Scientific
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Lupinus microcarpus Growing in Arsenic—Agricultural Soils from Chile: Toxic Effects and It Potential Use as Phytoremediator Plant

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Abstract

Arsenic (As) is the most important contaminant of the environment in northern Chile. The purpose of the present work is to study As-toxicity symptoms on *Lupino microcarpus* (lupine), an annual legume plant that constitutes part of the desert community of the pre-Andean area of the Antofagasta Region, Chile. This plant species is cultivated in As-agricultural soil collected from Chiu Chiu (northern Chile) which is classified as arid soils. Control soil (0 - 20 cm depth) is collected from an area located in the central zone of Chile, which is classified as molli soil. The main physic-chemical characteristics of As-soil and the control soil are determined. Eighteen plastic pots of 1.6 L (fifteen for experimental and three for control) are filled with As-soil and control soil treatments. Two plants are cultivated in each pot and then separated leaves and roots for As-analysis. Visual As-toxicity symptoms such as foliar chlorosis, necrosis of the leaf tips and margins, leaf wilting and stunted are determined. Total As concentrations in soils where lupine is cultivated, reach levels between 5.3 - 14.2 mg·kg⁻¹ d.w. (control soil As-level: 3.1 mg·kg⁻¹). Roots show higher As-concentration than leaves, both experimental plants as control plants (2.28 - 9.1 mg·kg⁻¹ d.w., and 0.76 mg·kg⁻¹ d.w., respectively) and low values of transport index (TI) (0.16 - 0.34). All of visual As-toxicity symptoms determined is showed by lupin cultivated in

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As-agricultural soils. Neither control lupin plant suffers any toxicity symptoms. The results indicate that lupine plants do not resist contamination and accumulated higher levels of As in roots. Lupine can be used in the phytostabilisation of As immobilizing it by microbial activity in agricultural soil.

Keywords

Lupinus microcarpus, **Arid As-Soil, As-Toxicity, Phytostabilization**

1. Introduction

Natural hydrogeological characteristics and the presence of volcanoes and mining activity are the most important sources of arsenic (As) pollution in the water and soil in the Antofagasta Region of Chile [1].

The average concentration of As is estimated in soil varies between 5 and 8 mg·kg^{-1} [2]. Most naturally occurring As has been transported in particular form from weathered rocks [3]. However, near smelting operations and around older orchards where arsenicals pesticides are used soil levels of 100 to 2500 mg·kg^{-1} As have been found [4]. Although it is estimated that about 80% of the total amount of anthropogenic (or man-made) As releases into the environment resides in soil, most As compounds remain in particulate form and adsorb to soil particles being transported via leaching only short distance in the soil [4] [5]. The As adsorption capacity of the soils is positively correlated with the free Fe oxides, MgO, Al_2O_3 and clay content of the soil [4].

It has been known for decade that soils with elevated As levels (*i.e.* >20 mg·kg^{-1}) produce plants with increased As levels [6]. In addition, concentrations of As may be 10 to 1000 times greater in soil than in plants growing on that soil. Moreover, the distribution of As among various plant parts is highly variable, with seed and fruits having lower As concentrations than leaves, stems or roots [7]. Roots and tubers generally have the highest As concentrations with the skin having higher concentrations than the inner flesh [8]. The edible portions of vegetables seldom accumulate high concentrations of As because most plants will be killed or severely stunted long before the As concentration in their tissues reaches concentrations that pose a health risk [9]. However it should be recognized that the As content found in plants will also depend on soil conditions. Plants generally absorb the least amount of soil As at neutral soil pH and increasing soil organic matter has been found to reduce plant uptake As of soil. However, adding phosphate amendments to high-As soils has been found to increase plant uptake As of soil [8]. This phenomenon can be because of the chemical similarity between P and As, which are in the same column of the periodic table.

There are many studies related with plant tolerance to As under hydroponic conditions [10]-[13], whereas there are only a few studies performed in As-contaminated soils (without artificially spiking with As) [14].

Many hydroponic studies have used much higher concentrations of As than those found in soil solutions, and their environmental relevance has been questioned [15]. On the other hand, water availability is a major limiting factor for the establishment of arid plant species. Moisture conditions immediately prior to and during germination play a dominant role in regulating germination in these dry environments [16].

Low and erratic precipitation characterizes the climate of the arid desert region of Northern Chile. This in combination with the rapid drying out of the soil due to intense sunlight contributes to extremely limited water availability throughout most of the year and creates conditions that greatly affect seed germination and seedling survival in these regions [17].

