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Novel method to determine element concentrations in foliage of poplar and willow cuttings

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ABSTRACT

Measuring the uptake of the chemical elements by plants usually requires the destructive harvest of the plants. Analyzing individual leaves is unsatisfactory because their elemental concentration depends on their age and position on the branch or stem. We aimed to find an easy method to determine the elemental concentrations using a few suitable single leaves along the main shoot of poplar (*Populus monviso*) and willow (*Salix viminalis*) cuttings at the end of the first season. Using Ca, Cd, Mn, Fe, K, P, Pb, and Zn concentrations, measured in selected leaves along the main shoots of the cuttings, mathematical functions were derived, which described best their distribution. Elemental allocation patterns were independent of the soil characteristics and soil element concentrations. Based on these functions, three leaves from specific positions along the main shoot were selected, which could accurately describe the derived functions. The deviation of the calculated average concentration, based on the 3-leaves method, was ≤15% in approximately 65% of the cases compared to the measured concentration. This method could be used to calculate element concentrations and fluxes in phytomanagement, biomonitoring, or biomass productions projects using one-season poplar or willow cuttings.

KEYWORDS
allocation; plant uptake; macro- and micronutrients; trace elements

Introduction

Poplar and willow are planted worldwide for soil conservation, riverbank protection, timber, bioenergy, supplementary stock fodder, and for the phytomanagement of contaminated sites Isebrands and Richardson (2014). Poplar and willow are used because they have a high biomass production (Klasnja, Kopitovic and Orlovic 2002; Evangelou *et al.* 2012), are easily propagated, establish rapidly, can be coppiced (Robinson *et al.* 2009) and accumulate trace elements (TEs), especially Cd and Zn (Dickinson and Pulford 2005; Unterbrunner *et al.* 2007; Dickinson *et al.* 2009). For these reasons, as well as their indeterminate growth, poplar and willow are also preferred experimental tree species for short (one season) experiments.

Knowledge on the elemental uptake by poplars and willows is critical when assessing their health, the likely nutritive value when used as stock fodder, and the ecological risks posed by them when planted on contaminated sites. The elemental uptake in the foliage is determined by harvesting the entire foliage per plant or a representative aliquot (Robinson *et al.* 2005). For the former, the elemental determination can only be performed at the end of the experiment. In this case, however, a determination of element fluxes during the growth period is not possible. For the latter, achieving a representative aliquot, could mean harvesting a great number of leaves which in turn could interfere with the growth of the cutting and thus alter the outcome of the experiment. Thus, to reduce the number of harvested leaves, one should harvest representative leaves. The best election of representative leaves along the main shoot of young woody plants with indeterminate growth is however not clear, because after allocation of the elements by the transpiration stream, later dislocations differ by the element for the transfer to younger leaves, to senescing leaves or to other plant parts (Laureysens *et al.* 2004; Vollenweider, Menard, and Günthardt-Goerg 2011). In addition, these processes change during the season (Robinson *et al.* 2005). Furthermore, knowledge to which extent the foliar element concentration depends on the soil type is scarce.

We aimed to determine the distribution patterns of leaf Ca, Cd, Mn, Fe, K, P, Pb, and Zn concentrations along the main shoots of poplar, *Populus monviso* (mother: *P. deltoides* 583 [Iowa, USA] x *P. trichocarpa* 196 [Oregon, U.S.A.], father: *P. nigra* 715–86 [Piemonte, Italy] *x P. nigra* 7 [Umbria, Italy]) and willow (*Salix viminalis* L.) cuttings grown on six soils with different TE contamination (four naturally TE-contaminated soils, a TE-spiked soil and an uncontaminated control soil). Specifically, we sought to a) determine the spatial distribution of the above-mentioned elements; b) determine its dependence from the soil type and c) establish a simple functional

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relationship between the minimum number of samples leaves needed to calculate the average element concentration of a cutting and the measured average element concentration.

Material and methods

Soils

The soils used in this study were allocated at four contaminated sites in Switzerland: Allmend (47°01′48″N, 8°18′30″E), Losone (46°10′21″N, 8°44′46″E), Dornach (47°28′36″N, 7°36′36″E) and Witzwil (46°58′60″N, 7°2′60″E). In addition we used a loamy soil from the area of Brugg that had been artificially contaminated with dust from a brass smelter and used in a previous lysimeter experiment at WSL Birmensdorf (Switzerland) (Menon *et al.* 2005), and an uncontaminated substrate (technosol) for control. It consisted of peat bark humus and clay pot mixture, as it is regularly used for growing seedlings in the tree nursery of WSL (47°21′40.53"N; 8°27′21.82"E). The chemical and physical properties of the soils are reported in Evangelou *et al.* (2013).

