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Arsenic accumulation and tolerance of *Cytisus scoparius* under controlled conditions.

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## Abstract

*Cytisus scoparius* is a native leguminous species which grows at a derelict arsenopyrite mine in NW Madrid, Spain. Among the species found in the area surrounding the mine, this plant has shown one of the highest arsenic bioaccumulation factors. For this reason, alongside with its ability to grow in a contaminated area and its high biomass, it was selected for an arsenate dose-response assay under controlled conditions in order to evaluate its potential resistance to arsenic. *C. scoparius* accumulated arsenic mainly in roots and this had a negative effect on root phosphorous concentration. Stress indicators, such as glutathione and synthesis of phytochelatins, and the lack of evidence of an increase in malondialdehyde when arsenate was supplied indicate that *C. scoparius* has a certain resistance to arsenic. According to our results, *C. scoparius* would be a good candidate to revegetate arsenic contaminated sites.

Keywords: arsenic, kinetics, stress indexes, *Cytisus scoparius*, revegetation

## 1 Introduction

Arsenic is a widely-distributed contaminant in the environment (Smedley et al., 2002), being mining activities one of the main anthropogenic sources, followed by fuel utilisation and applications of pesticides. Arsenic also occurs naturally in soils due to the weathering of the underlying parent rock. For example, in India and Bangladesh, the As presence in the water has led to around 60-75 million people suffering diseases from conditions related to As consumption in drinking water (Mukherjee and Bhattacharya, 2001).

Arsenic is a constituent of most plants but its concentration in tissues rarely exceeds  $1.5\text{ mg kg}^{-1}$  on non-contaminated soils (Kabata Pendias & Pendias, 2001). Soils affected by anthropogenic activities, such as mining, may promote higher arsenic and metals levels than those found in natural environments, their reclamation being a matter of concern. Local authorities (Autonomous Community of Madrid, Spain) have established reference values for total As and metals in soils for the protection of human health. In the case of arsenic, soils can be considered as contaminated when As total concentration in soils exceeds  $24\text{ mg kg}^{-1}$  (BOCM n°204, 28<sup>th</sup> August 2006).

In most plant species, As concentration is lower in the aerial part than in roots (Kabata-Pendias and Pendias, 2001). However, (hyper)accumulator species can concentrate greater amounts of As in their above-ground organs. For example, *Pteris vittata* was the first As hyperaccumulator species described (Ma et al., 2001). In the study of Tu et al. (2002), *P. vittata* accumulated  $6000\text{ mg As kg}^{-1}$  in its young fronds in soils contaminated containing  $98\text{ mg As kg}^{-1}$  eight weeks after transplanting. After 20 weeks the translocation factor (ratio of arsenic concentration in shoot to that in root) was 24.

Arsenate and phosphate are both transported into the cell by the phosphate uptake system in a wide range of plants (Asher and Reay, 1979; Meharg and Macnair, 1994). Arsenate is a strong competitor of phosphate in higher plants (Ullrich-Eberius et al., 1989) although the uptake system has a much-greater affinity for phosphate than for arsenate (Carbonell-Barrachina et al., 1998). Increasing the phosphate status in plant can lead to reduced arsenate uptake, through the suppression of the high affinity phosphate/arsenate uptake system (Meharg and Macnair, 1991, 1992). Thus, the study of kinetics of arsenate in plants grown with and without phosphorous can provide information about the regulation of the arsenate uptake by phosphate.

Under As exposure, the generation of reactive oxygen species (ROS) has been observed, leading to lipid peroxidation with the synthesis of products such as malondialdehyde (MDA). Toxic elements induce numerous responses in plants, such as root exudation, reduction of influx across the plasma membrane, chelation by ligands of toxic elements in the cytosol, transport and accumulation of metals in the vacuole and the synthesis of antioxidant molecules like glutathione (GSH). Plants can synthesise ligands, such as organic acids, phenolic compounds and oligopeptides that bind to and reduce the toxicity of some toxic elements. In particular, a class of oligopeptides called phytochelatins (PCs) with the basic structure  $(\gamma\text{-Glu-Cys})_n\text{-Gly}$  ( $n=2-11$ ) have been shown to play a crucial role in the detoxification of heavy metals and metalloids in plants (Cobbett and Goldsbrough, 2002). However, it has been suggested that in some plant species, such as *Silene paradoxa*, PCs are not involved in metal tolerance (Arnetoli, 2008).

