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Effect of genotype, Cr(III) and Cr(VI) on plant growth and micronutrient status in *Silene vulgaris* (Moench)

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Abstract

Chromium released into the environment from industrial activities has become an important environmental concern. *Silene vulgaris* has been proven to be tolerant to many heavy metals, so it is considered an interesting species in the revegetation and restoration of polluted soils, but no information is available about its response to Cr. The objective of this work was to study uptake and influence on plant growth of Cr(III) and Cr(VI) in six genotypes (four hermaphrodites and two females) of *S. vulgaris* from different sites of Madrid (Spain). Plants were treated for 12 days with 60 μM of Cr(III) or Cr(VI) in semihydroponics. Dry weights, soil-plant analysis development values (SPAD) reading with chlorophylls and micronutrient and total Cr concentrations were determined. Metal uptake was higher in presence of Cr(VI) than of Cr(III) and poorly translocated to the shoots. In both cases *S. vulgaris* did not show visual toxicity symptoms, biomass reduction, or differences among SPAD values as consequence of Cr additions. However genotypes SV36 and SV38 showed Fe and Mn imbalance. This is the first report on the relatively good performance of hermaphrodite and female *S. vulgaris* genotypes in Cr uptake and physiological traits, but further studies will be necessary to elucidate the mechanisms by which the gender may influence these variables. *S. vulgaris* presented high diversity at genotypic level; the treatment with hexavalent Cr increased the differences among genotypes so the use of cuttings from an homogeneous genotype seems to be an adequate method for the study of this species.

Additional key words: bladder campion; metal pollution; metal speciation; tolerance; nutrient balance.

Introduction

Chromium is a heavy metal used on a large scale in industry. In the last years its release into the enviroment from anthropogenic activities has become a major health and ecological hazard. On a worldwide basis, about 80% of the chromium mined goes to metallurgical applications, but it is also used in the manufacture of stainless steel, wood treatment, leather tanning and chrome plating due to its corrosion-resistant properties (Barnhart, 1997). Although Cr contamination does not pose a global risk, it could be a serious problem for the local environment (Bielicka *et al*., 2005).

Once it enters the environment, its toxicity is determined to a large extent by its chemical form, which is also responsible for its mobility and bioavailability (Kotas & Stasicka, 2000). The two most common and stable chemical species of Cr in the environment are Cr(III) and Cr(VI). The active-redox Cr(VI) is more water soluble and more bioavailable than Cr(III), and it has been classified as carcinogen of Group A by the EPA (United States Environmental Protection Agency; USEPA, 1998). The role of Cr(III) as essential anion in mammals and plants is under question because it also seems to be toxic at higher doses than Cr(VI) (Levina & Lay, 2008).

In plants grown in non-contaminated soils, Cr concentrations are usually less than 1 mg kg^{-1} dry weight, rarely exceed 5 mg kg^{-1} and are typically in the order of 0.02-0.2 mg kg^{-1} dry weight (Pezennec, 2007). To date, high concentrations of Cr have been detected in different species as *Brassica oleracea* (Zayed *et al*.,

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Abbreviations used: ANA (1-naftil acetic acid); ATP (adenosine triphosphate); BCF (bioconcentration factor); EPA (United States Environmental Protection Agency); SPAD (soil-plant analysis development values); TF (translocation factor).

1998), *Brassica juncea* (Kumar *et al*., 1995) and *Arabidopsis thaliana* (Salt *et al*., 1998). Accumulators have been reported in Gramineae family such as *Leersia hexandra* (Zhang *et al*., 2007) or *Miscanthus sinensis* (Arduini *et al*., 2006). High accumulations of Cr have been described in other species as *Salsola kali* (Gardea-Torresdey *et al*., 2005), *Convulvulus arvensis* (Gardea-Torresdey *et al*., 2004), *Polygonum hydropiperoides* (Qian *et al*., 1999) *Glycine max* and *Helianthus annuus* (Mei *et al*., 2002), *Azolla caroliana* (Bennicelli *et al*., 2004) and *Propopsis* spp (Aldrich *et al*., 2003). Genotypic differences in Cr uptake have been found in Indian mustard (Diwan *et al*., 2008) and rice (Zeng *et al*., 2010). In general, plants retain more Cr(VI) than Cr(III) (Zayed *et al*., 1998) with some exceptions, such as *Azolla caroliana* (Bennicelli *et al*., 2004), *Leersia hexandra* (Zhang *et al*., 2007) or *Glycine max* (Mei *et al*., 2002). Some researchers have suggested that plants could reduce Cr(VI) to Cr(III) through the action of the enzyme reductase Fe^{+3}. Under these conditions, Cr might enter into the plant as Cr(VI) and end up stored there as Cr(III).

