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Hydroponics as a valid tool to assess arsenic availability in mine soils

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Abstract

The low solubility of As in mine soils limits its phytoavailability. This makes the extrapolation of data obtained under hydroponic conditions unrealistic because the concentration in nutrient solution frequently overexposes plants to this metalloid. This work evaluates whether As supply in hydroponics resembles, to some extent, the As phytoavailable fraction in soils and the implications for phytoremediation. Phytotoxicity of As, in terms of biomass production, chlorophyll levels, and As concentrations in plants, was estimated and compared in both soils and hydroponics. In order for hydroponic conditions to be compared to soil conditions, plant exposure levels were measured in both cultures. Hydroponic As concentration ranging from 2-8 μM equated to the same plant organ concentrations from soils with 700-3000 mg kg^{-1}. Total and extractable As fractions exceeded those values, but As concentrations in pore water were bellow them. According to our results (i) hydroponics should include doses in the range 0-10 μM As to allow the extrapolation of the results to As-polluted soils, and (ii) phytoextraction of As in mining sites will be limited by low As phytoavailability.

Keywords: arsenate; dose-response; polluted soils; lupin plants; phytoremediation

1. Introduction

Arsenic is a trace element in soils that can pose significant risk to humans and the environment where it accumulates to high concentrations (Moreno-Jiménez et al., 2010). Human activities such as mining, coal burning or agriculture can increase As concentrations in soils. Pyritic mining is a major source of As, and soils impacted by this activity are relatively common (Ongley et al., 2007; Cattani et al., 2009; Moreno-Jiménez et al., 2010). Phytoremediation, an environmentally-friendly technique for soil reclamation, which can be competitive in derelict areas (Vangronsveld et al., 2009), is an emerging method of dealing with excessive concentrations of trace elements in soils. Within this technique phytoextraction consists of the uptake of As to the

harvestable parts of plants, after which they are removed, whilst phyto-stabilization and -immobilization are plant-aided stabilization techniques to reduce As mobility. One primary factor affecting plant accumulation is the available fraction of As in soils, and this availability has been of scientific interest for many years (McLaughin, 2001). Availability will affect both risk and plant uptake and, in the end, may determine the suitability or applicability of a particular soil remediation technology. Low availability of As in a soil will limit the extraction of significant amounts of the metalloid (Robinson et al., 2006). Low availability of As in mining soils is common because As is strongly retained in the most insoluble fractions (i.e., Fe oxides and sulphides) (Fitz and Wenzel, 2002; Beesley et al., 2010; Moreno-Jiménez et al., 2010). Single extractions have been frequently used as an index of trace element phytoavailability, although the accuracy to which this represents the true bioavailable fraction is doubted. In a previous pot experiment under controlled conditions, lupin plants were used for assessing As availability after single extractions with several extracting agents (Vázquez et al., 2008). Pore water has recently been effectively used as an indicator of As availability in some soil studies (Hartley et al., 2009; Clemente et al., 2010). Hydroponic cultures have been traditionally used in plant nutrition studies. The nutrient solution in hydroponics is prepared in controlled conditions so exact concentrations of elements can be modeled by specific software (i.e., V-MINTEQ) and manipulated. Phytoremediation is still an emerging technology under evaluation and many preliminary evaluations have been based in hydroponic experiments. Whilst hydroponics has already proved useful for screening interesting properties in plants (i.e., As resistance or accumulation) (Meharg, 2005), there is a limitation in many of these studies as they are useful from a physiological point of view but the tested doses are excessive in comparison with the available fraction of As in soils (Fitz and Wenzel, 2002). Additionally, there is evidence suggesting that plants respond in a different way when they are grown in hydroponics or soils (Zabłudowska et al., 2009). Despite these limitations, authors usually extrapolate results from hydroponic to field conditions and,

as a result, many studies are too optimistic and their conclusions unrealistic (Dickinson et al., 2009). For example, a plant species could be very efficient taking up As from nutrient solutions but this uptake will be limited under field conditions by As availability in soils. Total As is high in mine soils but the low available fraction largely limits the success of phytoextraction (Ernst, 2005).

Two experiments were carried out in parallel with different As doses: (i) a soil culture mixing two contrastingly As contaminated soils (ii) a hydroponic experiment using plants as indicators of As availability. The results attempt to indentify is the bioavailable fraction of As for plants in an As polluted mining site and whether results from hydroponics can be extrapolated to mining site soils.

