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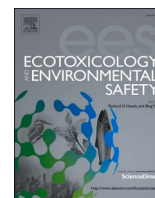
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Tolerance and trace elements extraction of *Alliaria petiolata* and *Salix aquatica grandis* under increased Cd(II) and Zn(II) exposure

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ABSTRACT

The use of accumulator species with high biomass production in phytoextraction scenarios is frequently studied, particularly in combination with treatments that increase the bioavailability of trace elements (TE) such as Cd(II) and Zn(II). In this context, this study aimed to test the potential of *Alliaria petiolata* and *Salix aquatica grandis* for phytoextraction through their tolerance and accumulation capacities in hydroponic conditions under increasing concentrations of Cd(II) or Zn(II). Both species showed high tolerance to these elements, in particular, *A. petiolata* showed few symptoms of phytotoxicity when grown under Cd(II) exposure. In addition, high maximum concentrations were observed in the leaves of *A. petiolata* (Cd(II): 332.1 mg kg⁻¹ DW; Zn(II): 1614.8 mg kg⁻¹ DW) and for *S. aquatica grandis* (Cd(II): 129.3 mg kg⁻¹ DW; Zn(II): 2227.2 mg kg⁻¹ DW). Our findings demonstrate that the extraction of Cd(II) and Zn(II) also increased with their concentrations in nutrient solution but is ultimately constrained by species-specific toxicity thresholds, highlighting an optimal range for assisted phytoextraction. The present study highlighted the potential and limitation of both *A. petiolata* and *S. aquatica grandis* for Cd(II) and Zn(II) extraction purpose when exposed to high concentrations. Field trials with these species are the next step in confirming their potential in a complex environment with environmental issues to consider.

1. Introduction

The management of soil contaminated with trace elements (TE) has become a major concern, given their persistence in the soil (Ali et al., 2019) and their impact when transferred to vegetation and humans (Peng et al., 2018; Goyal et al., 2020). Cd(II) and Zn(II) contaminations are often observed in co-contamination (Chaney, 2010) and frequently studied because of their recurrence in soils worldwide (Koopmans et al., 2008). Among the TE uptake by the plant, Zn(II) is an essential one that is involved in numerous metabolic functions (Vatanev et al., 2017; Kaur et al., 2023), while Cd(II) has no known functions (Huang et al., 2017). However, excessive accumulation of both elements could lead to toxic effects on plants (Cherif et al., 2011) as their bioavailability in the soil is relatively high and can vary easily under the impact of certain parameters, notably pH (Kashem and Singh, 2001; Spurgeon et al., 2006), compared with that of other TE, e.g., Pb(II) (Qasim, Motelica-Heino, 2014). Indeed, the presence of excess Cd(II) in the plants has several effects that impact growth. This element has a negative effect on the uptake of water and nutrients (NO₃, Ca, K, Mg, and P), inhibits the chlorophyll biosynthesis, and reduces the root and shoot

length (Asati et al., 2016). On the other hand, Zn(II) toxicity in plants is also reported by limiting the growth, causing chlorosis, and deficiencies of Mn and Cu (Nagajyoti et al., 2010).

Phytoremediation, which uses the natural abilities of plants to interact with chemical compounds, is increasingly being used to manage such contamination (Moosavi and Seghatoleslami, 2013). Phytoextraction is a method that focuses on the capacity of plants to take up TE from the soil and accumulate them in their aboveground parts (Chengatt et al., 2023). As a result, the sequestered contaminants in the plants can be harvested and extracted from the soil. Despite the ecological and financial benefits of phytoextraction, one of its main limitations is the treatment time required to significantly reduce contaminant concentrations at a site (Robinson et al., 2015). Therefore, improving the efficiency of phytoextraction is essential to making this method viable. There are two predominant key parameters that regulate extraction by plants, firstly, the concentration of the treated contaminant that the plant is capable of accumulating in its aboveground parts and secondly, the biomass produced by the plant (Bhargava et al., 2012). Among the plants commonly used for phytoextraction, hyperaccumulator plants can naturally accumulate vast amounts of TE in their aboveground parts

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(Pidlisnyuk et al., 2021). The threshold values required to consider a plant as a hyperaccumulator are 100 mg kg⁻¹ dry weight (DW) for Cd(II) and 3000 mg kg⁻¹ DW for Zn(II) (Krämer, 2010; Manara et al., 2020). However, hyperaccumulators plants (e.g. *Noccaea caerulea* and *Arabidopsis halleri*) have certain limitations, notably slow growth and biomass production, as well as selectivity for accumulated or even tolerated TE (Rascio and Navari-Izzo, 2011; Sheoran et al., 2016). On the other hand, accumulators, whether herbaceous or woody, which produce a large biomass, are also widely studied in the context of phytoextraction (Mayerová et al., 2017). Indeed, they compensate their lower TE accumulation compared to hyperaccumulators by their high biomass production, their extensive root systems, and their ability to accumulate and tolerate numerous contaminants and therefore offer a high extraction yield (Singh et al., 2020; Navazas et al., 2022).

Among the species of high-biomass accumulators used in phytoextraction, the *Salix* genus is one of the most studied. These species are widely used for their rapid growth and high biomass production (Kuzovkina and Volk, 2009). They also can tolerate and accumulate large quantities and panel of TE depending on the species and clones considered (Lachapelle et al., 2020; Cao et al., 2022), as these species had a high genetic variability (Amdoun et al., 2020). The species *Salix aquatica grandis* has been previously reported by Ciadamidaro et al. (2019) to accumulate Cd(II) and Zn(II) concentrations higher than 120 mg kg⁻¹ DW and 2500 mg kg⁻¹ DW in leaves, respectively, indicating that this species represents a good candidate for the phytoextraction, but no studies focused on its tolerance and extraction capacities when exposed to increasing bioavailability of TE. This is also the case for *Alliaria petiolata*, an herbaceous plant reported by Grabner et al. (2011) and Drozdova et al. (2019) as a Cd(II) and Zn(II) accumulator. Furthermore, *A. petiolata* was recently identified as one of the dominant species found on a multi-contaminated site, able to accumulate up to 18 mg kg⁻¹ DW of Cd(II) and 1750 mg kg⁻¹ DW of Zn(II) in its aboveground parts (Collot et al., 2023), supporting its potential for future phytoextraction investigations. Although the ability to accumulate Cd(II) and Zn(II) has been observed in *S. aquatica grandis* and *A. petiolata*, their tolerance to these elements depending on the concentrations present in the environment remains unknown. This variable is very important in the context of phytoextraction, particularly in methods aimed at increasing bioavailability to promote the extraction of contaminants.

The aims of this study were as follows: i) To evaluate the Cd(II) and Zn(II) accumulation and extraction in controlled condition of two species, *A. petiolata* and *S. aquatica grandis* by measuring the concentrations of Cd(II) and Zn(II) accumulated in the different plant parts and the bioconcentration and translocation factors when exposed to each elements. ii) To assess the tolerance and the extraction yield of these two species, particularly in the context of increasing TE exposure. The response of *A. petiolata* and *S. aquatica grandis* to Cd(II) and Zn(II) was monitored in a hydroponic experiment by measuring growth parameters, tolerance indices, and stress markers. This study therefore provides preliminary information on the physiological response and the potential use of *A. petiolata* and *S. aquatica grandis* in Cd(II) and Zn(II) phytoextraction methods that promote TE accumulation. The originality of this study lies in comparing the efficiency of Cd(II) and Zn(II) extraction based on the stress levels observed in two plants with distinct life cycles. This allows us to observe the optimal concentration of TEs in the plant environment to maximize their extraction based on the accumulation capacities and specific tolerance of the species considered. This subject has been poorly studied but is essential in the current drive to improve the yields of phytoextraction techniques. We hypothesized that both studied species exhibit significant tolerance capacity to Cd(II) and Zn(II), enabling them to increase the extraction of these TE when their concentration in the nutrient solution increases until phytotoxicity overcomes plant tolerance.

2. Materials and methods

2.1. Experimental design

A. petiolata was grown from seeds (Les semences du Puy nursery, Le Puy-en-Velay, France) that were sown in peat in autumn and watered three times a week until germination. Seedlings were obtained in early spring after stratification and were then transplanted into the same substrate and grown for two months before being used in the experiment. *S. aquatica grandis* was provided by the Soupe nursery (Châtillon-sur-Chalaronne, France), cuttings of about 8 cm high and 1.5 cm in diameter were placed in perlite-filled trays, watered three times a week, and grown for one month before being used in the experiment.

The experiment was carried out for 28 days in a growth chamber with the following controlled conditions: illumination intensity of 100 μmol m⁻² s⁻¹ (Vegeled Apollo LL300, COLASSE SA, Seraing, Belgium), day/night illumination duration of 14 h/10 h, day/night temperature of 22°C/18°C and humidity of 70%. The plants were placed in individual pots of 0.5 l filled with 25 g of perlite and placed in culture trays watered with a 0.25X Hoagland's solution (Hoagland and Arnon, 1950) adjusted to a pH of 6. The different treatments were replicated six times and consisted of adding increasing concentrations of Cd(II) (as CdCl₂) or Zn(II) (as ZnSO₄·7H₂O) in the nutrient solution (Table 1). The Cd(II) and Zn(II) treatments were chosen based on previous studies (Amdoun et al., 2020; Wang et al., 2021), as well as the tolerance of the chosen species, with *A. petiolata* being more tolerant to Cd(II) and Zn(II), an additional treatment was added.