Numerous studies on heavy metals phytoremediation, including phytoextraction and phytostabilisation on contaminated areas can be found in the literature [9] [18]. This phytotechnology seems to be an economically and ecologically feasible way of decontamination, especially for remaining toxic elements in soils [19].

Lupinus microcarpus (lupine, arvejilla) is an annual legume and an important native plant that constitute part of the desert scrub community of the pre-Andean area of the Antofagasta Region [7]. Arsenic concentrations in soils of this Region can vary from 50 to 70 mg·kg^{-1} [7] [20].

Previous studies with lupine, have allowed to consider *Lupinus microcarpus* and *Lupinus albus* (white lupine)

their use in phytoremediation and also as candidates for the revegetation of degraded landfill areas. Their N_2 fixation capacity, strong root system, and capacity to excrete citrate, through roots, among other properties, allow these plants to survive in poor and contaminated soils with As [7] [20].

In consequence, we selected *L. microcarpus* cultivated on As-contaminated agricultural arid soils with the aim to study the toxic effects on it development and then the possibilities of phytoremediation of agricultural soils affected with As pollution.

2. Materials and Methods

Zone of soil collection and determination of the main physicochemical properties. Surface soil samples (0 - 20 cm depth) were collected from three agricultural smallholdings located in Chiu Chiu community (22°20'57"S, 68°38'91"W, 2535 m.a.s.l.) 35 Km from the city of Calama, northern Chile (**Figure 1**). These soils have been classified as arid soils and used for agricultural purposes, especially vegetables. Agriculture is the inhabitants' main economic activity. The zone of Pre-Andean soil collection is influenced by the Loa River and is located prior to the confluence with the Salado River. The zone is characterized by scarce/poor plant cover, saline properties of soils and high concentrations of As of natural origin [14].

Control soil (0 - 20 cm depth) was collected from an area located in the central zone of Chile (33°26'S, 68°39'W, and 529 m.a.s.l.) which were classified as molli soils. The main physico-chemical and pH were determined in water extracts (1:5 v/v) using an electrode (WTW multi 340i). The organic matter content was determined using $K_2Cr_2O_7$, H_2SO_4 and saccharose solution as standard solution [7]. For total mineral elements determination, soil samples were dried at 40°C to constant weight, sieved to particle size of 2 mm and digested with aqua regia (HNO_3-HCl 1:3). Total Ca, K and Mg concentration were determined by atomic absorption spectrometry (AAS) with a Thermo Electronic Corporation AA Series apparatus.

Total As concentration in soils, was performed by dry ashing mineralization and quantification by flow injection hydride generation atomic absorption spectrometry (FI-HG-AAS) using a Perkin Elmer FIA 100 apparatus (detection limit 0.01 $\mu g \cdot As \cdot L^{-1}$) [7] [14]. Accuracy for Montana Soil (certified standard) found value = 98.19 ± 0.03 $\mu g \cdot g^{-1}$ (certified value = 105 ± 8 $\mu g \cdot g^{-1}$). Deionized water (18 M·Ω·cm) was used for the preparation of the reagents and standards. All chemical were of pro analysis quality and certified standard solution of As was used (MERCK Germany).
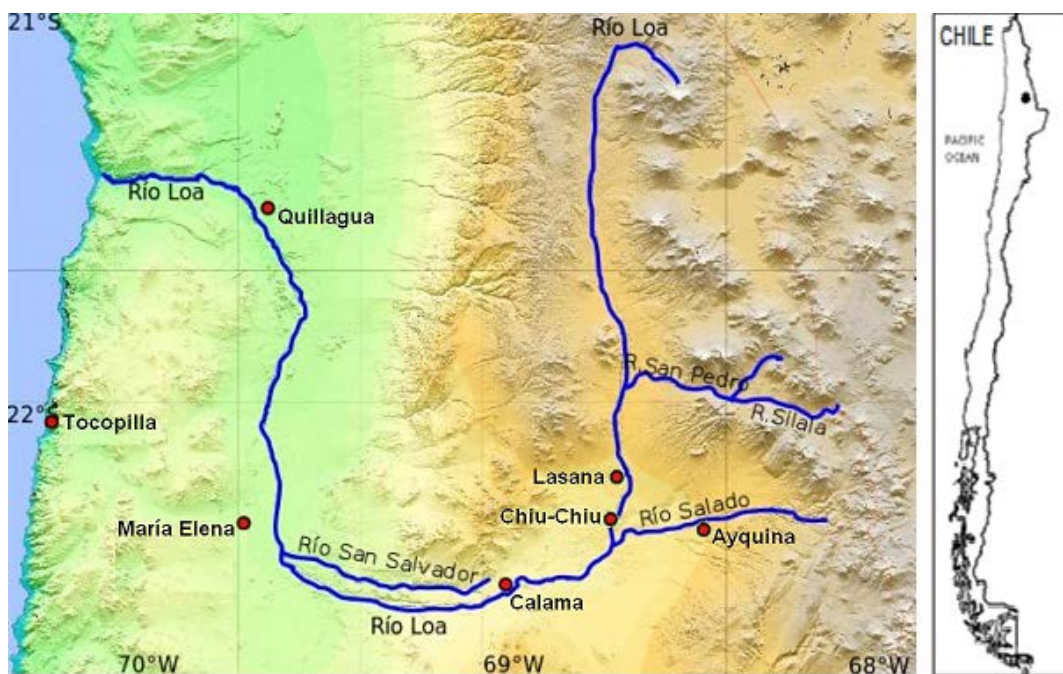