2. Materials and Methods

2.1. Plant growth

White lupin seeds (*Lupinus albus* L.) cv. Marta were surface-sterilized in 10% (v/v) sodium hypochlorite for 15 min, rinsed thoroughly with deionised water and germinated in darkness at 28 ºC for 3 days on water-moistened filter paper. Plant seedlings were then transferred to a container with moistened (distilled water) perlite for 3 days. Thereafter, plants were grown in a growth chamber (DYCOMETAL®) under the following conditions: night/day T 20/25 ºC, photoperiod 13/11 h, relative humidity of 40/60%, and photosynthetic photon flux density of 520 μmol m^{-2} s^{-1}.

2.1.1. Soil experiment

Un-polluted soil and mining polluted-soil were collected from a previously studied site (Moreno-Jiménez et al., 2010). The unpolluted soil (UPS) had < 5 mg As kg^{-1}, 3% organic matter, a sandy texture, and pH ~ 5.3. Mining-impacted soil (MIS) had 4500 mg As kg^{-1}, <1% organic matter, sandy texture, and pH ~ 4.1. Soil treatments were made by mixing different UPS:MIS ratios: 100:0; 80:20; 60:40 and 40:60 (w:w). The rationale

behind these ratios was to obtain a range of As in the substrate but preserving other physicochemical properties in a range appropriate for plant establishment. Plastic pots containing a fine layer of sand at the bottom were filled with 1.5 kg of the corresponding mixture. The walls of the pots were drilled to keep the substrate aerated. Four replicates were used for each treatment in the soil experiment. Pots were watered to 70% of the water holding capacity (WHC) every day for an equilibration period of 2 weeks. Soils were watered to 70% of the WHC by weighting so that water was retained in the soil-root interface and none was lost to leaching. Rhizon samplers (Eijkelkamp®) were inserted vertically in the substrate. Thereafter, plants were transferred to the pots and watered daily for 4 weeks. Red-ox potential above 370 mV and pH in the range 4.2-5.3 were maintained in all pots. This was monitored weekly with an 18.21 multi-parameter analyzer (Eijkelkamp®). One day before harvesting, pots were watered to 80% of the WHC, and pore water was collected with *rhizon* samplers 5 hours after irrigation, using vacuum tubes (Beesley et al., 2010). SPAD index, measured using a chlorophyll-meter (Minolta SPAD-502, Japan), which indicates the relative level of chlorophylls, was also measured in young, completely developed leaves (García-Marco et al., 2006). After 4 weeks of culture, plants were carefully removed from the pots and roots and shoots separated. Soil particles were manually removed from roots, then roots and shoots were washed in tap and distilled water and weighted. After that, roots and shoots were rinsed in distilled water for 2 min. Finally, plant material was dried at 60 ºC for 3 days and milled to a fine powder with a grinder. Soil from each pot was also sampled, air-dried for 10 days, disaggregated and sieved to 2 mm.

2.1.2. Hydroponic experiment

Plants were transferred to plastic containers (2 L) with a nutrient solution (pH 5) consisting of: 1.5 mM $Ca(NO_3)_2$; 1.5 mM KNO_3; 0.1 mM KH_2PO_4; 1.0mM $MgSO_4$; 0.75 mM K_2SO_4; 53.8 μM Fe-EDDHA; 27.3 μM $MnSO_4 \cdot H_2O$; 1.6 μM $ZnSO_4 \cdot 7H_2O$; 0.32 μM $CuSO_4 \cdot 5H_2O$; 46 μM H_3BO_3; 0.016 μM $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$. Nutrient solutions were

113 continuously aerated by an air-pump to keep it oxygenate and to maintain
114 homogeneous the solution. As P supply affects both, As uptake and phytotoxicity
115 (Meharg and Hartley-Whitaker, 2002), the P dose was calculated to equal that
116 measured in pore water in the un-polluted soil (1 mg L^{-1}). Arsenic treatments were
117 added as NaH_2AsO_4 at the following doses: 0, 2, 5, 10 and 20 μM As. Each treatment
118 was replicated three times. Nutrient solution was renewed weekly and the containers
119 were watered if necessary. One day before sampling, SPAD was measured in young,
120 completely developed leaves (García-Marco et al., 2006). Plants were sampled after 4
121 weeks of treatment. Roots and shoots were separated and weighted. Roots were
122 thoroughly rinsed in tap and distilled water. Then both shoots and roots were rinsed in
123 distilled water for 2 min. Plant material was dried at 60 ºC for 3 days and milled to a
124 fine powder with a grinder.
125