Control treatments corresponded to Hoagland's solution without the addition of Cd(II) or Zn(II). The solutions were entirely replaced twice a week. Plants were cultivated using a completely random design to minimize experimental bias and to ensure representative sampling of each treatment.

2.2. Plant growth

At the end of the experiment, the plant photosynthetic efficiency was evaluated by measuring the maximum photosystem II (PSII) quantum yield (Fv/Fm) on dark-adapted leaves after a full night phase (10 hr night period) (Maxwell and Johnson, 2000) using a photosynthesis yield analyser (MINI-PAM II, Walz, Effeltrich, Germany). Plant height was only measured for *S. aquatica grandis*, as the height of *A. petiolata* varies little during the first year of growth. Indeed, the leaves of *A. petiolata* remains in the form of a rosette during the first year, the stem only develops in the second year and was therefore absent when the plants were harvested. The plants were then harvested, the total leaf area was determined using ImageJ (Ver. 1.53), and the leaves of *A. petiolata* and *S. aquatica grandis* were freeze-dried before the biomass was measured. The length of the longest root was determined for each plant before

Table 1
Concentration of TE (Cd(II) or Zn(II)) added to the nutrient solution and code for each treatment.

Element	<i>Alliaria petiolata</i>		<i>Salix aquatica grandis</i>	
	TE Concentration (μM)	Code	TE Concentration (μM)	Code
Control	0	C	0	C
Cd(II)	1	Cd1	1	Cd1
	2.5	Cd2	2.5	Cd2
	5	Cd3	5	Cd3
	10	Cd4	10	Cd4
	30	Cd5		
Zn(II)	5	Zn1	5	Zn1
	10	Zn2	10	Zn2
	25	Zn3	25	Zn3
	100	Zn4	100	Zn4
	500	Zn5	500	Zn5
	1000	Zn6		

removing Cd(II) and Zn(II) adsorbed on the roots by shaking them for 20 min in 20 mM Na₂-EDTA and rinsing them 3 times in distilled water (Wang et al., 2021). The root (for *A. petiolata* and *S. aquatica grandis*) and stem (for *S. aquatica grandis*) biomasses were measured after oven-drying at 70°C. The tolerance index (TI) was determined based on the total dry biomass of the plants (corresponding to roots, stems and leaves for *S. aquatica grandis* and to roots and leaves for *A. petiolata*) at the end of the experiment as described in (Eq1) (Wang et al., 2021).

$$TI = \frac{\text{Dry weight in TE enriched solution (g)}}{\text{Dry weight in control solution (g)}} \quad (1)$$

All samples were then ground in a mixer mill (MM 200, Retsch, Eragny, France) at 30 rps for 3 min (leaves) or 5 min (stems and roots) prior to analyses.

2.3. Photosynthetic pigment concentration

The concentration of photosynthetic pigments was determined on samples of dried leaves. The metabolites were extracted using the protocol of López-Hidalgo et al. (2021). This method was chosen because it allows sensitive and reproducible results to be obtained with small amount of sample. Briefly, 5 mg of dry leaf samples (lyophilized) were homogenized for 30 s in 1 ml of 80% ethanol and centrifuged at 10,000g and 4°C for 10 min. The supernatant was recovered, diluted twice in 80% ethanol, and used for the measurement of Chlorophyll a (Chl a), Chlorophyll b (Chl b), and carotenoids (CAR), according to Lichtenthaler (1987). The optical density at 470, 649, and 664 nm was measured using a spectrophotometer (Spark multimode microplate reader, Tecan, Männedorf, Switzerland) on 150 µl of extract obtained in the previous step (blank corresponded to 80% ethanol). Each read of optical density was repeated three times. The concentrations of Chl a, Chl b, and CAR in µg ml⁻¹ were then determined following Eq2, 3, and 4, respectively, and finally expressed as µg mg⁻¹ DW.

$$Chla (\mu\text{g ml}^{-1}) = 13.36 \cdot Abs_{664nm} - 5.19 \cdot Abs_{649nm} \quad (2)$$

$$Chlb (\mu\text{g ml}^{-1}) = 27.43 \cdot Abs_{649nm} - 8.12 \cdot Abs_{664nm} \quad (3)$$

$$CAR (\mu\text{g ml}^{-1}) = \frac{(1000 \cdot Abs_{470nm} - 2.13 \cdot Chla - 97.63 \cdot Chlb)}{209} \quad (4)$$

With Abs the optical density measured in the extract.

2.4. Accumulation and extraction of TE

The concentration of Cd(II) or Zn(II) was determined in 5–25 mg of ground samples of roots, stems, and leaves using flame Atomic Absorption Spectroscopy (AAS) (240FS, Agilent technologies, Santa Clara, USA) or graphite furnace AAS (240Z, Agilent technologies, Santa Clara, USA) for low concentrations. The acid digestion was done using a mixture of 2 ml of 67% nitric acid and 0.5 ml of 35% hydrogen peroxide (Assad et al., 2017).

Bioconcentration factors (BCF) for roots (Eq5) and leaves (Eq6) were calculated on the basis of TE concentrations in the dried biomass and in the nutrient solution (Touati et al., 2019).

$$\text{Root BCF} = \frac{\text{TE concentration in dried root biomass (mg kg}^{-1}\text{)}}{\text{TE concentration in nutrient solution (mg l}^{-1}\text{)}} \quad (5)$$

$$\text{Leaf BCF} = \frac{\text{TE concentration in dried leaf biomass (mg kg}^{-1}\text{)}}{\text{TE concentration in nutrient solution (mg l}^{-1}\text{)}} \quad (6)$$

The translocation factor (TF) (Eq7) was calculated from concentration of TE in the biomass of dried leaves and roots (Touati et al., 2019).

$$TF = \frac{\text{TE concentration in dried leaf biomass (mg kg}^{-1}\text{)}}{\text{TE concentration in dried root biomass (mg kg}^{-1}\text{)}} \quad (7)$$

The mass of Cd(II) and Zn(II) extracted by the aboveground plant biomass was determined using concentration and dry biomass values and expressed as µg/plant. For AAS, the certified reference material used was oriental basma tobacco leaves (INCT-OBTL-5, LGC Standards, Molsheim, France). The recovery percentages were 89.9% (Cd(II)) and 85.2% (Zn(II)) for flame AAS and 98.8% (Cd(II)) and 94.6% (Zn(II)) for graphite furnace AAS.

2.5. Statistical analysis

The analyses were performed using R (ver. 4.3.0) with the package *agricolae* (De Mendiburu, 2023), *cowplot* (Wilke, 2023) and *ggplot2* (Wickham, 2016). All the tests performed were considered statistically significant at a p-value < 0.05. The normality of the data and the homogeneity of variances were assessed using Shapiro-Wilk and Levene tests, respectively, to determine the analysis to be performed (parametric or non-parametric). Comparisons of means were performed by analysis of variance (ANOVA) followed by Fischer's LSD post hoc test or by Kruskal-Wallis test when variables did not meet the requirement for parametric analysis. Concerning the data of maximum PSII quantum yield, leaf area, root lengths, height, dry biomass, TI, Chl a, Chl b, CAR, and mass of TE extracted, mean comparison between treatments were made for each plant and each element separately. For the data of TE concentrations in plant parts (roots, stems, and leaves), root BCF, leaf BCF, and TF, mean comparisons were made including all treatments (*A. petiolata* and *S. aquatica grandis*) for each element. Spearman's test was used to assess the correlation between Cd(II) and Zn(II) extracted or their concentration in plants parts and the concentration of TE in nutrient solution. Data visualizations were made using the *ggplot2* and *ggforce* packages.

3. Results

3.1. Plant growth, tolerance to TE, and response of photosynthetic pigments

After 28 days of growth, variable responses of growth parameters to TE exposure were observed, depending on the element, its concentrations, and the plant considered (Fig. 1). Concerning the Fv/Fm mean values (Fig. 1A and B), the addition of TE had an effect depending on its concentration. Indeed, for *A. petiolata* grown on Cd(II) enriched nutrient solution, the mean value for treatment C was higher than Cd3 and Cd5. A similar trend was observed for Zn(II) as the mean value for C treatment was statistically higher than Zn4 and Zn6. *S. aquatica grandis* growth was also impacted by the highest Cd(II) treatments as C, Cd1, and Cd2 had higher Fv/Fm mean values than Cd3 and Cd4. Concerning Zn(II), no significant differences were observed between C and all other treatments (except Zn2 that had a slightly higher value). However, Zn5 had a statistically lower value than all other Zn(II) treatments.

The leaf area of *A. petiolata* (Fig. 1C) in treatment C did not significantly differ from all the Cd(II) treatments that had values ranging from 13.6 to 34.2 cm². However, for Zn(II), the Zn5 and Zn6 treatments showed a significant negative effect, with values lower than C, Zn1, Zn2, and Zn3 treatments. The Zn4 treatment was not significantly different from all the other treatments. For *S. aquatica grandis* (Fig. 1D), the effects of TE on leaf area were more visible for both elements. Indeed, the mean value of treatments Cd3 and Cd4 decreased drastically compared to treatment C. The same trend was observed for Zn(II), with a mean value for treatment C that was higher than Zn4 and Zn5 that showed a strong negative effect (six fold lower than C) compared with all other treatments.

