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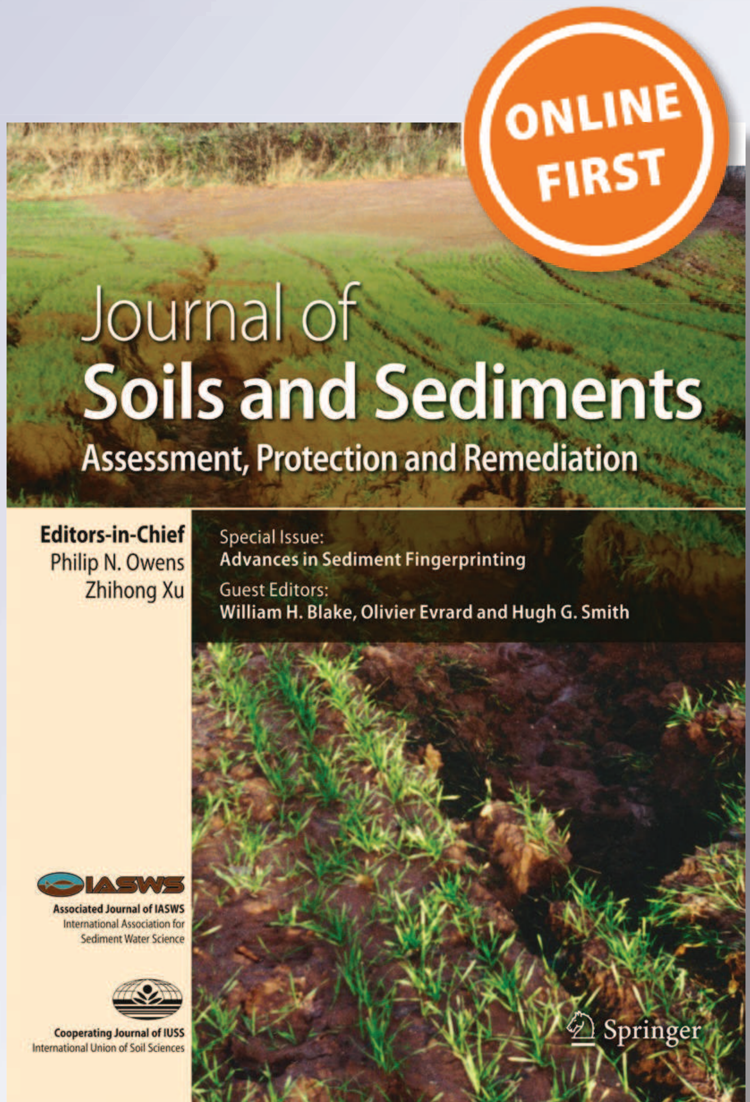

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Risk management and regeneration of brownfields using bioenergy crops

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Abstract

Purpose The potential of phytoremediation, as a cost-effective in situ alternative to conventional technologies for remediation of contaminated brownfields, has often been pointed out. Yet, phyto-technologies have failed to find widespread adoption in practice. To gain social and commercial acceptance of these technologies, there is a clear requirement of field studies that provide information on success and failures. The aim of this study was to investigate benefits and potential risks with phyto-stabilisation on brownfields using bioenergy-crops.

Materials and methods Two field trials with willow (*Salix Klara* and *Salix Inger*) were set up aiming for phyto-stabilisation on metal-contaminated sites. By the use of a tiered risk assessment approach, the cultivation's effect on ecological risks in different environmental compartments, such as soil, porewater and up-take to biota (including potential risks for wild grazers), was investigated *before* the cultivation was started and *during* following *three growth seasons*. Growth assessments were also made to evaluate the biomass' potential revenue.

Results and discussion The risks to the soil fauna proved to be unchanged or declining. The uptake in the plants was, as aimed for, low to moderate, and the growth rate depended on the soil texture rather than the contamination level of the sites. The low uptake indicated a negligible risk for wild grazers. The field trials were accomplished using no, or low, amounts of amendments, minimum soil interventions, no, or very simple, weeding control and conducted at sites with low annual temperature. Despite harsh conditions, the biomass production was high enough to potentially provide revenue.

Conclusions This study shows that cultivation of brownfields using phytostabilising willow clones can reduce the ecological risks, improve the soil quality of the site and provide revenue if the biomass is sold for e.g. bioenergy production. By choosing phytostabilisation willow clones, potential risks associated with phytoextraction of metals, such as biomass combustion and food chain transfer of metals, were eliminated. Consequently, using bioenergy crops for phytostabilisation on brownfields can contribute to preserve and improve ecosystem services, create economic regeneration of these areas and at the same time be a sustainable risk management option.

Keywords Bioenergy · Brownfields · Phytostabilisation · Risk-based land management · *Salix* · Short-rotation coppice

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1 Introduction

Land management practice has shown that remediation, aimed at revitalisation, is the core of achieving sustainable development (Volchko et al. 2013). At the same time, emerging regulatory requirements on soil advocate a holistic view on future management of contaminated sites (Rodrigues et al. 2009; Bone et al. 2010). Despite this, there are often little or no

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incitements to restore brownfield areas[1] as interventions based on conventional methods are considered uneconomic or unsustainable. For these sites, phytoremediation can be a beneficial and sustainable option. However, despite the potential benefits and despite many trials, phytoremediation has failed to find widespread adoption in commercial practice. The main reason for this failure is probably difficulties related to phyto-extraction, the most commonly trialled approach (Vangronsveld et al. 2009; Mench et al. 2010; Bardos et al. 2011; Van Slycken et al. 2013). Phyto-extraction relies on plants that can translocate large amounts of one or several target contaminants into the above-ground biomass, which then is removed from the site after cropping. This is often considered as a too slow process, with projected treatment times in the order of decades (Vangronsveld et al. 2009; Mench et al. 2010; Delplanque et al. 2013). Also, in the case of combining phytoremediation with the intention of producing biomass for bioenergy production, phyto-extraction is not a desirable option. This is due to the fact that, from a regulatory perspective, a biomass with elevated levels of contaminants may be regarded as “waste”. This would greatly reduce the economic value of the biomass, since its use would be restricted to specialised facilities with appropriate licensing and permitting (Bardos et al. 2011; Andersson-Sköld et al. 2014). Moreover, a crop that enriches in toxic elements as it grows may render a risk to herbivorous organisms if they consume the crop and may provide an exposure pathway for toxic elements to enter the food chain. Crops intended for bioenergy production, grown on brownfields, should hence be low accumulators and preferably act as stabilisers to immobilise the contaminants and prevent further spreading (Andersson-Sköld et al. 2014). So-called phytostabilisation can also have several other environmental and socio-economic benefits and help us preserve ecosystem services, such as carbon sequestration, water management and production of bioenergy and fibre. To certify that the intervention does not result in sub-optimisation, such as increased risks at the site or elsewhere, the risks and the potential soil functionality and ecosystem services at the site need to be assessed (Volchko et al. 2013; Andersson-Sköld et al. 2014). There is a lack of field studies where both the ecological risks and the potential benefits with this kind of land rehabilitation have been evaluated. Such knowledge and information is highly needed for sustainable regulations and to increase the acceptance of these often more sustainable risk-based management approaches, which are based on the effect of the contaminants, rather than on its total concentration in the soil.

