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ORIGINAL PAPER

Accumulation and toxic effects of chromium and zinc in *Iris pseudacorus* L.

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Abstract The aim of the present study was to examine the ability of *I. pseudacorus* L., an ornamental macrophyte of great potential for phytoremediation, to tolerate and accumulate Cr and Zn. Plants were grown in nutritive solution with $ZnCl_2$ or $CrCl_3 \cdot 6H_2O$ at 0, 10, 50, 100, and 200 μg ml^{-1} for 5 weeks; all survived and continued growing. The accumulation of Cr and Zn increased with increasing supply in all plant tissues, to reach 59.97 mg Cr and 25.64 mg Zn in roots. Leaves retained a remarkable amount of Zn (14.2 mg). Growth inhibition reached 65% and 31% (dry weight) in response to Cr and Zn, respectively. The root:shoot dry matter partitioning (*R*/*S*) increased 80% at 100 μg ml^{-1} $CrCl_3$. The most marked alterations in mineral content were in roots, where both metals decreased Al, Ca, Mg, Mn and S, and increased P concentration. No effect was noted on either leaf chlorophyll fluorescence kinetics (F_v/F_m and Φ_{PSII}), or photosynthetic pigment content, signifying that the light phase of photosynthesis was not impaired. Carbon isotope composition ($\delta^{13}C$) was only slightly heavier, indicating that the reduction of carbon fixation was not the main cause for growth decrease. This was attributed to the restricted mineral uptake and to the increased demand of carbohydrates of damaged roots. Biomass allocation to rhizomes (Cr) or roots (Zn) contributes to heavy metal tolerance by limiting transpiration and increasing metal–storing tissues and the surface for water and cation uptake. This species is a good candidate for Cr rhizofiltration and Zn phytoextraction.

Keywords Heavy metal · Abiotic stress · Toxicity · Phytoremediation · Macrophyte · Isotope

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Introduction

Fresh water resources have been steadily reduced in recent decades as a result of increasing human consumption, contamination, and climatic change. Anthropogenic pollution of water is currently a major environmental concern as it poses a serious hazard for humans and other organisms, and dramatically limits the uses of water. Among the toxic substances found in water bodies, heavy metals deserve special attention. They are highly toxic at low doses, strongly persistent in the environment and living tissues, and easily transferred to food chain. In addition, their monitoring and removal is costly. Cr and Zn, two of the most relevant heavy metals, are included in the US Environmental Protection Agency list of priority pollutants (USEPA 2005). Symptoms of Cr and Zn phytotoxicity include chlorosis, inhibited germination, stunted growth, reduced leaf number and area, reduced yield and flower production, inhibited photosynthesis, dysfunction of relevant enzymes, impaired nutrient uptake, plant wilting and altered water relations (Deng et al. 2006; Dhir et al. 2008; Prasad 2004; Shanker et al. 2005). The excess of metals has deleterious effects on the content and functionality of the photosynthetic pigments (Broadley et al. 2007; Shanker et al. 2005). This can be caused by the inhibition of the pigment synthesis (Prasad and Prasad 1987), the formation of metal-substituted chlorophylls of reduced functionality (Küpper et al. 1996), or the direct oxidative damage of the

pigments (Oláh et al. 2010). Several authors have reported damages on the reaction centres or the peripheral antennae complexes of PSII in response to high concentrations of metals (Janik et al. 2010; Vernay et al. 2007; Paiva et al. 2009). Todeschini et al. (2011) recently described the reduction of D1 and D2 expression in poplar exposed to high levels of Zn.

The phytoremediation of heavy metals by means of constructed wetlands constitutes a low cost, environmentally friendly alternative to conventional cleanup techniques (Salt et al. 1998). Furthermore, as metals accumulate mainly in roots, part of the biomass harvested from such wetlands has many potential uses in non-food industries. Some of these side-products that could yield substantial economic benefits for affected communities are biogas and compost (Malik 2007), fibres (Kuzovkina and Quigley 2005), and ornamental plants (Belmont and Metcalfe 2003). At present, few plant species with ornamental flowers have been evaluated for heavy metal removal in spite of their high market value. Several studies have revealed that *Iris lactea* var. *chinensis* (Fisch.) Koidz. Rank accumulates Cd in leaves and roots (Han et al. 2007), and *Lythrum salicaria* L. tolerates Pb (Uveges et al. 2002). However, greater research efforts are required to screen the performance of other suitable species.

Iris pseudacorus L. is native to Northern Africa, Western Asia and Europe, naturalized in Australia, New Zealand and North and South America, and cultivated worldwide as an ornamental plant. This plant displays a high rate of biomass production, tolerates polluted environments and is useful for water treatment purposes. Compared with *Acorus gramineus* Sol. in Aiton, *Acorus calamus* L., *L. salicaria* and *Reineckea carnea* (Andrews) Kunth, *I. pseudacorus* shows better performance in removing total nitrogen and phosphorus, COD, BOD, and heavy metals (Cr, Pb, Cd, Fe, Cu, and Mn) from sewage. In addition, it shows a high stress-tolerance response, which includes low lipid peroxidation, and increased proline levels and catalase activity (Zhang et al. 2007). *I. pseudacorus* plants exposed to high levels of Cd or Pb show decreased growth and chlorophyll content (Zhou et al. 2010), increased peroxidase, catalase, superoxide dismutase, and ascorbate peroxidase activity, and increased concentration of proline and malondialdehyde (Qiu and Huang 2008; Zhou et al. 2010). The roots are also able to form Cu nanoparticles in response to high levels of Cu (Manceau et al. 2008). *I. pseudacorus* shows a higher phenol concentration in roots than *Phragmites australis* (Cav.) Trin. ex Steud. and *Typha latifolia* L, which makes it more suitable for the treatment of metal polluted waters (Larue et al. 2010). However, there is insufficient information about heavy metal accumulation and distribution in *I. pseudacorus* and the effects of other metals on the parameters that condition biomass production, such as growth, chlorophyll synthesis, photosynthetic performance and plant nutritional status. These data are determinants in establishing the potential of this promising species for phytoremediation purposes. Here we assessed the physiological response of *I. pseudacorus* to a range of Cr or Zn concentrations, and evaluated the accumulation of these metals throughout the plant.