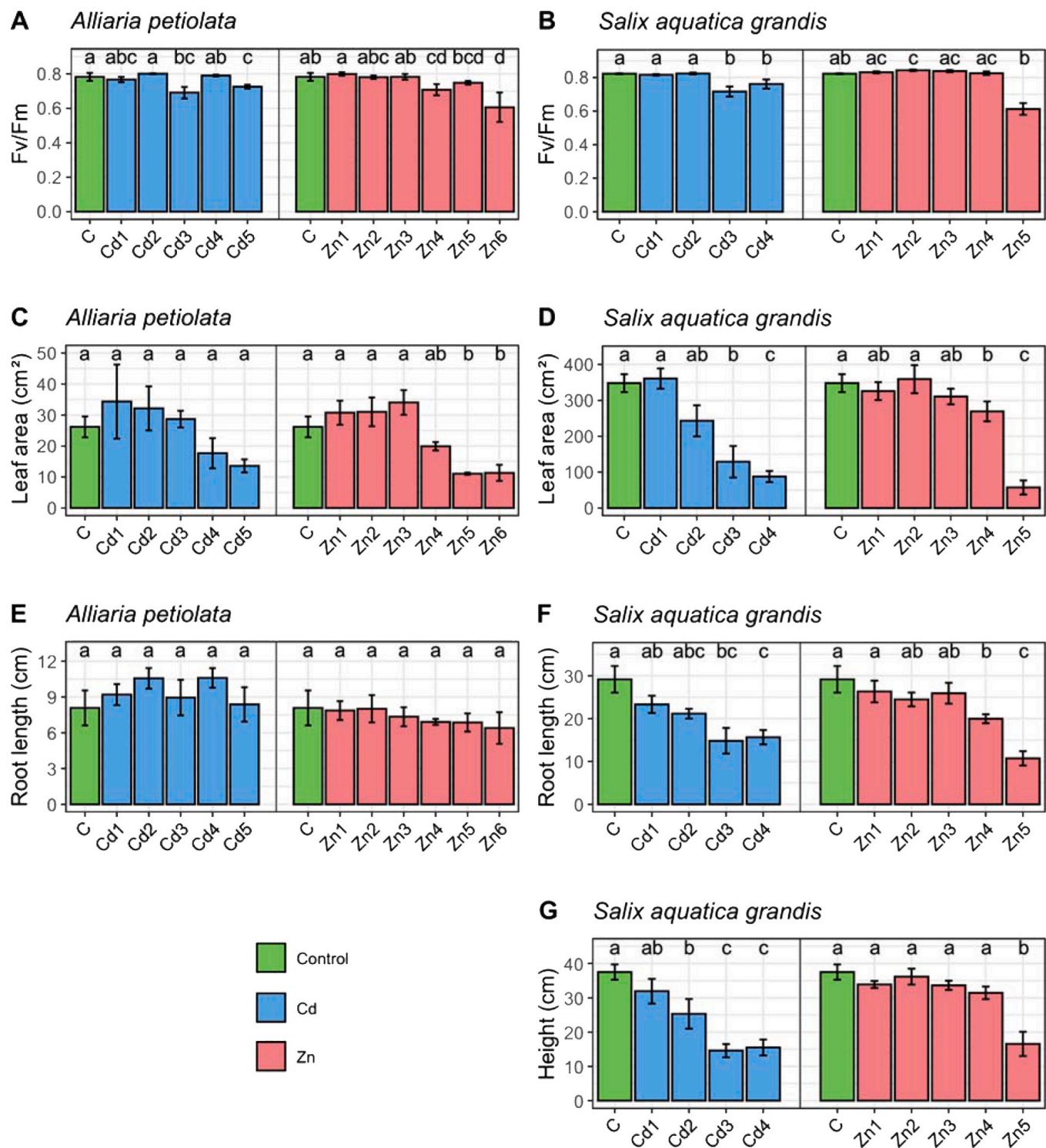


Fig. 1. Mean values of growth parameters measured on *Alliaria petiolata* (A, C and E) and *Salix aquatica grandis* (B, D, F and G) cultivated on Cd(II) (blue) or Zn(II) (red) enriched nutrient solution at the end of the experiment (n = 6). Control (green) correspond to non-enriched nutrient solution. The error bars represent standard errors. For each treatment, plant, and element, letters in common indicate statistically similar groups at p < 0.05 according to mean and post hoc pairwise comparison tests.

For *A. petiolata* (Fig. 1E), root length did not show any significant differences for all the treatments tested, regardless of the TE considered, with mean values that varied from 6.4 to 10.6 cm. Conversely, for *S. aquatica grandis* (Fig. 1F), the decreases observed for Fv/Fm and leaf area were also found for root length. Indeed, the mean value of the C treatment was almost twice higher than Cd3 and Cd4. For Zn(II), the mean value of C treatment was almost 1.5 times higher than Zn4 and 2.7

times higher than Zn5. Regarding the height of *S. aquatica grandis* (Fig. 1G), the addition of Cd(II) significantly reduced the mean values by 1.5 times in Cd2, 2.6 times in Cd3 and 2.4 times in Cd4 compared with C. Concerning Zn(II), the mean value for treatment C was 2.3 times higher than Zn5.

Concerning dry biomass production, no significant differences were observed when *A. petiolata* was exposed to Cd(II) on the roots and leaves

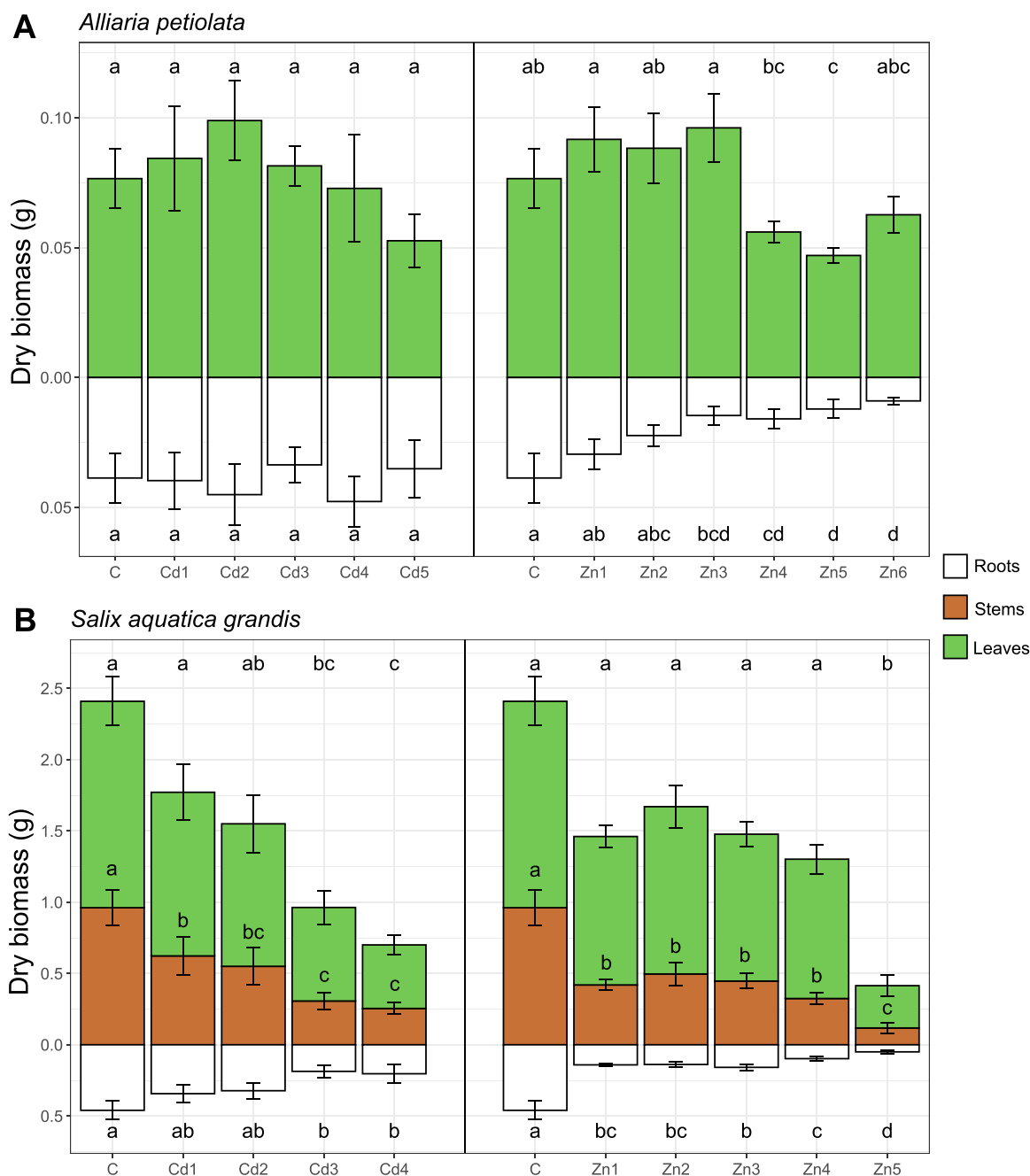


Fig. 2. Mean dry biomass of *Alliaria petiolata* (A) and *Salix aquatica grandis* (B) cultivated on Cd(II) or Zn(II) enriched nutrient solution at the end of the experiment (n = 6). Control correspond to non-enriched nutrient solution. The root (white), stem (brown, only for *Salix aquatica grandis*) and leaf (green) biomass are separated, and the error bars represent standard errors. For each treatment, plant, and element, letters in common indicate statistically similar groups at p < 0.05 according to mean and post hoc pairwise comparison tests.

