage represents that part of the aboveground biomass which is most susceptible to herbivory. Even when the trace metals in the soil are not highly available, their uptake by accumulator plants can make them available to insects, grazers and soil organisms. This was shown on a
disposal site for dredged sediment covered with willow. The high uptake of Cd in the
leaves of the willows was thought to be the cause of high Cd concentrations in the
small mammals living on the site (Mertens et al. 2001).

We certainly want to encourage the use of plant analysis to describe plant and
ecosystem functioning (implying biological productivity, soil quality, etc.). Resources
should be invested in improving the interpretation of plant analysis in terms of
environmental quality. Therefore, the chemical composition of plants or plant parts
should be linked to ecophysiological characteristics that give information on biological
productivity or plant functioning (see for example Luyssaert et al. 2005). If this step is
made, plant analysis can become an essential part of assessing environmental quality
including soil quality.

In conclusion, foliage sampling and analysis is a useful tool for studying the effects of
metals and for monitoring atmospheric pollution. However, we believe that it has little
value for the straightforward biomonitoring of soil pollution.

**Phytoextraction**

**Metal extraction ratio**

Phytoextraction aims to remove trace metals from the soil by repeatedly harvesting
plant biomass from a polluted site. Harvesting continues until metal concentrations in
the soil have reached a predefined target concentration. Target concentrations are
usually a legally defined threshold. The discovery of metal-accumulating plant species
lead to the idea of phytoextraction. Web of Science, a web based reference tool, has
recorded 86 articles concerning “Phytoextraction and metal*” published in 2005 and
more than two decades have passed since the idea of phytoextraction was developed.
Yet is phytoextraction as promising as many authors would have us believe? Up until now, the scientific basis for the practical application of phytoextraction has been limited (Keller & Hammer 2004). Accumulating plants are still being sought and few studies have evaluated the extraction capacity of hyperaccumulators under field conditions (McGrath and Zhao 2003). How can the extraction capacity of a plant be evaluated? Some authors define accumulating plants as plants that have higher concentrations than other species. Many authors define it as plants which have higher tissue concentrations of the metals concerned than the concentrations found in the soil (McGrath & Zhao 2003). This means that the bioconcentration factor (BCF), defined as the ratio of metal concentration in plant shoots to metal concentration in soil, should be larger than 1.

In one of our studies, the woody biomass of willows had rather high Cd concentrations (3.2 mg kg\(^{-1}\)) and a corresponding bioconcentration factor of 1.2 (unpublished results). Based on these data the willow stand studied would be described an accumulator and suitable for phytoextraction. However, when evaluating the possibilities for phytoextraction it is meaningless to compare plant concentrations to normal ranges or to the soil concentrations. As well as the trace metal concentrations in the soil and plants, an evaluation of the extraction capacity should take into account the depth of the rooting zone, the density of the soil and the biomass production and mortality of the aboveground biomass components harvested. Expressing the extraction capacity as a ratio which takes into account the produced biomass and the soil volume to be cleaned would be more informative. We therefore propose the metal extraction ratio (MER), which is calculated as follows:

\[
MER = \frac{C_{plant} \times M_{plant}}{C_{soil} \times M_{rooted\ zone}} \times 100
\]

Where, \(M_{plant}\) is the mass of the harvestable aboveground biomass produced in 1 harvest, \(C_{plant}\) is the metal concentration in the harvested component of the plant biomass, \(M_{rooted\ zone}\) is the mass of the soil volume rooted by the species under study, and
Chapter 6

$C_{soil}$ is the metal concentration in the soil volume. For example, a metal exchange ratio of 10% means that 10% of the soil metals can be removed in 1 harvest. For the aforementioned willow stand, the metal exchange ratio was 0.3%, and therefore only 0.3% of the metals would be removed by the concerned harvest. An even more informative ratio for evaluating the extraction capacity would take into account the target concentration of the trace metal in the soil. This gives the following formula for calculating the metal exchange ratio:

$$MER_{target\ conc} = \frac{C_{plant} \times M_{plant}}{C_{soil} - C_{target}} \times M_{rooted\ zone} \times 100$$