Figure 1. Study area near Loa river where soil samples were collected (Pre-Andean zone in Antofagasta Region, Chile).

2.1. Pot Experiments

L. microcarpus seeds were surface sterilized and germinated on moistened filter paper at ambient temperature in the dark for 3 days. Seedlings were placed on perlite in plastic containers for 15 days (**Figure 2**) and then transplanted in As-soil and control soil treatments. Eighteen plastic pots of 1.6 L (fifteen for experimental and three for control) were filled with As-soil and control soil treatments (**Figure 3**). Two plants were cultivated in each pot performed in the research greenhouse of the University of Santiago of Chile under ambient conditions and protected from rain. The plants were irrigated once a week with 200 mL of distilled water per container [21]. This study was conducted from March until September 2010. During the assay, the average minimum temperature was 7.9˚C ± 2.8˚C; average maximum temperature was 23.4˚C ± 4.6˚C and relative humidity was 60.3% ± 26.5% [14] [22].

Toxicity symptoms on *L. microcarpus*, such as foliar chlorosis, leaf wilting, necrosis of the leaf tips and margins, height (cm), stunted and number of leaves were observed and counted every month, from April to September.

2.2. Analysis of Vegetal Samples

L. microcarpus var. microcarpus is a species of lupine native to western North America from southwestern British Columbia to the Mojave Desert in California and Baja California and also a disjunct population in South America in Central Chile and western Argentina. It grows from sea level in the North of the range, up to 1600 m in southern California.

(a)   (b)

Figure 2. Seedlings of lupin on perlite substrate.

Figure 3. Small plant of lupin growing on As-soil collected from Chiu Chiu, Chile.

It is an annual plant growing to 80 cm tall. The leaves are palmetely compound with 5 - 11 leaflets 1 - 5 cm long and up to 1 cm broad. The flowers are generally pink to purple in color but can also be between white and yellow; they are produced in open whorls on an erect spike (**Figure 4**).

After 6 month from the beginning of the experiments, leaves and roots were separated and the soil particles were manually removed. Plant material rinsed under tap water for 5 min and submerged in distilled water for 2 min, dried at $60°C \pm 5°C$ until constant weight and then ground and sieved. Later, samples were calcined ($425°C \pm 25°C$) for 12 h. Once the ash had cooled, 5 mL of 10% HNO_3 was added, the mixture was evaporated in the sand bath, and the calcination process was repeated until white ash was obtained. The white ash was dissolved in 5 mL of 50% v/v HCl and 5 mL of reducing solution (5% m/v KI + 5% m/v ascorbic acid). After 30 mins, the resulting solution was diluted to volume with 50% v/v HCl and filtered through Whatman N° 1 filter paper into a 25 mL volumetric flask. The filtered acid digest were analyzed for t-As concentrations by FI-HG-AAS. All results are expressed in dry matter [7] [20]. The bioconcentration factor (BCF) and transport index (TI) of As to the leaf were calculated using the following equations: BCF = As concentration in leaf/total As concentration in soil ($mg\cdot kg^{-1}$), and TI = As concentration in leaf/As concentration in root ($mg\cdot kg^{-1}$) [7] [23].

