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Does the origin of cuttings influence yield and phytoextraction potential of willow in a contaminated soil?

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ABSTRACT

Plant survival and growth are essential parameters in monitoring the effectiveness of phytoremediation protocols for brownfields decontamination. The ability of different willow cultivars to survive and adapt to contamination in polluted soils can vary according to the nature and concentration of the contaminants. The objectives of this study were to identify which cultivars had the highest yields, and to determine whether the origin of the cuttings influences survival, growth and phytoextraction potential of willow cultivars when grown on polluted soil. Survival, growth and phytoextraction of trace elements (Cd, Cr, Cu, Ni, Pb, Zn) were compared for ten willow cultivars planted in a highly organic-contaminated industrial environment versus in uncontaminated soil. Variations in cultivar response according to cutting origin (contaminated site or not) were also analysed for each site. Results show that, *Salix nigra* '5005' was very productive in an extremely contaminated environment. *Salix eriocephala* 'S25' also performed exceptionally well, as it produced large root biomass (nearly twice that the other cultivars). The highest concentration of cadmium was found in *S. x dasyclados* 'SV1'; cultivars *S. purpurea x S. miyabeana* 'Millbrook' and *S. eriocephala* 'S25' exhibited the highest concentrations of chromium. Cultivar 'S25' was also the most effective at concentrating copper, and *S. miyabeana* 'SX67' was the most efficient at phytoextracting zinc. The origin of the cuttings did not have a significant influence on survival, growth or phytoextraction, except for 'Millbrook': individuals originating from the contaminated environment produced significantly more aboveground biomass when grown in a contaminated environment, compared to individuals who had never been in contact with contaminants. These results could help build more effective phytoremediation protocols for highly contaminated environments by identifying willow cultivars and cutting selection techniques appropriate for specific environments.

1. Introduction

Brownfields are land on which soil is highly contaminated due to past industrial activities and waste. Although brownfields are common in urban areas, they often remain vacant due to land-use regulations. Implementation of large-scale phytoremediation projects can represent an advantageous, low-cost method for rehabilitating such land. Willows are considered ideal candidates for use in phytoremediation due to their physiological characteristics: rapid establishment (Greger and Landberg, 1999), high transpiration rate (Trapp and Karlson, 2001), high tolerance of pollutants (Kuzovkina and Volk, 2009) and ability to resprout following coppicing (Labrecque and Teodorescu, 2003; Labrecque and Teodorescu, 2005). Willows are also a valuable perennial species for growers, producing biomass over several growing seasons (Pitre et al., 2010).

It is well known that long-term exposure to contaminants can affect the genome of plants, including genotoxic effects and DNA alteration (Labra et al., 2004; Ünyayar et al., 2006; Seth et al., 2012). This effect on genome might affect plant phenotype, but, to our knowledge, the physiological response of willows growing in highly contaminated environments is poorly documented. Willow survival and growth in some types of contaminated soils have been assessed (Vyslouzilova et al., 2003; Guidi Nissim et al., 2013; Grenier et al., 2015), but crucial information about willow physiology in such extreme environments is still lacking. For example, we do not know which willow cultivars have better survival, growth and phytoextraction rates in highly contaminated brownfields. The adaptive potential of plants was studied in mining environments in the mid-20th century, a period during which mineral exploitation increased considerably (Gregory and Bradshaw, 1965; Wu and Bradshaw, 1972; Antonovics, 1975). These studies showed that some herbaceous plants could adapt to inorganic contaminants in mining environments heavily contaminated with trace elements. Plants that adapted to the contaminated environment became more productive and tolerant in the presence of contaminants,

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compared to ”natural” individuals (*i.e.* that were never in contact with the contaminant). This suggests that phenotypic plasticity might be an important mechanism for survival (Bradshaw, 1965). More recently, several studies have highlighted the role of epigenetics, a mechanism of transgenerational inheritance (Becker and Weigel, 2012), in plant response to stress (Gutzat and Scheid, 2012). Thus, the origin of planting material (whether it originates from contaminated land or not) might have direct consequences on plant traits (*e.g.* biomass, phytoextraction). Regardless of the cause of plant stress adaptation, from the perspective of phytoremediation, it would be useful to know whether willows growing in contaminated environments can display such tolerance. This could become an additional criterion for evaluating which cultivars are the most efficient for extracting, degrading, and immobilizing contaminants.

Different willow cultivars can be used for phytoremediation purposes (Tharakan et al., 2005). Cultivars originating from *Salix miyabeana* ('SX61', 'SX64' and 'SX67') (Labrecque and Teodorescu, 2005; Tharakan et al., 2005; Grenier et al., 2015) produced high amounts of biomass in non-contaminated soils in various areas of southern Quebec. In a moderately contaminated environment, 'SX61' maintained high biomass yields, but 'SX67' seemed more affected by contaminants. *S.nigra* '5005' was not as productive as the *S. miyabeana* cultivars, but remains a potential candidate for use in phytoremediation, because it produces almost equal amounts of biomass whether planted in healthy or moderately contaminated environments (Grenier et al., 2015).