The use of woody plants has been recommended for trace elements immobilisation in soils. Their planting may provide an effective form of phytostabilisation (French et al., 2005).

Arsenic concentrations in twenty-one plant species collected in an abandoned arsenopyrite mine (Bustarviejo, NW Madrid, Spain) were analysed by Moreno-Jiménez et al. (2010). Among them, *Cytisus scoparius* plants were found in several locations in this area: close to the mining dumps, close to the streams which pass through the dumps and in natural soil not affected by contamination. Soil total As concentration in the areas near the mining dumps reached 1900 mg As·kg<sup>-1</sup>. Among the species collected, *C. scoparius* showed the highest bioaccumulation factor ([As]<sub>tissue</sub>/[extractable As]<sub>soil</sub>).

The aim of this work is to study the behaviour of *C. scoparius* when exposed to As in a hydroponic culture under controlled conditions, in order to evaluate its tolerance to As. The influence of phosphate on arsenate uptake by this plant was also studied.

## 2 Materials and Methods

### 2.1 Plant culture

One-year-old *C. scoparius* plants were obtained from a nursery; 12 of them were selected for the experiment. *C. scoparius* is a leguminous shrub, which proceeds originally from southern Europe where it grows as a native plant.

Plant roots were rinsed with tap and distilled water. Then, the plants (one per pot) were transferred to 20-L pots containing 15 L of perlite. The nutrient solution composition (pH 5.5-6.5) was 1.5 mM KNO<sub>3</sub>, 1.5 mM Ca(NO<sub>3</sub>)<sub>2</sub>, 1 mM KH<sub>2</sub>PO<sub>4</sub>, 1 mM MgSO<sub>4</sub>, 0.75 mM K<sub>2</sub>SO<sub>4</sub>, 56.76 µM Fe-EDDHA, 27.3 µM MnSO<sub>4</sub>, 0.32 µM CuSO<sub>4</sub>, 0.77 µM ZnSO<sub>4</sub>, 20 µM H<sub>3</sub>BO<sub>3</sub> and 0.016 µM (NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub> (Moreno-Jiménez et al., 2008), and it was renewed weekly. Three As treatments were applied: 0, 50 and 250 µM As, added as Na<sub>2</sub>HAsO<sub>4</sub>·7H<sub>2</sub>O, as arsenate is the dominant form of arsenic in aerobic conditions

and toxicity studies have focused mainly on it. Arsenic concentrations were chosen according to previous works with other Mediterranean woody plant species, whose resistance to arsenic was evaluated (Moreno-Jimenez et al., 2008, 2009). The experiment took place in a greenhouse for eight weeks under the following conditions: 10-28°C and 60-80% relative humidity. Each treatment was replicated four times. The plants were harvested after eight weeks. They were divided into roots and shoots. Each part was washed with tap and distilled water and the fresh weight was determined. Samples of the different plant organs were divided into two fractions: one was dried at 60°C for three days and the other one was frozen in liquid N<sub>2</sub> and stored at -80°C.

## 2.2 Analytical determinations

An acid mineralisation of the plant material was carried out with the addition of 4 mL H<sub>2</sub>O milli-Q, 1.5 mL HNO<sub>3</sub> (65%) and 1 mL H<sub>2</sub>O<sub>2</sub> (30%) to 0.25 g dry weight of plant material in an autoclave (1.5 KPa, 125°C, 30 minutes, Lozano-Rodríguez et al., 1995). The resulting solution was filtered and diluted to 15 mL. In the extract, As concentration was determined by atomic fluorescence spectroscopy and phosphorous concentration by colorimetry (MAPA, 1994).

Chlorophylls (Chl), MDA, GSH and PCs were analysed as stress indicators in the fresh material.

Chlorophylls were determined colorimetrically. Fresh material (0.25 g) was washed with acetone (80%) until the samples were totally decolourised. Then they were diluted to 25 mL with acetone and the absorbance was measured at 645 and 663 nm. The concentrations of chlorophyll were estimated according to the equations developed by Wellburn (1994).