[1] Brownfields are abandoned or underused industrial and commercial facilities that may have real or perceived contamination problems and require interventions to be brought back to beneficial use (Ferber et al. 2006). Only within the European Union there are estimated to be close to one million potential brownfield sites that may be contaminated and unexploited (Oliver et al. 2005).

Here, we present the results from two field trials where willow (*Salix* spp.) has been cultivated on metal-contaminated brownfields. The specific objectives of the study were to assess (i) the ecological risks to soil living species, (ii) the risks to grazing animals and (iii) the potential of phyto-stabilisation simultaneously providing other ecosystem services such as carbon sequestration and biomass production on these sites. The primary aim being to demonstrate that bioenergy crop cultivation on brownfields can have several environmental benefits and can be an integral part of land rehabilitation and risk management of brownfields in the long term.

2 Materials and methods

2.1 Site description, sampling plan and initial soil analyses

2.1.1 Field trial A

The field trial A was located in Kallinge, in the south of Sweden (Electronic Supplementary Material, Fig. S1). The site has previously been used as an industrial landfill area, where slag from a local metal industry and soil fillings from excavation works have been deposited. The surface soil consisted of a mix of metal slag lumps and sandy soil. Soil samples were collected from the layers 0–5 and 25–30 cm throughout the experimental site (750 m^2) and analysed in field by X-ray fluorescence (XRF) spectrometry to determine the distribution of metals within the site. The XRF-screening indicated elevated levels of chromium (Cr), copper, (Cu), nickel (Ni), lead (Pb) and zinc (Zn) when compared to soil quality benchmarks (Table S1 in the Electronic Supplementary Material). Based on the results of the XRF screening, five sampling squares (A2, A4, A5, A8 and A9), each of the size 2.5 m×2.5 m, representing a gradient of contamination (from low to high metal concentration) and one reference square to be used as field control (A7-ref) were selected for detailed characterisation. Soil sampling for ecological risk assessment (ERA) was made prior to cultivation and again after 2 years of cultivation to investigate possible changes in ecological risks caused by the plantation of willow.

As a complement, four out of the six squares (A2, A5, A9 and A7-ref) were used for further sampling to study metal concentrations in soil porewater (C_{pw}) and biota (C_{biota}, willow leaves and twigs) during the time frame of 3 years. Samples of porewater and leaves were taken in 2011, 2012 and in 2014, and samples of twigs were taken in 2012 and in 2014. All samples were composite samples for the different squares.

2.1.2 Field trial B

The field trial B was located in Timrå, in the central part of Sweden (Electronic Supplementary Material, Fig. S2). The site has been used for numerous industrial purposes (shipyard, sawmill, wood impregnation, production of sulphate, boards and fine paper). Previous investigations of the site identified heavy metals as the main contaminants (unpublished data). Reported concentrations for the specific area chosen for this field trial (2.5 ha) were, however, below the Swedish guideline values for less sensitive use of land. At the time for cultivation, additional composite soil sampling was performed, and the content of metals was determined (Sect. 2.3).

Higher tier ERA was not justified for this field trial, due to low soil contamination. The focuses of this field trial were instead to study the biomass production to confirm the expected low plant uptake and risk for grazers with and without sludge amendment and to demonstrate cultivation of willow on a brownfield site located at rather northern latitude.

Sewage sludge was applied in a randomised block design (15 t/ha, eightfold replication) to include investigation of the impact of a fertiliser. The sludge had been anaerobically stabilised and dehydrated before delivered to the site. The metal content of the sludge was below the Swedish guidelines values (SFS 1998:944) and the content of tot-P and tot-N were 29 and 46 g/kg dry weight (d.w.). Sampling of soil and biota (willow leaves and twigs) was performed as composite samples from areas treated with sludge (B_{WS}) and without sludge (B_{WOS}). Samples of leaves were taken in 2011 and 2012. Sample of twigs was taken in 2014.

2.2 Plant establishment

The two field trials were established in June 2010 by plantation of two different hybrids of willow (*Salix Inger* and *Salix Klara*). The selection was based on willow being commercially available, potential for use as biofuel and clones not extracting but stabilising soil contaminants, applying the Rejuvenate decision support tool (Bardos et al. 2011; Andersson-Sköld et al. 2014). The genotypes used and other field trial details are summarised in Table 1. Both trials were established using 500 mm un-rooted cuttings, hand-planted in rows with 100 cm between the rows and 50 cm between the plants. Apart from adding a mineral fertiliser (YaraMila[2], 0.1 kg/m^2), the field trial A was not managed before plantation. Hand-weeding and irrigation were carried out regularly on field trial A. Despite the weed control, the weeds were strongly competing with the willow plants, and in the autumn of 2011, two thirds of the plants were not viable. Hence, plantation of 800 new cuttings was made in October 2011 to replace non-viable plants. Prior to re-plantation, the soil was cultivated using a rotary tiller, viable plants were coppiced (i.e. cut back to ground level to encourage the growth of multiple stems), and a permeable geo-textile (MyPecks VICONA®) was applied to the experimental area to suppress weed in the future. After re-planting, each plant was mulched individually with bark mulch to maintain soil moisture.

The experimental site of field trial B was harrowed to a depth of 40 cm before the sewage sludge was spread on the predetermined areas. The sludge covered areas were harrowed once more after spreading. Neither irrigation nor weeding was performed at field trial B.

[2] YaraMila®: 11 % N (of which 4.4 % nitrate and 6.6 % ammonium), 4.6 % P (of which 3.5 % water-soluble), 17.6 % K (water-soluble), 1.6 % Mg (of which 1.1 % water-soluble), 10 % S (water-soluble), 1 % Ca, 0.05 % B, 0.08 % Fe, 0.03 % Cu, 0.25 % Mn, 0.002 % Mb, 0.04 % Zn and <1 % Cl.

2.3 Soil, porewater and biota analyses

Soil concentrations, C_{soil}, of As, Ba, Cd, Co, Cr, Cu, Ni, Pb, V and Zn were determined by ICP-AES (EN ISO 11885-1) following Aqua-Regia digestion (EN 13657). The water content of the soil (and the dry matter content) was analysed according to ISO 11465. The easily soluble fraction of P, K, Mg, Ca, Al and Fe (P-AL, K-AL, Mg-AL, Ca-AL, Al-AL, Fe-AL) was determined by extraction with ammonium lactate-acetate (AL), and the storage fraction (K-HCl, P-HCl, Cu-HCl) was determined by extractions with HCl. NO_3-N and NH_4-N were analysed according to ISO 11732, tot-N according to ISO 13878 and tot-C according to ISO 10694. Further characterisation of the soils' content of organic matter (NEN 5754), clay (fraction<2 μm) and chalk (measured as carbonates), and pH (ISO 10390) was also made. For the evaluation of the nutrient status in soil, composite soil samples were taken in 2012.

Soil porewater, C_{pw}, samples were collected by in situ samplers (Rhizon MOM, Rhizosphere Research Products, Wageningen, The Netherlands) installed at 25-cm depth. Macro and micro elements were analysed by ICP-AES and ICP-SFMS. tot-N and total organic carbon (TOC) were analysed according to EN 12260 and EN 1484.

