Accumulation and tolerance of cadmium in a non-metallicolous ecotype of *Silene vulgaris* Garcke (Moench)

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**Resumen**

Acumulación y tolerancia a cadmio en un ecotipo no metalífero de *Silene vulgaris* Garcke (Moench)

En este estudio, se analizó el efecto de diferentes concentraciones de Cd²⁺ sobre un ecotipo de *Silene vulgaris* Garcke (Moench). La concentración de 60 μM de Cd²⁺ provocó una ligera inhibición del crecimiento de las plantas mientras que la concentración más alta (120 μM) redujo drásticamente la biomasa y la elongación de la raíz y los brotes. Además, se detectaron altos niveles de Cd²⁺ en las plantas, un coeficiente de bioacumulación elevado en las raíces y un bajo factor de translocación indicando que el ecotipo de *S. vulgaris* empleado en este estudio presenta una alta capacidad de acumulación de Cd²⁺ en las raíces y sería un buen candidato para la fitostabilización, lo que contribuiría a reducir los niveles de Cd²⁺ en el suelo. Además, los resultados obtenidos indican que se debe tener precaución con el origen de esta planta, ya que podría representar una fuente adicional de Cd²⁺ en la dieta humana.

**Palabras clave:** Metal pesado, Fitoestabilización, Caryophyllaceae.

**Abstract**

In this study, a pot experiment was developed using a non-metallicolous ecotype of *Silene vulgaris* Garcke (Moench) exposed to 0, 60 and 120 μM Cd²⁺ for 13 days. The dose of 60 μM Cd²⁺ had little effect on the growth of *S. vulgaris* plants, whereas the highest dose produced a drastic reduction in biomass, and root and shoot elongation. The high internal Cd²⁺ concentration together with the high bioaccumulation coefficient in roots and the low translocation factor indicated that this ecotype could be a good candidate for the phytostabilisation of Cd²⁺-contaminated soils. In view of the widespread use of this plant in popular medicine and the cuisine of Mediterranean countries, the results obtained also suggest that caution needs to be taken concerning its origin since it could represent an additional source of Cd²⁺ in the human diet.

**Key words:** Heavy metal, Phytostabilisation, Caryophyllaceae.
Introduction

Cadmium (Cd\textsuperscript{2+}) is a widespread, highly toxic heavy metal that enters the environment mainly from industrial processes and fertilisers, resulting in the pollution of water, air and soil (Gallego et al. 2012). Several human disorders have been attributed to the ingestion of Cd, including learning disabilities in children (Marlowe et al. 1985), neurological disorders (Chen et al. 2011), the impairment of bone metabolism and increased cancer rates (Järup & Akesson 2009).

Cultivated and wild edible plants are the main source of heavy metal intake in humans (McLaughlin et al. 1999, Clemens 2006). In fact, the young shoots and tender leaves of Silene vulgaris, which is a perennial herb belonging to the Caryophyllaceae, are widely consumed as vegetables in many Mediterranean countries (Conforti et al. 2011, Cakilcioglu et al. 2011). S. vulgaris plants are also used in traditional and folk medicine as antianemic (Conforti et al. 2011) and anti-inflammatory (Cakilcioglu et al. 2011) agents. Moreover, these plants are an interesting source of polysaccharides -silenan- that exhibit macrophage immunomodulatory properties (Popov et al. 1999).

On the other hand, the occurrence of plants on naturally metal-enriched soils indicates that these plant species have evolved to develop metal resistance mechanisms under several environmental conditions (Ernst et al. 2000; Martínez-Iñigo et al. 2009). Silene vulgaris Garcke (Moench) is a facultative metallophyte that shows multiple tolerance and co-tolerance to heavy metals (Ernst et al. 2000). In fact, several authors have reported the effectiveness of some metallicolous ecotypes of S. vulgaris in the revegetation of contaminated soils (Mohtadi et al. 2012, Pérez-Sanz et al. 2012). Taking into account the traditional uses of S. vulgaris and its pharmaceutical potential, it is therefore of considerable interest to understand how non-metallicolous ecotypes of S. vulgaris, which are not genetically adapted to grow on metal-enriched soils, incorporate and accumulate heavy metals. Consequently, in this work, the effect of different concentrations of Cd\textsuperscript{2+} on biomass production, plant growth and yield, as well as the distribution of Cd\textsuperscript{2+} in roots and leaves in a non-metallicolous ecotype was studied.