126 **2.2. Analytical procedures**

127 Plant material (500 mg) was submerged in 10 mL mili-Q water, 3 mL HNO_3 (65%) and
128 2 mL H_2O_2 (33%), digested at 125 ºC under 1.25 kPa for 30 min in an autoclave
129 (Autester-G, Selecta), filtered and diluted to 25 mL with mili-Q water (Lozano-
130 Rodriguez et al., 1995). Soil (500 mg) was digested in an autoclave (Autester-G,
131 Selecta) at 125 ºC under 1.25 kPa for 30 min with 6 mL mili-Q water, 6 mL HNO_3 (65%)
132 and 4 mL H_2O_2 (33%), filtered and diluted to 50 mL (Vázquez et al., 2008).
133 Four solutions were used to extract As from soils:
134 -$CaCl_2$ (Vázquez et al., 2008): 2 g soil in 20 mL of 0.01 M $CaCl_2$ shaken for 3 h.
135 -$(NH_4)_2SO_4$ (Vázquez et al., 2008): 2 g soil in 20 mL of 0.1 M $(NH_4)_2SO_4$ shaken for 4 h.
136 -Low weight organic acids solution (LWOA) (Vázquez et al., 2008): 2 g soil in 20 mL of
137 LWOA solution, total concentration of acetic, lactic, citric, malic and formic acids was
138 0.01 M; their molar ratio was 4:2:1:1:1 (c/c), shaken for 16 h.
139 -EDTA (Lakanen and Erviö, 1971): 2 g soil in 20 mL of 0.02 M Na-EDTA in a buffered
140 solution 0.5 M CH_3COOH/CH_3COONH_4, shaken for 2 h.

All the extracts were filtered prior to analysis.

Arsenic in plant and soil extracts was analyzed by atomic fluorescence (Millenium Excalibur System, P S Analytical®, Kent, UK).

Certified reference materials (CTA-VL2, tobacco leaves, 0.97 µg As g^{-1}; CMR048-050, soil, 150 mg kg^{-1}) were also digested and analyzed. These were found to contain 0.94 µg As g^{-1} and 133 mg As kg^{-1}, respectively, with a coefficient of variance of <5%.

2.3. Statistical analysis, data processing and calculations

Influence of the As dose in either hydroponic or soil culture was evaluated by ANOVA with SPSS® and the statistical significance is shown in the results. Bivariate analysis was used to evaluate the correlation between As concentration in soil extracts and plant organs (Pearson's coefficient, *r*).

Arsenic concentration in plant organs as a function of the As dose in the growth media was represented and fitted to a curve using SigmaPlot®. Curve adjustment was evaluated by R^2. Arsenic concentrations in soil cultured plants were interpolated within hydroponic curves to calculate the corresponding soluble As dose (µM).

Potential calculated concentration of As (µM) in soil solution was calculated supposing that all the element extracted by the extracting solutions could be solubilized in the soil solution. This means soil As concentration for each extractant (in µg As kg^{-1} soil) is being transformed to As concentration in the soil solution (in µM). For pore water, it was not necessary to transform with neither soil weight nor water volume. Thus, equations are as follows:

Potential calculated concentration of As (µM) = $\frac{[As]_{extr}(\mu g\ As\ kg^{-1}) \times 1.5\ kg}{0.6\ L \times 75\ \mu g\ As\ \mu mol\ As^{-1}}$; or

$\frac{[As]_{pw}(\mu g\ As\ L^{-1})}{75\ \mu g\ As\ \mu mol\ As^{-1}}$, where $[As]_{extr}$ are extractable As fractions in soil for each extracting solution and $[As]_{pw}$ is concentration of As in pore water. Soil weight (1.5 kg) and volume of soil solution in each pot (0.6 L) were used for the calculation.

3. Results

3.1. Effects of As dose in plants growing under hydroponics or soil conditions

Arsenic concentration in plant shoots and roots progressively increased with the As dose either in the nutrient solution or in the soil (Figure 1A-D, $P<0.001$ for all the cases). For soil culture (Fig 1B,D), As total concentrations in each substrate were used. Arsenic levels in plants reached values of 1047 and 15.2 mg As kg^{-1} in hydroponics, and 600 and 12.2 mg As kg^{-1} in soil culture for roots and shoots, respectively. Hyperbolic curves were successfully fitted for As concentration in shoots and roots. Shoot-to-root ratios were calculated to compare As translocation (Table 1), and their values were in the range 0.015-0.035 and decreased when As increased in the growth media. Arsenic in nutrient solution decreased plant growth by up to 60% in roots and 64% in shoots, whilst in the soil a decrease of up to 20% and 47% was found in roots and shoots, respectively. SPAD values also decreased when As dose increased in the growth media. This index suggested chlorophylls levels reduced to 21% in hydroponics and to a 17% in soil culture in comparison to 0 μM As and un-polluted soil pots, respectively.