Samples of willow leaves and twigs were dried at 50 °C and finely pulverised (shredded and grinded) prior analyses. Pulverised samples were digested with HNO_3 and H_2O_2 (mixture of 10:1) in a closed microwave-assisted system. Metals were quantified by ICP-AES and ICP-SFMS, and tot-N was analysed by the Kjeldahl method. To investigate the effect of the deposition of airborne contaminants (or dust) on leaves, both unwashed and washed samples (rinsing in deionised water) were analysed. The deposition of airborne contaminants, or dust, was found to be of minor importance for all studied contaminants (Electronic Supplementary Material, Table S2).

2.4 Ecological risk assessment

An ERA of the soil at field trial A was assessed before planting the *willow*, and again after 2 years of cultivation, in order to investigate the cultivation's effect on ecological risks. The ERAs were performed using the TRIAD approach (Jensen and Mesman 2006; Mesman et al. 2011; Ribé et al. 2012;

Table 1 Summary of field trial details

	Field trial A	Field trial B
Location	Latitude: 56.2	Latitude: 62.5
	Longitude: 15.3	Longitude: 17.3
Aim	Study cultivation of bioenergy crop on metal-contaminated soil of different pollution grade. ERA performed, using the TRIAD approach,[a] before and after 2 years of cultivation.	Investigate the biomass production and plant uptake of contaminants in areas with and without sewage sludge amendment.
	Investigate the biomass production.	
	Investigate plant uptake of contaminants.	
Willow genotype	*Salix Inger* (*Salix triandra*×*S. viminalis*)	*Salix Klara* (*Salix viminalis*×*Salix schwerinii*×*Salix dasyclados*)
Planted area	750 m^2	2.5 ha
Main contaminants	Cu, Pb and Zn	Heavy metals (below Swedish guideline values for less sensitive use of land)
Soil analyses	Metals, organic content, pH, $CaCO_3$ Microtox (on soil leachates), Nematodes	Metals, organic content, pH,
Soil water analyses	Metals, nutrients, organic content, pH, conductivity	–
Biota analyses	Metals in leaves	Metals in leaves
	Metals in twigs	Metals in twigs
	Biomass growth assessment	Biomass growth assessment

[a] Described in report of Jensen and Mesman (2006)

Chapman 2013; Sorvari et al. 2013). This methodology is based on three independent types of assessments, so-called lines of evidence (LoE): (i) chemical characterisation, (ii) toxicity characterisation and (iii) ecological surveys (Jensen and Mesman 2006). Each of the LoEs can comprise multiple analyses which are combined into LoE-specific risk estimates. For the final judgment, the risk estimates of each LoE were used to produce integrated risk estimates (IREs) for each studied sample square (see Sect. S6 in Electronic Supplementary Material for more information). The TRIAD analysis was performed according to the principles stated in the report of RIVM (National Institute of Public Health and Environment in the Netherland) (Mesman et al. 2011) and comprised chemical studies combined with benchmark comparisons, assessments of toxic pressure (TP) based on soil concentrations of pollutants, an ecotoxicity test (microtox) and studies on soil animals (nematodes).

The ERA of the less contaminated brownfield (field trial B) was only based on chemical characterisation, i.e. soil concentrations were compared with soil quality benchmarks (BM), see Sect. 2.4.2.

2.4.1 Soil sampling

Composite soil samples were collected from all sampling squares at 0–30-cm depth for chemical and toxicological analyses, while topsoil (0–10-cm depth) was used for the ecological analyses (nematodes) for each sampling square.

2.4.2 Chemistry LoE

To identify Contaminants of Potential Ecological Concern (COPEC) and to estimate the magnitude of hazards they may pose to biota, hazard quotients (*HQs*) were calculated:

$$HQ_i = \frac{C_{i,soil}}{BM_i} \tag{1}$$

where $C_{i,\ soil}$ is the concentration (mg/kg in d.w.) of the COPEC "i" in the soil, and BM_i is the soil quality benchmark (mg/kg d.w.) for the COPEC "i" (i.e. a concentration limit not considered to cause significant adverse effects on the terrestrial biota). The Swedish generic guideline values for less sensitive land use, E_{MKM} (set to protect 50 % of the species in the soil), were chosen as *BM*s for both field trials. These values are based on no-observed effect concentration (NOEC) (Swedish-EPA 2009). As a preliminary investigation, the COPEC-specific *HQs* were aggregated to generate sample-specific hazard indexes (HI) in order to assess the differences in toxicity between the samples.

Second, the overall TP for each sample was calculated, using the Dutch web-based Sanscrit model (www.risicotoolboxbodem.nl). The TP expresses, in one value, the fraction of species that is expected to be locally exposed beyond a selected effect level due to the mixture of contaminants (Posthuma and Suter 2011). It is calculated from species sensitivity distributions (SSDs), for the studied contaminants, based on EC50 values from the literature (effect concentration demonstrating 50 % effect in a toxicity test), and is obtained from mixture modelling using models for concentration addition (CA) and response addition (RA) (De Zwart and Posthuma 2005; Rutgers et al. 2008), for equations see Electronic Supplementary Material, Eq. S1–Eq. S4. The following interpretation of TP-intervals was used: TP<0.25 indicate no risk, 0.25<TP<0.50 indicate low risk and TP>0.50 indicates high risk.

2.4.3 Toxicology LoE

Standardised Microtox test (measuring inhibitory effects on bacterial luminescence using the bacteria *Vibrio fischeri*) was used to study the toxicity of the different soil samples. Soil samples were extracted with aqueous solution and mixed with rehydrated bacteria (90 % extract). The inhibitory concentration (EC50) was determined after 30 min, relative to a control, by testing a concentration series of each extract.

2.4.4 Ecology LoE

The test procedure for the nematode analysis was based on the standard EN ISO 23611-4. The nematode analyses consisted of determining the total number of nematodes per 100 g of soil, as well as the number of different species, number of nematodes per species, number per feeding group (e.g. plant feeding, fungal feeding, bacterial feeding etc.), number per life strategy group (coloniser-persister (cp) scale) and the maturity index (MI) (Bongers 1990). For more information, see Electronic Supplementary Material Sect. 5.

2.4.5 Combining the results of separate LoEs and calculation of IREs

In order to calculate LoE-specific risk estimates and IREs, the results of the different methods had to be transformed into a uniform scale running from 0 (no effects) to 1 (full effects of the pollution). The scaling was based on comparisons of effects with the site-specific reference soil (A7-ref). For more details and equations, see Electronic Supplementary Material, Sect. 6 (Eq. S5–Eq. S11).

2.5 Risks to grazers

The translocation of metals from soil to plants was evaluated using bioconcentration factors ($BCF=C_{\mathrm{biota}}/C_{\mathrm{soil}}$), and the risks to grazers were calculated as risk quotients (R) using Eqs. (2) and (3). Cows and sheep were used as model organisms, due to lack of data for wild animals.

$$R(cow) = \frac{C_{biota} * X_{cow}}{Body\ weight * NOEC} \tag{2}$$

$$R(sheep) = \frac{C_{biota} * X_{sheep}}{ADI} \tag{3}$$

in which

C_{biota}	concentration of metal in biota (mg/kg d.w.)
X_{cow}	daily food intake for cow (kg d.w.)
X_{sheep}	daily food intake for sheep (kg d.w.)
$NOEC$	no observed effect concentration (mg/kg body weight/day)
ADI	acceptable daily intake (mg/day).