Biomass reduction and leaf chlorosis are the first toxicity symptoms shown by plants when they grow in soil with high doses of metal and hence in Cr. Among other reasons, this is caused by an imbalance in micronutrient status (Shanker *et al*., 2005). The Fe accumulation was reduced in leaves in presence of Cr(III) or Cr(VI) in *B. oleracea* (Pandey & Sharma, 2003), *Spinacia oleracea* (Chatterjee & Chatterjee, 2000) or *Zea mays* (Mallick *et al.,* 2010). Concentrations of other micronutrients, such as Cu, Zn and specially Mn, were greatly affected. Micronutrients concentration decreased with regard to the reference levels.

Silene vulgaris (Moench) Garcke, the bladder campion, is a perennial weed widely distributed in Europe, North America, Asia and North Africa. It occupies a great variety of habitats, including metalliferous soils. Flower phenology and insect pollination have lead to a predominantly outcrossing habit; therefore it exhibits a high level of genetic variability (Prentice & Giles, 1993). Spanish natural populations of *S. vulgaris* have been characterized by morphological traits and molecular markers (Alarcón *et al.*, 2008) that show a high genetic diversity. This species has a gynodioecious mating system characterized by the co-ocurrence of female and hermaphrodites individuals within populations (Taylor *et al*., 1999). Individuals of these populations are scattered along road sides and agricultural fields; thus their ecology is likely to be affected by anthropogenic factors associated with roadside maintenance as well as natural processes (Olson *et al*., 2005).

Theoretical studies suggest that because females cannot gain fitness through pollen, they must increase their investment in seed quantity and/or quality in order to coexist with hermaphrodites (Charlesworth & Laporte, 1998). This increase in female fitness has been observed in most gynodioecious species, but the few studies that investigated physiological differences between genders in gynodioecious species have produced inconsistent results (Poot *et al*., 1996; Caruso *et al*., 2003; Schultz, 2003).

The tolerance of many *S. vulgaris* ecotypes has been proven for most of heavy metal and their combinations, especially Cd, Cu, Fe, Mn, Pb, Zn and Hg (Paliouris & Hutchinson, 1991; Ernst & Nelissen, 2000; Ciarkowska & Hanus-Fajerska, 2008). Data from these studies show *S. vulgaris* to be an interesting species in the early stages of revegetation and soil restoration. Regarding genotypes, Cu has been the only metal studied to evaluate differences to metal uptake among genotypes of *S. vulgaris* (Price & Abrahams, 1994). There is no information about physiological traits and heavy metal tolerance between genders of *S. vulgaris* and about the ability of this species to tolerate and accumulate Cr.

The objectives of this study were: a) to assess whether the presence of low concentrations of Cr(III) or Cr(VI) in nutrient solution led to differences in Cr uptake by *S. vulgaris* at genotype level; b) to evaluate the effectiveness of the genotypes using the classical approach of the growth efficiency of wild plants based on micronutrient status and state of chlorophylls.

Material and methods

Plant material and growth conditions

Six genotypes of *S. vulgaris* (Moench) were chosen from different populations of Madrid, Spain (Table 1). A permanent 10 × 10 m plot (divided into 1 m^2 quadrats) was established to vegetative propagate in Alcalá de Henares, Madrid (Spain). Hermaphrodites and female individuals were easily discriminated by watching for the presence or absence of mature anthers. Cuttings of each juvenile growth of mature genotype were collected in March 2009. The base of the cuttings was dipped in 0.1% hormone Inavarplanté 1(indol-3-butyric acid (A,B), 0.1% 1-naftil acetic acid (ANA), 4% ziram for 5 min, and transferred into floating

Table 1. Geographical location and sex of *Silene vulgaris* genotypes

Genotype	Sex	Locality from Madrid	Altitude (m)	Latitude	Longitude	Lithology
SV19	Female	Cadalso de los Vidrios	808	401808	42638	Granite
SV21	Hermaphrodite	Rozas de Puerto Real	867	401842	42933	Granite
SV27	Hermaphrodite	Pinilla del Valle	1,096	405562	34840	Limestone
SV30	Hermaphrodite	Orusco	665	401712	31239	Limestone
SV36	Hermaphrodite	Brea de Tajo	738	401350	30636	Loam
SV38	Female	Valdemaqueda	860	403044	41812	Arkose

polystyrene trays. Twenty five cuttings per tray were induced to root in tap water, and set in a growth chamber under controlled environmental conditions (temperature 20/16°C, 164.527 µmol E m^{-2} s^{-1}, 16/8 hour photoperiod). They were allowed to root for 3 weeks until their roots reached a length of 2.0 ± 0.5 cm (Wierzbicka & Panufnik, 1998).