Where $C_{target}$ is the target concentration as defined by, for example, environmental legislation. In our example the metal exchange ratio conditional on the target concentration is 1.0%, which means that one harvest removes 1% of the amount of metals that have to be removed. Although the bioconcentration factor was greater than 1, both indexes for the metal exchange ratio indicate that in our example, the prospects for phytoextraction are limited. However, the metal exchange ratio should be used with care. As the bioconcentration factor decreases with increasing soil concentrations, (Efroymson et al. 2001), the metal exchange ratio will also change if soil concentrations change.

**Feasibility**

The amounts of Cd that could be exported annually ($M_{exp}$) using poplars ranged from about 20 g ha$^{-1}$ (Chapter 1) to 90 g ha$^{-1}$ (Chapter 4, outlet plot). Previous calculations of the amount of Cd offtake from Salix harvest using direct evidence from field trials at contaminated sites (Klang-Westin and Eriksson 2003; Pulford et al. 2002, Berndes et al. 2004) were also low with a range of 2.6–76.7 g ha$^{-1}$ year$^{-1}$. In a recent study with willow on dredged sediment, Meers et al. (2005) extracted annually 83 g Cd ha$^{-1}$. This means that our data are consistent with phytoextraction studies with *Salix* spp.
The highest MER that could be calculated from the field data of the previous chapters was 0.35%. This MER accounted for the inlet plot on the site of chapter three and means that annually, 0.35% of the amount of Cd in the upper 1 m layer of the soil could be extracted annually. For Zn, the highest MER found was 0.12% while for the other elements MER’s were disappointingly low. A MER of 0.35% means that, assuming that growth, uptake and soil characteristics remain constant, about 30 years are needed to decrease the soil concentration with 10%.

Above calculation is however an overestimation as the assumptions of constant growth, uptake and soil characteristics are not realistic. It is known that Cd availability in the soil can decrease with time but the rate of this decline is poorly known. In willow stands on dredged sediment, the ammonium acetate extractable Cd and Zn concentration seemed to decrease with time, as did the concentration in the biomass (Mertens et al. 2006). The extractable concentration on 1- and 2-year-old willow plots on dredged sediment was about 17-18% of the total concentration, while on a 4-year-old plot it was 13%, and on a 6-year-old plot it was only 6%. For the other elements, the extractable fraction was very similar among the plots of different ages. Eriksson and Ledin (1998) found that long-term cropping of Salix resulted in a 30-40% decrease in plant-available Cd, although the effects on concentrations of total Cd were negligible. This decrease in available Cd might partly be compensated because metal availability in the vicinity of willow roots can increase compared to unrooted bulk soil (Vervaeke et al. 2004). Pulford et al. (2002) also showed that the concentrations of EDTA-extractable Cd, Cu, Ni, and Zn in sewage-sludge amended soil were higher under willows than in unplanted areas. This topic certainly needs further investigation as it is important for the feasibility of phytoremediation.

Furthermore, it is doubtful that biomass production will remain constant for every rotation period. From 3 to 6 year rotations it can be expected that this will be true, based on reports on traditional short rotation forests (SRF) practices (Willebrand et al. 1993). Short rotation forests managed this way are generally characterized with
constant biomass production for every rotation as long as soil fertility is maintained. Only toward the end of the 20 year period in which SRF stands are generally operated can a decline in production be expected as a result of root and stump dieback. For the reduction of the risk of metal spreading to the environment, leaves and wood should be harvested annually before leaf fall. Reducing the rotation time to one year can, however, result in a decline of biomass production (Kopp et al. 2001).

A practical limitation is that if soil concentration is above the legislation limit, it needs to be cleaned until background values are achieved, not until the legislation limit is achieved.