The daily food intake (X) was assumed to 10 kg d.w. for cow and 1.35 kg d.w. for sheep. The body weight of a cow was assumed to be 600 kg.

2.6 Growth assessment

In 2014, the growth of willows at the two field trials was estimated based on method described in Verwijst and Nordh (1992). Seven trees at field trial A and 24 trees at field trial B (randomly picked) were cut at 5 cm above the soil surface. The trees' diameters (or cross sectional area) were measured at 55-cm height. The trees were dried (85 °C, 72 h), and the relation between d.w. of biomass and cross sectional area was determined for each site. For field trial A, the diameters of all trees in each sampling squares were measured in order to calculate the total biomass weight of each square. For field trial B, the diameters of 32 randomly picked trees, on either sludge-treated areas or untreated areas, were measured, and the biomass weight of each tree was calculated according to the equation obtained from the relation between the cross-sectional area and the d.w. of biomass. An average tree weight for the areas with and the areas without sludge was calculated, and together with the total amount of trees, the total biomass weights were estimated.

2.7 Potential adapted gross income

A potential adapted gross income (AGI) from cultivation of Salix was estimated for the two different scenarios (field trial A and field trial B):

$$AGI = Revenues - Costs \tag{4}$$

A template of potential costs and revenues of willow for energy purpose and traditional agricultural crops in Sweden (Rosenqvist 2010) was used for the AGI estimates.

3 Results

3.1 Field trial A

3.1.1 Soil properties

Soil properties are presented in Table 2. The soil of field trial A had a clay content ranging from 3.0 to 7.2 %. Prior cultivation, the pH ranged from 7.2 to 7.6 for all sampling squares but A2 which was more alkaline (pH=8.3). The organic matter content was rather low (between 1.6 and 4.7 %), and the chalk content ranged between 1.3 and 3.2 g $CaCO_3$/kg, indicating a rather low buffer capacity of the site. After 2 years of cultivation, the organic matter content had increased in all sample squares (ranging from 3.0 to 6.3 %), but the chalk content had decreased to non-detectable quantities (<0.5 g $CaCO_3$/kg). The pH was unchanged or slightly reduced (by 0.4 pH units at the most).

3.1.2 Metal concentrations in soil samples

The chemical soil analysis of Cu, Pb and Zn (prior cultivation) showed similar results to the once obtained with the initial screening analysis, using XRF (Table 2 and Table S1, in the Electronic Supplementary Material). However, Cr, Ni and V were found in much lower concentrations when determined with aqua regia digestion, below the Swedish generic guideline value for sensitive land use, E_{KM} (set to protect 75 % of the species in the soil). The COPECs identified for field trial A were hence Cu, Pb and Zn; however, HI and TP calculations were based on data for all the metals presented in Table 2. The HIs were found to be between 0.8 and 4.0, with the following order of potential toxicity of the squares: A7-ref<A8<A9≤A4<A2<A5 (Table 2). Concentrations above the corresponding *BM* value were found in A2 for Cu and Zn and in A5 for Pb (Table 2). Square A4 showed only concentration of Cu exceeding E_{KM}. The other investigated squares (A7-ref, A8 and A9) contained low metal concentrations (below corresponding ecological *BM*s), and the concentrations found in A7-ref were in line with background concentrations found in Swedish moraine soils (Swedish-EPA 2009); hence, A7-ref was chosen as the reference sample in the TRIAD evaluation.

Metal concentrations in soil samples collected 2 years after cultivation did not indicate any significant differences from those analysed prior cultivation (Table 2), except for Pb in Square A5, for which the concentration was found to be one order of magnitude lower at the second sampling occasion. The reduced concentration of Pb may partly be caused by off-site transfer and/or containment but is most probably related to a very large heterogeneity of the contamination found in this square, as the soil concentrations are not expected to change significantly during only two growing seasons.

3.1.3 Results from the separate TRIAD LoE

LoE chemistry—toxic pressure (TP) The calculated TP of the different sampling squares is given in Table 3 for sampling made before and after 2 years of cultivation. The highest TP (=0.67) was found for A2 before cultivation had started. After 2 years of cultivation, the TP's of the individual squares were found to be in the same range as the initial values, or lower.

LoE toxicology—microtox None of the soil leachates of the contaminated samples (including the reference sample) showed any inhibitory effects on the bacterial luminescence in the Microtox test at any of the sampling occasions (Table 3).

LoE ecology—nematode analysis The nematode populations deviated strongly from the reference sample, especially in 2010, when the number of nematodes and species was lowest in the reference sample (Electronic Supplementary Material, Table S3). No correlation was found between the TP and the nematode population. This means that the effects that have been observed in the sample are not caused by the pollution but by another external unknown factor. In 2012, the deviation in the nematode population was less pronounced, and the final risk estimates (LoE_e) were all found to be lower, or in the same range, compared to the 2010 calculation (Table 3), indicating better ecological conditions (at least for nematodes) at the site after 2 years of cultivation.

3.1.4 Metal concentrations in porewater

The concentrations of COPECs in porewater from the squares A2, A5, A9 and A7-ref were found to be low or declining during the cultivation (Fig. 1). The most contaminated squares (A2 and A5) showed decreasing trends of lead concentration, C_{pw}(Pb), reduction from 2.7 to 0.6 and 0.7 to 0.3 μg/L (initial values of 2011 compared to the means of 2012 and 2014). C_{pw}(Pb) in the other squares (A7-ref and A9) was already at the start of the sampling (autumn 2011) very low (below or equal to the detection limit of the analysis (0.2 μg/L)).

The concentration of Cu and Zn was found to be low and fluctuating in the porewaters of all squares. The mean value of C_{pw}(Cu) and C_{pw}(Zn) found in A2 (the square with the highest soil concentration of these metals) was not significantly different from that obtained for A7-ref, which contained Cu and Zn amounts in level with Swedish background values (Swedish-EPA 2009). Consequently, the porewater results indicate no/low risk for spreading of the COPECs identified for this site.

3.1.5 Metal concentrations in biota

No correlation of metal concentration in willow leaves with soil concentration could be found (Fig. 2). Replicates (n=3) collected for A7-ref showed relative standard deviations (RSD) of 3–28 % for the studied elements (shown as error bars in Fig. 2).

Significantly higher concentrations of Pb and Zn were found in leaves sampled in October 2011 compared to the other sampling occasions. This is probably due to the very slow growth rate 2011 (due to weeding problems) and accumulation of these elements in the willow leaves over a growth season (Dinelli and Lombini 1996; Maxted et al. 2007). It should also be noted that due to the replantation made in the autumn 2011, samples collected in 2012–2014 are not all from the same plants as in 2011.