Materials and methods

Plant material and treatments

Iris pseudacorus L. plants were purchased from a local nursery (Bioriza, Breda, Spain) in 300-ml multipot containers holding a peat–perlite 50/50 substrate. Plants were then root-washed in tap water to remove the original substrate, weighed, and placed in a pure hydroponics system in individual 4-L pots containing diluted Hoagland nutritive solution at pH 6.5. This solution comprised 130.25 mg l^{-1} NO^{3-}, 5.5 mg l^{-1} NH^{4+}, 28.5 mg l^{-1} $PO_4{}^{2-}$, 35.5 mg l^{-1} K^{+}, 24.5 mg l^{-1} Ca^{2+}, 4 mg l^{-1} Mg^{2+}, 14.25 mg l^{-1} $SO_4{}^{2-}$, 0.325 mg l^{-1} Fe, 0.240 mg l^{-1} Mn, 0.09 mg l^{-1} Zn, 0.030 mg l^{-1} B, 0.090 mg l^{-1} Cu, 0.028 mg l^{-1} Mo, and 0.005 mg l^{-1} Co. After an acclimation period of 2 weeks, individual plants were selected within a small range of initial fresh weight (104.0 ± 5.2 g expressed as average ± standard error). The nutritive solution was then amended with $ZnCl_2$ or $CrCl_3{\cdot}6H_2O$ at 0, 10, 50, 100, and 200 μg ml^{-1}, which correspond to Zn ion concentrations of 0.07, 0.4, 0.7 and 1.5 mM, and to Cr ion concentrations of 0.04, 0.2, 0.4, and 0.8 mM. The Cr ion concentrations were approximately half those of Zn, to compensate for the higher toxicity of Cr(III) for plants (Hara and Sonoda 1979). Five replicates (plants) of each treatment were randomly distributed and grown under glasshouse conditions for 5 weeks in June and July. The average temperature was 36–18°C (day/night), the relative humidity 31–59%, the maximum global solar irradiance 1,353 W m^{-2}, and the transmission of the greenhouse covers 51%. Nutritive solution was renewed regularly.

In vivo measurements

Before collecting the plants, in vivo measurements were taken. Chlorophyll content on leaf area basis was measured at the base, centre and tip of four representative mature pre-bloom leaves per plant using a portable chlorophyll meter (SPAD-502 Minolta, Illinois, USA), following Krugh et al. (1994). A reading checker of 72.4 ± 0.3 was used to calibrate the apparatus. Chlorophyll fluorescence was measured with a modulated fluorometer (Hansatech

Fluorescence Monitoring System FMS2, Norfolk, UK) to obtain estimates of maximum quantum yield (F_v/F_m) after 30 min of dark adaptation and of relative quantum yield (Φ_{PSII}) measured at environmental light (Genty et al. 1989). Plants were then thoroughly washed in tap water, gently wrapped in absorbent paper to remove excess water and weighed to record the increase in biomass. Each plant was divided into leaves, rhizomes and roots, and each section was weighed separately. The underground organs were not desorbed to preserve the fraction of metal adsorbed to cell walls, which would also be collected after harvest in phytoremediation systems. A portion of each fresh sample was ground in liquid nitrogen and stored at −80°C until analysis. The remaining fresh sample was oven-dried at 60°C until constant weight, ground in an agate mortar and passed through a 0.05-mm sieve.

Photosynthetic pigment content

The chlorophyll and carotenoid concentration of leaves was measured on extracts of frozen leaf samples in 80% acetone. Pigment contents were calculated from absorbance at wavelengths 663.2, 646.8, and 470.0 nm, as described by Lichtenthaler (1987). The absorbance values were measured in the extracts by means of a UV-160 spectrophotometer.

Element composition

Two replicates of each frozen sample were digested overnight at 90°C in a HNO_3–H_2O_2 mixture 1:1 v/v. The Al, Ca, Cu, Fe, K, Mg, Mn, S and P content of the extracts was determined by inductively coupled plasma optical emission spectrometry (ICP-OES) and by inductively coupled plasma mass spectrometry (ICP-MS) using a Perkin Elmer Optima-3200RL and a Perkin Elmer Elan-6000 apparatus, respectively. A blank and a sample of aquatic plant (*Trapa natans* L, CRM 596) or sea lettuce (*Ulva lactuca* L, CRM 279) certified reference material from the Community Bureau of Reference (BCR®), were processed in the same way and analysed per 12 samples. Element content determination was performed in the technical services of the University of Barcelona (Serveis Científicotècnics). Ash content was determined by furnacing samples at 500°C for 6 h or until constant weight.

Stable isotope composition

For each plant, a sample of dried leaf, rhizome, and root tissue was ground into a fine powder, and 1 mg was weighed in tin cups. The total C and N content of samples was analysed using an Elemental Analyser (EA, Carlo Erba 2100, Milan, Italy), which was interfaced with an Isotope Ratio Mass Spectrometer (IRMS, Thermo-Finnigan Deltaplus Advantage, Bremen, Germany) to analyse $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios. Results were expressed as $\delta^{13}C$ and $\delta^{15}N$ values, using a secondary standard calibrated against Vienna Pee Dee Belemnite calcium carbonate (VPDB) for C, and air for N. Analytical precision was of 0.1‰. All analyses were undertaken at the Colorado Plateau Stable Isotope Laboratory (CPSIL, Northern Arizona University). $\delta^{13}C$ and $\delta^{15}N$ were calculated as:

$$\delta^{13}C = \left(\left(\frac{^{13}C/^{12}C_{sample}}{^{13}C/^{12}C_{standard}}\right) - 1\right) \times 1{,}000$$

$$\delta^{15}N = \left(\left(\frac{^{15}N/^{14}N_{sample}}{^{15}N/^{14}N_{standard}}\right) - 1\right) \times 1{,}000.$$

Statistical methods

ANOVA (analysis of variance) was performed on the basis of a one-factor design using SPSS (Statistical Package for the Social Sciences) version 14.0 for Windows. Logarithmic transformation was used when data did not meet the assumption of equal variances. Student–Newman–Keuls post hoc tests were performed to assess the differences between groups. Sigma Plot version 10.0 was used for graphic edition. Cluster analysis was done using Gene Cluster (Standford University, USA) on standardized averages, and distances between clusters were established by average linkage clustering.

Results

Biomass, water content and chlorophyll

Plant growth was strongly impaired by heavy metal stress. Both Cr and Zn decreased fresh weight increment (ΔFW) and dry weight increment (ΔDW) of the whole plant after 5 weeks of treatment (Table 1). The treatment with $ZnCl_2$ did not disturb plant growth at low concentrations, but at 100 μg ml^{-1} ΔFW and ΔDW fell dramatically (59 and 65%, respectively). The effect of $CrCl_3$ was gradual, to reach a decrease of 61% (ΔFW) and 44% (ΔDW) at 200 μg ml^{-1}.

To determine whether growth was equally inhibited in all plant organs, final fresh weight (FW) and final dry weight (DW) of leaves, rhizomes and roots were recorded separately (Table 1). Increasing Zn and Cr reduced the FW of leaves (Table 1) up to 48% (200 μg ml^{-1} $ZnCl_2$) and 56% (100 μg ml^{-1} $CrCl_3$). The FW and the DW of all organs showed strong decrease when supplied with high concentrations of metals, but these variations were only significant in leaves due to the high variability of the