MDA was analysed by adding 1 mL of reagent containing trichloroacetic acid (15%)-thiobarbituric acid (0.37%)-HCl (0.25 M) and butylated hydroxytoluene (0.01%) to 0.1 g fresh weight of plant material. Samples were shaken at 90°C for 30 minutes. Then, they were centrifuged at 12000 *g* for 10 minutes and cooled in an ice container. The absorbance was determined at 535 and 600 nm. An extinction coefficient of  $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$  was used to calculate the concentrations of MDA (Heath and Packer, 1968).

GSH and PCs were determined by high performance liquid chromatography (HPLC, Agilent Technologies 1200) by comparison with GSH and PCs standards (Peptide 2.0, USA). Fresh material (0.1 g) was homogenised with 0.25 M HCl, adding 5 mM N-acetyl-cysteine (15  $\mu\text{l}$ ) as internal standard. The homogenates were centrifuged for 15 minutes at 14000 *g* and 4°C. The supernatant was filtered through a VecaSpin Micro centrifuge tube containing a nylon filter (0.2  $\mu\text{m}$ ) and centrifuged again. The filtered samples were injected in the HPLC system (100  $\mu\text{l}$ ). GSH and PCs were separated in a C18 Spherisorb 5 $\mu\text{m}$  ODS2 (250x4.6 mm; Waters), being eluted with a gradient of solvent A (98:2 H<sub>2</sub>O: acetonitrile (v/v) plus 0.05% trifluoroacetic acid) and solvent B (2:98 H<sub>2</sub>O: acetonitrile (v/v) plus 0.05% trifluoroacetic acid). The gradient program for % solvent B was: 2 min, 0%; 25 min, 25%; 26 min, 50%; 30 min, 50%; 35 min, 0%, 40 min, 0%. A derivation post-column with 1.76 mM Ellman reagent (5, 5'-dithiobis-2-nitrobenzoic acid in 0.3 M phosphate buffer at pH 7.5) was applied. Absorbance was read at 412 nm in UV-visible detector.

The data were analysed using one-way ANOVA and Duncan's test for comparison of means at  $P < 0.05$  with SPSS 15.0.

## 2.3 Kinetics of arsenate uptake



One-year-old plants of *C. scoparius* were grown in hydroponics with (1 mM  $\text{KH}_2\text{PO}_4$ ) and without phosphate under controlled conditions, as described above, for eight weeks. For the P-deficient plants (-P plants), 1.25 mM  $\text{K}_2\text{SO}_4$  was added to the nutrient solution instead of potassium phosphate.

The short-term influx of arsenate was determined by incubation (20 minutes, 25°C) of excised roots of *C. scoparius* in arsenate solutions with 5, 10, 20, 50, 100 or 200  $\mu\text{M}$  As, added as  $\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$ , at room temperature. Four replicate samples of young excised roots were used. The uptake solutions also contained 0.5 mM  $\text{Ca}(\text{NO}_3)_2$  and 5 mM 2-(N-morpholino)ethanesulfonic acid (MES) (pH 5). Afterwards, the excised roots were rinsed twice in an ice-cold solution containing 1 mM  $\text{KH}_2\text{PO}_4$ , 0.5 mM  $\text{Ca}(\text{NO}_3)_2$  and 5 mM MES adjusted to pH 5 with KOH, firstly for three minutes and then for ten minutes in a fresh solution, to remove the adsorbed arsenate from the root free space (Meharg and Macnair, 1994; Abedin et al., 2002; Esteban et al., 2003). The roots were then blotted dry and their fresh weights recorded.

The fresh material was mineralised in an autoclave and the total As determined as described above. Kinetic parameters were calculated using SigmaPlot 11.0.  $V_{\text{max}}$  represents the maximum influx achieved by the uptake system, at saturating substrate concentrations. The Michaelis constant  $K_m$  represents the substrate concentration at which half  $V_{\text{max}}$  is reached.

