# Accumulation of Elements in *Salix* and Other Species Used in Vegetation Filters with Focus on Wood Fuel Quality

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# Abstract

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Woody or herbaceous perennials used as vegetation filters for treatment of different types of wastes can be suitable for production of solid biofuels when their aboveground harvestable biomass yield is sufficiently high and when biomass contains appropriate concentrations of minerals with regard to fuel combustion processes. The concentrations of nitrogen (N), potassium (K) and heavy metals (especially Zn and Cd) in fuel should be low and calcium (Ca) concentrations high to avoid technical problems and environmentally harmful emissions during combustion. Since soil supplementation with essential elements improves biomass yield, a conflict might arise between yield and quality aims. There are various possibilities to influence fuel quality during the growing phase of the life cycle of perennial biomass crops.

This study assessed the suitability of two deciduous woody perennials (*Salix* and *Populus*) and two summer green herbaceous perennials (*Phragmites* and *Urtica*) for phytoremediation in terms of growth and nutrient allocation patterns. *Salix* and *Populus* proved suitable as vegetation filters when nutrients were available to plants in near-optimal proportions, but when unbalanced nutrient solutions (wastewater) were applied, stem biomass fraction was strongly reduced. *Phragmites* was more tolerant to wastewater treatment in terms of plant biomass production and nutrient allocation patterns, so if the N:P ratio of the wastewater is suboptimal, a vegetation filter using *Phragmites* could be considered.

In further studies, a method was developed to determine the proportions of nutrient-rich bark in coppiced *Salix*, while heavy metal phytoextraction capacity was assessed in two *Salix* vegetation filters. The relevance of proportion of bark on wood fuel quality and element removal from vegetation filters was also investigated. The concentrations of the elements studied in harvestable *Salix* shoot biomass were higher, meaning lower wood fuel quality, in plantations where proportion of bark was high. Removal of elements increased with biomass yield. As proportion of nutrient-rich bark decreases with increasing yield, longer cutting cycles should be considered, in order to improve fuel quality and nutrient removal potential.

*Keywords:* ash, domestic wastewater, growth form, heavy metals, landfill leachate, municipal sludge, nitrogen use efficiency, phosphorus use efficiency, short-rotation coppice

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***To my family***

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# Preface

## Papers I-IV

The present doctoral thesis is based on the following papers, referred to in the text by their Roman numerals:

I Adler, A., Karacic, A. & Weih, M. Biomass allocation and nutrient use in fast-growing woody and herbaceous perennials used for phytoremediation. (Submitted to *Basic and Applied Ecolog*y).

II Adler, A., Verwijst, T. & Aronsson, P. 2005. Estimation and relevance of bark proportion in a willow stand. *Biomass and Bioenergy* 29, 102-113.

III Dimitriou, I., Eriksson, J., Adler, A., Aronsson, P. & Verwijst, T. 2006. Fate of heavy metals after application of sewage sludge and wood-ash mixtures to short-rotation willow coppice. *Environmental Pollution* 142(1), 160-169.

IV Adler, A., Dimitriou, I., Aronsson, P., Verwijst, T. & Weih, M. Wood fuel quality of two *Salix viminalis* stands fertilised with sludge, ash and sludge-ash mixtures. (Submitted to *Biomass and Bioenergy*).

For Paper I, Dr. Almir Karacic and I planned the experiment and collected data, while I analysed the data and wrote the paper, consulting with Dr. Martin Weih on theoretical issues. For Paper II, Prof. Theo Verwijst and Dr. Pär Aronsson did the planning, sampling and preliminary data analysis, while I was responsible for the final data analysis and writing of the manuscript. For Paper III, all authors were involved in planning the experiment, while the sampling of willow shoots to obtain data on bark proportions as well as the statistical analyses related to bark proportions were my responsibility. Dr. Ioannis Dimitriou was responsible for the rest of the statistical analyses and for writing the paper. Paper IV was based on the same experiment as Paper III, where I was partly responsible for planning the experiment and collecting data. I wrote the paper and had main responsibility for data analysis.

# Introduction

In the life cycle of biomass fuels, three phases can be distinguished: a growing phase, a supply phase and an energetic phase (van Loo & Koppejan, 2003). The present study focuses on the growing phase of some perennial energy crops, mainly willow (*Salix* spp.). The characteristics that can be influenced during the growing phase of willow biomass fuel are mainly the yield, the proportion of bark and the chemical composition.

## Perennial energy crops

Suitable perennial crops for production of solid biofuels are characterised by high lignin and cellulose concentrations. In such energy crops, the whole aboveground biomass is harvested and used for combustion. Typical perennial energy crops are either trees that produce aboveground stem biomass (such as willows and poplars (*Populus* spp.) or perennial grasses that produce belowground stem biomass (rhizomes). Stalky aboveground biomass (straw) of perennial grasses and annual crops such as cereals can also be used as biofuel. The present thesis focuses only on perennial crops.

In addition to forest residues, short rotation woody crops such as *Eucalyptus*, *Populus* and *Salix* have been used as wood fuel or as industrial wood for centuries (Mitchell *et al.*, 1992; Balatinecz *et al.*, 2001). In Sweden, *Salix* plantations cover about 14 300 ha at present (SCB, 2005) and produce biomass at rates of up to 4.5 t DM $ha^{-1}$ $yr^{-1}$ during 2- to 15-year-long rotations in commercial plantations (Mola-Yudego & Aronsson, 2007). A few commercial plantations with *Populus* have also been established in southern Sweden and harvested after the first 9 - 14 years, with a biomass production of approx. 9 t DM $ha^{-1}$ $yr^{-1}$ (Karačić *et al.*, 2003).

Rhizomatous grasses and deciduous woody perennials can significantly contribute to sustainable biomass production due to their high production capacity (Lewandowski *et al.*, 2003). Yields of more than 30 t DM $ha^{-1}$ $yr^{-1}$ have been measured in experimental plantations of swithgrass (*Panicum virgatum*), miscanthus (*Miscanthus* spp.) and giant reed (*Arundo donax*) in southern and central Europe, and up to 12 t DM $ha^{-1}$ $yr^{-1}$ for reed canary grass (*Phalaris arundinacea*) in Scandinavia. Reed canary grass is the lowest yielding of the most important biomass grasses, but it is the only perennial rhizomatous grass that can be produced in regions with short growing seasons and cold winters, such as northern Europe.

## Environmental impact of production of perennial energy crops

Perennial woody and herbaceous crops have several ecological benefits. Contrary to annual crops, the need for soil tillage in perennial crops is limited to the year of establishment. Long periods without tillage mean a reduced risk of soil erosion and nutrient leaching, as well as a likely increase in soil carbon content (Börjesson, 1999; Ma & Wood, 1999). Since perennial crops have few natural pests, they may also be produced with little or no pesticide use (Lewandowski *et al.*, 2000).

With perennial energy crops, not only the end use but also the production of biomass could lead to benefits for the environment. Despite some negative effects caused, for instance, by application of herbicides and fertilisers, the production and use of biomass is more beneficial compared with fossil fuel use as far as the environment is concerned (Demirbas, 1998; Marosvölgyi *et al.*, 1999; Rafaschieri *et al.*, 1999). As many of the activities related to the procurement of biomass fuels involve use of fossil fuels (*e.g.* transportation), it is not fully correct to define biomass fuels as $CO_2$-neutral when considering the complete life cycle from procurement, transportation, storage and conversion to discharge and handling of the ash (van Loo & Koppejan, 2003). However, procurement of biomass fuels in general consumes less energy than that of fossil fuels, and combustion of fossil fuels causes the highest emissions of $CO_2$, $NO_x$ and $SO_x$ (Flyver Christiansen & Fock, 2000).