Table 1 Growth reduction in response to metals

	Control	$ZnCl_2$ (μg ml^{-1})				$CrCl_3$ (μg ml^{-1})				% Sum of squares	
		10	50	100	200	10	50	100	200	Treatment	Error
Leaf											
FW	318.4 ± 43.7a	236.2 ± 55.8a	287.6 ± 38.5a	167.5 ± 44.5a	166.6 ± 39.8a	266.4 ± 27.7a	258.2 ± 47.5a	140.6 ± 42.6a	179.4 ± 27.9a	33.4*	66.6
DW	39.4 ± 7.0a	31.1 ± 7.9a	34.5 ± 4.3a	20.2 ± 6.1a	20.8 ± 5.8a	34.1 ± 4.3a	35.6 ± 7.6a	17.3 ± 6.4a	30.1 ± 5.0a	26.4	73.6
Rhizome											
FW	139.8 ± 14.0a	101.4 ± 18.5a	128.1 ± 12.4a	90.3 ± 17.4a	84.8 ± 17.4a	114.8 ± 10.1a	112.6 ± 15.4a	94.2 ± 14.8a	104.1 ± 19.2a	22.5	77.5
DW	11.1 ± 2.1a	7.6 ± 1.7a	10.1 ± 0.7a	7.6 ± 2.3a	7.0 ± 2.1a	10.0 ± 1.4a	9.9 ± 2.1a	9.0 ± 2.2a	12.6 ± 1.6a	17.4	82.6
Root											
FW	135.3 ± 16.1a	113.2 ± 24.3a	133.5 ± 5.4a	84.5 ± 19.0a	101.6 ± 18.1a	110.7 ± 11.0a	97.9 ± 16.1a	92.6 ± 21.3a	79.7 ± 22.3a	21.1	78.9
DW	6.0 ± 1.0a	4.7 ± 1.2a	6.0 ± 0.4a	3.5 ± 1.1a	4.7 ± 1.0a	4.7 ± 0.5a	4.7 ± 1.5a	4.1 ± 1.4a	4.0 ± 1.5a	10.7	89.3
Plant											
ΔFW	488.2 ± 55.9b	449.5 ± 32.6b	436.8 ± 42.7b	199.8 ± 27.8a	213.6 ± 50.7a	385.5 ± 31.5b	337.7 ± 69.9ab	315.3 ± 26.7ab	192.8 ± 16.8a	61.4***	38.6
ΔDW	46.5 ± 8.0b	43.9 ± 4.5b	40.2 ± 4.2b	16.3 ± 1.9a	17.5 ± 4.3a	38.1 ± 4.0ab	36.1 ± 9.3ab	32.2 ± 3.0ab	26.0 ± 2.7ab	49.6***	50.4

Values given are the mean of $n = 5$ replicates. Different letters indicate statistically different groups according to Student–Newman–Keuls post hoc test. % sum of squares corresponds to an ANOVA of eight degrees of freedom

FW final fresh weight, *DW* final dry weight, Δ*FW* increase of FW, Δ*DW* increase of DW. All are expressed g

* p value < 0.05; *** p value < 0.005

response. Nevertheless, it is of note that high Zn (100 or 200 μg ml^{-1} $ZnCl_2$) reduced DW up to 48% (leaves), 37% (rhizomes) and 22% (roots). In contrast, DW was 33% lower in roots and 24% lower in leaves, but 14% greater in rhizomes treated with high concentrations of Cr, than in controls. Water content (WC) calculated as a percentage was 5% lower in leaves and rhizomes treated with 200 μg ml^{-1} $CrCl_3$, and remained stable in all the other treatments (results not shown).

The biomass allocation was altered (Fig. 1) as a result of the marked reduction of leaf DW, which accounts for most of the total biomass of *I. pseudacorus*. The root:shoot dry matter partitioning (*R*/*S*) was affected from 100 μg ml^{-1} $CrCl_3$ upwards (Fig. 1b). ANOVA performed on the same data confirmed the effect of Cr treatment on *R*/*S* (p value = 0.03). The *R*/*S* was proportional to the external Zn concentration (Fig. 1a), but the tendency was not significant (p value = 0.18).

No significant effect of metal concentration in growth media was found on either F_v/F_m, Φ_{PSII} or photosynthetic pigment content. The means of plants treated with the highest metal concentrations scarcely differed from those of control plants (Table 2).

Metal concentration and extraction

Metal concentration in leaf, rhizome and root tissues increased with increasing concentration in the growth media, both for Cr and Zn treatments (Fig. 2). The roots achieved the highest concentrations (4.8 mg g^{-1} Zn and 10.1 mg g^{-1} Cr), followed by rhizomes (2.1 mg g^{-1} Zn and 0.7 mg g^{-1} Cr). Leaves showed a remarkable capacity to retain Zn, reaching 0.6 mg g^{-1}. Chromium concentration was lower than Zn concentration in leaves and rhizomes, but higher in roots.

Despite the reduction of plant growth at high metal concentrations (Table 1), the amount of Cr and Zn extracted (calculated as the amount of metal extracted per unit of biomass) continued to increase as a result of the rising concentration in tissues. Chromium extraction (Table 3) was greater in roots than in rhizomes and leaves. The portion extracted by leaves was very small. The average total extraction per individual plant at the maximum Cr supply was 70.6 mg. Similarly, Zn extraction was greater in roots than in rhizomes and leaves, which extracted a similar amount. The average total extraction per individual plant at the highest Zn supply was 56.8 mg, thus 24% lower than in the highest Cr treatment. This in spite of the Zn ion concentration supplied being twice as much as that of Cr (1.5 mM Zn vs. 0.08 mM Cr for 200 μg ml^{-1} treatments). However, the amount of Zn extracted by leaves was 10-fold that of Cr (1.4 mg Cr vs. 14.2 mg Zn).

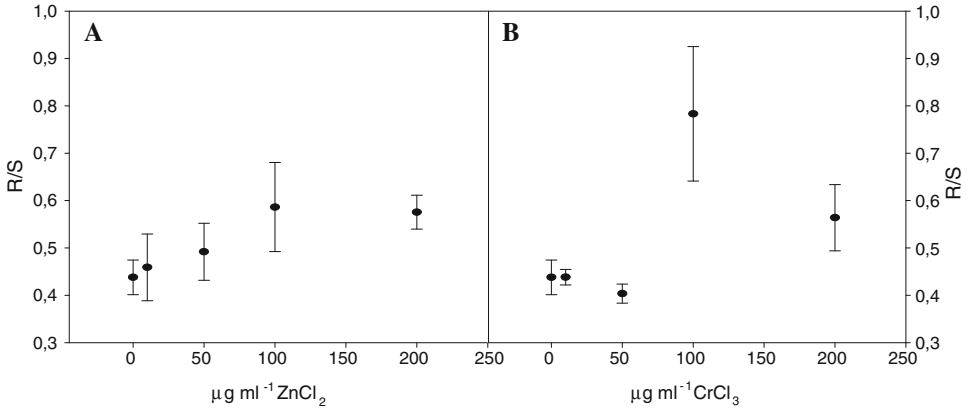

Fig. 1 Effect of treatments on biomass distribution ratio. Plants were grown in nutritive solution containing $ZnCl_2$ or $CrCl_3 \cdot 6H_2O$ at 0, 10, 50, 100, and 200 µg ml^{-1}. Root to shoot ratio (*R/S*) was calculated from final DW data, where "Shoot" designates the biomass of the emerged tissues, and "Root" the biomass of submerged tissues, with rhizomes and roots summed together. Values are the average of $n = 5$ replicates; *error bars* indicate the standard errors. The respective ANOVA p values were of 0.18 for **a**, and of 0.03 for **b**

Table 2 Chlorophyll fluorescence and content of photosynthetic pigments of controls and plants treated with the highest metal concentrations (200 µg ml^{-1} of the chloride salts)