3.2. Equivalence between hydroponic and soil cultures

The equivalent soluble As concentration was calculated for soils by interpolating As concentration in plants growing in soils to the fitted curves obtained for hydroponics (Fig. 1 A,C), as shown by the arrows in the figure. Although some pots had total As levels of almost 3000 mg As kg^{-1}, the highest corresponding soluble dose of As was 8 μM (Table 2).

3.3. Phytoavailable fraction of As in soils

194 Chemical extractions were performed in soils using four different methods commonly
195 used to assess the bioavailable fraction. Pore water was also sampled in the pots. The
196 results are shown in Table 3, along with pH, and were used as primary data for further
197 calculations. The extractability followed this order: $CaCl_2$ < $(NH_4)_2SO_4$ < EDTA <
198 LWOA. All the extractions were significantly correlated, with the highest Pearson's
199 coefficient for $(NH_4)_2SO_4$ and LWOA (Table 4).
200 Potential arsenic in soil solution was theoretically calculated for each extractant (Table
201 5, see Section 2.4.). All the extractions exceeded the corresponding soluble As
202 concentration, apart from pore water, where As concentrations were bellow the
203 calculated value.
204
205 **4. Discussion**
206 Similar or milder effects of As toxicity were obtained in plants grown on soil than in
207 those grown on nutrient solution. Thus, soil values can be compared to hydroponics
208 and the effects can be interpolated. Previous studies have shown changes in plant
209 response to arsenic when growing in soil and in hydroponic cultures, as a different
210 shoot-to-root translocation of As for example (Zabłudowska et al., 2009). However, our
211 study showed shoot-to-root As ratio values in the same range for both soil and
212 hydroponics, indicating that As accumulation by lupin plants was independent of the
213 kind of culture. Under the present experimental conditions of pH and redox potential
214 (see 2.1.1. and 2.1.2.) soluble arsenic is probably mainly speciated as $H_2AsO_4^-$ in both
215 hydroponics and soil cultures (Sadiq, 1997). Therefore, this experimental design seems
216 to be a suitable tool to compare As supply between soil and hydroponics. A
217 concentration of 3000 mg As kg^{-1} soil started to induce clear toxicity symptoms in
218 plants, and the calculated corresponding As soluble concentration was, on average,
219 7.4 μM. Similarly, plants growing in hydroponics started to show stress symptoms
220 somewhere between 5 and 10 μM. The interference and induced stress promoted by
221 As in plant tissues is a well known effect of exposure to this toxic element and can

include plant disorders such as metabolic and mineral disturbances, oxidative stress and depletion on chlorophyll level (Moreno-Jiménez et al., 2008).

Hydroponic solution is a defined media, allowing As availability and speciation to be controlled. Therefore, the calculated equivalent doses serve as an indicator of As phytoavailability. In soils, the element is taken up by plants from the soil solution, so we can compare hydroponic solution with potential soil solution and pore water underestimated the As equivalent dose (Table 5). This can be explained because plants (especially lupin plants) can mobilize nutrients such as iron and phosphorus in the rhizosphere (Neumann et al., 2000) and the mechanisms involved this process mobilize As in soluble to a similar extent as the nutrients (Fitz and Wenzel, 2002). *Rhizon* pore water samplers were positioned to extract soil solution from bulk soil and not specifically target the root-soil interface. Therefore soil solution under the influence of mobilization mechanisms of lupin roots was probably not fully represented by the soil solution extraction procedure employed in the present study. Chemical extraction procedures assess both current solubility of As and the likely re-supply from the labile fractions to soil solution in a short to medium period of time. Among all the extractions, $CaCl_2$ represents most accurately the corresponding soluble As concentration than the other methods. Assessing both pore water and $CaCl_2$ in soils therefore could provide an idea about the fraction that is immediately soluble and that is potentially phytoaccessible in the medium term. This information could also indicate the optimum As concentrations that should be used in hydroponic experiments to resemble exposure doses in contaminated soils.