## Phytoremediation with perennial energy crops

Plants used to remove nutrients and pollutants from wastes are called vegetation filters (Perttu, 1993) and such waste improvement by plants is called phytoremediation. Vegetation filters employ fast-growing woody (*e.g. Salix*, *Populus*) and/or herbaceous perennials (*e.g. Phragmites australis*) for treating contaminated water and/or soil. Vegetation filters can consist of constructed treatment wetlands or arable and riparian fields where perennial species are planted. The main difference between constructed wetlands and field vegetation filters is that the former systems are isolated from the groundwater.

The removal of pollutants by plants is based on their capability to maintain high growth rates and element accumulation into their tissues, *i.e.* phytoextraction, in moderately contaminated environments. Alternatively, rhizome and/or root systems of plants can prevent the migration of elements and contaminants through the soil profile, thereby reducing risks of further environmental degradation, *i.e.* rhizostabilisation (Salt *et al.*, 1998).

Fast-growing deciduous woody perennials (*Salix*, *Populus*) have been commercially cultivated for energy since the 1980s in Sweden. Due to their high biomass production and capacity for accumulation of nutrients and even pollutants from wastewaters and sludge, short rotation willow coppices in particular have also proven advantageous for use as vegetation filters (Perttu, 1993; Perttu & Kowalik, 1997; Aronsson, 2000; Aronsson & Perttu, 2001). Organic waste products used as fertilisers must meet the nutrient demands of *Salix*, without exceeding the limits for maximum heavy metal loading rates on agricultural land in Sweden (Hansson *et al.*, 1999). Fertilisation of energy crops with organic wastes is associated with reduced costs, since nutrient removal at municipal treatment plants is not necessary (Rosenqvist *et al.*, 1997). However, the nutrient concentrations are normally low in organic wastes, which increases the volumes to be transported to the field and thereby the logistic costs (Hansson *et al.*, 1999). Some of the products may also contain rather high rates of heavy metals (Brady &

Weil, 2002) and other pollutants. On the other hand, cultivation of energy crops can often be located in the vicinity of the local wastewater treatment plants in small and medium-sized municipalities.

The vegetation of *Phragmites australis* is often used as a filter in treatment wetlands (Kadlec & Knight, 1996) due to its ability to adapt to a wide range of environments (Ksenofontova, 1988; Amsberry *et al*., 2000).

## Quality of biomass fuels

Several aspects determine the suitability of perennial crops for production of solid biofuels. The first criterion in producing such crops is a high yield of energy, which is the product of the energy content in the biomass and the unit-area yield. Perennial energy grasses and deciduous trees contain energy-rich compounds in high proportions (Table 1). There are different quality criteria for solid biofuels. Low water content in the harvested biomass is a prerequisite for safe storage without self-ignition (Clausen, 1994; Jirjis, 2005) and a high net heating value (Lehtikangas, 1999; van Loo & Koppejan, 2003).

Table 1. *Heating value and content of energy-rich compounds in energy crops*

| Compound | Heating value (MJ $kg^{-1}$)[1] | Perennial energy grasses[2] | Deciduous woody perennials[3] | | *Phragmites*[4] | |
|---|---|---|---|---|---|---|
| | | | Bark | Wood | Leaf-blades | Culms and leaf sheaths |
| Lignin | 25-29 | 15-30% | 40-50% | 18-25% | 10-26% | 9-18% |
| Cellulose | | 32-42% | | | 28-34% | 48-56% |
| Hemi-cellulose | 17 | | 32-47% | 74-80% | 25-34% | |
| Extractives | 35 | | 5-12% | 2-9% | | |

[1]Sjöström, 1993; [2]Ververis *et al*., 2004; [3]Hakkila, 1989; [4]Dinka *et al*., 2004

The variation in proportion of bark among species/clones can have pronounced effects on the characteristics of the wood fuel. The proportion of bark in one-year-old stems of different *Salix* and *Populus* species and clones varies between 18 and 43% (Geyer, 1981; Anderson *et al.*, 1984; Krigstin, 1985; Sennerby-Forsse, 1985). It is apparent that the bark of small-diameter stems can form a large proportion of the composite biomass produced in short rotation crops. In the upper part of the stem, bark is thinner but makes up a larger proportion of the stem weight and volume. The bark comprises 30% of the stem tissue in the upper half of trees 12-14 m in height, compared with 10-15% in the lower half (Kenney *et al.*, 1990). According to Kenney *et al.* (1990), proportion of bark can be manipulated by silvicultural treatments, or by the selection or breeding for clones that allocate aboveground biomass to few large stems or many small stems.

Bark has significantly higher amounts of inorganic elements than wood (Sjöström, 1993; Meszaros *et al.*, 2004). Therefore, a comparative analysis of different biomass fractions is essential for an understanding of the quality of wood fuel. High ash content of bark (approx. 5%), perennial energy grasses and straw lowers the heat transfer during combustion (van Loo and Koppejan, 2003;

Obernberger, Brunner & Bärnthaler, 2006). When combusting biomass, the $NO_x$-emissions increase with the nitrogen (N) concentration of the biomass (Becher, 1998). However, the N concentration of the fuel is not the only determining factor for $NO_x$-emissions. Other factors include *e.g.* the type of combustion installation, combustion conditions and post-combustion removal of $NO_x$ from the flue gas (Lewandowski & Kauter, 2003). However, the N concentration is a less important fuel characteristic compared with the concentrations of alkali metals and chlorine (Cl). Potassium (K) and Cl are involved in corrosion processes, K and sodium (Na) in slagging processes and these elements can therefore cause technical complications during power plant operation (Scott *et al.*, 2000; Müller-Hagedorn *et al.*, 2003). There is an inverse relationship between elevated K concentrations, which typically occur in stalky graminaceous biomass, and ash melting temperature. Calcium (Ca) and magnesium (Mg) usually increase the ash melting point, while K decreases it (Obernberger, 1997; van Loo and Koppejan, 2003; Obernberger, Brunner and Bärnthaler, 2006). Ash melting in straw-type biomass typically occurs at temperatures below optimal operation temperatures of combustion plants. This leads to slagging and fouling of the combustion chamber and the heat exchanger surfaces. Slag impedes the heat transfer and favours high-temperature corrosion. It has to be removed manually because technical solutions to cope with slagging are very expensive (Lewandowski & Kauter, 2003). High concentrations of zinc (Zn) and cadmium (Cd) in biomass fuels can cause problems in ash recycling, since these metals accumulate in the ash during combustion or are emitted to the atmosphere as particulates (van Loo & Koppejan, 2003).

The chemical composition of biomass material also varies with timing of sampling occasion (Ernst, 1995). In the present thesis, willows were harvested and concentrations of mineral elements in these were analysed in early autumn (Paper I) and in late winter (Papers II-IV). To calculate the element content of willow shoots in this study, a method of weighted averages was used, where the concentrations of elements in bark and in wood in different parts of shoots were multiplied by the respective dry weight proportion of the shoot section (Papers II-IV).

## Using wastes as fertiliser

Municipal wastewater is the wastewater from a community. The characteristics of the wastewater vary with location depending on the population served. Domestic wastewater includes typical wastes from the kitchen, bathroom, laundry and car washing as well as that discharged from commercial, institutional, and similar facilities (Kadlec & Knight, 1996). Chemically, wastewater is composed of organic and inorganic compounds as well as various gases. Organic components may include carbohydrates, proteins, fats, surfactants, oils, pesticides, phenols, *etc*. Inorganic components may include heavy metals, nitrogen, phosphorus, sulphur, chlorides, alkalis, toxic compounds, *etc*. In domestic wastewater, the organic and inorganic portions make up approximately 50% each. Gases commonly dissolved in wastewater include hydrogen sulphide, methane, ammonia, oxygen, carbon dioxide and nitrogen. The first three gases result from the decomposition of organic matter present in the wastewater (Kadlec & Knight, 1996).