	Control	Zn	Cr
Φ_{PSII}	0.75 ± 0.01	0.74 ± 0.02	0.76 ± 0.01
F_v/F_m	0.84 ± 0.00	0.84 ± 0.01	0.84 ± 0.01
Chl_a	0.83 ± 0.05	0.68 ± 0.14	0.85 ± 0.06
Chl_b	0.32 ± 0.02	0.28 ± 0.05	0.34 ± 0.03
$Chl_{(a+b)}$	1.16 ± 0.07	0.95 ± 0.19	1.19 ± 0.08
Car	0.19 ± 0.01	0.16 ± 0.03	0.21 ± 0.02
Chl_a/Chl_b	2.60 ± 0.04	2.42 ± 0.05	2.51 ± 0.04
$Chl_{(a+b)}$/Car	6.08 ± 0.11	5.63 ± 0.21	5.88 ± 0.18
SPAD	60.04 ± 2.90	56.10 ± 5.5	54.04 ± 3.36

Values are the average of $n = 5$ replicates ± standard error. Quantum yield of PSII photochemistry (Φ_{PSII}), maximum quantum yield (F_v/F_m) and SPAD values are dimensionless. Pigment content is displayed in mg g^{-1}. None of the treatments induced significant differences according to ANOVA (p values not shown)

C and N content and isotopic composition

In response to the addition of either of the two metals, but particularly Cr, leaves, rhizomes and roots became isotopically heavier (Table 4). However, the increment of $\delta^{13}C$ did not attain significance in rhizomes due to the high variability between samples. Increasing Cr also augmented the C/N ratio, more markedly in roots (32.4%) than in rhizomes (21.7%). In agreement, %N decreased 32.1% in roots and 19.4% in rhizomes of Cr-exposed plants. The %C and $\delta^{15}N$ remained stable (results not shown).

Element content

The trees generated from element content cluster analysis (Fig. 3) clearly separated controls from treatments, and Zn

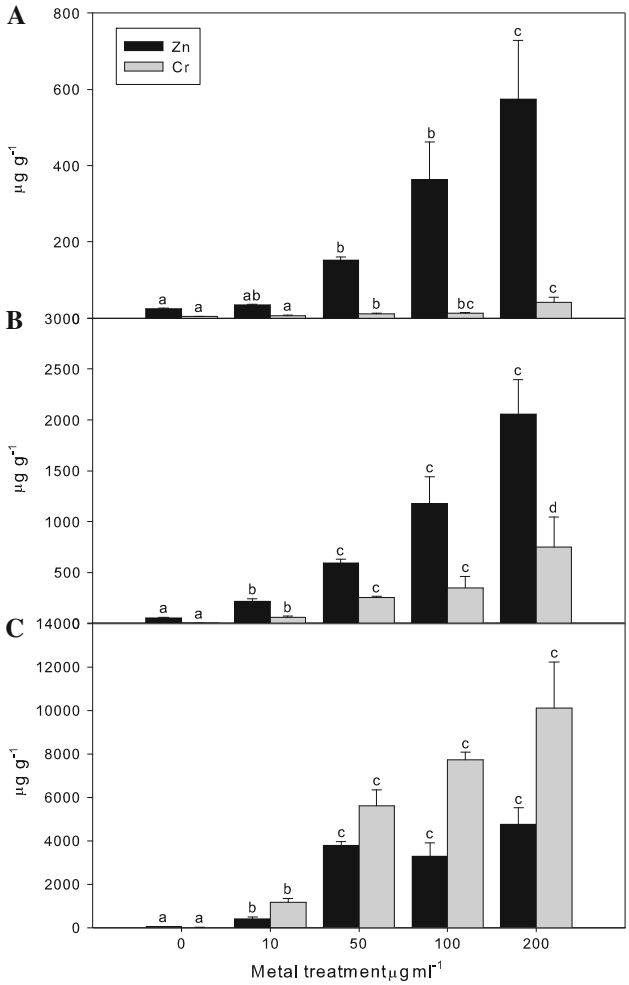

Fig. 2 Concentration of metals in *I. pseudacorus* leaves (**a**), rhizomes (**b**) and roots (**c**). Values are the average of $n = 5$ replicates. *Different letters* indicate significant differences between groups according to Student–Newman–Keuls post hoc test. Data transformation $\log(y + 1)$ was conducted to meet the equal variances assumption

Table 3 Accumulation of heavy metals in tissues, calculated as the metal concentration multiplied by the biomass of each plant section

	Control	Metal treatment (μg ml^{-1})				% Sum of squares	
		10	50	100	200	Treatment	Error
$ZnCl_2$							
Leaf	1.0 ± 0.2a	1.4 ± 0.1a	5.2 ± 0.7b	8.5 ± 0.5c	14.2 ± 1.1d	93.6***	6.4
Rhizome	0.6 ± 0.1a	2.2 ± 0.2ab	6.0 ± 0.6bc	9.1 ± 1.5c	17.0 ± 2.7d	81.1***	18.9
Root	0.3 ± 0.1a	2.7 ± 0.6a	22.7 ± 2.2b	16.8 ± 5.2b	25.6 ± 3.3b	75.8***	24.2
$CrCl_3$							
Leaf	0.1 ± 0.0a	0.2 ± 0.1a	0.3 ± 0.0a	0.4 ± 0.0a	1.4 ± 0.6b	45.1**	54.9
Rhizome	0.1 ± 0.0a	0.6 ± 0.1a	2.0 ± 0.1a	4.7 ± 1.6ab	9.3 ± 4.1b	42.8**	57.2
Root	0.0 ± 0.0a	5.6 ± 1.1a	17.9 ± 3.1ab	38.6 ± 8.7ab	59.9 ± 24.8b	46.7**	53.3

Values are means of $n = 5$ replicates, expressed in mg. Different letters indicate significant differences between groups according to Student–Newman–Keuls test. % of the sum of squares corresponds to an ANOVA of four degrees of freedom

** p value < 0.01; *** p value < 0.005

from Cr treatments. Except for leaves, treatments with high concentrations of metals (100 and 200 μg ml^{-1}) were closer to each other than to those applying low concentrations (10 and 50 μg ml^{-1}), which grouped together.

Both Cr and Zn stress induced diverse changes in the elemental composition and ash content (m_a) of plants (Tables 5, 6, 7). Chromium and Zn stress had a similar effect on some elements. Both caused an increment in Mn (leaves), and P content (roots), together with reduced Al (rhizomes) and Cu (rhizomes and leaves) content and Al, S, Mn, Mg and Ca content (roots). The other effects detected were of an opposite sign under Cr and Zn stress. In leaves, Cr decreased Fe, S, and Ca content, whereas Zn increased P and Ca. The quantification of Mg, Fe, S, and Al in leaves in the 100 μg ml^{-1} $ZnCl_2$ treatment was inconsistent with the other Zn treatments, and must thus be interpreted with caution. In rhizomes, high Cr diminished the concentration of Ca, Fe, Mg and K, whereas Zn had the opposite effect on Ca and K. In roots, high Zn decreased Fe, whereas all the Zn treatments increased Cu and K. High Cr decreased Cu and K.