### 3. Results and discussion

#### 3.1 Effects of As on plant growth

To the best of our knowledge, this is the first report about the resistance of *C. scoparius* growing under As exposure. Significant differences in fresh weight were observed between control plants and plants grown with arsenate ( $p < 0.05$ ). The biomass of both shoots and roots declined with As dose. At 50  $\mu\text{M}$  As, the percentage of decrease in comparison with the control treatment is lower than the one for the 250  $\mu\text{M}$  dose (30 and 30% in the 50  $\mu\text{M}$  As and 47 and 53% for the 250  $\mu\text{M}$  As dose for shoots and roots, respectively). Although plants exposed to As generally undergo a reduction in their biomass, a positive effect in terms of biomass production with low As doses has been observed in other studies, such as in red clover with 5 and 10  $\text{mg kg}^{-1}$  (Mascher et al., 2002). However, in the present study, the fresh weight reduction is in agreement with results concerning toxic effects of As on other plant species, such as the Mediterranean shrubs *Retama sphaerocarpa*, *Myrtus communis* and *Arbutus unedo* and *Lupinus albus* (Moreno-Jimenez et al. 2008; Vazquez et al., 2005).

*C. scoparius* plants did not show necrosis or whitened leaves, some of the common effects in plants exposed to As (Carbonell-Barrachina et al., 1998), but they showed less root development compared to the control plants.

### 3.2 As concentration and accumulation in plants

Arsenic concentration in *C. scoparius* plants (Table 1) increased with the arsenate dose applied in the growth medium, the roots being the plant organ which accumulated higher levels of As for both arsenate doses. Root As concentration was significantly higher at 250  $\mu\text{M}$  As than at 50  $\mu\text{M}$ . This is in agreement with reports about other Mediterranean shrubs (Moreno-Jimenez et al., 2008; 2009) grown in a similar culture system; but, in all these cases, the As concentrations in the plant organs were higher than in *C. scoparius*. Arsenic translocation from roots to shoots was low (Table 1).

Root to shoots ratios for *C. scoparius* are in the same range found for other species: 0.001-0.014 for *Miscanthus x giganteus* (Hartley et al. 2009), <0.03 in lupin (Vazquez et al., 2005) and 0.01-0.31 in rice (Zhang et al., 2011). Thus, *C. scoparius* behaves as a shoot excluder. Non-hyperaccumulator species accumulate As mainly in roots, as seen for *Pistacia lentiscus* and *Tamarix gallica* (Moreno-Jimenez et al., 2009), red clover (Mascher et al., 2002), white lupin (Vazquez et al., 2005) and *Pisum sativum* (Päivöke and Simola, 2001), while hyperaccumulator species, like *Pteris vittata*, could accumulate more than 6000 mg kg<sup>-1</sup> in eight weeks in fronds, with As concentration in roots 20-30 times lower (Tu et al., 2002).

With regard to As accumulation (Table 1), there were no significant differences between the two As treatments (p<0.05) for either of the two plant organs. Total As accumulated in the roots was 82% (50 µM As dose) and 88% (250 µM As dose) with respect to the total amount of As in the plant. As accumulation values in shoots and roots were lower than the ones found in other Mediterranean species mentioned above (Moreno-Jimenez et al., 2008; 2009), especially with the 250 µM As dose. *C. scoparius* would then be more interesting for phytostabilisation rather than for phytoextraction, due to the low As accumulation in shoots. Accordingly, the risk of contaminant transfer to the food chain would be limited.

### 3.3 Phosphorus concentration and P/As ratio

Phosphorus concentrations in the plants are shown in Table 2 and the P/As molar ratio (the P molar concentration divided by the As molar concentration) is shown in parentheses. Arsenate supply to plants decreased the P concentration in *C. scoparius* shoots, being more than 7-fold higher in control plants than at the 250 µM As dose. The P/As molar ratio was lower for the 250 µM As treatment than for the 50 µM dose.

The toxicity of As can be explained by the substitution of P by As in biochemical processes, along with the generation of ROS. Phosphorus has been reported to play a partial role in the protection of membranes against As-induced oxidative stress and growth inhibition: increasing the external P supply alleviates As toxicity (Wang et al., 2009), as in plants of chickpea (Gunes et al., 2009), wheat (Pigna et al., 2009) and *Arabidopsis* (Lee et al., 2003). The P/As molar ratio in plant tissues may be an indicator of As effects on plants (Tu and Ma, 2005). In other Mediterranean species, P/As ratios < 90 in shoots or < 15 in roots seemed to be associated with toxicity in terms of growth inhibition and oxidative stress (Moreno-Jimenez et al., 2008). In the case of *C. scoparius*, the values were higher than those found by these authors because of the lower As concentrations found in plant tissues for similar As doses, and no effects on oxidative stress were observed.