Nevertheless, phytoextraction has some clear advantages above the commonly used soil remediation techniques. Although current technologies for cleaning contaminated sites such as isolation and containment, mechanical/pyrometallurgical separation, or chemical treatment are efficient, they are usually expensive, labor intensive, and soil disturbing (Mulligan et al. 2001) Phytoremediation, on the other hand, can be accomplished in situ, is relatively inexpensive, environmentally friendly, and the soil can be utilized immediately after treatment application (Gardea-Torresdey et al. 2005). Further research is therefore needed. If the exported amount of metals can be significantly increased, there might be some possibilities to clean Cd and Zn polluted soils. In further research attention should be paid to:

1. the sustainability in terms of biomass production and soil quality of short rotation forestry that annually harvests wood and leaves

2. the practical possibilities for phytoextraction of harvesting the root bole at the end of a period of remediation
3. further selection and further improvement of species aimed at increasing the biomass production and biomass metal concentrations. Eventually, the technique can be improved with biotechnology.

**Conclusions**

Plants can definitely be a suitable tool for monitoring soil quality by means of for example the occurrence or abundance of particular species, mortality, growth or the chemical composition of plant tissues. The existence of a correlation between the chemical composition of plants and soils is not a sufficient condition for using the chemical composition of foliage as a biomonitor. In our opinion, a biomonitor should not reproduce the information already contained in soil analyses but should provide additional information on the environmental quality, of which soil quality is an essential part.

When evaluating the possibilities for phytoextraction it is meaningless to compare plant concentrations to normal ranges or to the soil concentrations. It was concluded that, to know whether a species is suitable for phytoextraction on a specific site, the amount of metals that are annually removed should be considered. The metal extraction ratio (MER) was proposed as the ratio of the amount of metals in the harvestable plant biomass to the amount to be extracted from the soil. This MER was thought to give more useful information for evaluating phytoextraction projects. On disposal sites for dredged sediments, phytoextraction of metals using trees seems not a realistic option for soil decontamination using the present best available techniques.
Chapter 6

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General discussion and conclusions

Sites for dredged sediment are often afforested as a measure to embed the sites in the landscape and to put these, otherwise useless sites, to a beneficial use. By doing so, the forested area increases (Flanders is a poorly forested region), soil and pollution are stabilized, recreational area might be provided, wood can be produced and buffer areas between hard to combine land uses might be established. Sites for dredged material are often polluted with a wide array of pollutants and the risk for spreading of pollutants in the environment should be avoided. As it is known that biota might play an important role in pollutant cycling, the main objective of this study was to investigate the species specific influence of afforestation of dredged sediments on the fate of metals. Specific aims were i) to evaluate and compare different tree species within the scope of reclamation and phytoremediation of metal polluted dredged sediment derived soils and ii) to quantify the reallocation of soil metals and ecosystem on the short and longer term under different tree species. This work compiles results of in situ studies from 2 to 33-years-old plantations with different tree species.

In the next paragraphs, the main results, in relation to the above objectives, are outlined and discussed, with special attention to the effect of tree species on the metal redistribution in the system and to the possibilities of trees to be used for phytoremediation (extraction or stabilization).
Main findings

Following conclusions were drawn:

Tree species can be divided in two groups based on the uptake patterns

When the uptake of metals in the aboveground biomass was considered, two groups could be distinguished. The biomass compartments of most tested tree species did not reveal elevated concentrations of metals compared to reference data while willows and poplars revealed elevated concentrations of Cd and Zn in their aboveground tissue. This holds as well for the young as for the old plantations, and the leaf concentrations on both young and old plantations were similar (see Chapter 1, 2 and 5). Hereafter, the possibilities and eventual risks of the elevated uptake of Cd and Zn by willows and poplars will be discussed first, followed by discussion of the tree species effects not related to uptake of metals.