The response of m_a to Zn varied. This parameter increased in leaves (Table 5) while in rhizomes and roots (Tables 6, 7) it decreased; however, none of these deviations were higher than 12%. In contrast, Cr decreased the m_a of leaves (5.4%), rhizomes (38.2%) and roots (23.7%).

Discussion

Iris pseudacorus was highly tolerant to Zn and Cr stress, as all plants survived the high metal concentrations supplied. Both metals were accumulated preferentially in roots, especially Cr, but Zn was also exported to leaves, in agreement with the literature (Deng et al. 2006; Mazej and Germ 2009; Qian et al. 1999). According to the definition by Baker and Brooks (1989), a plant must concentrate Cr to a minimum 1,000 μg g^{-1}, and Zn to a minimum 10,000 μg g^{-1} in its leaves to be considered a hyperaccumulator. These levels are much higher than that observed in the present experiment. Samecka-Cymerman and Kempers (2001) analysed leaves of *I. pseudacorus* naturally growing in a Polish anthropogenic lake with 120 μg g^{-1} Cr and 11 μg g^{-1} Zn in the sediment. These levels are comparable to our treatments with 10–50 μg ml^{-1} $ZnCl_2$ (which contain 4.8–24.0 μg ml^{-1} Zn, respectively), but are approximately threefold our highest Cr treatment (39.0 μg ml^{-1} Cr). The concentration of Zn in leaves in those conditions reached 21 μg g^{-1}, which is comparable to our 35 μg g^{-1} at 10 μg ml^{-1} $ZnCl_2$. However, the concentration of Cr was clearly inferior, only 4 μg g^{-1} versus our 41 μg g^{-1}. This discrepancy is possibly the result of the limited solubility of Cr in lake sediments, as described by Polyák and Hlavay (1999).

Our results demonstrated a specific response of the biomass acquisition and water content of plant sections to Zn and Cr, owing to their being essential and nonessential nutrient, respectively. The pattern shown by Zn-treated plants is consistent with the typical growth response to essential nutrients, which enhance growth at a sub-optimal or optimal concentration, but become toxic above a critical level (Marschner 1995). This threshold would lie between 50 and 100 μg ml^{-1} $ZnCl_2$ for *I. pseudacorus*. In contrast, Cr is a nonessential element that promotes growth only at very low doses (Bonet et al. 1991). At the Cr concentrations used in our study, growth was not enhanced but gradually inhibited as the external Cr concentration increased.

Table 4 Isotopic composition and total content of C and N

	Control	$ZnCl_2$ (μg ml^{-1})				$CrCl_3$ (μg ml^{-1})				% Sum of squares	
		10	50	100	200	10	50	100	200	Treatment	Error
Leaf											
$\delta^{13}C$	−29.6 ± 0.1a	−29.1 ± 0.2a	−29.2 ± 0.1a	−29.0 ± 0.4a	−28.5 ± 0.1a	−29.5 ± 0.3a	−29.0 ± 0.3a	−28.4 ± 0.1a	−28.4 ± 0.4a	40.1*	59.9
%N	2.7 ± 0.1a	2.5 ± 0.1a	2.7 ± 0.1a	2.5 ± 0.2a	2.4 ± 0.2a	2.7 ± 0.1a	2.5 ± 0.1a	2.6 ± 0.1a	2.2 ± 0.1a	27.4	72.6
C/N	14.8 ± 0.6a	15.9 ± 0.4a	14.6 ± 0.4a	15.9 ± 1.3a	16.9 ± 1.2a	15.0 ± 0.5a	16.2 ± 0.9a	15.9 ± 1.0a	18.6 ± 1.2a	29.6	70.4
Rhizome											
$\delta^{13}C$	−28.7 ± 0.1a	−27.9 ± 0.4a	−28.2 ± 0.2a	−27.9 ± 0.5a	−27.8 ± 0.2a	−28.5 ± 0.3a	−27.8 ± 0.3a	−27.5 ± 0.1a	−27.4 ± 0.5a	26.4	73.6
%N	3.1 ± 0.3a	3.3 ± 0.3a	3.0 ± 0.1a	3.3 ± 0.1a	3.2 ± 0.3a	3.0 ± 0.2a	3.0 ± 0.1a	3.0 ± 0.2a	2.5 ± 0.1a	26.1	73.9
C/N	12.6 ± 1.1ab	11.6 ± 0.9a	12.7 ± 0.4ab	11.6 ± 0.5a	12.4 ± 1.5ab	13.2 ± 0.8ab	12.9 ± 0.4ab	13.0 ± 0.9ab	16.1 ± 0.7b	33.9*	66.1
Root											
$\delta^{13}C$	−28.5 ± 0.1a	−28.1 ± 0.2a	−28.0 ± 0.1a	−27.9 ± 0.3a	−27.5 ± 0.1a	−28.4 ± 0.4a	−27.5 ± 0.2a	−27.6 ± 0.2a	−27.5 ± 0.3a	38.7*	61.3
%N	2.8 ± 0.3ab	2.7 ± 0.2ab	2.6 ± 0.2ab	2.7 ± 0.3ab	2.5 ± 0.2ab	2.9 ± 0.2b	2.7 ± 0.1ab	2.3 ± 0.2ab	1.9 ± 0.1a	31.6	68.4
C/N	14.3 ± 1.2ab	14.8 ± 1.9ab	14.8 ± 0.8ab	15.3 ± 2.4ab	16.5 ± 2.2ab	12.9 ± 1.1a	14.3 ± 1.2ab	17.0 ± 1.2ab	21.0 ± 1.3b	33.4*	66.6

Plants were grown in nutritive solution containing $ZnCl_2$ or $CrCl_3{\cdot}6H_2O$ at 0, 10, 50, 100, and 200 μg ml^{-1}. Values are means of $n = 5$ replicates. Different letters indicate significant differences between groups according to Student–Newman–Keuls post hoc test. % of the sum of squares corresponds to an ANOVA of eight degrees of freedom

* p value < 0.05

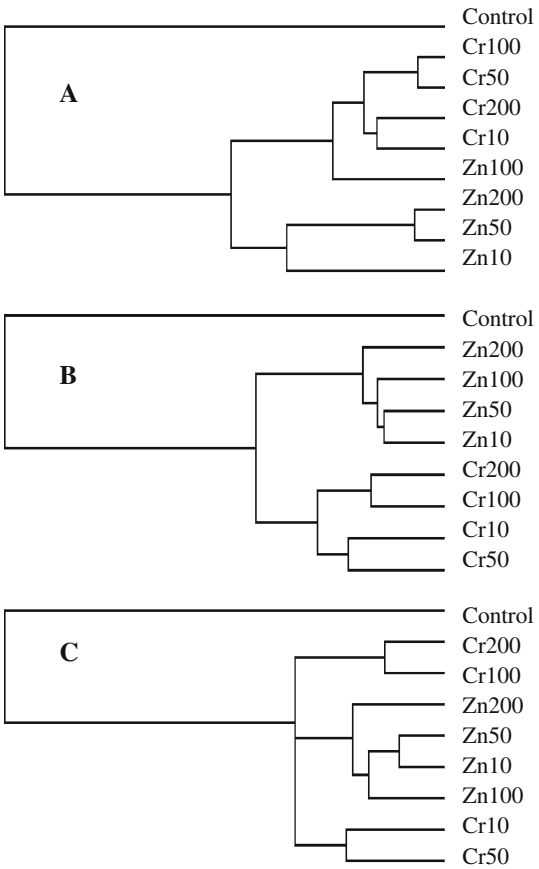

Fig. 3 Cluster analysis for the mineral content of leaves (**a**), bulbs (**b**) and roots (**c**). Elements analysed were Al, Ca, Cu, Fe, K, Mg, Mn, S and P. Plants were grown in nutritive solution containing $ZnCl_2$ or $CrCl_3{\cdot}6H_2O$ at 0, 10, 50, 100, and 200 μg ml^{-1}. Values were the average of $n = 5$ replicates