Pot experiment

Pot experiments were conducted from May to September in the WSL tree nursery at Birmensdorf (47°21′48″ N, 8°27′23″ E, 545 m a.s.l.) under ambient climate conditions. The average (+standard deviation) monthly temperature during the experiment was 13.1 (2.6) °C and the average solar radiation was 4.8 (0.8) $kWh/m^2/d$. Ten-L plastic pots with six small bottom holes each were filled with approximately 9 kg air-dried soil that had been sieved to <1 cm grain size fraction. A tray was placed under each pot to collect any leachate, which was recycled to ensure that there was no loss of macronutrients or TE from the pots. The soils were fertilized using Osmocote 6 M at rates recommended by the manufacturer, resulting in the addition of 500 mg N, 100 mg P and 200 mg K kg^{-1} of soil. Pots were irrigated to field capacity with tap water 3–4 times per week. For each soil type, three replicate pots were planted with one willow (*Salix viminalis*) cutting and three replicates with one poplar (*Populus monviso*) cutting per pot. The poplar and willow were planted as un-rooted cuttings of approximately 300 mm length (dry weight of 20.1 ± 2.5 g and 9.5 ± 1.5 g) respectively. All cuttings successfully established, although some cuttings in soils from Brugg and Dornach showed severe chlorosis and necrosis. The pots were positioned in a completely randomized design. Every 2–3 weeks the length of the main shoot was determined. There were no visible signs of pathogen activity during the experimental period.

Leaf harvest, biomass and analysis

After 4 months of growth, first every fifth leaf of every tree was collected starting with the 1st leaf at the bottom of the main shoot and the last grown leaf at the top, approximately 6–8 leaves for poplar and 11–18 leaves for willow. Afterwards all remaining leaves were harvested and pooled. Stems were not analyzed because their TE concentration is small compared to stems (Marmiroli *et al.* 2012).

Analysis of individual leaves followed the method of Gramlich *et al.* (2011). All leaves were subsequently washed with tap and deionized water and were dried at 60°C until a constant weight was obtained. Leaves with a weight > 0.2 g, were cut longitudinally along the main vein. Each leaf or leaf portion was crushed using a mortar and pestle and approximately 0.2 g, was digested in 15 mL of HNO_3 (65%), at 120°C for 1 h using a digestion block (DigiPREP MS, SCP-Science), and diluted to 25 mL with Millipore™ H_2O. The extracts were analyzed using ICP-OES (Varian, Vista-MPX CCS simultaneous). The remaining dried pooled leaves of the main shoot were ground using a Retsch ZM-200 centrifugal titanium mill. Pooled leaf samples had sufficient biomass for X-ray fluorescence (XRF) analyses following the method of Marmiroli *et al.* (2012). TEs were determined using a Spectro X-lab 2000 (Germany) XRF. For quality assurance, we analyzed certified plant reference material (Virginia tobacco leaves CTA-VTL-2, Polish Reference Material). Recoveries were for Ca, Cd, Mn, Fe, K, P, Pb, and Zn were within 10% of their certified values.

Mathematical calculation of average concentration

Based on the elemental concentrations of the individually analyzed leaves (every 5th leaf from bottom to top of the cutting) along the main shoot, we selected function $f(x)$ (e.g., logarithmic, exponential etc.), which gave the best description of the distribution using the least square method (Wolberg 2005). For example, for uptake of Zn in *Salix viminalis* the model function was $f(x) = a{\cdot}e^{bx}$, where a,b are parameters. These functions are given in Table 1. The average elemental concentration of the main shoot of the cutting was estimated by utilizing $f(x)$ in the following way (1):

$$c_e = \frac{1}{i-1}\int_{i_b}^{i_t} f(x)dx \qquad (1)$$

where c_e, is the total concentration of element e. Counting every fifth leaf from bottom to top, i_b is the count of the first leaf ($i_b =$ 1) and i_t is the count of the last leaf. Thereby $f(x)$ was parameterized by means of the least square method using every fifth leaf.