2.3. Statistics Analysis

The statistical analysis was based on the mean values concentrations of As, standard deviation, variation coefficient for pH values, electrical conductivity, organic matter, Ca, K and Mg, and standard error. All statistical tests were carried out with the SPSS 13.0 and the GraphPad software packages.

3. Results and Discussion

3.1. Total as Concentration in Soil, Seeds, Leaves and Roots of *L. microcarpus* Bioconcentration Factor (BCF) and Transport Index (TI)

Total As concentrations in soils was variable, being higher in soil 2 and 3 than in soil 1. These values are also higher than in control soil (**Table 1**). Arsenic in seeds was not detected.

Figure 4. Adult plant of *Lupinus microcarpus* specie growing in it natural environment.

Table 1. Mean As-concentrations (mg·kg^{-1}, dry weight, d.w.) in soil samples, leaves and roots of *L. microcarpus*.

Soil Sample	As (mg·kg^{-1} d.w.)			BCF	TI
	Soil	Leaf	Root		
Soil 1	5.3	1.44	9.08	0.27	0.16
Soil 2	13.1	0.78	2.28	0.06	0.34
Soil 3	14.2	0.94	3.53	0.07	0.27
Soil Control	3.1	0.19	0.76	0.06	0.25

Bioconcentration Factor (BCF) and Transport Index (TI).

Arsenic concentration found in roots of *L. microcarpus* was higher than in leaves, especially in soil 1. In this soil was obtained the lowest As concentration with respect to soils 2 and 3. However, *L. microcarpus* cultivated in 2 and 3 soils showed lower As concentrations both roots and leaves with respect to the plants cultivated on soil 1. Bioconcentration factor (BCF) was higher in leaves corresponding to lupine cultivated in soil 1 than the species cultivated in soils 2 and 3, included control soil. Transport Index (TI) showed higher values in roots of lupine cultivated in soils 2 and 3 than in soil 1 and control soil.

These results show that lupine plants cultivated in soil 1 (low As concentration with respect to soil 2 and 3, **Table 1**) accumulate high levels of the metalloid in their leaves (1.44 mg·kg^{-1}) which is supported by the high BCF value obtained (0.27) also showing a higher As concentration in root (9.1 mg·kg^{-1}), which correspond with a lowest value of TI (0.16) in relation to plants cultivated in soils 2 and 3. With respect to the control soil, this shows lowest levels of arsenic in both leaves and roots (0.19 and 0.76 mg·kg^{-1}, respectively). BCF and TI values were similar to obtained in plants cultivated on experimental soil.

Highest As-concentration measured in roots of *L. microcarpus* cultivated both experimental soil and control soil with respect to As-concentrations measured in leaves, show that this plant species acts as phytostabilizator on contaminated As-soils.

These results indicate that some agricultural soils in Chiu Chiu are contaminated with As, being that its concentration is higher than the average for natural soils of 5 to 6 mg·kg^{-1} [2] [3]. In farming As-soil samples collected in the villages of Chiu Chiu and Lasana (Antofagasta Region, Chile), the As concentration ranged between 50 - 70 mg·kg^{-1} [20]. In another work conducted by Díaz [7], As levels found in agricultural soils collected in Chiu Chiu were of 53 ± 9.91 mg·kg^{-1} dry weight (d.w.) and several of the main physic-chemical soil characteristics measured in those soils were similar to results obtained by Díaz *et al.* op. cit.

More recently, Tapia *et al.* [14] measured mean As-concentration (n = 3) in soil samples collected in Chiu Chiu, equivalent to 111 ± 19 mg·kg^{-1}, d.w., much higher than those found in the present study (**Table 1**). The As levels (n = 3) obtained in the control soil collected from an area located in the central zone of Chile, similar to our work, reached 12.7 ± 1.1 mg·kg^{-1} d.w., higher than those obtained in our work. These results indicate that the As-concentrations in soils from Chiu Chiu, are variable and varies from place to place.

It has been known for decades that soils with elevated As levels (*i.e.* ≥20 mg·kg^{-1}) produce plants with increased As levels. However, it should be recognized that many of these studies were conducted with soils containing > 500 mg·kg^{-1}, whereas most soils contain ≤ 10 mg·kg^{-1} [6] [24]. In addition, concentrations of As may be 10 to 1000 times greater in soil than in plants growing on that soil. Moreover, the distribution of As among various plant parts is highly variable, with seeds and fruits having lower As concentration than leaves, stems, or roots. Roots and tubers generally have the highest As concentrations [3] [8] [21], similar to our results (**Table 1**).

