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Phytoremediation of landfill leachate by irrigation to willow short-rotation coppice

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Abstract

Willow short rotation coppice (SRC) is a perennial agricultural crop used for the production of biomass for energy in Sweden. Environmental applications of willow SRC became of interest in the last years, such as the use of willow phytoremediation systems for the treatment of landfill leachate. Landfill leachate is generated after percolation of water through waste deposited in a landfill. The resulting effluent is a hazardous mixture of dissolved organic matter, inorganic components such as ammonium and chloride, heavy metals and xenobiotic compounds. This study investigates the long-term impact of landfill leachate application on the accumulation of heavy metals, as well as the carbon and nitrogen content in soil. In 2005 a field trial was established on an arable field next to a landfill operated by Ragnsells Avfallsbehandling AB, at Högbytorp, Upplands-Bro (Sweden). Two different *Salix sp.* clones (Gudrun and Tora) were planted in double rows in sixteen 400 m^2 square plots. The treatments consisted of three different concentrations of landfill leachate and a control. In 2011 soil samples were taken from every treatment plot and from the surrounding grassland and analysed for heavy metal concentrations and total carbon and nitrogen content. Moreover, samples of willow shoots were taken from the treatments and analysed for heavy metal concentrations and nitrogen content. Cd, Pb and Ni concentrations were significantly lower in the topsoil of the willow treatments compared to the reference. Moreover, Zn tended to decrease in the topsoil of the treatments compared to the reference. The total carbon and nitrogen content in the topsoil was significantly lower in the willow treatments compared to the surrounding grassland. Although willows showed potential for the accumulation of some heavy metals, biomass production is important for an efficient treatment. Plant growth was negatively affected by the leachate overload on the treatment with the highest supply resulting in low offtake of heavy metals. Although no substantial accumulation of heavy metals was reported in this study, application of heavy metals with the leachate should be within recommended application loads in order to prevent accumulation of metals that might not be taken up by the plants.

Keywords: heavy metals, landfill leachate, phytoremediation, willow short-rotation coppice

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1. Introduction

1.1 Willow short-rotation coppice (SRC) as a system for energy

Willow short-rotation coppice (SRC) is a perennial agricultural crop currently grown on about 13.000 hectares of agricultural land for biomass production in Sweden (Dimitriou et al., 2011). Several characteristics make willows (*Salix sp.)* suitable for environmental applications: willows are pioneer species, they grow on unfavorable conditions, can be propagated vegetatively by using cuttings and grow fast once they are established, which makes them suitable for biomass production (Kuzovkina & Volk 2009). Frequently used plant material for willow SRC in Sweden is taken from different clones and hybrids of *Salix viminalis*, *S. dasyclados* and *S. schwerinii* (Dimitriou & Aronsson, 2005).

The whole procedure of planting, management and harvesting of a willow SRC plantation is fully mechanized. Planting takes place early in the spring as soon as weather and ground conditions are suitable. Cuttings are planted in double rows with around 13.000 willow cuttings per hectare. The expression ‘short rotation’ refers to the frequency of harvesting, which is every 3-4 years. The life-span of a willow SRC plantation is about 25 years. Thus, a plantation can be harvested up to 6-8 times (Gustafsson et al., 2009). Harvest takes place during winter in order to prevent damage to the soil from the heavy machinery. The harvested biomass is processed on site and the chips are either stored or directly transported to heat plants or combined heat and power plants (Dimitriou et al. 2011). After harvest the plants regenerate from the coppiced stools and therefore replanting is not necessary (Kuzovkina & Volk 2009). Dependent on the site conditions and management, the production of willow plantations in Sweden is about 6-12 tonnes per hectare and year (Dimitriou & Aronsson, 2005).

Besides the use of the biomass for heat and power generation, several environmental applications of SRC became of interest in the last years. Biomass production can be combined with waste management using willows as a vegetation filter for the treatment of municipal wastewaters, sewage sludge, wood ash, as well as for the treatment of landfill leachate (Mirck et al., 2005).

1.2 Landfill leachate

Landfill leachate is generated after percolation of water (rain, snow melt or the waste itself) through waste deposited in a landfill (Dimitriou & Aronsson, 2007). The resulting effluent is a hazardous mixture of dissolved organic matter, inorganic components such as ammonium and chloride, heavy metals and xenobiotic compounds. The composition of landfill leachate is site-specific and depends on the landfilling technology, the type of waste and the degree of waste degradation. In general, a landfill is characterized by an initial aerobic phase, followed by an anaerobic phase as the oxygen is depleted. In this acidic stage the leachate contains high concentrations of easily degradable organic compounds, such as volatile fatty acids and alcohols. With time the fraction of degradable organic carbon decreases and more recalcitrant high molecular weight compounds, such as fulvic and humic acids are present. Moreover, in this methanogenic phase the leachate is characterized by a high pH (Kjeldsen et al., 2002).

The release of untreated landfill leachate can pose a risk to the environment, e.g. eutrophication of waters or contamination from hazardous compounds in the leachate (Dimitriou & Aronsson, 2007). The in general high chloride and ammonium concentrations in the leachates are of major concern if leachate is released in the near environment. Typical concentrations of dissolved organic matter, ammonium-nitrogen and chloride in landfill leachates are listed in Table 1. According to studies from Sweden, United Kingdom, Poland and Slovenia the ammonium-nitrogen concentrations in landfill leachate can vary a lot (see Table 1) even within a single landfill (Dimitriou & Aronsson, 2010). Moreover, landfill leachate can contain high concentrations of heavy metals. Typical heavy metals found in leachate are cadmium, chromium, copper, lead, nickel and zinc (Kjeldsen et al., 2002). Average concentrations of different compounds in leachates collected from several landfills in Sweden are presented in Table 2.

Table 1. Chemical composition of landfill leachates

Compound	Concentration (mg/l)	Reference
NH_4-N	17	Dimitriou & Aronsson, 2010
	86	Godley et al., 2004a
	102	Bialowiec et al., 2003
	205	Dimitriou & Aronsson, 2010
	327	Zupančič Justin & Zupančič, 2009
Cl	889	Godley et al., 2004a
	961	Zupančič Justin & Zupančič, 2009
	1093	Zalesny et al., 2008
	1540	Bialowiec et al., 2003
	3611	Tyrrel et al., 2002
COD	660	Zalesny et al., 2008
	1508	Zupančič Justin & Zupančič, 2009
	1930	Tyrrel et al., 2002
	3900	Bialowiec et al., 2003

Table 2. Average metal concentration in Swedish landfills (Öman & Junestedt, 2008)

Metal	Concentration (µg/l)
Cd	0,44
Cr	15,3
Cu	23
Ni	31
Pb	4,4
Zn	66

1.3 SRC and landfill leachate

Landfill leachate is usually treated in wastewater treatment plants, which involves transport and relatively costly treatment (Dimitriou & Aronsson, 2005). However, in many cases, the leachate can be treated locally in the landfill area in a more efficient and cost-effective way. Alternatives comprise phytoremediation systems, such as constructed wetlands (Bulc, 2006; Justin & Zupančič, 2009) or the irrigation of energy crops, such as willow SRC, on either restored landfill caps (Nixon et al., 2001; Godley et al., 2004a;) or on arable land adjacent to the landfill site (Aronsson et al., 2010). The combination of biomass production for energy with waste management makes willow SRC more economically profitable (Rosenqvist & Ness, 2004).

Phytoremediation is the use of plants in order to degrade, extract or inactivate potentially hazardous compounds in contaminated soil, air or water. Phytoremediation involves different mechanisms. On the one hand, pollutants can be metabolized by plants (phytodegradation). On the other hand, microorganisms associated with the plant in the rhizosphere can degrade pollutants in soil (rhizodegradation). Moreover, plants contribute to the purification of contaminated sites through extraction of pollutants from the soil and accumulation in the plant tissue (phytoextraction), immobilization of pollutants in the root zone and hydraulic control by the root system (phytostabilisation). In addition, transpiration promotes removal of hazardous compounds from soil or water into the atmosphere (EPA, 2000).

In the cases where willow SRC is used as a phytoremediation system for treatment of landfill leachate, the leachate has been collected and stored in ponds for aeration (oxidation of organic compounds and ammonium) and is afterwards applied to the SRC plantations (Dimitriou & Aronsson, 2007). Different processes in the soil-plant system contribute to the purification of leachate. Soil particles filter solids and adsorb dissolved substances contained in the leachate. Moreover, microorganisms metabolize and stabilize organic compounds and perform nitrification of ammonium. In addition, plants take up nutrients applied with the leachate and reduce the leachate volume through transpiration (Hasselgren, 1992). Willows have high evapotranspiration rates, which allow the application of big volumes of leachate. Moreover, transpiration correlates with biomass growth and depends therefore on the vitality of the plant (Bialowiec et al., 2007).

As discussed above, the use of willow phytoremediation systems for the treatment of landfill leachate has many advantages, such as relatively cheap treatment in the vicinity of the landfill and the combination of biomass production and waste management. There are several examples of large-scale phytoremediation systems successfully treating landfill leachate with willows. A three-year project on landfill leachate treatment by willow SRC was carried out on a landfill cell in Hatfield in the UK (Godley et al., 2004a). Tree growth and survival was high throughout the project. Moreover, it was shown that leachate treatment was beneficial for plant growth, resulting in higher yields on irrigated plots compared to water- and non-irrigated plots.

Moreover, long-term field studies were carried out in Sweden. Aronsson et al. (2010) investigated plant growth, treatment efficiency and the impact of leachate on groundwater quality in a three-year field study on arable land adjacent to a landfill. It was indicated that leachate application did not have negative influence on plant growth. However, the relative high nitrogen loads applied resulted in leaching from the system and therefore elevated nitrogen concentration in the groundwater. On the other hand, a field trial of willow SRC in southwest Sweden revealed a rather small impact of nitrogen leaching after leachate application on groundwater quality (Aronsson et al., 2000). However, concentrations of nitrogen applied with the leachate were much lower than in the previous study.