Then, four cuttings of each genotype with root lengths of 2.0 ± 0.5 cm were transferred into polyethylene containers of 4 L provided with a polystyrene floating plate with modified Hoagland's nutrient solution (3 mM KNO_3, 2 mM $Na(NO_3)_2 \cdot 4H_2O$, 1 mM $NH_4H_2PO_4$, 0.5 mM $MgSO_4 \cdot 7H_2O$, 50 mM NaCl, 25 mM H_3BO_3, 2 mM $ZnSO_4 \cdot 7H_2O$, 2 mM $MnSO_4 \cdot H_2O$, 0.1 mM $CuSO_4 \cdot 5H_2O$, 0.5 mM $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 20 mM Fe(EDDHA). Plants were acclimated for 2 weeks by a progressive increasing of nutrient solution concentration. Afterwards plants were randomly selected to be treated as follows: (a) Control, no Cr addition; (b) 60 µM Cr(VI) as $K_2Cr_2O_7$ (ACS grade Aldrich) and (c) 60 µM Cr(III) as $CrCl_3 \cdot 6H_20$ (RT grade Aldrich). The applied dosages of Cr were chosen according to Zhang *et al.* (2007) and Zayed *et al.* (1998) and hormesis responses determined by Gardea-Torresdey *et al.* (2005).

Four trays, with four cuttings of each genotype in each, were used as independent replicates of each treatment. The pH of the solutions was buffered with 2 mM of MES and adjusted to 5.5 with KOH. Nutrient solution was replenished daily and completely changed every 3 days. Aliquots (20 mL) of nutrient solution were collected before and after each change to check pH and the oxidation state of Cr. Plants were treated for 12 days.

The concentration of Cr(VI) in nutrient solution was determined just after changing the nutrient solution by UV-Vis light spectrophotometer (Thermo Spectronic Helios Alpha) using the EPA method 7196A (USEPA, 1992). The total Cr concentration was measured in the samples of nutrient solution previously acidified and then, by atomic absorption spectrophotometry (VARIAN fast sequential, model AA240FS). The total concentration of Cr(III) was calculated by subtracting the concentration of Cr(VI) from the total Cr concentration. Results from Cr speciation analysis indicated that the transformation among Cr species was not significant during the experiment.

Plant analysis

SPAD (soil-plant analysis development) index was measured to estimate the chlorophyll state. This is a green colour index related to chlorophyll content. The average of six determinations per leaf was recorded in the four plants of each genotype per tray using a Minolta Chlorophyll Meter SPAD-502. All plants were harvested. The roots and aerial parts were separated and washed thoroughly with MilliQ water. A full sample was made with four plants of the same genotype in each tray, thus there were four trays and a total of four independent replicates per treatment and genotype were analysed. The vegetal material was dried in a forced air oven for 48 h at 70°C. Subsequently, the dry weights were recorded and aerial parts and roots were ground separately. Dried samples (0.25 mg) were digested in an Anton Paar Microwave Reaction System 3000 by adding 6 mL of 65% HNO_3 and 2 mL of 33% H_2O_2. After cooling, the digests were filtered (Whatman filter paper nº 541) and brought up to a volume of 25 mL. Total concentrations of Cr, Fe, Mn and Zn were measured by flame atomic absorption spectrophotometer (Varian fast sequential model AA240FS) and Cu concentration by Sequential ICP-AES Liberty AX.

Tobacco leaves were used as certified reference materials (CTA-VL2, tobacco leaves). The recovery percentages were 88% in Fe, 99% in Zn, 93% in Cu, 88% in Mn and 109% in Cr.

Parameters of chromium accumulation in the plant

The following indexes were calculated to compare Cr uptake and translocation to the shoot as result of the different Chromium treatments. The translocation factor (TF) is defined as the ratio between the total metal concentration of shoots and roots. It shows the plant's ability to translocate heavy metals from the roots to the harvestable aerial part (Mattina *et al*., 2003):

$$TF = \frac{C_{aerial}}{C_{root}} \quad [1]$$

where C_{aerial} and C_{root} are the total Cr concentration in aerial part and roots respectively.