### 3.4 Kinetics of arsenate uptake

Fig. 1 shows the arsenate uptake kinetics for *C. scoparius*, with data fitted to Michaelis-Menten hyperbolas. Table 3 shows the kinetics parameters obtained after fitting Michaelis-Menten curves to experimental influx values (shown in Fig. 1). There was no evidence, through a better fit using a double hyperbola equation, for the existence of both a high-affinity transporter (working at low As external concentrations) and a low-affinity transporter (working at higher As external concentrations). Despite this, a better fit was obtained with the data for external As concentrations below 50  $\mu\text{M}$ , so the kinetic parameters have been calculated using only the arsenate influx values below 50  $\mu\text{M}$ , where a minimal contribution of the low-affinity uptake system could occur. After 8 weeks of growth, arsenate uptake was higher for -P plants than for +P plants. The  $V_{\text{max}}$  values obtained for the two P treatments are in the same range, but  $K_m$  is 8-times

higher for +P plants, suggesting a higher affinity of the cell membrane transporter for the substrate (arsenate) in –P plants.

Arsenate uptake is related to phosphorus nutrition, since phosphate and arsenate are taken up by the same transport system in a wide range of plants (Asher and Reay, 1979; Meharg et al., 1994) – so that increased phosphate status reduces arsenate uptake. The arsenate uptake kinetics (Fig. 1) shows this behaviour also. The same pattern occurred in *Cytisus striatus*, where root As concentration was higher for plants treated with 10  $\mu$ M P than for plants treated with 100  $\mu$ M P (Bleeker et al., 2002). Wang et al. (2002) observed a slow depletion of arsenate in the uptake solution of P sufficient plants (*P. vittata*), while a rapid decrease to a minimal concentration of arsenate was observed for plants grown without P.

Esteban et al. (2003) reported, for white lupin, a 4-fold higher  $K_m$  value for +P than for –P plants, the  $V_{max}$  values being in the same range for both +P and –P plants as in *C. scoparius*. In arsenate tolerant clones of *Holcus lanatus*, a 2- fold higher  $K_m$  value for +P than for –P plants was observed, being the  $V_{max}$  values very similar in both phosphate treatments (Meharg and Macnair, 1992). So, down-regulation of As uptake by phosphate may also take place in *C. scoparius* plants, confirming that the increase in phosphate concentration in nutrient solution can alleviate As toxicity.

### 3.5 Stress indexes

Chlorophyll *a* concentration decreased with the arsenate dose, 23% and 28% for 50 and 250  $\mu$ M As, respectively, with respect to control plants ( $p<0.01$ ), while there were no significant differences in chlorophyll *b* concentration (Table 4). For many plant species, the presence of As in the growth medium caused a decrease in chlorophyll

concentration (Mascher et al., 2002, Moreno-Jimenez et al., 2008). This occurred also for *Lemna minor* (Duman et al., 2010), but increases have been observed in *Brassica rapa* L. var. *pervirdis* (Shaibur and Kawai, 2009) and *Azolla filiculoides* (Sanchez-Viveros et al., 2011). In *Pisum sativum* (Päivöke and Simola, 2001) an increase in total chlorophyll was observed after 32 days of As exposure, but the chlorophyll a/b ratio decreased. The Chla/Chlb ratio in *C. scoparius* decreased significantly at the highest As dose, indicating that changes in photosynthesis could take place in the long term.

Under As exposure, ROS can be generated and they may induce lipid peroxidation of unsaturated fatty acids in membranes, leading to the formation of products like MDA (Singh et al., 2007). However, there were no significant differences ( $p < 0.05$ ) among treatments in MDA concentration (Table 4), although increased MDA levels have been observed under As exposure in many plant species, such as cucumber (Czeck et al., 2008) and *Holcus lanatus* (Hartley-Whitaker et al., 2001a). Probably, the threshold As concentrations to elicit oxidative stress have not yet been reached, accordingly with the high P/As ratios observed.