To validate our method, the calculated average elemental concentration was compared to the average elemental concentration (mean value from the every 5th leaf) as well as to the pooled foliage. We then selected just three leaves to determine the parameters for the type of distribution function $f(x)$. We denote this newly fitted function as $f(x)_{3\ \mathrm{leaves}}$. To identify the required three leaves, the main shoot was divided into five equal parts. A leaf must be harvested at the bottom of the main shoot of the cutting, at the top as well as from the top of the bottom 6th (see graphic abstract). This is the case for all determined elements except for P, where a leaf must be harvested at the bottom of the main shoot of the cutting, at the top as well as from the bottom of the upper 6th. Again, the foliage concentration was determined using equation (1) but substituted $f(x)_{3\text{-}\mathrm{leaves}}$ for $f(x)$. For the method to work accurately, it is important that $f(x)_{3\text{-}\mathrm{leaves}}$ is nearly equal to $f(x)$. This similarity depends on the position of the three specific leaves. To determine the position with the largest similarity, we tested all 3-leaves combinations and compared the resulting functions.

For application in the field, the method can be simplified: instead of counting the leaves of the tree, it is possible to also use its height. Then equation (1) is adapted as follows:

$$c_e = \frac{1}{h}\int_b^t f(x)\,dx \qquad (2)$$

where c_e, is the total concentration in the main shoot of the cutting of the element e and where b is the height of the bottom leaf in cm, t is the height of the top leaf of the main shoot of the cutting in cm and $h = t\text{-}b$.

Results

Mathematical functions of element allocation

Independent of the soil, each tree species showed a unique distribution function of the leaf concentration of each element along the main shoot of the cutting (Table 1, Figure 1). For poplar, the element allocation was not influenced by the soil substrate, but it was specific for individual elements. For willow, Zn allocation followed a polynomial function (which was approximated by an exponential

Table 1 Element distribution function, calculated (3-leaves method) and XRF determined average element concentration (mg kg^{-1}), (n = 3 ± SD).