(Fig. 2A). However, exposure to the highest concentrations of Zn(II) led to negative effect on these parameters. Indeed, the root dry biomass in C was almost 2.6 times higher than Zn3, 2.4 times higher than Zn4, 3.25 times higher than Zn5 and 4.3 times higher than Zn6. For leaf dry biomass, the mean value of C was almost 1.4 times higher than Zn4 and 1.6 times higher than Zn5. No significant difference was observed between the treatment Zn6 and all other treatments, including C. Unlike *A. petiolata*, *S. aquatica grandis* (Fig. 2B) showed a significant reduction of leaf dry biomass when exposed to Cd(II) concentration with a mean value for the treatment C that was 2.2 times higher than Cd3 and 3.2 times higher than Cd4. The dry biomass of the stems statistically varied for even lower Cd(II) exposure, as was observed for the mean values of Cd1, Cd2, Cd3 and Cd4 that were respectively almost 1.5, 1.7, 3.1 and

3.8 times lower than for the treatment C. Regarding root dry biomass, the trend was similar to leaf dry biomass with a mean value for C that was almost 2.5 times higher than Cd3 and 2.3 times higher than Cd4. Under Zn(II) exposure, a negative effect on dry biomass production of *S. aquatica grandis* was observed, especially for Zn5, the highest concentration. Indeed, for leaf dry biomass, the mean value of Zn5 was statistically lower than that of all other treatments, and in particular, that of C (4.9 times lower). The mean value of treatment C for stems dry biomass was statistically higher than all other treatments. The values were almost 2.3 times greater than Zn1, 1.9 times greater than Zn2, 2.2 times greater than Zn3, 3 times greater than Zn4 and 8.1 times greater than Zn5. Finally, the dry biomass of roots followed a similar trend to that of stems with significantly higher values in treatment C than in the

other treatments. The values were almost 3.3 times greater than Zn1 and Zn2, 2.9 times greater than Zn3, 4.7 times greater than Zn4 and 9.2 times greater than Zn5.

As TI values are calculated from total dry biomass data, they summarise the results observed previously for leaf, stem, and root biomass data (Table 2). Indeed, as seen before for leaf and root dry biomass, no significant variation was observed for the mean TI values of *A. petiolata* growing on a Cd(II)-enriched nutrient solution. As these values were close to or even greater than 1, the presence of Cd(II) had no impact on dry biomass production for this species. For Zn(II), a similar trend was observed in the treatments Zn1, Zn2, and Zn3, where the mean TI values ranged from 0.96 to 1.05. However, these three treatments showed significantly higher values than Zn4, Zn5, and Zn6, which had values

Table 2

Mean values of TI and concentrations of Chl a, Chl b and CAR in the leaves of *Alliaria petiolata* and *Salix aquatica grandis* at the end of the experiment (n = 6). For each treatment, plant, and element, letters in common indicate statistically similar groups at p < 0.05 according to mean and post hoc pairwise comparison tests.

Plants	Treatments	TI	Chl a (µg mg ⁻¹) (µg mg ⁻¹ DW)	Chl b (µg mg ⁻¹) (µg mg ⁻¹ DW)	CAR (µg mg ⁻¹) (µg mg ⁻¹ DW)
<i>Alliaria petiolata</i>	C	/	2.05	0.98	0.39
	Cd1	1.08	± 0.10 a	± 0.05 a	± 0.02 a
		± 0.17 a	± 0.26 a	± 0.11 a	± 0.04 a
	Cd2	1.25	± 0.21 a	± 0.24 a	± 0.15 a
		± 0.21 a	± 0.24 a	± 0.20 a	± 0.15 a
	Cd3	1.00	± 0.10 a	± 0.15 b	± 0.09 b
		± 0.10 a	± 0.15 b	± 0.09 b	± 0.08 a
	Cd4	1.05	± 0.25 a	± 0.18 b	± 0.17 bc
		± 0.25 a	± 0.18 b	± 0.17 bc	± 0.16 a
	Cd5	0.76	± 0.17 a	± 0.04c	± 0.02c
<i>Salix aquatica grandis</i>	C	/	1.83	0.70	0.35
		± 0.09 a	± 0.11 a	± 0.05 a	
	Cd1	0.74	± 0.14 a	± 0.06 b	± 0.02 a
		± 0.14 a	± 0.06 b	± 0.02 a	± 0.01 a
	Cd2	0.65	± 0.13 ab	± 0.07c	± 0.03 ab
		± 0.13 ab	± 0.07c	± 0.03 ab	± 0.02 ab
	Cd3	0.40	± 0.07 bc	± 0.04 d	± 0.02 bc
		± 0.07 bc	± 0.04 d	± 0.02 bc	± 0.01 bc
	Cd4	0.31	± 0.02c	± 0.04 d	± 0.02c
		± 0.02c	± 0.04 d	± 0.02c	± 0.01c
<i>Alliaria petiolata</i>	C	/	2.05	0.98	0.39
		± 0.10 a	± 0.05 a	± 0.02 a	
	Zn1	1.05	± 0.12 a	± 0.13 ab	± 0.05 b
		± 0.12 a	± 0.13 ab	± 0.05 b	± 0.02 a
	Zn2	0.96	± 0.15 a	± 0.12 ab	± 0.09 bc
		± 0.15 a	± 0.12 ab	± 0.09 bc	± 0.08 a
	Zn3	0.96	± 0.11 a	± 0.13 bc	± 0.10c
		± 0.11 a	± 0.13 bc	± 0.10c	± 0.05 ab
	Zn4	0.62	± 0.05 b	± 0.06 cd	± 0.07 d
		± 0.05 b	± 0.06 cd	± 0.07 d	± 0.04 bc
<i>Salix aquatica grandis</i>	C	/	1.83	0.70	0.35
		± 0.09 a	± 0.11 a	± 0.05 a	
	Zn1	0.56	± 0.04 a	± 0.03 ab	± 0.02 a
		± 0.04 a	± 0.03 ab	± 0.02 a	± 0.01 a
	Zn2	0.63	± 0.09 a	± 0.04 ab	± 0.01 ab
		± 0.09 a	± 0.04 ab	± 0.01 ab	± 0.01 a
	Zn3	0.57	± 0.05 a	± 0.05 bc	± 0.02 ab
		± 0.05 a	± 0.05 bc	± 0.02 ab	± 0.01 ab
	Zn4	0.49	± 0.05 a	± 0.05 cd	± 0.02 bc
		± 0.05 a	± 0.05 cd	± 0.02 bc	± 0.01 bc
<i>Salix aquatica grandis</i>	C	/	1.83	0.70	0.35
		± 0.09 a	± 0.11 a	± 0.05 a	
	Zn1	0.56	± 0.04 a	± 0.03 ab	± 0.02 a
		± 0.04 a	± 0.03 ab	± 0.02 a	± 0.01 a
	Zn2	0.63	± 0.09 a	± 0.04 ab	± 0.01 ab
		± 0.09 a	± 0.04 ab	± 0.01 ab	± 0.01 a
	Zn3	0.57	± 0.05 a	± 0.05 bc	± 0.02 ab
		± 0.05 a	± 0.05 bc	± 0.02 ab	± 0.01 ab
	Zn4	0.49	± 0.05 a	± 0.05 cd	± 0.02 bc
		± 0.05 a	± 0.05 cd	± 0.02 bc	± 0.01 bc
<i>Salix aquatica grandis</i>	C	/	1.83	0.70	0.35
		± 0.09 a	± 0.11 a	± 0.05 a	
	Zn1	0.56	± 0.04 a	± 0.03 ab	± 0.02 a
		± 0.04 a	± 0.03 ab	± 0.02 a	± 0.01 a
	Zn2	0.63	± 0.09 a	± 0.04 ab	± 0.01 ab
		± 0.09 a	± 0.04 ab	± 0.01 ab	± 0.01 a
	Zn3	0.57	± 0.05 a	± 0.05 bc	± 0.02 ab
		± 0.05 a	± 0.05 bc	± 0.02 ab	± 0.01 ab
	Zn4	0.49	± 0.05 a	± 0.05 cd	± 0.02 bc
		± 0.05 a	± 0.05 cd	± 0.02 bc	± 0.01 bc
<i>Salix aquatica grandis</i>	C	/	1.83	0.70	0.35
		± 0.09 a	± 0.11 a	± 0.05 a	
	Zn1	0.56	± 0.04 a	± 0.03 ab	± 0.02 a
		± 0.04 a	± 0.03 ab	± 0.02 a	± 0.01 a
	Zn2	0.63	± 0.09 a	± 0.04 ab	± 0.01 ab
		± 0.09 a	± 0.04 ab	± 0.01 ab	± 0.01 a
	Zn3	0.57	± 0.05 a	± 0.05 bc	± 0.02 ab
		± 0.05 a	± 0.05 bc	± 0.02 ab	± 0.01 ab
	Zn4	0.49	± 0.05 a	± 0.05 cd	± 0.02 bc
		± 0.05 a	± 0.05 cd	± 0.02 bc	± 0.01 bc
<i>Salix aquatica grandis</i>	C	/	1.83	0.70	0.35
		± 0.09 a	± 0.11 a	± 0.05 a	
	Zn1	0.56	± 0.04 a	± 0.03 ab	± 0.02 a
		± 0.04 a	± 0.03 ab	± 0.02 a	± 0.01 a
	Zn2	0.63	± 0.09 a	± 0.04 ab	± 0.01 ab
		± 0.09 a	± 0.04 ab	± 0.01 ab	± 0.01 a
	Zn3	0.57	± 0.05 a	± 0.05 bc	± 0.02 ab
		± 0.05 a	± 0.05 bc	± 0.02 ab	± 0.01 ab
	Zn4	0.49	± 0.05 a	± 0.05 cd	± 0.02 bc
		± 0.05 a	± 0.05 cd	± 0.02 bc	± 0.01 bc

ranging from 0.51 to 0.62. For *S. aquatica grandis*, the negative effect of Cd(II) was visible as the mean TI mean values decreased gradually. The treatment Cd1 had a statistically higher value than Cd3 and Cd4. Regarding Zn(II) values, the treatment Zn1 showed a mean TI value that was statistically similar to Zn2, Zn3 and Zn4. As observed previously for leaf, stem, and root dry biomass, the treatment Zn5 had a drastically lower value than all other treatments.