Investigations on heavy metal concentrations in soil after landfill application did not show alarming accumulation (Godley et al., 2004b; Zalesny et al., 2007; Zupančič Justin & Zupančič, 2009). Moreover, it was reported that total metal concentrations in soil decreased in the course of the leachate application, suggesting leaching of substantial amounts of heavy metals from the soil (Zupančič Justin & Zupančič, 2009). Application periods in the above mentioned studies were short ranging from a couple of months (Zalesny et al., 2007; Zupančič Justin & Zupančič, 2009) up to three years (Godley et al., 2004b). Moreover, amounts of heavy metals applied with the leachate were rather low. However, as mentioned earlier, the composition of landfill leachate is very variable. High variation of heavy metal concentrations in leachate during a three-year study were reported by Aronsson et al. (2010). In that study, concentrations of some heavy metals in the leachate were well above the permitted level of metal application with sewage sludge in Sweden. Thus, the possibility of heavy metal accumulation when landfill leachate is applied should not be underestimated. Long-term studies of wastewater irrigation have shown that heavy metals tend to accumulate in soil with increasing application time (Cajuste et al., 2002). Although not many studies on heavy metal accumulation in soil after landfill leachate application were found, several authors reported accumulation of heavy metals in soil after the application of sewage sludge (Labrecque et al., 1995; Hooda et al., 1997; McBride et al., 2004). Heavy metal contamination in soil is a potential risk to both human health and the environment (Pulford et al., 2002). Therefore, possible accumulation of heavy metals has to be taken into consideration when intensive irrigation of landfill leachate is carried out over a longer time period.

The composition of the leachate is often not consistent with the nutrient demand of the crop, resulting in an inefficient fertilization effect and consequences on the treatment efficiency. Especially the low phosphorus content of the leachate can be a limiting factor for growth. Pot experiments showed great differences in the performance of plants irrigated with leachate compared to the control, suggesting either high ionic strength of the leachate or nutrient insufficiency being possible reasons for the difference in growth (Dimitriou et al., 2006). There were no big differences in plant growth between the different concentrations of leachate in the above mentioned study, showing that the concentration of the pollutants is less important for plant performance.
The negative influence of chloride concentrations on willow growth were shown in other studies (Stephens et al., 2000). It was reported that Cl^- concentrations greater than 2500 mg/l deteriorated growth, caused defoliation and death of the plants. Although average concentrations of leachate might not exceed 2500 mg/l, short term changes in the leachate composition can influence treatment efficiency. Moreover, a large negative osmotic potential of the leachate can expose plants to an increased water deficit, causing leave senescence and desiccation. Studies on hybrid poplar showed a reduction in stomatal conductance, transpiration and photosynthesis of the plants treated with leachate in excess (Cureton et al., 1991). Moreover, the choice of willow variety is important, since different clones vary in resistance and growth when landfill leachate is applied (Dimitriou et al., 2006; Zalesny et al., 2007). On the one hand, an efficient willow phytoremediation system depends on sufficient growth and biomass production of the plants. Therefore, the above mentioned findings should be considered in order to maintain a healthy plant cover. On the other hand, phytoextraction of heavy metals from soil depends on the ability of the plant to accumulate these heavy metals in the plant tissue.

Several studies suggest that *Salix* has a high potential of accumulating Cd in its aboveground plant parts (Riddell-Black, 1994; Klang-Westin & Perttu, 2002; Klang-Westin & Eriksson, 2003; Vysloužilová et al.,2003), as well as Zn (Nissen & Lepp 1997; Vysloužilová et al. 2003). Cd and Zn are among the most mobile heavy metals in soil, readily transferred to the above-ground plant parts, making it suitable for removal from the soil by harvest (Labrecque et al. 1995; Nissen & Lepp 1997). Moreover, it is suggested that concentrations of Cd and Zn tend to be higher in leaves than in stems (Riddell-Black, 1994; Vysloužilová et al., 2003). Common practice implies harvesting of willow SRC during winter after leaf fall. However, regarding a phytoextraction system for heavy metals in soil the offtake of heavy metals could be increased by harvesting the shoots with the leaves. Nevertheless, the total amount of Cd is suggested to be higher in the stems and harvest earlier in the year before leave fall might cause considerable loss in growth.

The ability of heavy metal accumulation varies between willow species, hybrids and clones. Several willow clones are considered high accumulators, whereas other clones show very low accumulation of metals. Landberg & Greger (1996) reported big differences among *Salix viminalis* and *S. dasyclados* clones in uptake and tolerance of heavy metals such as Cd, Cu and Zn. Screening of several willow clones revealed differences in resistance and uptake of different heavy metals among species as well as among clones of single species. Moreover, willow species differ in the ability to transport heavy metals from the roots to the shoots and in the compartmentalization of heavy metals (Pulford et al., 2002).

However, not all kinds of heavy metals can be treated by phytoextraction. Ni, for example, is a phytotoxic element shown to be excluded by several willow varieties growing on a heavy metal contaminated soil. Varieties which excluded Ni were healthier and produced more biomass compared to varieties accumulating Ni in the bark (Pulford et al., 2002). Moreover, other heavy metals such as Cr and Pb are not taken up in the plant shoots or only in small amounts (Labrecque et al., 1995; Dimitriou et al., 2006). If certain heavy metals are excluded by the willows they might accumulate in soil in the course of the application of landfill leachate, since they cannot be taken out with the harvest.

The selection of specific genotypes with high phytoremediation ability and biomass production is crucial for an effective treatment. However, an effective treatment also depends on the nutrient content in the leachate, on concentrations of salts and heavy metals, on the chemical oxygen demand and on the irrigation loads. Thus, all these factors have to be considered specifically for individual cases in order to evaluate the phytoremediation efficiency of a system and the reasons behind success or failure.

2. Aim

This study deals with a willow field trial initiated in 2005 on an arable field next to a landfill operated by Ragnsells Avfallsbehandling AB, at Högbytorp, Upplands-Bro. RagnSells is a landfill operator that has established a willow phytoremediation system in their premises treating their landfill leachate. The leachate is collected in big ponds where denitrification takes place and is then applied to a willow plantation grown in and near the landfill area. The field trial involved four different treatments (three different supply dosages of landfill leachate and a control) on two different willow clones, Tora and Gudrun and two replicates of each clone. Leachate irrigation started in 2005 and was carried out until 2010. This study covers the treatment years 2008-2010, whereas a study for the previous years for this field trial was carried out by Aronsson et al. (2010).

The aim of this project work was to evaluate the long-term impact of landfill leachate application on the accumulation of heavy metals, carbon and nitrogen in the soil when different landfill leachate amounts were applied on willow plants of two different clones. Moreover, the uptake of heavy metals in the willow shoots was analyzed and evaluated.

3. Materials and methods

3.1 Site and plants

The field trial was established on an arable field adjacent to a large, commercial landfill operated by Ragnsells Avfallsbehandling AB at Högbytorp, Upplands-Bro, Sweden. The soil was a heavy clay soil with 34–42% clay content, a humus content of 14–25% in the topsoil and a pH of 6,7. The trial comprised sixteen 400 m^2 square plots (Figure 1). On 18-19 May 2005, cuttings of two varieties of willow, Tora and Gudrun, were planted manually in a double-row system, similar to establishment of commercial willow plantations in Sweden. Spacing between the rows was 0,75 m and the distance between double rows was 1,5 m. Spacing between single plants in the rows was 0,6 m. Tora is a hybrid between *Salix schwerinii* and *S. viminalis*, and Gudrun is a pure *S. dasyclados* variety with partly Siberian origin, making it more frost tolerant than Tora. The field was prepared for planting during 2004 by chemical weed control during autumn followed by ploughing. One week before planting, the soil was cultivated with a rotary cultivator. After planting, mechanical weeding was carried out repeatedly from June onwards. A sprinkler irrigation system for tap water was established in order to prevent drought damage before the onset of leachate irrigation. Four treatments were applied, three with landfill leachate (x1, x2 and x3), and a control, each with two replicates for each of the two willow varieties tested. Drip irrigation pipes were laid out in every double row for distribution of the landfill leachate. Leachate irrigation was carried out for six years, starting in 2005 until 2010. The control treatment was not irrigated in the first growing season (i.e. 2005), but in the years from 2006 to 2009 it was irrigated with tap water in amounts corresponding to treatment x1. In 2010, all plots received landfill leachate amounts equal to x1 treatment. This means that the treatments were not kept as in the years before and that the control plots received leachate as well, which was a mistake and not intended. The irrigation loads for the different treatments are presented in Table 3. The concentrations of different elements in the leachate and the loads applied through irrigation are presented in Table 4. In Table 5 mean concentrations of heavy metals in the leachate and the amounts of heavy metals applied with the leachate in the respective treatment are shown. In this study data from the years 2008 to 2010 is presented. Data from the years 2005 to 2007, such as irrigation loads, amounts of elements applied and their concentrations in the leachate, can be found in the previous study for this trial by Aronsson et al. (2010).

Table 3. Irrigation loads during three years of treatment

	Irrigation loads (mm)			
Year	Treatment 1	Treatment 2	Treatment 3	Control
2008	198	396	594	198+
2009	198	396	594	198+
2010	203	203	203	203*

+The control was irrigated with tap water

*In 2010, the control received landfill leachate as well

Table 4. Amounts of elements applied through leachate irrigation during three years of treatment and mean concentrations in the leachate. COD indicates chemical oxygen demand

		Application loads (kg/ha)			
Element	Year	Treatment 1	Treatment 2	Treatment 3	**Mean leachate conc. (mg/l)**
Tot-N	2008	271	542	813	137
	2009	172	344	517	87
	2010	71	71	71	35
NO_3-N	2008	137	273	410	69
	2009	51	103	154	26
	2010	20	20	20	10
NH_4-N	2008	6,7	13,5	20,2	3,4
	2009	5,3	10,7	16,0	2,7
	2010	1,6	1,6	1,6	0,8
Chloride	2008	1930	3859	5789	975
	2009	2296	4592	6888	1160
	2010	2283	2283	2283	1124
COD	2008	1330	2660	3990	672
	2009	1273	2545	3818	643
	2010	1144	1144	1144	563
Tot-P	2008	4,75	9,50	14,25	2,4
	2009	5,34	10,69	16,03	2,7
	2010	3,86	3,86	3,86	1,9

Table 5. Amounts of heavy metals applied through leachate irrigation during three years of treatment and mean concentrations in the leachate. Application loads exceeding the maximum annual heavy metal supply with sludge on agricultural land according to the Swedish board of agriculture (2012) are highlighted with an asterisk