The bioconcentration factor (BCF) or phytoextraction rate was described as the heavy metal concentration in total plant divided by heavy metal concentration in nutrient solution (Kumar *et al*., 1995).

$$BCF = \frac{C_{plant}}{C_{solutiion}} \quad [2]$$

where $C_{solution}$ is the Cr concentration in the nutrient solution.

Statistical treatment

Statistical analyses were performed used the statistical package SPSS version 16.0. Data from all variables considered were analysed by general lineal model (GLM) with doses of Chromium (no Cr, 60 μM Cr(III) or 60 μM Cr(VI)) and *S. vulgaris* genotypes (SV19, SV21, SV27, SV30, SV36 and SV38) as experimental factors at $\alpha = 0.05$ using the F-test. GLM was followed by a post hoc Duncan test to assess the significance of differences among treatments and among genotypes for each parameter.

Results

The statistical values (*F*) and empirical *p*-values of both factors and their interaction for all variables considered are presented in Table 2.

Chromium uptake

Fig. 1 gives the total Cr concentration in the studied genotypes of *S. vulgaris*. Chromium uptake was strongly affected by the source of Cr in nutrient solution ($p = 0.000$), but not by the genotype. Chromium mainly accumulated in the roots of the genotypes exposed to Cr(VI) and Cr(III). The highest Cr concentrations were achieved in roots of plants growth with Cr(VI) with values between 374 and 481 mg Cr kg^{-1} dw.

Concentration of Cr in the shoots of *S. vulgaris* genotypes only increased in treatments with Cr(VI), which ranged between 6 and 20 mg Cr kg^{-1} dw. The

Table 2. Testing of general hypothesis in general lineal model (GLM) for variables studied in *S. vulgaris* at $\alpha = 0.05$ (df: degrees of freedom, F: statistical value, *p*: empirical significance level)

Variable[1]	Dose			Genotype			Dose × Genotype		
	df	*F*	*p*	df	*F*	*p*	df	*F*	*p*
TF	2	9.785	0.000***	5	0.833	0.533	10	0.602	0.804
BCF	2	35.946	0.000***	5	0.223	0.313	10	0.815	0.616
dw shoots	2	1.963	0.144	5	4.050	0.002**	10	1.601	0.113
dw roots	2	0.476	0.622	5	6.653	0.000***	10	0.668	0.753
SPAD	2	2.853	0.066	5	7.493	0.000***	10	0.581	0.822
[Cr] shoots	2	31.100	0.000***	5	1.286	0.284	10	1.547	0.150
[Cr] roots	2	162.403	0.000***	5	1.197	0.324	10	0.395	0.943
[Fe] shoots	2	6.593	0.003**	5	0.653	0.660	10	0.826	0.606
[Fe] roots	2	6.478	0.003**	5	1.610	0.175	10	3.248	0.003**
[Zn] shoots	2	4.127	0.022*	5	4.517	0.002**	10	0.736	0.687
[Zn] roots	2	0.318	0.729	5	4.661	0.001**	10	2.166	0.036*
[Cu] shoots	2	6.744	0.002**	5	5.476	0.000***	10	0.639	0.773
[Cu] roots	2	4.898	0.012*	5	3.834	0.005**	10	2.072	0.045*
[Mn] shoots	2	17.181	0.000***	5	6.707	0.000***	10	0.209	0.995
[Mn] roots	2	4.053	0.024*	5	4.463	0.002**	10	1.637	0.124

[1] TF: translocation factor. BCF: bioconcentration factor. dw: dry weight. SPAD: soil-plant analysis development values.