Although Cd(II) exposure had no impact on the dry biomass of *A. petiolata*, a negative effect was observed on the concentrations of photosynthetic pigments, which gradually decreased as the TE concentration in the nutrient solution increased (Table 2). The Chl a and Chl b mean concentrations showed the same trend as the values were statistically similar between treatments C, Cd1, and Cd2 but higher compared to Cd3, Cd4 and Cd5. In addition, average CAR concentrations did not vary significantly in all treatments, with values ranging between 0.11 and 0.53 µg mg⁻¹ DW. For Zn(II), the mean concentration of Chl a in C was statistically higher than Zn3, Zn4, Zn5 and Zn6. The negative effect was even more visible for Chl b as the mean concentration of C was statistically higher than all other treatments that had values gradually decreasing from 0.73 µg mg⁻¹ DW in Zn1–0.18 µg mg⁻¹ DW in Zn6. For the CAR mean concentrations, the value of treatment C was significantly higher than Zn3, Zn4, Zn5 and Zn6. *S. aquatica grandis* showed a similar trend to *A. petiolata*, with mean values that progressively decreased as the TE concentrations increased in the nutrient solution. For Chl a on Cd (II), the mean concentration of treatment C was significantly higher than all other treatments, where values steadily decreased from 1.14 µg mg⁻¹ DW for Cd1–0.24 µg mg⁻¹ DW for Cd4. Chl b showed a less visible effect on the lowest Cd(II)-concentrated treatments as the mean value for C was only higher than Cd3 and Cd4. CAR mean concentration values followed the same trend as Chl b with a lower value for Cd3 and Cd4 in comparison to C. Regarding the photosynthetic pigment concentrations for Zn(II) exposure in *S. aquatica grandis*, Chl a mean values for treatment C were statistically higher than Zn3, Zn4 and Zn5. For Chl b, as for Cd(II), a less visible effect was found as the mean concentration value for treatment C was only greater than Zn4 and Zn5. The trend was the same for CAR as for Chl b, with a significantly lower value for Zn4 and Zn5 than for C.

3.2. Concentration, accumulation, and translocation of Cd(II) and Zn(II)

Increasing the concentration of TE in the nutrient solution led to a gradual increase in the concentrations of TE accumulated in the different parts of the plants. Indeed, the mean Cd(II) concentration in roots (Fig. 3A) of *A. petiolata* were statistically lower in C than in Cd2, Cd3, Cd4 and Cd5. The average Cd(II) concentration observed in the roots of *S. aquatica grandis* varied the same way as for *A. petiolata*, C treatment showed a significantly lower value than Cd2, Cd3 and Cd4. With regards to Zn(II) concentrations in the roots (Fig. 3B), the values also increased with the concentration of TE in the nutrient solution, but only for the most concentrated treatments. Indeed, for *A. petiolata*, the mean Zn(II) concentration in C was statistically lower than Zn4, Zn5 and Zn6. This trend was even more visible for *S. aquatica grandis*, as the only treatment significantly higher than C was Zn5 that showed a strong augmentation of Zn(II) root concentration. Concerning the Cd(II) concentrations in stems of *S. aquatica grandis* (Fig. 3C), the mean value of the treatment C was statistically lower than Cd2, Cd3 and Cd4. The trend was similar for Zn(II) (Fig. 3D) as the mean value of C was lower than Zn2, Zn3, Zn4 and Zn5. Regarding the Cd(II) concentrations in leaves (Fig. 3E), the mean values observed for *A. petiolata* revealed that the treatment C was significantly lower than Cd2, Cd3, Cd4 and Cd5. The *S. aquatica grandis* concentrations varied the same way, with a higher value for Cd2, Cd3, and Cd4 in comparison to C. The maximal Cd(II) concentration observed in leaves in the experimentation was 332.1 mg kg⁻¹ DW (Cd5) for *A. petiolata* and 129.3 mg kg⁻¹ DW (Cd4) for *S. aquatica grandis*. For Zn (II) (Fig. 3F) in *A. petiolata* leaves, no significant variations in the treatments C, Zn1, and Zn2 were found. Still, these treatments were

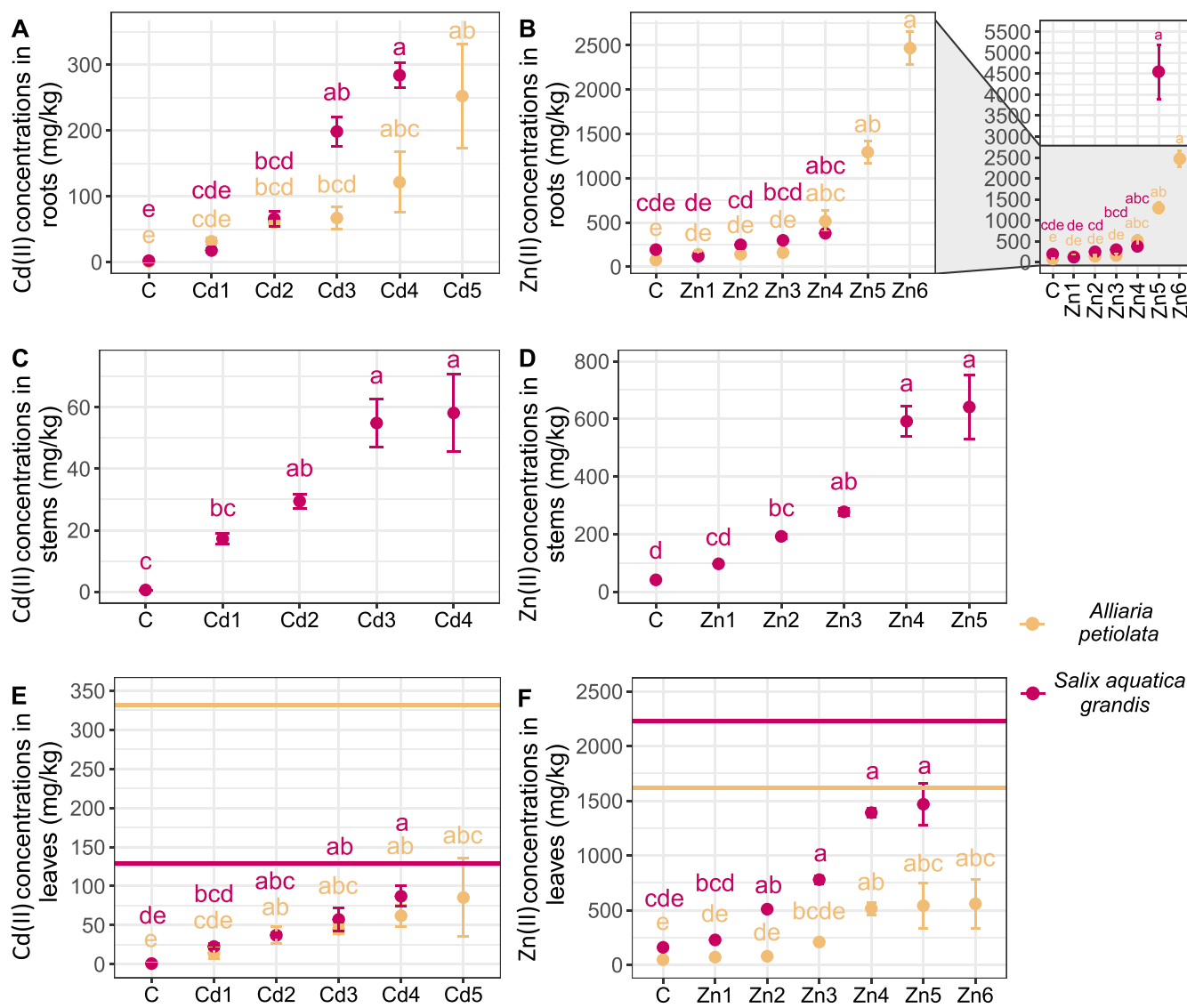


Fig. 3. Mean concentration of Cd(II) and Zn(II) in roots (A and B), stems (C and D, only for *Salix aquatica grandis*) and leaves (E and F) of *Alliaria petiolata* (yellow) and *Salix aquatica grandis* (red) at the end of the experiment (n = 6). For E and F, the coloured line indicates the maximal concentrations of Cd(II) or Zn(II) observed in *Alliaria petiolata* (yellow) or *Salix aquatica grandis* (red). For each treatment and element, letters in common indicate statistically similar groups at p < 0.05 according to mean and post hoc pairwise comparison tests.

lower than Zn4, Zn5 and Zn6. Zn3 had an intermediate value as it did not differ from any other treatments. Finally, mean Zn(II) concentrations in leaves of *S. aquatica grandis* were significantly lower in C than in Zn2, Zn3, Zn4 and Zn5. Spearman's test highlighted strong positive correlation between the concentration of Cd(II) and Zn(II) in roots and leaves of *A. petiolata* and the concentrations of the corresponding TE in the nutrient solution (r value ranging from 0.67 to 0.86) (see supplementary informations). The same trend is observed for the concentration of Cd(II) and Zn(II) in roots, stems and leaves of *S. aquatica grandis* and the concentrations of the corresponding TE in the nutrient solution (r value ranging from 0.84 to 0.97). The maximal Zn(II) concentrations observed in leaves of the plants in the experimentation were 1614.8 mg kg⁻¹ DW (Zn6) for *A. petiolata* and 2227.2 mg kg⁻¹ DW (Zn5) for *S. aquatica grandis*.