Element	Year	**Application loads (g/ha)** Treatment 1	Treatment 2	Treatment 3	**Mean leachate conc. (mg/l)**
As	2008	23,8	47,5	71,3	0,012
	2009	27,7	55,4	83,1	0,014
	2010	26,4	26,4	26,4	0,013
Cd	2008	0,59	1,19*	1,78*	0,0003
	2009	0,40	0,79*	1,19*	0,0002
	2010	0,41	0,41	0,41	0,0002
Co	2008	35,6	71,3	106,9	0,0180
	2009	33,6	67,3	100,9	0,0170
	2010	27,4	27,4	27,4	0,0135
Cr	2008	160*	321*	481*	0,081
	2009	135*	269*	404*	0,068
	2010	124*	124*	124*	0,061
Cu	2008	51,5	102,9	154,4	0,026
	2009	47,5	95,0	142,5	0,024
	2010	44,7	44,7	44,7	0,022
Hg	2008	0,20	0,40	0,59	0,0001
	2009	0,20	0,40	0,59	0,0001
	2010	0,20	0,20	0,20	0,0001
Ni	2008	127*	253*	380*	0,064
	2009	129*	257*	386*	0,065
	2010	108*	108*	108*	0,053
Pb	2008	8,5	17,0	25,5*	0,0043
	2009	7,5	15,0	22,6	0,0038
	2010	7,5	7,5	7,5	0,0037
Zn	2008	238	475	713*	0,120
	2009	129	257	386	0,065
	2010	126	126	126	0,062

Table 6. Maximum supply of heavy metals with sludge and maximal concentrations of heavy metals in the topsoil prior to sludge application in Sweden (Swedish board for agriculture, 2012)

	Cd	**Cr**	**Cu**	**Hg**	**Ni**	**Pb**	**Zn**
Max. supply to soil (g/ha/yr)	0.75	40	300	1.5	25	25	600
Max. conc. in topsoil (mg/kg DM)	0.4	60	40	0.3	30	40	100

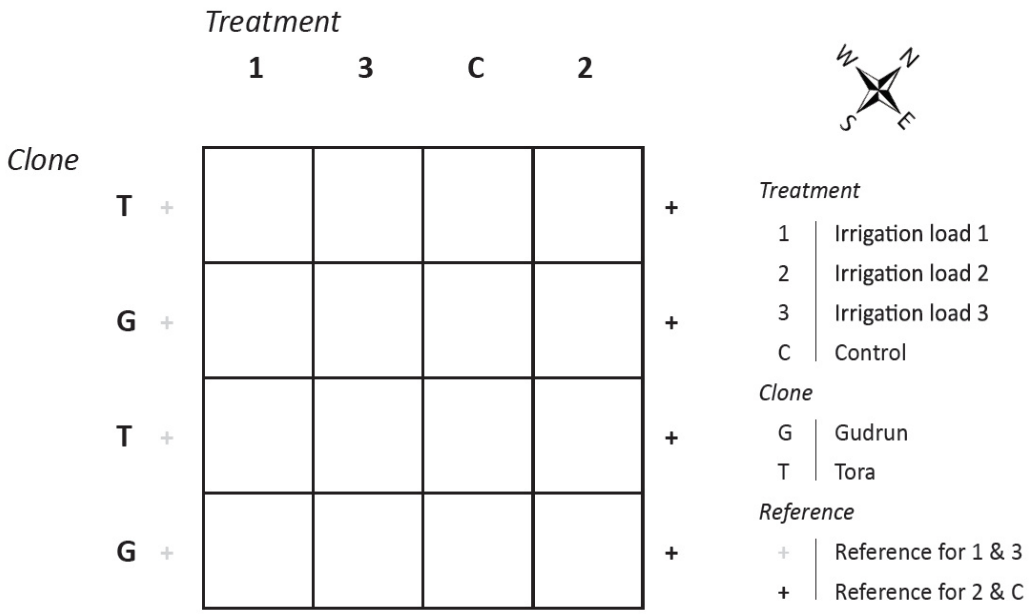


Figure 1. Field trial design showing plots with the different treatments 1-3 and the control, as well as the different clones, Gudrun and Tora. Soil samples from the adjacent grassland were taken as a reference.

3.2 Sampling and analyses

On 4 May 2011 soil samples from the topsoil (0-20 cm) and subsoil (40-60 cm) were taken from each plot using a soil auger. Each topsoil sample consisted of six auger subsamples pooled together. These samples were taken across three willow double rows in the centre of the plot (two from between plants, two from between rows, and two from between double rows). The subsoil samples were taken in the same way and in the same plots as the topsoil samples. However, in that case only four soil cores were taken and pooled together into one bulk sample. Moreover, topsoil and subsoil samples were taken from the adjacent grassland. In that case, the subsamples were taken within a circle with a radius of 2 m from ca. 7 m distance from the willow field. Samples taken from the grassland adjacent to treatment 1 and 3 were taken as a reference for those treatments, whereas samples taken from the grassland adjacent to the control and treatment 2 were taken as a reference for those treatments (as indicated in Figure 1). Shoot samples from three-year-old willows grown in all plots were taken from the center of every plot; three whole shoots of representative diameter from three different rows that were not damaged were put together into one sample.

The soil samples were dried at 30-40°C until constant weight. Soil pH was measured in a suspension of 5 ml soil + 25 ml deionized water. The suspension was shaken for 15 min, left standing overnight, and then, immediately before measurement on the next day, the suspension was again shaken for 1 min. Total C and total N were measured using an elemental analyzer (LECO CH-2000) in which 1 g of sample was heated to 1250°C for 5 min. Organic C was considered to equal total C since pH was lower than 7. Pseudototals of Cd, Cr, Cu, Ni, Pb, and Zn were determined after extraction with 7 M nitric acid (2,5 g soil, 20 ml acid) at 120°C for 2 h on a Tecator heating block. Trace element concentrations were measured with ICP-MS (Perkin Elmer Elan 6100 DRC). Shoot samples were comminuted and ground to pass a 0,2-mm sieve (RETSCH Ultra Centrifugal Mill ZM 100). The ground samples were dried under vacuum at 65°C for 48 h, then left to cool at room temperature.

Subsamples of the plant material were wet-ashed (heating block 150°C) in a mixture of 10 ml concentrated HNO_3 and 1 ml conc. $HClO_4$. The acids were evaporated until 0,5 ml of perchloric acid residue remained, which was diluted with H_2O to a final volume of 35 ml. The extracts were analysed for Cd, Cr, Cu, Ni, Pb and Zn on a JY-70 Plus ICP Emission Spectrometer.

3.3 Statistical analysis

The Minitab Statistical Software (Release 16, Minitab Corp., State College, PA) was used to describe and analyse the datasets. Differences between treatments and reference as regards all measured responses were tested for significance by ANOVA GLM. Paired sample t-tests were used to analyse differences between willow treatments and reference for significance. All willow treatments as well as individual treatments have been compared with the respective references. Due to the character of the paired sample t-test only four samples taken in the adjacent grassland closest to the treatments were used as a reference. The reference values on the southwest side of the field have been compared with treatment 1 and treatment 3, respectively. The reference values on the northeast side of the field have been compared with the control and treatment 2, respectively.

4. Results

4.1 Heavy metal concentrations in soil

Figure 2 shows the concentration of heavy metals in soil after six years of treatment for the respective clone and treatment at a depth of 0-20 cm and 40-60 cm. Heavy metal concentrations in the grass field adjacent to the experimental plots are referred to as reference, whereas the control refers to plots with willows irrigated with tap water.

Concentrations of Cd in the reference grass field indicated that Cd concentrations in the soil of the experimental site followed a gradient from southwest to northeast. Thus, the Cd concentrations in treatment 1 and 3 were lower than in treatment 2 and the control in the upper 20 cm. Comparing all willow treatments with the respective reference using a paired t-test (paired t (15)= 3,07, p= 0,008) showed that Cd concentrations in the topsoil were significantly lower in the willow treatments. More detailed statistical analysis between single treatments and the respective references indicated that significant differences occurred only between treatment 2 and the reference (paired t (3)= 6,36, p= 0,008) and between the control and the reference (paired t(3)= 3,18, p= 0,050).

Comparisons between Ni and Pb concentrations in all willow treatments with the respective reference showed that concentrations were significantly lower in the willow treatments for Ni (paired t (15)= 2,30, p= 0,036) and Pb (paired t (15)= 3,91, p= 0,001). The Zn concentrations in the topsoil tended to decrease in all willow treatments, though no significant differences were shown (paired t (15)= 2,09, p= 0,055). Concentrations of Ni in the reference grass field showed a gradient from southwest to northeast, as described for Cd.

The concentrations of As, Cu, and Hg in the topsoil of the reference grassland showed an increase from southwest to northeast of the site, as it was the case for Cd. Concentrations for Hg at a depth of 40-60 cm were below detection limit in all samples and are therefore not presented. The As concentrations in the topsoil were similar among the willow treatments and seemed to increase in treatments 1 and 3 compared to the reference, although not significantly, whereas there was a decrease in concentration indicated on the control. Cu and Hg concentrations in the topsoil seemed to be higher compared to the reference in some willow treatments, although no significant differences were evident.

Concentrations of Co, Cr and V in the topsoil did not vary much among the different treatments and seemed to decrease slightly on treatment 1 and treatment 3, but not significantly, compared to the concentrations in the reference.