Element	Cutting		Soils: Allmend	Dornach	Losone	Witzwil	Technosol
Zn	willow	Function	$y = a e^{-bx}$	$y = a e^{-bx}$	$y = a e^{-bx}$	$y = a e^{-bx}$	$y = a e^{-bx}$
		Calc. conc.	515 ± 80.1[20]	797 ± 130[30]	278 ± 7.2[5]	414 ± 26[30]	107 ± 22.0[15]
		XRF conc	615 ± 81.1	1290 ± 125	268 ± 8.1	565 ± 7.2	127 ± 13.1
	poplar	Function	$y = a e^{-bx}$	$y = a e^{-bx}$	$y = a e^{-bx}$	$y = a e^{-bx}$	$y = a e^{-bx}$
		Calc. conc.	255 ± 33.3[5]	385 ± 15.3[5]	120 ± 7.94[30]	320 ± 8.42[15]	56.7 ± 4.79[15]
		XRF conc	255 ± 46.4	376 ± 34.1	93.2 ± 14.1	275 ± 39.9	50.7 ± 2.32
Pb	willow	Function	$y = a x^{-b}$	n.d.	n.d.	n.d.	n.d.
		Calc. conc.	46.8 ± 8.08[10]				
		XRF conc	51.2 ± 2.95	b.d	b.d.	b.d.	b.d.
	poplar	Function	$y = -a \ln(x) + b$	n.d.	n.d.	n.d.	n.d.
		Calc. conc.	(29.5)				
		XRF conc	31.6 ± 1.1	b.d.	b.d.	b.d.	b.d.
Cd	willow	Function	n.d.	$y = -a x + b$	n.d.	n.d.	n.d.
		Calc. conc.		16.9 ± 2.12[5]			
		XRF conc	6.77 ± 0.85	16.4 ± 2.95	3.07 ± 0.65	3.70 ± 0.14	b.d.
	poplar	Function	n.d.	n.d.	n.d.	n.d.	n.d.
		Calc. conc.					
		XRF conc	b.d.	b.d.	b.d.	b.d.	b.d.
P	willow	Function	$y = a e^{bx}$	$y = a e^{bx}$	$y = a e^{bx}$	$y = a e^{bx}$	n.d.
		Calc. conc.	1410 ± 230[15]	2350 ± 280[5]	1480 ± 100[10]	1980 ± 290[10]	
		XRF conc	1600 ± 380	2450 ± 270	1580 ± 140	2200 ± 400	2400 ± 140
	poplar	Function	$y = a e^{bx}$	$y = a e^{bx}$	$y = a e^{bx}$	$y = a e^{bx}$	n.d.
		Calc. conc.	3800 ± 120[30]	2300 ± 100[20]	1550 ± 130[30]	3500 ± 450[30]	
		XRF conc	2320 ± 270	1950 ± 120	2210 ± 110	2300 ± 180	3100 ± 110
Fe	willow	Function	$y = a x^{-b}$	n.d.	$y = a x^{-b}$	$y = a x^{-b}$	$y = a x^{-b}$
		Calc. conc.	76.0 ± 7.8[10]		95.6 ± 6.11[10]	69.8 ± 5.62[5]	63.9 ± 4.82[15]
		XRF conc	69.7 ± 4.2	65.7 ± 29.6	86.9 ± 5.22	72.4 ± 7.31	74.7 ± 11.8
	poplar	Function	$y = a x^{-b}$	$y = a x^{-b}$	$y = a x^{-b}$	$y = a x^{-b}$	$y = a x^{-b}$
		Calc. conc.	58.8 ± 0.42[5]	57.8 ± 2.21[30]	52.3 ± 1.63[5]	55.1 ± 4.82[10]	48.1 ± 1.73[5]
		XRF conc	57.2 ± 1.82	42.3 ± 4.22	49.9 ± 0.64	50.3 ± 4.22	50.2 ± 7.64
Mn	willow	Function	$y = a x^{-b}$	$y = a x^{-b}$	$y = a x^{-b}$	$y = a x^{-b}$	$y = a x^{-b}$
		Calc. conc.	142 ± 17.9[15]	90 ± 11.1[30]	139 ± 12.1[5]	148 ± 46.7[5]	124 ± 17.3[20]
		XRF conc	160 ± 3.6	128 ± 12.1	139 ± 13.9	146 ± 17.9	156 ± 8.92
	poplar	Function	$y = a e^{-bx}$	$y = a e^{-bx}$	$y = a e^{-bx}$	$y = a e^{-bx}$	$y = a e^{-bx}$
		Calc. conc.	95.6 ± 7.63[25]	60.5 ± 5.72[10]	65.3 ± 4.24[20]	78.4 ± 6.44[10]	110 ± 12.5[15]
		XRF conc	124 ± 10.9	75.3 ± 11.5	79.8 ± 4.53	83.7 ± 12.5	127 ± 22.1
K	willow	Function	$y = a x^{b}$	$y = a x^{b}$	$y = a x^{b}$	$y = a x^{b}$	$y = a x^{b}$
		Calc. conc.	10500 ± 1200[20]	36700 ± 2000[10]	14400 ± 200[20]	23100 ± 1500[10]	22400 ± 4100[15]
		XRF conc	13000 ± 1300	40200 ± 1900	17900 ± 370	20900 ± 2250	19700 ± 380
	poplar	Function	$y = a x^{b}$	$y = a x^{b}$	$y = a x^{b}$	$y = a x^{b}$	n.d.
		Calc. conc.	17200 ± 1450[10]	19000 ± 2000[5]	22400 ± 1100[5]	22100 ± 1100[10]	
		XRF conc	18800 ± 1500	19000 ± 2100	23300 ± 300	24300 ± 1900	21500 ± 500
Ca	willow	Function	$y = a e^{-bx}$	$y = a e^{-bx}$	$y = a e^{-bx}$	$y = a e^{-bx}$	$y = a e^{-bx}$
		Calc. conc.	22300 ± 3300[30]	47100 ± 4300[5]	18900 ± 2400[30]	22000 ± 5100[30]	17000 ± 1550[30]
		XRF conc	14700 ± 1400	46000 ± 6700	13000 ± 390	17300 ± 1140	11600 ± 1100
	poplar	Function	$y = a e^{-bx}$	$y = a e^{-bx}$	$y = a e^{-bx}$	$y = a e^{-bx}$	$y = a e^{-bx}$
		Calc. conc.	15000 ± 2200[15]	24000 ± 600[30]	18300 ± 1200[30]	15000 ± 4700[5]	13600 ± 2000[5]
		XRF conc	13400 ± 6000	19000 ± 800	14500 ± 900	14600 ± 2200	12900 ± 2000

5, 10, 15, 20, 25 $<$ 5%, 10%, 15%, 20%, 25% deviation of the calculated concentration with 3-leaf method from XRF concentration, 30 ≈30% deviation of the calculated concentration with 3-leaf method from XRF concentration