The greater growth reduction observed in Zn-treated leaves is coherent with the higher amount of Zn transported to leaves. Zinc molar concentration is also twice as high as that of Cr in equivalent treatments (1.5 vs. 0.8 mM at 200 μg ml^{-1}). The reduction of growth caused by Cr was due both to poor dry matter acquisition, which affected the roots more severely, and to reduced plant water content. Both effects are derivable from root damage and consistent with the preeminent role of roots in Cr retention and the restricted exportation of this metal to leaves. The decrease in m_a in Cr-stressed roots was lower than expected; most probably because the high amounts of Cr accumulated there (Table 3) partially compensated the decrease of other elements. A similar process might have occurred in Zn-stressed plants.

There is ample evidence of the deleterious effects of Cr and Zn at various stages of photosynthesis and biosynthesis of chlorophyll (Ali et al. 2006; Chandra and Kulshreshtha 2004; Küpper et al. 1996; Oláh et al. 2010; Prasad and Strzałka 2002; Todeschini et al. 2011). Nevertheless, our

Table 5 Elemental composition of leaves

	Control	$ZnCl_2$ (μg ml^{-1})				$CrCl_3$ (μg ml^{-1})				% Sum of squares	
		10	50	100	200	10	50	100	200	Treat (df8)	Error
Al	29.0 ± 4.2a	30.4 ± 5.4a	28.3 ± 4.1a	16.6 ± 2.2a	32.4 ± 3.7a	16.4 ± 2.3a	23.1 ± 4.2a	23.5 ± 3.3a	24.4 ± 5.8a	30.6	69.4
Cu	5.7 ± 0.6a	5.0 ± 0.6a	5.2 ± 0.4a	3.8 ± 0.5a	4.7 ± 0.5a	3.8 ± 0.4a	3.6 ± 0.3a	3.5 ± 0.2a	4.4 ± 0.7a	34.2*	65.8
Fe	65.2 ± 8.7a	76.1 ± 22.3a	65.8 ± 2.6a	48.5 ± 11.2a	65.8 ± 8.2a	59.4 ± 6.8a	66.6 ± 8.4a	55.7 ± 3a	54.7 ± 9.7a	12.0	88.0
Mn	47.4 ± 5.9a	55.8 ± 11.9a	104.5 ± 9b	108.1 ± 16.6b	87.9 ± 13ab	52.0 ± 6.7a	60.3 ± 6.0a	53.9 ± 3.8a	62.3 ± 15.1a	51.8***	48.2
Ca	11.8 ± 0.8ab	12.5 ± 0.9ab	14.3 ± 0.4ab	10.8 ± 1.7ab	15.8 ± 1.7b	11.4 ± 1.7ab	12.7 ± 1.2ab	12.5 ± 0.6ab	9.6 ± 1.6a	31.0	69.0
K	41.4 ± 1.4a	44.3 ± 6.1a	49.1 ± 1.8a	46.7 ± 6.1a	48.5 ± 6.1a	43.7 ± 0.6a	45.8 ± 3.1a	50.1 ± 3.0a	46.8 ± 2.5a	9.8	90.2
Mg	2.4 ± 0.2a	2.3 ± 0.4a	2.3 ± 0.1a	2.1 ± 0.3a	2.9 ± 0.2a	2.1 ± 0.1a	2.2 ± 0.1a	2.3 ± 0.1a	2.1 ± 0.0a	23.4	76.6
P	5.2 ± 0.5a	4.8 ± 0.7a	5.9 ± 0.6a	5.8 ± 0.7a	6.2 ± 0.4a	5.4 ± 0.2a	4.9 ± 0.1a	4.5 ± 0.3a	4.8 ± 0.8a	21.4	78.6
S	2.3 ± 0.4a	2.2 ± 0.2a	2.5 ± 0.3a	1.9 ± 0.1a	2.4 ± 0.2a	2.1 ± 0.3a	2.1 ± 0.2a	2.2 ± 0.3a	1.9 ± 0.2a	12.8	87.2
m_a	12.8 ± 0.5ab	13.5 ± 0.7ab	14.0 ± 0.3ab	13.8 ± 0.4ab	14.3 ± 0.7b	12.4 ± 0.2ab	12.6 ± 0.3ab	12.8 ± 0.4ab	12.1 ± 0.4a	37.2*	62.8

Plants were grown in nutritive solution containing $ZnCl_2$ or $CrCl_3 \cdot 6H_2O$ at 0, 10, 50, 100, and 200 μg ml^{-1}. Values are means of $n = 5$ replicates, provided in μg g^{-1} (Al, Cu, Fe and Mn) or mg g^{-1} (Ca, K, Mg, P, and S), m_a is the ash content of the dry matter in percentage. Different letters indicate significant differences between groups according to Student–Newman–Keuls post hoc test. % of sum of squares corresponds to an ANOVA with eight degrees of freedom