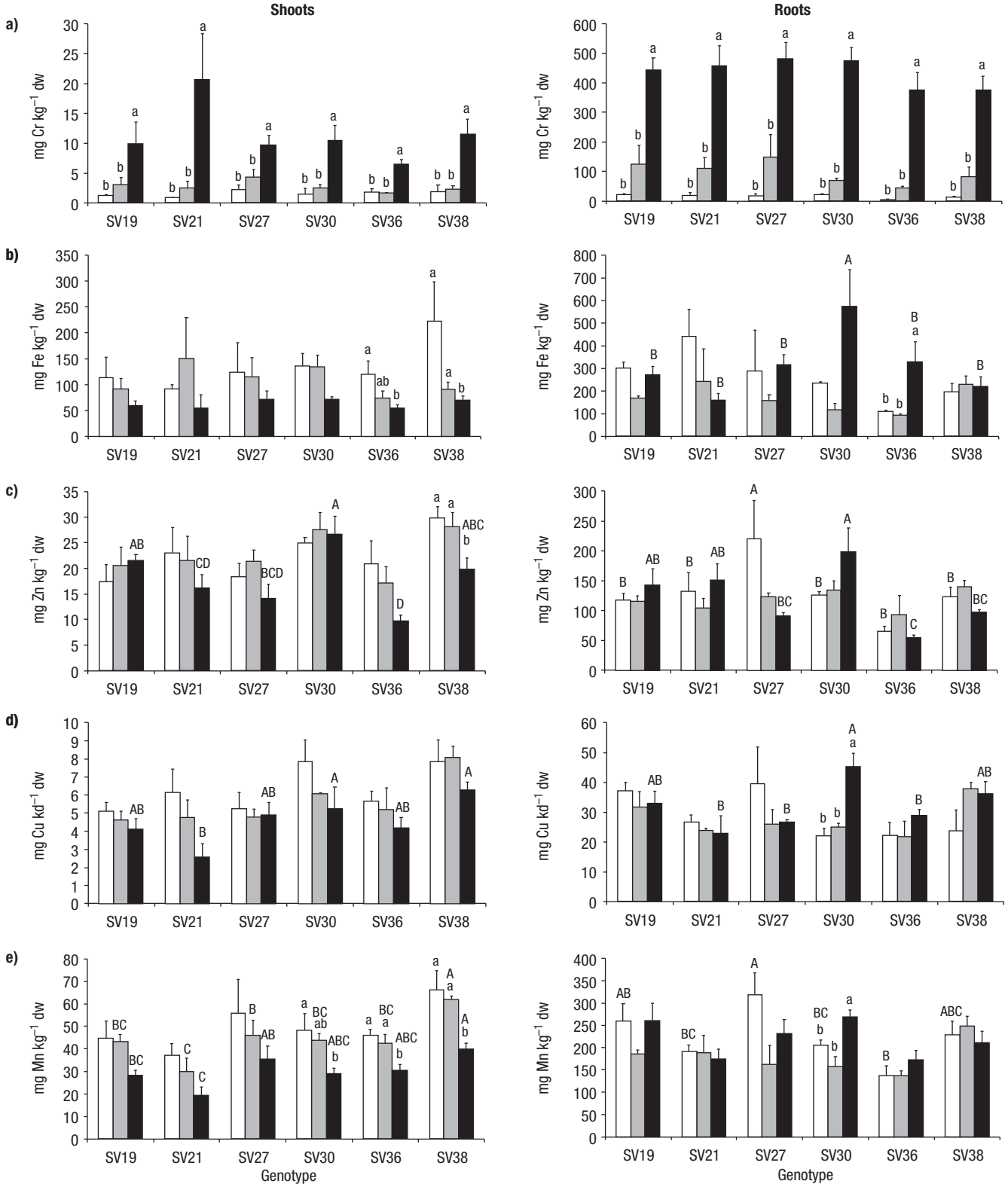

Figure 1. Total metal concentrations (mg kg^{-1} dw) in shoots and roots of *Silene vulgaris* genotypes: a) chromium, b) iron, c) zinc, d) copper and e) manganese. Control, white bars; Cr(III) treatment, grey bars and Cr(VI) treatment, black bars. Different lowercase and capital letters mean significant differences among treatments and genotypes, respectively (Duncan's test, $p < 0.05$, mean ± SE, n = 4).

Table 3. Translocation factor (TF, ratio between the total metal concentration of shoots and roots) and bioconcentration factor (BCF, heavy metal concentration in total plant divided by heavy metal concentration in nutrient solution) of *Silene vulgaris* genotypes after 12 days of treatment with 60 µM of Cr(III) or Cr(VI)

Genotype	SV19	SV21	SV27	SV30	SV36	SV38
TF						
Cr(III)	0.015 ± 0.005^{b}	0.021 ± 0.003^{b}	0.031 ± 0.005^{b}	0.027 ± 0.005	0.040 ± 0.008^{b}	0.022 ± 0.001^{b}
Cr(VI)	0.026 ± 0.013^{a}	0.031 ± 0.004^{a}	0.021 ± 0.004^{a}	0.026 ± 0.006	0.018 ± 0.002^{a}	0.030 ± 0.005^{a}
BCF						
Cr(III)	3 ± 2	4 ± 2^{b}	10 ± 5^{b}	2.6 ± 0.3^{b}	2.6 ± 0.7^{b}	3.6 ± 1.2^{b}
Cr(VI)	13 ± 1	31 ± 14^{a}	23 ± 2^{a}	13 ± 2^{a}	20 ± 4^{a}	21 ± 3^{a}

Different lowercase letters mean significant differences between Cr treatments (Duncan's test, $p < 0.05$, mean ± SE, n = 4).

translocation factor (TF) presented very low values for all the genotypes, independently of Cr status in the nutrient solution (Table 3).