Possibilities of uptake

Phytoextraction of metals using trees seems not realistic on disposal sites for dredged sediments using the present best available techniques

The high Cd and Zn concentrations in Salicaceae have been reported before and have lead to the idea that these species can be used for cleaning the soil by phytoextraction of Cd and Zn. This work showed that the possibilities for phytoextraction by trees on dredged sediments are limited (as was also shown by Vervaeke et al. 2003). Only Cd and Zn are taken up in high amounts, but the amounts were too small and remediation of the soil would take several hundreds of years. The amount of metals that can be exported from the soil by yearly harvesting wood and leaves is about 60 g of Cd and
3500 g Zn per ha (Chapter 1). Hundred years of annual harvesting would decrease the soil concentration with less than 0.5 mg kg\textsuperscript{-1} Cd and 27 mg kg\textsuperscript{-1} Zn, supposed that metals were extracted only from the upper 1 m of the soil (soil concentrations in this study were about 1-15 mg Cd kg\textsuperscript{-1} and 300-1500 mg Zn kg\textsuperscript{-1}).

Several authors claim however that there are possibilities for the use of willows to remediate soils from Cd pollution, in cases where the concentration of Cd in a soil is just above guideline values (Meers et al. 2005; Dickinson and Pulford 2005). Nevertheless, in practice, some remarks have to be made. Firstly, sites that are only polluted with Cd will not be easy to find, especially not dredged sediments. Secondly, according to Flemish legislation the soil has to be cleaned till background values. In case of a slight exceedance of limits it might be possible to decrease the concentration of Cd below the limit in a ‘realistic’ timescale, but decrease till the background values will require an excessive amount of time. The third problem is a problem that was shown to be very relevant namely the risk of spreading the metals through uptake in the leaves. It is therefore said that it might be better to harvest both wood and leaves, although it is not yet sure if site productivity will be sustainable in that way.

The metal extraction ratio (MER) was suggested for the evaluation of phytoextraction capacity of plant species

The evaluation of the phytoextraction capacity of plants is often made by comparing the biomass concentrations to reference concentrations or by calculating the ratio of the plant to soil concentration (=BCF). However, when evaluating the possibilities for phytoextraction it is meaningless to compare plant concentrations to normal ranges or to the soil concentrations. It was concluded that, to know if a species is suitable for phytoextraction on a specific site, the amount of metals that are removed annually should be considered. The metal extraction ratio (MER) was proposed as the ratio of the amount of metals in the harvestable plant biomass to the amount to be extracted from the soil (Chapter 6). This MER was thought to give more useful information.
General discussion and conclusions

Possibilities for biomonitoring

Accumulation of Cd and Zn from the soil in the aboveground biomass of willows and poplars and a correlation between the soil and plant concentration do not mean that the chemical composition of foliage of these species is suitable for biomonitoring trace metal concentrations in the soil (Chapter 6). The chemical composition of foliage can, however, provide additional information to the traditional soil samples.

Metal mobilization due to uptake

Sites for disposal of dredged sediment might cause a risk for metal transfer to the ecosystem

Cd levels, but not Zn, were elevated in small mammals living on disposal sites for dredged sediment of about 10-years-old, as compared with background levels (Chapter 3). No significant differences were found between sites in Cd or Zn levels in animals. In contrast, there was inter-site variation in contaminant levels in soil and in willow leaves. Small mammals were not found to accumulate Zn, probably because of the effect of homeostasis. Cd, on the other hand, was clearly accumulated, even on the site where soil and leaf analysis did not indicate an important pollution problem. The calculations using the BIOMAG model indicated that the Cd in the soil of the investigated disposal facilities probably caused low risk for predators. However, the elevated Cd and Zn concentrations in the willow leaves on the polluted sites and the high Cd concentrations in the small mammals on all the sites indicated that ecosystem development should be considered carefully.

Poplars can cause elevated Cd and Zn concentrations in the upper soil layer

The accumulation of Cd and Zn in the aboveground biomass of poplars mobilizes these
metals to the crown canopy, the forest floor and the surface soil layer. Redistribution of the metals via litter fall can cause the metal concentrations in the upper soil layer to increase. This is only possible when the estimated leaf concentrations in the decomposed litter were substantially higher than the concentrations in the soil. This occurred in poplar stands for Cd and Zn on the least polluted part of the 10-years-old site in Menen (Chapter 4) and on the 33-years-old site in Rotterdam (Chapter 5). Such redistribution might even cause the Cd or Zn concentrations for this horizon to exceed the legislative limits. These findings are relevant as the surface soil layer has most biological activity. Nevertheless, the soil layer that showed elevated concentrations was only about 3-5 cm thick, which might implicate that the risk and the extent of this process are rather limited.