Landfill leachate refers to the liquid that seeps through a landfill site and is usually characterised by high concentrations of ammonium ($NH_4$) and high ionic strength caused by high concentrations of chloride (Cl) and sodium (Na). This liquid may already be present in the material dumped in the landfill, or it may be the result of rainwater entering the landfill, filtering through the waste material and picking up additional chemicals before leaking out into the environment. The leachate contains also water from decomposition processes in landfill. Leachate passing through landfill is most likely to eventually mix with the groundwater near the site (Williams, 2005).

Sewage sludge is the main solid waste produced by wastewater treatment processes. It is essential to utilise this material in a sustainable and beneficial way. Following EU legislation, the disposal of sewage sludge in landfills in Sweden was banned from 2005 (Swedish EPA, NFS 2004:4). Using sewage sludge as an organic fertiliser in short rotation willow coppice has become an acceptable alternative to spreading of sludge on agricultural land. In Sweden, there are strict regulations concerning both the total sludge amounts applied and the maximum permissible heavy metal concentrations in soils after sludge application (EEC, 1986; Swedish EPA, 2004). Sludge application to agricultural land increases soil organic matter content and provides the crops with nutrients. In comparison with inorganic fertilisers, sewage sludge is generally low in nutrients, especially as regards K (Brady & Weil, 2002), the concentration of which is low because most K present is in soluble form and remains in the effluent during treatment. The phosphorus (P) content is higher, because advanced sewage treatment is designed to remove P from the effluent and deposit it in the sludge component. If the treatment precipitates P by reactions with iron or aluminium compounds, the P in the sludge is likely to have a low availability to plants.

Wood ash is the inorganic and organic residue remaining after the combustion of wood or unbleached wood fibre. Calcium is the most abundant element in wood ash and gives the ash properties that are similar to those of agricultural lime. Today there is no legislation regarding ash recycling on agricultural land in Sweden. Wood ash treatment has been shown to increase soil pH, causing a mineralisation pulse and an increase in nitrate-reductase activity in the fine roots (Genenger *et al.*, 2003a). An unsustainable base-cation balance in forest soils, resulting from whole-tree harvesting and acid deposition or both, can be counteracted by recirculation of wood ash (Lundborg, 1998; Saarsalmi *et al.*, 2001). Given its alkalising character, the effects of wood ash on the soil environment and biota are to some extent similar to the effects of liming. Lime has been widely used to counteract soil acidification, although there is a risk of increased leaching of nitrate (Lundell, Johannisson & Högberg, 2001). The changes in pH and microbial biota in soils are related to the dose and form of the wood ash applied (Perkiomäki & Fritze, 2002; Genenger *et al.*, 2003b). Compacted wood ash dissolves more slowly than loose ash, and therefore the latter can induce more changes at the same application rate. Stabilisation of wood ash before spreading is necessary to avoid the risks of dust, salt effects, pH shock and burn damage to vegetation (Steenari & Lindqvist, 1997). Positive effects of hardened wood ash treatment in aboveground organs of trees, such as increased concentrations of P, Ca and K in the needles of *Picea abies*, have been demonstrated (Arvidsson & Lundkvist, 2002). Effects of wood ash treatment on soil chemistry (pH, base saturation) and on fine roots have been reported to occur mainly in the upper 0-5 cm of the soil (Clemensson-Lindell & Persson, 1995; Arvidsson & Lundkvist, 2003). A long-term effect of wood ash on fine roots of *Picea abies* has been reported in deeper mineral soil layers (Püttsepp, 2004).

## Objectives and hypotheses

- To assess the suitability of two deciduous woody perennials (*Salix* and *Populus*) and two summer green herbaceous perennials (*Phragmites* and *Urtica*) for use as vegetation filters (**Paper I**). Two major hypotheses tested were that: (1) species combining high relative growth rate (RGR), low nutrient productivity (A) and long mean residence time (MRT) of nutrients would be the most effective in accumulating nutrients; and (2) vegetation filters of deciduous woody perennials would be more effective in nutrient removal compared with summer green herbaceous perennials, due to extensive above ground perennial parts that easily can be harvested.
- To develop a method for estimation of bark proportions in stands of *Salix* used for biomass production and/or as vegetation filter (**Paper II**).
- To assess the effects of different proportions of bark in stands of *Salix* fertilised with sludge and wood ash on wood fuel quality and above ground nutrient accumulation (**Papers III, IV**). The hypothesis tested was that wood fuel with a higher proportion of bark would contain more essential elements and heavy metals than wood fuel with a lower proportion of bark (**Paper IV**).

# Outline of experimental work and methods

## Growth study (Paper I)

Information concerning partitioning of growth among roots and perennial and seasonal aboveground plant parts is necessary for an understanding of how application of wastewater may affect growth. An outdoor experiment was therefore conducted at Ultuna, central Sweden, to study the suitability of species from two different growth forms for use in vegetation filters (Table 2). Aboveground stem fragments (cuttings) of deciduous woody perennials (*Salix* and *Populus*) and belowground stem (*i.e*. rhizome) fragments of summer green herbaceous perennials (*Phragmites australis* and *Urtica dioica*) were planted in 4-litre pots. The growth response of the plants was studied at genet level (Fig. 1). A genet is an independent plant individual originating from one seed – a genotype (Harper, 1977). An individual genet may be a tiny seedling, a tree or a clone of a rhizomatous plant. Several aboveground shoots that are interconnected with each other by rhizomes are thus also a genet. Studying plants at genet level allows comparison of biomass and nutrient allocation into perennial and seasonal tissues between these growth forms. After establishment, all the plants were fertilised weekly during the growing season. The aim was to compare the plants treated with municipal wastewater and landfill leachate with the plants grown in optimal growth conditions (balanced nutrient solution) and those in the treatment where no nutrients were added (pure water). The aim was to assess how much of the potential of the species to grow and accumulate nutrients was realised in the municipal wastewater and landfill leachate treatments.

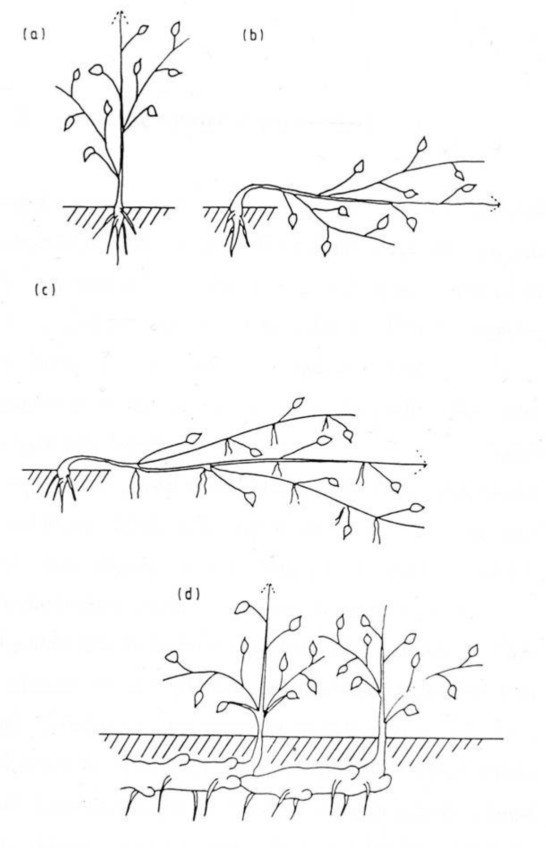


*Figure 1.* Continuum of modular growth from woody perennials to rhizomatous perennials. a) The main axis is vertical, rooting nodes are missing; b) the main axis is horizontal, rooting nodes are missing; c) the main axis is horizontal, rooting nodes are present; d) vertical shoots develop on a horizontal main axis, that can be a sympodium or monopodium (from Clegg, 1978)