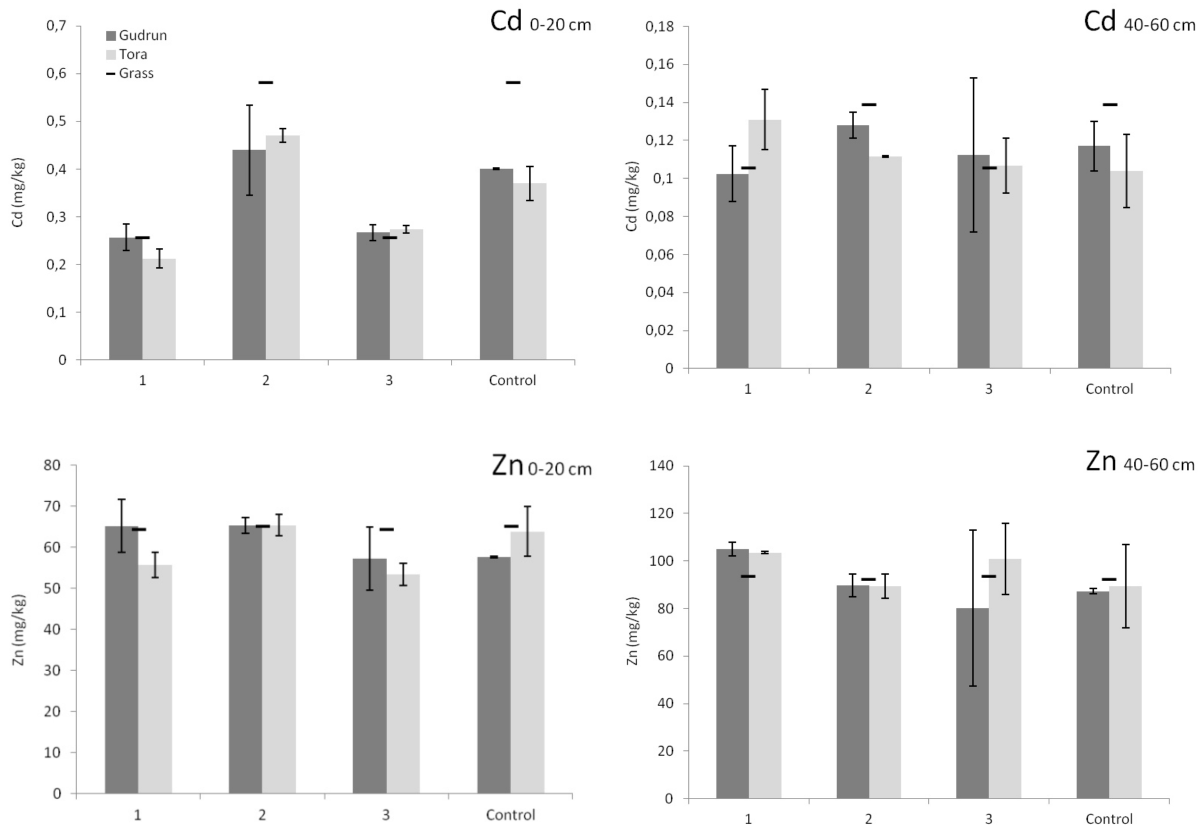


Figure 2. Average concentration of heavy metals in soil at a depth of 0-20 cm and 40-60 cm for different leachate treatments and *Salix* clones Gudrun and Tora. Error bars indicate the standard error of the mean (SEM) (n=2). “Grass” refers to heavy metal concentrations in soil (n=4) in the grassland adjacent to the willow treatments.

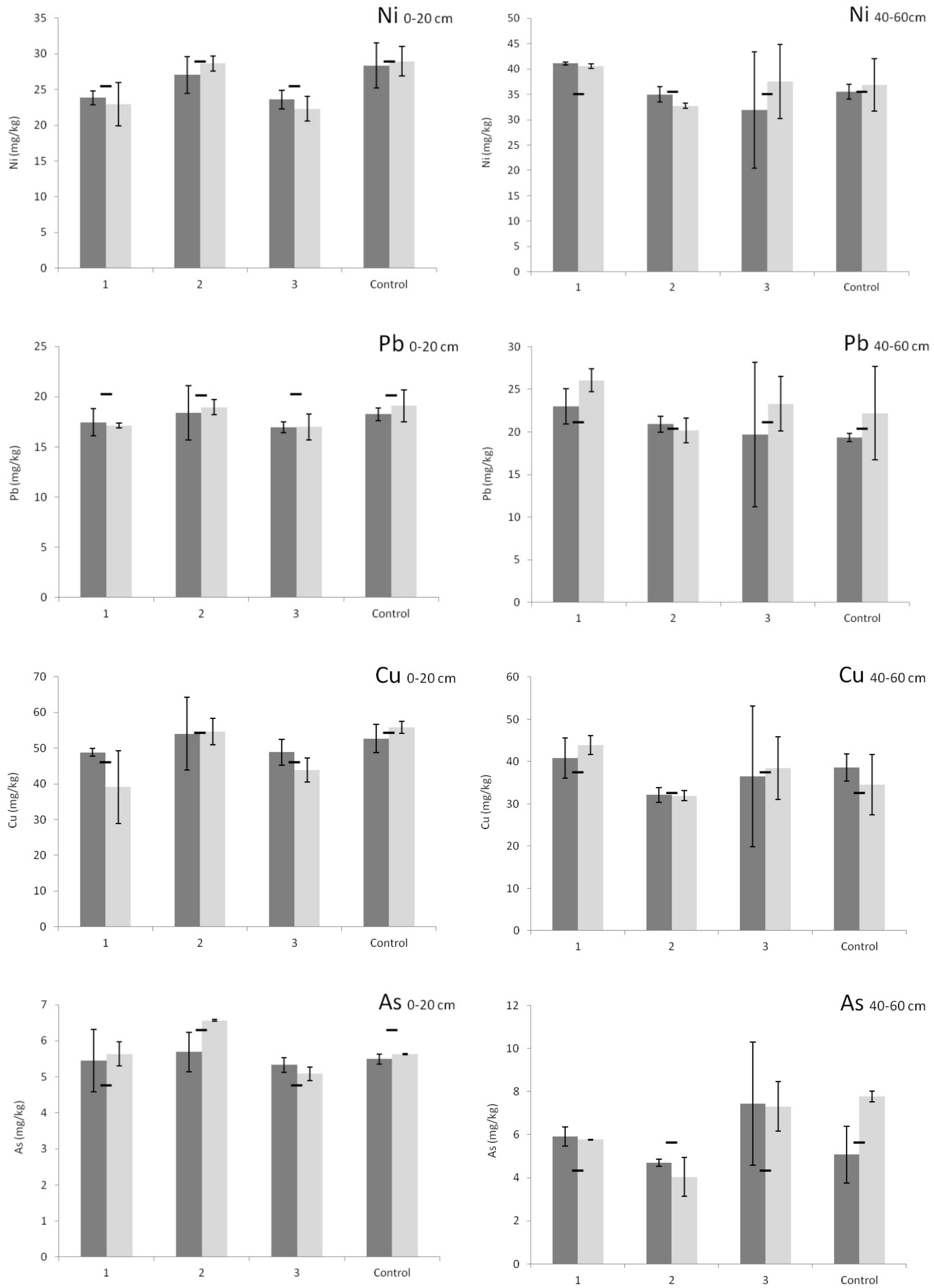


Figure 2. continued

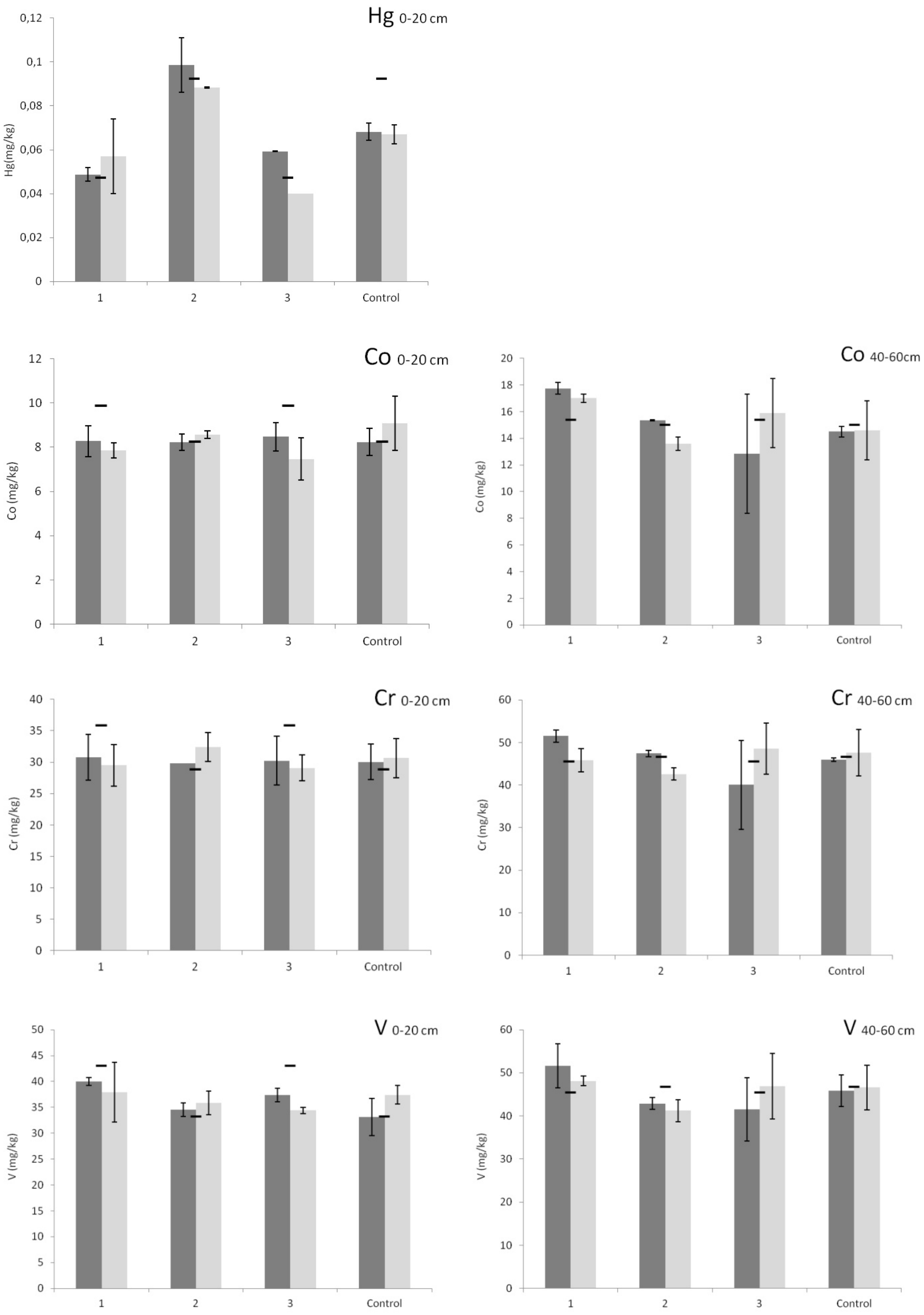
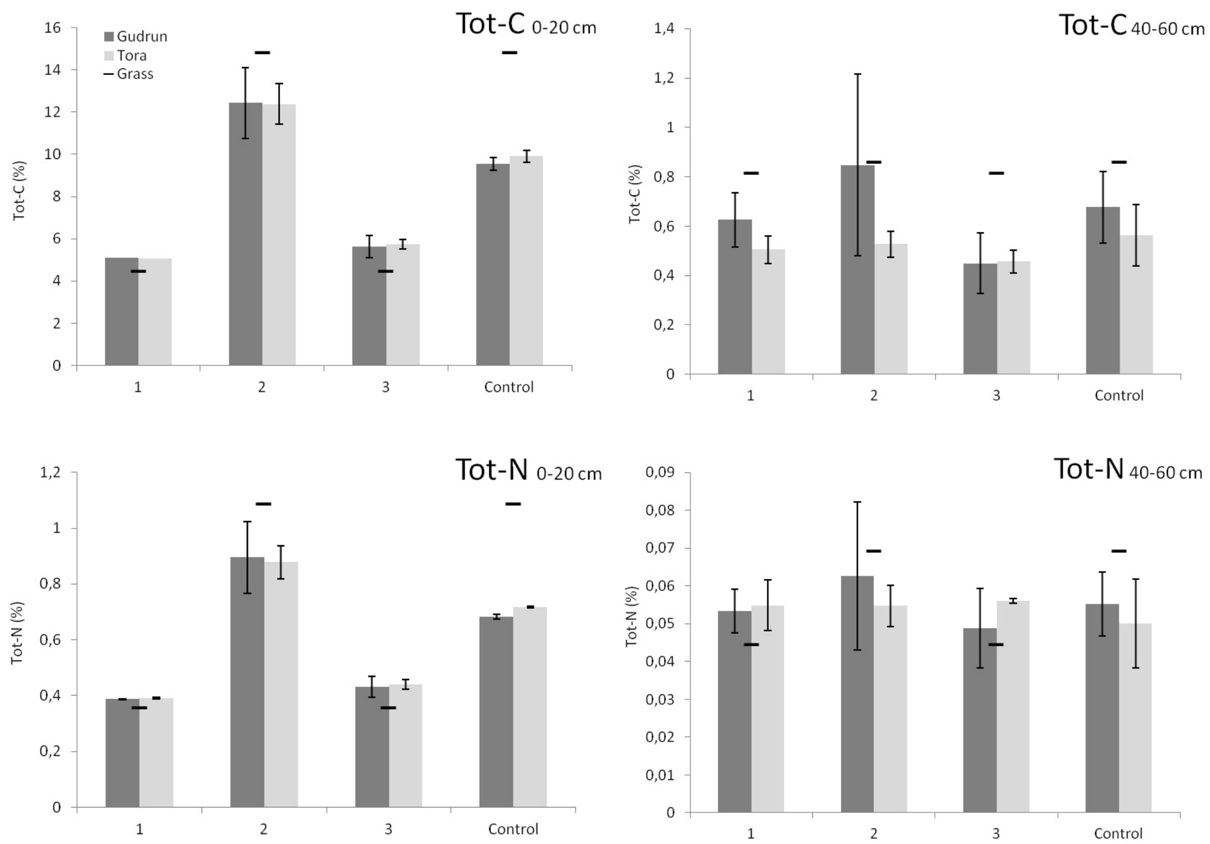