* p value < 0.05; *** p value < 0.005

Table 6 Element composition of rhizomes

	Control	$ZnCl_2$ (μg ml^{-1})				$CrCl_3$ (μg ml^{-1})				% Sum of squares	
		10	50	100	200	10	50	100	200	Treat (df8)	Error
Al	61.1 ± 12.0a	50.4 ± 8.4a	40.7 ± 8.7a	43.8 ± 8.5a	24.2 ± 13.1a	74.0 ± 18.8a	53.2 ± 5.0a	53.8 ± 13.7a	29.1 ± 5.9a	29.6	70.4
Cu	15.2 ± 2.2a	18.4 ± 3.4a	15.7 ± 3.6a	11.9 ± 2.4a	9.3 ± 1.7a	9.5 ± 1.4a	8.6 ± 0.6a	11.3 ± 3.1a	10.0 ± 2.4a	29.6	70.4
Fe	84.6 ± 6.6a	78.5 ± 4.1a	58.9 ± 8.7a	75.5 ± 10.5a	109.5 ± 30.0a	109.6 ± 21.2a	131.0 ± 35.9a	94.9 ± 21.1a	57.0 ± 12.8a	25.9	74.1
Mn	24.1 ± 0.7ab	24.1 ± 3.3ab	26.9 ± 4.0ab	36.8 ± 4.3ab	24.7 ± 2.3ab	41.5 ± 7.2b	25.3 ± 8.0ab	17.1 ± 5.7a	24.1 ± 5.2ab	32.5	67.5
Ca	13.9 ± 1.4ab	17.2 ± 0.8b	14.6 ± 1.2ab	17.0 ± 2.4b	17.9 ± 4.1b	14.3 ± 1.3ab	11.9 ± 0.9ab	13.1 ± 2.4ab	6.8 ± 0.9a	39.0*	61.0
K	39.4 ± 7.0a	48.0 ± 1.7a	44.2 ± 3.1a	49.1 ± 4.3a	43.4 ± 3.9a	43.1 ± 4.1a	43.1 ± 2.8a	33.2 ± 1.0a	31.1 ± 7.6a	29.4	70.6
Mg	2.6 ± 0.1ab	2.9 ± 0.1b	2.5 ± 0.3ab	2.7 ± 0.5ab	2.5 ± 0.7ab	2.2 ± 0.2ab	1.9 ± 0.2ab	2.1 ± 0.3ab	1.2 ± 0.2a	34.1*	65.9
P	6.2 ± 0.7a	8.6 ± 0.4a	7.1 ± 0.7a	7.1 ± 0.5a	7.2 ± 0.4a	6.9 ± 0.4a	6.6 ± 0.5a	7.2 ± 0.7a	6.6 ± 0.7a	24.0	76.0
S	2.8 ± 0.3a	3.1 ± 0.2a	2.8 ± 0.2a	2.5 ± 0.2a	3.2 ± 0.3a	2.4 ± 0.1a	2.8 ± 0.3a	2.8 ± 0.3a	2.3 ± 0.3a	23.0	77.0
m_a	13.6 ± 1.1b	13.0 ± 1.0ab	13.5 ± 0.7b	12.2 ± 1.2ab	12.5 ± 1.3ab	10.8 ± 0.6ab	11.9 ± 0.6ab	9.4 ± 1.3ab	8.4 ± 1.2a	40.6***	59.4

Plants were grown in nutritive solution containing $ZnCl_2$ or $CrCl_3 \cdot 6H_2O$ at 0, 10, 50, 100, and 200 μg ml^{-1}. Values are means of n = 5 replicates, provided in μg g^{-1} (Al, Cu, Fe and Mn) or mg g^{-1} (Ca, K, Mg, P, and S), m_a is the ash content of the dry matter in percentage. Different letters indicate significant differences between groups according to Student–Newman–Keuls post hoc test. % of sum of squares corresponds to an ANOVA with eight degrees of freedom

* p value < 0.05; *** p value < 0.005

Table 7 Element composition of roots

	Control	$ZnCl_2$ (μg ml^{-1})				$CrCl_3$ (μg ml^{-1})				% Sum of squares	
		10	50	100	200	10	50	100	200	Treat (df8)	Error
Al	328.4 ± 47.5b	121.5 ± 39.5a	108.2 ± 19.8a	202.4 ± 22.2ab	130.6 ± 7.4a	188.3 ± 16.1ab	246.6 ± 49.9ab	236.5 ± 46.5ab	209.8 ± 32.2ab	48.0***	52.0
Cu	18.4 ± 2.8a	23.4 ± 6.5a	25.1 ± 3.4a	28.6 ± 3.7a	23.2 ± 2.4a	18.1 ± 2.9a	17.6 ± 2.8a	15.9 ± 3.7a	17.2 ± 2.8a	24.5	75.5
Fe	420.6 ± 53.2a	488.4 ± 59.9a	479.3 ± 78.7a	495.4 ± 79.1a	282.3 ± 25.2a	378.2 ± 54.4a	466.6 ± 73.3a	479.9 ± 112.5a	460.8 ± 66.8a	17.7	82.3
Mn	143.8 ± 24.3ab	141.4 ± 31.9ab	58.3 ± 12.3a	92.1 ± 21.6a	56.4 ± 2.7a	172.5 ± 41.1ab	265.8 ± 58.3b	158.0 ± 47.9ab	39.8 ± 5.0a	51.7***	48.3
Ca	9.1 ± 0.4ab	9.9 ± 1.3b	8.0 ± 0.6ab	7.2 ± 0.8ab	5.2 ± 0.5a	9.7 ± 1.8b	9.0 ± 0.9ab	8.7 ± 1.2ab	5.2 ± 0.1a	42.5**	57.5
K	38.2 ± 3.4ab	48.2 ± 4.0ab	46.5 ± 6.7ab	44.3 ± 7.2ab	60.3 ± 1.4b	39.3 ± 3.3ab	45.0 ± 5.7ab	30.1 ± 6.7a	30.2 ± 9.2a	37.1*	62.9
Mg	3.2 ± 0.2d	2.3 ± 0.3bc	2.6 ± 0.3 cd	1.6 ± 0.3ab	1.2 ± 0.1a	2.4 ± 0.2bc	1.7 ± 0.2ab	1.6 ± 0.2ab	0.9 ± 0.1a	70.2***	29.8
P	4.1 ± 0.2a	5.9 ± 0.5abc	5.3 ± 0.8ab	7.0 ± 0.8bc	8.0 ± 0.3c	5.9 ± 0.1abc	7.6 ± 0.2bc	6.2 ± 0.7abc	6.7 ± 0.7bc	52.3***	47.7
S	6.2 ± 1.0b	4.9 ± 0.4ab	6.1 ± 0.6b	2.9 ± 0.1a	4.1 ± 0.8ab	4.8 ± 0.5ab	4.5 ± 0.3ab	3.8 ± 0.3ab	3.5 ± 0.5a	45.1***	54.9
m_a	13.5 ± 0.8ab	15.5 ± 0.5b	14.7 ± 1.2ab	12.2 ± 1.2ab	12.9 ± 1.3ab	13.5 ± 0.8ab	12.1 ± 1.1ab	11.7 ± 1.0ab	10.3 ± 1.2a	34.2*	65.8

Plants were grown in nutritive solution containing $ZnCl_2$ or $CrCl_3{\cdot}6H_2O$ at 0, 10, 50, 100, and 200 μg ml^{-1}. Values are means of $n = 5$ replicates, provided in μg g^{-1} (Al, Cu, Fe and Mn) or mg g^{-1} (Ca, K, Mg, P, and S), m_a is the ash content of the dry matter in percentage. Different letters indicate significant differences between groups according to Student–Newman–Keuls post hoc test. % of sum of squares corresponds to an ANOVA with eight degrees of freedom

* p value < 0.05; ** p value < 0.001; *** p value < 0.005

results showed no harmful effect of Cr or Zn either on photosynthetic pigment content or on chlorophyll fluorescence. This observation implies that the efficiency of the light phase of photosynthesis was preserved. Despite the high concentration of metals supplied, PSII appeared fully functional: Φ_{PSII} values were high, in agreement with the low intensity of environmental light, and F_v/F_m values were optimal. F_v/F_m is widely accepted as a rigorous measure of photo-inhibition, whereas Φ_{PSII} is a measure of photochemistry, which is related to electron transport (effective quantum yield) and thus to photosynthesis (Maxwell and Johnson 2000). The correlation of Φ_{PSII} with CO_2 fixation in C_3 plants is not always linear, but can be modified depending on the electron fractionation between photosynthesis and photorespiration (Krall and Edwards 1992). Dhir et al. (2008) studied the photosynthetic performance of *Salvinia natans* L. in response to Cr and Zn stress, and reported that pigment content and RuBisCo activity was decreased by both metals, while F_v/F_m was decreased only by Zn. A reduction in CO_2 fixation as a result of heavy metal stress is therefore not necessarily reflected in chlorophyll fluorescence, and a decay of assimilation cannot be excluded from our results, even if pigment content was not affected.