_Elevated Cd and Zn concentration in the litter might cause retardation of the litter decomposition rate and acidification_

The upper soil layer under poplars in the 33-years-old site for dredged sediment showed not only elevated Cd and Zn concentrations, but also high organic carbon contents and low pH, as compared to the other tree species (Chapter 5). Litter decomposition was supposed to be retarded under poplars due to the high metal concentrations in the litter. Retarded litter decomposition causes higher acidification. The pH under poplar was indeed lower than expected. This might enhance the risk posed by the high soil concentrations because acidification tends to mobilize metal mobility.

It was not investigated if the high uptake of Cd and Zn by willows and poplars also caused higher risk for the ecosystem and uptake in the food web (see further), but in anticipation of further research, it seems that willows and poplars are not suited to be planted on sites for dredged sediments.
Metal mobilization not related to uptake

_Oak causes highest acidification and highest leaching from the surface soil layer_

Trees can also influence metal mobility in other ways than through uptake of metals. After 33-years of tree growth, we found a redistribution of metals in the soil profile that was dependant on the tree species (Chapter 5). Two major processes seemed to determine the soil concentration in the upper soil layer: the metal concentration of the leaves and species specific soil acidification. Oak seemed to acidify the surface soil layer more than ash and maple. The metal concentrations in this surface layer were lower under oak than under ash and maple because acidification increases metal mobility. Again, the surface soil layer was very thin and it is not sure if risks are higher when growing oak or ash and maple. The metal concentrations in the soil profile under ash seemed to have changed less of all species.

**Implications and research needs**

_Phytostabilization_

Although willow and poplar species are well adapted to the sediment derived soils, these species are not suited for phytostabilization. According to Ross (1994), the input of metals into the food web is potentially more harmful than metal leaching into the groundwater. Therefore, uptake of metals in the leaves should be avoided and species that do not take up metals such as maple, ash, oak, Robinia and alder should be chosen to be planted on these sites. If acidifying species, such as oak, are chosen, metals will leach from the surface soil layer. On the dredged sediment derived soils, this
acidification and leaching will be limited because of the high buffering capacity of these soils.

Further investigation should indicate to what extent acidification of the upper soil layer is desirable. Acidification of the soil will increase the risk for leaching of metals to the groundwater. On the other hand, the metal concentrations in the surface soil layer will decrease due to acidification, possibly causing the risk for uptake in the food web to decrease. The ecotoxicological and environmental risks involved with acidification should be investigated and weighed against the risk when soil was not acidified. Because of the high buffering capacity and low risk for leaching on dredged sediments (Tack et al. 1999), oaks might be more suited as the metal concentration in the surface soil layer decreases.

Other research needs concerning phytostabilization are i) the effects of afforestation on metal distribution should be compared to the effect of other vegetation types or other practices such as covering the sites with different materials. ii) Mobilizing and immobilizing processes should be investigated more in detail and need to be weighed against each other. Examination of the biota effects on these processes and process interactions will provide information for site and risk management.

Phytoextraction

Phytoextraction of soil metals using trees did not seem a realistic option due to the moderate and selective metal uptake. Unless metal accumulation in willow or poplar biomass or the biomass production itself can be drastically increased, the prospect of willow or poplar based phytoextraction systems becoming an interesting option for the remediation of contaminated sediments is small. More research might be needed to find species or clones with higher metal extraction ratios. A higher MER means that the
metal concentrations in the harvestable biomass are higher and/or biomass production is higher.