The presence of arsenate induced the synthesis of GSH and PCs in roots and shoots of *C. scoparius* (Fig. 2). There were significant differences in GSH concentration in roots among treatments, but it did not occur in shoots ( $p < 0.05$ ). The concentration of GSH in plant roots treated with 250  $\mu$ M As was twice the concentration of GSH in the control treatment. With regard to PCs in shoots, only PC2 was observed in the As treatments while no PCs were detected in the 0  $\mu$ M As treatment (control). When arsenate was added, PCs of larger size (PC3 and PC4) were observed in roots, besides PC2. This also occurred in plants of *Silene vulgaris*, which synthesised PCs of higher molecular weight when higher doses of As were applied (Sneller et al., 1999). There were marked increases in PC3 and PC4 concentrations in roots grown with 250  $\mu$ M As

with respect to 50  $\mu$ M, being 6- and 11-times greater, respectively. In addition, GSH levels did not decrease in plant roots treated with arsenate despite the synthesis of PCs (Fig. 2b), which is noteworthy considering the protective role of GSH as an antioxidant (Foyer and Noctor, 2005). Detoxification with PCs has been reported as an essential mechanism for plants in order to cope with As toxicity (Hartley-Whitaker et al., 2002). In *Cytisus striatus* and *Holcus lanatus*, the level of PCs increased under arsenate exposure, in both tolerant and non-tolerant plants (Bleeker et al., 2003; Hartley-Whitaker et al., 2001a). However, it has been suggested that the tolerance of metalicolous plants of *Silene paradoxa* is dependent on mechanisms of complexation that do not involve PC production (Arnetoli et al., 2008), as in the case of hyperaccumulator species (Zhao et al., 2003).

No significant decrease was observed in roots or shoots for the GSH:As ratio as the arsenate dose increased. For the PC:As ratio in roots, there was a significant increase at the highest arsenate dose (Table 5). In shoots, the GSH:As ratio was higher than the PC:As ratio. The –SH:As ratio reflects the relative abundance of GSH and PCs which could complex As. Most reports show a role for PCs in the detoxification of As, with –SH:As ratios > 3 (Sneller et al., 1999, Schömoger et al., 2000, Hartley-Whitaker et al., 2001b, 2002, Vazquez et al., 2005). Ratios > 3 imply that there are enough thiol groups available to complex As, while ratios < 3 indicate that not all the As can be complexed by thiol groups. According to our results, there are enough thiol groups (mainly from GSH) available to complex arsenic in shoots. On the contrary, low ratios in roots of *C. scoparius* indicate that there are not enough thiol groups available to complex all the arsenic present in the roots, despite the increase in the levels of PCs.

## 4 Conclusions

*Cytisus scoparius* accumulated arsenic mainly in roots. Stress indicators, such as glutathione and synthesis of phytochelatins, and the lack of evidence of an increase in malondialdehyde when arsenate was supplied indicate that *C. scoparius* has a certain tolerance to arsenic. So, its use in phytostabilisation is promising, considering the high biomass production even with As supply. According to these results, the use of *Cytisus scoparius* in the revegetation and remediation of As-polluted soils could be recommended but further research would be needed growing this plant species in soils contaminated with arsenic to evaluate the effectiveness of the process.

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**Table 1** As concentrations in the roots and shoots of *C. scoparius* ( $\mu\text{g As/g dry weight (DW)}$ ) with As accumulation in parentheses ( $\mu\text{g As/plant}$ ) after eight weeks of As supply (mean $\pm$ SE, n=4). Different letters indicate significant differences among treatments for each plant organ. (p<0.01)

As dose ( $\mu\text{M}$ )	Root ( $\mu\text{g/plant}$ )	Shoot ( $\mu\text{g/plant}$ )
0	n.d	n.d
50	71.48 $\pm$ 7.24 b (402 $\pm$ 94.3)	2.43 $\pm$ 0.97 a (56.0 $\pm$ 21.2)
250	118.24 $\pm$ 7.28 a (596 $\pm$ 96.7)	3.69 $\pm$ 0.83 a (68.2 $\pm$ 10.5)

As dose ( $\mu\text{M}$ )	Root	Shoot
0	1.15 $\pm$ 0.19 a	0.99 $\pm$ 0.10 a
50	1.76 $\pm$ 0.32 a (63)	0.74 $\pm$ 0.11 a (1133)
250	1.47 $\pm$ 0.25 a (30)	0.13 $\pm$ 0.03 b (9308)

**Table 3** Kinetic parameters for arsenate uptake by *C. scoparius* plants grown with 1 mM phosphate (+ P) or without phosphate (– P) (mean $\pm$ SE, n=4).