## Methodological study (Paper II)

The method developed to estimate proportion of bark in willow stands was based on the shoots of clone 77-683, collected in an experimental plantation at Ultuna, near Uppsala (Table 2). The general idea of the method was to calculate proportion of bark in the whole stand on the basis of proportion of bark in sample pieces from a small number of shoots (destructive measurements)(Fig. 2) and the shoot size distribution in the whole stand (non-destructive measurements of all shoots in randomly selected stools)(Fig. 3). In the study, weighted averages of the proportion of bark in different age fractions of entire shoots were used to calculate the proportion of bark in fifteen destructively sampled shoots. Thereafter the relationship between $D_{55}$ and proportion of bark in the destructively measured shoots (Fig. 2) was calculated.

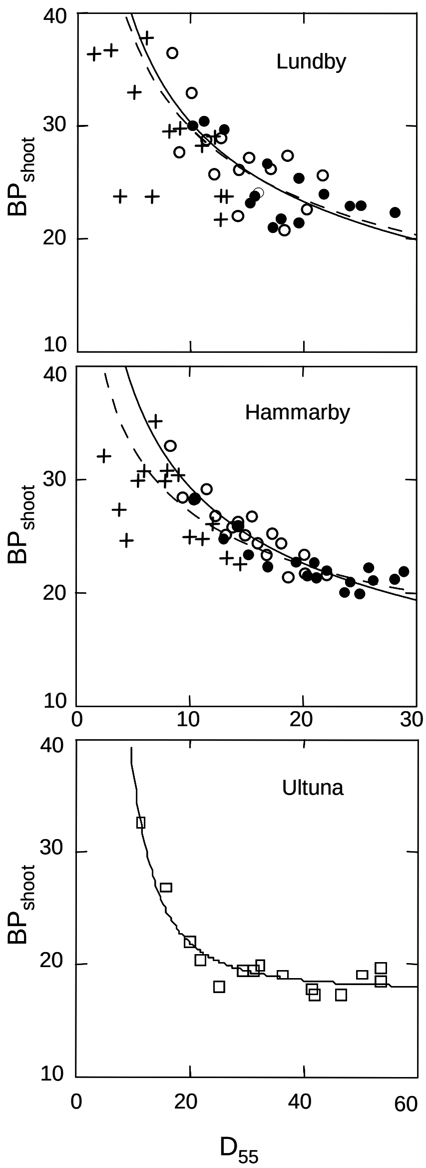


*Figure 2.* Relationship between D55 (shoot diameter at 55 cm height) and proportion (%) of bark ($BP_{shoot}$) for 1-year-old (crosses), 2-year-old (open circles, solid line) and 3-year-old shoots (filled circles, dashed line) of *Salix viminalis* (clone 78-021) grown at two sites in central Sweden (part a and b) and for 5-year-old shoots (open squares, solid line) of *Salix viminalis* (clone 77-683) grown at Ultuna in central Sweden (part c).

a) 2-year-old shoots: $BP_{shoot}=-2.0+73.0 \times D55^{(-0.35)}$, $R^2=0.54$; 3-year-old shoots: $BP_{shoot} =-2.0+66.60 \times D55^{(-0.32)}$, $R^2=0.60$,

b) 2-year-old shoots: $BP_{shoot}=-2.0+70.14 \times D55^{(-0.35)}$, $R^2=0.86$; 3-year-old shoots: $BP_{shoot} =-2.0+52.48 \times D55^{(-0.25)}$, $R^2=0.81$,

c) 5-year-old shoots: $BP_{shoot}=0.2+32.8 \times D55^{(-2.22)}$.

## Field study (Papers III and IV)

A field experiment was designed to investigate the accumulation of heavy metals (Paper III) and nutrients (Paper IV) in willow shoots (*Salix viminalis* clone 78-021) in response to fertilisation with dewatered sewage sludge and/or loose wood fuel ash (Table 1). The two study sites were located in central Sweden. The aim of the study was to assess whether application of sewage sludge and wood fuel ash as fertiliser for energy willow plantations is a sustainable practice. A particular question addressed was whether willow plants accumulated similar amounts of heavy metals into their stems as was supplied to the experimental plots by application of sludge and/or ash. Thus, the phytoextraction potential of willow plantations was addressed (Paper III). In addition, the relevance of proportion of bark and yield for element removal and wood fuel quality was studied (Paper IV).

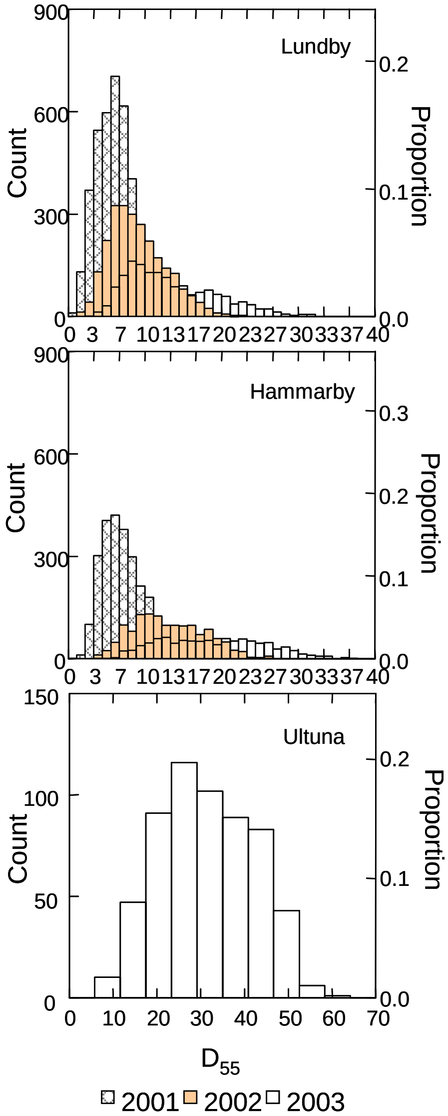


*Figure 3.* Shoot size distribution of non-destructively measured shoots in three willow plantations in Central Sweden. Diameters of 55 cm height ($D_{55}$) of all shoots in 240 stools after 1st, 2nd and 3rd year of cutting cycle a) at Lundby and b)at Hammarby, and c) $D_{55}$ of all shoots in 142 randomly selected stools after 5th year of cutting cycle at Ultuna.

Table 2. *Summary of the studies included in this thesis*

| Study | Species | Experimental conditions | Site/s | Experimental design | Treatment/s | Replicates | Age of plants |
|---|---|---|---|---|---|---|---|
| Growth response of perennials (I) | *Salix, Populus, Urtica, Phragmites* | Outdoor pot experiment | Ultuna | Randomised block design | Wastewater, Leachate, Balanced nutrient solution, Pure water | 4 | One growing season |
| Methodological study (II) | *Salix* | Research plantation | Ultuna, one willow plantation | - | - | - | 5-year-old shoots on 12-year-old roots |
| Phytoextraction studies (III, IV) | *Salix* | Field experiment | Linnes Hammarby, Lundby Gård; Two commercial willow plantations | Randomised block design | Wood ash, sludge 2 doses of sludge-ash mixtures, control | 4 | 1-year-old shoots and 3-year-old shoots on 4-year-old roots |

# Results and Discussion

## Growth and nutrient partitioning of plants treated with wastewater or landfill leachate

For healthy growth, plants need essential nutrients in optimal proportions (Ingestad, 1971; Ingestad & Ågren, 1995). Municipal wastewater and landfill leachate contain nutrients in proportions far from optimal for plant growth. Treatments with these wastes affect plant growth and nutrient allocation pattern in a way that cannot be predicted by conventional plant growth models.