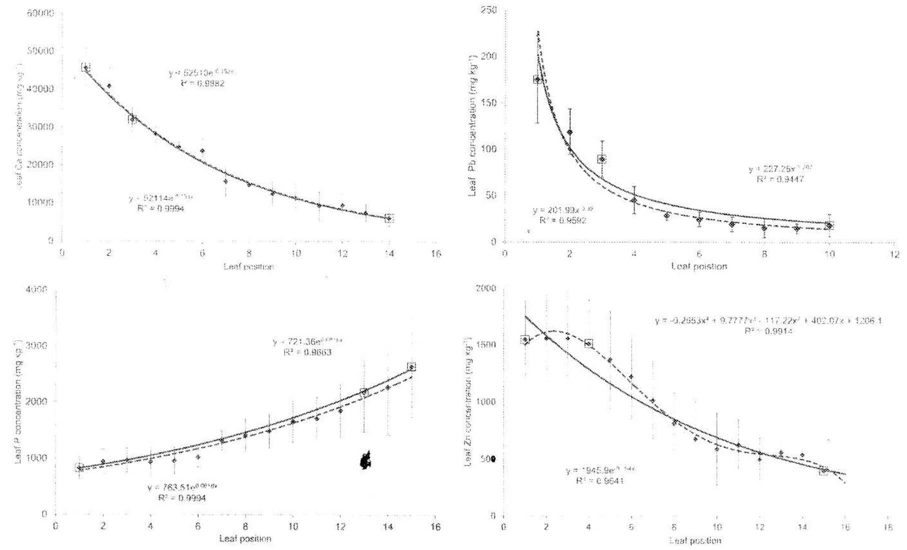

Figure 1: Examples of various element distribution functions, encountered in this study. (mean of standard deviation, n=3). The examples are taken from willow. The dashed line (—) shows the element distribution function when all concentrations of all leaves were considered; the solid line (—) shows the element distribution function when only 3-leaves were considered (3-leaves method). The square (□) shows the leaves chosen for the 3-leaves method.

function), while Ca and P followed exponential vertical distributions and Fe, K, Mn, and Pb followed power functions. The elemental allocation did not follow a specific pattern (not shown) in cutting showing symptoms of severe toxicity, such as severe leaf chlorosis and necrosis and severely reduced growth (SI Figure 1). This was the case for the poplars and willows growing on the artificially contaminated Brugg soil, and the willows growing on the Dornach soil. In the case of the Brugg, in contrast to the Dornach soil, the toxicity symptoms were so severe that the cuttings lost the majority of their leaves, so that it was impossible to derive an element distribution function. The types of distribution function differed between the two species for some elements, such as for Mn, while they were the same in for others such as P (Table 1).

Calculation of average elemental concentrations from allocation functions

Based on the fitted element and cutting specific distribution functions, we calculated the average element concentrations in the foliage of the respective cuttings. With the 3-leaves method, it was possible to calculate, in approximately 65% the cuttings, the average concentration in foliage with a deviation $\leq$15% compared to the XRF-determined concentration, and in 75% with a deviation $\leq$20% (Table 1). The error exceeded 30% in only 10% of the trees (six cases). Five of these cases were found for the elements Ca and P. In these cases, the deviation from the total concentration ranged between 31 and 39%. Zinc showed a polynomial allocation pattern, which was approximated with an exponential function, giving satisfactory results with deviation from the total foliage ranging between 2 and 24%, except for willow growing on Dornach soil, where the deviation was 38%. For poplar, the Pb allocation pattern was best approximated with a logarithmic function. In this case the deviation was 7%.

Discussion

Usability and precision of the proposed 3-leaves-method

The elemental allocation from bottom to top was specific for each tree species and each element and independent of the soils' physicochemical characteristics and element concentrations in all cases where we did not observe stress related symptoms in the plants. Thus, it is possible to predict the elemental allocation from bottom to top for the elements Ca, Cd, Mn, Fe, K, P, Pb, and Zn in young (one season) poplars and willows, with rather high reliability by sampling just three leaves at well-defined positions from the main axis of the cutting.

The main deviation of the average concentration calculated from the average concentration determined via XRF using the 3-leaves method occurred with Ca and at P for poplar. In contrast to willow leaves, the weight of poplar leaves at the bottom and the top of the cutting can be substantially different. Thus, as the proposed calculation does not include a correction for leaf weight, the error was higher for P, where two of the three leaves were collected from the top 6th of the cutting, than for the other elements Ca, Cd, Mn, Fe, Pb, and Zn.