Table 2 Soil concentrations for field trials A and B, before and after 2 years of cultivation

Before cultivation

	E_{KM}/E_{MKM}[a]	Background[b]	Field trial A soil conc.[c]						Field Trial B soil conc.[c]	
			A2	A4	A5	A7-ref	A8	A9	B_{WS}	B_{WOS}
Cr	80/150	29.8	62	22	36	19	27	31	18	21
Cu	80/200	28.5	240	89[d]	77	49	50[d]	28	16	14
Ni	70/120	22.1	16	13	11	6,1	14	15	12	13
Pb	200/400	15.6	190	83	1,000	22	40	76	11	25
V	100/200	38.7	29	<LOD[d]	100	29	<LOD[d]	30	27	22
Zn	250/500	60.4	530	170	130	72	120	180	65	56
HI			**3.4**	**≥1.2**	**4.0**	**0.8**	**≥0.9**	**1.2**	**0.6**	**0.6**
dw		%	87	87	84	84	84	86	74	79
Organic matter		% d.w.	2.8	4.7	3.7	1.6	3.5	3.7	14	6.4
pH-H_2O			8.3	7.3	7.6	7.2	7.3	7.5	6.6	7.2
$CaCO_3$ content		g/kg d.w.	2.8	3.2	3.0	1.4	1.3	2.0	NA	NA
Fraction<2 µm		%	3.0	3.6	4.6	6.2	7.2	4.3	<1	<1

After 2 years of cultivation

		Field trial A						Field Trial B	
		A2	A4	A5	A7-ref	A8	A9	B_{WS}	B_{WOS}
Cr		46	19	44	23	30	20	12	9.3
Cu		200	55	110	15	19	34	11	7.3
Ni		23	19	13	7.3	17	15	7.7	8.0
Pb		230	99	94	33	42	58	6.2	8.0
V		32	38	130	34	28	44	13	9.8
Zn		600	190	150	130	120	160	55	58
HI		**3.4**	**1.4**	**2.1**	**0.8**	**0.9**	**1.1**	**0.4**	**0.2**
dw	%	87	80	82	80	79	81	NA	NA
Organic matter	% d.w.	6.0	6.3	6.0	3.0	4.2	4.8	NA	NA
pH-H_2O		7.9	7.4	7.6	7.0	6.9	7.3	NA	NA
$CaCO_3$-content	g/kg d.w.	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	NA	NA

Ecological benchmark values (E_{KM} and E_{MKM}) and background concentrations in Swedish soils are listed for comparisons. All soil concentrations in the table are given in milligrams per kilogram dry weight (d.w.)

NA not analysed, *HI* hazard index, *LOD* limit of detection, B_{WS} composite sample from areas treated with sludge, B_{WOS} Composite sample from areas treated without sludge

HIs (Hazard indexes) are marked in bold to highlight the potential differences in toxicity between the samples

[a] Swedish ecological guideline values for sensitive (E_{KM}) and less sensitive (E_{MKM}) land use (Swedish-EPA 2009)

[b] Background concentrations reported for Swedish soils (moraine) as 90th percentile (Swedish-EPA 2009)

[c] Extractions with aqua regia

[d] XRF analysis (mean value of samples taken at 5- and 30-cm depths)

Table 3 Summary of results from Triad analysis performed 2010 (before cultivation) and 2012 (after 2 years of cultivation)

	Sample square and Triad analysis year											
	A2		A4		A5		A7-ref		A8		A9	
Triad analysis parameters	2010	2012	2010	2012	2010	2012	2010	2012	2010	2012	2010	2012
Environmental chemistry												
Toxic pressure (TP)	0.67	0.64	0.23	0.18	0.44	0.34	0.06	0.04	0.08	0.03	0.09	0.09
Toxicology												
Effect (Microtox EC_{20})	0	0	0	0	0	0	0	0	0	0	0	0
Effect (Microtox EC_{50})	0	0	0	0	0	0	0	0	0	0	0	0
Ecology												
Effect (Nbr of nematodes)	0.82	0.22	0.79	0.01	0.26	0.18	0	0	0.84	0.47	0.12	0.56
Effect (Nbr of species)	0.56	0.05	0.69	0.1	0.81	0.2	0	0	0.44	0.35	0.44	0.25
Effect ($MI_{(2–5)}$)	0.09	0.03	0.04	0.14	0	0.08	0	0	0	0.13	0.10	0.11
Risk assessment												
Judgement LoE chemistry	0.67	0.64	0.23	0.18	0.44	0.34	0.06	0.04	0.08	0.03	0.09	0.09
Judgement LoE toxicology	0	0	0	0	0	0	0	0	0	0	0	0
Judgement LoE ecology	0.58	0.10	0.60	0.08	0.48	0.15	0	0	0.55	0.33	0.24	0.34
IRE[a]	0.48	0.31	0.33	0.09	0.34	0.18	0.02	0.01	0.26	0.14	0.11	0.15
Deviation	0.63	0.59	0.53	0.16	0.46	0.29	0.06	0.02	0.52	0.31	0.21	0.30

[a] No risk (<0.25), low risk (0.26–0.5), and high risk (>0.5)

Metal concentrations in twigs, sampled in November 2012 and June 2014, were low, and no trend of accumulation of Cu, Pb or Zn could be seen with increasing soil contamination of the studied squares (Electronic Supplementary Material, Table S4). The metal concentrations in the twigs of the different squares were all lower than, or in the same range as, the average leave concentration of each square (Table S4, Electronic Supplementary Material). This finding is consistent with previous reports on metal concentrations in leaves, stems and twigs (Klang-Westin and Eriksson 2003; Maxted et al. 2007).

3.1.6 Risk quotients (R-values) and BCFs

Risk quotients are presented in Table 4, and *BCF*s for Cr, Cu, Ni, Pb, V and Zn can be found in the Electronic Supplementary Material, Table S5. The *BCF*s and the *R*-values were found well below 1 for all metals except for Zn (BCF_{Zn} ranged between 0.3 and 3.7 for the different sampling squares).

3.1.7 Growth assessment

The biomass growth (Table 5) was assessed after two growing seasons, i.e. 2 years after the replantation and the coppice of viable plants. The trees were growing very well in all sampling squares except in A2, which only had a biomass of 0.75 t d.w./ha. Possible reasons for this low yield are discussed in Sect. 4.3.1. Highest production was found for the reference square (A7-ref; 23 t d.w./ha), while the other two studied squares produced about half of this amount: 9.4 and 11 t d.w./ha. The mean productivity for field trial A was assessed to 11 t d.w./ha, which is in line with expected productivity for commercial operations; trees are normally ready for harvesting 3 to 4 years after planting, and yields of first harvest are reported to be approx. 20–25 t d.w./ha (SalixEnergi 2013).

3.2 Field trial B

3.2.1 Soil properties metal concentrations in soil

The results from the soil analyses from field trial B and the calculated HI values are presented in Table 2. All studied compounds, in all samples, were well below the soil quality benchmarks (E_{MKM} and E_{KM}) and in line with reported background soil levels (Swedish-EPA 2009), and hence, no COPECs were identified for this brownfield. The clay content was low <1 %, and the organic content varied between 6 and 14 %, and pH between 6.6 and 7.2.