Materials and methods

Plant material

The seeds used in this study were provided by the germplasm bank of the Universidad Politécnica de Cartagena, registered as accession UPCT-01-313. The seeds were collected from a non-polluted soil of Cartagena (37°41’50"N, 1°0.5’05"W) in which Cd levels are assumed to be relatively low. S. vulgaris seeds were surface-sterilised for 2 min in 70% ethanol, transferred to 10% NaOCl for 10 min, rinsed three times with sterile distilled water, and placed on 150 x 25 mm Petri dishes containing filter paper moistened with distilled H\textsubscript{2}O. The Petri dishes were incubated in the dark at 25 °C for 72 h. Then, the seed germination rate was scored, and seedlings were transplanted into vermiculite in polyethylene containers (15 x 15 x 20 cm, one plant per pot) and grown under a 16 h photoperiod with 24/22 ºC day/night temperature, with a photon flux density of 120 µmol photons m\textsuperscript{-2}s\textsuperscript{-1}, and 65% relative humidity. The seedlings were watered with one-quarter-strength Hoagland solution (Sigma-Aldrich, Spain), adjusted to pH 6.0, for 1 week and then, full strength medium for another week. Seventeen-day-old plants of uniform height and number of leaves were used in the Cd\textsuperscript{2+} treatments.

Cadmium treatments

Moderate levels of Cd\textsuperscript{2+} pollution in soil solutions have a Cd\textsuperscript{2+} concentration range of between 0.32 and 1 µM (Sanità di Toppi & Gabrielli 1999), and so to induce an acute Cd\textsuperscript{2+} stress, doses of 60 and 120 µM Cd\textsuperscript{2+} were chosen. Seventeen-day-old plants were split into in four groups and the assay was started by the addition of 60 or 120 µM Cd\textsuperscript{2+} (in the form of Cd(NO\textsubscript{3})\textsubscript{2}. 4 H\textsubscript{2}O; Sigma-Aldrich, Spain) for the Cd\textsuperscript{2+} treatments. As control, plants were watered with 120 and 240 µM KNO\textsubscript{3}.

Growth and biomass determinations

Plant growth was measured by an assessment of shoot height, root length, fresh weight and the oven-dry weight (60°C, 24 h) of roots and leaves. Root length was determined as the distance between the root-shoot junction and the tip of the main root. Plants were harvested after 13 days of Cd\textsuperscript{2+} exposure.
**Cadmium determination**

The roots of the Cd$^{2+}$-treated plants were immersed in 2 mM Na$_2$EDTA for 15 min to remove Cd$^{2+}$ adhered to the root surface (Liu et al. 2009) and then the roots, stems and leaves were separated. Dried samples were ashed in a muffle furnace (Select-Horn furnace P-Selecta) at 450 °C for 8 h. The ashes were digested with an acid oxidative mixture H$_2$O:HNO$_3$ (65%):H$_2$O$_2$ (30%) (3:2:5, v/v/v). The concentration of Cd$^{2+}$ in the samples was determined by Inductively Coupled Plasma Emission Spectroscopy (ICP, Agilent 7500CE).

**Determination of the Tolerance Index, Bioaccumulation Coefficient and Translocation Factor**

The Tolerance Index (TI) at different individual concentrations of Cd$^{2+}$ was calculated by dividing the root length at the different metal concentrations by that obtained in the control treatments (Wilkins 1978), using the following equation: TI (%) = 100 x (root length in metal treatment)/(root length in the control). The Bioaccumulation Coefficient (BAC) was calculated according to the formula: BAC = metal concentration (µg g$^{-1}$ dry weight) in leaves or roots/metal concentration (µg ml$^{-1}$) in nutrient solution. The Translocation Factor (TF), which indicates the ability of plants to translocate heavy metals from the roots to the shoots (Liu et al. 2009), was calculated as the relation between metal concentration in leaves and metal concentration in roots.

**Statistical analysis**

Data were analysed by one-way analysis of variance (ANOVA) followed by Tukey’s HSD test in order to examine the significance of the observed differences using the SPSS package (SPSS Inc., Chicago, USA) version 19.0, and P values <0.05 were considered as statistically significant.