Tapia *et al.* [14] in a research conducted in two Atriplex species (*A. halimus* and *A. atacamensis*) cultivated in As-soil from Chiu Chiu, determined that the concentration of As in the leaves of *A. halimus* was significantly higher than *A. atacamensis*, whilst the concentrations of As in stems and roots were similar between two species. After 90 days, *A. halimus* cultivated in As-soil maintained the concentration of As in the leaves, increased the concentration of As in stems and roots, which was the highest concentration of As found in that assay (16.3 ± 3.3 mg·kg^{-1}). *A. atacamensis* maintained the As concentration in the roots and showed similar levels of As in the leaves and stems between plants grown in As-soil, indicating that this specie did not translocate the As to the aerial part, similar to our results. In control soil, the concentrations of As in leaves, stems and roots at 30 and 90 days, did not show significant differences between two species.

However, Díaz *et al.* [7] found higher As levels in leaves of *L. microcarpus* (9.7 ± 1.6 mg·kg⁻¹, d.w.) collected from Ayquina (Antofagasta Region, Chile), than stem and roots. Nevertheless, As concentrations in Ayquina soils were lowest (5.6 ± 1.04 mg·kg⁻¹) similar that obtained in soil 1 (**Table 1**). Other researchers have also found high As levels in plants collected from soils with low As levels [25].

The authors estimate that these results are uncommon because most plants preferentially accumulate As in their roots and, even if they absorb high levels, transport of the metalloid to the aerial parts is minimal [26]. This fact agrees with the results obtained in the present study. The transport index (TI) is an important feature for characterizing plant capacity in phytoremediation techniques [27] [28]. TI values lower than 1 for plants indicates that arsenic transportation to the shoots is limited and that could be due to high phosphate concentrations [29] [30].

With equivalent soil As concentrations, plants grown on sands or sandy loam soil usually have higher total As contents than those grown on heavier-textured soils. Plants generally absorb the least amount of soil-As at neutral soil pH and increasing soil organic matter by adding compost, manures, or other organic soil amendments, it has been found a decrease in plant uptake of soil-As [3]. This seems to be the principal explanation for the lower As levels on plants cultivated in control soil (pH: mean 7.3; organic matter: 6.8%) than in experimental soil (pH: mean 7.8; organic matter 2.7%) values presented in **Table 2**.

Table 2. Main physico-chemical soil characteristics.

Soil Sample	pH	Electric Conductivity (dS·m⁻¹) Extract 1:10	Organic Matter (%)	Ca	K	Mg
					(mg·kg⁻¹)	
	8.0	1.19	2.77	6.66	7.87	1.51
Soil 1	7.6	4.65	2.46	5.58	4.39	1.26
	7.5	1.86	2.87	5.66	5.11	1.08
Average	7.7	2.57	2.70	5.97	5.79	1.28
SD	0.25	1.84	0.21	0.60	1.84	0.22
CV	3.25	71.53	7.89	10.12	31.74	16.98
	7.8	1.04	3.83	7.48	4.79	1.63
Soil 2	8.3	0.38	4.34	7.71	6.97	2.09
	7.2	2.26	4.07	7.71	6.54	1.94
Average	7.8	1.23	4.08	7.63	6.10	1.89
SD	0.58	0.95	0.25	0.13	1.16	0.24
CV	7.48	77.70	6.19	1.76	18.95	12.57
	8.2	0.63	1.70	6.00	4.22	0.99
Soil 3	7.7	4.70	2.06	5.59	4.56	0.95
	7.4	1.14	3.87	6.07	6.91	2.03
Average	7.8	2.16	2.54	5.89	5.23	1.32
SD	0.40	2.22	1.17	0.26	1.46	0.61
CV	5.13	103.10	45.90	4.41	27.97	46.20
	7.2	0.46	6.66	8.26	2.72	0.14
Control Soil	7.7	0.61	6.19	7.99	2.57	0.11
	7.0	0.57	7.69	7.61	2.62	0.15
Average	7.3	0.55	6.85	7.95	2.64	0.13
SD	0.37	0.08	0.76	0.33	0.08	0.02
CV	5.04	14.93	11.17	4.12	2.89	15.31

The main mechanisms responsible for the pH in the rhizosphere have been classified by Hinsinger *et al.* [31]. Among these one of the most important is the release of large amounts of citrate by lupine roots [22] [32]. The pK for citrate $^{2-}$/citrate $^{3-}$ is 6.40 which is feasible for reacting with free H$^+$ in the soil solution at relative low pH. Hence in acidified soils (pH ≤ 5.5) the presence of citrate could contribute to an increase in the rhizosphere pH [31]. The decrease in the concentrations of soluble elements in the presence of plants could be due to either plant uptake or plant interaction with the soil components or both, being the pH the most important factor [19] [21].