Risk assessment and monitoring

Risk assessment and monitoring should not only account for the concentrations in the soil, but also for the amounts of metals in the biota and the redistribution caused by the biota. We certainly want to encourage the use of plant analysis to describe plant and ecosystem functioning (implying biological productivity, soil quality, etc.). Resources should be invested in improving the interpretation of plant analysis in terms of environmental quality. Therefore, the chemical composition of plants or plant parts should be linked to ecophysiological characteristics that give information on biological productivity or plant functioning (see for example Luyssaert et al. 2005). If this step is made, plant analysis can become an essential part of assessing environmental quality including soil quality.
Summary

As a consequence of former river water pollution, dredged sediment is often polluted with trace metals. Dredged sediment sites, disposal sites as well as for example mounds constructed of dredged sediments, are often overgrown by trees, spontaneously developed or planted by men. Recently, the potential use of trees for the remediation of metal contaminated land has received increasing attention. Trees are said to be potentially suited for phytoextraction of metals or for phytostabilization. Phytoextraction aims at removal of the metals from the site by repeated coppicing of the trees, phytostabilization aims at fixation of metals in the soil. The central aim of this dissertation was to investigate the short and long term influence of tree growth on the reallocation of metals present in the dredged sediment and particularly to investigate the differences between tree species. This reallocation was measured in aboveground biomass compartments, soil compartments and small mammals living on the study sites.

A first survey was performed to explore the uptake of metals by different tree species and to evaluate the prospects of the use of trees for site reclamation by means of phytoextraction of metals or phytostabilization. The biomass concentrations of five tree species (black alder, white poplar, sycamore maple, common ash and black locust) growing on a dredged sediment mound were assessed.

Except for white poplar, the other tested species showed normal metal concentrations in their leaves. It was found that white poplar accumulated high concentrations of Cd (8.0 mg kg\(^{-1}\)) and Zn (465 mg kg\(^{-1}\)) in its leaves. Despite this elevated concentrations, phytoextraction of metals from the soil by harvesting stem and/or leaf biomass of white
Summary

Poplar is not a realistic option because it will require an excessive amount of time to be effective. If woody biomass is harvested together with the leaves, at most 0.12% of the total Cd stock in the upper 1 m soil could be removed with annual harvest. Furthermore, the high metal concentrations in its leaves cause risks of Cd and Zn dispersal in the food web of the ecosystem because of autumn litter fall. Therefore, this species was evaluated as being unsuitable for planting on dredged sediment sites.

Black alder, sycamore maple, common ash and black locust contained normal concentrations of Cd, Cu, Pb and Zn in their foliage, as compared to literature data. As a consequence these species cause low risk of metal dispersal in the ecosystem and are therefore more suitable species for phytostabilization under the given conditions.

The next step was to evaluate the risk for metal dispersal in the food chain. Therefore, the uptake of Cd and Zn by small mammals and willow trees was assessed on three sites of about 10-years-old with a different pollution degree. Willow species showed high concentrations of Cd and Zn in the aboveground biomass, similar to poplar species. The Cd concentrations in small mammals were elevated compared to background levels whereas Zn concentrations were rather low. There were no significant differences between sites in Cd or Zn levels in animals. Leaf analysis showed a clear response to the pollution degree of the sites. The calculations using the BIOMAG model indicated that the Cd of the soil caused low risk for predators. However, the elevated Cd and Zn concentrations in the willow leaves on the polluted sites and the high Cd concentrations in the small mammals on all sites indicated that ecosystem development should be carefully considered.

Further, the impact of tree growth on the soil metal concentrations was evaluated after 10 years of poplar growth. When the estimated concentrations of the decomposed litter were higher than the soil concentrations, the concentrations in the upper soil layer increased due to redistribution of the metals via litter fall. This occurred for Cd and Zn on the least polluted part of the site and the increase caused the Cd concentrations for
this horizon to exceed the legislative limits set by the Flemish authorities. When the metal concentration in the decomposed litter was lower than the concentration in the soil, no increase of the soil concentration occurred. Risk assessment is traditionally based on the soil concentrations but these results give a clear indication that risk assessment should not only account for the concentrations in the soil, but also for the amounts of metals in the biota and the redistribution caused by the biota.