Accumulation of nutrients in plant parts that are subject to biomass harvest is an important N and P removal mechanism in vegetation filters. In Paper I, the experimental treatments strongly affected plant growth and nutrient accumulation. Across the species, municipal wastewater and landfill leachate treatments (*i.e.* phytoremediation treatments) resulted in greatly reduced (by 75%) total plant biomass compared with the balanced nutrient solution treatment and up to threefold increase in total plant biomass compared with plants in the pure water treatment (Fig. 2 and Table 4 in Paper I). During the short period of the experiment (one growing season), *Phragmites, Populus* and *Salix* had accumulated larger plant nutrient pools compared with *Urtica* at September harvest. The largest proportion of N and P was found in seasonal tissues in all species in balanced nutrient solution and phytoremediation treatments (Fig. 4). *Phragmites* accumulated a larger amount of biomass and nutrients (N and P) into its belowground stems in phytoremediation treatments compared with the biomass and nutrient accumulation into stems of deciduous woody perennials (Fig. 4). *Salix* and *Populus* responded to the phytoremediation treatments by allocating less biomass into stems and more biomass into roots. If the aboveground biomass is harvested at this time in the autumn, *i.e.* before the leaf senescence, a considerable amount of N and P could be removed from the vegetation filter (Toet *et al.*, 2005). Since biomass that is harvested as green has high nutrient and water concentrations, it can be used to produce biogas and to provide agricultural land with nutrients via biodigestate application (Hansson & Fredriksson, 2004). In addition, increasing nutrient levels in lakes, marshes and brackish bays are contributing to environmental problems such as eutrophication and overgrowth by emergent macrophytes, especially *Phragmites*. By harvesting of these `natural´ reed beds and producing biogas from the biomass, nutrients that have leached to lakes, could be returned to agricultural land via biodigestate application.

During autumn senescence, considerable amounts of nutrients are retranslocated into perennial tissues and roots. In addition to this autumn nutrient retranslocation, woody perennials may also take up considerable amounts of nutrients from soil during warm periods in late autumn or early winter (Weih & Karlsson, 1997; Weih, 2000). The concentrations to which plants can reduce their N and P in leaves prior to abscission (resorption proficiency) are species-specific (Killingbeck, 1996; Fig. 5) and also depend on site conditions (Richardson *et al.*, 2005; Fig. 5). Using the resorption proficiency data (Fig. 5), the retranslocation of N and P was calculated in Paper I. The potential removal of N and P after the retranslocation of the nutrients was higher in *Phragmites* than in other species in phytoremediation treatments (Fig. 7 in Paper I).

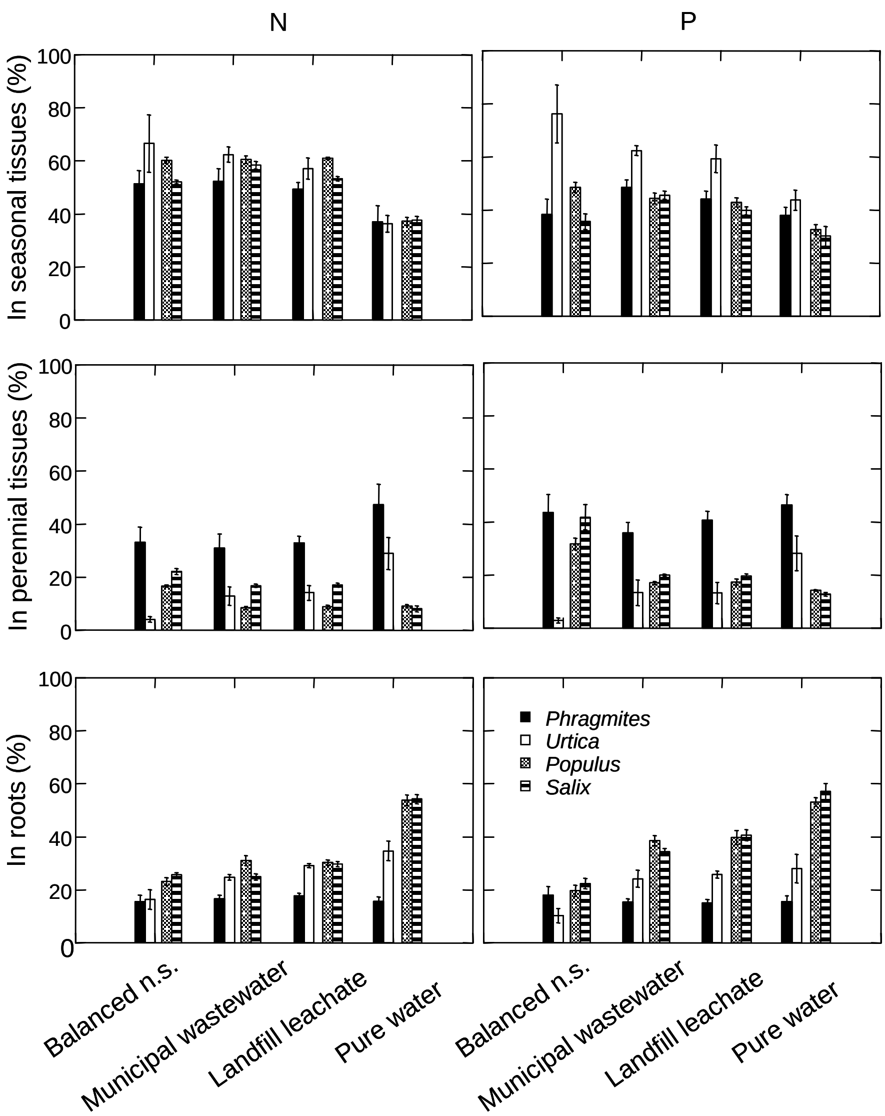


*Figure 4.* N and P partitioning in summer green herbaceous perennials (*Phragmites* and *Urtica*) and deciduous woody perennials (*Salix* and *Populus*) between seasonal and perennial plant parts and roots at September harvest. Treatments: balanced nutrient solution, municipal wastewater, landfill leachate and pure water. Data from Paper I.