**Results**

**Growth and metal tolerance in S. vulgaris plants**

When S. vulgaris plants were treated with Cd$^{2+}$, all the growth parameters analysed (shoot and root size, fresh and dry weights of leaves and roots) decreased significantly as Cd$^{2+}$ concentration increased compared to controls (Table 1). The adverse effects of Cd$^{2+}$ were more pronounced on shoot growth than on root growth. Thus, when 120 µM Cd$^{2+}$ was added to the medium, 79 % reduction in leaf fresh weight and 60 % reduction in root fresh biomass were observed. Root and shoot elongations were also reduced by 50 % and 56 %, respectively (Table 1). Moreover, at this concentration, browning of the root-tips and chlorosis, which are specifically symptoms of Cd$^{2+}$ toxicity, were observed (data not shown). In contrast, the dose of 60 µM Cd$^{2+}$ had little effect on the growth of S. vulgaris plants, and the plants showed no visual phytotoxic symptoms. The TI, based on root length for the different Cd$^{2+}$ doses, indicated

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cd$^{2+}$ concentration in the medium</th>
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<tbody>
<tr>
<td></td>
<td>0 µM</td>
</tr>
<tr>
<td>Root elongation (mm)</td>
<td>16 ± 2a</td>
</tr>
<tr>
<td>Shoot elongation (mm)</td>
<td>91 ± 7a</td>
</tr>
<tr>
<td>Root fresh weight (mg/plant)</td>
<td>100 ± 8a</td>
</tr>
<tr>
<td>Root dry weight (mg/plant)</td>
<td>10.2 ± 0.8a</td>
</tr>
<tr>
<td>Leaf fresh weight (mg/plant)</td>
<td>560 ± 54a</td>
</tr>
<tr>
<td>Leaf dry weight (mg/plant)</td>
<td>62.0 ± 3.2a</td>
</tr>
<tr>
<td>TI</td>
<td>88 %</td>
</tr>
</tbody>
</table>

Data are means of n = 10 (± SE). Different letters indicate significant differences at p<0.05 according to the Tukey HSD test.

**Tabla 1.** Tamaño de raíz y brote, masa e índice de tolerancia de plantas de Silene vulgaris expuestas a 0, 60 y 120 µM de Cd$^{2+}$. Las plantas se recolectaron después de 13 días de tratamiento. Los valores entre paréntesis representan el porcentaje de inhibición respecto a los grupos de control.

**Table 1.** Root and shoot size, mass and tolerance index of Silene vulgaris plants exposed to 0, 60, and 120 µM Cd$^{2+}$. Plants were collected after 13 days of treatment. Values in brackets are % inhibition from their respective control groups.
that *S. vulgaris* could tolerate a relative excess of Cd²⁺ (60 µM, TI > 85 %) but was more sensitive to the dose of 120 µM (TI < 50 %) (Table 1).

**Concentration and accumulation of Cd²⁺ in root and leaves of *S. vulgaris* plants**

The Cd²⁺ concentration in *S. vulgaris* tissues increased significantly in both leaves and roots as Cd²⁺ concentration in the medium increased (Table 2). However, most of the Cd²⁺ absorbed by the plants was found in the root tissues. In fact, when plants were exposed to 60 and 120 µM Cd²⁺, the concentration of accumulated Cd²⁺ in root tissues was 203.3 ± 17.8 and 750.4 ± 9.2 µg g⁻¹ dry weight, respectively, while the concentration in leaves was 7.0 ± 0.9 and 19.1 ± 1.3 µg g⁻¹ dry weight, respectively. In addition, in *S. vulgaris* roots, BACs were 12.43 and 20.27, with 60 and 120 µM Cd²⁺, respectively, while BACs in leaves were in the range of 0.38-0.51, in the presence of 60 and 120 µM Cd²⁺, respectively. Moreover, the tendency to translocate Cd from the roots to the leaves, as estimated by TF, was of 0.034 and 0.025 in the presence of 60 and 120 µM Cd²⁺, respectively (Table 2).

**Discussion**

In this work, a non-metallicolous ecotype of *S. vulgaris* was used to assess the effects of different concentrations of Cd²⁺ on its growth, TI, metal uptake and accumulation. It is well established that both growth inhibition and a reduction of biomass production are part of a generic stress-induced morphogenic response that allow plants to decrease stress exposure (Potters et al. 2007). Furthermore, the most commonly used method for monitoring Cd²⁺ toxicity is based on root elongation (Prasad 1995). In the present study, leaf biomass was more sensitive to Cd²⁺ than other measured growth parameters, including root and shoot lengths. The decrease in leaf biomass observed in *S. vulgaris* plants under acute Cd²⁺ stress can be explained, at least in part, by the direct effects of Cd²⁺ on the inhibition of both cell elongation and division rates (Prasad 1995, Sanità di Toppi & Gabbrielli 1999, Fusconi et al. 2006). Nevertheless, since Cd²⁺ also interferes with several physiological processes, such as photosynthesis, plant water status, and mineral nutrition (Prasad 1995, Gallego et al. 2012), an effect on leaf biomass production could not be excluded.