Finally, on a 33-years-old disposal site for dredged material, the reallocation of metals was investigated to determine the species effect on the longer term and to distinguish the processes responsible for the metal reallocation. The reallocation in the soil depended on the tree species (poplar - *Populus* ‘Robusta’, oak - *Quercus robur*, ash - *Fraxinus excelsior* and maple - *Acer pseudoplatanus*). The thriving processes responsible for this reallocation were found to be accumulation of metals in the leaves and soil acidification due to tree growth. The typically high uptake of Cd and Zn by poplar caused a significant increase of Cd and Zn concentrations in the surface soil layer. Oak and poplar seemed to acidify the soil which caused a decrease of the concentration of metals in the upper soil layer due to leaching. The low pH under poplar, associated with high carbon concentrations, was unexpected and was assigned to retardation of the litter decomposition due to the elevated concentrations of Cd and Zn in the litter and upper soil layer. Leaching of metals from the upper soil layer might cause a risk for contamination of the ground water. On the other hand, the risks for uptake in the food web and for biomagnification might become smaller. At the moment, our results indicate that, within the scope of risk minimizing, ash and maple are most suited species for planting sites for dredged material. These species cause least reallocation of the metals in the soil and biomass compartments, making them most suitable for phytostabilization of polluted dredged sediment sites.
Samenvatting

Baggerterreinen, zowel baggerstorten als bijvoorbeeld baggedijken, zijn vaak begroeid met bomen, spontaan ontwikkeld of aangeplant. Sinds enkele jaren kan het gebruik van bomen voor remediatie van vervuilde terreinen rekenen op een verhoogde belangstelling. Bomen worden verondersteld mogelijk geschikt te zijn voor fytoextractie van metalen of voor fytostabilisatie. Fytoextractie heeft tot doel het verwijderen van metalen uit de bodem door herhaaldelijk oogsten van met zware metalen aangerijkte biomassa, fytostabilisatie heeft tot doel het vastleggen van de vervuiling in de bodem. De algemene doelstelling van dit onderzoek was de korte en lange termijn invloed van boomgroei op de reallocatie van de in het slib aanwezige metalen te onderzoeken. Hierbij werd in het bijzonder gefocust op het verschil tussen boomsoorten. De reallocatie van de metalen werd gemeten in de bovengrondse biomassa, de bodem compartimenten en op een aantal terreinen ook in muizen die op deze terreinen leefden.

Op een pas aangeplante site werden de verschillen in metaalopname tussen de soorten onderzocht. Aan de hand van deze gegevens werden de mogelijkheden voor remediatie van baggerterreinen via fytoextractie of fytostabilisatie onderzocht. De aangeplante soorten op dit terrein waren: abeel (Populus alba L.), zwarte els (Alnus glutinosa L. GAERTN.), esdoorn (Acer pseudoplatanus L.), gewone es (Fraxinus excelsior L.) en robinia (Robinia pseudoacacia L.). Al deze soorten, met uitzondering van abeel, vertoonden normale metaalconcentraties in hun bladeren. Abeel accumuleerde hoge concentraties Cd (8,0 mg kg⁻¹) en Zn (465 mg kg⁻¹) in zijn bladeren. Niettegenstaande deze hoge concentraties bleek dat fytoextractie van metalen uit de bodem door middel van het regelmatig oogsten van de stam- en bladbiomassa geen realistische optie was vanwege onaanvaardbaar lange remediatietermijnen. Door het oogsten van blad- en stambiomassa kan jaarlijks maximaal 0.12% van de totale Cd hoeveelheid uit de bovenste meter bodem verwijderd worden. Voor de andere metalen ligt dit percentage
nog veel lager. Daarbovenop zouden de verhoogde Cd en Zn concentraties in de bladeren een risico kunnen veroorzaken voor verspreiding van deze elementen in het ecosysteem. Daarom wordt abeel niet aangeraden om te planten op deze terreinen.

De andere soorten vertoonden normale concentraties Cd, Cu, Pb en Zn in hun bladeren, vergeleken met literatuurgegevens. Deze soorten veroorzaken bijgevolg een lager risico voor verspreiding van metalen naar de omgeving. Deze soorten lijken ook meer geschikt voor fytostabilisatie in de gegeven omstandigheden.