The bioconcentration factor (BCF), which evaluates the plant ability to take up the metals from the nutrient solution, is given in Table 3 for each genotype at the end of the experiment. The highest values were achieved when Cr(VI) was added to the nutrient solution. This factor decreased by 80% for all the genotypes treated with Cr(III) compared to Cr(VI). In relation to genotypes, there were no significant differences in Cr concentrations or BCF values. Though not statistically significant, there is a tendency of female individuals to accumulate less Cr in their tissues than hermaphrodites. The genotype SV21 seemed to be the most efficient in Cr uptake, as it showed the highest bioconcentration factor.

Plant growth and SPAD index

Table 4 presents the dry weight for roots and shoots of the *S. vulgaris* genotypes after 12 days of exposure to the different treatments. Significant differences were mainly due to genotypes ($p = 0.002$ for shoots dry weights and $p = 0.000$ for roots) rather than to Cr treatments. As regards treatment with Cr(VI), the Duncan test showed that the hermaphrodite genotype SV36 presented the highest dry weight in shoots and roots while the female genotypes (SV38 and SV19) showed growth inhibition. To compare the effect of Cr treatments on chlorophyll content, SPAD values were measured for each genotype of *S. vulgaris* after 12 days of treatment started. The results are given in Table 5.

Table 5. SPAD index values of *Silene vulgaris* genotypes after 12 days of treatment with 60 µM Cr(III) or Cr(VI)

Genotype	Control	Cr(III)	Cr(VI)
SV19	34.125^{B}	36.150^{AB}	31.275^{C}
SV21	42.325^{A}	45.925^{A}	37.713^{AB}
SV27	40.525^{AB}	38.47^{AB}	39.400^{A}
SV30	40.925^{AB}	44.167^{AB}	38.763^{A}
SV36	40.525^{AB}	43.175^{AB}	41.950^{A}
SV38	34.250^{B}	33.000^{B}	31.887^{BC}

Significant differences among genotypes are indicated by different capital letters (Duncan's test, $p < 0.05$, mean ± SE, n = 4).

Table 4. Dry weight (mg plant^{-1}) of *Silene vulgaris* genotypes after 12 days. Control (no chromium), 60 µM Cr(III) and 60 µM Cr(VI)

Genotype	Aerial part			Roots		
	Control	Cr(III)	Cr(VI)	Control	Cr(III)	Cr(VI)
SV19	332 ± 48^{ABC}	408 ± 42^{B}	275 ± 51^{AB}	21 ± 5^{C}	25 ± 5^{B}	22 ± 5^{B}
SV21	337 ± 77^{ABC}	586 ± 126^{A}	359 ± 76^{AB}	53 ± 15^{AB}	45 ± 13^{B}	62 ± 28^{AB}
SV27	353 ± 56^{AB}	335 ± 70^{BC}	415 ± 84^{B}	35 ± 9^{AB}	97 ± 39^{A}	57 ± 17^{AB}
SV30	251 ± 50^{BC}	371 ± 37^{B}	348 ± 76^{AB}	17 ± 3^{C}	33 ± 6^{B}	33 ± 10^{B}
SV36	439 ± 53^{A}	447 ± 58^{AB}	454 ± 60^{A}	73 ± 23^{A}	73 ± 19^{AB}	79 ± 12^{A}
SV38	171 ± 41^{C}	186 ± 23^{C}	202 ± 27^{C}	24 ± 6^{C}	21 ± 3^{B}	31 ± 6^{B}

Significant differences among genotypes are indicated by different capital letters (Duncan's test, $p < 0.05$, mean ± SE, n = 4).

In this setting, there were no significant differences in treatments ($p = 0.066$) but once again, there were differences among genotypes ($p = 0.000$). The leaves of female genotypes (SV19 and SV38) displayed the lowest SPAD values. Note that this trend is not the same as biomass yield, where the maximum and minimum values were kept for the same genotypes regardless of treatment. The SPAD index values showed differences among genotypes due to the effect of Cr(VI). The females (SV19 and SV38) presented the lowest SPAD when plants were treated with Cr(VI).