	$V_{\text{max}}$ nmol As $\cdot$ (g FW $\cdot$ h) $^{-1}$	$K_m$ ( $\mu\text{M}$ )	$R^2$	P-values
-P	216.1 $\pm$ 29.91	9.73 $\pm$ 3.92	0.959	<0.05
+P	270.8 $\pm$ 18.18	72.8 $\pm$ 7.36	0.992	<0.05

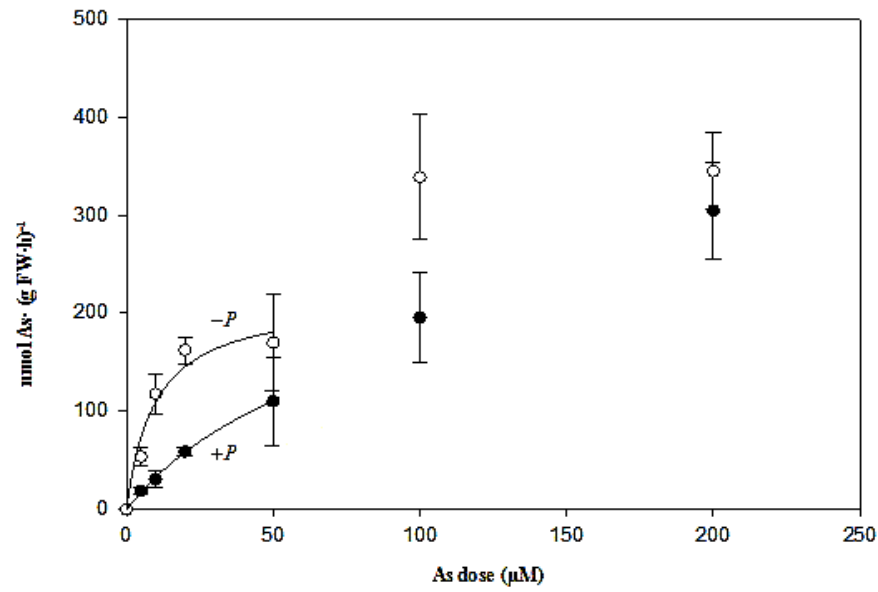
**Table 4** MDA concentrations in the roots and shoots, shoot chlorophyll concentration of *C. scoparius* and ratio Chla/Chlb. Different letters show significant differences among treatments for MDA, ( $p<0.01$ ) chlorophylls ( $p<0.01$ ) and ratio Chla/Chlb ( $p<0.05$ ) (mean $\pm$ SE, n=4) (FW: fresh weight)

As ( $\mu$ M)	nmol MDA $\cdot$ g <sup>-1</sup> FW		mg Chl $\cdot$ g <sup>-1</sup> FW		Ratio Chla/Chlb
	Root	Shoot	Chla	Chlb	
0	12.80 $\pm$ 1.87a	33.56 $\pm$ 2.54a	0.60 $\pm$ 0.01 a	0.12 $\pm$ 0.01a	4.95 $\pm$ 0.101 a
50	14.35 $\pm$ 2.43a	37.06 $\pm$ 3.13a	0.46 $\pm$ 0.03 b	0.10 $\pm$ 0.01a	4.63 $\pm$ 0.122 a
250	14.78 $\pm$ 1.80a	33.64 $\pm$ 2.77a	0.43 $\pm$ 0.04 b	0.11 $\pm$ 0.01a	4.07 $\pm$ 0.163 b