3.2. Toxicity Symptoms on *L. microcarpus* Due to As Concentration in Soils

Figure 5(a) shows variation of height of lupine plants during the assay period. Experimental plant 1 and 2, grew during the first 4 month of the assay period and then stop their growth, decreasing during the last month, compared to the control which showed a steady growth especially in control plant 2, in the last 3 months of the study.

Regarding to the number of leaves, **Figure 5(b)** shows the increase of leaves in the experimental plants 1 and 2 during the first month of assay and then an increase in plant 1 until the end of the study. With respect to the control lupine plants 1 and 2, the number of leaves increase progressively until the final of the study especially in control plant 1.

These results demonstrate that the arid soils with high As-levels where lupine grew are reduced both, growth and foliar biomass. At higher concentrations, As interfere with plant metabolic process and can inhibit growth, often leading to death [33].

Other authors have also reported an increase in growth of rice (hydroponic culture) after treatments with dimethylarsinic acid [34]. Carbonell-Barrachina *et al.* [35], reported an increase in tomato plant growth after arsenite and arsenate treatments at concentrations of 2 and 5 mg·L^{-1}. However, this author also reported a significant decrease in the total biomass production of *Spartina patens* compared to the control when plants were grown in hydroponic culture with 2 mg·L^{-1} of arsenate during 30 days. Probably *S. patens* plants could have been also more dramatically affected if the study had had a longer duration. In this direction, Del Río *et al.* [36] observed that after 8 weeks of growth affected by As treatments a significant decrease in the total biomass production in *A. blitoides* compared to the control and reached its minimum at the highest treatment of As (10 mg·kg^{-1}). Similar results on plant growth in *M. communis*, *A. unedo* and *R. sphaerocarpa* which was reduced when As concentrations increased in plant organs [37]. A greater growth inhibition and toxicity in roots, compared to shoots, has been reported as a common effect of As supply in other plant species [38].

Studies on arsenate (the dominant form of As phytoavailable in aerobic soils) toxicity have shown that plant species not resistant to As like lupine, suffer considerable stress upon exposure to arsenate, with symptoms ranging from inhibitions of root growth through to death [39] [40]. As(III) has a high toxicity on radicular membranes, because it reacts with sulphyldryl groups of proteins, leading to the disruption of root functions, and even cellular death [35]. There is significant evidence that exposure to inorganic As-species results in the gene-
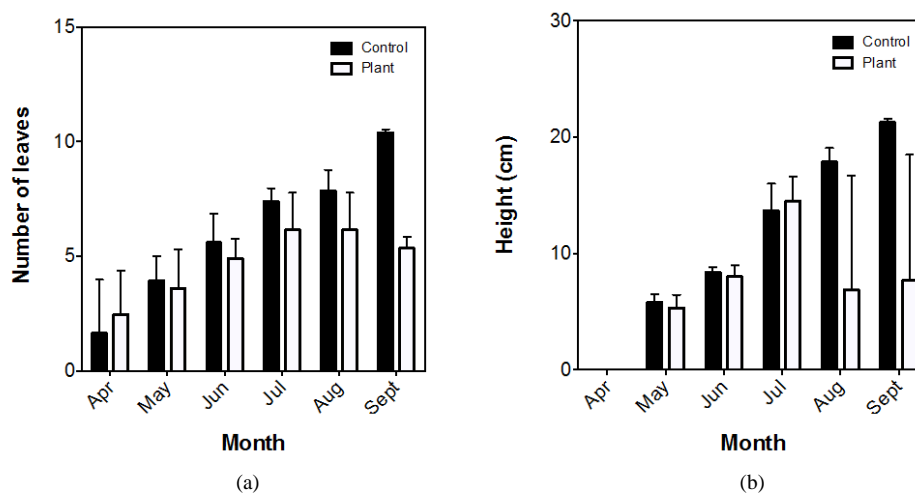