Traditionally, the way to evaluate whether a method can evaluate phytoavailability has been by using the correlation coefficient between extractable element and As concentration in shoots (Feng et al., 2005; Vázquez et al., 2008). The best method (with the highest *r*) was the extraction with $(NH_4)_2SO_4$ (Table 4), which is in agreement with previous studies (Vázquez et al., 2008). These results show again the difficulties

to handle with availability, which is an exceptionally complex concept that can only be estimated.

All the results indicate a low availability of As in the mining soil, which limits plant uptake. Equivalent As dose in the soil experiment was low (<8 μM). This is in agreement with previous data involving different soils (Fitz and Wenzel, 2002). In a similar mining soil, not only labile As concentrations were low but also the re-supply of As to soil solution was slow (Cattani et al., 2009).

5. Conclusions

Unrealistic doses of As under hydroponic conditions have previously limited interpretation of this data with regards to field applications (Dickinson et al., 2009). The results of the present study suggest low As availability, which should limit As phytoextraction in this kind of mine polluted soil, making phytostabilization a feasible prospect. Therefore, we propose that hydroponic studies are valid in such circumstances but should not just use high doses of As, but also doses in the range 0-10 μM, which includes As levels that plants are exposed to in soils. In this respect the results of hydroponics can be confidently extrapolated to soil conditions.

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343 **Table 1.** Plant fresh weight, SPAD index and $[As]_s$:$[As]_r$ ratio in lupin plants growing

344 either on soil or in hydroponics. Mean ± SE (*n*=3-4).

As dose		**Fresh weight (g)**		**SPAD**	**$[As]_s$:$[As]_r$**
		Root	**Shoot**		
Hydroponics					
(μM)	0	9.0 ± 0.6	12.4 ± 1.7	52 ± 1	-
	2	8.4 ± 0.3	10.4 ± 0.3	48 ± 1	0.035
	5	8.0 ± 0.6	8.6 ± 1.0	45 ± 2	0.025
	10	4.3 ± 0.8	5.3 ± 0.7	44 ± 1	0.015
	20	3.9 ± 0.3	4.5 ± 0.1	41 ± 2	0.015
ANOVA		<0.001	<0.001	<0.001	-
Soil culture					
(mg As kg^{-1})	2	1.7 ± 0.3	5.2 ± 0.2	47 ± 1	-
	745	1.9 ± 0.1	4.4 ± 0.2	44 ± 2	0.027
	1751	1.9 ± 0.1	4.7 ± 0.3	41 ± 2	0.026
	2937	1.5 ± 0.1	2.8 ± 0.1	40 ± 3	0.020
ANOVA		0.150	0.111	<0.001	-

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347 **Table 2.** Equivalent As dose in soils, interpolated from Fig. 1A,C. Mean (*n*=4).

As soil	**Equivalent As dose (μM)**	
(mg As kg^{-1})	**Root**	**Shoot**
745	2.4	2.8
1751	3.7	5.0
2937	6.7	8.0

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350 **Table 3.** Total As concentration in different treatments of the soil culture, As extracted
351 by several methods, pH, and As in pore water: Mean (*n*=4). Soil treatment was
352 obtained by mixing an unpolluted soil with an As-polluted soil at different ratios, UPS:
353 unpolluted soil; MIS: mining impacted soil.

UPS:MIS (w:w)	**Total (mg As kg^{-1})**	**$CaCl_2$**	**$(NH_4)_2SO_4$**	**EDTA**	**LWOA**	**pH**	**PW (mg As L^{-1})**
100:0	2	n.d.	n.d.	n.d.	n.d.	5.2	n.d.
80:20	745	0.36	1.7	1.0	4.2	4.9	0.033
60:40	1751	0.92	3.7	3.7	7.4	4.7	0.045
40:60	2937	0.95	5.2	7.9	11.4	4.5	0.066
ANOVA	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.01

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357 **Table 4.** Pearson's correlation coefficients (*r*) between As extracted by different
358 solutions from soil and As concentration in roots and shoots of lupin (*n*=16). All the
359 coefficients were statistically significant (*P*<0.001).

	$CaCl_2$	**$(NH_4)_2SO_4$**	**EDTA**	**LWOA**	**PW**
$[As]_{shoot}$	0.88	0.93	0.81	0.92	0.87
$[As]_{root}$	0.85	0.93	0.91	0.95	0.92

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362 **Table 5.** Potential concentration of As in soil solution: (i) extracted by 0.01 M $CaCl_2$, 0.1
363 M $(NH_4)_2SO_4$, EDTA solution and low weight organic acid solution (LWOA); (ii) in pore
364 water (PW); and (iii) averaged* from equivalent doses (EqD) for roots and shoots (see
365 Table 2).