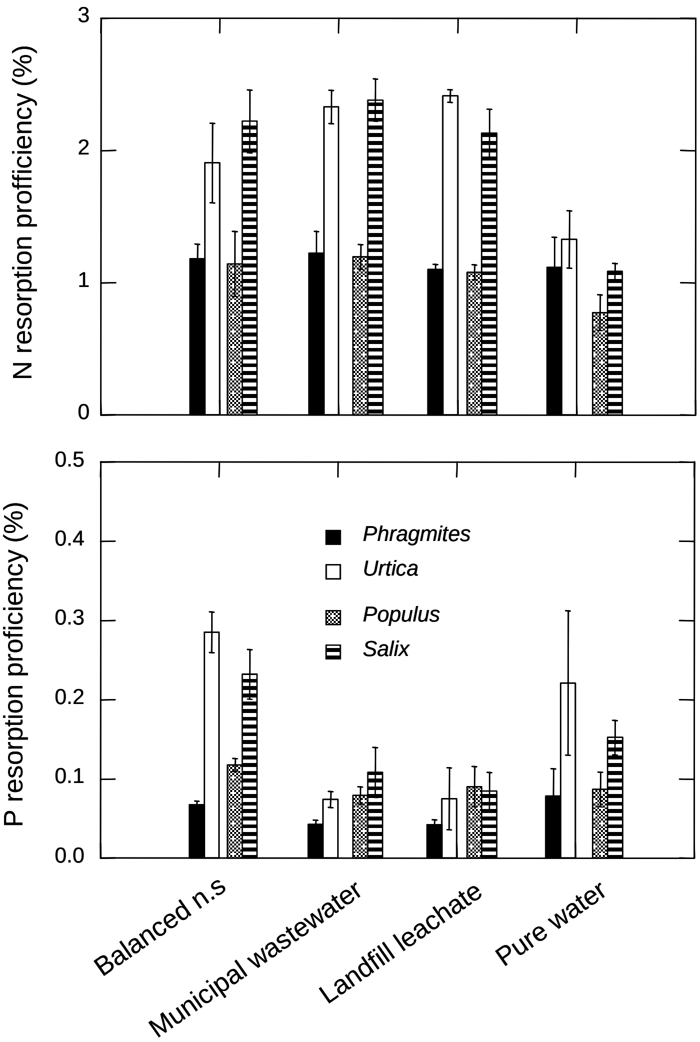


*Figure 5.* N and P resorption proficiency in summer green herbaceous perennials (*Phragmites* and *Urtica*) and deciduous woody perennials (*Salix* and *Populus*) in different treatments in an outdoor pot experiment. Data from Paper I.

The partitioning of N and P into belowground stems of summer green herbaceous perennials was higher than that into aboveground stems of deciduous woody perennials (Fig. 7 in Paper I). In the optimal nutrient treatment, removal of N (by harvest of aboveground plant parts) was high both in *Salix* and *Phragmites*, while removal of P was significantly higher by *Salix* than *Phragmites*. In phytoremediation treatments, biomass allocation into *Salix* and *Populus* roots was increased at the expense of biomass allocation into stems. In *Urtica*, rhizome biomass fraction increased at the expense of seasonal biomass fraction. Such growth responses are characteristic when deciduous trees or clonal herbaceous perennials are experiencing their environment as nutrient-poor (Ericsson, 1981;

Ericsson, 1995; de Kroon & van Groenendael, 1997). As confirmation, nutrient deficiency symptoms became visible in *Salix*, *Populus* and *Urtica* plants in late August in some treatments of the pot experiment (Paper I). The plants in the phytoremediation treatments exhibited purple coloration due to accumulation of anthocyanins, which is a typical phosphorus/nitrogen deficiency symptom (Raven *et al*., 1999). No changes in colouration or biomass allocation pattern were observed in *Phragmites* in this experiment.

## Herbaceous perennials vs. deciduous woody perennials in vegetation filters

The vegetation itself functions as a temporary store of nutrients. *Phragmites* accumulated more biomass, N and P into its perennial tissues compared with the deciduous tree species *Salix* and *Populus* in phytoremediation treatments (Fig. 7 in Paper I). This implies that in the beginning of growth, *Phragmites* was more efficient at accumulating nutrients from wastewater than the trees, due to rapid rhizome development. However, summer green herbaceous perennials complete their life cycle faster than deciduous woody perennials. Rhizome senescence in *Phragmites* starts approx. 6 years after establishment (Asaeda, 2005). Nutrients from older rhizomes are thereafter translocated into younger rhizomes and biomass of perennial tissues of a genet does not increase, meaning that the perennial nutrient pool does not increase either after this period. Trees need a longer time to establish, with the perennial biomass of deciduous trees, for example *Populus*, increasing over several decades (Karacic *et al*., 2003), meaning that their life cycle is considerably longer. This implies that a single *Salix* or *Populus* tree is capable of accumulating more biomass and nutrients during its life cycle than a single *Phragmites* genet. Besides the realisation of P adsorption capacity (Kadlec & Knight, 1996), the shorter life cycle of genets of herbaceous perennials compared with woody perennials may also contribute to the ageing of wetland vegetation filters.

If vegetation filters are not harvested, many of the nutrients in seasonal plant parts end up in the litter compartment. In autumn and winter, a large proportion of the nutrients is then gradually released again through leaching and organic matter mineralisation. The microbes decomposing the litter may take up (immobilise) large amounts of nutrients from the wastewater and release these several months to years later (Verhoeven & Meuleman, 1999). In most wetland vegetation filters, a proportion of the organic matter is broken down at such a slow rate that it accumulates as soil organic matter. The N and P contained in this organic matter accumulate along with it and this represents a significant nutrient removal process in many wastewater wetlands (Verhoeven & Van der Toorn, 1990).

Other N removal processes in vegetation filters are $NO_3$ production (nitrification), $N_2O$ production (denitrification) and $N_2$ production (denitrification), while removal of P occurs via adsorption of phosphates to soil particles (Verhoeven & Meuleman, 1999). The adsorption capacity is dependent on the presence of iron, aluminium or calcium in clay minerals or bound to the soil organic matter (Verhoeven & Meuleman, 1999). Adsorption is subject to

saturation. Each soil has only a certain adsorption capacity and as soon as all adsorption sites are occupied, no further adsorption occurs (Kadlec, 1985).
High concentrations of heavy metals were found in senescent leaves in the two willow vegetation filters studied (Fig. 4 in Paper III). The abscised leaves formed a considerable sink for temporarily bound heavy metals. The concentrations of heavy metals in these abscised leaves were at least as high as, or higher than, those in the bark of winter-harvested shoots.

If the vegetation is harvested, the amounts of nutrients released in autumn and winter are substantially lower. Harvesting grasses, especially *Phragmites*, before retranslocation of nutrients to belowground plant parts has occurred can substantially contribute to the nutrient removal capacity of a vegetation filter (Kadlec & Knight, 1996; Toet *et al*., 2005) and does not affect the long-term vitality of that vegetation (Meuleman *et al*., 2002). In contrast, several studies have indicated that harvesting of natural reed stands in early summer may negatively affect their long-term vitality (Mook & Van der Toorn, 1982; Weisner, 1987; Graneli *et al*., 1992; Tanner, 2001; Asaeda *et al*., 2006).

Perennial energy grasses and *Phragmites* can stand upright at low water contents. Therefore their biomass can dry `on the stem´ and a winter harvest for improved biomass quality is possible (Hartmann, 2001). Winter harvested perennial energy grasses and *Phragmites* can be compressed into bales, pellets or briquettes and burnt in combustion units for straw and other solid fuels (Graneli, 1984; Björndahl, 1985; Allirand & Gosse, 1995; http://www.pilliroog.ee).

Winter harvest is a common practice in short rotation coppices today. As approximately half of the N retranslocated from leaves remains in willow stems and the other half is retranslocated to roots (Bollmark, 1999), a considerable amount of N is removed even by winter harvest of willows. Accumulation of nutrients and heavy metals in bark and wood is also a matter of allocation. Clones with many thin stems, giving a high proportion of bark, are a suitable choice for effective element removal. Clones with few thicker stems and small proportion of branches, meaning a low proportion of bark, are suitable if the plantation is being established mostly for wood fuel production.

## Estimation of proportion of bark in *Salix*

Proportion of bark is high in thinner shoots and low in thicker shoots (Kenney *et al*., 1990; Paper II). Similarly, younger parts of shoots (thinner) have a higher proportion of bark while older parts of shoots (thicker) have a lower proportion (Paper II). The advantage of the method developed (Paper II) and used in this thesis to assess the proportions of bark (Paper II and III) is that the dry weight proportion of each age/size fraction is considered. A simple relationship between proportion of bark and shoot $D_{55}$ (Fig. 2) provides a reliable tool for estimations of proportion of bark in entire shoots and plantations, based only on non-destructive shoot diameter measurements. In addition, estimating the concentrations of different elements separately for bark and wood in different age fractions (Paper II) gives more reliable values of these elements in the harvestable biomass.