The correlation of $\delta^{13}C$ with intercellular CO_2 concentration (C_i) has been extensively demonstrated (Farquhar 1983). The mild increase of $\delta^{13}C$ in response to the high metal concentrations supplied indicates that the stomatal aperture was restricted to some extent, which could affect intrinsic CO_2 fixation. However, the changes in $\delta^{13}C$ were too subtle, in our opinion, to be the only cause of the notable reduction in growth detected. Wei et al. (2008) observed that $\delta^{13}C$ was slightly affected by Cd exposure in mangrove (*Aegiceras corniculatum* (L.) Blanco) and roots were more sensitive than leaves. These authors observed that $\delta^{13}C$ also differed between plant parts, with assimilating organs showing lower values than non-assimilating or storage organs. In our study, leaves were isotopically lighter than rhizomes and roots, but $\delta^{13}C$ was equally responsive in all tissues. The $\delta^{13}C$ values were between −30.58 and −25.88‰, which is within the range of C_3 plants (Boutton et al. 1998). Average values across all plant parts and growing conditions ranged from 29.5 to −27.4‰, which again is usual for C_3 plants. The WC and m_a reduction noted in response to Cr is therefore best attributed to other causes than the subtle inhibition of transpiration, such as restrained water and nutrient uptake induced by severe root damage. Water uptake is directly connected to the deposition and absorption of minerals (Bakker and Elbersen 2005).

Zinc and Cr disturbed not only plant growth, but also biomass allocation. The most plausible explanation for this is a source-to-sink carbohydrate relocation from leaves to

non-assimilating tissues. The growth decrease per plant section was ranked leaf > rhizome > root, which caused the constant increase of *R*/*S* with increasing Zn concentration. This finding is consistent with the Zn accumulation pattern and suggests that roots were the most relevant sink tissue. In contrast, although roots accumulated higher amounts of Cr and showed a stronger growth inhibition, *R*/*S* increased abruptly at high Cr treatments as a result of the weight increase of the rhizomes. This observation points to rhizomes as the most demanding sink tissue in Cr-stressed plants. The roots treated with Cr accumulated a significantly higher amount of metal and showed more symptoms of damage, which may explain a restricted unload of carbohydrates.

The carbohydrate requirements of the roots and rhizomes of a plant under heavy metal stress might increase as a result of active detoxification mechanisms, such as ROS scavenging, compartmentalization, damage repair, cell wall thickening, or the synthesis of secondary metabolites. Some authors report increased dark respiration and ATP in response to heavy metal exposure, which may exert a protective role (Pavlovič et al. 2006; Romanowska et al. 2002). This notion is also in agreement with the reduced growth and increased P concentration, especially in roots, where the demand for ATP in order to neutralize the negative effects of excess Zn or Cr should be most augmented. P is assimilated as ATP, the chemical energy storage of the cell. The decrease in P content in Cr-treated leaves could be interpreted as relocation to P-demanding roots. Stobrawa and Lorenc-Plucińska (2007) found no evidence of increased respiration in the fine roots of *Populus nigra* L. growing in a site polluted by multiple metals; however, sucrose breakdown was activated and the level of soluble carbohydrates lowered. Those authors proposed that sucrose is used for the synthesis of cell wall polysaccharides (callose or cellulose) or secondary metabolites.

A high *R*/*S* has also been described in response to nutrient or water deficiency (Hermans et al. 2006; Price et al. 2002), as a tolerance mechanism that reduces transpiration and redirects carbohydrates to increase the surface available for water and nutrient uptake. Chromium (III) and Zn are passively taken up and retained by cation exchange sites in cell walls (Marschner 1995; Skeffington et al. 1976). An excessive concentration of these metals can compete with other polyvalent cations such as Mg, Mn, and Ca for the formation of coordination complexes, and induce mineral nutrient deficiency. This would explain the decreased levels of Al, Mg, Mn, and Ca in Zn-treated roots and of Al, Mg, Mn, Ca, and K in Cr-treated roots in our study, and is in agreement with the response of m_a. Increased P concentration and decreased S concentration have been described in cauliflower plants under Cr stress (Chatterjee and Chatterjee 2000). Sulphur is absorbed by plant roots as sulphate by means of a proton symporter (like nitrate and phosphate), and stored in vacuoles (Buchner et al. 2004). Sulphur may be displaced from metal-occupied vacuoles of roots and rhizomes, where Cr, Zn and other heavy metals are compartmentalized to prevent their toxic effects on cell metabolism. This notion is coherent with previous data from TEM microanalysis of the vacuoles of Cr-exposed *I. pseudacorus* rhizomes (Caldelas et al. 2012), which showed high of S and Cr contents. In summary, a greater number of elements showed a tendency to reduce their contents in Cr-stressed plants than in Zn-stressed plants, and roots were more affected by this decrease than leaves and rhizomes. However, when considering the previous results, that WC was lower in Cr–stressed rhizomes and roots must be taken into account. This lower WC might have caused the concentration of some elements to be higher in these tissues, and does not imply that uptake has increased or relocation taken place. Moreover, reductions in the element contents of these samples might pass unnoticed if they are small, or appear less relevant than they truly are.

Conclusions

Iris pseudacorus shows a great capacity to tolerate and accumulate both Zn and Cr, which displayed distinct distribution patterns, thereby leading to specific physiological responses. Chromium was retained mainly in roots, causing greater root damage than Zn. Zinc was partially exported to the rest of the plant, and consequently caused a higher decrease in the growth of photosynthetic tissues, but less root malfunction. The functionality of the PSII persisted in all the treatments, and the stomatal aperture was only partially limited by Cr.

We conclude that the reduction of growth in *I. pseudacorus* in response to exposure to Cr and Zn is due to the restricted mineral and water uptake and to the increased demand of carbohydrates of damaged roots, rather than to the direct effects of these metals on photoassimilating tissues. Biomass allocation to rhizomes (Cr) or roots (Zn) may contribute to heavy metal tolerance in this species by reducing transpiration and increasing metal-accumulating tissues and/or the surface for water and mineral uptake.

The high biomass production and metal extraction capacity makes this species a good candidate for Cr rhizofiltration and Zn phytoextraction, as reflected by the level of exportation of each metal to leaves. The reduced exportation of Cr to leaves can be advantageous for flower production, yield of emerged parts, and human safety. Metal extraction would also be higher than in environments polluted by Zn, as long as the whole plant is collected. After harvest the metal-enriched biomass must be disposed

of safely, a technical issue which remains partially unsolved. A variety of techniques under development, i.e. composting, compacting, pyrolysis, or biogas production (Ghosh and Singh 2005; Rai 2009), will remove this limitation in future and allow for a wider use of phytoremediation.

Author contribution C. Caldelas designed and performed the experiments, obtained the analytical data, interpreted the results and wrote the manuscript. J.L. Araus, J. Bort and A. Febrero, acting as thesis advisors, supervised the conception and development of the experiments, discussed the interpretation of the results, and reviewed the proofs of the manuscript.

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