Micronutrient concentration

Fig. 1 gives the concentrations of Fe, Zn, Cu and Mn in shoots and roots of *S. vulgaris* genotypes. Iron concentrations in shoots of genotypes SV36 and SV38 have decreased by 55% and 68% respectively, due to Cr(VI) in the nutrient solution. This fact was accompanied by increasing of Fe concentration in roots and significant to SV36 genotype, in which Fe increased by 160% over the control. Genotypes SV38, SV30, and SV36 treated with Cr(VI) have reduced by 40% Mn concentration in aerial parts compared to controls. These genotypes showed increments in Mn concentrations of roots, but not statistically significant except for genotype SV30 (up to 30% more than the control). Zinc and Cu concentrations in roots and aerial parts are not consistent enough to show if there is any influence of Cr uptake.

It should be noted that differences in micronutrient concentrations were found among genotypes. But the trend showed that hermaphrodite individuals had micronutrient concentrations lower than females. This is the case of hermaphrodite SV36 which presented the lowest micronutrient concentrations, significant only for Zn and Mn concentration when plants were treated with Cr(VI). Maximum micronutrient concentrations were reached by females and both hermaphrodites SV30 and SV27, especially for SV30, that achieved the highest concentrations of Zn, Cu and Fe when plants were treated with Cr(VI).

Discussion

The genotypes of *S. vulgaris* grown in nutrient solution did not show any visual or physiological toxicity symptoms after being treated with 60 μM of Cr(III) or Cr(VI) during 12 days. With the exception of Cr uptake, which was higher in the plants treated with Cr(VI) than with Cr(III), the main differences in the plant development that were found related more to the genotypes than to the Cr forms in the nutrient solution.

Total Cr concentrations in dry tissues of *S. vulgaris* exposed to Cr(III) and Cr(VI) were in the same range as other species treated with similar doses and time of exposure as *Helianthus annuus* and *Glycine max* (Mei *et al*., 2002), *Salsola cali* (Gardea-Torresdey *et al*., 2005) or *Vigna radiata* (Shanker *et al*., 2004).

As shown in Cr tissue concentrations and bioconcentration factor, all genotypes of *S. vulgaris* studied here presented a significantly greater uptake of Cr(VI) than Cr(III). These differences have already been explained based on a different Cr uptake mechanism by the plant. Skeffington *et al*. (1976) carried out inhibitor studies with barley seedlings which demonstrated that Cr(III) and Cr(VI) do not share a common uptake mechanism. Cr(VI) is actively taken up in metabolically driven processes, in contrast to Cr(III) which is passively taken up and retained by cation exchange sites of the cell wall. This fact explained why plants take up more Cr(VI) than Cr(III). This was later confirmed by other authors (Zayed *et al*., 1998; Gardea-Torresdey *et al*., 2005).

The six genotypes of *S. vulgaris* presented TFs < 1 of Cr in all treatments. Chromium was mainly accumulated in roots and poorly translocated to aerial parts, as was previously reported by McGrath (1982) and Sharma *et al*. (1995). The authors proposed that poor translocation of Cr to the shoots could be due to sequestration of Cr in the vacuoles of the root cells to render it non-toxic. Furthermore, Han *et al*. (2004) and Arduini *et al*. (2006) showed that Cr is only translocated at toxic levels well above the dose applied in this study.

Among the effects shown to be caused by Cr toxicity to the plant are the detriment of dry weight in both root and shoots and leaf chlorosis. None of these symptoms were shown in *S. vulgaris* genotypes at the applied dose. It is remarkable that previous studies found biomass decreases in *Z. mays*, *Solanum lycopersicum* and *B. oleracea* (di Toppi *et al*., 2002) treated with Cr(VI) at 5 mg L^{-1} and in *Sorghum bicolour* at 50 μM (Shanker & Pathmanabhan, 2004). Similar doses also affected the chlorophyll contents of other species like *Vigna radiata* (Samantary, 2002), *Salvinia maritima* (Nichols *et al*., 2000) and *Z. mays* (Sharma *et al*., 2003).