Despite the fact that a non-metallicolous ecotype of *S. vulgaris* was used, this ecotype can be considered as tolerant to relatively high Cd²⁺ concentrations, as shown by its TI values. Several authors (De Knecht et al. 1994, Schat et al. 2000) evaluated Cd²⁺ tolerance in non-metallicolous and metallicolous populations of *S. vulgaris*, and reported that both Cd²⁺-imposed root growth inhibition (Schat et al. 2000) and Cd²⁺ accumulation in root tips (De Knecht et al. 1994) were similar in both populations of *S. vulgaris*. In this study, the internal Cd²⁺ concentration in roots was much higher than that found in leaves, and increased as the external Cd²⁺ levels increased. These results agree with previous investigations reporting that there is normally more Cd²⁺ in roots than in leaves (Clemens 2006, and references herein). The accumulated Cd²⁺ in root tissues was several times above the threshold values found in shoots of Cd²⁺-hyperaccumulator plants (100 µg Cd g⁻¹ dry weight) (Maestri et al. 2010).

In addition, in order to qualify the heavy metal accumulation efficiency in *S. vulgaris* plants, the BAC or phytoextraction rate, must be taken into consideration. On the basis of BAC, plants can be classified into four groups according to their capacity to accumulate heavy metal: “non-accumulator”, species or plant part with BAC < 0.01;

<table>
<thead>
<tr>
<th>Cd²⁺ concentration in the medium</th>
<th>Cd²⁺ concentration in plant tissues (µg g⁻¹ Dry Weight)</th>
<th>BAC</th>
<th>TF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaves</td>
<td>Roots</td>
<td>Leaves</td>
</tr>
<tr>
<td>0 µM</td>
<td>nd</td>
<td>nd</td>
<td>0.025</td>
</tr>
<tr>
<td>60 µM</td>
<td>7.0 ± 0.9</td>
<td>203.3 ± 17.8</td>
<td>0.38</td>
</tr>
<tr>
<td>120 µM</td>
<td>19.1 ± 1.3</td>
<td>750.4 ± 9.2</td>
<td>0.51</td>
</tr>
</tbody>
</table>

nd, not detected. Data are means of n = 3 (± SE)

**Tabla 2.** Concentración de Cd²⁺, Coeficiente de Bioacumulación (BAC) y Factor de Translocación (TF) en hojas y raíces de plantas de *Silene vulgaris* tratadas con 0, 60 y 120 µM Cd²⁺ durante 13 días.

**Table 2.** Concentration of Cd²⁺, Bioaccumulation Coefficient (BAC), and Translocation Factor (TF) in leaves and roots of *Silene vulgaris* plants treated with 0, 60 and 120 µM Cd²⁺ for 13 days.
“low accumulator”, with BAC values between 0.01-0.1; “moderate accumulator”, 0.1-1.0; and “hyperaccumulator”, BAC >1 (Sekabira et al. 2011). In *S. vulgaris* roots, BACs were always higher than 1. However, the tendency to translocate Cd$^{2+}$ from the roots to the leaves, as estimated by TF, was low. Nevertheless, BACs in leaves were in the range of 0.38-0.51, indicating that this plant had a good potential for accumulating Cd$^{2+}$ in leaf tissues. Plants that over-accumulate heavy metals in their roots, excluding or limiting translocation to shoots, can be regarded as efficient to phytostabilise heavy metals in soils (MacGrath & Zhao 2003, Maestri et al. 2010). Thus, this species could be used for phytostabilisation of soils with low Cd$^{2+}$ bioavailability, to avoid high accumulation in leaves.

On the other hand, given the continued accumulation of Cd$^{2+}$ in many fertilized agricultural soils (McLaughlin et al. 1999, Bhat et al. 2010), and taking into account the ethnobotanical relevance of this species in Mediterranean countries, the results obtained suggest that caution has to be taken about its use, since it could represent an additional source of Cd in the human diet whose maximum levels allowed are 7 µg kg$^{-1}$ human body weight.

In conclusion, based on its good growth, its TI, BAC and TF under acute Cd$^{2+}$ stress, these results suggest that this non-metallicolous ecotype of *S. vulgaris* has a high tolerance towards Cd$^{2+}$. The high internal Cd$^{2+}$ concentration, the high BAC in roots, and the low TF indicate that this plant could be a good candidate for phytostabilisation of Cd$^{2+}$-contaminated soils.

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**References**