As soil (mg As kg^{-1})	**745**	**1751**	**2937**
	Calculated dose (μM As)		
$CaCl_2$	12	31	32
$(NH_4)_2SO_4$	57	124	174
EDTA	33	122	382
LWOA	134	248	265
PW	0.4	0.7	1.0
EqD*	2.6	4.3	7.4

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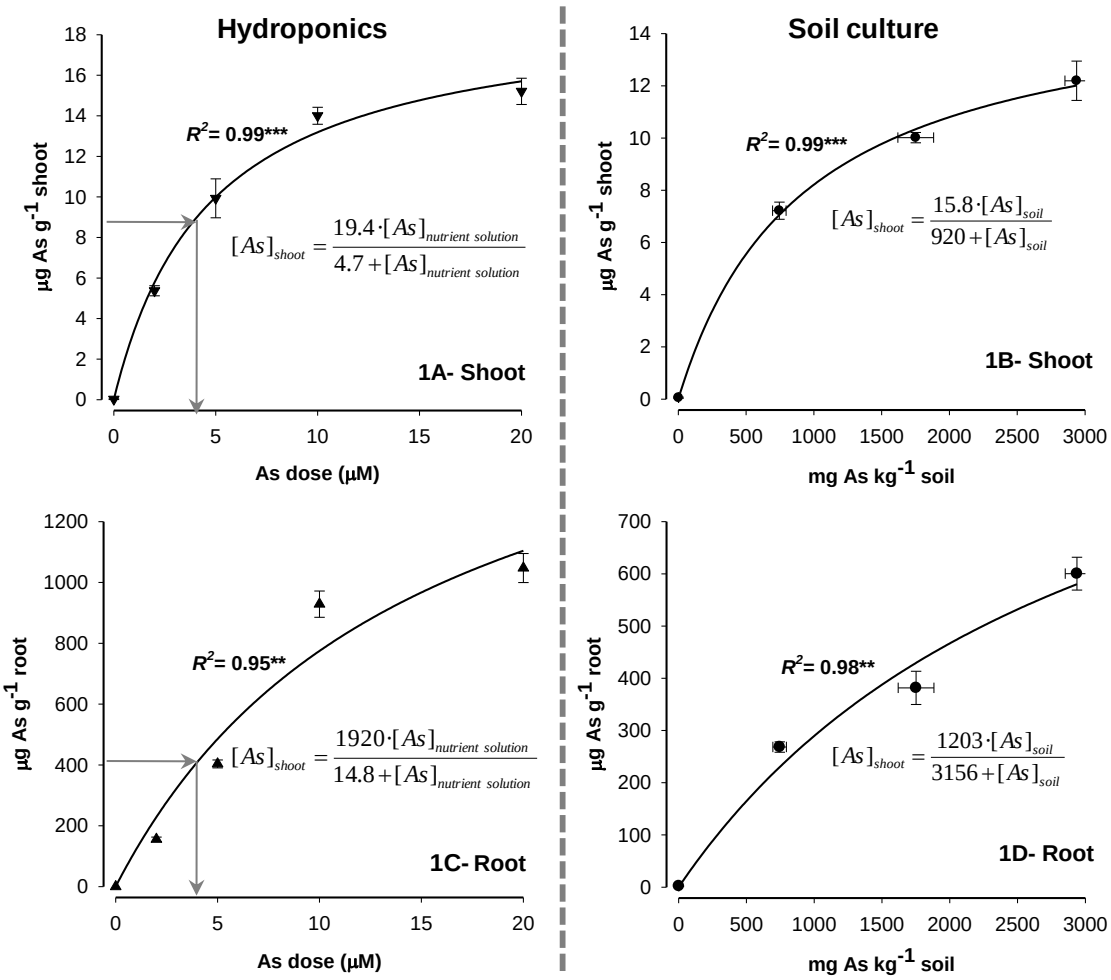

Figure 1. Arsenic concentration in roots and shoots of lupin plants growing in hydroponic (A,C) and soil culture (B,D) with different As doses. Mean ± SE (*n*=3-4). Data were fitted to a hyperbolic curve and R^2 and curve equation are shown in the graph. Grey arrows* show the way data have been interpolated in the curve, by using As concentration in plants from the soil experiment to obtain the corresponding soluble As concentration. *Note: the arrows are just simulating the way data were interpolated in the curve, but they do not correspond to any treatment.