Chromium, due to its structural similarity with some essential elements, can affect mineral nutrition of plants in a complex way. Competition mechanisms for transport bindings in the plant resulting in a decrease in micronutrient uptake and translocation have also been described (Shanker *et al*., 2005). Results from Cr(VI) treatment showed Mn decrease in the shoots of genotypes SV30, SV36 and SV38. They also showed a decrease of Fe in SV36 and SV38, which in case of SV36 is accompanied by a greater accumulation of Fe in roots. These results agree with those of Gardea-Torresdey *et al*. (2004) and Gopal *et al*. (2009) that reported decreases of Mn and Fe contents in stems and leaves of cauliflower and spinach respectively. Gardea-Torresdey *et al*. (2004) found that Fe was significantly concentrated in the root of Cr(VI)-treated plants of *Convultus arvensis* as in genotype SV36. It must be taken into account that treatment with Cr(VI) increased the differences in micronutrient concentrations among genotypes. No effect has been found related to Cr(III) treatment.

The imbalance in Fe and Mn found in Cr(VI) treatment does not happen in all genotypes and is not translated in biomass or SPAD values reductions, suggesting that *S. vulgaris* presents a relatively high tolerance to Cr compared with other species treated with similar doses. The lack of toxicity symptoms could be related to the low dosage applied (close to hormesis). It should be taken into account that this experiment was conducted using Cr concentrations far less than those that cause toxicity because the objective was to evaluate the influence of the Cr speciation in the genotype development at environment concentrations.

Based on results obtained, there is a high level of variability among *S. vulgaris* genotypes. Each genotype studied presented differences in all variables studied in this work, except in Cr uptake. It could be difficult to choose the most efficient genotype in a possible treatment of Cr contaminated sites. Considering results of biomass and micronutrients concentrations, the common definition of "efficiency" for wild plants could be used. Efficiency is defined as the quantity of dry matter produced per gram of nutrient and it is simply the inverse of tissue concentration (Small, 1972). Given this definition, hermaphrodite genotypes and especially SV21 and SV36 would be more efficient than females because they present higher biomass and lower nutrient concentrations, especially in Cr(VI) treatment. Tissue concentration may be affected by processes like luxury consumption or substantial storage. Therefore a more useful measure of efficiency might be respiration, photosynthetic or net assimilation rate (Shanker *et al*., 2004; Arduini *et al*., 2006).

In relation to SPAD values, like the measure of the state of chlorophylls, the hermaphrodites, and especially SV21, could be considered more efficient also because they presented higher values than females. In general, hermaphrodite genotypes showed similar behaviour in Cr uptake and tolerance, but SV21 seems to be the best candidate to be used in future assays. SV21 presented the highest BCF and it was the genotype that showed the highest concentrations of Cr in the aerial part without any alteration in its micronutrient balance. On the other hand, female genotypes were less efficient in all variables studied here, especially SV38. Both SV38 and SV19 seem to have different behaviour. SV38 translocated more Cr than other genotypes in relation with its poor uptake. This leads to a decrease in the levels of Fe, Mn and Zn in the aerial part. On the other hand SV19 did not accumulate great amounts of Cr and it did not show any micronutrients imbalance probably due to an exclusory mechanism. Some plants simply avoid toxicity by preventing uptake of the metal as have been reported by De Vos *et al*. (1991) in Cu-treated *Silene cucubalus* plants.

Comparison of physiological traits between genders is scarce for gynodioecious species, and the results are generally inconsistent, with some suggesting that females have higher photosynthetic rates (Caruso *et al*., 2003) and others suggesting the opposite (Schultz, 2003). At least three physiological mechanisms might underlie such sex differences: i) higher carbon demand in developing fruit; ii) greater nitrogen demand in developing seeds and iii) greater rate of active translocation of resources from leaves to developing fruit. All require a limited pool of resources (carbon, nitrogen, ATP) shared between the developing reproductive structures and supporting leaf tissue. As metal uptake is controlled by many physiological traits, it was expected to find sex differences in Cr uptake, but further studies will be necessary to elucidate the mechanisms by which the gender may influence metal uptake in *S. vulgaris*.

Uptake and effects of Cr are related to the dose and time of exposure and more studies are necessary to better understand tolerance and Cr uptake of *S. vulgaris* and to select the most effective genotype in field.

To our knowledge, this is the first report on the relative good performance of hermaphrodite and female *S. vulgaris* genotypes in Cr uptake and physiological

traits. *S. vulgaris* uptakes mainly the hexavalent form of Cr and accumulates it mainly in roots. This species presents relative high tolerance to Cr at the applied dose.

This work confirms the high variability in physiological traits of *S. vulgaris,* even in genotypes from neighbouring areas. It seems that the treatment with hexavalent Cr increases the differences among genotypes; hence the use of cuttings from a homogeneous genotype seems to be an adequate method for the study of metal behaviour in this species.

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