Although the TE concentrations observed in the different plant parts increased under increasing TE concentrations in the nutrient solution, the translocation and bioconcentration of these elements did not evolve in the same way. When exposed to Cd(II)-enriched nutrient solutions,

the root BCF of *A. petiolata* showed a decrease as Cd1 and Cd2 had a similar value but were significantly higher than Cd3, Cd4 and Cd5 (Table 3).

However, the values for *S. aquatica grandis* varied differently as the root BCF in Cd1 was lower than Cd3 and Cd4. In addition, the Cd3 treatment had a higher value than Cd4. These differences between plants were also highlighted by the higher root BCF value for *A. petiolata* than *S. aquatica grandis* in Cd1, while the opposite was observed for treatments Cd3 and Cd4. However, both plants followed the same trend for the Cd(II) leaf BCF, as *A. petiolata* showed a lower value in Cd5 than in Cd1 and Cd2. For *S. aquatica grandis*, a lower value was observed in Cd4 than Cd1. *S. aquatica grandis* had a higher leaf BCF than *A. petiolata* in the treatment Cd1. Regarding the TF of Cd(II) for *A. petiolata*, the value in the treatment C was significantly higher than that of the Cd1, Cd2, and Cd5 treatments. The treatments Cd3 and Cd4 were similar to all other treatments (except Cd3 that was significantly higher than Cd5). For *S. aquatica grandis*, the treatment Cd1 had significantly higher values than C, Cd3 and Cd4. Few differences were observed between plants,

Table 3

Mean values of root and leaf BCF and TF of *Alliaria petiolata* and *Salix aquatica grandis* at the end of the experiment (n = 6). For each treatment and element, letters in common indicate statistically similar groups at p < 0.05 according to mean and post hoc pairwise comparison tests.

Plants	Treatments	Root BCF	Leaf BCF	TF
<i>Alliaria petiolata</i>	C	/	/	5.92 ± 4.28 a
	Cd1	482.8 ± 67.8 ab	215.0 ± 102.2 ab	0.44 ± 0.21 bc
	Cd2	367.2 ± 12.8 bc	229.4 ± 68.9 abc	0.61 ± 0.17 bc
	Cd3	205.0 ± 51.1 d	137.1 ± 18.2 abd	0.83 ± 0.15 ab
	Cd4	185.7 ± 70.0 d	94.9 ± 21.2 bd	1.45 ± 0.63 abc
<i>Salix aquatica grandis</i>	Cd5	128.6 ± 40.2 d	43.6 ± 25.4 d	0.36 ± 0.15c
	C	/	/	0.64 ± 0.18 bc
	Cd1	270.8 ± 45.4 cd	347.0 ± 51.6 c	1.36 ± 0.14 a
	Cd2	404.8 ± 69.6 bc	227.1 ± 18.0 ac	0.63 ± 0.09 abc
	Cd3	606.8 ± 67.9 a	175.5 ± 44.5 abc	0.33 ± 0.10 bc
<i>Alliaria petiolata</i>	Cd4	434.1 ± 28.9 b	133.1 ± 20.2 abd	0.32 ± 0.07 bc
	C	/	/	1.70 ± 1.04 abc
	Zn1	251.2 ± 52.4 ab	125.2 ± 11.1 abc	0.59 ± 0.12 abd
	Zn2	126.1 ± 31.8 abc	69.6 ± 8.5 bde	0.90 ± 0.29 abd
	Zn3	57.3 ± 17.1 cde	74.4 ± 6.4 bcd	2.53 ± 1.19 acef
	Zn4	45.9 ± 10.8 cde	45.9 ± 5.0 bdef	1.27 ± 0.29 abce
	Zn5	23.0 ± 2.2 e	9.6 ± 3.7 ef	0.40 ± 0.14 bd
<i>Salix aquatica grandis</i>	Zn6	22.0 ± 1.6 e	5.0 ± 2.0 f	0.22 ± 0.08 d
	C	/	/	0.91 ± 0.14 abc
	Zn1	213.8 ± 22.6 ab	405.4 ± 18.4 a	1.96 ± 0.13 cef
	Zn2	220.9 ± 17.5 a	452.8 ± 17.0 a	2.11 ± 0.16cef
	Zn3	106.5 ± 12.1 abcd	277.2 ± 12.4 ac	2.80 ± 0.36 ef
	Zn4	33.7 ± 0.8 de	123.8 ± 3.5 abc	3.69 ± 0.15 f
<i>Salix aquatica grandis</i>	Zn5	80.9 ± 11.6 bcd	26.1 ± 3.4 def	0.40 ± 0.12 bd

except for C treatment that was higher in *A. petiolata* than in *S. aquatica grandis*, and Cd1 that was higher in *S. aquatica grandis* than in *A. petiolata*.

For Zn(II), the root BCF decreased when the concentration in the nutrient solution increased for both species. *A. petiolata* presented a mean value in Zn1 that was significantly higher than treatments Zn3, Zn4, Zn5 and Zn6. For *S. aquatica grandis*, the treatment Zn4 was significantly lower than Zn1 and Zn2, and the treatment Zn5 was significantly lower than Zn2. Regarding the leaf BCF, the *A. petiolata* treatments Zn5 and Zn6 were statistically similar but presented lower values than Zn1, Zn2 and Zn3. The treatment Zn4 had intermediate values and did not differ from all the other treatments. Concerning *S. aquatica grandis*, the treatments Zn1, Zn2, Zn3, and Zn4 were statistically similar but all higher than Zn5. The only difference observed between plants was that the leaf BCF of *S. aquatica grandis* was significantly higher than *A. petiolata* for Zn2 treatment. Finally, few variations were observed for the Zn(II) TF of *A. petiolata*. Indeed, the mean value of the treatment C was significantly higher than Zn6. The value of Zn3 was also higher than in Zn5 and Zn6. For *S. aquatica grandis*, the TF gradually

increased until treatment Zn4 and then sharply decreased in the treatment Zn5. Indeed, the mean value in C was statistically similar to Zn1, Zn2, and Zn5 but lower than Zn3 and Zn4. In addition, Zn1, Zn2, Zn3, and Zn4 had significantly higher mean value than Zn5. When comparing the two plants, *S. aquatica grandis* had a higher Zn(II) TF value than *A. petiolata* for Zn1, Zn2, and Zn4 treatments.

3.3. Extraction of Cd(II) and Zn(II)

Despite the continuous increase observed in Cd(II) and Zn(II) concentration in stems and leaves as a function of treatment, the extraction of these TE followed a different trend since the values of Cd(II) and Zn(II) extracted reached a maximum observed between Cd1 and Cd2 for Cd(II) and between Zn2 and Zn3 for Zn(II). Indeed, the mean value of the mass of Cd(II) extracted by *A. petiolata* (Fig. 4A) in C was significantly lower than all other treatments except Cd1. The mean values of Cd2, Cd3, Cd4 and Cd5 were statistically similar with a maximal mean value observed in Cd4. For *S. aquatica grandis* (Fig. 4B), the Cd(II) extracted in C was significantly lower than all other treatments. Concerning Zn(II) extracted by *A. petiolata* (Fig. 4C), the mean value of C was similar to that of Zn1 and Zn2, additionally these treatments were significantly lower than the rest of treatments. Finally, for Zn(II) extracted by *S. aquatica grandis* (Fig. 4D) the mean values were similar between C and Zn1, but these treatments were lower than Zn2, Zn3 and Zn4. The value of the treatment Zn5 was similar to all other treatments except Zn4 that was almost 3.2 times higher. Spearman's test highlighted positive correlation between the concentration of Cd(II) and Zn(II) extracted by *A. petiolata* and the concentrations of the corresponding TE in the nutrient solution (r value of 0.52 for Cd(II) and of 0.76 for Zn(II)). A similar trend is observed between the concentration of Cd(II) and Zn(II) extracted by *S. aquatica grandis* and the concentrations of the corresponding TE in the nutrient solution (r value of 0.61 for Cd(II) and of 0.53 for Zn(II)) (supplementary informations). These positive correlations showed a similar evolution than the concentration of Cd(II) and Zn(II) in the plant parts but the correlation coefficient was lower for TE extracted.