Several studies have compared the performance of several willow cultivars growing in moderately contaminated soil and the consequences of this stressful habitat on their physiology. For example, *Salix alba* can easily tolerate up to 1000 mg/kg of diesel in a hydroponic environment without a noticeable decline in water transpiration rate (Trapp et al., 2001). It has also been shown that willows can tolerate up to 7.5 mg/L of chromium (Yu and Gu, 2007) and more than 100 μM of cadmium in a hydroponic environment (Cosio et al., 2006). Despite the fact that willows are highly tolerant to contamination, there is a limit beyond which toxic effects can be observed. Leaf necrosis (Trapp et al., 2001; Cosio et al., 2006; Bialowiec and Randerson, 2010), slower growth (Trapp et al., 2000), decreased transpiration rate (Larsen et al., 2005; Ucisik and Trapp, 2007), leaf chlorosis and even death (Yu et al., 2005) have been observed under extreme conditions.

In the present study, we assessed biomass production, survival rate, and phytoextraction capabilities in regard to six trace elements (Cd, Cr, Cu, Ni, Pb, and Zn) in ten willow cultivars originating from a highly contaminated site (mixed contamination) previously occupied by a petrochemical plant, and compared their performance to that of individuals of the same cultivars originating from a non-contaminated environment. For willows growing on the contaminated site, we expected higher biomass production, survival rates and phytoextraction capabilities from cuttings originating from contaminated soil. Among the selected cultivars, we expected 'SX61' and '5005' (formerly known as S05; *Salix nigra*) to display the highest survival rate and biomass yield in the contaminated environment, since this pattern was previously observed in a slightly contaminated environment *(*Labrecque and Teodorescu 2005; Tharakan et al., 2005; Grenier et al., 2015*)*.

2. Materials and methods

2.1. Study site

The study site is located south of Montreal, Québec, Canada (45.69 N, −73.43 W), along the St. Lawrence River. On average, the area receives 954 mm of precipitation annually (*i.e.* 167 mm of snow and 767 mm of rain) (Guidi Nissim et al., 2013) and the average annual temperature is 6.4 °C (Labrecque and Teodorescu, 2005). The experiment was set up on the site where Pétromont and Co. LP. previously engaged in primary petrochemical activities as a major producer of low-density polyethylene in Canada. The plant was shut down in 2008 and the site was converted to experimental soil rehabilitation activities.

Table 1
Soil characteristics of the contaminated and uncontaminated sites.

Organic contaminants	Soil origin		
	Contaminated	Non-contaminated	Units
PAHs			
Total	572.73 ± 1065.21	< 0.20	mg/kg
HPs			
C10-C50	5792.00 ± 7539.12	< 100.00	mg/kg

Trace elements	Soil origin		
	Contaminated	Non-contaminated	Units
Cd	0.65 ± 0.01	0.0020 ± 0.0004	mg/kg
Cr	135.35 ± 37.56	0.45 ± 0.04	
Cu	53.10 ± 32.88	0.43 ± 0.04	
Ni	65.80 ± 4.42	0.12 ± 0.01	
Pb	19.30 ± 14.47	0.010 ± 0.001	
Zn	133.90 ± 59.41	0.06 ± 0.02	

Soil properties	Soil origin		
	Contaminated	Non-contaminated	Units
Cation exchange capacity	29.77	24.60	$cmol_c$/kg
pH	7.43	6.65	
Organic matter content	5.65	6.10	%

Modified from: Bell et al. (2014); F. Courchesne (personal communication); Desjardins et al. (2014); Grenier et al. (2015); Cloutier-Hurteau et al. (2014).

The decantation basins (45mx35m) used by Pétromont and Co. LP. to hold wastewater generated during petrochemical activities were emptied in 2008, and the bottom of one of these basins, composed of industrial waste, was used for this experiment. Several studies have described this site previously (Desjardins et al., 2014; Grenier et al., 2015). Briefly, this soil contains a mixture of contaminants including polycyclic aromatic hydrocarbons (PAHs), petroleum hydrocarbon (HPs), oils, and many trace elements (Table 1). Despite these extremely contaminated conditions, a native vegetation cover has been able to establish in parts of the basins (Desjardins et al., 2014) and a willow plantation was set up there by our research group in 2011 (*unpublished results*).

2.2. Experimental design layout

In May 2014, a reciprocal transplant experiment was established on the former petrochemical site, using ten willow cultivars (Table 2). The cultivars selected for the study encompassed a large phylogenetic (Lauron-Moreau et al., 2015) and functional diversity (Grenier et al., 2015).

Table 2
Willow cultivars used in the project.

Name	Species	Origin	Characteristics
SX67	*Salix miyabeana*	Asia	High biomass production
SX61	*Salix miyabeana*	Asia	
5069	*Salix acutifolia*	Europe	
5044	*Salix alba*	Europe	
5027	*Salix viminalis*	Europe	
Fish Creek	*Salix purpurea*	North America	
Millbrook	*Salix purpurea* X *Salix miyabeana*	Hybrid	
5005	*Salix nigra*	Canada	Indigenous species
S25	*Salix eriocephala*	Canada	
SV1	*Salix dasyclados*	Canada	