3.2.2 Metal concentrations in biota

Concentrations of most of the elements in leaves and twigs were similar between the years, and no differences were found between areas with and without sewage sludge (mean values and RSD are shown in Table S4, in the Electronic Supplementary Material). The biota

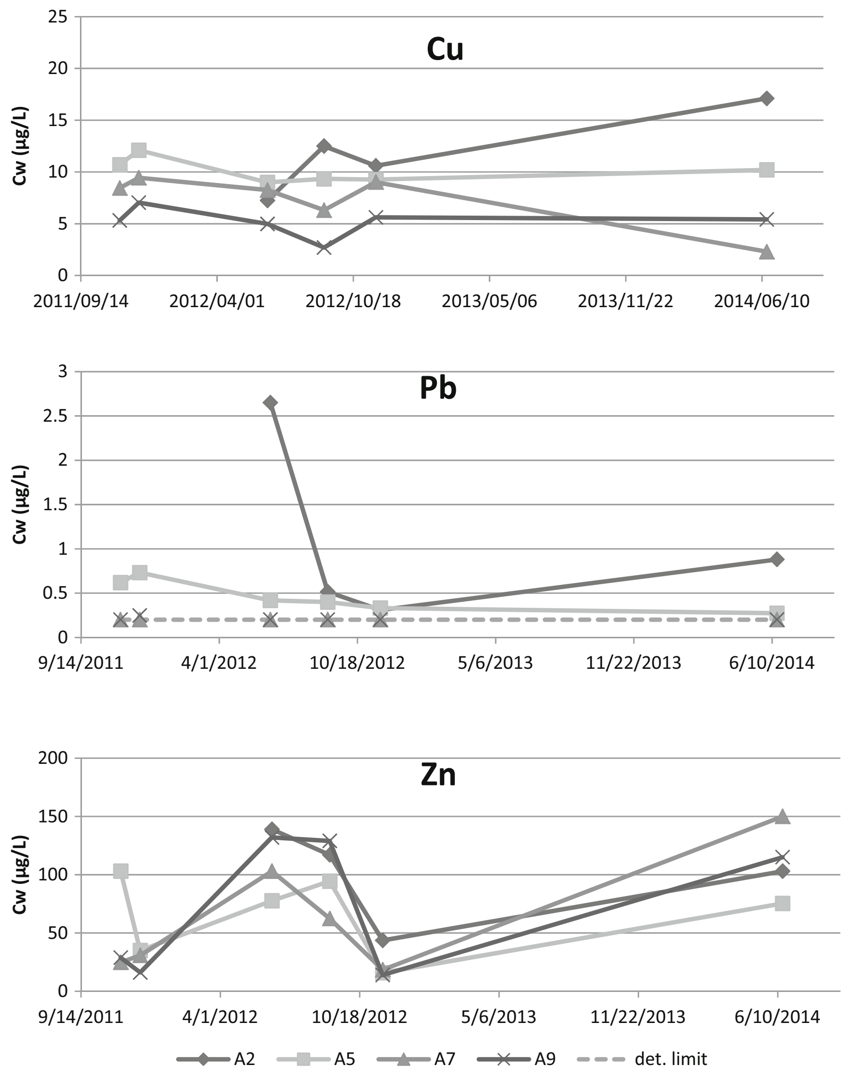

Fig. 1 Fluctuation of the porewater concentration during cultivation in sample squares A2, A5, A7-ref and A9. Monitoring of A5, A7-ref and A9 started in September 2011 (i.e. approx. 1 year after plantation), while the first sample of A2 was collected in June 2012, due to sampling device problems of this square

concentrations found in willow leaves in field trial A (*Salix Inger*) and field trial B (*Salix Klara*) were of similar magnitude despite the deviations in soil concentrations. The major difference found was that the clone *Salix Klara* had higher Zn accumulation (630–750 mg/kg) than *Salix Inger* (190–430 mg/kg).

3.2.3 Risk quotients (R-values) and BCFs

As for field trial A, calculated *R*-values (Table 4) and *BCF*s (Table S5, in the Electronic Supplementary Material) for *Salix Klara*, grown on field trial B, were well below 1 for all metals except Zn (BCF_{Zn} ranged from 4.8 to 13).

3.2.4 Growth assessment

The growth of willows (measured after three growing seasons) was found uneven at field trial B and was independent of whether sludge was added or not; the biomass production of *Salix Klara* was found to be at average 4.8 t d.w./ha for sludge areas and 4.3 t d.w./ha for non-sludge areas. Due to the northern location of this site, the growth rate is expected to be slower (due to the shorter growth season), and lower annual yield will be produced. The annual growth in the region is expected to be just above 5 t d.w./ha (Rosenqvist 2010).

3.3 Potential AGI

For Nordic Swedish conditions (field trial B scenario), the average annual harvest is predicted to 5.4 t d.w./ha/year over 20 years (Rosenqvist 2010). The revenue for energy product, sold directly to a thermal plant, is expected to be about 70 €/t d.w. based on the prize for forest chips and the energy yield of 4.4 kWh/t d.w. For new plantations, there is also a national state investment grant (500 €/ha). Hence, the annual average revenue adds up to approx. 400 €/year (for a 20-year cultivation scenario). The costs are related to plantation, weed control, applied fertilisers, transports, the

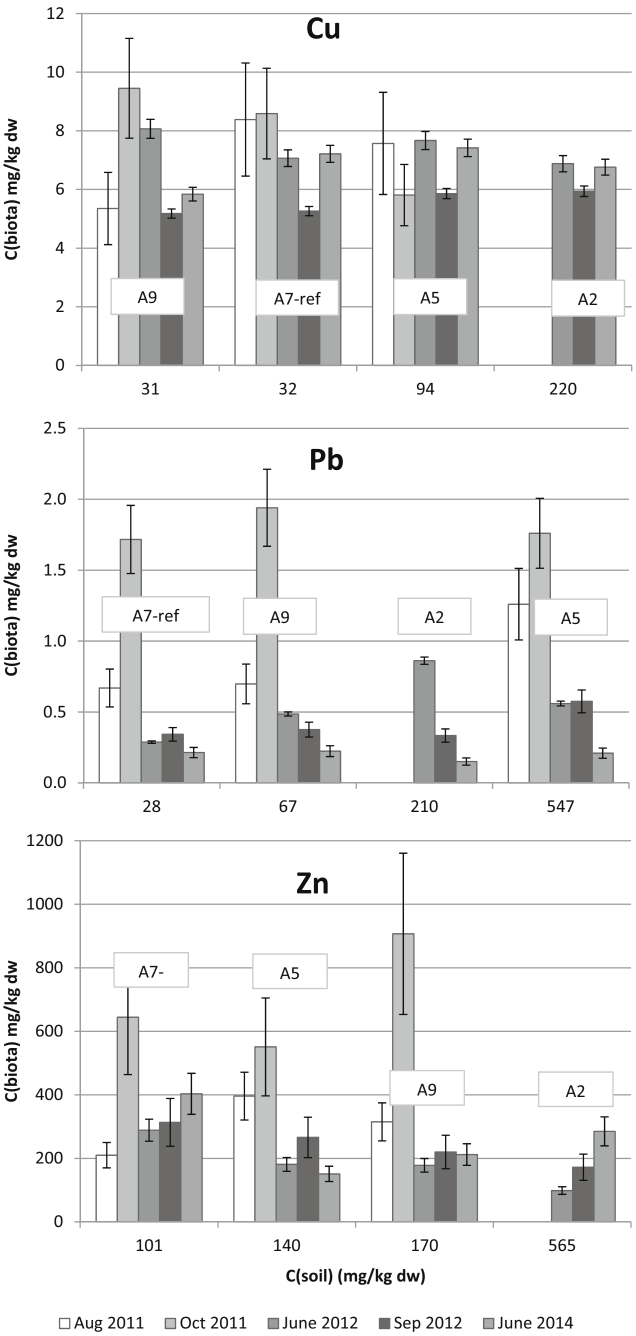

Fig. 2 Metal concentrations in *Salix* leaves (mg/kg d.w.) at five sampling occasions versus mean soil concentration of each sampling square (in mg/kg d.w. soil). Data for A2 for 2011 is lacking due to much suppressed growth. Means for $C_{(\mathrm{biota})}$ and the standard deviation were calculated for A7-ref (n=3) for each sampling occasion, here presented as error bars for A7-ref. *Error bars* for the other samples are set to the same RSD percentage as found for A7-ref