Various authors have used different methods to estimate proportion of bark, which makes comparisons difficult and requires careful interpretation of results. For example, Sennerby (1979) obtained bark percentages of 1-year-old willow shoots from 20 different clones as the ratio between fresh weight of bark and wood by using 10-cm long sample pieces taken from the lower 30% of the stem height. Tharakan *et al.* (2003) obtained bark percentages of 3-year-old willow and poplar shoots of several different clones using 10-cm long sections from the centroid (balancing point) of each harvested stem. Bark percentages in that study were calculated using the 'green' weight of bark and wood. Pulford *et al.* (2002) argue that it is important to consider the ratio of bark to wood when the overall pattern of metal uptake in harvested willow biomass is estimated. For this purpose they calculated the weighted averages of bark and wood metal concentrations in 2-year-old willow shoots. Sander & Ericsson (1998) documented increased concentrations of P, K, Ca, Mg, S, Mn, Zn, Cu, Ni and Cd with willow shoot height and suggested that this was a consequence of increased proportion of bark. Results in this thesis support these findings (Fig. 3, Tables 1 and 2 in Paper II). In addition, an age effect of bark and wood on concentrations of some elements was observed. The concentrations of some elements (N, P, K, Mg, Cu and Zn) were significantly higher in twigs than in the older fractions of shoots (Paper II). Laureysens *et al.* (2004, 2005) estimated proportion of bark in 2-year-old poplar shoots as average proportion of bark of dry sample pieces from three different heights per shoot (40%, 60% and 80% of stem height). Doing so may result in an overestimation, since the proportion of bark in those three different heights gets a similar weighting. The proportion of bark at the shoot base should have more weighting in such calculations, since the majority of biomass is allocated to the oldest age fraction of a shoot, where the proportion of bark is low (Paper II). When amounts of nutrients and heavy metals in harvestable biomass are of interest, weighting the concentrations in terms of the dry weight proportion of the respective age fraction of the shoot is extremely important. These calculations should be performed separately for bark and wood, since concentrations of macronutrients and some heavy metals (Cd, Zn and Co in Paper II; Cd, Zn, Cu, Ni in Papers III and IV) are significantly higher in bark than in wood (see also Pulford *et al.*, 2001; Pulford *et al.*, 2002; Tharakan *et al.*, 2003; Laureysens *et al.*, 2004; Laureysens *et al.*, 2005).

## Is the proportion of bark important for element removal?

The proportion of bark in the whole stand ($BP_{stand}$) decreased with increasing stand age (Fig. 6). After the third growing season, the harvestable shoot biomass was significantly higher at Hammarby compared with Lundby (Fig. 5 in Paper III; Table 6 in Paper IV). The plantations at Hammarby and Lundby had different stand structure. High proportions of small-sized shoots ($D_{55}$) in harvestable biomass contributed to high proportions of bark at Lundby plantation (Fig. 3). $BP_{stand}$ decreased with increasing harvestable shoot biomass (yield) per hectare (Fig. 3 in Paper IV).

The major differences in mineral element concentrations were between bark and wood and between sites (Table 3). Higher concentrations of heavy metals, P and K in willow bark and wood (the effect of site in Table 3; Paper III and IV), but lower total biomass yield at Lundby compared with Hammarby resulted in a similar amount of element (P, K, Cu and Ni) removal by harvest (Table 4). In contrast, the removal of Cd and Zn was higher at Lundby in spite of the lower yield. This is explained by higher proportion of bark and high Cd and Zn concentrations in the bark at Lundby compared to Hammarby.

The removal of N was significantly higher at Hammarby, where the biomass yield was higher (Table 4). The small differences in N concentration between bark and wood did not affect the content of N accumulated in the composite biomass. These results are in agreement with those of Adegbidi *et al*. (2001) who also found that the variability in average removal of nutrients was determined more by biomass production than by variation in concentration of nutrients. Labrecque & Teodorescu (2003) found that the biomass productivity and N, P and K contents in harvestable biomass of *S. viminalis* grown on a clayey soil were more than twice the yield and N, P, K removal obtained on a sandy soil (62 vs. 29 t DM $ha^{-1}$, respectively) on abandoned farmland sites in southern Quebec in Canada.

Soil (*e.g*. the effect of site in Table 3) plays an important role in determining element concentrations in biomass. The higher concentrations of Zn, Cd and Ni in the clayey soil at Lundby resulted in higher concentrations of these metals in the bark and wood of willows compared with those at Hammarby (Paper III).

Table 3. *Effects of various factors on concentrations of elements in willow shoots. * significant at 0.05 > p > 0.01, ** significant at 0.01 > p > 0.001, *** significant at p < 0.001; + effect positive, -effect negative*

| Paper | Factor | Effect on | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | N | P | K | Ca | Mg | Zn | Cd | Ni | Cu |
| | | Concentrations in shoots | | | | | | | | |
| II[a] | Age | ** | * | * | n.s. | *** | * | n.s. | n.s. | * |
| II[a] | Tissue (bark/wood) | *** | *** | *** | *** | *** | *** | *** | n.s. | n.s. |
| IV[b] | Tissue (bark/wood) | *** | *** | *** | *** | *** | *** | *** | *** | *** |
| III,IV [b] | Site | * | *** | *** | | | *** | *** | * | n.s. |
| III,IV [b] | Treatment | n.s. | n.s. | * | | | +* | n.s. | * | * |
| | | | | | | | Concentrations of plant-available fractions in soil | | | |
| III | Site | | | | | | * | *** | *** | *** |
| | Depth | | | | | | - ; + | ** | +** | -*** |
| | Treatment | | | | | | ** | *** | *** | * |
| | Ash | | | | | | - | - | - | + |
| | | Concentrations in senescent leaves | | | | | | | | |
| I[c] | Treatment | *** | *** | | | | | | | |
| III[b,c] | Site | | | | | | *** | *** | ** | ** |
| III | Year of cutting cycle (1-3) | | | | | | - | - | n.s. | - |

[a]5-year-old shoots, [b]3-year-old shoots, [c]the age of the shoots is one growing season

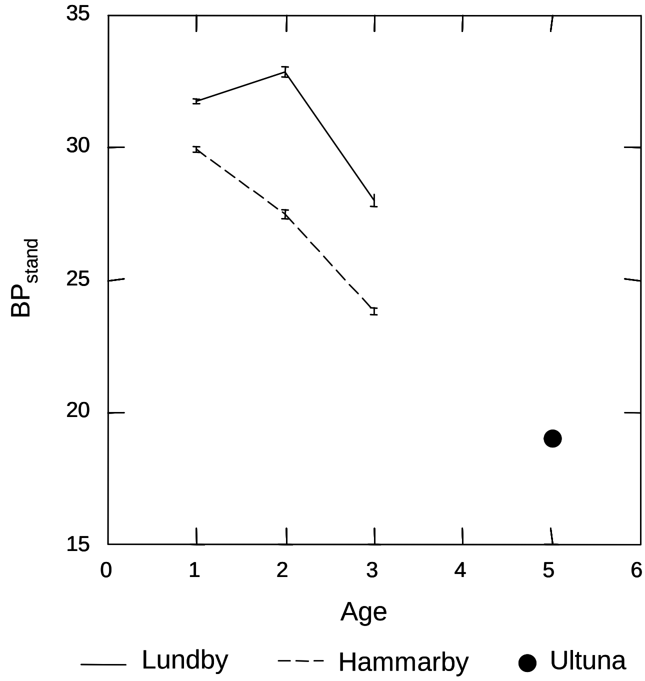


*Figure 6*. Proportion of bark (%) in willow stands of different ages grown at Lundby, Hammarby (clone 78-021) and Ultuna (clone 77-683). Standard errors of estimation are shown for the mean values of 5 treatments. Proportion of bark did not differ significantly between treatments (Papers II and IV).