4. Discussion

4.1. Tolerance of *A. petiolata* and *S. aquatica grandis* to Cd(II) and Zn(II)

The hypothesis regarding the high tolerance of these two accumulator species to Cd(II) and Zn(II) is confirmed by measurement of plant growth parameters. In particular, *A. petiolata*, showed high tolerance to the presence of Cd(II) and Zn(II) in the nutrient solution, as the plants showed little or no effect (compared to the control) on most of the parameters when the lowest concentrations of TE were applied. These elements still have a negative impact, depending on their concentrations, that was stronger on *S. aquatica grandis* than on *A. petiolata*, especially for Cd(II) that only influenced *A. petiolata* for the highest Cd(II) concentrations tested (Cd3, Cd4, and Cd5). As *S. aquatica grandis* plants grow faster than *A. petiolata*, part of these differences can be explained by the fact that a modification in the growth of *S. aquatica grandis* would therefore be more easily visible, even statistically, than for *A. petiolata*. Nevertheless, it appears that *S. aquatica grandis* was more sensitive to these elements, as observed under the Zn5 treatment, which revealed severe growth inhibition. The water evaporation and TE accumulation of several *Salix* species growing on a Mn-, Fe- and S-contaminated site have been assessed by Malabad et al. (2022) and they reported that the species *S. aquatica grandis* had a high stomatal conductance and that it could explain the accumulation of Mn in this plant. Therefore, in our experiment, the high water flux of *S. aquatica grandis* could have increased its sensitivity to Cd(II) and Zn(II) through a higher uptake rate than in *A. petiolata*. *A. petiolata* has already been reported to easily grow and colonise sites contaminated with TE, such as Cd(II) and Zn(II)

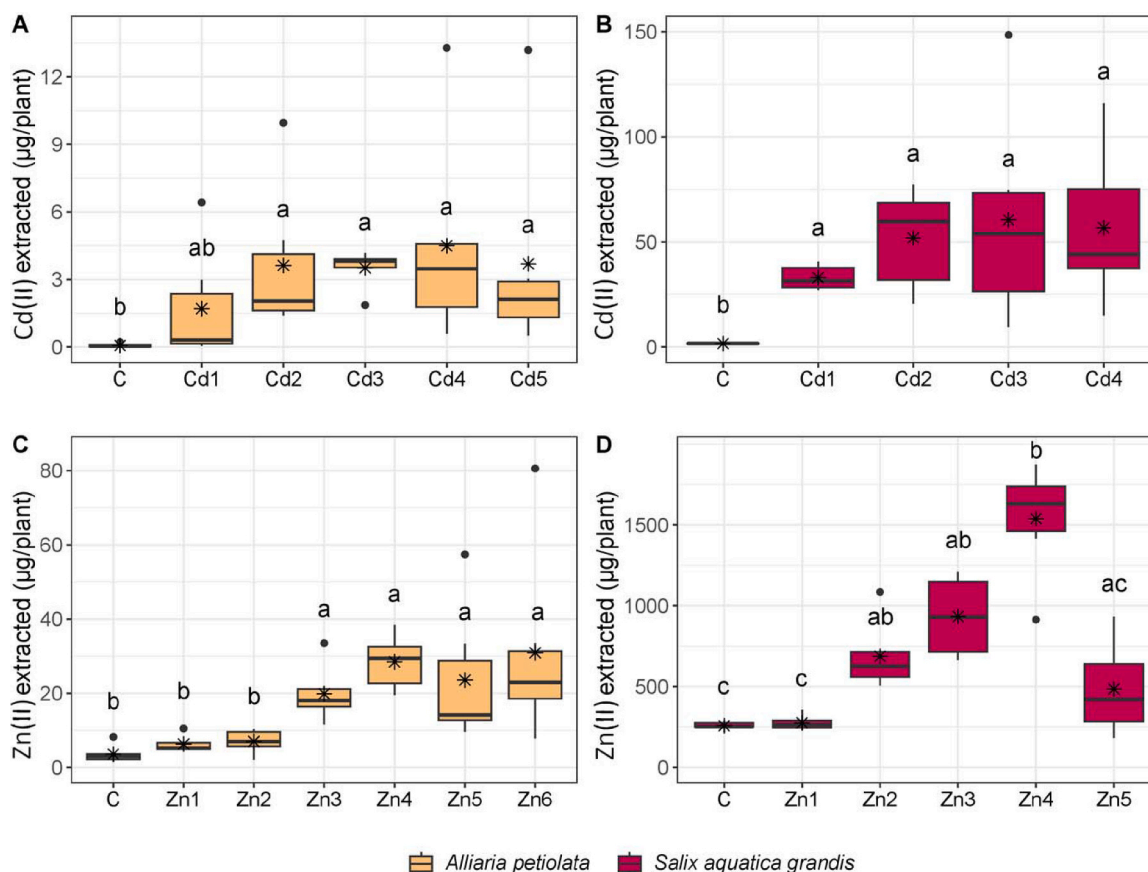


Fig. 4. Boxplots of Cd(II) (A and C) and Zn(II) (B and D) extracted from nutrient solution by *Alliaria petiolata* (yellow) and *Salix aquatica grandis* (red) at the end of the experiment, expressed as μg of element per plant ($n = 6$). The mean value of TE extracted is represented by stars in the boxplots. For each treatment, plant, and element, letters in common indicate statistically similar groups at $p < 0.05$ according to mean and post hoc pairwise comparison tests.

(Grabner et al., 2011; Collot et al., 2023). Other mechanisms could, therefore, occur in this species, explaining its high tolerance to Cd(II) and Zn(II), such as efficient sequestration of TE via the overexpression of genes encoding for metal transporters such as those from CDF or HMA families that are involved in the transport of Cd(II) and Zn(II) from the cytosol to the vacuole (Jan and Parray, 2016; Singh et al., 2016). These mechanisms are observed in hyperaccumulator species such as *Arabidopsis halleri* and *Noccaea caerulea*, enabling them to maximise their tolerance and the accumulation of TE in their aerial parts (Tibbett et al., 2021).

The exposure of plants to the highest concentrations of Cd(II) and Zn(II) led to a negative effect on both plants studied via reduction of dry biomass, Fv/Fm value, photosynthetic pigment concentrations, total leaf area, and, for *S. aquatica grandis*, root length, and height. The exposure of plants to high levels of TE has often been reported to have toxic effects on their growth through modification of the rate of cell division and elongation (Wani et al., 2018), disturbance of the photosynthesis process (Das et al., 2015) or decreases of photosynthetic pigments (Hajar et al., 2014). Behtash et al. (2024) work revealed similar observations during a hydroponic experiment on the species *Lactuca sativa* exposed to increasing concentrations of Cd. They observed a significant decrease in biomass produced and a decrease in Chl a, Chl b, and CAR concentrations for Cd(II) concentrations in the nutrient solution of 22 μM (between Cd4 and Cd5) and 44 μM (higher than Cd5). A similar experiment conducted by Ciriello et al. (2022) on two basil cultivars exposed to increasing concentrations of Zn(II) up to 50 μM (between Zn3 and Zn4) also illustrates the phytotoxicity of this element. Leaf area, total dry biomass, Fv/Fm value, and total chlorophyll concentration were generally reduced by the addition of Zn(II); however, the parameters affected and the level of toxicity were specific to each cultivar, as

observed in our experiment between the two species. Among the parameters measured in this study, the length of *A. petiolata* roots was the only parameter that did not vary for all the treatments tested, even when the root biomass of this plant was significantly reduced in the Zn3 to Zn6 treatments. This highlighted that this parameter does not appear relevant for assessing the metal stress to which the plant was exposed.

4.2. Accumulating capacities of *A. petiolata* and *S. aquatica grandis*

The concentrations of Cd(II) and Zn(II) in the different parts of the plants increased following the concentration in the nutrient solution as highlighted by the significant increase of TE concentration in plant parts and the positive correlation observed, which is a result of the higher availability of TE in solutions as observed in soils (Muszyńska and Labudda, 2019). However, a decrease in root BCF was observed, affecting leaf BCF, when TE levels in the nutrient solution increased, as already reported by Touati et al. (2019) when hydroponically growing *Salix alba* and *Salix viminalis* on increasing Cd(II) concentration. This phenomenon highlights a limit to the passage of Cd(II) and Zn(II) through the plant that could be explained by the saturation of the TE adsorbed onto the roots, that is the first step for their uptake (Song et al., 2017; Shahid et al., 2017), and therefore reducing the transfer rate from soil solution to roots, which led to a reduction in BCF. Indeed, the metal ions in the soil solution are adsorbed by the plant by exchanging with the H^+ at the root surface, before being absorbed by the plant. A high concentration of ions in the solution will saturate the exchange sites and limit their flow into the plant (Muszyńska and Labudda, 2019; Zhang et al., 2024). The inhibition of biomass observed on plants can also be involved in this phenomenon by reducing the amount of TE that can be adsorbed and therefore the rate of TE uptake by the plants.