Figure 2. continued

4.2 Carbon and nitrogen content in soil

Figure 3 shows the total carbon and nitrogen content in soil after six years of treatment at a depth of 0-20 cm and 40-60 cm. The total carbon content in the topsoil in the reference grass field indicates a gradient from southwest to northeast of the experimental field, as it was shown for Cd and other heavy metals. Comparisons between individual willow treatments and the corresponding reference plots indicated significant differences in the total carbon content in the topsoil. The total carbon content in soil was lower in treatment 2 and the control compared to the respective reference (paired t (3)= 4,71, p=0,018 and paired t (3)= 6,57, p=0,007 for treatment 2 and the control, respectively). Comparison of the carbon content in treatment 3 and the respective reference showed a significant higher content in the willow treatment (paired t (3)= 3,36, p=0,04). Comparing all willow treatments with the respective reference showed that the carbon content in the subsoil was significantly lower in the treatments (paired t (15)= 2,49, p=0,025).

The total amount of nitrogen in the topsoil over all treatments was significantly lower in the willow treatments compared to the reference grass land (paired t(15)= 2,33, p=0,034). More detailed statistical analysis of the total nitrogen content in the topsoil in the different treatments showed the same pattern as for carbon in the topsoil. The nitrogen content in the topsoil was significantly lower in treatment 2 and the control than in the respective reference (paired t(3)= 4,94, p=0,013 and paired t(3)= 8,08, p=0,004 for treatment 2 and the control, respectively). The amount of nitrogen in treatment 3 was slightly higher than in the reference, although not significantly (paired t(3)= 3,15, p=0,051).



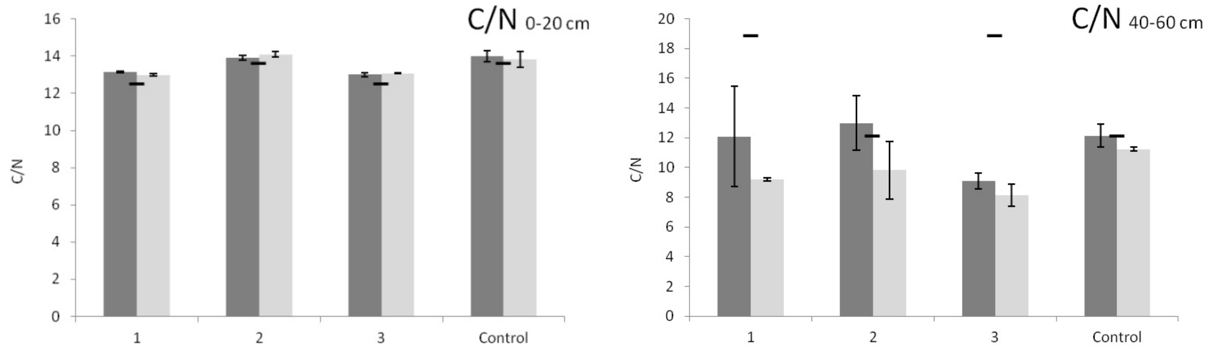


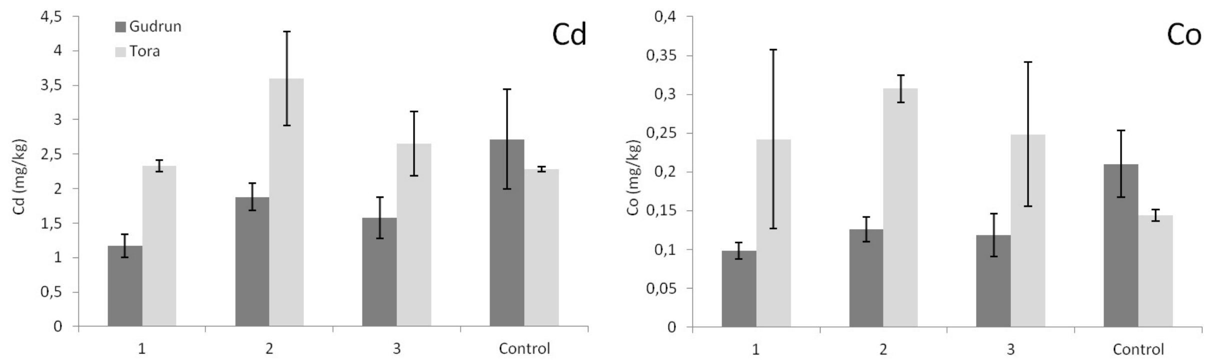
Figure 3.Total carbon and nitrogen content and C/N ratio in soil at a depth of 0-20 cm and 40-60 cm for different leachate treatments and *Salix* clones Gudrun and Tora. Error bars indicate SEM (n=2). “Grass” refers to total carbon and nitrogen content and C/N ratio in soil (n=4) in the grassland adjacent to the willow treatments.

4.3 Heavy metal concentrations and total nitrogen content in willow shoots

Concentrations of heavy metals in the shoots are presented in Figure 4. There are differences in the accumulation of some compounds between the two clones. Tora shoots seemed to accumulate higher concentrations of Cd, Co, Mn, Pb and Zn on the leachate treatments. However, Gudrun showed highest concentrations of all metals on the control. Tora accumulated more Cd, Co and Zn when grown on treatment 2 compared to the other treatments. Moreover, concentrations of Ni on the leachate treatments were higher in Gudrun than in Tora shoots.

The different leachate treatments did not seem to influence the concentration of Cr and Cu in any of the two clones. However, the concentration of these metals in Tora shoots in the control was lower compared to the corresponding ones of treatments 1-3.

The total N content in the shoots was about the same in the different clones, except for a higher concentration in Gudrun on treatment 2 and the control.