use of working machineries and indirect costs. The major costs are cuttings and costs for machineries, where the latter depends on availability. The average annual costs for a cultivation scenario of 20 years add up to 350 €–400 €/year depending on availability of own/low cost machineries or if high machinery costs (rental, hiring or investments) are to be expected. Consequently, for an average harvest of 5.4 t d.w./ha/year, the annual average AGI will vary from being very low negative to very low positive. In Sweden, however, crops produced for energy purposes are supported by subsidies (approx. 400 €/ha/year) resulting in positive AGI already at yields of 5.4 t d.w./ha/year. For Central Europe climate conditions (field trial A scenario), at least the double yield can be expected (SalixEnergi 2013) providing an annual revenue of 800 €/ha without, and 1200 €/ha with, energy crop subsidies, resulting in an annual average AGI of ca 400 €/ha without, and ca 800 €/ha with, energy crop subsidies.

4 Discussion

4.1 Ecological risk assessment

4.1.1 TRIAD analysis of field trial A

Two years after the cultivation started, the TP's were found to be in the same range as the initial values, or lower, no leachate inhibitory effects were found and the nematode analysis indicated improved ecological conditions. The IREs of 2012 were all lower, or in the same magnitude, as the ones obtained in 2010, prior cultivation (Table 3, for equations, see Eq. S5 – Eq. S11 in the Electronic Supplementary Material). Consequently, the ecological risks were judged to be unchanged or even reduced at the site. However, the obtained IREs, especially from the sampling before cultivation, are associated with rather high deviations indicating uncertainties of the results and that the three LoEs point at somewhat different risk levels. In the TRIAD procedure, the obtained results have to be related to corresponding results derived from an uncontaminated reference soil (here A7-ref). The reference soil should be similar to the other investigated soil samples, concerning e.g. soil type, properties and vegetation. A7-ref had no elevated concentrations of the studied metals, and the organic matter content was comparable to the other studied samples (Table 2). Even though the fraction <2 mm was slightly higher in A7-ref (Table 2), the soil conditions were still more or less comparable, and hence, the available soil information cannot be used to explain the deviation found in the ecological LoE. Both the toxicological and the ecological LoEs were based on only one test: the Microtox and the nematode test. Ideally, more tests could be used to improve the judgements and lower the uncertainty of the risk assessment. Furthermore, the lack of toxic effects in the here performed Microtox analyses can in some ways be regarded as unfortunate, since these results precluded the possibility to see any differences in toxicity to terrestrial biota, which maybe a more sensitive test method would have allowed. Other indicators, such as extractability and

Table 4 Risk quotients for cows and sheep based the maximum metal concentration (in d.w.) found in *Salix* leaves and twigs

	R(cows) *Salix* leaves	*R*(cows) *Salix* twig	*R*(sheep) *Salix* leaves	*R*(sheep) *Salix* twig	NOEC[a] (mg/kg bw/d)	ADI[b] (mg/d)
Cu	0.26	0.15	0.70	0.40	0.67	20
Pb	0.06	0.01	–	–	0.6	–
Zn	0.41	0.11	**3.0**[c]/**2.3**[d]/1.0[e]	**1.2**[c]/1.0[d]/0.5[e]	37	410

[a] No observed effect concentration (mg/kg body weight/day) (Ma et al. 2001)

[b] Acceptable daily intake (mg/day) (NRC 2005)

[c] *R* for Zn, based on maximum leave or twig concentration found at the two brownfields (Zn_{leaves}=907 mg/kg and Zn_{twigs}=350 mg/kg dw)

[d] *R* for Zn, based on the mean concentration in *Salix Klara*, field trial B (Zn_{leaves}=690 mg/kg dw and Zn_{twig}= 310 mg/kg dw; mean of all sampling occasions)

[e] *R* for Zn, based on the mean concentration in *Salix Inger*, field trial A (Zn_{leaves}=309 mg/kg dw; Zn_{twig}= 158 mg/kg dw)

additional bioindicators, have been suggested recently by Kumpiene et al. (2014). Extractability tests (leaching tests) were also performed here. According to those, the leachability in all squares of field trial A was very low (close to or below the detection limit) and consequently in agreement with the TRIAD results.

4.2 Risks to grazing animals

Reported values of *BCF* for bioenergy crops such as willow are generally low. There are, however, a large number of species and hybrids of willow, and the variability of uptake is varying both dependent on clone and on type of contaminants (Landberg and Greger 1996; Pulford and Watson 2003; Van Slycken et al. 2013). High metal uptake by plants could possibly affect herbivores, and in the study by Ohlson and Staaland (2001), high concentrations of e.g. Cd (9 ppm in twigs) in naturally grown willow in Norway have been reported to cause toxic metal intake by moose. Our results showed negligible accumulations of metals in both leaves and twigs of both field trials, except for Zn (Table S5, in the Electronic Supplementary Material) indicating a low risk to grazers. Calculated *R*-values using maximum concentrations found in willow leaves and twigs also confirmed this (Table 4). Based on this worst-case scenario (using maximum concentrations), it was concluded that it is safe for bigger grazing animals to graze on willow leaves and twigs; *R*-values for all studied metals were well below 1. For smaller animals, the model indicates that there may be a risk of too high concentrations of Zn (risk quotients for sheep=3.0 (leaves) and 1.2 (twigs)). However, recalculation using mean values obtained for *Salix Inger* reduced the risk quotients to 1.0 (leave) and 0.5 (twigs) for field trial A. The same recalculation for *Salix Klara*, grown on field trial B, reduced the *R*-value to 1.0 for twigs but still indicated a risk for smaller animals when consuming leaves as their main food source (*R*=2.3). It is unlikely, though, that grazing animals would have willow leaves as their single food source all year around (leaves will be shred during the autumn), and hence, this risk quotient is most likely overrated.

Table 5 Estimated developed biomass of the studied sample squares, as means, for field trial A

Filed trial A		
Sample square	TP[a]	Biomass (tonne dw)/ha
A7-ref	0.04	23
A9	0.09	11
A5	0.34	9.4
A2	0.64	0.75
Mean (*n*=4)		11±9

The toxic pressure (TP) of the squares of field trial A is shown for comparison

[a] TP=calculated from total content of COPECs measured in the second TRIAD analysis (2010)

In addition, the concentrations in the vegetation above ground can be expected to decrease with time (as the biomass yield/year increases), which would reduce the risk even further. Pulford et al. (2002), for example, reported, based on 20 varieties of willow, decreased or similar metal concentrations in bark and wood in the second year of harvest compared to the first. The risk for spreading of soil contaminants, the uptake into soil organisms and the plant uptake depend on the mobility and bioavailability. The reduction of Pb in the porewater during the studied time frame (18 and 24 months for A2 and A5, respectively) indicates a reduced availability of this metal and possible sorption/containment of Pb to the willow roots. This indicates that the subsequent plant uptake and thereby the risk for grazers, as well as other organisms, may decrease with time. However, the sorption/containment cannot be verified since metal accumulation to roots was not included in the study.