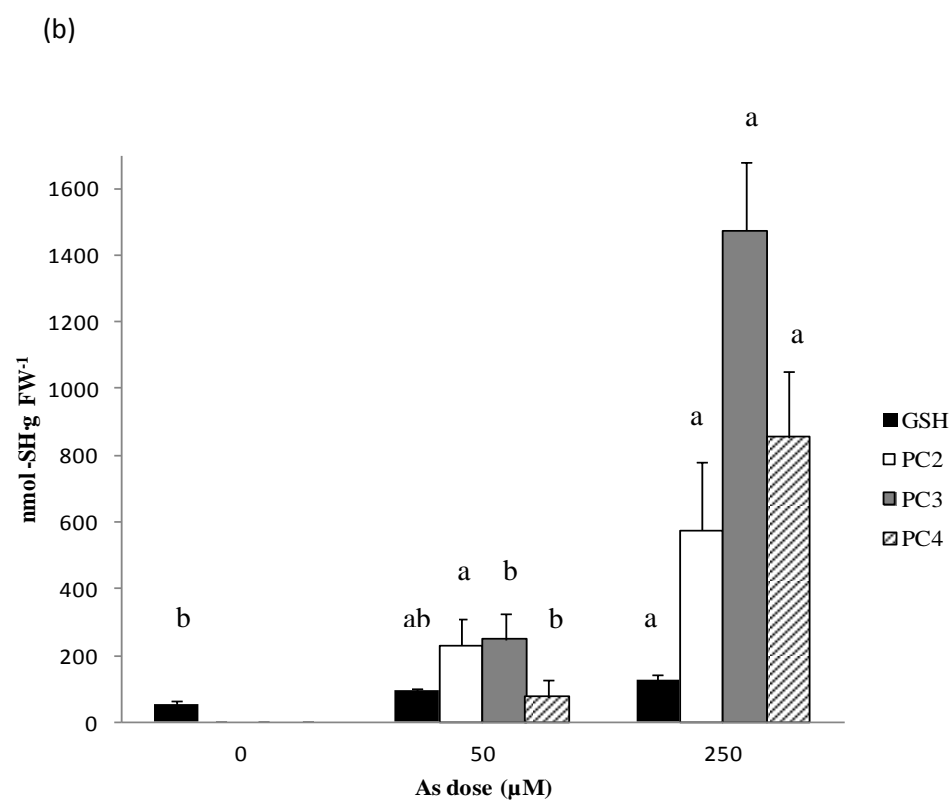
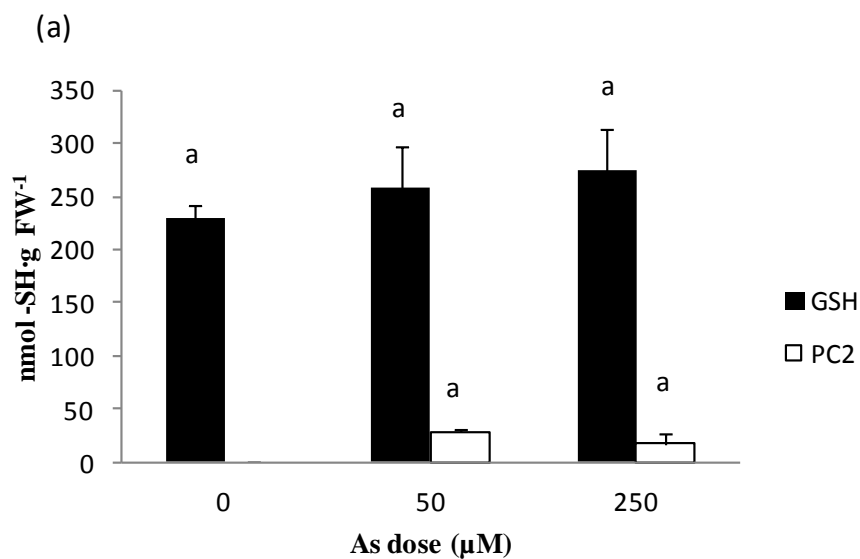
**Table 5** The -SH (GSH and PCs):As ratios for the shoots and roots of *C. scoparius*. Different letters show significant differences between treatments for each plant organ ( $p<0.01$ ) (mean $\pm$ SE, n=4)

As dose ( $\mu$ M)	Shoot		Root	
	GSH:As	PC:As	GSH:As	PC:As
50	14.19 $\pm$ 5.71 a	0.905 $\pm$ 0.24 a	0.103 $\pm$ 0.008 a	0.508 $\pm$ 0.144 b
250	6.17 $\pm$ 1.08 a	0.285 $\pm$ 0.147 a	0.076 $\pm$ 0.006 a	1.85 $\pm$ 0.263 a





**Fig 1** As uptake by excised roots of *Cytisus scoparius* plants grown without phosphate (open circles) and with 1mM phosphate (closed circles) for 8 weeks (mean±SE, n=3)



**Fig 2** Concentrations of GSH and PCs (nmol-SH referred to the internal standard N-acetyl-cysteine·g FW<sup>-1</sup>) in shoots (a) and roots (b) of *C. scoparius*. Different letters show significant differences among treatments ( $p<0.05$ ).