Figure 5. Height (a) and number of leaves (b) of lupin during the assay period.

ration of reactive oxygen species (ROS) [41]. This probably occurs through the conversion of arsenate to arsenite, a process which readily occurs in plants, and leads to the synthesis of enzymatic antioxidants such as superoxide dismutase (SOD), catalase and glutathione-S-transferase [9] [41] [42]. Inorganic As-species are generally highly toxic to plants. Arsenate act as a phosphate analogue and is transported across the plasma membrane via phosphate cotransport systems. This competition results in a reduction on their absorption by soil and an increase in solution concentration [43]. Once inside the cytoplasm it competes with phosphate, for example replacing phosphate in ATP to form unstable ADP-As and leads to the disruption of energy flows in cells [44]. However, arsenate will not normally have enough cytoplasmatic concentrations to exert toxicity-groups (-SH) of enzymes and tissue proteins leading to inhibition of cellular function and death [9] [45].

Probably, the biochemical mechanisms before mentioned could have been involved in the As-toxic effects observed in *L. microcarpus*, including the death in the majority of the experimental plants analyzed at the end of the assays. As was established by Carbonell-Barrachina *et al.* [46] both, As (used as $NaAsO_2$) and salinity (used as NaCl) influenced plant growth negatively, although the As effects were more evident.

To avoid influences from chemical fertilizers and other factor, in this research, lupine plants were grown without fertilizer addition, indicating the importance of nitrogen fixation by root nodules that would be reduced by the presence of As. This would partly explain the reduction in biomass, which was demonstrated by Carpena *et al.* [47].

Figure 6 shows the results of visual As-toxicity symptoms analyzed: foliar chlorosis, necrosis of the leaf tips and margins, leaf wilting and stunting. Lupine plants that grew on As-contaminated soils, showed all toxicity symptoms above indicated, throughout the study period, especially foliar chlorosis (**Figure 6(a)**). Other toxicity symptoms studied such as leaf wilting (**Figure 6(b)**), leaf necrosis (**Figure 6(c)**) and stunting (**Figure 6(d)**). It is important to point out that neither of control lupine plants suffered any toxicity symptoms.

Arsenic toxicity has been described by Marin *et al.* [34] as consisting of root plasmolysis and leaf wilting followed by root discoloration and necrosis of the leaf tips and margins. On the present study, experimental plants growing in As-soils were stunted with foliar chlorosis and necrosis of leaf tips and margins. These symptoms suggest a restriction in water movements into the plant, showing that these As-treated plants, were suffering from water stress [46]. This situation is not observed on plants species resistant to As. Tapia *et al.* [14] studied the xerophytic and halophytic shrubs *Atriplex halimus* and *Atriplex atacamensis* cultivated in As-soil which grew normally and did not show visual symptoms of toxicity. Additionally, neither the dry weight nor height on both plants was negatively affected. *A. halimus* cultivated in As-soil showed a dry weight and height significantly higher than the plants cultivated in control soil. At the end of the assay there were no significant difference between the weight and height of *A. atacamensis* cultivated in the control soil compared to *A. halimus* cultivated in the As-soil. Therefore, it may be assumed that As-resistant plants either compartmentalize and/or transform As to other less phytotoxic As-species, to withstand high cellular As-burdens [48].

The toxicity of As is dependent on its speciation, with inorganic arsenicals thought to be more toxic than organic forms [9] [49]. In this sense, a decrease in chlorophylls levels has been previously reported for arsenate, due to the inhibition of pigment biosynthesis. In this sence, the chlorophyll suffered a small decrease of a 23% in *R. sphaerocarpa* for the highest As-levels, while in *A. unedo* decreased up to 32% from the 50 µM As dose [37].

4. Conclusions

Lupinus microcarpus grown in natural soil showed visual symptoms of toxicity and exhibiting decreased growth and foliar biomass. Foliar chlorosis, necrosis of the leaf tips and margins, leaf wilting and stunted, are the main arsenic-toxic effect observed. The results indicate that this plant does not resist contamination and accumulated higher levels of As in roots. Lupine plants can be used to remediate agricultural soil polluted with As by mechanisms other than phytoextraction. Lupine plants can be used in the phytostabilization of As immobilizing it mainly in roots during each culture cycle. In addition, it is a N_2-fixing legume and increases microbial activity in soils. The harvesting of the plants (at the adequate time), produces a little As-extraction that can contribute to the general improvement in agricultural soil affected by high arsenic concentration.