To summarise, the higher *BCF*- and *R*-values for Zn found for field trial B (*Salix Klara*) compared to A (*Salix Inger*) clearly show the importance of keeping in mind that different willow species have considerable different metal uptake capabilities, and species with low uptake to shoots and twigs, but with metal accumulation in the roots, are the most suitable ones in an ecological point of view for growing on brownfield sites.

4.3 Potential effects on biomass growth

4.3.1 Field trial A—effects of contaminants on growth?

The biomass yield increased with decreasing TP (Table 5). However, the C_{pw} of COPECs found at field trial A (measured in the root zone) were low compared to reported toxic concentrations to Salix. For example, Kuzovkina et al. (2004) showed that growth and transpiration of different willow species were not decreased by 5 μM Cu solutions (which are approx. 25 times higher concentration than the highest C_{pw} found in our experiments). Salix tolerance to Pb is less investigated than tolerance to e.g. Cd, Cu and Zn. Studies by Zhivotovsky et al. have, however, reported high degree of tolerance for several willow varieties in both hydroponic screening tests (Zhivotovsky et al. 2011a) and pot/field experiments (Zhivotovsky et al. 2011b) and their smallest reported EC_{50} threshold value (measured on above ground tissue, using nutrient film technique) was more than three orders of magnitude larger than the maximum C_{pw} of Pb recorded in A2 during the field trial (EC_{50}=91.3 μM compared to $C_{max,pw}$= 0.013 μM=2.6 μg/L). Furthermore, the C_{pw} of Zn in A2 was not different from the concentrations recorded in the other squares with very good biomass production. Consequently, it seems very unlikely that the metal concentrations are the single or main cause to the suppressed biomass growth of A2. The much lower production in the most contaminated square (A2) is more likely a result of weeding problems and the nature of the soil, as the soil at this location was very compact, containing bricks and rocks (slag lumps). In addition, the soil-water content of A2 was consequently lower compared to the other squares. No correlation was found between the growth and soil nutrients or pH.

4.3.2 Field trial B—effects of sludge on growth?

The growth of *Salix Klara* at field trial B was independent of whether sludge was added or not. In several other studies (Simon et al. 1991; Labrecque et al. 1997; Labrecque and Teodorescu 2001), the addition of sludge significantly increased the growth of willows, although genotypic variation exists (Simon et al. 1991). However, in the study by Cogliastro et al. (2001), the growth of willow did not vary with sludge quantity. When the field trial B was visually examined, it was discovered that the areas with lower growth either were sandier or contained more (heavy textured) bark residues than the soils with higher growth. Thus, it seems that the growth rate depends more on the texture in soil. This is in agreement with Labrecque and Teodorescu (2001) and Stolarski et al. (2011) who found that sandy and heavy textured silt soil was a limiting factor for growth.

Nevertheless, field trial B demonstrates that cultivation can be made with limited resources and little management efforts (no irrigation and no weeding at all) on brownfields located at rather northern latitudes. However, due to climate effects, the growth rate will be considerably slower, and frost tolerant clones must be chosen, like the one here tested (*Salix Klara*).

4.4 Potential revenues and ecosystem services provided by phytostabilisation

The net income from cultivation of biomass on brownfield areas depends on the produced amount, but also on how the product is regarded. A net gross income is provided if the product is regarded as biofuel that can be used for energy production. However, a net gross cost is provided if the product is regarded as waste or if the crop is left at the site (Bardos et al. 2011). The low concentrations of metals found in the biomass of both *Salix Inger* and *Salix Klara* prove that these willow clones grown on moderate to low contaminated brownfields can be used for energy production without further restriction. The production of biomass was high enough to provide revenue even under the harsh conditions investigated here. In more nutrient-rich and warmer climate areas, reported revenues are even higher (DEFRA 2007; Milovanović et al. 2011). Our field trials were accomplished using no, or low, amounts of amendments, minimum soil interventions, no, or very simple, weeding control (i.e. the use of permeable geotextile) and conducted at sites with low annual temperature. However, to achieve revenues, the market must be open. Sweden has a fully commercial biofuel market, a well-developed infrastructure for utilisation of bioenergy from various sources (Andersson-Sköld et al. 2009), and interviews with producers of biofuel in the regions showed that there is an interest of purchasing biocrops from brownfields, as long as there is no risk for high contaminant concentrations.

During cultivation, other uses of the land will not be possible. Other land uses than cultivation could provide higher revenues, depending on the site-specific context. Both trial areas in this study are, however, rather remote, and there are no exploitation pressures. Other uses may demand faster remediation. In Sweden, the remediation extent depends on the criteria for the planned land use: sensitive land use (E_{KM}), i.e. there are no land use limitations or restrictions, and less sensitive land use (E_{MKM}) with land use restrictions for uses such as offices, roads and industries (E_{KM} and E_{MKM} criteria for individual metals can be found in Table 2). The remediation costs would include costs for machineries (e.g. excavators) and landfill costs, or other equipment depending of remediation method. Moreover, compared to conventional remediation options, phytostabilisation is reported to be a technique with very low, or no, environmental impacts, due to less transportation and less use of land and energy resources (Suer and Andersson-Sköld 2011). Hence, the cultivations can be seen as both ecological and economical sustainable options to conventional risk management of these sites. In addition, several wider benefits and ecosystem services, such as contribution to increased biodiversity, water-holding capacity, carbon sequestration and other soil improvements, are expected to be gained during the continuing growth (Bardos et al. 2011; Suer and Andersson-Sköld 2011; Volchko et al. 2013). Furthermore, the cultivation can also contribute to perceived increased aesthetical value (Andersson-Sköld et al. 2009; Andersson-Sköld et al. 2015) due to the increased greenery in the area. There are a growing demand of all these ecosystem services and an increasing awareness of their importance to human well fare; however, loss of biodiversity and degradation of ecosystems still continue on a large scale (TEEB 2010; Haines-Young and Potschin 2013). Fundamental changes are needed in the way land use, ecosystems and their services are viewed and valued by society. Our study shows that using bioenergy crops for phytostabilisation on brownfields can contribute to preserve and improve ecosystem services and at the same time be a sustainable risk management option.

5 Conclusions

This study proves that abandoned, moderately contaminated, industrial land can safely be used for bioenergy crop production by the use of phytostabilisation willow clones *(Salix Klara* and *Inger)*. The uptake in the plants was, as aimed for, low to moderate, and the growth rate depended on the soil texture rather than the contamination level of these sites.

The low uptake of contaminants into biota indicated a negligible risk for grazing animals. Furthermore, the ecological risk assessment, made for field trial A, indicated lower risks after 2 years of cultivation and over the longer term, the risks are expected to continue to decrease as a result of the way that cultivation improves the soil quality. This was also confirmed by the reduced availability of the contaminants in the porewater of this site. The biomass production was high enough to provide revenue, despite harsh conditions. Hence, this study shows that phytostabilisation with bioenergy-crops on brownfields has, besides the environmental benefits, potential to turn these areas from uneconomical into beneficial ones.

It should be kept in mind though that only for willow, there are several hundred species and hybrids and the crops capability of uptake depends both on clone and type of contaminants. Hence, in order to succeed, a relevant crop, which meets all of the site's objectives (including phytostabilisation, soil improvement and potential use of biomass), must be selected.

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