Neither XRF, nor ICP-OES were found to be methods of choice for determining Ca concentrations, as the measurement error was large and, consequently, also the deviation between the two determination methods; XRF and 3-leaves method via ICP-OES, was large. With the exception of Ca and P, the healthier (determined by biomass and leaf chlorosis and necrosis) the cuttings (on contaminated soil) were (SI Figure 1), the smaller the deviation of the average concentration calculated via 3-leaves method was from the average concentration determined via XRF.

Limitations of the 3-leaves-method

The validity of our method needs to be validated for a wider range of conditions. At present, it is limited to the conditions covered by our pot trial with respect to a) plant species, b) growing conditions, c) TE toxicity and d) developmental stage of the plants. In the current study, only the two plant species, poplar (*Populus monviso*) and willow (*Salix viminalis*) were used, and among these plant species only two varieties from the numerous that exist. We chose poplar (*Populus monviso*) because of its higher biomass production compared to other poplar varieties (Pannacci, Bartolini, and Covarelli 2009) and willow (*Salix viminalis*) because it is the most common used willow clone (Dickinson and Pulford 2005; Cosio, Vollenweider and Keller 2006; Adler *et al.* 2008). The pot trial was conducted under homogeneous soil conditions and the sufficient supply of water as well as nutrients. Under field conditions, the distribution of growth-limiting soil resources such as nutrients and water is typically heterogeneous. Many plants can adapt to such conditions through specific responses in the development of their root systems such as precision foraging (de Kroon and Mommer 2006; McNickle, St Clair, and Cahill 2009). Although such adaptations in root foraging behavior can counteract resource limitations, restrictions still occur, which can influence the element distribution along the main shoot. Furthermore, in the field, surface deposition of windborne dust from contaminated soil can occur, which may have a strong influence on the results of our method (Laureysens *et al.* 2004; Robinson *et al.* 2008). Therefore, the allocation patterns as well as our model need further validation under field conditions.

The effect of TE toxicity and other types of stress conditions such as high salinity, diseases etc. on elemental allocation patterns have rarely been studied. One of the few exceptions is the study of (Cosio *et al.* 2006). They found that stress due to TE contamination in soil that led to visible leaf injury and growth reduction changed the TE allocation pattern. Thus, in such cases application of the 3-leaves method may not be possible.

In the current study the leaves were sampled and the element concentrations in the leaves were determined only once (after 4 months of growth). These results provided the data basis for deriving the distribution functions $f(x)$ on which we based our 3-leaves function $f(x)_{3\text{-leaves}}$. However, the concentration of elements along the main shoot of a cutting does not only depend on the position of the leaves (Cosio *et al.* 2006), but also on their age and on factors such as the time of sampling during the growing season (Laureysens *et al.* 2004; Laureysens *et al.* 2005).

The distribution of the elements along the main shoot is mainly regulated physiologically by three processes: a) the translocation in the xylem, b) re-translocation in the phloem (e.g., during senescence) and c) exchange between xylem and phloem (Marschner 1995; Laureysens *et al.* 2005). The extent to which these processes affect spatial distribution along the shoot of a plant differs among elements. The phloem mobility of Ca and Mn is low, Zn is moderate and K and P are high (Marschner 1995; Riesen and Feller 2005). Thus, over time, the foliar concentrations of elements such as Ca, Fe, Mn, and Zn tend to increase, while for elements such as K and P they tend to decrease (Vollenweider *et al.* 2011). On the other hand, Laureysens *et al.* (2005) found no substantial variation in distribution patterns over time during a growing season, indicating that the derived functions in this study may be used for a whole growing season.

Due to commonalities in tree physiology (Dreyer 1989), it is likely that the results from this study would also be applicable to other deciduous dicotyledonous plants. However, evergreen and monocotyledonous species have distinct uptake patterns (Robinson *et al.* 2009) and would require the development of a new model. Due to interspecific differences, or even differences between clones (Granel *et al.* 2002), a database of parameters is required to make this method broadly applicable.

Conclusions

The 3-leaves method for average element concentrations determination of a poplar or willow cutting gives comparable results compared to the commonly used technique of harvesting all leaves from a cutting. This method could assist researchers in determining elemental fluxes and average concentrations of a cutting over a season in pot and possibly field experiments without influencing the growth of the experimental plants. This could be used to assess plant TE-uptake in phytomanagement, biomonitoring, and biomass production projects. The results of this study were used to build a calculator been generated (trecc.ethz.ch), offering users the possibility to calculate the average concentration of their poplar or willow cutting.

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