These results confirm abnormalities in plant growth with As-contaminated soils, where the phytotoxic effect is observed. Hence, a need exists to develop a mathematical model for predicting dynamic uptake, translocation, accumulation, and mobilization of arsenic in the soil-plant system.
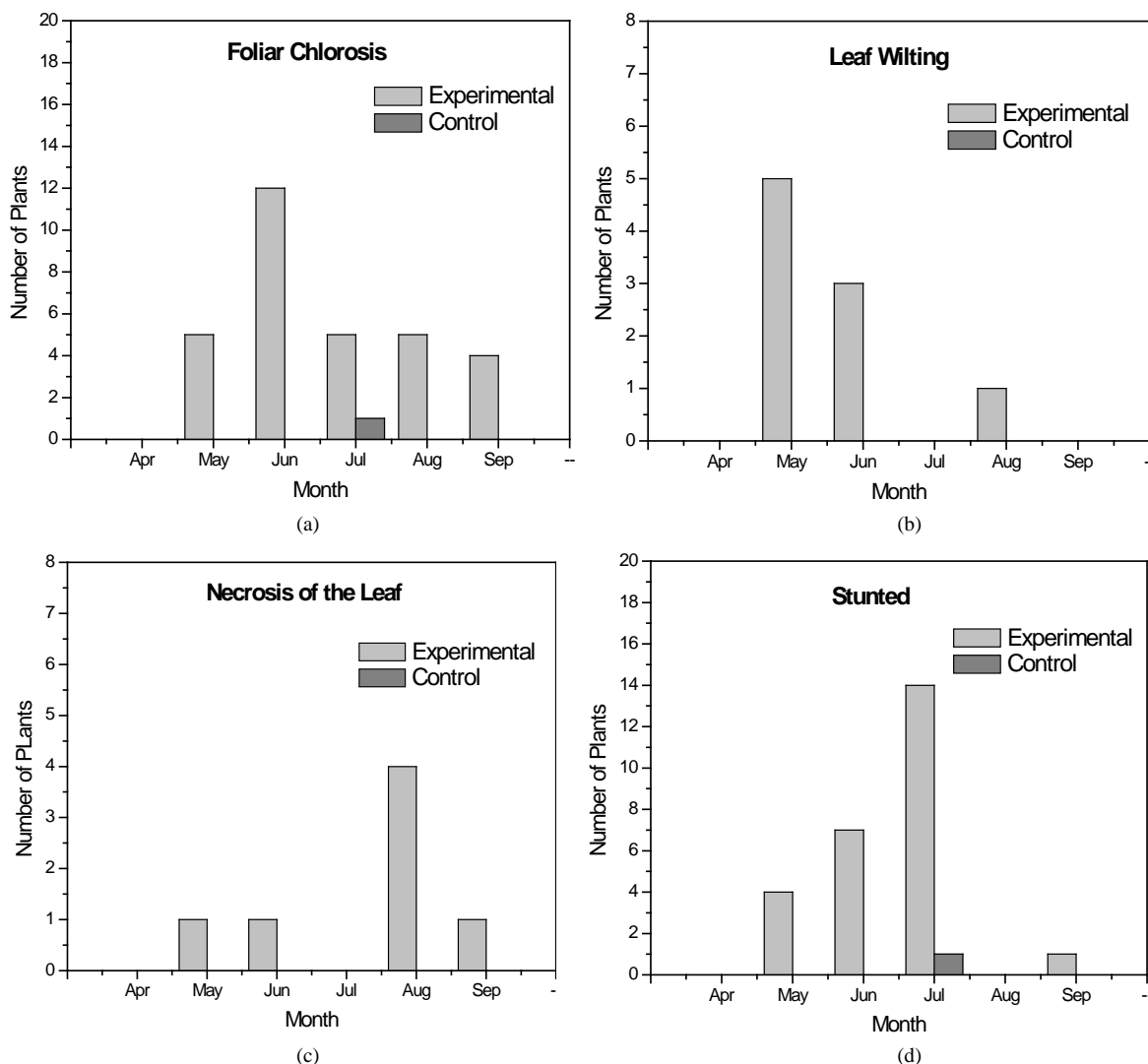
Figure 6. As-toxic effects in lupin.

Acknowledgements

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