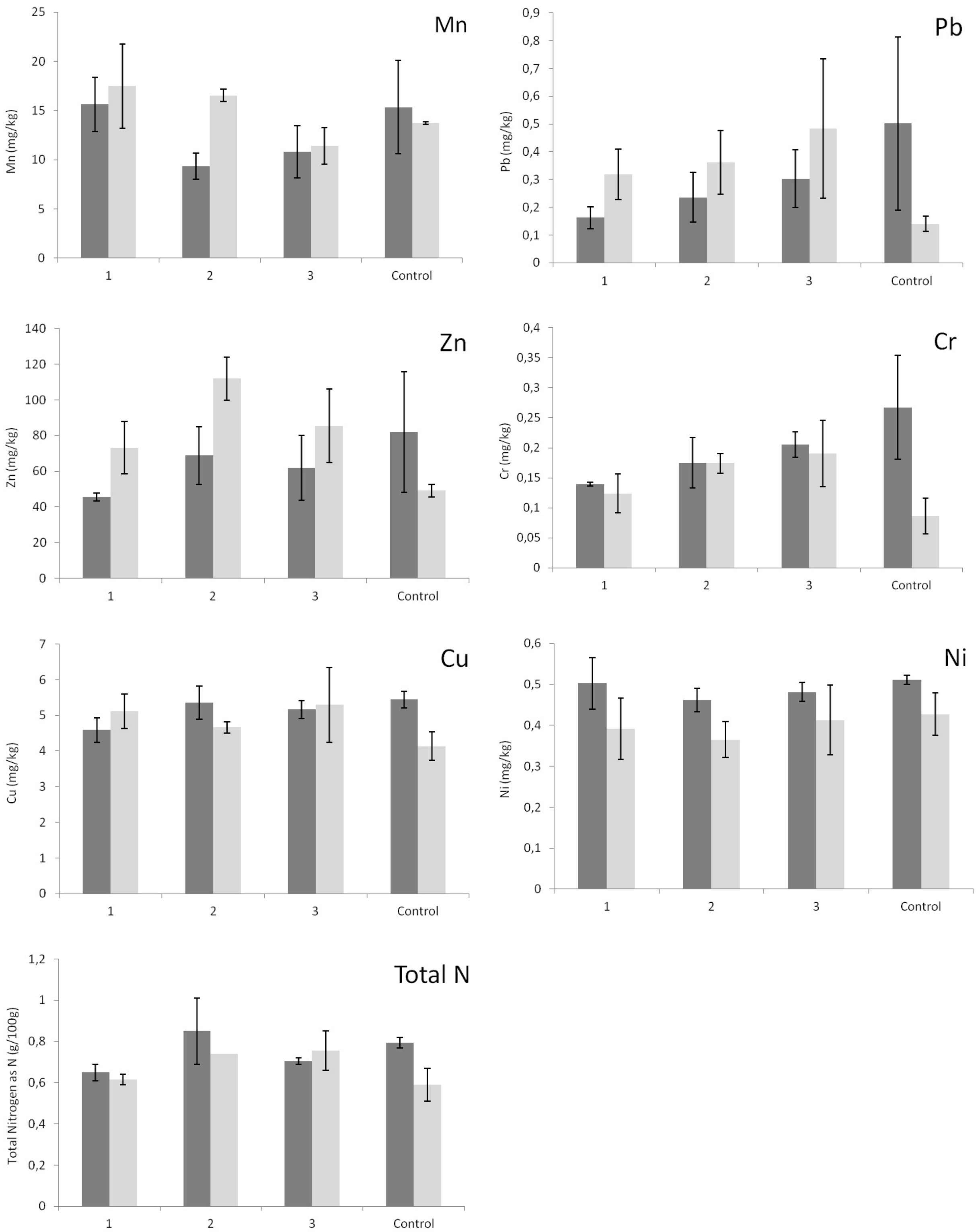


Figure 4. Concentration of heavy metals and nitrogen content in the shoots of *Salix* clones Gudrun and Tora for different leachate treatments. Error bars indicate SEM (n=2).

4.4 Biomass estimations

Detailed measurements of growth and biomass production were not made after harvest. Thus, growth performance was estimated from the appearance of the plants on the different treatments at the time of sampling, following a visual ranking on a relative basis (pers. comm., I. Dimitriou, 2013). Biomass estimations are presented in Figure 5. In general, control plants grew much better than plants irrigated with landfill leachate. Tora grew better than Gudrun on the control, but seemed to suffer more from high leachate applications than Gudrun. Within the different treatments, plants were most affected by the leachate on treatment 3, resulting in very poor growth. Tora was damaged by leaf beetles on one plot with treatment 2 and production was low on treatment 1 in the northwest corner of the field. Growth was similar between Tora and Gudrun on the other plots with treatment 1 and treatment 2.

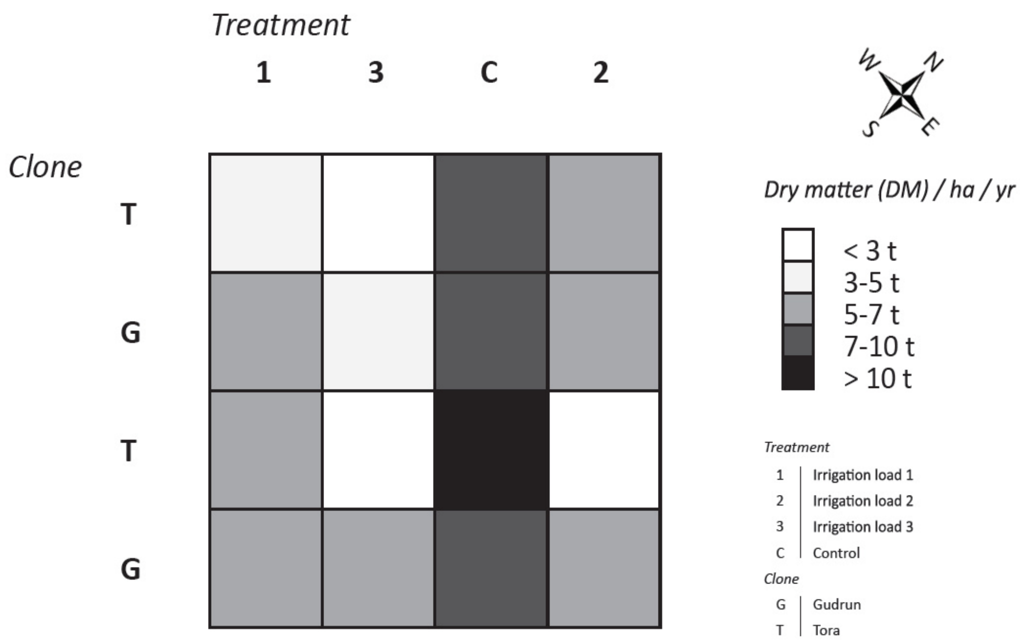


Figure 5. Biomass estimations in tons of dry matter per hectare and year. Estimations are based on the appearance of the plants at the time of sampling.

5. Discussion

5.1 Treatment efficiency in regard to heavy metal accumulation

The amount of Cd applied with the leachate over six years of treatment was low compared to the amount of Cd present in the topsoil (Table 7), suggesting that no accumulation in soil has occurred in the course of the experiment. In fact, Cd concentrations in the topsoil were significantly lower in treatment 2 and the control in comparison to the reference. Concentrations of Cd in the shoots were highest in Tora growing on treatment 2 with 3,6 mg/kg. Table 7 shows that the potential offtake of Cd with the willow shoots after three years of treatment outweighs the application with the leachate, except in Tora on treatment 3, which can be attributed to the very bad growth on these plots. Offtakes are theoretical values, since the amount of heavy metals that is mobile and bioavailable and therefore can be taken up by the plant can change with time and therefore also the uptake might decrease with time. The significant lower concentrations in the control compared to the grassland indicate that Cd is taken up by willows even when no supply occurs, provided that growth is satisfactory. Plants grew well in the control, probably due to the low amount of leachate that was applied only in 2010. This shows that a healthy plant cover is important in order to achieve an efficient treatment.

Besides the application with leachate other mechanisms might contribute to an increase in Cd in the topsoil. For example, willows might have taken up Cd from deeper soil layers. A study by Klang-Westin & Perttu (2002) suggested that significant amounts of Cd accumulated in willow stems are taken up from the subsoil. Harvest of willow plantations takes usually place during winter when the leaves have fallen in order to recycle nutrients to promote re-sprouting in spring (Greger & Landberg, 1999) and to prevent damage to the soil by the heavy machinery (Gustafsson et al., 2009). Therefore, the Cd accumulated in the leaves is not removed with the harvest. Cd taken up from the subsoil and accumulated in the leaves can be reallocated in the topsoil after leaf fall, as suggested in other studies (Eriksson & Ledin, 1999; Berndes et al., 2004). Klang-Westin & Eriksson (2003) assumed that 0-30% of the amount of Cd in willow leaves origin from the subsoil and suggested that the annual input of Cd from the subsoil with the leaves is about 2 g/ha and year. Assuming an addition of about 12 g/ha Cd by the leaves in the topsoil over the 6-year-period of this study, the balance for Cd would result in a theoretical net accumulation in the topsoil on treatment 3 (Table 7). Nevertheless, it is not likely that uptake from the subsoil causes accumulation instead of depletion of Cd in the topsoil since a high uptake of Cd from the subsoil is rather unlikely given that most willow roots are found in the topsoil (Rytter & Hansson, 1996).

However, as mentioned earlier, concentrations of Cd in the surrounding grassland showed a gradient from southwest to northeast of the experimental site, which is from left to right in Figure 1. Statistical analysis of Cd concentrations revealed significant lower concentrations in all the willow treatments compared to the respective references, but detailed comparison between individual treatments and the respective reference showed lower concentrations of Cd only in treatment 2 and the control. Therefore, it is possible that the differences shown by the statistical analysis source from the higher concentrations of Cd in the reference on the northeast part of the area, closest to treatment 2 and the control. The reference might not have described the situation in the soil before the treatment. Therefore, samples from the willow treatments before the start of the treatment might have been a more reliable reference. In general, the experimental design of this study could be optimized. Due to practical reasons, e.g. application of different leachate dosages, the treatments were not fully randomized which reduced the reliability of the statistical analysis of treatment effects. Moreover, the described gradient could have been accounted for in the experimental design, if it would have been recognized before. In this field trial the treatments go across the gradient (at a right angle to the gradient). Thus, each treatment is not placed along the gradient but only in one part of the gradient. In order to make this gradient a systematic source of variation the treatments should have been distributed along the gradient.

Table 7. Comparison between total amounts of heavy metals in the topsoil after six years of treatment, the supply over three years and the potential offtake of heavy metals with the shoots after three years

		Total amount in the topsoil, supply and offtake of heavy metals (g/ha)							
		Treatment 1		Treatment 2		Treatment 3		Control	
		Tora	*Gudrun*	*Tora*	*Gudrun*	*Tora*	*Gudrun*	*Tora*	*Gudrun*
Cd	Amount in soil [a]	553	668	1222	1143	712	694	962	1041
	Supply	9	9	17	17	26	26	0,41	0,41
	Offtake [b]	56	35	65	56	16	38	117	114
Co*	Amount in soil	20436	21515	22269	21385	19409	22022	23595	21424
	Supply	97	97	166	166	235	235	27	27
	Offtake	3	2	6	4	2	3	7	9
Cr	Amount in soil	76700	79950	84240	77480	75660	78650	79690	78130
	Supply	918	918	1712	1712	2506	2506	124	124
	Offtake	3	4	3	5	1	5	4	11
Cu	Amount in soil	101660	127010	141960	140530	114270	127140	145210	137020
	Supply	303	303	562	562	821	821	45	45
	Offtake	123	138	84	161	32	124	211	228
Ni	Amount in soil	59670	62010	74490	70330	57980	61360	75270	73710
	Supply	790	790	1473	1473	2156	2156	108	108
	Offtake	9	15	7	14	3	12	22	22
Pb	Amount in soil	44590	45370	49270	47840	44200	44070	49660	47450
	Supply	54	54	101	101	147	147	7,5	7,5
	Offtake	8	5	3	7	3	7	7	21
Zn	Amount in soil	144950	169520	170040	169910	138970	148850	166010	149760
	Supply	958	958	1791	1791	2623	2623	126	126
	Offtake	1758	1364	2016	2066	513	1487	2504	3448

[a] Total amounts of heavy metals in the topsoil were calculated assuming a bulk density of 1,3 g/cm³.