De volgende stap in het onderzoek was het evalueren van het risico voor verspreiding van metalen door verhoogde opname van metalen. Hiervoor werd de opname van Cd en Zn door wilgen en muizen begroot op drie baggerstortterreinen van ongeveer 10 jaar oud met een verschillende vervuilingsgraad. De wilgen vertoonden eveneens hoge Cd en Zn concentraties in de bovengrondse biomassa. De concentraties in de bladeren waren gecorreleerd met de vervuilingsgraad van de terreinen. De Cd concentraties in de muizen waren vrij hoog vergeleken met achtergrondwaarden uit de literatuur terwijl de Zn concentraties vrij laag waren. Deze verschillen in concentraties tussen de muizen die op de verschillende terreinen gevangen werden waren niet significant. Berekeningen met het BIOMAG model gaven aan dat de Cd in de bodem van het meest vervuilde terrein slechts een laag risico veroorzaakte voor predatoren. De hoge Cd en Zn concentraties in de wilgenbladeren op de meer vervuilde sites en de hoge Cd concentraties in de muizen op alle terreinen tonen echter aan dat natuurontwikkeling goed moet overwogen worden.

Verder werd ook de impact van boomgroei op de metalconcentraties in de bodem geëvalueerd op een 10 jaar oude populierenaanplant. Waar de geschatte metaalconcentratie van het strooisel na afbraak hoger was dan de bodemconcentratie bleek de concentratie in de bovenste bodemlaag gestegen omwille van redistributie van de metalen via bladval. Dit fenomeen deed zich voor voor Cd en Zn op het minst vervuilde gedeelte van het terrein en veroorzaakte zelfs een overschrijding van de
bodemmaaningsnormen voor Cd voor deze bodemlaag. Het risico van vervuilde bodems wordt traditioneel ingeschat vertrekkende van de bodemconcentraties maar de resultaten van dit onderzoek gaven een duidelijke indicatie dat risico-bepaling niet enkel rekening moet houden met bodemconcentraties maar ook met hoeveelheid metalen in de biota en met de door de biota veroorzaakte redistributie van de metalen.

Finaal werd ook de reallocatie van metalen onderzocht op een 33 jaar oud baggerstort bebost met populier (Populus ‘Robusta’), zomereik (Quercus robur), gewone es (Fraxinus excelsior) en esdoorn (Acer pseudoplatanus). De reallocatie van metalen in de bodem was duidelijk afhankelijk van de boomsoort. De sturende processen voor de reallocatie bleken opname van metalen door de bomen te zijn en soortspecifieke bodemverzuring. De hoge opname van Cd en Zn door populier veroorzaakte ook hier een significante stijging van de concentratie van deze elementen in de bovenste bodemlaag onder deze soort. Eik en populier bleken de bovenste bodemlaag meer te verzuren dan es en esdoorn wat dan weer een daling van de metaalconcentraties in deze laag tot gevolg had door de hiermee gepaard gaande hogere uitspoeling. De lage pH onder de populieren, die gerelateerd was aan een hoog gehalte aan organisch materiaal, was niet helemaal verwacht en zou kunnen veroorzaakt zijn door vertraagde afbraak van het strooisel omwille van de hoge Cd en Zn concentraties in het strooisel en de bovenste bodemlaag. Uitspoeling van metalen vanuit de bovenste bodemlagen kan eventueel een risico veroorzaken voor vervuiling van het grondwater. Van de andere kant zal uitspoeling uit de bovenste bodemlagen er ook voor zorgen dat het risico voor verspreiding in het voedselweb en voor biomagnificatie kleiner zal worden. Op dit ogenblik duiden onze resultaten er op dat, met het oog op zo laag mogelijke risico’s, es en esdoorn de meest geschikte soorten zijn voor het beplanten van baggerterreinen. Deze soorten zullen de minste reallocatie van metalen veroorzaken in de bodem en de biomassacompartimenten. Dit maakt hen van de geteste soorten meest geschikt voor fyto-stabilisatie van metaal vervuilde baggerterreinen.
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