Both species showed Cd(II) and Zn(II) accumulation in their above-ground tissues with similar Cd(II) concentrations whereas Zn(II) concentrations were higher in *S. aquatica grandis* than in *A. petiolata* for several treatments. The Cd(II) accumulation of *S. aquatica grandis* was comprised in the values observed for the leaf concentrations of several species of *Salix* by Dos Santos Utmazian et al. (2007), that ranged from 11.9 to 315 mg kg⁻¹ DW after 30 days of growth on a nutrient solution enriched with 4.45 µM (close to the treatment Cd3 in our experiment). Regarding Zn(II), *S. aquatica grandis* presented higher leaf concentration values than those reported by McBride et al. (2016) for several other *Salix* species growing on Zn(II) concentration of 200 µM (between Zn4 and Zn5 in our experiment) and which reached values ranging from 679 to 1020 mg kg⁻¹ DW, after 18 days of growth. Ciadamidaro et al. (2019) reported Cd(II) and Zn(II) concentrations in leaves of *S. aquatica grandis* that reached 120 and 2500 mg kg⁻¹ DW, respectively, for plants that grew for several years in TE multi-contaminated sites in field settings. All these values underlined that there is significant variability in Cd(II) and Zn(II) accumulation in the *Salix* genus. However, the values observed in our experiment showed that *S. aquatica grandis* accumulated these elements that were within the range of that observed in controlled and field conditions. For *A. petiolata*, few studies were conducted on its capacities to accumulate TE. However, Drozdova et al. (2019) reported that this species showed TF higher than 1 for Cd(II) and Zn(II) in contaminated sites, as observed in our experiment in C, Cd4, Zn3, and Zn4 treatments. Therefore, our results confirm the interest of *A. petiolata* for phytoextraction. Under field setting, Collot et al. (2023) found leaf concentrations of Cd(II) and Zn(II) that reached 18 and 1750 mg kg⁻¹ DW, respectively, in a TE multi-contaminated site. These values are difficult to compare with our data as they were observed in natural conditions. However, the Zn(II) concentrations values observed *in-situ* on older plants (over one-year-old) were higher than those observed in our hydroponics experiment, showing that exposure time is also an important factor that could enhance the extraction of this element by *A. petiolata*. Overall, for both *A. petiolata* and *S. aquatica grandis*, Cd(II) concentrations in leaves exceeded 100 mg kg⁻¹ DW depending on the treatment, which is the threshold value considered to qualify a plant as hyperaccumulator of Cd(II) (Krämer, 2010; Manara et al., 2020). The characterisation of a species as hyperaccumulator is only possible for values observed under natural conditions, which is not the case in our study. However, exceeding this value illustrates the interest of these two species as potential candidates for Cd(II) accumulation.

For *S. aquatica grandis*, the TF value for the different treatments reached a maximum (observed in Cd1 and in Zn3-Zn4) before falling back to a value equivalent to or even lower than that of the control. The same phenomenon is observed for *A. petiolata* exposed to Zn. This could be linked to the stress response of the plants that would favour the translocation of TE into the aboveground parts to maximise their sequestration in the plant and, therefore, its tolerance to these elements. Indeed, the accumulation of TE in the aboveground parts of the plant can reduce the stress linked to these contaminants, notably by mechanisms of chelation in the cytosol or sequestration in the vacuoles (Rascio and Navari-Izzo, 2011; Tibbett et al., 2021). The drop of TF values observed for highly concentrated treatments would therefore be due to the limit of this defence mechanism, which is unable to further increase the transport and sequestration of Cd(II) and Zn(II). The translocation of Cd(II) and Zn(II) is induced by heavy metal-transporting ATPases. These proteins are overexpressed in accumulator species, both in the roots and in the aerial parts, indicating an upregulation of translocation (Singh et al., 2016). Root damage linked to excessive metal stress could explain the drastic decrease in TF observed in our experiment. Indeed, TE stress leads to the production of reactive oxygen species (ROS) in plants. When these ROS accumulate, they target vital biomolecules such as DNA, lipids and proteins, altering cell functions and ultimately leading to cell death (Choudhary et al., 2020). Consequently, the accumulation of ROS can damage plant tissues, particularly roots where TE concentrations observed in our experiment were high, altering the pathway for ions

transport and therefore their translocation.

4.3. Extraction of Cd(II) and Zn(II)

Our study focused on the TE extraction of *A. petiolata* and *S. aquatica grandis* grown in hydroponic. It revealed that both species showed relevant capacities for Cd(II) and Zn(II) extraction as they showed increasing Cd(II) and Zn(II) accumulation when exposed to high concentrations of these elements in nutrient solution and, for *A. petiolata*, a higher tolerance. The extraction potential of Cd(II) and Zn(II) by *S. aquatica grandis* were in the ranges reported by Dos Santos Utmazian et al. (2007) for several other *Salix* species growing on Cd(II) (4.45 µM, similar to Cd3) or Zn(II) (76.5 µM, comprised between Zn3 and Zn4) for 30 days, with extraction values ranging from 5 to 170 µg/plant for Cd(II) and from 40 to 2410 µg/plant for Zn(II). On the other hand, our values were higher than those observed by Wang et al. (2016) on *Salix fragilis*, *Salix matsudana*, and *Salix babylonica* where Cd(II) extraction was comprised between 2.73 and 7.60 µg/plant after 28 days of growth on a cocktail of TE composed of 20 µM of Cd(II), Cu, Pb and Zn(II) (Cd(II) concentration twice higher than Cd4). However, these lowest values could be partially explained by the presence of a multi-contamination that could induce a stronger effect on plants (Alia et al., 2015) and that can reduce the accumulation of each single contaminant (Dos Santos Utmazian et al., 2007).

The biomass production and the accumulation of TE in the above-ground parts of the plants are two major parameters in the phytoextraction process (Mayerová et al., 2017) given that they directly influence the amount of contaminants extracted by the plants. Therefore, the increase in the concentration of Cd(II) and Zn(II) in the nutrient solution, had two opposite effects. First, it led to increases in the TE concentration in aboveground part of the plants that is a positive effect for phytoextraction purpose. However, it also led to a decrease in the biomass production (except for *A. petiolata* growing on Cd(II)) which can reduce the yield of phytoextraction. Additionally, the extraction of Cd(II) and Zn(II) increased rapidly under lower TE concentrations and then stabilized at the highest TE concentrations to a maximal value where the effect observed on biomass counterbalanced the increase of TE concentration in aboveground parts. These observations are supported by correlation analysis, which showed a positive correlation between TE concentrations in the nutrient solution and TE concentrations in plants that was greater than the correlation between TE concentrations in the nutrient solution and extracted TE concentrations. Furthermore, in the case of Zn(II) extraction by *S. aquatica grandis*, the inhibition of growth observed under the Zn5 treatment clearly impacted the extraction of this element compared to the previous treatment (Zn4) which exhibited the highest value. Similar observations were previously reported on soil experiments that aimed at increasing the TE availability by reducing soil pH and leading to higher TE accumulation but simultaneously impact the biomass of *Brassica juncea*, *Helianthus annuus*, *Salix viminalis* (Kayser et al., 2001) or *Zea mays* (Cui et al., 2004; Sun et al., 2020). However, except when reaching solubilised Zn(II) concentration equivalent to Zn5 (500 µM) for *S. aquatica grandis*, our study highlighted the possibility of improving extraction of Cd(II) and Zn(II) by both species studied when increasing concentrations in the nutrient solution. The initial assumptions assumed that both plants had a high tolerance to Cd(II) and Zn(II) and that it would be possible to increase the mass of extracted elements to a certain extent. These hypotheses were confirmed during our experiment, which identified an optimal concentration range that increases extraction from our plants, as long as sufficient above-ground biomass is produced. Consequently, optimising phytoextraction by increasing concentrations in the plant's environment is a method that requires carefully managing the balance between promoting the accumulation of elements by the plant and maintaining biomass production.

The comparison of both species in terms of Cd(II) and Zn(II) extraction showed that *S. aquatica grandis* presented higher values for these elements than *A. petiolata*. Given the low differences observed in

terms of concentration in aboveground parts of both species, the extraction is mainly influenced by the biomass production. Indeed, the studies conducted on *Salix* genus for phytoextraction purpose frequently reported an interest of these species because of their high biomass production (Varga et al., 2009; Cloutier-Hurteau et al., 2014). However, in the case of a field experiment, the planting density of *A. petiolata*, an herbaceous plant species, would be higher than *S. aquatica grandis* and could therefore compensate for this difference. In addition, these species could be used in a system of woody and herbaceous co-cropping, which has already shown promising results regarding the phytoextraction process by increasing either the TE accumulation or the biomass produced on a given area (Zeng et al., 2019; Lachapelle et al., 2020).

5. Conclusions

This study highlighted the tolerance, accumulation, and extraction capacities of a plant recently identified on contaminated sites as potentially suitable for phytoremediation, *A. petiolata*, and *S. aquatica grandis* for the extraction of Cd(II) and Zn(II). Both species showed an accumulation of Cd(II) and Zn(II) in their aboveground parts, which increased in parallel with the degree of exposure to each of these elements in the nutrient solution. It was assumed that both species would show significant tolerance to Cd(II) and Zn(II), however, we were able to demonstrate much higher tolerance in *A. petiolata*, particularly when exposed to Cd, as the plant showed only few symptoms of phytotoxicity at the maximum exposure level (30 μ M of Cd(II)) and maintained stable root and leaf biomass production. The main conclusions drawn from this study are that it is possible for both species to maximise the extraction of Cd(II) and Zn(II) when the concentrations in the nutrient solution are increased. However, this process has a species-specific limit related to each species tolerance and biomass production under TE stress conditions. These results therefore illustrate both the potential and the limitations of *A. petiolata* and *S. aquatica grandis* and pave the way for field phytoextraction trials to determine the performance of these species, particularly in a more complex environment with multi-contaminated sites, and the treatment times that will enable the possibility of using these species in the rehabilitation of contaminated sites to be assessed.

CRedit authorship contribution statement

CHALOT Michel: Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **ARSENOV Danijela:** Writing – original draft, Investigation, Conceptualization. **BINET Philippe:** Writing – original draft, Validation, Investigation, Conceptualization. **COLLOT Jordan:** Writing – original draft, Visualization, Investigation, Conceptualization.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2026.120074.

Data availability

Data will be made available on request.

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Glossary

- AAS: Atomic Absorption Spectroscopy
 BCF: Bioconcentration factor
 CAR: Carotenoids
Chl a: Chlorophyll a
Chl b: Chlorophyll b
 DW: Dry weight
 PSII: Photosystem II
 TE: Trace element
 TF: Translocation factor
 TI: Tolerance index