[b] Potential offtakes were calculated by multiplying the concentrations of heavy metals in the three year old shoots (Figure 4) with the estimated biomass (Figure 5).

The application of Zn with the leachate was low compared to the total amount of Zn in the topsoil (Table 7). Moreover, the offtake on treatment 1, treatment 2 and the control suggests a potential net uptake of the plants, suggesting that plants took up more Zn than was applied with the leachate. Another possible explanation for a decline in Zn concentrations in soil might be leaching of Zn to deeper soil layers, which was also shown in another study (Zupančič Justin & Zupančič, 2009). Moreover, leaching of Zn and other heavy metals to the groundwater was reported in the previous study for this trial by Aronsson et al. (2010). Zn concentrations in the subsoil tended to increase on treatment 1 and on treatment 3 with Tora, which could derive from the topsoil. However, in treatment 2 and the control, Zn concentrations tended to decrease throughout the soil profile. However, the statistical analysis showed no significant differences in Zn concentrations in the topsoil on the willow treatments compared to the reference (p=0,055). Zn concentrations in the reference field did not show the above discussed gradient from southwest to northeast of the field. Nevertheless, reference from inside the actual treatments would have been more reliable.

The application of Ni with the leachate was high during the years 2008-2011, exceeding the guidelines for application of heavy metals with sludge on all treatments (Table 5). Nevertheless, statistical analysis showed that concentrations of Ni were significantly lower in the treatments compared to the reference. Ni concentrations in the shoots were low which is consistent with a similar study by Godley et al. (2004a) and the potential offtake with the shoots was low compared to the supply (Table 7). Concentrations in the shoots did not vary much between the treatments. Thus, only the increased biomass production in the control contributed to a higher offtake of Ni compared to the leachate treatments. Since the accumulation of Ni in the shoots was low in this trial, Ni might have been accumulation in the roots instead of the upper plant parts. Again, the reference taken from the adjacent grassland might not have reflected the initial situation in the experimental field.

Application of Cu with the leachate was low compared to the amount of Cu present in the topsoil (Table 7). However, potential offtakes on the leachate irrigated treatments were low, which might result in an accumulation in the long run. On the contrary, a potential net-uptake of Cu was indicated on the control, which might be attributed to good plant growth. Studies by Pulford et al. (2002) showed that Tora accumulated high amounts of Cu in the bark. However, comparing potential offtakes of Cu, amounts were higher in Gudrun than in Tora. Studies by Nissen & Lepp (1997) showed low mobility of Cu in plants and that willows tend to exclude Cu from the shoots. Thus, Cu might potentially accumulate in the soil over a longer treatment period.

Concentrations of Pb in soil in the willow treatments were significantly lower than in the reference soil. However, compared to the supply with the leachate potential offtakes with the willow shoots were rather low (Table 7). Concentrations of Pb in the shoots were low, which is consistent with a similar study by Godley et al. (2004a). Other studies indicated that there is no or very low uptake of Pb into willow shoots (Pulford et al., 2002; Dimitriou et al., 2006). Thus, Pb might have been accumulated in the roots of the plants instead of the shoots. Pot experiments with application of sewage sludge to willows showed that the highest amounts of Pb were accumulated in the roots compared to amounts measured in stems and leaves (Labrecque et al., 1995). Although the accumulated Pb in the roots is not taken out with the harvest, Pb is immobilized in the roots and can be taken out of the system with the final harvest.

5.2 Clone differences

Fertilization and irrigation experiments with varying concentrations of nitrogen showed that both Gudrun and Tora grew best under a high irrigation and high fertilization regime, with higher shoot biomass and accumulation of nitrogen in Tora (Weih & Nordh, 2002). Therefore, Tora seems most suitable for the application with landfill leachate with high concentrations of nitrogen. In this study, however, Gudrun was more resistant to the high application loads in treatment 3 and total nitrogen applications in treatment 3 were much higher than in the study mentioned above. In general, growth was highest in the control for both clones, suggesting that a possible fertilization effect of the leachate on the treatments was outweighed by substances toxic to the plants, such as the high chloride content or the high chemical oxygen demand in the leachate. Moreover, an overload with leachate and the resulting anaerobic conditions in soil might have influenced growth.

Heavy metal concentrations in the shoots vary between the two different clones. Concentrations of Cd, Co, Mn, Pb and Zn were higher in Tora on the leachate treatments, whereas Gudrun showed higher concentrations of all heavy metals in the control. Lower concentrations in Tora in the control plots might derive from a biological dilution effect as growth increases. Klang-Westin & Perttu (2002) investigated Cd concentrations and amounts in different willow clones dependent on different fertilization regimes. Concentrations of Cd in stems were significantly higher in willows at a low nutrient level and therefore lower biomass production. Higher concentrations of some heavy metals in Tora on the leachate treatments might result from a higher bark:wood ratio. The bark:wood ratio is higher for slim shoots whereas stronger shoots have a higher portion of wood. It was shown that Cd and Zn are accumulated in higher amounts in the bark than in the wood (Dimitriou et al. 2006). Thus, the elevated concentrations of some heavy metals in Tora might be a result of an increasing bark proportion due to bad growth on the leachate treatments.

5.3 Carbon and nitrogen accumulation

Comparisons between the total carbon content in the treatments and the reference showed that the carbon content in the topsoil in treatment 2 and the control and the carbon content in the subsoil in all treatments were lower in willows than in grass, suggesting that carbon sequestration is more efficient in grassland than in willow stands. The total nitrogen content in soil followed the same pattern as carbon in the topsoil. Since most willow roots are located in the topsoil (Rytter & Hansson, 1996) the effect of carbon sequestration might be lower in the subsoil compared to grasslands. Grasslands are suggested to maintain a higher carbon content in soil compared to SRC land-use management systems. Nevertheless, perennial crops, such as willow SRC, are believed to improve carbon storage in soils compared to annual crops, which is mainly attributed to the management, e.g. tillage (Ostle et al., 2009). Carbon is added to the soil by root turnover in deeper soil layers and decaying of litter and other plant parts on the topsoil (Lemus & Lal, 2005). Plant growth on treatment 3 was bad and the carbon content in the topsoil increased compared to the grassland. Bad growth might be connected to high applications of leachate leading to anaerobic conditions in the soil and therefore slower decomposition of the organic material.

6. Conclusion

The application of landfill leachate to willow short-rotation coppice over a time span of six years did not lead to an alarming accumulation of heavy metals in the soil. It has been shown that willows are able to take up substantial amounts of some heavy metals and therefore counteract an accumulation of these. The accumulation of Cd was potentially high in the plants and concentrations of Cd in soil in the willow treatments significantly decreased compared to the concentrations in the grass reference. However, in order to improve the evaluation of the treatment efficiency of the system, a reliable reference is needed. Thus, primarily concentrations of heavy metals should have been analysed in each plot before landfill leachate was applied. The set-up of the field trial was influenced by practical restrictions, such as the supply of defined irrigation loads over several years. Nevertheless, the experimental design could be improved. For example, the gradient that was shown for Cd and the carbon and nitrogen content in the topsoil could have been included in the experimental design by distributing the treatments along the gradient.

Efficiency of phytoremediation is a combination of concentration of heavy metals and biomass production of the plants. Thus, for moderate concentrations of e.g. Cd in *Salix* high biomass is required in order to achieve a high output of the metal. An overload of leachate with a negative effect on growth is unfavorable for a successful treatment. Application loads corresponding to treatment 3 in this study affected plant health negatively and decreased biomass production, whereas plants grew well under medium application loads. Although no substantial accumulation of heavy metals was reported in this study, applications of heavy metals with the leachate should be within recommended application loads in order to prevent long-term accumulation of metals that might not be taken up by the plants, such as for example Cu in this study. However, concentrations of Pb and Ni in the treatments significantly decreased and a potential uptake of Zn by the plants was indicated. Willow SRC is a perennial crop and therefore believed to improve carbon sequestration in soil compared to annual crops. Lower amounts of carbon and nitrogen in soil in the willow treatments compared to the surrounding grassland suggest that grass is more effective in carbon and nitrogen storage.

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References

Aronsson, P., Dahlin, T. & Dimitriou, I. (2010). Treatment of landfill leachate by irrigation of willow coppice--plant response and treatment efficiency. *Environmental pollution*, vol. 158(3), pp.795–804. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19883963 [2013-01-31].

Aronsson, P.G., Bergström, L.F. & Elowson, S.N.E. (2000). Long-term influence of intensively cultured short-rotation Willow Coppice on nitrogen concentrations in groundwater. *Journal of Environmental Management*, vol. 58(2), pp.135–145. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0301479799903195 [2013-02-13].

Berndes, G., Fredrikson, F. & Börjesson, P. (2004). Cadmium accumulation and Salix-based phytoextraction on arable land in Sweden. *Agriculture, Ecosystems & Environment*, vol. 103, pp.207–223. Available at: http://www.sciencedirect.com/science/article/pii/S0167880903003335 [2013-03-11].

Bialowiec, a., Wojnowska-Baryla, I. & Hasso-Agopsowicz, M. (2003). Effectiveness of leachate disposal by the young willow sprouts Salix amygdalina. *Waste Management & Research*, vol. 21(6), pp.557–566. Available at: http://wmr.sagepub.com/cgi/doi/10.1177/0734242X0302100608 [2013-03-13].

Bulc, T.G. (2006). Long term performance of a constructed wetland for landfill leachate treatment. *Ecological Engineering*, vol. 26(4), pp.365–374. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0925857406000425 [2013-06-01].