The doses of sludge and/or ash applied to willow vegetation filters were sustainable only in terms of phytoextraction of Cd, *i.e.* willows accumulated more Cd in their harvestable shoot biomass than was supplied by sludge and/or ash (Table 7 in Paper III). The potential removal (output) of heavy metals in last-mentioned table was underestimated due to 3 % lower value of proportions of bark used in calculations. This lower value represented proportion of bark in 15 destructively measured shoots, while the proportion of bark from non-destructively measured shoots in 240 stools was 28% after the 3rd year in cutting cycle (Fig. 6). However, the potential removal of heavy metals at Lundby was within the standard deviation of the mean removals, presented in Table 4, where the 28% proportion of bark is used in calculations. Accumulation of Cd in particular in aboveground biomass of willow has been reported previously (Perttu & Göransson, 1998; Klang-Westin and Eriksson, 2003) and is supported by the results in Paper III.

The amounts of Zn, Ni and Cu accumulated in the willow shoots were smaller compared with the amounts of these metals applied to soil by sludge and/or ash (Paper III).

## Relevance of the proportion of bark for wood fuel quality

The proportion of bark significantly affected the wood fuel quality only in terms of Zn concentration per tonne harvestable shoot biomass (Table 5). The site factor affected significantly the wood fuel quality in terms of P and K, as well as Zn, Ni, Cd and Cu concentrations (Table 5). Treatment had a significant effect on wood fuel quality in terms of the concentrations of the four heavy metals and K (Table 5).

Table 4. *Mean (±SD) element concentrations (part a) and amounts (part b) in harvestable shoot biomass of Salix at Lundby and Hammarby in central Sweden (t-test n=20 shoots per site, p denotes p-value)(Data from Paper IV)*

| Site | Effect on | | | | | | |
|---|---|---|---|---|---|---|---|
| | N | P | K | Zn | Cd | Ni | Cu |
| | a) Concentrations in wood fuel (kg $t^{-1}$/g $t^{-1}$) | | | | | | |
| L | 4.3±0.4 | 0.85±0.05 | 2.7±0.3 | 58±4 | 1.3±0.1 | 1.3±0.3 | 3.0±0.2 |
| H | 4.3±0.1 | 0.56±0.02 | 2.1±0.3 | 33±3 | 0.5±0.04 | 1.0±0.1 | 2.7±0.2 |
| *p* | n.s. | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| | b) Removal by harvest of willow shoots (kg $ha^{-1}$/g $ha^{-1}$) | | | | | | |
| L | 64±13 | 13±3 | 41±9 | 878±206 | 19±4 | 19±5 | 45±11 |
| H | 81±15 | 11±2 | 39±10 | 620±136 | 9±2 | 18±3 | 51±11 |
| *p* | <0.01 | n.s. | n.s. | <0.001 | <0.001 | n.s. | n.s. |

Table 5. *Effects of various factors on a) wood fuel quality of 3-year-old willow shoots in terms of element concentrations in composite shoot biomass, and b) removal of elements by willow shoots. * significant at 0.05 > p > 0.01, ** significant at 0.01 > p > 0.001, *** significant at p < 0.001 (Paper IV)*

| Factor | Effect on | | | | | | |
|---|---|---|---|---|---|---|---|
| | N | P | K | Zn | Cd | Ni | Cu |
| | a) Concentrations in wood fuel (kg $t^{-1}$/g $t^{-1}$) | | | | | | |
| Site | n.s. | *** | ** | *** | *** | * | * |
| Yield | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |
| Treatment | n.s. | n.s. | ** | *** | * | *** | ** |
| $BP_{stand}$ | n.s. | n.s. | n.s. | *** | n.s. | n.s. | n.s. |
| Adj. $R^2$ | 0.18 | 0.96 | 0.74 | 0.99 | 0.98 | 0.67 | 0.52 |
| | b) Removal by harvest of willow shoots (kg $ha^{-1}$/g $ha^{-1}$) | | | | | | |
| Site | n.s. | *** | *** | *** | *** | * | * |
| Yield | *** | *** | *** | *** | *** | *** | *** |
| Treatment | n.s. | n.s. | *** | *** | n.s. | *** | *** |
| $BP_{stand}$ | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |
| Adj. $R^2$ | 0.94 | 0.94 | 0.86 | 0.98 | 0.96 | 0.68 | 0.93 |

The age of the plantations studied influenced the element concentrations in the harvestable crop due to the different yields and the varying proportion of wood and bark. The 5-year-old plantation at Ultuna contained 3.3 kg N $t^{-1}$ of dry mass (Paper II), while the 3-year-old plantations at Lundby and Hammarby contained 3.7-4.7 and 4.0-4.3 kg N $t^{-1}$ of dry mass, respectively (Paper IV).

The problems with emission of nitrogen oxides can easily be avoided in larger heat and power plants by installation of flue gas filters. In Hässelby Heat and Power Plant (AB Fortum Värme, Sweden), bark pellets are used as fuel for boilers as long as $NO_x$ emissions to the ambient air do not exceed 75 mg $MJ^{-1}yr^{-1}$ (Per Ytterberg, pers. comm.). After this limit is reached, only wood pellets are used as fuel.

Although the concentrations of Zn were higher in the plantation with the high proportion of bark, it was not possible to test the hypothesis that wood fuel with a higher proportion of bark contains more essential elements and heavy metals compared with wood fuel with a lower proportion of bark. Site conditions, stand structure and treatment played a more important role in determining the concentrations of elements in composite harvestable biomass than proportion of bark (Table 5). To test this hypothesis, assessment of more sites with similar yields and more variable proportions of bark is needed.

# Conclusions and implications for practice

Before the establishment of a new vegetation filter is considered, the N:P ratio of wastewater and site soil should be examined. If the N:P ratio is optimal for plants, vegetation filters planted with deciduous woody perennials should be used, due to considerable accumulation of harvestable aboveground perennial tissues. If the N:P ratio of the wastewater is suboptimal, a vegetation filter with *Phragmites* could be considered. However, harvest of belowground stems of *Phragmites* is not a common practise. Consequently, the large fractions of nutrients accumulated in the belowground stems represent a temporary removal of the nutrients.

The developed method for measuring the proportions of bark in willow plantations appeared to be easily applicable in practice. The destructive measurements of some shoots to establish the relationship between their diameter at 55 cm shoot height ($D_{55}$) and the proportion of bark, and the non-destructive measurements of $D_{55}$ in large number of shoots gave a representative estimate of the proportion of bark in willow stands.

The proportion of bark in a willow stand depends on the shoot size frequency distribution of the stand and can be influenced by the management regime. For example, extension of cutting cycle should decrease the proportion of bark and consequently the concentrations of nutrients and some heavy metals in the wood fuel. With longer cutting cycles, removal of elements would increase due to higher harvestable biomass yields.

Plant breeding and genetic improvements of energy crops form one possibility to optimise yield and fuel quality in the future, *e.g.* to grow *Salix* species with high yields and low concentrations of alkalis and heavy metals and other elements (S and Cl) that have negative effects on wood fuel quality.

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