Cajuste, L.J., Vazquez-A., A. & Miranda-C., E. (2002). Long-Term Changes in the Extractability and Availability of Lead, Cadmium, and Nickel in Soils Under Wastewater Irrigation. *Communications in Soil Science and Plant Analysis*, vol. 33(15-18), pp.3325–3333. Available at: http://www.tandfonline.com/doi/abs/10.1081/CSS-120014526 [2013-06-07].

Cureton, P.M., Groenevelt, P.H. & McBride, R.A. (1991). Landfill leachate recirculation: Effects on vegetation vigor and clay surface cover infiltration. *Journal of Environmental Quality*, vol. 20, pp. 17-24.

Dimitriou, I. & Aronsson, P. (2005). Willows for energy and phytoremediation in Sweden. *Unasylva 221*, vol. 56, pp. 47-50.

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*, vol. 142(1), pp.160–9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16278041 [2013-05-07].

Dimitriou, I., Aronsson, P. & Weih, M. (2006). Stress tolerance of five willow clones after irrigation with different amounts of landfill leachate. *Bioresource technology*, vol. 97(1), pp.150–7. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16154512 [2013-02-13].

Dimitriou, I. & Aronsson, P. (2007). Landfill leachate treatment on short-rotation willow coppice: Resource instead of waste? In: Ernest C. Lehmann (ed.), Landfill Research Focus. Nova Science Publishers, Inc., pp. 55-85.

Dimitriou, I., Rosenqvist, H. & Berndes, G. (2011). Slow expansion and low yields of willow short rotation coppice in Sweden; implications for future strategies. *Biomass and Bioenergy*, vol. 35(11), pp.4613–4618. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0961953411004600 [2013-07-27].

Godley, A., Alker, G., Hallett, J., Marshall, R. & Riddell-Black, D. (2004a). Landfill leachate nutrient recovery by willow short rotation coppice I. Yield, tissue composition and wood quality. *Arboricultural Journal*, vol. 27, pp. 281-295.

Godley, A., Alker, G., Hallett, J., Marshall, R. & Riddell-Black, D. (2004b). Landfill leachate nutrient recovery by willow short rotation coppice II. Soil quality. *Arboricultural Journal*, vol. 28, pp. 45-65.

Gustafsson, .J, Larsson S., Nordh N.E. (2009). Manual for SRC Willow Growers. *Lantmännen Agroenergi*, Sweden. 18 pp.

Hasselgren, K. (1992). Soil–plant treatment system. In: Christensen, T.H., Cossu, R., Stegmann, R. (Eds.), Landfilling of Waste: Leachate. Elsevier, Barking, UK, pp. 361–380.

Hooda, P.S., McNulty, D., Alloway, B.J. & Aitken, M.N. (1997). Plant Availability of Heavy Metals in Soils Previously Amended with Heavy Applications of Sewage Sludge. *Journal of the Science of Food and Agriculture*, vol. 73(4), pp.446–454. Available at: http://onlinelibrary.wiley.com/doi/10.1002/(SICI)1097-0010(199704)73:4%3C446::AID-JSFA749%3E3.0.CO;2-2/abstract [2013-03-11].

Jones, D.L., Williamson, K.L. & Owen, a G. (2006). Phytoremediation of landfill leachate. *Waste management*, vol. 26(8), pp.825–37. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16168631 [2013-02-13].

Kjeldsen, P., Barlaz, M.A., Rooker, A.P., Baun, A., Ledin, A. & Christensen, T. H. (2002). Present and Long-Term Composition of MSW Landfill Leachate: A Review. *Critical Reviews in Environmental Science and Technology*, vol. 32(4), pp.297–336. Available at: http://www.tandfonline.com/doi/abs/10.1080/10643380290813462 [2013-04-15].

Klang-Westin, E. & Eriksson, J. (2003). Potential of Salix as phytoextractor for Cd on moderately contaminated soils. *Plant and Soil*, vol. 249, pp.127–137. Available at: http://link.springer.com/article/10.1023%2FA%3A1022585404481 [2013-03-15].

Klang-Westin, E. & Perttu, K. (2002). Effects of nutrient supply and soil cadmium concentration on cadmium removal by willow. *Biomass and Bioenergy*, vol. 23, pp.415–426. Available at: http://www.sciencedirect.com/science/article/pii/S0961953402000685 [2013-03-15].

Kuzovkina, Y. A. & Volk, T. A. (2009). The characterization of willow (Salix L.) varieties for use in ecological engineering applications: Co-ordination of structure, function and autecology. *Ecological Engineering*, vol. 35(8), pp.1178–1189. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0925857409000810 [2013-02-10].

Labrecque, M., Teodorescu, T.I. & Daigle, S. (1995). Effect of wastewater sludge on growth and heavy metal bioaccumulation of two Salix species. *Plant and Soil*, vol. 171(2), pp.303–316. Available at: http://link.springer.com/10.1007/BF00010286 [2013-07-25].

Lemus, R. & Lal, R. (2005). Bioenergy Crops and Carbon Sequestration. *Critical Reviews in Plant Sciences*, vol. 24(1), pp.1–21. Available at: http://www.tandfonline.com/doi/abs/10.1080/07352680590910393 [2013-08-09].

McBride, M.B., Richards, B.K. & Steenhuis, T. (2004). Bioavailability and crop uptake of trace elements in soil columns amended with sewage sludge products. *Plant and Soil*, vol. 262(1/2), pp.71–84. Available at: http://link.springer.com/10.1023/B:PLSO.0000037031.21561.34 [2013-03-11].

Mirck, J., Isebrands, J.G., Verwijst, T., Ledin, S. (2005). Development of short-rotation willow coppice systems for environmental purposes in Sweden. *Biomass and Bioenergy*, vol. 28, pp.219–228. Available at: http://www.sciencedirect.com/science/article/pii/S096195340400159X [2013-03-11].

Nissen, L.R. & Lepp, N.W. (1997). Baseline concentrations of copper and zinc in shoot tissues of a range of salix species. *Biomass and Bioenergy*, vol. 12(2), pp.115–120. Available at: http://www.sciencedirect.com/science/article/pii/S0961953496000657 [2013-07-25].

Nixon, D.J., Stephens, W., Tyrrel, S.F., Brierley, E.D.R. (2001). The potential for short rotation energy forestry on restored landfill caps. *Bioresource Technology*, vol. 77, pp.237–245. Available at: http://www.sciencedirect.com/science/article/pii/S096085240000081X [2013-03-05].

Öman, C.B. & Junestedt, C. (2008). Chemical characterization of landfill leachates--400 parameters and compounds. *Waste management*, vol. 28(10), pp.1876–91. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17869498 [2013-04-02].

Ostle, N.J., Levy, P.E., Evans, C.D., Smith, P. (2009). UK land use and soil carbon sequestration. *Land Use Policy*, vol. 26, pp.S274–S283. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0264837709000945 [2013-08-09].

Pulford, I.D., Riddell-Black, D. & Stewart, C. (2002). Heavy Metal Uptake by Willow Clones from Sewage Sludge-Treated Soil: The Potential for Phytoremediation. *International journal of phytoremediation*, vol. 4(1), pp.59–72. Available at: http://www.tandfonline.com/doi/abs/10.1080/15226510208500073#.UgTgI9JM-So [2013-03-11]

Riddell-Black, D. (1994). Heavy metal uptake by fast growing willow species. In: Aronsson P., Perttu K., editors. Willow vegetation filters for municipal wastewaters and sludges. A biological purification system. Uppsala: Swedish University of Agricultural Sciences; 1994. p. 145–151.

Rosenqvist, H. & Ness, B. (2004). An economic analysis of leachate purification through willow-coppice vegetation filters. *Bioresource technology*, vol. 94(3), pp.321–329. Available at: http://www.sciencedirect.com/science/article/pii/S0960852404000094 [2013-07-27].

Rytter, R.-M. & Hansson, A.-C. (1996). Seasonal amount, grothw and depth distribution of fine roots in an irrigated and fertilized Salix viminalis L. plantation. *Biomass and Bioenergy*, vol. 11, pp.129–137. Available at: http://www.sciencedirect.com/science/article/pii/0961953496000232 [2013-08-09].

Stephens, W., Tyrrel, S. & Tiberghien, J. (2000). Irrigating short rotation coppice with landfill leachate: constraints to productivity due to chloride. *Bioresource Technology*, vol. 75, pp.227–229. Available at: http://www.sciencedirect.com/science/article/pii/S0960852400000651 [2013-02-21].

Swedish Board of Agriculture (jordbruksverket) (2012). Riktlinjer för gödsling och kalkning 2013. Available at: http://www2.jordbruksverket.se/webdav/files/SJV/trycksaker/Pdf_jo/jo12_12.pdf [2013-08-07].

Tyrrel, S.F., Leeds-Harrison, P.B. & Harrison, K.S. (2002). Removal of ammoniacal nitrogen from landfill leachate by irrigation onto vegetated treatment planes. *Water research*, vol. 36(1), pp.291–9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11766806 [2013-02-21].

U.S. EPA, United States Environmental Protection Agency (2000). Introduction to phytoremediation. EPA/600/R-99/107.

Vysloužilová, M., Tlustoš, P. & Száková, J. (2003). Cadmium and zinc phytoextraction potential of seven clones of Salix spp . planted on heavy metal contaminated soils. *Plant, soil and environment*, vol. 49(12), pp.542–547. Available at: http://www.agriculturejournals.cz/publicFiles/52952.pdf [2013-04-02].

Zalesny, R.S. & Bauer, E.O. (2007). Evaluation of Populus and Salix continuously irrigated with landfill leachate II. Soils and early tree development. *International Journal of Phytoremediation*, vol. 9, pp. 307-323.

Zalesny, J. A., Zalesny, R., Wiese, A. H., Sexton, B., Hall, R. B. (2008). Sodium and chloride accumulation in leaf, woody, and root tissue of Populus after irrigation with landfill leachate. *Environmental pollution*, 155(1), pp.72–80. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18069106 [2013-02-13].

Zupančič Justin, M. & Zupančič, M. (2009). Combined purification and reuse of landfill leachate by constructed wetland and irrigation of grass and willows. *Desalination*, 246(1-3), pp.157–168. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0011916409004305 [2013-04-02].